Funded by
the European Union

ERASMUS+ / ADULT EDUCATION

BIOMED ADU

Learning materials

Raising the awareness and increasing the competences of Adult learners on Microbiome applications

---

Project reference
2023-2-MT01-KA210-ADU-000177505

Action
Small-scale partnership in adult education (KA210-ADU)

Project period
1 January 2024 to 30 June 2025

Coordinator
The BioArte Limited, Malta

Project partners
University of Siena and Siena Bio Active Srl, Italy

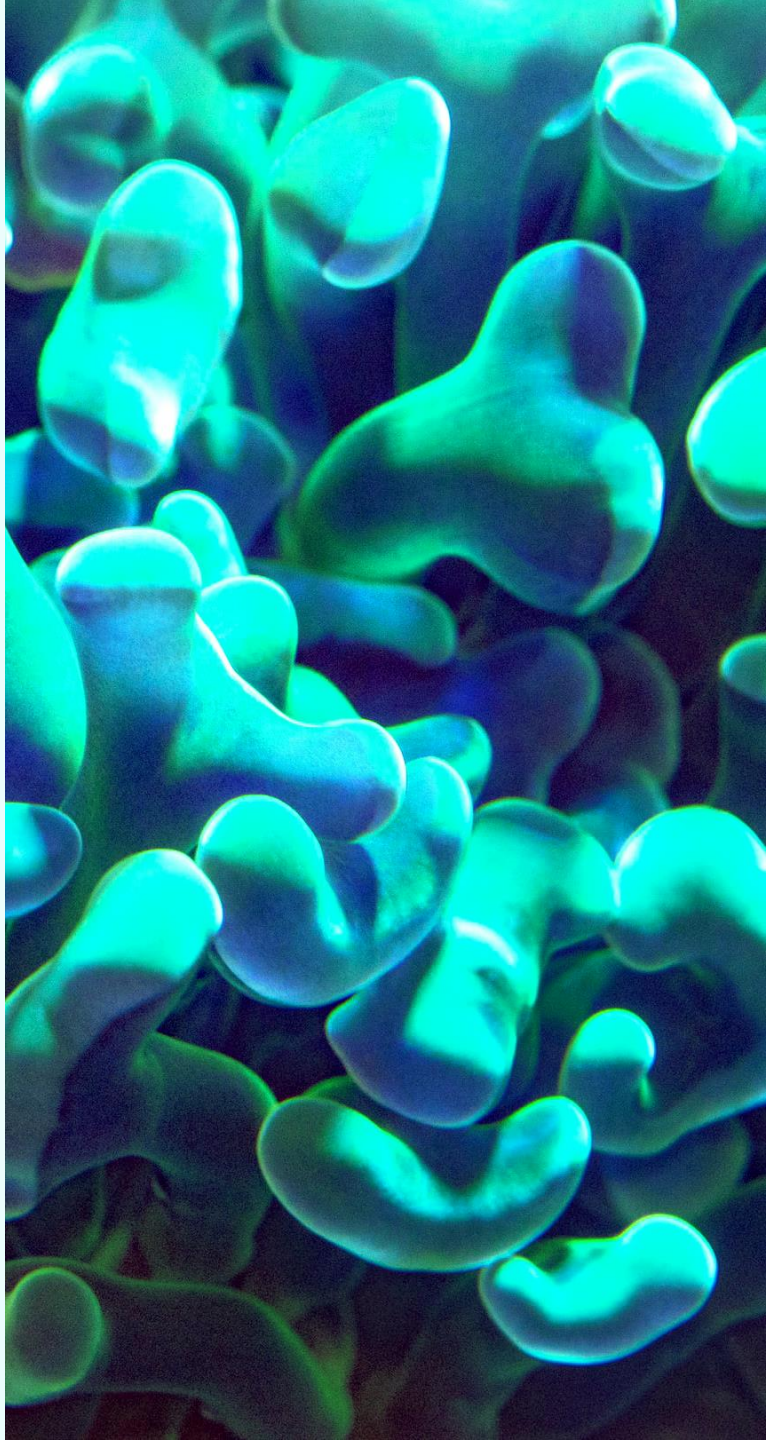
Granting authority
European Union Programmes Agency (EUPA), Malta

Funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Union Programmes Agency (EUPA). Neither the European Union nor the granting authority can be held responsible for them.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Post-graduate certificate level 7 (30 ECTS)

BREATH TESTS



Malta Further & Higher Education Authority

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Table of content

- Introduction – What are breath tests?
- Procedure of the examination
- Usage of breath tests for GI conditions
- Examples explained with figures
- Techniques and interpretation of results
- Limitations of Breath tests
- Conclusion and take away message

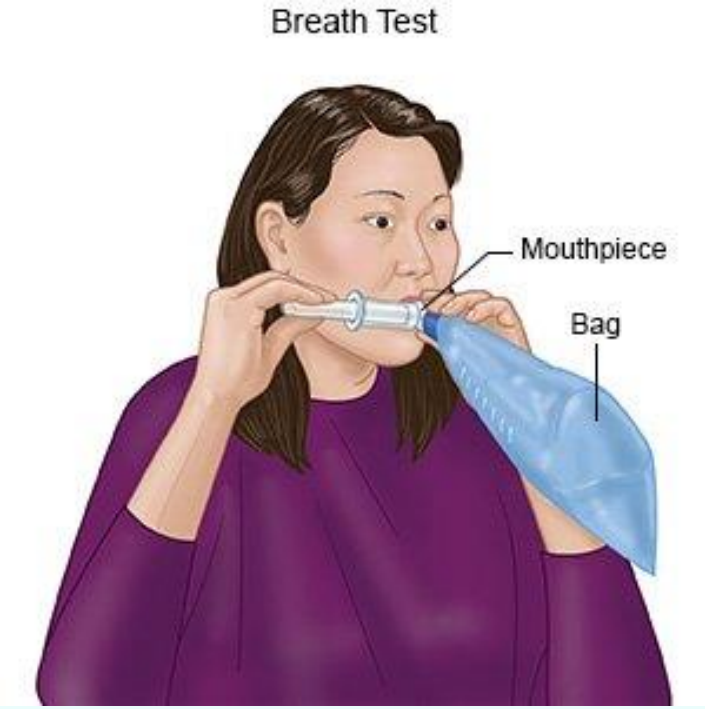
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

What are breath tests?

- A non-invasive way that measures the levels of certain gases in your breath to determine the presence of various digestive disorders.

- Prior to the test the patient has to follow specific guidelines.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



BEFORE THE EXAMINATION

- ✓ Patients typically follow a **specific diet** before the test.
- ✓ During the test, patients drink a substrate.
- ✓ Th test begin, exhale into the bag for collection of gases.
- ✓ These breath samples are sent to a laboratory for analysis.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

What conditions can we detect?

- H. pylori Infection → urea breath test → CO_2
- Carbohydrate Intolerance → lactose or fructose intolerance detecting mainly H, CH_4
- Bacterial Overgrowth Syndrome → (H, CH_4)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

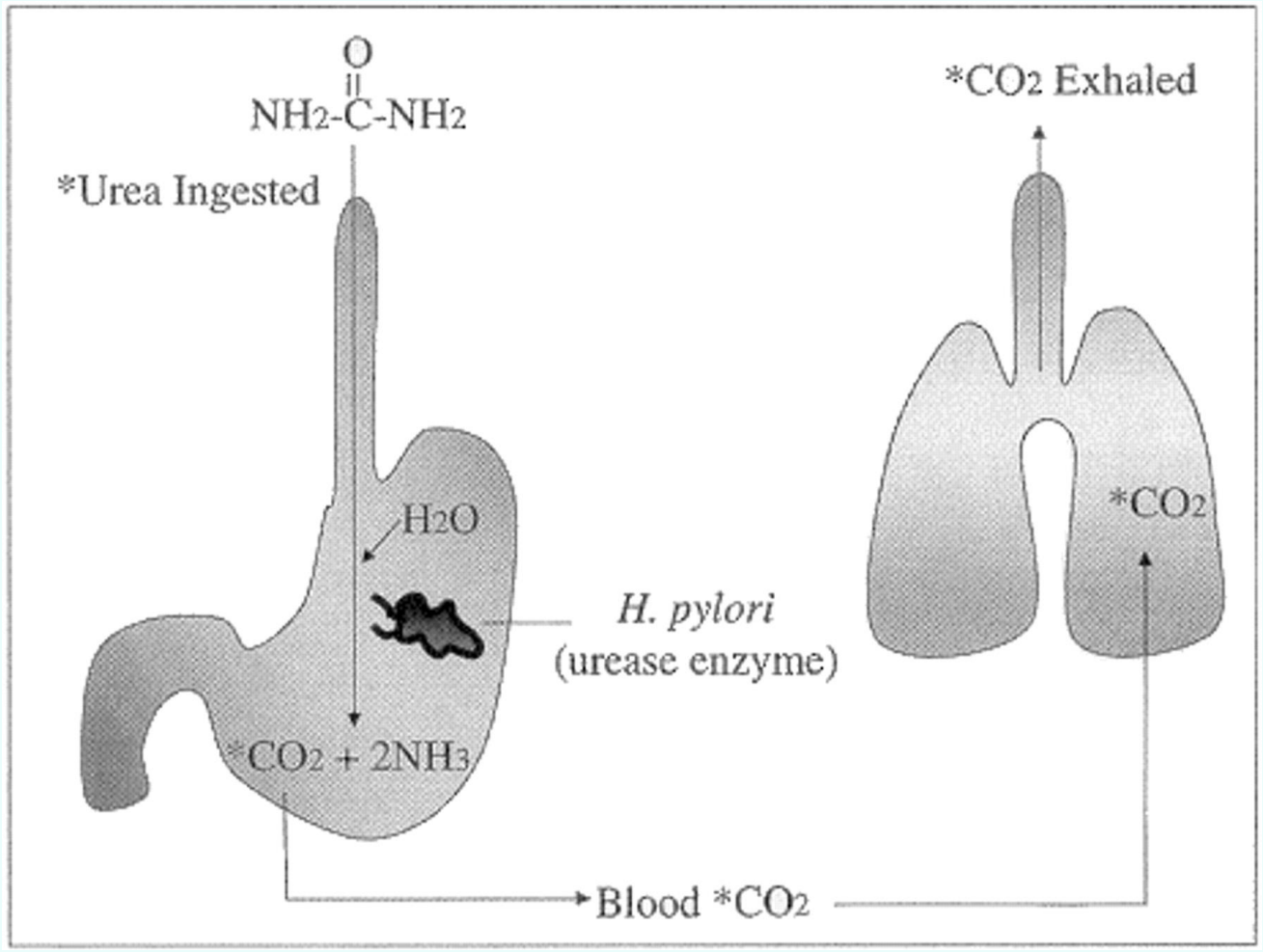


Figure 1. The urea breath test for *Helicobacter pylori* infection: taking the wind out of the sails of endoscopy
Carlo A. Fallone, Sander J.O. Veldhuyzen van Zanten, Naoki Chiba
CMAJ Feb 2000, 162 (3) 371-372;

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Test sugar (liquid solution with lactose)

Bacterial fermentation produce Hydrogen and Methane gas

Gases absorbed in bloodstream → to the lungs

Concentrations of exhaled breath Hydrogen and Methane are measured

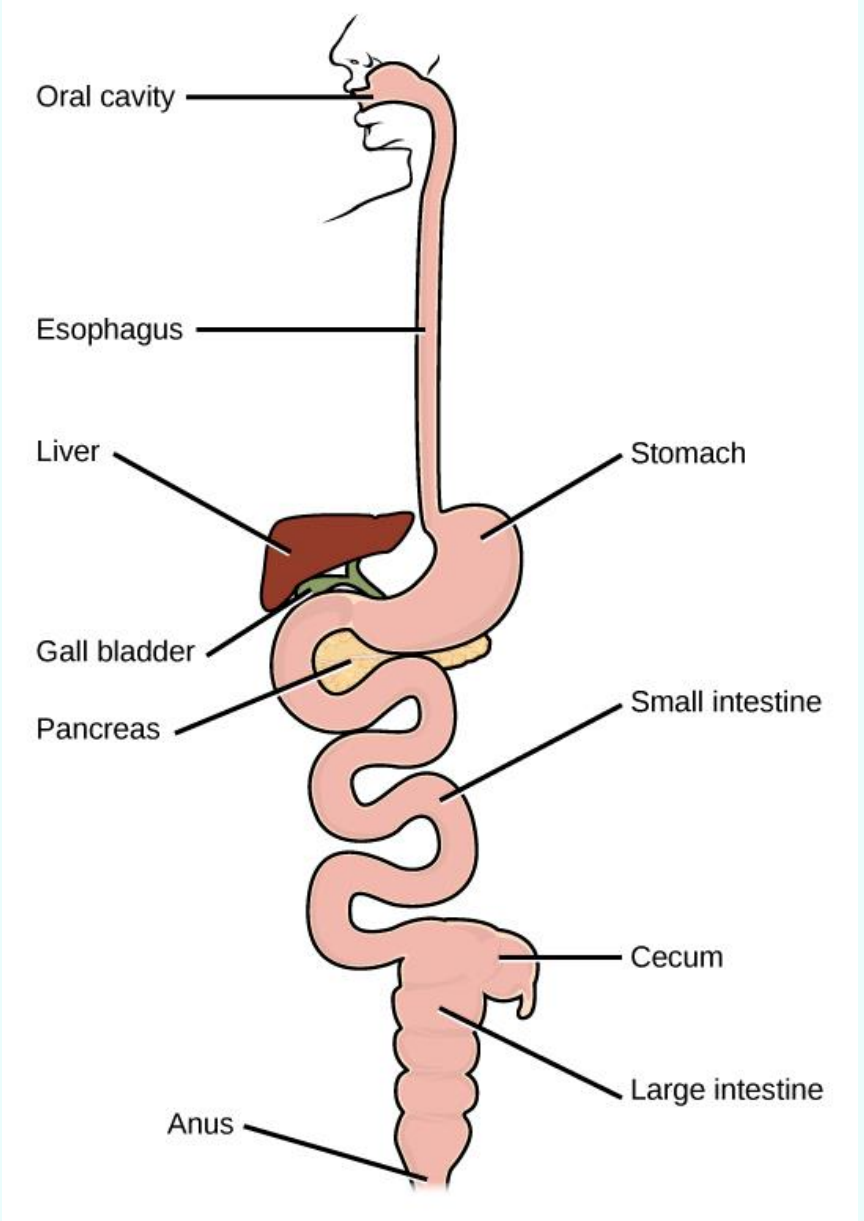
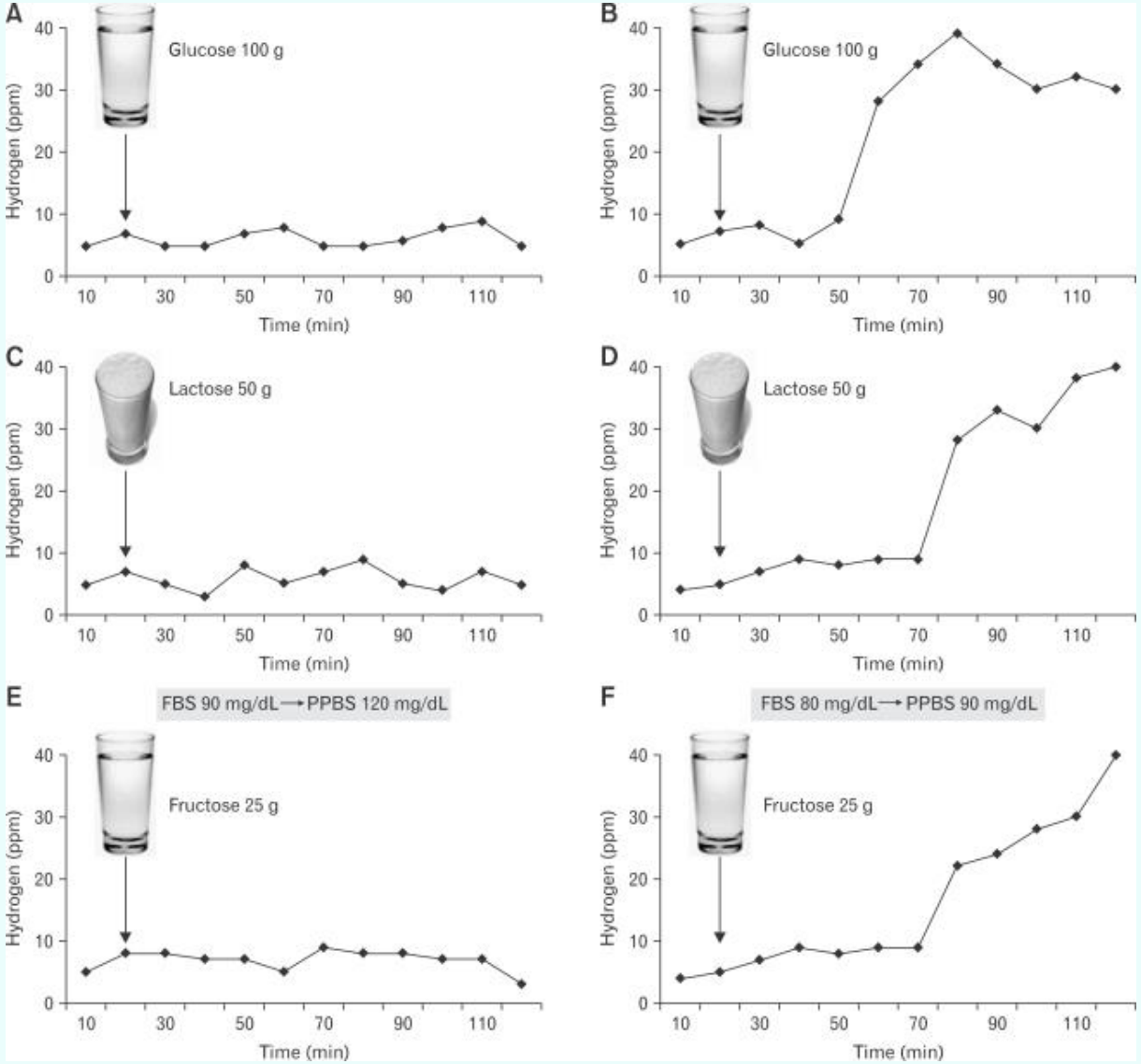


Figure 2. GI tract parts (own source)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Ghoshal UC. How to interpret hydrogen breath tests. J Neurogastroenterol Motil. 2011 Jul;17(3):312-7. doi: 10.5056/jnm.2011.17.3.312. Epub 2011 Jul 14. PMID: 21860825; PMCID: PMC3155069.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Techniques used and interpretation of results

- A 20–30 cc volume of end-expiratory air is immediately collected and submitted for analysis using a *gas chromatography* to detect *hydrogen, methane, and, if available, carbon dioxide*.
- Gas concentration data are reported in parts per million (ppm).
- *UBT (urea breath test)* relies on the ability of H. pylori to convert into carbon dioxide urea that has been labelled with isotopes and then ingested by the patient. The difference in carbon dioxide levels between the baseline breath sample and the post-administration breath sample is detected by *mass spectrometer or infrared spectrophotometer*.
- The ^{13}C isotope is not radioactive and thus is more frequently used compared to ^{14}C.

- H. pylori → Following 30 min of administration (50 mg 13C isotope labeled urea with 80–100 mL of water.) a delta over baseline (DOB) value of 4% or above was regarded as a positive indicator of H. pylori infection.
- Carbohydrate intolerance → A rise of ≥20 ppm from baseline in hydrogen during the test is generally considered positive for carbohydrate intolerance (fructose and lactose breath tests).
- SIBO → A hydrogen gas rise of ≥20 ppm from baseline by 90 minutes as a positive test result for SIBO in both clinical and research settings. A methane gas rise ≥10 ppm at any time during the study period is also defined as positive breath test for SIBO.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Limitations of breath tests

- Indirect assessment of microbial overgrowth
- Patient's adherence to guidelines and rules
- Variations in oro-cecal transit time (OCTT) affects the interpretation of SIBO test
- Conditions that impair delivery of the carbohydrate substrate to the small intestine can result in *false-negative results*
- Lack of Standardization
- Limited Clinical Adoption

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Conclusion

- As with any medical examination, breath testing has its constraints, demanding careful consideration and contextual interpretation of results. Nonetheless, it presents numerous merits. Its capacity to discern unique breath test patterns not only aids in forecasting treatment outcomes but also facilitates the customization of antibiotic regimens.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Take away messages

- Breath tests represent a **valuable diagnostic tool** in the realm of medicine.

- As technology advances and research continues to expand, breath tests are poised to further **contribute to early disease detection**.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

- The urea breath test for Helicobater pylori infection: taking the wind out of the sails of endoscopy. Carlo A. Fallone, Sandor J.O. Veldhuyzen van Zanten, Naoki Chiba CMAJ Feb 2000, 162 (3) 371-372

- Best LM, Takwoingi Y, Siddique S, Selladurai A, Gandhi A, Low B, Yaghoobi M, Gurusamy KS. Non-invasive diagnostic tests for Helicobacter pylori infection. Cochrane Database Syst Rev. 2018 Mar 15;3(3):CD012080. doi: 10.1002/14651858.CD012080.pub2. PMID: 29543326; PMCID: PMC6513531.

- Ghoshal UC. How to interpret hydrogen breath tests. J Neurogastroenterol Motil. 2011 Jul;17(3):312-7. doi: 10.5056/jnm.2011.17.3.312. Epub 2011 Jul 14. PMID: 21860825; PMCID: PMC3155069.

Lim J, Rezaie A. Pros and Cons of Breath Testing for Small Intestinal Bacterial Overgrowth and Intestinal Methanogen Overgrowth. Gastroenterol Hepatol (N Y). 2023 Mar;19(3):140-146. PMID: 37706108; PMCID: PMC10496284.

- Tansel A, Levinthal DJ. Understanding Our Tests: Hydrogen-Methane Breath Testing to Diagnose Small Intestinal Bacterial Overgrowth. Clin Transl Gastroenterol. 2023 Apr 1;14(4):e00567. doi: 10.14309/ctg.0000000000000567. PMID: 36744854; PMCID: PMC10132719.

- Ling D. Carbon-13 urea breath test for Helicobacter pylori infection in patients with uninvestigated ulcer-like dyspepsia: an evidence-based analysis. Ont Health Technol Assess Ser. 2013 Oct 1;13(19):1-30. PMID: 24228082; PMCID: PMC3818027.

- Wang, X., Zhang, S., Chua, E.G. et al. A re-testing range is recommended for 13C- and 14C-urea breath tests for Helicobacter pylori infection in China. Gut Pathog 13, 38 (2021). https://doi.org/10.1186/s13099-021-00435-3

STUDY MATERIAL

- https://academic.oup.com/clinchem/article/52/5/800/5626826

- Breath Analysis: An Approach for Smart Diagnostics | SpringerLink

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thank you for your kind attention!

Breath tests 25/03/2024

Donjeta Shala

- **Institute of Microbiome and Applied Sciences**
- **For admin questions contact: shala.dnjta@gmail.com**
- **Type of course: Post-graduate certificate level 7 (30 ECTS)**
- **Breath tests**
- **Dietitian Donjeta Shala, MSc in Food Safety and Quality Engineering**

1. Introduction

Breath tests offer a noninvasive method for diagnosing various conditions by analyzing specific gases present in a patient's breath. Prior to the test, individuals may be required to follow specific instructions such as consuming particular liquids and refraining from certain activities. These tests can aid in the diagnosis of conditions such as carbohydrate intolerance (lactose or fructose), bacterial overgrowth syndrome, and H. pylori infection, providing a quick and straightforward diagnostic approach.

In this discussion, we'll delve into understanding the mechanics of breath tests, highlighting the significance of gases in the diagnostic process. We'll also underscore the merits and drawbacks of utilizing breath tests, along with exploring their potential future advancements.

Breath tests serve a range of diagnostic purposes, including identifying:

H. pylori Infection: Breath tests diagnose H. pylori infection by detecting specific compounds like carbon dioxide or urea in exhaled breath following the ingestion of a labeled substrate.

Carbohydrate Intolerance: These tests diagnose conditions like lactose or fructose intolerance by measuring the levels of hydrogen and methane gases, which increase when carbohydrates ferment due to gut bacteria.

Bacterial Overgrowth Syndrome: They detect bacterial overgrowth in the small intestine by measuring hydrogen and methane levels, indicative of bacterial fermentation of carbohydrates.

Through analyzing exhaled breath composition, these tests provide a noninvasive and user-friendly approach for diagnosing various gastrointestinal conditions.

Understanding how the breath test works

- ✓ Patients typically follow a specific diet before the test.
- ✓ During the test, patients drink a substrate (usually a specific type of sugar or other compounds).
- ✓ They then exhale into a collection tube at regular intervals to provide breath samples.
- ✓ These breath samples are sent to a laboratory for analysis.

- ✓ Certified laboratory technicians analyze the samples to detect specific gases or compounds.

2. ***Role of gut microbiome for breath tests***

As we breathe out, numerous chemical compounds are expelled into the air through your breath. Among these are volatile organic compounds (VOCs), which are carbon-based chemicals formed from metabolic processes in your body. Intriguingly, a portion of these compounds originates from the metabolic activities of the vast array of microbial species residing within us, numbering over 10,000. These microbial metabolites essentially mirror shifts in our metabolic processes, offering a snapshot of our physiological state. A recent study showed that investigated the relation between volatiles in breath and gut microbiome in active and quiescent Crohn's disease (CD) via a multivariate statistical approach showed that Volatile organic compounds present in breath are linked, either directly or indirectly, to activities within the gut microbiome (Smolinksa et al., 2018). This relationship is sensitive to changes observed in active and inactive states of gastrointestinal diseases implying that there are multiple underlying mechanisms involved.

3. ***Clinical evidences***

UBT (Urea Breath Test) in diagnosis of H.pylori

Helicobacter pylori is a bacterium that can cause stomach infections. Helicobacter pylori (H. pylori) is characterized by its ability to thrive in low oxygen environments. It is a gram-negative, spiral-shaped bacillus that infects over 50% of the global population. The primary method for confirming the presence of H. pylori is esophagogastroduodenoscopy (EGD) with biopsy examination, which is considered the gold standard. However, EGD is invasive and costly, particularly for younger patients with dyspepsia and no alarming symptoms. As an alternative, the urea breath test (UBT) is valuable for diagnosing H. pylori and for assessing post-treatment status. H. pylori possesses urease, an enzyme absent in mammalian cells, which it uses to break down urea into ammonia and carbon dioxide (CO_2). This ammonia aids H. pylori in neutralizing stomach acid, facilitating its colonization. The UBT capitalizes on this by administering urea containing a labeled carbon (C) isotope. When H. pylori hydrolyzes the urea, the labeled carbon is incorporated into CO_2, which is subsequently exhaled via the lungs. UBT typically employs two carbon isotopes: radioactive ^{14}C and stable non-radioactive ^{13}C. Both versions of the test exhibit similar performance, boasting sensitivities exceeding 95% and specificities surpassing 93% (Mj Best et al., 2018) Prior to undergoing the urea breath test (UBT), patients are advised to stop taking antibiotics and bismuth compounds for at least four weeks, and proton pump inhibitors (PPIs) and sucralfate for at least two weeks. These medications can lower the urease activity of H. pylori, potentially leading to false negative test results. If a positive result is obtained, it can be reliably considered as truly positive, indicating the need to initiate treatment. However, if the result is negative, patients should discontinue these medications as instructed and repeat the UBT.

Hydrogen Breath test for detection of lactose and fructose maldigestion

Lactose malabsorption occurs when there is a decrease in the production or effectiveness of lactase enzyme in the small intestine. This condition is prevalent and results in lactose intolerance where individuals experience abdominal discomfort such as nausea, bloating, and pain after consuming dairy products while fructose is a naturally occurring sugar present in various fruits, vegetables, and sweeteners an in recent years, there has been a notable increase in fructose consumption, potentially contributing to stomach-related issues. During a hydrogen breath test patient will breathe into a balloon-type container that measures the amount of hydrogen in your breath then he/she will drink a flavored liquid that contains lactose and breathe into the container again 30 minutes later, and every 30 minutes after that for three to four hours. Patient's hydrogen levels will be measured each time. Studies have found that unlike lactose, breath tests (BTs) for other sugars like fructose and sorbitol don't provide significant diagnostic benefits in guiding the management of these carbohydrates compared to simply adjusting the diet (Sata et al., 2021). However, conducting a blind sugar challenge remains the most effective way to objectively show a clinical intolerance to carbohydrates, even though it's challenging to implement in clinical settings.

Hydrogen/Methane for detection of SIBO (Small Intestinal Bacterial Overgrowth)

In general, when there's bacterial overgrowth in the small intestine, as in Small Intestinal Bacterial Overgrowth (SIBO), excessive fermentation of carbohydrates leads to a significant increase in hydrogen gas. In patients with Small Intestinal Bacterial Overgrowth (SIBO), higher levels of hydrogen (H_2) and methane (CH_4) gases are anticipated because of impaired digestion or absorption, leading to increased fermentation of ingested substances by bacteria. The preferred method for diagnosing Small Intestinal Bacterial Overgrowth (SIBO), which involves directly culturing the contents of the small intestine, is challenging due to its technical complexity, invasiveness, high cost, and uncertainties regarding the appropriate thresholds for bacterial counts. Consequently, hydrogen/methane breath tests are favored for diagnosing SIBO due to their practicality and effectiveness. The hydrogen diffuses into the bloodstream and is exhaled through the lungs. During a hydrogen breath test, patients fast overnight and follow a low-fiber diet for a day. Similar as above they exhale into a tube connected to a bag to establish baseline hydrogen levels before consuming a fermentable substrate, such as glucose, lactulose, or xylose. Breath samples are then collected every 15 minutes for three hours. Among various hydrogen breath tests, the *lactulose hydrogen breath test* is the most commonly used for diagnosing SIBO. After ingesting lactulose, which is fermented by intestinal flora, breath samples are collected at 15-minute intervals for three hours. The criteria for a positive breath test are not standardized, but typically involve observing a significant increase in hydrogen levels (greater than 20 parts per million) along with a double peak on the breath hydrogen expiration graph. The first peak indicates bacterial overgrowth in the small intestine, while the second peak results from lactulose fermentation by cecal flora. It's generally accepted that the first rise in hydrogen occurs within 90 minutes of lactulose ingestion. (Sachdev and Pimentel, 2013). Although the criteria for a positive test are not fully validated, hydrogen breath tests are still widely used due to their simplicity and cost-effectiveness. They are useful for both screening and monitoring therapy for conditions like SIBO.

4. ***Critical thinking. What can be learned from what has been discovered so far?***

Generally speaking, early detection of diseases is vital for managing patients effectively. Nevertheless, current diagnostic methods encounter issues like patient compliance, causing delays in diagnosis. That's why it's crucial to create non-invasive diagnostic tools. Breath analysis shows to be a promising method is detecting gas-based biomarkers such as the volatile organic compounds and inorganic gases in exhaled

breath. Analytical techniques and the sensor technology are often used for identifying gas patterns, and the recent studies in research are focused on integrating advanced nanomaterials into sensor development to increase performance for various applications, including selective and cross-reactive detection.

5. Future perspectives

Current breath testing methods allow for assessing various gastrointestinal conditions such as gastric emptying, H. pylori infection, small intestinal bacterial overgrowth (SIBO), and malabsorption of lactose and fructose. However, studying the small intestine is challenging due to its location, inaccessible by conventional endoscopic procedures. New volatile organic compound (VOC)-based methods could greatly aid in comprehending small intestinal bacterial overgrowth (SIBO) and characterizing bacterial overgrowth by analyzing the types of bacteria present and the VOC profile they produce. This could offer insights into why SIBO recurs rapidly after antibiotic treatment in certain individuals, posing challenges for eradication efforts. Looking ahead, breath tests offer several future opportunities: 1) Expanding volatile compound detection: innovations like Owlstone Medical's Breath Biopsy® technology could enhance the volatile compound detection, potentially transforming digestive health assessment. 2) Personalized medicine: discovering more VOCs linked to specific gastrointestinal conditions may enable personalized breath profiles, guiding individualized interventions. 3) Early disease detection: routine breath tests could facilitate early detection of gastrointestinal diseases, allowing for prompt treatment. 4) Monitoring treatment efficacy: regular breath tests during treatment could evaluate response and inform adjustments to optimize treatment outcomes. 5) integration with gut microbiome research: connecting breath biomarkers with gut microbiome composition may improve diagnostic accuracy and deepen understanding of gastrointestinal conditions and lastly 6) Gastric cancer screening: breath analysis holds promise for screening gastric cancers, potentially streamlining diagnostic procedures for at-risk patients.

As a conclusion, breath testing provides a simple, non-invasive approach to explore gastrointestinal health. As thresearch progresses, we anticipate more accurate and comprehensive diagnostic tools for digestive disorders in the future.

References

Best LM, Takwoingi Y, Siddique S, Selladurai A, Gandhi A, Low B, Yaghoobi M, Gurusamy KS. Non-invasive diagnostic tests for Helicobacter pylori infection. Cochrane Database Syst Rev. 2018 Mar 15;3(3):CD012080. doi: 10.1002/14651858.CD012080.pub2. PMID: 29543326; PMCID: PMC6513531.

Usai-Satta P, Oppia F, Lai M, Cabras F. Hydrogen Breath Tests: Are They Really Useful in the Nutritional Management of Digestive Disease? Nutrients. 2021 Mar 17;13(3):974. doi: 10.3390/nu13030974. PMID: 33802839; PMCID: PMC8002624.

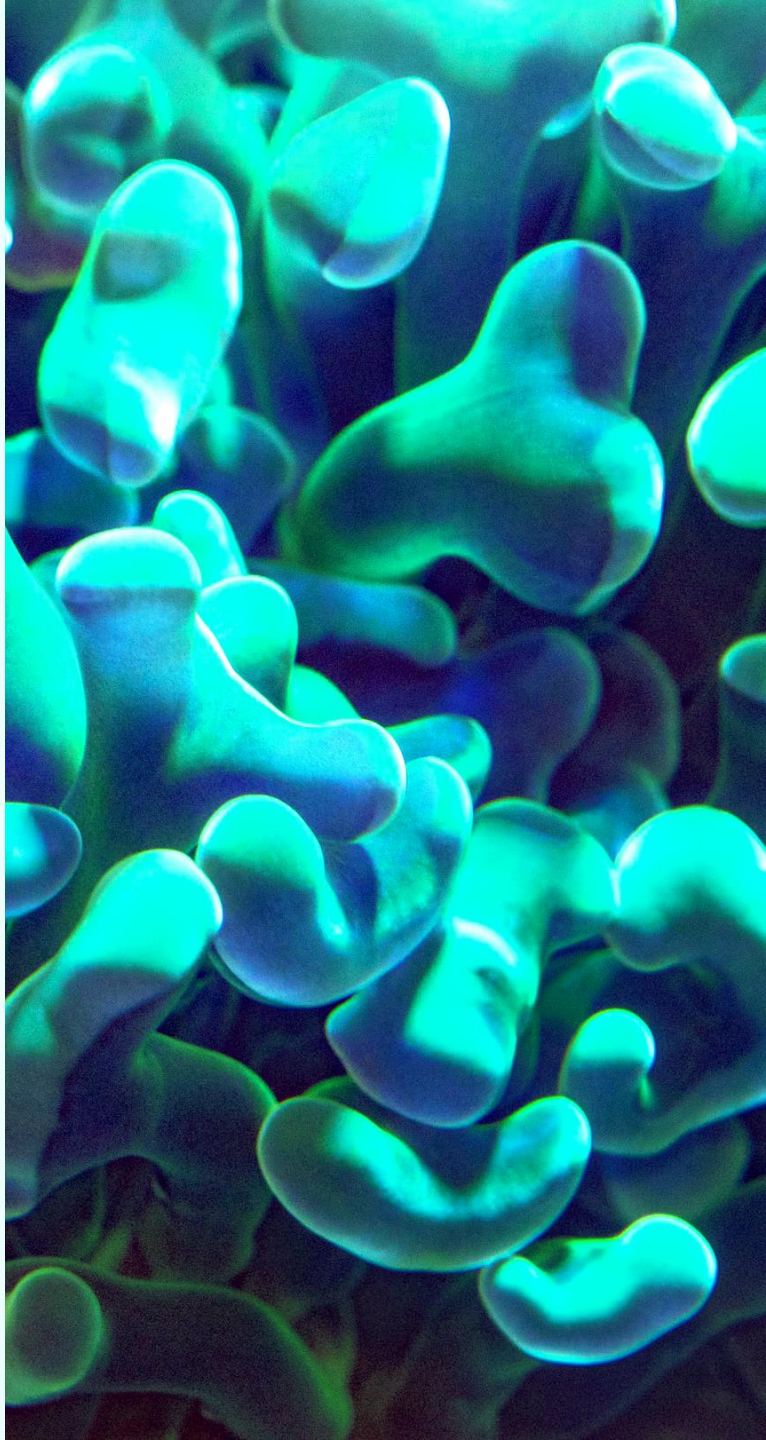
Sachdev AH, Pimentel M. Gastrointestinal bacterial overgrowth: pathogenesis and clinical significance. Ther Adv Chronic Dis. 2013 Sep;4(5):223-31. doi: 10.1177/2040622313496126. PMID: 23997926; PMCID: PMC3752184.

Smolinska A, Tedjo DI, Blanchet L, Bodelier A, Pierik MJ, Masclee AAM, Dallinga J, Savelkoul PHM, Jonkers DMAE, Penders J, van Schooten FJ. Volatile metabolites in breath strongly correlate with gut microbiome in CD patients. Anal Chim Acta. 2018 Sep 26;1025:1-11. doi: 10.1016/j.aca.2018.03.046. Epub 2018 Mar 30. PMID: 29801597

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Post-graduate certificate level 7 (30 ECTS)

CHRONIC CONSTIPATION AND DIARRHEA



Malta Further & Higher Education Authority

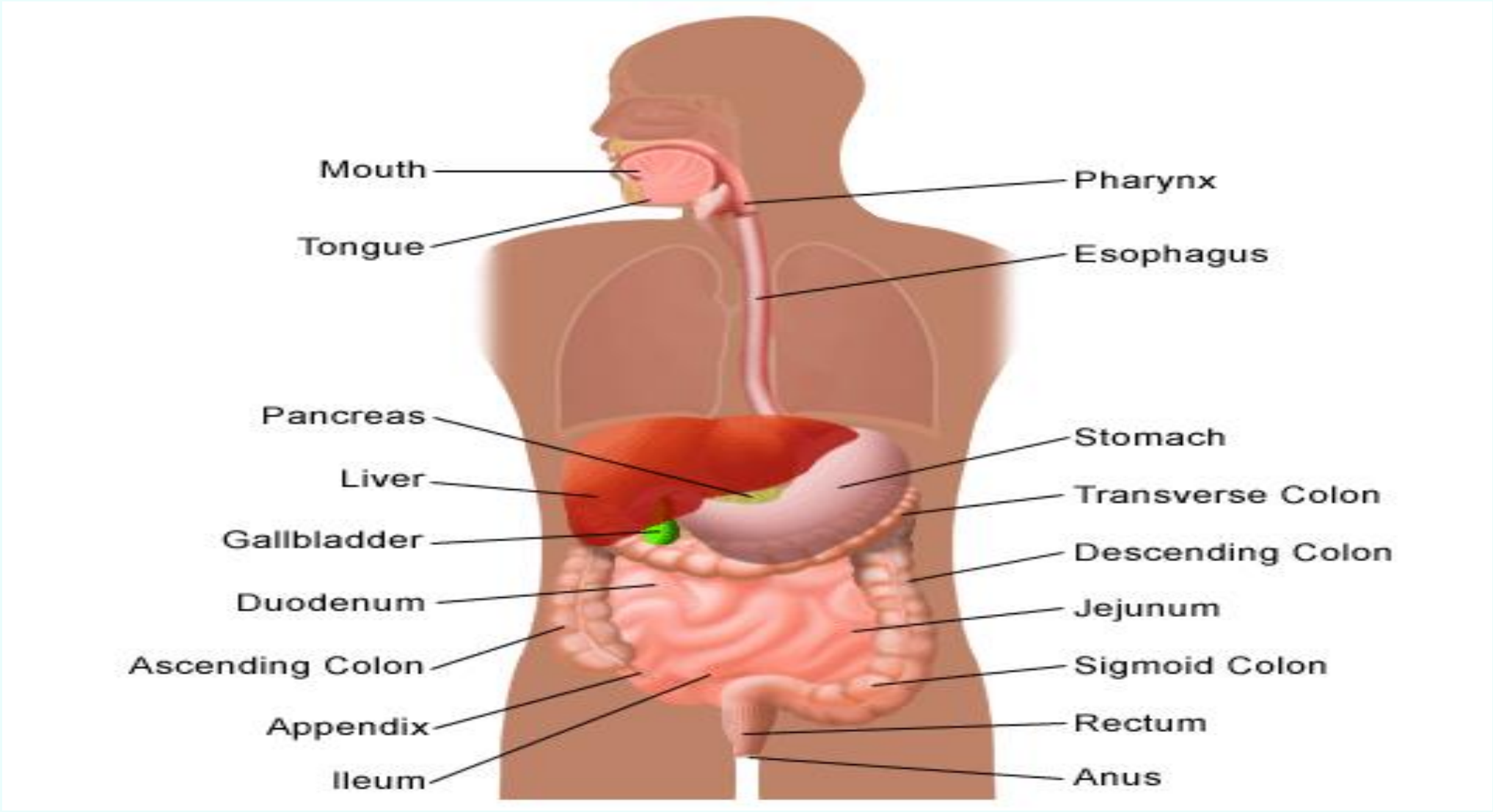
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation and diarrhea

Illustration of digestive system

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

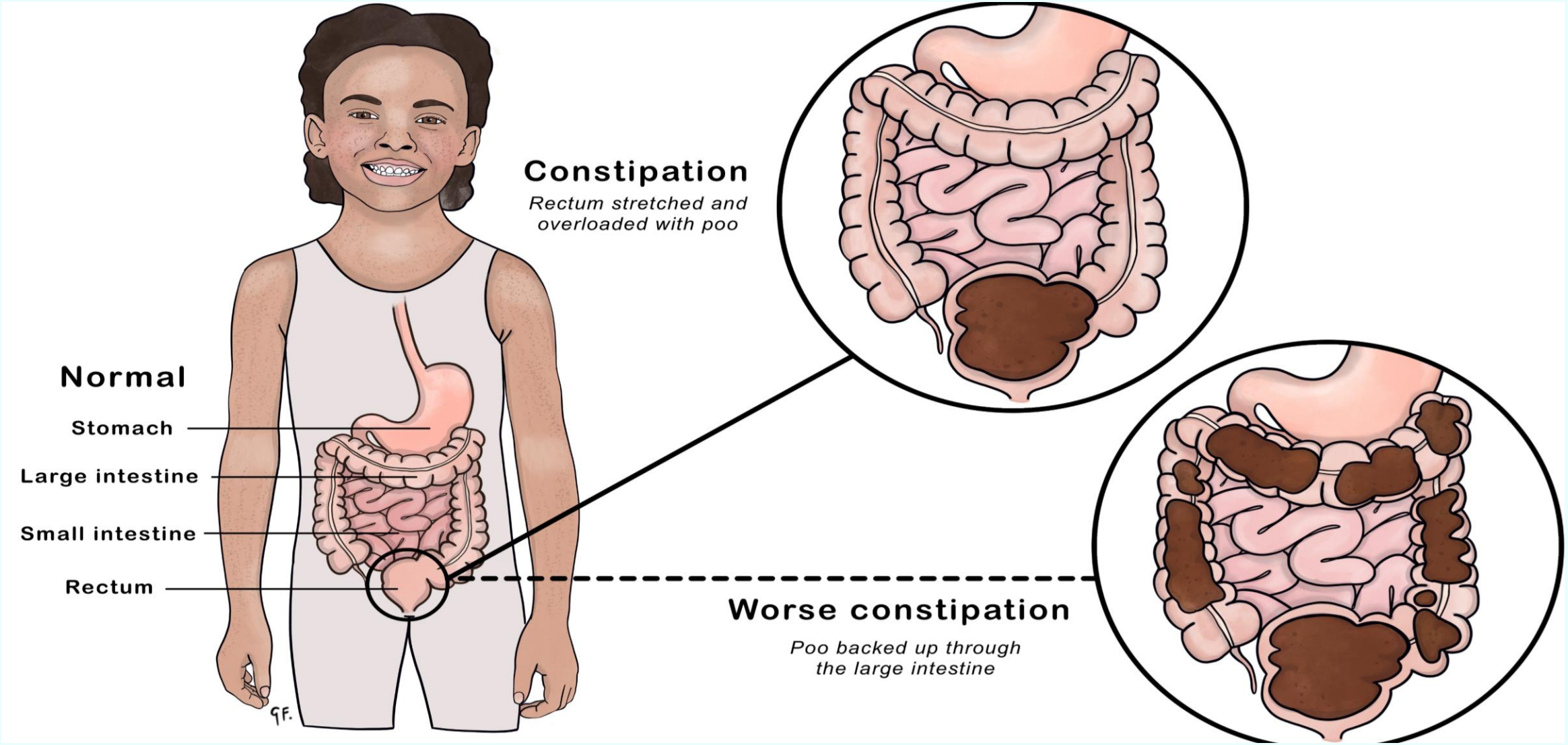
Illustration of digestive system



- Constipation and diarrhea are common gastrointestinal disorder with multifactorial pathophysiology.
- Constipation involve hard stools, straining with defecation, and infrequent large bowel movements, otherwise referred to as bowel disturbances.
- In children, there may be vomiting, abdominal pain, anorexia, or encopresis (i.e., involuntary passage of stool or fecal soiling) (Benniga et al., 2004).
- In adults, there are more concern with hard stools, incomplete evacuation, and difficulty passing stools than just the infrequency of bowel movements.

- The normal stool weight in adult is about 100–200 g daily, and normal frequency could range from one stool every 3 days to three times per day.
- Normal transit time through the gastrointestinal (GI) tract ranges approximately from 18–48 hours.
- There are normally more frequency stools in children, ranging from an average of two to three stools daily for the first few months of life to approximately one and a half bowel movements daily at age 3.
- Individuals who consume a diet that contains the recommended amounts of dietary fiber in the form of fruits, vegetables, and whole-grain breads and cereals tend to have larger, softer stools that are relatively easy to pass.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Pathophysiology/classification of chronic constipation

- Constipation may be primary (idiopathic) or secondary. The primary constipation involves the following:

Normal transit constipation (NTC)

- This is the most common class of constipation.
- Although, stool traverses at a normal rate through the colon and with normal stool frequency, it is characterized with difficult evacuation, bloating, abdominal pain or discomfort and hard stools.

Slow-transit constipation (STC)

- Individuals with STC experience and/or report of prolonged intestinal transit time, with significant impaired propulsive colonic motor activity and diminished colonic responses after a meal and on awakening in the morning.
- Slow transit may be an indicative of an underlying defecation disorder. The STC is common in women

Pelvic floor dysfunction or defecatory disorders (DD)

- This class of chronic constipation is an indicative of difficult expelling of stool from the rectum, characterized by impaired rectal contraction, paradoxical anal contraction, or inadequate anal relaxation.

- However, lack of coordination, or dyssynergia, of muscles involved in defecation is thought to be the most likely cause.

- Biofeedback therapy is thought to be beneficial in recent controlled trials in patients with pelvic floor dysfunction.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Secondary constipation involves the following:

Organic	Colorectal cancer, extra-intestinal mass, post-inflammatory, ischemic or surgical stenosis
Endocrine or metabolic	Diabetes mellitus, hypothyroidism, hypercalcemia, porphyria, chronic renal insufficiency, panhypopituitarism, pregnancy
Neurological	Spinal cord injury, Parkinson's disease, paraplegia, multiple sclerosis, autonomic neuropathy, Hirschsprung's disease, chronic intestinal pseudo-obstruction
Myogenic	Myotonic dystrophy, dermatomyositis, scleroderma, amyloidosis, chronic intestinal pseudo-obstruction
Anorectal	Anal fissure, anal strictures, inflammatory bowel disease, proctitis
Drugs	Opiates, antihypertensive agents, tricyclic antidepressants, iron preparations, anti-epileptic drugs, anti-Parkinsonian agents (anticholinergic or dopaminergic)
Diet or lifestyle	Low fiber diet, dehydration, inactive lifestyle

Diagnosis of chronic constipation: This will be done using Rome III criteria below.

Criteria fulfilled for the last 3 months and symptom onset at least 6 months prior to diagnosis

Presence of ≥2 of the following symptoms:

- Lumpy or hard stools in ≥25% of defecations
- Straining during ≥25% of defecations
- Sensation of incomplete evacuation for ≥25% of defecations
- Sensation of anorectal obstruction/blockage for ≥25% of defecations
- Manual maneuvers to facilitate ≥25% of defecations (digital manipulations, pelvic floor support)
- <3 evacuations per week

Loose stools rarely present without the use of laxatives

Insufficient criteria for irritable bowel syndrome

Prevalence of constipation

- The median prevalence of constipation is 16% in adults, 33.5% in older adults (60–101 years). It is also greater in people who are not white, in institutionalized people, while the median prevalence ratio is 1.5:1, women to men.
- The global prevalence of chronic constipation is approximately 14%, with higher rates observed in older adults and females. Prevalence rate by region showed that, American population recorded 12–19% prevalence; Asia 16–25% prevalence; Africa 10–20% prevalence; Europe 10 – 20% prevalence.

Risk factors of chronic constipation

- Increasing age, female sex, lower socioeconomic status, lower parental education rates, less self-reported physical activity, certain medications, stressful life events, physical and sexual abuse, and depression.

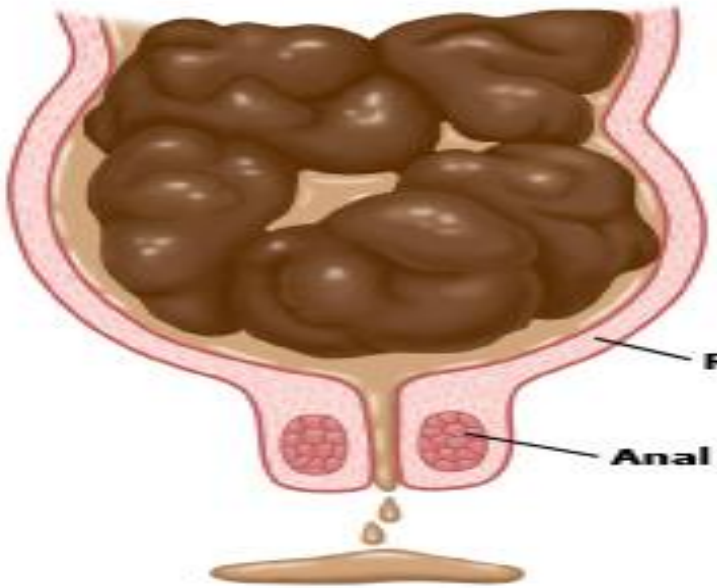
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Treatment options

Laxative type	Examples	Proposed mode of action	Potential limitation
Dietary fiber; bulking agents	Wheat bran Pysllium seed husk Methylcellulose	Luminal water binding increases stool bulk and reduces consistency	Flatulence and abdominal distension Stool impaction (rarely) Not recommended in frail, immobile, or palliative care patients.
Osmotic laxatives **Undigestible disaccharides and sugar alcohols** **Synthetic macromolecules**	Lactulose Sorbitol PEG Polycarbophil Magnesium hydroxide (e.g., milk of magnesia) Magnesium citrate Magnesium sulfate Sodium phosphate	Luminal water binding by creating an osmotic gradient Luminal water binding Luminal water binding Increases fluid excretion	Bloating, flatulence Boating Electrolyte imbalance
Stimulant laxatives **Diphenylmethane derivatives**	Bisacodyl, sodium picosulfate	Act locally to stimulate colonic motility, decrease water absorption from large intestine	Abdominal discomfort and cramps
Anthraquinones	Senna, aloe, cascara	Act locally to stimulate colonic motility, decrease water absorption from large intestine	Abdominal discomfort and cramps

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Constipation



- Child holds in stool because of pain
- Stretched out nerves and muscle that don't work well
- Buildup of hard stool
- Soft stool may move around hard stool and leak out

Consistent emptying of colon (for months)
- Laxatives
- Toilet-sitting

Recovery

- Thicker, stronger muscle that works well
- Nerves able to sense need to go
- Sphincters able to prevent leaking

Personalized dietary management of constipation/medical nutrition therapy

- Primary nutrition therapy for constipation is consumption of adequate amounts of both soluble and insoluble dietary fiber. Fiber increases colonic fecal fluid, microbial mass, stool weight and frequency, and the rate of colonic transit.
- The recommended amount of dietary fiber is about 14 g/1000 kcal. The diet of an adult women should contain about 25 g of fiber daily; that of a man, about 38 g daily, while children about 19–25 g daily.
- The sources of fiber include whole grains, fruits, vegetables, legumes, seeds, and nuts.
- These sources of fiber are also rich in nutrients, healthful phytochemicals and resistant starches, and may serve as prebiotics to maintain the desired colonic microflora.

Bran and powdered fiber supplements may be helpful in persons who cannot or will not eat sufficient amounts of fibrous foods.

Consumption of at least 8-oz glasses (2 L) of fluids daily is recommended to facilitate the effectiveness of a high-fiber diet intake.

High-fiber diet

Dietary fiber refers primarily to edible plant materials not digested by the enzymes in the upper digestive tract of humans. It consists of cellulose, hemicellulose, pectins, gums, lignin, starchy materials, and oligosaccharides that are partially resistant to digestive enzymes.

Personalized guideline for high-fiber diets

- Increase consumption of whole grain breads and cereals and other whole grain products to 6–11 servings daily.
- Increase consumption of vegetables, legumes, and fruits, nuts, and edible seeds to 5–8 servings daily.
- Consume high-fiber cereals, granolas, and legumes as needed to bring fiber intake to 25 g in women or 38 g in men or more daily.
- Increase consumption of fluids to at least 2 L daily.

The above guideline should be allowed for 1 to 2 weeks to give the GI tract time to adjust and thus, minimize symptoms of discomfort or gas.

contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Examples of foods rich in fiber

Cereals and starchy

Porridge, Weetabix, Whole-meal bread, potato with skin, brown rice.

Fruits and vegetables

Apple, prunes, banana, carrots, broccoli, sweet corn.

Pulses

Baked beans, red kidney beans, peas.

Nuts and seeds

Almond, peanut, linseed (flaxseeds).

- Gradual initiation of a high-fiber diet may help reduce unpleasant side effects such as increased flatulence, intestinal rumbling, cramps, or diarrhea.

- The GI disturbances associated with initial fiber ingestion usually decrease within 4 to 5 days, but some increase in flatulence is normal with a high-fiber diet intake.

- The high-fiber diet is most effective when consumed continuously for several months.

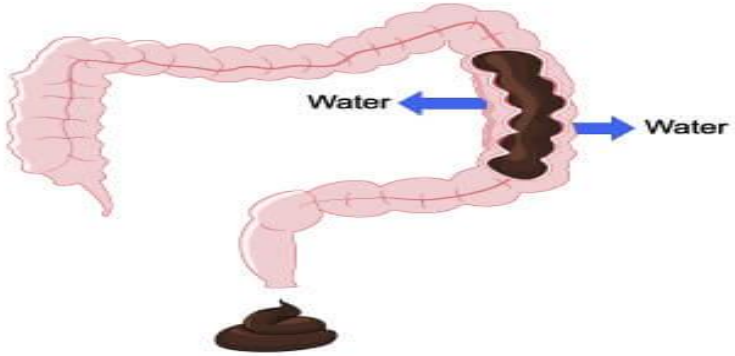
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

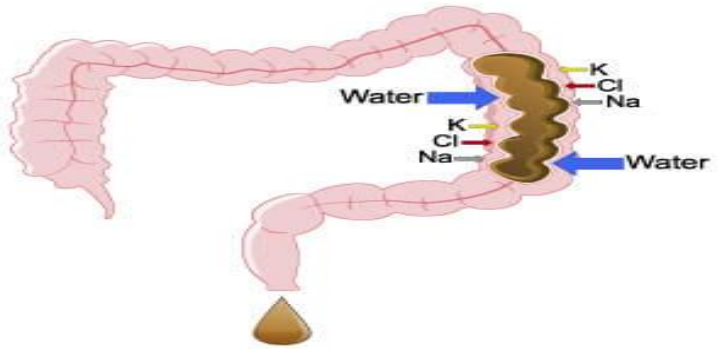
Infographic tips to prevent chronic constipation

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

What is Diarrhea and How Does It Work

Diarrhea is a condition characterized by having 2–3 or more loose or watery bowel movements each day. It is often acute and only lasts for up to a week. If it lasts more then 2–3 weeks it is considered chronic diarrhea.





In diarrhea, the physiological mechanisms of the gut are impaired and it results in fluid and electrolytes not being reabsorbed over the large intestine.

When individuals experience diarrhea they have trouble absorbing nutrients and lose a lot of fluid and electrolytes which can lead to dehydration and malnourishment.

DrJockers.com SUPERCHARGE YOUR HEALTH

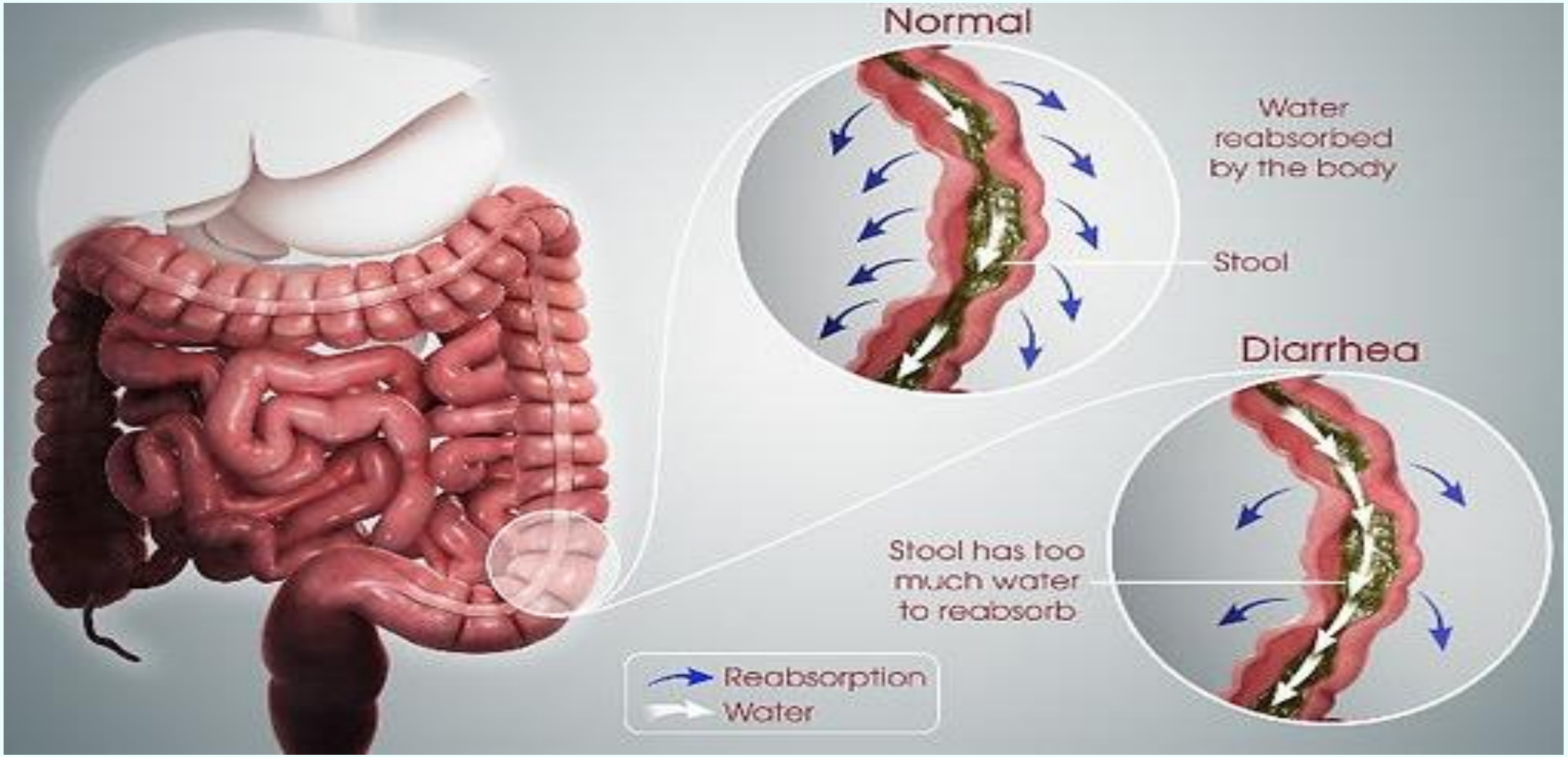
Diarrhea

- Diarrhea disease is the third leading cause of death among under-five children.

- It can last several days and can leave the body without the water and salts that are necessary for survival.

- Children who are malnourished or have impaired immunity, as well as people living with HIV, are most at risk of life-threatening diarrhea.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Pathophysiology

Diarrhea is defined by the frequent evacuation of watery stools, which typically surpass 300 ml and are accompanied by an excessive loss of fluid and electrolytes, particularly sodium and potassium.

It occurs when there is excessively rapid transit of intestinal contents through:

- the small intestine
- decreased enzymatic digestion of foodstuffs
- decreased absorption of fluids and nutrients
- increased secretion of fluids into the GI tract, or exudative losses.

- ➢ Inflammatory disease; medications; infections with fungal, bacterial, or viral agents; overconsumption of sugars; insufficient or damaged mucosal absorptive surface; or GI resections or malnutrition; may lead to diarrhea episode.

Osmotic diarrheas occur when osmotically active solutes are present in the intestinal tract and are poorly absorbed.

- ✓ Examples, diarrhea that accompanies dumping syndrome and that which follows lactose ingestion in the person with a lactase deficiency.

Secretory diarrheas are the result of active intestinal secretion of electrolytes and water by the intestinal epithelium, resulting from bacterial exotoxins, viruses, and increased intestinal hormone secretion.

- ➢ Unlike osmotic diarrhea, fasting does not relieve secretory diarrhea.

Exudative diarrheas are always associated with mucosal damage, which leads to an outpouring of mucus, fluid, blood, and plasma proteins, with a net accumulation of electrolytes and water in the gut.

✓ Prostaglandin and cytokine release may be involved.

The diarrheas associated with Crohn's disease, ulcerative colitis, and radiation enteritis are typically exudative, but secretory and osmotic diarrheas may also occur.

Medication-induced diarrheas may be caused by several medications, but especially antibiotics.

➢ Antibiotics can reduce the usual 'salvage' by colonic bacteria of the small amounts of foodstuffs that escape digestion and absorption.

- Broad-spectrum antibiotics can greatly reduce the numbers of colonic bacteria that normally convert osmotically active carbohydrates and amino acids to gases and short-chain fatty acids (SCFAs).
- The SCFAs are normally absorbed from the lumen of the colon as long as the amount produced is close to normal.
- Absorption of the SCFAs facilitates absorption of electrolytes and water from the colon.
- However, eradication of the bacteria from the colon results in accumulation of osmotically active molecules and reduced absorption of electrolytes and water.

Mal-absorptive diarrhea is often complicated by steatorrhea (lipid malabsorption) and mal-digestion of other macronutrients or micronutrients.

- It occurs when there is not enough healthy absorptive area or there is rapid transit of chime, such as what might occur in inflammatory bowel disease or after extensive bowel resection.

Prevalence of diarrhea

- Diarrheal disease is the third leading cause of death in under-five children (1–59 months).

- It both preventable and treatable. In each year, diarrhea kills about 443 832 under-five children, and an additional 50 851 children aged 5–9 years.

- Globally, there are nearly 1.7 billion cases of childhood diarrhea disease annually, and it is a leading cause of malnutrition in under-five children.

Treatment

- The first step in medical treatment is to identify and treat the underlying problems, since diarrhea is a symptom of a disease state.
- The next priority is to manage fluid and electrolyte replacement. Losses of electrolytes, particularly potassium and sodium, should be corrected early by using oral glucose electrolyte solutions with added potassium.
- Infant and young child with intractable diarrhea would need parenteral feeding.
- Parenteral nutrition is necessary if exploratory surgery is anticipated or if the patient is not expected to resume full oral intake within 5–7 days.

Personalized dietary management of diarrhea/medical nutrition therapy

- Fluids and electrolytes replacement is the first point of call in managing diarrhea, with the use of electrolyte solutions, soups and broths, vegetable juices, and other isotonic liquids.
- Cereals, breads, low-fat meats, and small amounts of vegetables and fruits will follow and then, lipids.
- The vital aim of dietary management is to limit large amounts of hyperosmotic carbohydrates that may be mal-digested or mal-absorbed, foods that stimulate secretion of fluids, and foods that speed the rate of GI transit.

- Modest amounts of fat can be used if digestive mechanisms for lipid are intact.
- Sugar alcohols, lactose, fructose, and large amounts of sucrose may worsen osmotic diarrheas, because the activity of the disaccharidases and transport mechanisms may be decreased during inflammatory and infectious intestinal disease.
- Limit sugar intake especially in children.
- A minimum-residue diet may be started as the acute episode resolves.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Food to limit in a low- or minimum-residue diet

Food	Comments
Lactose (in lactose mal-absorbers)	6–12 g normally tolerated in healthy lactase-deficient individuals, but may not be in some individuals
Fiber (quantities > 20 g)	Modest amounts (10–15 g) may help maintain normal consistency of GI contents and normal colonic mucosa in healthy states and GI disease
Resistant starch (especially raffinose and stachyose found in legumes) Sorbitol, mannitol, and xylitol (excess, > 10 g/day) Fructose (excess, 20–25 g/meal) Sucrose (excess, >25–50 g/meal)	Well tolerated in moderate amounts, large amounts may cause hyperosmolar diarrhea or decreased fecal pH with fermentation to short-chain fatty acids
Caffeine	Increases GI secretions, colonic motility
Alcoholic beverages (especially wine and beer)	Increases GI secretions

- Prebiotics foods including oats, banana flakes and chicory may actually help to control or, treat diarrhea, by maintaining lactobacillus and bifidus microbes and may also prevent the overgrowth of potentially pathogenic organisms.

- Chronic diarrhea is accompanied by dehydration and electrolyte depletion.

- There will be malabsorption of vitamins, minerals, and protein or lipid, if chronic diarrhea is associated with prolonged infectious, immunodeficiency, or inflammatory disease.

- The nutrients may need to be replaced parenterally or enterally. Importantly, the loss of potassium alters bowel motility, encourages anorexia, and can introduce a cycle of bowel distress.

- Fiber-rich diet should be added gradually as the diarrhea begins to resolve in order to restore normal mucosal function, increase electrolyte and water absorption, and increase the firmness of the stool.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Oral rehydration solution for treating diarrhea in infants and children, as recommended by the World Health Organization (WHO) (1986).

Element	Composition
Glucose (g/100 mL)	20
Sodium (mEq/L)	90
Potassium (mEq/L)	20
Chloride (mEq/L)	80
Bicarbonate (mEq/L)	30
Osmolarity (mOsm/L)	330
Recipe To 1 L of water add the following 3.5 g sodium chloride 2.5 g sodium bicarbonate 1.5 g potassium chloride 20 g glucose The solution should be made fresh every 24 hr	

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiota-mediated Diarrhea and its Mechanism

- The relationship between diarrhea and gut microbiota is one aspect that is currently receiving a lot of attention.
- First, the number of beneficial bacteria in the GI tract decreases as a result of invasive pathogenic bacteria inhibiting the growth of normal bacteria.
- Diarrhea result due to toxic substances produced by pathogens, which also disrupt immunological responses and gut function.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiota-mediated Diarrhea and its Mechanism

Bacteria and Diarrhea

- Enteric bacterial pathogens are the primary cause of infectious diarrhea, and bacterial-pathogenic diarrhea is a global health concern, particularly in developing nations.

- The most common pathogens associated with diarrhea are thought to be the following:

✓ *Escherichia coli* (*E. coli*) – (facultative gram-negative bacteria)
✓ *Shigella* – *(*gram-negative bacteria)
✓ *Salmonella* – (facultative gram-negative bacterium)
✓ *Clostridium difficile* (gram +ve anaerobic bacteria)
✓ *Campylobacter* and *Aeromonas*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiota-mediated Diarrhea and its Mechanism

Fungus and diarrhea

- As a significant component of gut microbiota, fungi have been shown to be strongly linked to diarrhea in certain fungal communities (Sangster et al., 2016).

- Candida is a reliable fungus that causes diarrhea on its own terms.

- The most common fungus in mammal intestines is Candida albicans (C. albicans), a conditional pathogen (Noble & Johnson, 2015).

Gut microbiota-mediated Diarrhea and its Mechanism

Virus and diarrhea

- The complex community nature of viral microbiome, which is made up of bacteriophages, DNA viruses, and eukaryotic RNA viruses, is crucial to preserving both human and animal health.

- Certain viruses cause diarrhea and manifest clinically as fever, vomiting, and diarrhea (Goodgame, 2001).

- More than 200,000 cases of diarrhea occur yearly among infants and young animals that contract a common diarrheal pathogen (rotavirus) (Ramig, 2004).

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiota-mediated Diarrhea and its Mechanism

Probiotics and Diarrhea

- Probiotics are bacterial and yeast microbes. Yeast probiotics such as *Saccharomyces boulardii* (*Sb*) are different from bacterial probiotics.

- Probiotics are beneficial to the host's health and contain a sufficient amount of nonpathogenic specific live bacteria and yeast.

- ✓ Example of bacteria probiotics are: *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and *Bacillus*

- ✓ Example of yeast probiotics include: *Saccharomyces boulardii* (*Sb*)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiota-mediated Diarrhea and its Mechanism

- Probiotics have been reported to treat pathogens-caused diarrhea by maintaining or improving the balance of gut microbiota.

- Its mechanism is linked to the inhibitory effect on the colonization of harmful bacteria by competing for nutrients and producing antibacterial compounds (Bron et al., 2017).

- Mechanism of action include the following pathways:

✓ Regulation of the balance of gut microbiota;
✓ Improvement immunity;
✓ Manipulation of intestinal defense barrier; and
✓ metabolites

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiota-mediated Diarrhea and its Mechanism

- Probiotics have been widely considered to maintain gut microbial hemostasis and a healthy gut microbiota community predicate a low incidence of diarrhea.

- It can produce organic acids in the metabolic process and lower the pH of the gut cavity, thereby inhibiting the growth of pathogens (Peters et al., 2019).

- Probiotic strains serve as a key activator for the gut innate and adaptive immune systems, thereby signaling antimicrobial and inflammatory responses and enhancing diarrhea resistance.

Gut microbiota-mediated Diarrhea and its Mechanism

- Probiotics can stimulate the production and secretion of mucin, cathelicidins, and defensins from goblet cells and epithelial cells to form an immune barrier, preventing the invasion of pathogenic bacteria.

- *Saccharomyces boulardii* is a nonpathogenic yeast first isolated from lychee fruits in Indonesia and used in France to treat diarrhea.

- It is able to stimulate an immune response in the intestinal mucosa, and has a trophic effect by enhancing the metabolic function of the mucosa.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiota-mediated Diarrhea and its Mechanism

- *Saccharomyces boulardii* releases polyamines, which are implicated in stimulating the enzymatic activity of the colonic mucosa.
- It is a promosing agent for the amelioration of the course of acute diarrhea in children.
- Studies have shown that children treated with *Sb* had a decreased number of episodes of diarrhea in subsequent months.
- Another study reported reduction in number of episodes of diarrhea in the post-treatment follow-up period of two months, thereby stimulate the increase of IgA, and enhance the trophic activity of the mucosa.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiota-mediated Diarrhea and its Mechanism

Prebiotics and Diarrhea

- Prebiotics are substances that are selectively utilized by host organisms to confer health benefits (Gibson et al., 2017).

- Consumption of prebiotics can improve the gut microbiota, which is beneficial to health

- Recent study has shown that prebiotic can increase the production of short chain fatty acid, play an important role in maintaining the intestinal barrier (Azad MAK et al., 2020; Snelson et al., 2021).

Gut microbiota-mediated Diarrhea and its Mechanism

- Prebiotics such as fructo-oligosaccharide, inulin, etc., can resist the colonization of pathogen by acting as soluble decoy receptors that mimic the binding site of pathogens, thereby promoting the elimination of pathogens from the intestine (Pujari & Banerjee, 2021).

- Previous studies have shown that prebiotics can shorten the duration of acute watery diarrhea and has a good therapeutic effect on diarrhea (Rigo-Adrover et al., 2017).

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Stool chart showing constipation and diarrhea

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Conclusion/Summary

- Abnormalities in intestinal motility, pelvic floor function, and neurological signaling play crucial roles, resulting in chronic constipation. Dysfunctions such as slow transit, pelvic floor dyssynergia, and enteric nervous system abnormalities disrupt normal bowel movements, leading to symptoms of incomplete evacuation and hard stools.
- Diarrhea involves disruptions in fluid and electrolyte balance, intestinal inflammation, and alterations in gut microbiota composition.
- Factors such as increased fluid secretion, mucosal inflammation, and microbial dysbiosis contribute to watery stools and gastrointestinal discomfort.
- Understanding the pathophysiological processes and mechanisms underlying chronic constipation and diarrhea is essential for developing targeted management strategies and improving patient outcomes.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

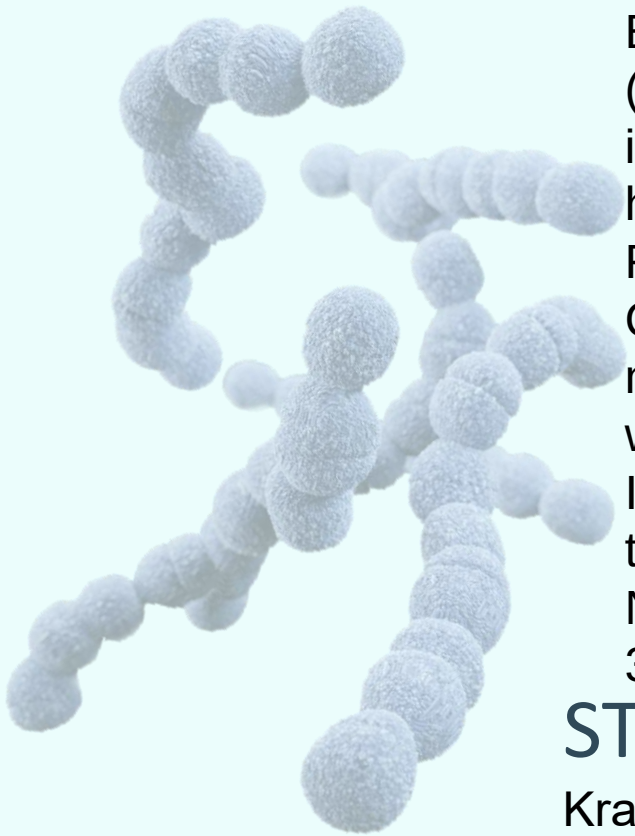
Learning Outcomes

- Each student will be able to understand and describe constipation and diarrhea;
- Upon completion of the course, students will be able to understand the concept and pathophysiology of constipation and diarrhea;
- Students will be able analyze, evaluate and apply the knowledge of personalize nutrition in order to manage patients living with constipation and diarrhea;
- Students will be able to know foods high in fiber, and apply it in order to reduce unpleasant side effect of constipation; and
- Students will be able to differentiate between constipation and diarrhea, particularly the stool types.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

Bajaj, J. S. (2022). Gut microbiota alterations in diarrheal diseases: Insights into pathophysiology and therapeutic implications. Nature Reviews Gastroenterology & Hepatology, 19(1), 32-45.
Bassotti, G., Fred G. U, Bella Mae I. U., Paul T. H and Arnold H. J. (2023). Enteric nervous system abnormalities in chronic constipation: Pathophysiological insights and therapeutic implications. Neurogastroenterology & Motility, 35(1), e14463.
Benninga, M.A. et al. (2004). Childhood constipation: Is there new light in the tunnel? J. Pediar. Gastroenteral Nutr. 39: 448.



Bharucha, A. E. (2020). The microbiome and chronic constipation: a review. Gut Microbes, 11(5), 1331-1340.
Buret, A. G., and Olson, M. E. (2021). Pathophysiology of infectious diarrhea: Microbial and host factors. Clinical Microbiology Reviews, 34(3), e00214-20.
Chen, X. (2023). Dysregulation of neurotransmitter signaling in patients with chronic constipation: Implications for pathophysiology and therapeutic targeting. Neurogastroenterology & Motility, 36(1), e14456.

STUDY MATERIAL

Krause's Food & Nutrition Therapy, 12th Edition
Understanding Normal and Clinical Nutrition, 8th Edition
Thematic Review on Gastroenterological Diseases – Chronic Constipation, Mayo Clinic

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

- Chronic constipation and diarrhea
- Institute of Microbiome and Applied Sciences
- For admin questions contact@imas.edu.eu
- Type of course: Post-graduate certificate level 7 (30 ECTS)
- Chronic constipation and diarrhea
- Dr. Ijioma Okorie, PhD in Human and Experimental Nutrition, MSc in Applied Human Nutrition

Introduction

Constipation and diarrhea are common gastrointestinal disorder with multifactorial pathophysiology. Constipation involve hard stools, straining with defecation, and infrequent large bowel movements, otherwise referred to as bowel disturbances.

In children, there may be vomiting, abdominal pain, anorexia, or encopresis (i.e., involuntary passage of stool or fecal soiling) (Benniga et al., 2004).

In adults, there are more concern with hard stools, incomplete evacuation, and difficulty passing stools than just the infrequency of bowel movements.

The normal stool weight in adult is about 100–200 g daily, and normal frequency could range from one stool every 3 days to three times per day.

Normal transit time through the gastrointestinal (GI) tract ranges approximately from 18–48 hours.

There are normally more frequency stools in children, ranging from an average of two to three stools daily for the first few months of life to approximately one and a half bowel movements daily at age 3.

Individuals who consume a diet that contains the recommended amounts of dietary fiber in the form of fruits, vegetables, and whole-grain breads and cereals tend to have larger, softer stools that are relatively easy to pass.

Pathophysiology/classification of chronic constipation

Constipation may be primary (idiopathic) or secondary. The primary constipation involves the following:

Normal transit constipation (NTC)

This is the most common class of constipation. Although, stool traverses at a normal rate through the colon and with normal stool frequency, it is characterized with difficult evacuation, bloating, abdominal pain or discomfort and hard stools.

Slow-transit constipation (STC)

Individuals with STC experience and/or report of prolonged intestinal transit time, with significant impaired propulsive colonic motor activity and diminished colonic responses after a meal and on awakening in the morning.

Slow transit may be an indicative of an underlying defecation disorder. The STC is common in women

Pelvic floor dysfunction or defecatory disorders (DD)

This class of chronic constipation is an indicative of difficult expelling of stool from the rectum, characterized by impaired rectal contraction, paradoxical anal contraction, or inadequate anal relaxation. However, lack of coordination, or dyssynergia, of muscles involved in defecation is thought to be the most likely cause.

Biofeedback therapy is thought to be beneficial in recent controlled trials in patients with pelvic floor dysfunction.

Secondary constipation involves the following:

Organic	Colorectal cancer, extra-intestinal mass, post-inflammatory, ischemic or surgical stenosis
Endocrine or metabolic	Diabetes mellitus, hypothyroidism, hypercalcemia, porphyria, chronic renal insufficiency, panhypopituitarism, pregnancy
Neurological	Spinal cord injury, Parkinson's disease, paraplegia, multiple sclerosis, autonomic neuropathy, Hirschsprung's disease, chronic intestinal pseudo-obstruction
Myogenic	Myotonic dystrophy, dermatomyositis, scleroderma, amyloidosis, chronic intestinal pseudo-obstruction
Anorectal	Anal fissure, anal strictures, inflammatory bowel disease, proctitis
Drugs	Opiates, antihypertensive agents, tricyclic antidepressants, iron preparations, anti-epileptic drugs, anti-Parkinsonian agents (anticholinergic or dopaminergic)
Diet or lifestyle	Low fiber diet, dehydration, inactive lifestyle

Diagnosis of chronic constipation: This will be done using Rome III criteria below.

Criteria fulfilled for the last 3 months and symptom onset at least 6 months prior to diagnosis
Presence of ≥2 of the following symptoms:

- Lumpy or hard stools in ≥25% of defecations
- Straining during ≥25% of defecations
- Sensation of incomplete evacuation for ≥25% of defecations
- Sensation of anorectal obstruction/blockage for ≥25% of defecations

- Manual maneuvers to facilitate ≥25% of defecations (digital manipulations, pelvic floor support)
- <3 evacuations per week

Loose stools rarely present without the use of laxatives

Insufficient criteria for irritable bowel syndrome

Prevalence of constipation

The median prevalence of constipation is 16% in adults, 33.5% in older adults (60–101 years). It is also greater in people who are not white, in institutionalized people, while the median prevalence ratio is 1.5:1, women to men.

The global prevalence of chronic constipation is approximately 14%, with higher rates observed in older adults and females. Prevalence rate by region showed that, American population recorded 12–19% prevalence; Asia 16–25% prevalence; Africa 10–20% prevalence; Europe 10 – 20% prevalence.

Risk factors of chronic constipation

Increasing age, female sex, lower socioeconomic status, lower parental education rates, less self-reported physical activity, certain medications, stressful life events, physical and sexual abuse, and depression.

Treatment options

Laxative type	Examples	Proposed mode of action	Potential limitation
Dietary fiber; bulking agents	Wheat bran Pysllium seed husk Methylcellulose	Luminal water binding increases stool bulk and reduces consistency	Flatulence and abdominal distension Stool impaction (rarely) Not recommended in frail, immobile, or palliative care patients.
Osmotic laxatives Undigestible disaccharides and sugar alcohols Synthetic macromolecules	Lactulose Sorbitol PEG Polycarbophil Magnesium hydroxide (e.g., milk of magnesia) Magnesium citrate Magnesium sulfate Sodium phosphate	Luminal water binding by creating an osmotic gradient Luminal water binding Luminal water binding Increases fluid excretion	Bloating, flatulence Boating Electrolyte imbalance
Stimulant laxatives Diphenylmethane derivatives	Bisacodyl, sodium picosulfate	Act locally to stimulate colonic motility, decrease water absorption from large intestine	Abdominal discomfort and cramps
Anthraquinones	Senna, aloe, cascara	Act locally to stimulate colonic motility, decrease water absorption from large intestine	Abdominal discomfort and cramps

The first approach to treatment of mild and functional constipation is to ensure adequate dietary fiber, fluid, and exercise and to advise the patient to heed the urge to defecate.

Patients dependent on laxatives are usually encouraged to use milder products and reduce the dose until withdrawal is complete.

Personalized dietary management of constipation/medical nutrition therapy

Primary nutrition therapy for constipation is consumption of adequate amounts of both soluble and insoluble dietary fiber. Fiber increases colonic fecal fluid, microbial mass, stool weight and frequency, and the rate of colonic transit.

The recommended amount of dietary fiber is about 14 g/1000 kcal. The diet of an adult women should contain about 25 g of fiber daily; that of a man, about 38 g daily, while children about 19–25 g daily.

The sources of fiber include whole grains, fruits, vegetables, legumes, seeds, and nuts. These sources of fiber are also rich in nutrients, healthful phytochemicals and resistant starches, and may serve as prebiotics to maintain the desired colonic microflora.

Bran and powdered fiber supplements may be helpful in persons who cannot or will not eat sufficient amounts of fibrous foods.

Consumption of at least 8-oz glasses (2 L) of fluids daily is recommended to facilitate the effectiveness of a high-fiber diet intake.

High-fiber diet

Dietary fiber refers primarily to edible plant materials not digested by the enzymes in the upper digestive tract of humans. It consists of cellulose, hemicellulose, pectins, gums, lignin, starchy materials, and oligosaccharides that are partially resistant to digestive enzymes.

Personalized guideline for high-fiber diets

- Increase consumption of whole grain breads and cereals and other whole grain products to 6–11 servings daily.
- Increase consumption of vegetables, legumes, and fruits, nuts, and edible seeds to 5–8 servings daily.
- Consume high-fiber cereals, granolas, and legumes as needed to bring fiber intake to 25 g in women or 38 g in men or more daily.
- Increase consumption of fluids to at least 2 L daily.

The above guideline should be allowed for 1 to 2 weeks to give the GI tract time to adjust and thus, minimize symptoms of discomfort or gas.

Examples of foods rich in fiber

Cereals and starchy

Porridge, Weetabix, Whole-meal bread, potato with skin, brown rice.

Fruits and vegetables

Apple, prunes, banana, carrots, broccoli, sweet corn.

Pulses

Baked beans, red kidney beans, peas.

Nuts and seeds

Almond, peanut, linseed (flaxseeds).

Gradual initiation of a high-fiber diet may help reduce unpleasant side effects such as increased flatulence, intestinal rumbling, cramps, or diarrhea.

The GI disturbances associated with initial fiber ingestion usually decrease within 4 to 5 days, but some increase in flatulence is normal with a high-fiber diet intake.

The high-fiber diet is most effective when consumed continuously for several months.

Diarrhea

Diarrhea disease is the third leading cause of death among under-five children. It can last several days and can leave the body without the water and salts that are necessary for survival.

Children who are malnourished or have impaired immunity, as well as people living with HIV, are most at risk of life-threatening diarrhea.

Pathophysiology

Diarrhea is defined by the frequent evacuation of watery stools, which typically surpass 300 ml and are accompanied by an excessive loss of fluid and electrolytes, particularly sodium and potassium.

It occurs when there is excessively rapid transit of intestinal contents through:

- the small intestine
- decreased enzymatic digestion of foodstuffs
- decreased absorption of fluids and nutrients
- increased secretion of fluids into the GI tract, or exudative losses.

Inflammatory disease; medications; infections with fungal, bacterial, or viral agents; overconsumption of sugars; insufficient or damaged mucosal absorptive surface; or GI resections or malnutrition; may lead to diarrhea episode.

Osmotic diarrheas occur when osmotically active solutes are present in the intestinal tract and are poorly absorbed. Examples, diarrhea that accompanies dumping syndrome and that which follows lactose ingestion in the person with a lactase deficiency.

Secretory diarrheas are the result of active intestinal secretion of electrolytes and water by the intestinal epithelium, resulting from bacterial exotoxins, viruses, and increased intestinal hormone secretion.

Unlike osmotic diarrhea, fasting does not relieve secretory diarrhea.

Exudative diarrheas are always associated with mucosal damage, which leads to an outpouring of mucus, fluid, blood, and plasma proteins, with a net accumulation of electrolytes and water in the gut.

Prostaglandin and cytokine release may be involved.

The diarrheas associated with Crohn’s disease, ulcerative colitis, and radiation enteritis are typically exudative, but secretory and osmotic diarrheas may also occur.

Medication-induced diarrheas may be caused by several medications, but especially antibiotics. Antibiotics can reduce the usual ‘salvage’ by colonic bacteria of the small amounts of foodstuffs that escape digestion and absorption.

Broad-spectrum antibiotics can greatly reduce the numbers of colonic bacteria that normally convert osmotically active carbohydrates and amino acids to gases and short-chain fatty acids (SCFAs).

The SCFAs are normally absorbed from the lumen of the colon as long as the amount produced is close to normal.

Absorption of the SCFAs facilitates absorption of electrolytes and water from the colon.

However, eradication of the bacteria from the colon results in accumulation of osmotically active molecules and reduced absorption of electrolytes and water.

Mal-absorptive diarrhea is often complicated by steatorrhea (lipid malabsorption) and mal-digestion of other macronutrients or micronutrients.

It occurs when there is not enough healthy absorptive area or there is rapid transit of chime, such as what might occur in inflammatory bowel disease or after extensive bowel resection.

Prevalence of diarrhea

Diarrheal disease is the third leading cause of death in under-five children (1–59 months). It both preventable and treatable. In each year, diarrhea kills about 443 832 under-five children, and an additional 50 851 children aged 5–9 years.

Globally, there are nearly 1.7 billion cases of childhood diarrhea disease annually, and it is a leading cause of malnutrition in under-five children.

Treatment

The first step in medical treatment is to identify and treat the underlying problems, since diarrhea is a symptom of a disease state.

The next priority is to manage fluid and electrolyte replacement. Losses of electrolytes, particularly potassium and sodium, should be corrected early by using oral glucose electrolyte solutions with added potassium.

Infant and young child with intractable diarrhea would need parenteral feeding. Parenteral nutrition is necessary if exploratory surgery is anticipated or if the patient is not expected to resume full oral intake within 5–7 days.

Personalized dietary management of diarrhea/medical nutrition therapy

Fluids and electrolytes replacement is the first point of call in managing diarrhea, with the use of electrolyte solutions, soups and broths, vegetable juices, and other isotonic liquids.

Cereals, breads, low-fat meats, and small amounts of vegetables and fruits will follow and then, lipids.

The vital aim of dietary management is to limit large amounts of hyperosmotic carbohydrates that may be mal-digested or mal-absorbed, foods that stimulate secretion of fluids, and foods that speed the rate of GI transit.

Modest amounts of fat can be used if digestive mechanisms for lipid are intact.

Sugar alcohols, lactose, fructose, and large amounts of sucrose may worsen osmotic diarrheas, because the activity of the disaccharidases and transport mechanisms may be decreased during inflammatory and infectious intestinal disease.

Limit sugar intake especially in children.

A minimum-residue diet may be started as the acute episode resolves.

Food to limit in a low- or minimum-residue diet

Food	**Comments**
Lactose (in lactose mal-absorbers)	6–12 g normally tolerated in healthy lactase-deficient individuals, but may not be in some individuals
Fiber (quantities > 20 g)	Modest amounts (10–15 g) may help maintain normal consistency of GI contents and normal colonic mucosa in healthy states and GI disease
Resistant starch (especially raffinose and stachyose found in legumes) Sorbitol, mannitol, and xylitol (excess, > 10 g/day) Fructose (excess, 20–25 g/meal) Sucrose (excess, >25–50 g/meal)	Well tolerated in moderate amounts, large amounts may cause hyperosmolar diarrhea or decreased fecal pH with fermentation to short-chain fatty acids
Caffeine	Increases GI secretions, colonic motility
Alcoholic beverages (especially wine and beer)	Increases GI secretions

Source: Rummessen & Gudman-Hoyer (1998).

Prebiotics foods including oats, banana flakes and chicory may actually help to control or, treat diarrhea, by maintaining lactobacillus and bifidus microbes and may also prevent the overgrowth of potentially pathogenic organisms.

Chronic diarrhea is accompanied by dehydration and electrolyte depletion. There will be malabsorption of vitamins, minerals, and protein or lipid, if chronic diarrhea is associated with prolonged infectious, immunodeficiency, or inflammatory disease.

The nutrients may need to be replaced parenterally or enterally. Importantly, the loss of potassium alters bowel motility, encourages anorexia, and can introduce a cycle of bowel distress.

Fiber-rich diet should be added gradually as the diarrhea begins to resolve in order to restore normal mucosal function, increase electrolyte and water absorption, and increase the firmness of the stool.

Oral rehydration solution for treating diarrhea in infants and children, as recommended by the World Health Organization (WHO) (1986).

Element	Composition
Glucose (g/100 mL)	20
Sodium (mEq/L)	90
Potassium (mEq/L)	20
Chloride (mEq/L)	80
Bicarbonate (mEq/L)	30
Osmolarity (mOsm/L)	330
Recipe To 1 L of water add the following 3.5 g sodium chloride 2.5 g sodium bicarbonate 1.5 g potassium chloride 20 g glucose The solution should be made fresh every 24 hr	

Gut microbiota-mediated diarrhea and its mechanism

As much as environmental living conditions have improved and vaccination rates have increased, diarrhea remains a global issue, especially for young children (Stockmann et al., 2017). Dysbiosis, a condition marked by the dominance of bacteria, fungi, and viruses, is primarily found in cases of diarrhea in both humans and animals. The relationship between diarrhea and gut microbiota is one aspect that is currently receiving a lot of attention. First, the number of beneficial bacteria in the gastrointestinal tract decreases as a result of invasive pathogenic bacteria inhibiting the growth of normal bacteria (Fan & Pedersen, 2020). Diarrhea is then brought on by toxic substances produced by pathogens, which also disrupt immunological responses and gut function (Ward et al., 2016).

Bacteria and diarrhea

Enteric bacterial pathogens are the primary cause of infectious diarrhea, and bacterial-pathogenic diarrhea is a global health concern, particularly in developing nations. As of right now, the most common pathogens associated with diarrhea are thought to be *Escherichia coli* (*E*. *coli*), *Shigella*, *Salmonella*, *Campylobacter*, *Clostridium difficile* (*C*. *difficile*), and *Aeromonas* (Hodges & Gill, 2010; Kaaakoush et al., 2015; Levine et al., 2020).

Escherichia coli (*E*. *coli*) is a type of facultative anaerobic gram-negative bacteria to cause diarrhea. Different diarrhea *E*. *coli* strains show different epidemiology and have been described as enteropathogenic *E*. *coli* (EPEC, the main cause of infant diarrhea), enterohemorrhagic *E*. *coli* (EHEC/STEC, the cause of hemorrhagic colitis and hemolytic uremic disease), enteroaggregative *E*. *coli* (EAEC), enterotoxigenic *E*. *coli* (ETEC, the main cause of travelers' diarrhea and infant diarrhea) and entero-invasive *E*. *coli* (EIEC, the main cause of dysentery) according to the pathological types, colonization sites, virulence mechanisms, and clinical symptoms (Gomes et al., 2016).

Following infection, *E coli* produces toxins and causes pathogenic effects by adhering to intestinal epithelial cells via adherent fimbriae (Mirhoseini et al., 2018; Liu et al., 2020). Multidrug resistant diarrheal *E*. *coli* typically make it challenging to stop the spread of pathogens (Broujerdi et al., 2018).

Salmonella is also a facultative anaerobic gram-negative bacterium, and it is the third most common cause of diarrhea causing death (Troeger et al., 2017). *Salmonella* infection is characterized by gastroenteritis and diarrhea (De Jong et al., 2012), and can cause fever, pancreatitis and bleeding (Knodler & Elfenbein, 2019).
Also, abnormal use of antibiotics has led to an increase in antimicrobial resistance, and the emergence of multidrug resistance in *Salmonella* serotypes has also led to an increase in the severity of *Salmonella* infections. Thus, humans are more prone to *Salmonella* gastroenteritis after antibiotic treatment.

The gram-positive, anaerobic bacteria *Clostridium difficile* (*C*. *difficile*) is often found in both human and animal intestines and has the ability to generate spores. Globally, diarrhea caused by antibiotic usage is mostly caused by *C*. *difficile* infection (Peng et al., 2018; Mileto et al., 2020). Clinical signs of a *C*. *difficile* infection can range from carriers who show no symptoms to different levels of diarrhea and even death. Enterotoxin A and cytotoxin B are released when *C*. *difficile* colonizes and takes over in the large intestine, disrupting the normal gut microbial population (Androga et al., 2015).

According to Czepiel et al. (2019), both enterotoxin A and cytotoxin B cause severe intestinal inflammation, diarrhea, and pseudomembranous colitis by further damaging the cytoskeleton of epithelial cells. By altering the local microbial community, *C*. *difficile* can produce indole while also preventing the growth of helpful indole-sensitive bacteria like *Bacteroides* and *Edwardsiella*. This alteration affects the diversity and abundance of bacterial communities in the colon (Darkoh et al., 2019).

A kind of gram-negative bacteria that can cause diarrhea in both humans and animals is called *Shigella* (Liu et al., 2019). An estimated 125 million cases of diarrhea and 160,000 fatalities are attributed to *Shigella* each year, with young children accounting for a third of these cases (Baker and The, 2018). Shigella causes aggressive watery, mucus-like, or bloody diarrhea by producing serotype toxin 1 and *Shigella* enterotoxin, which further penetrate and damage the large intestine's epithelium (Nisa et al., 2020).

Fungus and diarrhea

As a significant component of gut microbiota, fungi have been shown to be strongly linked to diarrhea in certain fungal communities (Sangster et al., 2016). Although the mechanism by which *Candida* causes diarrhea is still unknown, it is generally accepted to be a reliable cause of diarrhea (Awoyeni et al., 2017). In a clinical setting, it is generally accepted that *Candida* may cause diarrhea on its own terms.

The most common fungus in mammal intestines is *Candida albicans*, (*C*. *albicans*), a conditional pathogen (Noble & Johnson, 2015). Diarrhea and *C*. *albicans* has long been associated with controversy. It was discovered that C. albicans could exacerbate the severity of DSS-induced colitis and induce intestinal dysbiosis in a mouse model (Panpetch et al., 2020).

Virus and diarrhea

The complex community nature of viral microbiome, which is made up of bacteriophages, DNA viruses, and eukaryotic RNA viruses, is crucial to preserving both human and animal health. Past research revealed that certain viruses can cause diarrhea in both humans and animals. Viral diarrhea manifests clinically as fever, vomiting, and diarrhea (Goodgame, 2001). More than 200,000 occur each year among infants and young animals that contract rotavirus, which is a common diarrheal pathogen (Ramig, 2004).

Rotavirus is a double RNA virus without envelope, that infects intestinal epithelial cells, thereby inducing diarrhea (Tafazoli et al., 2001; Crawford et al., 2017). It has been extensively documented that rotavirus infection reduces the intestinal epithelium's capacity for absorption, which damages and kills intestinal cells both *in vivo* and *in vitro*. Furthermore, Norovirus, Enterovirus, Astrovirus, and Boca virus are among the viruses that can cause diarrhea, whereas, Rotavirus infection is known to be more severe than other sources of infection (Crawford et al., 2017).

Probiotics and Diarrhea

Probiotics are bacterial and yeast microbes. Yeast probiotics such as *Saccharomyces boulardii* (*Sb*) are different from bacterial probiotics. Probiotics are beneficial to the host's health and contain a sufficient amount of nonpathogenic specific live bacteria (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Bacillus*) and yeast (*Sb*) preparations. Probiotics have been reported to treat pathogens-caused diarrhea by maintaining or improving the balance of gut microbiota and the mechanisms may be associated with the inhibitory effect on the colonization of harmful bacteria by competing for nutrients and producing antibacterial compounds (Bron et al., 2017).

The anti-diarrheal mechanisms of probiotics have not yet been fully elucidated, the currently considered mechanism of action of probiotics mainly rely on the following pathways: regulation of the balance of gut microbiota; improvement immunity; manipulation of intestinal defense barrier; metabolites.

Firstly, probiotics have been widely considered to maintain gut microbial hemostasis and a healthy gut microbiota community predicate a low incidence of diarrhea. In addition,

probiotics can produce organic acids in the metabolic process and lower the pH of the gut cavity, thereby inhibiting the growth of pathogens (Peters et al., 2019).

Secondly, probiotic strains serve as a key activator for the gut innate and adaptive immune systems, thereby signaling antimicrobial and inflammatory responses and enhancing the diarrhea resistance (Kawashima et al., 2018).

Thirdly, probiotics can stimulate the production and secretion of mucin, cathelicidins, and defensins from goblet cells and epithelial cells to form an immune barrier, preventing the invasion of pathogenic bacteria (Do Carmo et al., 2018).

For example, numerous probiotic agents have been studied for the management and prevention of various forms of diarrhea, including antibiotic-associated diarrhea. However, the use yeast such as *S*. *boulardii* have been implicated in the treatment and prevention of diarrhea.

Saccharomyces boulardii is a nonpathogenic yeast first isolated from lychee fruits in Indonesia and used first in France to treat diarrhea, in the beginning of the 1950s. a lyophilized form is in clinical use in Europe, Asia, Africa, and Central and South America. *Saccharomyces boulardii* is able to stimulate an immune response in the intestinal mucosa. It has a trophic effect by enhancing the metabolic function of the mucosa. *Saccharomyces boulardii* releases polyamines, which are implicated in stimulating the enzymatic activity of the colonic mucosa.

Saccharomyces boulardii is a promising agent for the amelioration of the course of acute diarrhea in children. Studies have shown that children treated with S. boulardii had a decreased number of episodes of diarrhea in subsequent months.

Another study reported reduction in number of episodes of diarrhea in the post-treatment follow-up period of two months, thereby stimulate the increase of IgA, and enhance the trophic activity of the mucosa. This explains the long term effect of *S*. *boulardii*.

Prebiotics and Diarrhea

Prebiotics are substances that are selectively utilized by host organisms to confer health benefits (Gibson et al., 2017). Consumption of prebiotics can improve the gut microbiota, which is beneficial to health.

Recent study has shown that prebiotic can increase the production of short chain fatty acid, play an important role in maintaining the intestinal barrier (Azad MAK et al., 2020; Snelson et al., 2021).

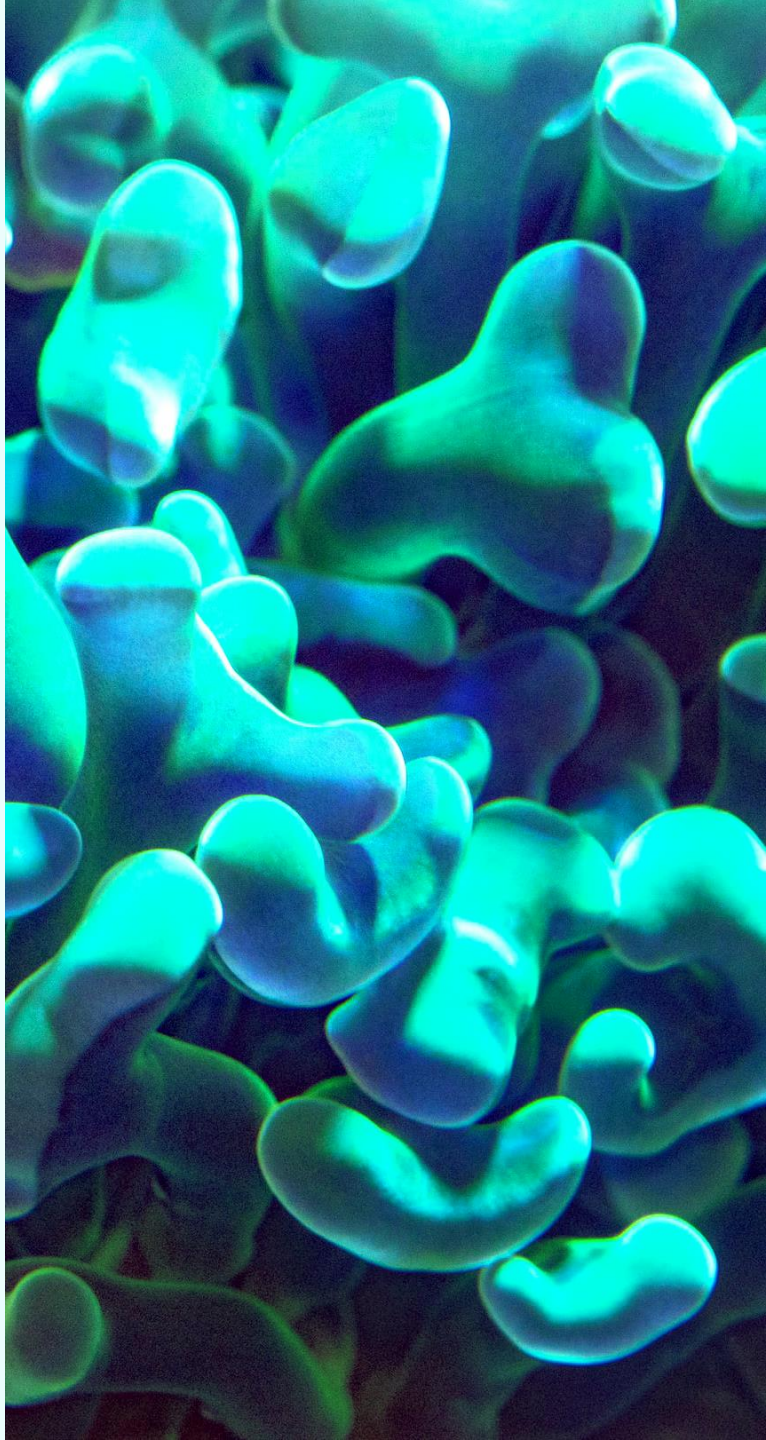
The gut lymphoid tissue can induce secrete cytokines and antimicrobial peptides such as β-defensins, to defend against the invasion of microorganisms (De Santis et al., 2015).

Some prebiotics such as fructo-oligosaccharide, inulin, pectin-oligosaccharides, etc., can resist the colonization of pathogen by acting as soluble decoy receptors that mimic the binding site of pathogens, thereby promoting the elimination of pathogens from the intestine (Pujari & Banerjee, 2021).

Previous studies have shown that prebiotics can shorten the duration of acute watery diarrhea and has a good therapeutic effect on diarrhea (Rigo-Adrover et al., 2017).

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Constipation – Severe & Chronic Causes & Remedies



Malta Further & Higher Education Authority

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Learning outcomes

- Physiology of normal bowel function
- Classification of constipation
- Causes of severe and chronic constipation
- Diagnosis of severe and chronic constipation
- Management and treatment
- Real clients case studies
- Complications and prognosis

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Constipation

- Characterised by infrequent bowel movements and symptoms like incomplete evacuation, bloating, straining, and hard stools.
- Most prevalent in Western countries, more in women than men.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Prevalence of constipation		
Demographic features	%	95% CI
Overall	15.6	13.2–18.0
Sex		
Men	10.6	7.6–13.6
Women	19.7	16.2–23.3
Age groups		
18–38 years	23.5	18.6–28.3
39–54 years	13.3	9.5–17.2
55–80 years	9.9	6.4–13.3
Educational level		
Primary	11.7	6.8–16.6
Secondary	14.8	11.1–18.5
Tertiary	18.3	14.2–22.3
Living environment		
Rural	13.5	9.7–17.3
Urban	16.7	13.7–19.8
Body mass index		
Underweight	12.5	−1.8–26.8
Normal weight	16.9	13.3–20.5
Overweight	12.2	8.4–15.9
Obese	18.9	12.7–25.0
Dietary factors		
Water intake < 1.5 L/day	21.5	16.3–26.6
Vegetables < 3 spoons/day	22.5	16.9–27.9
Fruits < 2 pieces/day	16.7	13.3–20.1
Whole grain bread < 3 slices/day	17.8	13.3–22.1

Reference: Verkuijl, S.J., Meinds, R.J., Trzpis, M. *et al., 2020*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

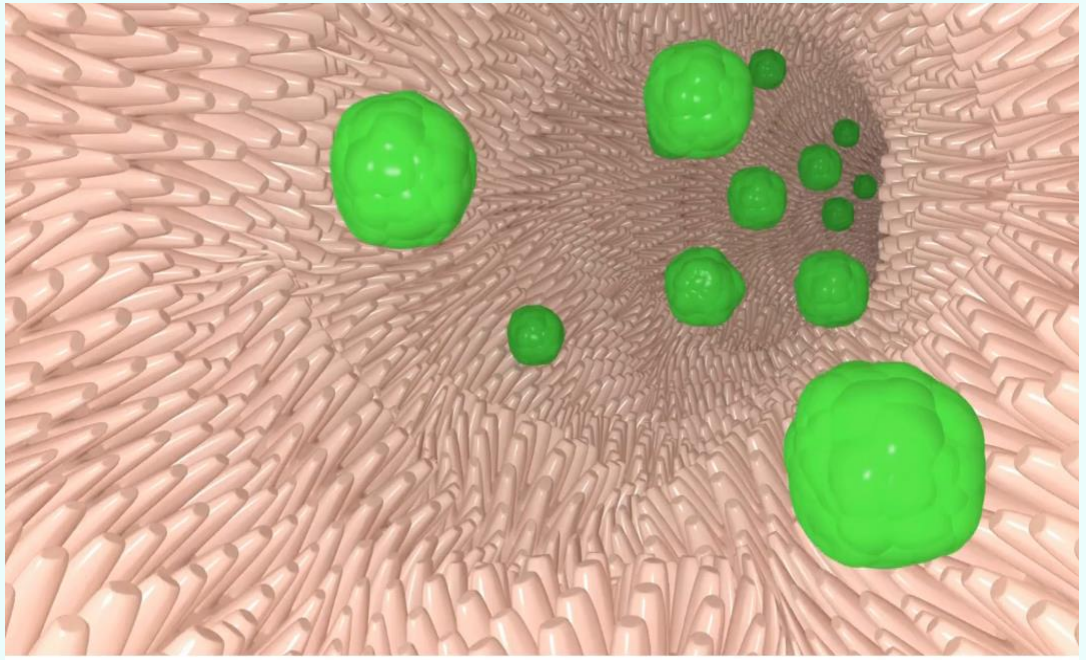
Constipation

- Can be categorised into primary (e.g., IBS-C, functional constipation) and secondary forms (from metabolic, medication-related, or neurological factors).
- Challenges in defining constipation
- Untreated constipation weighs on daily life and may lead to further health conditions

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Physiology of normal bowel function

- Bowel function: involves coordinated peristalsis, rectal filling, muscle actions, and sensory mechanisms



- Key factors include anal sphincters, puborectalis muscles, and rectoanal angle
- Factors include anal sphincters, pelvic floor muscle relaxation, and rectal anatomy

(Bharucha, 2006)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Physiology of normal bowel function

- High amplitude propagated contractions (HAPCs) occur 6 times a day, they propel stool forward triggered by eating/drinking
- Decreased HAPC frequency often associated with constipation
- Effective anorectal function relies on sensory perception and muscle coordination, including reflex relaxation triggered by rectal distension and voluntary control of defecation
- Normal colonic transit varies from 20 to 72 hours in adults

Bharucha, 2006, Southwell & Clarke et al., 2019

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Physiology of normal bowel function

Peristalsis and MMC

- Peristalsis is coordinated by enteric nervous system and autonomic nervous system
- Migrating Motor Complex (MMC) consists of four distinct phases
- Serotonin mediates peristalsis via myenteric plexus
- Autonomic nervous system modulates gut motility through Vagus nerve

Camilleri, Ford 2017; Keller & Gröger et al. 2011; Konturek & Brzozowski et al. 2011

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Physiology of normal bowel function

Peristalsis and MMC

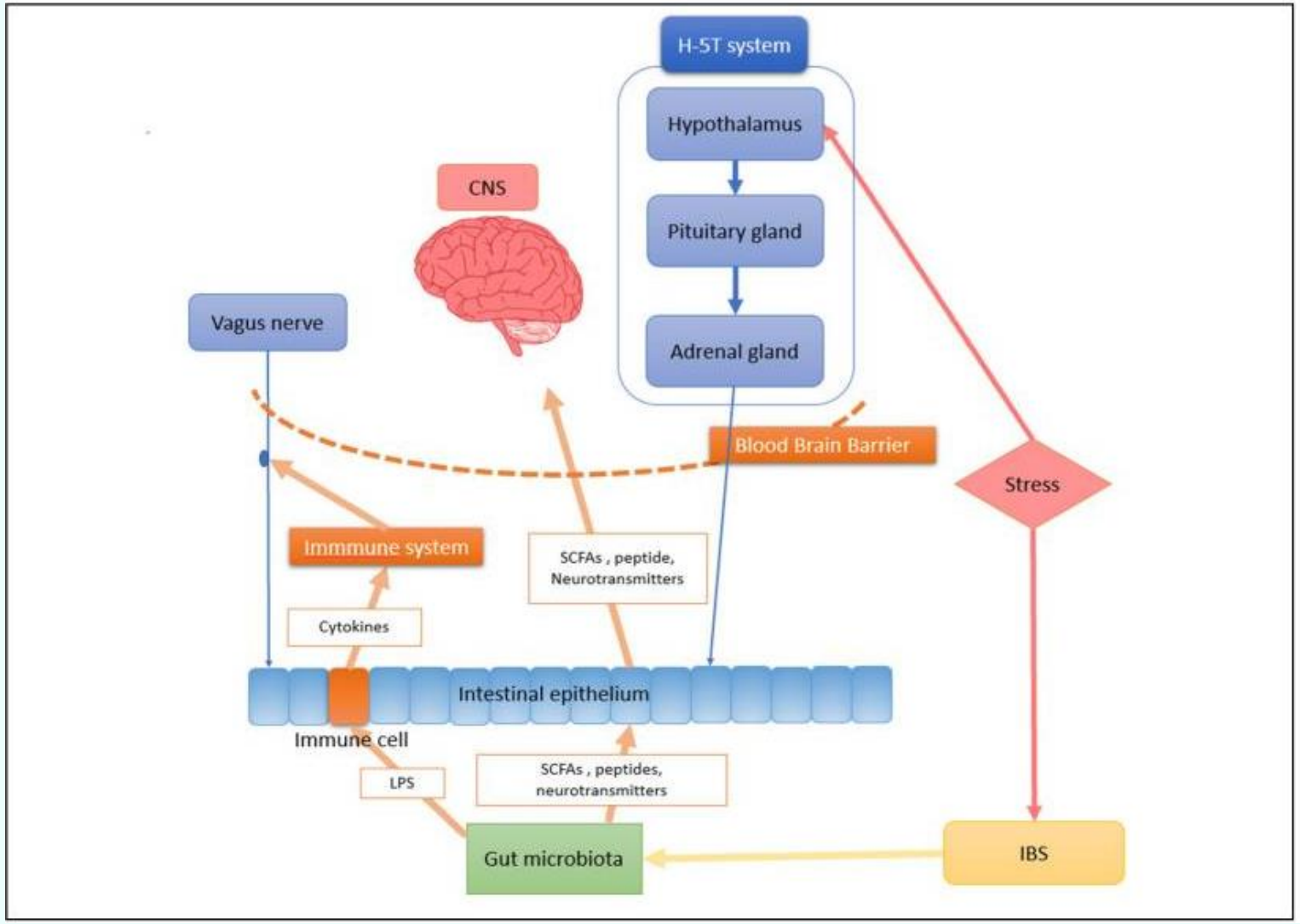
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut-Brain Axis and HPA Axis

- Gut-brain axis integrates gut functions with emotional and cognitive centres
- HPA axis coordinates stress response, releasing cortisol
- Cortisol affects brain and intestinal cells, influencing gut function
- Circadian rhythm disruption affects gastrointestinal functions

Carabotti, M., Scirocco et al. 2015; Qi, Z., Yan-Yan, C., Xu, D. Q., et al. 2021

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Reference: Zhu F, Tu H, Chen T. 2022

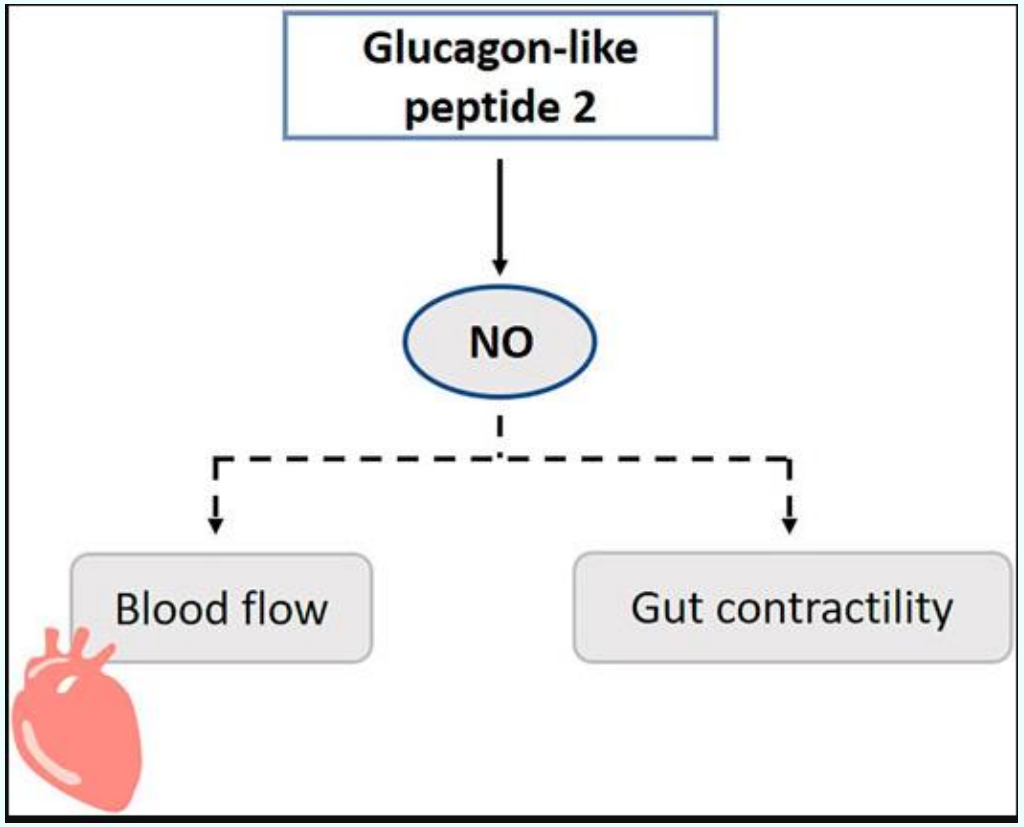
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

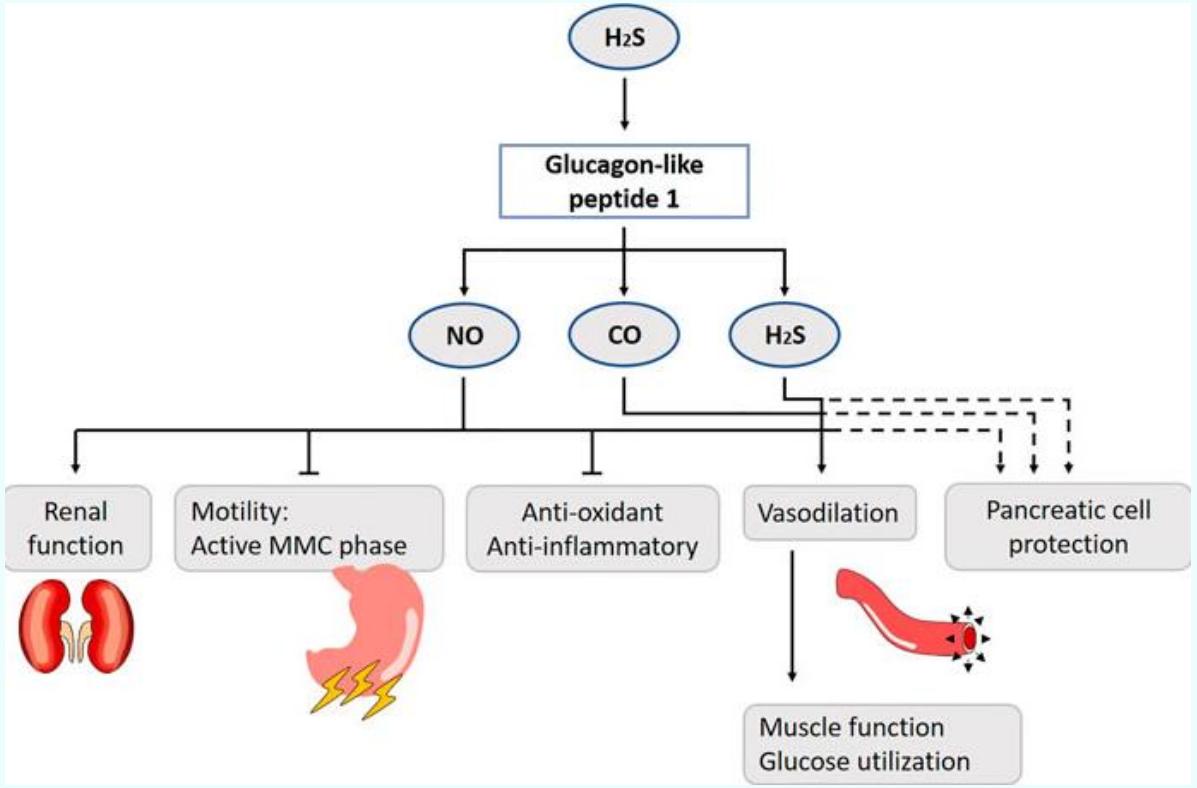
Regulation by Neurotransmitters

- Intestinal neurotransmitters categorised as either inhibitory or excitatory, play crucial roles in regulating gastrointestinal motility and secretion.
- VIP, NO, ATP, and H2S relax smooth muscles and reduce intestinal sensitivity, potentially exacerbating constipation.
- Excitatory neurotransmitters like serotonin (5-HT), acetylcholine (Ach), and substance P (SP) stimulate intestinal muscle contraction and enhance peristalsis

Zhao, Q., Chen, Y. Y., Xu, D. Q et al. 2021

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



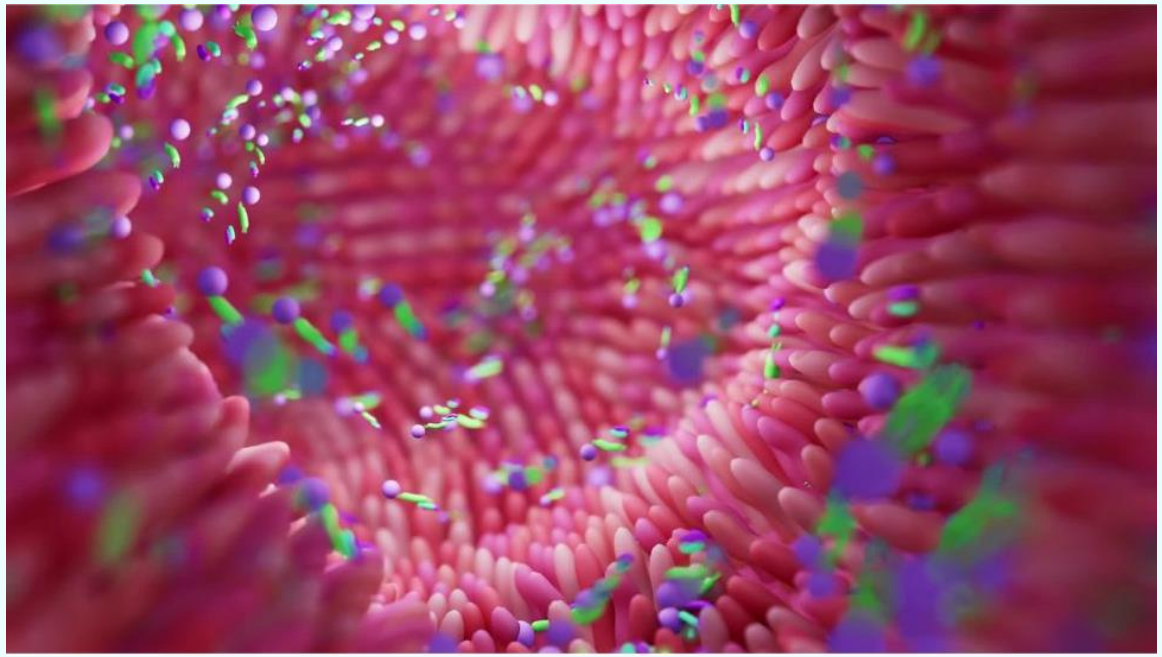


Reference: Verbeure, W., van Goor, H., et al. (2021).

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiota and Gut Motility

- Gut microbiome controls a development of constipation
- Bacteroides, Bifidobacteria, and Lactobacilli levels impact bowel emptying
- Short-chain fatty acids (SCFAs), endotoxins impact gut motility & gut brain axis



Chen Y, Xu J, Chen Y, 2021; Dimidi et al., 2017; Fukumoto et al., 2003; Segers et al., 2019

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Hydrochloric Acid and Digestive Enzymes

- Hydrochloric acid (HCL) is crucial for triggering the release of digestive enzymes from the pancreas, facilitating proper digestion in the small intestines
- Plays a role in preventing the overgrowth of pathogens - reducing the risk of SIBO
- Insufficient levels of HCL or digestive enzymes can impair digestion, leading to prolonged transit time
- Disrupted circadian rhythm also affects GI functions like digestive secretions, and motility

Lukowicz & Lacy, 2017

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Classification of constipation

Acute Constipation	Chronic Constipation
Sudden onset of constipation symptoms, typically lasts for a few days to a few weeks.	Persists for at least three months.
Causes may include dietary changes, dehydration, stress, medication side effects, or travel-related factors.	Can result from intrinsic colonic issues, organic diseases, or medication usage.
Management often involves lifestyle and diet modifications, occasional laxatives	Can result from intrinsic colonic issues, underlying diseases, long-term poor diet or medication usage.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Causes of severe constipation

- **Dietary Factors:** Insufficient fibre and water intake, excessive coffee, tea, or alcohol consumption can disrupt the microbiome, leading to acute constipation

- **Decreased Physical Activity:** Sedentary lifestyle impacts colonic motility and gut transit, while exercise stimulates the Vagus nerve and enhances GI hormone release, promoting bowel movement

Chan AO, Leung et al. 2007; Chin& Paw, 2006

contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

- **Stress:** Activation of the HPA axis and the release of CRF during stress can directly affect bowel function and gut microbiota

- **Pregnancy:** Elevated levels of progesterone and oestradiol during pregnancy, coupled with pressure from the growing uterus on the intestines

- **Travel:** Disrupted circadian rhythms due to travel can affect colonic pressure activity and bowel movement timing

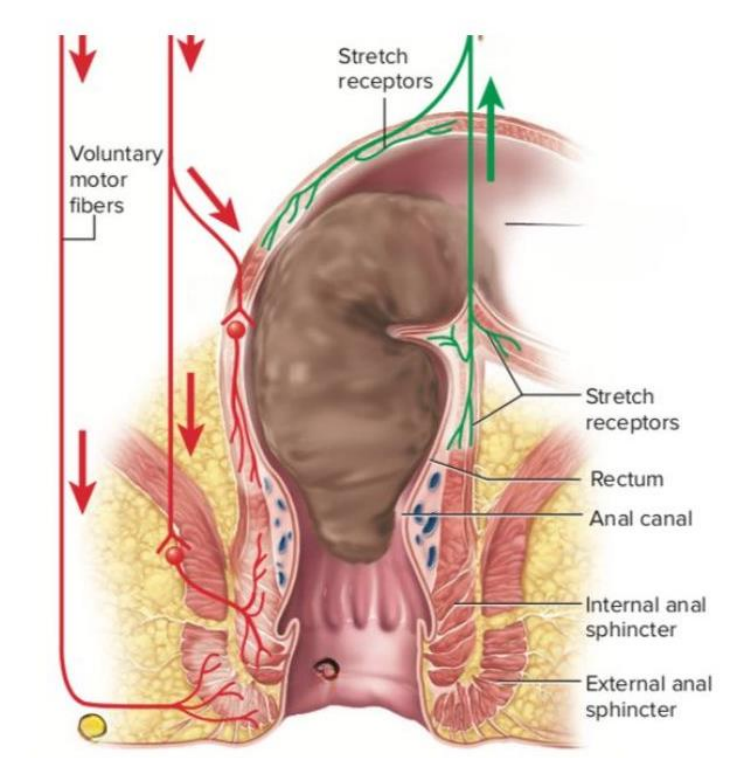
Czimmer J, Million, 2006; Hoogerwerf, 2006

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Causes of chronic constipation

Anorectal motor and sensory functions

- Normal defecation involves a complex interplay of colorectal motor and sensory functions
- Defecation disorders manifest as abnormalities like anismus, hypertonic internal anal sphincter, inadequate rectal propulsion, rectoceles, rectal prolapse, or sensory-motor decoupling



Reference: healthjade.com

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation – physiological factors

Motility Disorders:

- Delayed colonic transit correlates strongly with stool consistency, water content, and transit time. It can result from impaired motor activity, voluntary stool retention, or neuromuscular disorders like reduced HAPCs

- Increased dietary fibre intake may alleviate symptoms in individuals with normal colonic transit but may not help constipated patients with delayed colonic transit and defecation disorders

Andrews, C. N., & Storr, M., 2011

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Motility Disorders:

- Interstitial cells of Cajal (ICCs) regulate gastrointestinal motility by generating and propagating electrical slow waves to coordinate smooth muscle contractions

- Substance P is involved in motility regulation and expressed in myenteric plexus neurons

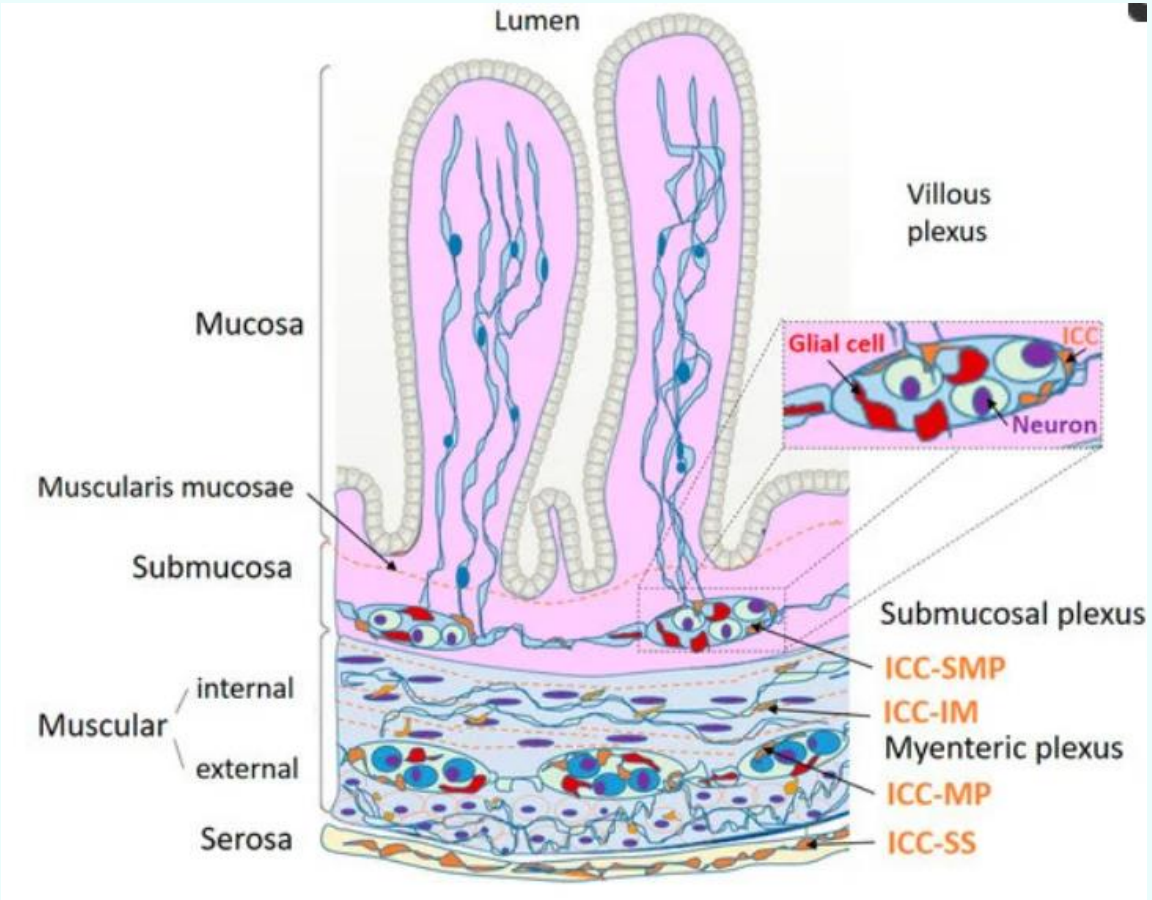
- The slow transit may result from impaired motor activity, decreased ICCs, or altered myenteric plexus neurons

- Variations in transit time may impact bacterial mass

Al-Shboul O. A. 2013

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

A representation of interstitial cells of Cajal at submucosal and myenteric plexuses in the digestive tube



Reference: López-Pingarrón, Almeida & Soria-Aznar et al. 2023

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

Impact of hormones

- Sex hormones, such as oestrogen and progesterone, play a significant role in regulating stool transit, modulate colonic motor functions, with oestrogen inhibiting colonic propulsion and progesterone receptor overexpression impairing gut motility.

contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

- Hormones influence microbial composition and metabolism, while gut microbes can impact hormone levels, leading to alterations in circulating hormonal profiles.

- Oestrogen receptor (ER) signalling within colonic mucosa modulates intestinal activity and gut motility, further impacting microbial composition.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

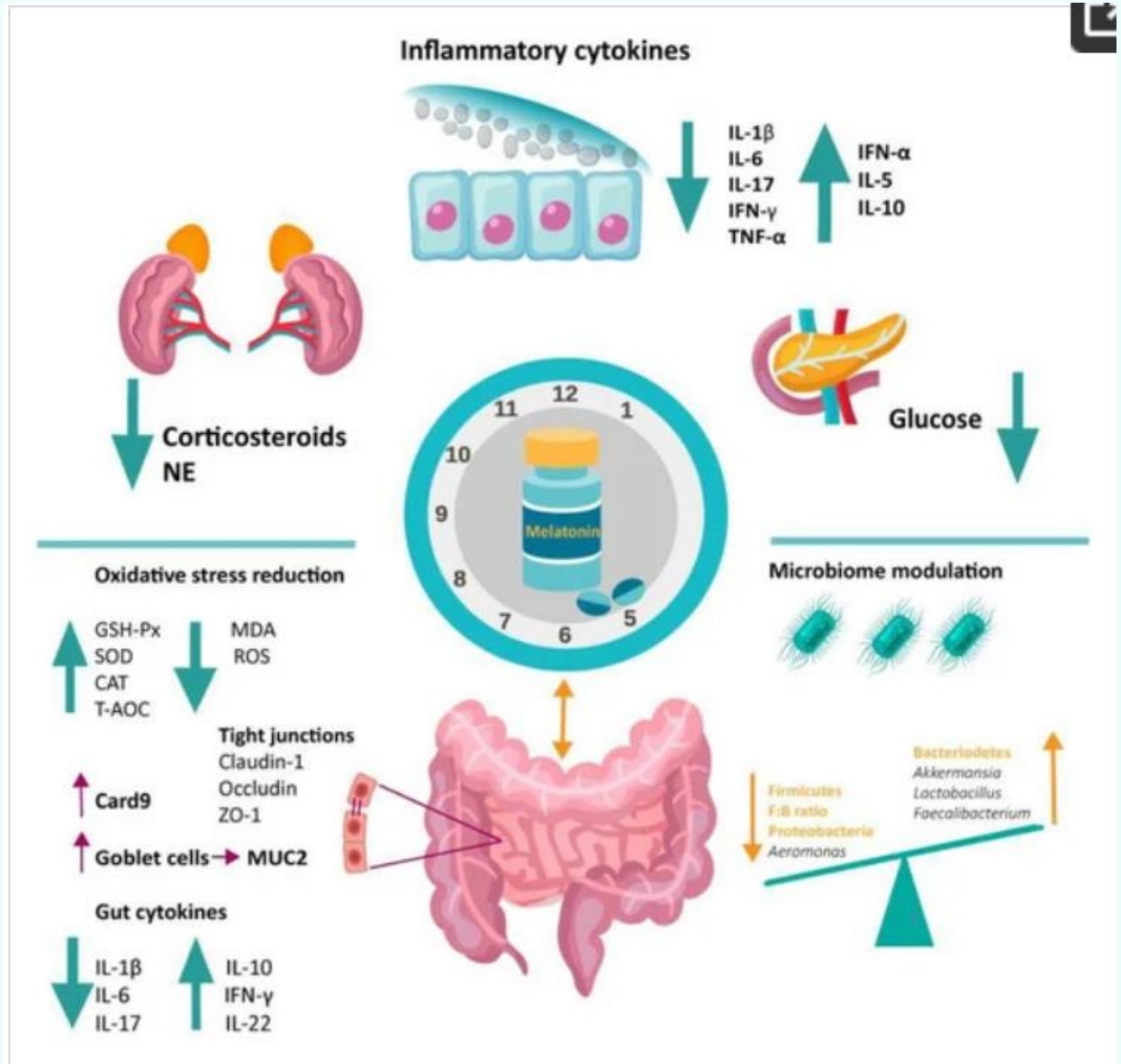
Circadian rhythm - workshifts

- Disruption of the circadian rhythm affects key gastrointestinal functions, including secretion, enzyme activity, motility, and nutrient absorption

- Melatonin, a hormone involved in regulating the sleep-wake cycle, may play a role in preserving intestines integrity and gut motility

Duboc H, Coffin et al. 2020

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Reference: Iesanu MI, Zahiu CDM & Dogaru I-A, et al. 2022

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

Gut Microbiota, Intestinal Secretion, and Constipation

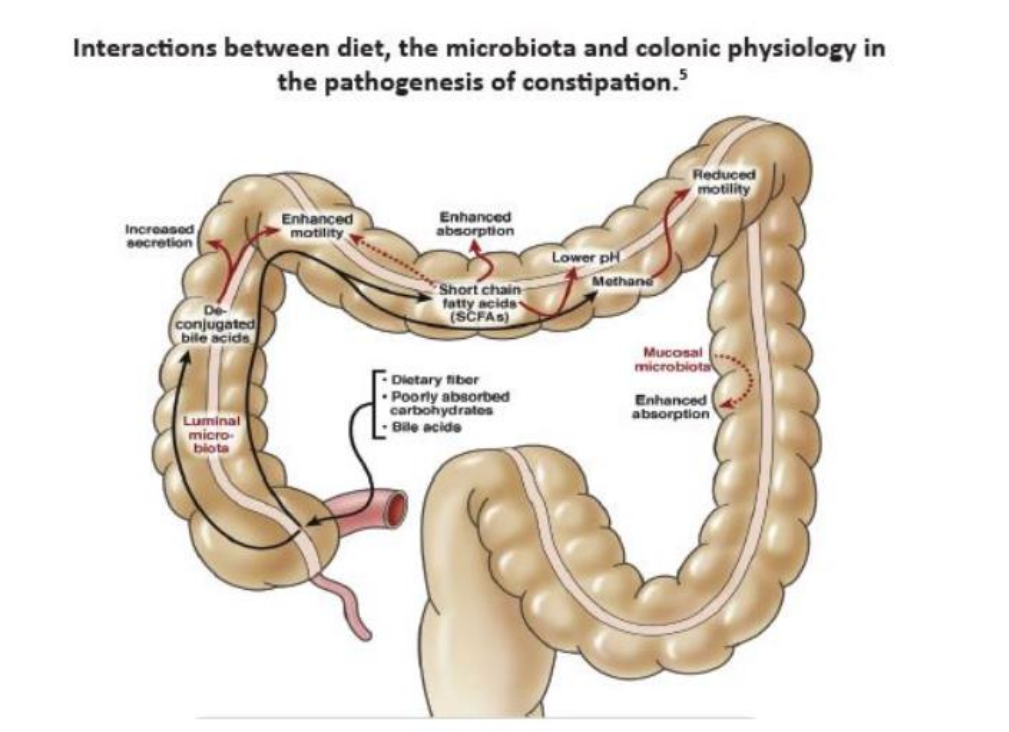
- Decreased beneficial bacteria (Lactobacillus, Bifidobacterium, Prevotella)
- Increased harmful microorganisms (Bacteroides sp., Enterobacteriaceae)
- Elevated levels of butyrate-producing genera (Coprococcus, Roseburia, Faecalibacterium)

Gerritsen et al. 2011; Kirgizov et al. 2001

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

- SCFA influence gut motility by stimulating ileal propulsive contractions
- Dysbiosis leads to greater water retention and decreased faecal water content
- Bacterial endotoxins delay gastric emptying

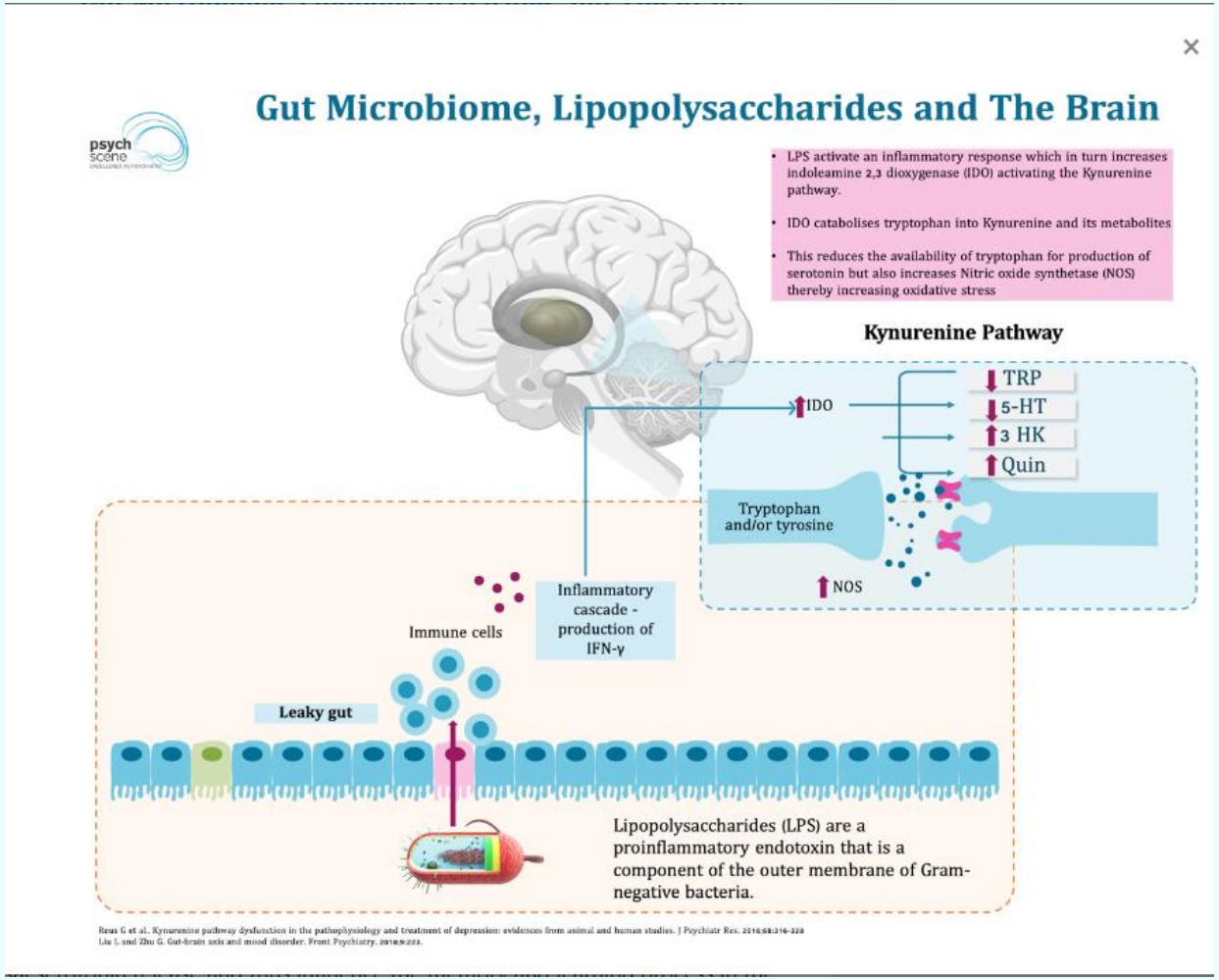


Reference: Quigley & Spiller 2016

- Bile acids activate TGR5 receptor, releasing 5-HT and calcitonin gene-related peptide, initiating bowel peristaltic reflex

Gou, X., Qin, L., Wu et al. 2023

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Reference: Réus, G. Z., Jansen, K. & Titus, et al. (2015)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

SIBO

- SIBO can impair small intestinal motility and delay small bowel transit time
- Human colonic gases, such as methane (CH4) and hydrogen sulfide (H2S), influence gut motility, with methane production potentially slowing gut transit and altering faecal microbiota composition
- Hypothyroidism can be potentially contributing to SIBO

Dukowicz, A. C., Lacy, B. E., & Levine, G. M. 2007

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

Mould

- Mold exposure is linked to higher prevalence of hypothyroidism, reducing gut motility
- Mycotoxins alter microbiome composition, increase inflammation, and reduce beneficial bacteria like Lactobacillus reuteri and SCFA
- Mycotoxins may interfere with liver detoxification, impacting bile acid release and clearance, worsening gut health and microbiome status

Pinton and Oswald, 2014

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

Low serotonin

- Reduced serotonin levels lead to decreased colonic transit

- The gut microbiota influences the growth of colon enterochromaffin cells, leading to the upregulation of 5-HT-positive cells

- In constipation, the gut microbiota elevates serotonin transporter expression, resulting in decreased colonic transit due to increased removal of 5-HT from the gut

Wei, L., Singh, R., Ro, S., & Ghoshal, U. C. 2021

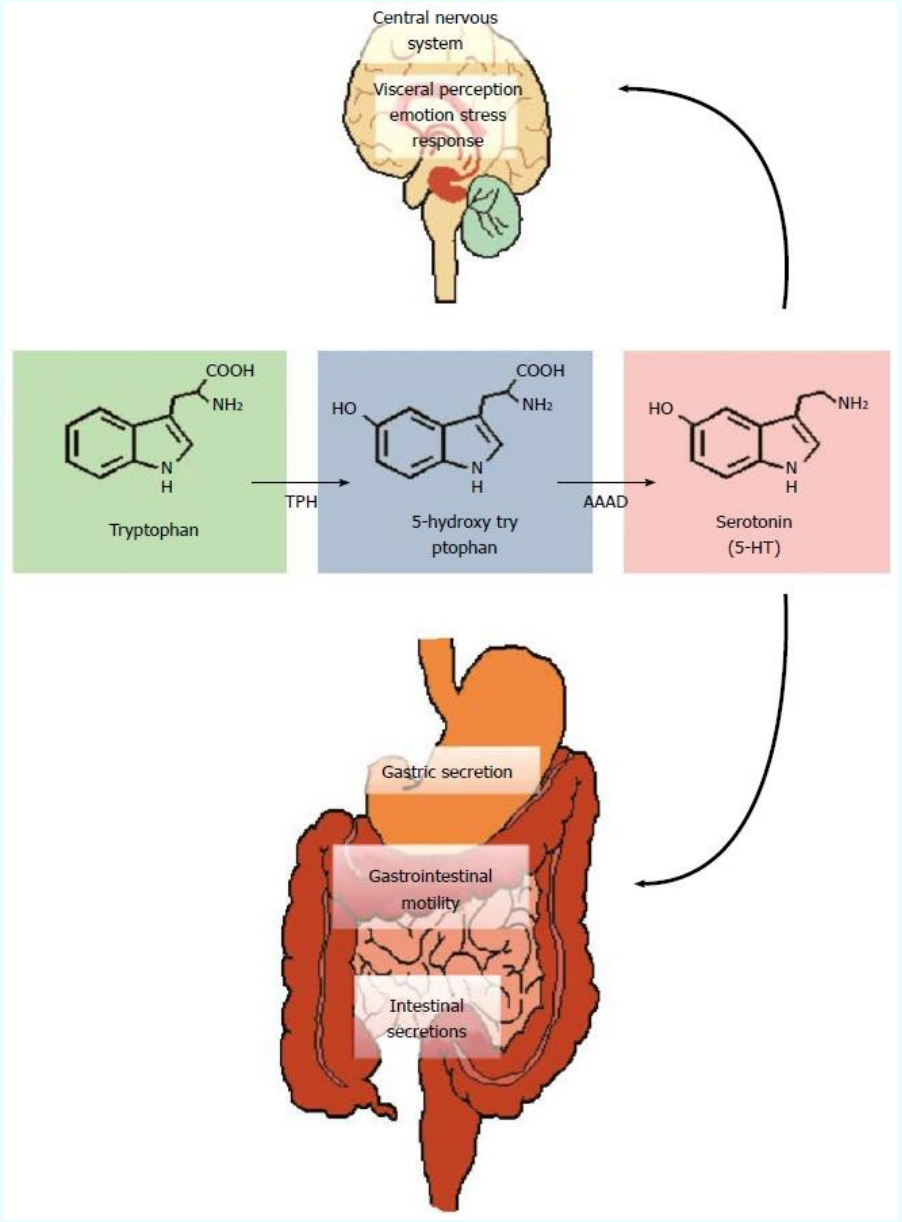
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

Low serotonin

- Microbiota may impact 5-HT production by modulating tryptophan metabolism in the gut
- Decreased relative abundance of gut microbiota such as *Firmicutes* and increased *Bacteroidetes* impacts serotonin levels
- Serotonin is synthesised by Clostridium spp. and Escherichia coli, influence peristalsis and motility by interacting with the enteric nervous system, thus low levels can impact constipation development

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Reference: Kennedy, Cryan & Dinan et al. 2014

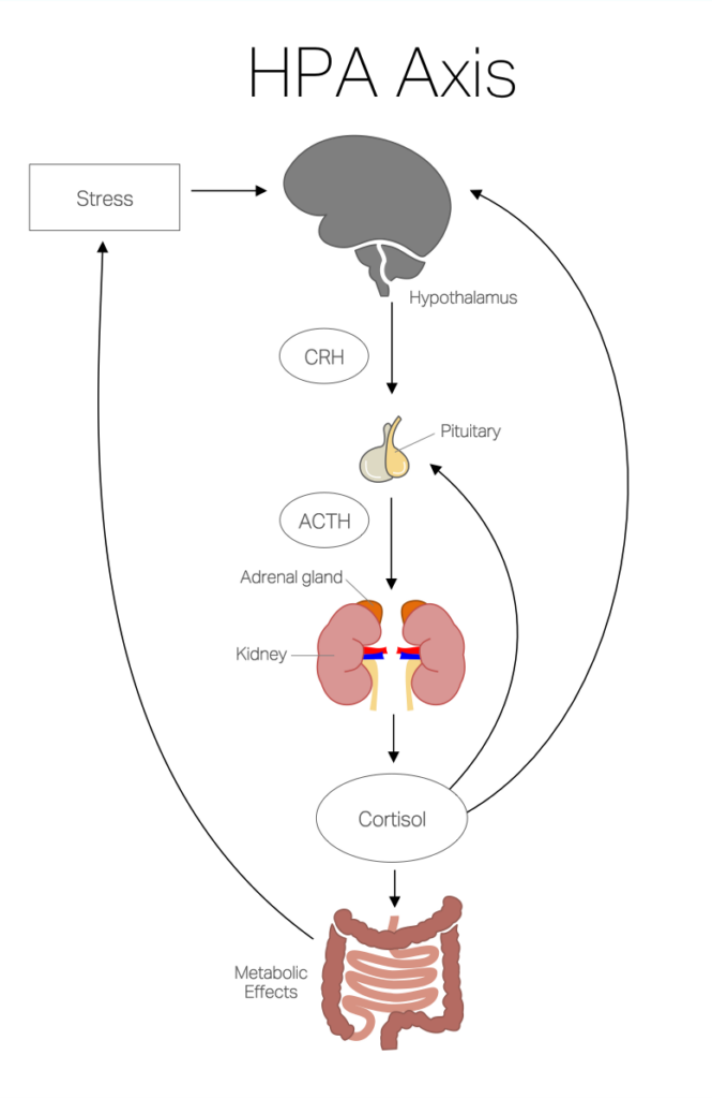
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

Chronic anxiety, stress

- Cortisol can increase intestinal permeability, leading to inflammation and bowel dysfunction

- Diminished expression of MUC2, a key mucin protein, reduces mucin release, compromising the epithelial barrier and exacerbating constipation-induced dysbiosis



Source: bellalindenmann

Cao H., Liu X., An et al. 2017

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

Neurological conditions:

- Conditions like spinal cord injury, multiple sclerosis (MS), and Parkinson's disease are associated with chronic constipation
- The pathophysiology of constipation in MS involves neurological dysfunction affecting gut motility
- In Parkinson's disease, both central and peripheral mechanisms contribute to constipation

Andrews, C. N., & Storr, M. 2011

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

Medications

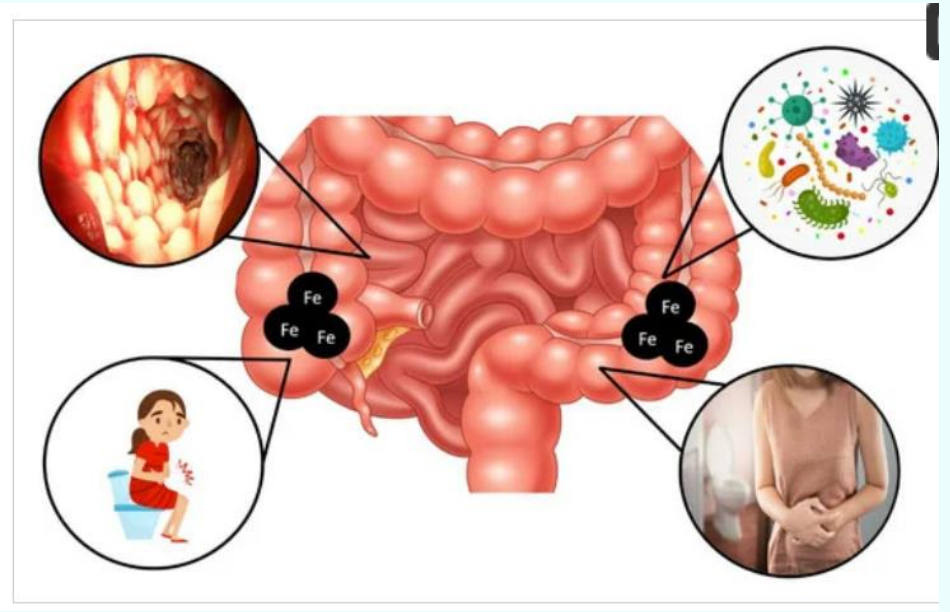
- Antihypertensive medications such as clonidine, calcium antagonists, and ganglionic blockers can decrease smooth muscle contractility
- Antidepressants are often associated with constipation

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Chronic constipation –

- Oral iron supplements can cause constipation by irritating the gastric mucosa and prompting water movement into the intestinal lumen via osmotic forces

- Aluminium-containing medications like sucralfate and antacids can contribute to constipation, with proton pump inhibitors serving as potential alternatives.



Bloor SR, Schutte, 2021; Yiannikourides A, Latunde-Dada, 2019

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Diagnosis of severe and chronic constipation

Stool and Medical History

- A thorough medical history is crucial in diagnosis of severe and chronic constipation using the Rome IV criteria

- Metabolic tests such as thyroid panel, serum glucose, creatinine, and calcium levels, along with colonoscopy

Table 2

ROME IV Criteria

ROME IV criteria for functional constipation(must have at least 2 of 6):
• Fewer than 3 bowel movement per week
• Straining during >25% of the time
• Lumpy or hard stools > 25% of the time
• Sensation of anorectal obstruction > 25% of the time
• Sensation of incomplete evacuation > 25% of the time
• Manual maneuvers required to aid defecation > 25% of the time

Basilisco, Coletta, 2013; Jani B, Marsicano E., 2018

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Bristol Stool Chart

	Type 1	Separate hard lumps	SEVERE CONSTIPATION
	Type 2	Lumpy and sausage like	MILD CONSTIPATION
	Type 3	A sausage shape with cracks in the surface	NORMAL
	Type 4	Like a smooth, soft sausage or snake	NORMAL
	Type 5	Soft blobs with clear-cut edges	LACKING FIBRE
	Type 6	Mushy consistency with ragged edges	MILD DIARRHEA
	Type 7	Liquid consistency with no solid pieces	SEVERE DIARRHEA

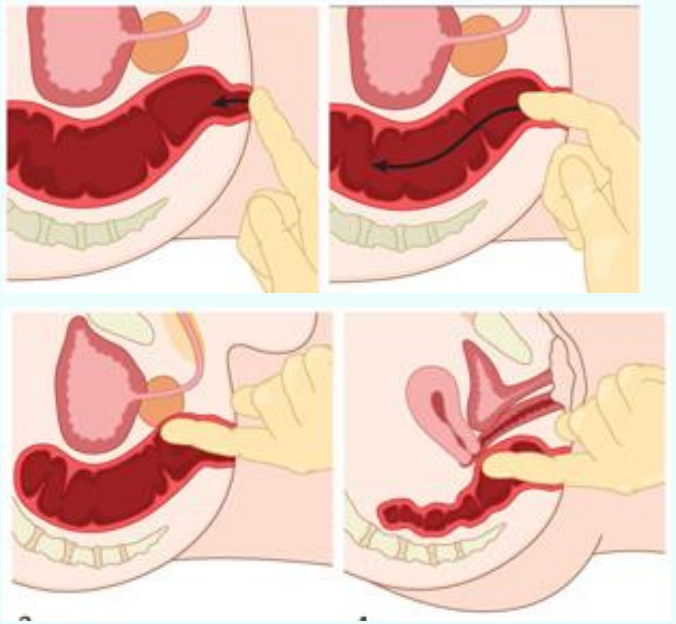
Reference: Kumar, Jayant & Kumar, et al. (2023)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Digital Rectal Examination (DRE)

- Essential in physical assessment, aiding in identifying pelvic floor dyssynergia with high specificity and sensitivity compared to standard manometry

- It helps exclude alarm symptoms indicating severe gastrointestinal conditions and detects polyps, masses, haemorrhoids, rectal prolapse, mucosal injury, inflammation, ulcers, or fissures.



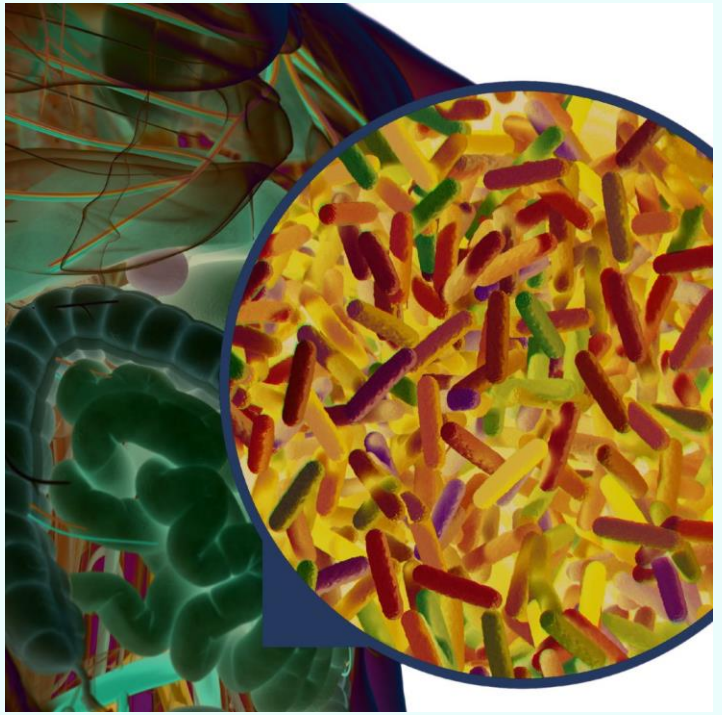
Liu, J., Lv, C., Huang, Y., Wang, et al. 2021

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Diagnosis of severe and chronic constipation

Stool test

- Stool microbiome testing provides insights into the composition and diversity of bacteria in the gut, offering information about the underlying causes of constipation
- Offers a comprehensive assessment of the gut ecosystem, from SCFA, to pathogenic bacteria or parasites



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Management and treatment of severe and chronic constipation

Non-pharmacological interventions - Diet

- Soluble fibres like Psyllium are effective in increasing defecation frequency, stool consistency, and reducing tenderness in defecation
- Insoluble fibre, such as bran, improves intestinal transit time
- Gradually increase fibre intake, up to a maximum of 35 g per day

Pannemans, & Masuy, 2020

Considerations and Side Effects

- Fibre intake may lead to side effects such as discomfort, flatulence, or bloating due to gas production

- An imbalance of gut bacteria such as primarily Bacteroides, Ruminococcus, and Roseburia responsible for carbohydrate metabolism may produce significant amounts of hydrogen gas in the colon – flatulence

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Major gas producing microbes present in the human gut microbiome

Phylum	Genus and/or species	Gas
Bacteroidetes	*Parabacteroides*	H_2 & CO_2
	Alistipes	H_2 & CO_2
	Bacteroides	H_2 & CO_2
Firmicutes	*Enterococcus*	H_2 & CO_2
	Dorea	H_2 & CO_2
	Clostridium spp.	H_2 & CO_2
	Roseburia intestinalis	H_2 & CO_2
	Ruminococcus	H_2 & CO_2
	Anaerostipes caccae	H_2 & CO_2
	Eubacterium rectale	H_2 & CO_2
	Blautia	H_2 & CO_2
	Veillonella	H_2 & CO_2
	Victivallis vadensis	H_2 & CO_2
	Desulfotomaculum	H_2S

Reference: Mutuyemungu, Singh & Liu et al. 2023

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

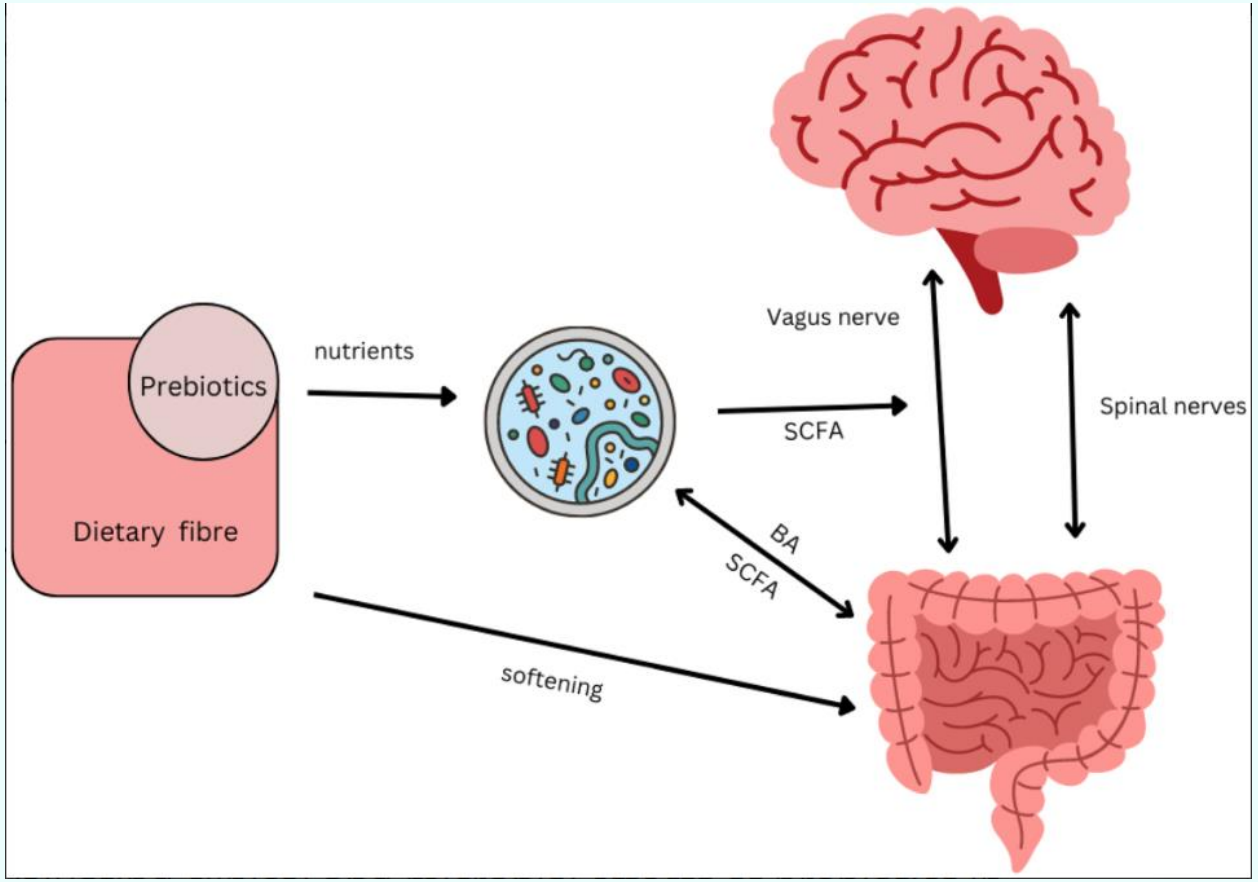
Prebiotics

- Prebiotics undergo bacterial metabolism in the colon, producing lactic and short-chain fatty acids and other beneficial bacteria
- Examples include fructo-oligosaccharides, galacto-oligosaccharides, and inulin.
- Prebiotics such as GOS and FOS increase bifidobacteria and inulin impacts intestinal microbiota, normalizing stool frequency
- Polyphenols increase Bifidobacteria and Lactobacillus aiding in symptom relief.

Linetzky Waitzberg et al. 2012

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Interacting pathways between microbiota, prebiotics, dietary fibre, gut and brain affecting constipation

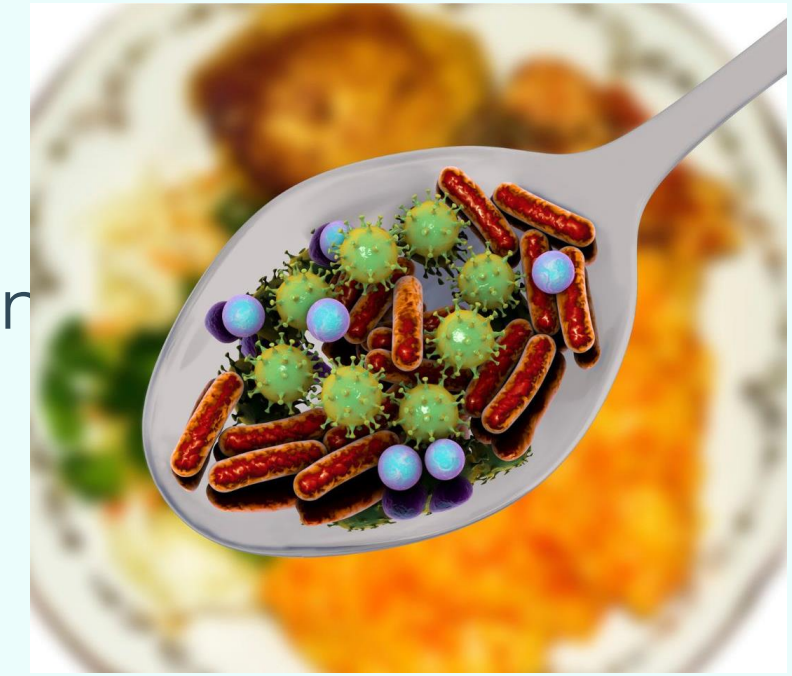


References: Erhardt R, Harnett JE et al. 2023

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Probiotics

- Probiotics may modify altered intestinal microbiota, gut sensation and motility function, and regulate the intraluminal environment, contributing to constipation relief
- Prevotella is correlated with softer stools
- Bifidobacteria and Lactobacilli produce SCFAs, stimulate intestinal peristalsis, and increase faecal moisture



Waller et al. 2011

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Probiotics

- Specific strains like Lactobacillus plantarum, Bifidobacterium breve, Bifidobacterium lactis, and L. reuteri alleviate evacuation difficulties and enhance colonic motility

- Butyrate-producing bacteria like Coprococcus and Roseburia stimulate gut motility

- Combining probiotics and prebiotics (synbiotics) offers synergistic benefits, enhancing probiotic survival and rebalancing intestinal microbiota, thereby aiding in constipation management

Barbara et al., 2005; Del Piano et al., 2010; Ojetti et al., 2014;

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Management and treatment of severe and chronic constipation

Elimination diet

- Eliminating inflammation-triggering foods is crucial for promoting healing and regeneration of the gut microbiome
- Even commonly known healthy foods can exacerbate the symptoms and cause imbalance, testing is best!
- Poor absorption of high-FODMAP diets leads to fermentation in the colon, resulting in GI symptoms

Thomas, A., Thomas, A., & Butler-Sanchez, M. 2021

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Elimination diet

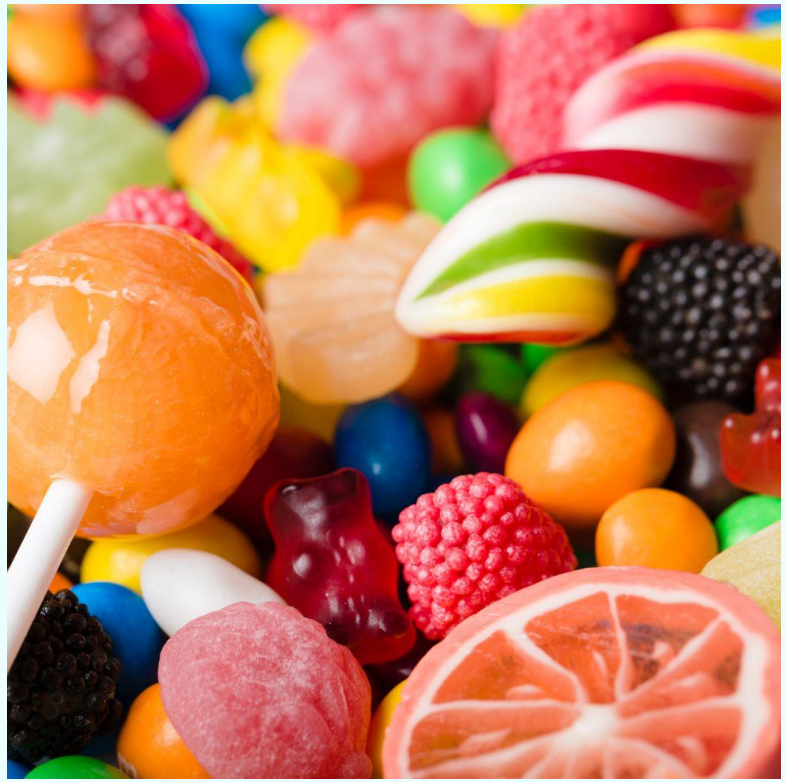
- The elimination diet comprises three phases: restriction, reintroduction/rechallenge, and personalisation/maintenance

- Gluten-free and low-FODMAP diets reduce gut bacterial concentrations of Bifidobacterium adolescentis and Faecalibacterium prausnitzii

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Elimination diet

- Excessive sugar or starch consumption can lead to Candida = dysbiosis
- Artificial food ingredients for instance maltodextrin and polysorbate-80 are linked to gut dysbiosis and intestinal inflammation, highlighting the importance of eliminating processed foods from the diet



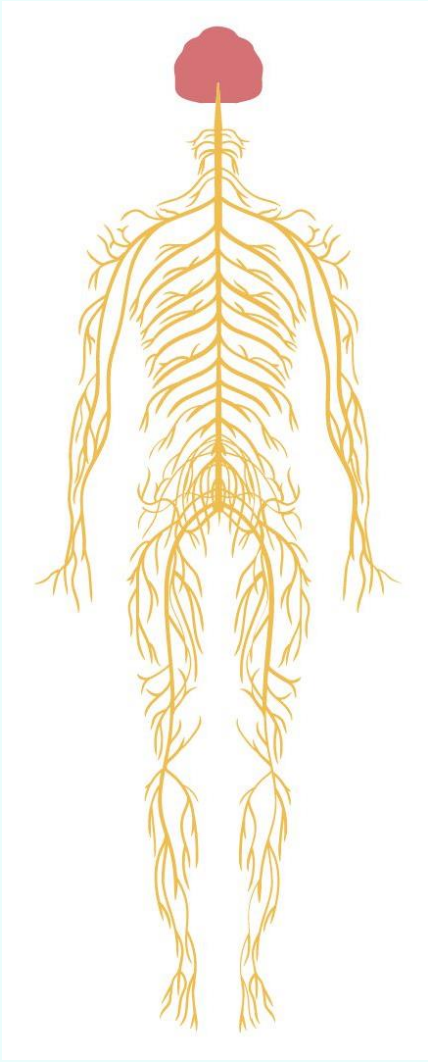
Liu, C., Zhan, S., Tian et al. 2021

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut-brain axis modulation

- The Vagus nerve (VN) is key in gut-brain communication, housing receptors that interact with gut microbes and their metabolites

- Treatment with Lactobacillus rhamnosus can alter GABA mRNA in the brain and, together with Lactobacillus reuteri, the excitability of intestinal sensory neurons

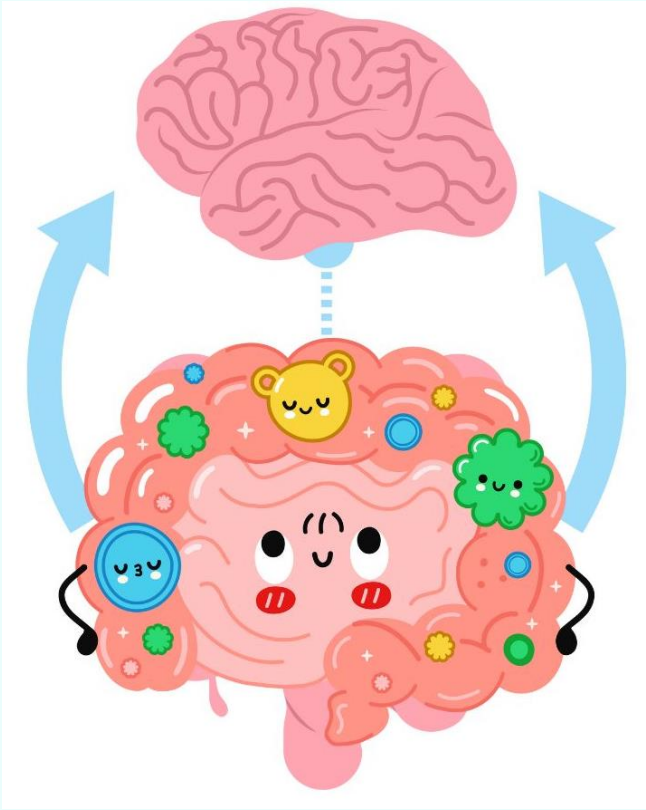


Carabotti, M., Scirocco, A., Maselli, M. A et al. 2015

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut-brain axis modulation

- Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 restore tight junction barrier integrity
- SCFAs activate vagal afferent fibres, impacting gut-brain communication
- Enhancing the parasympathetic nervous system and stimulating the Vagus nerve by lifestyle practices can impact gut motility



Han, Y., Wang, B., Gao et al. 2022

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Medications

- Bulk laxatives like psyllium (Metamucil) are usually well-tolerated for ongoing use
- Stimulant laxatives such as senna and bisacodyl (Dulcolax) may cause cramping
- Osmotic agents, including PEG-based solutions, magnesium citrate, sodium phosphate products retain water in the colon through an osmotic effect

Ikarashi et al., 2011

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Herbal Supplements

- Aloe Vera: Barbaloin, a key compound increases water content in the intestinal lumen, providing a laxative effect.

- Triphala: protect against intestinal injuries, improve epithelial cell integrity, and reduce gut mucosa enzyme levels.

- Marshmallow root : contains mucilage, contributing to its therapeutic effects in treating constipation

Hong, S. W., Chun, J., Park, et al. 2018; Tarasiuk, A., Mosińska, P., & Fichna, J., 2018

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Surgery

- Considered when pharmacological treatments are ineffective
- Options include ileostomy, total colectomy with ileorectal anastomosis, or cecostomy with antegrade enemas
- Segmental colonic resection or total colectomy may be beneficial
- Correction of pelvic floor dysfunction is prioritised
- Common procedures include the Malone procedure and percutaneous cecostomy (PEC).

Włodarczyk, J., Waśniewska, A., Fichna et al. 2021

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies

Janelle, 40 years of age, school teacher— does not enjoy job

Presents with: Bloating, constipation, sugar cravings and fatigue
Diet: High in refined carbohydrates, low protein, trans fats, snacks frequently.
Drinks 2–3 bottles of white wine per week. 700 ml water / day.
History: On / off antibiotics for 3 years
Stool test: low *Faecalibacterium*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies:

Normally, not knowing client's microbiome test, we would switch her from processed diet and put her on high fibre, fruit and vegetable diet.

However, seeing her stool test result we cannot give a healthy meal containing quinoa, beans, mushrooms and spinach or oats with dried fruits and pumpkin seeds as these will further decrease *Faecalibacterium*, which is responsible for stabilising gut microbiota.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies:

Recommendations:
Remove processed foods
Consume steamed apples with cranberries 3x a week.
Carrot and butternut squash soup
Increase pre- and probiotics foods like leeks, dandelion greens or miso
Healthy fat & protein: Salmon, Herring

Supplements:
Magnesium and probiotic blend

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies:

Kate, 38 years, buyer for a grocery chain, 50 hours / week.

Presents with: 4 months — stress and anxiety, difficulty falling asleep, constipation
Diet: Coffee x 3 / day milk +1 sugar, water x 4 glasses, wine x 2 glasses most nights to 'wind down'. Often skips breakfast. Sandwiches (cheese or egg), mostly vegetarian, few legumes or nuts. Rice or pasta and fresh veg for dinner
Tests: increased Firmicutes to Bacteroidetes (F/B) ratio

Case studies:

An imbalance in the Firmicutes to Bacteroidetes (F/B) ratio, particularly concerning the gut-brain axis, thus affecting her mood and serotonin synthesis.

First thing before assessing her stool test is giving her a substitute to her regular sandwich breakfast with chia or flax seed pudding, however in this case they could increase the ration and not help which can make her give up.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies:

Focusing on foods: Cod, Halibut, Herring, Mackerel, Salmon, Trout, Oysters, Nori, peas, mushrooms Greek yogurt, egg yolk, parmesan,
Ganoderma lucidum- Reishi that is also calming

Adding calming practices that stimulate Vagus nerve and calm the NS:

- Magnesium salt baths
- Deep breathing

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies

Agnes, 42 years, retail assistant, average 45h/a week

Presents with: constipation, anxiety, depression, hormonal issues- oestrogen dominance
Diet: 3 cups of coffee, monotonous diet, eats a lot of dairy produce, pesto pasta most days, used to drink excess alcohol and smoke
History: lives in a mouldy flat, took rounds of antibiotics
Test: Low *Akkermansia,* high *Enterococcus*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies:

If we have clients with oestrogen dominance we usually focus on their detox and healthy hormonal support, adding plant protein: lentils, quinoa, or seed cycling: pumpkin seeds, flax, chia seeds to reduce inflammation.

However, in this case, the stool test shows us that consuming chia, lentils, quinoa, salmon or spinach may potentially cause an increase in *Enterococcus*.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies:

Recommendations:

Increase red pigment fruits and vegetables such as: blueberry, cranberry, pomegranate.

Remove foods that will increase *Enterococcus* and decrease Akkermansia.

Reduce your coffee intake- which can increase cortisol

Diversify your meals

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies:

Examples of foods:

AVOID	CONSUME
Cuttle fish	Clams
Herring	King Crab
Salmon	Octopus
Trout	Lettuce, Bitter
Tuna	Cabbage
Quinoa	Berries
Pumpkin & Sunflower Seeds	Buckwheat
Prunes	Kefir
Avocado	Fermented foods

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies:

Alex, 55y old., office worker, average 50 hours/a week
Presents with: bloating, constipation
Diet: Processed foods, ready-made sandwiches, diet cokes, microwaved meals
History: Smoker, few beers after work x3 a week
Test: high *Lachnospiraceae* and low *Prevotellaceae*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case studies:

In this client's case we discovered he could not tolerate garlic; hangover-like feeling the next day. This is probably due to fructan, alliin and other organosulfur derivatives.

Most of the healthy foods would increase his *Lachnospiraceae* and decreasing *Prevotellaceae* bacteria increasing constipation, effectively.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

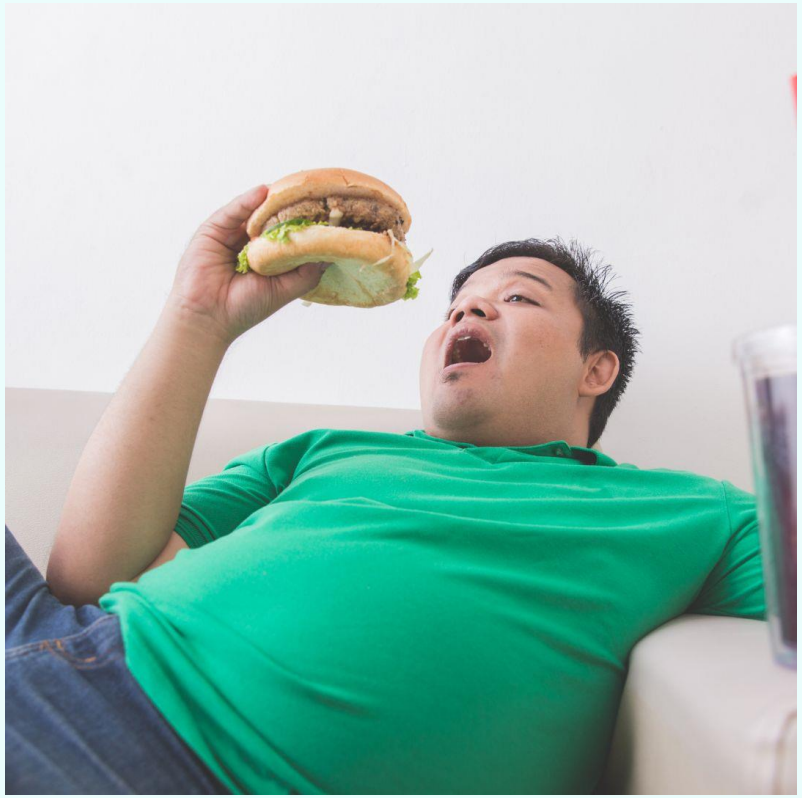
Case studies:

AVOID	CONSUME
Garlic	Broccoli
Apples	Kiwi
Leeks	Peppers
Beetroot	Navy beans
Berries	Brussels sprouts
Coffee	Buckwheat
Black beans	Chicken breast

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Prognosis

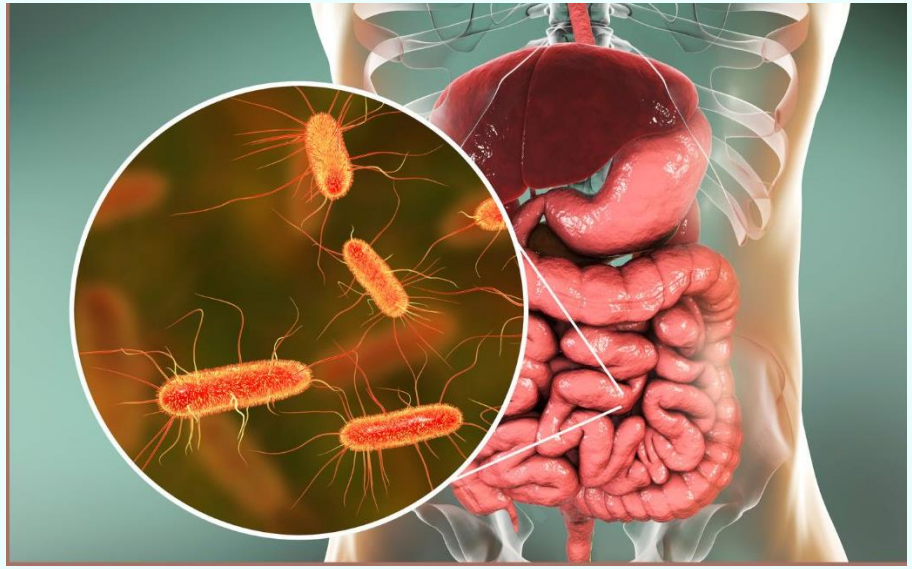
- Factors like gender, age, and delay in treatment initiation affect the treatment
- Some treatments may be ineffective or offer short-lived benefits
- Recurrence common due to non-compliance with dietary or lifestyle recommendations.



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Prognosis

- Microbiome modulation offers promising method in treating constipation
- Stool testing is a non-invasive method, offers a personalised approach and more effective treatments



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Common Complications

- Abdominal discomfort or cramps
- Decreased quality of life
- Haemorrhoids
- Anal fissures
- Pelvic floor damage
- Faecal incontinence
- Urinary retention
- Stercoral perforation
- Rectal prolapse

Diaz S, Bittar K, Hashmi MF, et al. 2023

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Conclusion

- Constipation is seen in patients of all ages, and the causes are diverse
- There is no one or quick solution and a microbiome testing followed by a personalised approach are best to manage the disorder
- Patients' education empowers them to manage their condition effectively, enables prevention and reduces complication risks

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

Andrews, C. N., & Storr, M. (2011). The pathophysiology of chronic constipation. Canadian Journal of Gastroenterology = Journal Canadien de Gastroenterologie, 25(Suppl B), 16B–21B.

Al-Shboul O. A. (2013). The importance of interstitial cells of cajal in the gastrointestinal tract. *Saudi journal of gastroenterology : official journal of the Saudi Gastroenterology Association*, *19*(1), 3–15.

Basilisco, G., & Coletta, M. (2013). Chronic constipation: A critical review. Digestive and Liver Disease, 45(11), 886–893.

Bharucha, A. E. (2006). Pelvic floor: Anatomy and function. Neurogastroenterology and Motility, 18, 507–519.

Camilleri, M., Ford, A. C., Mawe, G. M., Dinning, P. G., Rao, S. S., Chey, W. D., et al. (2017). Chronic Constipation. Nature Reviews Disease Primers, 3, 17095..

Cao, H., Liu, X., An et al. (2017). Dysbiosis contributes to chronic constipation development via regulation of serotonin transporter in the intestine. Gastroenterology, 7, 10322.

Carabotti, M., Scirocco, A., Maselli, M. A et al. (2015). The gut-brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Annals of gastroenterology*, *28*(2), 203–209.

Chan, A. O., Leung, G., Tong, T., & Wong, N. Y. (2007). Increasing dietary fiber intake in terms of kiwifruit improves constipation in Chinese patients. World Journal of Gastroenterology, 13(35), 4771–4775.

Chen, Y., Xu, J., & Chen, Y. (2021). Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders. Nutrients, 13(6), 2099

Chin, A., Paw, M. J., van Poppel, M. N., & van Mechelen, W. (2006). Effects of resistance and functional-skills training on habitual activity and constipation among older adults living in long-term care facilities: A randomized controlled trial. BMC Geriatrics, 6, 9.

Czimmer, J., Million, M., & Tache, Y. (2006). Urocortin 2 acts centrally to delay gastric emptying through sympathetic pathways while CRF and urocortin 1 inhibitory actions are vagal dependent in rats. American Journal of Physiology. Gastrointestinal and Liver Physiology, 290(3), G511–G518.

Diaz S, Bittar K, Hashmi MF, et al. Constipation. [Updated 2023 Nov 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-.

Duboc, H., Coffin, B., & Siproudhis, L. (2020). Disruption of Circadian Rhythms and Gut Motility: An Overview of Underlying Mechanisms and Associated Pathologies. Journal of Clinical Gastroenterology, 54(5), 405–414.

Dukowicz, A. C., Lacy, B. E., & Levine, G. M. (2007). Small intestinal bacterial overgrowth: A comprehensive review. Gastroenterology & Hepatology, 3(2), 112–122.

Erhardt R, Harnett JE, Steels E, Steadman KJ. Functional constipation and the effect of prebiotics on the gut microbiota: a review. *British Journal of Nutrition*. 2023;130(6):1015-1023.

Gou, X., Qin, L., Wu, et al. . (2023). Research Progress of Takeda G Protein-Coupled Receptor 5 in Metabolic Syndrome. *Molecules (Basel, Switzerland)*, *28*(15), 5870

Gerritsen, R. J. S., & Band, G. P. H. (2018). Breath of Life: The Respiratory Vagal Stimulation Model of Contemplative Activity. *Frontiers in human neuroscience*, *12*, 397

Han, Y., Wang, B., Gao, H., He, C., Hua, R., Liang, C., Zhang, S., Wang, Y., Xin, S., & Xu, J. (2022). Vagus Nerve and Underlying Impact on the Gut Microbiota-Brain Axis in Behavior and Neurodegenerative Diseases. *Journal of inflammation research*, *15*, 6213–6230..

Hoogerwerf, W. A. (2006). Biologic clocks and the gut. Current Gastroenterology Reports, 8, 353–359.

Hong, S. W., Chun, J., Park, et al. (2018). *Aloe vera* Is Effective and Safe in Short-term Treatment of Irritable Bowel Syndrome: A Systematic Review and Meta-analysis. *Journal of neurogastroenterology and motility*, *24*(4), 528–535

Iesanu MI, Zahiu CDM, Dogaru I-A, Chitimus DM, Pircalabioru GG, Voiculescu SE, Isac S, Galos F, Pavel B, O'Mahony SM, et al. Melatonin–Microbiome Two-Sided Interaction in Dysbiosis-Associated Conditions. *Antioxidants*. 2022

Jani, B., & Marsicano, E. (2018). Constipation: Evaluation and Management. Missouri Medicine, 115(3), 236–240.

Keller, J., Gröger, G., Cherian, L., et al. (2001). Circadian coupling between pancreatic secretion and intestinal motility in humans. American Journal of Physiology. Gastrointestinal and Liver Physiology, 280, G273–G278.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

Kennedy, P., et al. (2014). Irritable bowel syndrome: A microbiome-gut-brain axis disorder? World Journal of Gastroenterology : WJG, 20(39), 14105–14125

Konturek, P. C., Brzozowski, T., & Konturek, S. J. (2011). Gut clock: Implication of circadian rhythms in the gastrointestinal tract. Journal of Physiology and Pharmacology, 62, 139–150.

Kumar, Jayant, Kumar, et al. (2023). Outcome of preventive interventions on the prevalence of constipation in hip fracture patients admitted in orthopaedic ward of a tertiary care hospital in eastern India. International Journal of Research in Orthopaedics, 9, 1029–1032.

Liu, J., Lv, C., Huang, Y., et al. (2021). Digital Rectal Examination Is a Valuable Bedside Tool for Detecting Dyssynergic Defecation: A Diagnostic Study and a Meta-Analysis. *Canadian journal of gastroenterology & hepatology*, *2021*, 5685610.

Liu, C., Zhan, S., Tian, Z., Li, N., Li, T., Wu, D., Zeng, Z., & Zhuang, X. (2022). Food Additives Associated with Gut Microbiota Alterations in Inflammatory Bowel Disease: Friends or Enemies?. *Nutrients*, *14*(15), 3049.

López-Pingarrón, L., Almeida, H., Soria-Aznar, M., Reyes-Gonzales, M. C., Rodríguez-Moratinos, A. B., Muñoz-Hoyos, A., & García, J. J. (2023). Interstitial Cells of Cajal and Enteric Nervous System in Gastrointestinal and Neurological Pathology, Relation to Oxidative Stress. *Current issues in molecular biology*, *45*(4), 3552–3572.

Mutuyemungu, E., Singh, M., Liu, S., & Rose, D. J. (2023). Intestinal gas production by the gut microbiota: A review. Journal of Functional Foods, 100, 105367.

Narducci, F., Bassotti, G., Granata, M. T., et al. (1986). Colonic motility and gastric emptying in patients with irritable bowel syndrome. Effect of pretreatment with octylonium bromide. Digestive Diseases and Sciences, 31, 241–246.

Nurielohayon, M., Neuman, H., Ziv, O., Belogolovski, A., Barsheshet, Y., Bloch, N., Uzan, A., Lahav, R., Peretz, A., & Frishman, S. (2019). Progesterone Increases Bifidobacterium Relative Abundance during Late Pregnancy. Cell Reports, 27, 730.

Quigley EM, Spiller RC. Constipation and the Microbiome: Lumen Versus Mucosa! Gastroenterology. 2016 Feb;150(2):300-3. doi: 10.1053/j.gastro.2015.12.023. Epub 2015 Dec 19. PMID: 26710987.

Pannemans, J., Masuy, I., & Tack, J. (2020). Functional Constipation: Individualising Assessment and Treatment. Drugs, 80, 947–963.

Pinton, P., & Oswald, I. P. (2014). Effect of deoxynivalenol and other Type B trichothecenes on the intestine: A review. Toxins, 6, 1615–1643.

Qi, Z., Yan-Yan, C., Xu, D. Q., et al. (2021). Action Mode of Gut Motility, Fluid and Electrolyte Transport in Chronic Constipation. Frontiers in Pharmacology, 12.

Réus, G. Z., Jansen, K. & Titus, et al. (2015). Kynurenine pathway dysfunction in the pathophysiology and treatment of depression: Evidences from animal and human studies. *Journal of psychiatric research*, *68*, 316–328.

Southwell, B. R., Clarke, M. C., Sutcliffe, J., & Hutson, J. M. (2009). Colonic transit studies: Normal values for adults and children with comparison of radiological and scintigraphic methods. Pediatric Surgery International, 25, 559–572.

Tarasiuk, A., Mosińska, P., & Fichna, J. (2018). Triphala: Current applications and new perspectives on the treatment of functional gastrointestinal disorders. Chinese Medicine, 13, 39.

Verbeure, W., van Goor, H., Mori, H., van Beek, A. P., Tack, J., & van Dijk, P. R. (2021). The Role of Gasotransmitters in Gut Peptide Actions. *Frontiers in pharmacology*, *12*, 720703.

Wei, L., Singh, R., Ro, S., & Ghoshal, U. C. (2021). Gut microbiota dysbiosis in functional gastrointestinal disorders: Underpinning the symptoms and pathophysiology. *JGH open : an open access journal of gastroenterology and hepatology*, *5*(9), 976–987

Włodarczyk, J., Waśniewska, A., Fichna, J., Dziki, A., Dziki, Ł., & Włodarczyk, M. (2021). Current Overview on Clinical Management of Chronic Constipation. Journal of Clinical Medicine, 10(8), 1738.

Zhao, Q., Chen, Y. Y., Xu, D. Q et al. (2021). Action Mode of Gut Motility, Fluid and Electrolyte Transport in Chronic Constipation. *Frontiers in pharmacology*, *12*, 630249.

Zhu, F., Tu, H., & Chen, T. (2022). The Microbiota-Gut-Brain Axis in Depression: The Potential Pathophysiological Mechanisms and Microbiota Combined Antidepression Effect. *Nutrients*, *14*(10), 2081

Topic : Constipation : severe and chronic. Causes and remedies.

1. **Introduction**

Slide 3: Constipation, a prevalent gastrointestinal disorder in Western countries, is characterised by infrequent bowel movements and various accompanying symptoms such as incomplete evacuation, bloating, straining, and hard stools. Its aetiology categorises it into primary and secondary forms.

Slide 4 : In a study from 2020, it was found that in comparison to men, women were more than twice as likely to suffer from constipation, the likelihood of constipation was significantly lower in the two older age groups compared to the youngest age group of 18 to 38-year-olds. Also, the likelihood of constipation was significantly higher in patients with low water and vegetable intake and a BMI classification of ‘obese’.

Slide 5: Primary constipation encompasses conditions like IBS-C and functional constipation, while secondary constipation can stem from metabolic, medication-related, or neurological factors.
Secondary constipation may be a result of metabolic disorders, example: hypercalcemia, medications - examples: calcium channel blockers or opiates, primary colonic disorders (e.g., cancer, proctitis) and neurologic disorders.

Defining constipation still remains challenging, often relying on self-reported symptoms and formal criteria.
Addressing constipation is crucial for maintaining overall health, as if untreated, it can result in symptoms beyond digestive discomfort, such as fatigue, headaches, skin conditions like eczema, psoriasis, and neurological conditions Alzheimer, Parkinson’s impacting daily functioning and quality of life.

In our presentation, we'll delve into chronic and severe constipation definitions, emphasising their impact on overall health and exploring the influence of diet on the microbiome.

Physiology of normal bowel function

Slide: 6 Peristalsis:

Normal defecation and maintaining continence are complex processes involving rectal filling, awareness, and coordinated muscle action. Key anatomical factors include the anal sphincters, puborectalis muscles, and rectoanal angle, which act as passive barriers.

Normal bowel function is essential for maintaining faecal continence, relying on coordinated peristalsis and muscle actions, including factors such as anal sphincters, pelvic floor muscle relaxation, and rectal anatomy. Additionally, the gastrocolonic response, triggered by eating, facilitates bowel movements through propagated contractions, crucial for healthy defecation. Understanding these mechanisms is fundamental for comprehending gastrointestinal health and effectively addressing constipation-related concerns.

Slide: 7 Repetitive non propulsive contractions aid in absorption and content mixing, while larger, coordinated contractions known as high-amplitude propagated contractions (HAPCs) occur approximately six times per day, propelling stool forward in mass movements from the ascending to the left colon. Typically occurring in the morning and possibly triggered by activities like eating or drinking, HAPCs reflect the common urge to defecate during this time. Notably, colonic motility diminishes during sleep to prevent incontinence, making nocturnal bowel movements or leakage abnormal. Distension upon stool entry into the rectum triggers the urge to defecate, with the rectum temporarily storing stool if inappropriate, dissipating the urge and initiating propagating contractions. Variations in normal colonic transit, spanning from 20 to 72 hours in adults, are observed, with decreased HAPC frequency often associated with constipation, highlighting their significant role in its pathophysiology.

Physiological -
The internal anal sphincter, mainly smooth muscle, maintains resting anal tone, while the external anal sphincter (EAS), made up of striated muscle, enables voluntary control. The puborectalis muscle regulates the rectoanal angle and contracts during squeezing. Rectal distension triggers the reflex relaxation of the internal sphincter, known as the rectoanal inhibitory reflex (RAIR), controlled by the enteric nervous system.

During defecation, stool distension signals the urge to defecate. Sensory mechanisms in the anal canal distinguish between gas and stool. If necessary, voluntary contraction of the EAS inhibits defecation. As the rectum relaxes, the urge to defecate subsides, allowing stool accommodation. When appropriate, coordinated muscle actions, such as diaphragmatic contraction and EAS relaxation, facilitate stool expulsion.

Sensory perception and physiological coordination are essential for effective anorectal function.

Slide 8 : MMC / Peristalsis

The migrating motor complex (MMC) consists of waves of electrical activity starting in the stomach and moving along the gut, stimulating peristaltic contractions of contractions and relaxation. They spread along the intestinal tract in response to stimulation. The neural control hierarchy for peristalsis is structured as follows: the enteric nervous system (ENS) serves as the primary regulator, followed by the autonomic nervous system (ANS), and then the central nervous system (CNS). Moreover, neurotransmitters, gastrointestinal hormones, and microbiota collaborate to regulate peristalsis.

The ENS coordinates peristalsis through interconnected circuits involving different neuron and neurotransmitters that interact with intestinal neurons and contribute to the regulation of gut motility. They are crucial in maintaining normal intestinal electric ion transport and secretory motor function.

The MMC consists of distinct phases: phase I (40-60% of total time) marked by quiescence, phase II (20-30%) - increased action potentials and muscle contractility, phase III (5-10 min) showing peak activity, and phase IV with declining activity. Nighttime cycles feature more motor quiescence, while daytime cycles have longer periods of increased contractility to facilitate bowel movements. In the colon, there's no MMC, but sleep inhibits colonic motor activity

Motilin and ghrelin hormones contribute to generating MMC, while hormones like gastrin, cholecystokinin, and serotonin are involved in generating propagation sequence spikes. These processes lead to peristaltic or segmental contractions in both the small (duodenum, jejunum, ileum) and large intestines (colon).

On a molecular level, peristalsis is mediated by serotonin binding to different neurons (5-hydroxytryptamine [5HT]). This signal transmission through the myenteric plexus triggers intestinal smooth muscle contraction, essential for propulsion. Disorders like carcinoid syndrome, characterised by 5HT overproduction, can lead to diarrhoea.

Slide 10: Fight or flight Gut brain axis

The autonomic nervous system, which includes both the sympathetic and parasympathetic branches, serves as the intermediary between the central nervous system and the enteric nervous system, regulating gut motility. Sympathetic nerves release norepinephrine, inhibiting intestinal motility during times of stress, while the

parasympathetic nervous system, primarily via the vagus nerve, predominantly stimulates gut activity.

Gut motility modulation occurs through the autonomic nervous system's transmission via the vagus nerve, relaying intestinal signals to the central nervous system. Additionally, central nervous system signals influence gastrointestinal motility via hormonal pathways, such as the hypothalamus–pituitary–adrenal (HPA) axis, and hormones involved in the neuroendocrine stress response. For example, central preganglionic neurons release neurotensin, which further stimulates peristalsis by enhancing substance P release.

The "gut-brain axis" integrates gut functions with emotional and cognitive brain centers through various mechanisms, including immune activation and hormonal signaling mediated by neuro-immuno-endocrine pathways. The HPA axis acts as the primary stress response system, coordinating the body's reactions to stressors. Environmental stress and pro-inflammatory cytokines trigger this system, leading to cortisol release from the adrenal glands. Cortisol, a key stress hormone, affects various organs, including the brain, facilitating communication between the brain and intestinal cells and influencing immune response and gut function.

The absense of microbial colonisation is associated with altered expression and turnover of neurotransmitters in both nervous systems and changes in gut sensory-motor functions, resulting in delayed gastric emptying and intestinal transit.

Slide: 11 This shows the response to internal or external stressors, and the HPA axis activation. This prompts the hypothalamus to release corticotrophin-releasing hormone (CRH), which stimulates the pituitary gland to release adrenocorticotrophic hormone (ACTH). Consequently, the adrenal gland releases cortisol into the bloodstream. Cortisol, a key hormone, influences intestinal functions such as integrity, motility, and mucus production, thereby affecting the composition of gut microbiota.

This hyperactivity of the system often manifests as irritable bowel syndrome (IBS) and disturbances in gut microbiota. Microbial byproducts like short-chain fatty acids (SCFAs) and microbial neurotransmitters such as catecholamines, histamines, and GABA can interact with intestinal epithelial cells and nerves, ultimately stimulating the central nervous system via the vagus nerve.

Furthermore, certain SCFAs and peptides have the ability to traverse the blood-brain barrier and directly affect the central nervous system. Lipopolysaccharide (LPS), found on the surface of Gram-negative bacteria, plays a pivotal role in mediating the effects of immune cells and the vagus nerve on the brain. This intricate interplay

between the gut and brain involves various biochemical pathways and contributes to the complex nature of depression.

Slide 12: Neurotransmitter Regulation in Constipation

Intestinal neurotransmitters categorised as either inhibitory or excitatory, play crucial roles in regulating gastrointestinal motility and secretion.

Inhibitory neurotransmitters, such as vasoactive intestinal peptide (VIP), nitric oxide (NO), adenosine triphosphate (ATP), and hydrogen sulfide (H2S), induce smooth muscle relaxation and suppress intestinal sensitivity.

VIP, a key neurotransmitter in the enteric nervous system (ENS), prevents intestinal circular muscle contraction, maintaining relaxation and possibly influencing fluid secretion. Reduced VIP levels may lead to decreased fluid secretion, potentially worsening constipation.

VIP also activates nitric oxide synthase (NOS), promoting the production of NO, which further relaxes smooth muscle cells and reduces gastrointestinal motility. H2S, a gasotransmitter, regulates gut motility and inhibits pacemaker activity in interstitial cells of Cajal, leading to smooth muscle relaxation. Additionally, high H2S concentrations can hinder gut motility by affecting neural pathways.

Excitatory neurotransmitters like serotonin (5-HT), acetylcholine (Ach), and substance P (SP) stimulate intestinal muscle contraction and enhance peristalsis. 5-HT, released from enterochromaffin cells and serotonergic neurons, acts as both a paracrine signaling molecule and neurotransmitter, influencing gastrointestinal function. Ach triggers muscle contraction and increases peristalsis, while SP exerts contractile effects on gastrointestinal smooth muscle by regulating ICCs through the tachykinin NK1 receptor.

Interstitial cells produce and transmit electrical signals that regulate the excitement and inhibition of smooth muscle. Mast cells can regulate peristalsis and fluid secretion through bidirectional interactions between the enteric nervous system (ENS) and the central nervous system (CNS). When mast cells are activated, they can stimulate excitatory neurons and activate the ENS network, leading to enhanced propulsive motility. Additionally, histamine and prostaglandin D2 released by mast cells can modulate smooth muscle activity and fluid secretion.

Picture: Slide: 13 - In this picture we can see how gasotransmitters play pivotal roles in regulating the physiological functions of both glucagon-like peptide 2 (GLP-2) and glucagon-like peptide 1 (GLP-1). Dotted lines denote initial evidence for pathway

presence, while solid lines represent well-established evidence supported by blockers and substrate administration.

GLP-2 is associated with promoting nitric oxide (NO) release, potentially enhancing blood flow and gut contractility. On the other hand, H2S stimulates the release of GLP-1. The effects of GLP-1, mediated by NO, include suppressing gut motility, reducing nephropathy risk factors, increasing muscle microvasculature, reducing reactive oxygen species, and producing vasoconstrictive mediators. Additionally, H2S and carbon monoxide (CO) contribute to vasodilation.

Furthermore, gasotransmitters may play a protective role in governing pancreatic function in cases of glucotoxicity. Key abbreviations include NO (Nitric oxide), CO (Carbon monoxide), H2S (Hydrogen sulfide), and MMC (Migrating motor complex).

Slide 14 : Microbiota

Interstitial cells generate and propagate electrical signals that modulate both the stimulation and inhibition of smooth muscle activity. This regulation involves two main types of interstitial cells: interstitial cells of Cajal (ICCs) and platelet-derived growth factor receptor α positive (PDGFRα+) cells. Alterations in the structure and morphology of ICCs could potentially be associated with constipation.

There is a significant link between intestinal microbiota and constipation. Higher levels of Bacteroides and lower counts of Bifidobacteria and Lactobacilli may impact bowel emptying (Dimidi et al., 2017). Metabolites like short-chain fatty acids (SCFAs) and endotoxins, produced during microbial metabolism, can influence gut motility (Segers et al., 2019). SCFAs regulate the release of gut peptides from enteroendocrine cells and the synthesis of gut-derived serotonin from enterochromaffin cells, impacting gut-brain hormonal communication. Endotoxins can slow motility by decreasing myoelectric spiking activity and deluing gastric emptying and inducing sphincteric dysfunction. SCFAs can also stimulate 5-HT3 receptors on vagal sensory fibres, leading to muscle contraction (Fukumoto et al., 2003).

Slide: 15 Hypochloridia and digestive enzymes

Hydrochloric acid plays a crucial role in numerous physiological functions, one of them is the release of digestive enzymes from the pancreas by triggering the release of hormones like gastrin when an acidic chyme reaches the small intestines. Moreover, HCL prevents upper gastrointestinal tract pathogen overgrowth, making hypochlorhydria a risk factor for small intestinal bacterial overgrowth (SIBO). Additionally, HCL aids in protein digestion, reducing the risk of food allergies associated with incomplete protein breakdown. If digestion is impaired due to

insufficient hydrochloric acid or inadequate release of digestive enzymes, transit time may be prolonged, leading to slower bowel movements and potentially constipation.

Disruption of the circadian rhythm alters the timing of eating and normal gastrointestinal functions, including gastric, bile, and pancreatic secretions, enzyme activity, intestinal motility, and nutrient absorption rates. Melatonin acts on the muscularis mucosae or the myenteric plexus, is involved in regulating colonic motility, and has a bidirectional effect on gut motility.

Slide: 16 Classification of constipation

Constipation can be severe or chronic. Acute constipation is a sudden onset of bowel movements, occurring over a short period. It can be caused by changes in diet, lifestyle - stress, travel, or illness. Acute constipation is usually temporary and can often be resolved with lifestyle modifications or short-term interventions.

Chronic constipation is a persistent difficulty with bowel movements that lasts for an extended period. It may require ongoing management and treatment.

Constipation causes are categorised into two groups: primary and secondary causes. Primary causes are intrinsic problems of colonic or anorectal function, whereas secondary causes are related to organic disease, systemic disease or medications.

Causes for acute constipation

Slide: 17 Acute:

Diet - Constipation can stem from dietary factors such as insufficient fibre and water intake, excessive consumption of coffee, tea, or alcohol, and lack of physical activity. They can all cause an imbalance in the microbiome which can result in further health conditions and limit stool formation.

Decreased physical activity:
Physical activity impacts colonic motility, influencing gut transit. This can be linked to vagus nerve stimulation and/or reduced blood flow to the gastrointestinal tract, leading to increased release of crucial GI hormones. Additionally, mechanical stimulation of the gut during exercise—such as bouncing, maintaining an upright posture, gravity, and contraction of the abdominal muscles—helps propel stools into the rectum, further enhancing stimulation.

Slide 18: Stress
The hypothalamus-pituitary-adrenal axis largely governs physiological reactions to stress. Alongside this axis, the release of corticotropin-releasing factor (CRF) induced by stress can contribute to bowel dysfunction. CRF can affect the bowel directly, changing the composition of gut microbiota and delaying gastric emptying.

Pregnancy:
Constipation often occurs during the second and third trimesters of pregnancy due to significantly elevated levels of progesterone and estradiol. Additionally, the growing uterus puts pressure on the intestines, exacerbating the issue.

Travel:
Irregular circadian rhythms within the gastrointestinal tract have been associated with constipation due to a variation in colonic pressure activity and timing of bowel movements.

Slide 19 : Physiological factors

Anorectal motor and sensory functions

Chronic:
Chronic constipation, persisting for at least three months, may arise from intrinsic colonic issues or be secondary to organic diseases or medication usage. Its multifactorial pathogenesis involves genetic predisposition, diet, fluid intake, physical activity, hormonal imbalances, and anatomical considerations.

Normal defecation involves a complex interaction of colorectal motor and sensory functions, synchronised with appropriate behaviour. This includes loading the rectosigmoid with normal stools, sensing rectal distension, and performing voluntary defecation actions (such as abdominal contraction and relaxation of the puborectalis and anal muscles) at the right moment.

Defecation disorders often exhibit various abnormalities, such as failure of anal sphincter relaxation (anismus), hypertonic internal anal sphincter, inadequate rectal propulsion, rectal hyposensitivity, anatomical changes like rectoceles or rectal prolapse, excessive perineal descent, and decoupling of sensory signals and colon motor activity. These dysfunctions may coexist and their primary or secondary nature to constipation remains unclear. Additionally, these alterations are often nonspecific and may be found in healthy individuals, with assessment influenced by defecation technique variability.

Slide 20 : Motility

It can involve reduced high-amplitude propagated contractions (HAPCs), indicating a neuromuscular disorder.

Increasing dietary fibre intake may decrease symptoms in individuals with normal colonic transit and anorectal function, yet it does not provide relief for constipated patients with delayed colonic transit and defecation disorders.

Delayed colonic transit, often associated with hard, small stools, is challenging to pass and correlates strongly with stool consistency, water content, and transit time.

Slide 21: The slow transit can result from impaired motor activity, voluntary stool retention, defecation disorders, or inadequate caloric intake, with potential contributions from abnormalities in interstitial cells of Cajal or progesterone receptor overexpression.

As we know, interstitial cells of Cajal play a crucial role in regulating gastrointestinal motility by generating and propagating electrical slow waves, which coordinate smooth muscle contractions. Any decrease in the number or dysfunction of ICCs can disrupt this coordinated movement, leading to constipation.

Similarly, alterations in myenteric plexus neurons expressing substance P, a neurotransmitter involved in the transmission of pain signals and regulation of gastrointestinal motility, can contribute to chronic constipation. Substance P is known to modulate smooth muscle contractility and sensory perception in the gastrointestinal tract. Changes in the expression or function of substance P neurons in the myenteric plexus may disrupt the normal balance of motility and sensory functions, leading to constipation.

Prolonged transit fosters water absorption and can influence bacterial mass, affecting colonic absorption and secretion. Moreover, variations in transit time may impact bacterial mass, potentially influencing colonic absorption and secretion.

Slide 22: This image shows interstitial cells of Cajal (ICC) within the gastrointestinal tract. The enteric nervous system spans the digestive system and comprises two key plexuses: the myenteric plexus, situated between the longitudinal and circular muscle layers, and the submucosal plexus, found within the submucosal layer between the mucosa and inner muscular layer.
The myenteric plexus primarily regulates muscle contraction, while the submucosal plexus controls secretory functions and blood flow. Within these plexuses, three main cell types are present: neurons, glial cells, and the ICCs.

Slide 23: Hormones

The high prevalence of constipation in women underscores the significant role of sex hormones, like oestrogen and progesterone, in regulating prolonged stool transit.. Hormones influence microbial composition and metabolism, while gut microbes can impact hormone levels, particularly oestrogen-bacterial genes involved in oestrogen metabolism, such as β-glucuronidase. This regulation leads to oestrogen reabsorption and further alteration in the circulating hormonal profile.
In addition, increased levels of progesterone cause alterations in community structure, characterised by enhanced abundances of *Blautia* and *Bifidobacterium*, and reduced abundances of *Dehalobacterium* and *Bacteroidales*. Microbial composition is also influenced by estrogen receptor (ER) signalling within colonic mucosa, which modulates intestinal activity and gut motility

Steroid hormones also modulate colonic motor functions. Oestrogen inhibits colonic propulsion via GPERs and nuclear ERs, while progesterone's receptor overexpression, rather than excess levels, impairs gut motility. These hormonal effects may explain the high constipation prevalence during pregnancy.

Slide 25 Circadian rhythm - workshifts
Disruption of the circadian rhythm affects the timing of eating and key gastrointestinal functions, including gastric, bile, and pancreatic secretions, enzyme activity, intestinal motility, and nutrient absorption rates. Studies show that shift workers often experience gastrointestinal issues, with nighttime workers particularly reporting constipation. Changes in gastrointestinal physiology and/or gut microbiota could contribute to gastric disorders by raising gastrointestinal permeability and heightening the risk of injury and inflammation. Evidence suggests melatonin may play a role in preserving gut wall integrity.

Slide 26: This image shows the beneficial effects of melatonin on gastrointestinal functions in sleep disorders. In the gut, melatonin: restores the intestinal microbiome by rebalancing the Firmicutes to Bacteroidetes ratio and reducing Proteobacteria at the phylum level. It promotes the growth of beneficial bacteria such as Akkermansia, Lactobacillus, and Faecalibacterium, while inhibiting harmful bacteria like Aeromonas at the genus level, enhances barrier integrity by upregulating tight junction proteins (claudin-1, occludin, ZO-1) and increasing goblet cell numbers (MUC2), thereby reducing oxidative stress through elevation of antioxidant enzymes (GSH-Px, SOD, CAT) and suppression of ROS. It also diminishes the inflammatory state by decreasing proinflammatory cytokines (IL-1ß, IL-6, IL-17) and increasing anti-inflammatory cytokines (IL-10, IFN-γ, IL-22).
At the systemic level, melatonin reduces stress hormones (corticosterone, NE).

(ii) Modulates the systemic inflammatory state by decreasing inflammatory cytokines (IL-1β, IL-6, IL-17, TNF-α, INF-γ) and increasing anti-inflammatory cytokines (IL-5, IL-10, IFN-α).

Slide 27 Gut Microbiota, Intestinal Secretion, and Constipation

Microbiome dysbiosis

Patients with chronic constipation often demonstrate alterations in their intestinal microbiota. This includes a decrease in beneficial bacteria such as Lactobacillus, Bifidobacterium, and Bacteroides spp., alongside a notable increase in Bacteroides sp. and Enterobacteriaceae. Additionally, potentially harmful microorganisms like Pseudomonas aeruginosa and Campylobacter jejuni may elevate.

Furthermore, there's a decrease in Prevotella abundance and an increase in Coprococcus, Ruminococcus, Blautia, Anaerotruncus, and Clostridium, which are associated with constipation. Prevotella plays a crucial role in fibre metabolism, and its decreased abundance correlates with a low-fibre diet. Conversely, constipated individuals often exhibit heightened levels of butyrate-producing genera like Coprococcus, Roseburia, and Faecalibacterium.

These alterations could affect intestinal motility and secretory functions by changing the availability of biologically active substances and metabolic conditions within the gut. Dysbiosis contributes to increased water retention in the colon and reduced faecal water content. Moreover, the gut microbiota influences aquaporin expression; the Prevotella (P) enterotype is believed to enhance faecal water content and accelerate gut transit.

Slide 28: The bacterial endotoxin lipopolysaccharide (LPS) may delay gastric emptying and cause sphincteric dysfunction. In contrast, deconjugated bile salts, a bacterial metabolite, can trigger colonic motor responses leading to bile salt-related diarrhoea.
Short-chain fatty acids stimulate ileal propulsive contractions by evoking prolonged propagated and clustered contractions, influencing the release of intestinal serotonin (5-HT) and directly stimulating smooth muscle contractility.

Butyrate, for instance, inhibits mucin secretion by intestinal goblet cells and reduces stool volume by enhancing water and electrolyte absorption in the colon. Lactate-utilizing bacteria, significantly reduced in patients with constipation, also play a role in this process.

Bile acids

Bile acids activate the G protein-coupled bile acid receptor (TGR5), triggering the release of 5-HT and calcitonin gene-related peptide from enterochromaffin cells and intrinsic primary afferent neurons, thereby initiating the bowel peristaltic reflex.
Bile acids regulate colonic epithelial cells by inducing chloride secretion while suppressing sodium absorption through modulation of ion pumps, exchangers, and transporters.
Bile acids are initially conjugated with glycine or taurine in hepatocytes before reaching the colon, where they undergo deconjugation by bacterial bile salt hydrolase influencing colonic transit. Changes in the microbiota can affect gastrointestinal transit by altering bile acid deconjugation.

Slide 29:
The image presents the relationship between inflammation triggered by LPS and its disruptive effect on the kynurenine pathway. In this pathway, heightened inflammation leads to an increase in immune cell activity while concurrently reducing tryptophan (Trp) levels and subsequently diminishing serotonin (5-HT) production. Furthermore, the elevation of 3-hydroxykynurenine (3-HK) and nitric oxide synthase (NOS) exacerbates the inflammatory response. This cascade of events is accompanied by the development of a leaky gut and disturbances in motility.

Slide 30
Small intestinal bacterial overgrowth (SIBO), characterised by abnormal bacterial growth in the small intestines, presents with a variety of clinical symptoms, including abdominal discomfort, bloating, diarrhoea, and nutritional deficiencies. SIBO can impair small intestinal motility and delay small bowel transit time.

SIBO, characterised by abnormal bacterial growth in the small intestine, can impair small intestinal motility and delay transit time. On the other hand, slow motility can create conditions conducive to bacterial overgrowth.

Human colonic gases, including methane (CH_4) and hydrogen sulfide (H_2S), influence gut motility, with methane production associated with changes in faecal microbiota composition and potential slowing of gut transit.

It has been reported that SIBO may be present with hypothyroidism. Hypothyroidism decreases gastric motility by decreasing frequency of rhythmic colonic activity. It can impact bacterial overgrowth, poor bile release, consequently leading to dysbiosis impacting intestinal clearance.

Slide: 31 Mould/ Mycotoxins:
Mold-exposed individuals experience a higher prevalence of hypothyroidism, thus reduced motility. Mycotoxins can alter the microbiome composition and increase inflammation. The reduction of *Lactobacillus reuteri* and SCFA is observed in these individuals.

They induce a reduction in the number of goblet cells secreting mucin, a key component of the gut barrier function. This disruption compromises the integrity of the intestinal epithelium, facilitating the entry of pathogens into the gut lumen, which can contribute to conditions like constipation. Without protection from beneficial bacteria, the growth of pathogens will expand rapidly and produce a high level of LPS. Mycotoxins can also affect liver function, interfering with its detoxification processes. This disruption can lead to alterations in the release and clearance of bile acids, further exacerbating the impact on gut health and microbiome status.
As seen in the picture, mould and mycotoxins can be found anywhere around us, from a previous leak in the flat or frequent condensation to damp shower tiles, poorly ventilated areas, and even contaminated food sources. An important note is to never cut off the ‘mould-free’ part of food if some was already contaminated.

Slide 32: Low serotonin
The gut microbiota influences the growth of colon enterochromaffin cells, specialised cells found in the GI tract that produce and release serotonin (5-HT), which in turn stimulates colonic motility. Microbial metabolites such as bile acids and SCFAs may induce the release of 5-HT from enterochromaffin cells. In constipation, the gut microbiota elevates serotonin transporter expression, leading to decreased colonic transit due to increased removal of 5-HT from the gut.

Microbiota may impact 5-HT production by modulating tryptophan metabolism in the gut, enhancing indole and kynurenine production from tryptophan, thereby reducing the substrate available for 5-HT production, which leads to constipation.

One study showed that the faecal samples from patients with IBS-C had slowed gut transit, upregulated 5-HT transporter, and reduced serotonin levels, which were supported by decreased relative abundance of gut microbiota such as *Firmicutes* and increased *Bacteroidetes*. Furthermore, microbiota-derived SCFAs may impact colonic serotonin and motilin-containing enteroendocrine cells, potentially regulating colonic physiology. Products of the gut microbiota, notably the neurotransmitter serotonin synthesised by Clostridium spp. and Escherichia coli, have been demonstrated to influence peristalsis and motility by interacting with the enteric nervous system.

Slide 34: In this pathway, tryptophan is initially converted to (5-HTP) by tryptophan hydroxylase (TPH). Subsequently, aromatic amino acid decarboxylase (AAAD) converts 5-HTP into serotonin (5-HT). These reactions occur not only in the central nervous system, where serotonin regulates functions like emotion, cognition, stress response, and visceral perception but also in the enteric nervous system, which influences gastrointestinal motility and secretion.

Therefore, a healthy microbiome is essential for optimal tryptophan metabolism and gut motility. The gut microbiota can modulate tryptophan availability and metabolism.

Slide 35: Anxiety, stress
Stress can increase intestinal permeability and thus bowel dysfunction. Constipated patients have a higher rate of mood, depression and anxiety disorders.

Chronic stress can also increase intestinal permeability and thus bowel dysfunction. This leads to increased exposure to intestinal epithelial bacteria and, subsequently, to promoting gut inflammation. The diminished expression of MUC2 reduces mucin release, indicating that constipation-induced dysbiosis compromises the epithelial barrier.

About the picture: Cortisol, often referred to as the "stress hormone," plays a crucial role in the body's response to stress through its influence on the hypothalamic-pituitary-adrenal (HPA) axis. In times of stress, the hypothalamus signals the pituitary gland to release adrenocorticotropic hormone (ACTH), which stimulates the adrenal glands to produce cortisol. Elevated cortisol levels activate the sympathetic nervous system, the body's "fight or flight" response, while simultaneously suppressing the parasympathetic nervous system, responsible for rest and digestion. Prolonged stress and elevated cortisol levels can disrupt normal gastrointestinal function, leading to decreased gut motility, increased intestinal permeability, and alterations in gut microbiota composition, all of which contribute to the development or exacerbation of constipation.

Slide 36: Neurological conditions:

Neurological conditions like spinal cord injury, multiple sclerosis, and Parkinson’s disease are often linked with chronic constipation, driven by a complex interplay of autonomic and pelvic nerve dysfunction alongside dietary changes, limited activity, and psychological issues, like anxiety or depression. Medications used for these conditions can also contribute to constipation.

In MS, studies suggest impaired colonic motility - pelvic floor spasms as a major contributor to constipation.

Similarly, Parkinson’s disease the pathophysiology involves both central and peripheral mechanisms, including changes in the dorsal vagal nucleus function and loss of dopaminergic neurons.

Slide 37: Medications:

Many medications can lead to constipation, therefore it is important to gather a comprehensive medication history during the initial evaluation.

Antihypertensive medications like clonidine, calcium antagonists, and ganglionic blockers can decrease smooth muscle contractility, potentially leading to constipation.

Additionally, antidepressants, particularly tricyclic antidepressants, are often linked to constipation. These medications have anticholinergic properties, which means they block the action of acetylcholine, a neurotransmitter involved in smooth muscle contraction and GI motility.

Slide 38: Oral iron supplements commonly lead to constipation causing irritation and inflammation of the gastric mucosa, via the production of ROS and enhanced growth of pathogenic species and a decrease in beneficial bacterial species. This could be explained by the high amount of iron in a supplement that the body cannot cope with. It is believed that an abundance of iron ions in the stomach may prompt the movement of water into the intestinal lumen via osmotic forces. This leads to the extraction of water from the lower gastrointestinal tract to maintain the acid-base equilibrium throughout the gastrointestinal system, ultimately resulting in the hardening of stool and subsequent constipation.

Aluminium-containing medications like sucralfate and antacids can also cause constipation, and proton pump inhibitors may be used instead.

Moreover, medications for Parkinson's, antiepileptic, and antipsychotic drugs are linked to constipation because of their anticholinergic and dopaminergic effects, warranting caution or combination with regular laxative usage.

Slide 39: Diagnosis of severe and chronic constipation

History
It is hard to test for constipation, therefore diagnosis relies on the Rome criteria IV criteria -at least 2 of 6 criteria for diagnosis.

Table.
A thorough medical history should assess stool consistency, frequency, size, feelings of incomplete evacuation, abdominal bloating, straining, and other relevant factors like changes in living conditions, medications, and symptom onset. For a diagnosis, the patient must experience one of the following conditions for over 6 months and exhibit two or more of the following within the past 3 months: feeling of straining during over 25% of bowel movements, passing lumpy or hard stools during

over 25% of bowel movements (Bristol stool types 1 and 2), sense of incomplete evacuation during over 25% of bowel movements, sensation of anorectal obstruction/blockage during over 25% of bowel movements, needing manual manoeuvres for over 25% of bowel movements, and having fewer than three spontaneous bowel movements per week.

Identifying the specific distressing symptoms in each patient is crucial, in guiding the initial treatment focus.
Are they troubled by irregularity or texture of bowel movements, or is the act of defecating itself challenging? If a patient is feeling incomplete evacuation or perceived obstruction, they can be linked to defecation difficulties. Furthermore, any adverse life events, psychological disturbances, eating disorders, or behavioural modifications that may influence the act of defecation should be investigated.

When assessing patients with constipation, metabolic tests such as thyroid-stimulating hormone, serum glucose, creatinine, and calcium levels as well as colonoscopy are frequently conducted to exclude other conditions. Clinicians may order chemistry panels, thyroid function tests or complete blood tests to exclude or confirm metabolic diseases as a cause of constipation.

In chronic constipation cases, a rectal examination is essential to assess potential causes of anal discomforts, such as fissures or thrombosed haemorrhoids. Anal contraction upon gentle stimulation suggests intact sacral nerve pathways.
Resting sphincter tone evaluation helps gauge evacuation difficulties. Additionally, assessing rectocele presence and perineal descent during straining is vital.

If constipation persists despite treatment, experts recommend referring the patient for anorectal function tests and colonic transit studies to determine the underlying cause, whether it's a defecation disorder or slow transit constipation.

Slide 40: Bristol stool chart Commonly used classification tool for stools according to consistency.

Slide 41: Digital rectal examination (DRE) is a crucial part of physical assessment. It helps identify pelvic floor dyssynergia with 87% specificity and 75% sensitivity when compared to standard manometry. Additionally, it aids in excluding alarm symptoms indicating severe gastrointestinal conditions. DRE can reveal polyps, masses, haemorrhoids, and rectal prolapse, which may not always be visible in the left lateral position and may require a change in position for proper examination. It can also detect signs of mucosal injury, inflammation, ulcer, or fissure. Sphincter tone and incontinence should also be assessed. If a patient's condition improves with over-the-counter laxatives or fibre
supplements, further examination may not be necessary.

Slide 42: Stool test as an assessment for constipation

Stool microbiome testing offers valuable insights into the underlying causes of constipation. By analysing the composition and diversity of bacteria in the gut, this test can identify specific dysbiosis, an imbalance in the gut microbiota, which is often associated with constipation. Additionally, stool tests can detect the presence of pathogenic bacteria or parasites, which could be contributing to gastrointestinal symptoms. Furthermore, it provides information about bacterial metabolites, such as short-chain fatty acids, which play a crucial role in gut health and motility.
Overall, stool microbiome testing offers a comprehensive assessment of the gut ecosystem, helping to uncover **the root causes** of constipation and guiding personalised treatment strategies.

Slide 43: Management and treatment of severe and chronic constipation
Non-pharmacological interventions

The first-line management in constipation involves educating patients about diet, fibre and fluid intake, physical activity, and toilet training.
Fibre may be soluble and insoluble, whereas soluble fibres are more effective, such as Psyllium. Its effectiveness is found in increasing defecation frequency, weight and consistency of stool, and lower tenderness of pain in defecation, however Psyllium does not improve colon transit
Insoluble fibre, e.g., bran, improves intestinal transit time. In the treatment of constipation, the recommendation is to gradually increase fibre intake, to a maximum of 35 g per day.

Mechanism of Action of Fibre: It promotes the excretion of intestinal mucin by enhancing mucosal protein synthesis. In the proximal colon, dietary fibre is broken down, providing energy for microbial fermentation and stimulating the growth of intestinal microbiota, which increases stool dry weight. Additionally, fibre aids in the excretion of bacterial fermentation products like short-chain fatty acids (SCFAs), known for their pro-motility effects. Moreover, fibre serves as a significant source of gas in the colon, including hydrogen, methane, and carbon dioxide, which can increase stool bulk and enhance colonic transit. Evidence suggests that dietary fibres, such as wheat bran and pea fibres, can modulate intestinal microbiota by promoting beneficial bacterial species and suppressing pathogenic ones

Slide 44: Unfortunately, fibre can lead to side effects such as discomfort, flatulence, or bloating due to gas production. It may be caused by dysbiosis characterised by

decreased abundance of fibre-degrading bacteria, such as Bacteroidaceae and Lachnospiraceae, which lead to reduced fermentation and slower transit time.

Accordingly, breath H2 has been used as a primary marker for diagnostic testing of carbohydrate malabsorption or small intestinal bacterial overgrowth. Fermentative metabolism by gut bacteria creates the potential for large quantities of H2 gas to be produced within the gut. The most abundant bacterial genera responsible for H2 production in the colon are Bacteroides, Ruminococcus, and Roseburia.

Slide: 45: This table represents major gas-producing microbes present in the human gut microbiome

Slide 46: Prebiotic

Prebiotics promote the selective growth of specific beneficial bacteria already present in the colon, such as Lactobacilli and Bifidobacteria. Examples of prebiotics include fructo-oligosaccharides, galacto-oligosaccharides, and inulin.
Prebiotics undergo bacterial metabolism in the colon, converting them into lactic and short-chain fatty acids. Studies suggest that galacto-oligosaccharides may enhance intestinal peristalsis and alleviate constipation. Inulin can impact intestinal microbiota and stimulate bowel movements normalising stool frequency in constipated patients by decreasing the amount of pathological bacteria of the Clostridium genera.
Prebiotics such as GOS and FOS have been shown to improve microbial profiles by increasing bifidobacteria and decreasing *E. coli.*
The effects of prebiotic therapy also depend on the individual's starting microbial profile. In a study comparing FOS, sorghum and arabinoxylan, equally high SCFA production was observed in volunteers whose microbiota was dominant in fibre-utilising Prevotella, but Bacteroides-dominated individuals showed different SCFA levels in response to each fibre.
Polyphenols can change the makeup of gut bacteria beneficially by increasing the concentrations of *Bifidobacteria* and *Lactobacillus*, thus aiding in symptoms.

Slide 47: The picture shows the interconnected pathways linking microbiota, prebiotics, dietary fibre, gut function, and brain activity play a pivotal role in influencing constipation. Various pathways impact gut motility, including bidirectional communication between the gut and brain via the vagal and spinal nerves, with serotonin acting as a key neurotransmitter. SCFA produced by the microbiota, can directly stimulate nerve cells in the gut or indirectly trigger peristaltic reflexes by stimulating enteroendocrine cells to produce serotonin. Moreover, SCFA can directly interact with receptors on the vagus nerve. Bile acids generated by the microbiota can influence gut motility. The gut's microbial composition is modulated by the enteric nervous system through immunological defence secretions, permeability, or

motility. Transit time affects the microbiota's composition by exposing them to water and nutrients. Prebiotics like galacto-oligosaccharides can enhance the growth of SCFA-producing Bifidobacterium spp. and improve motility in constipated individuals. Additionally, non-fermentable dietary fibres such as coarse wheat bran and psyllium husks soften stools through mechanisms not directly involving the microbiota but by altering the stool's environment

Slide 48: Probiotics:

Mechanisms of Action:
Probiotics have demonstrated beneficial effects in patients with constipation, making them increasingly used as alternative treatment options. Several mechanisms have been proposed by which probiotics may benefit chronic constipation: probiotics may modify the altered intestinal microbiota in patients with constipation; probiotic metabolites may alter gut sensation and motility function; some probiotics may regulate the intraluminal environment, such as increasing the end products of bacterial fermentation, reducing luminal pH.*Prevotella* correlated with softer type stools,

Slide 49 : Bifidobacteria and Lactobacilli play a beneficial role in producing SCFAs, stimulating intestinal peristalsis, and increasing faecal moisture. Intake of Lactobacillus plantarum, Bifidobacterium breve, or Bifidobacterium lactis has been shown to significantly alleviate evacuation difficulties and hard stools in constipated patients. Evidence suggests that L. reuteri can also enhance both the frequency and velocity of the colonic myoelectric motility complex.

Supplementation with probiotic strain *L. rhamnosus* GG has been shown to decrease microbial overgrowth, restore mucosal integrity, reduce microbial translocation, and ameliorate alcohol-induced liver injury.
Butyrate-producing taxa, such as *Coprococcus* and *Roseburia* could be beneficial for constipation due to faster colonic transit -the motility-stimulating effect exerted by butyrate in the gut and by induction of serotonin release.

The probiotic strains that are found to be effective in reducing IBS symptoms, if used alone or as a multistrain formulation, are Bifidobacterium species (Bifidobacterium lactis, Bifidobacterium infantis, etc), Lactobacillus species, Streptococcus species, Saccharomyces boulardii (yeast), and Lactobacillus rhamnosus GG.

IBS participants on an exclusion diet had a greater abundance of genera such as Lachnospira compared with those not on an exclusion diet. Lachnospira are short-chain fatty acid producers, which can have positive effects on immune function, intestinal barrier integrity, and mucus production.28 Increased abundance of Lachnospira has been associated with increased vegetable intake and plant-based diet

Incorporating both probiotics and prebiotics, known as synbiotics, can offer synergistic benefits. Synbiotic therapy has the potential to enhance probiotic survival and rebalance intestinal microbiota, thereby aiding in the management of constipation.

Slide 50: Elimination diet

Eliminating specific food products known to trigger inflammation is crucial for promoting the healing and regeneration of the microbiome. However, in cases of dysbiosis, the addition of fibre, probiotics, or certain foods may exacerbate the condition rather than improve it. Therefore, careful attention to dietary choices is essential for restoring balance to the gut microbiota.

Microbiome testing is highly recommended as it can assess an individual's sensitivities and imbalances, allowing for a tailored treatment plan to address their specific needs.

Regarding carbohydrate digestion and absorption in the small intestine, various factors come into play, including the number of hydrolase enzymes, the presence of digestive diseases, transit time, and dosage consumed. The absence of luminal enzymes capable of hydrolyzing glycosidic bonds in complex carbohydrates is reported as a contributing factor to poor absorption in high-FODMAP diets. The undigested and unabsorbed carbohydrates enter the large intestine, where they undergo fermentation. In the colon, particularly fructans and oligosaccharides, lead to increased production of hydrogen and methane, resulting in gas accumulation and luminal distension, causing functional GI symptoms.

Patients with IBS develop severe symptoms due to a low density of gut endocrine cells. A diet low in fermentable carbohydrates and insoluble fibre has been shown to increase the number of endocrine cells in the gut, leading to greater improvement in digestive symptoms. However, long-term adherence to an elimination/low-FODMAP diet is not recommended due to the risk of micronutrient deficiencies. Therefore, patients should closely collaborate with clinicians to navigate each phase.

Slide 51: The implementation of a new diet/eliminating certain products into three phases: the restriction phase, the reintroduction/rechallenge phase, and the personalization and maintenance phase.

Furthermore, a significant reduction in gut bacterial concentrations of Bifidobacterium adolescentis and Faecalibacterium prausnitzii is observed with a gluten-free and low-FODMAP diet."

Slide 52: Excessive sugar or starch consumption can lead to *Candida* dysbiosis which has intrinsic resistance to the fungistatic effect of SCFAs. Interestingly, *Candida* overgrowth diet therapy involves antifungals and avoiding foods high in simple sugars and starch, cured and fatty meats, milk and dairy products, and alcohol

Artificial food ingredients, such as maltodextrin and polysorbate-80, have been linked to gut dysbiosis. Maltodextrin, a common additive, facilitates the adherence of invasive E. coli strains to intestinal cells, promoting biofilm formation and inflammation. Similarly, polysorbate-80, an emulsifier found in processed foods, enhances the movement of pathogenic E. coli strains across colon cells. These emulsifiers have been shown to reduce microbial diversity, decrease beneficial Bacteroidales, and increase mucolytic Ruminococcus gnavus and pro-inflammatory Proteobacteria. Additionally, they lead to a reduction in mucus thickness and bacterial encroachment in the gut.into the normally sterile inner mucus layer. Microbiota encroachment has been implicated in IBD and metabolic syndrome.

Slide 53: Behavioural therapies, gut-brain axis modulation

The vagus nerve (VN) plays a crucial role in gut-brain communication, expressing receptors that interact with gut microbes and their metabolites.

Chronic treatment with Lactobacillus rhamnosus induced alterations in GABA mRNA in the brain. Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 restored tight junction barrier integrity and attenuated HPA axis and autonomic nervous system activities, assessed through plasma cortisol and catecholamine measurements.

Slide 54: Probiotics like Lactobacillus reuteri (LR) and Lactobacillus rhamnosus (JB-1) affect the excitability of intestinal sensory neurons, as suggested by recent studies. LR intake increases the intrinsic excitability of myenteric IPANs and promotes sensory responses, possibly by inhibiting calcium-dependent potassium channels. Short-chain fatty acids (SCFAs), such as sodium oleate and sodium butyrate, activate vagal afferent fibres, affecting gut-brain communication. Long-chain fatty acids like oleate stimulate afferent fibres via a CCK-mediated mechanism, while short-chain fatty acids like butyrate act directly on afferent terminals.

By understanding the intricate connections between the gut and the brain, we can explore methods to stimulate the vagus nerve, thus enhancing this bidirectional communication and potentially alleviating issues like constipation.

Through synaptic connections, they transmit vital information from the intestines to the brain, influencing gut-brain communication. Additionally, receptors within the gut can detect microbial metabolites, further modulating vagal signalling and enhancing this communication pathway. By stimulating the vagus nerve, either through specific techniques or interventions, we can potentially improve constipation relief and overall gastrointestinal function positively impacting both the nervous system and the gut-brain axis.
You can help enhance the parasympathetic nervous system and stimulate the vagus nerve by deep breathing, singing, humming, yoga, and cold exposure.

Slide 55: Medications

In managing constipation, laxatives are often necessary. For ongoing use, bulk laxatives like psyllium (Metamucil) and PEG, Miralax are typically well-tolerated. Stimulant laxatives like bisacodyl and sodium picosulfate, senna, aloe, cascara sagrada are common for patients experiencing prolonged periods without bowel movements (typically 2-3 days). They trigger colonic High-Amplitude Propagating Contractions (HAPCs).

Osmotic agents, including PEG-based solutions, magnesium citrate, sodium phosphate products (e.g., Fleets), and non-absorbable carbohydrates like sorbitol, work by retaining water in the colon through an osmotic effect. Osmotic laxatives are substances that are either poorly absorbed or not absorbed at all. They create osmotic gradients within the intestinal lumen, prompting the secretion of water and electrolytes. As a result, there is an accumulation of water in the intestine, leading to softer stools and easier passage. Senna and bisacodyl (Dulcolax) may cause cramping.

Magnesium sulfate, a saline laxative, elevates intestinal osmotic pressure, hindering water absorption in the colon, increasing intestinal contents, and prompting intestinal peristalsis, resulting in rapid and intense catharsis. Elevated intracellular magnesium ions activate adenylate cyclase (AC), triggering an increase in cyclic AMP (cAMP) levels. This rise in cAMP concentration subsequently activates protein kinase A (PKA), facilitating CREB phosphorylation and transcription of the AQP3 gene. Overuse of saline laxatives can lead to electrolyte imbalances, making them unsuitable for the elderly and individuals with impaired kidney function.

Slide 56: Herbals and supplements

Aloe vera: Barbaloin, a key compound in Aloe vera (AV), acts as a potent laxative. Once metabolized by intestinal bacteria, it transforms into aloe-emodine-9-anthrone (AE-anthrone). AE-anthrone promotes intestinal motility and enhances paracellular permeability by inhibiting Na+/K+-adenosine triphosphatase at the colonic mucosa. Additionally, it influences the release of prostaglandin-like substances and stimulates mucus secretion in the colon. These actions lead to increased water content in the intestinal lumen, resulting in a laxative effect in humans. Consequently, AV shows promise as a potential therapeutic option for patients with constipation-predominant irritable bowel syndrome (IBS) or functional constipation.

Triphala: Different formulations of TLP solutions have been shown to protect against intestinal injuries by improving the integrity of epithelial cell tight junctions and restoring the brush border membrane of the intestine. Additionally, they decrease the levels of myeloperoxidase and xanthine oxidase in the gut mucosa. In one study, a 1-week treatment with TLP increased average weekly bowel frequency by 64.4%, rising to 79.5% after 2 weeks.

TLP also exhibits inhibitory effects on the growth of pathogenic microorganisms in the intestines, which can exacerbate intestinal symptoms. Furthermore, the polyphenols present in TLP can modulate the human gut microbiota by promoting the growth of beneficial Bifidobacterium and Lactobacillus species while inhibiting the growth of undesirable intestinal residents.

Marshmallow root

Marshmallow root offers a soothing effect and is frequently included in medicinal teas due to its laxative properties. This herb’s therapeutic properties are partly attributed to the mucilage it contains for treating constipation. Marshmallow root is commonly ingested in capsule, tincture, or tea form.

Slide 57: Surgery

When pharmacological treatments fail patients with outlet rectal obstruction syndrome may require rectocele repair or surgery for rectal intussusception. For individuals with combined dyssynergic defecation and slow-transit constipation, segmental colonic resection or total colectomy could be beneficial, with correction of pelvic floor dysfunction as a priority. An ileostomy can offer relief for severe, uncorrectable pelvic floor dysfunctions. However, segmental colectomy, while a short-term solution, may lead to constipation relapse.

Antegrade continence enemas provide a means for forward irrigation of the colon, allowing for flushing of fluids with or without laxative agents through an external-

opening aperture into the colonic lumen. Common procedures for establishing this opening include the Malone procedure (via an appendiceal conduit) and percutaneous cecostomy (PEC). PEC is preferred over colectomy for patients with significant comorbidities and higher surgical risk and can be performed under conscious therapy or local anaesthesia. However, approximately 30% of patients who undergo Malone appendicectomy experience complications.

While surgery often results in greater patient satisfaction, about half of patients report experiencing abdominal pain afterwards. The successful implementation of surgical procedures necessitates an individualised approach and the expertise of a surgeon skilled in the chosen technique.

Slide 59: Case studies– Read from what's on the slide.

Slide 71: Prognosis

While most patients with constipation respond well to holistic treatments and experience improvement, a small percentage of adults may suffer from severe debilitation due to this condition. Additionally, at least 30% of children with constipation may continue to experience symptoms into adulthood.

Several factors are associated with a poorer prognosis, including female gender, older age of onset, longer duration between symptom onset and initiation of therapy, and longer colonic transit time. In some cases, treatments may prove ineffective, and even when successful, their benefits may be short-lived.

Recurrence of constipation is common in many patients, often due to non-compliance with dietary recommendations. For those significantly impacted by chronic constipation, their quality of life may be significantly diminished. In select cases where medical management fails, total abdominal colectomy may be considered; however, careful patient selection is crucial for optimal outcomes.

The most challenging cases involve patients dependent on laxatives, who resist lifestyle changes and persist in using various laxatives.

Slide 72: Microbiome approach

The gut microbiome is pivotal in constipation, interacting with the immune, enteric, and central nervous systems, and regulating intestinal secretion and hormones.

Focusing on a microbiome-based approach is promising to regulate and reduce a type of dysbiosis specific to an individual. By targeting the gut microbiota, with interventions such as dietary modifications, or elimination diet, one can restore

microbial balance and improve gastrointestinal function, stool frequency, consistency, and overall bowel habits.

Given the uniqueness of each individual's microbiome, a personalised approach is crucial in constipation treatment. Microbiome tests serve as valuable tools in pinpointing imbalances and uncovering the root causes of a patient's condition. This can also help to create a protocol with probiotics and prebiotics that are best for the particular case.

The analysis of faecal flora presents a promising method for swiftly screening and identifying biomarkers linked to chronic constipation. Faecal sample collection is noninvasive and does not necessitate specialised clinical procedures.

Slide 73: Complications

The impact of constipation on a patient's life varies depending on its severity and underlying causes. Some common complications of constipation include:

- Abdominal discomfort or cramps
- Decreased quality of life
- Hemorrhoids
- Anal fissures
- Pelvic floor damage
- Fecal incontinence
- Urinary retention
- Stercoral perforation
- Rectal prolapse
- Volvulus
- Fistula-in-ano

While microbiome-based interventions for constipation are generally well-tolerated, some potential complications are worth consideration. Dietary modifications, such as increasing fibre intake, may exacerbate symptoms in some individuals, leading to bloating, gas, and abdominal discomfort. Additionally, the use of probiotics and prebiotics carries the risk of adverse effects, including allergic reactions, gastrointestinal disturbances, and bacterial overgrowth. It is recommended to work with a health practitioner, who will gather the whole medical history, when undergoing treatment.

Slide 74: Conclusion:

Constipation manifests across all age groups and stems from various causes, impacting millions worldwide with treatment often falling short. Healthcare providers must evaluate and address constipation, through patient assessments, physical

examinations, and diagnostic test interpretations. Given constipation's chronic nature, ongoing care coordination is vital, ensuring treatment efficacy monitoring and adaptive strategies.

Getting into the root cause of the issue proves to bring promising results. Microbiome tests reflect unique interactions between the host and specific bacterial species or their metabolic products - SCFAs. Understanding the connection is essential, as it holds potential for future therapeutic interventions and further exploration into the pathogenesis of constipation.

Patient education is essential in managing constipation, enabling individuals to understand and actively address their condition. Pharmacists play a pivotal role in patient education on laxative risks and benefits, while nurses administer medications with proficiency. Clinicians must grasp medication nuances, monitor for adverse effects, and address concerns promptly.

Given constipation's chronic nature, ongoing care coordination is vital, ensuring treatment efficacy monitoring and adaptive strategies.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Post-graduate certificate level 7 (30 ECTS)

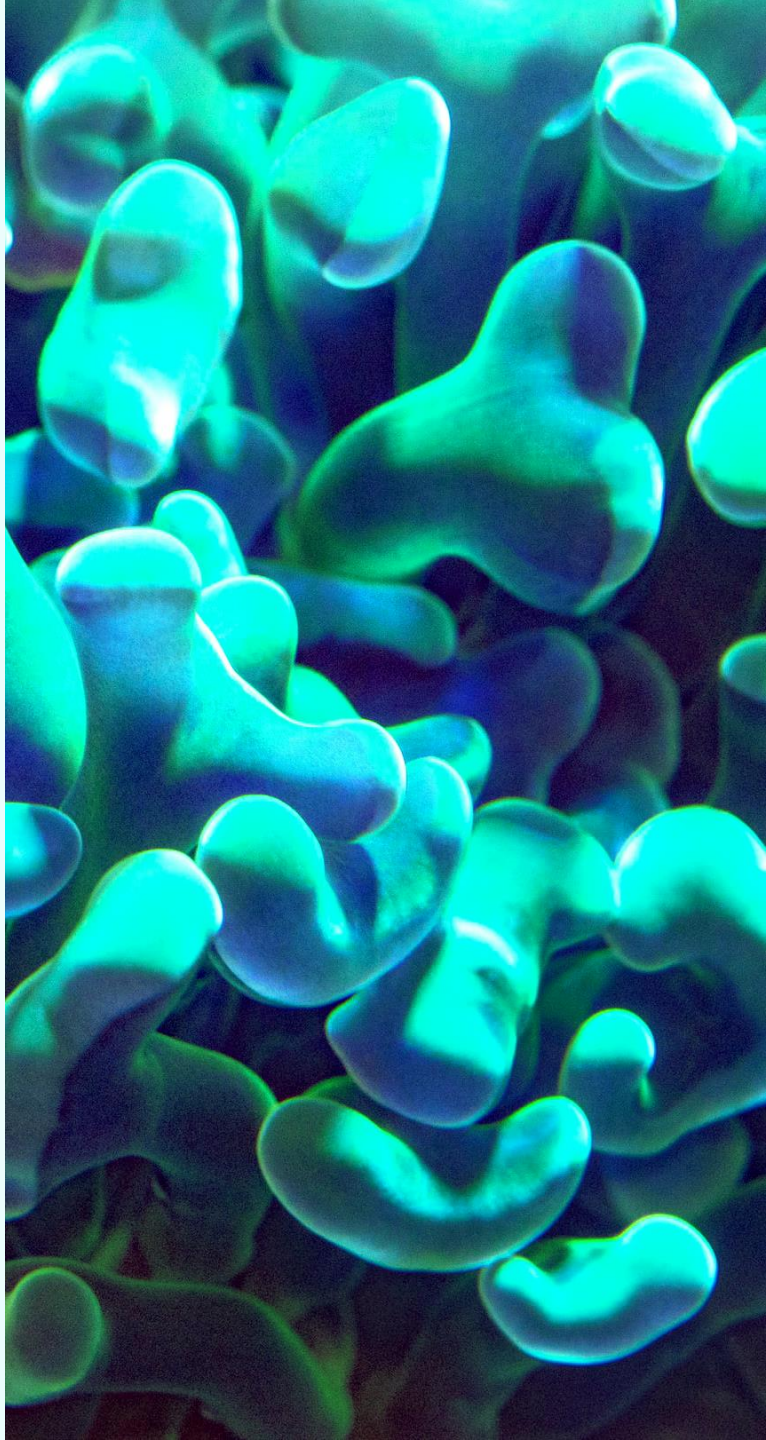
The Estrobolome

Its Role in Estrogen Metabolism

Dr. Eman Zaky
MBBCh., MSc., MPH., RNutr.

lecturer@imas.edu.mt

Malta Further & Higher Education Authority



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Objective: To Explore the Relationship between Gut Microbiota and Hormonal Balance

Explore Estrogen-Microbiota Interaction: Bidirectional relationship between estrogen and gut microbiota.

Define Estrobolome: Key role in estrogen metabolism and hormonal balance.

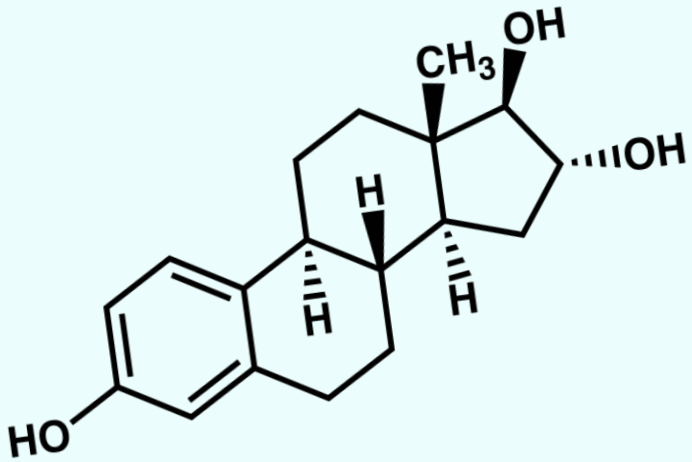
Highlight Health Effects of Estrogen Microbiome Interaction: Recognize the link between dysregulation of the estrobolome and various estrogen-related health conditions

Strategies to Improving Gut Health and Hormonal Balance: Dietary changes and lifestyle modifications, including exercise, and cautious use of antibiotics and minimising disrupting agents.

Investigate New Therapeutic Interventions: Explore HRT, gmGUS inhibitors, and fecal microbiota transplantation (FMT) for estrogen-related health conditions.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Estrogen Overview:



Vital for reproductive organs and female characteristics.

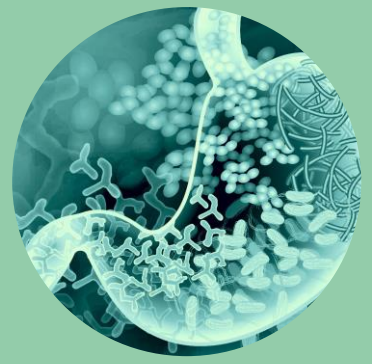
Produced in ovaries, adrenal glands, and adipose tissue.

Influences metabolic processes and cardiovascular health.

The Hormone Estrogen

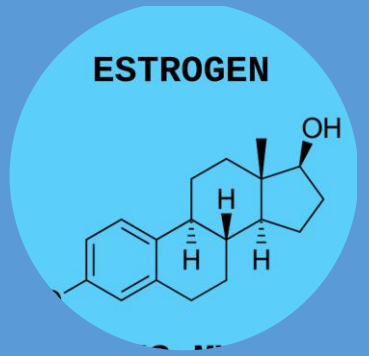
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Estrogen-Microbiota Interaction



The Gut Microbiota

- Functions as a mature endocrine organ.
- Influences estrogen metabolism and homeostasis.
- **Dysbiosis** or imbalanced gut microbiome due to (e.g., poor diet, antibiotics, or stress) can disrupt estrogen metabolism, leading to either estrogen excess or deficiency.



Estrogen Influence on Gut Microbiota:

- **Direct Effects**
  - Estrogen receptors in the gut modulate gut permeability, immune responses, and microbial composition. Low estrogen cause leaky gut leading to dysbiosis.
- **Indirect Effects**
  - Estrogen influences bile acid production, which shapes microbial communities and their metabolic activities.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

What is The Estrobolome?

The Estrobolome

- It is a group of enteric bacterial genes responsible for estrogen metabolism, which significantly impact estrogen levels in the body.

Function:

- Determines whether estrogens are reabsorbed or excreted.
- Mediates overall body **estrogen** levels.

Mechanism:

- GUS gene encodes Gut microbial **β-glucuronidase (gmGUS).**
- Converts conjugated estrogen to free form for reabsorption.

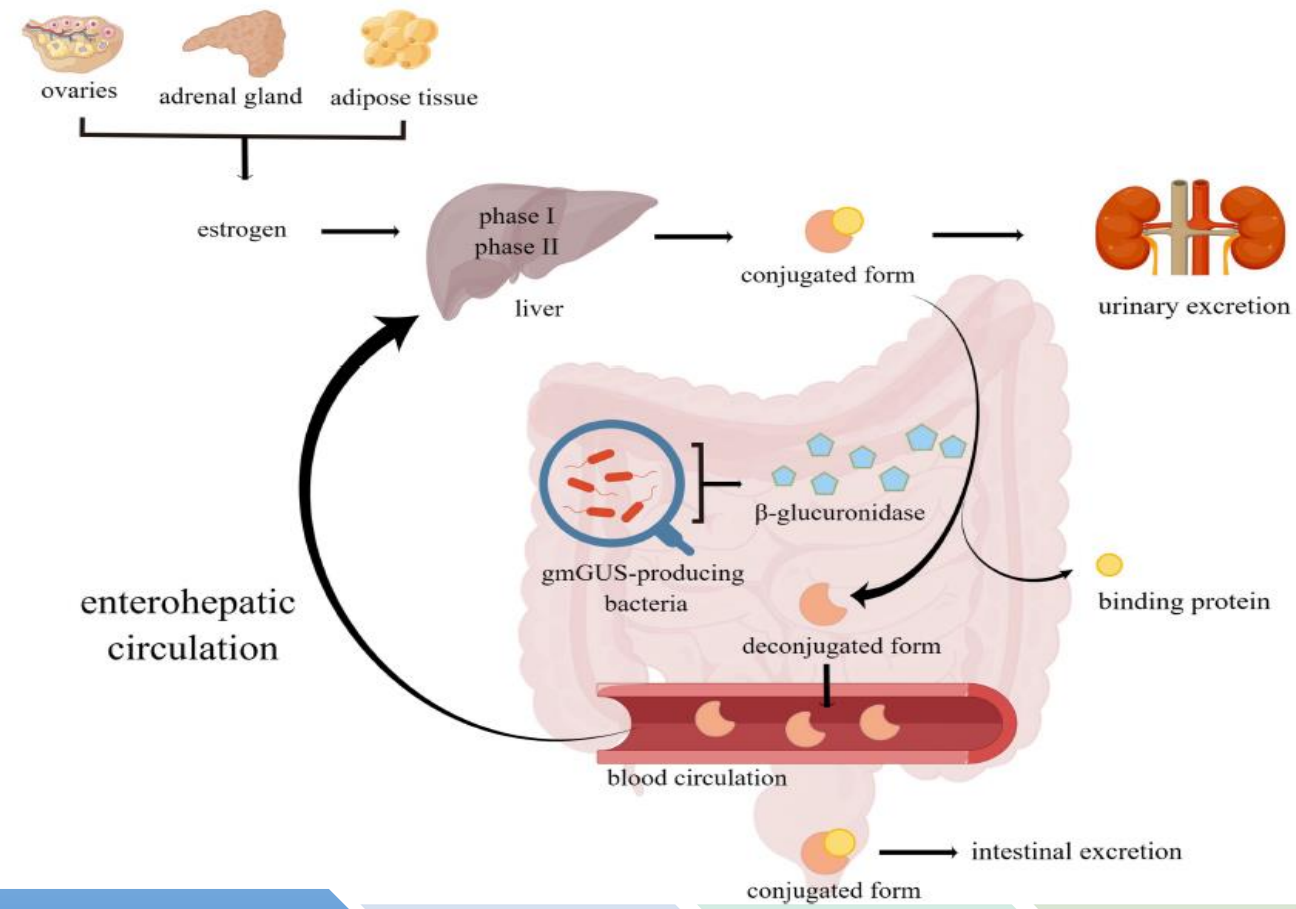
Bidirectional Interaction:

- Estrogen levels can influence gut microbiota composition and diversity, while gut microbes, through enzymes like (gmGUS), can modulate systemic estrogen levels

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Estrogen Metabolism Process:

(S. Hu et al., 2023)



Estrogen Metabolism Process

- Metabolized in the liver.
- Excreted into bile and enters the intestine.
- Reabsorbed in the intestines and returned to the liver.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Human Microbiome Project Findings: GUS Genes

GUS genes are involved in the breakdown of complex carbohydrates, which is crucial for digestion and nutrient absorption.

The identification of these genes suggests a rich **enzymatic capacity** within the gut microbiota and correlate it with estrogen levels.

HMP Identified 279 GUS genes based on structural characteristics and biocatalytic properties.

Taxonomic classification:

- **Bacteroidetes (52%)**
- **Firmicutes (43%)**
- **Verrucomicrobia (1.5%)**
- **Proteobacteria (0.5%)**

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Analyzing Estrobolome

Methodologies:

- **Metagenomic Sequencing:** 16S rRNA gene sequencing
- **Enzymatic Activity Assays:** measure GUS activity
- **Metabolomic Profiling:** analyzing metabolites levels

Importance:

- Informs therapeutic strategies for estrogen-related conditions.
- Potential for dietary interventions and developing functional probiotics.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Health Effects of Estrogen-Microbiota Interaction
Microbial Influence:

	Function	Health Implication
Key Mechanisms of Microbial Influence	**Regulates estrogen levels**:	**Dysbiosis:** affects β-glucuronidase activity altered estrogen metabolism, which is associated with various estrogen-related diseases.
	Inflammatory response :	**Leaky gut:** increases permeability and systemic inflammation disrupting estrogen balance.
	Carbohydrate metabolism:	**Metabolite pathway:** Gut microbiota produces metabolites impacting disease progression.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Health Effects of Estrogen-Microbiota Interaction

Hormonal Influence:

Pathological Implications

Hyper-estrogenic Pathologies

- Endometriosis and its symptoms.
- (PCOs) polycystic ovarian syndrome
- Estrogen-dependent cancers.
- Premenstrual Syndrome (PMS) and gut health.

(S. Hu et al., 2023)

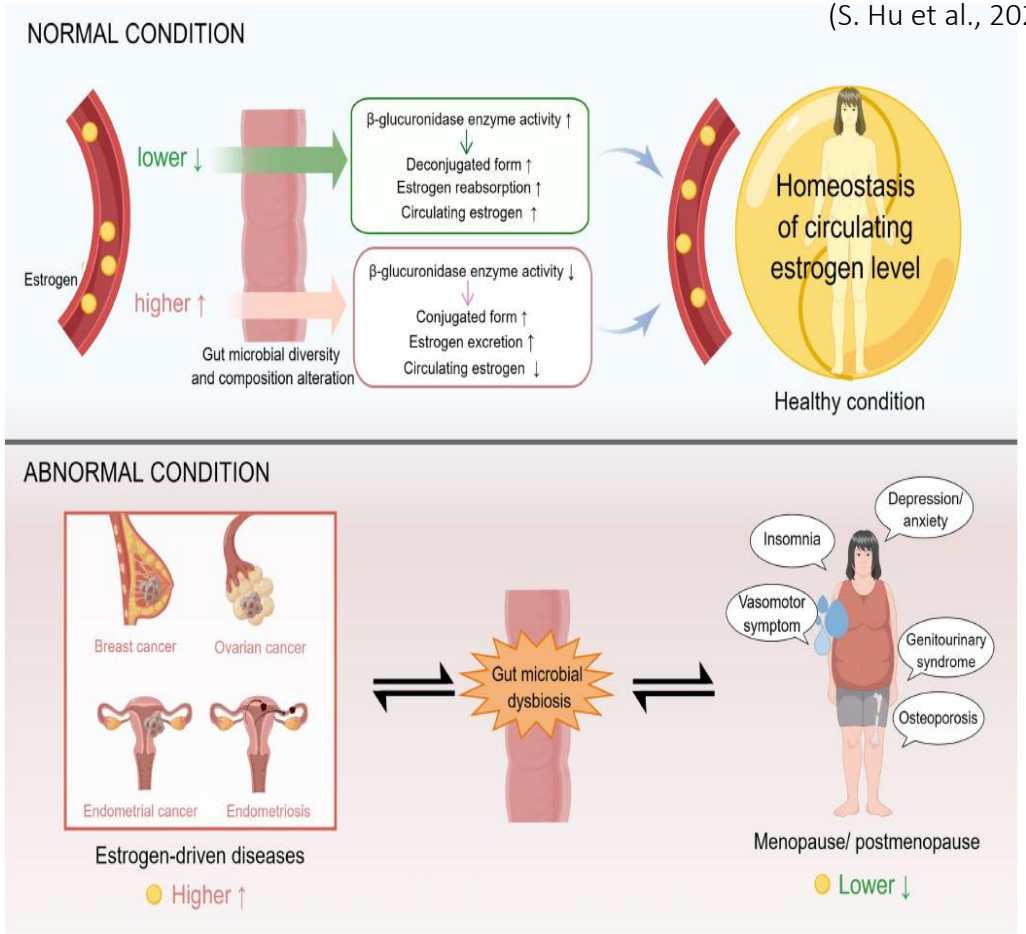


Figure 2. Higher systemic estrogens affect gut microbiome diversity and composition, thereby reducing β-glucuronidase availability and increasing estrogen excretion; when estrogen levels reduce, it also reshapes the gut microbiome, thereby increasing β-glucuronidase activity, promoting estrogen reabsorption and maintaining systemic homeostasis of estrogen levels. Thus, abnormalities in either of them may break this homeostasis and cause the occurrence of estrogen-driven diseases.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Health Effects of Estrogen-Microbiota Interaction
Hyper Estrogen Impact on Gut Microbiota

(Wang MY et al. 2024)

Hyper-estrogenic Disorder	Increased Gut Microbiota	Decreased Gut Microbiota
Polycystic Ovarian Syndrome (PCOS)	Lachnoclostridium, Fusobacterium, Coprococcus_2, Tyzzerela 4, Bacteroides, Escherichia/Shigella, Streptococcus	Tenericutes, Firmicutes/Bacteroides ratio, Akkermansia, Ruminococcaceae
Endometriosis	Bacteroides, Parabacteroides, Oscillospira, Coprococcus	Paraprevotella, Lachnospira, Clostridia, Clostridiales, Lachnospiraceae, Ruminococcus, Ruminococcaceae
Breast Cancer	Firmicutes, Clostridium cluster IV, cluster XIVa	Bacteroidetes, Bacteroidetes phylum, Verrucomicrobia phylum
Cervical Cancer	Prevotella, Porphyromonas, Dialister, Succinivibrio, Ruminococcus, Morganella, Shewanella, Proteus	Bacteroides, Alistipes, Lachnospiracea, Phascolarctobacterium, Halomonas
Ovarian Cancer	Proteobacteria	Actinobacteria, Bifidobacterium, Coprococcus

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Health Effects of Estrogen-Microbiota Interaction

Hormonal Influence:

Pathological Implications

Hypo-estrogenic Pathologies

- **Menopause**
- **Obesity & Metabolic Syndrome**
- **Cardiovascular Disease & Cognitive Decline.**
- **Osteoporosis**

(S. Hu et al., 2023)

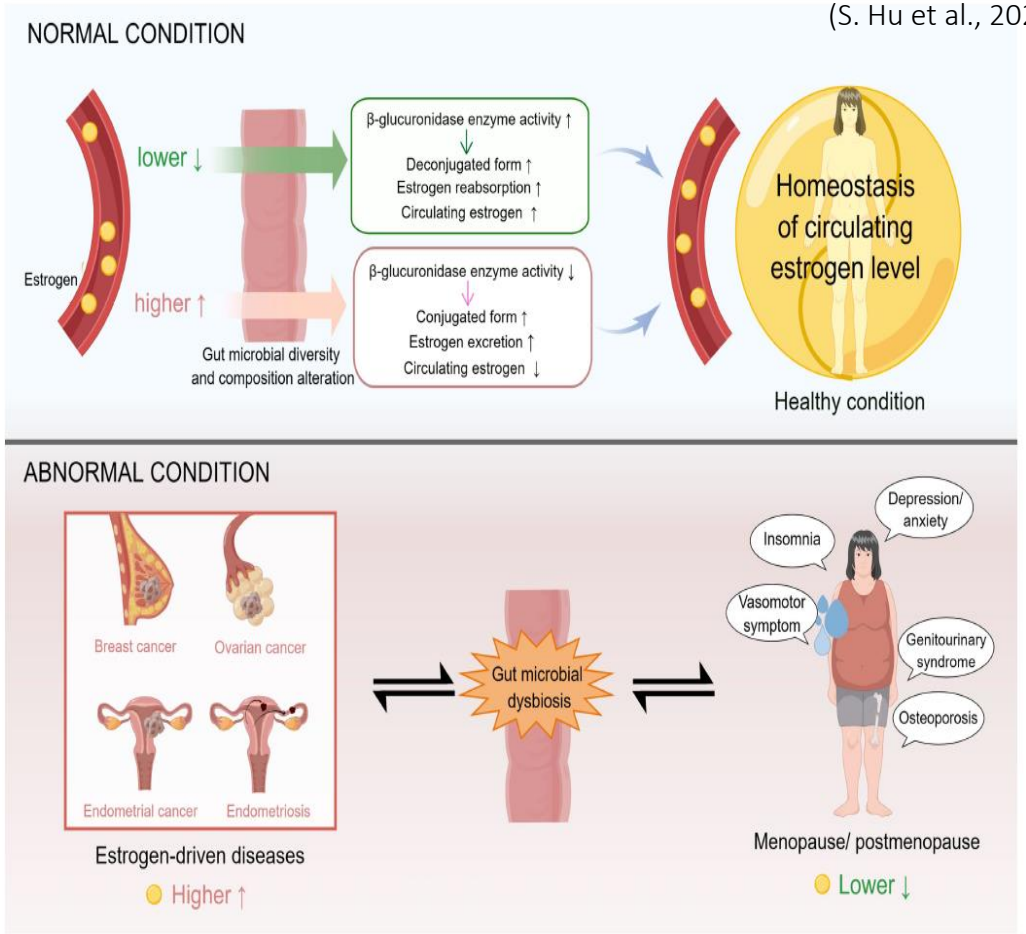


Figure 2. Higher systemic estrogens affect gut microbiome diversity and composition, thereby reducing β-glucuronidase availability and increasing estrogen excretion; when estrogen levels reduce, it also reshapes the gut microbiome, thereby increasing β-glucuronidase activity, promoting estrogen reabsorption and maintaining systemic homeostasis of estrogen levels. Thus, abnormalities in either of them may break this homeostasis and cause the occurrence of estrogen-driven diseases.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Health Effects of Estrogen-Microbiota Interaction

Hypo-Estrogen Impact on Gut Microbiota

Understanding the Impact of Menopause on Gut Health

- **Diversity Changes In Gut Microbiota**
- **Dominant Bacterial Taxa Shifts**
- **Dysbiosis in Gut Microbiota**

(Wu, S., et al., 2024)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

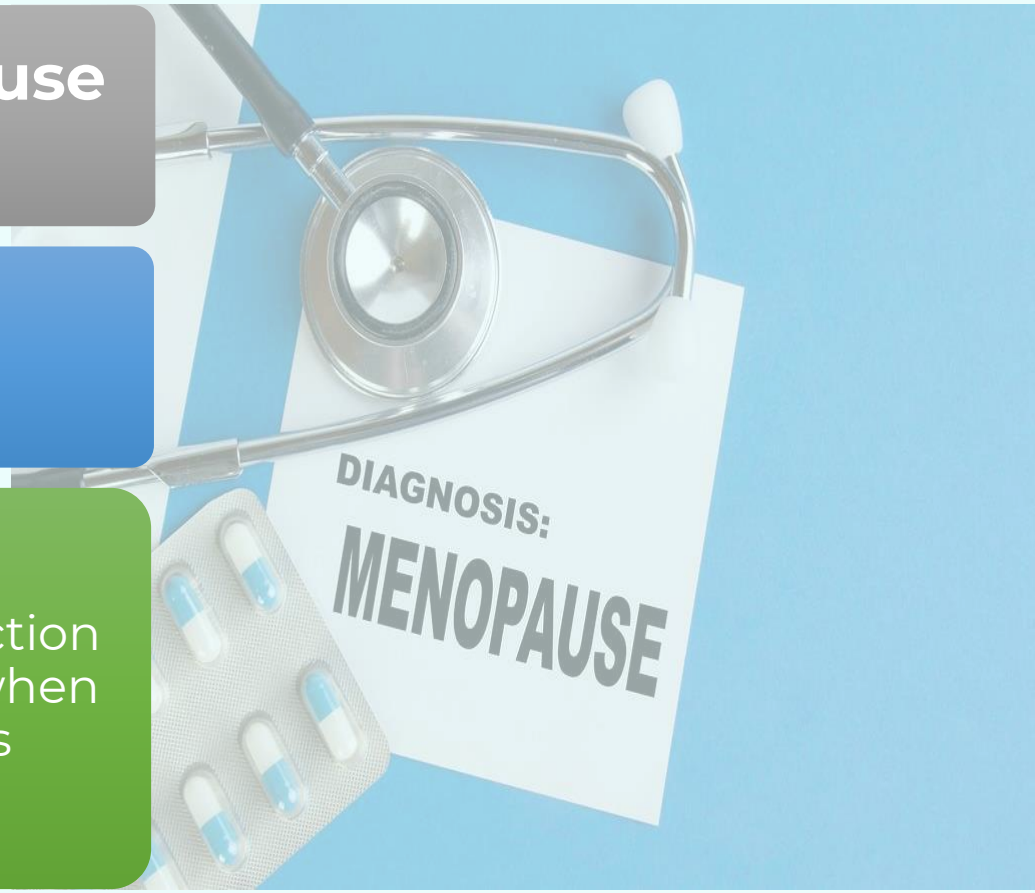
Health Effects of Estrogen-Microbiota Interaction

Hormonal Influence:

Understanding the Impact of **Menopause** on Gut Health

Diversity Changes in Gut Microbiota

Postmenopausal women exhibit a significant reduction in both **alpha and beta gut microbiota diversity** when compared to their premenopausal counterparts



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

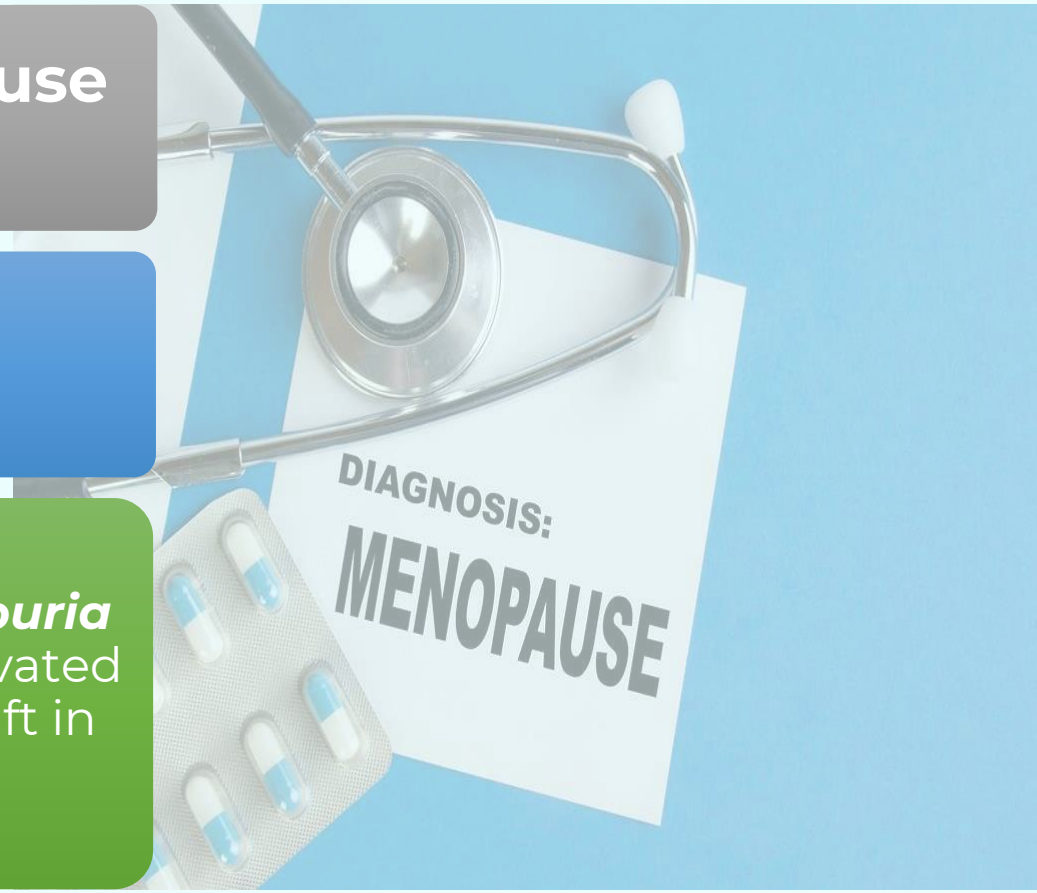
Health Effects of Estrogen-Microbiota Interaction

Hormonal Influence:

Understanding the Impact of **Menopause** on Gut Health

Dominant Bacterial Taxa Shifts

postmenopausal women have lower levels of ***Roseburia*** *Prevotellaceae and Firmicutes*, while displaying elevated levels of the phylum *Bacteroidetes*, indicating a shift in bacterial composition that may impact health.



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

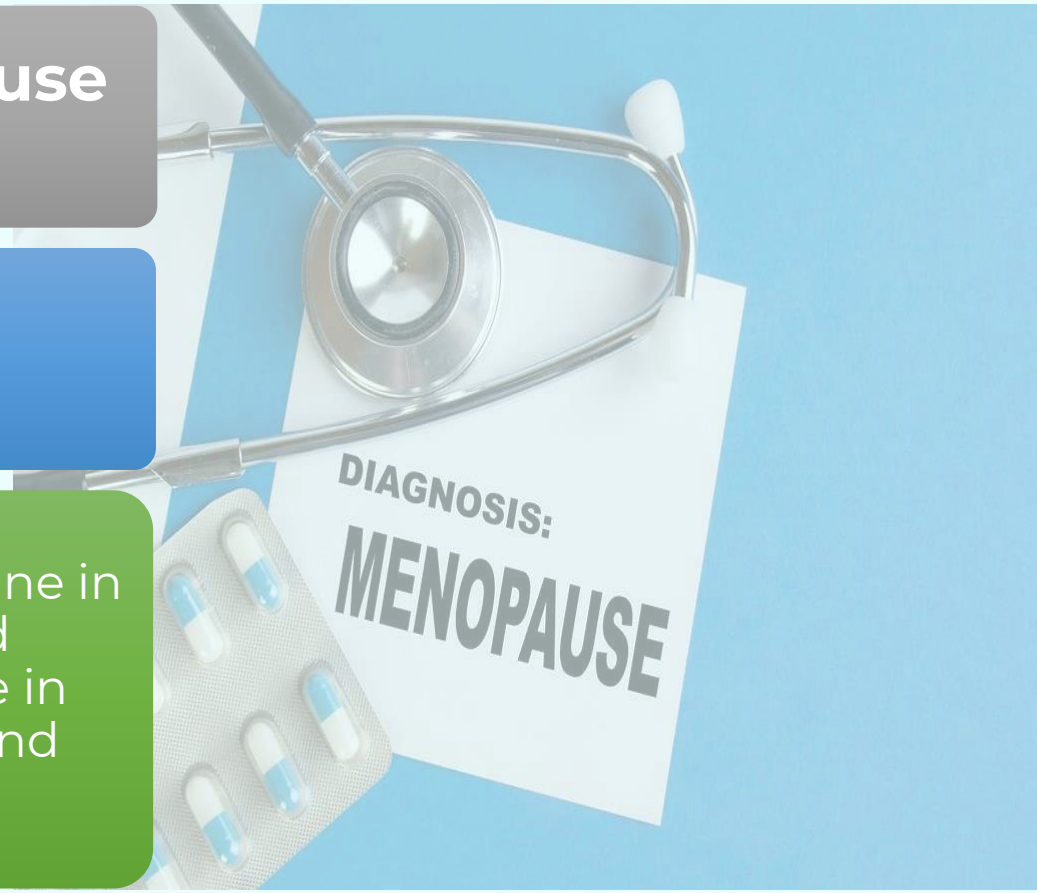
Health Effects of Estrogen-Microbiota Interaction

Hormonal Influence:

Understanding the Impact of **Menopause** on Gut Health

Dysbiosis in Gut Microbiota

Postmenopausal transitions characterized by a decline in beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*. Simultaneously, there is an increase in potentially harmful species, including Klebsiella and Clostridiodes difficile.



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance:

Key Strategies:

Dietary Changes:

- Whole Food
- Prebiotics
- Probiotics

Lifestyle Modifications:

- Regular Exercise
- Stress Management

Avoiding Antibiotics

OTC Medications

Minimize Toxins

Hormonal Replacement Therapies

Alternative Therapies

Fecal Microbiota Transplantation (FMT)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance:

Dietary Changes for Gut Health

Balanced diet rich in **omega-3s, antioxidants**, and **vitamins**.

Incorporation of fiber-rich foods as a **prebiotic** for microbiome diversity.

Fermented Foods: yogurt, kefir, and kimchi are rich in **probiotics** that help restore and maintain gut flora balance

Probiotics and Prebiotics Supplements

Prebiotic supplements, such as inulin, can support gut health and balance estrogen levels.

Probiotics containing strains like *Lactobacillus* and *Bifidobacterium* may enhance estrogen metabolism.

Lifestyle Modifications

Regular **physical activity** enhances gut health.

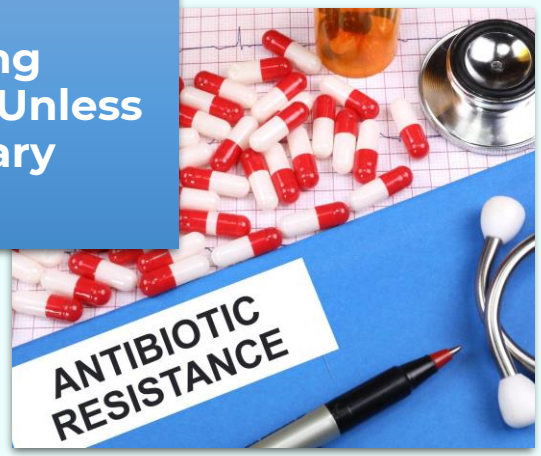
Stress management techniques (yoga, deep sleep, meditation)

Medications: Melatonin, SSRIs or Gabapentin may be prescribed depending on the symptoms.

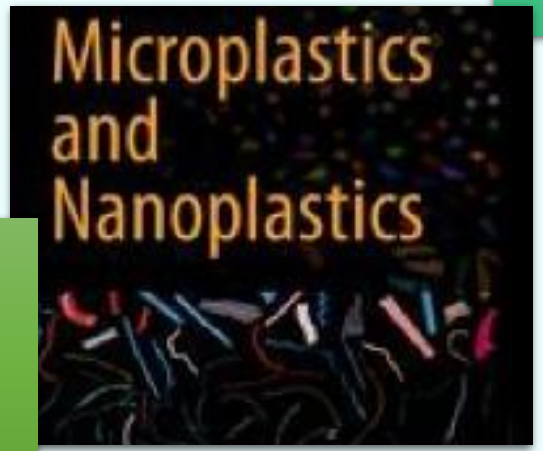
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance: Avoiding Disrupting Agents

Avoiding Antibiotics Unless Necessary



Avoiding Over-the-Counter Pain Relievers



Minimize Toxins:

- **Reduce Exposure**
- **Choose Organic Foods**

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance: Avoiding Disrupting Agents

Antibiotics

Effects of Antibiotics	**Antibiotics can disrupt the diversity of gut microbiota,** potentially leading to a decrease in beneficial bacteria that produce GUS. This disruption can damage the metabolism of estrogens, exacerbating conditions related to hormonal imbalance.
Bacterial Genera	Certain bacterial genera like *Bacteroides* and *Lactobacillus* are known to contain genes encoding GUS. A decrease in these populations due to antibiotic treatment may result in lower GUS activity and altered estrogen metabolism.
Clinical Implications	Managing gut health through **probiotics** and/or **prebiotics** might help mitigate some adverse effects of antibiotics on hormonal balance.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance: Avoiding Disrupting Agents

Medications

1- Over-the-Counter Pain Relievers

- **Examples**:
  - Ibuprofen
  - Acetaminophen
- **Impact**:
  - May alter gut microbiota composition.
  - Can increase intestinal permeability, affecting gut health.
  - Influences gmGUS activity.

2- Hormonal Contraceptives

- **Examples**:
  - Birth control pills (estrogen and/or progestin)
- **Impact**:
  - Hormonal changes influence gut microbiota diversity.
  - Estrogen affects gmGUS activity, potentially increasing estrogen reactivation.

3-Antidepressants (SSRIs)

- **Examples**:
  - Fluoxetine
  - Sertraline
- **Impact**:
  - May alter gut microbiota composition.
  - Changes in microbiota can influence gmGUS activity and estrogen metabolism.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance: Avoiding Disrupting Agents

Toxins and Microplastic

Dysbiosis : Environmental toxins and plastics can disrupt gut microbiome balance.

Metabolism of Estrogens: Impaired gut function affects estrogen metabolism.

Health Risks: Increased risk of hoarmone-related diseases (e.g., breast and cervical cancer).

Reducing Exposure to Endocrine-Disrupting Chemicals

Identify sources include plastics and household products.

Practical Steps:

- Use glass or stainless-steel containers.
- Avoid microwaving plastic.
- Choose natural cleaning products.
- Reduces pesticide exposure.
- Prioritize organic for the "Dirty Dozen" (high pesticide produce).
- Support local organic farms when possible.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance: New Therapeutic Intervention

Hormonal Replacement Therapy

HRT Purpose:

- Treatment to relieve menopause symptoms, menstrual disorders, low estrogen levels, and Premature Ovarian Insufficiency by replenishing estrogen.

Administration:

- Estrogen can be used locally or systemically to balance hormone levels and alleviate symptoms.

Common Symptoms Treated

- Hot flashes
- Night sweats
- Vaginal dryness
- Mood swings
- Hormonal acne

Fluctuations in Estrogen Levels

- **Influence of Gut Microbes**: microbial enzyme activity can affect individual responses to HRT.
- **Probiotics**: Combining probiotics with HRT may enhance the effectiveness of bioactive estrogen and reduce cancer risks.

Clinical Considerations

- **Long-term Effects**: increase cancers risk
- **Usage**: While commonly used for menopausal symptoms, HRT has lower clinical use due to associated cancer risks.

Research Focus Areas

- Investigating the relationship between gut microbiota and hormonal therapies.
- Exploring implications for conditions like breast cancer.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

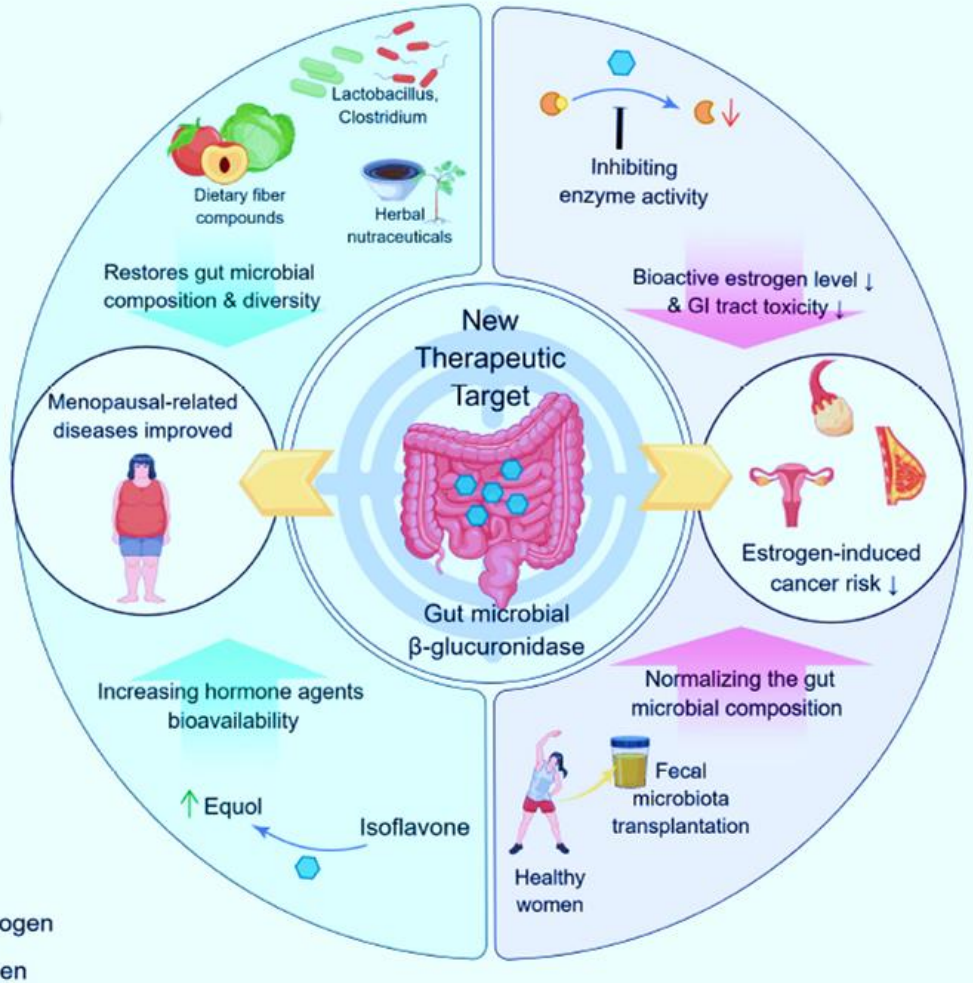
Strategies for Improving Gut Health and Hormonal Balance: New Therapeutic Intervention

(S. Hu et al., 2023)

Gut β-Glucuronidase (gmGUS)

Role in Menopausal Disorders

- Normalization of gut microbiota composition and diversity:
  - Dietary fiber intake
  - Herbal nutrients
  - Beneficial gut microbiota
- Increased bioavailability of estrogenic agents:
  - Soy isoflavones converted to estradiol



Role in Gynecological Cancers

- **Inhibition** of gmGUS enzyme activity:
- Lowers biologically active estrogen levels
- Reduces intestinal side effects of anti-cancer drugs
- **Synergistic effects**: Suppresses progression of gynecological cancers

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance: New Therapeutic Intervention

New Treatment Method:

The gmGUS inhibitor

Study on GUS Enzymes: Research examined GUS enzymes in the gut of healthy women and **breast cancer patients**, finding that adjusting probiotics could lower breast cancer risk by inhibiting reactivation ofestrobolome-associated protein

Structural Modifications: Changes to the structure of gmGUS can help inhibit its activity, which may prevent estrogen reactivation.

Effective Inhibitors:

- **D-glucaro-1,4-lactone (1,4-GL)**: This inhibitor may help fight cancer by affecting hormone production.
- **TCH-3511**: A specific gmGUS inhibitor that can prevent gut bacteria imbalances caused by cancer-causing substances.

Role of Probiotics and Prebiotics: These can lower the risk of estrogen-related cancers by reducing β-glucuronidase activity in the intestines. For example, substances like **lactulose** and **inulin** can significantly decrease this enzyme's activity.#

Potential New Therapy: Combining gmGUS inhibitors with anticancer drugs like **irinotecan** could reduce intestinal toxicity and enhance anti-tumor effects potentially improving outcomes for patients.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance: Fecal Microbiota Transplantation (FMT)

Definition:

- FMT involves transferring gut microbiota from a healthy donor to a recipient.

Beneficial Effects of FMT:

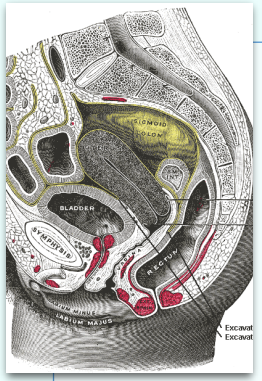
- Modulation of immune response.
- Reduction of inflammation.
- **Influence on Gut Microbiota**: FMT can alter gut microbiota composition, potentially alleviating symptoms of ovarian insufficiency and menopause-related issues, endometriosis and PCOS.

Therapeutic Potential:

- FMT may provide new treatment avenues for gynaecological disorders.

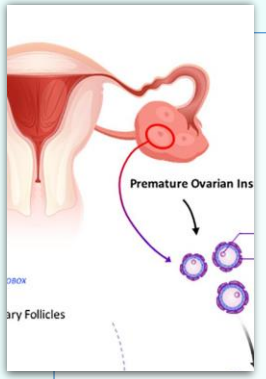
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Strategies for Improving Gut Health and Hormonal Balance: FMT Research Insights



Impact on Vaginal Health:

- **Research Insights**:
- FMT from ovary-intact *fecund* females significantly lessened vaginal epithelial atrophy in ovariectomized (OVX) mice.
- **Implication**: potential for treating vaginal health issues associated with menopause.



Research on Ovarian Function:

- **Iatrogenic Ovarian Aging**:
  - OVX mice exhibited significant differences in gut microbiota Notable alterations in the *Firmicutes/Bacteroidetes* ratio
- **OVX Mouse Models**: FMT demonstrated improvement in ovarian function by restoring gut microbiota composition.

Polycystic Ovaries Syndrome (PCOS):

- **Animal studies:** Rats with PCOS showed lower beneficial bacteria (e.g., Lactobacillus, Clostridium).

  Post-FMT: Improved estrogen cycles, reduced androgen levels, normalized ovarian morphology

Association with Antiaging:

- **Antiaging Agents**:

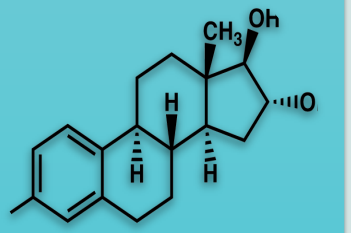
  Coenzyme Q10 (CoQ10), Melatonin, Resveratrol.
- **Effects** : CoQ10 linked to preservation of ovarian follicles and improved oocyte quality.
- These agents may interact with gut microbiota to enhance ovarian function.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Future Research Direction:

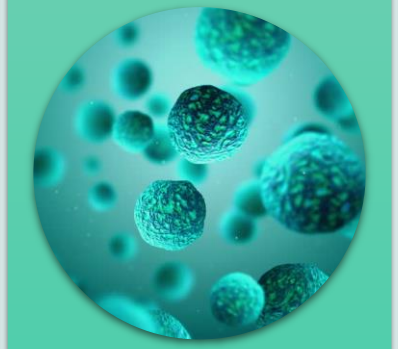
Multi-Omics Integration:

Combine genomics, metabolomics, and proteomics to study gut microbiota and host hormone interactions.



Estrogen Regulation:

Investigate how gut microbiome influences estrogen levels in different stages of life for potential therapeutic applications.



Comparative Microbial Studies:

Compare microbial signatures in healthy women versus those with diseases like breast and cervical cancer.

Biomarker Identification:

Identify unique microbial profiles and their metabolites as biomarkers for disease risk and progression.

Therapeutic Research:

Focus on restoring gut microbiota and hormonal balance in conditions like endometriosis and dysbiosis-related cancers.

Individualized Probiotics

Develop tailored functional probiotics based on gut microbiome profiles.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Summary:

Gut Microbiota as Endocrine Organ:	• **Gut microbiota** produce enzymes to metabolise and recycle estrogen.
Role of Estrobolome:	• Crucial for **regulating estrogen levels**. • Determines reabsorption or excretion of estrogens, impacting hormonal balance.
GUS Gene Function:	• Encodes enzyme (**gmGUS**) that converts conjugated estrogen to free forms, aiding reabsorption.
Impact of Dysbiosis:	• **An Imbalance** in gut microbiota disrupts estrogen metabolism in a vicious cycle. Linked to conditions like endometriosis, obesity, and certain cancers.
Strategies for Enhancing Gut Health:	• **Dietary changes**: Whole foods, prebiotics, probiotics. • **Regular exercise** and stress management. • **Cautious use** of antibiotics, and chemicals disrupting hormonal balance.
Future Research Directions:	• **Investigate** specific microbial taxa, develop functional probiotics, and determine the long-term effects of new therapeutic interventions (e.g. HRT, gmGUS inhibitor, FMT) for managing estrogen-related health conditions.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

1- Hu, S., Ding, Q., Zhang, W., Kang, M., Ma, J. and Zhao, L., 2023. Gut microbial beta-glucuronidase: a vital regulator in female estrogen metabolism. *Gut Microbes*, *15*(1), p.2236749.

2- Baker, J.M., Al-Nakkash, L. and Herbst-Kralovetz, M.M., 2017. Estrogen–gut microbiome axis: Physiological and clinical implications. *Maturitas*, *103*, pp.45-53.

3- Wang, M.Y., Sang, L.X. and Sun, S.Y., 2024. Gut microbiota and female health. *World Journal of Gastroenterology*, *30*(12), p.1655.

4- Huang, F., Cao, Y., Liang, J., Tang, R., Wu, S., Zhang, P. and Chen, R., 2024. The influence of the gut microbiome on ovarian aging. *Gut microbes*, *16*(1), p.2295394.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

Estrobolome

Overview

Estrogen, plays a vital role in a woman's life, promoting reproductive organs and maintaining female sexual characteristics. It also impacts the metabolic process, cardiovascular system, and bone growth. Estrogen metabolism regulates circulating estrogen levels, and abnormalities can lead to estrogen-related diseases and disruption of estrogen levels.

The Gut Microbiota, a mature endocrine organ, is crucial for estrogen metabolism, with evidence suggesting estrogen levels influence gut microbiome homeostasis.

The Estrobolome is a vital aspect of the gut microbiome.

Definition and Function

- **Definition**: The estrobolome is a group of enteric bacterial genes responsible for estrogen metabolism, which can significantly impact estrogen levels in the body.
- **Function**: The GUS gene of estrobolome encodes Gut microbial **β-glucuronidase (gmGUS)** which converts conjugated estrogens back to their free forms, facilitating their reabsorption into the bloodstream, a process part of the enterohepatic circulation. **(1)**
- **The bidirectional interaction** Estrogen levels can influence gut microbiota composition and diversity, while gut microbes, through enzymes like gmGUS, can modulate systemic estrogen levels. (**1**)

Estrogen Metabolism

- **Production**: Estrogens are produced in the ovaries, adrenal glands, and adipose tissue.
- **Metabolism**: After being metabolized in the liver, estrogens are excreted into bile, which enters the intestine. reabsorbed in the intestines, and returned to the liver

- **Influence of Estrobolome**: The estrobolome can determine whether estrogens are reabsorbed into the bloodstream or excreted, it is an important mediator in remaining body estrogen levels impacting overall hormonal balance.

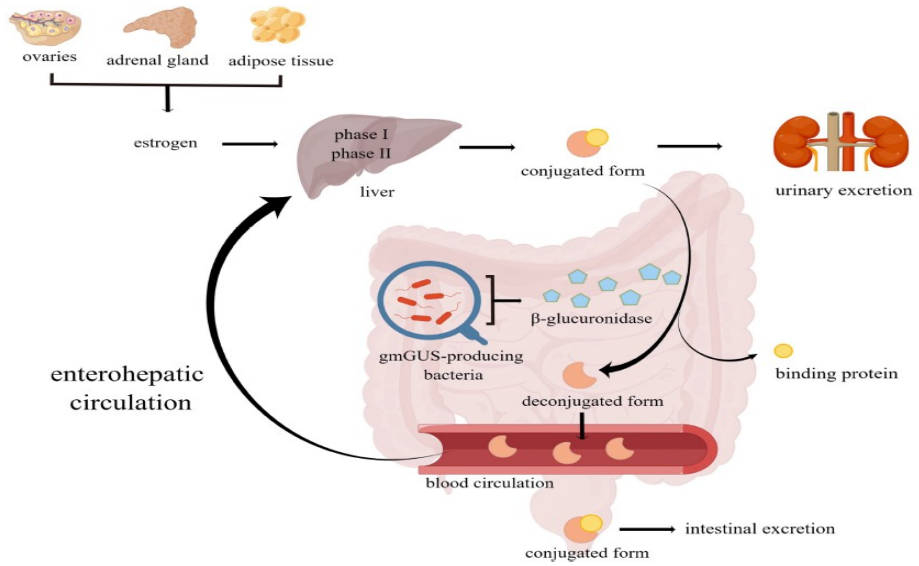


Figure 1. Estrogen produced by the ovaries, adrenal glands, and adipose tissue is metabolized in the liver within two phases to form a biologically inactive conjugated form, and the conjugated estrogen is deconjugated by β-glucuronidase encoded by gut microbiota and changes to a deconjugated form with biological activity, some of which is excreted in the stool and urine, while some of which is reabsorbed into the blood circulation and returned to the liver and this process is called enterohepatic recycling of estrogen.

The Human Microbiome Project GI database has identified 279 GUS genes, with 93.5% taxonomically classified as Bacteroidetes (52%), Firmicutes (43%), Verrucomicrobia (1.5%), and Proteobacteria (0.5%) based on structural characteristics and biocatalytic properties.

Effect on Health:

Estrogen-gut microbiome interactions play a significant role in various physiological and clinical outcomes.

The gut microbiome regulates estrogen levels, inflammatory response, and carbohydrate metabolism. Dysbiosis can lead to Dysregulation of **gmGUS** activity can lead to altered estrogen metabolism, which is associated with various estrogen-related diseases. (**2**)

- **Mechanisms of Influence: (3)**

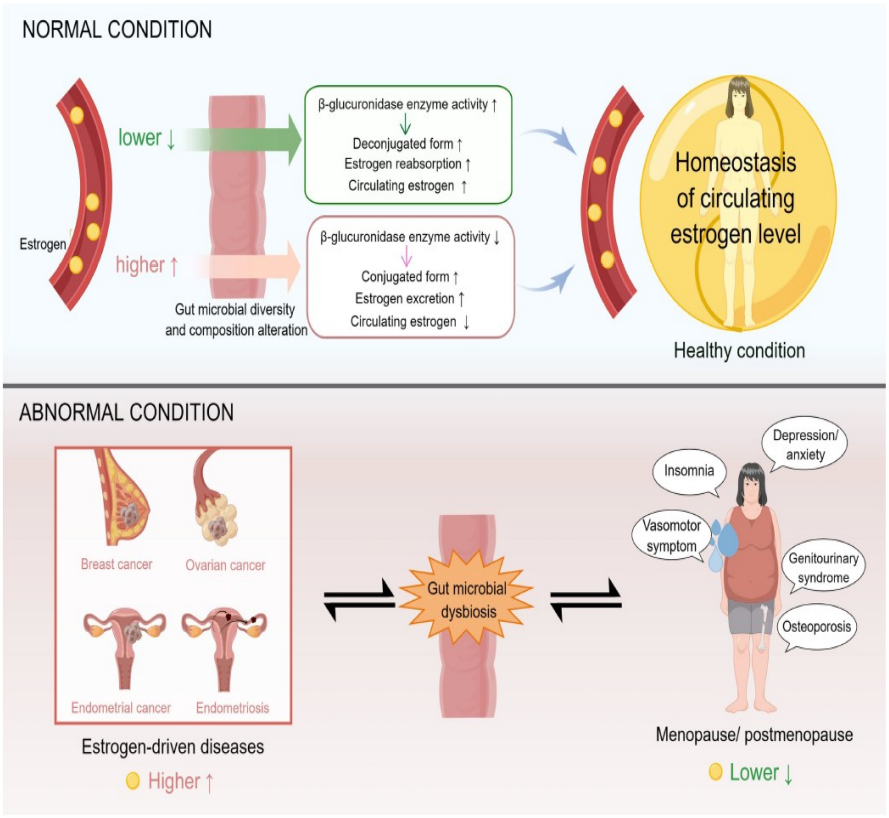


Figure 2. Higher systemic estrogens affect gut microbiome diversity and composition, thereby reducing β-glucuronidase availability and increasing estrogen excretion; when estrogen levels reduce, it also reshapes the gut microbiome, thereby increasing β-glucuronidase activity, promoting estrogen reabsorption and maintaining systemic homeostasis of estrogen levels. Thus, abnormalities in either of them may break this homeostasis and cause the occurrence of estrogen-driven diseases.

1- **Leaky gut** increase gut permeability, and lead to systemic inflammation, which disrupt estrogen balance, impacting diseases like ovarian cancer.

2- **Gut Microbiota-Estrogen Axis: Dysbiosis**, a decrease in gut microbiota diversity, negatively impacts the estrobolome. This leads to decreased β-glucuronidase activity, affecting estrogen receptor activation. Lower estrogen levels can lead to hypoestrogenic pathologies like obesity. Conversely, increased β-glucuronidase-producing bacteria can cause hyperestrogenic conditions, leading to elevated estrogen levels associated with certain diseases and cancers. (**2**)

3- **Metabolite Pathway**: Gut microbiota produces metabolites such as short-chain fatty acids (SCFA) and bile acids that impact inflammation,

metabolism, and disease progression.

- **Pathological Implications: (2)**
- **Hormonal Influence:** Gut microbiota alterations in hyperestrogenic pathologies like: polycystic ovarian syndrome endometriosis, breast cancer, cervical cancer, and ovarian cancer of the human studies **(3)**

1. **Hyper estrogenic Pathologies:**

- **Endometriosis**: High estrogen levels promote the proliferation of endometrial tissue outside the uterus, leading to pelvic pain and infertility. Dysbiosis may exacerbate this condition by increasing β-glucuronidase-producing bacteria.

- **Cancers**: Increased estrogen levels driven by gut microbiota can also contribute to the development of estrogen-dependent cancers

- **Premenstrual Syndrome (PMS):** fluctuations in estrogen and progesterone, symptoms can be affected by gut health which can change neurotransmitter levels in the brain, leading to mood-related symptoms.

Hyper-estrogenic Disorder	Increased Gut Microbiota	Decreased Gut Microbiota
Polycystic Ovarian Syndrome (PCOS)	Lachnoclostridium, Fusobacterium, Coprococcus_2, Tyzzerela 4, Bacteroides, Escherichia/Shigella, Streptococcus	Tenericutes, Firmicutes/Bacteroides ratio, Akkermansia, Ruminococcaceae
Endometriosis	Bacteroides, Parabacteroides, Oscillospira, Coprococcus	Paraprevotella, Lachnospira, Clostridia, Clostridiales, Lachnospiraceae, Ruminococcus, Ruminococcaceae
Breast Cancer	Firmicutes, Clostridium cluster IV, cluster XIVa	Bacteroidetes, Bacteroidetes phylum, Verrucomicrobia phylum
Cervical Cancer	Prevotella, Porphyromonas, Dialister, Succinivibrio, Ruminococcus, Morganella, Shewanella, Proteus	Bacteroides, Alistipes, Lachnospiracea, Phascolarctobacterium, Halomonas
Ovarian Cancer	Proteobacteria	Actinobacteria, Bifidobacterium, Coprococcus

2. **Hypoestrogenic Pathologies:**

- **Polycystic Ovary Syndrome (PCOS):** characterized by elevated levels of androgens and lower estrogen levels, which can impact overall health and metabolic function.

- **Obesity and Metabolic Syndrome:** Low circulating estrogen levels are prevalent in postmenopausal women, contributing to obesity and metabolic syndrome.
- **CVD and Cognitive Decline:** Altered estrogen receptor activation due to reduced circulating estrogens may lead to cardiovascular issues and cognitive impairments**.**

- **Menopause and influence of microbiota**:

1- **<u>Diversity Changes:</u>**

- A systematic review and meta-analysis confirm that gut microbiota shifts occur before and after menopause.
- Postmenopausal women show a significant decrease in both alpha and beta gut microbiota diversity compared to premenopausal women, with **90** differentially abundant taxa identified. (**4**)

2- **<u>Dominant Bacterial Taxa:</u>**

- The genus **Roseburia** has been highlighted as a particularly significant bacterial taxon that differentiates premenopausal women from postmenopausal women.
- Postmenopausal women exhibit lower levels of **Roseburia** and **Firmicutes** and higher levels of the phylum **Bacteroidetes**. (**4**)

3- **<u>Dysbiosis in Gut Microbiota:</u>**

- There is often a decrease in beneficial bacteria such as **Bifidobacterium** and **Lactobacillus**.
- Concurrently, there is an increase in potentially harmful species like **Klebsiella** and **Clostridiodes difficile**. (1)
- **This dysbiosis is linked to various menopausal symptoms, including:**
    - Vasomotor symptoms
    - Genitourinary syndrome
    - Insomnia Emotional disturbances such as depression and anxiety (1).

How to analyse it? (2)

When analyzing the estrobolome, consider employing the following methodologies:

- **Metagenomic Sequencing:** Techniques such as 16S rRNA gene sequencing can identify and quantify microbial populations involved in estrogen metabolism.
- **Enzymatic Activity Assays:** To measure GUS activity and correlate it with estrogen levels. Specific bacterial genera like **Bacteroides**, **Escherichia**, and **Lactobacillus** contain genes encoding GUS.
- **Metabolomic Profiling:** Analyzing metabolites produced by gut microbiota SCFA and bile acid levels in relation to hormonal status.

Why to analyse is?

- Understanding the estrobolome can inform therapeutic strategies for conditions influenced by estrogen levels, such as endometriosis, metabolic syndrome, and certain cancers. Manipulating gut microbiota through dietary interventions or probiotics may enhance estrogen metabolism and improve health outcomes.

Strategies for Improving Gut Health and Hormonal Balance:

Achieving optimal gut health and hormonal balance, particularly concerning estrogen metabolism, involves several effective strategies that positively influence the gut microbiome. Here are key approaches to consider: **(1)(2) (3)**

1. Dietary Changes

- **Balanced Diet:** A diet rich in whole foods while minimizing processed foods can support gut health and hormonal balance. This includes adequate intake of omega-3 fatty acids, antioxidants, and vitamins.
- **Prebiotics:** Fiber-Rich Foods: Incorporating a variety of fruits, vegetables, whole grains, and legumes enhances gut microbiome diversity, which is essential for maintaining a healthy estrobolome—the gut microbial gene pool responsible for metabolizing estrogens.
- **Probiotics:** Fermented Foods: Foods such as yogurt, kefir, sauerkraut, and kimchi are rich in probiotics that help restore and maintain gut flora balance, promoting better estrogen metabolism.

2. Probiotics and Prebiotics Supplements

- **Probiotics** containing strains like **Lactobacillus** and **Bifidobacterium** may enhance estrogen metabolism.
- **Prebiotic** supplements, such as inulin, can support gut health and balance estrogen levels.

3. Lifestyle Modifications:

- **Regular Physical Activity:** Engaging in regular exercise positively influences gut microbiome composition and enhances overall metabolic health, contributing to hormonal balance.
- **Manage Stress Level:** Chronic stress negatively impacts gut health. Techniques such as deep sleep, mindfulness, yoga, and meditation can help manage stress and promote a healthier gut environment.

- **Medications:** Over-the-counter pain relievers, hormonal contraceptives, or antidepressants e.g. SSRIs or Gabapentin may be prescribed depending on the severity of symptoms.

4. Avoiding Antibiotics Unless Necessary: Overuse of antibiotics can disrupt gut microbiota diversity. It is crucial to use them with caution to maintain a balanced microbiome**. (3)**

Effects of Antibiotics	Antibiotics can disrupt the diversity of gut microbiota, potentially leading to a decrease in beneficial bacteria that produce GUS. This disruption can damage the metabolism of estrogens, exacerbating conditions related to hormonal imbalance.
Bacterial Genera	Certain bacterial genera like Bacteroides and Lactobacillus are known to contain genes encoding GUS. A **decrease** in these populations due to antibiotic treatment may result in lower GUS activity and altered estrogen metabolism.
Clinical Implications	managing gut health through probiotics or prebiotics might help mitigate some adverse effects of antibiotics on hormonal balance.

5. Avoiding OTC: Over-the-counter pain relievers (Ibuprofen, acetaminophen), Some studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) can increase intestinal permeability, which may affect gmGUS activity.

6. Minimize Toxins: (3)

- The presence of environmental toxins and plastics in the diet can further exacerbate dysbiosis and negatively impact the gut microbiome's ability to metabolize estrogens. This may lead to increased risks for hormone-related diseases such as breast and cervical cancer
- Reduce exposure to endocrine-disrupting chemicals found in plastics and household products.

- Choose organic foods, when possible, to limit pesticide exposure.

7. Hormonal Replacement Therapies: In some cases, especially menopause or conditions like Premature Ovarian Insufficiency (POI), hormonal therapies, such as estrogen, may be used locally or systemically to effectively balance hormone levels and ease the symptoms. (**4**)

- **HRT is commonly used for menopausal symptoms but has lower clinical use due to cancer risks.**
- **Gut microbial enzyme activity can influence individual responses to HRT.**
- **Combining probiotics with HRT may increase the effectiveness of bioactive estrogens and reduce cancer risks.**

8. Alternative Therapies:

- **New Treatment Method**: The gmGUS inhibitor is a new way to manage diseases and medication therapy.
- **Study on GUS Enzymes**: Research examined GUS enzymes in the gut of healthy women and breast cancer patients, finding that adjusting probiotics could lower breast cancer risk by inhibiting certain proteins related to estrogen.
- **Effective Inhibitors**:
    - **D-glucaro-1,4-lactone (1,4-GL)**: This inhibitor may help fight cancer by affecting hormone production.
    - **TCH-3511**: A specific gmGUS inhibitor that can prevent gut bacteria imbalances caused by cancer-causing substances.
- **Structural Modifications**: Changes to the structure of gmGUS can help inhibit its activity, which may prevent estrogen reactivation.
- **Improving Cancer Treatment**: Inhibiting β-glucuronidase may help reduce side effects of anticancer drugs, like irinotecan, which is used for various cancers but can cause gastrointestinal (GI) toxicity.

- **Gut Health and Cancer**: Inhibiting gmGUS can help maintain a healthy gut microbiome and reduce the side effects of cancer treatments, potentially improving outcomes for patients.
- **Potential New Therapy**: Combining gmGUS inhibitors with anticancer drugs like irinotecan could reduce intestinal toxicity and enhance anti-tumor effects.
- **Role of Probiotics and Prebiotics**: These can lower the risk of estrogen-related cancers by reducing β-glucuronidase activity in the intestines. For example, substances like lactulose and inulin can significantly decrease this enzyme's activity.
- **Natural Inhibitors**: Certain natural compounds, like flavonoids found in Mulberry bark, may effectively inhibit β-glucuronidase activity and could be used as dietary supplements to reduce gut toxicity.
- **Future Research**: Understanding how gmGUS activity is regulated could provide insights into how gut bacteria influence estrogen reactivation and cancer risk.

9. Fecal Microbiota Transplantation (FMT): has shown promise in restoring gut microbiota balance, especially in cases of dysbiosis linked to gynecological diseases. Future research may focus on "matching" donors and recipients to enhance treatment efficacy. (**3**)

Take home Message

the **Estrobolome** have a key role in the relationship between gut health and hormonal balance, particularly concerning estrogen metabolism emphasizing the importance of maintaining a diverse and functional gut microbiome.

By prioritizing gut health through targeted dietary and lifestyle changes, individuals can proactively enhance their hormonal well-being and potentially improve conditions associated with estrogen imbalance.

Regular check-ups on hormonal levels and consultation with healthcare professionals recommended for early identification of imbalances, treatment of

dysbiosis affecting gut health, and proper intervention.

Summary

- **Estrobolome Significance**: The estrobolome, a collection of gut microbial genes, plays a critical role in estrogen metabolism, influencing systemic estrogen levels and overall hormonal balance.
- **Gut Microbiota Interaction**: There is a bidirectional relationship between estrogen levels and gut microbiota composition, where dysbiosis can lead to altered estrogen metabolism and contribute to various estrogen-related diseases.
- **Health Implications**: Dysregulation of the estrobolome is linked to conditions such as endometriosis, obesity, metabolic syndrome, and certain cancers, highlighting the importance of gut health in managing these issues.
- **Strategies for Improvement**: Dietary changes (including prebiotics and probiotics), regular physical activity, stress management, and avoiding unnecessary antibiotics..
- **Future Research Directions**: Continued investigation into specific microbial taxa, dietary influences on the estrobolome, and therapeutic interventions like fecal microbiota transplantation may provide new insights into managing estrogen-related health conditions.

References:

1- Hu, S., Ding, Q., Zhang, W., Kang, M., Ma, J. and Zhao, L., 2023. Gut microbial beta-glucuronidase: a vital regulator in female estrogen metabolism. *Gut Microbes*, *15*(1), p.2236749.

2- Baker, J.M., Al-Nakkash, L. and Herbst-Kralovetz, M.M., 2017. Estrogen–gut microbiome axis: Physiological and clinical implications. *Maturitas*, *103*, pp.45-53.

3- Wang, M.Y., Sang, L.X. and Sun, S.Y., 2024. Gut microbiota and female health. *World Journal of Gastroenterology*, *30*(12), p.1655.

4- Huang, F., Cao, Y., Liang, J., Tang, R., Wu, S., Zhang, P. and Chen, R., 2024. The influence of the gut microbiome on ovarian aging. *Gut microbes*, *16*(1), p.2295394.

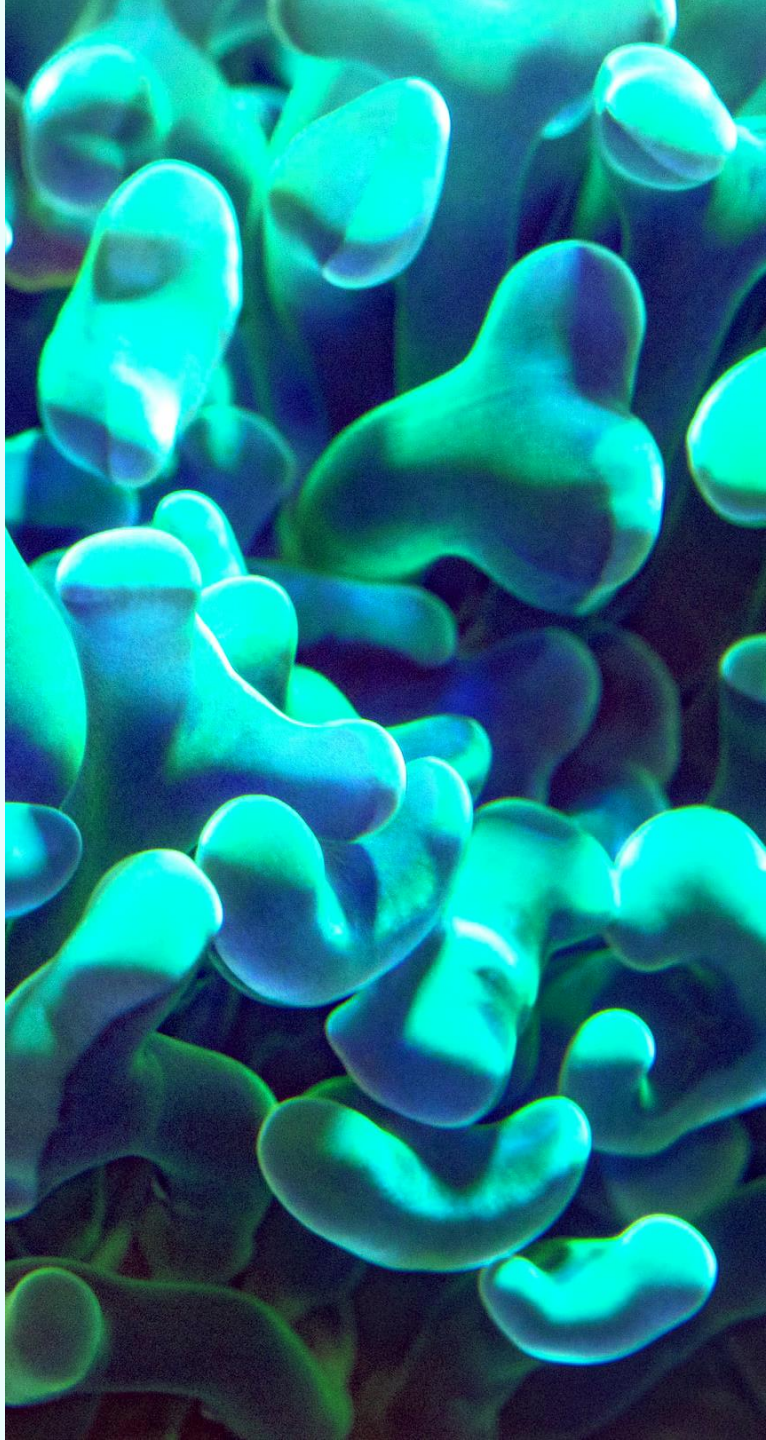
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Post-graduate certificate level 7 (30 ECTS)

Nutrition-Micorbiome Interaction in IBS

Eman Salah Zaky
MSc., MBBCH., RD
Clinical Nutrition and Health Consultant

Malta Further & Higher Education Authority



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Content and Learning Objectives

To have a clear understanding of :

- IBS management and First-line treatment
- Evidence supporting the effectiveness of a low-FODMAP diet
- Strategies to manage the gut-brain axis in IBS
- The link between IBS and the intestinal microbiome
- Dysbiosis as an etiology of IBS

Be aware of:

- Dietary changes alter the gut's microbiota and alleviate IBS symptoms.
- Evidence for using fibers, Pre-biotics and Probiotics in IBS
- Practical Considerations for Diet Changes

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

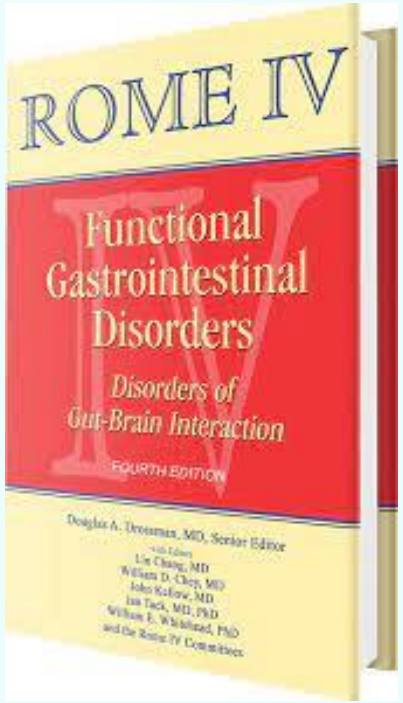
Irritable Bowel Syndrome (IBS)

IBS is a disorder of a Gut brain interaction

Worldwide Prevalence **3-5%**

Rome IV Criteria:
Recurrent abdominal pain at least 1 day per week, with at least 2 or more of the following

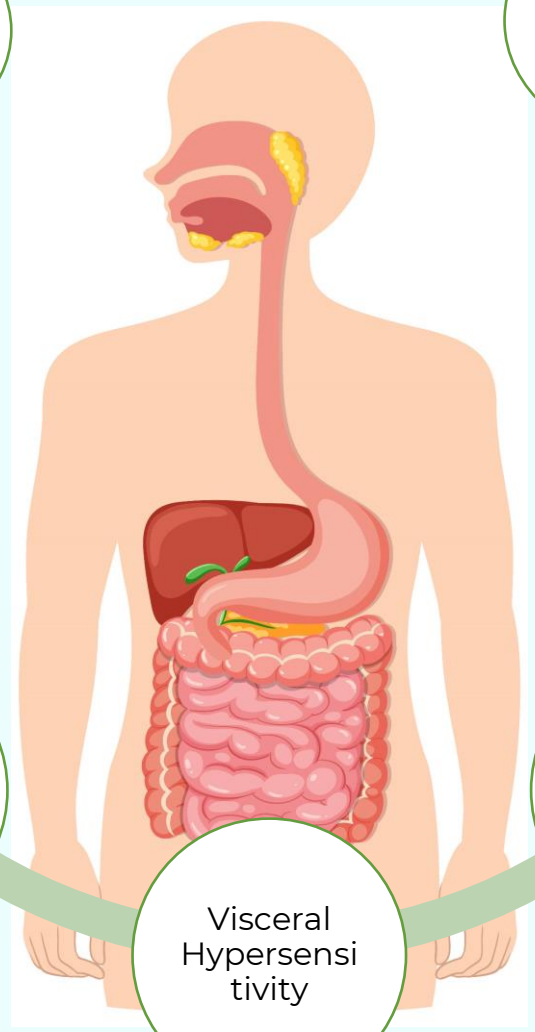
- Related to defecation
- Change ins tool frequency
- Change in stool consistency



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS)

Pathophysiology

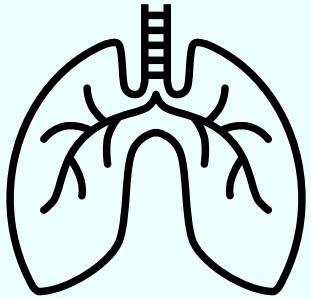


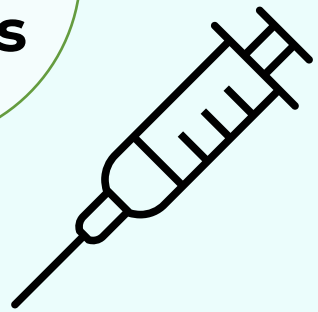
- Gut-Brain Interaction
- Altered Microbiome
- Increase Mucosal Permeability
- Altered Mucosal Immune Function
- Visceral Hypersensitivity
- Central Neuro-hormonal Dysregulation
- Enteric Neuroendocrine Abnormalities
- Diet

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Diagnosis

IBS Testing Options



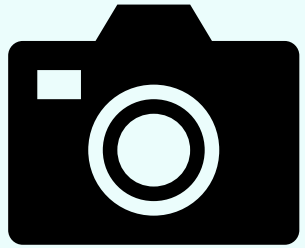


Breath Test
- Breath test for food intolerances or bacteria

Stool Test
- for bacteria, inflammation and blood

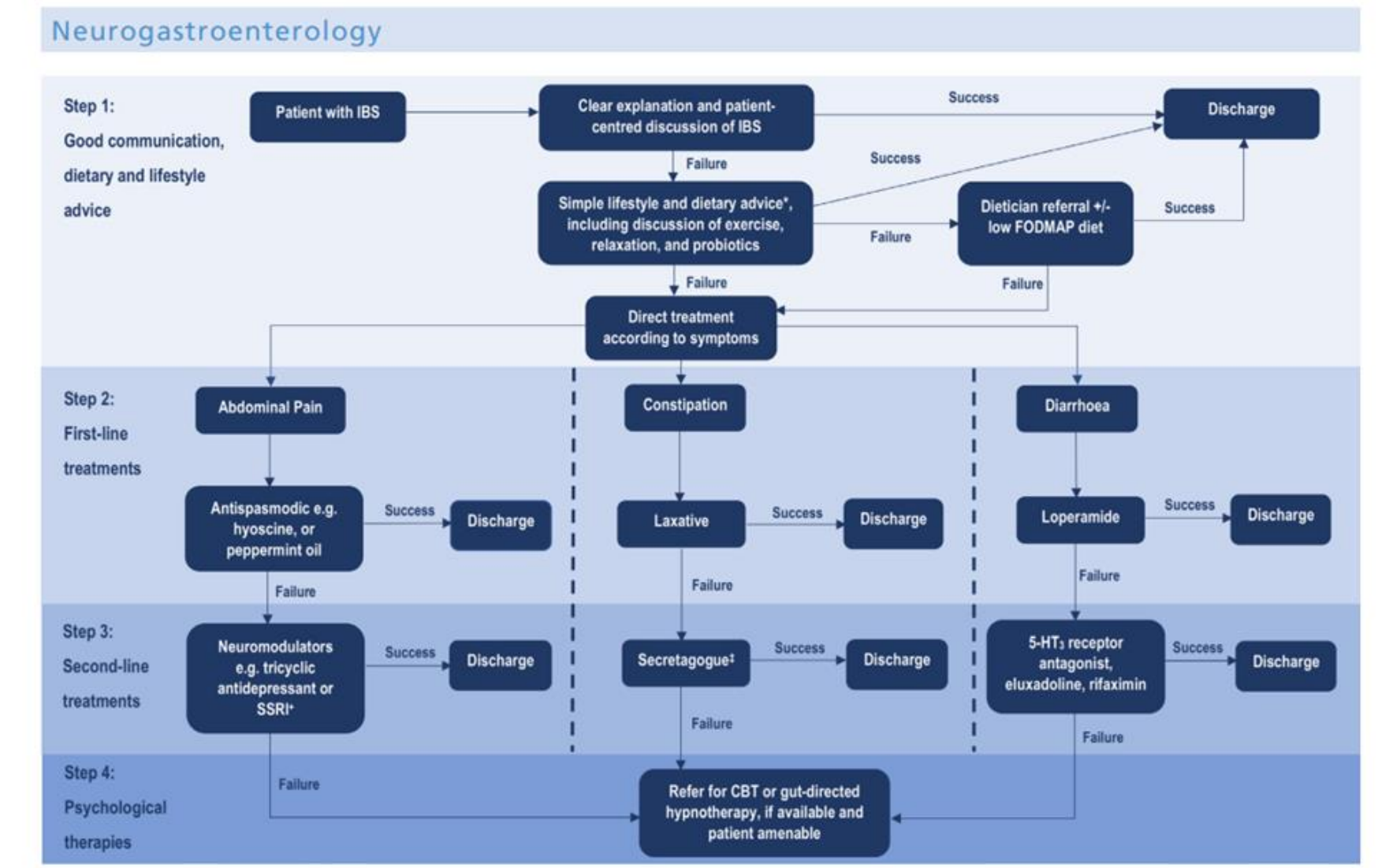
Blood Test
- IBS-smart for certain antibodies post infection or IBS or Celiac disease

Colonoscopy
- non-invasive camera to examine lower GIT



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Management



Black, C. J., & Ford, A. C. (2021). Best management of irritable bowel syndrome. *Frontline Gastroenterology*, *12*(4), 303-315

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Management

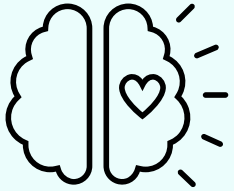
Lifestyle & Dietary Advice

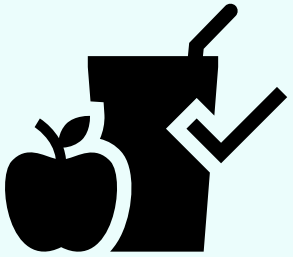
Yoga
- Studies show practicing meditation provides health benefits

Cognitive Behavioral Therapy
- 1/3 of people treated with CBT for IBS improve

Hypnosis
- May improve symptoms

Diet Changes
- 65% of IBS patients associate symptoms with food.





INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Dietary Management

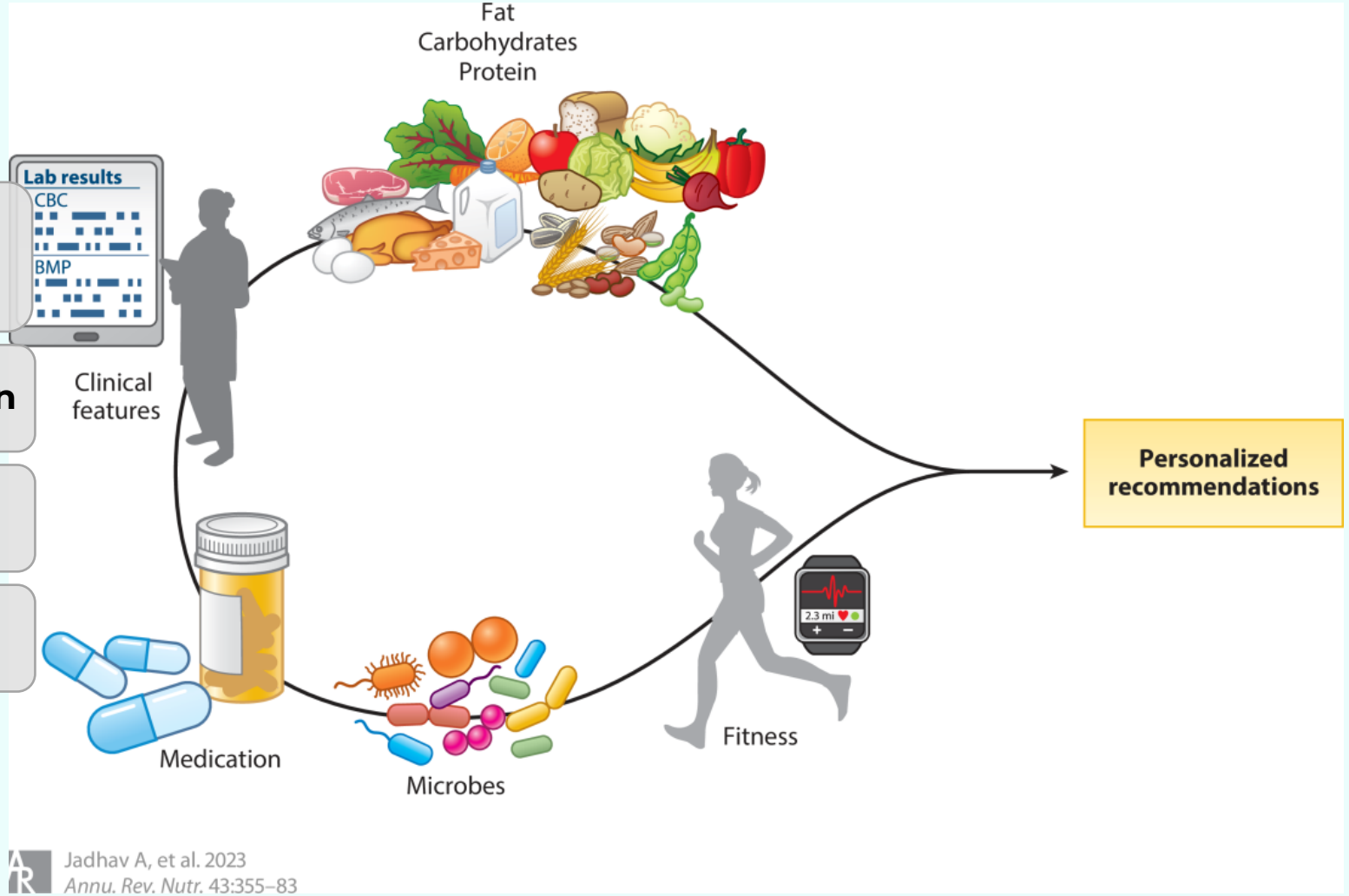
1st Line Dietary Advice:

1- Avoidance of dietary triggers
e.g. caffeine, alcohol, fatty foods, and spicy foods.

2- Milk and dairy restriction

3- Dietary fiber

4- Fluid intake



Jadhav A, et al. 2023
Annu. Rev. Nutr. 43:355–83

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Dietary Management

Excess Fructose
- Fruit: apple, mango, nashi, pear, canned fruit in natural juice, watermelon.
- Sweeteners: fructose, high fructose corn syrup, corn syrup, honey.
- Concentrated fructose: concentrated fruit, large servings of fruit, dried fruit, fruit juice.

Lactose
- Milk: milk from cows, goats, or sheep.
- Custard, ice cream
- Yogurt
- Cheese: soft, unripened cheeses like cottage, cream, mascarpone, ricotta

Fructans
- Asparagus
- Beetroot
- Broccoli
- Brussels sprouts
- Cabbage
- Eggplant
- Fennel
- Garlic
- Leek
- Okra
- Onion (all)
- Shallots
- Cereals: wheat and rye in large amounts (e.g. bread, crackers, cookies, couscous, pasta)
- Fruit: custard apple, persimmon, watermelon
- Misc: chicory, dandelion, inulin

Galactans
- Legumes: Beans, baked beans, chickpeas, kidney beans, lentils

Polyols
- Apple
- Apricot
- Avocado
- Blackberry
- Cherry
- Lychee
- Nashi
- Nectarine
- Peach
- Pear
- Plum
- Prune
- Watermelon
- Vegetables: Green bell pepper, mushroom, sweet corn
- Sweeteners: sorbitol (420), mannitol (421), isomalt (953), maltitol (965), xylitol (967)

Fruit
- Banana
- Blueberry
- Boysenberry
- Canteloupe
- Cranberry
- Durian
- Grape
- Grapefruit
- Honeydew melon
- Kiwi
- Lemon
- Lime
- Mandarin
- Orange
- Passionfruit
- Pawpaw
- Raspberry
- Rhubarb
- Rockmelon
- Star anise
- Strawberry
- Tangelo

Misc
- Sweeteners - sucrose, glucose, artificial sweeteners not ending in "-ol", and sugar in

Vegetables
- Alfalfa
- Artichoke
- Bamboo shoots
- Beat shoots
- Bok choy
- Carrot
- Celery
- Choko
- Choy sum
- Endive
- Ginger
- Green beans
- Lettuces
- Olives
- Parsnip
- Potato
- Pumpkin
- Red bell pepper
- Silver beet
- Spinach
- Summer squash (yellow)
- Swede
- Sweet potato
- Taro
- Tomato
- Turnip
- Yam
- Zucchini

Starch
- Gluten free bread or cereal products
- 100% spelt bread
- Rice
- Oats
- Polenta
- Other: arrowroot, millet, psyllium, quinoa, sorgum, tapioca

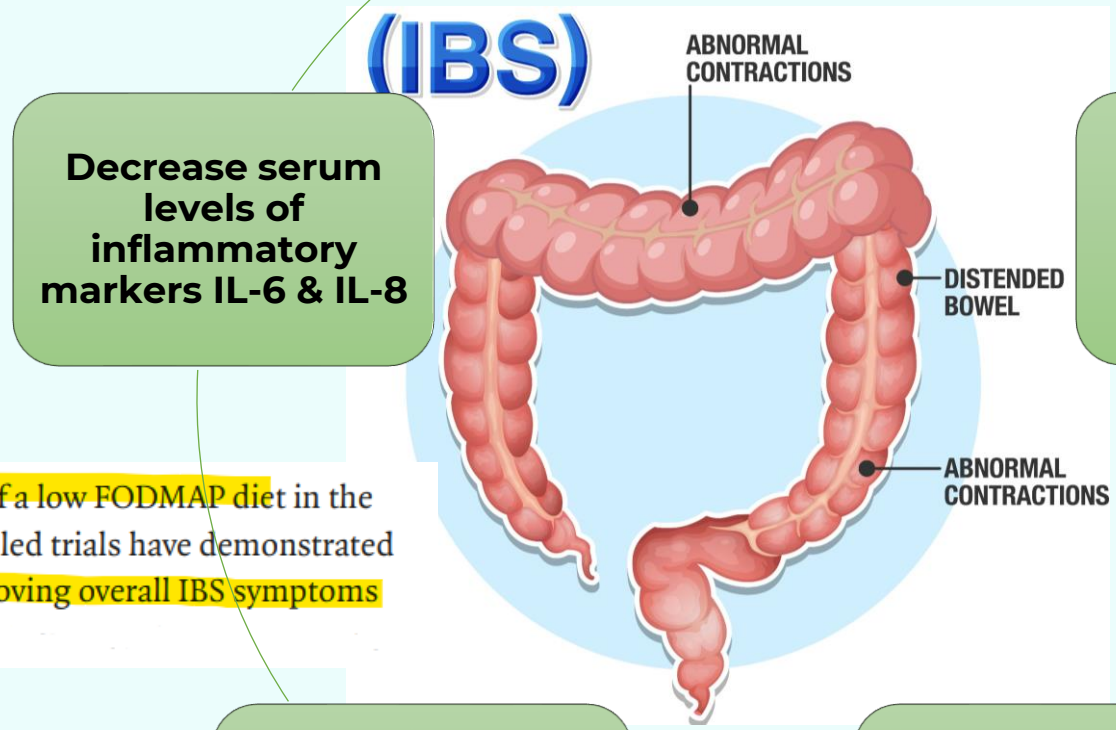
Dairy
- Milk - lactose-free milk, oat milk, rice milk, soy milk (check for additives)
- Cheeses - hard cheeses, brie, and camembert
- Yogurt (lactose free)
- Ice cream substitutes - gelati, sorbet
- Butter substitutes (e.g. olive oil)

2nd Line Dietary Advice:
Low FODMAPs Diet

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Dietary Management: Low FODMAPS Diet

There has been growing insight into the beneficial effects of a low FODMAP diet in the management of IBS symptoms. Several randomized controlled trials have demonstrated the efficacy of a reduced high FODMAP food intake in improving overall IBS symptoms such as visceral pain, bloating, and quality of life [79,83-89]

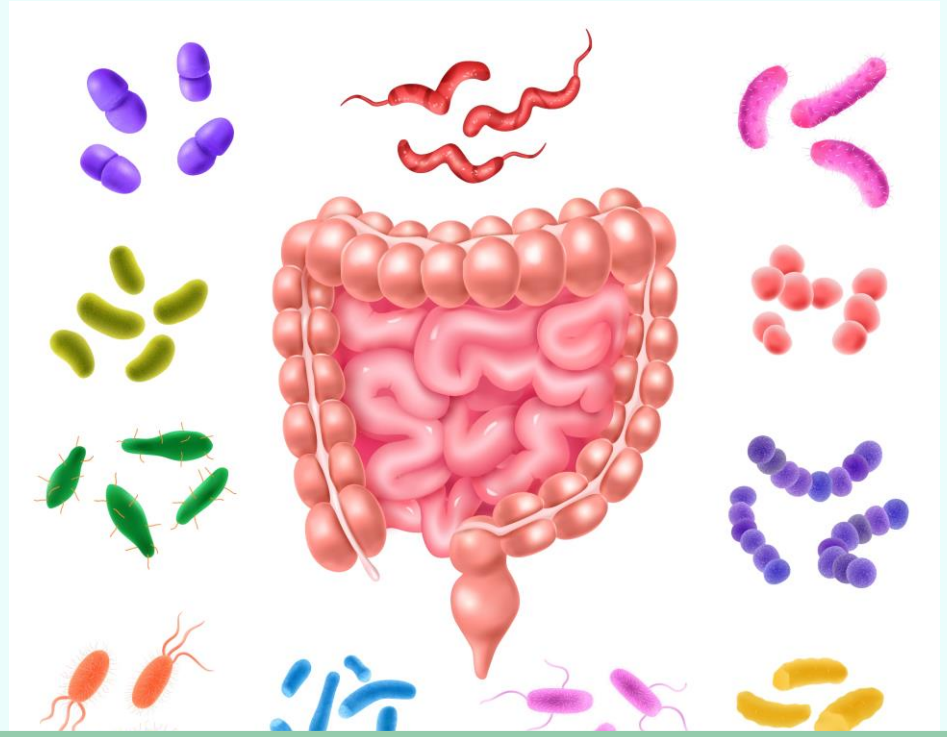


- Improve visceral pain
- Lower symptom severity
- Reduced bloating
- Improved quality of life
- Decrease serum levels of inflammatory markers IL-6 & IL-8

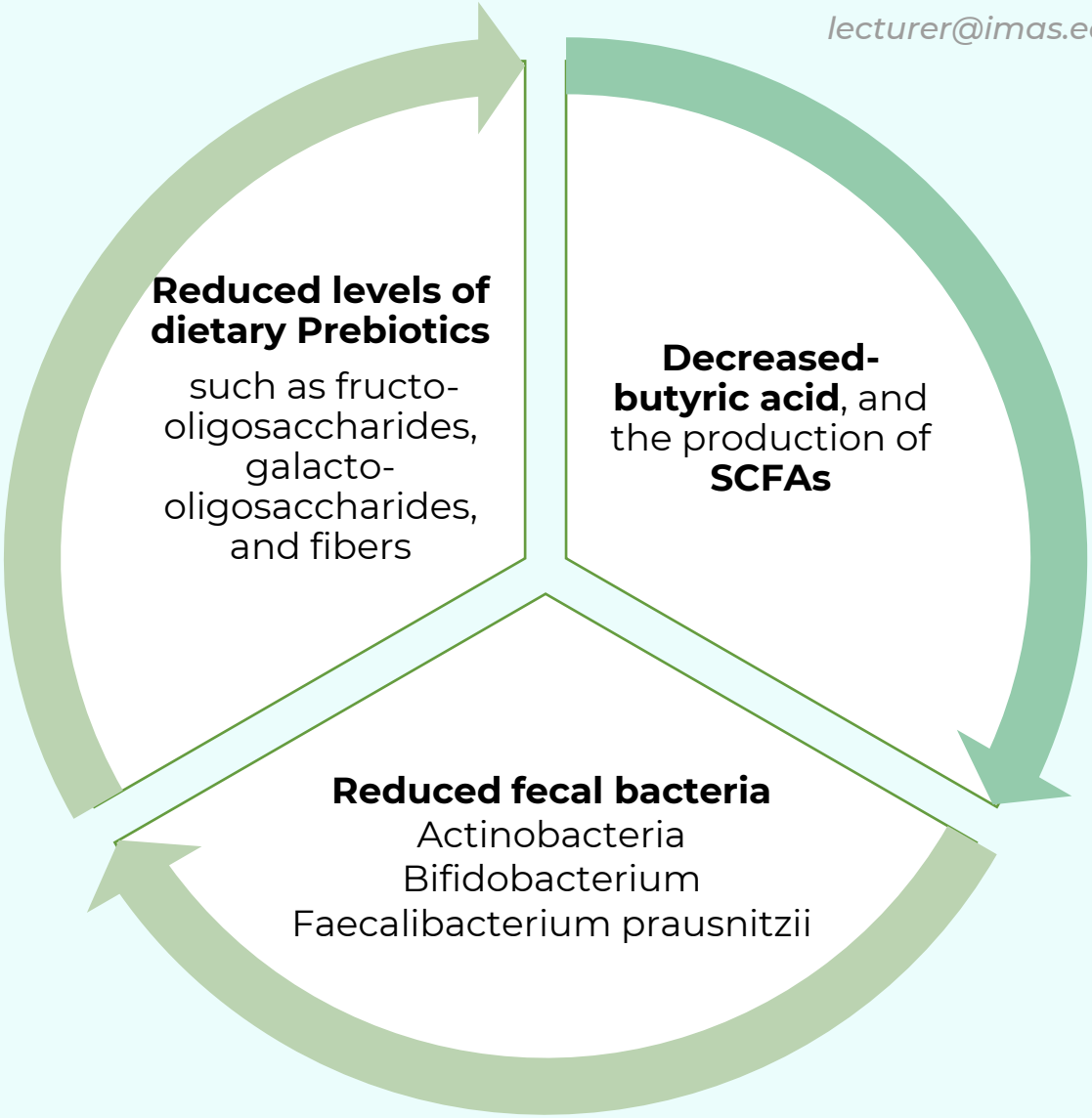
Kim, M. Y., & Choi, S. W. (2021). Dietary modulation of gut microbiota for the relief of irritable bowel syndrome. Nutrition Research and Practice, 15(4), 411.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Dietary Management: Low FODMAPS Diet



Low FODMAP Diet Can Alter Gut Microbiota Profile



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Irritable Bowel Syndrome Dietary Management: Low FODMAPS Diet

Clinical trials investigating the effect of the low FODMAP diet on the microbiota in IBS

Study design	Subjects	Dietary intervention	Intervention period (wk)	Method	Findings in microbiota	Ref.
RCT, double blind, crossover study	Adults with IBS (Rome III) (n = 20)	Low FODMAP vs. high-FOS diet	3	qPCR	Reduction in *Faecalibacterium prausnitzii*, *Actinobacteria*, and *Bifidobacterium* in low FODMAP diet vs. high-FOS diet	[90]
RCT, single blind, crossover study	Adults with IBS (Rome III) (n = 27) and healthy subjects (n = 6)	Habitual diet vs. low FODMAP or Australian diet (control)	3	qPCR	· Lower absolute abundance of total bacteria, bifidobacteria, *F. prausnitzii*, *Clostridium* Cluster IV in low FODMAP diet vs. habitual and Australian diet · Lower relative abundance of bifidobacteria in low FODMAP diet vs. habitual and Australian diet · Decreased abundance of *Clostridium* cluster XIVa in low FODMAP vs. habitual and Australian diet	[63]
RCT, unblind	Adults with IBS (Rome III) (n = 35)	Low FODMAP diet (n= 19) vs. habitual diet (n = 22)	4	FISH	Lower absolute and relative abundance of bifidobacteria in low FODMAP vs. habitual diet	[92]
RCT, single blind	Adults with IBS (Rome III) (n = 104)	Sham diet (n = 53) vs. low FODMAP diet (n = 51) and co-administration of multistrain probiotics · Sham diet/placebo (n = 27) · Sham diet/probiotic (n = 26) · Low FODMAP diet/placebo (n = 24) · Low FODMAP diet/probiotic (n = 27)	4	qPCR and 16S rRNA sequencing	· Lower abundance of *Bifidobacterium* species in low FODMAP vs. sham diet · No effect on microbial diversity in low FODMAP diet	[139]
RCT, single blind	Adults with IBS (Rome III) (n = 95)	Sham diet (n = 48) vs. low FODMAP diet (n = 47) and co-administration of a probiotic · Sham diet/placebo (n = 24) · Sham diet/probiotic (n = 24) · Low FODMAP diet/placebo (n = 21) · Low FODMAP diet/probiotic (n = 26)	4	16S rRNA gene sequencing	· Higher *Bacteroides* and lower *Bifidobacterium* in low FODMAP vs. sham diet · Higher *Lactobacillus* and *Streptococcus* abundance after probiotic supplementation vs. placebo	[140]
RCT, single blind	Adults with IBS (Rome III) (n = 40)	Low FODMAP diet (n = 19) vs. high FODMAP diet (n = 18)	3	16S rRNA gene sequencing	· Increased richness and diversity of *Acinetobacter* in low FODMAP diet in all types of IBS · Higher bacterial richness of *Actinobacteria*, *Firmicutes*, Clostridiales and increased diversity of *Acinetobacter* in low FODMAP diet in IBS-M and IBS-D · Decreased the relative abundance of bacteria involved in gas consumption in high FODMAP diet · Increased the family *Bifidobacteriaceae* and unclassified genera within the family *Lachnospiraceae* in high FODMAP diet	[82]
Uncontrolled study, unblind	Children with IBS (Rome III) (n = 8)	Low FODMAP diet	1	454 pyrosequencing	· No difference in richness or diversity after low FODMAP diet · Trend for higher Clostridiales and lower *Bacteroidetes* in low FODMAP vs. habitual	[94]

- Kim, M. Y., & Choi, S. W. (2021). Dietary modulation of gut microbiota for the relief of irritable bowel syndrome. Nutrition Research and Practice, 15(4), 411.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Dietary Management: Low FODMAPS Diet

The “**Low FODMAP** diet" is Not a “**No FODMAP diet**" or a “**Lifetime diet**.“

It's recommended to **follow for 6-8 weeks**, followed by a **Dietician's review** to advise on gradually **reintroducing** certain foods and amounts

This Photo by Unknown Author is licensed under CC BY-NC

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

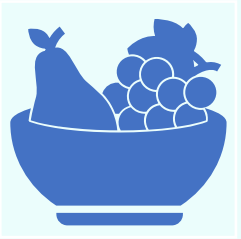
Irritable Bowel Syndrome (IBS): Summary of Dietary Management

Confirming IBS diagnosis after ruling out food intolerance, IBD and Coeliac disease :

1st line :
1- Healthy eating and lifestyle changes:
2- Milk and Dairy Restriction
3- Dietary fiber
4- Fluid intake

Advise➔ Review➔ No improvement➔ 2nd line

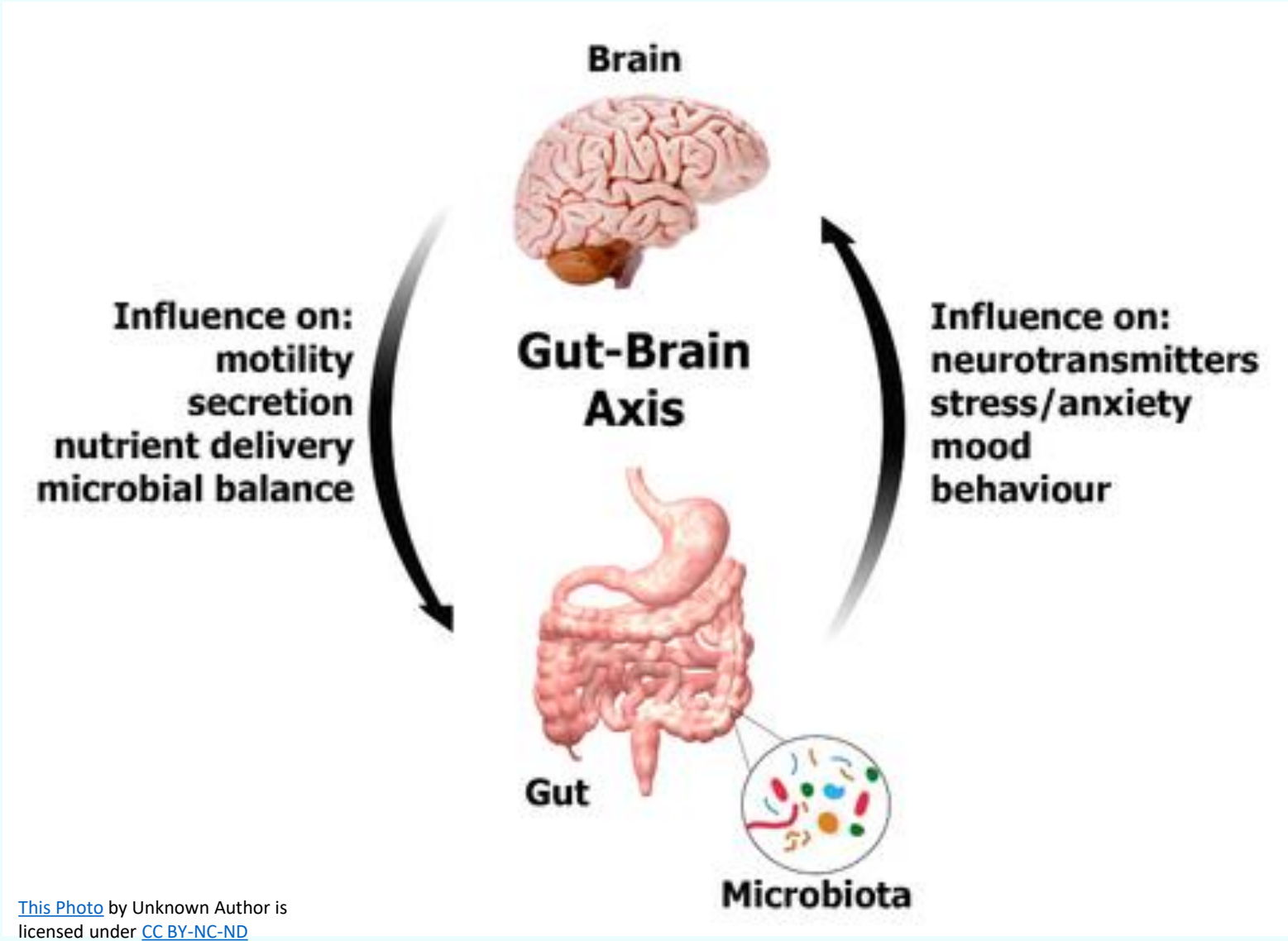
2nd line:
A food and symptom diary can help identify if **a low FODMAP Diet** is likely to be effective in symptomatic relief of IBS.



Low FODMAP diet unlikely to be effective unless first-line guidance is addressed concurrently

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Gut-Brain Axis

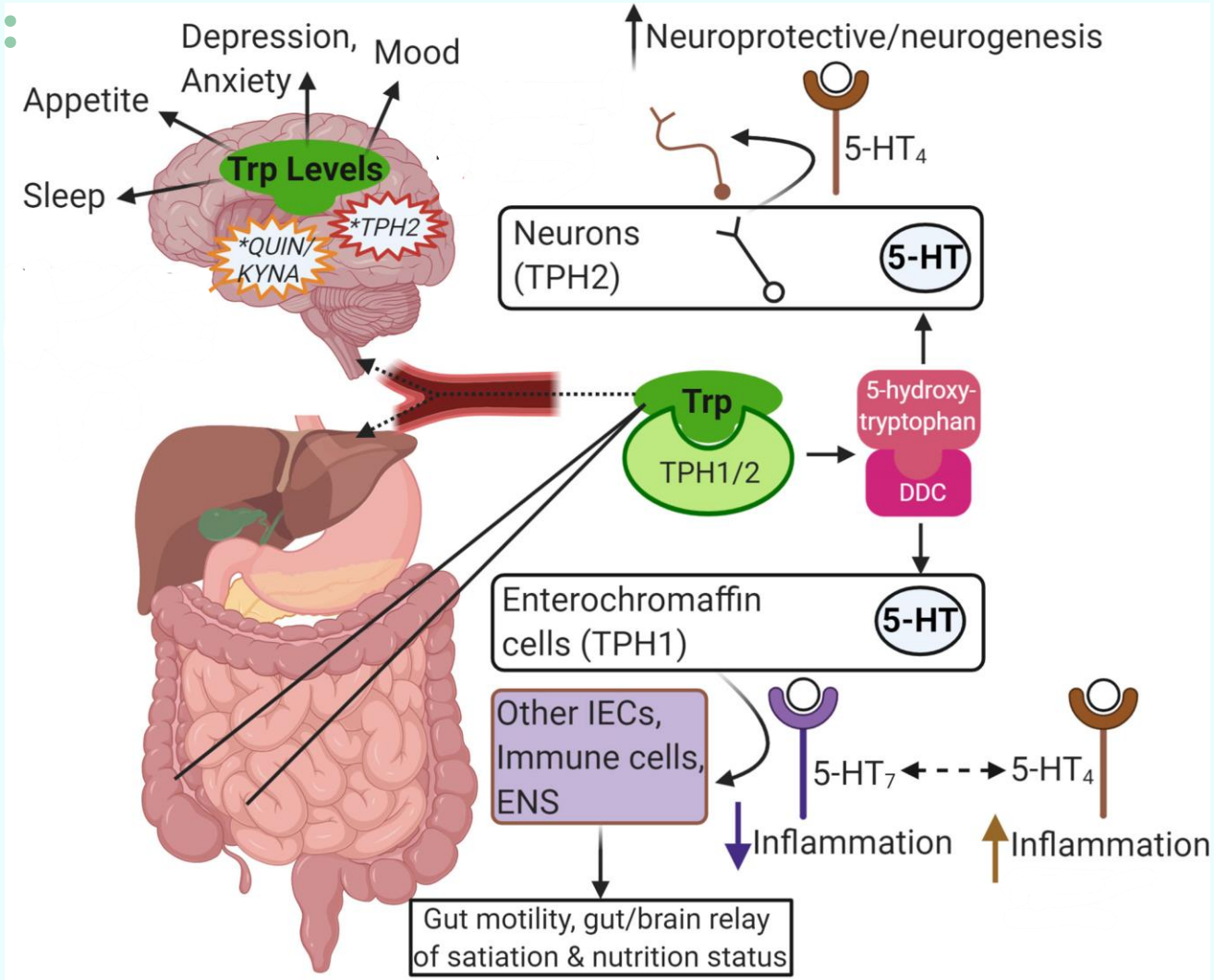


This Photo by Unknown Author is licensed under CC BY-NC-ND

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Gut-Brain Microbiota Axis

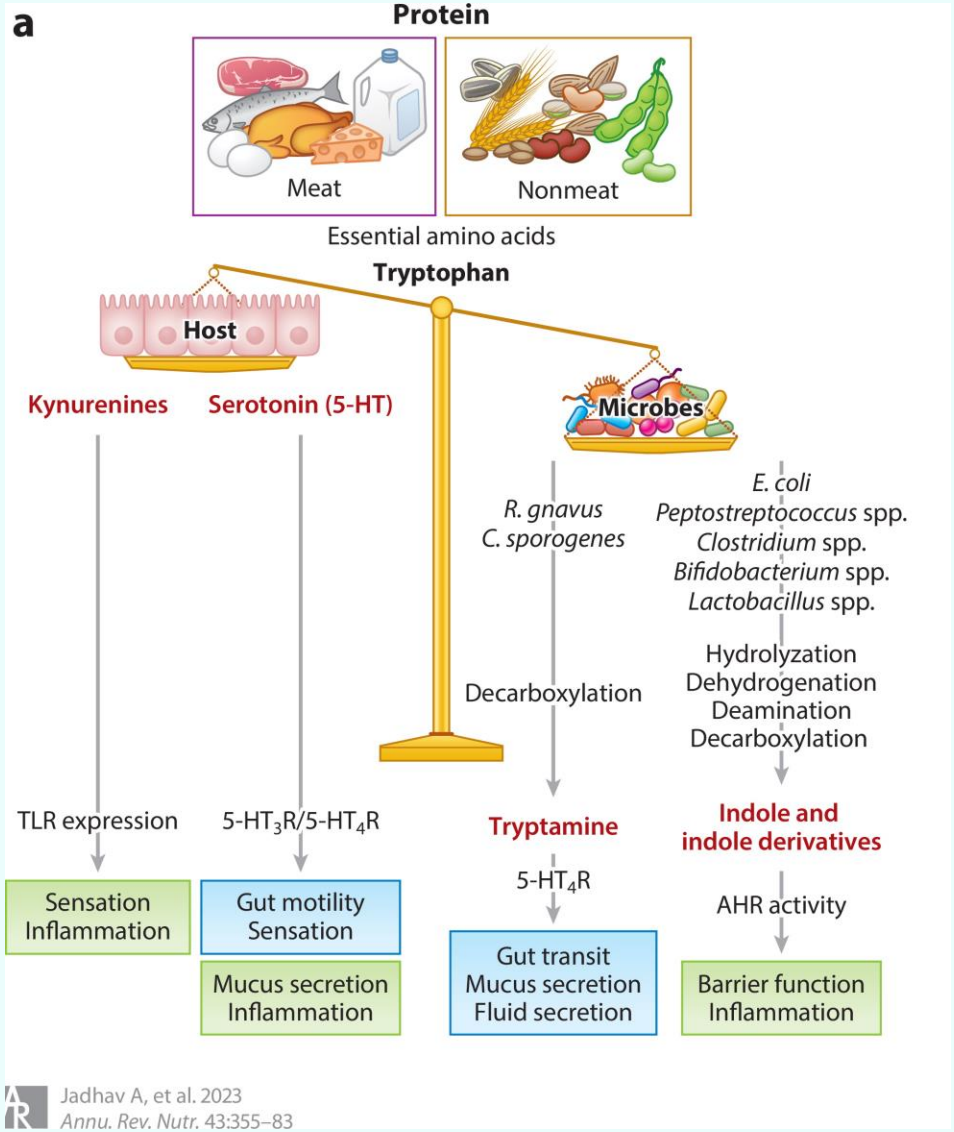
IBS symptoms may be influenced by
Impaired Tryptophan Metabolism



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Gut-Brain Microbiota Axis



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Dysbiosis

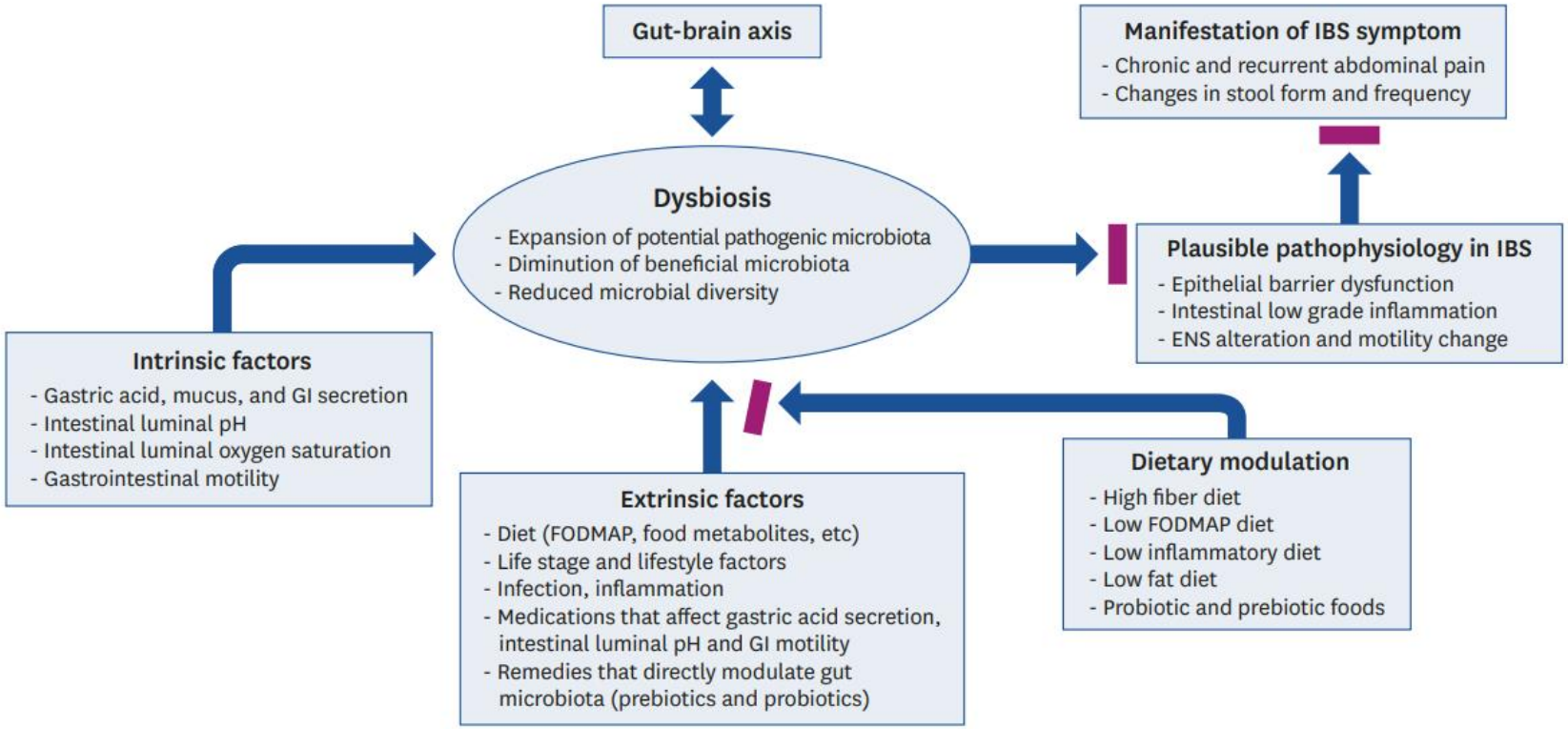


Fig. 1. Interrelation between IBS and dysbiosis of the intestinal microbiota. Many extrinsic and intrinsic factors can influence the dysbiosis of intestinal microbiota, which has been suggested as a novel pathophysiologic mechanism of IBS. A hypothesis is proposed that dietary modulation may improve IBS symptoms by mitigating the dysbiosis.
ENS, enteric nervous system; FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; GI, gastrointestinal; IBS, irritable bowel syndrome.

- Kim, M. Y., & Choi, S. W. (2021). Dietary modulation of gut microbiota for the relief of irritable bowel syndrome. Nutrition Research and Practice, 15(4), 411.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Dysbiosis

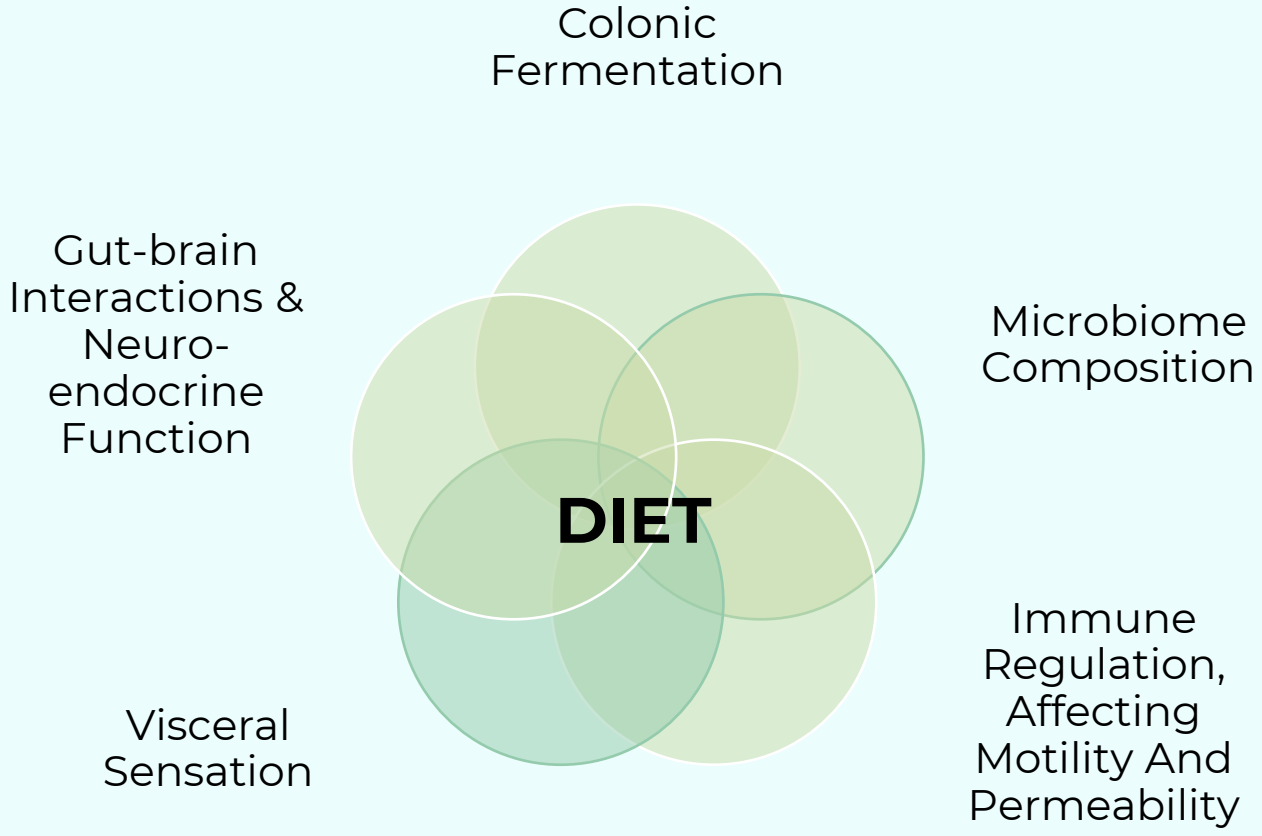
Higher Abundance
Increased
Bacteroidetes
Firmicutes
Proteobacteria

Lower Abundance
Decrease beneficial bacteria
lactobacilli
Bifidobacterium
Severe IBS
Prevotella
Methanobacteriales

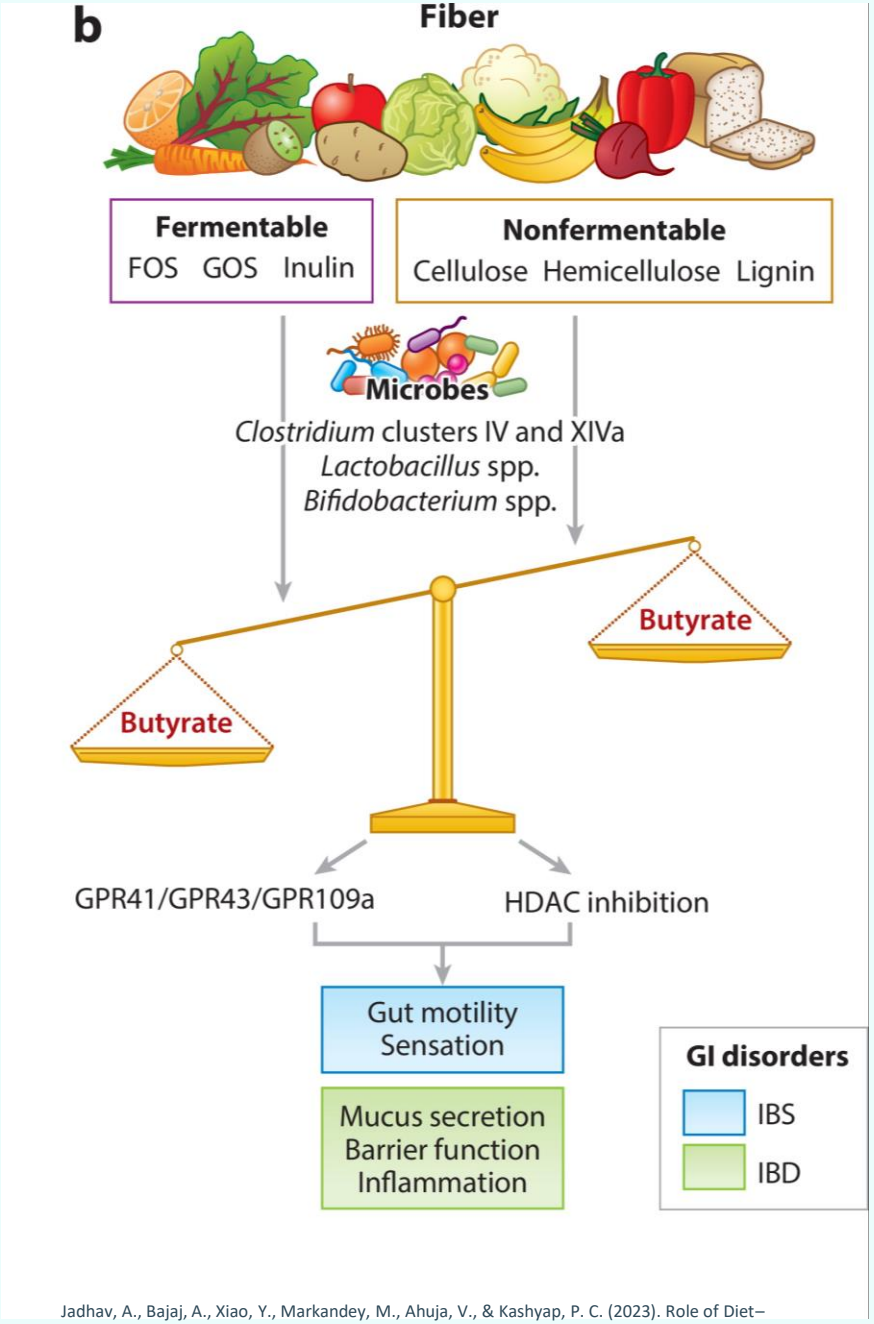
The Dysbiosis of Intestinal Microbiota in IBS

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Irritable Bowel Syndrome (IBS): Diet and Microbiota



Diet Pattern Significantly Impact The Gut Microenvironment



Jadhav, A., Bajaj, A., Xiao, Y., Markandey, M., Ahuja, V., & Kashyap, P. C. (2023). Role of Diet–Microbiome Interaction in Gastrointestinal Disorders and Strategies to Modulate Them with Microbiome-Targeted Therapies. Annual review of nutrition, 43, 355-383.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

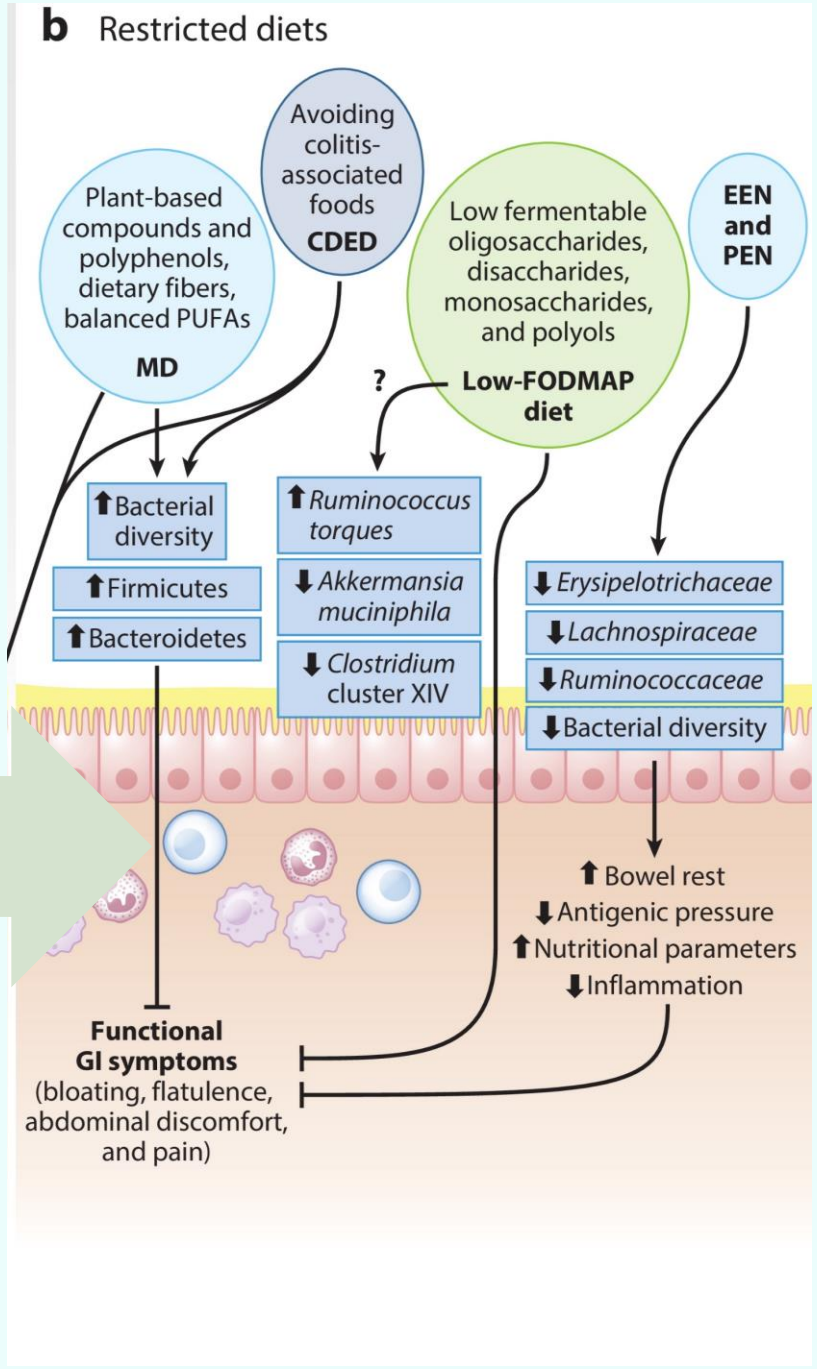
Irritable Bowel Syndrome (IBS): Diet and Microbiota

The study suggests that a cost-effective approach to manage IBS symptoms involves:

1- Reducing triggering foods, and a high-fibre diet

2- Modifying gut microbiota through regular meals

3- This can reduce colonic fermentation & antigen activation of the gut immune system



Jadhav, A., Bajaj, A., Xiao, Y., Markandey, M., Ahuja, V., & Kashyap, P. C. (2023). Role of Diet–Microbiome Interaction in Gastrointestinal Disorders and Strategies to Modulate Them with Microbiome-Targeted Therapies. Annual review of nutrition, 43, 355-383.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Diet and Microbiota

Diets	Microbiota changes	IBS symptoms
High fiber diet	Fibers are the main source of microbiota-accessible carbohydrates that increases the gut microbiota diversity and produces beneficial metabolites such as short-chain fatty acid.	Soluble fibers relive symptoms by softening the stool. High fiber diet has been a standard care particularly for IBS-C.
Low FODMAP diet	*Actinobacteria*, *Bifidobacterium*, and *Faecalibacterium prausnitzii* were decreased by low FODMAP diet.	Improves overall IBS symptoms such as visceral pain, bloating and quality of life.
Low inflammatory diet	*Bacteroidetes* phylum was more abundant in vegans and vegetarians.	Alleviates chronic low-grade mucosal inflammation, which helps enterocyte healing and nutritional support to the microbial environment.
Low fat diet	Increases bacterial diversity and beneficial butyrate-producing bacteria. Nuts and other plant-based fat increases the genera *Bifidobacterium*, *Roseburia*, and *Faecilibacterium*.	Beneficial effects on intestinal inflammation.

IBS-C, irritable bowel syndrome-constipation-predominant; FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols.

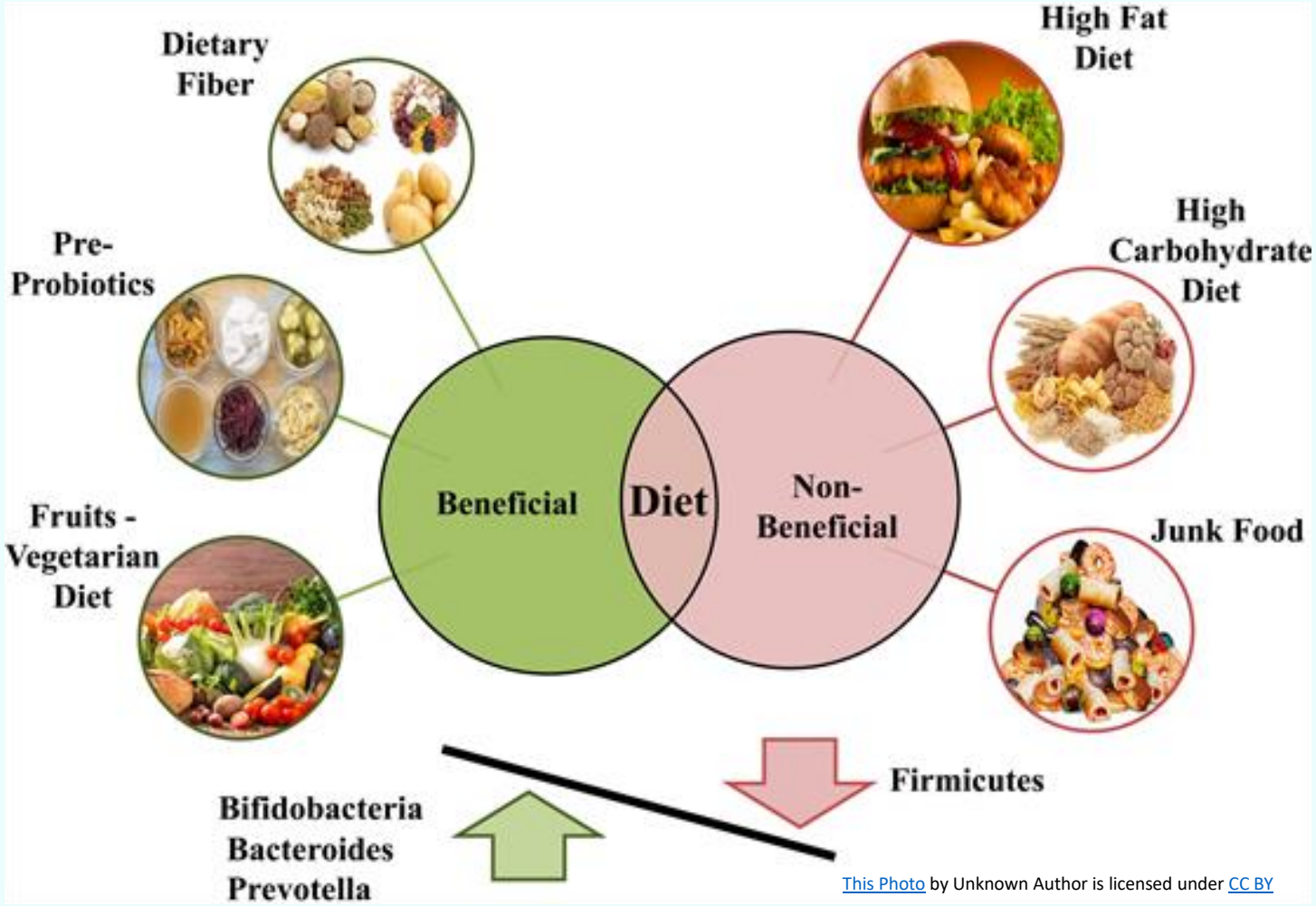
Dietary Modulation Of Gut Microbiota For IBS Symptom Relief

- Kim, M. Y., & Choi, S. W. (2021). Dietary modulation of gut microbiota for the relief of irritable bowel syndrome. Nutrition Research and Practice, 15(4), 411.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS):
Diet Supplementation

1- Dietary Fibers



This Photo by Unknown Author is licensed under CC BY

This Photo by Unknown Author is licensed under CC BY-NC

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Diet Supplementation

2- Prebiotics:

Food compound that feed microbiota

+

Probiotics:

Live micro-organisms

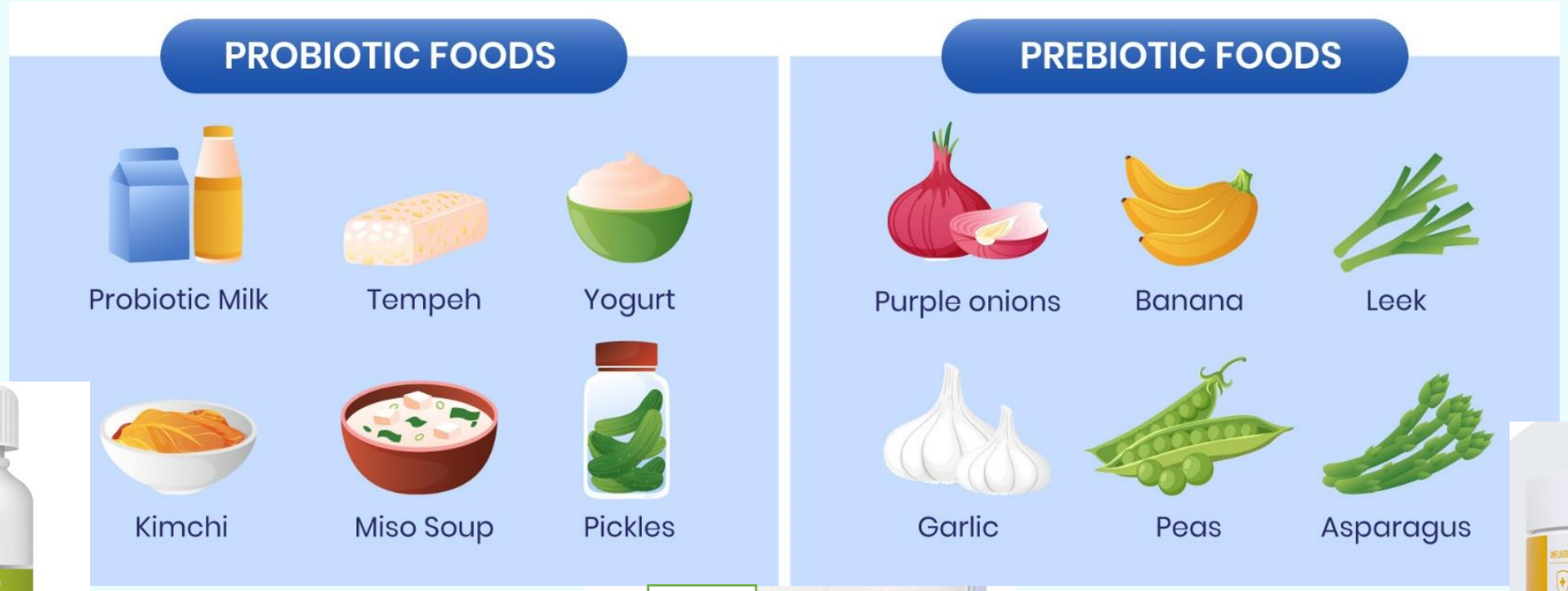
Can improve microbial homeo-stasis

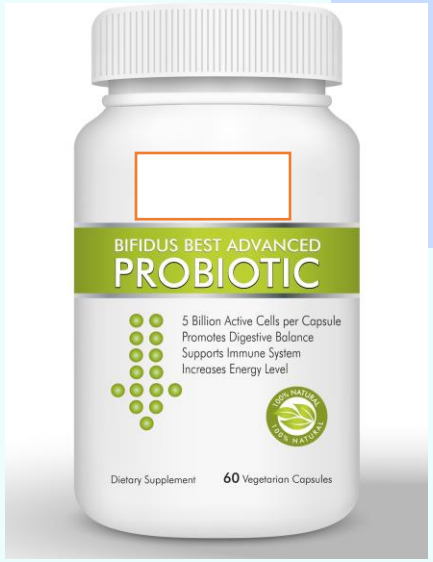
Synbiotics:

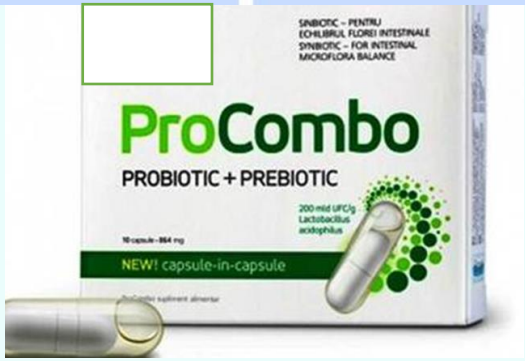
Combination of both pre and probiotics

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Diet Supplementation





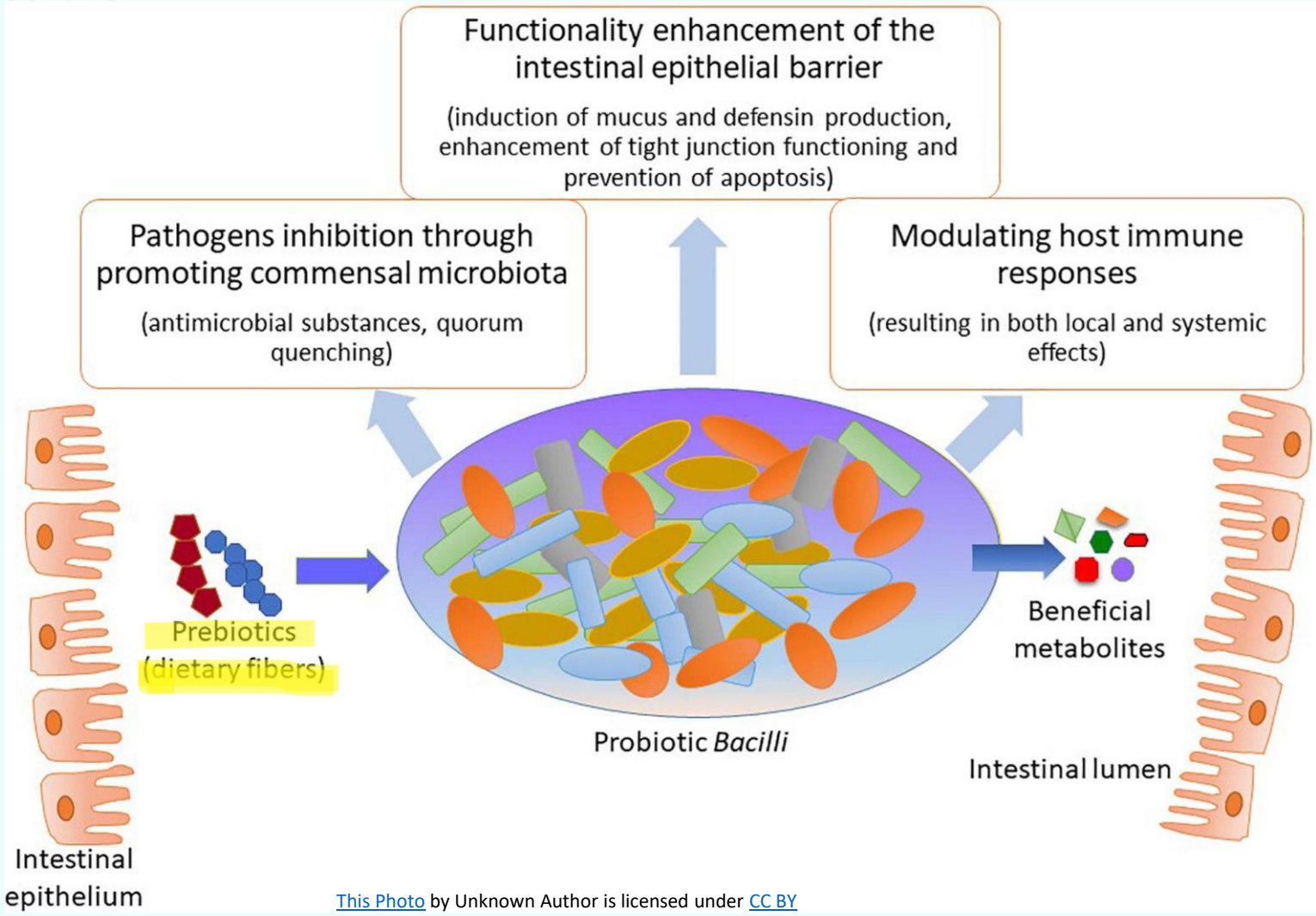


This Photo by Unknown Author is licensed under CC BY-SA
This Photo by Unknown Author is licensed under CC BY-NC-ND

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Diet Supplementation

Prebiotics:

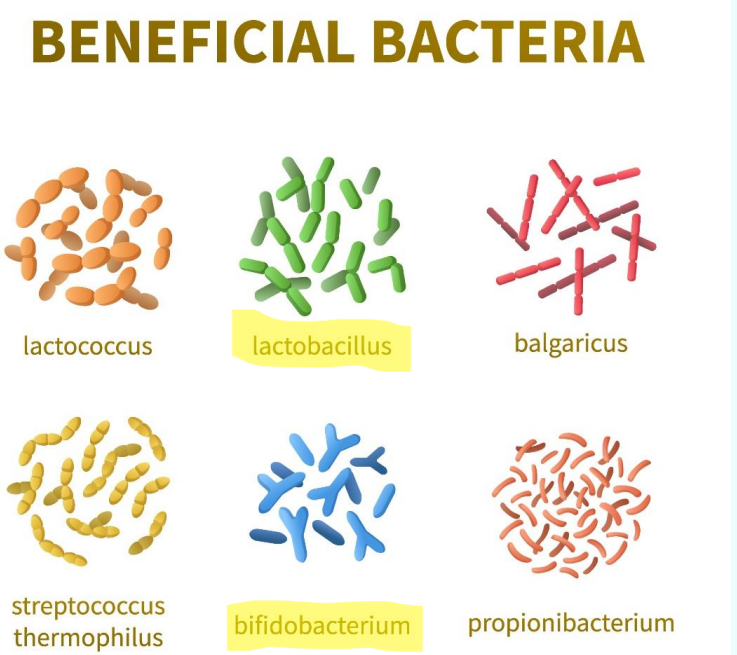


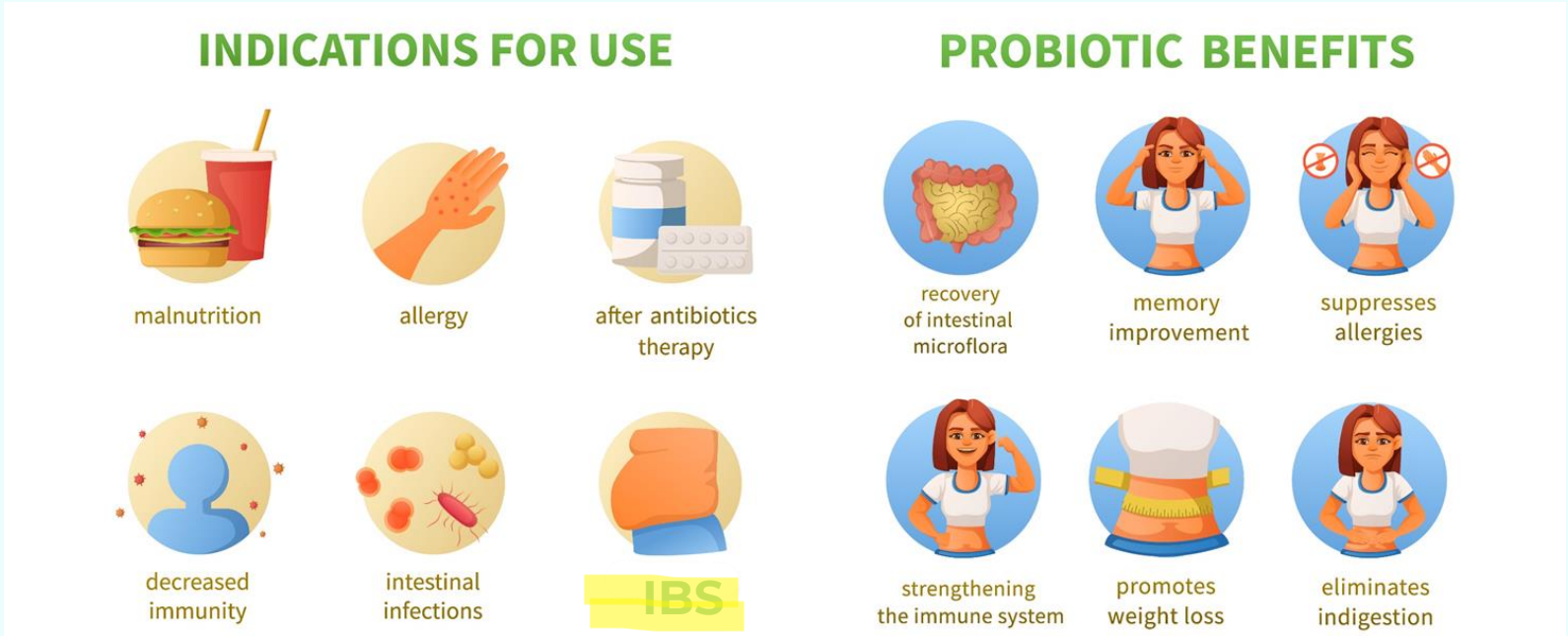
This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Diet Supplementation

Probiotics:

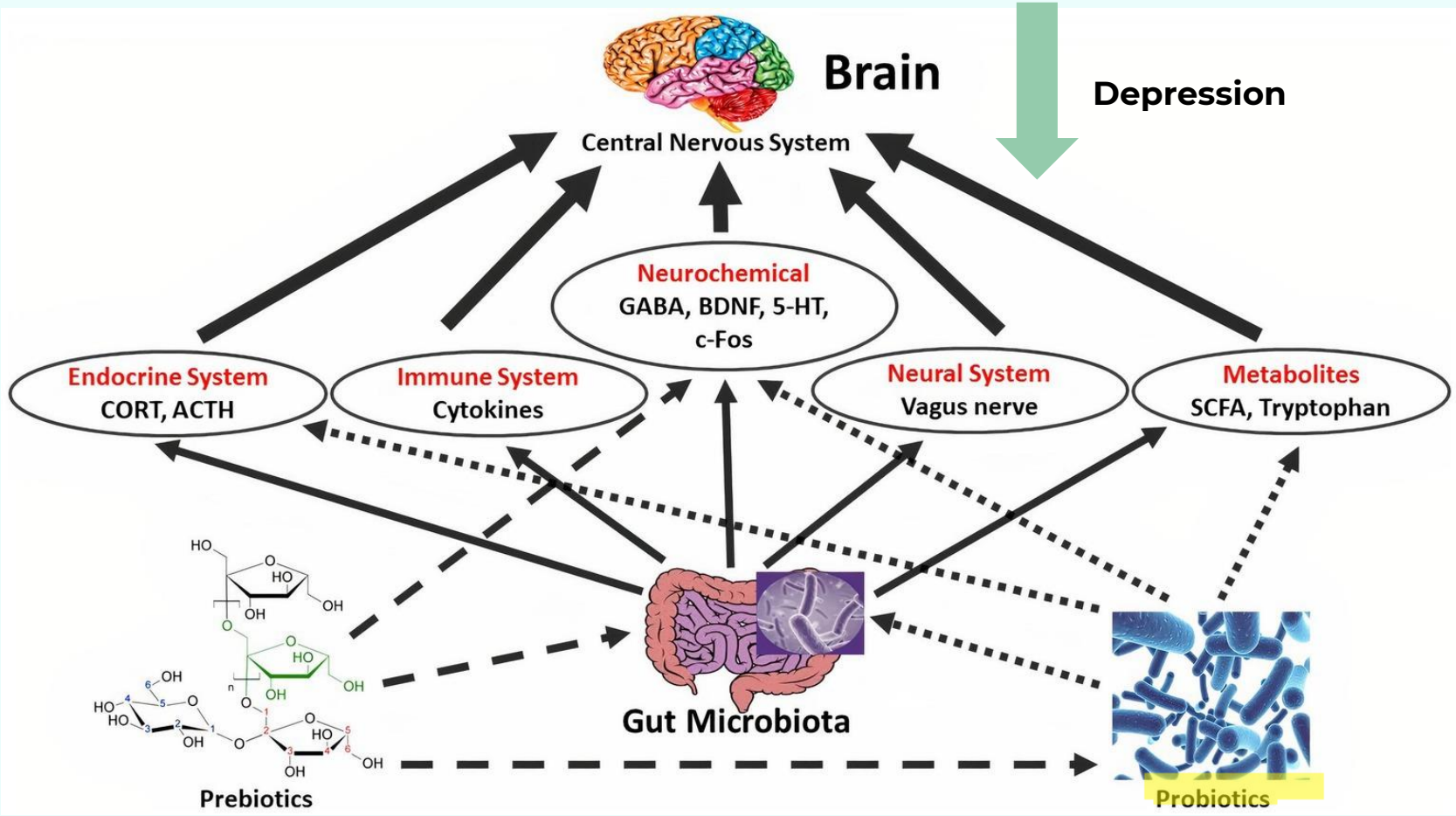




INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Diet Supplementation

Probiotics:



This Photo by Unknown Author is licensed under CC BY

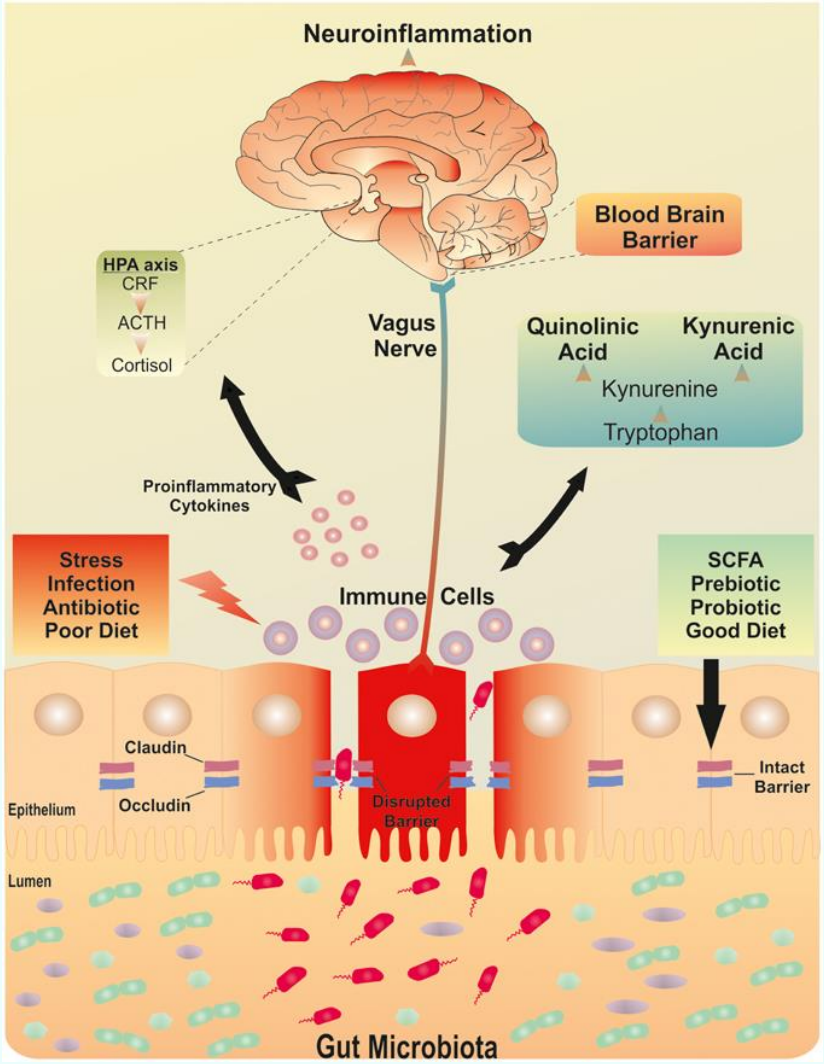
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS): Summary

Human intestinal microbiota coexists with host cells in **Symbiotic relationship**, participating in metabolism and maintaining homeostasis.

The etiology of IBS is unclear, but gut microbiota alterations may contribute to its development.

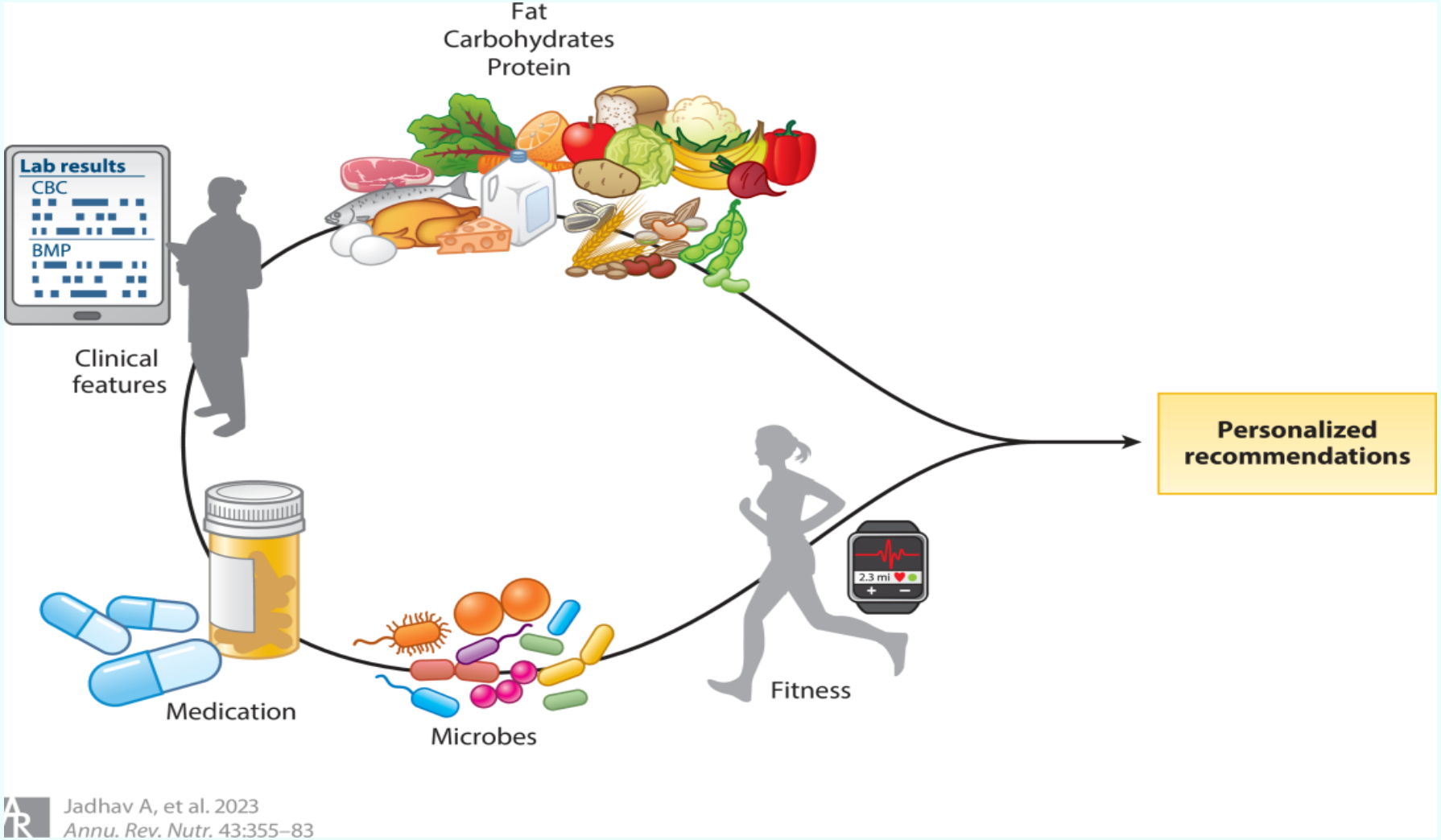
Modulating a daily diet that improves the microbial environment may be the most effective strategy for treating IBS.



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Irritable Bowel Syndrome (IBS):
Practical Consideration for Dietary Changes



Jadhav A, et al. 2023
Annu. Rev. Nutr. 43:355–83

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Take Home Message

The study on diet-host-gut microbiota interactions in IBS suggests a link between the gut microbiome and IBS aetiology and pathophysiology. However, no specific microbiome has been identified.

Future research is needed to determine effective microbiome-related interventions, as a one-size-fits-all approach is insufficient. Personalised medicine may unlock the potential of microbiome-targeted therapies.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

1- Kim, M. Y., & Choi, S. W. (2021). Dietary modulation of gut microbiota for the relief of irritable bowel syndrome. Nutrition Research and Practice, 15(4), 411.

2- Black, C. J., & Ford, A. C. (2021). Best management of irritable bowel syndrome. Frontline Gastroenterology, 12(4), 303-315.

3- Jadhav, A., Bajaj, A., Xiao, Y., Markandey, M., Ahuja, V., & Kashyap, P. C. (2023). Role of Diet–Microbiome Interaction in Gastrointestinal Disorders and Strategies to Modulate Them with Microbiome-Targeted Therapies. Annual review of nutrition, 43, 355-383.

4- Shaikh, S. D., Sun, N., Canakis, A., Park, W. Y., & Weber, H. C. (2023). Irritable bowel syndrome and the gut microbiome: a comprehensive review. Journal of Clinical Medicine, 12(7), 2558.

STUDY MATERIAL

https://aboutibs.org/

https://www.bda.uk.com/resource/irritable-bowel-syndrome-diet.html

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

Content and learning objectives: To have a clear understanding of :

- First-line treatment of IBS
- Evidence supporting the effectiveness of a low-FODMAP diet
- Discuss treatment options for subtypes of IBS
- Strategies to manage the gut-brain axis in IBS
- The link between IBS and the intestinal microbiome

Be aware of:

- Dietary changes alter the gut's microbiota and alleviate IBS symptoms.
- Evidence for using prebiotics and probiotics in IBS
- Practical Considerations for Diet Changes

Body

1. IBS Overview: IBS is a disorder of a Gut brain interaction

1. **The prevalence** and symptoms of irritable bowel syndrome (IBS) vary widely among individuals, making it difficult to diagnose. However, it is estimated that around 3–5% of people worldwide suffer from IBS. It is a chronic condition that can significantly impact a person's quality of life and remains widely underdiagnosed and undertreated.
2. **Symptoms: Rome IV criteria** any of the following symptoms for at least six months: abdominal pain or discomfort, bloating, or change in bowel habit. In addition to physical symptoms, IBS can also cause emotional distress and anxiety.
   It is classified into diarrhea-predominant, IBS-D; constipation-predominant, IBS-C; mixed type of diarrhea and constipation, IBS-M;

or unclassified, IBS-U, according to the characteristics of the predominant symptoms.

Pathophysiology:

Many underlying pathophysiologic factors have been proposed. However, the exact etiology of IBS has not been fully determined.

1: including altered GI motility

2: Visceral hypersensitivity

3- infection

4: low-grade mucosal inflammation

5-intestinal permeability change

6: food intolerance

7-bile acid malabsorption

8: *central neurohormonal dysregulation; enteric neuroendocrine abnormalities*

9: immunologic or psychologic factors

10 genetic factors.

11- **Diet** The role of diet cannot be overlooked because diet is an important external factor that influences the distribution and composition of the intestinal microbiota.

Diagnosis

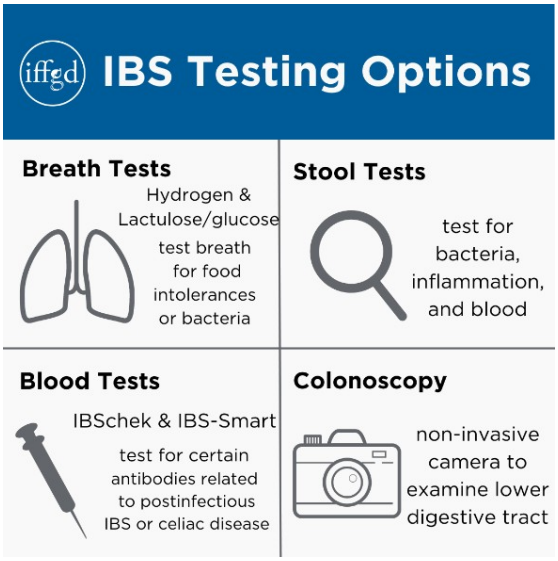
IBS should be considered only if there is recurrent abdominal pain at least 1 day per week that is either relieved by defecation or associated with a change in bowel habit. At least two of the four symptoms listed below should be present:

- Altered stool passage (straining, urgency, incomplete evacuation)
- Abdominal bloating (more common in women than men), distension, tension, or hardness
- Symptoms made worse by eating
- Passage of mucus

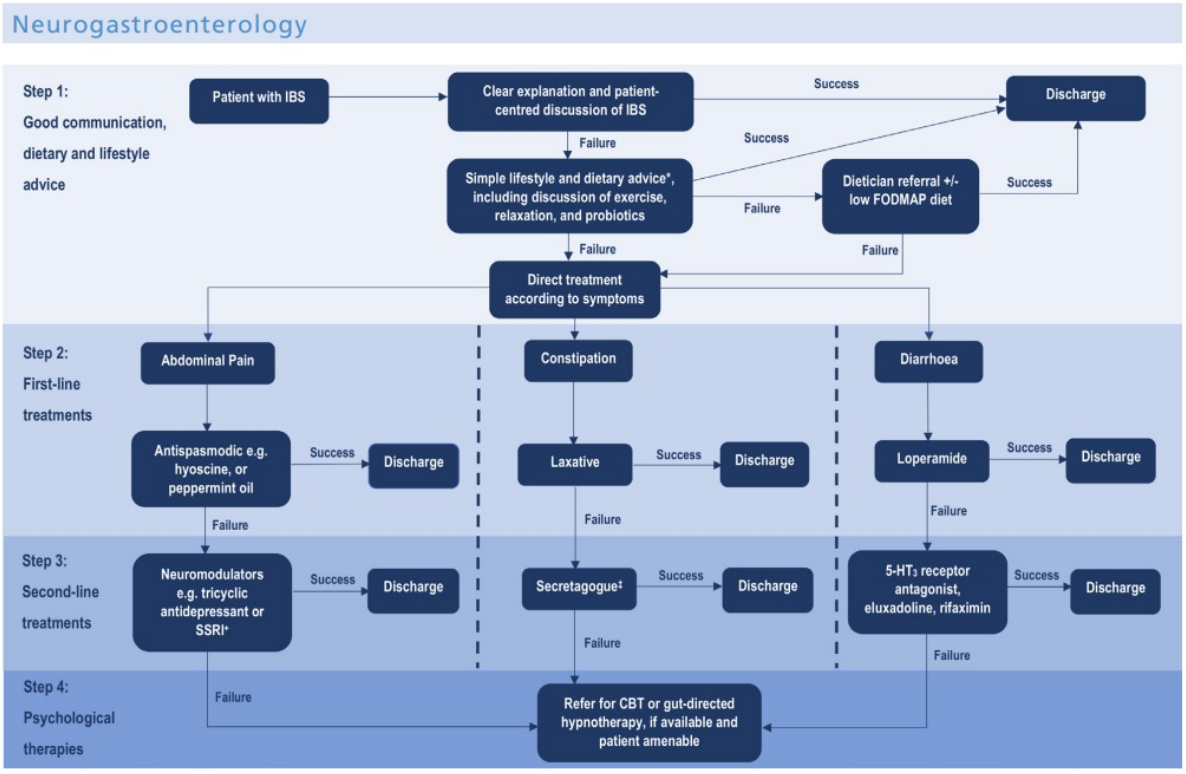
Other features of IBS include lethargy, nausea, backaches, and bladder issues. A confirmed diagnosis is crucial, and other conditions like **coeliac** and **inflammatory bowel disease** should be ruled out.

IBS Testing Options:

Breath Tests, Stool Tests, Blood Tests, and Colonscopy



Management



Lifestyle changes are the cornerstone of treatment, regardless of symptom severity, and are likely to be sufficient in the management of mild symptoms.

- **Dietary management of IBS**: 1st and 2nd lines

1st line dietary advice: 1- Dietary advice for IBS patients included the avoidance of dietary triggers e.g. caffeine, alcohol, fatty foods, and spicy foods. 2- Milk and dairy restriction 3- Dietary fiber

If symptoms include bloating and wind:

• Limit fruit juice to one small glass per serving.

• Limit intake of gas producing foods, e.g., beans and pulses, broccoli, Brussels sprouts, cabbage, cauliflower, and also sugar-free mints and chewing gum.

• Lactose can sometimes cause wind, bloating, and IBS-type symptoms. Trial using lactose free dairy products.

If symptoms include constipation,

• Ensure a good fluid intake—about eight glasses or mugs (two liters) a day.

• Increase your fiber intake gradually; any sudden increase may make symptoms worse.

• Try adding one tablespoon per day of brown or golden linseeds (whole or ground) to breakfast cereal, yogurt, soup, or salad.

• Avoid eating extra wheat bran.

If symptoms include diarrhea,

• Ensure a good fluid intake—about eight glasses or mugs (two liters) a day. • Limit fruit juice to one small glass a day.

• Limit caffeine intake from tea, coffee, and soft drinks to three drinks per day.

• Try reducing whole-wheat breakfast cereals and breads and choosing white versions instead.

• Lactose can sometimes cause diarrhea and IBS-type symptoms. Trial using lactose free diary products for two to four weeks.

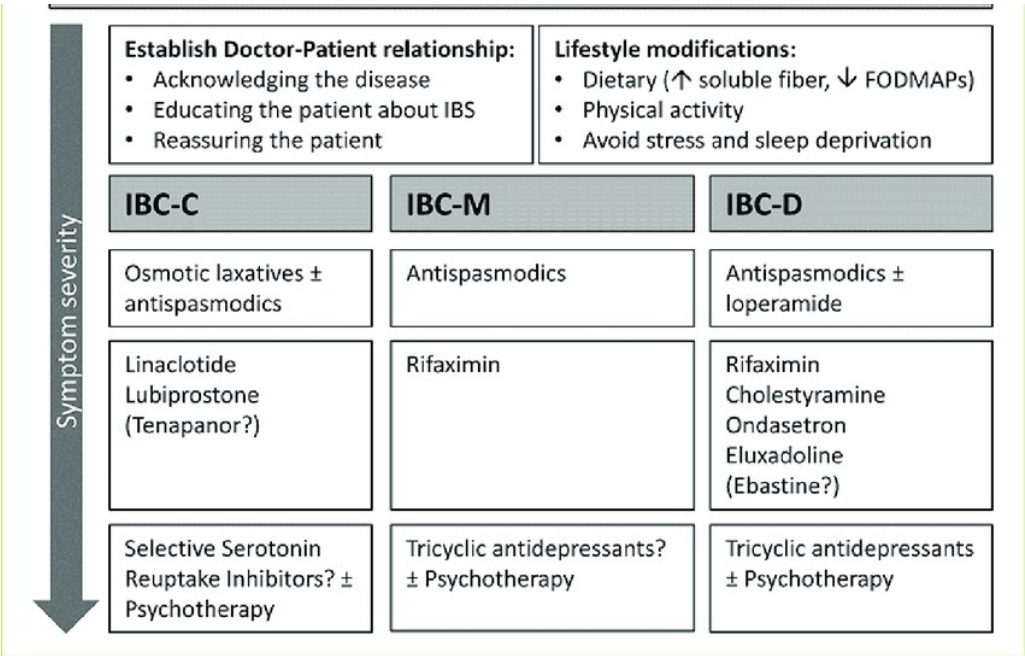
• Avoid sugar-free sweets, mints, and gum containing sorbitol, mannitol, and xylitol.

2nd line dietary advice : a low FODMAP (fermentable oligosaccharides, disaccharides, monosaccharides, and polyols) diet.

- A meta-analysis of 22 studies revealed that low-FODMAP diet adherents experienced lower symptom severity, improved quality of life, and reduced abdominal pain.(4)
- A 9-week clinical trial found that a low FODMAP diet alleviated IBS symptoms and decreased serum levels of inflammatory cytokines (IL)-6, IL-8.(1)
- A low FODMAP diet may have an adverse effect on the gut microbiota because it has reduced levels of dietary prebiotics, total saturated fatty acids (SCFAs), n-butyric acid, and fecal bacteria (Actinobacteria, Bifidobacterium, and Faecalibacterium prausnitzii) suggesting that low FODMAP can alter gut microbiota profile.
- A series of studies investigating the effect of low FODMAP on gut microbiota and its metabolites have shown mixed and sometimes conflicting results in contrast to the symptomatic improvement by low FODMAP diet.
- The "low FODMAP diet" is not a "no FODMAP diet" or a "lifetime diet." It's recommended to follow for 6-8 weeks, followed by a dietician's review to advise on gradually reintroducing certain foods and amounts.

Treatment options for IBS vary according to the predominant symptoms and their severity.

- For **moderate symptoms**, pharmacological therapies may be added and aim to relieve predominant bowel habits and visceral pain.
- For **severe symptoms** and patients with refractory symptoms, psychopharmacologic agents and CBT can be used.

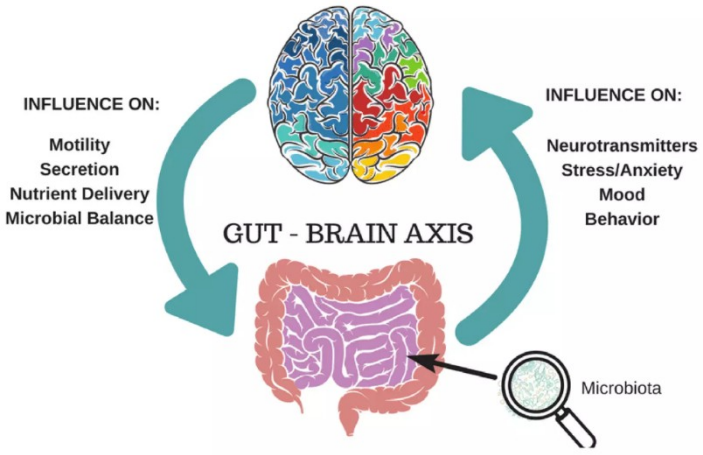


2: Gut-Brian Axis: Numerous mechanisms that underlie the gut microbiota's impact on neurodevelopment and pathogenesis remain unclear.

A. Gut-Brian axis

The nervous system is a **bidirectional** network comprising the central nervous system, autonomic nervous system, enteric nervous system, and hypothalamic pituitary adrenal axis. It regulates gut motility, digestive enzyme secretion, and communication with other organs using

neurotransmitters. The enteric nervous system regulates gut motility, secretion, the immune system, and intestinal homeostasis.



B. Gut Brain Microbiota Axis:

In the upcoming part, will study microbiota-dependent mechanisms linked to **Irritable Bowel Syndrome Pathophysiology**

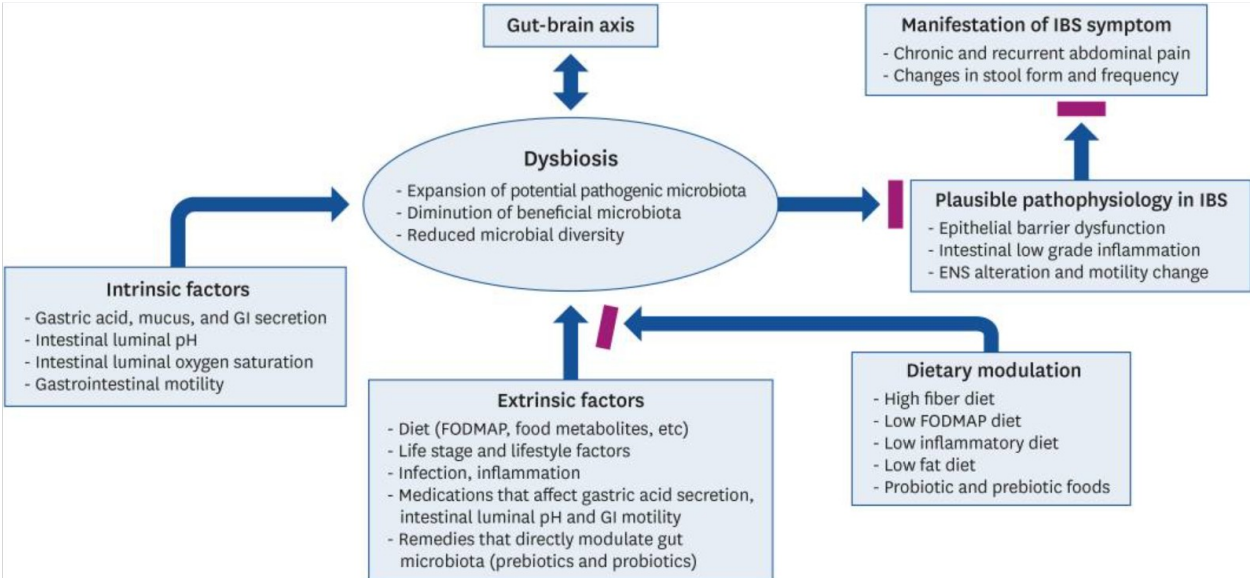
- The dysregulation of the GBA through interactions with the gut microbiota has been suggested as an etiology of IBS.
- The gut microbiota and its metabolites can affect GI motility; its alteration is a hallmark of IBS.
- IBS symptoms may be influenced by impaired tryptophan metabolism, which can affect the GBA and the local and central nervous systems. Tryptophan, a key amino acid in IBS, is essential for serotonin (5-hydroxytryptamine [5-HT]) production and is involved in functional gastrointestinal disorders. Changes in 5-HT homeostasis may lead to dysmotility. Depletion of tryptophan can reduce the

proliferation of *Lactobacillus*, which can be selectively induced by restoring tryptophan levels.

C. Dysbiosis refers to gut microbial imbalance characterized by one or more of three types: the expansion of pathogenic microbes, the reduction of beneficial microbiota, or a decrease in microbial diversity.

IBS can compromise the composition and activities of beneficial bacteria like *lactobacilli* and *Bifidobacterium*, while severe IBS patients experience reduced *Prevotella* and *Methanobacteriales*, along with increased Bacteroides.

The relationship between IBS and intestinal microbiota dysbiosis is influenced by various extrinsic and intrinsic factors, suggesting a new pathophysiologic mechanism for IBS:



3. Diet as an important determinant of the gut microbiome

- Dietary patterns significantly impact the gut microenvironment:
  - colonic fermentation
  - microbiome composition
  - immune regulation, affecting motility and permeability
  - visceral sensation
  - gut-brain interactions and neuroendocrine function in IBS.
- Diet is a modifiable factor that can modulate the gut microbiome, making it a potential therapeutic intervention for dysbiosis-related conditions.
- Consumption of plant-based proteins and fiber-rich diets boosts commensal organisms like *Bifidobacterium* and *Lactobacillus*, Low-fat/high-fiber diets have higher gut biodiversity.
- While animal-based proteins increase *Bacteroides*, *Alistipes*, and *Bilophila*, while high-fat diets increase gut *anaerobes* and *Bacteroides*.

4: Dietary modification of the gut microbiota to relieve IBS symptoms

The study suggests that a cost-effective approach to managing IBS symptoms involves modifying gut microbiota through regular meals, reducing causable foods, and a high-fiber diet.
This can reduce colonic fermentation and antigen activation of the gut immune system. Additional prebiotics and probiotics can also improve

symptoms, with live microorganisms like *Lactobacillus* and *Bifidobacterium* being particularly beneficial.

Dietary modulation of gut microbiota for IBS symptom relief

Diets	Microbiota changes	IBS symptoms
High fiber diet	Fibers are the main source of microbiota-accessible carbohydrates that increases the gut microbiota diversity and produces beneficial metabolites such as short-chain fatty acid.	Soluble fibers relive symptoms by softening the stool. High fiber diet has been a standard care particularly for IBS-C.
Low FODMAP diet	*Actinobacteria*, *Bifidobacterium*, and *Faecalibacterium prausnitzii* were decreased by low FODMAP diet.	Improves overall IBS symptoms such as visceral pain, bloating and quality of life.
Low inflammatory diet	*Bacteroidetes* phylum was more abundant in vegans and vegetarians.	Alleviates chronic low-grade mucosal inflammation, which helps enterocyte healing and nutritional support to the microbial environment.
Low fat diet	Increases bacterial diversity and beneficial butyrate-producing bacteria.	Beneficial effects on intestinal inflammation.
	Nuts and other plant-based fat increases the genera *Bifidobacterium*, *Roseburia*, and *Faecilibacterium*.	

5: Supplementing Diet

1- Fiber intake:

- Fibers, found in fruits, vegetables, whole grains, and legumes, are a source of microbiota-accessible carbohydrates. They are fermented by enteric bacteria in the colon to produce short-chain fatty acids (SCFAs) and gases, which are believed to have beneficial effects on gut microbiota, including preserving gut epithelial integrity, energy homeostasis, and inflammatory signal inhibition and immunity.
- The standard treatment for IBS-C patients is a high-fiber diet, with no serious side effects and potential benefits on gut microbiota. Fiber softens stool but doesn't alleviate pain. In clinical settings
- Psyllium, a soluble fiber supplement, is effective for relieving symptoms in IBS, while insoluble fiber found in foods like wheat bran

does not. fiber supplements should be started at a low dose and gradually increased to avoid aggravating symptoms.

2- Prebiotics, Probiotics and Synbiotics:

- Prebiotics, food compounds that feed microbiota, and probiotics, live microorganisms like *Lactobacillus* and *Bifidobacterium*, can improve microbial homeostasis and symptom improvement. Synbiotics are a combination of both pre and probiotics that have beneficial effect on IBS symptoms.
- Several studies have investigated prebiotics and synbiotics, but there is insufficient data to make recommendations. However, studies show that prebiotics can improve flatulence severity at doses ≤ 6 g/d, while inulin-type fructo-oligosaccharides worsen the symptom.
- Diet variations in prebiotic type and dose may affect the improvement or exacerbation of flatulence.
- A randomized study that compared an MD-type diet and a prebiotic supplement to a low-FODMAP diet, and a placebo xylose supplement discovered that both groups improved symptoms. But prebiotic supplementation improved the gut microbiota profile, implying that food supplementation and modification may be more effective than restrictive diets.

- The efficacy and long-term effectiveness of probiotics in treating IBS remain uncertain, Nevertheless, probiotics have been used to

improve overall abdominal symptoms and bloating. Patients should take a combination for up to 12 weeks.

- Probiotics influence the gut-brain axis. The probiotic Bifidobacterium longum NCC3001 was shown in randomized, double-blind, placebo-controlled research among individuals with IBS to lower depression scores and fMRI responses. However, anxiety and quality of life were unchanged. Although there are no guidelines for utilizing probiotics in depression therapy, a meta-analysis found that probiotics had a substantial favorable impact when combined with antidepressants.
- IBS patients' symptoms have not consistently improved with pre- and pro-biotics, fecal microbiota transplants, highlighting the need for further research into precision medicine and metabolomics for individual treatment.

Summary

- The most effective and physiological strategy for treating IBS is to modify a daily diet that improves the microbial environment.
- IBS symptoms can be treated by limiting triggering foods while retaining those that improve them and adjusting the microbiota profile with a high-fiber diet. However, long-term nutritional consequences of restricted foods should be investigated to prevent essential nutrient deficiency in patients.

- Low FODMAP diets and pre- and probiotic foods have been tried to improve IBS symptoms, but it's crucial to determine their long-term impact on microbial balance, and effectiveness in IBS patients.

Practical considerations for dietary changes include:

- Keep a food and symptom diary while you are making changes so you can see what has helped.
- Take time to eat meals; chew your food well.
- Take regular exercise such as walking, cycling, or swimming.
- Take time to relax and destress; relaxation tapes, yoga, aromatherapy, or massage may help.
- Make one change at a time so that you can see what has helped.
- Despite the lack of evidence supporting the advice of fluids in IBS, practical considerations include drinking 1.5 to 3 L daily to increase stool frequency and reduce the need for laxatives in IBS-C.

Take home message

The study on diet-host-gut microbiota interactions in chronic GI diseases is in its early stages, but it suggests a link between gut microbiome and IBS. However, no specific microbiome has been identified, and treatments like pre- and pro-biotics have not consistently improved symptoms. Future research should consider these factors for personalized dietary recommendations.

References:

1- Kim, M. Y., & Choi, S. W. (2021). Dietary modulation of gut microbiota for the relief of irritable bowel syndrome. *Nutrition Research and Practice*, *15*(4), 411.

2- Black, C. J., & Ford, A. C. (2021). Best management of irritable bowel syndrome. *Frontline Gastroenterology*, *12*(4), 303-315.

3- Jadhav, A., Bajaj, A., Xiao, Y., Markandey, M., Ahuja, V., & Kashyap, P. C. (2023). Role of Diet–Microbiome Interaction in Gastrointestinal Disorders and Strategies to Modulate Them with Microbiome-Targeted Therapies. *Annual review of nutrition*, *43*, 355-383.

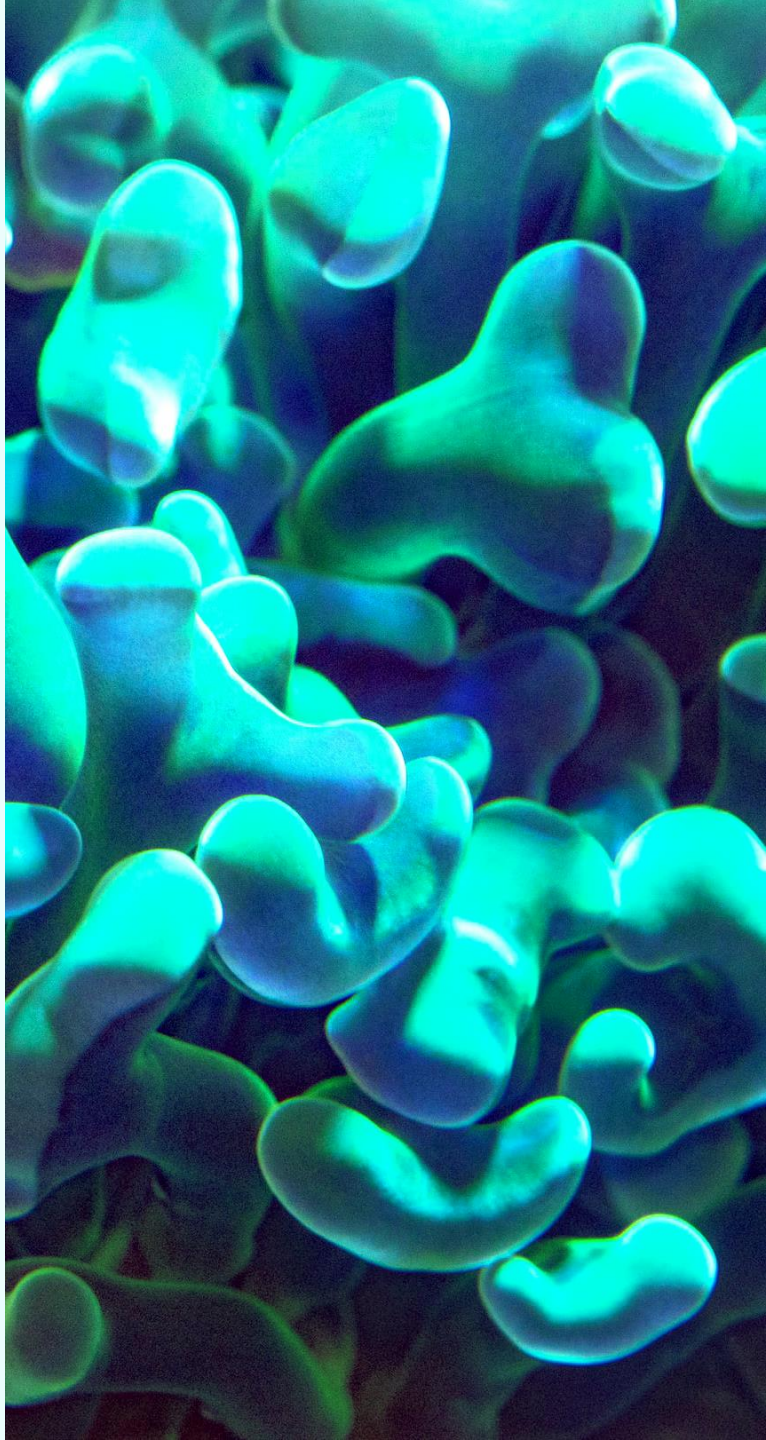
4- Shaikh, S. D., Sun, N., Canakis, A., Park, W. Y., & Weber, H. C. (2023). Irritable bowel syndrome and the gut microbiome: a comprehensive review. *Journal of Clinical Medicine*, *12*(7), 2558.

5- https://aboutibs.org/

6- https://www.bda.uk.com/resource/irritable-bowel-syndrome-diet.html

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Microbiome and clinical implications



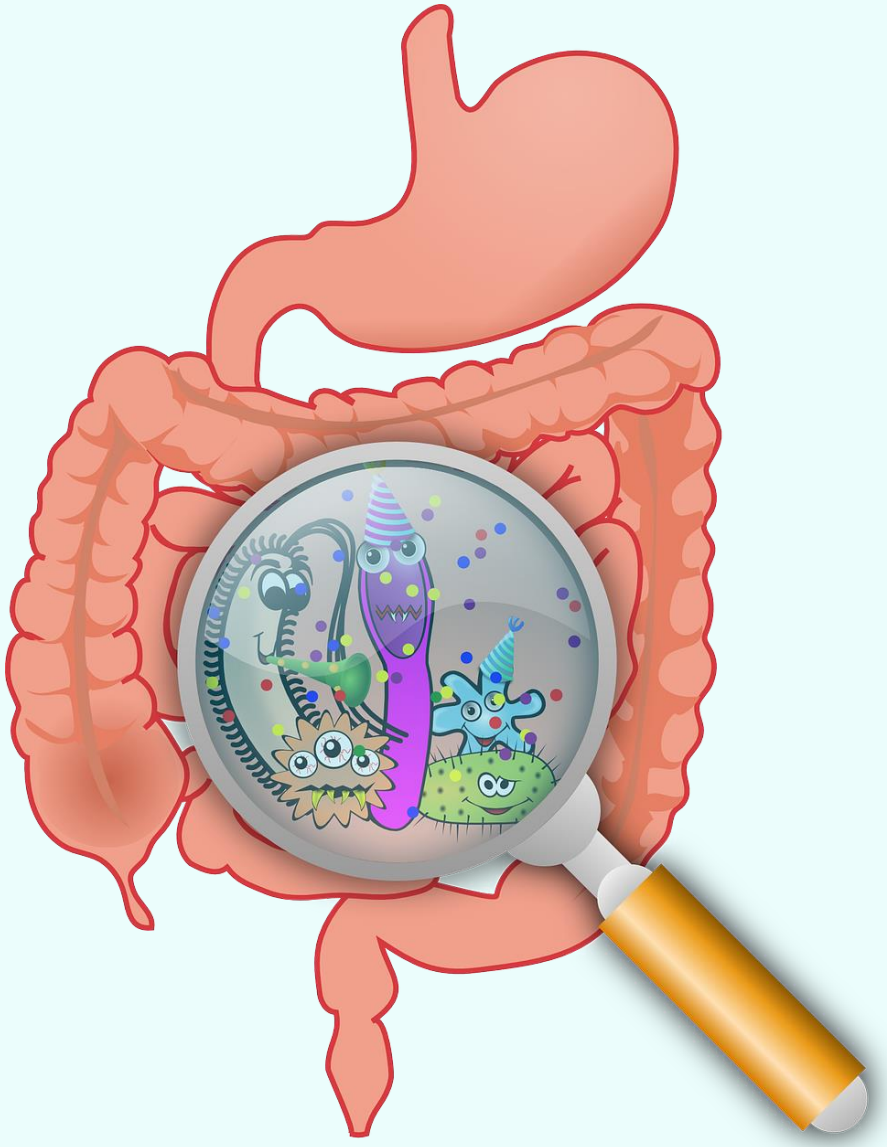
Malta Further & Higher Education Authority

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

What is a microbiome?

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

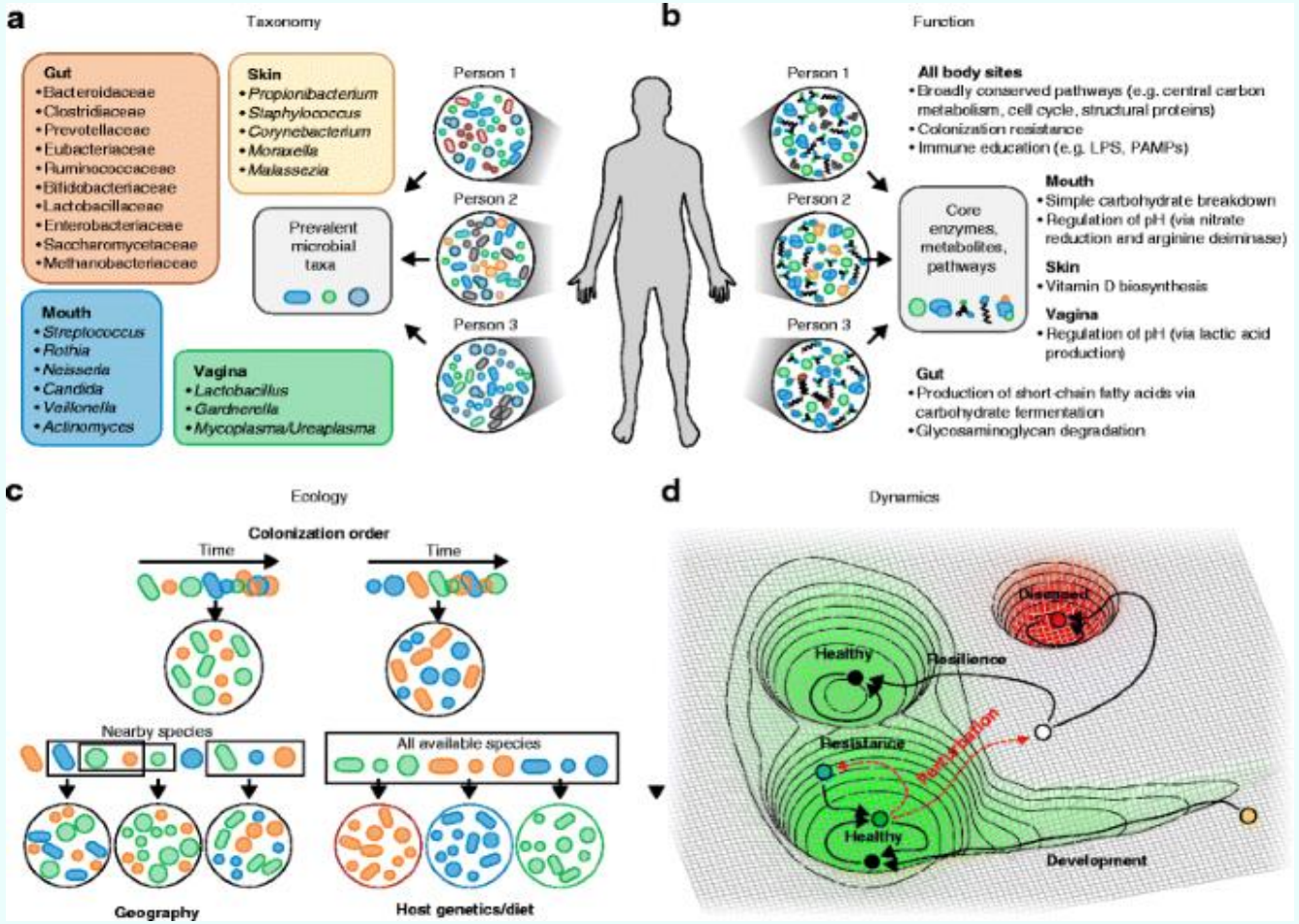
kalaubek.ainur98@gmail.com



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

The healthy GUT microbiome

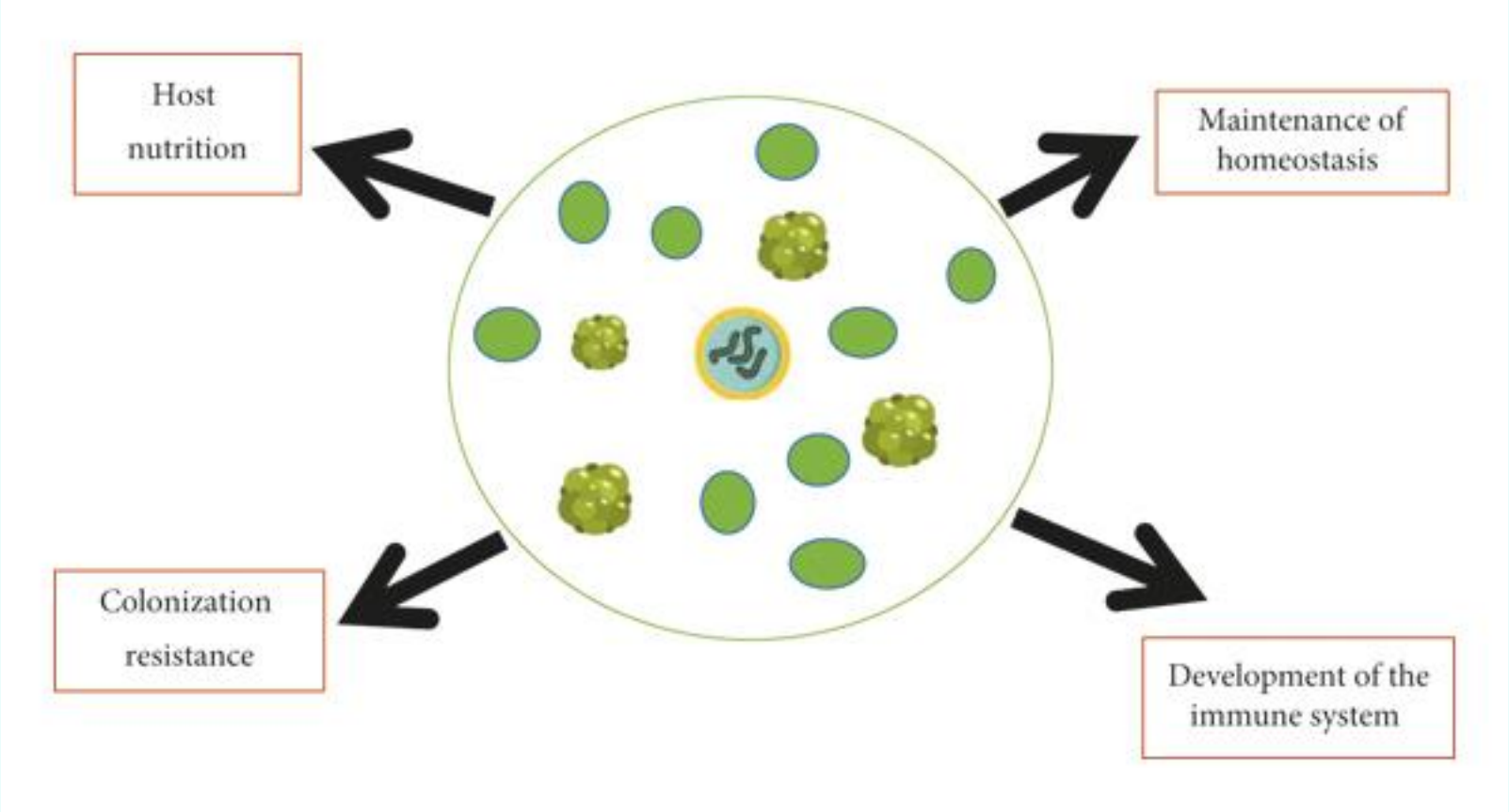
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
Kalaubek.ainur98@gmail.com



Lloyd-Price, J., Abu-Ali, G., & Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine*, *8*(1). https://doi.org/10.1186/s13073-016-0307-y

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
Kalaubek.ainur98@gmail.com

Symbiotic interaction between eubiotic flora and the human body results in the maintenance of homeostasis, regulation, and development of the immune system, hosts nutrition, and colonization resistance.



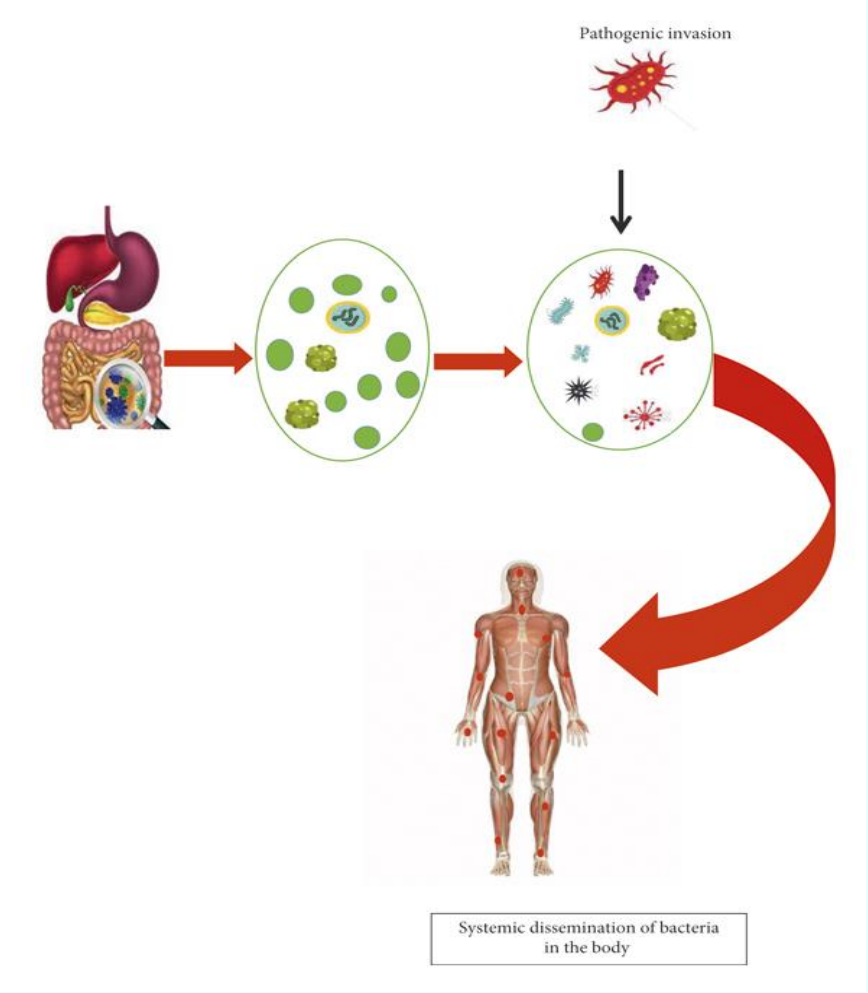
Ogunrinola, G. A., Oyewale, J. O., Oshamika, O. O., & Olasehinde, G. I. (2020). The human microbiome and its impacts on health. *International Journal of Microbiology*, *2020*, 1–7. https://doi.org/10.1155/2020/8045646

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

The human microbiome and disease

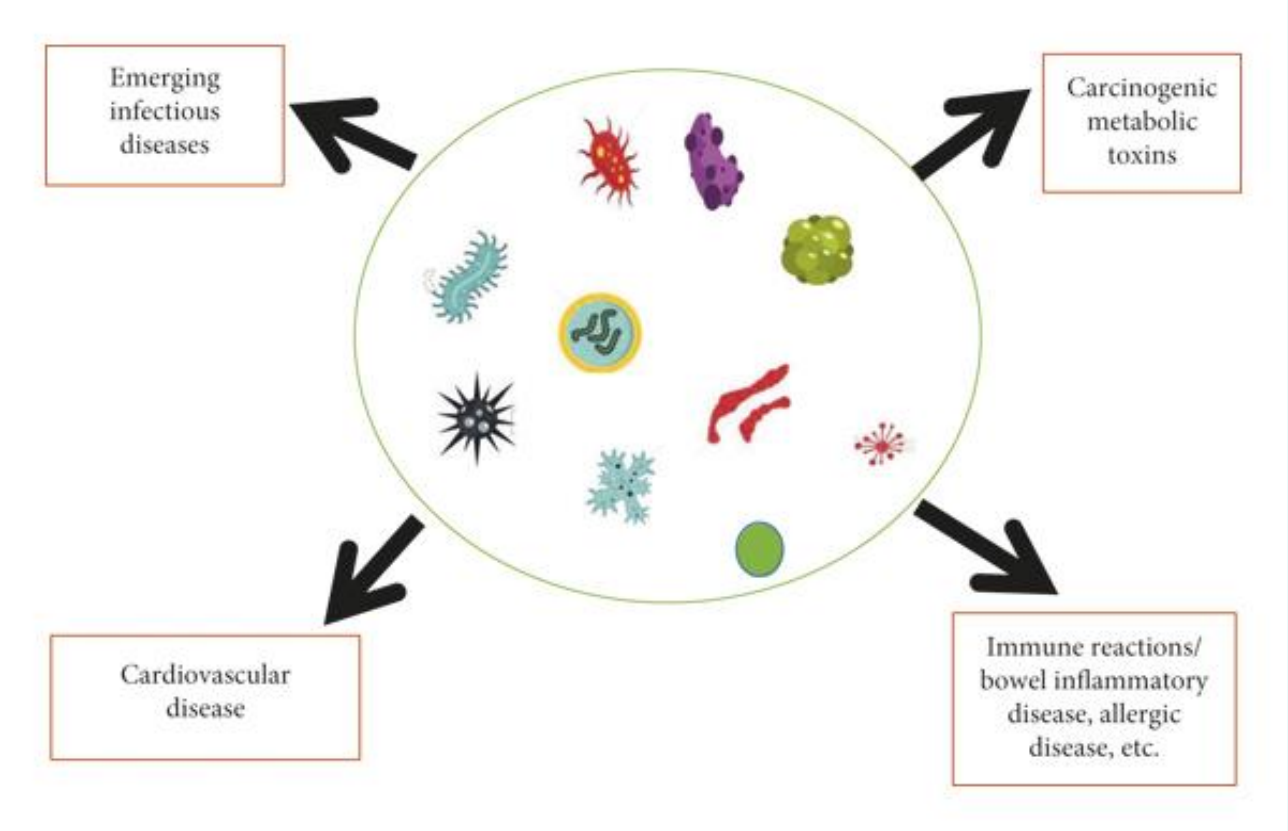
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

An alteration in the gut microbiota leads to systemic translocation of organisms from damaged gut epithelium to other extraintestinal sites.



Ogunrinola, G. A., Oyewale, J. O., Oshamika, O. O., & Olasehinde, G. I. (2020). The human microbiome and its impacts on health. *International Journal of Microbiology*, *2020*, 1–7. https://doi.org/10.1155/2020/8045646

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



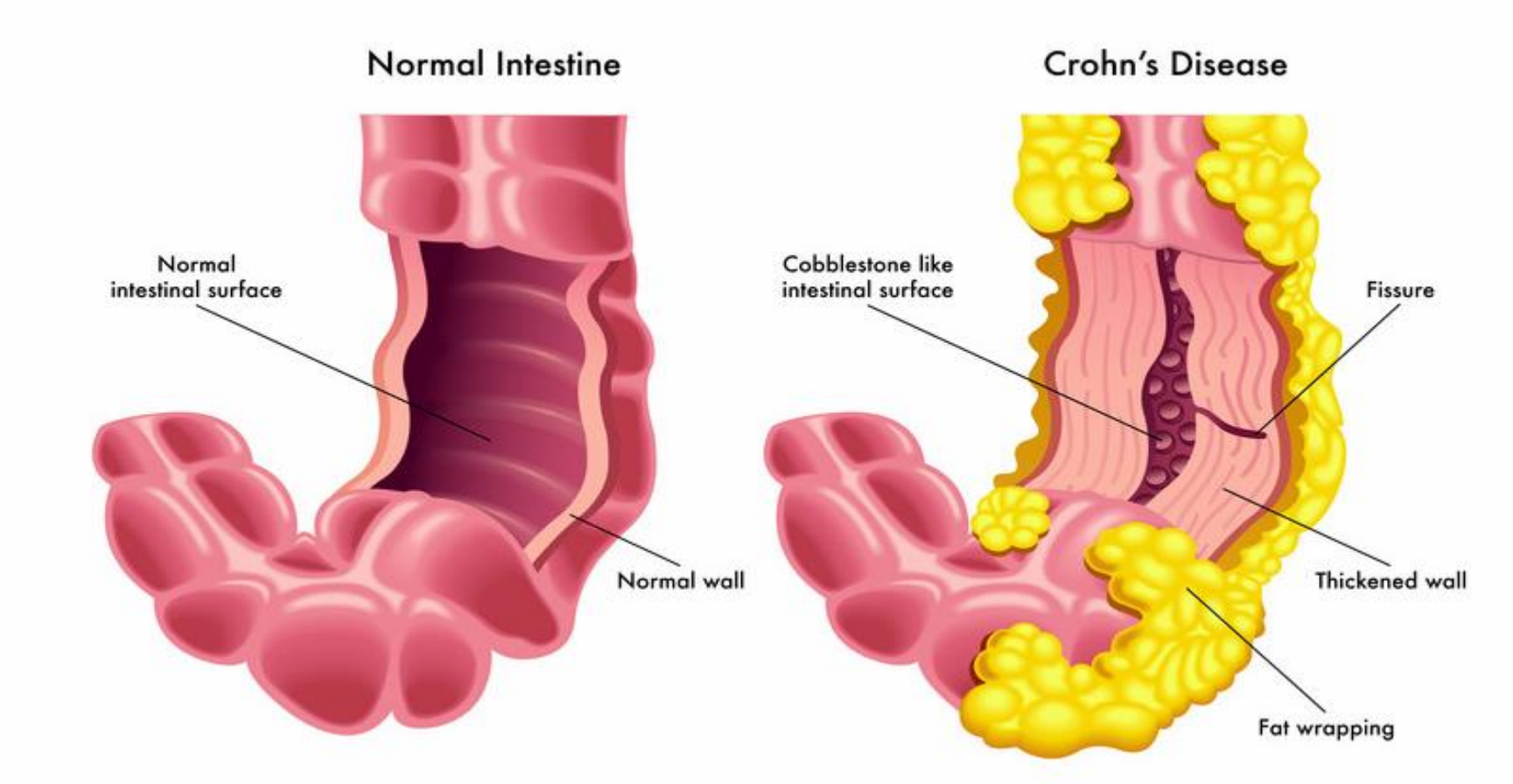
Dysbiotic flora and its impact on human health. Carcinogenic metabolic toxins produced from dysbiotic flora may trigger the progression of cancer and immune reaction in the gastrointestinal tract. In addition, hepatic oxidation of trimethylamine to trimethylamine N-oxide contributes to cardiovascular and emerging diseases.

Ogunrinola, G. A., Oyewale, J. O., Oshamika, O. O., & Olasehinde, G. I. (2020). The human microbiome and its impacts on health. International Journal of Microbiology, 2020, 1–7. https://doi.org/10.1155/2020/8045646

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

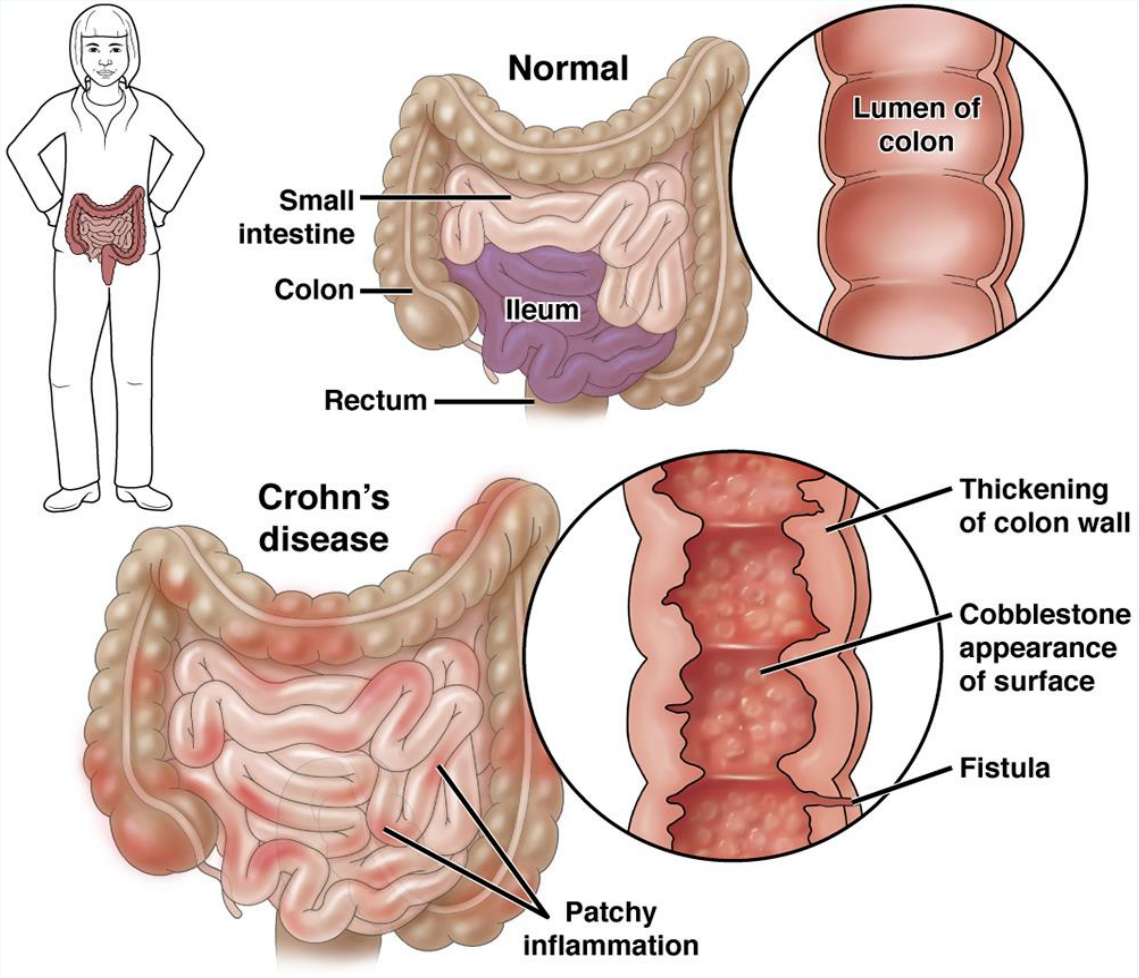
Crohn’s disease

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



Harvard Health. (2023, March 15). *Crohn's disease*. https://www.health.harvard.edu/a_to_z/crohns-disease-a-to-z

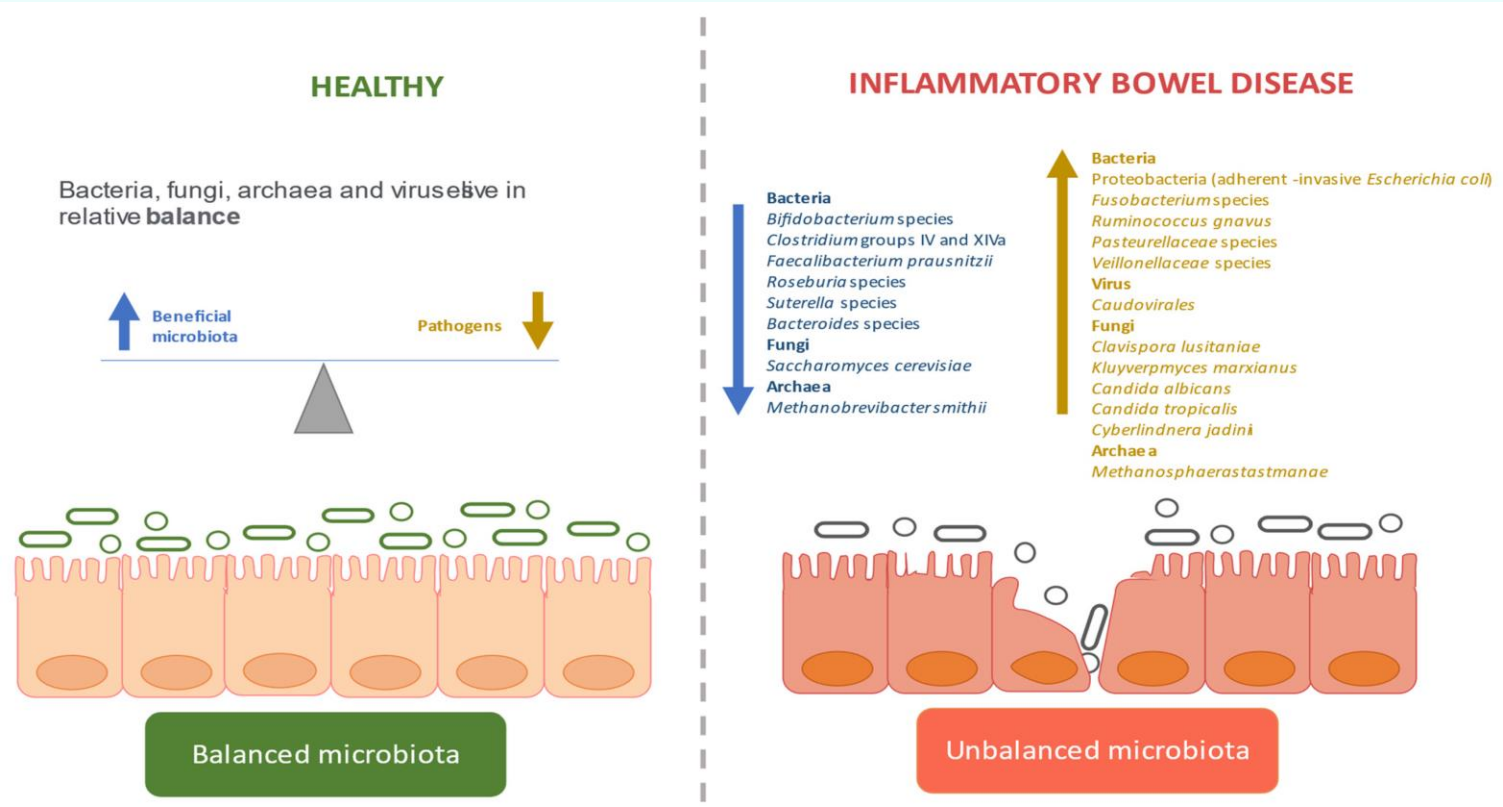
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



Stone, C. (2022, June 13). *Crohn's disease - AGA GI Patient Center*. AGA GI Patient Center. https://patient-staging.gastro.org/crohns-disease/

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

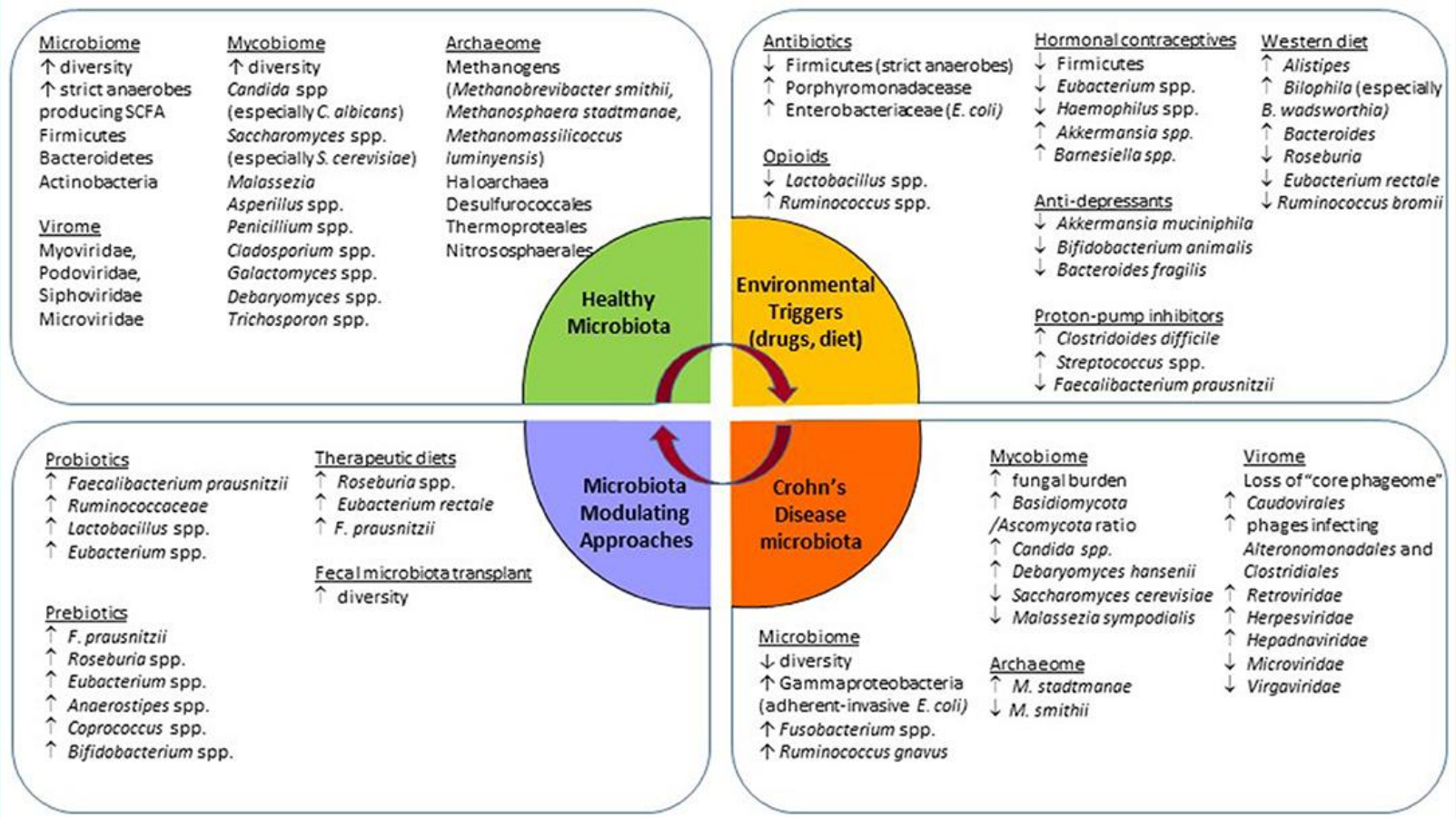
Gut microbiota disturbance in inflammatory bowel disease compared to healthy individuals. Upward arrow indicates increase and downward arrow decrease.



Aldars-García, L., Chaparro, M., & Gisbert, J. P. (2021). Systematic Review: The gut microbiome and its potential Clinical application in inflammatory bowel disease. *Microorganisms*, *9*(5), 977. https://doi.org/10.3390/microorganisms9050977

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

Schematic summary of observed gut microbiota changes associated with Crohn's disease



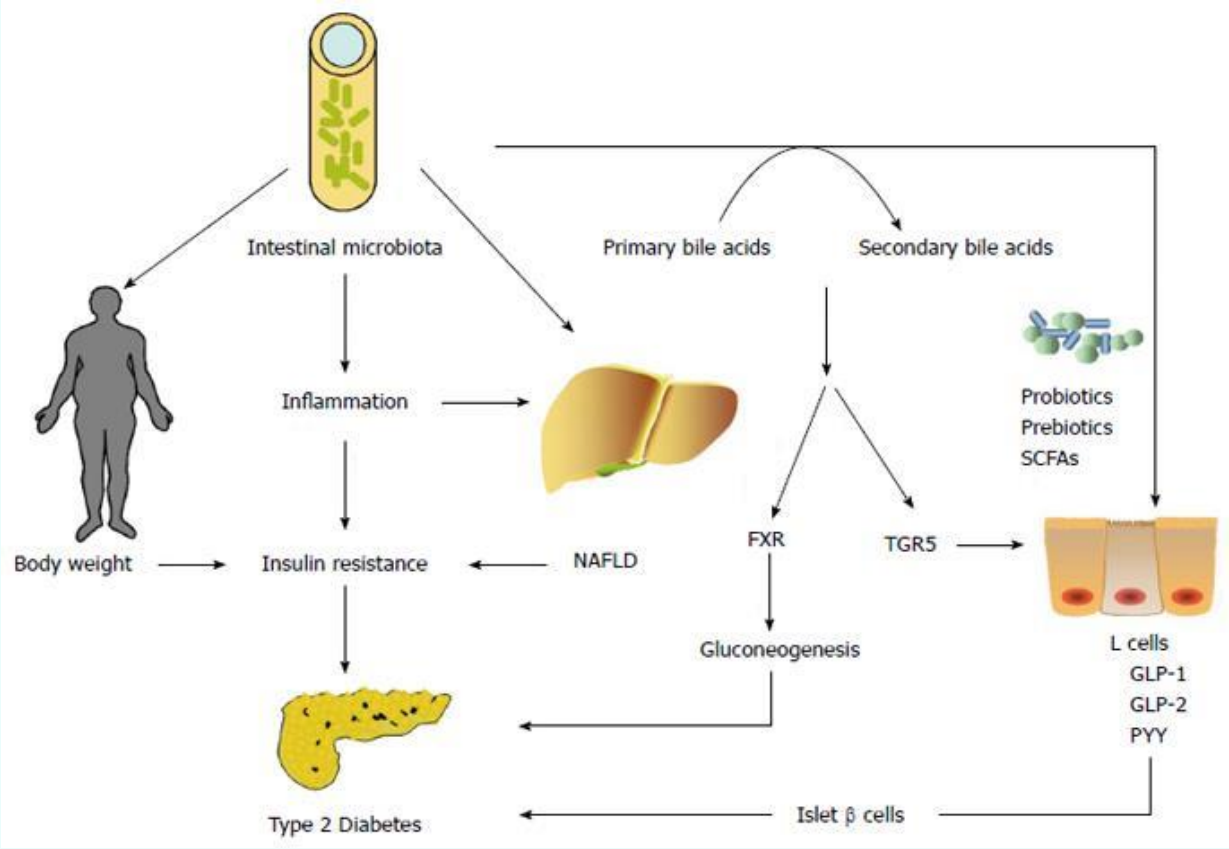
Rashed, R., Valcheva, R., & Dieleman, L. A. (2022). Manipulation of gut microbiota as a key target for Crohn's disease. *Frontiers in Medicine*, *9*. https://doi.org/10.3389/fmed.2022.887044

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

Type 2 Diabetes

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

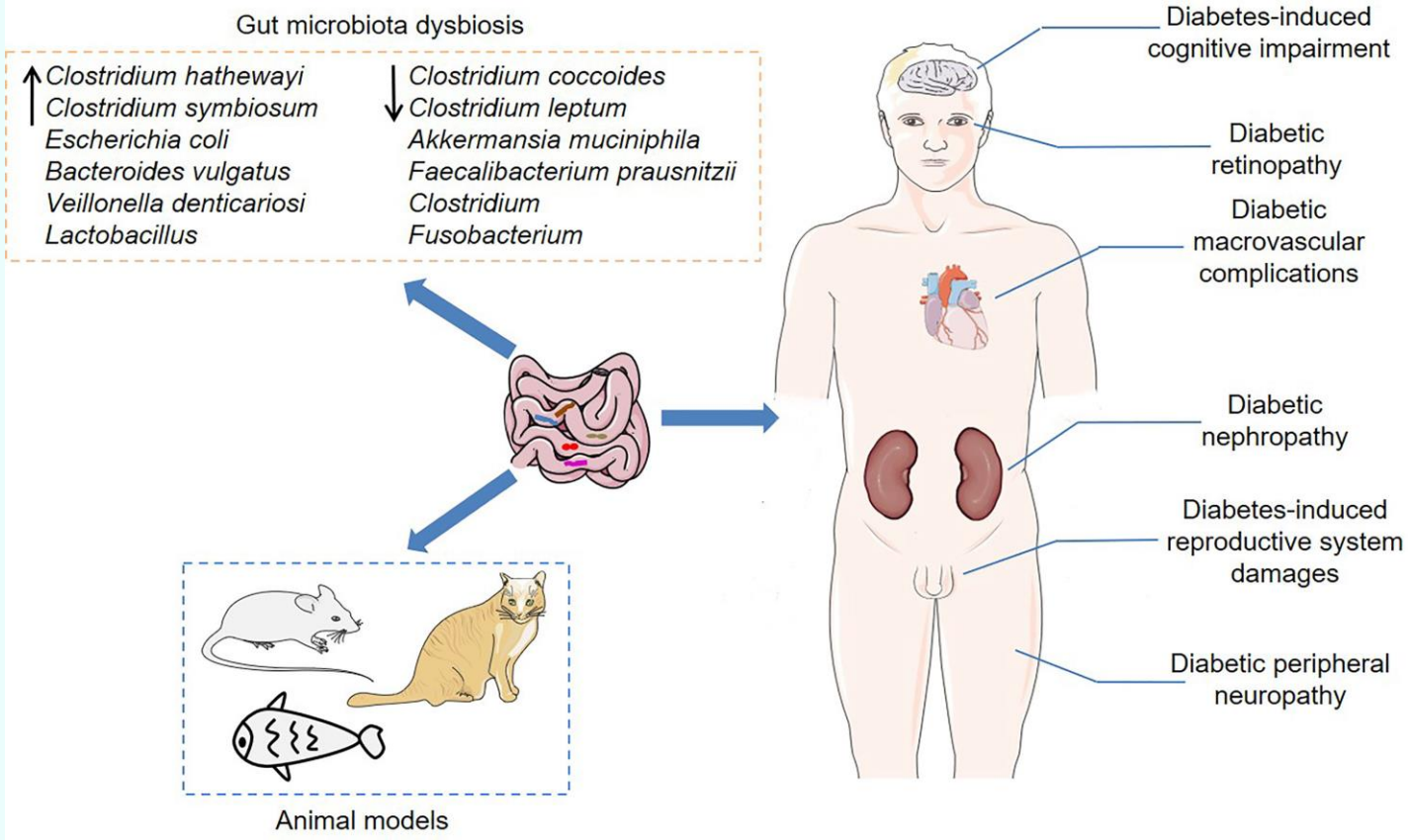
Role of the intestinal microbiota in the pathogenesis of type 2 diabetes.



Han, J., & Lin, H. (2014). Intestinal microbiota and type 2 diabetes: From mechanism insights to therapeutic perspective. *World Journal of Gastroenterology*, *20*(47), 17737–17745. https://doi.org/10.3748/wjg.v20.i47.17737

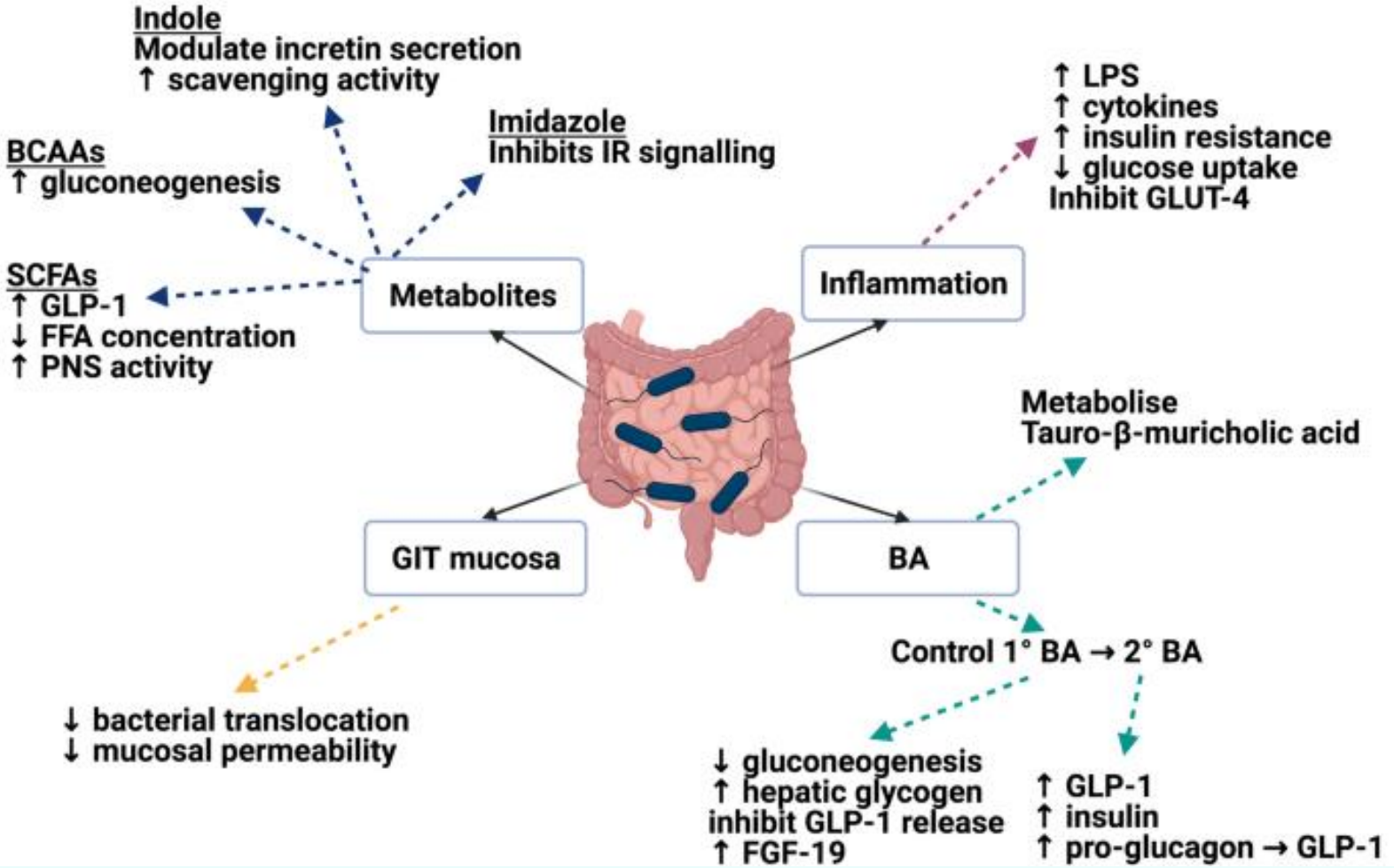
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

The association between the gut microbiota and T2DM



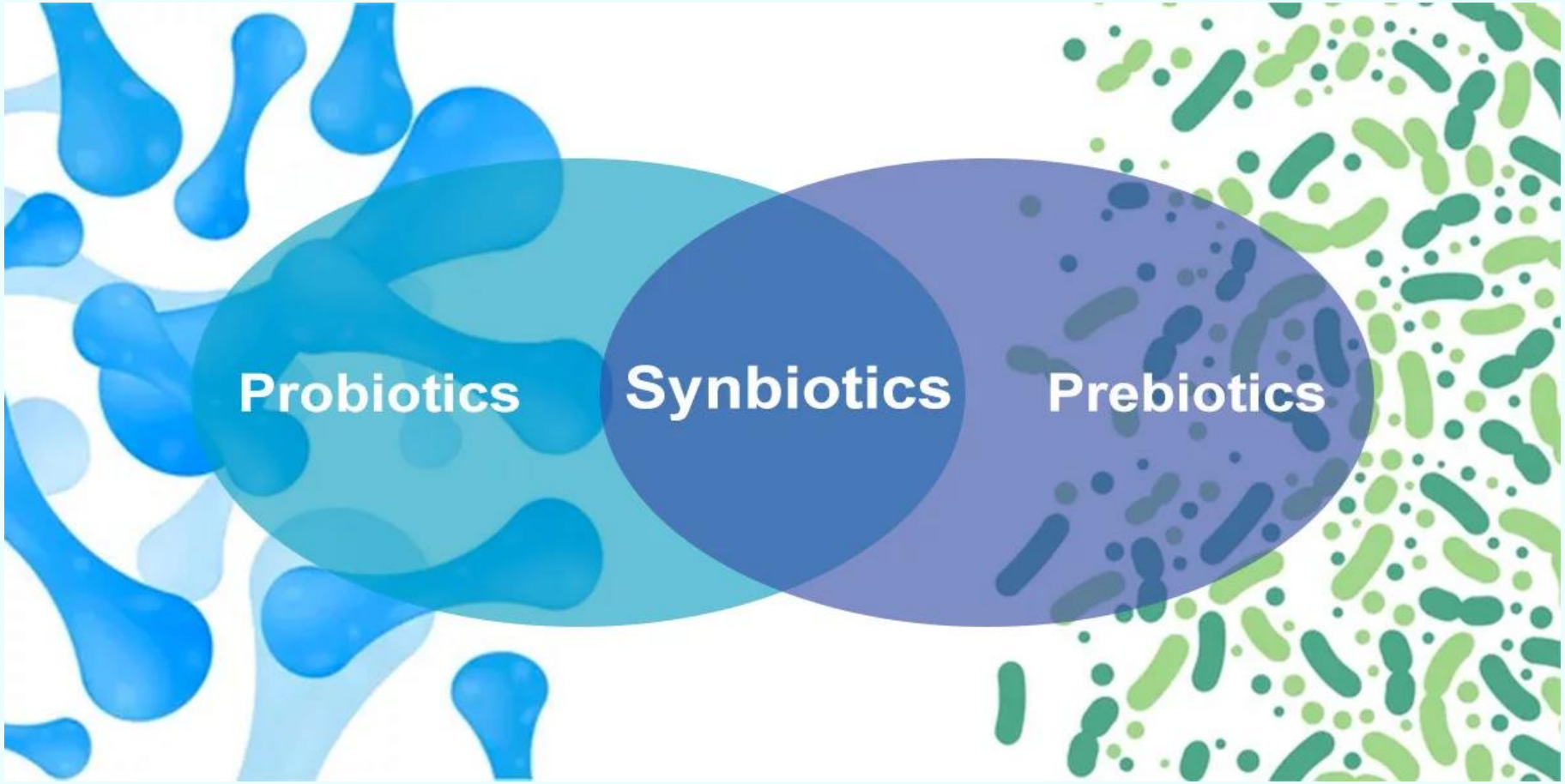
Zhou, Z., Sun, B., Yu, D., & Zhu, C. (2022). Gut Microbiota: An Important Player in Type 2 Diabetes Mellitus. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.834485

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



Cunningham, A., Stephens, J. W., & Harris, D. A. (2021). Gut microbiota influence in type 2 diabetes mellitus (T2DM). *Gut Pathogens*, *13*(1). https://doi.org/10.1186/s13099-021-00446-0

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

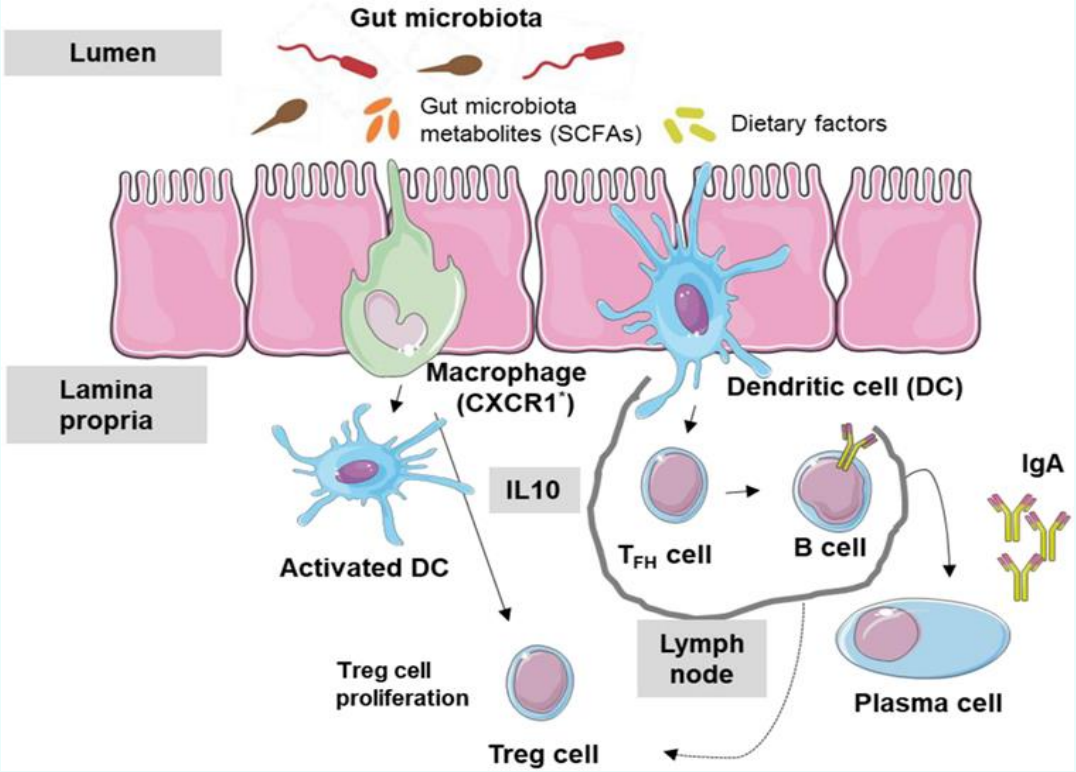
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

Allergic Diseases

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

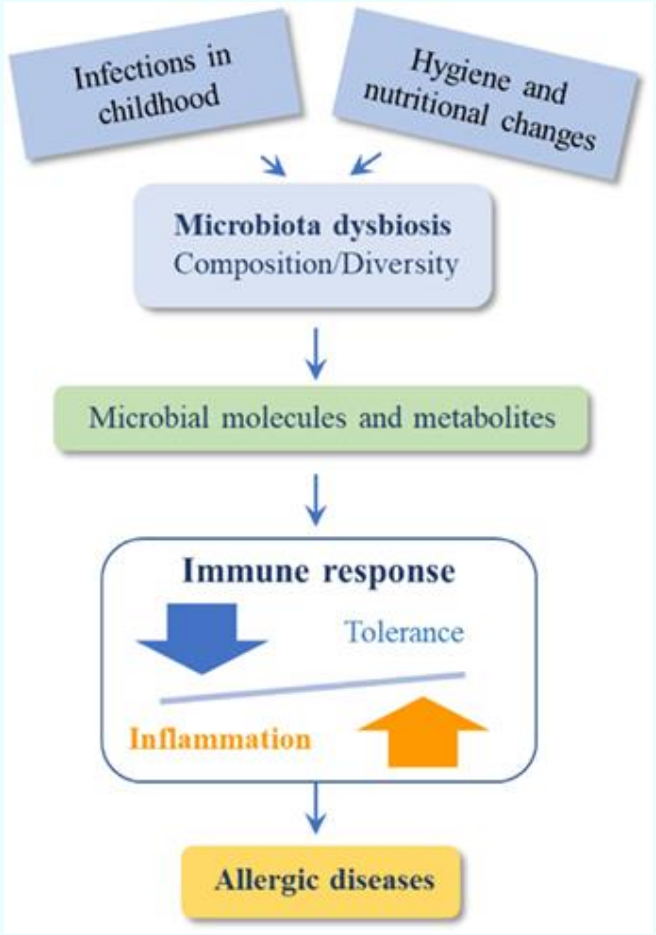
Interaction between gut microbiota and immune system.



Pascal, M., Pérez-Gordo, M., Caballero, T., Escribese, M. M., Longo, M., Luengo, O., Manso, L., Matheu, V., Seoane, E., Zamorano, M., Labrador, M., & Mayorga, C. (2018). Microbiome and allergic diseases. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.01584

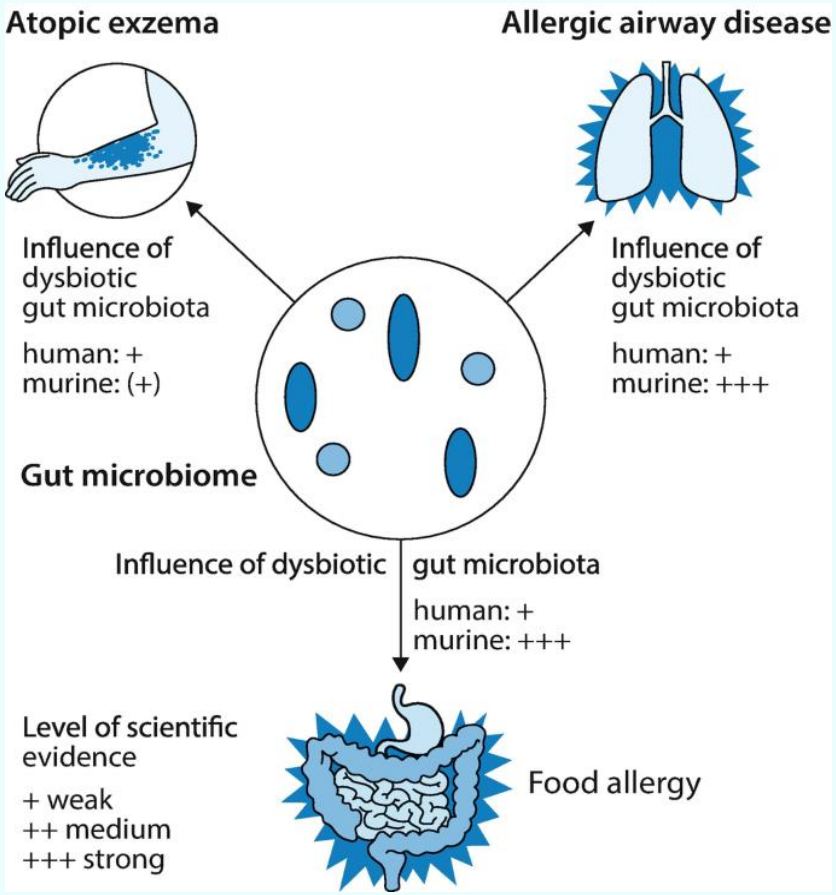
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

Dysbiosis induce qualitative and quantitative changes in the microbiota that directly affect immunological mechanisms leading to allergic diseases.



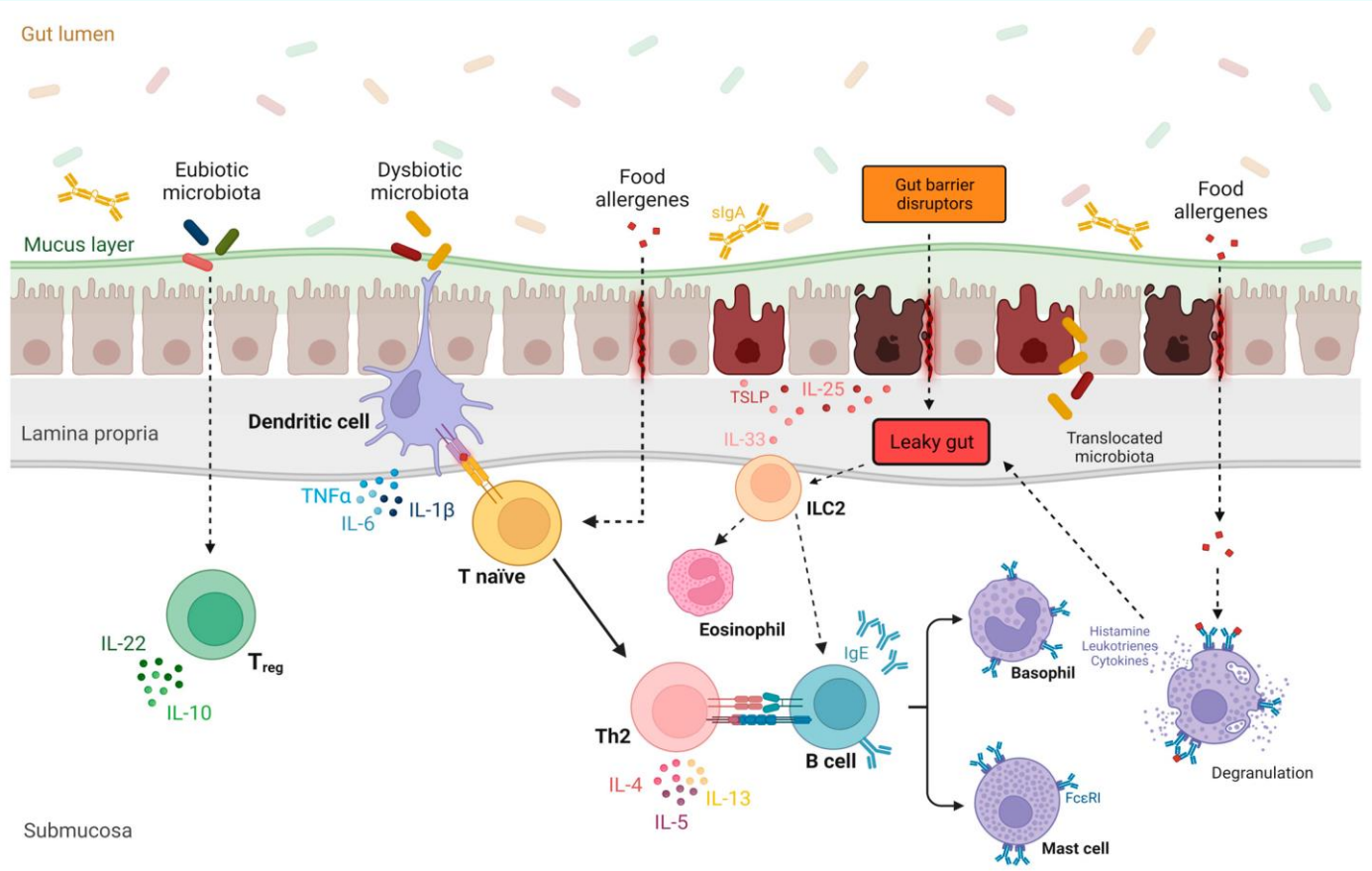
Pascal, M., Pérez-Gordo, M., Caballero, T., Escribese, M. M., Longo, M., Luengo, O., Manso, L., Matheu, V., Seoane, E., Zamorano, M., Labrador, M., & Mayorga, C. (2018). Microbiome and allergic diseases. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.01584

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



Wölbing, F., Köberle, M., Volz, T., & Biedermann, T. (2018). Microbiome and diseases: allergy. In *Springer eBooks* (pp. 175–194). https://doi.org/10.1007/978-3-319-90545-7_12

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

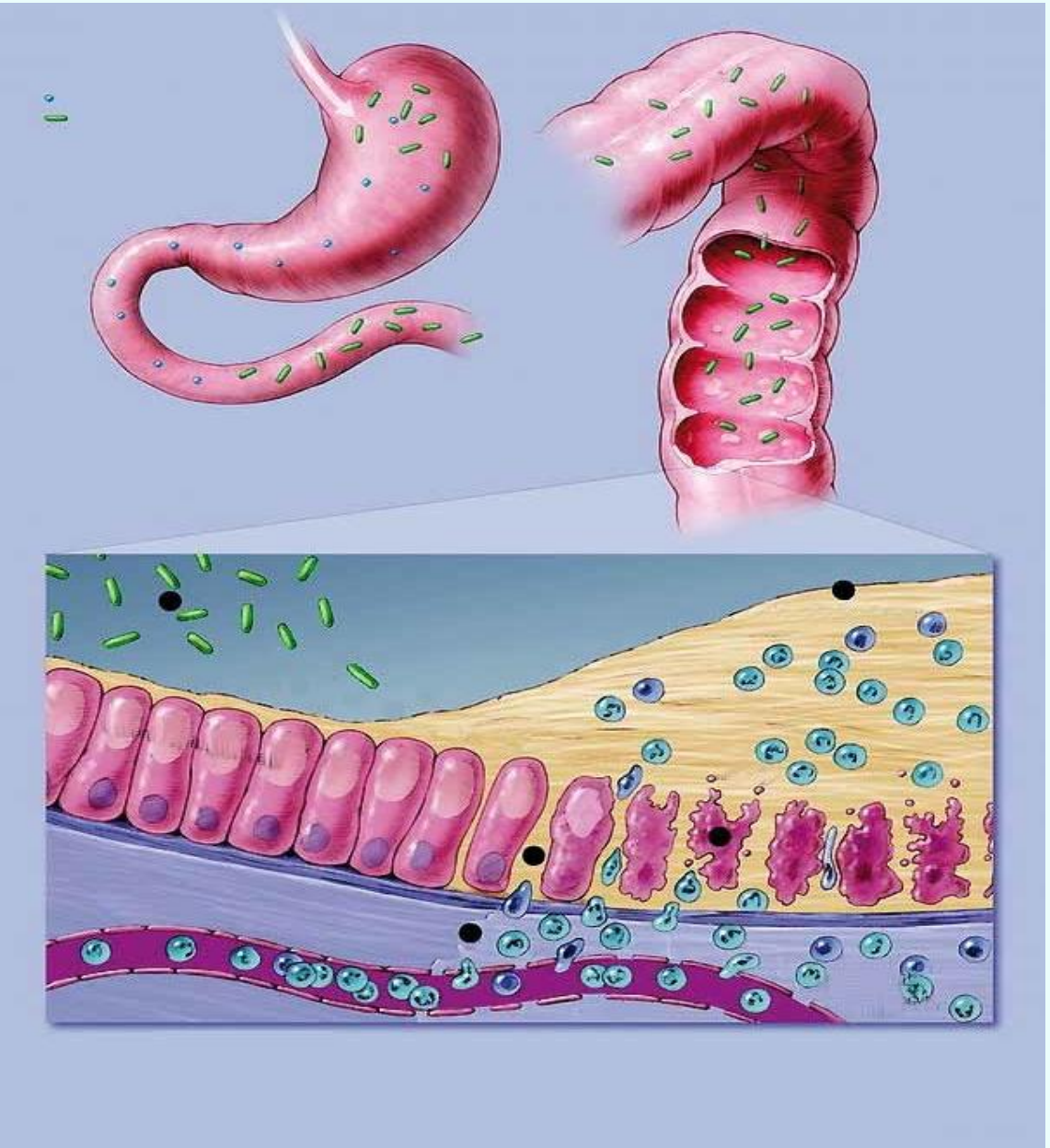


Poto, R., Fusco, W. G., Rinninella, E., Cintoni, M., Kaitsas, F., Raoul, P., Caruso, C., Mele, M. C., Varricchi, G., Gasbarrini, A., Cammarota, G., & Ianiro, G. (2023). The role of gut microbiota and leaky gut in the pathogenesis of food allergy. *Nutrients*, *16*(1), 92. https://doi.org/10.3390/nu16010092

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

Clostridium difficile-associated diarrhoea (CDAD) and stool trasplantation

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



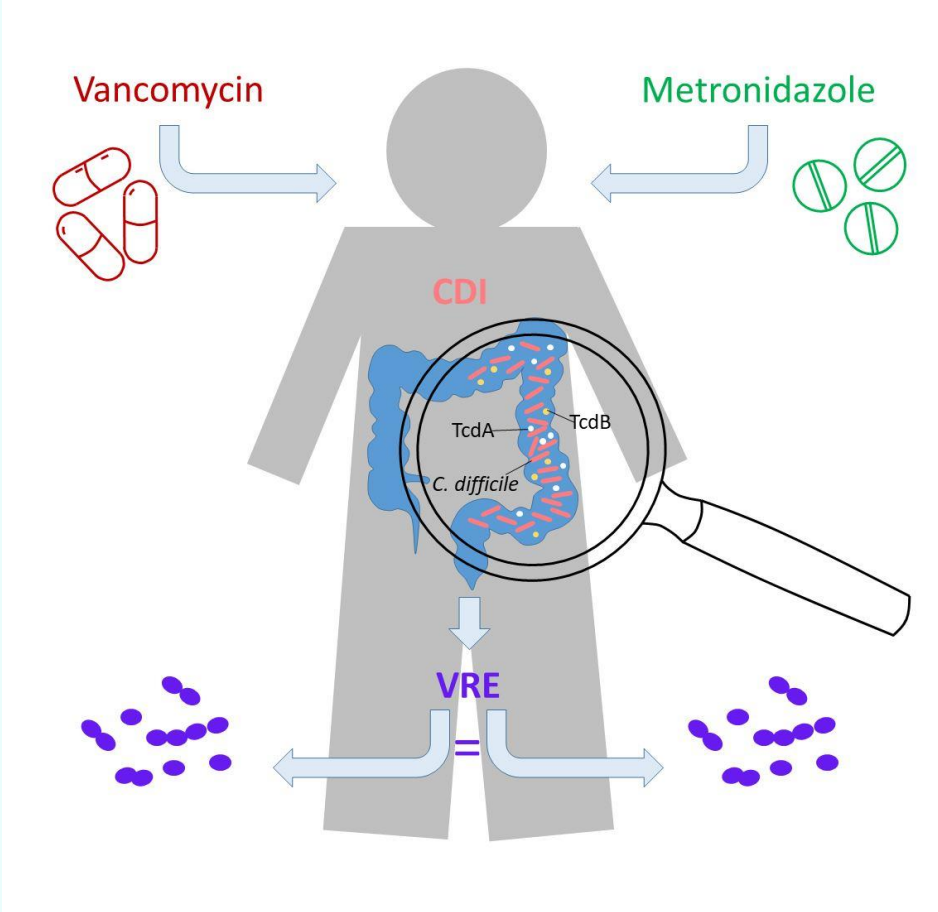
Pathogenesis of Clostridium difficile-associated diarrhoea.

From Poutanen and Simor (2004)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

Clostridium difficile

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

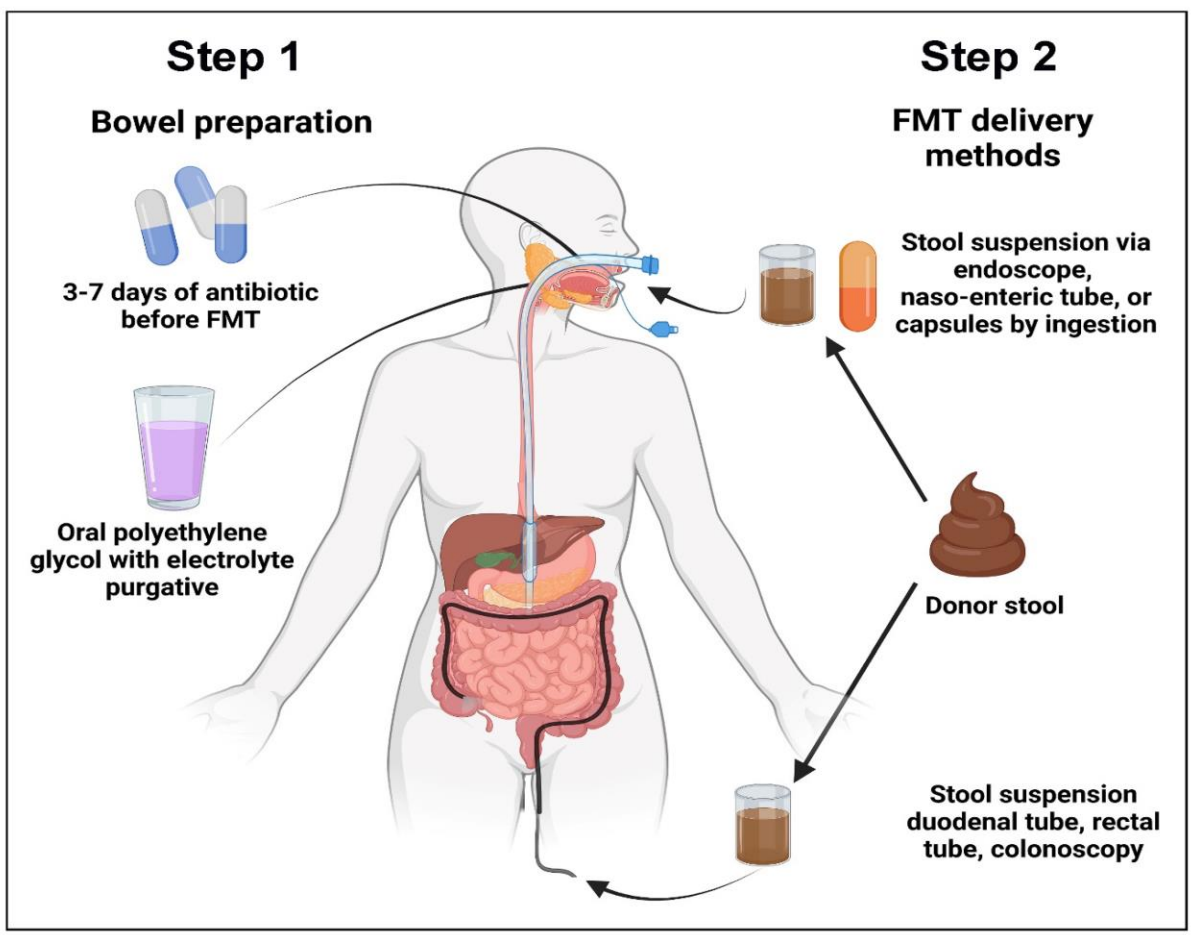


Correa-Martínez, C. L., Hagemeier, N. C. J., Froböse, N. J., & Kampmeier, S. (2021). Impact of Clostridioides difficile Therapy on Nosocomial Acquisition of Vancomycin-Resistant Enterococci. *Pharmaceuticals*, *14*(11), 1066. https://doi.org/10.3390/ph14111066

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

UK case series

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



Zerdan, M. B., Niforatos, S., Nasr, S., Nasr, D., Ombada, M., John, S., Dutta, D., & Lim, S. H. (2022). Fecal microbiota transplant for hematologic and oncologic diseases: Principle and practice. *Cancers*, *14*(3), 691. https://doi.org/10.3390/cancers14030691

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

Outcomes

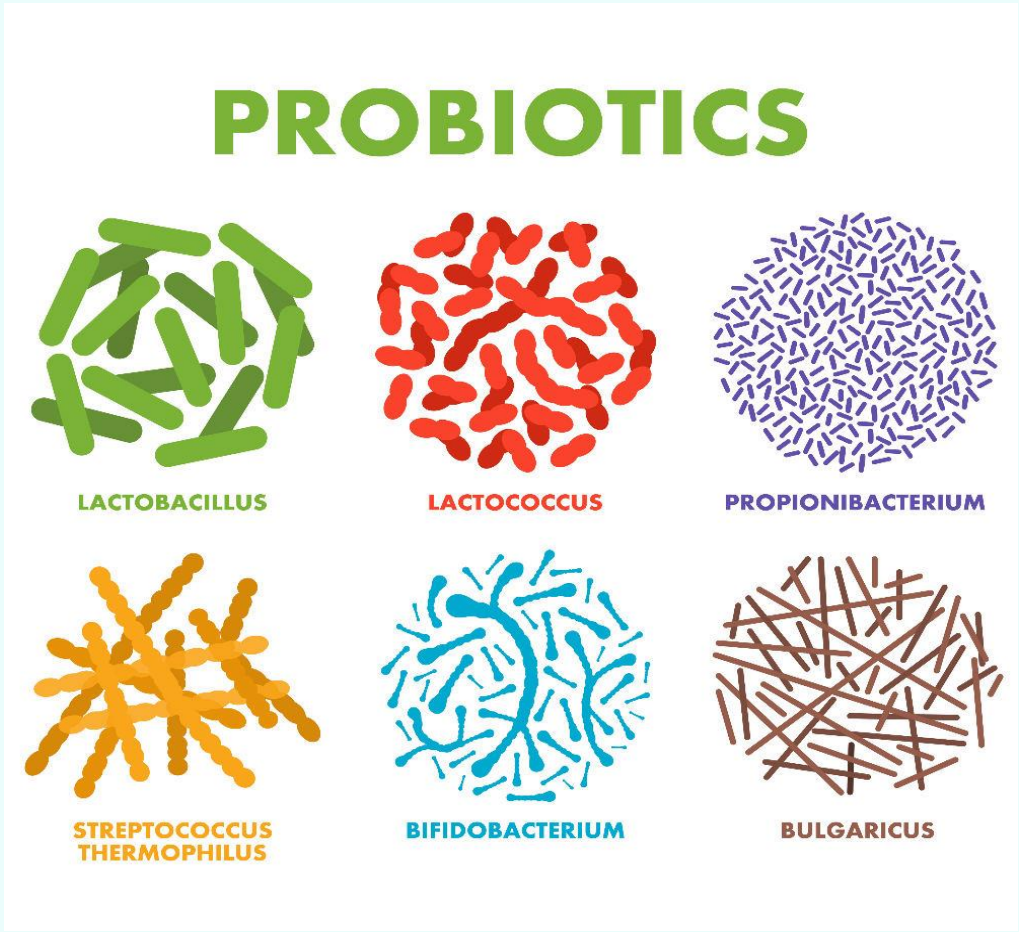
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

Probiotics and Clostridium Difficile cases. Systematic review

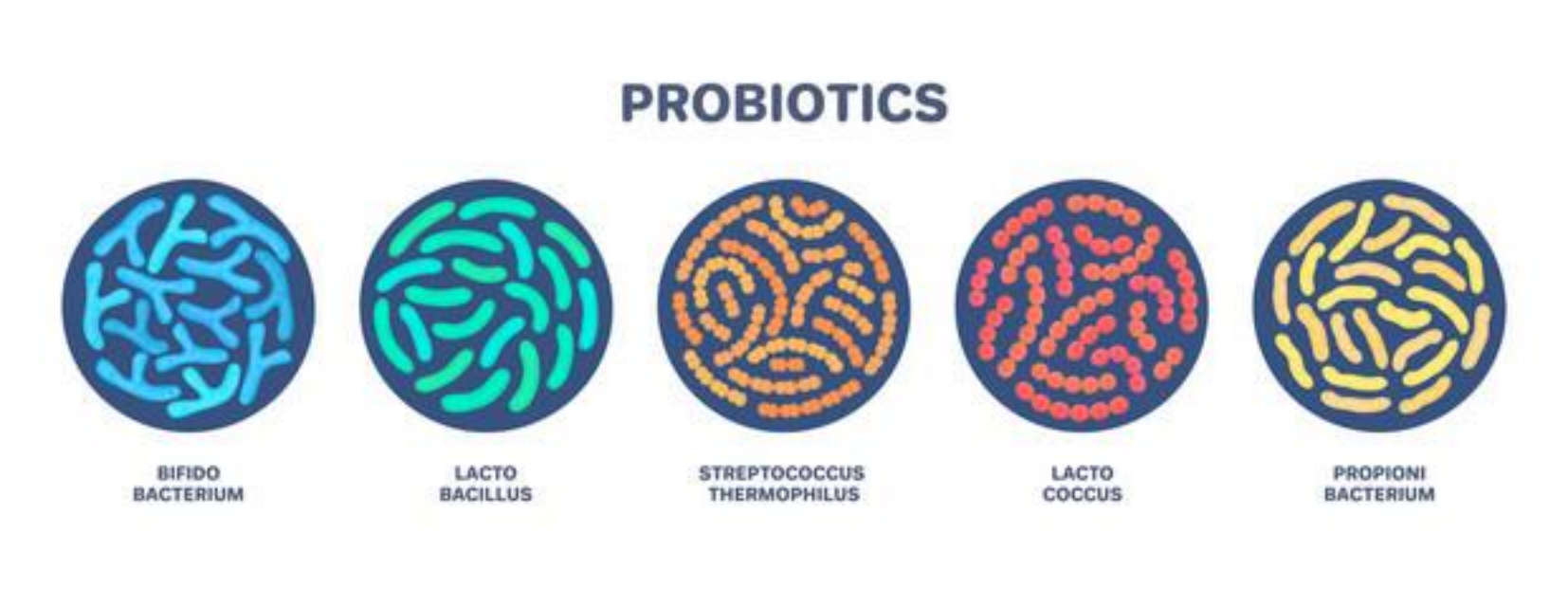
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

A 72-year-old male was admitted to the hospital for severe right lobe consolidation of the lung and was receiving treatment with fluoroquinolones and intravenous therapy (IV). On his fourth day of admission, he complained of severe abdominal pain and was passing watery stools five times a day. His blood work showed increased leukocytes and his stool culture for the Clostridium difficile (C. difficile) toxin was positive.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com

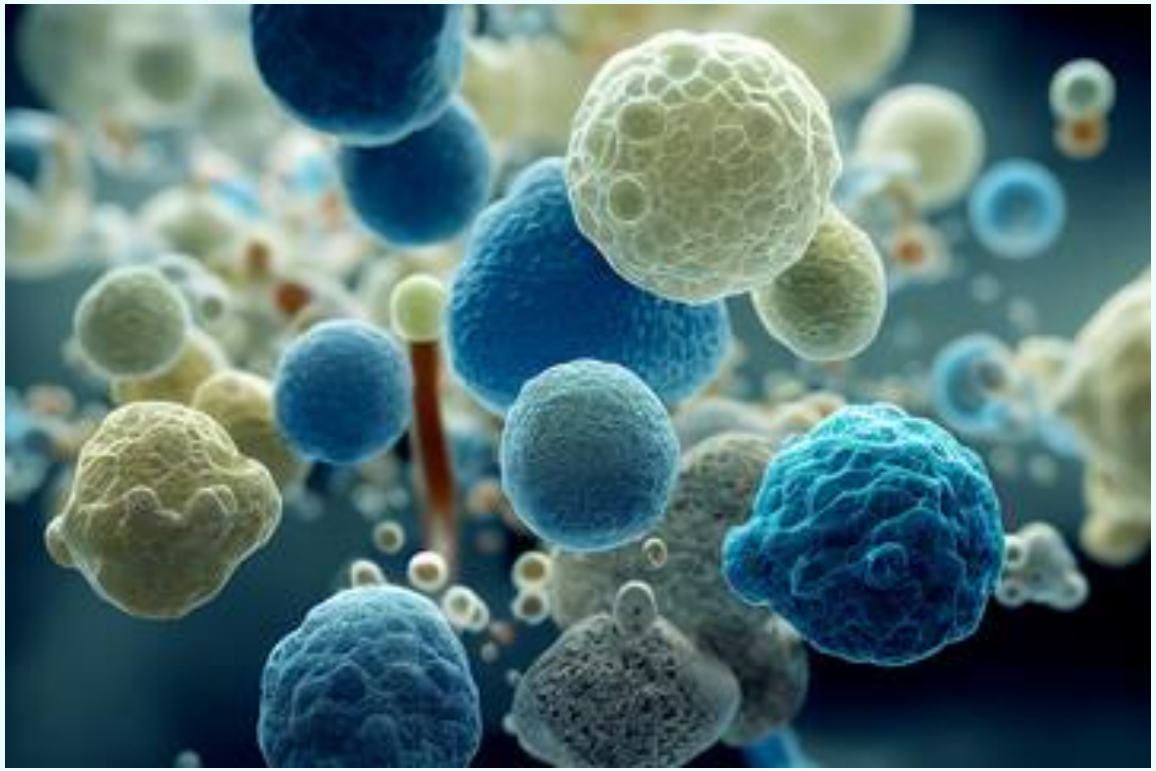


INSTITUTE OF MICROBIOME AND APPLIED SCIENCES

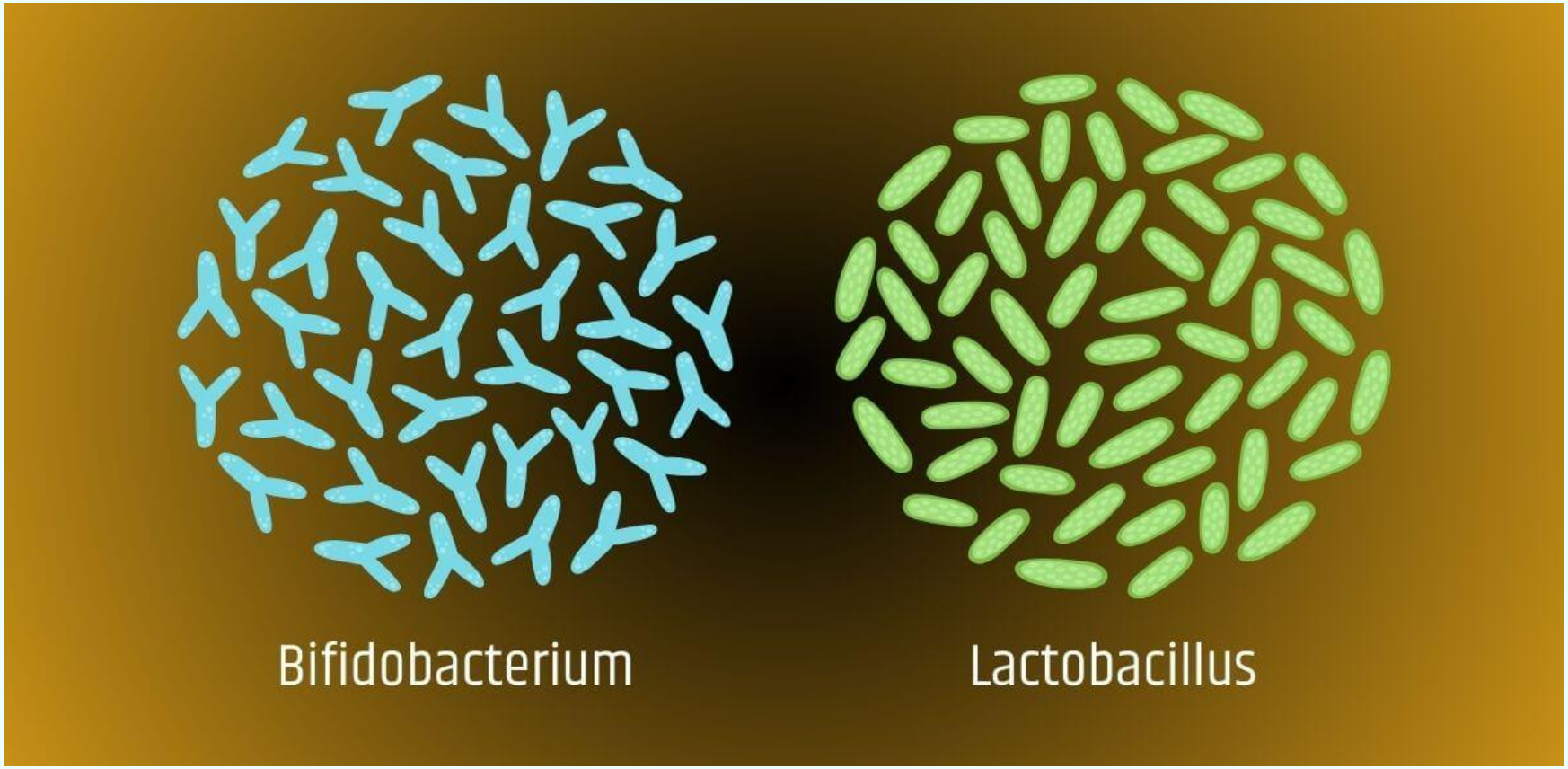
contact@imas.edu.eu

+356 21383758

kalaubek.ainur98@gmail.com



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
kalaubek.ainur98@gmail.com



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

- O'Neill LAJ , Golenbock D , Bowie AG . The history of Toll-like receptors — redefining innate immunity. Nat Rev Immunol 2013;13:453–60.doi:10.1038/nri3446 CrossRefPubMedGoogle Scholar
- Chu H , Mazmanian SK . Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nat Immunol 2013;14:668–75.doi:10.1038/ni.2635 CrossRefPubMedGoogle Scholar
- Ximenez C , Torres J . Development of microbiota in infants and its role in maturation of gut mucosa and immune system. Arch Med Res 2017;48:666–80.doi:10.1016/j.arcmed.2017.11.007
- Mowat AM , Agace WW . Regional specialization within the intestinal immune system. Nat Rev Immunol 2014;14:667–85.doi:10.1038/nri3738 CrossRefPubMedGoogle Scholar

- Hooper LV , Macpherson AJ . Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol 2010;10:159–69.doi:10.1038/nri2710
- Pascal, M., Pérez-Gordo, M., Caballero, T., Escribese, M. M., Longo, M., Luengo, O., Manso, L., Matheu, V., Seoane, E., Zamorano, M., Labrador, M., & Mayorga, C. (2018). Microbiome and allergic diseases. *Frontiers in Immunology*, *9*. https://doi.org/10.3389/fimmu.2018.01584
- Zhou, Z., Sun, B., Yu, D., & Zhu, C. (2022). Gut Microbiota: An Important Player in Type 2 Diabetes Mellitus. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.834485

STUDY MATERIAL

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

Microbiome and clinical implication

1. Introduction

Every human being carries between 10 trillion and 100 trillion microbial cells in a symbiotic connection. Some studies indicate that there are around ten times more microbial cells in the body than human cells, while others believe the ratio is closer to 1:1.

This symbiotic interaction helps both bacteria and hosts as long as the body is in good condition. The human microbiota may include approximately 1,000 distinct types of bacteria, according to various estimates. The mouth cavity, sexual organs, skin, gastrointestinal system, and respiratory tract are all rich in microbial cells. However, the types, levels, and functions will differ by place.

In this discourse, we explain the complex world of the microbiome and its important clinical applications. We will look at how the composition and variety of microbes in our bodies affects human health and disease. Real-life examples will be explored to demonstrate practical uses in healthcare conditions.

The gut microbiota is increasingly recognized as a key collaborator of human cells, interacting with nearly all human cells. According to Patrice D Cani, many mechanisms detect and monitor the existence of microbes in the body. For example, in the gastrointestinal tract (GI), epithelial cells serve as guardians, translating crucial information to immune cells in the lamina propria. In reality, the innate immune system recognizes and monitors bacteria primarily through pattern recognition receptors (PRRs) such as toll-like receptors (TLRs) and NOD-like receptors (NLR) (Luke A.J. O'Neill).

TLRs are transmembrane receptors found on cell surfaces (TLR-2, 4, 5) or in endolysosomal compartments (TLR-3, 7, 8, 9, or 13), whereas NOD receptors are cytosolic proteins. These receptors work together to recognize pathogen-associated molecular patterns (PAMPs) from microbes (for example, lipopolysaccharides, peptidoglycans, lipoteichoic acid, flagellin, and muramyldipeptide) as well as danger-associated molecular patterns from injured tissues. Thus, the GI tract not only contains the great majority of microorganisms found in the human

body, but it also likely houses the largest pool of immune cells (Mowat AM, Agace WW). Notably, the immune system has a significant impact on the makeup of the microbiota.

Outline:

1.Introduction

2. General Features of the Infant Gut Microbiota

3. Microbial activity, metabolites and metabolism

4. What matters: quality, quantity or activity of microbes?

5. Microbial diversity as a biomarker for health

6. Presence and abundance of bifidobacteria as a biomarker for health

7. Microbial lipids and proteins as Modulators of gut health

8. Microbiota modulation through prebiotics – I don’t know if we need this ?

9. Cases

10. Conclusion and perpspectives

References

1. O’Neill LAJ , Golenbock D , Bowie AG . The history of Toll-like receptors — redefining innate immunity. Nat Rev Immunol 2013;13:453–60.doi:10.1038/nri3446 CrossRefPubMedGoogle Scholar

2. Chu H , Mazmanian SK . Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nat Immunol 2013;14:668–75.doi:10.1038/ni.2635 CrossRefPubMedGoogle Scholar

3. Ximenez C , Torres J . Development of microbiota in infants and its role in maturation of gut mucosa and immune system. Arch Med Res 2017;48:666–80.doi:10.1016/j.arcmed.2017.11.007

4. Mowat AM , Agace WW . Regional specialization within the intestinal immune system. Nat Rev Immunol 2014;14:667–85.doi:10.1038/nri3738 CrossRefPubMedGoogle Scholar

5. Hooper LV , Macpherson AJ . Immune adaptations that maintain homeostasis with the intestinal microbiota. Nat Rev Immunol 2010;10:159–69.doi:10.1038/nri2710

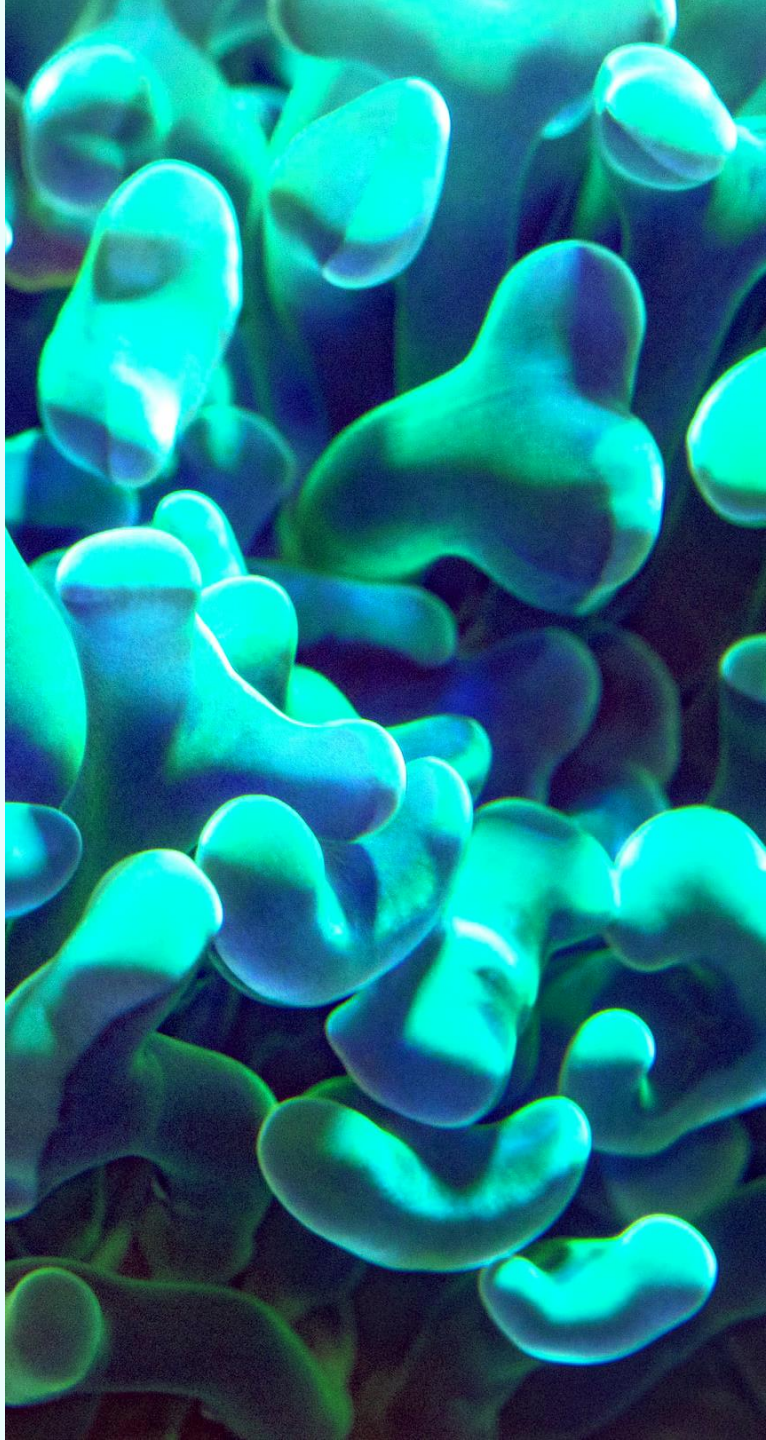
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Post-graduate certificate level 7 (30 ECTS)

UTILITY OF MICROBIOME IN OSTEOPATHY

Donjeta Shala
BSc Dietitian, MSc Food safety and Quality Engineer
shala.dnjta@gmail.com

Malta Further & Higher Education Authority



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Table of content

- Introduction – What is Osteopathy?
- Diseases and Osteopathy
- Evidence based research for Osteopathy
- Gut microbiome in musculoskeletal system
- Limitations of Osteopathy
- Conclusion and take away message

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

What is Osteopathy

- Alternative medicine that emphasizes physical manipulation of the body's muscle tissue and bones.
- The practice of this alternative medicine is by osteopaths.
- Five main key principles; holistic approach, body structure and function, manual therapy, self-healing and preventive care.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Diseases and Osteopathy

- Wide range of conditions.

  - Musculoskeletal issues
    - Osteoporosis
    - Fibromyalgia
    - Arthritis
    - Migraines
    - Neck pain
    - Sport injuries or recovery

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Evidence based research for Osteopathy

- More research is needed to be conducted.

Musculoskeletal Disorders:

- Osteopathic manipulative treatment shows *promising results* in reducing pain and improving functional status for acute and chronic *non-specific low back pain (NSLBP) and chronic non-specific neck pain (CNSNP) and it's also effective in managing chronic non-cancer pain (CNCP).*
- However, evidence for *pediatric conditions, primary headaches, and for digestive issues such as irritable bowel syndrome (IBS) it remains inconclusive.*

Neurological Conditions:

- Integrated OCF–OMT treatments *positively* affected gait kinematics in patients with *Parkinson's disease*.
- OCF treatments have been associated with pain reduction and improved functionality in patients with chronic pain.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Evidence based research for Osteopathy

Mental Health:

- osteopathy is commonly used to *treat musculoskeletal disorders, including back pain*.
- It has been *proposed for inclusion in chronic pain management guidelines*.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiome in musculoskeletal system

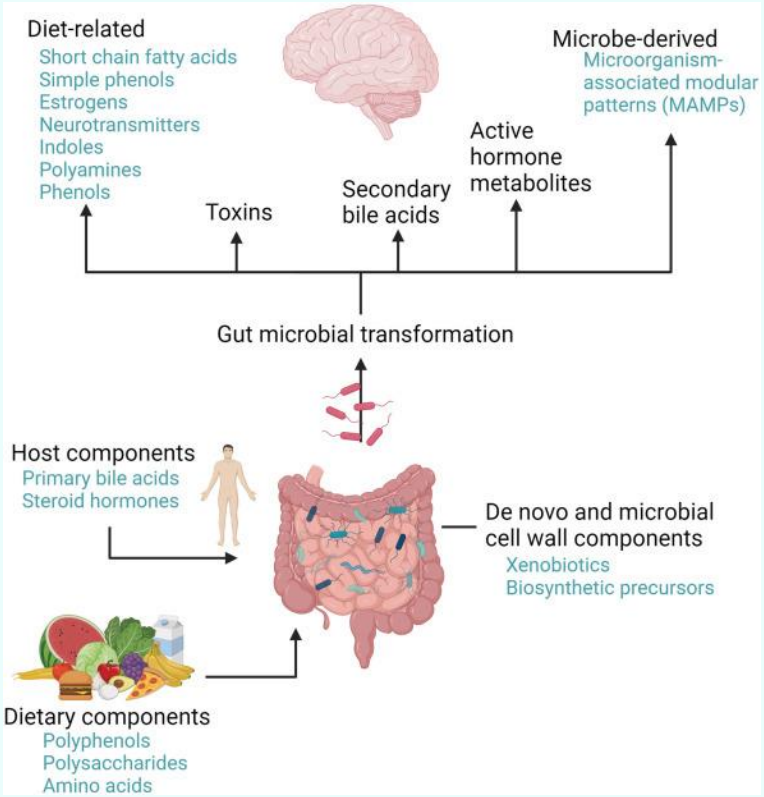
- The mechanisms by which the gut microbiota influences muscle physiology are a topic of recent research. The primary metabolites studied in this context are short-chain fatty acids (SCFAs).
- Certain bacteria are associated with bone density and microarchitecture.
- Gut microbes modulate bone strength, density, and composition in powerful ways.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut microbiome in musculoskeletal system

A study led by Harvard Medical School researchers found links between specific gut bacteria and bone health.

- For instance, bacteria like Akkermansia and Clostridiales bacterium DTU089 were negatively associated with bone density and composition. Interestingly, some bacterial types were negatively associated with bone density but positively associated with bone size, suggesting an influence on bone growth as people age.



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Limitations of Osteopathy

Osteopathy, like any medical practice, has its limitations. Understanding these limitations can help patients make informed decisions about their healthcare.

- Limited Scope
- Limited Research
- Not Recommended in Certain Cases
- Side Effects

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Conclusion and take away message

- Osteopathy can be a valuable part of a comprehensive health care plan, particularly for managing musculoskeletal pain and promoting overall well-being. However, it should not replace conventional medical care for serious or systemic conditions.

- Always consult with a healthcare provider to determine the best approach for your individual health needs and ensure that osteopathic treatment is appropriate for your specific situation.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

- Barsotti N, Casini A, Chiera M, Lunghi C, Fornari M. Neurophysiology, Neuro-Immune Interactions, and Mechanobiology in Osteopathy in the Cranial Field: An Evidence-Informed Perspective for a Scientific Rationale. Healthcare (Basel). 2023 Nov 28;11(23):3058. doi: 10.3390/healthcare11233058. PMID: 38063626; PMCID: PMC10705895.

- Bagagiolo D, Rosa D, Borrelli F. Efficacy and safety of osteopathic manipulative treatment: an overview of systematic reviews. BMJ Open. 2022 Apr 12;12(4):e053468. doi: 10.1136/bmjopen-2021-053468. PMID: 35414546; PMCID: PMC9021775.

- Bohlen L, Shaw R, Cerritelli F, Esteves JE. Osteopathy and Mental Health: An Embodied, Predictive, and Interoceptive Framework. Front Psychol. 2021 Oct 27;12:767005. doi: 10.3389/fpsyg.2021.767005. PMID: 34777176; PMCID: PMC8578726.

- Li G, Jin B, Fan Z. Mechanisms Involved in Gut Microbiota Regulation of Skeletal Muscle. Oxid Med Cell Longev. 2022 May 18;2022:2151191. doi: 10.1155/2022/2151191. PMID: 35633886; PMCID: PMC9132697.

- The Gut Microbiome's Role in Skeletal Health | Harvard Medical School.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thank you for your kind attention!

“Utility of microbiome in osteopathy”

11/06/2024

Donjeta Shala

- **Institute of Microbiome and Applied Sciences**
- **For admin questions contact: shala.dnjta@gmail.com**
- **Type of course: Post-graduate certificate level 7 (30 ECTS)**
- **Utility of microbiome in osteopathy**
- **Dietitian Donjeta Shala, MSc in Food Safety and Quality Engineering**

1. Introduction

Nowadays alternative treatments are being more and more explored, one of them includes the osteopathy.

Osteopathy includes hands-on manipulations of different body structures to increase systemic homeostasis and total patient well-being. The practice of this alternative medicine is by osteopaths. An osteopath is a licensed physician who practices medicine using both conventional treatments and osteopathic manipulative medicine, focusing on relieving pain and tension in the musculoskeletal system. They receive special training in treating people as a whole instead of targeting specific systems and symptoms by emphasizing the body’s innate principles of self-healing and modification to achieve homeostasis. This is done mainly by osteopaths through massages, stretching stiff joints, articulation and high-velocity thrusts which are short, sharp movements to the spine, creating a clicking noise similar to cracking your knuckles.

Although osteopathy has been practiced for quite a long time more research is needed to confirm its effectiveness.

2. Treatment of diseases with osteopathy

Osteopathic physicians can treat the full spectrum of medical conditions. However, the practice of osteopathic manipulative treatment OMT is thought to be particularly useful in treating difficulties or problems involving the musculoskeletal system, such as;

- ✓ Osteoporosis

- ✓ Fibromyalgia
- ✓ Arthritis
- ✓ Migraines
- ✓ Neck pain
- ✓ Sport injuries or recovery

Osteoporosis

Osteoporosis is a widespread metabolic bone disorder marked by bone density reduction and bone tissue's microstructure degradation, leading to increased bone fragility and a higher risk of fractures. As the global population ages osteoporosis has become a more significant concern impacting individuals and families. Although it can affect people of all genders and ages, it is most prevalent among postmenopausal women. When we're younger our body makes new bone faster than it breaks down old bone and the bone mass increases. After the early 20s this process slows down, and most people reach their peak bone mass by age 30. As people age, bone mass is lost faster than it's created. Thus, how likely we are to develop osteoporosis depends partly on how much bone mass we attained in our youth. Peak bone mass is partly inherited and varies as well by ethnic group. The higher the peak bone mass, the more bone we have and the less likely we are to develop osteoporosis as we age. The link between osteoclast, macrophage colony stimulating factor and pro-inflammatory cytokines, especially tumor necrosis factor-alpha and interleukin-1 explain the association between inflammation and osteoporosis.

Fibromyalgia

Fibromyalgia is a chronic (long-lasting) disorder that causes pain and tenderness throughout the body, as well as fatigue. Scientists do not fully understand what causes it, but people with the disorder have an increased sensitivity to pain. A multidimensional approach is required for the management of FM, including pain management, pharmacological therapies, behavioral therapy, patient education, and exercise. There is no cure for fibromyalgia, but doctors and other health care providers can help manage and treat the symptoms. Treatment typically involves a combination of exercise or other movement therapies, psychological and behavioral therapy, and medications.

Brain imaging studies and other research have uncovered evidence of altered signaling in neural pathways that transmit and receive pain in people with fibromyalgia. These changes may also contribute to the fatigue, sleep troubles, and "fibro fog" issues that many people with the disorder experience. Recent studies have suggested that autoimmunity and neuroinflammation may play a role in fibromyalgia. Some people with fibromyalgia may also have small-fiber neuropathy, which is nerve damage in the skin.

Arthritis

Arthritis is characterized by the swelling and tenderness of one or more joints. Its primary symptoms include joint pain and stiffness, which generally become more severe with age. The inflammatory pathway occurs when the body's immune system attacks healthy joint tissue causing pain, stiffness, and swelling in the joints. There are several types of inflammatory arthritis, including rheumatoid arthritis, juvenile idiopathic arthritis, and gout. The two most prevalent forms of arthritis are osteoarthritis and rheumatoid arthritis, each affecting the joints differently. Osteoarthritis, the most common type, results from the degradation of cartilage—the firm, smooth tissue that covers the ends of bones in a joint. This type of arthritis is associated with wear-and-tear damage to the cartilage, which normally cushions the bone ends and allows for nearly frictionless joint movement. When the cartilage deteriorates significantly, bones may grind directly against each other, leading to pain and limited mobility.

Migraines

A migraine is a headache that can cause severe throbbing pain or a pulsing sensation, usually on one side of the head. It's often accompanied by nausea, vomiting, and extreme sensitivity to light and sound. Migraine attacks can last for hours to days, and the pain can be so bad that it interferes with your daily activities. For some people, a warning symptom known as an aura occurs before or with the headache. An aura can include visual disturbances, such as flashes of light or blind spots, or other disturbances, such as tingling on one side of the face or in an arm or leg and difficulty speaking. During a migraine attack, the levels of cytokines and CRP heighten, which can cause symptoms like fatigue, nausea, headaches, and brain fog. This is because most individuals with migraine already have a higher baseline of inflammation than the general population, and any further elevation of inflammation can become a problem1. To reduce inflammation and improve migraine, you can try nonsteroidal anti-inflammatory drugs (NSAIDs), stress busters, moderate exercise, high-quality sleep, and an anti-inflammatory diet.

Neck pain

Neck pain, sometimes called cervicalgia, is pain in or around your spine beneath your head. Your neck is also known as your cervical spine. Neck pain is a common symptom of many different injuries and medical conditions. It might be an axial neck pain (felt mostly in your neck) or radicular neck pain (pain that shoots into other areas, such as your shoulders or arms). It can be acute (lasting from days to six weeks) or chronic (lasting longer than three months). Neck pain can interfere with your daily activities and reduce your quality of life if it's not treated. Fortunately, most causes of neck pain aren't serious and improve with

conservative treatments, like pain medicine, exercise and stress management. Regarding the inflammatory pathway, there is evidence of raised inflammatory markers in the blood of patients with musculoskeletal pain including low back pain (LBP). Increased CRP and raised macrophage inflammatory protein (MIP)-1β and IL-12 have also been found in people with chronic (median 6 years) work-related neck and shoulder pain.

Sport recovery/injuries

Generally speaking, injuries are broadly defined as tissue damage. When an individual performs activity, their body is exposed to various forces. For simplicity, we will refer to the sum of these forces as load. Load causes tissues to undergo deformation. Upon deformation, tissue cells try to keep or restore themselves to their original state, causing an internal resistance known as stress. Loads beyond a tissue's load capacity (or load tolerance) will cause excessive stress leading to tissue damage. Fatigue is included when we speak about sport injuries, namely fatigue is the loss of tissue strength upon repeated loading and associated microdamage. While not considered an injury in itself, it results in decreased load capacity, which can make a tissue more susceptible to injury. Muscle fatigue specifically refers to a reduction in the maximal force or ability to maintain maximal force of the muscles due to repeated use, and is reversible with rest. It occurs due to impairments in the contractile proteins and structures that allow muscles to generate force, which can be considered as damage at the cell cytoskeleton level.

3. Diet and supplementation for musculoskeletal system

Lifestyle modifications are consistently recommended to musculoskeletal injuries/diseases. Key strategies include; adequate intake of calcium and vitamin D, engaging in regular exercise, quitting smoking and avoiding excessive alcohol consumption

Dietary changes and exercise are among the most effective and conservative treatment methods. Research in rodents has shown promising outcomes from incorporating high-fiber diets, which boost the gut microbiome's production of short-chain fatty acids (SCFA), particularly butyrate. Butyrate is a primary metabolite produced by microbial fermentation in the gut and has shown potential benefits for bone health. We can add that almost all kinds of diets are fermented by the gut microbiome, where fibers are broken down into short-chain fatty acids (SCFAs), lowering the intestinal pH and impacting bone minerals. Although this explanation is very simple, the underlying mechanisms are way more complex.

Increased calcium intake supplemented with vitamin D has been proven to slow down bone mineral loss without negatively impacting the intestinal microbiota. Patients with insufficient dietary calcium should take supplemental elemental calcium (typically 500 to 1000 mg/day) in divided doses with meals, aiming for a total daily calcium intake of around 1200 mg. The

combined intake of calcium from diet and supplements should generally not exceed 2000 mg/day due to potential adverse effects, such as kidney stones, cardiovascular issues, indigestion, interference with iron and thyroid hormone levels, and constipation. However, calcium supplementation alone may not significantly reduce fracture risk.

The appropriate dosage of vitamin D is debated, but vitamin D3 (Cholecalciferol) is known to enhance calcium absorption in the small intestine. Therefore, maintaining adequate vitamin D levels is essential and may require supplementation after evaluating the risks and benefits.

Additionally, prebiotics and probiotics have shown benefits for various chronic inflammatory conditions and are increasingly recognized for their positive effects on calcium metabolism and bone health. Prebiotics, especially non-digestible oligosaccharides (NDOs), selectively stimulate and activate beneficial bacteria in the gastrointestinal tract. This process inhibits the growth of harmful bacteria, enhances the metabolism of beneficial bacteria, and increases the secretion of SCFAs, such as butyric acid, in the gut. This, in turn, promotes calcium absorption.

Overall, a recommend a diet with adequate total calcium intake (1000 mg/day for men aged 50–70 years; 1200 mg/day for women ≥ 51 years and men ≥ 71 years), incorporating calcium supplements if intake is insufficient.

4. The role of gut microbiome in musculoskeletal system

By which mechanisms the gut microbiota is modulating muscle physiology is a recent research area. The main metabolites studied in this context are *short-chain fatty acids*. Other bacterial metabolites that may play a role in muscle physiology are emerging, including *bile acids, bacterial amino acid metabolites, and metabolites originating from the bacterial transformation of polyphenols.* Lastly, we can envision that the gut microbiome impacts muscle physiology indirectly, through a modulation of third-party tissues, such as the adipose tissues.

SCFAs, such as acetic acid, propionic acid, and butyric acid, are the main byproducts of bacterial fermentation in the colon. Butyric acid, in particular, serves as a crucial energy source for colonic epithelial cells. Studies suggest that probiotics can enhance the production of SCFAs by breaking down dietary fiber, thereby promoting calcium absorption in the intestinal wall and increasing bone mass which is beneficial for overall health regarding the musculoskeletal system. The rapid development of modern molecular biotechnology has enabled the continuous discovery of the human microbiome, and can provide more data on early events about the pathogenesis of osteoporosis.

One of the first demonstrations of the protective role of commensals during acute injury was provided by the observation that, in the gut, TLR activation by commensals was required to promote tissue repair and host survival. Up-and-coming research suggests a strong connection between gut microbiota and the regulation of bone and muscle metabolism, though the really exact mechanisms are not yet fully understood.

5. Clinical evidences and future perspectives

The innate immune response is essential for maintaining a balanced environment by eliminating harmful bacteria and regulating the adaptive response to the microbiota. This regulation involves factors like secretory IgA (sIgA), toll-like receptor 5 (TLR5), autophagy, and inflammasomes. For example, sIgA can bind to commensal bacteria, forming complexes that selectively present bacterial components to tolerogenic dendritic cells. As an anti-inflammatory molecule, sIgA helps reduce the inflammatory response that could be triggered by the large bacterial load in the organs. However, an imbalance in the microbiota, known as dysbiosis, can disrupt the sIgA response, leading to uncontrolled bacterial growth. Hapfelmeier et al. demonstrated that germ-free (GF) mice exhibited a microbiota-specific sIgA response using a reversible microbial colonization system. This induction of sIgA was confirmed as a gradual response to the current bacterial exposure, indicating communication between the microbiota and the immune system.

The adaptive immune response is also crucial for maintaining a healthy microbiota and immune balance. This response is shaped by the differentiation and maturation of B and T cells and the establishment of immune tolerance to microbiota. Depending on the bacterial species, CD4 T cell responses vary significantly, leading to differentiation into distinct subsets and the subsequent release of pro-inflammatory cytokines such as interferon-γ and interleukin IL-17A.

Additionally, the gut microbiota influences the regulation of neurotransmitter secretion, such as serotonin. For instance, *Bifidobacterium infantis* affects central serotonin transmission by increasing plasma tryptophan, a precursor to serotonin. It has also been reported that various bacterial species synthesize neurotransmitters, including acetylcholine, dopamine, noradrenaline, and serotonin. Microbiota-derived metabolites, including vitamins, neuroactive metabolites like short-chain fatty acids (SCFAs), and neurotransmitters, mediate bidirectional interactions in the microbiota-gut-brain axis (MGBA) to modulate host neurophysiology and immunity, regarding positively for the relationship between the immunity and musculoskeletal system.

From another study the results from a systematic review showed a preliminary low level of evidence that osteopathy manipulation treatment is effective in the management of headache. Nevertheless, more studies with more rigorous designs and methodology are needed to strengthen these evidences.

Given the methodological differences in much of the existing research in this field, establishing standard operating procedures is necessary to facilitate better comparisons between studies and expedite research and development. In the future, this area of research will likely depend on the combined efforts of experts in nutrition, epidemiology, medicine, immunology, and microbiology. This interdisciplinary collaboration will be crucial for gaining a comprehensive understanding of the intricate interactions between the host and gut microbiota.

References

Seely KD, Kotelko CA, Douglas H, Bealer B, Brooks AE. The Human Gut Microbiota: A Key Mediator of Osteoporosis and Osteogenesis. Int J Mol Sci. 2021 Aug 31;22(17):9452. doi: 10.3390/ijms22179452. PMID: 34502371; PMCID: PMC8431678.

Foppa C, Rizkala T, Repici A, Hassan C, Spinelli A. Microbiota and IBD: Current knowledge and future perspectives. Dig Liver Dis. 2024 Jun;56(6):911-922. doi: 10.1016/j.dld.2023.11.015. Epub 2023 Nov 25. PMID: 38008696.

Cerritelli F, Lacorte E, Ruffini N, Vanacore N. Osteopathy for primary headache patients: a systematic review. J Pain Res. 2017 Mar 14;10:601-611. doi: 10.2147/JPR.S130501. PMID: 28352200; PMCID: PMC5359118.

Sorboni SG, Moghaddam HS, Jafarzadeh-Esfehani R, Soleimanpour S. A Comprehensive Review on the Role of the Gut Microbiome in Human Neurological Disorders. Clin Microbiol Rev. 2022 Jan 19;35(1):e0033820. doi: 10.1128/CMR.00338-20. Epub 2022 Jan 5. PMID: 34985325; PMCID: PMC8729913.

Hou K, Wu ZX, Chen XY, Wang JQ, Zhang D, Xiao C, Zhu D, Koya JB, Wei L, Li J, Chen ZS. Microbiota in health and diseases. Signal Transduct Target Ther. 2022 Apr 23;7(1):135. doi: 10.1038/s41392-022-00974-4. PMID: 35461318; PMCID: PMC9034083.

Collins FL, Rios-Arce ND, Schepper JD, Parameswaran N, McCabe LR. The Potential of Probiotics as a Therapy for Osteoporosis. Microbiol Spectr. 2017 Aug;5(4):10.1128/microbiolspec.BAD-0015-2016. doi: 10.1128/microbiolspec.BAD-0015-2016. PMID: 28840819; PMCID: PMC5710820.

Abboud M, Papandreou D. Gut Microbiome, Probiotics and Bone: An Updated Mini Review. Open Access Maced J Med Sci. 2019 Feb 11;7(3):478-481. doi: 10.3889/oamjms.2019.047. PMID: 30834022; PMCID: PMC6390135.

Weaver CM. Diet, gut microbiome, and bone health. Curr Osteoporos Rep. 2015 Apr;13(2):125-30. doi: 10.1007/s11914-015-0257-0. PMID: 25616772; PMCID: PMC4996260.

Ding K, Hua F, Ding W. Gut Microbiome and Osteoporosis. Aging Dis. 2020 Mar 9;11(2):438-447. doi: 10.14336/AD.2019.0523. PMID: 32257552; PMCID: PMC7069453.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

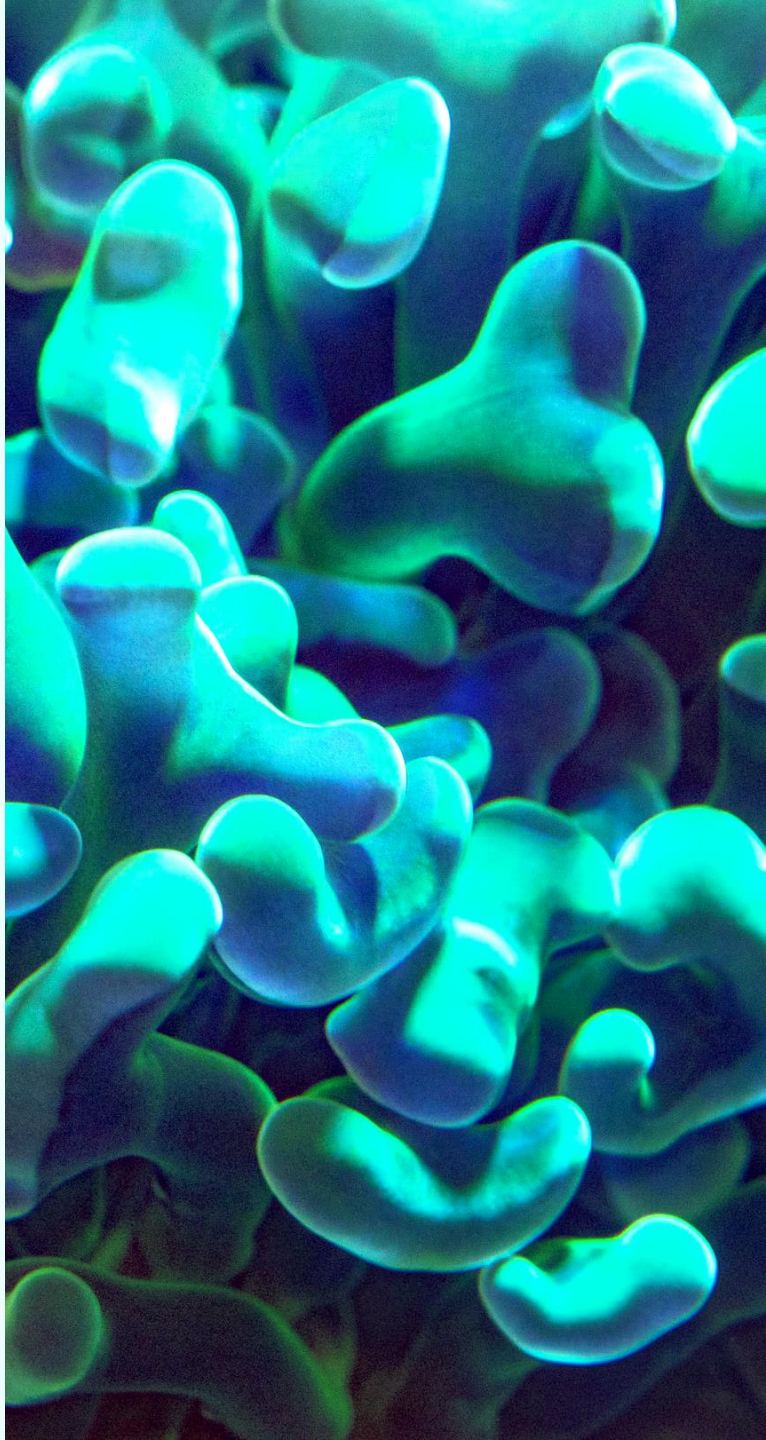
Post-graduate certificate level 7 (30 ECTS)

The Microbiome Transplantation

Lana Novković
MSc. Nutr.

lananovkovic147@gmail.com

Malta Further & Higher Education Authority



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Summary

lananovkovic147@gmail.com

- This webinar will provide an in-depth exploration of **human microbiota transplantation**, focusing on its applications in treating a range of health conditions linked to microbial imbalances. The session will highlight **fecal microbiota transplantation** (FMT), which has gained significant traction in treating recurrent Clostridioides difficile infections (rCDI). We will cover the mechanics of how FMT restores microbial diversity and promotes gut health, making it a powerful alternative to conventional antibiotic treatments.
- Beyond rCDI, the webinar will delve into the emerging use of FMT for conditions such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and metabolic syndrome. We will explore the role of microbiome restoration in reducing inflammation, improving immune function, and addressing metabolic dysfunctions. Additionally, we will discuss donor selection strategies, including the concept of super donors, and how matching the donor's microbiota to the recipient can influence treatment success.
- The session will also introduce and **skin microbiota transplantation** (SMT) for skin conditions such as atopic dermatitis and acne and **vaginal microbiota transplantation** (VMT) focusing on its promising role in treating recurrent bacterial vaginosis (BV). These emerging fields offer new possibilities for managing conditions where traditional treatments often fall short.
- Finally, the webinar will address the **challenges and future** of microbiota transplantation, including regulatory considerations and ong-term safety, as well as the role of omics technologies in advancing this personalized approach to healthcare. This session will provide a comprehensive overview of the current state and future potential of microbiota transplantation in clinical practice.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Learning objectives

1. Understand the concept and applications of microbiota transplantation, including fecal, vaginal, and skin microbiota transplantation.
2. Recognize the effectiveness of FMT in treating recurrent *Clostridioides difficile* infections (rCDI), with a success rate of over 90%.
3. Explore the potential of FMT for managing chronic conditions such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), and understand the factors influencing its success rates.
4. Identify the mechanisms of action behind FMT, including restoring microbiome balance, promoting colonization resistance, and modulating immune responses.
5. Examine the emerging applications of microbiota transplantation for extraintestinal diseases and systemic conditions such as autoimmune disorders.
6. Learn the importance of donor selection and recipient factors in determining the success of microbiota transplantation procedures.
7. Evaluate the challenges and considerations in microbiota transplantation, such as long-term safety, regulatory aspects, and the need for personalized approaches.
8. Understand the role of omics technologies in advancing microbiota transplantation and their potential for enhancing future personalized medicine treatments.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Index

1. Introduction

- Human Microbiome
- Gut vs. Vaginal vs. Skin Microbiota

2. The Microbiota Transplantation

- Definition
- General steps of the process

3. Fecal Microbiota Transplantation (FMT)

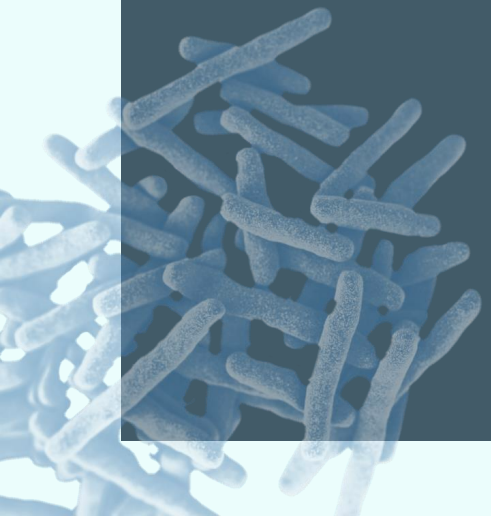
- Selection of a healthy donor
- Sample processing – Fecal preparation
- Recipient preparation
- Fecal microbiota transfer techniques
- Most common application of FMT (rCDI, IBD, IBS, metabolic syndrome, neurodegenerative disorders)

4. Skin Microbiota Transplantation (SMT) – Atopic Dermatitis

5. Vaginal Microbiota Transplantation (VMT) – Bacterial Vaginosis

6. Challenges and Future Directions

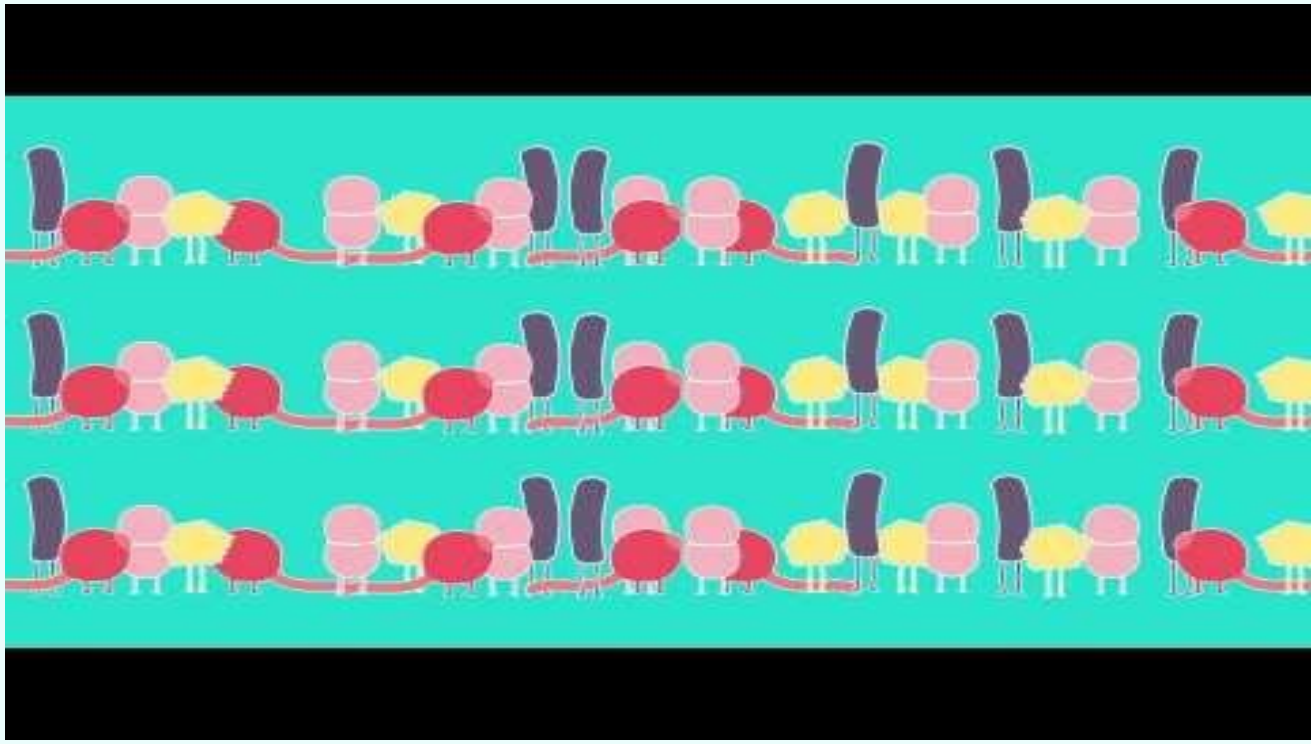
7. Conclusions



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

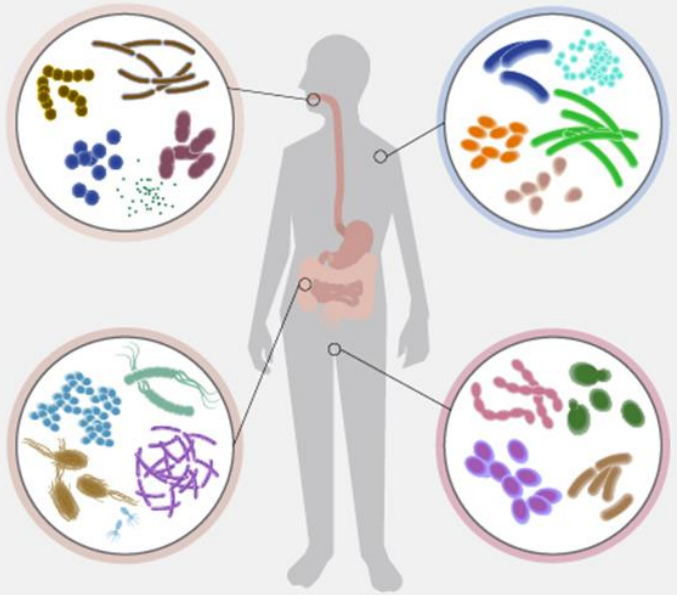
1. Introduction – Human Microbiome

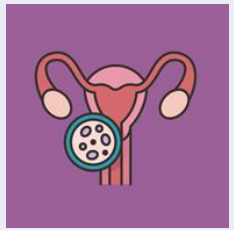
- Microbiota = the microorganisms (the actual bacteria, viruses, fungi, etc.)
- Microbiome = the collection of their genetic material and their combined impact on the host environment
- The human microbiome is a complex and dynamic ecosystem comprising distinct microbiota at various sites of the body:
  - **Gut Microbiota**: Digestion and immunity.
  - **Skin Microbiota**: Protection and regulation of skin health.
  - **Oral Microbiota**: Oral hygiene and immune defense.
  - **Vaginal Microbiota**: Reproductive health and infection prevention.
  - **Respiratory Microbiota**: Protection against respiratory pathogens.
  - **Urogenital Microbiota**: Urinary and reproductive health.
  - **Placental and Fetal Microbiota**: Influences fetal development.
  - **Breast Milk Microbiota**: Transfers beneficial bacteria to infants.
  - **Eye Microbiota**: Protects eye health.
  - **Scalp Microbiota**: Regulates scalp and hair health.



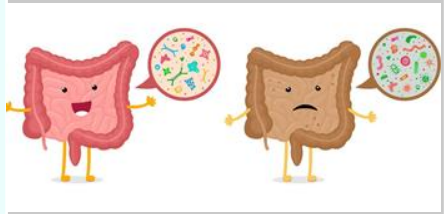
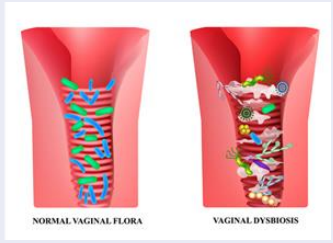
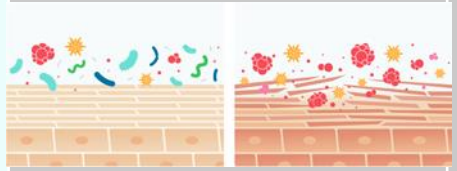
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

1. Introduction – Gut vs. Vaginal vs. Skin Microbota



Region of Human Microbiome	Common composition	Functions	Environmental modulating factors
Gut Microbiota	Primarly bacteria (phyla *Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria),* viruses, fungi and archea	Digestion, Immune modulation, metabolism	Diet, antibiotics use, stress, lifestyle, environmental changes
Vaginal Microbiota 	*Lactobacillus* species domination, other bacteria *Gardnerella*, *Atopobium* and various anaerobes, and yeast *Candida* spp.	Low pH maintanance (protection against pathogens); immune defense	Hormonal fluctuations, sexual activity, hygene practices
Skin Microbiota	*Staphylococcus*, *Corynebacterium*, and *Propionibacterium.* Varies significantly across different skin sites (e.g., oily vs. dry areas)	Barrier protection; Immune system interaction; Sebum metabolism	Skin type, hygene practices, climate, exposure to pollutants

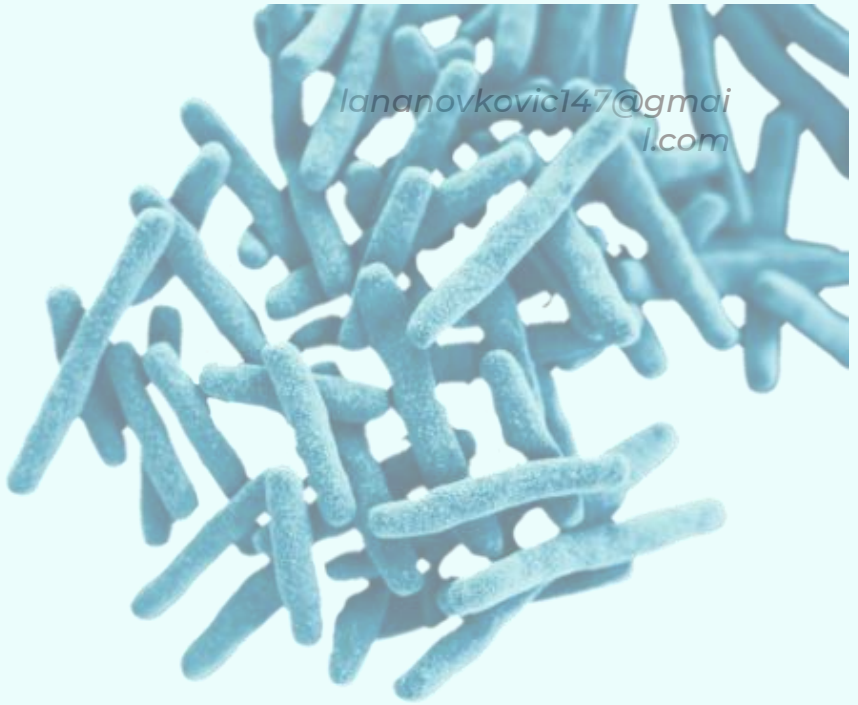
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Type of Microbiota Dysbiosis	State of dysbiosis	Associated health disorders	Type of treatment and prevention
	↓ microbial diversity, ↑ pathogens or opportunistic microbes (e.g. *Clostridium difficile*), ↓ beneficial microbes (e.g. *Lactobacillus*, *Bifidobacterium*), a shift in microbial metabolic activity (SCFA and proinflammatory molecules production)	Inflammatory bowel disease (IBD), irritable bowel syndrome (IBS); metabolic syndrome; allergies and asthma; autoimune diseases; neurological disorders	Dietary and lifestyle adjustments, probiotics, prebiotics, limiting antibiotic use, **fecal microbiome transplantation**
	↓ Lactobacillus spp. dominance and ↑ anaerobes, leading to an increase of pH value (pH > 4,5)	Bacterial vaginosis; yeast infections; urinary tract infections; infertility and pregnancy complications	Antimicrobials/antibiotics treatment, probiotics, prebiotics, **vaginal microbiome transplantation**
	↓ microbial diversity, ↑ specific pathogens (e.g., *Staphylococcus aureus)*, ↓ beneficial microbes (e.g., beneficial strains of *Staphylococcus* or *Corynebacterium*)	Acne; Eczema (Atopic Dermatitis); Psoriasis, Rosacea	Hygene and skin care, diet and lifestyle, probiotics and prebiotics, topical treatments, **skin microbiome transplantation**

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

lananovkovic147@gmail.com

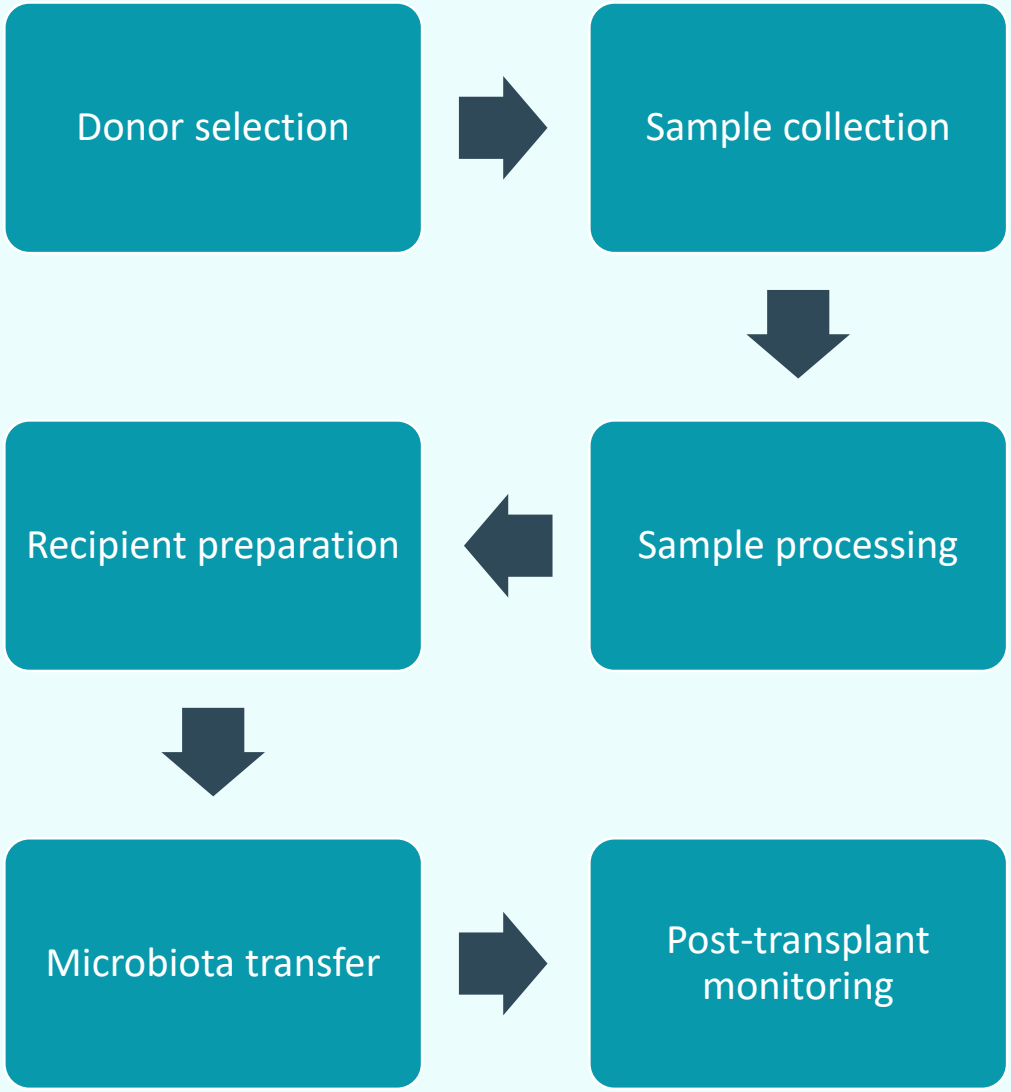
2. The Microbiota Transplantation

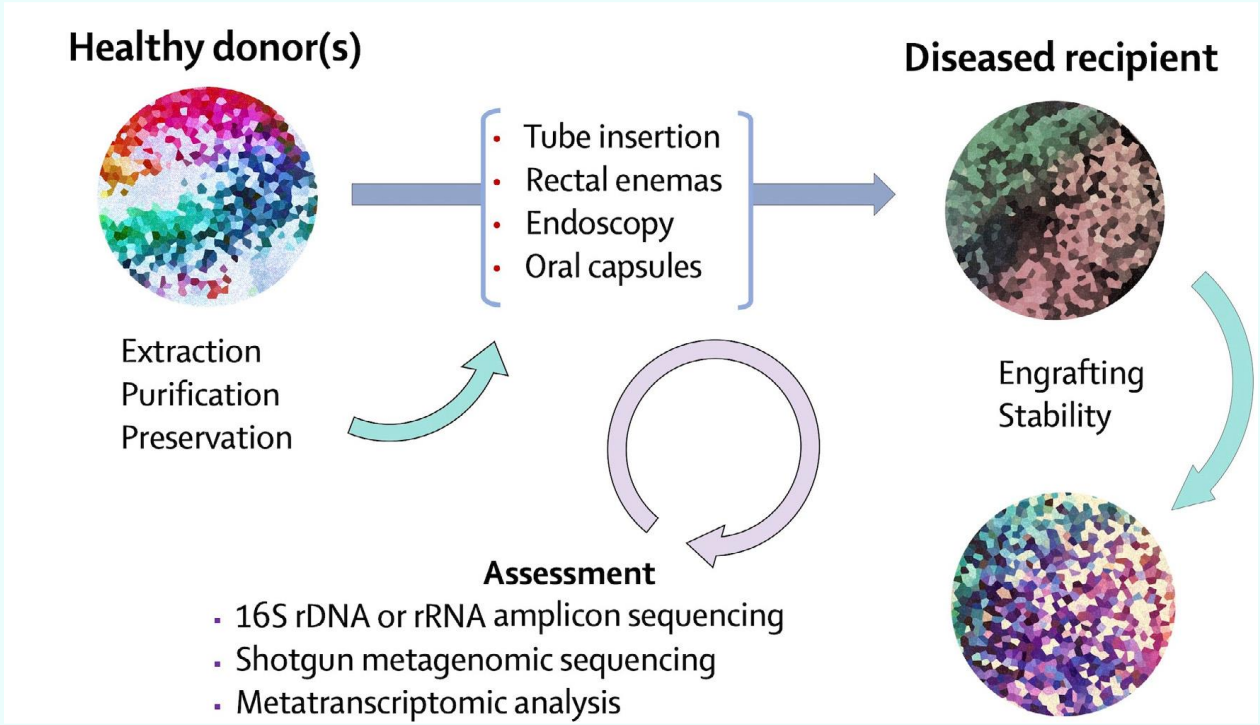


- The process of transferring the microbiome of a healthy donor to a diseased recipient individual
- Key challenges:
  1. identifying the specific components of the microbiome that provide health benefits
  2. Identifying the pathobiome and understanding „pathofunctions”
- Most common: fecal, skin and vaginal microbiome transplantation

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

The Microbiota Transplantation – general steps of the process

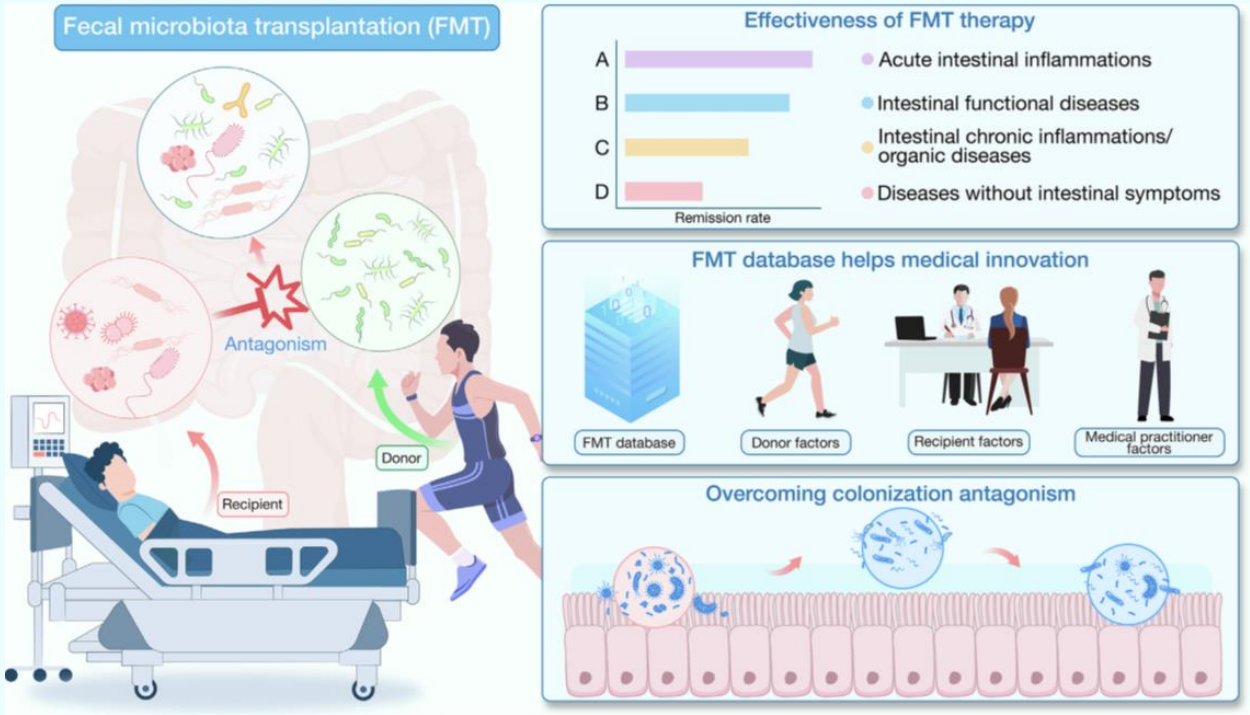




Junca, Howard, Dietmar H. Pieper, and Eva Medina. "The emerging potential of microbiome transplantation on human health interventions." *Computational and structural biotechnology journal* 20 (2022): 615-627.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

3. Fecal Microbiome Transplantation (FMT)



Tian, Hongliang, et al. "Fecal microbiota transplantation in clinical practice: Present controversies and future prospects." *hLife* (2024).

- FMT transfers stool from a healthy donor to restore gut microbiota in a recipient
- Effectiveness of FMT
  - **rCDI**: Over 90% success rate in treating recurrent *Clostridium difficile* infections
  - **Chronic conditions**: Lower success rates in IBD, IBS (~50-60%)
  - **Extraintestinal diseases**: Moderate success (around 48%) for conditions with gut involvement
  - **Success in systemic diseases**: About 40% in autoimmune and other non-gastrointestinal diseases
- Mechanism of FMT
  - **Restores microbiome balance**: Replaces harmful bacteria with a healthy microbial population
  - **Colonization resistance**: New bacteria outcompete pathogens, preventing infections
  - **Immunomodulation**: Regulates immune response, reduces inflammation
- Main factors influencing FMT success:
  1. **Donor selection**
  2. **Recipient factors**
  3. **Colonization resistance**

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Selection of a healthy donor

Box 1 Key issues to select potential donors at the preliminary interview

Known history of, or risk factors for, infectious diseases

- History of HIV, hepatitis B or C viruses, syphilis, human T-lymphotropic virus I and II
- Current systemic infection
- Use of illegal drugs
- High-risk sexual behaviour
- Previous tissue/organ transplant
- Recent hospitalisation or discharge from long-term care facilities
- High-risk travel/engaged in medical tourism
- Recent (≤6 months) needle stick accident
- Recent (≤6 months) body tattoo, piercing, earring, acupuncture
- Recent (≤2 months) enteric pathogen infection
- Recent (≤2 months) acute gastroenteritis with or without confirmatory test
- History of receiving growth hormone, insulin from cows or clotting factor concentrates
- Recent (≤2 months) history of vaccination with a live attenuated virus, if there is a possible risk of transmission

Disorders potentially associated with perturbation of the gut microbiota

- Personal history of chronic gastrointestinal disease, including functional gastrointestinal disorders; inflammatory bowel disease; coeliac disease; other chronic gastroenterological diseases
- Personal history of systemic autoimmune disorders
- Personal history of cancer, including gastrointestinal cancers or polyposis syndrome
- Recent abnormal gastrointestinal symptoms (eg, diarrhoea, haematochezia, etc)
- Personal history of neurological/neurodegenerative disorders
- Personal history of psychiatric/neurodevelopmental conditions
- Obesity (body mass index >30) and/or metabolic syndrome/diabetes
- First-degree family history of premature colon cancer or first-degree family history of early onset polyposis syndromes

Drugs that can alter gut microbiota

- Recent (≤3 months) exposure to systemic antimicrobial drugs, immunosuppressant agents, chemotherapy
- Chronic treatment (≥3 months) with daily use of proton pump inhibitors

Box 2 Donor blood and stool testing

Blood testing

- Hepatitis A, hepatitis B, hepatitis C and hepatitis E viruses
- HIV-1 and HIV-2
- *Treponema pallidum*
- Nematodes (*Strongyloides stercoralis*)
- Complete blood cell count with differential
- Creatinine
- Aminotransferases, bilirubin

Stool testing

- *Clostridioides difficile*
- Common enteric pathogens, including Salmonella, Shigella, Campylobacter, shiga toxin-producing *Escherichia coli*, Yersinia and *Vibrio cholerae*
- Antibiotic-resistant bacteria (ARB), including vancomycin-resistant Enterococci, meticillin-resistant *Staphylococcus aureus*), Gram-negative ARB including extended-spectrum β-lactamase-producing Enterobacteriaceae, and carbapenem-resistant Enterobacteriaceae/carbapenemase-producing Enterobacteriaceae
- Norovirus, rotavirus, adenovirus
- *Giardia lamblia*, *Cryptosporidium* spp, Isospora and Microsporidia
- Protozoa and helminths/va and parasites (including *Blastocystis hominis* and *Dientamoeba fragilis*)
- *Helicobacter pylori* faecal antigen (for upper route of FMT delivery)

Cammarota, Giovanni, et al. "International consensus conference on stool banking for faecal microbiota transplantation in clinical practice." *Gut* 68.12 (2019): 2111-2121.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

What is a „Super-donor”?

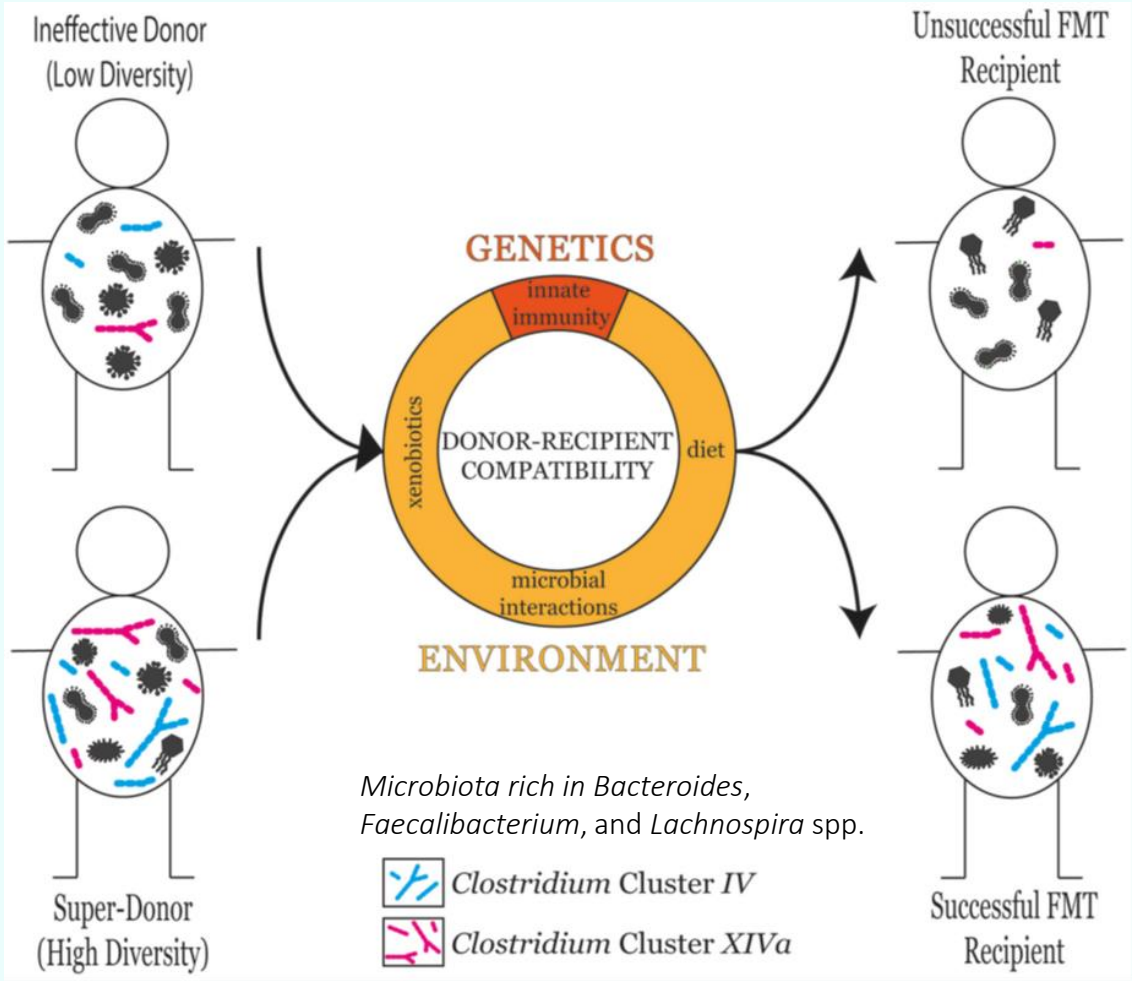
„One stool DOES NOT fits all”

An increase of the microbial diversity – multi-donor

The restoration of the specific metabolic disturbances associated with recipient’s particular disease phenotype

The donor-recipient matching approach

„Super donor” identification



Wilson, Brooke C., et al. "The super-donor phenomenon in fecal microbiota transplantation." *Frontiers in cellular and infection microbiology* 9 (2019): 2.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

The donor-recipient matching approach

1. **Microbial Profile Matching**

   Ensuring the donor's microbiome closely resembles the recipient's needs to improve FMT effectiveness.

2. **Personalized Health Factors**

   Donor selection based on recipient-specific factors like age, immune status, and underlying health conditions.

3. **Genetic and Metabolic Compatibility**

   Considering genetic and metabolic similarities between donor and recipient to enhance transplant success.

4. **Risk Minimization**

   Matching aims to minimize potential risks of adverse reactions or disease transmission.

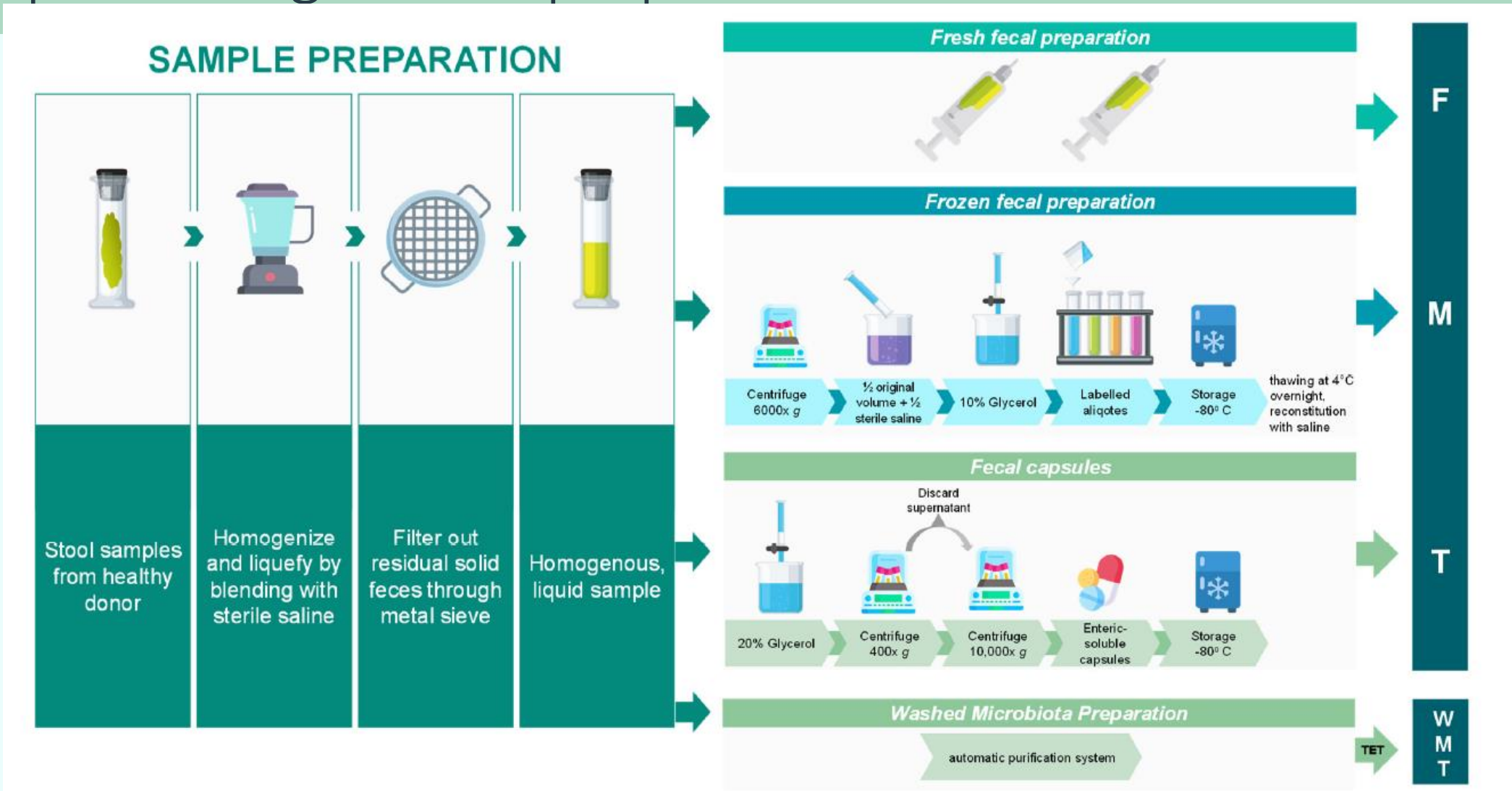
5. **Optimizing Long-term Outcomes**

   A tailored match can lead to better long-term health results, including gut health and immune response improvements.

*Post – MT donor's and recipient's microbiome composition are never the same – MICROBIAL INTERACTIONS
*Long-term effects not only influenced by the right microbiome donor – lifestyle adjustments are also required (diet, antibiotics,..)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Sample processing – Fecal preparation



Biazzo, Manuele, and Gabriele Deidda. "Fecal microbiota transplantation as new therapeutic avenue for human diseases." *Journal of Clinical Medicine* 11.14 (2022): 4119

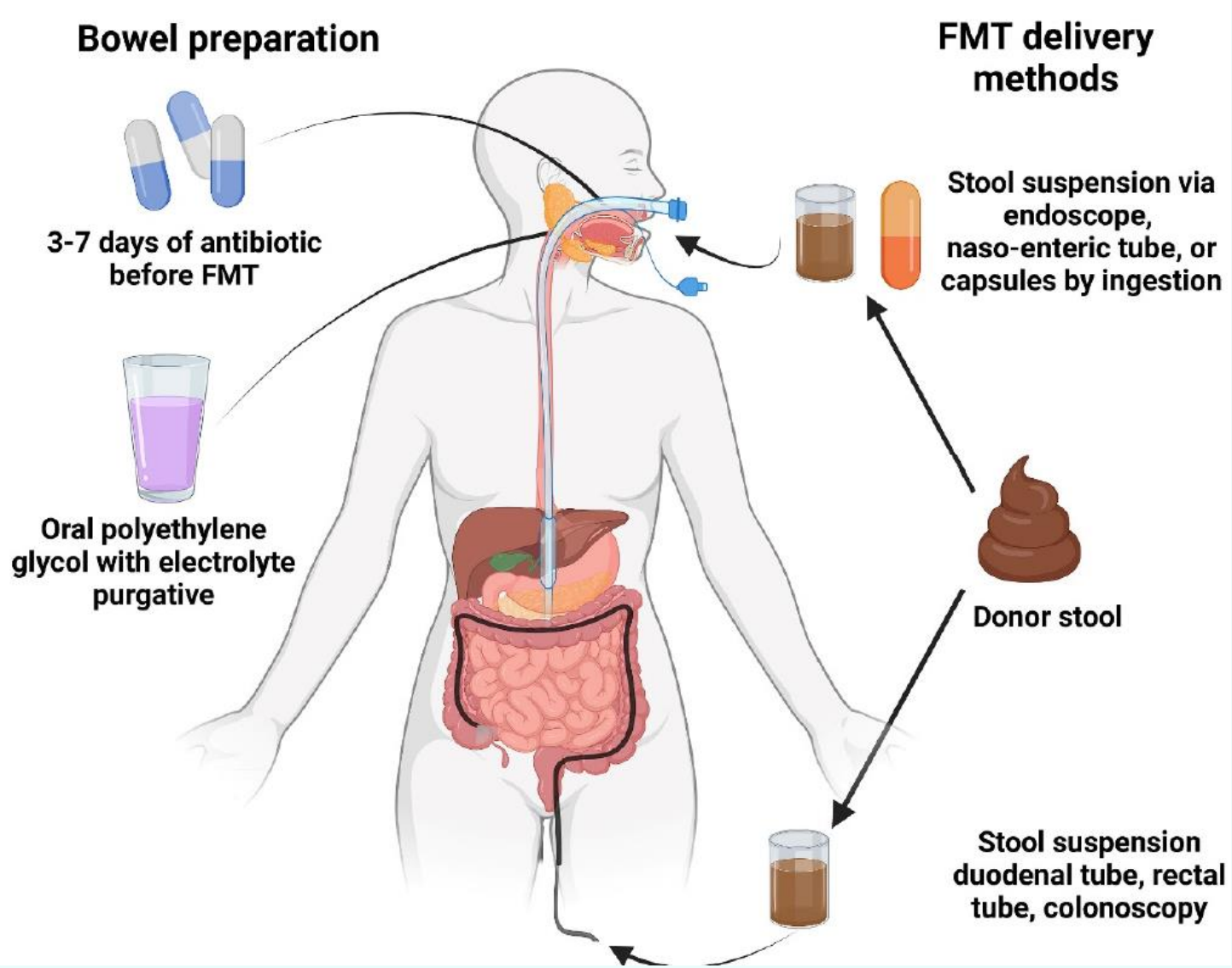
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Recipient preparation

- Antibiotic use depends on recipient's disorder
- Similar to a colonoscopy preparation process – bowel cleansing

Fecal microbiota transfer techniques:

- upper GI route (via esophagogastroduodenoscopy (EGD), nasogastric, nasojejunal, or nasoduodenal tube)
- lower GI route (via colonoscopy or retention enema)
- oral capsule

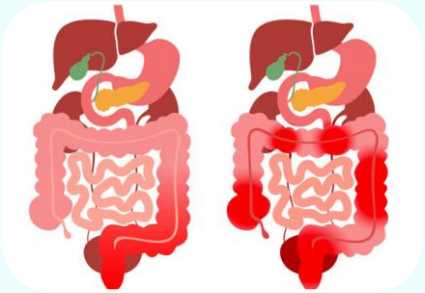


Bou Zerdan, Maroun, et al. "Fecal microbiota transplant for hematologic and oncologic diseases: principle and practice." *Cancers* 14.3 (2022): 69

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Most common application of FMT

1) Clostridioides difficile infection (CDI)



2) Inflammatory Bowel Disease (IBD)

3) Irritable Bowel Disease (IBD)

4) Metabolic Syndrome

5) Neurological disorders

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

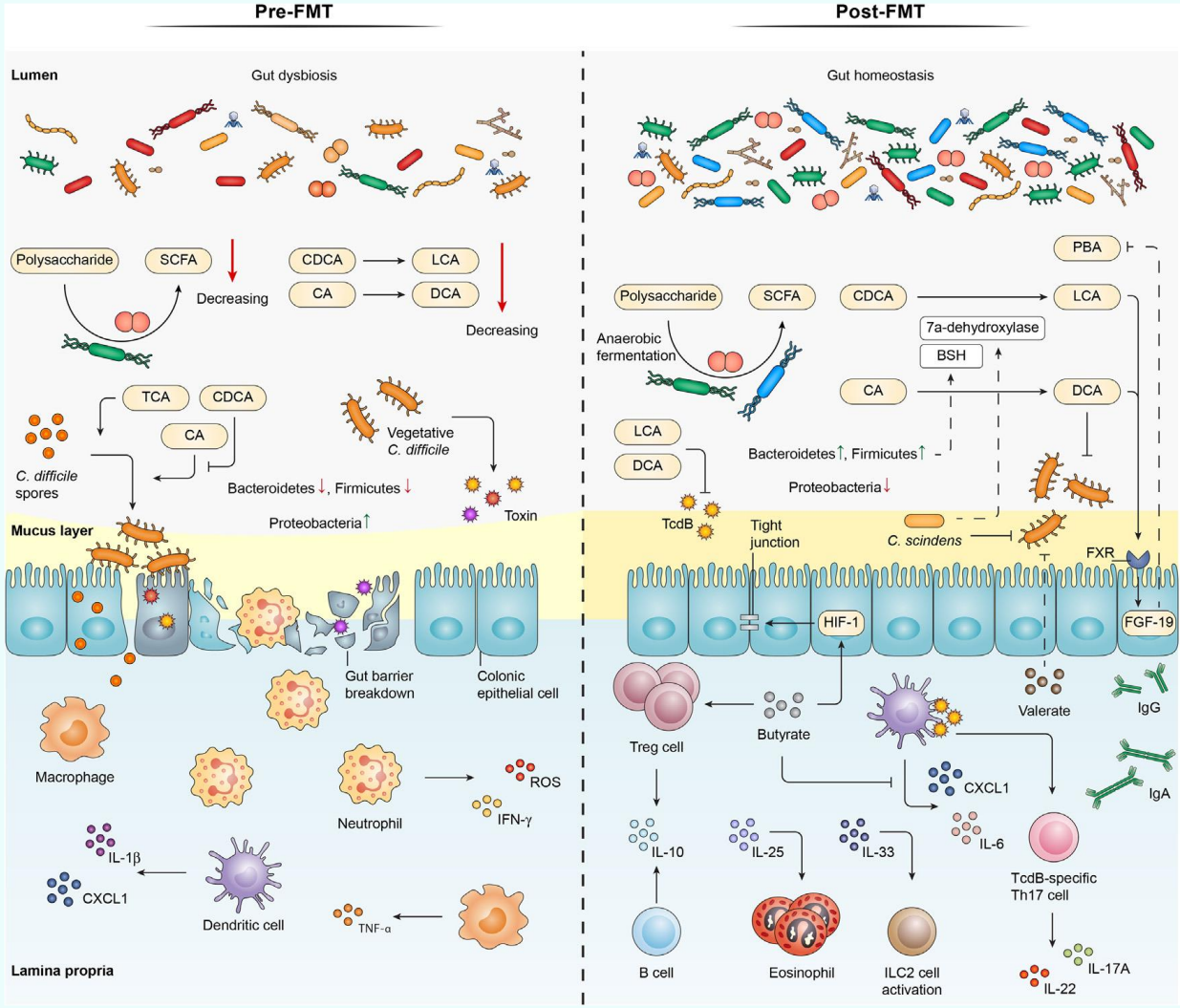
1) FMT and recurrent CDI

- Fecal microbiota–based therapies should be given upon completion of a course of standard of care antibiotics for recurrent CDI. **FMT is method of reccurency prevention, not cure.**

Effects of FMT on gut microbiota

Increased: Microbial diversity; *Bacteroidaceae*, *Lachnospiraceae* and *Ruminococcaceae;* SCFA production

Decreased: E*nterobacteriaceae, Lactobacillaceae, Enterococcaceae* and pathogenic *Clostridiales;* proinflammatory metabolites and molecules production



Yadegar, Abbas, et al. "Beneficial effects of fecal microbiota transplantation in recurrent Clostridioides difficile infection." Cell host & microbe 31.5 (2023): 695-711.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

FMT and recurrent CDI – general clinical practice

lananovkovic147@gmail.com

What is rCDI?

Need for proper diagnosis of recurrent CDI (confirmatory positive test within 8 weeks of completing antibiotics for CDI)

FMT – when?

After the second recurrence (third episode) of CDI or in select patients at high risk of either recurrent CDI or a morbid CDI recurrence

FMT – how?

- Correct selection of the donor stool
- 1-3 days before FMT the use of antibiotics should be stopped
- Conventional FMT; Live-JSLM (colonoscopy, enema, oral capsules); Spores Live-BRPK (oral capsules)
- delivered via multiple routes. Insufficient evidence to recommend a specific route.

FMT response

- after single dose > 80 %
- after repeated dose > 90 %

* Patients with severe or fulminant CDI usually need multiple FMT (colonscopy, enema or capsule)

Post-FMT effects

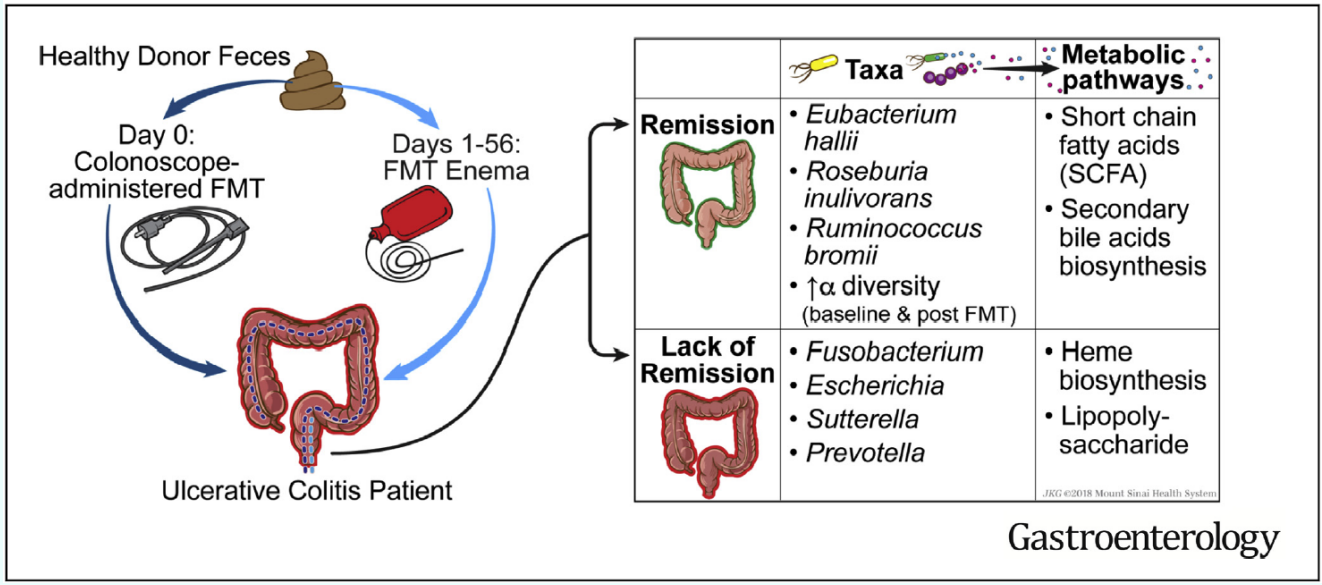
- First 24 h: bloating, flatulence, and loose stools – go away after few days
- Common IBS and lactose intolerance
- Early FMT failure is far more common than late FMT failure

FMT widely recommended in clinical guidelines for primary and recurrent CDI

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

2) FMT and IBD (Chron's disease and ulcerative colitis)

- Multiple FMT procedure more effective than single one, particuralrly in UC
- First administration: FMT through lower GIT (colonscopy, enema) more successful than through upper GIT
- Follow-up FMT: through capsules or less invasive methods
- Remission rate:
  ~ 45 % (Chron's patients > UC patients)
- Potential advantage over the drug treatment methods

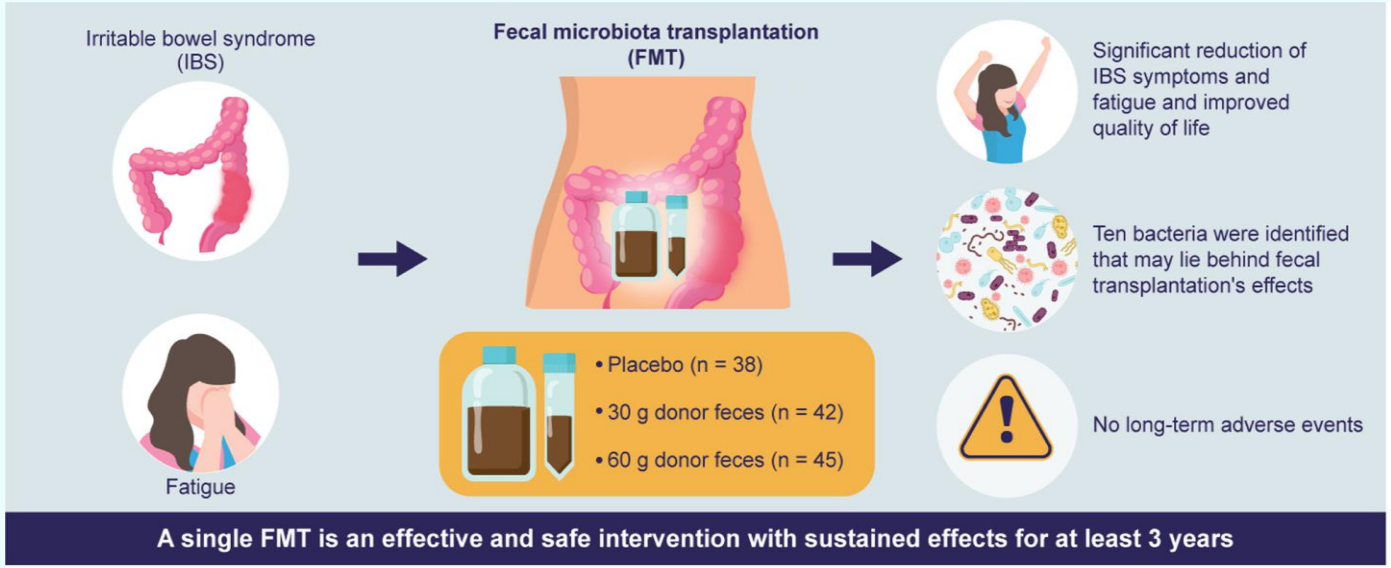


Paramsothy, Sudarshan, et al. "Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis." *Gastroenterology* 156.5 (2019): 1440-1454.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

3) FMT and IBS (Irritable Bowel Syndrome)

- IBS patients – increased specific bacterial species linked to inflammation and gut permeability, which can contribute to the symptoms
- Post-FMT: incresed diversity and beneficial alternations of gut microbiota
- Symptoms relief rate: 30 – 50 %
- Beneficial FMT re-treatment



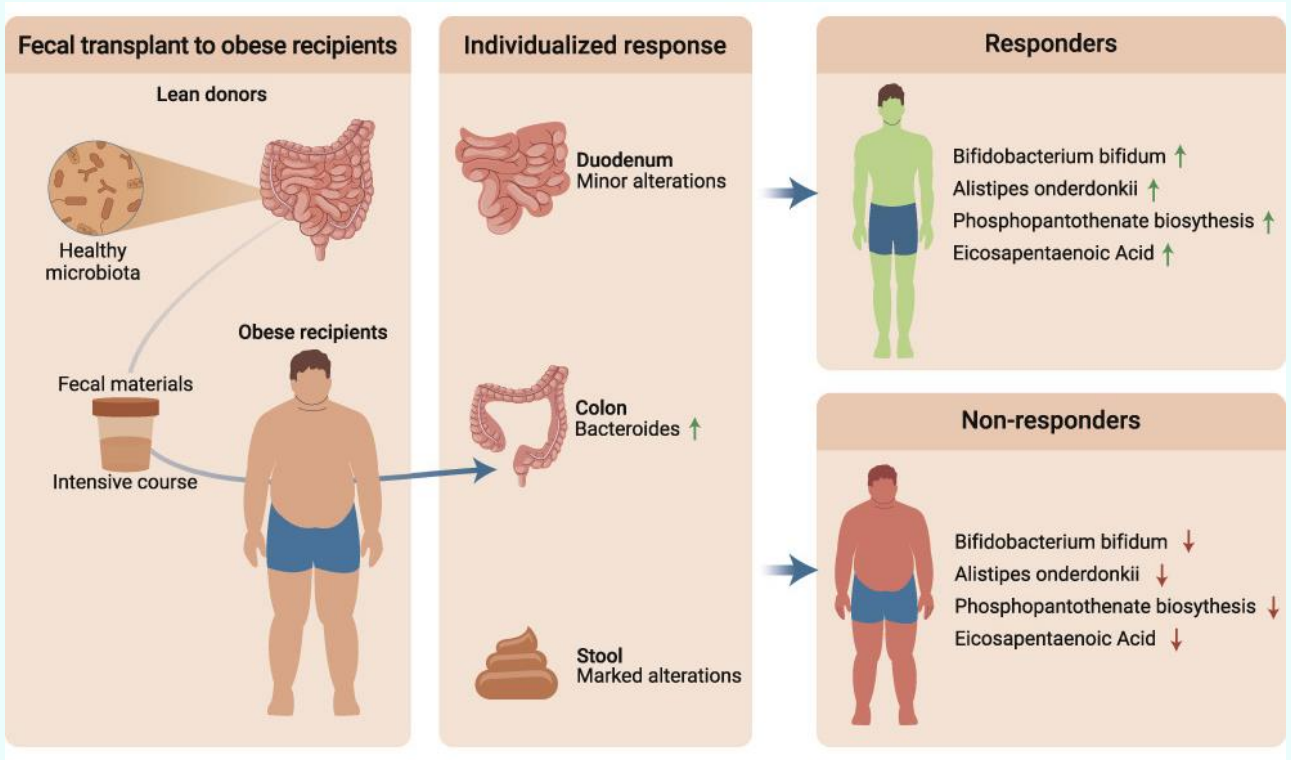
El-Salhy, Magdy, et al. "Efficacy of fecal microbiota transplantation for patients with irritable bowel syndrome at 3 years after transplantation." *Gastroenterology* 163.4 (2022): 982-994.

Key bacteria such as *Alistipes, Faecalibacterium prausnitzii, and Parabacteroides* (butyrate-producing bacteria) were linked to symptom relief

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

4) FMT and Metabolic Syndrome/Obesity

- Limited evidence
- Short-term > long-term effects
- More resilient gut microbiota in obese (duodenum microbiota especially)
- Personalized microbial engraftment – specific need for „super donors”



Zhang, Fen, et al. "Multi-omic analyses identify mucosa bacteria and fecal metabolites associated with weight loss after fecal microbiota transplantation." *The Innovation* 3.5 (2022).

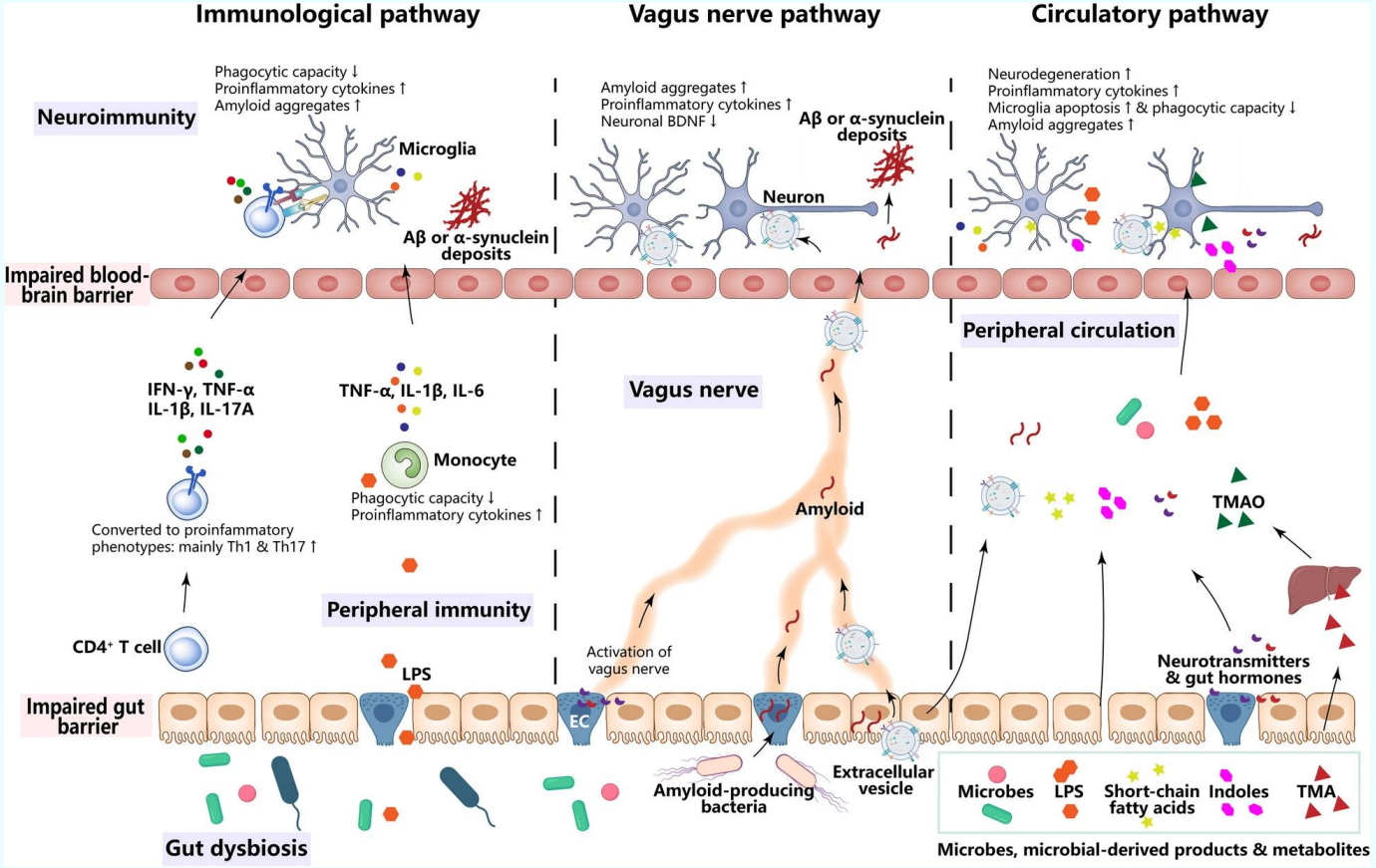
Improved insulin sensitivity associated with: *Ruminococcus bromii* and *Roseburia intestinali* (butyrate-production) + *Clostridium* spp. and *Akkermansia muciniphila* (gut barierr function)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

5) FMT and Neurodegenerative Disorders

(Alzheimer's disease, Parkinson's disease, multiple sclerosis)

- Depends on gut-brain axis
- Human studies still in early stages
- Main aim: modulation of immune responses, neuroinflammation and promotion of neuroprotective metabolites
- Potential in long-term effects



Ma, Yuan-Yuan, et al. "Therapeutics for neurodegenerative diseases by targeting the gut microbiome: from bench to bedside." *Translational Neurodegeneration* 13.1 (2024): 12.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Video: FMT effects on mice with induced Parkinson's disease

Zhao, Zhe, et al. "Fecal microbiota transplantation protects rotenone-induced Parkinson's disease mice via suppressing inflammation mediated by the lipopolysaccharide-TLR4 signaling pathway through the microbiota-gut-brain axis." *Microbiome* 9 (2021): 1-27.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

4. Skin Microbiome Transplantation

Donor selection
- No skin conditions, infections, diseases
- Overall healthy

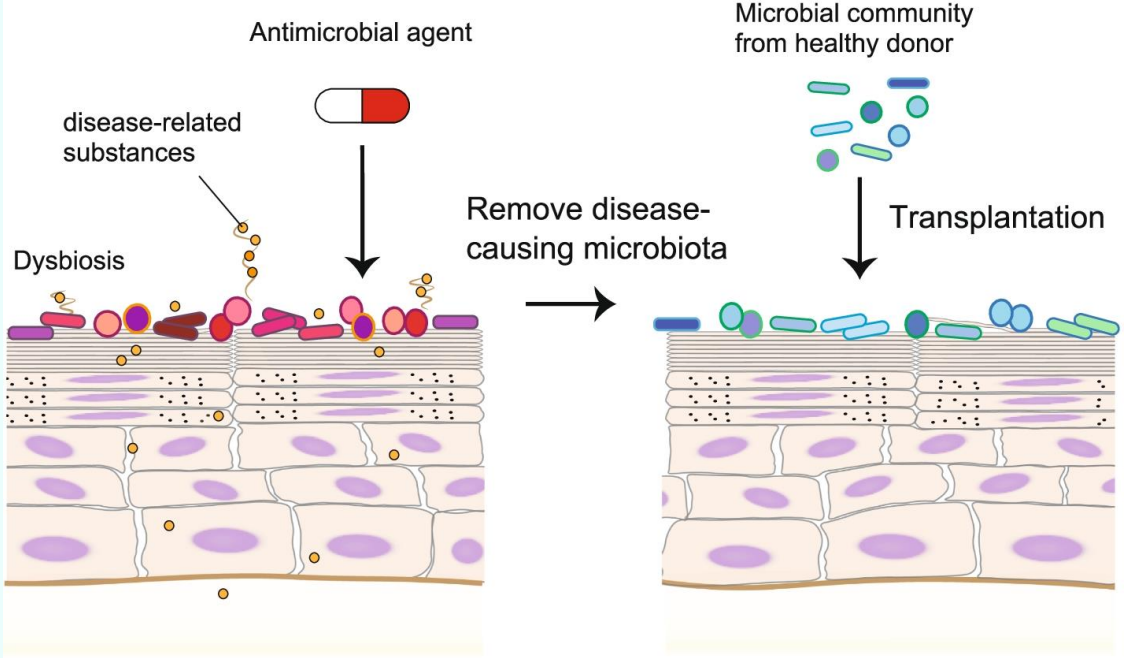
→ **Donor sample collection:**
- Swabing (forearm, behind the ear)
- Skin scrapings

Processing and quality control of donor's skin microbiota sample

→ **Before-SMT**
- ensuring recepient's cleaned skin – antiseptic soloutions

Aplication method
- Direct
- Through a specialized formulations that deliver the microbes

→ **Post-SMT**
- Monitornig and evaluation

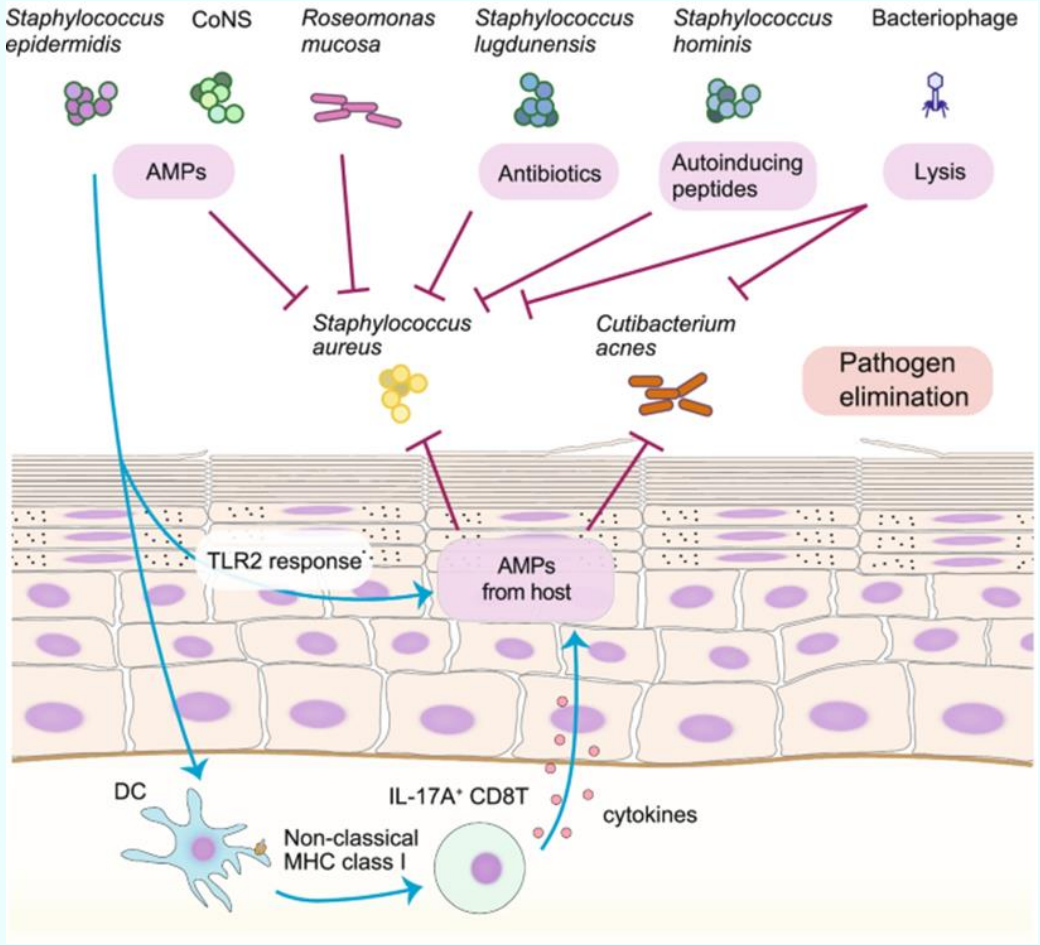


Ito, Y., Amagai, M. Controlling skin microbiome as a new bacteriotherapy for inflammatory skin diseases. *Inflamm Regener* **42**, 26 (2022). https://doi.org/10.1186/s41232-022-00212-y

- Promising method for a potential improvement of skin disorders like: **atopic dermatitis, acne, rosacea, psoriasis**
- Transfer of the entire skin microbiota or specifically curated mixture of microorganisms from a healthy donor to a recipient's skin

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

SMT and Atopic Dermatitis (AD)



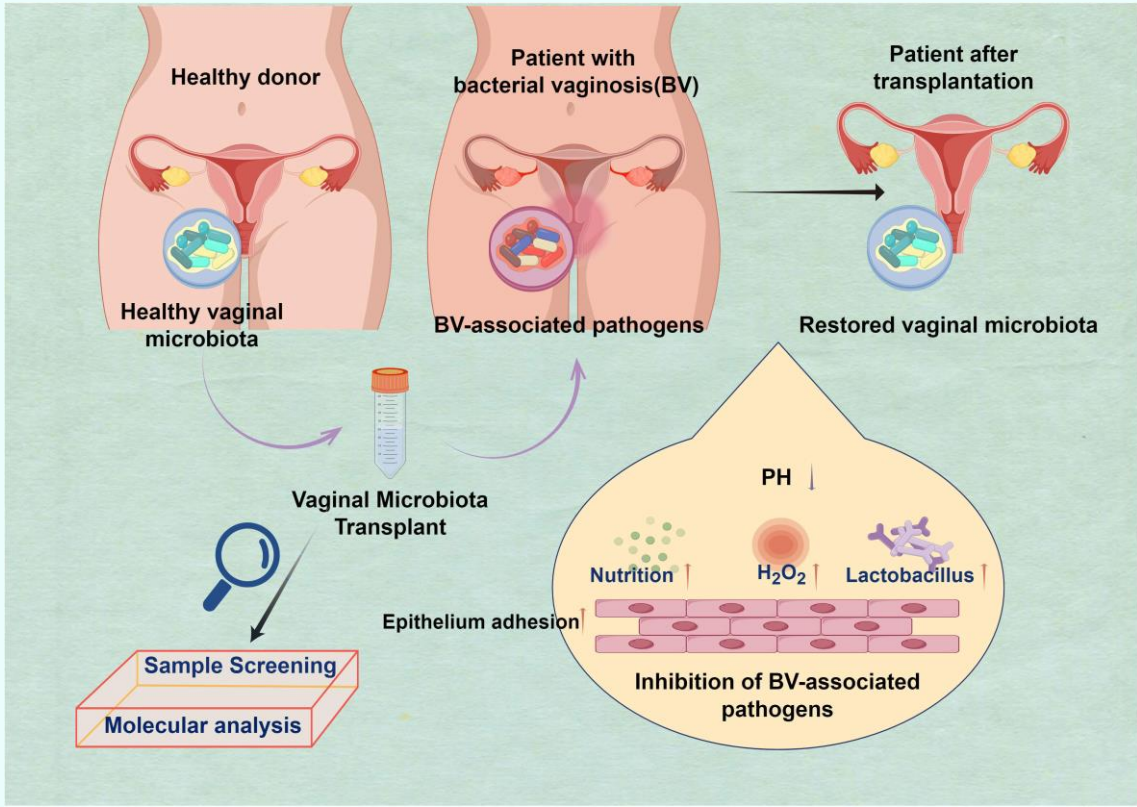
Ito, Y., Amagai, M. Controlling skin microbiome as a new bacteriotherapy for inflammatory skin diseases. *Inflamm Regener* **42**, 26 (2022). https://doi.org/10.1186/s41232-022-00212-y

- Mechanism of action of SMT:
  - Restoring healthy and diverse skin microbiota
  - Pathogenic colonisation resistance
  - Enhancement of the immune response
  - Reestablishment of skin's barrier function
  - Production of beneficial bioactive compounds
- Atopic dermatitis = overgrowth of *Staphylococcus aureus* and reduced microbial diversity
- The success of treatment varies among studies based on used bacterial strains or whole donor's microbiota for the SMT
- Beneficial bacteria for the AD treatment:
  - Coagulase-negative staphylococci (CoNS) - *Staphylococcus hominis* A9
  - *Staphylococcus epidermidis*
  - *Roseomonas mucosa*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

5. Vaginal Microbiome Transplantation

- A novel method to restore healthy vaginal microbiota from a healthy donor's vaginal fluid or swabs
- The aim – regain the dominance of *Lactobacillus* spp. (eubiosis)
- mechanisms of action:
  - Enhancing H2O2 priduction and low vaginal pH
  - Incresed competition for nutrients
  - Improved epithelial adhesion
- Potential use:
  - Recurrent BV and VVC
  - Infertility
  - Urinary tract infections



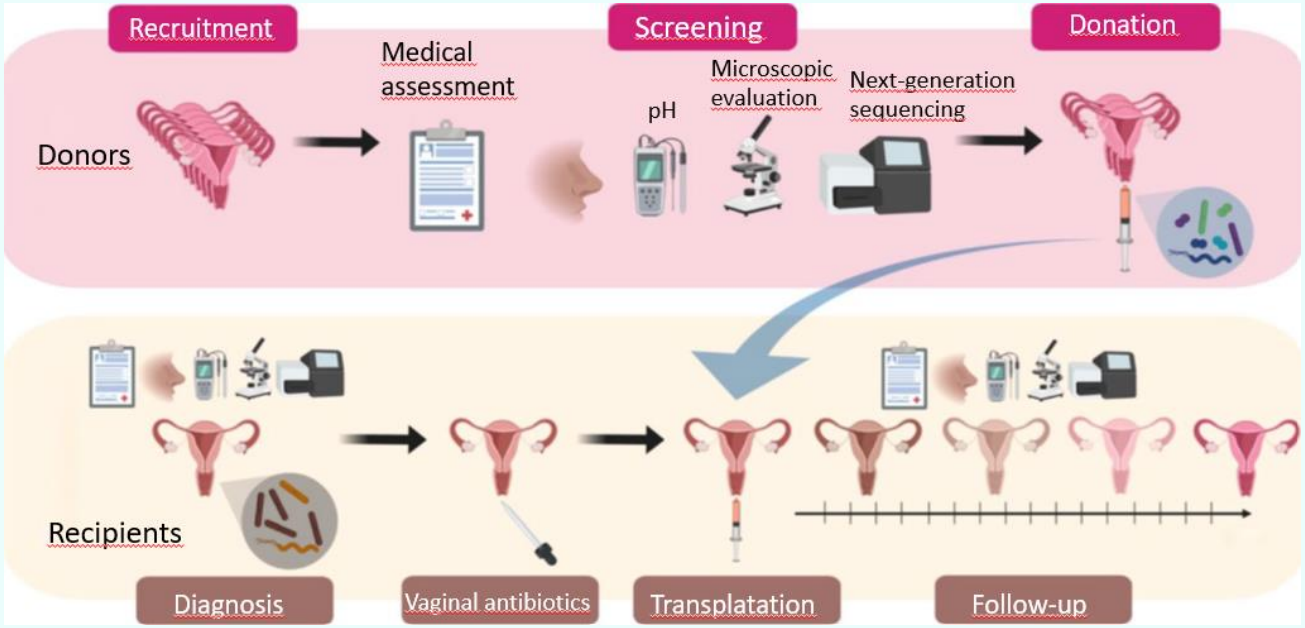
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

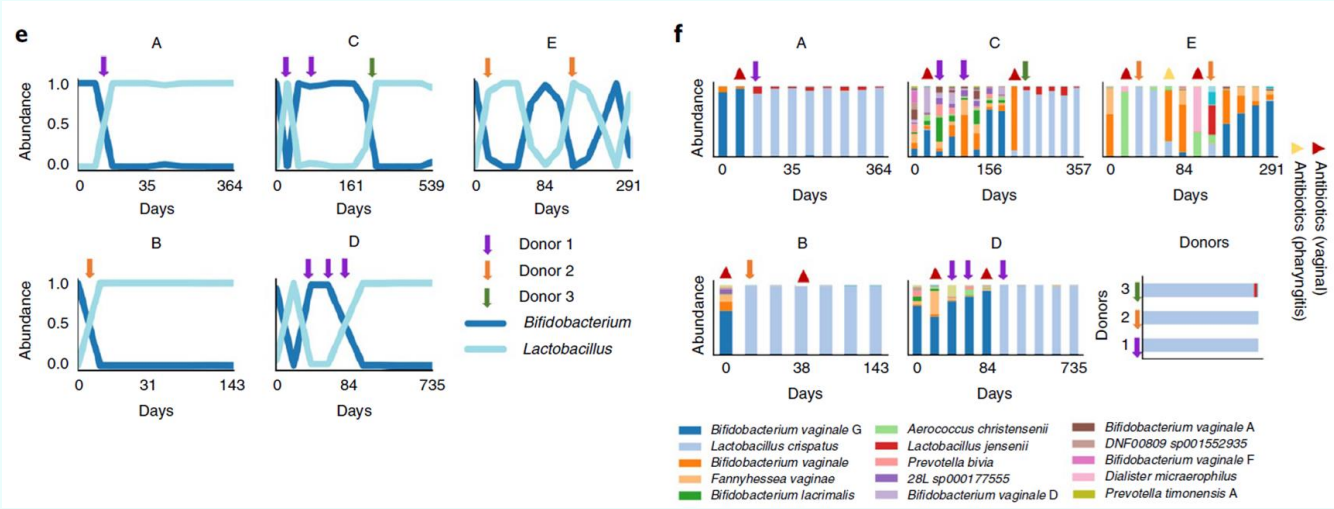
lananovkovic147@gmail.com

Steps of VMT in Recurrent Bacterial Vaginosis (BV)

Lev-Sagie *et al*., 2019: „Vaginal microbiome transplantation in women with intractable bacterial vaginosis"

1. Suitable donors: general health evaluation; no history of rBV /no BV in the last 5 years; 1 week abstinence of sexual activity
2. Recipiet preparation: 1 week of antibiotic treatment before VMT
3. sample processing
4. Application methods: direct inoculation or insertion of devices
5. Engraftment success evaluation: Amsel's criteria, metagenomic sequencing
6. Monitoring and follow-up are essential
7. Repeated process of VMT in cases of symptom recurrence





Figures: Metagenomic compositional assessment of the vaginal microbiome following VMT.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

6. Challenges of the Microbiome transplantation

- **Complexity of the Human Microbiome**
- **Standardization issues**: Need for uniform donor screening, preparation, and administration protocols.
- **Donor variability**: Super donor concept; finding and identifying consistently effective donors is a challenge.
- **Colonization antagonism**: Difficulty in overcoming resistance from recipient's existing microbiota.
- **Long-term effects and safety**: A need to conduct extensive longitudinal studies to monitor the recipients for any delayed or unforeseen adverse effects.
- **Microbiota profiling**: Limitations of current 16S rRNA Gene Sequencing

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

6. Future directions in the Microbiome transplantation

- Elucidating Mechanisms of FMT
- Expanding Clinical Applications
- Developing Stool Microbiota Banks
- Enhancing FMT Response with Diet-assisted Strategies
- Artificial feces development
- Engineered bacteria/phages
- Personalized precision treatment
- Advancing Skin Microbiota Transplantation
- Exploring Vaginal Microbiota Transplantation

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

7. Conclusions

1. **Effective Therapeutic Approach**: Fecal microbiota transplantation (FMT) successfully restores microbial balance, particularly effective for treating recurrent Clostridioides difficile infections (rCDI) and potentially beneficial for IBD, IBS, and metabolic syndrome.
2. **Expanding Applications**: Promising developments in vaginal microbiota transplantation (VMT) for recurrent bacterial vaginosis and skin microbiota transplantation for conditions like atopic dermatitis and acne.Challenges and Innovations:
3. **Key challenges:** include donor selection and variability in long-term outcomes. The concept of "super donors" with diverse microbiomes aims to address these issues.
4. **Future Directions**: Ongoing research, regulatory advancements, and omics technologies will refine FMT, enhancing its role in personalized medicine and offering innovative treatment solutions.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Take-home Messages

- Human microbiota transplantation is a powerful tool for treating dysbiosis-related conditions, particularly fecal microbiota transplantation (FMT), which is highly effective in treating recurrent Clostridioides difficile infections (rCDI).
- FMT shows promise in managing other health conditions such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), and metabolic syndrome, though outcomes can vary depending on factors like donor-recipient matching and the diversity of the microbiota.
- Donor selection is critical for the success of microbiota transplantation. The concept of super donors with highly diverse microbiomes has improved transplantation outcomes, but genetic, dietary, and environmental factors remain important.
- Emerging areas of microbiota transplantation, such as vaginal microbiota transplantation (VMT) for recurrent bacterial vaginosis (BV) and skin microbiota transplantation (SMT) for conditions like atopic dermatitis and acne, show promising early results, offering new ways to treat these persistent conditions.
- Regulation and safety remain central challenges in microbiota transplantation, with ongoing research needed to establish standardized protocols and ensure long-term benefits.
- Microbiota transplantation is poised to become a key component of personalized medicine, with future advancements in omics technologies and microbiome research enhancing its potential for broader therapeutic applications.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

- Biazzo, Manuele, and Gabriele Deidda. "Fecal microbiota transplantation as new therapeutic avenue for human diseases." *Journal of Clinical Medicine* 11.14 (2022): 4119
- Tian, Hongliang, et al. "Fecal microbiota transplantation in clinical practice: Present controversies and future prospects." hLife (2024).
- Lev-Sagie, A., Goldman-Wohl, D., Cohen, Y. *et al.* Vaginal microbiome transplantation in women with intractable bacterial vaginosis. *Nat Med* **25**, 1500–1504 (2019). https://doi.org/10.1038/s41591-019-0600-6
- Callewaert C, Knödlseder N, Karoglan A, Güell M, Paetzold B. Skin microbiome transplantation and manipulation: Current state of the art. Comput Struct Biotechnol J. 2021 Jan 4;19:624-631. doi: 10.1016/j.csbj.2021.01.001. PMID: 33510866; PMCID: PMC7806958.
- Boxberger, M., Cenizo, V., Cassir, N. *et al.* Challenges in exploring and manipulating the human skin microbiome. *Microbiome* **9**, 125 (2021). https://doi.org/10.1186/s40168-021-01062-5
- Paller, Amy S., et al. "The microbiome in patients with atopic dermatitis." *Journal of Allergy and Clinical Immunology* 143.1 (2019): 26-35.
- Junca, Howard, Dietmar H. Pieper, and Eva Medina. "The emerging potential of microbiome transplantation on human health interventions." *Computational and structural biotechnology journal* 20 (2022): 615-627.
- D. Goldenberg, Simon, and Blair Merrick. "The role of faecal microbiota transplantation: Looking beyond Clostridioides difficile infection." *Therapeutic advances in infectious disease* 8 (2021): 2049936120981526.
- Yadegar, Abbas, et al. "Beneficial effects of fecal microbiota transplantation in recurrent Clostridioides difficile infection." *Cell host & microbe* 31.5 (2023): 695-711.
- Goll, Rasmus, et al. "Effects of fecal microbiota transplantation in subjects with irritable bowel syndrome are mirrored by changes in gut microbiome." *Gut microbes* 12.1 (2020): 1794263.
- Zhang, Zhengxiao, et al. "Impact of fecal microbiota transplantation on obesity and metabolic syndrome—a systematic review." *Nutrients* 11.10 (2019): 2291.
- Matheson, Julie-Anne T., and RM Damian Holsinger. "The role of fecal microbiota transplantation in the treatment of neurodegenerative diseases: a review." *International Journal of Molecular Sciences* 24.2 (2023): 1001.
- Vendrik, Karuna EW, et al. "Fecal microbiota transplantation in neurological disorders." *Frontiers in cellular and infection microbiology* 10 (2020): 98.
- Wilson, Brooke C., et al. "The super-donor phenomenon in fecal microbiota transplantation." *Frontiers in cellular and infection microbiology* 9 (2019): 2.
- Cammarota, Giovanni, et al. "International consensus conference on stool banking for faecal microbiota transplantation in clinical practice." *Gut* 68.12 (2019): 2111-2121.
- Ito, Y., Amagai, M. Controlling skin microbiome as a new bacteriotherapy for inflammatory skin diseases. *Inflamm Regener* **42**, 26 (2022). https://doi.org/10.1186/s41232-022-00212-y
- Paramsothy, Sudarshan, et al. "Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis." *Gastroenterology* 156.5 (2019): 1440-1454.
- Ma, Yuan-Yuan, et al. "Therapeutics for neurodegenerative diseases by targeting the gut microbiome: from bench to bedside." *Translational Neurodegeneration* 13.1 (2024): 12.
- Zhao, Zhe, et al. "Fecal microbiota transplantation protects rotenone-induced Parkinson's disease mice via suppressing inflammation mediated by the lipopolysaccharide-TLR4 signaling pathway through the microbiota-gut-brain axis." *Microbiome* 9 (2021): 1-27.
- El-Salhy, Magdy, et al. "Efficacy of fecal microbiota transplantation for patients with irritable bowel syndrome at 3 years after transplantation." *Gastroenterology* 163.4 (2022): 982-994

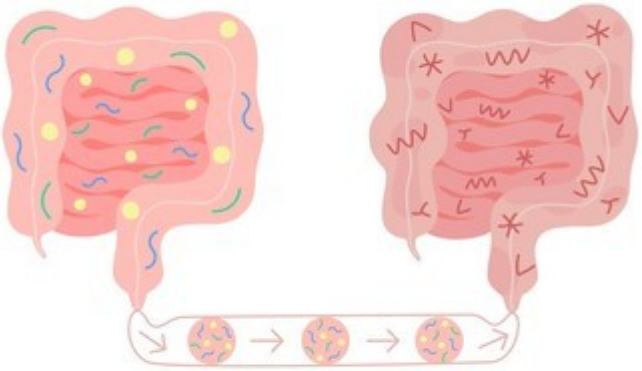
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

Msc. Nutr. Lana Novković

THE MICROBIOME TRANSPLANTATION

A Mini-review



e-mail: lananovkovic147@gmail.com

Table of contents

1. INTRODUCTION....1
2. The Microbiome Transplantation....2
3. Fecal Microbiota Transplantation (FMT)....4
3.1. FMT and recurrent Clostridioides difficile infection (CDI)....5
3.2. FMT and Inflammatory Bowel Disease (IBD)....6
3.3. FMT and Irritable Bowel Syndrome (IBS)....6
3.4. FMT and Metabolic Syndrome....7
3.5. FMT and Neurodegenerative Disorders....7
4. Skin Microbiota Transplantation (SMT) – Atopic Dermatitis (AD)....9
5. Vaginal Microbiota Transplantation (VMT) – recurrent Bacterial Vaginosis....10
6. CLINICAL EVIDENCE....11
7. CRITICAL THINKING....12
8. FUTURE DIRECTIONS....13
9. REFERENCES:....14

1. INTRODUCTION

In recent years, the human microbiome has emerged as a critical player in maintaining overall health and well-being. Comprising trillions of microorganisms, including bacteria, viruses, fungi, and other microbes, the microbiota inhabits various parts of the human body, with the gut, skin, and vagina being primary areas of interest. These microorganisms, through their collective genetic material—known as the microbiome—play essential roles in digestion, immune system regulation, and even mental health through mechanisms such as the gut-brain axis. However, disturbances or imbalances in this microbial ecosystem, known as dysbiosis, have been linked to a range of chronic and pathological health conditions, from gastrointestinal disorders to autoimmune diseases and even neurodegenerative disorders (Kim *et al*., 2013).

Microbiota transplantation has emerged as a revolutionary therapeutic approach to restore microbial balance. This involves transferring a microbiome sample from a healthy donor to a patient suffering from dysbiosis-related disorders. The most well-established form of this treatment is fecal microbiota transplantation (FMT), which has been extensively used for conditions like recurrent *Clostridium difficile* infections (rCDI). Beyond FMT, research is exploring the potential of skin microbiota transplantation (SMT) for conditions like atopic dermatitis and acne, as well as vaginal microbiota transplantation (VMT) for treating bacterial vaginosis and other vaginal infections. With promising clinical outcomes, microbiota transplantation is rapidly gaining attention for its potential to address some of the most challenging health issues where conventional treatments often fail (Junca *et al*., 2022).

Despite its promise, this field remains in its early stages, facing challenges such as donor selection, long-term safety concerns, and regulatory hurdles. However, ongoing advances in sequencing technologies, bioinformatics, and personalized medicine are likely to expand the scope and effectiveness of microbiota transplantation, making it a cornerstone in future healthcare practices. This review aims to explore the different forms of microbiota transplantation, the conditions they target, and the future potential of this therapeutic approach.

2. The Microbiome Transplantation

Microbiome transplantation is a therapeutic procedure aimed at modifying the recipient's microbial ecosystem by introducing a balanced and healthy microbiota from a donor. This approach has gained traction in treating dysbiosis-related conditions, where the patient's natural microbial community is either imbalanced or deficient in beneficial organisms. The procedure is grounded in the idea that restoring microbial diversity can promote better health outcomes by re-establishing a stable and healthy microbiome, which performs vital functions such as immune modulation, nutrient absorption, and protection against pathogens (Junca *et al*., 2022).

A microbiome transplantation involves several key steps, regardless of the body area targeted:

Donor Selection: Healthy individuals are carefully screened to ensure they do not carry infectious diseases or other health conditions that could adversely affect the recipient. The donor must have a well-balanced and diverse microbiota, which is critical for successful transplantation.

Sample Collection: Microbial samples are collected from the donor. For fecal microbiota transplantation (FMT), stool samples are obtained, whereas skin and vaginal transplants involve swabbing the respective body areas to collect microbiota.

Sample Processing: The collected samples undergo processing to isolate the microbiota from larger particles and contaminants. This can involve filtration, dilution, and sometimes freezing for future use. For FMT, this could mean freezing the sample in glycerol for storage in stool banks.

Recipient Preparation: Before receiving the transplantation, the recipient undergoes preparation, which may include taking antibiotics or cleansing procedures (such as bowel cleansing for FMT). This is done to minimize the native microbiota and create an optimal environment for the new microbiota to colonize.

Microbiota Transfer: The processed microbiota sample is introduced to the recipient via various methods. In FMT, this might involve colonoscopy, enema, or oral capsules, while SMT may involve topical applications or swabs, and VMT is typically performed using swabs or syringes to introduce the sample into the vaginal cavity.

Post-Transplant Monitoring: Following the procedure, the recipient is closely monitored to assess the success of the transplant and to check for any adverse reactions. Periodic tests may be conducted to evaluate the engraftment of the transplanted microbiota and improvements in health outcomes (Biazzo and Deidda, 2022; Callewaert *et al*., 2021; Lev-Sagie *et al*., 2019).

Super Donor Concept

A key concept in microbiome transplantation is the idea of the "super donor." Not all donors' microbiota is equally effective for all recipients. A super donor is an individual whose microbiota has shown exceptional therapeutic efficacy when used in transplantation. These donors typically possess a highly diverse and stable gut microbiota, often enriched with beneficial bacterial species like *Bacteroides*, *Faecalibacterium*, and *Lachnospira* (Junca *et al*., 2022; Wilson *et al*., 2019).

Super donors play a critical role in improving the success rates of microbiota transplantation. Studies have demonstrated that microbiota from super donors can lead to significantly better clinical outcomes, such as higher remission rates in patients with ulcerative colitis and recurrent *C. difficile* infections. However, the exact factors that contribute to the success of super donors are not fully understood, and research is ongoing to better define and identify these individuals. Factors such as genetic profile, lifestyle, and diet may influence a donor's microbiome diversity and stability, making them more or less effective in certain transplantation scenarios (Bibbo *et al*., 2020; Wilson *et al*., 2019).

In some cases, "multi-donor" strategies are employed, where samples from multiple healthy donors are combined to increase microbial diversity and improve treatment efficacy. This approach can be particularly useful in treating conditions with complex underlying causes, such as metabolic syndrome or neurodegenerative diseases, where a more diverse microbiota may be needed to address multiple physiological pathways (Junca *et al*., 2022).

3. Fecal Microbiota Transplantation (FMT)

Fecal microbiota transplantation (FMT) is the most widely researched and clinically applied form of microbiota transplantation. It involves the transfer of stool from a healthy donor into the gastrointestinal (GI) tract of a recipient. FMT restores the natural balance of the gut microbiota, which can be disrupted due to antibiotic use, infections, or chronic diseases. Unlike probiotics, which introduce only specific bacterial strains, FMT delivers an entire microbial ecosystem, making it a more comprehensive approach to re-establishing a healthy gut environment (Biazzo and Deidda, 2022).

One of the main advantages of FMT is its ability to outcompete harmful bacteria, enhance immune function, and improve metabolic processes. The diverse array of microbial species provided by FMT can increase the production of beneficial metabolites like short-chain fatty acids (SCFAs), which help reduce inflammation and maintain the integrity of the gut barrier. FMT is a promising treatment for conditions involving gut dysbiosis, particularly those that have not responded well to conventional treatments (Goldenberg *et al*., 2021).

Fecal Microbiota Transplantation (FMT) has demonstrated varying levels of effectiveness depending on the condition being treated. For recurrent *Clostridium difficile* infections (rCDI), FMT boasts a success rate of over 90%, making it the gold standard treatment for this condition. However, its effectiveness in chronic conditions such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) is comparatively lower, with success rates around 50-60%. For extraintestinal diseases with gut involvement, such as metabolic or neurodegenerative disorders, the success rate of FMT hovers around 48%. In systemic diseases, including autoimmune disorders and other non-gastrointestinal conditions, the success rate decreases to approximately 40% (Tian *et al*., 2024).

The specific procedures of FMT treatment, which involve all the steps mentioned as basic steps of microbiome transplantation previously, are shown in the Figure 1.

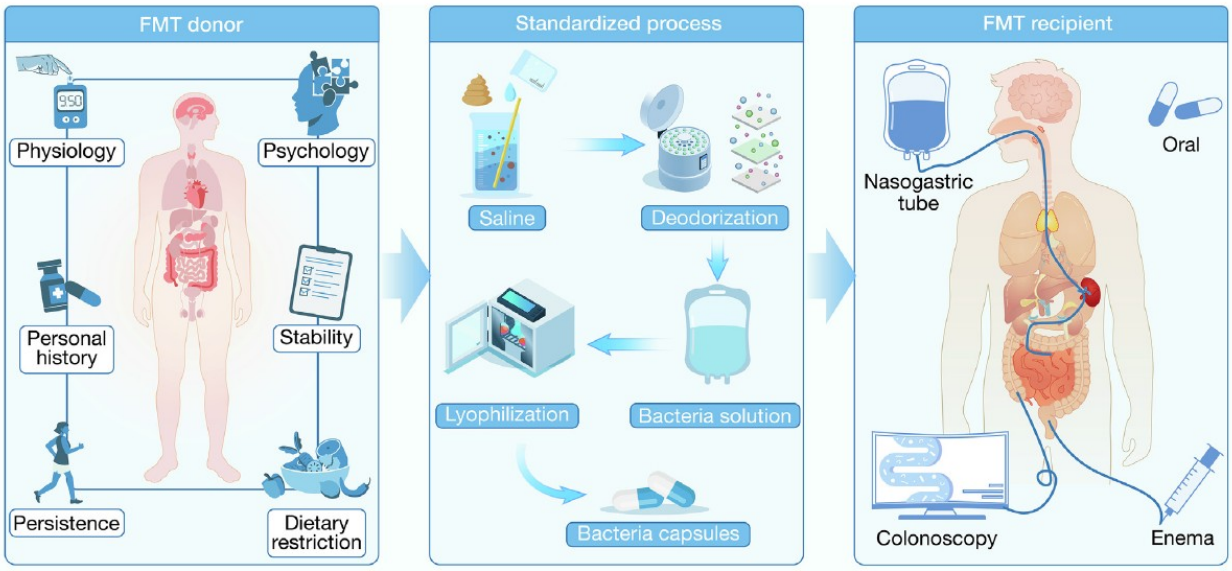


Figure 1. **The procedures of FMT treatment**

The recruitment of donors follows a meticulous “five-step, six-dimensional” plan. Graft preparation adheres to a standardized procedure. The choice of the transplant route is determined based on the recipient’s treatment plan. Abbreviation: FMT, fecal microbiota transplantation (Tian *et al*., 2024).

3.1. FMT and recurrent Clostridioides difficile infection (CDI)

Recurrent *Clostridium difficile* infection (rCDI) is the most established indication for FMT. Antibiotic use often disrupts the gut microbiota, leading to the overgrowth of *C. difficile* and recurring bouts of diarrhea, colitis, and inflammation. Antibiotic treatments for rCDI, such as vancomycin and fidaxomicin, have limited long-term success because they fail to restore a balanced gut microbiota (Yadegar *et al*., 2023).

Fecal microbiota-based therapies should be given after completing standard antibiotics for recurrent CDI, as they aim to prevent recurrence, not treat active infections. Suppressive antibiotics like vancomycin can be used beforehand, but ideally, they should be stopped 1–3 days before FMT. FMT can be administered through colonoscopy, enema, or oral capsules, though no single method is definitively preferred (Peery *et al*., 2024).

In a wide range of observational studies and trials, a single dose of FMT cures over 80% of recurrent C. difficile infection (R-CDI) cases. Repeat dosing increases the cure rate to over 90%, particularly for patients with severe or fulminant CDI, who often require multiple treatments. By introducing a healthy microbial community from a donor, FMT can suppress *C. difficile* overgrowth, restore microbial diversity, and enhance gut barrier function. This process reduces the risk of relapse and leads to long-term improvements in gut health. Studies have shown that FMT is particularly effective after two or more recurrences of *C. difficile* infection, where standard antibiotic therapies have failed (Kelly *et al*., 2018).

3.2. FMT and Inflammatory Bowel Disease (IBD)

Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are characterized by chronic inflammation of the gastrointestinal tract. Dysbiosis, or an imbalance in the gut microbiota, plays a significant role in the pathogenesis of these conditions. Patients with IBD often show reduced levels of beneficial bacterial species, such as *Faecalibacterium prausnitzii*, and an overgrowth of harmful bacteria like *Proteobacteria* and *Enterobacteriaceae* (Goldenberg *et al*., 2021).

While FMT has shown some promise in treating IBD, its success has varied significantly between patients and conditions. For Crohn's disease and ulcerative colitis, dysbiosis is a key factor, with patients often having a reduced diversity of beneficial bacteria. FMT aims to restore this balance, yet differences in donor microbiota and the complexity of IBD pathogenesis mean that results can be inconsistent. Multiple FMT sessions are often needed, and the treatment's effectiveness may depend on specific donor-recipient compatibility and microbial composition (Biazzo and Deidda, 2022).

Additionally, studies have suggested that FMT introduces beneficial bacterial species like *Faecalibacterium* and *Bacteroides*, which help in reducing inflammation and maintaining gut barrier integrity. The success of FMT in IBD appears closely tied to its ability to increase the production of short-chain fatty acids (SCFAs) like butyrate, which play a critical role in reducing inflammation and promoting colon health (Paramsothy *et al*., 2019).

3.3. FMT and Irritable Bowel Syndrome (IBS)

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder characterized by abdominal pain, bloating, and altered bowel habits, which can include diarrhea (IBS-D), constipation (IBS-C), or a mix of both (IBS-M). IBS is often linked to dysbiosis in the gut microbiota, where an imbalance in bacterial populations contributes to gut permeability, immune activation, and altered motility (Goll *et all*., 2020).

FMT has been investigated as a treatment for IBS, with studies showing mixed results. Some patients, particularly those with IBS-D, have reported significant symptom improvement following FMT, while others have shown little or no change. The variability in outcomes may be attributed to differences in donor microbiota, the method of FMT delivery, and the patient’s baseline microbiome, as well as, the specific IBS subtype, and the timing of the treatment (Goll

et al., 2020). A study by El-Sachy and colleagues showed that FMT can provide long-term relief for up to three years in IBS patients who respond positively to the treatment. Post-FMT changes in gut bacteria were linked to improvements in IBS symptoms and fatigue. Key species like *Alistipes,* and *Parabacteroides* contributed to symptom relief, with increased levels of butyrate-producing bacteria, such as *Faecalibacterium prausnitzii,* providing additional benefits for gut health (El-Salhy *et al*., 2022).

3.4. FMT and Metabolic Syndrome

Metabolic syndrome encompasses a group of conditions, including obesity, insulin resistance, hypertension, and dyslipidemia, that increase the risk of cardiovascular disease and type 2 diabetes. Gut dysbiosis has been linked to metabolic syndrome, with studies showing a reduction in microbial diversity and a shift in bacterial populations including an increase in *Firmicutes* and a decrease in *Bacteroidetes,* that promote inflammation and fat storage (Goldenberg *et al*., 2021).

FMT from lean donors to patients with metabolic syndrome has shown promise in improving insulin sensitivity, reducing body fat, and modifying gut microbial composition. Research has shown that specific bacterial species, introduced via FMT, such as *Akkermansia muciniphila, Ruminococcus bromii* and *Roseburia intestinalis* and *Clostridium* species, have been shown to improve gut barrier function and modulate glucose metabolism. These bacteria play a role in reducing low-grade inflammation and improving the body's response to insulin, both of which are critical in managing metabolic syndrome and reducing the risk of type 2 diabetes. Despite these early promising results, challenges remain in achieving lasting weight loss and metabolic improvements. Factors such as the resilience of the recipient’s microbiota, dietary habits, and lifestyle can influence the effectiveness of FMT. Studies suggest that combining FMT with dietary interventions, such as increasing fiber intake, may enhance its benefits for metabolic syndrome patients (de Groot *et al*., 2020; Zhang *et al*., 2019).

3.5. FMT and Neurodegenerative Disorders

FMT has gained interest as a potential treatment for neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD) and multiple sclerosis (MS) through its influence on the gut-brain axis. Preclinical studies have shown that FMT can reduce neuroinflammation, improve cognitive function, and alleviate motor symptoms. Though human studies are still in early stages, animal models suggest that restoring gut microbial balance could be a viable

therapeutic strategy for reducing the progression of neurodegenerative conditions by targeting the gut-brain interaction (Matheson and Holsinger, 2023).

Gut dysbiosis has been linked to an increased risk of neurodegenerative diseases, with changes in microbial composition affecting the production of neuroactive compounds and inflammatory responses. FMT restores beneficial gut bacteria that can modulate the immune system and improve the gut-brain communication, leading to potential reductions in the build-up of harmful proteins like amyloid-beta and *α-synuclein* in the brain (Vendrik *et al*. 2020).

Studies are also exploring the potential of FMT to enhance the production of short-chain fatty acids (SCFAs), such as butyrate, which have been shown to exert neuroprotective effects. While much of the evidence is still preclinical, these findings open up new possibilities for using FMT as part of a multi-modal treatment strategy for Alzheimer's, Parkinson's, multiple sclerosis and other neurodegenerative diseases (Matheson and Holsinger, 2023; Zhao et al., 2021).

4. Skin Microbiota Transplantation (SMT) – Atopic Dermatitis (AD)

The skin microbiome varies across different areas, categorized as sebaceous, moist, or dry. *Cutibacterium* species are common in sebaceous regions, while *Staphylococcus* and *Corynebacterium* dominate moist areas. The skin microbiota protects against harmful microbes through mechanisms like colonization resistance or antimicrobial peptides. Dysbiosis is linked to conditions such as acne and atopic dermatitis. Skin microbiota transplantation, similar to fecal microbiota transplantation, may help treat these disorders, though research is still in its early stages. This involves transferring either a full microbiota or a curated mix of microbes to the recipient's skin. (Callewaert *et al*., 2021).

SMT is emerging as a novel therapeutic option for atopic dermatitis (AD), a condition often linked to an overgrowth of *Staphylococcus aureus*. SMT aims to restore a healthy microbial balance on the skin by introducing beneficial bacteria like *Staphylococcus hominis* or *Roseomonas mucosa*. Early trials have shown significant reductions in AD symptoms and inflammation, with patients experiencing improved skin barrier function and reduced need for corticosteroids (Paller *et al*., 2019). The mechanism of action for SMT involves introducing beneficial bacteria that compete with pathogenic strains, such as *S. aureus*, reducing the inflammation and skin barrier damage typically seen in AD. These transplanted microbes help improve moisture retention and bolster the skin’s immune responses, preventing future flare-ups (Boxberger *et al*., 2021).

In clinical studies, patients receiving SMT showed a substantial reduction in the use of corticosteroids and reported improvements in quality of life. A 2018 study applying *Roseomonas mucosa* to patients with atopic dermatitis (AD) demonstrated significant improvements. Around 60% of patients showed reduced eczema severity, improved quality of life, and decreased use of corticosteroids after six weeks of treatment (Myles *et al*., 2018). Similarly, a trial using *Staphylococcus hominis A9*, which inhibits *Staphylococcus aureus*, revealed notable reductions in AD symptoms, including decreased skin inflammation and *S. aureus* colonization. As research in SMT continues, this approach could become a more standardized therapy for treating AD, particularly for patients who are unresponsive to conventional treatments like topical steroids or immunosuppressants (Junca et al., 2022).

5. Vaginal Microbiota Transplantation (VMT) – recurrent Bacterial Vaginosis

The vagina hosts various microorganisms, with *Lactobacillus* species dominating in healthy women. These bacteria produce lactic acid, keeping the environment acidic to prevent harmful pathogens. Hormonal changes and sexual activity can disrupt this balance, leading to vaginal dysbiosis, increasing the risk of bacterial vaginosis (BV), preterm labor, and sexually transmitted infections. BV, caused by reduced *Lactobacillus* and overgrowth of harmful bacteria like *Gardnerella* and *Prevotella*, often recurs despite antibiotic treatment. Probiotics containing Lactobacillus strains are being studied to restore balance, but mixed results highlight the need for better delivery methods, such as fibers or 3D-printed scaffolds (Barrientos-Durán *et al*., 2020).

Vaginal microbiota transplantation (VMT) offers a promising solution for women with recurrent, antibiotic-resistant bacterial vaginosis (BV). The goal of VMT is to restore a healthy vaginal flora by transferring vaginal fluid from a healthy donor. Studies have demonstrated long-term remission in patients, with improvements in microbial diversity and reduced recurrence rates. VMT is still an emerging therapy, but its potential to offer a sustainable solution for women suffering from chronic BV is encouraging (Sadeghpour Heravi, 2024).

Research into VMT has shown that the procedure is well-tolerated and leads to significant improvements in vaginal health. For example, one study where the donors were healthy premenopausal volunteers (aged 35–48) with no BV history in the past 5 years, followed specific behaviors, such as abstaining from sexual activity for one week before donating. After, recipients administered intra-vaginal antibiotics for a week, donor's vaginal fluid was collected from the upper vagina and transferred to recipients within 60 minutes. Repeat VMTs were done if symptoms or Amsel criteria reappeared. The outcomes were positive, with four patients improving after 1–3 VMT sessions and one showing partial remission. Metagenomic sequencing showed increased Lactobacillus and decreased BV-associated genera like Gardnerella. The partially responsive patient had a different dominant *Lactobacillus* strain (*L. gasseri*). The study showed VMT's potential as a long-term treatment for recurrent BV, but long-term effects are unknown. Further trials are needed to confirm its efficacy (Lev-Sagie *et al*., 2019).

6. CLINICAL EVIDENCE

Microbiota transplantation, including FMT, SMT, and VMT, has shown potential across various conditions linked to microbial imbalances. FMT is particularly effective for recurrent *Clostridium difficile* infection (rCDI), achieving success rates over 90%. It has also been explored for inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), with moderate success, especially in ulcerative colitis and long-term IBS symptom relief (Yadegar *et al*., 2023; El-Sahy *et al*., 2022; Paramsothy *et al*., 2019).

In metabolic syndrome, FMT from lean donors has shown promise in improving insulin sensitivity, though weight loss effects have been modest (Zhang *et al*., 2019). FMT's role in neurodegenerative diseases like Parkinson's is emerging, with early studies indicating improvements in gut-brain communication and reductions in neuroinflammation (Zhao *et al*., 2021).

SMT has shown encouraging results in treating atopic dermatitis (AD), where restoring skin microbial balance can reduce inflammation and reliance on corticosteroids (Paller *et al*., 2019). VMT, though still experimental, offers a promising solution for recurrent bacterial vaginosis (BV), with early evidence suggesting long-term remission in cases where antibiotics have failed (Lev-Sagie *et al*., 2019).

In summary, while more research is needed to standardize these therapies, early clinical evidence supports microbiota transplantation as a promising treatment across several dysbiosis-related conditions.

7. CRITICAL THINKING

Microbiota transplantation shows great potential for treating various conditions, but several key challenges need to be addressed. One of the primary issues is the variability in donor microbiota and the recipient's response. The idea of a "super donor" has emerged, but finding donors with a consistently high microbial diversity remains difficult, and success depends on many factors, including the recipient's own microbiome and health status. More research is needed to optimize donor-recipient matching, which involves tailoring transplants to individual genetic and environmental factors, such as diet and immune status.

Another critical issue is the long-term safety of microbiota transplantation. While short-term results have been promising, the long-term consequences of introducing foreign microbiota into the body are still unknown. Extended monitoring and longitudinal studies are necessary to assess potential risks, such as the development of infections or chronic conditions triggered by the new microbial population. Colonization resistance—where a recipient's existing microbiota rejects the transplanted microbes—also needs further exploration to improve treatment success.

Regulatory frameworks and ethical considerations must evolve to accommodate the growing use of microbiota transplantation. Currently, regulatory guidance varies widely between regions, and there are no universally accepted protocols for donor selection, sample preparation, or transplantation methods. Standardizing these protocols is essential to ensure safety and efficacy across different clinical settings. Ethical concerns also arise regarding donor privacy, informed consent, and the potential commercialization of microbiota products. The development of microbiota banks, which would offer standardized treatments for various conditions, could help address some of these challenges.

8. FUTURE DIRECTIONS

The future of microbiota transplantation is moving towards more personalized, targeted therapies, driven by advances in omics technologies like metagenomics and metatranscriptomics. These tools are improving our understanding of microbial functions and their impact on human health, allowing for the development of tailored microbiota-based treatments for individual patients (Biazzo and Deidda, 2022).

Another promising direction is artificial microbiota and engineered bacteria, designed to address specific dysbiosis patterns. These synthetic solutions could overcome colonization resistance, providing more consistent outcomes by genetically manipulating microbial communities for reliable therapeutic benefits. Standardizing donor screening, microbiota preparation, and administration methods will also be crucial as the practice expands, including creating microbiota banks with "super donors" to provide reliable, diverse microbial communities (Tian et al., 2024; Almeida et al., 2022; Bibbo et al., 2020).

Additionally, integrating microbiota transplantation with personalized nutrition and prebiotics could enhance its effectiveness, especially in conditions like inflammatory bowel disease (IBD) and metabolic syndrome, by promoting healthier gut environments and improved microbial colonization (Tian et al., 2024; Almeida et al., 2022).

In conclusion, microbiota transplantation is set to become a powerful tool in personalized medicine, offering innovative treatments for a wide range of dysbiosis-related diseases as research progresses (Biazzo and Deidda, 2022).

9. REFERENCES:

Almeida, Cátia, et al. "Current trends and challenges of fecal microbiota transplantation—an easy method that works for all?." *Biomedicines* 10.11 (2022): 2742.

Biazzo, Manuele, and Gabriele Deidda. "Fecal microbiota transplantation as new therapeutic avenue for human diseases." *Journal of Clinical Medicine* 11.14 (2022): 4119

Bibbò, Stefano, et al. "Fecal microbiota transplantation: screening and selection to choose the optimal donor." *Journal of Clinical Medicine* 9.6 (2020): 1757.

Barrientos-Durán, Antonio, et al. "Reviewing the composition of vaginal microbiota: inclusion of nutrition and probiotic factors in the maintenance of eubiosis." Nutrients 12.2 (2020): 419.

Boxberger, M., Cenizo, V., Cassir, N. *et al.* Challenges in exploring and manipulating the human skin microbiome. *Microbiome* **9**, 125 (2021). https://doi.org/10.1186/s40168-021-01062-5

Callewaert C, Knödlseder N, Karoglan A, Güell M, Paetzold B. Skin microbiome transplantation and manipulation: Current state of the art. Comput Struct Biotechnol J. 2021 Jan 4;19:624-631. doi: 10.1016/j.csbj.2021.01.001. PMID: 33510866; PMCID: PMC7806958.

D. Goldenberg, Simon, and Blair Merrick. "The role of faecal microbiota transplantation: Looking beyond Clostridioides difficile infection." *Therapeutic advances in infectious disease* 8 (2021): 2049936120981526.

de Groot, Pieter, et al. "Donor metabolic characteristics drive effects of faecal microbiota transplantation on recipient insulin sensitivity, energy expenditure and intestinal transit time." *Gut* 69.3 (2020): 502-512.

El-Salhy, Magdy, et al. "Efficacy of fecal microbiota transplantation for patients with irritable bowel syndrome at 3 years after transplantation." *Gastroenterology* 163.4 (2022): 982-994

Goll, Rasmus, et al. "Effects of fecal microbiota transplantation in subjects with irritable bowel syndrome are mirrored by changes in gut microbiome." *Gut microbes* 12.1 (2020): 1794263.

Junca, Howard, Dietmar H. Pieper, and Eva Medina. "The emerging potential of microbiome transplantation on human health interventions." *Computational and structural biotechnology journal* 20 (2022): 615-627.

Kim, Bong-Soo, Yoon-Seong Jeon, and Jongsik Chun. "Current status and future promise of the human microbiome." *Pediatric gastroenterology, hepatology & nutrition* 16.2 (2013): 71-79.

Kelly, Brendan J., and Pablo Tebas. "Clinical practice and infrastructure review of fecal microbiota transplantation for Clostridium difficile infection." *Chest* 153.1 (2018): 266-277.

Lev-Sagie, A., Goldman-Wohl, D., Cohen, Y. *et al.* Vaginal microbiome transplantation in women with intractable bacterial vaginosis. *Nat Med* **25**, 1500–1504 (2019). https://doi.org/10.1038/s41591-019-0600-6

Matheson, Julie-Anne T., and RM Damian Holsinger. "The role of fecal microbiota transplantation in the treatment of neurodegenerative diseases: a review." *International Journal of Molecular Sciences* 24.2 (2023): 1001.

Myles, Ian A., et al. "First-in-human topical microbiome transplantation with Roseomonas mucosa for atopic dermatitis." *JCI insight* 3.9 (2018).

Paller, Amy S., et al. "The microbiome in patients with atopic dermatitis." *Journal of Allergy and Clinical Immunology* 143.1 (2019): 26-35.

Paramsothy, Sudarshan, et al. "Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis." *Gastroenterology* 156.5 (2019): 1440-1454.

Peery, Anne F., et al. "AGA Clinical Practice Guideline on Fecal Microbiota–Based Therapies for Select Gastrointestinal Diseases." *Gastroenterology* 166.3 (2024): 409-434.

Sadeghpour Heravi, Fatemah. "Host-vaginal microbiota interaction: shaping the vaginal microenvironment and bacterial vaginosis." *Current Clinical Microbiology Reports* (2024): 1-15.

Tian, Hongliang, et al. "Fecal microbiota transplantation in clinical practice: Present controversies and future prospects." hLife (2024).

Vendrik, Karuna EW, et al. "Fecal microbiota transplantation in neurological disorders." *Frontiers in cellular and infection microbiology* 10 (2020): 98.

Wilson, Brooke C., et al. "The super-donor phenomenon in fecal microbiota transplantation." *Frontiers in cellular and infection microbiology* 9 (2019): 2.

Yadegar, Abbas, et al. "Beneficial effects of fecal microbiota transplantation in recurrent Clostridioides difficile infection." *Cell host & microbe* 31.5 (2023): 695-711.

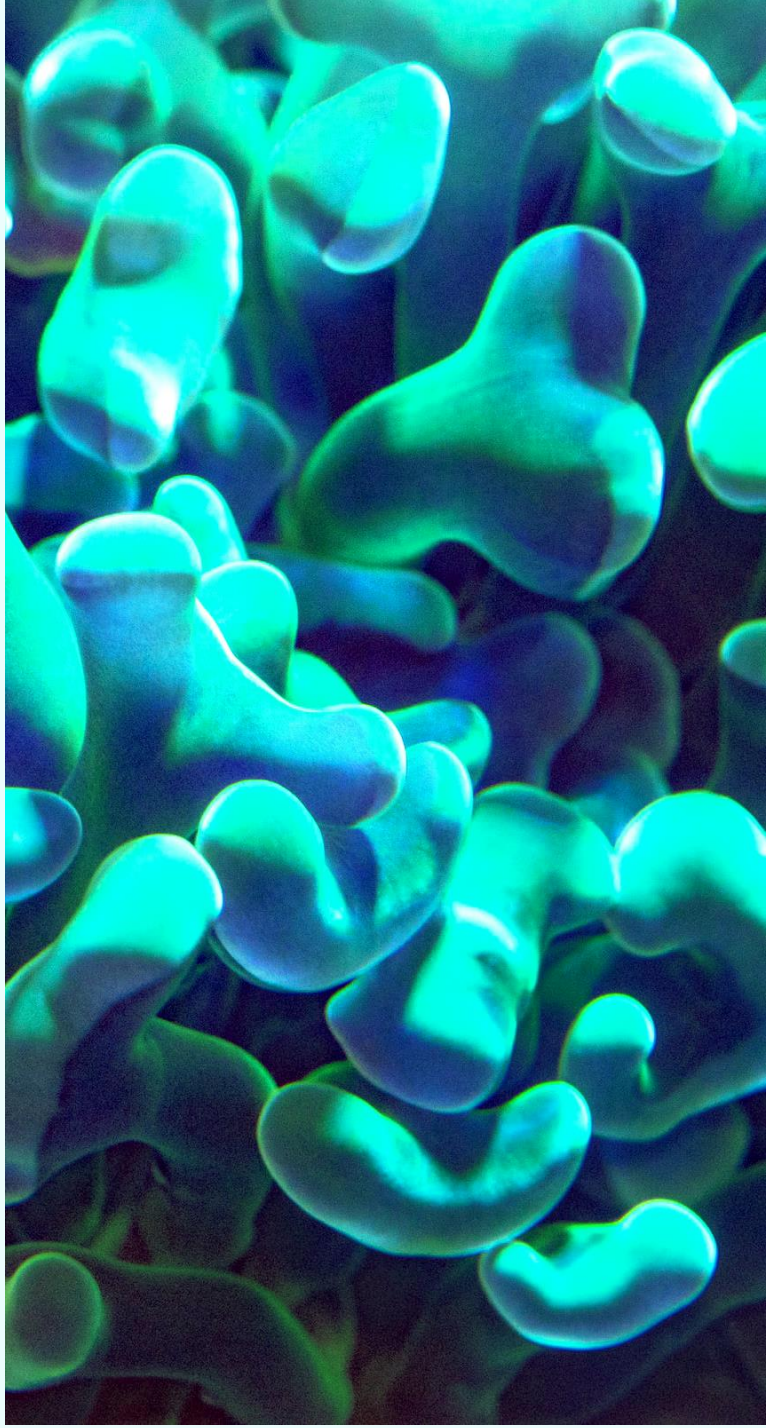
Zhang, Zhengxiao, et al. "Impact of fecal microbiota transplantation on obesity and metabolic syndrome—a systematic review." *Nutrients* 11.10 (2019): 2291.

Zhao, Zhe, et al. "Fecal microbiota transplantation protects rotenone-induced Parkinson's disease mice via suppressing inflammation mediated by the lipopolysaccharide-TLR4 signaling pathway through the microbiota-gut-brain axis." *Microbiome* 9 (2021): 1-27.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Post-graduate certificate level 7 (30 ECTS)

Gut and Vaginal Microbiota and Female Reproductive Health



Malta Further & Higher Education Authority

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Summary

The topic of present webinar is “Gut and Vaginal Microbiota and Reproductive Health”. On the one hand, this topic was developed as an understanding of the composition of individual microbiota and their role as well as the role of microbial metabolites in maintaining health. On the other hand, the causes and consequences of vaginal and gut microbiota dysbiosis, their interconnections, as well as the gut microbiota axis with extrogen and brain function in the development of reproductive disorders was covered.

Based on literature search process, common female reproductive health disorders that will be represented include vaginitis (encompassing bacterial vaginosis (BV), sexually transmitted infections (STI), and vulvovaginal candidiasis (VVC)), endometriosis, polycystic ovary syndrome (PCOS), and infertility. It will be addressed that vaginitis is typically linked to vaginal dysbiosis, while endometriosis and PCOS are more often associated with gut dysbiosis. These conditions can lead to infertility, which encompasses issues with conception, miscarriages, and pre-term birth

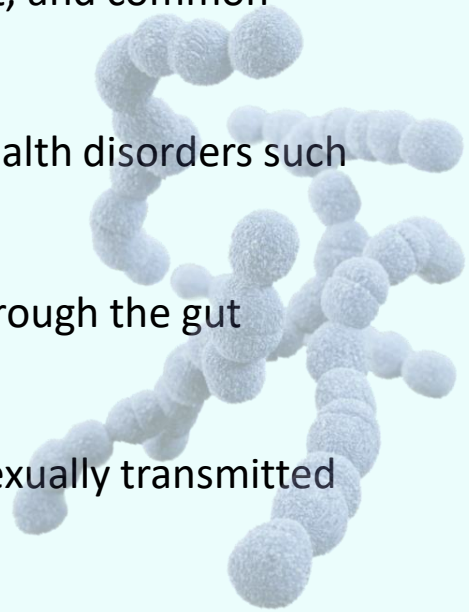
The origin, diagnosis, symptoms, alternations in the composition of the microbiota, as well as the pathogenesis associated with dysbiosis of the microbiota in the context of each reproductive disorder are presented separately for easier understanding and observation of their similarities. It will be shown that the mentioned disorders are often intertwined with other comorbidities, especially in endometriosis and PCOS, so there is a great number of risk factors causing their occurrence. All are important, but only most relevant to present topic will be described.

At the end of each section with one of the 4 listed disorders, potential treatments to alleviate symptoms and promote fertility will be listed. All proposed treatment strategies are directly or indirectly related to the modulation of the composition of the gut and vaginal microbiome. Case studies will demonstrate the potential of these treatments in managing vaginitis, endometriosis, PCOS, and infertility, offering valuable insights for future research.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Learning objectives

1. **Recognize Microbiota Composition and Dysbiosis**
   Understand the typical composition of gut and vaginal microbiota and their roles in maintaining health. Define gut and vaginal dysbiosis.
2. **Identify Modulators of Microbiota**
   Learn the main factors influencing the composition of gut and vaginal microbiota, with a focus on female physiological factors.
3. **Understand Female Reproductive Health**
   Define female reproductive health, its coordination, the parts of the female reproductive tract, and common reproductive health disorders.
4. **Acknowledge Gut-Vaginal Microbiota Crosstalk**
   Recognize the importance of gut-vaginal microbiota interaction and its role in reproductive health disorders such as vaginitis, endometriosis, PCOS, and infertility.
5. **Connect Hormonal Homeostasis and Microbiota**
   Understand the relationship between hormonal balance and reproductive health disorders through the gut microbiota-estrogen and gut microbiota-brain axes.
6. **Identify Causes and Symptoms of Disorders**
   Learn the main causes, symptoms, and diagnostic methods for vaginitis, bacterial vaginosis, sexually transmitted infections, vulvovaginal candidiasis, endometriosis, PCOS, and infertility.

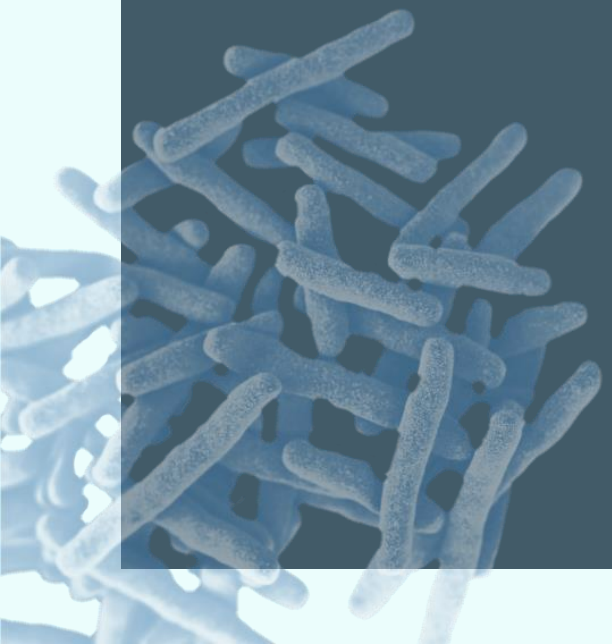


INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

7. **Observe Microbiota Alterations**
   Identify changes in gut and vaginal microbiota composition in relation to specific bacteria and metabolites in reproductive health disorders
8. **Differentiate Pathogenesis and Outcomes**
   Recognize differences and similarities in the pathogenesis and outcomes of vaginitis, endometriosis, PCOS, and infertility based on microbiota roles.
9. **Acknowledge Comorbidities**
   Understand that endometriosis and PCOS often co-occur with other comorbidities, necessitating individualized treatment approaches.
10. **Understand Dysbiosis and Infertility**
    Learn how vaginitis is linked to vaginal dysbiosis, while endometriosis and PCOS are more associated with gut dysbiosis, and how all three can lead to infertility
11. **Comprehend Complexity of Dysbiosis**
    Understand the challenge of determining whether dysbiosis is a cause or consequence of reproductive disorders and identify risk factors and treatments.
12. **Learn Treatment Strategies**
    Recognize conventional and alternative treatments for vaginitis, endometriosis, PCOS, and infertility, including probiotics, microbiome transplants, and dietary interventions.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Indeks



Introduction

1. Human gut microbiota
   - Composition
   - Functions of the Gut microbiota and Microbial Metabolites
   - Interaction of Gut Microbiome with Human Organic Systems
2. Vaginal Microbiota
   - Composition
   - The Role of *Lactobacillus* spp.
   - Vaginal Dysbiosis
   - Factors Affecting Vaginal Mcrobiota Composition
3. Female Reproductive Health
4. Three Main Crosstalks in Female Reproductive Health
   - Gut – Vaginal Microbiota Crosstalk
   - Gut microbiota – Estrogen Axis
   - Gut Microbiota – Brain Axis
5. Female Reproductive Disorders Associated with Gut and Vaginal Dysbiosis
   - Vaginitis – Bacterial Vaginosis, Sexually Transmitted Infections, Vulvovaginal Candidiasis
   - Endometriosis
   - Polycistic Ovary Syndrome (PCOS)
   - Female Infertility
6. Real Cases
7. Conclusions

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

1. Human Gut Microbiota: Composition

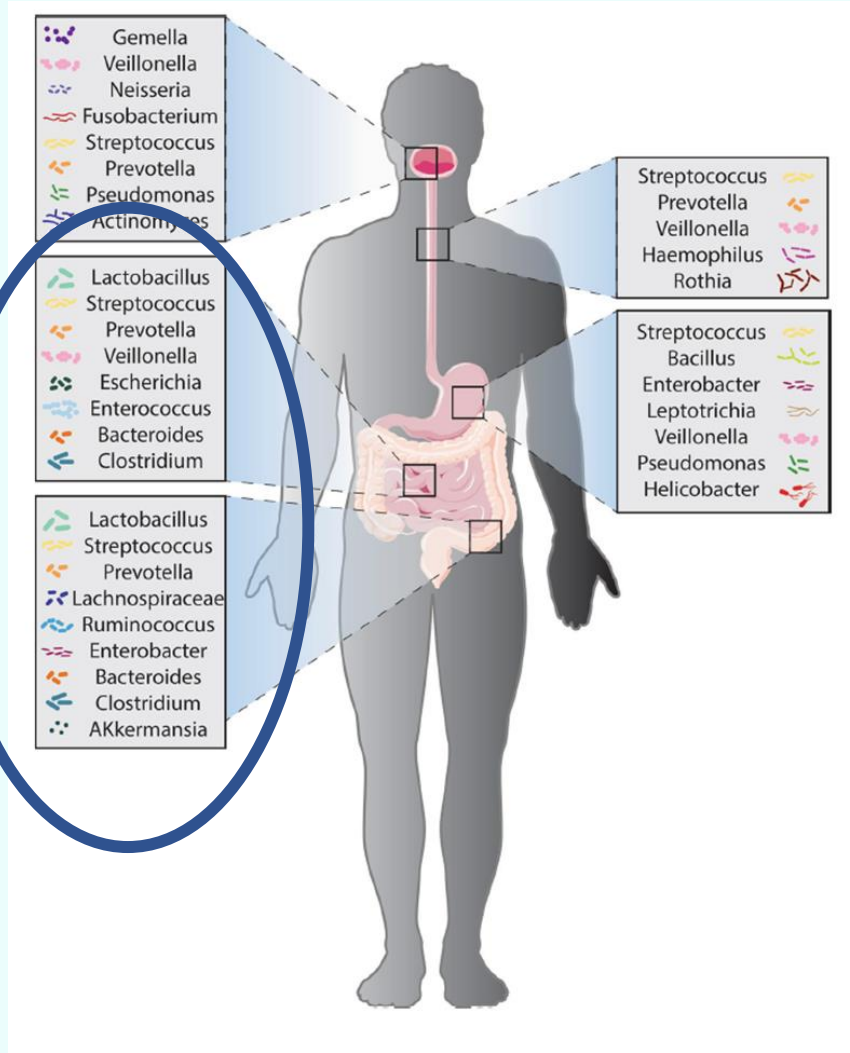
- Gut microbiota = Collection of microorganisms present in the gut environment
- Gut microbiome = The entire gut ecosystem, including all the microorganisms, their genomes, associated functions and actual activity and the surrounding environmental conditions

- structure varies among healthy individuals

Personal: genetics, birth mode, age, gender, migration

Transmission: vertical and horizontal

Environment and lifestyle: socio-economic, diet, medications,



- Female gut microbiota **more diverse**

Ruan, Wenly, et al. "Healthy human gastrointestinal microbiome: composition and function after a decade of exploration." *Digestive diseases and sciences* 65 (2020): 695-705.

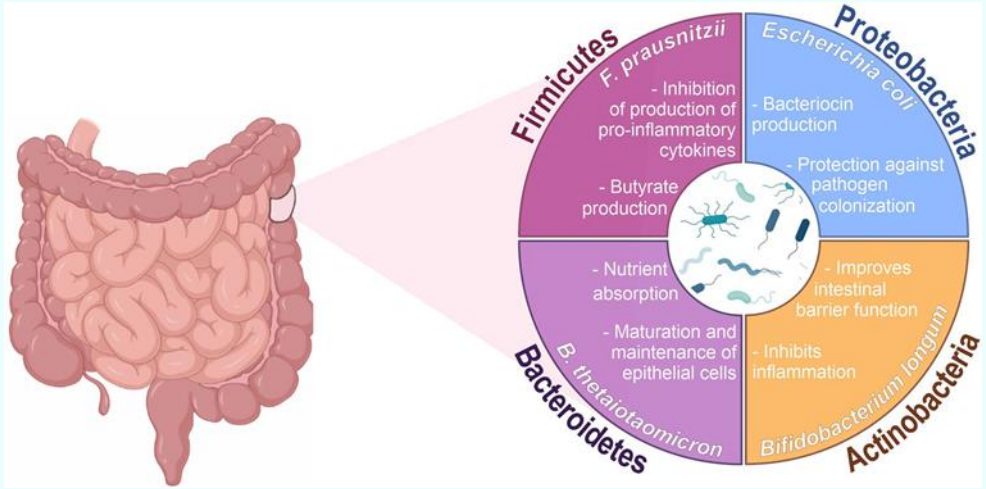
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

lananovkovic147@gmail.com

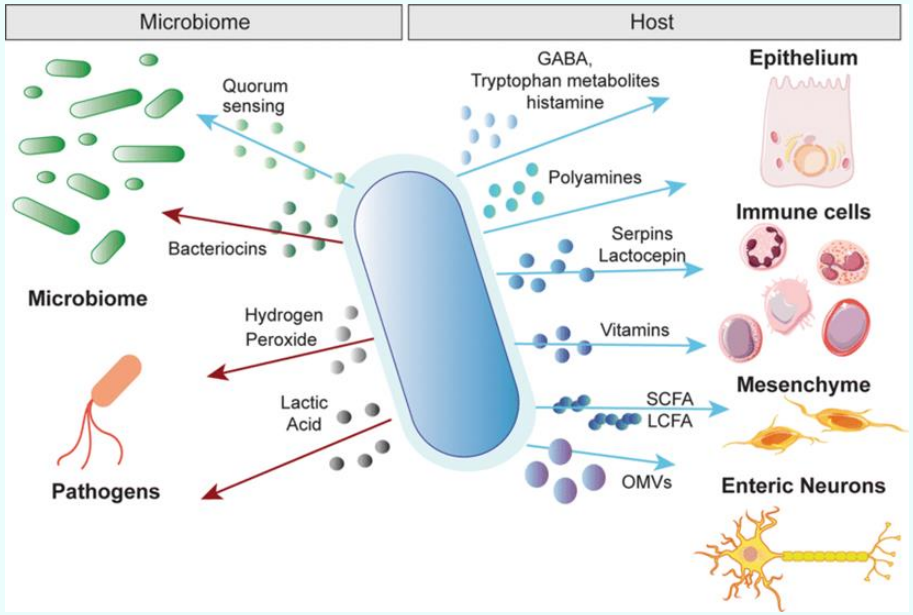
Functions of the Gut Microbiota and Microbial Metabolites

- Microbial functions well-conserved
- Basic functions in intestinal tract:
  - Promoting digestion
  - Health of intestinal tract
  - Barrier against pathogens and toxins
  - Defensive role in the immune system

- Important role in function – the generation of microbial metabolites – interaction with both microbes and host
- The main aim of microbial functions is to form:
  - **Diverse**
  - **Stable**
  - **Resilient** gut microbiome



Maciel-Fiuza MF, Muller GC, Campos DMS, do Socorro Silva Costa P, Peruzzo J, Bonamigo RR, Veit T and Vianna FSL (2023) Role of gut microbiota in infectious and inflammatory diseases. *Front. Microbiol.* 14:1098386. doi:10.3389/fmicb.2023.1098386



Ruan, Wenly, et al. "Healthy human gastrointestinal microbiome: composition and function after a decade of exploration." *Digestive diseases and sciences* 65 (2020): 695-705.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Interaction of Gut Microbiome with Human Organic Systems

- Diverse, stable and resilient gut microbiome = **eubiosis** of gut microbiota with many health benefits
- Gut microbiome functions interact with other human organs by forming particular axis (gut – vaginal microbiome axis) – **separate organ**

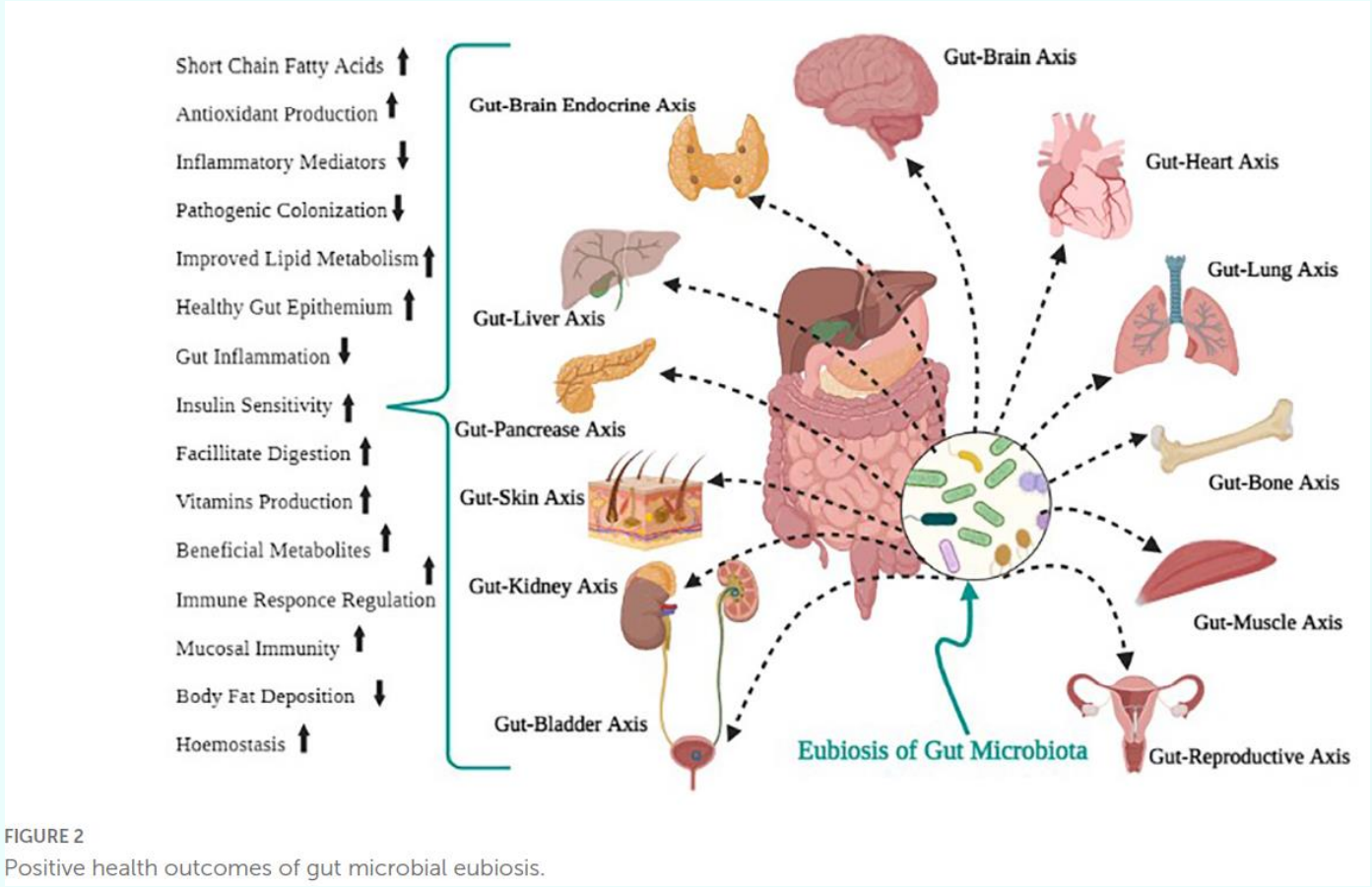


FIGURE 2
Positive health outcomes of gut microbial eubiosis.

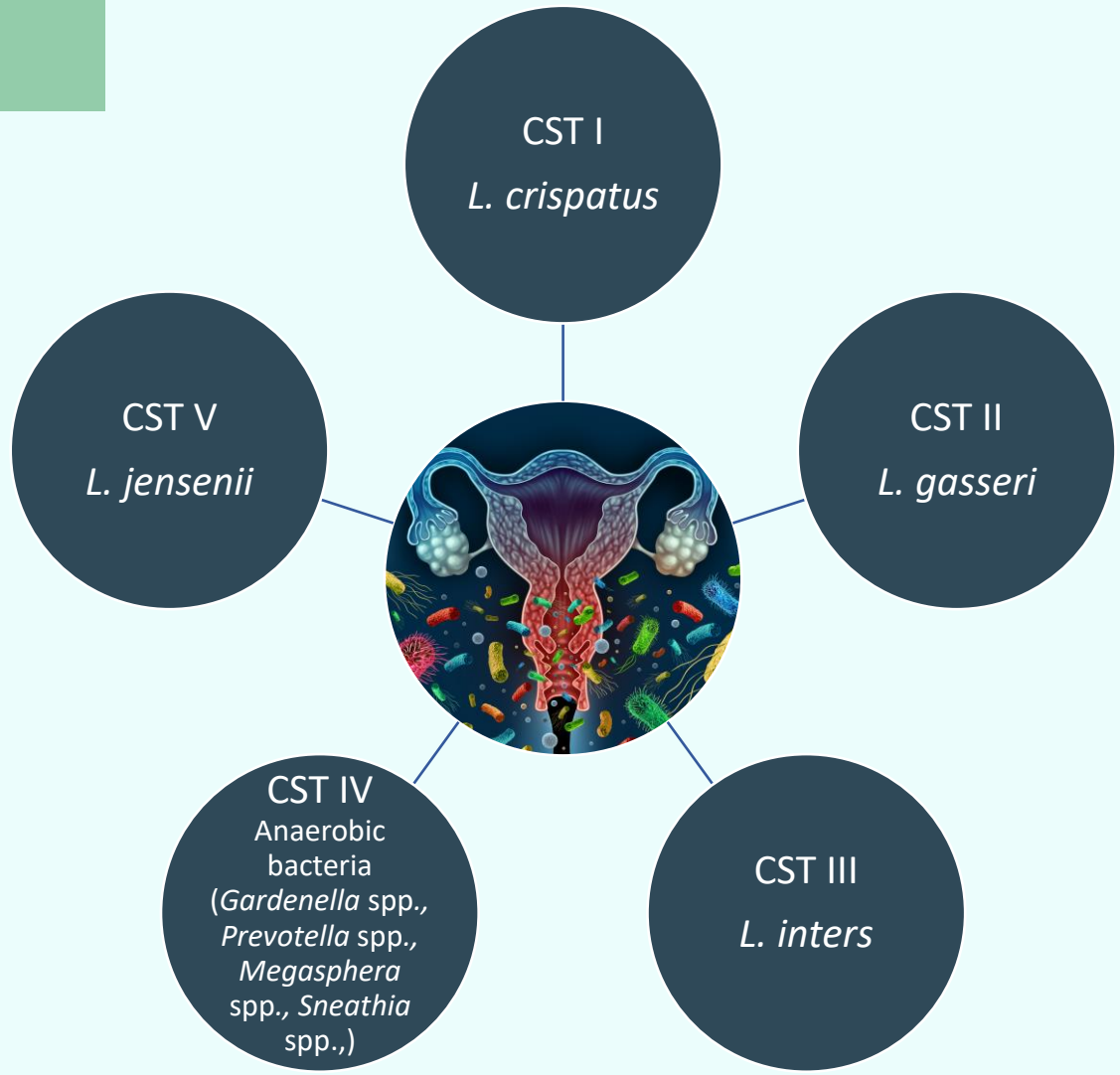
Afzaal, Muhammad, et al. "Human gut microbiota in health and disease: Unveiling the relationship." *Frontiers in microbiology* 13 (2022): 999001.

a change, a difference or an alteration of gut microbiota → Gut – organ dysbiosis → Health disorders: obesitiy, diabetes mellitus, hypertension, cardiovascular diseases, inflamatory bowel disease (IBD), cancer

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

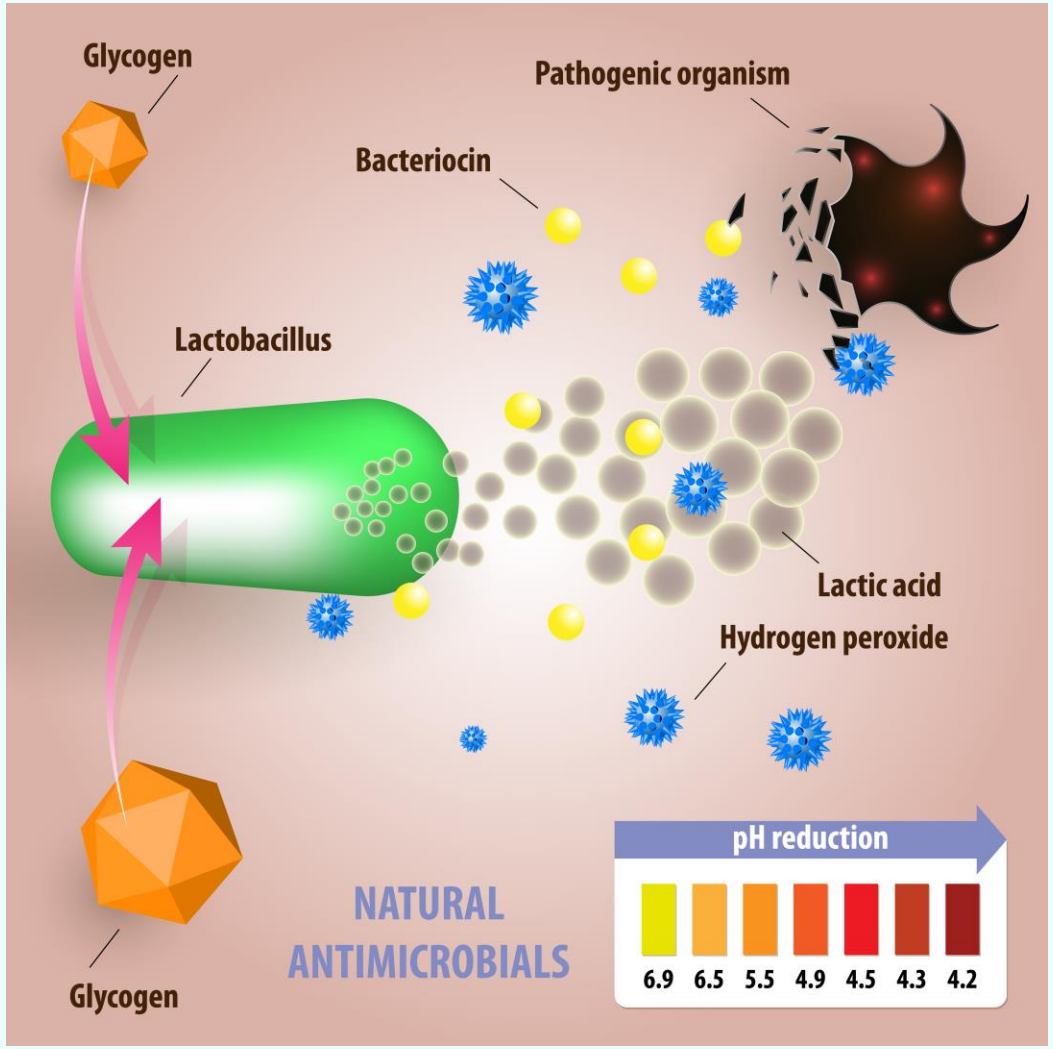
2. Vaginal Microbiota: Composition

- Balanced compositon = **low diversity** (c. 200 bacterial species) + **predominant** (70 %) ***Lactobacillus* spp.** (10^7 – 10^9 per gram vaginal fluid in reproductive healthy women)
- Vaginal bacterial community composition clustered into five specific community state types (CSTs)
- Vaginal mycobiota mostly consists of *Candida* species
- pH value **< 4,5** (varies from 4.0 ± 0.3 for CST I to 5.3 ± 0.6 for CST IV)



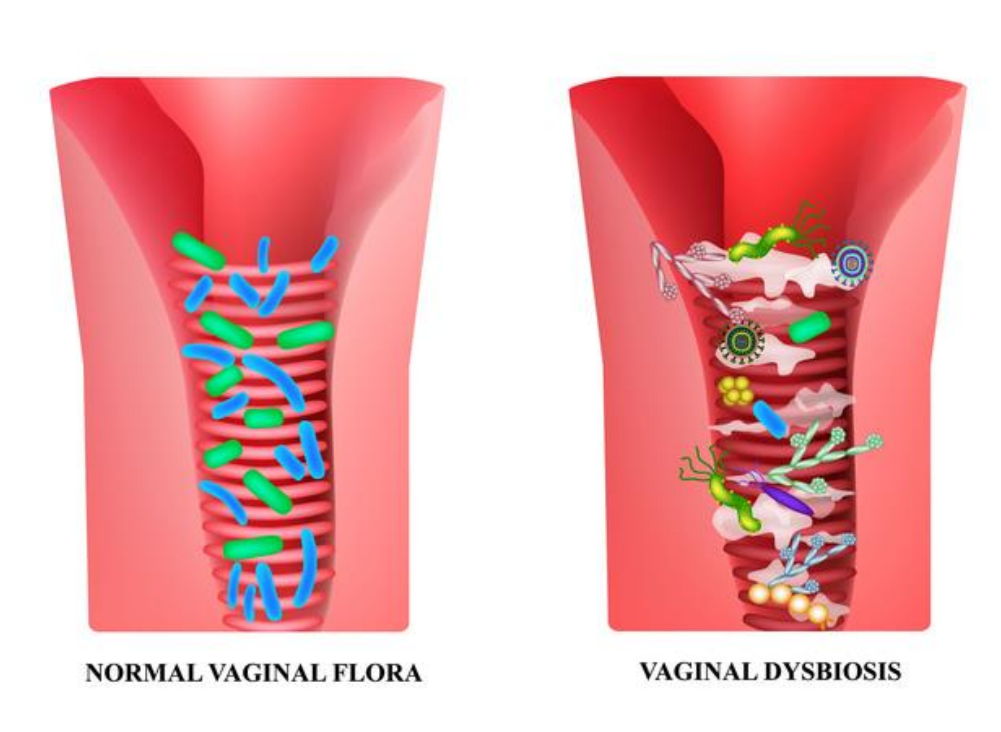
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

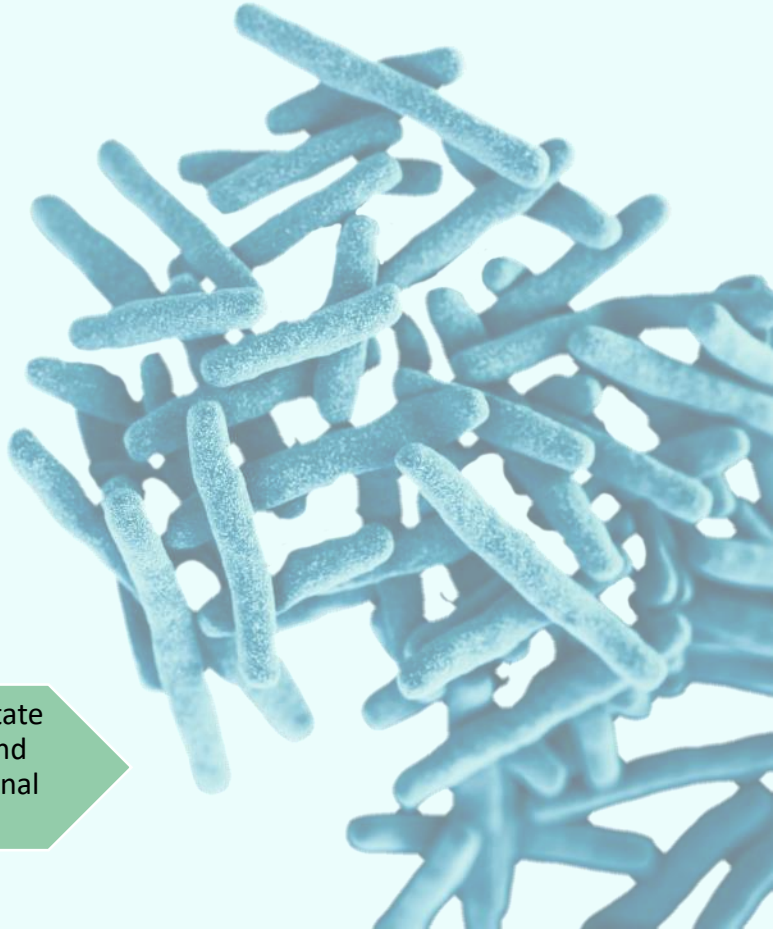
The Role of *Lactobacillus* spp.



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Vaginal Dysbiosis

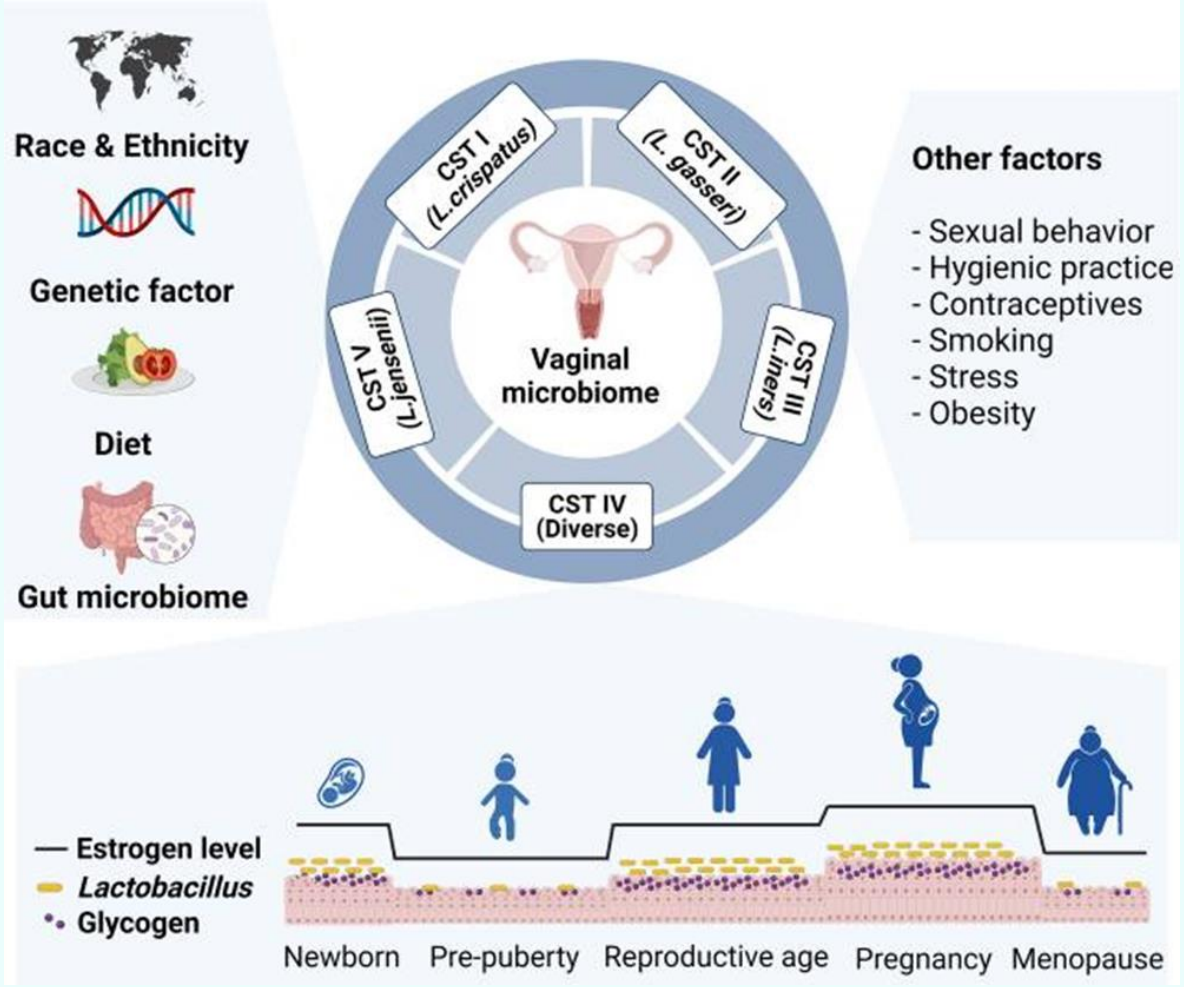




Higher microbiota diversity → Increase of the CST IV bacteria → Decrease of the *Lactobacilus* spp. dominance → Decreased lactate production and increased vaginal pH

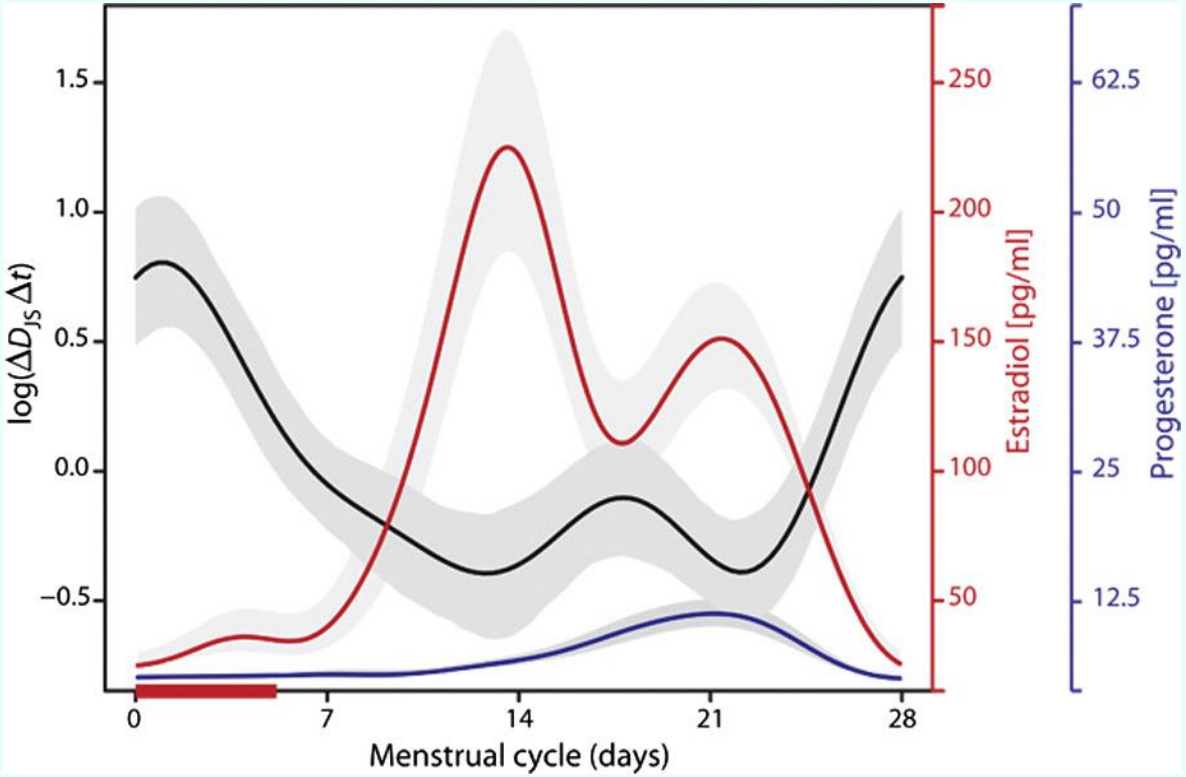
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Factors Affecting Vaginal Mcrobiota Composition

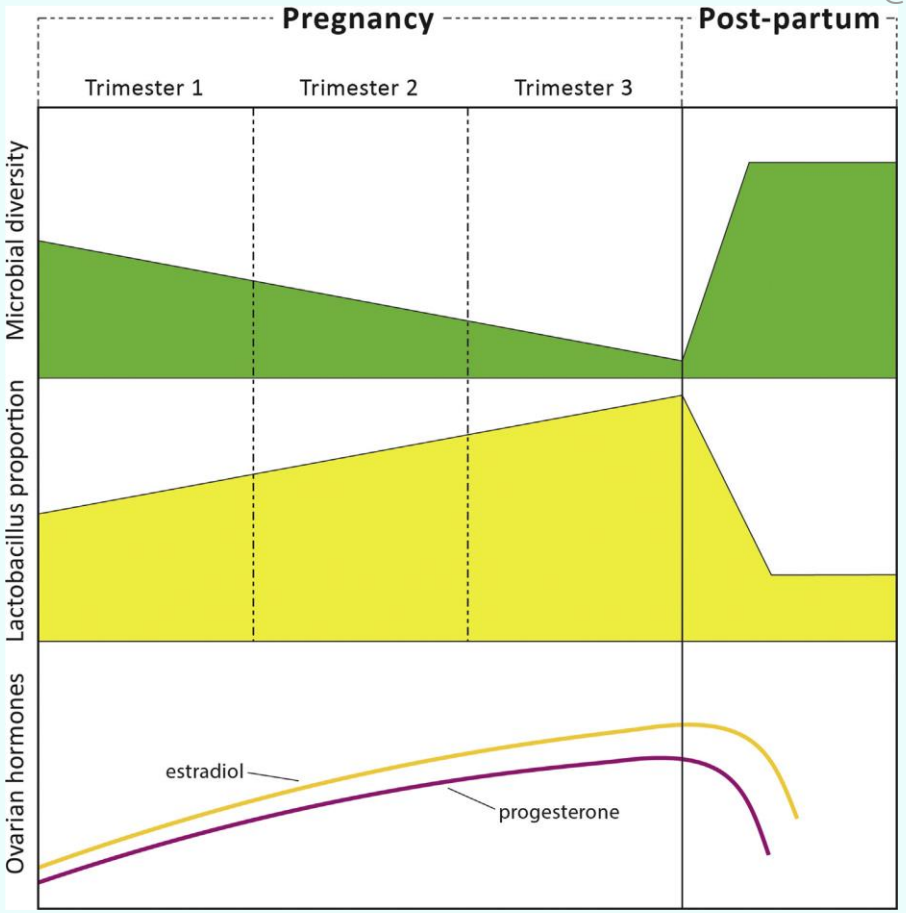


Kwon, Myoung Seung, and Heung Kyu Lee. "Host and microbiome interplay shapes the vaginal microenvironment." *Frontiers in immunology* 13 (2022): 919728.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com



- Vaginal microbiota composition during phases of menstrual cycle:
- Abundance of *Lactobacillus* spp. relatively stable
- Fluctuations between CST I, II, III and V
- Increase of diversity during period but not harmful



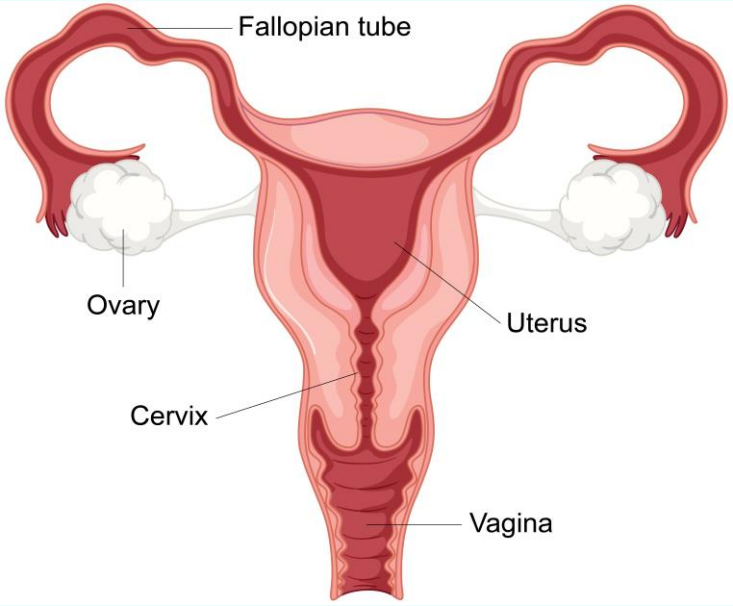
- Vaginal microbiota composition during pregnancy and pospartum:
- Strong shift in number of *Lactobacillus* spp. and diversity level followed by drop in hormones level in postpartum period

Both figures' source: Kroon, Samuel J., Jacques Ravel, and Wilhelmina M. Huston. "Cervicovaginal microbiota, women's health, and reproductive outcomes." *Fertility and sterility* 110.3 (2018): 327-

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

3. Female Reproductive Health

„Reproductive health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity, in all matters relating to the reproductive system and to its functions and processes. Reproductive health implies that people are able to have a satisfying and safe sex life and that they have the capability to reproduce and the freedom to decide if, when and how often to do so.” (WHO, https://www.who.int/westernpacific/health-topics/reproductive-health)



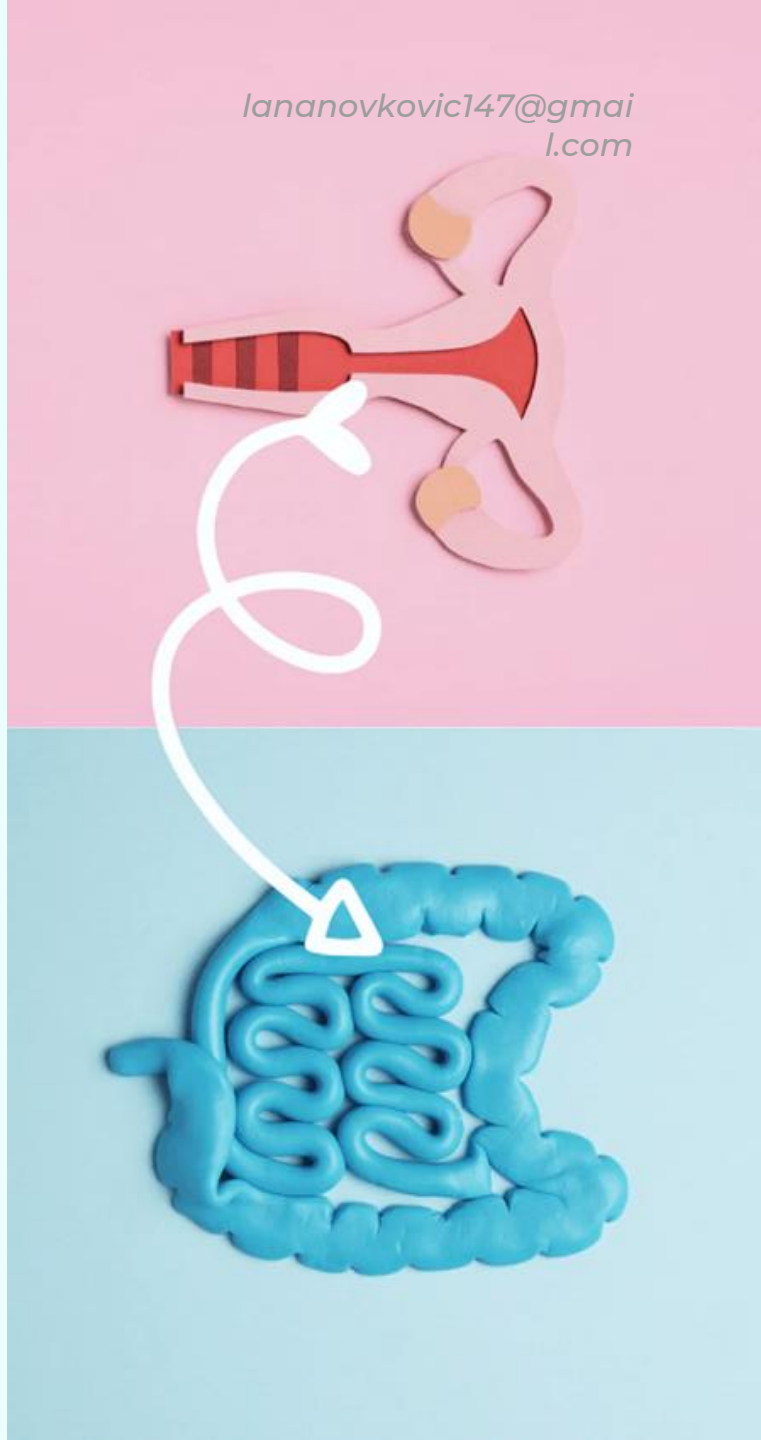
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

lananovkovic147@gmail.com

4. Three Main Crosstalks in Female Reproductive Health

Gut and Vaginal Microbiome Crosstalk

- Crosstalk via:
  - Vertical transmission of microbiota
  - Rectum – vaginal microbiota translocation
  - Gut – estrogen axis
- Vaginal *Lactobacillus* spp. originated from gut microbiota
- Disrupted crosstalk by gut dysbiosis alternates vaginal microbiota and vice versa



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Gut and Vaginal Microbiome Crosstalk – SCFA and Immune Response

Gut microbiota

- TNF-α
- NF-κB
- IL-1β, IL-6, IL-8, IL-12
+ PGE_2 IL-10, Foxp3+
- pH, CXCL9-CXCL11
- LPS translocation
- Bacterianemia
+ AMPs

- *Clostridium*
+ *Bacteroides*
- *Prevotella*
-*Proteobacteria*
+ *Bifidobacterium*
+ *Lactobacillus*

- Anti-inflammatory effects
- Stabile and resistant – maintaining homeostasis between gut epithelium, diet and comensal microbes

Prevention of „leaky gut" and preterm birth

SCFA:
Acetate
Butyrate
Propionate

Vaginal microbiota

- *Lactobacillus*
+ *Gardnerella*
+ *Prevotella*
+ *Mobiluncus*
+ *Firmicutes*
+ *Mycoplasma*

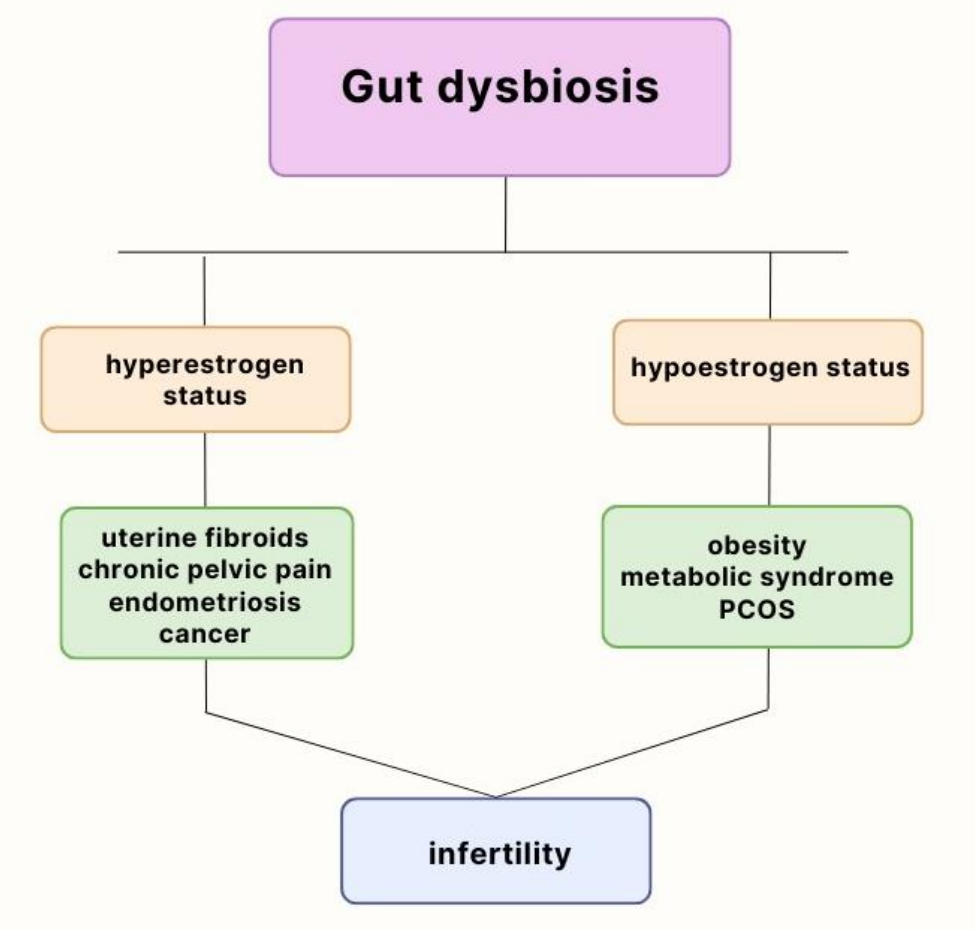
+ NF-κB
+ IL-1β
+ TNF-α
+ IL-6
+ IL-8
+ pH
-Lactate

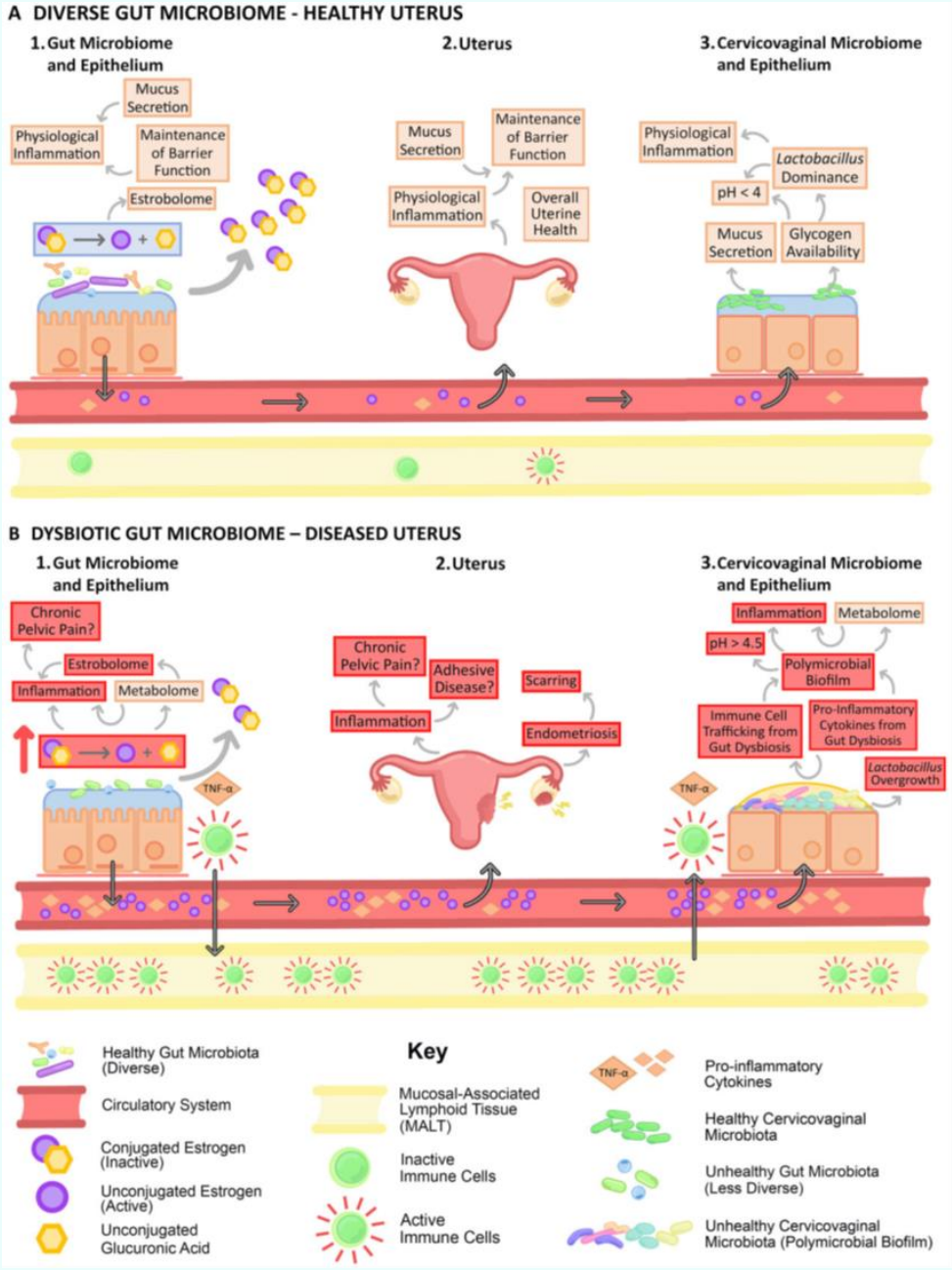
Vaginal dysbiosis – Bacteria vaginosis and/or vulvovaginal candidiasis

Intrauterine inflammation, infection - Failure of conception, miscarriage and preterm birth

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Gut Microbiota– Estrogen axis

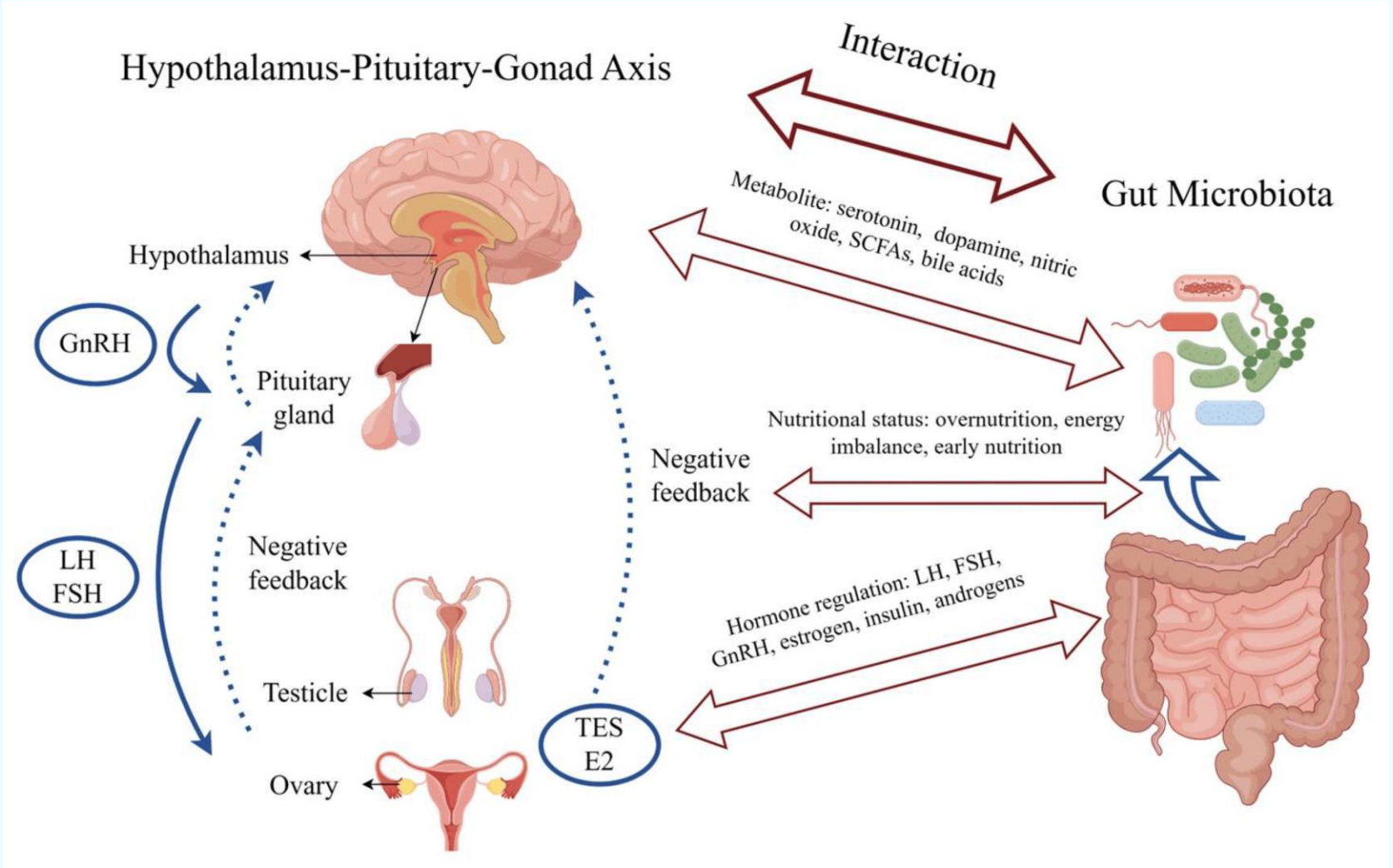




Salliss, Mary E., et al. "The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain." *Human reproduction update* 28.1 (2022): 92-131.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Gut Microbiota – Brain/HPG Axis

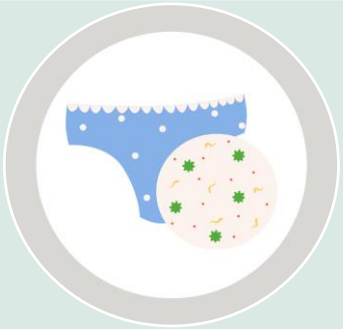


Yue, Min, and Lei Zhang. "Exploring the Mechanistic Interplay between Gut Microbiota and Precocious Puberty: A Narrative Review." *Microorganisms* 12.2 (2024): 323.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

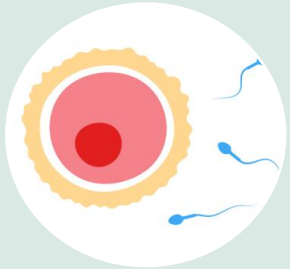
5. Female Reproductive Disorders Associated with Gut and Vaginal Dysbiosis

Bacterial Vaginosis/Candidasis/ STI



Endometriosis

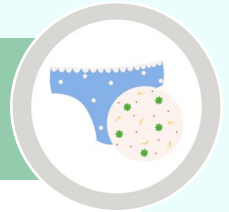
Polycistic Ovary Syndrom (PCOS)



Infertility

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Vaginitis (Bacterial vaginosis/candidiasis/STI)



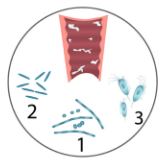
SYMPTOMS OF VAGINITIS

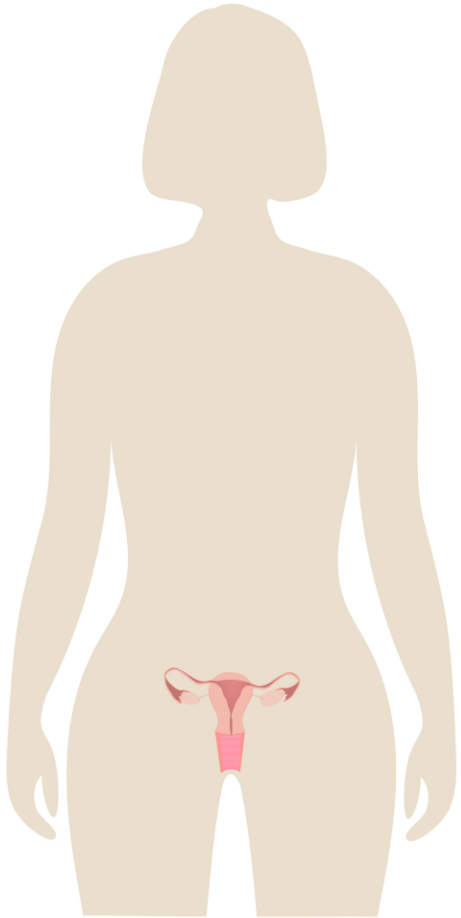
Weakening of immunity

- Change in color, odor or amount of discharge from vagina,
- Light vaginal bleeding or spotting

- Light vaginal bleeding or spotting

1. Candida vaginitis with thick white discharge that resembles cottage cheese;
2. Bacterial vaginitis with grayish-white, foul-smelling discharge;
3. Trichomoniasis with greenish-yellow, frothy discharge.





Decreased libido
Indignation disorders

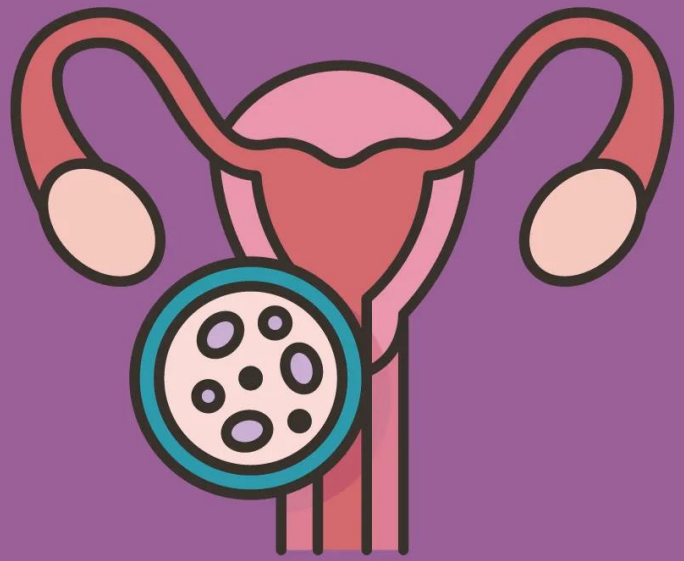
Painful urination
Pain during sex

Inflammation: irritation, redness, and swelling of the labia majora, labia minora, or perineal area

Vaginal itching
or irritation genital area

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Bacterial Vaginosis (BV)



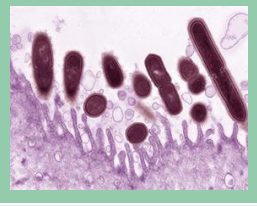
- Prevalence: low in Europe and Asia, intermediate in America, high in Africa
- Dominant BV- associated bacteria: *Gardnerella vaginallis*, *Prevotella* spp. (*P. bivia*, *P. disiens*), *Atopobium vaginae, Mobiluncus*
- Risk factors: multiple/new sexual partner, inconvenient vaginal hygene practice, smoking
- Gynecological complications: increased risk of pelvic inflammatory disease, sexually transmitted infections, including HIV infection spontaneous preterm labor, abortion, and endometriosis

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmai

Bacterial Vaginosis- Diagnosis Criteria

- Most commonly based on Amsel and Nugent criteria and molecular methods

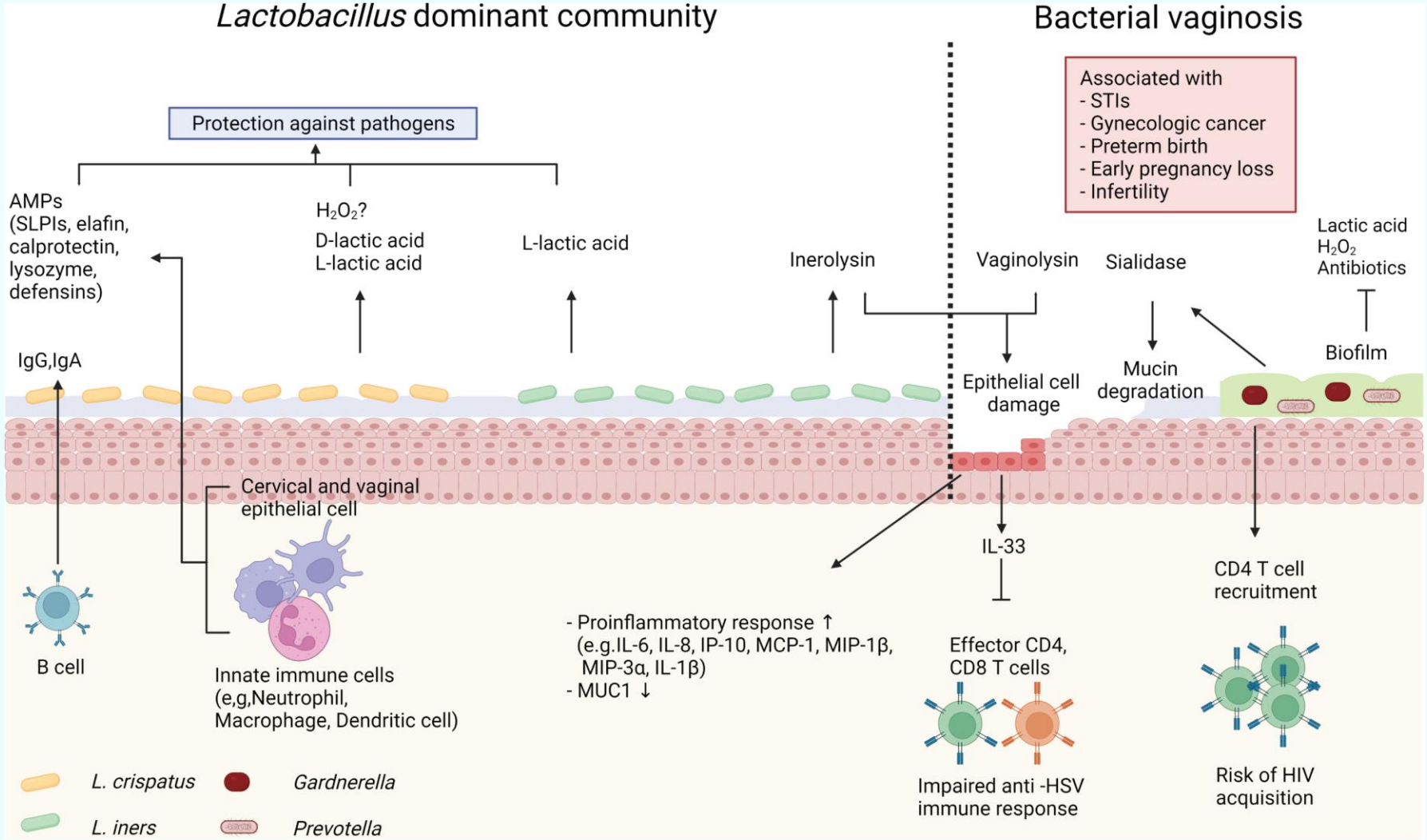
Amsel criteria	
Clinical signs of BV	3 – 4 confirmed clinical signes = BV
Vaginal pH > 4,5	
Presence of adherent white discharge	
Detection of „clue cells”	
Presence of amine odor after the addition of KOH	

Nugent scoring test		
Organism morphotype	Number/oil immersion field	Score
Lactobacillus spp. (gram-positive bacilli)	>30	0
	5 – 30	1
	1 – 4	2
	< 1	3
	0	4
Mobiluncus (curved gram-variable rods)	>5	2
	< 1 – 4	1
	0	0
Gardnerella/Bacteroides (gram – negative coccobacilli) 	>30	4
	5 – 30	3
	1 – 4	2
	< 1	1
	0	0
Total score: 0 – 3 = Normal; 4 – 6 = Intermediate (repeat test later); 7 – 10 = BV		

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

BV and Immune Response

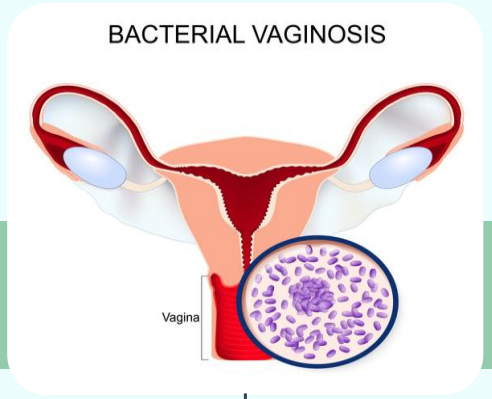
lananovkovic147@gmail.com



Kwon, Myoung Seung, and Heung Kyu Lee. "Host and microbiome interplay shapes the vaginal microenvironment." *Frontiers in immunology* 13 (2022): 919728.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Treatment of BV

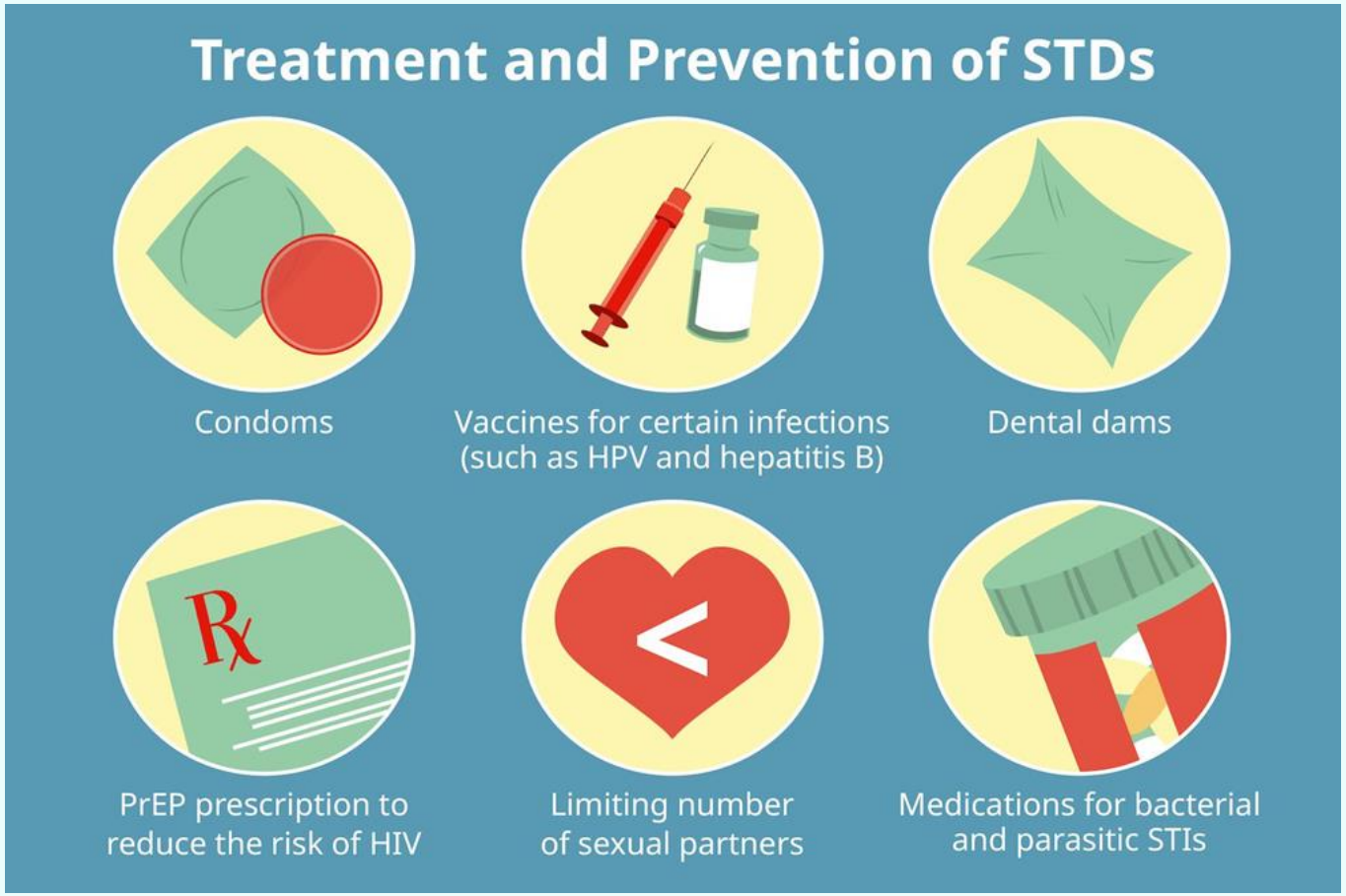


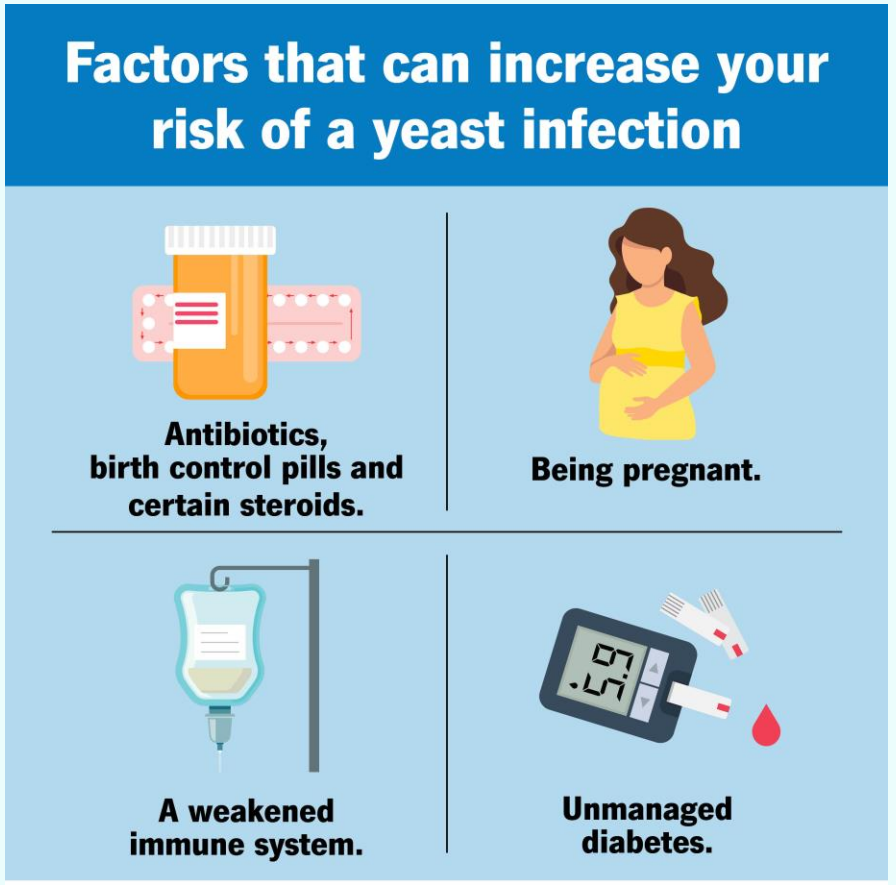
- Standard
  - Antimicrobials (Antibiotics)
    - Metronidazole
    - Clindamycin
    - Recurrence
- Alternatives
  - Act directly on bacteria
    - Other antimicrobials
    - Antiseptics
    - Surfactans
    - DNAses
  - Reestablish vaginal environment
    - probiotics
    - prebiotics
    - acidifying agents
- Innovative approach
  - Total replacement of microbiota
    - Vaginal microbiota transpalntation

Main Focus

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Sexually Transmitted Infection(STI) vs. Vulvovaginal Candidiasis (VVC)



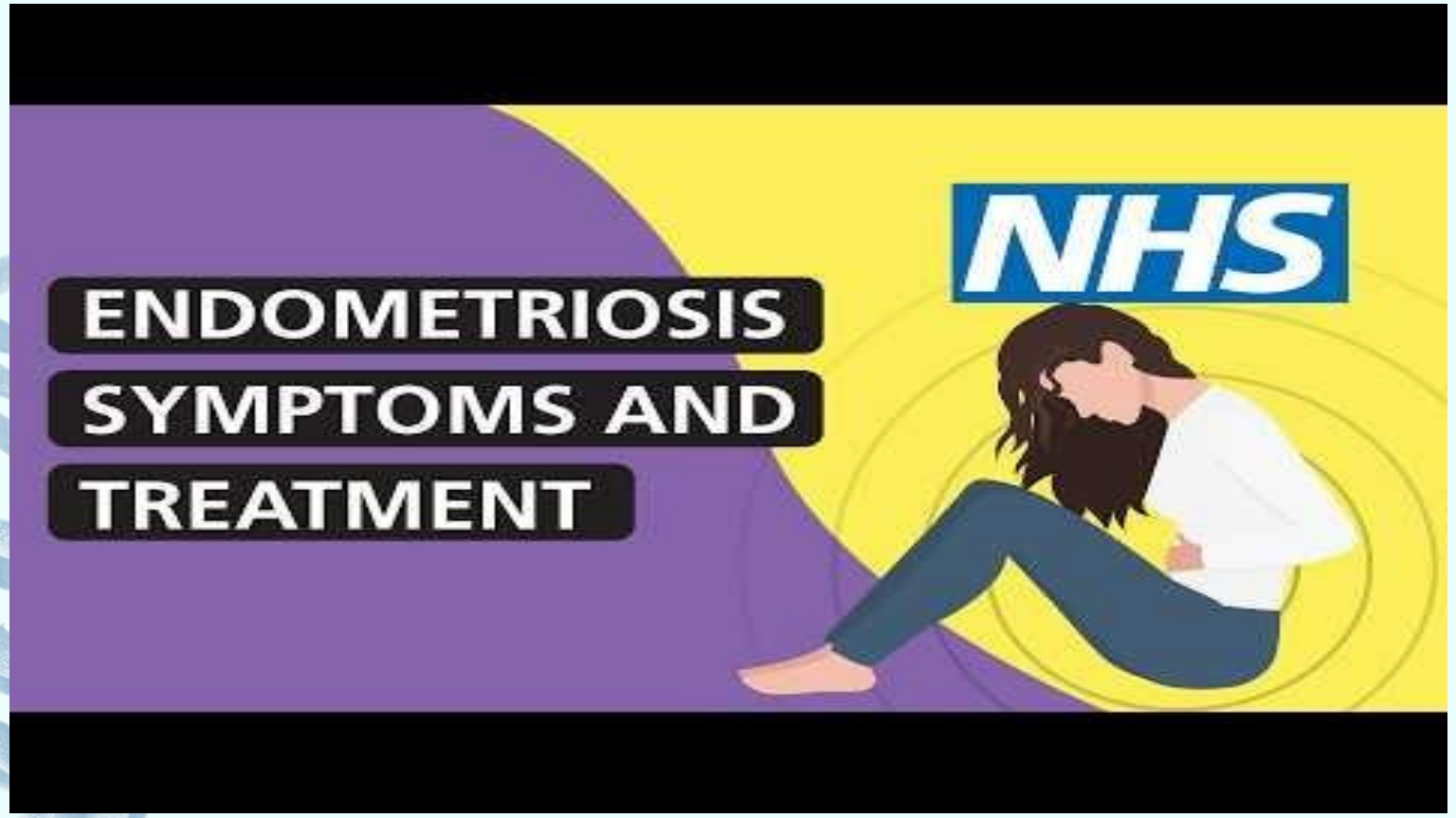


Zink, Christine. What Is a Sexually Transmitted Infection? (2022) available on: https://www.verywellhealth.com/sexually-transmitted-infection-overview-and-more-5206974

Cleveland clinic (2022) avaliable on: https://my.clevelandclinic.org/health/diseases/5019-vaginal-yeast-infection

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Endometriosis



Microbial Modulations of the Gut Microbiota Composition in the Patients with Endometriosis

		Function Characteristics	Endometriosis outcomes
Diversity changes	α-diversity reduced, β- diversity reduced, *Firmicutes/Bacteroidetes* ratio increased	Estrobolome and metabolism changes; increased β-glucuronidase activity	Endometriosis comorbidities, pelvic pain, infertillity
Increased strains	Phylium Proteobacteria; *genera Bifidobacterium, Escherichia, Streptococcus, Bacteroides, Parabacteroides, Shigella,*	LPS production; altered immune response; proinflammatory effect; increased β-glucuronidase activity	Chronic local and systemic inflammation, growth of endometrial stromal cells
Decreased strains	*Lachnospiraceae, Ruminococcus, Sneathia, Barnesella, Gardnerella, Atopobium, Barnesiella, Prevotella, Lactobacillus, Sneathia*	Reduced SCFA production	Growth of endometrial implants

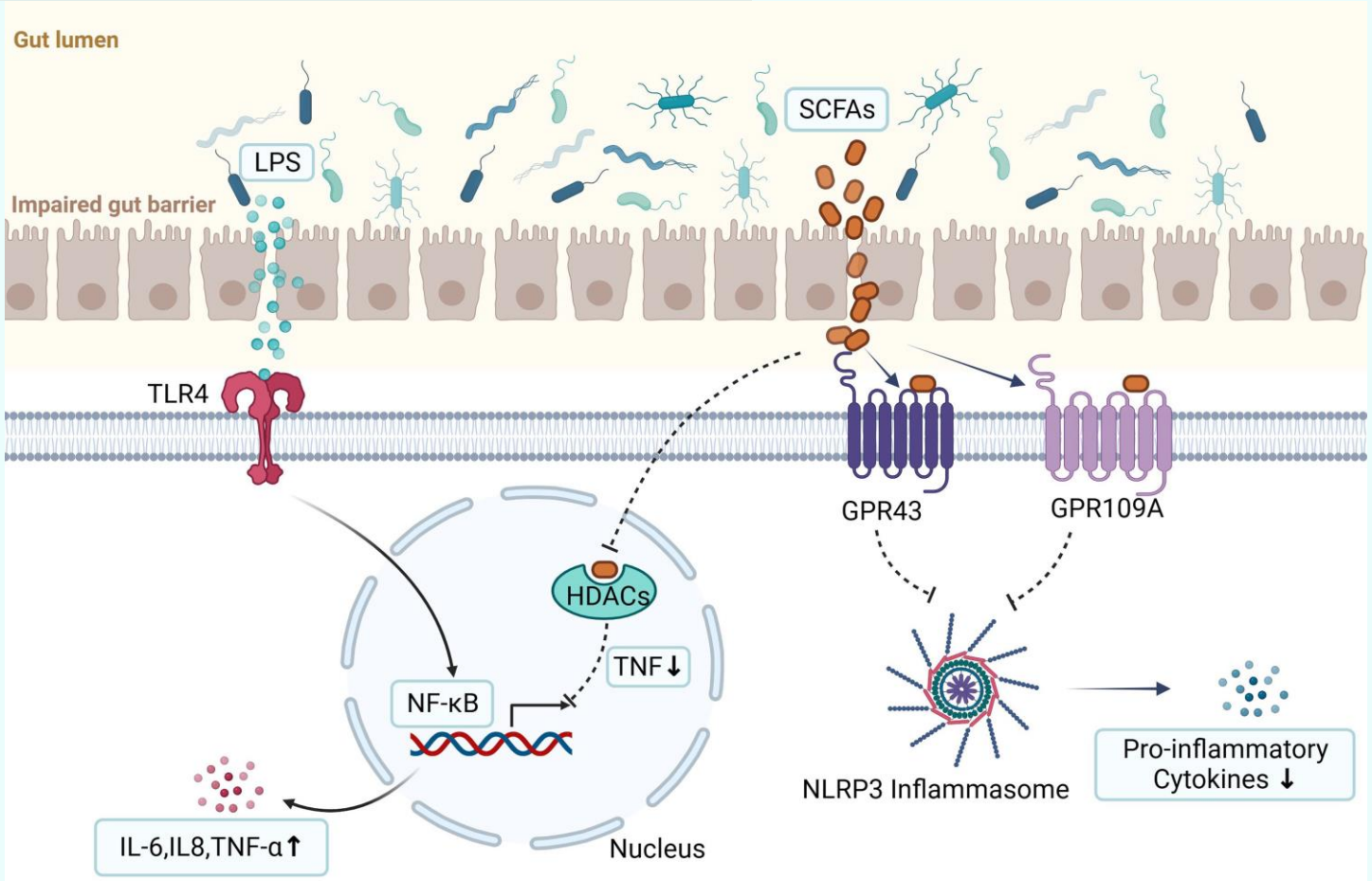
Vaginal/Cervical Microbiota Composition in Endometriosis

		Function Characteristics	Endometriosis outcomes
Diversity changes	Increased diversity; higher abundance of BV-associated organisms	Increased vaginal pH > 4,5 ; BV	Worsening symptoms
Increased strains	*Gardnerella, Streptococcus, Escherichia, Shigella*, and *Ureaplasma*	Opportunistic pathogens, chronic inflammation stimulators	Pelvic inflammatory disease, chronic endometritis
Decreased strains	*Lactobacillus* spp.	Bacteriocins production, keeping desirable pH value	Increased infertility

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

The Gut Dysbiosis Mechanism of Action in Endometriosis

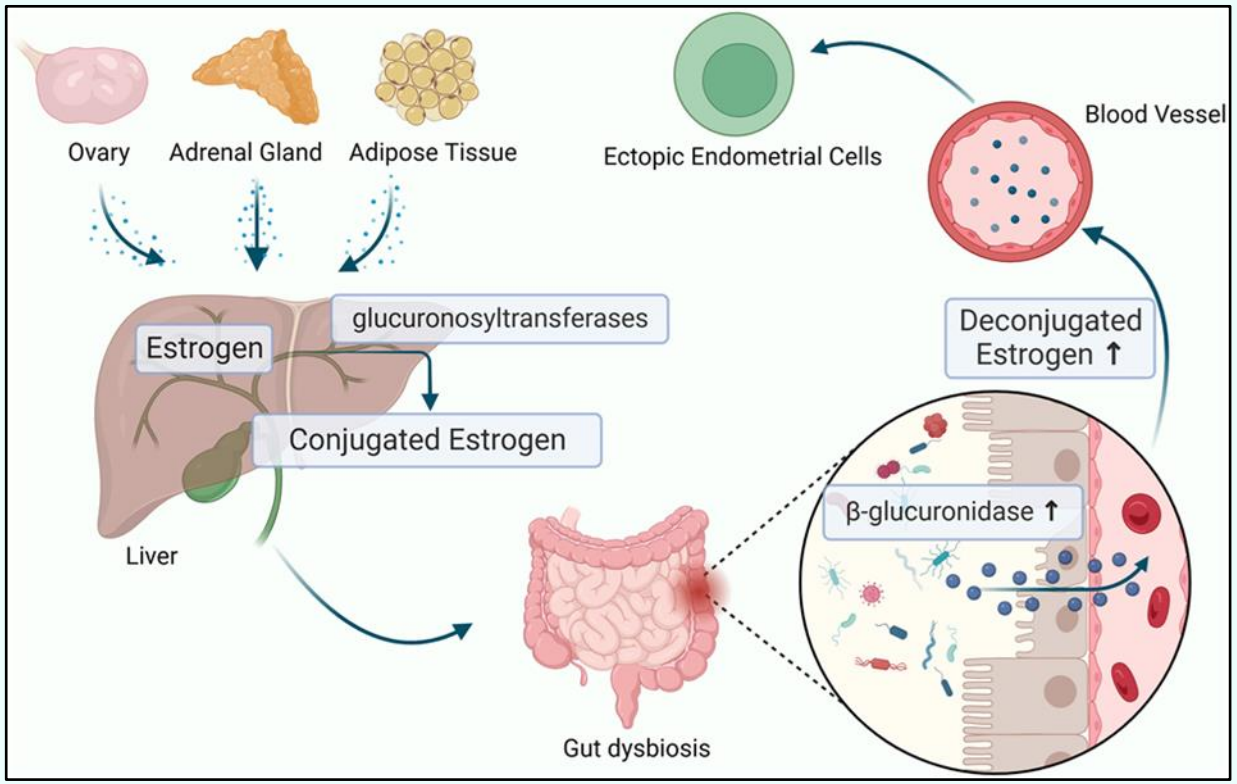
1. Imunnity response leading to **2**. chronic low inflammation.



Guo, Cuishan, and Chiyuan Zhang. "Role of The Gut Microbiota in The Pathogenesis of Endometriosis: A Review." *Frontiers in Microbiology* 15 (2024): 1363455.

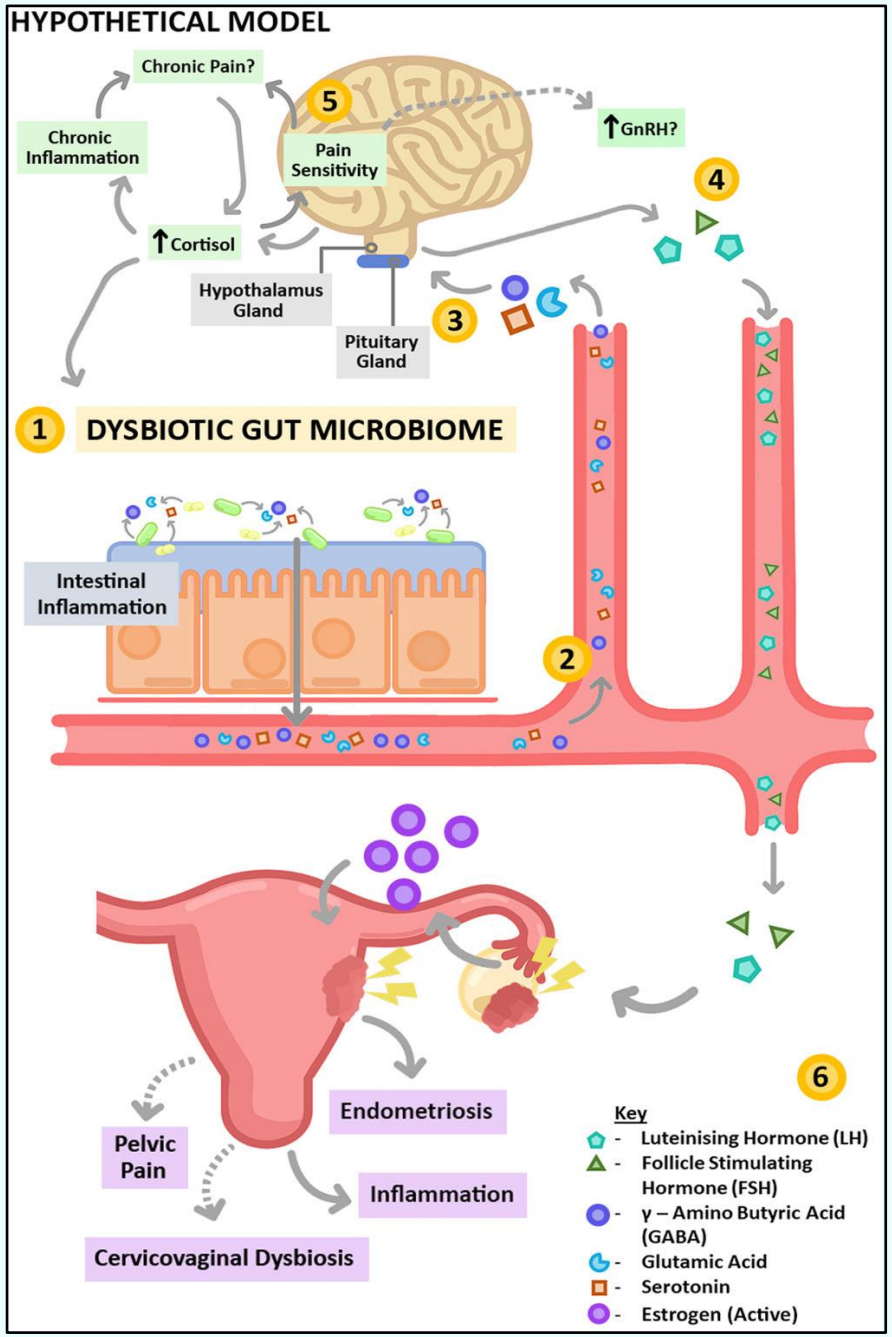
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

3. Gut microbiota-estrogen axis



Guo, Cuishan, and Chiyuan Zhang. "Role of The Gut Microbiota in The Pathogenesis of Endometriosis: A Review." *Frontiers in Microbiology* 15 (2024): 1363455.

4. Gut microbiota-brain axis



Salliss, Mary E., et al. "The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain." *Human reproduction updates* 28.1 (2022): 92-131.

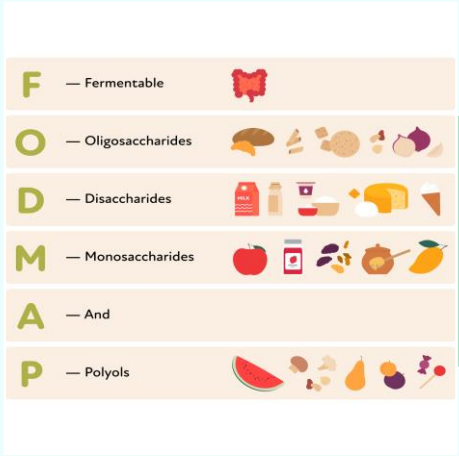
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

New Approaches in Endometriosis Treatment Strategies

1. Stimulate regeneration of gut eusbiosis
2. Promising potential in inhibition of further progression of endometriosis and associated pain reduction (chronic pelvic pain)
3. Still cannot replace conventional strategies

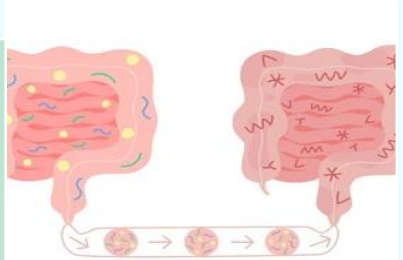
Probiotic supplementation

Omega-3 supplementation



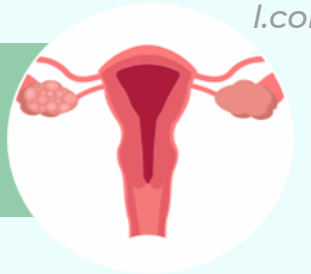
Low FODMAP diet

Fecal microbiota transplantation (FMT)



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

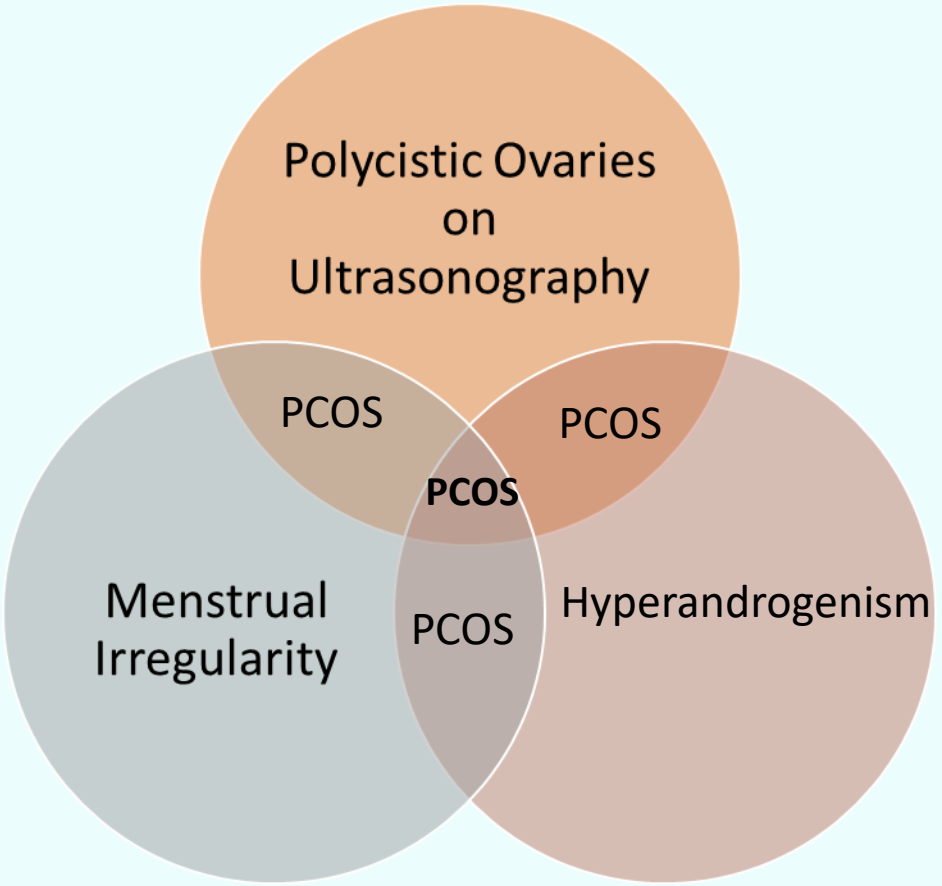
Polycistic Ovary Syndrome (PCOS)



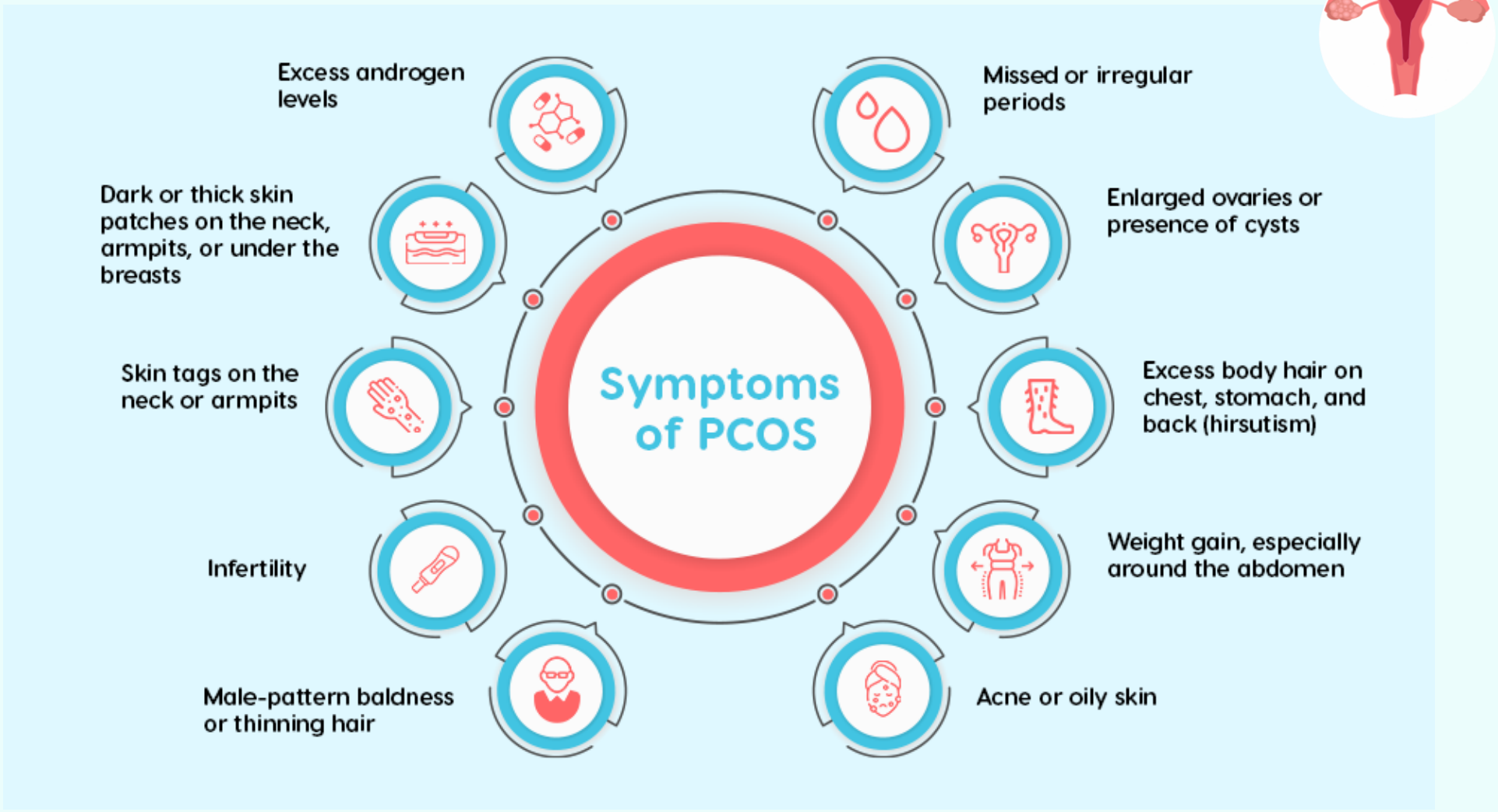
Risk factors:

- Genetic predisposition
- High insulin levels
- High androgen levels
- Lifestyle habits (poor dietary patterns; being overweight/obese; living a sedentary life:

Rotterdam criteria parameters

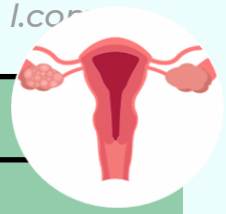


INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com



Available on: https://matrikaivf.in/pcos-symptoms-causes-risk-factors

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmai
l.cor

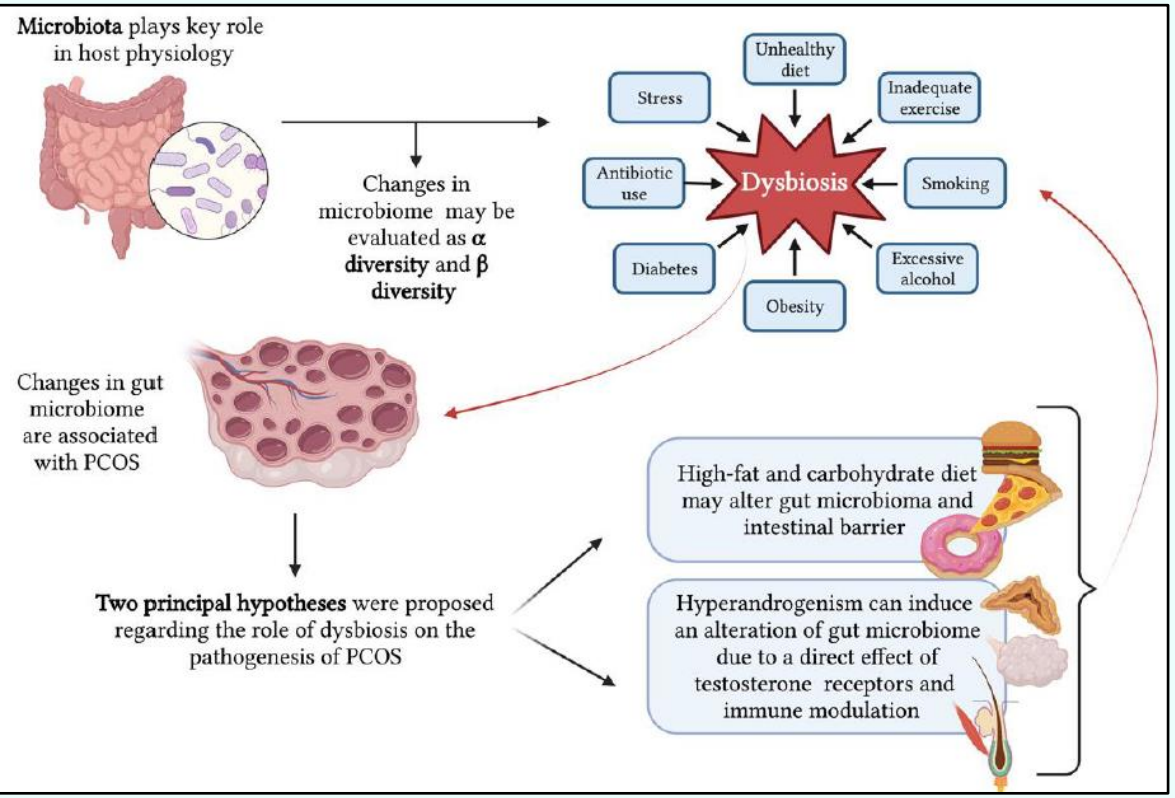


Microbial Modulations of the Gut Microbiota Composition in the PCOS Patients

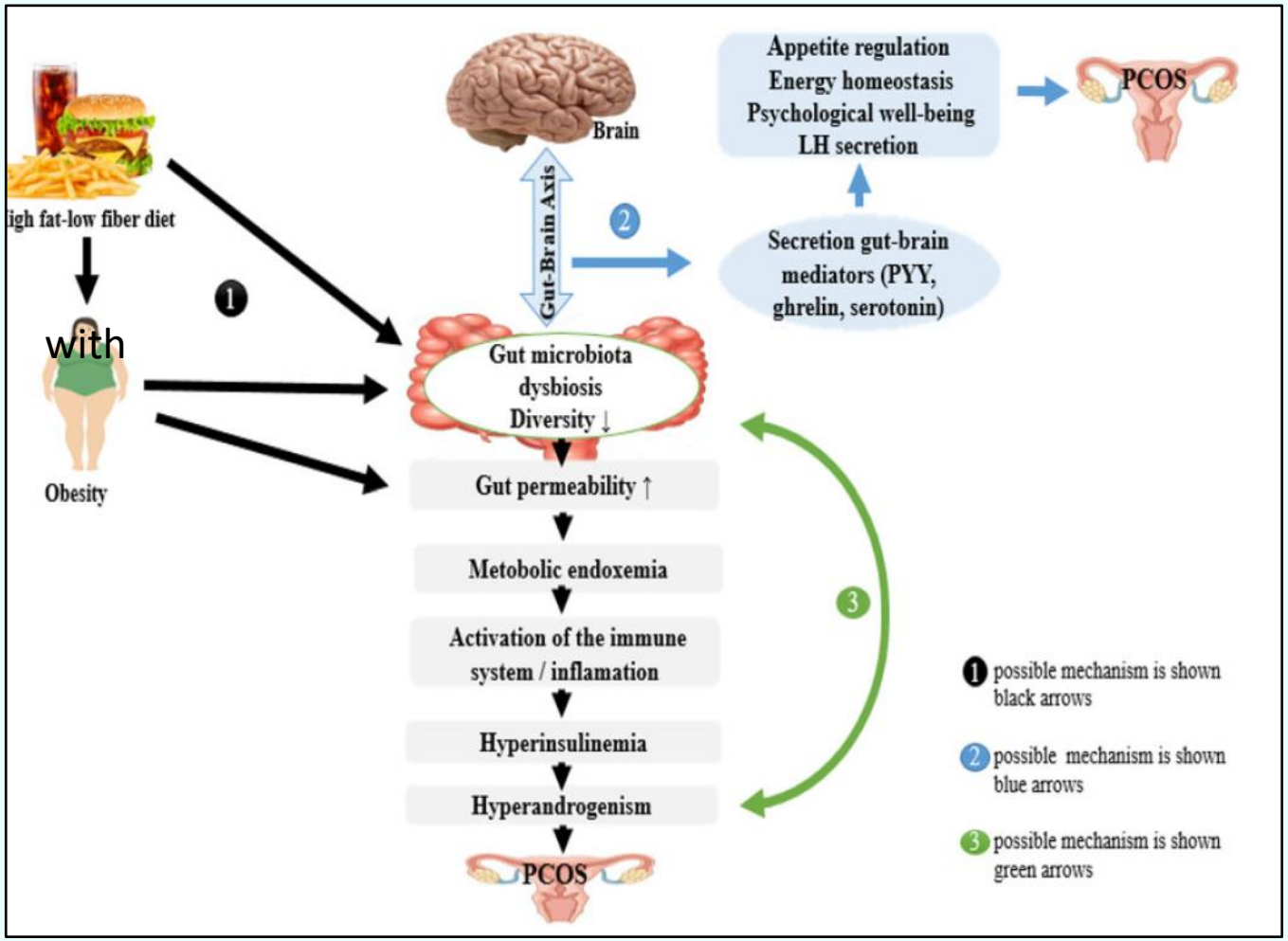
Strain abundance change	Function Characteristics	PCOS outcomes
decreased α-diversity, changed β-diversity	Altered intestinal function; activation of immune system	anovulation/menstrual disorder, hyperandrogenism (acne, hirsutism), and polycystic ovary occur, pre-diabetic status
Bacteroides, *Escherichia coli* (gram-negative bacteria) **increase**; *Escherichia*/*Shigella* ratio **increase**	LPS production; lower level of ghrelin and PYY in gut-brain axis	Chronic inflammatory state, insulin resistance, obesity, hormonal disturbances
B. vulgatus **increase**	Altered bile acid metabolism, reduced IL-22 secretion, BCAAs biosynthesis	Disruption of ovarian functions, insulin resistance, infertility
Prevotella spp. **increase**	BCAAs biosynthesis	Insulin resistance, higher testosterone levels
Roseburia, Akkermansia, Bifidobacterium, Tenericutes Faecalibacterium prausnitzii **decrease**	reduced SCFAs production; disturbances in gut barrier; altered immunomodulation	Chronic inflammatory state, insulin resistance, obesity
Vaginal/Cervical Microbiota Composition in PCOS Patients		
Lactobacillus spp. **decrease**, *G. vaginalis*, *Prevotella*, *Chlamidia trachomatis* **increase**	Changed pH values, lower estrogen utilization	Vaginal dysbiosis, induced levels of androgen

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmai

The Risk Factors and Gut Dysbiosis Mechanism of Action in PCOS



Calcaterra, Valeria, et al. "Probiotics and polycystic ovary syndrome: a perspective for management in adolescents with obesity." *Nutrients* 15.14 (2023): 3144.



Yurtdaş, Gamze, and Yasemin Akdevelioğlu. "A new approach to polycystic ovary syndrome: the gut microbiota." *Journal of the American College of Nutrition* 39.4 (2020): 371-382.

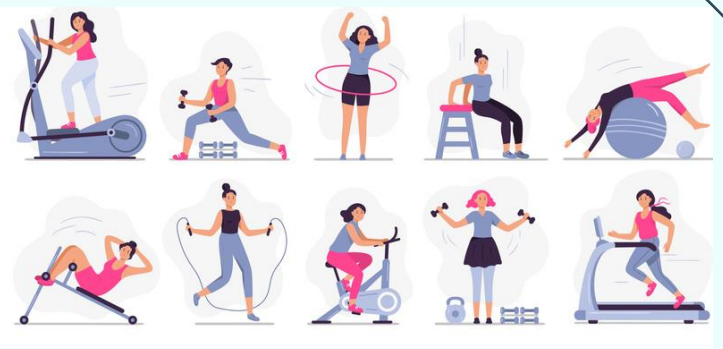
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
lananovkovic147@gmail.com

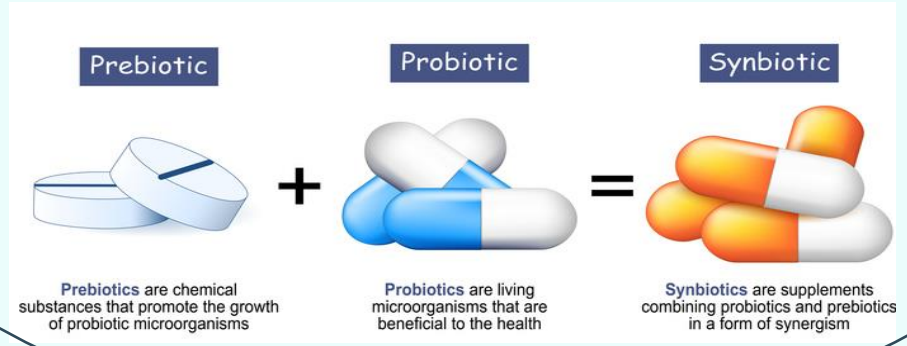
Alternative PCOS Treatments – Gut Dysbiosis Regulation

↑ Insulin sensitivity

✚ Blood lipid profile

✚ Hormonal balance





↓ Weight, BMI, waist circumference value

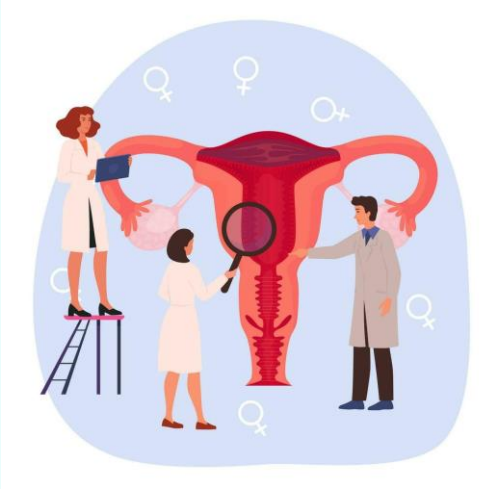
↓ Testosteron levels

↓ Hirsutism

lananovkovic147@gmail.com

Female Infertility

Video for better understanding of etiology of the infertility:
https://www.youtube.com/watch?v=KIdyK-V-0Sg



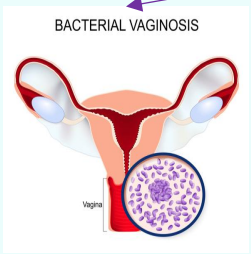
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.ec
+356 21383758

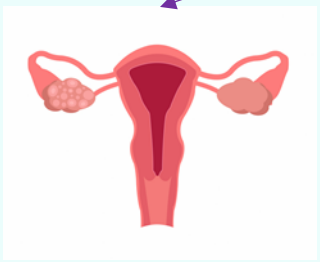
Gut Dysbiosis and Infertility

lananovkovic147@gmai
l.com

- Inflammation and Hormone Regulation
- Nutrient Absorption and Metabolism
- Gut-Estrogen Axis
- Gut-Brain Axis

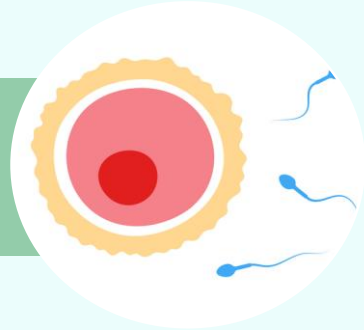
- Immune response
- Hormone/circulating estrogen imbalance
- Barierr disfunction
- Stress, depression, anxiety





INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

Vaginal Dysbiosis and Infertility



Bacterial Vaginosis and STD

- Increased vaginal microbiota diversity
- Decreased *Lactobacillus* spp.
- Increased genera *Gardnerella, Atopobium, Ureaplasma, Prevotella*
- Increased vaginal SCFA

Mechanism of action

- progression of bacterial pathogens to fallopian tubes affecting endometrial microbiome
- an excessive immune response

Infertility + postpartum complications

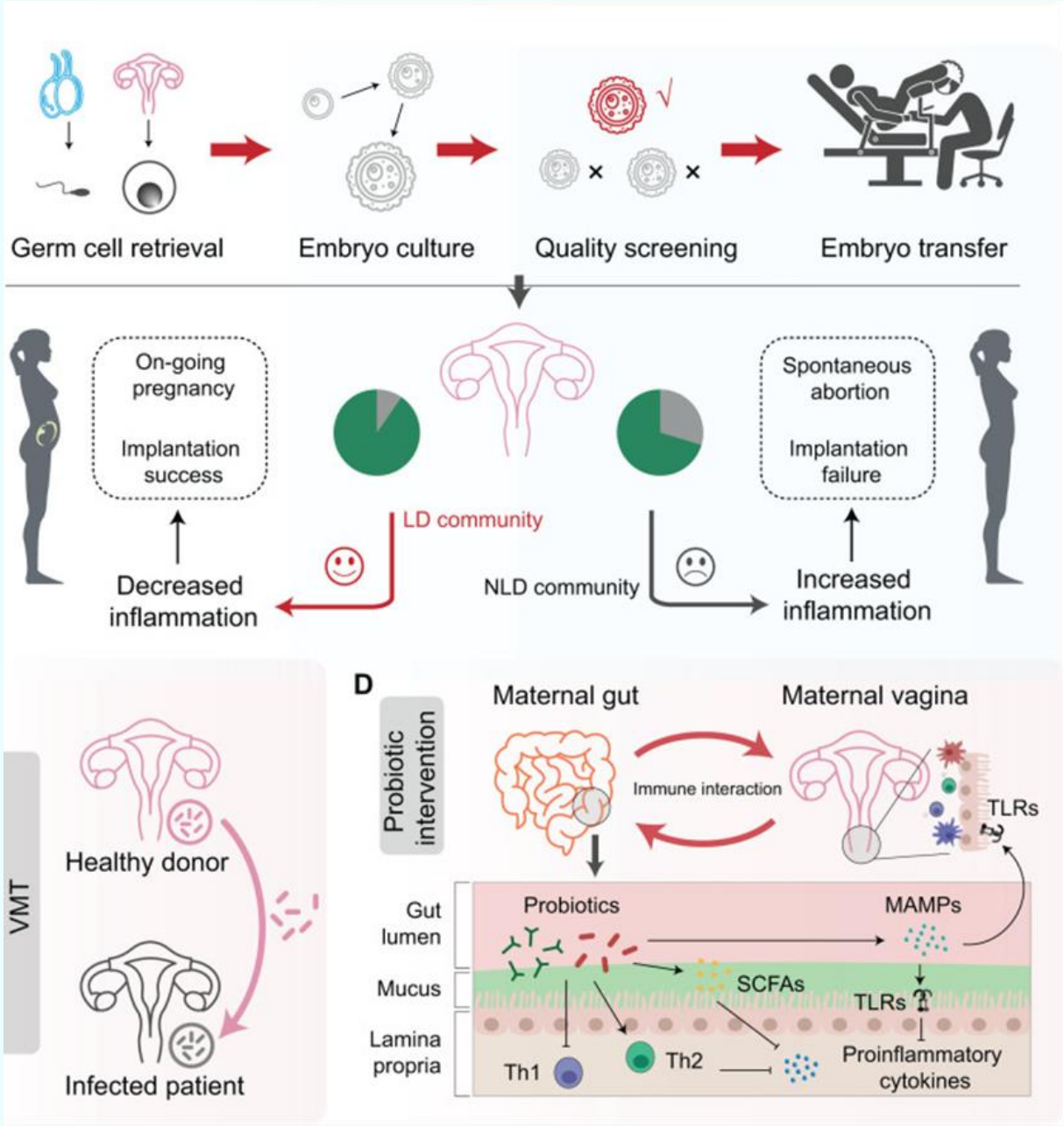
- Problems with endometrial receptivity
- Miscarriage
- Preterm delivery
- Low birth weight

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Assisted Reproductive Technologies (ART):

in vitro Fertilization (IVF)

- Vaginal dysbiosis negative impact on pregnancy rate after IVF
- the isolation of *Enterococci*, *Enterobacteriaceae*, *Streptococci*, *Staphylococci*, and/or Gram-negative bacteria from endometrium microbiota associated with poor implantation outcomes and increased number of miscarriages
- Strategies to enhance IVF and fertility in general:
  - Balanced diet + proper micronutrient supplementation
  - Probiotics and/or prebiotics supplementation
  - FMT
  - VMT



Xiao, Liwen, Zhenqiang Zuo, and Fangqing Zhao. "Microbiome in Female Reproductive Health: Implications for Fertility and Assist

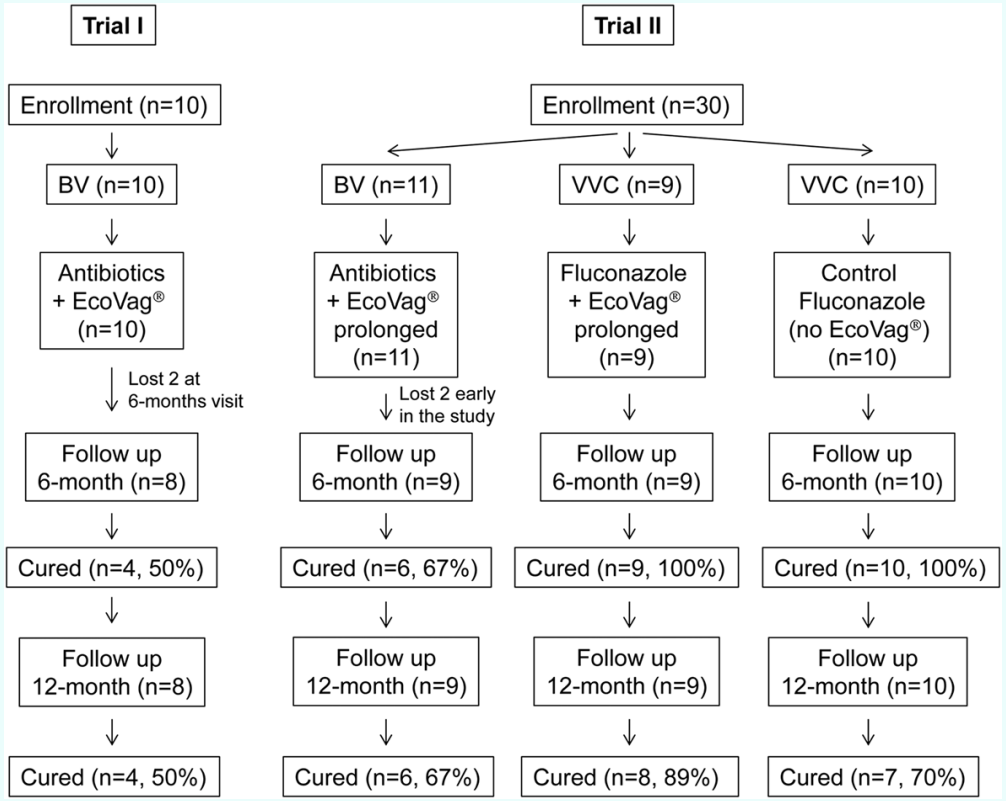
Reproductive Technologies." *Genomics, Proteomics & Bioinformatics* 22.1 (2024): qzad005.

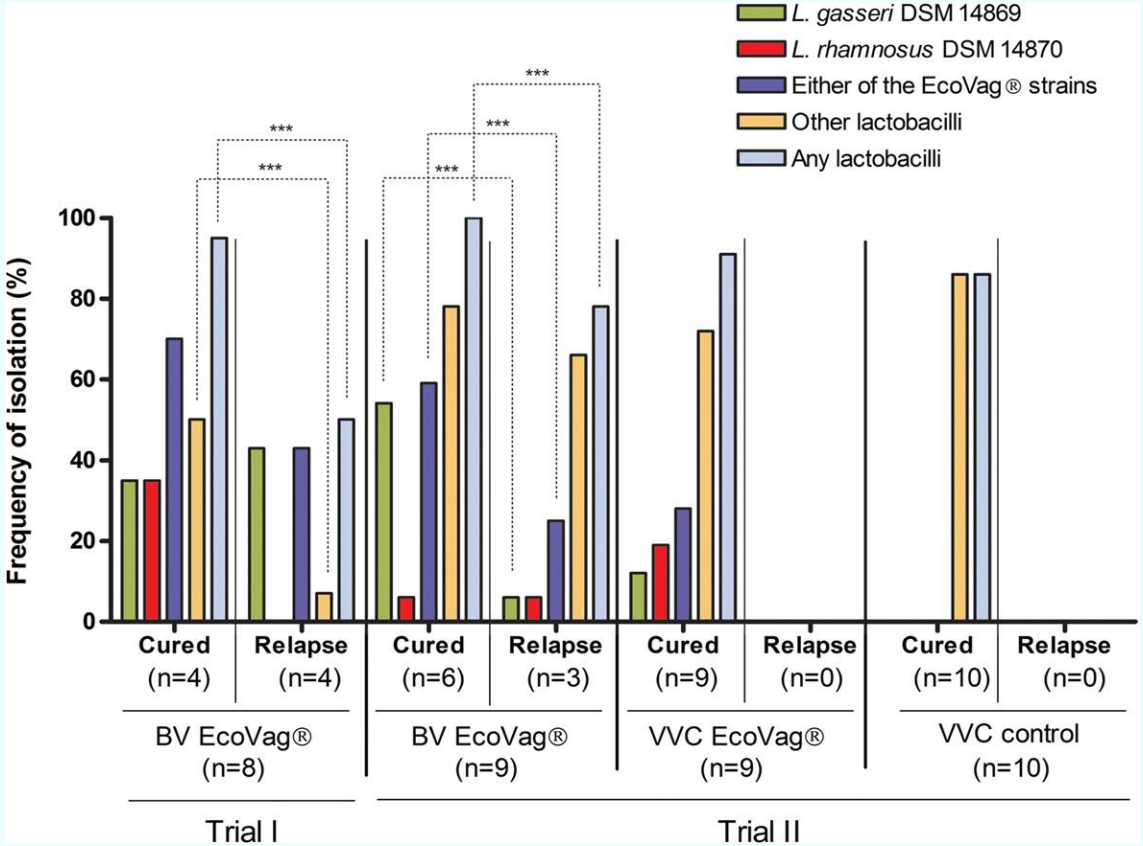
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

6. Real Cases

A) BV and VVC Treatment – antibiotic therapy + mixed probiotic supplementation (EcoVag®)

Pendhakar *et al*., 2015: „ Vaginal colonisation by probiotic lactobacilli and clinical outcome in women conventionally treated for bacterial vaginosis and yeast infection"

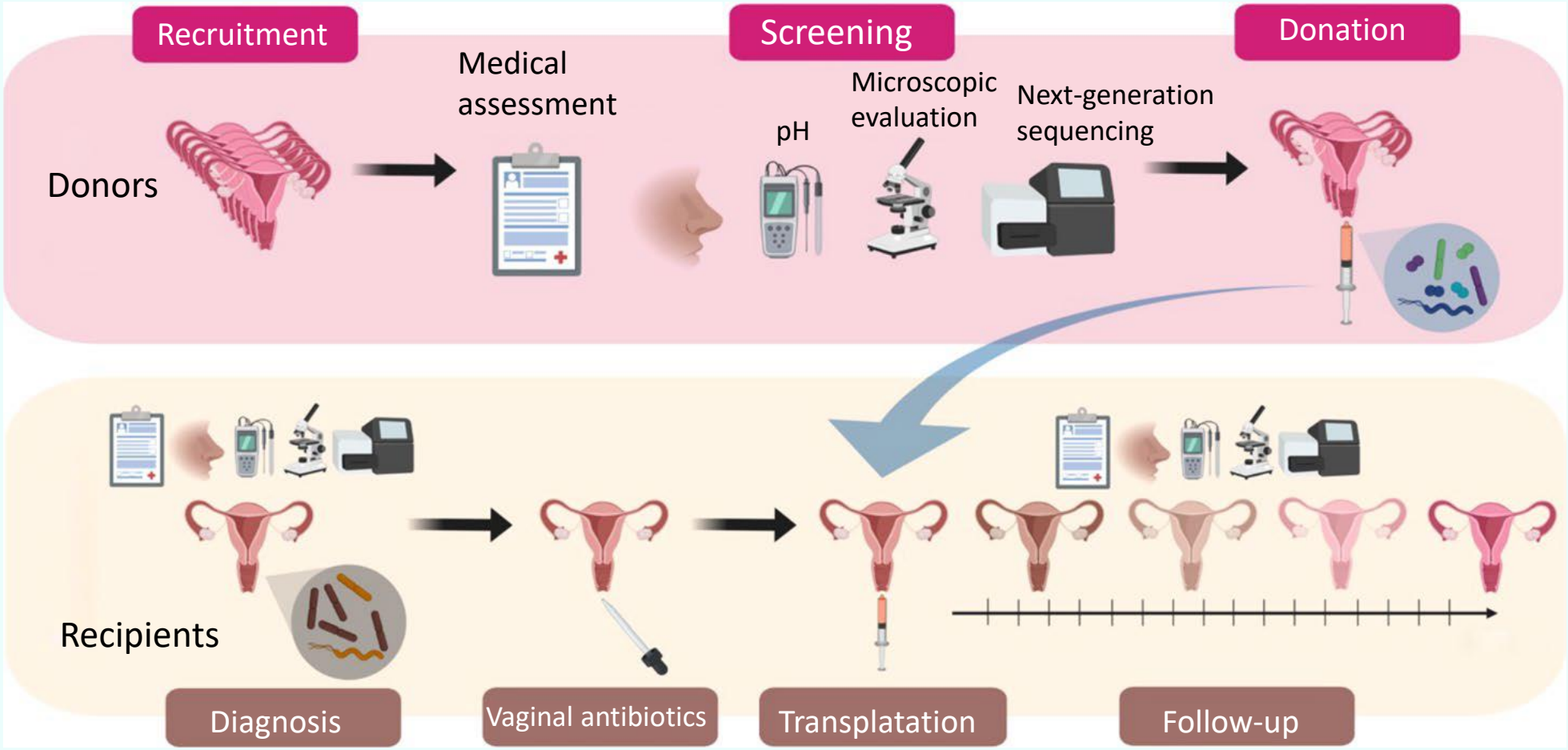




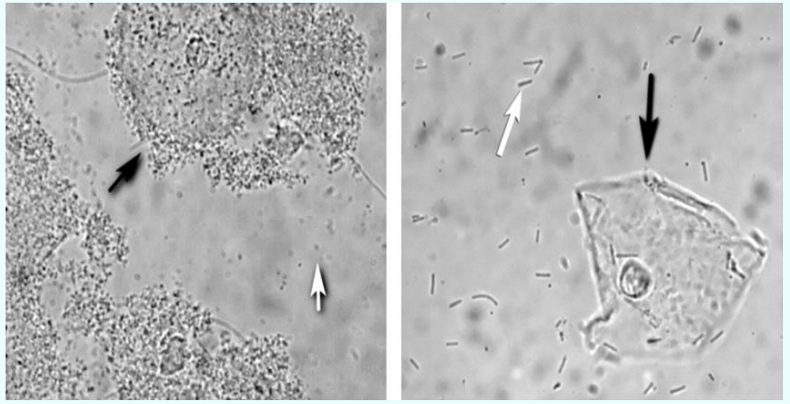
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

B)Reccurent BV and vaginal microbiome transplatation (VMT)

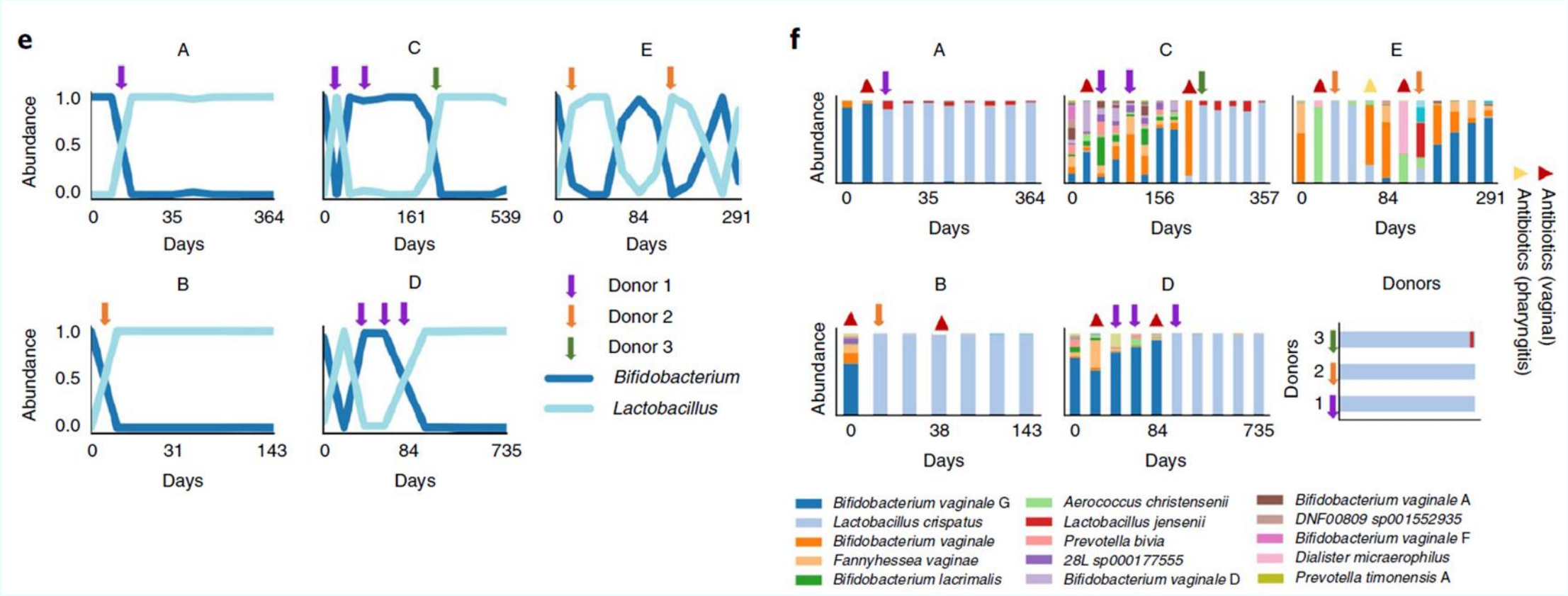
Lev-Sagie *et al*., 2019: „Vaginal microbiome transplantation in women with intractable bacterial vaginosis"



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com



Microscopic picture: Wet-mount microscopy before and after VMT

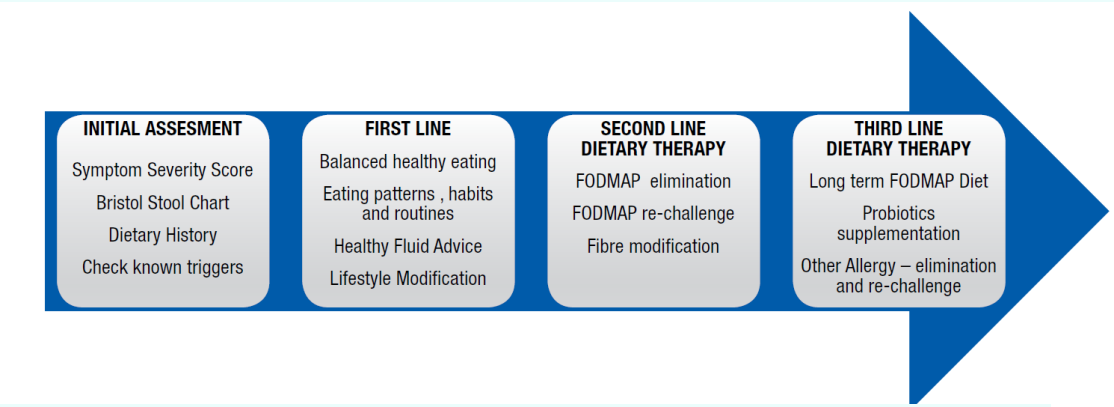


Figures: Metagenomic compositional assessment of the vaginal microbiome following VMT.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

C) Endometriosis + IBS and low FODMAP treatment

Jankovich and Watkins, 2017: Case Study: „The low FODMAP diet reduced symptoms in a patient with endometriosis and IBS"



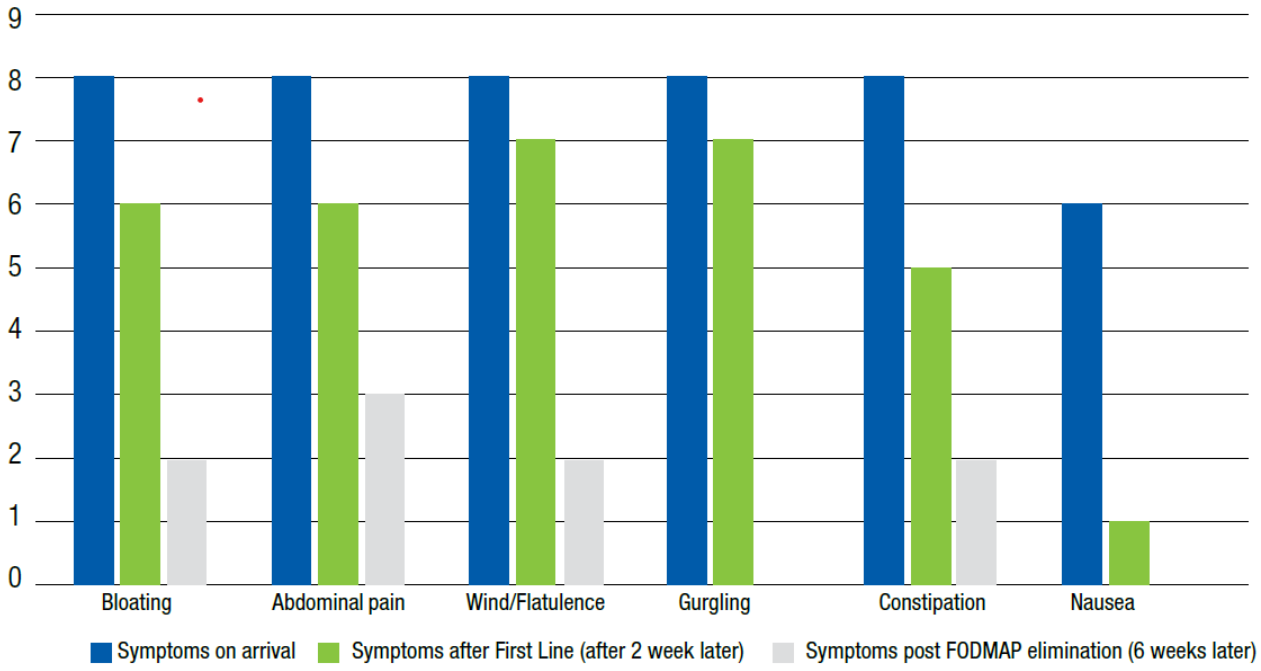
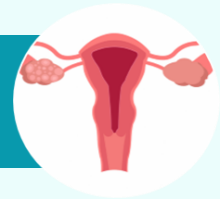


Figure 2: Ms. A - Change in symptom severity throughout the dietary process

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

D) PCOS and vitamin D + probiotic co-supplementation



Ostadmohammadi *et al*., 2019. „ Vitamin D and probiotic cosupplementation affects mental health, hormonal, inflammatory and oxidative stress parameters in women with polycystic ovary syndrome”

Table 2 Mental health parameters and metabolic profiles at baseline and after the 12-week intervention in women with polycystic ovary syndrome that received either vitamin D plus probiotic supplements or placebo

Variables	Placebo group ($n = 30$)		Vitamin D plus probiotic group ($n = 30$)		Difference in outcome measures between vitamin D plus probiotic and placebo groups[1]	
	Baseline	Week 12	Baseline	Week 12	β (95% CI)	P[2]
25-hydroxyvitamin D (ng/mL)	12.9 ± 3.2	13.3 ± 2.7	11.1 ± 4.1	24.4 ± 5.6	12.69 (11.09, 14.29)	< 0.001
BDI total scores	13.8 ± 3.6	13.2 ± 3.7	12.9 ± 4.1	11.9 ± 3.4	−0.58 (−1.15, − 0.02)	0.04
GHQ scores	42.7 ± 9.1	41.9 ± 8.9	40.4 ± 6.7	38.7 ± 6.8	−0.93 (−1.78, − 0.08)	0.03
DASS scores	82.5 ± 11.8	80.8 ± 12.4	81.5 ± 12.2	78.9 ± 12.3	−0.90 (−1.67, − 0.13)	0.02
PSQI	7.5 ± 2.8	6.5 ± 3.0	7.8 ± 2.7	5.8 ± 2.1	−0.73 (−2.11,-0.63)	0.28
Total testosterone (ng/mL)	1.1 ± 0.4	1.1 ± 0.3	1.0 ± 0.3	0.9 ± 0.2	−0.19 (− 0.28, − 0.10)	< 0.001
SHBG (nmol/L)	40.2 ± 5.0	40.3 ± 5.3	38.4 ± 5.9	39.6 ± 5.7	0.92 (−0.45, 2.31)	0.18
mF-G scores	13.9 ± 3.0	13.6 ± 3.0	14.98 ± 3.2	13.5 ± 3.3	−0.95 (−1.39, − 0.51)	< 0.001
hs-CRP (mg/L)	3.7 ± 1.0	3.8 ± 1.3	3.8 ± 1.1	3.2 ± 0.8	−0.67 (− 0.97, − 0.38)	< 0.001
NO (μmol/L)	33.3 ± 4.9	32.3 ± 5.5	34.2 ± 1.0	33.6 ± 0.8	0.37 (−0.48, 1.23)	0.38
TAC (mmol/L)	835.1 ± 101.7	830.9 ± 93.8	850.5 ± 82.7	919.2 ± 85.4	82.81 (42.86, 122.75)	< 0.001
GSH (μmol/L)	475.8 ± 71.7	491.7 ± 81.0	510.4 ± 99.3	556.5 ± 97.2	40.42 (4.69, 76.15)	0.02
MDA (μmol/L)	2.7 ± 0.5	2.6 ± 0.5	2.9 ± 0.3	2.5 ± 0.2	−0.25 (−0.40, −0.10)	0.001

Data are mean ± SDs

[1]"Outcome measures" refers to the change in values of measures of interest between baseline and week 12. β [difference in the mean outcomes measures between treatment groups (vitamin D plus probiotic group = 1 and placebo group = 0)]

[2]Obtained from multiple regression model (adjusted for baseline values of each biochemical variables, age and baseline BMI)

BDI, beck depression inventory; DASS, depression anxiety and stress scale; GHQ, general health questionnaire; GSH, total glutathione; hs-CRP, high-sensitivity C-reactive protein; mF-G, modified Ferriman Gallwey; MDA, malondialdehyde; NO, nitric oxide; PSQI, Pittsburgh Sleep Quality Index; SHBG, sex hormone-binding globulin; TAC, total antioxidant capacity

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

E) PCOS and lifestyle modifications + synbiotic supplementation

Chudzicka-Strugała, *et al*. 2021: „Effects of Synbiotic Supplementation and Lifestyle Modifications on Women Wit Polycystic Ovary Syndrome”

Table 2. Effects of lifestyle modifications and comparison of the placebo group with the synbiotic group

	Placebo group		Synbiotic group		
Effect	Change at 3 months	*P* value vs baseline	Change at 3 months	*P* value vs baseline	*P* value (between groups)
Weight (kg)	-4.74 ± 0.65 (-5%)	<0.0001	-7.46 ± 0.92 (-8.0%)	<0.0001	0.022
BMI (kg/m^2)	-1.74 ± 0.24 (-5%)	<0.0001	-2.68 ± 0.33 (-8.0%)	<0.0001	0.030
Body fat (%)	-2.71 ± 0.36 (-7%)	<0.0001	-3.92 ± 0.62 (-10%)	<0.0001	0.12
WHR	0.07 ± 0.05 (7%)	0.18	0.03 ± 0.03 (3%)	0.33	0.52
Waist (cm)	-6.3 ± 1.4 (-6%)	0.0008	-10.7 ± 1.2 (-10%)	<0.0001	0.03
Hip (cm)	-11.5 ± 3.1 (-11%)	0.001	-10.2 ± 2.1 (-10%)	0.0001	0.71
Thigh (cm)	-3.8 ± 1.1 (-6%)	0.003	-4.3 ± 0.6 (-7%)	<0.0001	0.74
Hirsutism (Ferriman/ Gallwey score)	-0.3 ± 0.1 (-4%)	0.19	-0.6 ± 0.3 (-6%)	0.06	0.60
Ovarian volume (both ovaries, mL)	-0.7 ± 1.7 (-3%)	0.71	-0.1 ± 1.2 (0%)	0.96	0.78
Total testosterone (ng/ mL)	-0.04 ± 0.03 (-6%)	0.33	-0.18 ± 0.02 (-32%)	<0.0001	0.016
DHEAS (µmol/mL)	-0.20 ± 0.93 (-2%)	0.83	1.02 ± 0.37 (14%)	0.012	0.21
SHBG (nmol/L)	6.8 ± 2.8 (17%)	0.49	2.4 ± 2.5 (6%)	0.35	0.25
LH (mIU/mL)	-2.5 ± 1.1 (-26%)	0.039	-0.3 ± 1.0 (-3%)	0.74	0.15
FSH (mIU/mL)	-0.8 ± 0.5 (-15%)	0.09	0.4 ± 0.6 (8%)	0.54	0.13
LH:FSH ratio	-0.16 ± 0.29 (-9%)	0.58	-0.26 ± 0.27 (-11%)	0.58	0.79
Fasting insulin (µU/mL)	-0.9 ± 1.3 (-6%)	0.49	-1.6 ± 1.5 (-11%)	0.31	0.72
Fasting glucose (mg/dL)	-2.4 ± 1.5 (-3%)	0.13	-1.1 ± 1.5 (-1%)	0.42	0.57
ISI (Matsuda)	0.0 ± 0.4 (0%)	0.97	0.7 ± 0.42 (23%)	0.12	0.23
Total cholesterol (mg/dL)	0 ± 5 (0%)	1.00	-6 ± 5 (-3%)	0.29	0.46
LDL cholesterol (mg/dL)	1 ± 5 (1%)	0.92	-1 ± 5 (-1%)	0.76	0.79
HDL cholesterol (mg/dL)	2.3 ± 4.4 (4%)	0.61	0.7 ± 1.5 (1%)	0.62	0.70
Triglycerides (mg/dL)	-16 ± 6 (-15%)	0.034	-28 ± 11 (-23%)	0.022	0.35

All values represent mean ± SEM; statistical analysis was performed using paired *t* test, or, in the absence of normal distribution, by nonparametric test (Wilcoxon signed rank).
DHEAS, dehydroepiandrosterone sulfate; HDL, high-density lipoprotein; ISI, insulin sensitivity index; LDL, low-density lipoprotein; WHR, waist-to-hip ratio.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

F) Infertility and probiotics

Fernández et al., 2021: „Application of *Ligilactobacillus salivarius* CECT5713 to Achieve Term Pregnancies in Women with Repetitive Abortion or Infertility of Unknown Origin by Microbiological and Immunological Modulation of the Vaginal Ecosystem

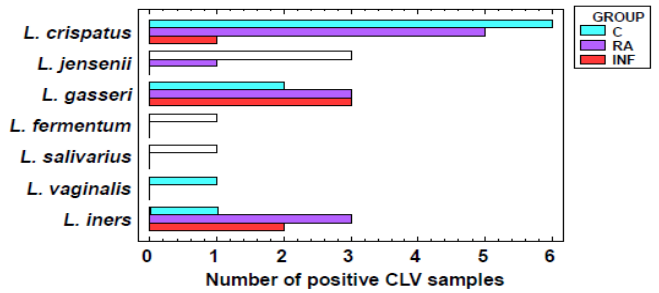


Figure 1. Dominant lactobacilli species (when lactobacilli could be isolated) in cervovaginal lavage (CVL) samples of fertile women (C, bluish green), women with repetitive abortion (RA, purple) and women with infertility of unknown origin (INF, red).

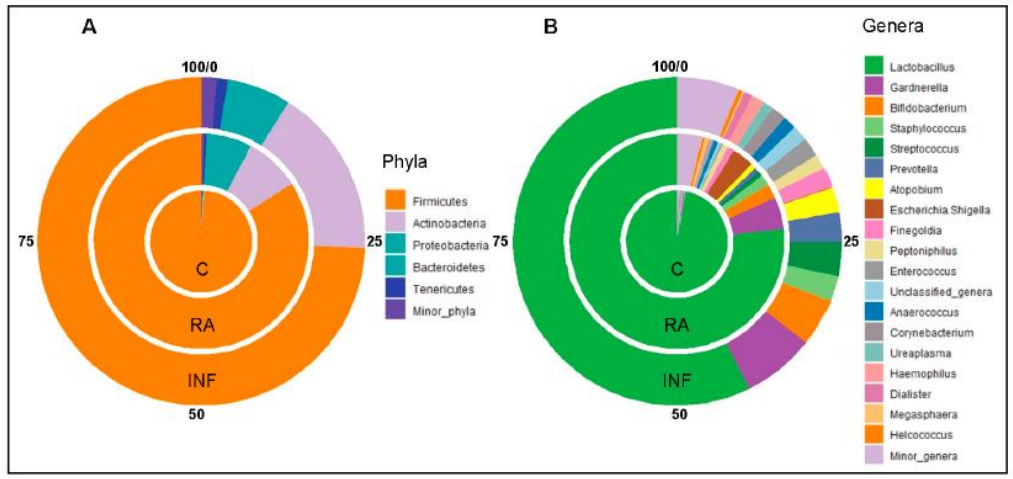


Figure 3. Pie charts showing the percentages of the relative abundances of the 5 most abundant phyla (**A**) and the 20 most abundant genera (**B**) in the CVL samples from healthy fertile women (inner pie charts; C group), women with a history of repetitive abortion (middle pie charts; RA group), and women with infertility of unknown origin (outer pie charts; INF group).

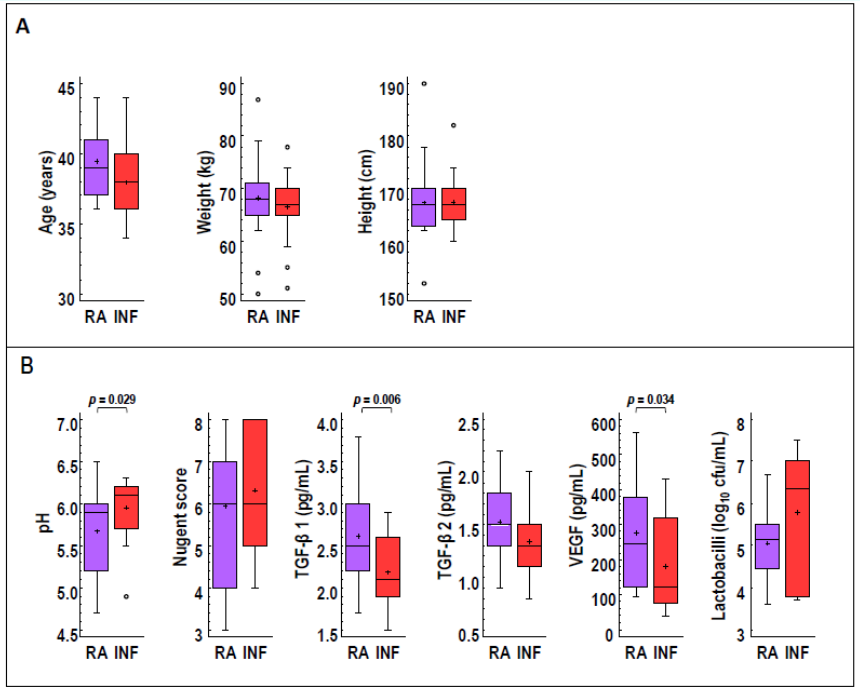


Figure 2. Comparison of selected baseline (**A**) demographic characteristics (age, weight and height) and (**B**) vaginal parameters (pH, Nugent score, TGF-β 1, TGF-β 2, and VEGF concentrations, and viable *Lactobacillus* counts) in CVL samples of women with repetitive abortion (RA, purple) and women with infertility of unknown origin (INF, red) at recruitment. For each boxplot, the line and the cross within the box represent the median and mean, respectively. The bottom and top boundaries of each box indicate the first and third quartiles (the 25th and 75th percentiles), respectively. The whiskers represent the lowest and highest values within the 1.5 interquartile range (IQR) and the dots outside the rectangles are suspected outliers (>1.5 × IQR). One-way ANOVA tests were used to compare both groups.

lananovkovic147@gmail.com

Table 7. Comparison of vaginal parameters (pH, Nugent score, TGF-β 1, TGF-β 2, and VEGF concentrations, and *Lactobacillus* counts) of all women who were able to complete a full-term pregnancy (n = 25) and of those who did not (n = 19) among all women with a history of repetitive abortion and with infertility of unknown origin (RA and INF groups) after the probiotic intervention with *L. salivarius* CECT5713 and vaginal parameters of fertile women (Control group; n = 14).

Vaginal Parameter	Probiotic Intervention Resulted in Pregnancy			
	Control (n = 14) Mean (95% CI)	Yes (n = 25) Mean (95% CI)	No (n = 23) Mean (95% CI)	p-Value
pH	4.53 (4.38–4.68) [a]	4.48 (4.39–4.58) [a]	5.78 (5.62–5.95) [b]	0.000 [1]
Nugent score	1.79 (1.27–2.30) [a]	2.36 (1.92–2.80) [a]	5.84 (5.35–6.33) [b]	0.000 [1]
TGF-β 1, pg/mL	4.83 (4.65–5.01) [a]	4.36 (4.20–4.52) [b]	2.27 (2.06–2.48) [c]	0.000 [1]
TGF-β 2, pg/mL	3.22 (3.10–3.34) [a]	2.88 (2.75–3.01) [b]	1.27 (1.15–1.40) [c]	0.000 [1]
VEGF, pg/mL	406.0 (322.0–490.0) [a]	755.0 (637.1–872.5) [b]	103.3 (82.4–124.1) [c]	0.000 [1]
Lactobacilli				
Positive women, n (%)	14 (100)	25 (100)	10 (43)	<0.001 [2]
Viable counts [3], $\log_{10}$ CFU/mL	7.24 (6.89–7.60) [a]	6.47 (6.22–6.72) [b]	4.87 (3.83–5.90) [c]	0.000 [1]

[1] One-way ANOVA tests were used to evaluate differences in mean values between groups. Values followed by different superscript letters within the same row indicate statistically significant differences between groups according to Scheffé post hoc comparison tests. [2] Freeman–Halton extension of the Fisher exact probability tests for a 2 × 3 contingency table were used to compute the (two-tailed) probability of obtaining a distribution of values of lactobacilli positive women. [3] Mean (95% CI) of *L. salivarius* qPCR (copies/mL) in lactobacilli-positive women. TGF-β 1, transforming growth factor-β 1; TGF-β 2, transforming growth factor-β 2; VEGF, vascular endothelial growth factor.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

7. Conclusions

- The female reproductive health is closely associated with both gut and vaginal microbiota. The gut disturbances of both microbiota composition are leading to a state of dysbiosis which may result in female reproductive health disorders such as BV, STI, VVC, endometriosis, PCOS and ultimately to infertility.
- The gut microbiota has role in immunomodulatory effects in the host and via imbalances of microbial production of beneficial SCFA and adverse LPS may lead to elevated intestinal permeability causing local or systematic low-grade chronic inflammation and affecting reproductive health. The established gut microbiota-estrogen axis and gut microbiota-brain/hypothalamic-pituitary-adrenal axis have role in sex hormones stimulation. The occurrence of gut dysbiosis and changed estrobolome, testrobolome and othe microbial activities may affect these axis and drive to hormone-depended reproductive disorders.
- The vaginal microbiota has a role in maintaining reproductive health through high dominance of *Lactobacillus* spp. and pH < 4,5. Composition of vaginal microbiota in reproductive women is characterized by ethnicity, genetics, hormonal changes through the menstruation cycle and pregnancy. Direct factors causing adverse reproductive health outcomes are multiple sexual partners, poor hygiene practices and inappropriate clothes.
- The supporting evidence have established the gut-vaginal crosstalk through mother-to-infant vertical transition and rectum – vaginal microbiota translocation. The gut microbiota-estrogen axis also plays a role via regulation of estrogen levels which is inevitable for vaginal *Lactobacillus* spp. growth. The adverse alternations of each microbiota may affect the other one, leading to dysbiosis.
- The risk factors influencing both of microbiota that can be modified are obesity, eating habits, smoking, alcohol, antibiotics, stress, insulin resistance.
- The vaginal dysbiosis is mostly caused by diversity, lower abundance of lactobacilli and increased presence of anaerobic *G. vaginalis*, *A. vaginae* and *Prevotella* spp. These biofilm-forming pathogens disrupt the host physiology via destroying the vaginal barrier and promoting the release of pro inflammatory chemokines and cytokines which cause BV, STI, VVC and infertility. The gut dysbiosis in reproductive health disorders is characterized by general decrease of microbial diversity, increased *Firmicutes*/*Bacteroides* and *Escherichia*/*Shigella* ratio and in PCOS, multiply observed increased abundance of *Bacteroides*, *B. vulgatus* respectively.
- Even though specific composition changes in gut and vaginal microbiota, their etymology and pathogenesis, particularly in endometriosis and PCOS and their effects in reproductive health are still not clear, it has been established that both primary vaginal dysbiosis associated infections like BV, STI and VVC and primary gut dysbiosis associated conditions, endometriosis and PCOS have an impact in infertility. Because infertility, including conceiving problems, miscarriage and pre-term birth, has become more prevalent and significantly affecting life quality, modulating microbiota composition has a great potential in alleviating the symptoms.
- Promising treatments for BV, STI, VVC, endometriosis, PCOS and infertility include pro-prebiotic supplementation, dietary interventions such as Mediterranean diet, low FODMAP diet in endometriosis coupled with IBS and fecal or vaginal microbiota transplantation. Treatment strategies should be individualised and supporting conventional methods.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lananovkovic147@gmail.com

End Summary

Throughout this webinar it was described how composition of gut and vaginal microbiota have a role in reproductive health via their crosstalk, gut microbiota-estrogen and gut microbiota-brain axis. The differences in defining vaginal and gut dysbiosis were stated. The alternations in their microbial activity, particularly regarding short fatty chain acids, lipopolysaccharides and proinflammatory cytokines stimulation were observed and associated with bacterial vaginosis, endometriosis, PCOS and infertility pathogenesis. The similarities and co-dependency between them were connected with the same risk factors for microbiota perturbations and the differences were related to the other co-existing non-reproductive disorders. It was indicated that the vaginal and gut microbiota with derived metabolites represent a new frontier in the management of reproductive tract diseases. In that manner, the alternative treatment strategies supporting or alleviating conventional methods were introduced for each reproductive disorder separately and the example of their potential were shown through the chosen case studies. In the end, it was concluded that because of great connection of these 2 microbiotas with women health, future studies based on microbiota modulations in female diseases should be continued.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Take-Home Messages

lananovkovic147@gmail.com

- There is an increase in women reproductive health disorders, especially regarding bacterial vaginosis, endometriosis, PCOS what is ultimately leading to infertility. The reason for this may be the more frequent occurrence of risk factors such as western lifestyle which has an established adverse effect in gut and vaginal microbiota.

- The crosstalk between gut and vaginal microbiota exists and both should be taken into consideration when treating female reproductive health disorders.

- The gut dysbiosis may affect gut microbiota-estrogen axis, gut microbiota-brain axis. Beneficial modulations of gut microbiota can balance those axes via suppressed inflammation process and alleviate the symptoms of hormone-associated reproductive health disorders, particularly endometriosis and PCOS.

- Positive modulations of vaginal dysbiosis may return lactobacilli dominance and value of pH < 4,5 what prevents the pathogen proliferation and keeps immune system resistant to bacterial vaginosis and further attempt of STI and VVC coupled with infertility.

- Pro-prebiotic supplementation and dietary interventions have promising potential in reproductive health disorders prevention and symptom reduction and should be seriously considered in combination with conventional therapy. Fecal and vaginal microbiota transplantations are still not well investigated, but further studies should be carried out.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

- Ruan, Wenly, et al. "Healthy human gastrointestinal microbiome: composition and function after a decade of exploration." Digestive diseases and sciences 65 (2020): 695-705..
- Maciel-Fiuza MF, Muller GC, Campos DMS, do Socorro Silva Costa P, Peruzzo J, Bonamigo RR, Veit T and Vianna FSL (2023) Role of gut microbiota in infectious and inflammatory diseases. Front. Microbiol. 14:1098386. doi:10.3389/fmicb.2023.1098386
- Afzaal, Muhammad, et al. "Human gut microbiota in health and disease: Unveiling the relationship." *Frontiers in microbiology* 13 (2022): 999001.
- Kwon, Myoung Seung, and Heung Kyu Lee. "Host and microbiome interplay shapes the vaginal microenvironment." Frontiers in immunology 13 (2022): 919728.
- Both sources: Kroon, Samuel J., Jacques Ravel, and Wilhelmina M. Huston. "Cervicovaginal microbiota, women's health, and reproductive outcomes." Fertility and sterility 110.3 (2018): 327-336.
- Salliss, Mary E., et al. "The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain." *Human reproduction update* 28.1 (2022): 92-131.
- Yue, Min, and Lei Zhang. "Exploring the Mechanistic Interplay between Gut Microbiota and Precocious Puberty: A Narrative Review." *Microorganisms* 12.2 (2024): 323
- Zink, Christine. What Is a Sexually Transmitted Infection? (2022) https://www.verywellhealth.com/sexually-transmitted-infection-overview-and-more-5206974
- Cleveland clinic (2022) https://my.clevelandclinic.org/health/diseases/5019-vaginal
- Guo, Cuishan, and Chiyuan Zhang. "Role of The Gut Microbiota in The Pathogenesis of Endometriosis: A Review." *Frontiers in Microbiology* 15 (2024): 1363455.
- https://matrikaivf.in/pcos-symptoms-causes-risk-factors
- Calcaterra, Valeria, et al. "Probiotics and polycystic ovary syndrome: a perspective for management in adolescents with obesity." *Nutrients* 15.14 (2023): 3144.
- Yurtdaş, Gamze, and Yasemin Akdevelioğlu. "A new approach to polycystic ovary syndrome: the gut microbiota." *Journal of the American College of Nutrition* 39.4 (2020): 371-382.
- Xiao, Liwen, Zhenqiang Zuo, and Fangqing Zhao. "Microbiome in Female Reproductive Health: Implications for Fertility and Assisted Reproductive Technologies." *Genomics, Proteomics & Bioinformatics* 22.1 (2024): qzad005.
- Pendharkar, S., Brandsborg, E., Hammarström, L., Marcotte, H., & Larsson, P. G. (2015). Vaginal colonisation by probiotic lactobacilli and clinical outcome in women conventionally treated for bacterial vaginosis and yeast infection. BMC infectious diseases, 15, 1-12.
- Lev-Sagie, A., Goldman-Wohl, D., Cohen, Y., Dori-Bachash, M., Leshem, A., Mor, U., ... & Elinav, E. (2019). Vaginal microbiome transplantation in women with intractable bacterial vaginosis. Nature medicine, 25(10), 1500-1504
- Jankovich, E., & Watkins, S. (2017). The low FODMAP diet reduced symptoms in a patient with endometriosis and IBS. South African Journal of Clinical Nutrition, 30(4), 32-36. Retrieved from http://www.sajcn.co.za/index.php/SAJCN/article/view/1301
- Ostadmohammadi, V., Jamilian, M., Bahmani, F., & Asemi, Z. (2019). Vitamin D and probiotic co-supplementation affects mental health, hormonal, inflammatory and oxidative stress parameters in women with polycystic ovary syndrome. Journal of ovarian research, 12, 1-8.
- Chudzicka-Strugała, I., Kubiak, A., Banaszewska, B., Zwozdziak, B., Siakowska, M., Pawelczyk, L., & Duleba, A. J. (2021). Effects of synbiotic supplementation and lifestyle modifications on women with polycystic ovary syndrome. The Journal of Clinical Endocrinology & Metabolism, 106(9), 2566-2573.
- Fernández, L., Castro, I., Arroyo, R., Alba, C., Beltrán, D., & Rodríguez, J. M. (2021). Application of Ligilactobacillus salivarius CECT5713 to achieve term pregnancies in women with repetitive abortion or infertility of unknown origin by
- Acharya, Ashwitha, and Shilpa S. Shetty. "Role of gut microbiota derived short chain fatty acid metabolites in modulating female reproductive health." *Human Nutrition & Metabolism* (2024): 200256.
- Brennan, Caitriona, et al. "Harnessing the power within: engineering the microbiome for enhanced gynecologic health." Reproduction and Fertility 5.2 (2024).
- Chadchan, S. B., Singh, V., & Kommagani, R. (2022). Female reproductive dysfunctions and the gut microbiota. *Journal of molecular endocrinology*, *69*(3), R81-R94.
- Cocomazzi, Giovanna, et al. "The impact of the female genital microbiota on the outcome of assisted reproduction treatments." *Microorganisms* 11.6 (2023): 1443.
- Elkafas, Hoda, et al. "Gut and genital tract microbiomes: Dysbiosis and link to gynecological disorders." Frontiers in Cellular and Infection Microbiology 12 (2022): 1059825.
- Lehtoranta, Liisa, et al. "Healthy vaginal microbiota and influence of probiotics across the female life span." Frontiers in microbiology 13 (2022): 819958
- Salliss, Mary E., et al. "The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain." Human reproduction update 28.1 (2022): 92-131.

STUDY MATERIAL

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

Msc. Nutr. Lana Novković

Gut and Vaginal Microbiota and Female Reproductive Health

A Mini-review

e-mail: lananovkovic147@gmail.com

TABLE OF CONTENTS

1. INTRODUCTION....1

2. HUMAN GUT MICROBIOTA....2

3. VAGINAL MICROBIOTA....3

4. FEMALE REPRODUCTIVE HEALTH....4

5. THREE MAIN CROSSTALKS IN FEMALE REPRODUCTIVE HEALTH....5

5.1. The Gut – Vaginal Microbiota Crosstalk 5

5.2. The Gut Microbiota – Estrogen Axis 6

5.3. The Gut Microbiota – Brain Axis 8

6. FEMALE REPRODUCTIVE DISORDERS ASSOCIATED WITH GUT AND VAGINAL DYSBIOSIS....9

6.1. Vaginitis – Bacterial Vaginosis, Sexually Transmitted Infections, Vulvovaginal Candidiasis....9

6.2. Endometriosis 12

6.3. Polycistic Ovary Syndrome (PCOS) 14

6.4. Infertility 15

7. CLINICAL EVIDENCES....16

8. CRITICAL THINKING....18

9. FUTURE PERSPECTIVES....19

10. REFERENCES....20

1. INTRODUCTION

According to the World Health Organization (WHO), reproductive health is defined as a state of complete physical, mental, and social well-being, not merely the absence of disease or infirmity, encompassing all aspects of the reproductive system and its functions and processes. The female reproductive system is a delicate and intricate network of hormone-dependent tissues essential for the continuation of life and the survival of the species. (Acharya *et al.*, 2024)

The human microbiota, comprising trillions of microorganisms, plays a crucial role in maintaining health across various bodily systems. Among these, the gut and vaginal microbiota have garnered significant attention due to their profound impact on reproductive health. The gut microbiota, residing primarily in the intestines, influences systemic immunity, metabolism, and hormonal balance, which are vital for reproductive health. The vaginal microbiota, predominantly composed of *Lactobacillus* species, is essential for protecting against infections and maintaining vaginal pH (Kroon *et al.*, 2018).

Disruptions in these microbial communities, known as dysbiosis, can lead to a variety of reproductive health issues, including bacterial vaginosis (BV), yeast infections, sexually transmitted infections (STI), hormone-associated disorders, such as endometriosis and PCOS, preterm birth, and infertility. Emerging research indicates that the gut and vaginal microbiota are not isolated ecosystems but are interconnected, influencing each other and the host's reproductive outcomes. This introduction delves into the complex interplay between these microbiota and reproductive health, highlighting their roles, mechanisms of interaction, and implications for clinical practice and future research (Amabebe *et al.*, 2020). Understanding this relationship is pivotal for developing microbiota-targeted therapies that could revolutionize the management of reproductive health disorders. As we advance our knowledge in

this field, the potential for improving reproductive outcomes through microbiota modulation offers a promising frontier in medical science (Ashonibare *et al.*, 2024).

2. HUMAN GUT MICROBIOTA

The human gut microbiota encompasses a complex ecosystem of microorganisms residing within the gastrointestinal tract, collectively known as the gut microbiome. This intricate community, akin to a fingerprint, varies among individuals but predominantly comprises bacteria from the *Firmicutes* and *Bacteroidetes* phyla, with fewer representatives from *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia* (Clarke *et al.*, 2019). Various factors influence the composition and diversity of the gut microbiome. Personal attributes such as genetics, birth mode, age, gender, and lifestyle choices including diet and medication intake play significant roles. Moreover, transmission routes, whether vertical (mother to infant) or horizontal (between individuals and their environments), shape microbial colonization patterns (Shanahan *et al.*, 2021). The gut microbiome serves pivotal functions essential for host health. Primarily, it aids in digestion by fermenting undigested food, producing essential metabolites like short-chain fatty acids (SCFAs), and facilitating nutrient absorption. Additionally, the microbiota contributes to the maturation of the digestive tract, host defense against pathogens, and plays a fundamental role in immune system development and regulation (Walther *et al.*, 2019).

Microbial metabolites, products of the gut microbiota's metabolic activities, exert profound effects on both microbial communities and host physiology. Among these metabolites, SCFAs emerge as key regulators of host health, and thus reproductive health, with diverse physiological benefits ranging from immunomodulation to cardiometabolic protection. Additionally, gut microbes synthesize essential vitamins and bioactive compounds, further underscoring their symbiotic relationship with the host (Acharya *et al.*, 2024).

The gut microbiome's interactions extend beyond the gastrointestinal tract, encompassing various organ systems. A diverse and stable microbiome, characterized by eubiosis, fosters mutualistic relationships with other organs, promoting overall health. On the other hand, dysbiosis, marked by microbial imbalance, correlates with inflammatory processes and numerous health disorders, including reproductive health disorders (Afzaal *et al.*, 2022).

3. VAGINAL MICROBIOTA

Vaginal microbiota in humans, characterized by *Lactobacillus* dominance and low diversity, contrasts with other mammals. Molecular techniques classify vaginal bacterial communities into community state types (CSTs), where CSTs I, II, and V signify health, while CST IV indicates dysbiosis, often associated with bacterial vaginosis (BV) (Lehtoranta et al., 2022; Kroon et al., 2018). Candida predominates in the vaginal mycobiota, with colonization more common in lactobacilli-dominated microbiota.

Lactobacillus spp. play pivotal roles in vaginal health, metabolizing glycogen, which amounts depends on circulating estrogen levels, into lactic acid, which maintains vaginal pH < 4.5. It also produces hydrogen peroxide, and bacteriocins, inhibiting pathogenic colonization and protecting against infections, what is crucial for health (Elkafas et al., 2022; Kroon et al., 2018). Diminished lactobacilli protection leads to vaginal dysbiosis, characterized by high microbial diversity (in contrast to gut dysbiosis) and reduced *Lactobacillus* dominance. This shift, often associated with elevated pH and Candida species, correlates with gynaecological and reproductive disorders, underscoring the significance of maintaining eubiosis for female genital tract health (van de Wijgert, 2017).

The composition of vaginal microbiota depends on several factors. Ethnicity and genetics impact microbiota composition, with lactobacilli dominance observed in Asian and White women, while Black or African-American and Hispanic

women tend to exhibit diverse microbial communities (Kwon and Lee, 2022; Lehtoranta et al., 2022). Reproductive age, controlled by hormonal fluctuations, profoundly shapes vaginal microbiota. Estrogen levels dictate glycogen deposition, affecting Lactobacillus abundance and pH. Menstrual cycle phases correlate with microbiota stability, with Lactobacillus predominance during estrogen peaks (Kwon and Lee, 2022; Punzon-Jimenez and Labarta, 2021). Pregnancy induces relative stability in the vaginal microbiota, characterized by increased *Lactobacillus* dominance, likely influenced by hormonal changes and mucosal barrier alterations. (Lehtoranta et al., 2022; Kroon et al., 2018).

Lifestyle factors, including hormonal contraceptives and vaginal hygiene practices, directly impact vaginal microbiota. Hormonal contraceptives promote Lactobacillus colonization but may affect gut microbiota diversity. Poor hygiene practices, particularly vaginal douching, elevate the risk of dysbiosis (Krog et al., 2022). Sexual behaviors such as multiple or new partners and unprotected intercourse contribute to lactobacilli depletion and increase susceptibility to vaginal infections. These factors disrupt the protective vaginal mucosa, predisposing individuals to gynecological diseases (Gupta et al., 2019). Understanding the interplay of these modulating factors is essential for elucidating vaginal microbiota dynamics and their implications for women's reproductive health.

4. FEMALE REPRODUCTIVE HEALTH

eproductive health, a concept outlined by the World Health Organization, encompasses the holistic well-being of both male and female reproductive systems across all life stages. In females, this intricate system includes vital organs such as the ovaries, oviducts, uterus, vagina, and mammary glands, all working in tandem to produce and combine germ cells—ovum (egg) from the female and sperm from the male—to facilitate conception and support fetal

development. Hormonal and neural signals from various sources, including the anterior hypothalamus, anterior pituitary, and gonads, orchestrate this complex process, collectively forming the hypothalamic-pituitary-gonadal (HPG) axis. This axis regulates the pulsatile release of key hormones like follicle-stimulating hormone (FSH) and luteinizing hormone (LH), crucial for ovarian function, ovulation, and menstrual cycle regulation (Acharya *et al.*, 2024).

The endocrine system intricately regulates the operations of the female reproductive tract, and disruptions within this system can lead to a myriad of disorders and diseases. Additionally, lifestyle factors such as body weight, smoking, alcohol consumption, stress, exercise habits, diet, and nutrition significantly influence reproductive health. Of particular importance, nutrition exerts a profound impact on various reproductive processes, from puberty onset to implantation and fetal development (NIEHS, 2024).

The effects of microbiota and reproductive health, specific disorders and diseases have gained significant attention in the science. Conditions such as bacterial vaginosis (BV), endometriosis, PCOS, and fertility-related issues often exhibit associations with alterations in both vaginal and gut microbiota compositions. Understanding the intricate interplay between microbiota and reproductive health is crucial for evaluating potential therapeutic interventions and preventive measures for these conditions. Thus, a comprehensive approach to reproductive health must consider not only hormonal and physiological factors but also the influence of microbial ecosystems on overall well-being.

5. THREE MAIN CROSSTALKS IN FEMALE REPRODUCTIVE HEALTH

5.1. The Gut – Vaginal Microbiota Crosstalk

Understanding the intricate interplay between the intestinal and vaginal microbiota, as well as their influence on women's reproductive health, is crucial for unraveling the complexities of host-microbe interactions. The gut and female

reproductive tract maintain constant communication via microbial metabolites, the immune system, and the circulatory system. Common bacterial phyla, such as *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Fusobacteria*, inhabit both niches, highlighting their connection.

Vertical transmission serves as an initial pathway illustrating the dependence of gut microbiota composition on the vaginal microbiota. Vaginal delivery plays an initial role in shaping the neonatal gut microbiota, with newborns acquiring microbial communities from their mothers' vaginal microbiota. Conversely, infants born via C-section exhibit reduced microbial richness and diversity, often with a skin-derived microbe and a deficiency in beneficial bacteria transmitted during vaginal delivery. Such alterations in early microbial colonization can impact immune development and metabolism, potentially predisposing individuals to chronic immune disorders (Coscia et al., 2021).

Rectum-vagina translocation of microbes underscores another facet of gut-vagina microbiota crosstalk, particularly relevant in conditions like recurrent bacterial vaginosis (BV). Microorganisms originating from the rectum, such as *Escherichia coli* and *Candida* species, may colonize the vagina, disrupting its microbial balance and leading to infections. The resemblance in composition between rectal and vaginal microbiota provides additional evidence suggesting that Lactobacillus species, which are abundant in the rectal microbiota, may have originated from this source. (El Aila et al., 2009).

Microbial metabolites, such as short-chain fatty acids (SCFAs), play a pivotal role in modulating immune responses and maintaining homeostasis within the gut and vaginal microbiota. While SCFAs produced in the gut contribute to intestinal integrity and anti-inflammatory effects, their accumulation in the cervicovaginal space is associated with decreased lactobacilli, increased vaginal pH, and a pro-inflammatory environment. This imbalance can lead to vaginal dysbiosis and increase the risk of intrauterine inflammation, infection, and adverse pregnancy outcomes (Ashonibare et al., 2024).

5.2. The Gut Microbiota – Estrogen Axis

Another interaction includes the gut microbiota impact on vaginal health related to fluctuations in hormone levels, particularly estrogens, which can influence the vaginal microbiota. The gut microbiota plays a role in estrogen metabolism, leading to their accumulation in the body instead of their elimination through feces. As previously mentioned, the presence of dominant *Lactobacillus* spp. in the vaginal microbiota relies on glycogen byproducts from an estrogen-rich vaginal epithelium and host amylases. However, this glycogen can also serve as a nutrient for pathogenic yeast like *Candida albicans*, heightening the risk of vulvovaginal candidiasis, especially in estrogen-favoring conditions such as pregnancy (Sallis et al., 2022).

The gut microbiota's influence on estrogen levels occurs through the estrobolome, a collection of genes encoding estrogen-metabolizing enzymes in the gut microbiome. These enzymes regulate estrogen elimination and circulation by modulating estrogens' enterohepatic circulation. Within the intestinal tract, specific gut bacteria, such as Escherichia coli, Bacteroides fragilis, and Streptococcus agalactiae, produce the enzyme β-glucuronidase, which converts conjugated estrogens to their deconjugated form. This process allows for estrogen reabsorption into the bloodstream, impacting target tissues like the vagina and uterus and stimulating cell proliferation (Elkafas et al., 2022).

In a healthy state, characterized by a diverse gut microbiota and *Lactobacillus* spp.-dominant cervicovaginal microbiota, circulating estrogen levels are regulated, inflammation is minimized, and barrier function is maintained. Elevated estrogen availability in the cervicovaginal mucosa supports *Lactobacillus* dominance, low pH, and minimal inflammation, crucial for cervicovaginal health. However, dysbiosis in the gut microbiome, disrupts this

balance, leading to higher estrogen levels, local and systemic inflammation, and dysbiosis in the cervicovaginal mucosa (Sallis et al., 2022).

Elevated estrogen levels can overstimulate endometrial tissue, potentially causing endometriosis and fertility issues. Meanwhile, systemic inflammation resulting from gut dysbiosis disturbs the cervicovaginal mucosa, shifting it away from *Lactobacillus* dominance towards disease-associated polymicrobial biofilms, raising the pH above 4.5, a condition linked to cervicovaginal diseases. On the other hand, dysbiosis and inflammation in the gut microbiome can reduce the function of β-glucuronidase-producing bacteria, leading to a reduction in free estrogen levels in circulation. This alteration in estrogen receptor activations can result in a hypoestrogenic state, contributing to conditions like obesity, metabolic syndrome, and polycystic ovary syndrome (PCOS), ultimately impacting fertility. Both hyper- and hypoestrogenic pathologies can lead to infertility (Graham et al., 2021)

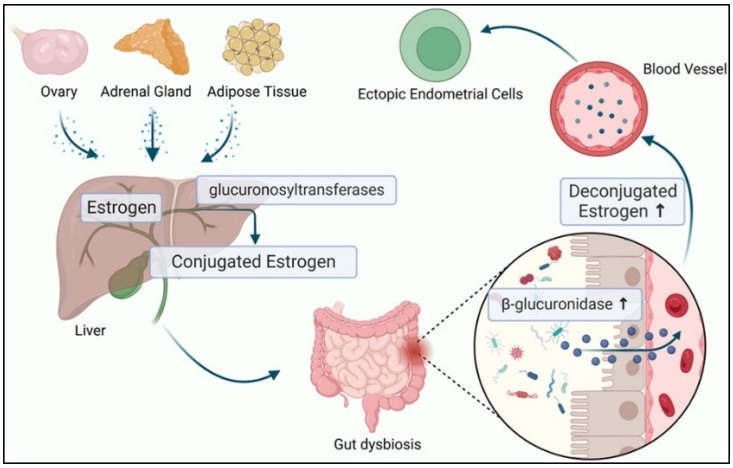


Figure 1. Gut Microbiota – Estrogen Axis (Guo & Zhang, 2024)

5.3. The Gut Microbiota – Brain Axis

The last axis involved in reproductive health outcomes is the gut microbiota-brain axis (MGB). The microbiome-gut-brain axis has gained increasing attention for its role in the bidirectional communication between gut microbes and the central nervous system. This axis is crucial in regulating immune, neural, endocrine, and metabolic pathways to maintain homeostasis. Dysregulation of the MGB axis has been implicated in various diseases, including irritable bowel syndrome (IBS), depression, pain, and polycystic ovary syndrome (PCOS). Gut microbiota can influence the production and secretion of neurotransmitters such as γ-aminobutyric acid (GABA), norepinephrine, dopamine, and serotonin. These neurotransmitters affect hormone levels in the host, linking gut bacteria closely to the hypothalamus-pituitary-adrenal (HPA) axis. Therefore, changes in gut microbiota composition may lead to alterations in host hormone levels including estrogen levels what is associated with endometriosis (Guo et al., 2019).

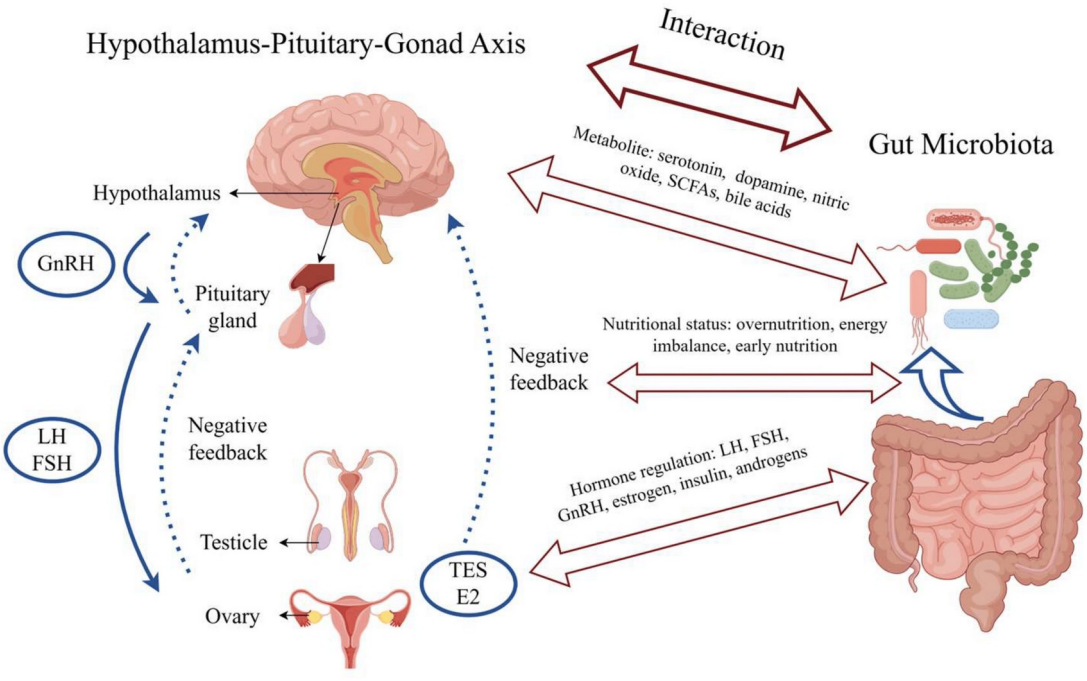


Figure 2. Gut Microbiota – HPG Axis (Yue & Zhang, 2024)

6. FEMALE REPRODUCTIVE DISORDERS ASSOCIATED WITH GUT AND VAGINAL DYSBIOSIS

6.1. Vaginitis – Bacterial Vaginosis, Sexually Transmitted Infections, Vulvovaginal Candidiasis

Bacterial Vaginosis (BV)

BV can occur at any reproductive age (15-44 years). Its prevalence varies globally, within countries, and among populations, influenced by ethnic origin and socioeconomic status. In the USA, the prevalence is about 29%, lower in Asia and Europe but high in Poland and Norway (Kenyon et al., 2013). In Africa, the prevalence is generally high due to the diverse vaginal microbiota, but there are exceptions with low BV rates in some African regions and high rates in certain Asian and European populations (Joseph et al., 2021). BV involves a shift from *Lactobacillus* spp. to a mixed flora including *Gardnerella vaginalis*, *Atopobium vaginae*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Prevotella*, *Peptoniphilus*, *Megasphaera*, *Mobiluncus*, and uncultured bacteria. These bacteria produce virulence factors like cytolysins and biofilms. Complications: BV is linked to gynecological issues, such as increased risk of pelvic inflammatory disease, STIs, including HIV, infertility, preterm labor, abortion, and endometriosis (Lopez-Moreno & Aguilera, 2021). During pregnancy, BV increases the risk of preterm labor, miscarriage, intrauterine fetal death, and postpartum infections (Abou Chacra et al., 2022).

BV depletes *Lactobacillus* spp., disrupting vaginal pH and allowing infections by biofilm-forming, antibiotic-resistant pathogens. BV-associated bacteria produce sialidase and vaginolysin, degrading mucin and damaging epithelial cells, respectively. This triggers a proinflammatory response, increasing the risk of HIV acquisition (Kwon & Lee, 2022; Joseph et al., 2021). *G. vaginalis*, a key

bacterium in BV, forms biofilms and produces cytotoxins, proteolytic enzymes, and malodorous amine compounds. It also creates "clue cells," a BV hallmark, and works with *Prevotella* spp. to degrade the mucin layer and promote pathogen adhesion (Abou Chacra et al., 2022). *G. vaginalis* infection can also stimulate gut microbiota dysbiosis and systemic inflammation (Amabebe & Anumba, 2020).

BV treatment focuses on halting BV-associated microorganism proliferation and restoring normal vaginal flora. Standard treatments include antibiotics like clindamycin and metronidazole (Abou Chacra et al., 2022). Due to recurrent BV, alternative strategies include probiotics to restore Lactobacillus spp. and maintain vaginal homeostasis (Joseph et al., 2021). Probiotics can be administered orally or vaginally, both showing efficacy (Lehtoranta et al., 2022). Prebiotics, like FOS and inulin, support beneficial microbiota growth, improving host health. In BV treatment, they stimulate Lactobacillus growth, lower vaginal pH, and enhance probiotics' effectiveness, helping maintain a healthy vaginal microbiome and prevent BV recurrence (Sadeghpour Heravi, 2024). Vaginal Microbiota Transplantation (VMT), transferring microbiota from a healthy donor to a BV patient, is emerging as a treatment to restore vaginal balance and reduce antibiotic use (Joseph et al., 2021). Further research in humans is needed to advance this promising therapy.

Sexually Transmitted Infection (STI) and Vulvovaginal Candidiasis (VVC)

Epidemiological studies link BV and vaginal microbiome dysbiosis to higher STI risk. STI include like *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis*, herpes simplex virus, HIV, and HPV. *Chlamydia* is more associated with *L. iners* (CST III) and CST IV, while *L. crispatus* (CST I) has antichlamydial and immune-enhancing properties (Kroon et al., 2018). Lactobacillus spp. (*L. gasseri*, CST II, and *L. jensenii*, CST V) can compete with N. *gonorrhoeae* for epithelial binding (Kroon et al., 2018). Women with vaginal dysbiosis are four times more likely to acquire HIV. Diverse microbiomes with

more CD4 T cells create an environment conducive to HIV transmission. Despite advances in antiretroviral therapy and pre-exposure prophylaxis (PrEP), HIV remains a major concern, especially in low- and middle-income countries (Brennan et al., 2024). Poor BV treatment and recurrence increase HIV risk (Greenbaum et al., 2018). Probiotic supplementation may help prevent STIs secondary to BV, though specific treatments are still necessary (Kwon & Lee, 2022; Greenbaum et al., 2018).

Vulvovaginal candidiasis (VVC), primarily caused by *Candida albicans*, manifests as a thick white discharge resembling cottage cheese. Over 75% of women experience Candida infections, with some prone to recurrent VVC due to C. albicans' biofilm formation, enhancing virulence and antifungal resistance (Lehtoranta et al., 2022). Risk factors for VVC include antibiotic use, high estrogen levels (from pregnancy, contraceptives, or hormone therapy), uncontrolled diabetes, and immune system impairment (e.g., HIV infection or corticosteroid therapy). Preventative measures include wearing cotton underwear, avoiding tight clothes, scented products, hot tubs, unnecessary antibiotics, and staying in wet clothes too long. Probiotic supplementation with antibiotics may help prevent VVC (Mayo Clinic, 2023).

For treatment, combining antifungal therapies (azoles) with probiotics is recommended, as specific probiotic strains can enhance protection against azole-resistant Candida (Barrientos-Durán et al., 2019).

6.2. Endometriosis

Endometriosis affects approximately 196 million people worldwide. Nearly half experience chronic pelvic pain, and 30-50% are infertile. Endometriosis might be better considered a “syndrome” due to its wide range of symptoms, including severe dysmenorrhea, heavy menstruation, bowel and bladder symptoms, fatigue, and depression. Retrograde menstruation is a leading cause, but only 10% of those with retrograde menstruation develop endometriosis. The American Society for Reproductive Medicine classifies endometriosis as stage I-IV based on lesion size, number, and adhesion presence (Chadchan et al., 2023).

The diagnosis and treatment of endometriosis are complex and often delayed by 4-11 years due to atypical symptoms and invasive diagnostic methods. Laparoscopic surgery with histologic examination is the gold standard for diagnosis, but less invasive biomarkers are urgently needed. Recent studies suggest that gut microbiota plays a significant role in chronic diseases, including endometriosis, by influencing inflammation, estrogen metabolism, and immunity (Guo and Zhang, 2024).

Gut dysbiosis is linked to endometriosis, with reduced α-diversity and β-diversity, an increased *Firmicutes*/*Bacteroidetes* ratio, and altered bacterial populations (Salliss et al., 2022). Women with endometriosis show increased gram-negative bacteria and decreased beneficial bacteria like *Lactobacillus*. This dysbiosis extends to vaginal microbiota, causing vaginal dysbiosis and higher bacterial vaginosis prevalence (Salliss et al., 2022).

Gut dysbiosis influences endometriosis through immune modulation, inflammation, and hormone regulation. It affects T lymphocyte differentiation and increases Th17 cells, promoting inflammation. Increased LPS from gram-negative bacteria leads to immune responses via TLR4/NF-κB pathways, promoting pelvic inflammation (Leonardi et al., 2019). Conversely, reduced

SCFA-producing bacteria disrupt Treg cell regulation, enhancing inflammation (Chadchan et al., 2023).

Inflammatory cytokines like NF-κB, IL-1, IL-8, and COX-2 are overexpressed in women with endometriosis, driven by bacteria like Streptococcus bovis. Elevated pro-inflammatory factors in the pelvic cavity promote endometrial adhesion and lesion formation (Guo and Zhang, 2024).

The gut-estrogen axis involves the estrobolome's β-glucuronidase activity, increasing circulating estrogen and promoting endometriosis. Dysbiosis alters β-glucuronidase-producing microbiota, enhancing estrogen reabsorption and inflammation (Graham et al., 2021). The gut-brain axis also plays a role, where dysbiosis affects HPA axis activity, leading to hormone imbalances that exacerbate endometriosis symptoms (Salliss et al., 2022).

Treatment includes a multimodal approach with surgery, NSAIDs, hormonal therapies, and new potential therapies like probiotics, omega-3 fatty acids, low-FODMAP diets, and fecal microbiota transplantation (FMT) (Uccello et al., 2012; Tomio et al., 2013). Probiotics like Lactobacillus gasseri can suppress ectopic endometrial cells, reduce menstrual pain, and improve gut health (Guo and Zhang, 2024). Omega-3 PUFAs reduce inflammation and modulate gut microbiota, aiding endometriosis management (Tomio et al., 2013). Low-FODMAP diets help manage gastrointestinal symptoms associated with endometriosis (Hustoft et al., 2017). FMT, though not yet tested for endometriosis, shows promise due to its success in treating gut-related disorders (Ni et al., 2020).

6.3. Polycistic Ovary Syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is a reproductive disorder not directly connected to vaginal microbiota but can lead to vaginal dysbiosis due to hormonal imbalances caused by gut dysbiosis. The Rotterdam criteria, which include polycystic ovaries on ultrasonography, menstrual irregularities, and hyperandrogenism, are commonly used for diagnosis; two out of three parameters confirm PCOS. Genetic predisposition, high insulin and androgen levels, poor diet, and sedentary lifestyle are risk factors that contribute to gut dysbiosis. Symptoms include hirsutism, weight gain, acne, fatigue, insulin resistance, and heavy bleeding, affecting 8–13% of reproductive-age women globally (Siddqui et al., 2022). Short-term complications include infertility and adverse pregnancy outcomes, while long-term risks encompass diabetes and heart disease (Sun et al., 2023).

The gut microbiome's role in PCOS includes insulin resistance, hyperandrogenism, and chronic inflammation, similar to endometriosis but via different pathways. Women with PCOS exhibit decreased α-diversity and altered β-diversity in gut microbiota, leading to metabolic abnormalities and immune system activation, causing hyperinsulinemia and preventing normal follicle development (Chadchan et al., 2022). Increased *Bacteroides* and *Escherichia*/*Shigella* ratios in PCOS patients elevate lipopolysaccharide levels (LPS), causing chronic inflammation, hyperandrogenism, and obesity (Yurtdas & Akdevelioglu, 2019).

Obesity linked to a high-fat, high-sugar diet exacerbates gut dysbiosis, insulin resistance, and hyperinsulinemia, contributing to androgen production and PCOS symptoms (Guo et al., 2021). Hyperandrogenism further alters gut microbiota, impacting testosterone levels and gut-brain axis mediators like GABA and serotonin, influencing appetite and hormonal regulation (Calcaterra et al., 2023).

Higher *Bacteroides vulgatus* and *Prevotella* spp. levels disrupt bile acid metabolism, affecting fat absorption and contributing to insulin resistance and hyperandrogenism (Ashonibare et al., 2024; Chadchan et al., 2022). Beneficial bacteria like *Roseburia* and *Akkermansia* are reduced in PCOS, leading to decreased SCFA production, reduced insulin sensitivity, which is also connected with the occurrence of vaginal dysbiosis (Guo et al., 2021). Treatments focus on lifestyle changes, including a Mediterranean diet and physical activity, and may include probiotics, prebiotics, and synbiotics to improve gut microbiota and PCOS symptoms (Brennan et al., 2024).

6.4. Infertility

Reproductive health disorders such as vaginitis, PCOS, and endometriosis can lead to infertility, difficulty during pregnancy, miscarriage, and premature birth. Infertility, as defined by the WHO, is a failure to conceive after 12 months or more of regular unprotected sex and can be due to male, female, or unexplained factors. Treatment often involves in-vitro fertilization (IVF) and other assisted reproductive technologies. Approximately one in six people worldwide experience infertility, with female infertility often caused by abnormalities in the ovaries, uterus, fallopian tubes, or endocrine system. Gut and vaginal microbiota significantly influence reproductive health, with imbalances linked to conditions like PCOS and endometriosis (Cocomazzi et al.,2023).

Dysbiosis in the gut can lead to inflammation and hormone dysregulation, affecting ovulation and menstrual. For instance, studies have shown that imbalances in gut microbiota composition can impair women's reproductive function and cause related diseases and conditions (Chadchan et al., 2022). Similarly, vaginal microbiota imbalances can lead to bacterial vaginosis, increasing the risk of STIs and affecting fertility (Lehtoranta et al., 2022).

Research indicates that a *Lactobacillus*-dominated vaginal microbiota is beneficial for fertility, while a decrease in *Lactobacilli* and an increase in pathogenic bacteria can lead to infertility. Additionally, assisted reproductive technologies (ART) have become crucial for those facing infertility, with the success of treatments like *In Vitro* Fertilisation (IVF) being influenced by the microbiota composition (Cocomazzi et al.,2023). Nutritional support, including a Mediterranean diet, and supplementation with probiotics can enhance reproductive health and IVF outcomes (Acharya et al., 2024). Probiotics, particularly *Lactobacilli,* can improve vaginal microbiota, potentially increasing the success of fertility treatments (Chadchan et al.,2022).

7. CLINICAL EVIDENCES

Probiotic Supplementation for BV and VVC

A study investigated the efficacy of probiotics (EcoVag® containing Lactobacillus rhamnosus DSM 14870 and Lactobacillus gasseri DSM 14869) in treating Bacterial Vaginosis (BV) and Recurrent Vulvovaginal Candidiasis (VVC). 40 Scandinavian women were divided into two trials: one for BV and another with three groups (BV, R-VVC, and extended fluconazole only). BV cure rates were 50% at 6 months in trial I and 67% at 6 and 12 months in trial II. VVC cure rates were 100% and 89% with probiotics and 100% and 70% with fluconazole only. Probiotics enhanced lactobacilli colonization, crucial for BV cure, though less effective for VVC without prior antibiotic treatment. The study highlighted probiotics' role in prolonging lactobacilli colonization post-antibiotics and suggested sexual activity as a BV risk factor (Pendharkar et al., 2015).

Vaginal Microbiome Transplantation (VMT) for Recurrent BV

A study explored VMT from healthy donors as a treatment for recurrent, antibiotic-resistant BV. Five women with severe BV underwent VMT, resulting in long-term symptom improvement for four patients and partial remission for one. VMT shifted the vaginal microbiome composition, increasing *Lactobacillus* and decreasing BV-associated bacteria like *Gardnerella*. The study confirmed VMT's potential but emphasized the need for rigorous donor screening to ensure safety and efficacy (Lev-Sagie et al., 2019).

Low FODMAP Diet for Endometriosis and IBS

A case report detailed a 23-year-old with endometriosis and IBS who benefitted from a low FODMAP diet. After dietary and lifestyle adjustments, she joined a 16-week FODMAP elimination and re-challenge program. This approach significantly improved her gastrointestinal symptoms, including bloating, abdominal pain, and constipation. Personalized dietary guidelines and gradual reintroduction of FODMAPs helped maintain symptom control. The study

highlighted the effectiveness of individualized dietary management for endometriosis and IBS (Jankovich and Watkins, 2017).

Vitamin D and Probiotic Supplementation for PCOS

A double-blind, placebo-controlled trial in Iran examined the effects of vitamin D and probiotics on PCOS symptoms. 60 women received either 50,000 IU of vitamin D biweekly and probiotics daily or a placebo for 12 weeks. Results showed significant improvements in depression, anxiety, stress, hirsutism, and testosterone levels. Additionally, there was a notable reduction in inflammatory markers. The synergistic effects of vitamin D and probiotics suggest benefits for mental health and hormonal balance in PCOS patients (Ostadmohammadi et al., 2019).

Lifestyle Changes and Synbiotics for PCOS

A study involving 65 overweight women with PCOS evaluated the impact of lifestyle changes combined with synbiotics. Over three months, participants followed a calorie-restricted diet and exercise regimen, with one group receiving synbiotics. The synbiotic group experienced an 8% BMI reduction, significantly more than the control group. Additionally, testosterone levels decreased by 32% in the synbiotic group. Synbiotics enhanced the effects of lifestyle modifications, potentially through gut microbiota modulation (Chudzicka-Strugała et al., 2021).

Probiotic Intervention for Infertility

A study assessed the impact of *Ligilactobacillus salivarius* CECT5713 on pregnancy rates in women with recurrent abortion or unexplained infertility. 58 women were divided into three groups: recurrent abortion, infertility, and healthy controls. Probiotic supplementation improved the cervicovaginal environment, enhancing lactobacilli presence and reducing pathogenic bacteria. This microbiome modulation was associated with higher pregnancy

rates in the probiotic group, suggesting probiotics' potential in reproductive health (Fernandez et al., 2021).

These case studies underscore the significant role of probiotics and dietary interventions in managing female reproductive health issues, highlighting their potential to improve treatment outcomes for conditions like BV, VVC, PCOS, endometriosis, and infertility. Further research with larger cohorts is necessary to confirm these findings and optimize treatment protocols.

8. CRITICAL THINKING

The mini-review successfully underscores the complex relationship between gut and vaginal microbiota and female reproductive health, offering valuable insights into potential therapeutic avenues such as probiotics and dietary interventions. The case studies on PCOS, endometriosis, and infertility illustrate the promising benefits of microbiota modulation, highlighting the significant potential for non-invasive treatment strategies. However, while the review presents a wealth of evidence, it is crucial to consider the challenges of generalizing findings from studies with relatively small sample sizes and short durations. Future research with larger, more diverse populations could provide a more comprehensive understanding of long-term outcomes and effectiveness. The review also touches on the intricate interplay between gut and vaginal microbiota, suggesting possible pathways through which these microbial communities influence reproductive health. Expanding mechanistic studies could further elucidate these pathways and solidify our understanding. Moreover, the review briefly acknowledges the socioeconomic and cultural factors affecting microbiota and reproductive health. A deeper exploration of these dimensions could enrich the context and applicability of the findings, ensuring that therapeutic strategies are inclusive and equitable. Overall, the review lays a robust foundation, advocating for the continued exploration of microbiota's role in reproductive health and the development of tailored, evidence-based interventions.

9. FUTURE PERSPECTIVES

Future research on gut and vaginal microbiota and their impact on female reproductive health should aim to address several key areas to enhance our understanding and therapeutic approaches. Large-scale, longitudinal studies are crucial to establish causal relationships and understand the long-term effects of microbiota modulation on reproductive health. Advanced genomic and metabolomic technologies could provide deeper insights into the specific microbial species and metabolic pathways involved in reproductive health and disease. Personalized medicine approaches, considering individual microbiota profiles, genetic background, and lifestyle factors, could optimize probiotic and dietary interventions, making treatments more effective and tailored. Additionally, the exploration of novel therapeutic strategies such as microbiota transplants, as seen in the promising results of vaginal microbiome transplantation for recurrent BV, should be expanded and standardized to ensure safety and efficacy. Integrating microbiota research with broader reproductive health initiatives could also address disparities in health outcomes influenced by socioeconomic and cultural factors. Ultimately, a multidisciplinary approach, combining microbiology, reproductive medicine, nutrition, and public health, will be essential to translate these scientific insights into practical health benefits for women globally.

10. REFERENCES

Abou Chacra, Linda, Florence Fenollar, and Khoudia Diop. "Bacterial vaginosis: what do we currently know?." *Frontiers in cellular and infection microbiology* 11 (2022): 672429.

Acharya, Ashwitha, and Shilpa S. Shetty. "Role of gut microbiota derived short chain fatty acid metabolites in modulating female reproductive health." *Human Nutrition & Metabolism* (2024): 200256.

Afzaal, Muhammad, et al. "Human gut microbiota in health and disease: Unveiling the relationship." *Frontiers in microbiology* 13 (2022): 999001.

Amabebe, Emmanuel, and Dilly OC Anumba. "Female gut and genital tract microbiota-induced crosstalk and differential effects of short-chain fatty acids on immune sequelae." *Frontiers in Immunology* 11 (2020): 553047.

Amabebe, Emmanuel, and Dilly OC Anumba. "Female gut and genital tract microbiota-induced crosstalk and differential effects of short-chain fatty acids on immune sequelae." *Frontiers in Immunology* 11 (2020): 553047.

Ashonibare, A., Presley, L. L., Ashorn, P., Dewey, K. G., Klein, N., Maleta, K., ... Kamwendo, D. (2024). Maternal gut microbiome influences maternal and infant nutritional status and pregnancy outcomes in rural Malawi. Scientific Reports, 14(1), 8157.

Ashonibare, Victory J., et al. "Gut microbiota-gonadal axis: the impact of gut microbiota on reproductive functions." *Frontiers in Immunology* 15 (2024): 1346035.

Barrientos-Durán, Antonio, et al. "Reviewing the composition of vaginal microbiota: inclusion of nutrition and probiotic factors in the maintenance of eubiosis." Nutrients 12.2 (2020): 419.

Calcaterra, Valeria, et al. "Probiotics and polycystic ovary syndrome: a perspective for management in adolescents with obesity." *Nutrients* 15.14 (2023): 3144.

Chadchan, S. B., Singh, V., & Kommagani, R. (2022). Female reproductive dysfunctions and the gut microbiota. *Journal of molecular endocrinology*, *69*(3), R81-R94.

Chudzicka-Strugała, I., Kubiak, A., Banaszewska, B., Zwozdziak, B., Siakowska, M., Pawelczyk, L., & Duleba, A. J. (2021). Effects of synbiotic supplementation and lifestyle modifications on women with polycystic ovary syndrome. *The Journal of Clinical Endocrinology & Metabolism*, *106*(9), 2566-2573.

Clarke, G., Sandhu, K.V., Griffin, B.T., Dinan, T.G., Cryan, J.F., Hyland, N.P. (2019) Gut Reactions: Breaking Down Xenobiotic–Microbiome Interactions. *Pharmacol. Rev.* **71**(2): 198-224.

Cocomazzi, Giovanna, et al. "The impact of the female genital microbiota on the outcome of assisted reproduction treatments." *Microorganisms* 11.6 (2023): 1443.

Coscia, A., Bardanzellu, F., Caboni, E., Fanos, V. (2021). Role of Maternal Microbiota and Metabolome in Newborn Gut Colonization. International Journal of Molecular Sciences, 22(18), 10079.

El Aila, N. A., Tency, I., Claeys, G., Saerens, B., De Backer, E., Temmerman, M., ... Verstraelen, H. (2009). Comparison of different sampling techniques and of different culture methods for detection of group B streptococcus carriage in pregnant women. BMC Infectious Diseases, 9(1), 103.

Elkafas, Hoda, et al. "Gut and genital tract microbiomes: Dysbiosis and link to gynecological disorders." Frontiers in Cellular and Infection Microbiology 12 (2022): 1059825.

Fernández, L., Castro, I., Arroyo, R., Alba, C., Beltrán, D., & Rodríguez, J. M. (2021). Application of Ligilactobacillus salivarius CECT5713 to achieve term pregnancies in women with repetitive abortion or infertility of unknown origin by microbiological and immunological modulation of the vaginal ecosystem. *Nutrients*, *13*(1), 162.

Graham, Madeline E., et al. "Gut and vaginal microbiomes on steroids: implications for women's health." *Trends in Endocrinology & Metabolism* 32.8 (2021): 554-565.

Greenbaum, Shirley, et al. "Ecological dynamics of the vaginal microbiome in relation to health and disease." *American Journal of Obstetrics and Gynecology* 220.4 (2019):

324-335.

Guo, Cuishan, and Chiyuan Zhang. "Role of The Gut Microbiota in The Pathogenesis of Endometriosis: A Review." Frontiers in Microbiology 15 (2024): 1363455.

Guo, Jingbo, et al. "Gut microbiota in patients with polycystic ovary syndrome: a systematic review." Reproductive Sciences (2022): 1-15.

Guo, Ran, et al. "Pain regulation by gut microbiota: molecular mechanisms and therapeutic potential." *British journal of anaesthesia* 123.5 (2019): 637-654.

Gupta, Akash, et al. "Combatting antibiotic-resistant bacteria using nanomaterials." Chemical Society Reviews 48.2 (2019): 415-427.

Hustoft, T. N., et al. "Effects of varying dietary content of fermentable short-chain carbohydrates on symptoms, fecal microenvironment, and cytokine profiles in patients with irritable bowel syndrome." Neurogastroenterology & Motility 29.4 (2017): e12969.

Jankovich, E., & Watkins, S. (2017). The low FODMAP diet reduced symptoms in a patient with endometriosis and IBS. *South African Journal of Clinical Nutrition*, *30*(4), 32-36. Retrieved from http://www.sajcn.co.za/index.php/SAJCN/article/view/1301

Joseph, Rebecca Jane, et al. "Finding a balance in the vaginal microbiome: how do we treat and prevent the occurrence of bacterial vaginosis?." *Antibiotics* 10.6 (2021): 719.

Kenyon, Chris, et al. "A dimorphic fungus causing disseminated infection in South Africa." *New England Journal of Medicine* 369.15 (2013): 1416-1424.

Krog, Maria Christine, et al. "The healthy female microbiome across body sites: effect of hormonal contraceptives and the menstrual cycle." Human Reproduction 37.7 (2022): 1525-1543.

Lehtoranta, Liisa, et al. "Healthy vaginal microbiota and influence of probiotics across the female life span." Frontiers in microbiology 13 (2022): 819958.

Kroon, Samuel J., Jacques Ravel, and Wilhelmina M. Huston. "Cervicovaginal microbiota, women's health, and reproductive outcomes." Fertility and sterility 110.3 (2018): 327-336.

Kwon, Myoung Seung, and Heung Kyu Lee. "Host and microbiome interplay shapes the vaginal microenvironment." Frontiers in immunology 13 (2022): 919728.

Lev-Sagie, A., Goldman-Wohl, D., Cohen, Y., Dori-Bachash, M., Leshem, A., Mor, U., ... & Elinav, E. (2019). Vaginal microbiome transplantation in women with intractable bacterial vaginosis. *Nature medicine*, *25*(10), 1500-1504.

López-Moreno, Ana, and Margarita Aguilera. "Vaginal probiotics for reproductive health and related dysbiosis: systematic review and meta-analysis." Journal of clinical medicine 10.7 (2021): 1461.

Mayo clinic, 2023, available on: https://www.mayoclinic.org/diseases-conditions/yeastinfection/symptoms-causes/syc-20378999

National Institute of Environmental Health Sciences (NIEH) 2024. available on: https://www.niehs.nih.gov/health/topics/conditions/repro-health

Ostadmohammadi, V., Jamilian, M., Bahmani, F., & Asemi, Z. (2019). Vitamin D and probiotic co-supplementation affects mental health, hormonal, inflammatory and oxidative stress parameters in women with polycystic ovary syndrome. *Journal of ovarian research*, *12*, 1-8.

Pendharkar, S., Brandsborg, E., Hammarström, L., Marcotte, H., & Larsson, P. G. (2015). Vaginal colonisation by probiotic lactobacilli and clinical outcome in women conventionally treated for bacterial vaginosis and yeast infection. *BMC infectious diseases*, *15*, 1-12.

Sadeghpour Heravi, Fatemah. "Host-vaginal microbiota interaction: shaping the vaginal microenvironment and bacterial vaginosis." *Current Clinical Microbiology Reports* (2024): 1-15.

Salliss, Mary E., et al. "The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain." Human reproduction update 28.1 (2022): 92-131.

Siddiqui, Ruqaiyyah, et al. "The gut microbiome and female health." Biology 11.11 (2022): 1683.

Sun, Yan, et al. "Gut microbiota dysbiosis in polycystic ovary syndrome: Mechanisms of progression and clinical applications." Frontiers in cellular and infection microbiology 13 (2023): 1142041.

Tomio, K., Kawana, K., Taguchi, A., Isobe, Y., Iwamoto, R., Yamashita, A., et al. (2013). Omega-3 polyunsaturated Fatty acids suppress the cystic lesion formation of peritoneal endometriosis in transgenic mouse models. PLoS One 8:e73085. doi: 10.1371/journal.pone.0073085

Uccello, M., Malaguarnera, G., Basile, F., D'agata, V., Malaguarnera, M., Bertino, G., et al. (2012). Potential role of probiotics on colorectal cancer prevention. BMC Surg. 12:S35. doi: 10.1186/1471-2482-12-S1-S35

Walther, Barbara, et al. "GutSelf: Interindividual variability in the processing of dietary compounds by the human gastrointestinal tract." *Molecular nutrition & food research* 63.21 (2019): 1900677.

Yue, Min, and Lei Zhang. "Exploring the Mechanistic Interplay between Gut Microbiota and Precocious Puberty: A Narrative Review." *Microorganisms* 12.2 (2024): 323.

Yurtdaş, Gamze, and Yasemin Akdevelioğlu. "A new approach to polycystic ovary syndrome: the gut microbiota." *Journal of the American College of Nutrition* 39.4 (2020): 371-382.

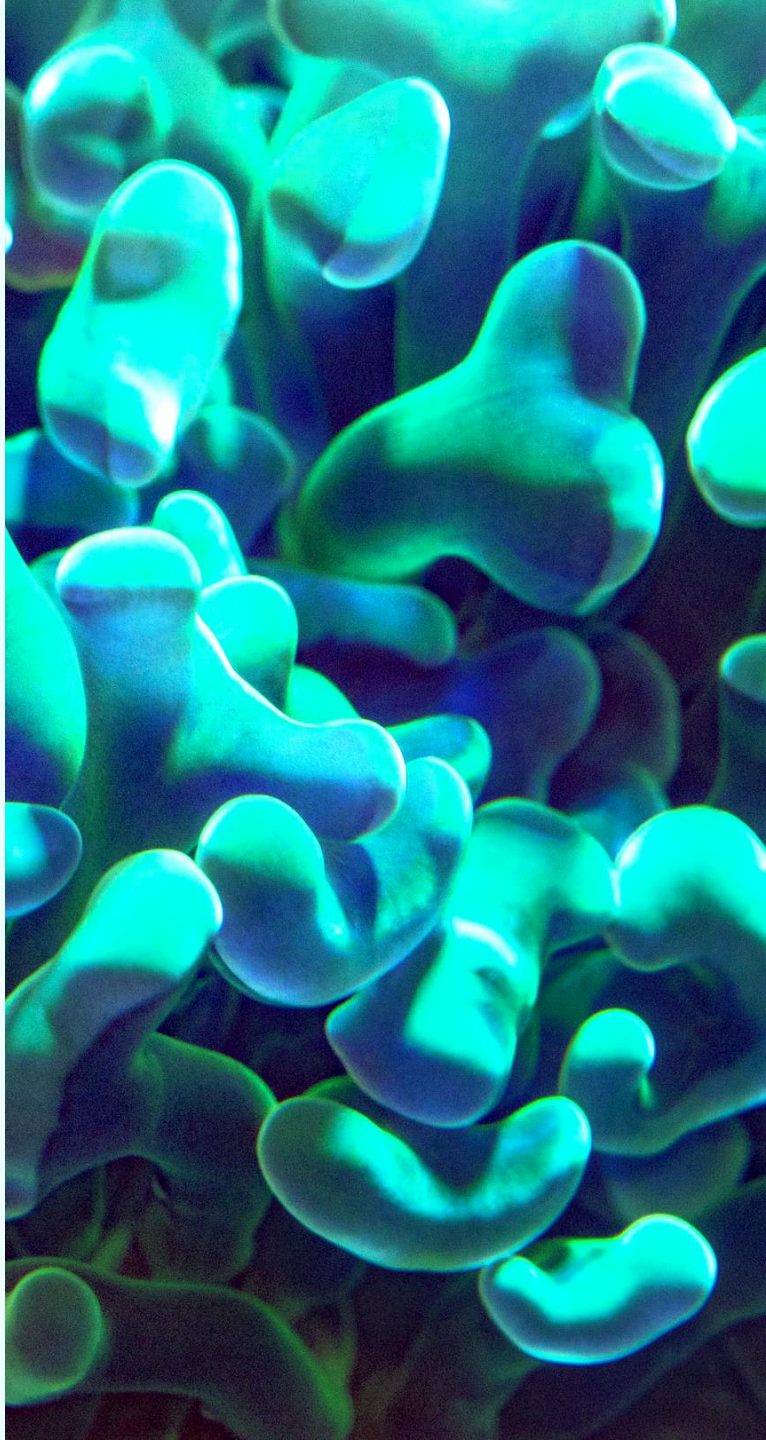
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Post-graduate certificate level 7 (30 ECTS)

Microbiome/Nutrition and Insulin Resistance & Type 2 Diabetes

Dr. Zeki Çağın Onbaşı
MSc & PhD in Nutrition and Dietetics

lecturer@imas.edu.mt



Malta Further & Higher Education Authority

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

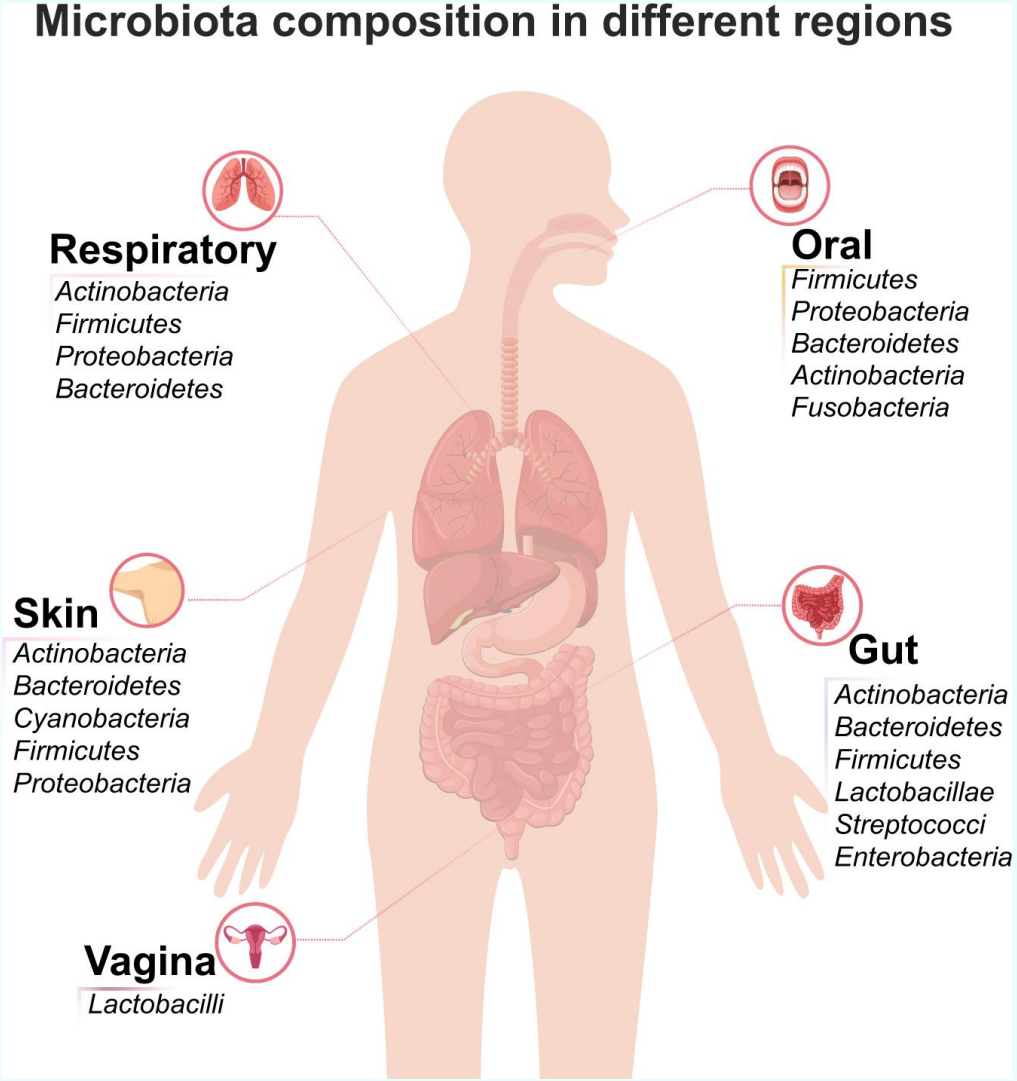
INDEX

✓ DEFINING OF MICROBIOTA
✓ CONCEPTS OF INSULINE RESISTANCE AND DIABETES
✓ RELATIONSHIP & INTERACTIONS BETWEEN MICROBIOTA INSULINE RESISTANCE AND TYPE 2 DIABETES
✓ MICROBIAL CHANGES IN PRE-DIABETIC PHASE AND INSULINE RESISTANCE
✓ INTESTINAL PERMABILITY
✓ POTENTIAL IMPLICATIONS FOR MANAGEMET AND TREATMENT

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

MICROBIOTA

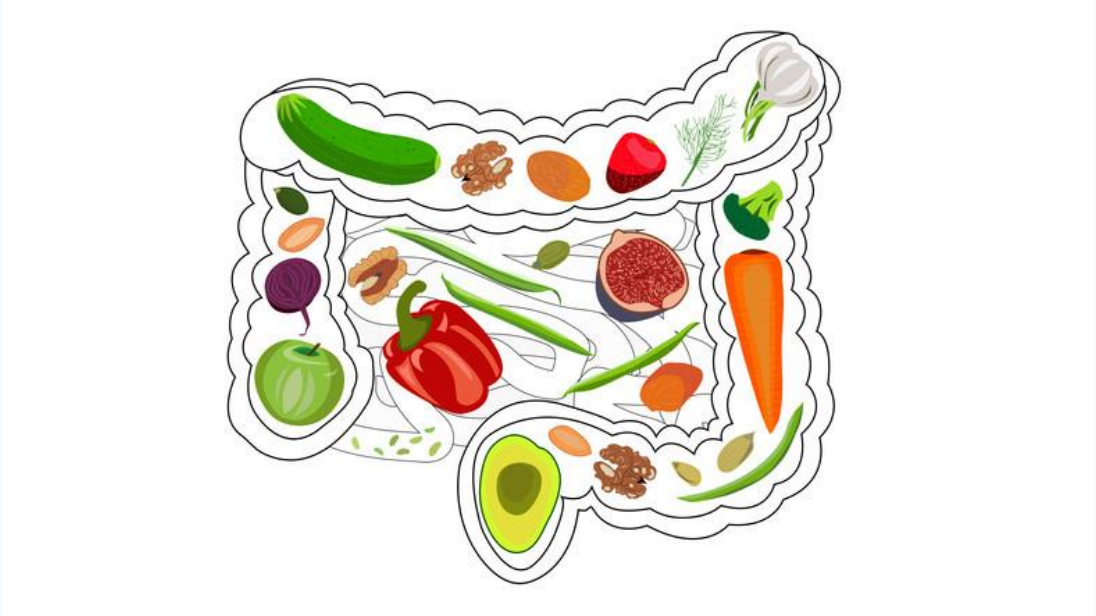
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



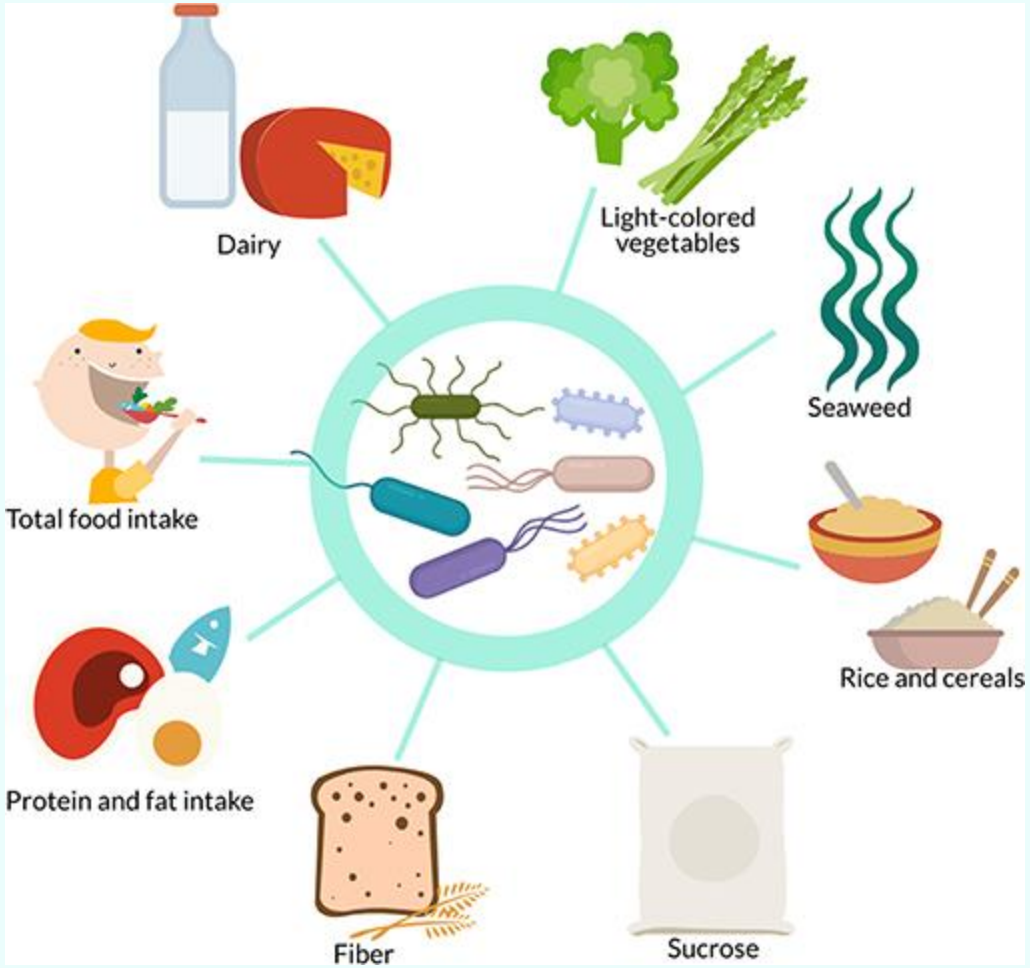
Hou, K., Wu, Z. X., Chen, X. Y., Wang, J. Q., Zhang, D., Xiao, C., ... & Chen, Z. S. (2022). Microbiota in health and diseases. Signal transduction and targeted therapy, 7(1), 1-28.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

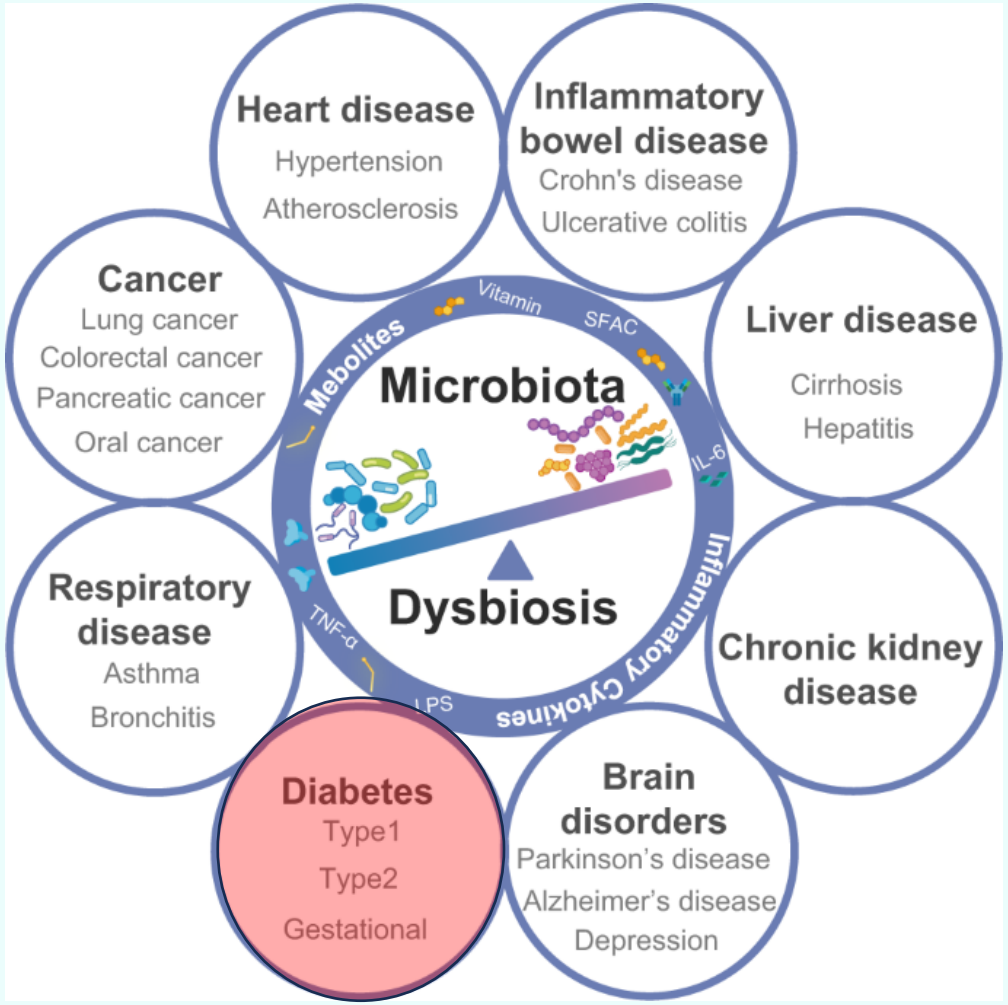
Microbiota and Nutrition



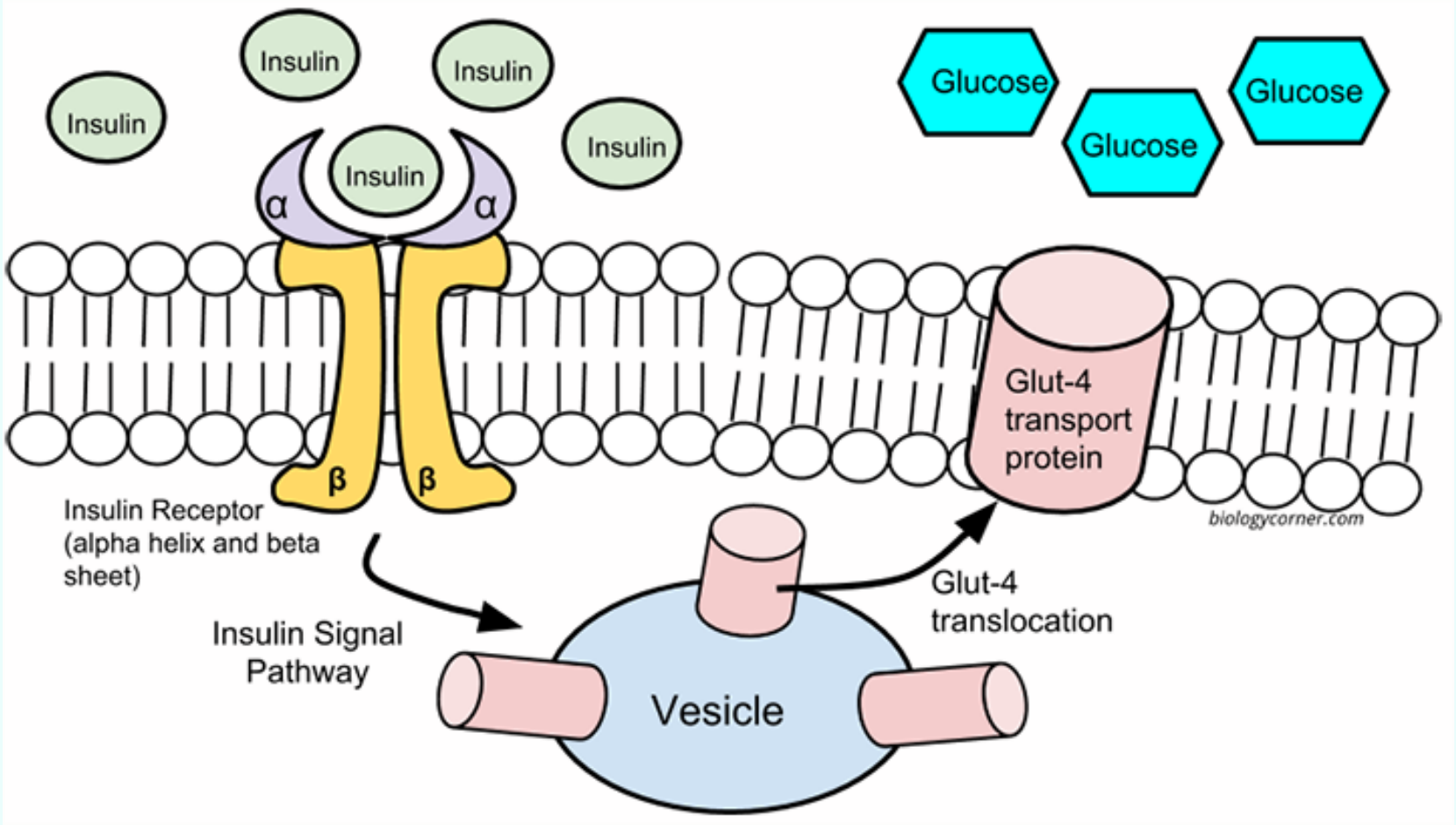
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



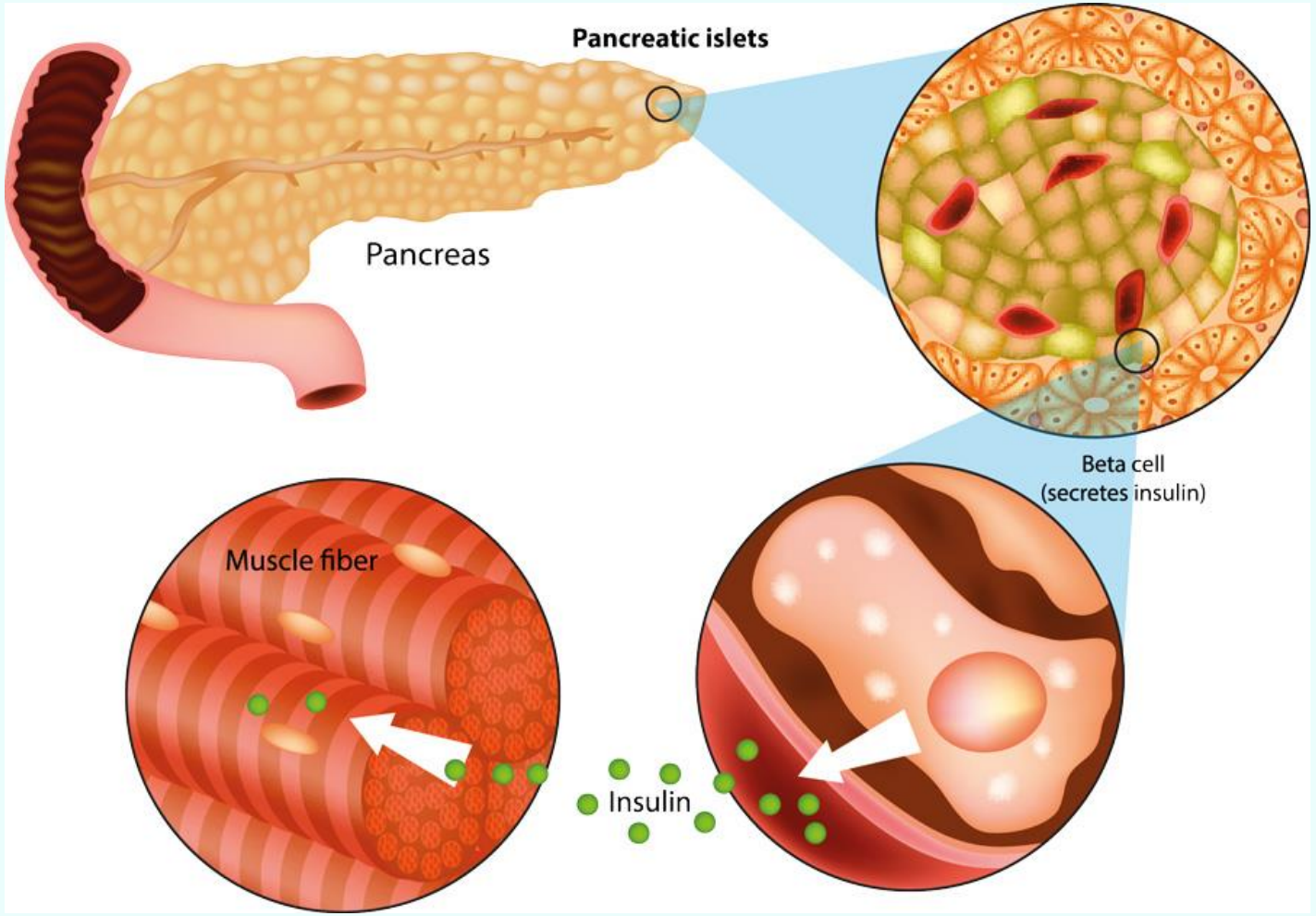
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



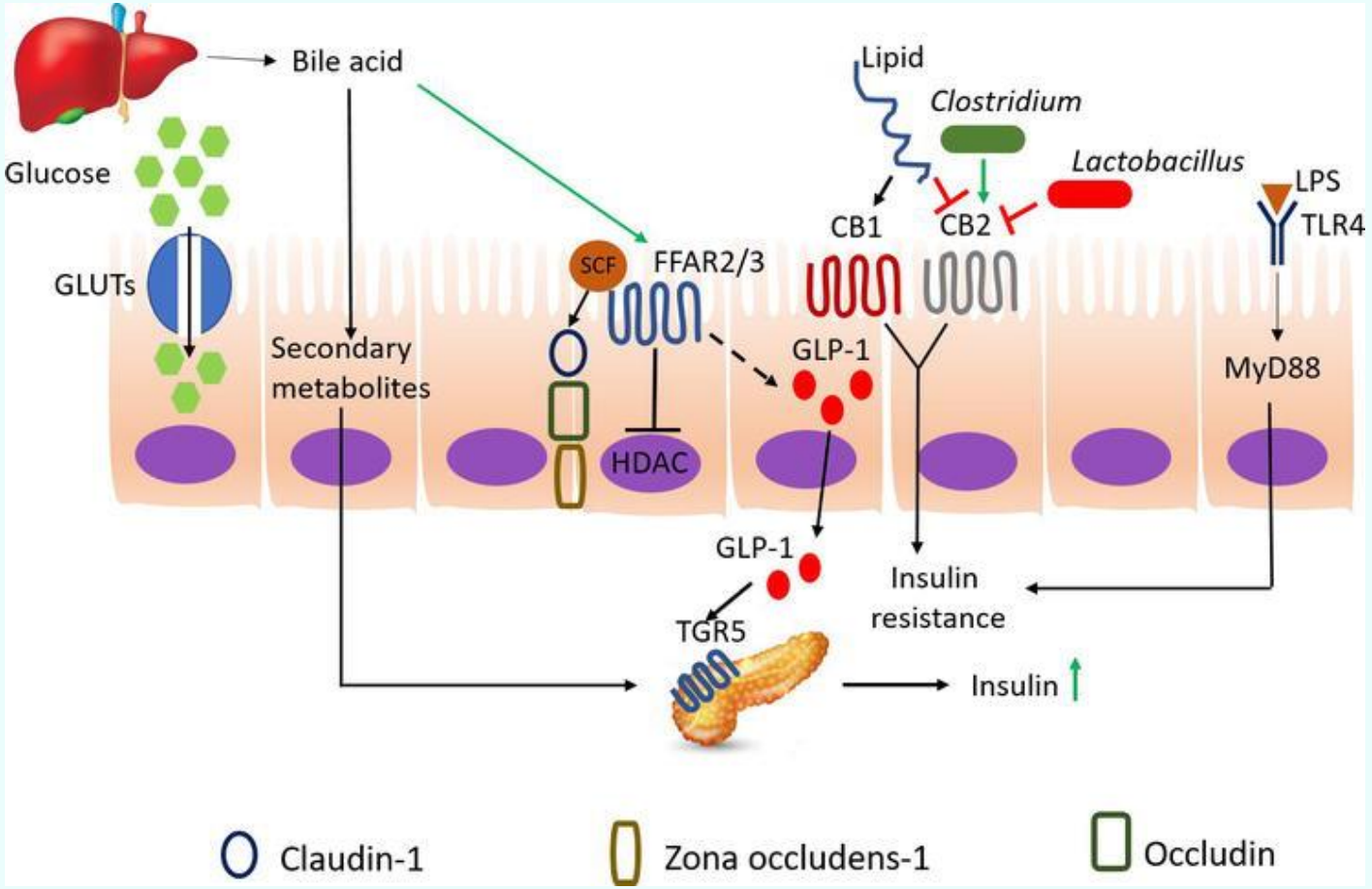
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiota and Insulin Resistance/Type 2 Diabetes

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Mukhuty, A., Fouzder, C., Das, S., & Chattopadhyay, D. (2021). An insight into the changing scenario of gut microbiome during type 2 diabetes. Parasitology and Microbiology Research.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

- Increase in endotoxemia
- Changes in incretin secretion related to insulin resistance and beta cell function
- Changes in butyrate production
- Changes in characteristics of brown adipose tissue
- Impact of secondary bile acids
- The role of choline and niacin as vitamins.
- Impact of drug treatment.
- Changes in gut microbiota

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiota Pattern of Individuals with Type 2 Diabetes / Insuline Resistance

Disease / Altered genus of bacteria	Type 2 diabetes	Nonalcoholic fatty liver disease	Angiocardiopathy	Chronic Nephropathy	Reference
Firmicutes	↑ Streptococcus mutans	↓ Ruminococcus obeum	↑ Ruminococcus gnavus	↑ Ruminococcus spp	[24],[39],[53],[72]
	↑ ↓ Clostridium spp.	-	↑ Streptococcus spp	↓ Clostridium spp.	[21],[53],[72]
	↓ Roseburia spp	↓ Roseburia spp	↓ Roseburia intestinalis	↓ Roseburia spp	[21],[39],[53],[72]
	↓ Eubacterium rectale	↑ ↓ Eubacterium rectale	-	↓ Eubacterium rectale	[21],[39],[72]
	↓ Faecalibacterium prausnitzii	↓ Faecalibacterium prausnitzii	↓ Faecalibacterium cf. prausnitzii	↓ Faecalibacterium prausnitzii	[21],[39],[53],[72]
	↑ ↓ Clostridiales	-	-	↑ Flavonifractor plautii	[21],[24],[72]
	↑ Lactobacillus gasseri	↑ Lactobacillus		-	[24],[37]
Bacteroidales	↑ Bacteroides spp.	↑ Bacteroides vulgatus	↓ Bacteroides spp	-	[21],[39],[53]
	↑ Alistipes	-	↓ Alistipes shahii	↑ Alistipes spp	[21],[53],[72]
	↑ Parabacteroides	-	↓ Prevotella copri	↓ Prevotella copri	[21],[53],[72]
Proteobacteria	↑ Escherichia coli	↑ Escherichia coli	↑ Escherichia coli	-	[21],[39],[53]
	-	-	↑ Enterobacter aerogenes	-	[53]
Verrucomicrobia	↑ ↓ Akkermansia muciniphila	-	-	-	[21]
Fusobacteria	-	-	-	↑ Fusobacterium spp	[72]
Actinobacteria	↑ Eggerthella lenta	-	↑ Eggerthella lenta	↑ Eggerthella lenta	[21],[53],[72]
	-	↑ Klebsiella pneumoniae	↑ Klebsiella spp.	-	[42],[53]
Corresponding changes in microbial functions	Branched-chain amino acid transport, butyrate metabolism	Amino acid metabolism, butyrate metabolism	Simple sugars and amino acids transport, butyrate metabolism, O-antigen of LPS synthesis	Aromatic amino acids degradation, secondary bile acids biosynthesis, butyrate metabolism	[21],[39],[53],[72]

Yang, G., Wei, J., Liu, P., Zhang, Q., Tian, Y., Hou, G., ... & Jiang, X. (2021). Role of the gut microbiota in type 2 diabetes and related diseases. Metabolism, 117, 154712.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Pre-Diabetic Phase

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

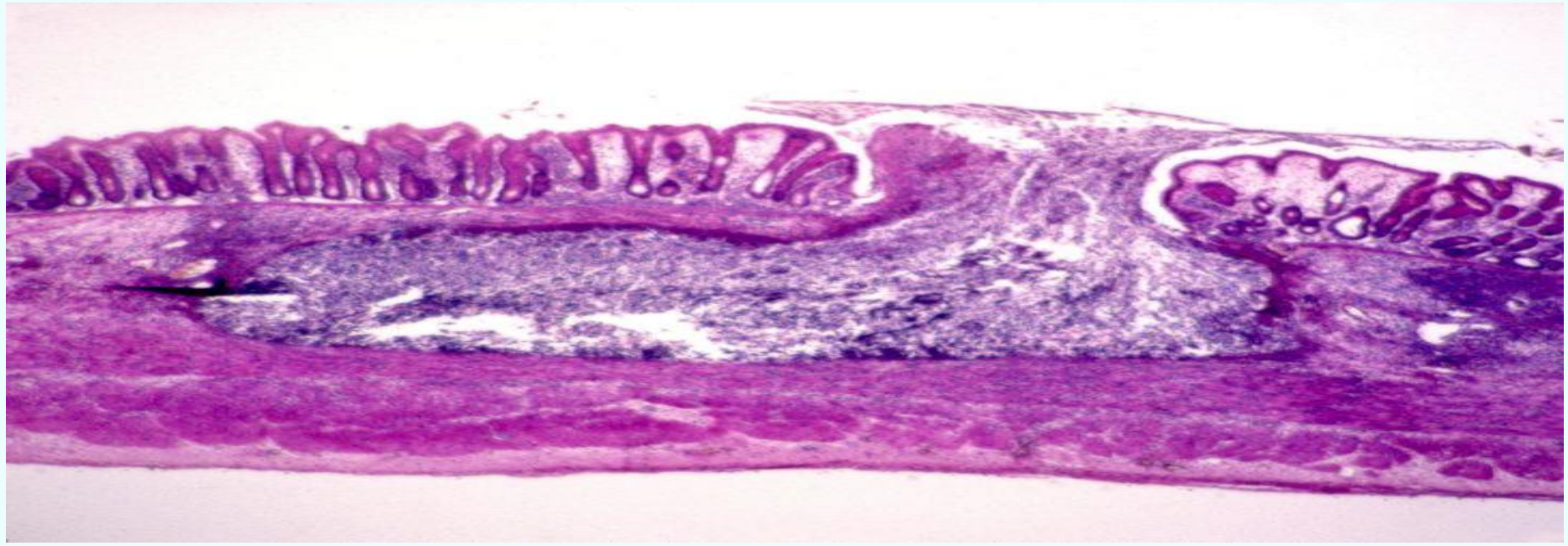
Pre-Diabetic Phase

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

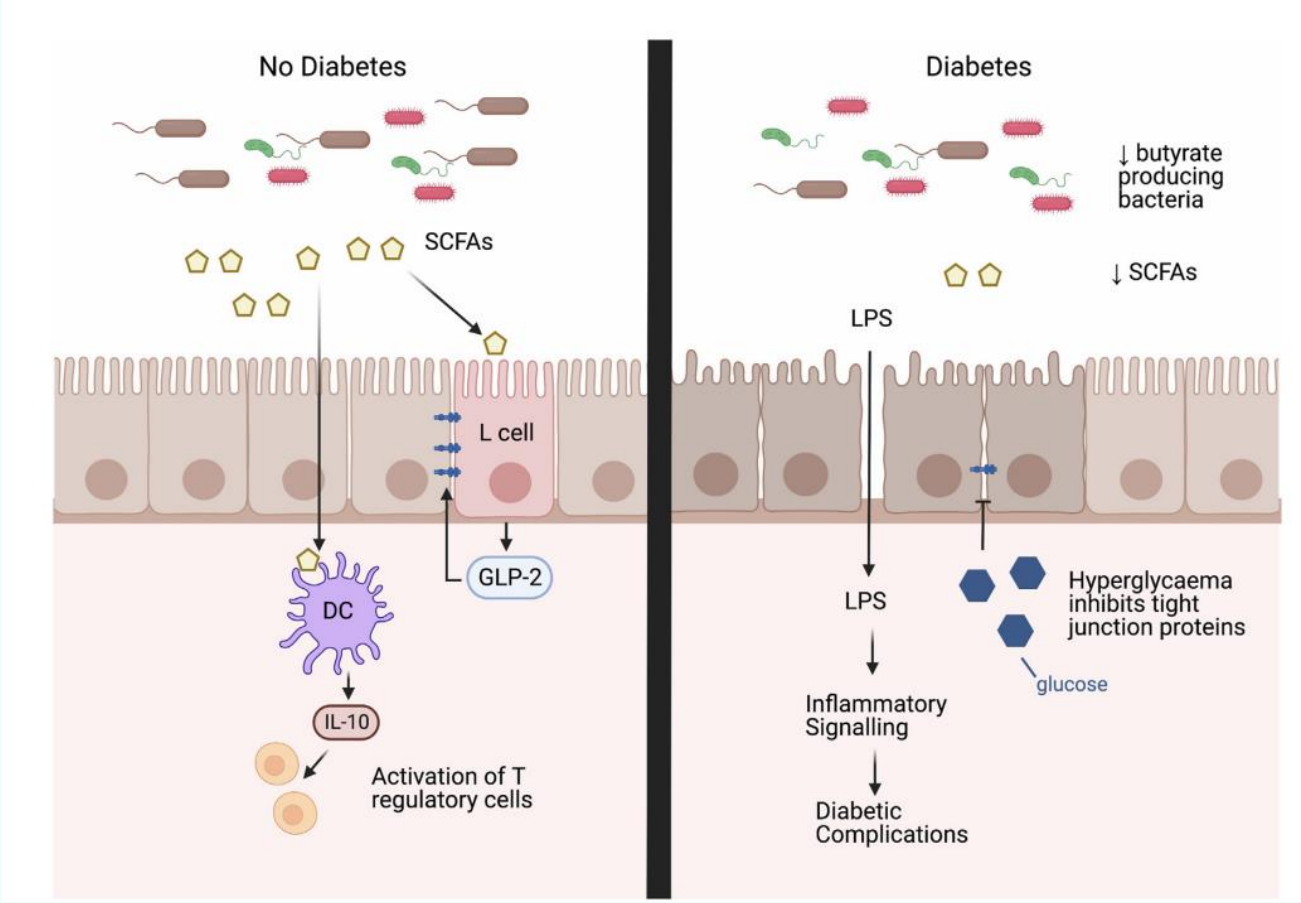
Insulin Resistance

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Intestinal Permability



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

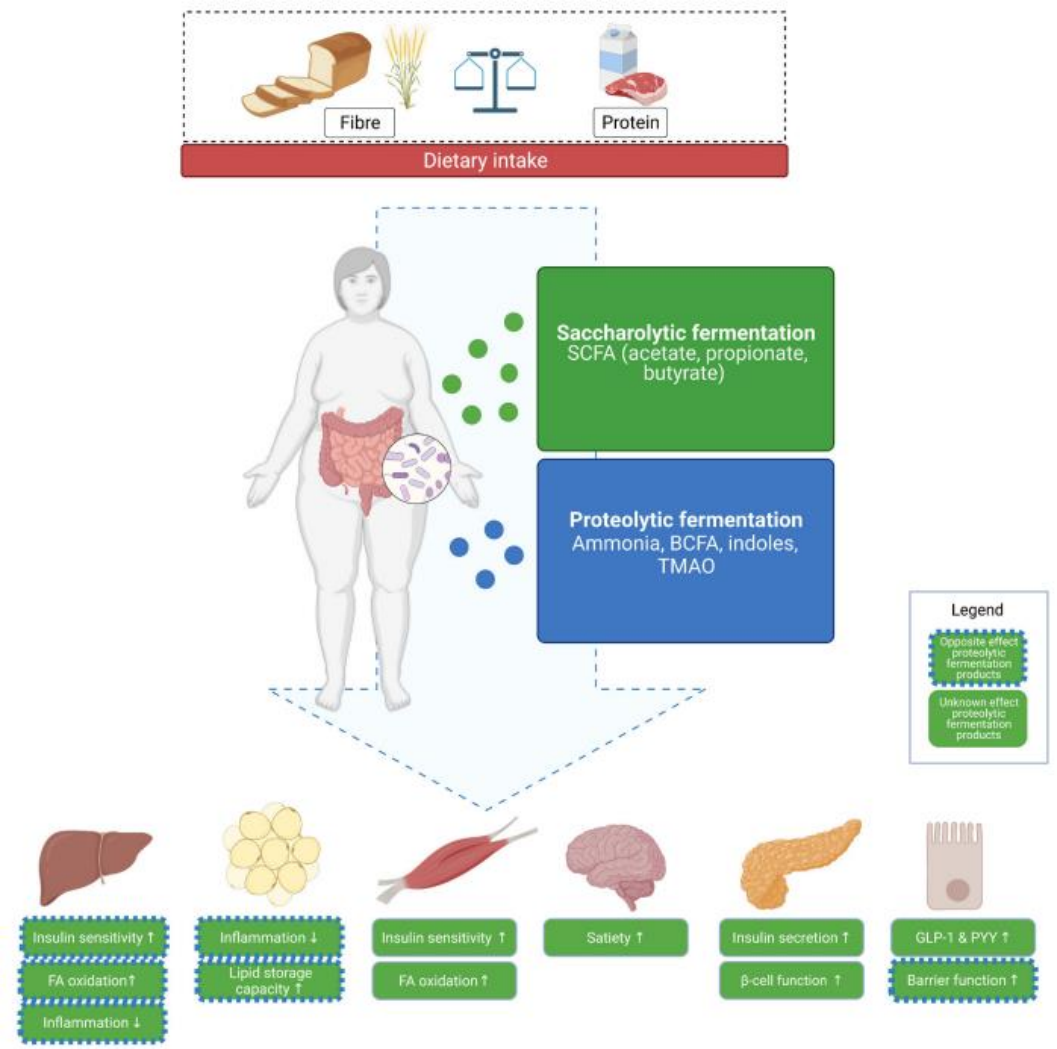


M. Snelson, C. de Pasquale, E.I. Ekinci et al, Best Practice & Research Clinical Endocrinology & Metabolism 35 (2021) 101507

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Effect of Nutrition and Probiotics on Improving Microbial Dysbiosis in Type 2 Diabetes/Insulin Resistance

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case Reports

Diet	Study design	Functional outcomes	Gut microbiota composition and functionality	Individuals
Dietary protein				
High protein/moderate CHO (HPMC) and HP/low CHO (HPLC)	28-day, randomised, cross-over design	HPMC and HPLC: increased faecal branched-chain fatty acids and concentrations of phenylacetic acid and N-nitroso compounds	HPMC: no significant changes HPLC: ↓ *Roseburia*, *Eubacterium rectale*, *Bacteroides* spp; no changes in *Faecalibacterium prausnitzii*	17 men with obesity (BMI: 30.0–48.5 kg/m^2)
LC/higher protein isocaloric	14 weeks, parallel design	Liver fat ↓ Hepatic lipid metabolism↑ Inflammation ↓	Rapid microbial alterations: *Streptococcus*, *Lactococcus* and *Eggerthella*↑ *Ruminococcus*, *Eubacterium*, *Clostridium* and *Bifidobacterium*↓	10 adults with obesity/NAFLD (BMI: 27–40 kg/m^2)
Dietary fibre and carbohydrates				
Barley kernel bread (37.6 g fibre/day)	3 days, randomised cross-over design (vs white bread reference 9.1 g fibre/day)	Increased plasma GLP-1, PYY, breath H_2 excretion, fasting serum SCFAs, improved insulin sensitivity index ($p<0.05$)	Increased *Prevotella*/*Bacteroides* ratio in responders versus non-responders (post hoc analysis)	39 healthy adults (BMI: 18–28 kg/m^2)
16 g oligofructose (8 g two times) per day	2 weeks, placebo-controlled (16 g dextrin maltose), parallel design	Reduced 2-hour postprandial glucose AUC ($p<0.05$) Increased plasma GLP-1 and PYY levels and increased breath–hydrogen excretion	Not determined	10 healthy adults (BMI mean±SD: 21.6±0.99 kg/m^2)
16 g FOS or GOS per day	2 weeks, cross-over design	Increased fasting glucose	*Bifidobacterium* ↑, FOS: butyrate-producing *Phascolarctobacterium* ↑, GOS: *Ruminococcus* ↓	35 healthy adults (BMI <18 kg/m^2)
RS versus NSP (resp. 16 g NSP, 25 g RS vs 42 g NSP, 2.5 g RS)	3 weeks, randomised cross-over design	Inverse relationship gut microbiota diversity–dietary responsiveness	↑ Ruminococcaceae and ↑ Lachnospiraceae	14 men with MetS (BMI: 27.9–51.3 kg/m^2)
30 g RS (10 g three times) per day	4 weeks, placebo controlled (20 g digestible starch), two-way crossover	Improved whole-body insulin sensitivity (euglycaemic–hyperinsulinaemic clamp) ($p<0.05$).	Not determined	10 healthy adults (BMI: 18.4–32.3 kg/m^2
16 g inulin/oligofructose mix (8 g two times) per day	12 weeks, parallel design, placebo controlled (16 g maltodextrin)	Reduced post-OGTT glucose response (7%, $p<0.01$); no effects on HOMA, fasting glucose and insulin and HbA1c	↑*Bifidobacterium* and *F. prausnitzii*, ↓ *Bacteroides intestinalis*, *B. vulgatus* and *Propionibacterium*	30 women with obesity (BMI, >30 kg/m^2)
15 g GOS (5 g, three times) per day	12 weeks, placebo-controlled (isocaloric maltodextrin), parallel design	No effects on glucose or insulin homeostasis, no increases in fasting SCFA plasma concentrations or faecal SCFAs	Fivefold increase in faecal *Bifidobacterium* ($p=0.009$, $q=0.144$); no effect microbial richness or diversity	44 adults with prediabetes andoverweight/obesity (BMI: 25–35 kg/m^2)
5.5 g GOS mixture 1×/day	12 weeks, placebo-controlled (5.5 g maltodextrin), two-way crossover	Decreased fasting insulin ($p<0.01$), triglyceride and C reactive protein plasma concentrations	Increased *Bifidobacterium* ($p<0.0001$)	45 adults with overweight/obesity (BMI >25 kg/m^2)
Dietary patterns				
Animal-based diet (very low fibre) versus plant-based diet (high fibre)	5 days, cross-over design	Altered bile acid metabolism, plant polysaccharide fermentation ↓	*Alistipes*, *Bilophila* and *Bacteroides* ↑ *Roseburia*, *E. rectale* and *Ruminococcus bromii* ↓	10 healthy, lean and overweight adults (BMI: 19–32 kg/m^2)
Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion	1 year	BMI, fasting glucose, glycated haemoglobin and triglycerides ↓, high-density cholesterol ↑	*Butyricicoccus*, *Haemophilus*, *Ruminiclostridium 5* and *E. hallii* ↓	400 adults with MetS (BMI ≥27 and ≤40 kg/m^2)

Jardon, K. M., Canfora, E. E., Goossens, G. H., & Blaak, E. E. (2022). Dietary macronutrients and the gut microbiome: a precision nutrition approach to improve cardiometabolic health. *Gut*, *71*(6), 1214-1226.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case Reports

Diet	Study design	Functional outcomes	Gut microbiota composition and functionality	Individuals
Dietary protein				
High protein/moderate CHO (HPMC) and HP/low CHO (HPLC)	28-day, randomised, cross-over design	HPMC and HPLC: increased faecal branched-chain fatty acids and concentrations of phenylacetic acid and N-nitroso compounds	HPMC: no significant changes HPLC: ↓ *Roseburia*, *Eubacterium rectale*, *Bacteroides* spp; no changes in *Faecalibacterium prausnitzii*	17 men with obesity (BMI: 30.0–48.5 kg/m^2)
LC/higher protein isocaloric	14 weeks, parallel design	Liver fat ↓ Hepatic lipid metabolism↑ Inflammation ↓	Rapid microbial alterations: *Streptococcus*, *Lactococcus* and *Eggerthella*↑ *Ruminococcus*, *Eubacterium*, *Clostridium* and *Bifidobacterium*↓	10 adults with obesity/NAFLD (BMI: 27–40 kg/m^2)
Dietary fibre and carbohydrates				
Barley kernel bread (37.6 g fibre/day)	3 days, randomised cross-over design (vs white bread reference 9.1 g fibre/day)	Increased plasma GLP-1, PYY, breath H_2 excretion, fasting serum SCFAs, improved insulin sensitivity index ($p<0.05$)	Increased *Prevotella*/*Bacteroides* ratio in responders versus non-responders (post hoc analysis)	39 healthy adults (BMI: 18–28 kg/m^2)
16 g oligofructose (8 g two times) per day	2 weeks, placebo-controlled (16 g dextrin maltose), parallel design	Reduced 2-hour postprandial glucose AUC ($p<0.05$) Increased plasma GLP-1 and PYY levels and increased breath–hydrogen excretion	Not determined	10 healthy adults (BMI mean±SD: 21.6±0.99 kg/m^2)
16 g FOS or GOS per day	2 weeks, cross-over design	Increased fasting glucose	*Bifidobacterium* ↑, FOS: butyrate-producing *Phascolarctobacterium* ↑, GOS: *Ruminococcus* ↓	35 healthy adults (BMI <18 kg/m^2)
RS versus NSP (resp. 16 g NSP, 25 g RS vs 42 g NSP, 2.5 g RS)	3 weeks, randomised cross-over design	Inverse relationship gut microbiota diversity–dietary responsiveness	↑ Ruminococcaceae and ↑ Lachnospiraceae	14 men with MetS (BMI: 27.9–51.3 kg/m^2)
30 g RS (10 g three times) per day	4 weeks, placebo controlled (20 g digestible starch), two-way crossover	Improved whole-body insulin sensitivity (euglycaemic–hyperinsulinaemic clamp) ($p<0.05$).	Not determined	10 healthy adults (BMI: 18.4–32.3 kg/m^2
16 g inulin/oligofructose mix (8 g two times) per day	12 weeks, parallel design, placebo controlled (16 g maltodextrin)	Reduced post-OGTT glucose response (7%, $p<0.01$); no effects on HOMA, fasting glucose and insulin and HbA1c	↑*Bifidobacterium* and *F. prausnitzii*, ↓ *Bacteroides intestinalis*, *B. vulgatus* and *Propionibacterium*	30 women with obesity (BMI, >30 kg/m^2)
15 g GOS (5 g, three times) per day	12 weeks, placebo-controlled (isocaloric maltodextrin), parallel design	No effects on glucose or insulin homeostasis, no increases in fasting SCFA plasma concentrations or faecal SCFAs	Fivefold increase in faecal *Bifidobacterium* ($p=0.009$, $q=0.144$); no effect microbial richness or diversity	44 adults with prediabetes andoverweight/obesity (BMI: 25–35 kg/m^2)
5.5 g GOS mixture 1×/day	12 weeks, placebo-controlled (5.5 g maltodextrin), two-way crossover	Decreased fasting insulin ($p<0.01$), triglyceride and C reactive protein plasma concentrations	Increased *Bifidobacterium* ($p<0.0001$)	45 adults with overweight/obesity (BMI >25 kg/m^2)
Dietary patterns				
Animal-based diet (very low fibre) versus plant-based diet (high fibre)	5 days, cross-over design	Altered bile acid metabolism, plant polysaccharide fermentation ↓	*Alistipes*, *Bilophila* and *Bacteroides* ↑ *Roseburia*, *E. rectale* and *Ruminococcus bromii* ↓	10 healthy, lean and overweight adults (BMI: 19–32 kg/m^2)
Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion	1 year	BMI, fasting glucose, glycated haemoglobin and triglycerides ↓, high-density cholesterol ↑	*Butyricicoccus*, *Haemophilus*, *Ruminiclostridium 5* and *E. hallii* ↓	400 adults with MetS (BMI ≥27 and ≤40 kg/m^2)

Jardon, K. M., Canfora, E. E., Goossens, G. H., & Blaak, E. E. (2022). Dietary macronutrients and the gut microbiome: a precision nutrition approach to improve cardiometabolic health. *Gut*, *71*(6), 1214-1226.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Case Reports

Diet	Study design	Functional outcomes	Gut microbiota composition and functionality	Individuals
Dietary protein				
High protein/moderate CHO (HPMC) and HP/low CHO (HPLC)	28-day, randomised, cross-over design	HPMC and HPLC: increased faecal branched-chain fatty acids and concentrations of phenylacetic acid and N-nitroso compounds	HPMC: no significant changes HPLC: ↓ *Roseburia*, *Eubacterium rectale*, *Bacteroides* spp; no changes in *Faecalibacterium prausnitzii*	17 men with obesity (BMI: 30.0–48.5 kg/m^2)
LC/higher protein isocaloric	14 weeks, parallel design	Liver fat ↓ Hepatic lipid metabolism↑ Inflammation ↓	Rapid microbial alterations: *Streptococcus*, *Lactococcus* and *Eggerthella*↑ *Ruminococcus*, *Eubacterium*, *Clostridium* and *Bifidobacterium*↓	10 adults with obesity/NAFLD (BMI: 27–40 kg/m^2)
Dietary fibre and carbohydrates				
Barley kernel bread (37.6 g fibre/day)	3 days, randomised cross-over design (vs white bread reference 9.1 g fibre/day)	Increased plasma GLP-1, PYY, breath H_2 excretion, fasting serum SCFAs, improved insulin sensitivity index ($p<0.05$)	Increased *Prevotella*/*Bacteroides* ratio in responders versus non-responders (post hoc analysis)	39 healthy adults (BMI: 18–28 kg/m^2)
16 g oligofructose (8 g two times) per day	2 weeks, placebo-controlled (16 g dextrin maltose), parallel design	Reduced 2-hour postprandial glucose AUC ($p<0.05$) Increased plasma GLP-1 and PYY levels and increased breath–hydrogen excretion	Not determined	10 healthy adults (BMI mean±SD: 21.6±0.99 kg/m^2)
16 g FOS or GOS per day	2 weeks, cross-over design	Increased fasting glucose	*Bifidobacterium* ↑, FOS: butyrate-producing *Phascolarctobacterium* ↑, GOS: *Ruminococcus* ↓	35 healthy adults (BMI <18 kg/m^2)
RS versus NSP (resp. 16 g NSP, 25 g RS vs 42 g NSP, 2.5 g RS)	3 weeks, randomised cross-over design	Inverse relationship gut microbiota diversity–dietary responsiveness	↑ Ruminococcaceae and ↑ Lachnospiraceae	14 men with MetS (BMI: 27.9–51.3 kg/m^2)
30 g RS (10 g three times) per day	4 weeks, placebo controlled (20 g digestible starch), two-way crossover	Improved whole-body insulin sensitivity (euglycaemic–hyperinsulinaemic clamp) ($p<0.05$).	Not determined	10 healthy adults (BMI: 18.4–32.3 kg/m^2
16 g inulin/oligofructose mix (8 g two times) per day	12 weeks, parallel design, placebo controlled (16 g maltodextrin)	Reduced post-OGTT glucose response (7%, $p<0.01$); no effects on HOMA, fasting glucose and insulin and HbA1c	↑*Bifidobacterium* and *F. prausnitzii*, ↓ *Bacteroides intestinalis*, *B. vulgatus* and *Propionibacterium*	30 women with obesity (BMI, >30 kg/m^2)
15 g GOS (5 g, three times) per day	12 weeks, placebo-controlled (isocaloric maltodextrin), parallel design	No effects on glucose or insulin homeostasis, no increases in fasting SCFA plasma concentrations or faecal SCFAs	Fivefold increase in faecal *Bifidobacterium* ($p=0.009$, $q=0.144$); no effect microbial richness or diversity	44 adults with prediabetes andoverweight/obesity (BMI: 25–35 kg/m^2)
5.5 g GOS mixture 1×/day	12 weeks, placebo-controlled (5.5 g maltodextrin), two-way crossover	Decreased fasting insulin ($p<0.01$), triglyceride and C reactive protein plasma concentrations	Increased *Bifidobacterium* ($p<0.0001$)	45 adults with overweight/obesity (BMI >25 kg/m^2)
Dietary patterns				
Animal-based diet (very low fibre) versus plant-based diet (high fibre)	5 days, cross-over design	Altered bile acid metabolism, plant polysaccharide fermentation ↓	*Alistipes*, *Bilophila* and *Bacteroides* ↑ *Roseburia*, *E. rectale* and *Ruminococcus bromii* ↓	10 healthy, lean and overweight adults (BMI: 19–32 kg/m^2)
Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion	1 year	BMI, fasting glucose, glycated haemoglobin and triglycerides ↓, high-density cholesterol ↑	*Butyricicoccus*, *Haemophilus*, *Ruminiclostridium 5* and *E. hallii* ↓	400 adults with MetS (BMI ≥27 and ≤40 kg/m^2)

Jardon, K. M., Canfora, E. E., Goossens, G. H., & Blaak, E. E. (2022). Dietary macronutrients and the gut microbiome: a precision nutrition approach to improve cardiometabolic health. *Gut*, *71*(6), 1214-1226.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Effects of Probiotic and Synbiotic Supplementation Among Diabetic Patients

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

ELSEVIER
Progress in Neuro-Psychopharmacology and Biological Psychiatry
Volume 84, Part A, 8 June 2018, Pages 50-55

The effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic status in type 2 diabetic patients with coronary heart disease: A randomized, double-blind, placebo-controlled trial

Fariba Raygan [a], Vahidreza Ostadmohammadi [b c], Fereshteh Bahmani [b], Zatollah Asemi [b 1]

Raygan, F., Ostadmohammadi, V., Bahmani, F., & Asemi, Z. (2018). The effects of vitamin D and probiotic co-supplementation on mental health parameters and metabolic status in type 2 diabetic patients with coronary heart disease: a randomized, double-blind, placebo-controlled trial. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 84, 50-55.

Diabetes/Metabolism Research and Reviews

RESEARCH ARTICLE

The beneficial effects of probiotic administration on wound healing and metabolic status in patients with diabetic foot ulcer: A randomized, double-blind, placebo-controlled trial

Sima Mohseni, Masomeh Bayani, Fereshteh Bahmani, Maryam Tajabadi-Ebrahimi, Mohammad Ali Bayani, Parvaneh Jafari, Zatollah Asemi

First published: 28 November 2017 | https://doi.org/10.1002/dmrr.2970 | Citations: 85

Mohseni, S., Bayani, M., Bahmani, F., Tajabadi-Ebrahimi, M., Bayani, M. A., Jafari, P., & Asemi, Z. (2018). The beneficial effects of probiotic administration on wound healing and metabolic status in patients with diabetic foot ulcer: a randomized, double-blind, placebo-controlled trial. Diabetes/Metabolism Research and Reviews, 34(3), e2970.

Probiotics & Antimicro. Prot. (2018) 10:496–503
DOI 10.1007/s12602-017-9313-7

CrossMark

The Effects of Synbiotic Supplementation on Pregnancy Outcomes in Gestational Diabetes

Maryam Karamali[1] · Nabiollah Nasiri[1] · Naemeh Taghavi Shavazi[1] · Mehri Jamilian[2] · Fereshteh Bahmani[3] · Maryam Tajabadi-Ebrahimi[4] · Zatollah Asemi[3]

Karamali, M., Nasiri, N., Taghavi Shavazi, N., Jamilian, M., Bahmani, F., Tajabadi-Ebrahimi, M., & Asemi, Z. (2018). The effects of synbiotic supplementation on pregnancy outcomes in gestational diabetes. Probiotics and antimicrobial proteins, 10, 496-503.

RESEARCH ARTICLES | SEPTEMBER 04 2014

Effects of Daily Consumption of Synbiotic Bread on Insulin Metabolism and Serum High-Sensitivity C-Reactive Protein among Diabetic Patients: A Double-Blind, Randomized, Controlled Clinical Trial

Topic Article Package: Topic Article Package: Diabetes

Subject Area: Endocrinology , Further Areas , Nutrition and Dietetics , Public Health

Maryam Tajadadi-Ebrahimi; Fereashteh Bahmani; Hossein Shakeri; Haleh Hadaegh; Mohammad Hijijafari; Fatemeh Abedi; Zatollah Asemi

Ann Nutr Metab (2014) 65 (1): 34–41.
https://doi.org/10.1159/000365153 Article history
PubMed:25196301

Tajadadi-Ebrahimi, M., Bahmani, F., Shakeri, H., Hadaegh, H., Hijijafari, M., Abedi, F., & Asemi, Z. (2014). Effects of daily consumption of synbiotic bread on insulin metabolism and serum high-sensitivity C-reactive protein among diabetic patients: a double-blind, randomized, controlled clinical trial. Annals of Nutrition and Metabolism, 65(1), 34-41.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Original Article

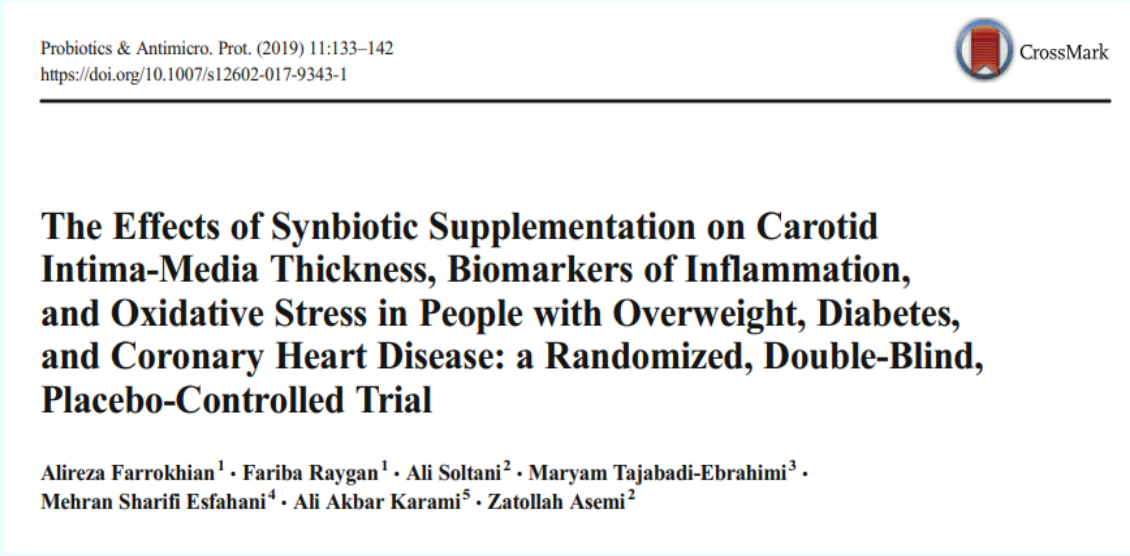
The effects of probiotic supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes

Bita Badehnoosh, Maryam Karamali, Mitra Zarrati, Mehri Jamilian, Fereshteh Bahmani, Maryam Tajabadi-Ebrahimi, ...show all

Pages 1128-1136 | Received 01 Dec 2016, Accepted 20 Mar 2017, Published online: 10 Apr 2017

Cite this article https://doi.org/10.1080/14767058.2017.1310193 Check for updates

Badehnoosh, B., Karamali, M., Zarrati, M., Jamilian, M., Bahmani, F., Tajabadi-Ebrahimi, M., ... & Asemi, Z. (2018). The effects of probiotic supplementation on biomarkers of inflammation, oxidative stress and pregnancy outcomes in gestational diabetes. The Journal of Maternal-Fetal & Neonatal Medicine, 31(9), 1128-1136.



Probiotics & Antimicro. Prot. (2019) 11:133–142
https://doi.org/10.1007/s12602-017-9343-1

CrossMark

The Effects of Synbiotic Supplementation on Carotid Intima-Media Thickness, Biomarkers of Inflammation, and Oxidative Stress in People with Overweight, Diabetes, and Coronary Heart Disease: a Randomized, Double-Blind, Placebo-Controlled Trial

Alireza Farrokhian[1] · Fariba Raygan[1] · Ali Soltani[2] · Maryam Tajabadi-Ebrahimi[3] · Mehran Sharifi Esfahani[4] · Ali Akbar Karami[5] · Zatollah Asemi[2]

Farrokhian, A., Raygan, F., Soltani, A., Tajabadi-Ebrahimi, M., Sharifi Esfahani, M., Karami, A. A., & Asemi, Z. (2019). The effects of synbiotic supplementation on carotid intima-media thickness, biomarkers of inflammation, and oxidative stress in people with overweight, diabetes, and coronary heart disease: a randomized, double-blind, placebo-controlled trial. Probiotics and antimicrobial proteins, 11, 133-142.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

REVIEW Open Access

Effects of probiotics on type II diabetes mellitus: a meta-analysis

Yun-Wen Tao[1], Ying-Luo Gu[1], Xin-Qi Mao[1], Lei Zhang[2,3*] and Yu-Fang Pei[1,3*]

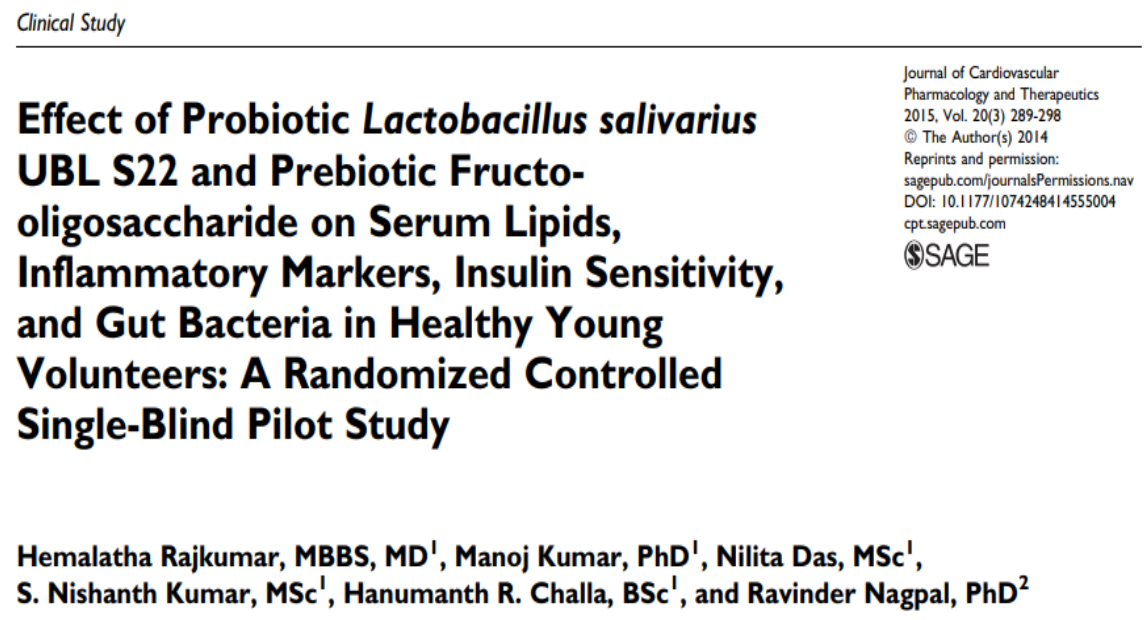
Study	Country	No. of cases (treatment/control)	Treatment group	Control group	Duration of treatment (week)
Razmpoosh E 2019	Iran	30/30	*Lactobacillus acidophilus* 2×10^9 CFU + *Lactobacillus casei* 7×10^9 CFU + *Lactobacillus rhamnosus* 1.5×10^9 CFU + *Lactobacillus bulgaricus* 2×10^8 CFU + *Bifidobacterium breve* 3×10^{10} CFU + *Bifidobacterium longum* 7×10^8 CFU + *Streptococcus thermophilus* 1.5×10^9 CFU 4 times/day	Placebo	6
Raygan F 2018	Iran	30/30	*Bifidobacterium bifidum* 2×10^9 CFU + *Lactobacillus casei* 2×10^9 CFU + *Lactobacillus acidophilus* 2×10^9 CFU/day	Placebo	12
Ejtahed HS 2012	Iran	30/30	*Lactobacillus acidophilus La5 and Bifidobacterium lactis Bb12* 4×10^9 CFU/day	Conventional yogurt	6
Shakeri H 2014	Iran	26/26	*Lactobacillus sporogenes* 1×10^8 CFU/1 g 40 g 3 times/day	Bread without probiotic bacteria and prebiotic inulin	8
Khalili L 2019	Iran	20/20	*Lactobacillus casei* 1×10^8 CFU/day	Placebo	8
Tajadadi-Ebrahimi M 2016	Iran	30/30	*Lactobacillus acidophilus* 2×10^9 + *Lactobacillus casei* 2×10^9 + *Bifidobacterium bifidum* 2×10^9 CFU/g	Placebo	12
Mohamadshahi M 2014	Iran	22/22	*Lactobacillus acidophilus* 3.7×10^6 CFU/g and B. lactic 3.7×10^6 CFU/g 300 g/day	Conventional yogurt	8
Tajadadi-Ebrahimi M 2014	Iran	27/27	*Lactobacillus sporogenes* 1×10^8 CFUs/g 3 times/day	Control bread	8
Kobyliak N 2018	Ukraine	31/22	*Lactobacillus* + *Lactococcus* 6×10^{10} CFU/g + *Bifidobacterium* 1×10^{10} CFU/g + *Propionibacterium* 3×10^{10} CFU/g + *Acetobacter* 1×10^6 CFU/g 10 g/day	Placebo	8
Mobini R 2016	Swedish	14/15	*Lactobacillus reuteri DSM 17938* 1×10^{10} CFU/day	Placebo	12
Firouzi S 2017	Malaysia	48/53	*Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis* 5×10^9 CFU + *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus lactis* 5×10^9 CFU 2 times/day	Placebo	12
Tonucci LB 2017	Brazil	23/22	*Lactobacillus acidophilus La-5* 1×10^9 CFU/day + *Bifidobacterium animalis* subsp. *lactis BB-12* 1×10^9 CFU/day	Conventional fermented milk	6
Hui Y 2018	China	53/55	*Lactobacillus paracasei N1115* 1×10^8 CFU/mL 200 g 2 times/day	Placebo	8
Asemi Z 2016	Iran	51/51	*Lactobacillus sporogenes 1 × 107 CFU* + 0.05 g beta-carotene 3 times/day	Placebo	6
Mazloom Z 2013	Iran	16/18	*Lactobacillus acidophilus* + *Lactobacillus bulgaricus* + *Lactobacillus bifidum* + *Lactobacillus casei*	Placebo	6

Tao, Y. W., Gu, Y. L., Mao, X. Q., Zhang, L., & Pei, Y. F. (2020). Effects of probiotics on type II diabetes mellitus: a meta-analysis. Journal of translational medicine, 18, 1-11.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Effects of Probiotic and Supplementation in Insulin Resistance

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



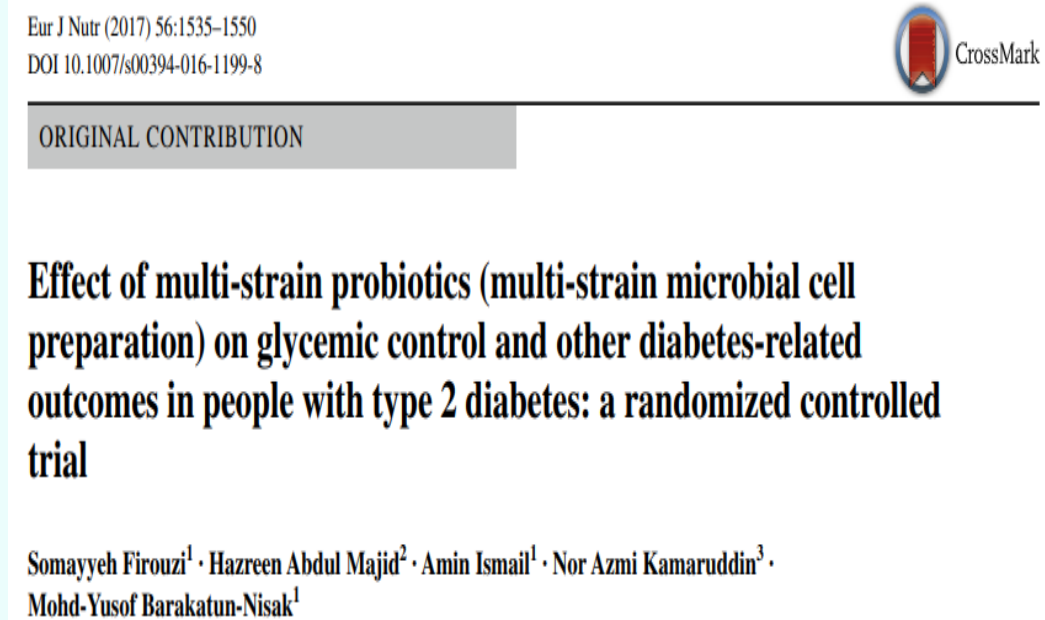
Clinical Study

Effect of Probiotic *Lactobacillus salivarius* UBL S22 and Prebiotic Fructo-oligosaccharide on Serum Lipids, Inflammatory Markers, Insulin Sensitivity, and Gut Bacteria in Healthy Young Volunteers: A Randomized Controlled Single-Blind Pilot Study

Journal of Cardiovascular Pharmacology and Therapeutics
2015, Vol. 20(3) 289-298
© The Author(s) 2014
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1074248414555004
cpt.sagepub.com
SAGE

Hemalatha Rajkumar, MBBS, MD[1], Manoj Kumar, PhD[1], Nilita Das, MSc[1], S. Nishanth Kumar, MSc[1], Hanumanth R. Challa, BSc[1], and Ravinder Nagpal, PhD[2]

Rajkumar, H., Kumar, M., Das, N., Kumar, S. N., Challa, H. R., & Nagpal, R. (2015). Effect of probiotic Lactobacillus salivarius UBL S22 and prebiotic fructo-oligosaccharide on serum lipids, inflammatory markers, insulin sensitivity, and gut bacteria in healthy young volunteers: a randomized controlled single-blind pilot study. Journal of cardiovascular pharmacology and therapeutics, 20(3), 289-298.



Eur J Nutr (2017) 56:1535–1550
DOI 10.1007/s00394-016-1199-8

CrossMark

ORIGINAL CONTRIBUTION

Effect of multi-strain probiotics (multi-strain microbial cell preparation) on glycemic control and other diabetes-related outcomes in people with type 2 diabetes: a randomized controlled trial

Somayyeh Firouzi[1] · Hazreen Abdul Majid[2] · Amin Ismail[1] · Nor Azmi Kamaruddin[3] · Mohd-Yusof Barakatun-Nisak[1]

Firouzi, S., Majid, H. A., Ismail, A., Kamaruddin, N. A., & Barakatun-Nisak, M. Y. (2017). Effect of multi-strain probiotics (multi-strain microbial cell preparation) on glycemic control and other diabetes-related outcomes in people with type 2 diabetes: a randomized controlled trial. European journal of nutrition, 56, 1535-1550.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study

Clara Depommier, Amandine Everard, Céline Druart, Hubert Plovier, Matthias Van Hul, Sara Vieira-Silva, Gwen Falony, Jeroen Raes, Dominique Maiter, Nathalie M. Delzenne, Marie de Barsy, Audrey Loumaye, Michel P. Hermans, Jean-Paul Thissen, Willem M. de Vos & Patrice D. Cani

Nature Medicine **25**, 1096–1103 (2019) | Cite this article

Depommier, C., Everard, A., Druart, C., Plovier, H., Van Hul, M., Vieira-Silva, S., ... & Cani, P. D. (2019). Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nature medicine, 25(7), 1096-1103.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Conclusion

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

- Ahmad, E., Lim, S., Lamptey, R., Webb, D. R., & Davies, M. J. (2022). Type 2 diabetes. The Lancet, 400(10365), 1803-1820
- Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatric clinics of North America. 2013;60(1):49- 74. Epub 2012/11/28. doi: 10.1016/j.pcl.2012.10.002. PubMed PMID: 23178060; PubMed Central PMCID: PMCPmc3586783
- Caricilli, A. M., & Saad, M. J. (2013). The role of gut microbiota on insulin resistance. Nutrients, 5(3), 829-851.
- Coppa GV, Bruni S, Morelli L, Soldi S, Gabrielli O. The fi rst prebiotics in humans: human milk oligosaccharides. Journal of clinical gastroenterology. 2004;38(6 Suppl):S80-3. Epub 2004/06/29. PubMed PMID: 15220665
- De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, et al. High-level adherence to a Mediterranean diet benefi cially impacts the gut microbiota and associated metabolome. Gut. 2015:gutjnl-2015-309957
- Delzenne, N. M., & Cani, P. D. (2011). Gut microbiota and the pathogenesis of insulin resistance. Current diabetes reports, 11, 154-159.
- Garbossa SG, Folli F. Vitamin D, sub-inflammation and insulin resistance. A window on a potential role for the interaction between bone and glucose metabolism. Rev Endocr Metab Disord 2017; 18: 243-58.
- Hsieh M-C, et al. The beneficial effects of Lactobacillus reuteri ADR-1 or ADR-3 consumption on type 2 diabetes mellitus: a randomized, double-blinded, placebo-controlled trial. Sci Rep. 2018;8(1):16791

STUDY MATERIAL

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

- Kasuga M. Insulin Resistance and Pancreatic β Cell Failure. J. Clin. Invest. 2006;116:1756-1760.
- Madan JC, Hoen AG, Lundgren SN, Farzan SF, Cottingham KL, Morrison HG, et al. Association of Cesarean Delivery and Formula Supplementation With the Intestinal Microbiome of 6-Week-Old Infants. JAMA pediatrics. 2016;170(3):212-9. Epub 2016/01/12. doi: 10.1001/jamapediatrics.2015.3732. PubMed PMID: 26752321; PubMed Central PMCID: PMCPmc4783194
- Munoz-Garach, A., Diaz-Perdigones, C., & Tinahones, F. J. (2016). Gut microbiota and type 2 diabetes mellitus. Endocrinología y Nutrición (English Edition), 63(10), 560-568.

- Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, et al. The NIH human microbiome project. Genome research. 2009;19(12):2317-23.
- Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. Physiol Rev 2013;93:993-1017
- Snelson M, Kellow NJ, Coughlan MT. Modulation of the gut microbiota by resistant starch as a treatment of chronic kidney diseases: evidence of efficacy and mechanistic insights. Advances in nutrition (Bethesda, Md). 2019;10(2):303–20.
- Szosland K, Lewiński A. In quest for method of insülin resistance assessment in everyday clinical practice – insülin resistance indices. Diabetes Metab Syndr Clin Res Rev 2016; 10S: S120-5.

STUDY MATERIAL

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

- World Health Organization. Obesity and Overweight Fact Sheet. WHO 2008;3110-17.
- Yang, G., Wei, J., Liu, P., Zhang, Q., Tian, Y., Hou, G., ... & Jiang, X. (2021). Role of the gut microbiota in type 2 diabetes and related diseases. Metabolism, 117, 154712.
- Zhang, Z., Tian, T., Chen, Z., Liu, L., Luo, T., & Dai, J. (2021). Characteristics of the gut microbiome in patients with prediabetes and type 2 diabetes. PeerJ, 9, e10952.
- M. Snelson, C. de Pasquale, E.I. Ekinci et al, Best Practice & Research Clinical Endocrinology & Metabolism 35 (2021) 101507

- Jardon, K. M., Canfora, E. E., Goossens, G. H., & Blaak, E. E. (2022). Dietary macronutrients and the gut microbiome: a precision nutrition approach to improve cardiometabolic health. Gut, 71(6), 1214-1226.
- Özdemir, A., & Demirel, Z. B. (2017). Beslenme ve mikrobiyota ilişkisi. Journal of Biotechnology and Strategic Health Research, 1, 25-33.
- Zhang X, Shen D, Fang Z, et al. Human gut microbiota changes reveal the progression of glucose intolerance. PLoS ONE. 2013;8(8):e71108

STUDY MATERIAL

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

Title: Microbiome/Nutrition and Insulin Resistance & Type 2 Diabetes

Brief summary of what will be showed.

This presentation will explore the intricate relationship between microbiota, insulin resistance, and type 2 diabetes, with a particular focus on the role of nutrition. Firstly, we will define microbiota as the collection of microorganisms residing within the body, primarily in the gut, and examine its connection with dietary habits. Moving on, insulin resistance and diabetes will be elucidated, highlighting insulin's role in glucose metabolism and the development of type 2 diabetes when cells become resistant to its effects.

The presentation will delve into the interplay between microbiota and insulin resistance, discussing recent research suggesting that specific microbiota profiles may influence insulin sensitivity. Furthermore, we will explore the relationship between microbiota and type 2 diabetes, underscoring how alterations in gut microbiota composition may contribute to the development of the disease.

Examining the microbiota patterns of individuals with insulin resistance and type 2 diabetes, we will identify common trends and differences compared to healthy individuals. Finally, strategies for improving microbial dysbiosis through dietary interventions in individuals with type 2 diabetes and insulin resistance will be explored, emphasizing the potential of probiotics, prebiotics, and fiber-rich foods to promote a healthy microbiota profile and mitigate metabolic dysfunction.

Overall, this presentation aims to provide insight into the complex interconnections between microbiota, insulin resistance, and type 2 diabetes, underscoring the importance of nutrition in modulating these relationships for improved metabolic health.

Learning objectives

- ✓ Basic information about microbiota will be learned.
- ✓ Information about insulin resistance and type 2 diabetes diseases and their basic mechanisms will be obtained.
- ✓ Learn about the relationship between microbiota and insulin resistance and type 2 diabetes.
- ✓ Information will be given about insulin resistance and the microbial composition of type 2 diabetes patients.
- ✓ Information will be obtained about the effects of nutritional interventions on insulin resistance and the microbial composition of individuals with type 2 diabetes.

Index

- ✓ Definition of microbiota and its relationship with nutrition
- ✓ Definition of insulin resistance and diabetes
- ✓ The relationship between microbiota and insulin resistance
- ✓ The relationship between microbiota and type 2 diabetes
- ✓ Microbiota pattern of individuals with insulin resistance and type 2 diabetes
- ✓ Effect of nutrition and probiotics on improving microbial dysbiosis of individuals with type 2 diabetes and insulin resistance.

Introduction

Type 2 diabetes and insulin resistance are the fastest-growing metabolic diseases in the world. Many clinical studies have found that type 2 diabetes and insulin resistance patients have metabolic disorders and chronic inflammatory states accompanied by disturbances in the gut microbiota. The gut microbiota plays an important role in body metabolism and immune regulation, and disturbances in the gut microbiota in conjunction with destruction of the intestinal barrier in type 2 diabetes and insulin resistance patients causes damage to multiple organs. Therefore, the gut microbiota may be a new therapeutic target for treating these patients.

In this course, we introduce the defitinon of the gut microbiota, insulin resistance and type 2 diabetes, the relationship between microbiota, insulin resistance and type 2 diabetes, microbiota pattern of individuals with insulin resistance and type 2 diabetes and lastly the effect of nutrition and probiotics on improving microbial dysbiosis in individuals with type 2 diabetes and insulin resistance.

(To be developed)

Body

In the light of scientific articles, intestinal microbiota dysbiosis in Type 2 diabetes and insulin resistance and ways to correct this situation with nutrition and probiotic supplements will be discussed.

Real cases

Studies examining dysbiosis in individuals with insulin resistance and type 2 diabetes and experimental studies investigating how to eliminate dysbiosis will be given in this section. The relevant cognacs are given below.

- ✓ Galié, S., García-Gavilán, J., Papandreou, C., Camacho-Barcía, L., Arcelin, P., Palau-Galindo, A., ... & Bulló, M. (2021). Effects of Mediterranean Diet on plasma metabolites and their relationship with insulin resistance and gut microbiota composition in a crossover randomized clinical trial. Clinical Nutrition, 40(6), 3798-3806.
- ✓ Gomes, A. C., Bueno, A. A., de Souza, R. G. M., & Mota, J. F. (2014). Gut microbiota, probiotics and diabetes. Nutrition journal, 13, 1-13.
- ✓ Delzenne, N. M., & Cani, P. D. (2011). Gut microbiota and the pathogenesis of insulin resistance. Current diabetes reports, 11, 154-159.
- ✓ Tian, W., Liu, L., Wang, R. et al. Gut microbiota in insulin resistance: a bibliometric analysis. J Diabetes Metab Disord (2024). https://doi.org/10.1007/s40200-023-01342-x

Conclusion

The relationship between type 2 diabetes and insulin resistance and gut microbiota has been scientifically demonstrated. Studies have shown that dysbiosis of individuals with Type 2 diabetes and insulin resistance can be corrected with nutritional interventions and probiotic supplements.

When the research is completed, suggestions on this subject will be added to this section.

Summary again and take home message

(To be developed)

Reference and suggested readings

- ✓ Caricilli, A. M., & Saad, M. J. (2013). The role of gut microbiota on insulin resistance. Nutrients, 5(3), 829-851.
- ✓ Munoz-Garach, A., Diaz-Perdigones, C., & Tinahones, F. J. (2016). Gut microbiota and type 2 diabetes mellitus. Endocrinología y Nutrición (English Edition), 63(10), 560-568.
- ✓ Jardine, M. (2016). Nutrition considerations for microbiota health in diabetes. Diabetes Spectrum, 29(4), 238-244.
- ✓ Diamant, M., Blaak, E. E., & De Vos, W. M. (2011). Do nutrient–gut–microbiota interactions play a role in human obesity, insulin resistance and type 2 diabetes?. Obesity reviews, 12(4), 272-281.
- ✓ Tian, W., Liu, L., Wang, R. et al. Gut microbiota in insulin resistance: a bibliometric analysis. J Diabetes Metab Disord (2024). https://doi.org/10.1007/s40200-023-01342-x

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

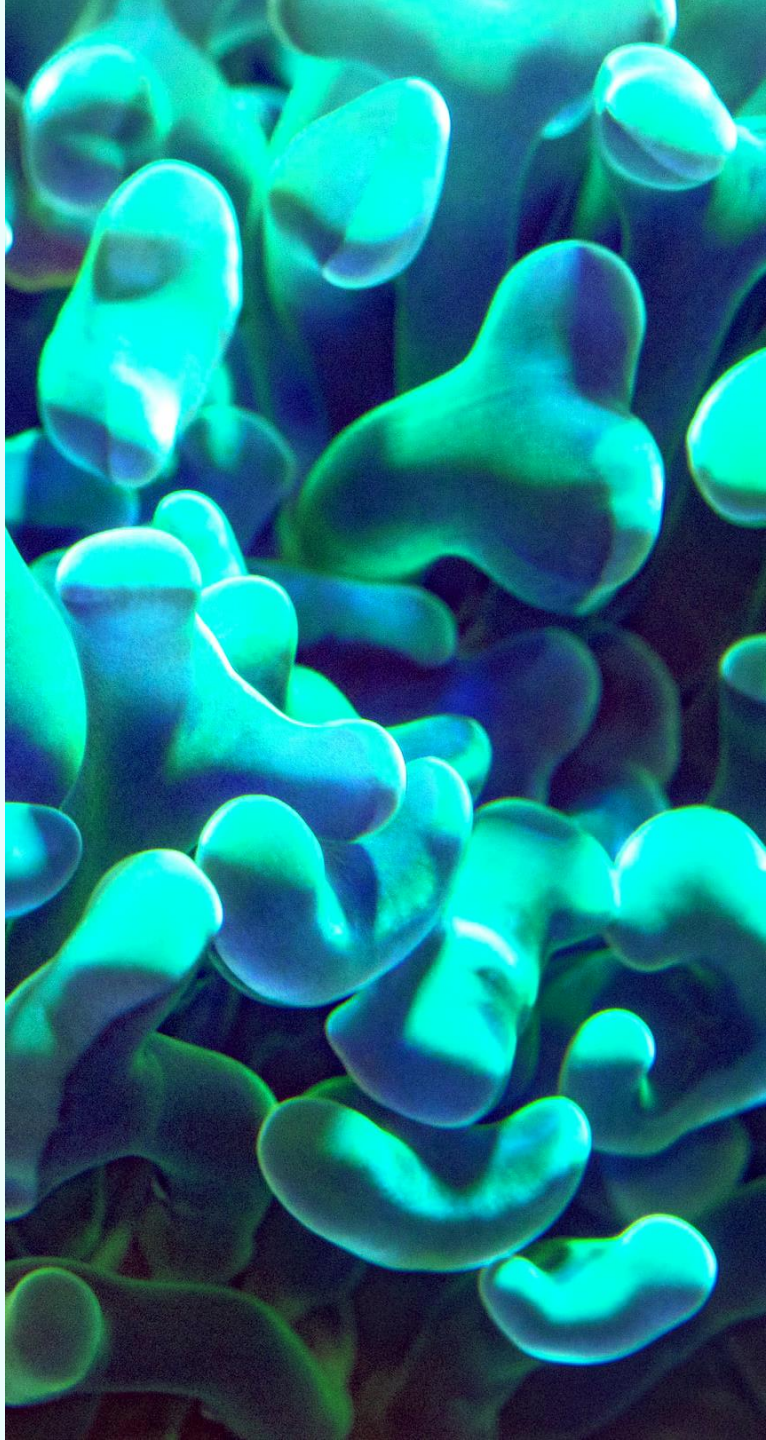
Post-graduate certificate level 7 (30 ECTS)

Exploring the Connection Between Sports, Microbiome and Nutrition: Impact on Health and Performance

Dr Eman Salah Zaky
MSc., MBBCH., RD

Clinical Nutrition and Health Consultant

Malta Further & Higher Education Authority



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Key Points:

This Photo by Unknown Author is licensed under CC BY

A- **Overview of Gut Microbiota Key Role in Health and Sports:**
- Physiological and pathological roles
- Gut-brain axis in sports
- Gut-muscle axis
- Role of microbiota in exercise

B- **Athletic Performance Impact on Gut Microbiota:**
- Impact on diversity and inflammation
- Mechanisms of exercise induced microbial modulation
- Risks of overtraining

C- **Nutrition:**
- Impact of diet on performance and gut microbiota
- Macronutrients: protein - carbs - fats
- Micronutrients: pre & probiotics - vitamins - supplements
- Dietary pattern and performance

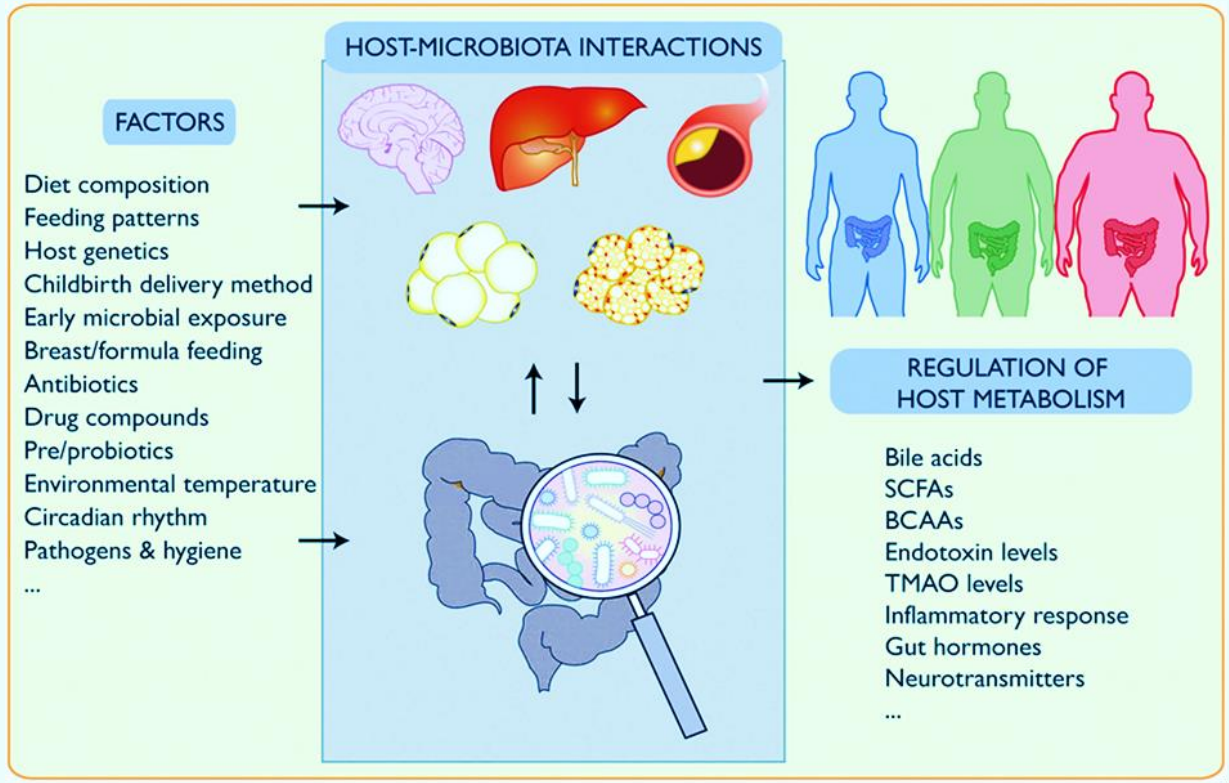
D- **Research and Future Directions**

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

A. Introduction to Gut Microbiome

The **Gut Microbiome** plays a crucial role in maintaining health and optimizing athletic performance. It acts as a **'conductor'** of various physiological processes, influencing metabolism, immune function, and even the brain-gut axis.

A diverse and balanced microbiota contributes to **Host Metabolism**: energy production, and recovery, while also modulating inflammation and stress responses, essential for athletes striving for peak performance.



This Photo by Unknown Author is licensed under CC BY

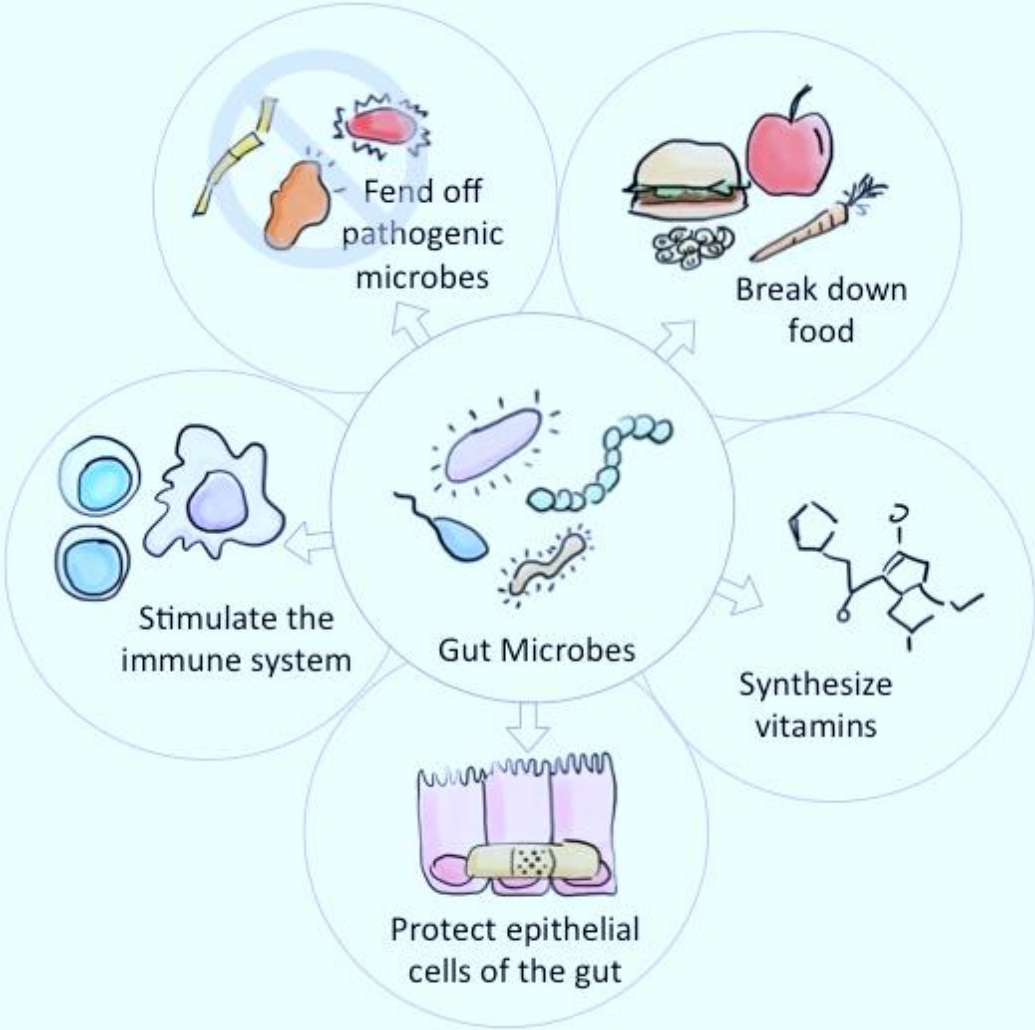
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut Microbiota Physiological Role:

Maintains **Intestinal Homeostasis** by forming a protective barrier against pathogens.

Enhances **Nutrient Absorption**, improving recovery and overall athletic performance.

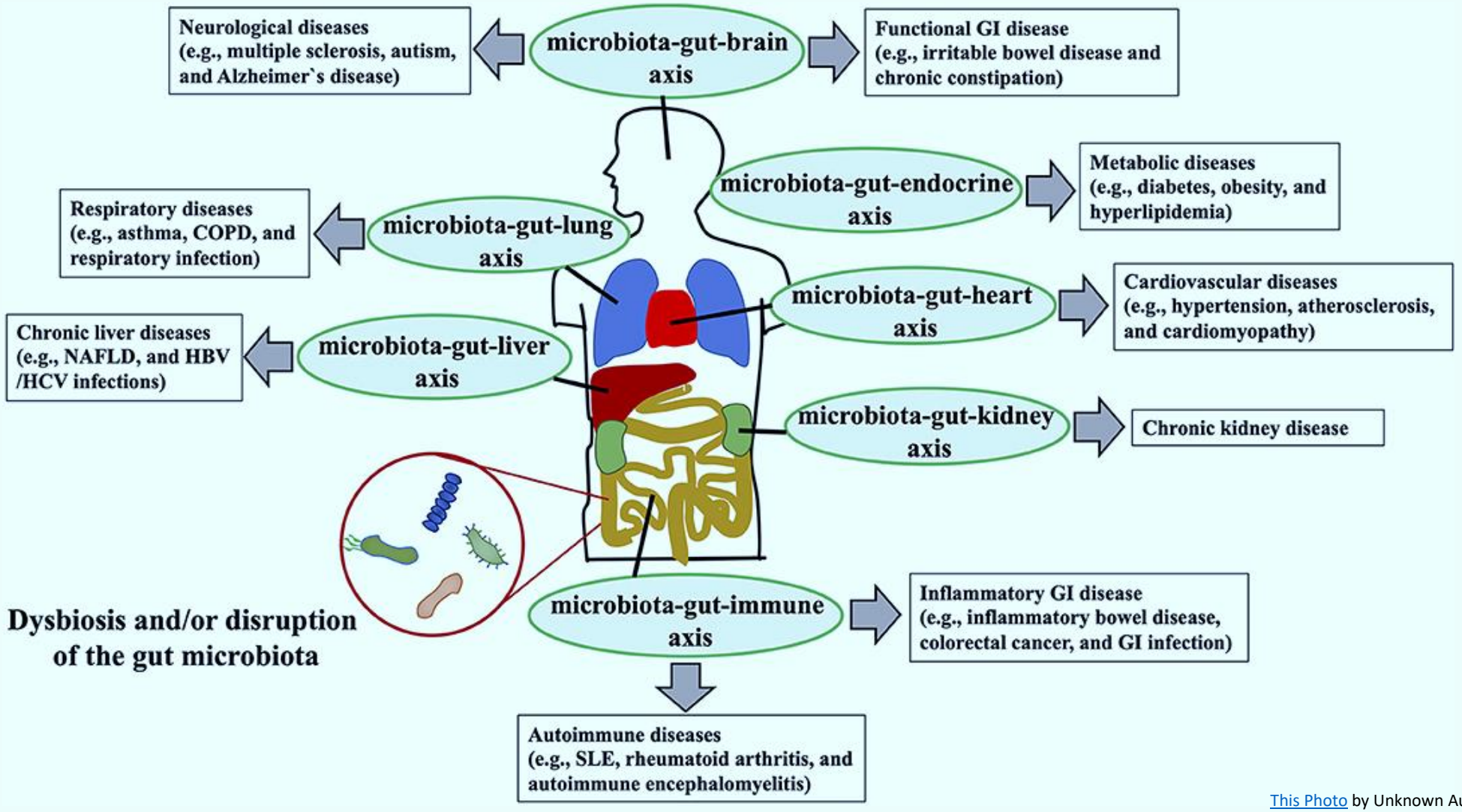
Regulates Metabolism, influencing energy expenditure and oxidative stress during exercise.



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut Microbiota Pathological Role:



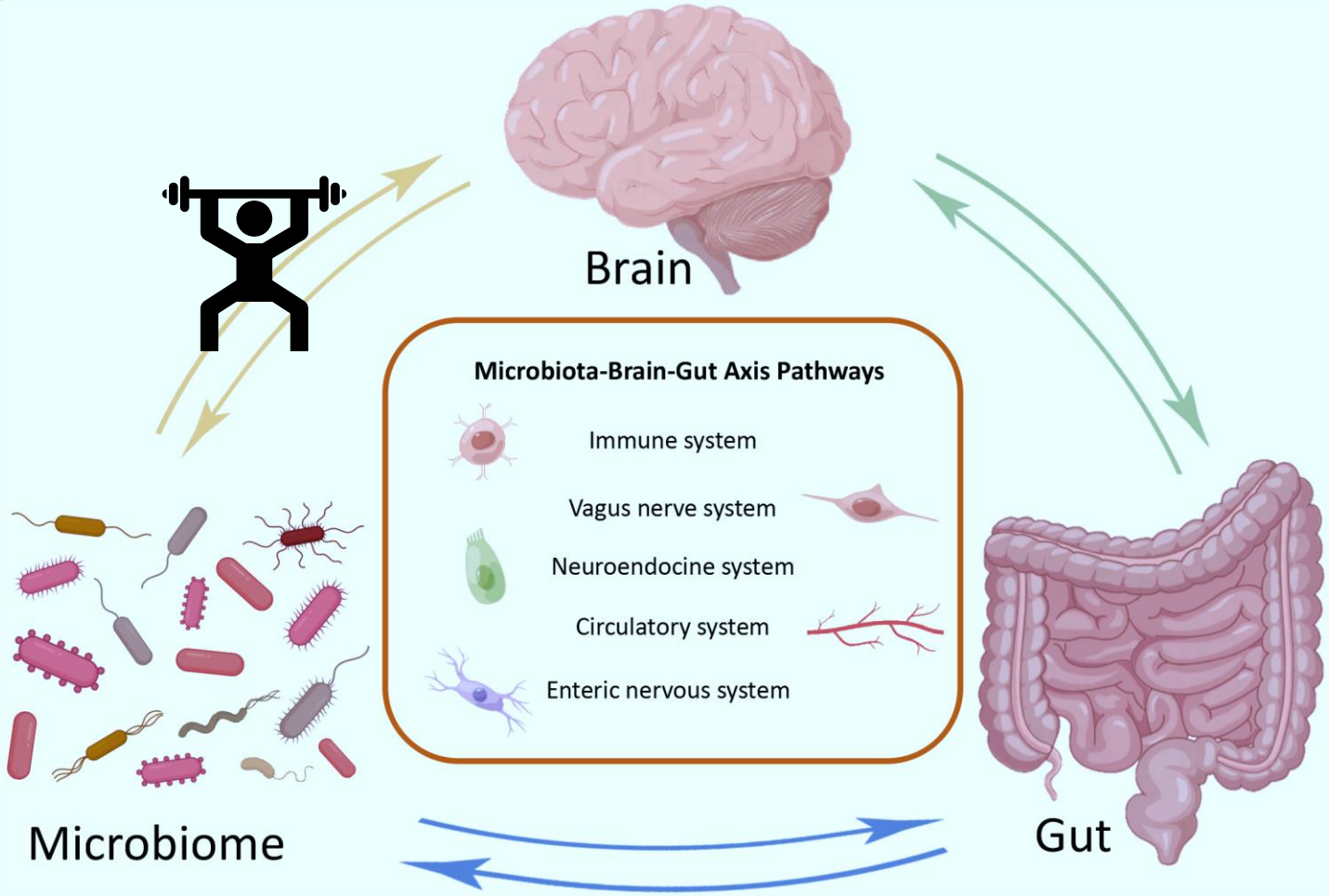
This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The Gut-Brain Axis in Sports:

Stress Response Modulation: The gut microbiota interacts with the gut-brain axis, influencing the body's stress response.

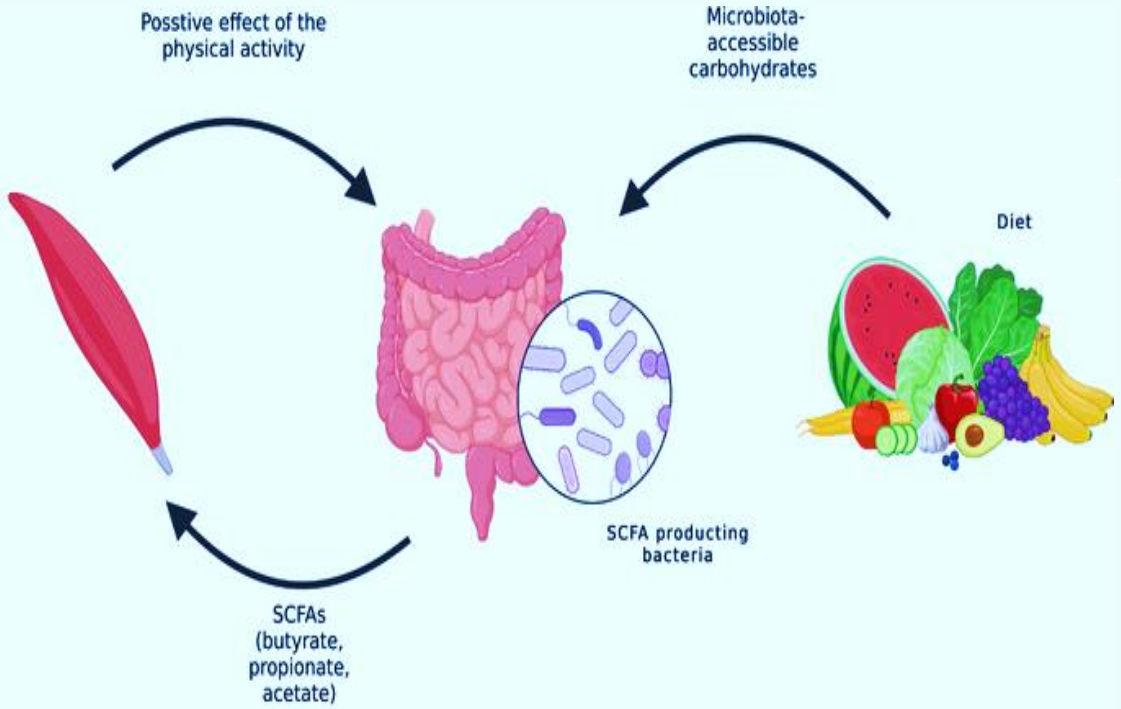
Exercise-Induced Stress can alter the composition of the microbiota, which can have an impact on hormone release, mood, and general mental health—all of which are essential for optimal performance.



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The Gut-Muscle Axis:



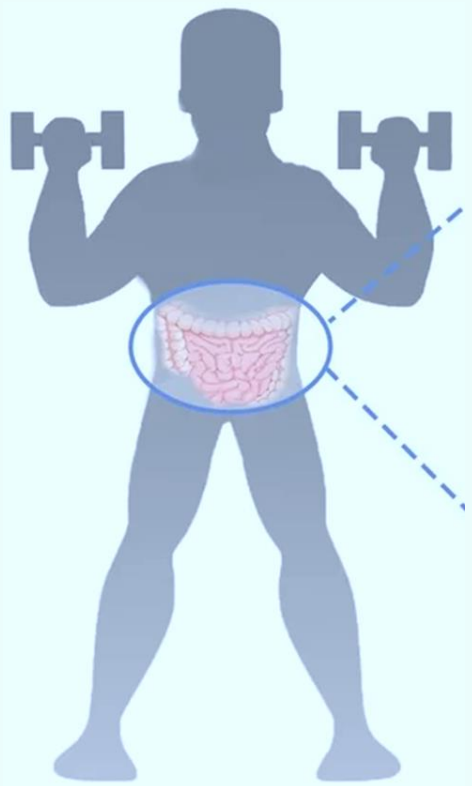
Microbiota Influence on Muscle Function
Gut bacteria regulate inflammatory and metabolic pathways essential for muscle growth and recovery, impacting overall performance.

Research on Muscle Atrophy
Studies reveal a link between gut microbiota depletion and muscle atrophy, showing reduced endurance and glucose homeostasis in mice.

Gut Microbiota and Skeletal Muscle Mechanisms of Interaction:

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The Gut-Muscle Axis:

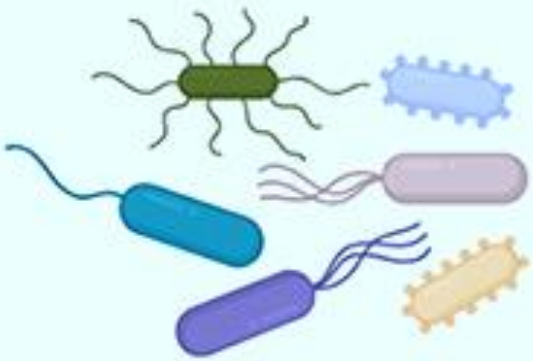


GM PRODUCTS	MAIN SUBSTRATES	MAIN BACTERIA TAXA INVOLVED	OTHER DIETARY SOURCES	POTENTIAL MAIN IMPACTS ON MUSCLES
Lactate	Complex carbohydrates (dietary fibers)	Fibrolytic bacteria, bifidobacteriales, lactic acid bacteria	Fermented milk products, wine, akebia fruit	Energy substrate[28]
Succinate	Complex carbohydrates (dietary fibers)	Bacteroidetes, for example, *P. copri*	Food additives and dietary supplements	↑ Insulin sensitivity and metabolism[15,29]
Imidazole propionate	Histidine	Bacteria with urocanate reductase activity, for example, *Streptococcus mutans* and *Eggerthella lenta*	/	Related to impairment of insulin signaling and glucose tolerance[27]
Short chain fatty acids (SCFAs)	Complex carbohydrates (dietary fibers) and proteins	Most bacteria, fibrolytic, glycolytic, and/or proteolytic	/	↓ Systemic insulin resistance and inflammation and appetite,[1] ↑ muscle insulin sensitivity, ↓ muscle atrophy, ↑ muscle strength and exercise capacity[16]
Branched-chain fatty acids	Branched-chain amino acids (BCAA)	Most bacteria, displaying a proteolytic activity	/	Related to insulin resistance[30,31]
Phenolic metabolites, in particular isovanillic acid 3-O-sulfate	Dietary phenolics (eg, from cereal brans and berry fruits)[19]	Butyrate-producing bacteria	/	↑ Glucose uptake and metabolism in the differentiated human skeletal muscle myoblast line[19]
Conjugated linoleic acid	Linoleic acid	*Bifidobacteria*[32]	Dairy products and meat	↑ Body mass and physical performance[20]
Secondary and tertiary bile acids (deoxycholic, lithocholic, and ursodeoxycholic acids)	Primary bile acids	*Clostridium*	/	↑ Systemic glucose homeostasis and energy expenditure[18]

(Gizard *et al.*, 2020)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Role of Microbiota in Exercise:



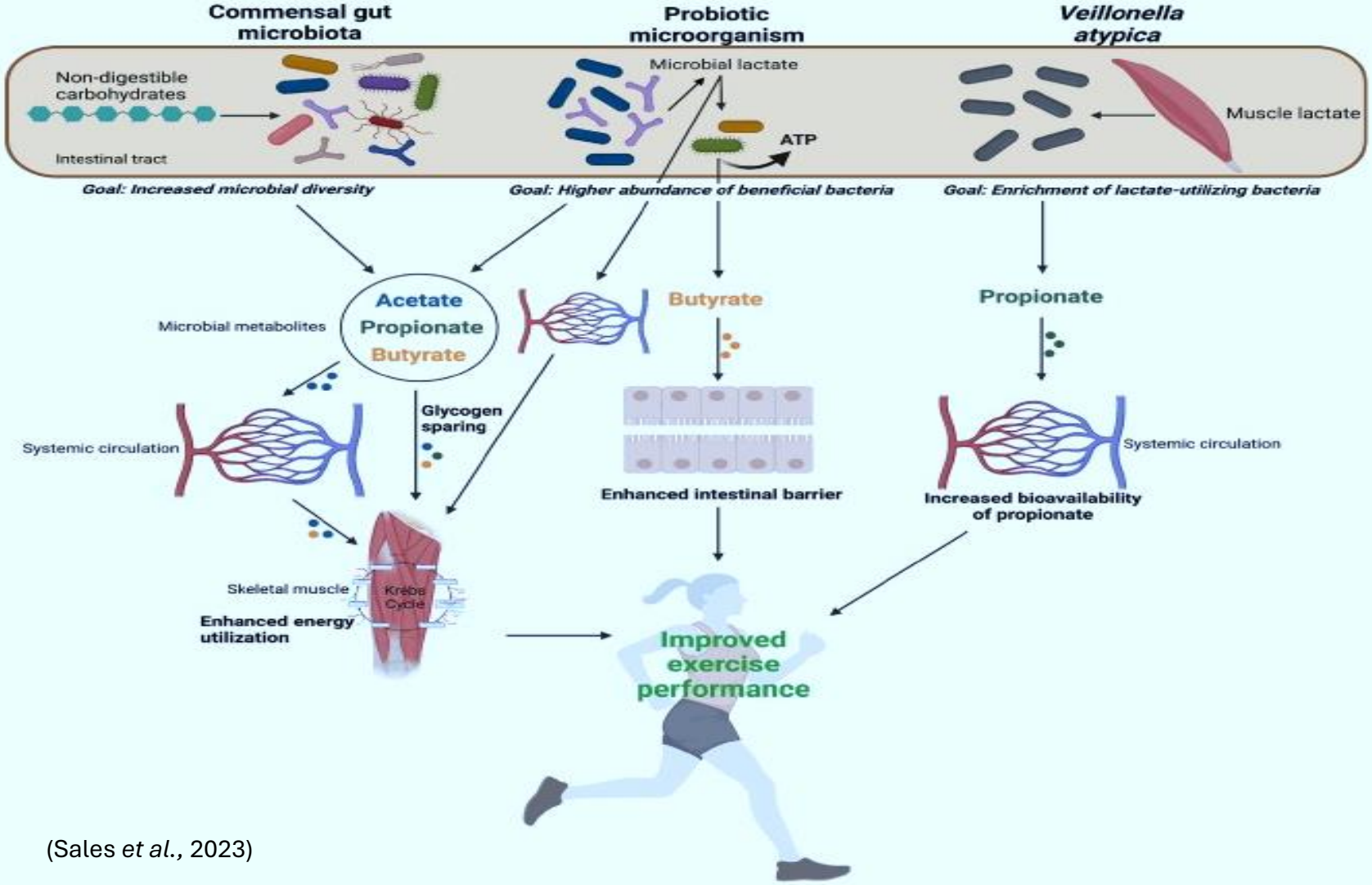
Mechanisms:
SCFA availability
Antioxidant enzyme activity
Immune system and inflammation
Gastrointestinal permeability
Glycogen storage/metabolism
Lactate metabolism
Carbohydrate/protein fermentation
Mitochondria cross-talk
Gut-brain axis

Performance measures:
VO2max/peak
Time-to-fatigue
Time trial
Lactate peak
Rep max loads
Vertical jump height
Wingate

This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

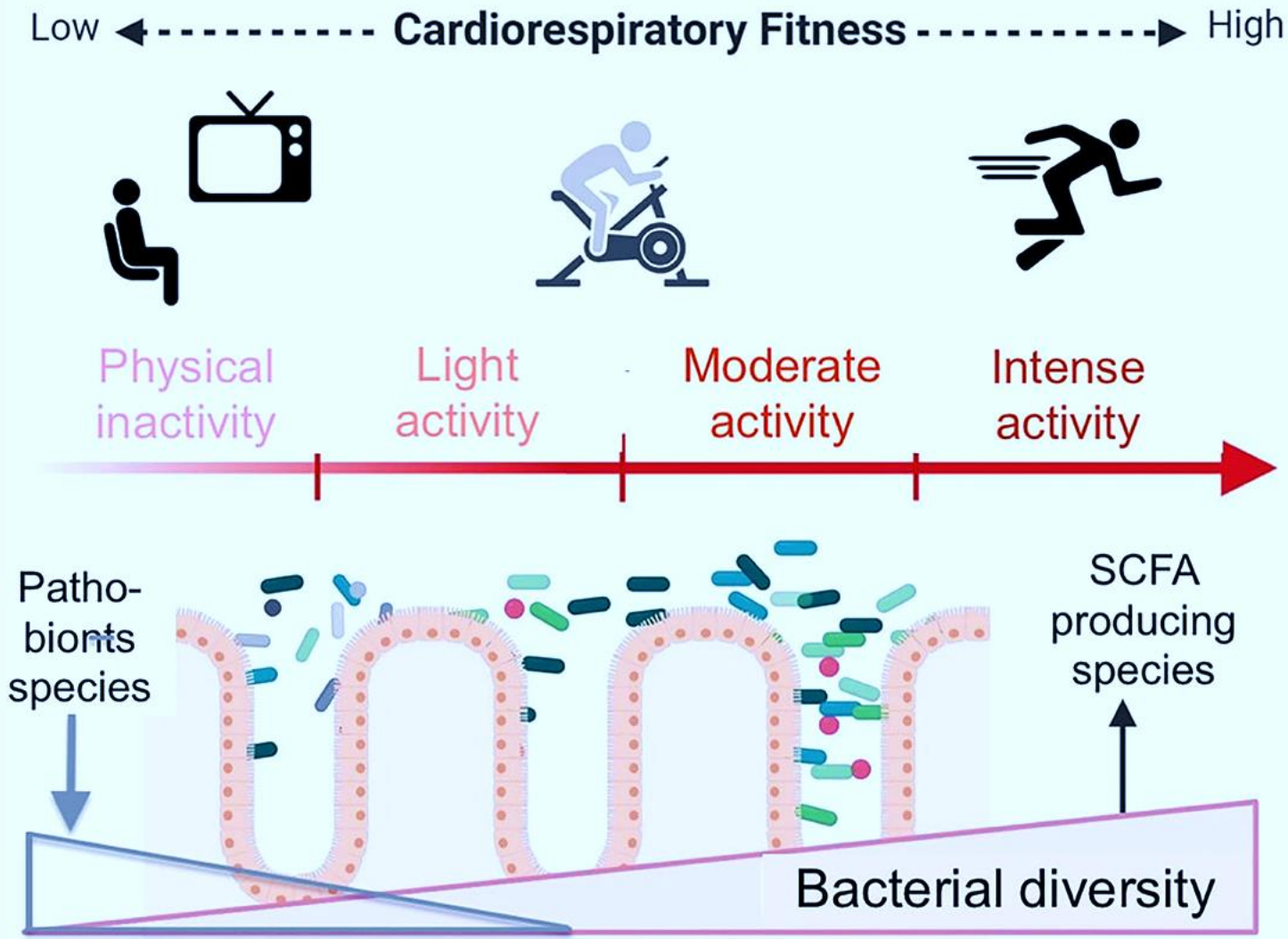
Role of Microbiota in Exercise:



(Sales *et al.*, 2023)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

B. Athletic Performance Impact on Gut Microbiota:

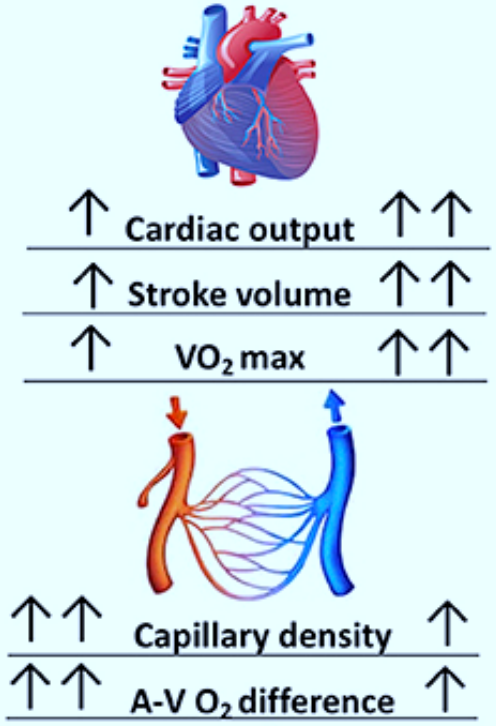


(Aya *et al.*, 2021)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

B. Athletic Performance Impact on Gut Microbiota:

MICT



HIIT/SIT

Spontaneous

↑*Parasutterella*
↑ *Lactobacillus johnsonii*

↑ *Subdoligranulum* (genus)

↑ *Bacteroidetes*

(Boisseau *et al.*, 2022)

Athletic Performance Impact on Gut Microbiota:

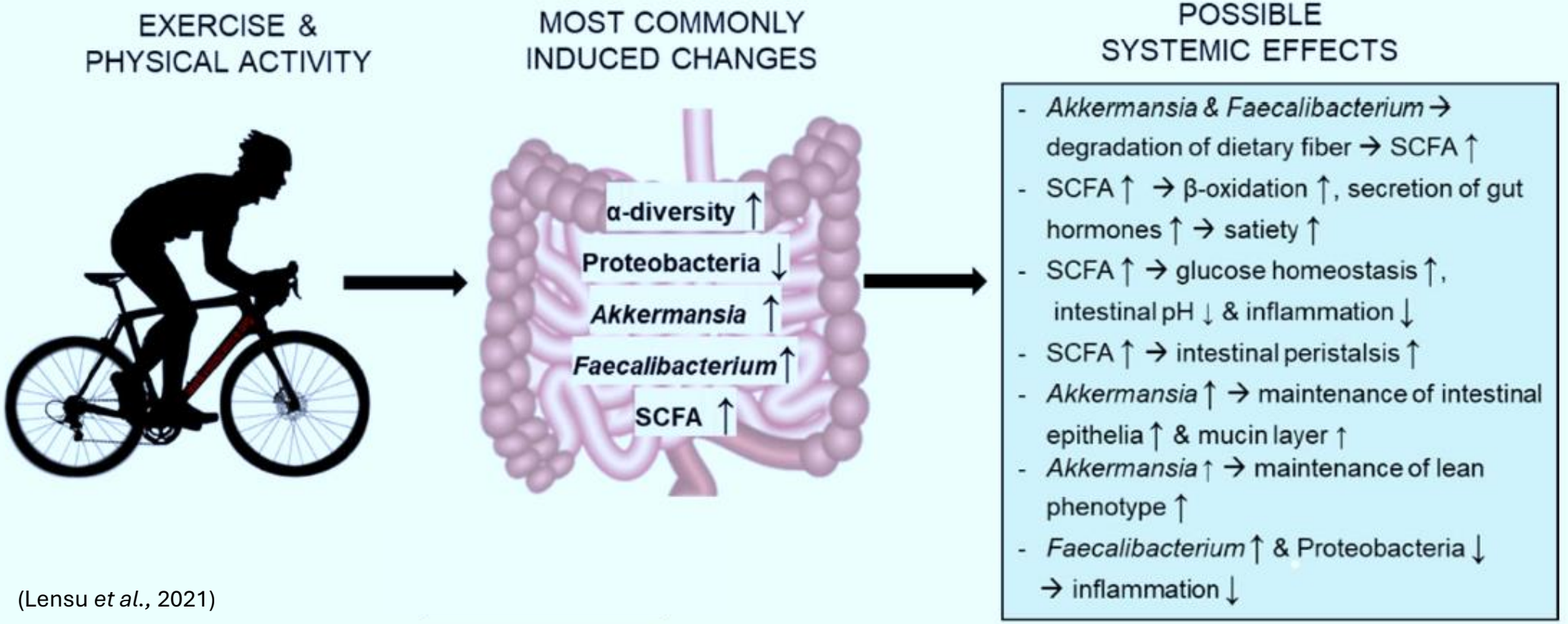
Sports Type/Activity Level	Main Results	Authors-Year
Professional rugby players High level	Gut microbiota with higher richness, decrease of the phylum Bacteroidetes and increase of the genus *Akkermansia* prevalence.	Clarke et al., 2014
39 healthy participants Various cardiorespiratory fitness levels with similar age, body mass index, and diets	VO_2 peak explained 20% of the variation in taxonomic richness. Increased abundances of key butyrate-producing taxa (Clostridiales, *Roseburia*, *Lachnospiraceae*, and *Erysipelotrichaceae*) in physically fit participants and increase in butyrate levels.	Estaki et al., 2016
Boston Marathon participants Treadmill-exercised C57BL/6 mice with acute supplementation of *Veillonella atypica*	Higher prevalence of *Veillonella atypica* in the athletes' fecal samples after the marathon. Inoculation of this strain in mice significantly increased exhaustive treadmill run time via its metabolic conversion of exercise-induced lactate into propionate.	Scheiman et al., 2019
Bodybuilders and distance runners compared to healthy sedentary men	Gut microbiota α and β diversity similar in the two athlete groups. At the genus and species level, differences between sport disciplines, but associated with diet variations.	Jang et al., 2019
37 elite athletes who competed in 16 different sports/Olympic level	The gut microbiome and metabolome differ among sports, classified in groups. Diet is not the driver of these differences.	O'Donovan et al., 2020

(Boisseau *et al.*, 2022)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Effects of Exercise on Gut Microbiota:

I. Impact on Diversity

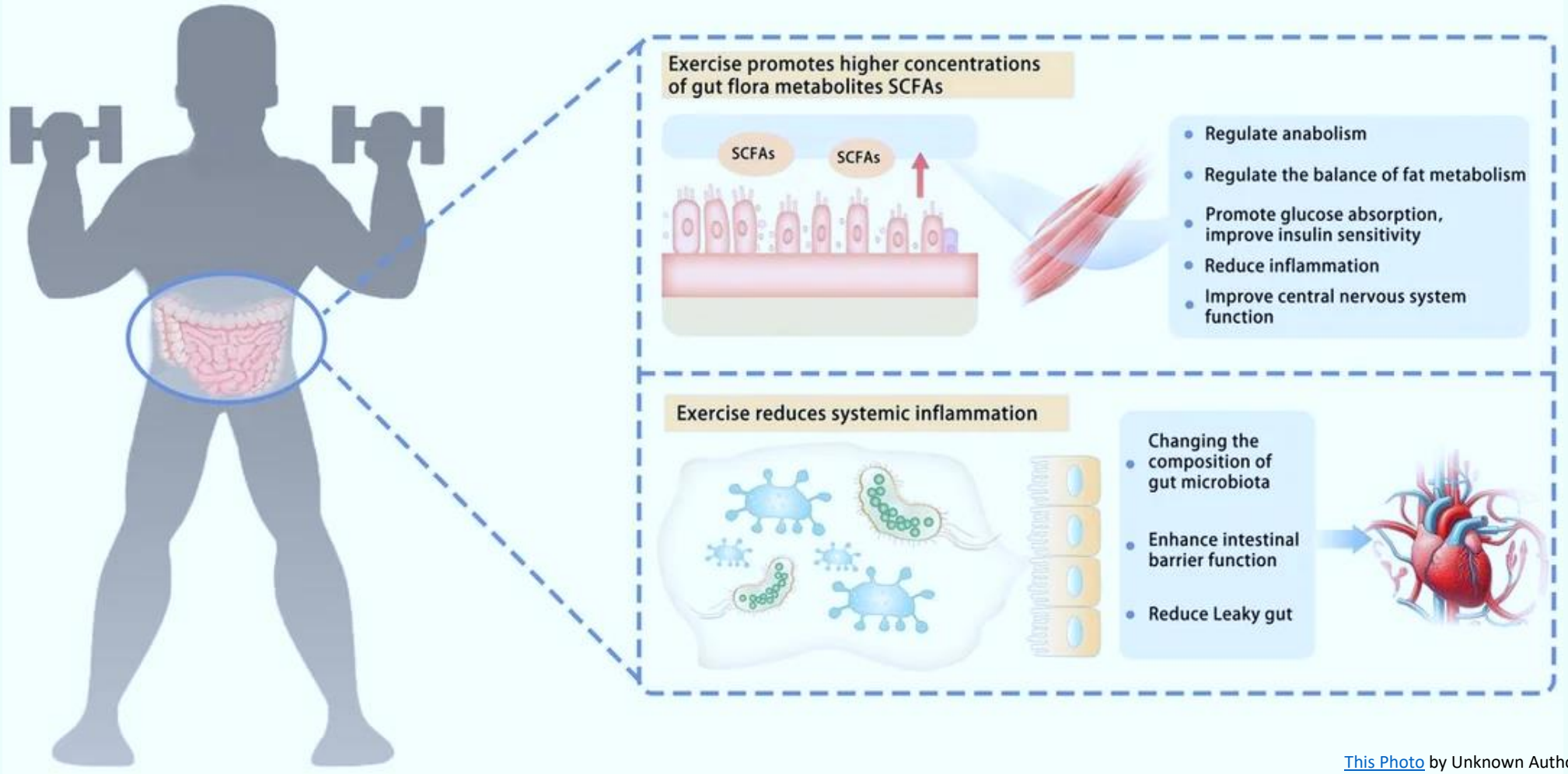


(Lensu *et al.*, 2021)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Effects of Exercise on Gut Microbiota Modulation

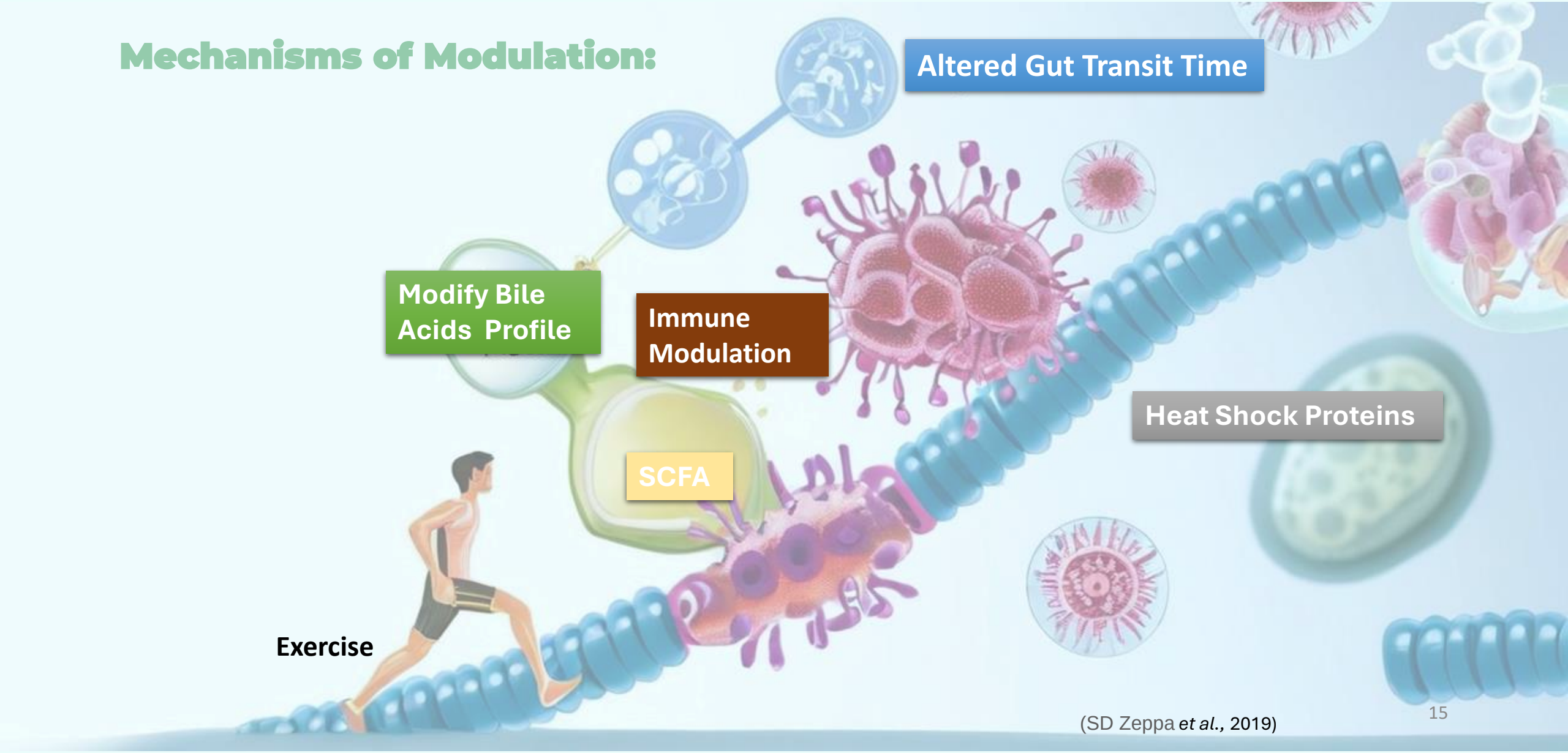
II. Impact on Inflammation



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Mechanisms of Modulation:



(SD Zeppa *et al.*, 2019)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Risks of Over Training:

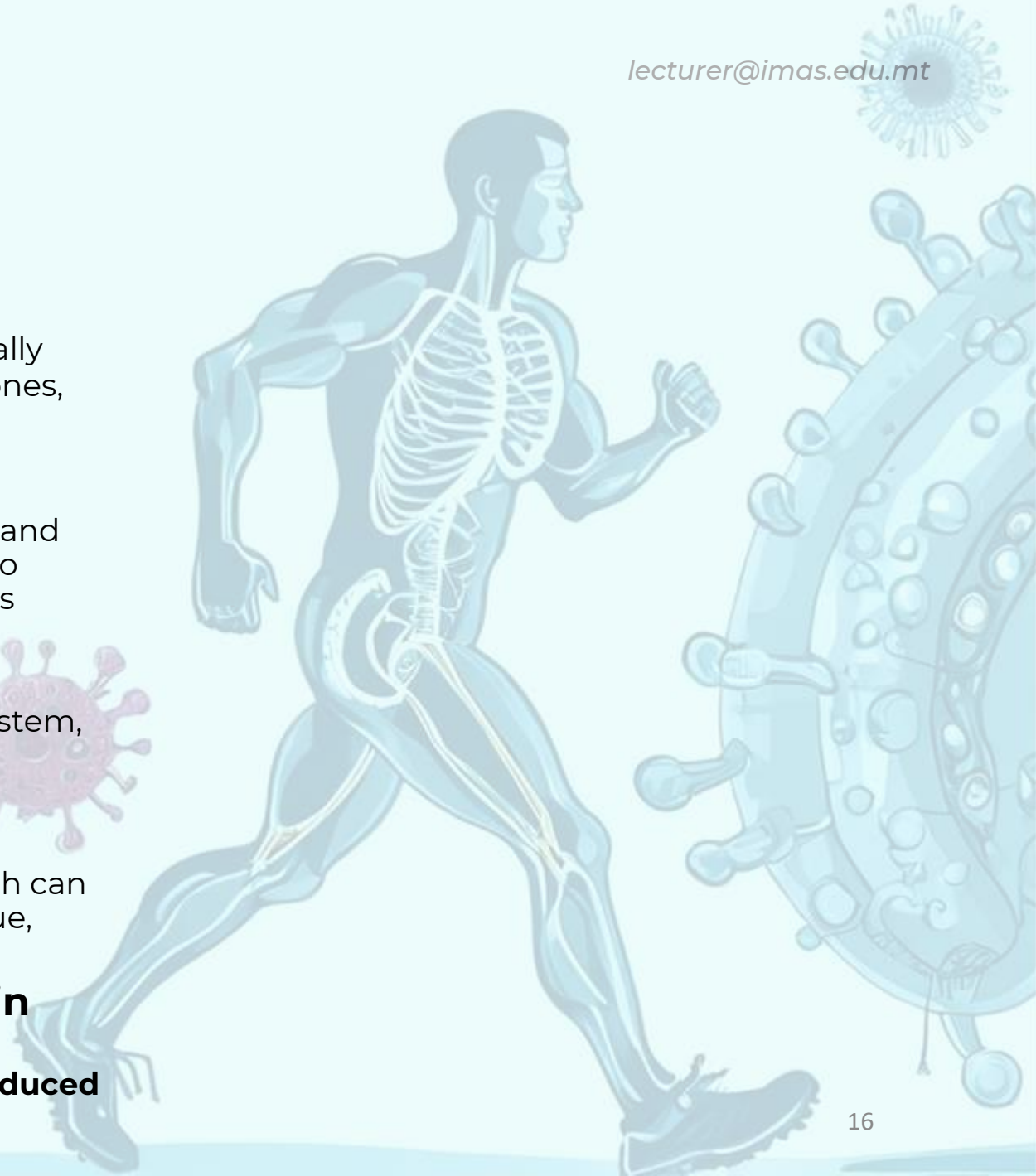
Disturbance of Gut Microbiota:
Prolonged HIT can disrupt the gut microbiota, potentially **reducing beneficial bacteria** and increasing harmful ones, affecting gut health and overall well-being.

Increased Intestinal Permeability:
Referred to as **"leaky gut."** This condition allows toxins and bacteria to enter the bloodstream, potentially leading to **systemic inflammation** and other health issues such as abdominal pain, nausea, and diarrhea.

Immunosuppression:
Intense and long training can suppress the immune system, making the body more **susceptible to infections.**

Altered Metabolic Function:
Overtraining may disrupt the metabolic functions which can cause **malabsorption**. This disruption can lead to fatigue, decreased performance, and other metabolic issues.

Psychological Effects: **Disrupted Gut-brain Axis**
Changes in microbiota composition due to **exercise-induced stress** can impact hormone release, mood, and overall mental health.

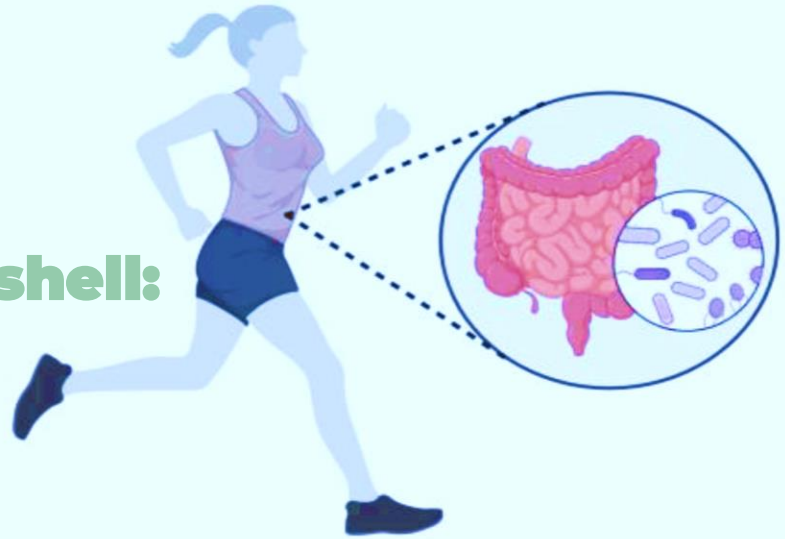


In A Nutshell:

Exercise on gut microbiota:

1. Increase microbiota diversity
2. Promote the production of SCFAs
3. Increase the abundance of beneficial bacteria

……



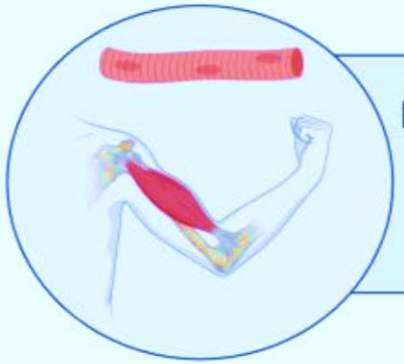
Other beneficial outcomes:

1. Promote nutrient absorption
2. Regulate energy metabolism
3. Improve gastrointestinal discomfort
4. Immunity enhancement

……

(Zhang *et al.*, 2024)

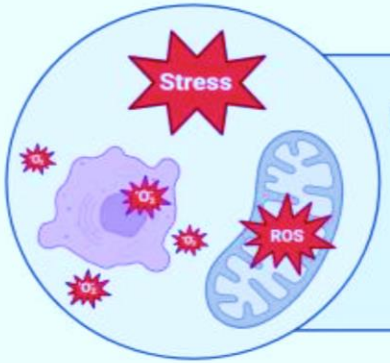
Role of Microbiota in Exercise:



1

Enhance skeletal muscle function

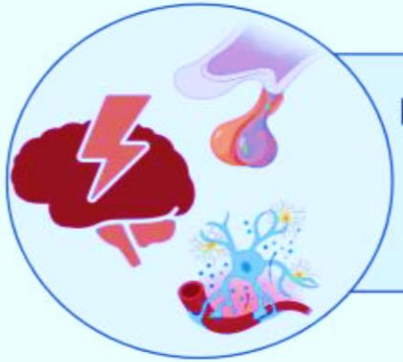
- Increase muscle mass
- Promote energy uptake
- Impact on protein synthesis



2

Reduce oxidative stress

- Alleviate oxidative stress
- Decrease ROS levels
- Increase antioxidant enzyme activity



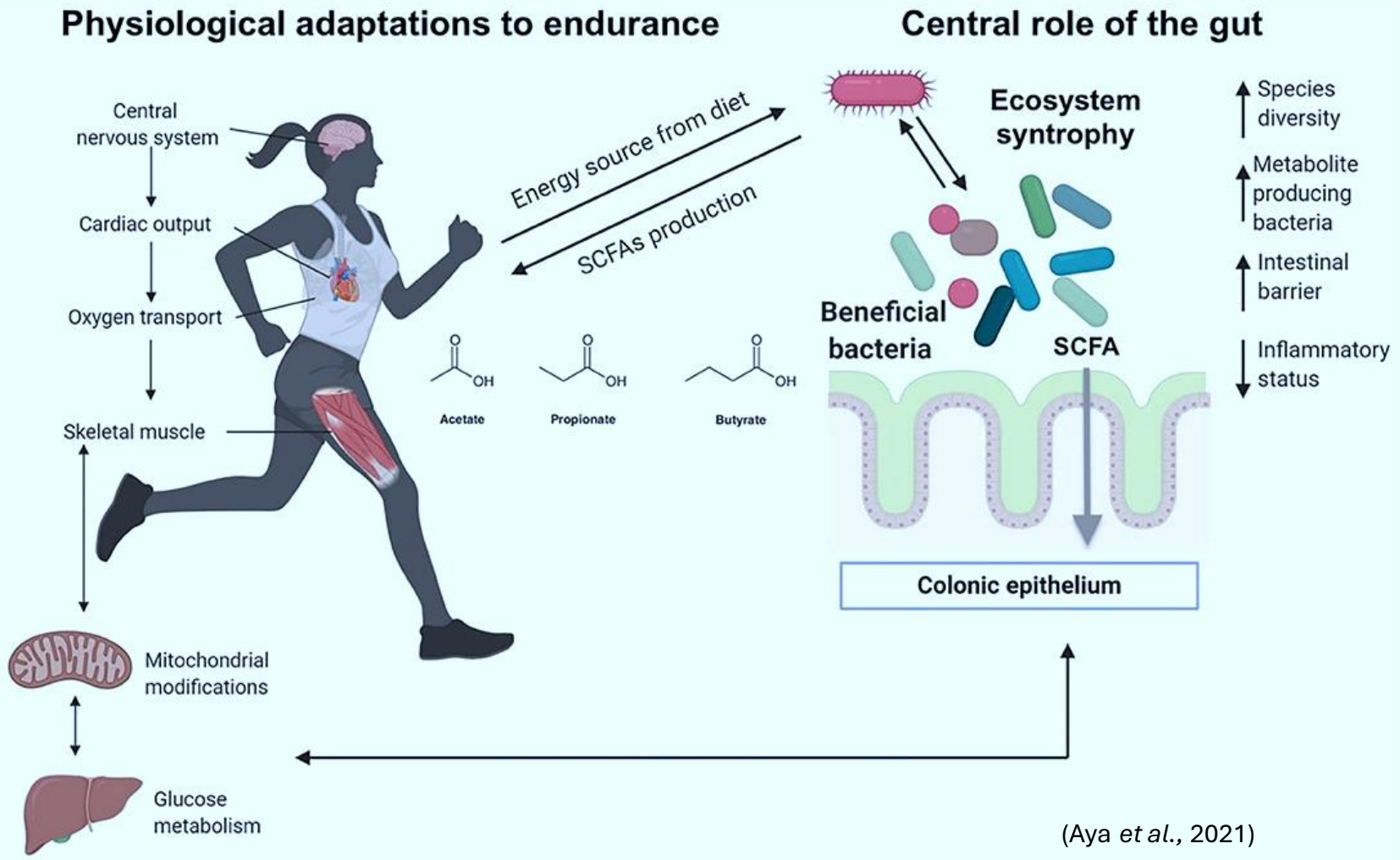
3

Regulate neuroendocrine system

- Reduce stress and anxiety
- Improve sleep quality
- Improve emotions

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

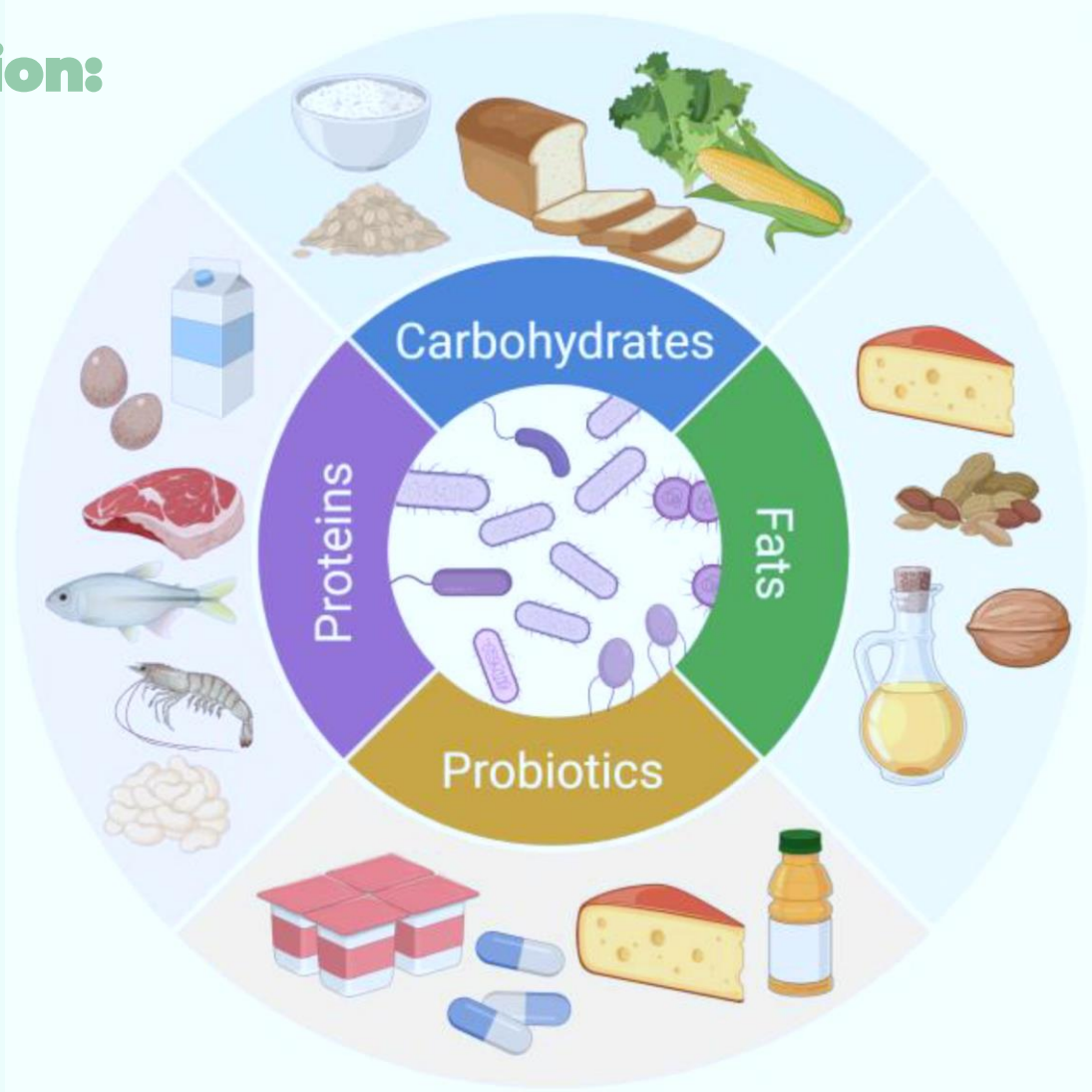
The Need for Balance



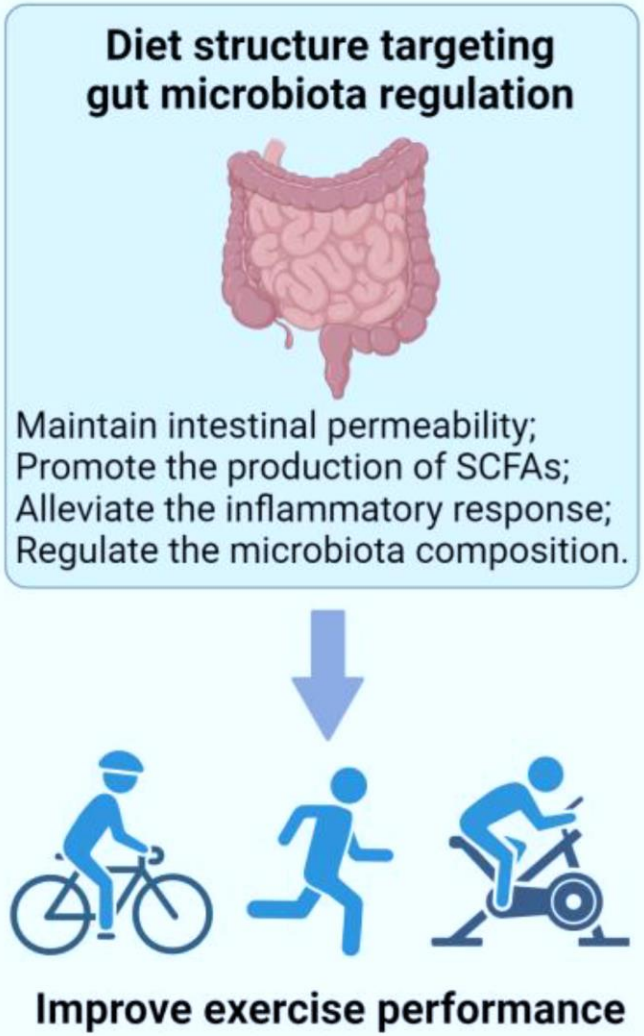
(Aya *et al.*, 2021)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

C. Nutrition:



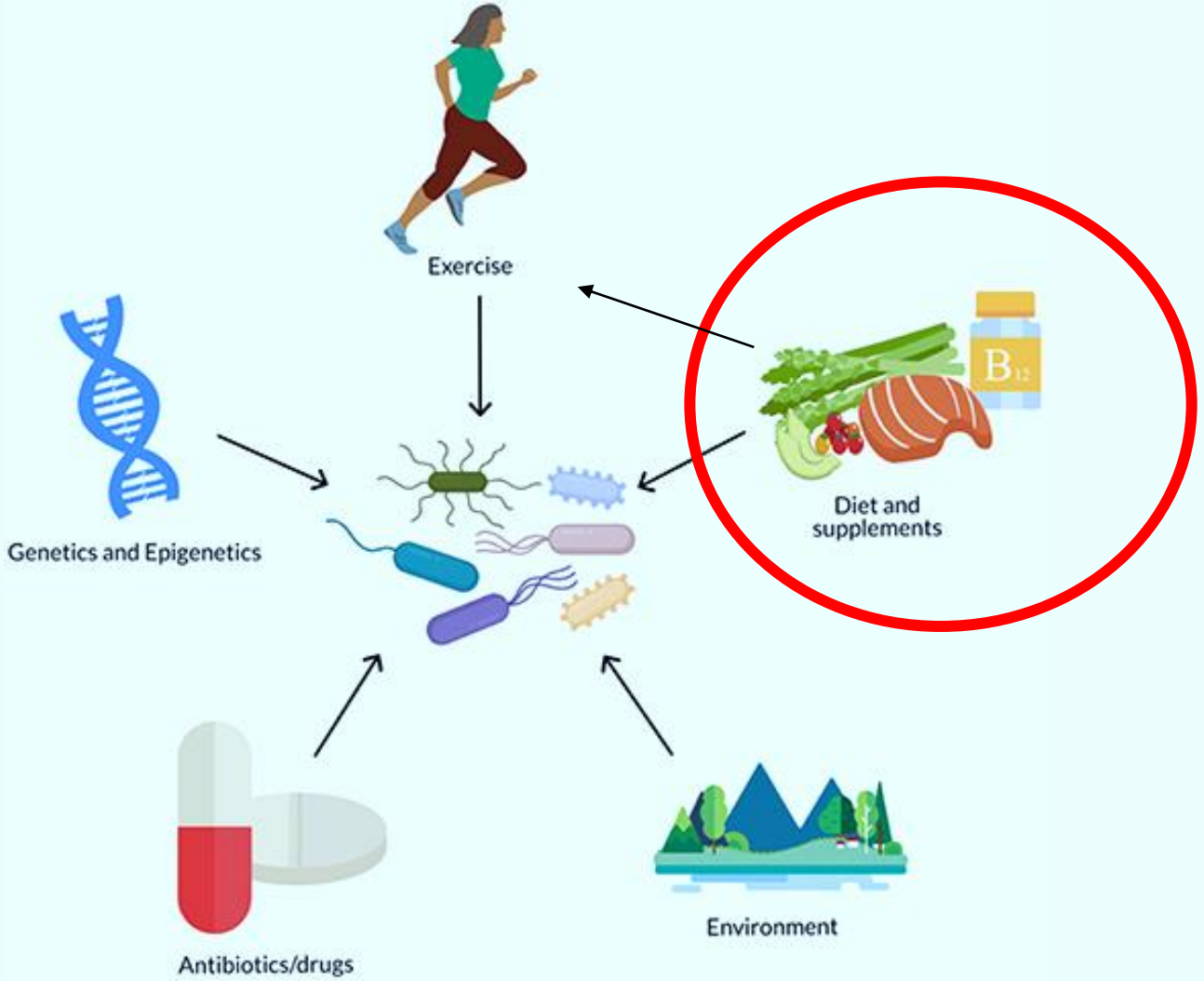
Dietary nutrient food sources



(Zhang *et al.*, 2024)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Impact of Diet on Performance and Gut Microbiota:



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

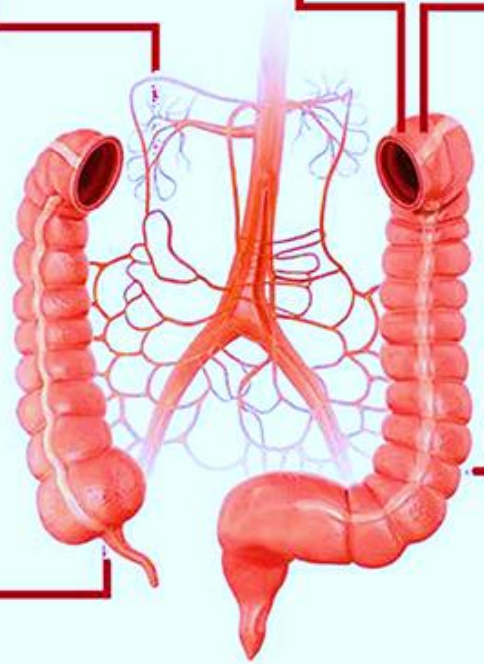
Macro - Micronutrients:

Protein | Fat | Fiber | Carbohydrates | Polyphenols

Bacteroidetes: ↓Rikenellaceae ↓Bacteroides spp ↑ Bactereoides enterotype
Firmicutes: ↓ Eubacterium rectale; ↑ Baltia coccoiudes; ↑ Ruminococcaceae; ↑ Bacili Erysispelotrichi; ↑ Roseburia spp
Proteobacteria:↓ Desulfovribrio

Bacteroidetes: ↑Bacteroides ↑ Prevotella spp.
Firmicutes: ↑ Bacili Erysispelotrichi, ↑ Roseburia spp. ↑ Clostridia; ↑ Enterococcus-Lactobacilus; ↑ Ruminococcus; ↑ F. prausnitzii; ↑ E. rectale coccoides
Actinobacteria: ↑ Biffidobacterium
Proteobacteria:↓ Desulfovribrio

Bacteroidetes:↑Bacteroides enterotype; ↓ Prevotella enterotype; ↑ Lactobacillus; ↑ Biffidobacterium, ↑ Rikenella; ↑ Peptostreptococcus;
Firmicutes: ↑ Clostridia
Deferribacteres:↑Mucisprillum
Proteobacteria:↑Desulfovribrio



Bacteroidetes: ↑ Prevotella enterotype
Firmicutes: ↑ Roseburia; Biffidobacteria; ↑ E. rectale; ↑ Ruminococcus ↑ Bromii; ↑ R. flavefaciens; ↑ R. alvus
Actinobacteria: ↑ Biffidobacterium

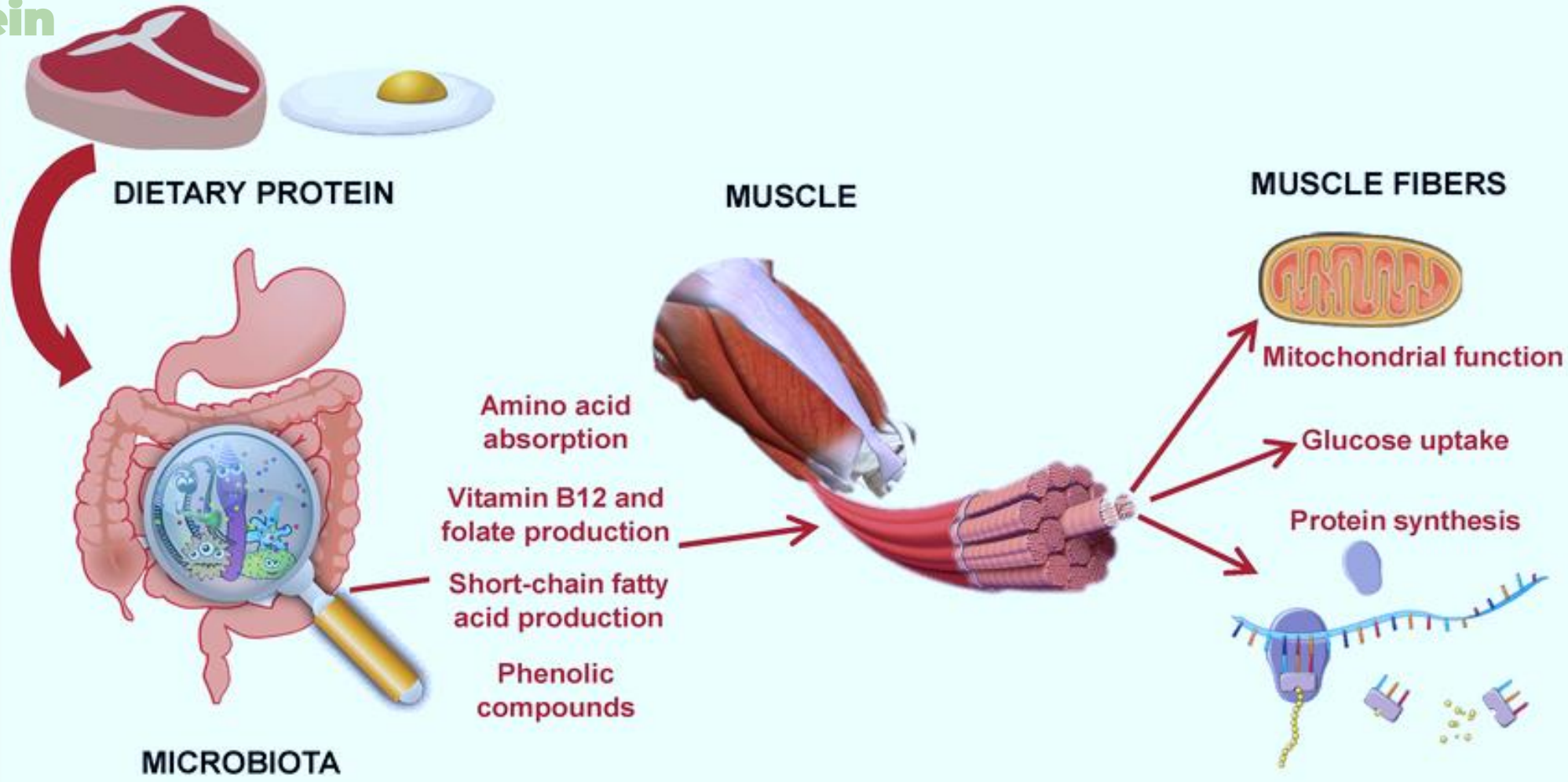
↑ Bacteroides spp.
↑ Lactobacillus spp.
↓ Clostridium Coccoides
↓ Eubacterium rectale
↓ E. coli
↑ Biffidobacterium spp

(Bessa *et al.*, 2021)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Macronutrients:

I. Protein



(Ticinesi *et al.*, 2019)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Macronutrients: II. Carbs

Small intestine

Host digestion *Host absorption*

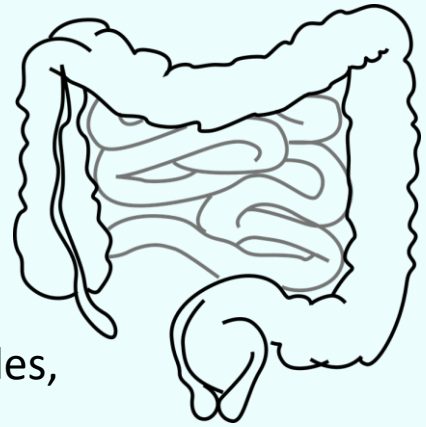
Digestible polysaccharides ➔ Monosaccharides (mostly glucose)
Ingested monosaccharides, lactose

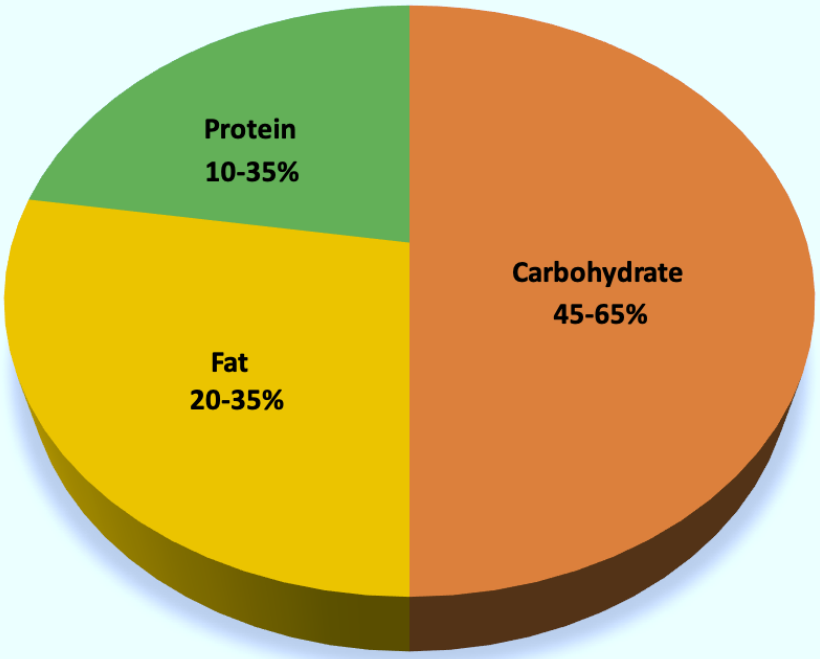
Large intestine

Microbial degradation *Host effects*

Fiber and resistant starch ➔ SCFA

HMOS ➔ Growth of Bacteroides, Bifidobacterium

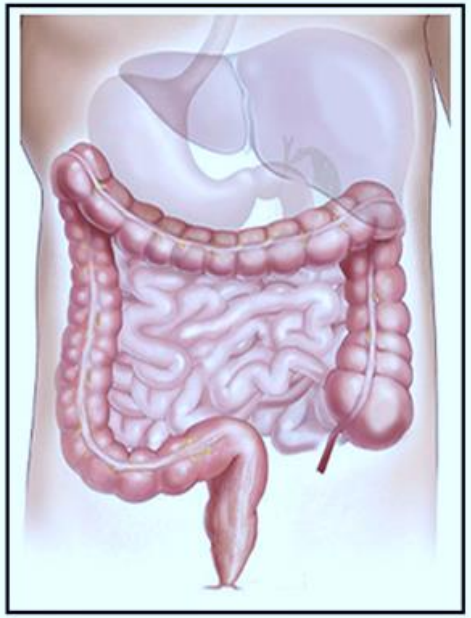




This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

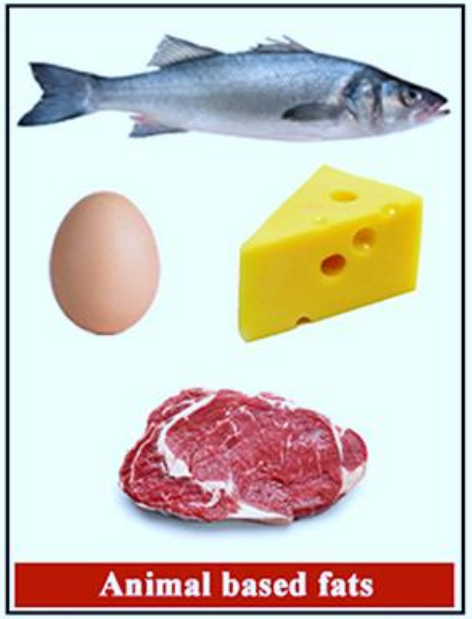
Macronutrients:
III. Fats



Human intestine

↑ *Bifidobacterium spp.*
Lactobbacillus spp.
SCFA producing bacteria ↓

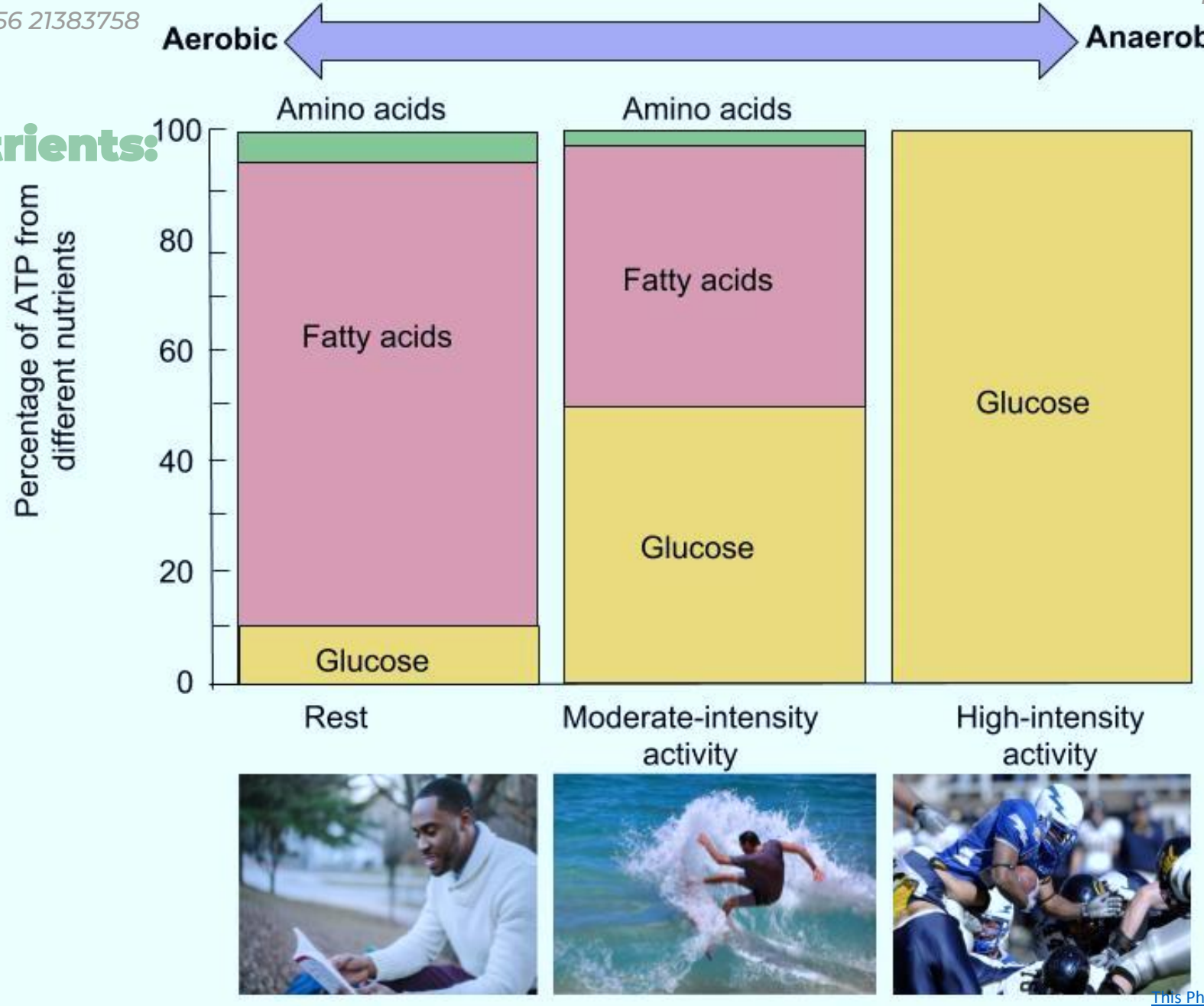
↓ *Clostridium perfringens*
Ruminococcaceae family
Enterococcus ↑



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Macronutrients:



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Micronutrients: I. Prebiotics & Probiotics

Probiotics

L. fermentum, L. plantarum, L. rhamnosus, L. acidophilus; B. bifidum, B. infantum:

- Modulate gut immunity
- Strengthen intestinal barrier

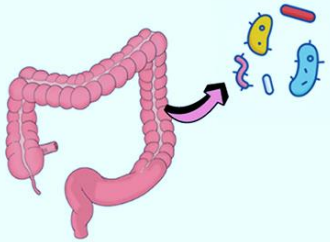
Dietary fiber

Butyrate:

Enhance metabolic health and exercise recovery by modulating gut microbiota, improving athletes' stress response and performance.

Mediate dietary effect on human body

- Fermentation of fiber to form butyrate



DIET GUT MICROBIOTA

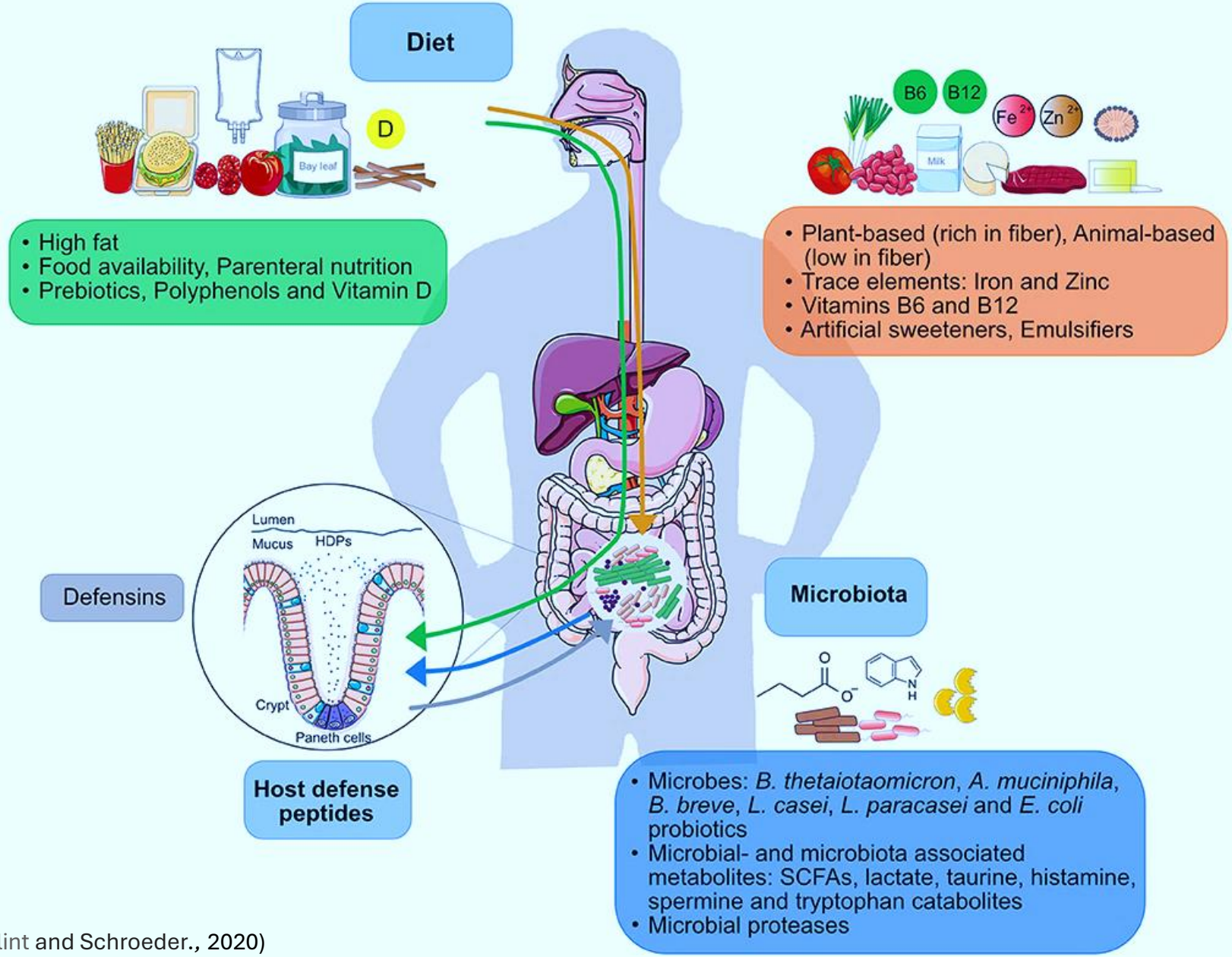
Exert dietary effect on human body

- Prevent colonisation by pathogenic bacteria
- Promote enrichment of Bacteroidetes, especially Prevotella
- Restore high-fat diet-induced dysbiosis, obesity and steatohepatitis

Optimal Athletic Performance

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Micronutrients: II. Vitamins



(Balint and Schroeder., 2020)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Micronutrients: III. Supplements

(SD Zeppa *et al.*, 2019)

Strong Evidence	Moderate or Emerging Evidence	Lack of Evidence
Antioxidants (Polyphenols) Probiotics Proteins	BCAA [1] L-Glutamine Sodium-Bicarbonate Vitamin D Omega-3 PUFAs [2] CHO [3]-Electrolytes Sport Drink L-Carnitine Caffeine	Creatine Taurine Beta-Alanine Beetroot Juice Collagen Glucosamine Vitamin C

[1] BCAA: Branched chain amino acids; [2] PUFAs: Polyunsaturated fatty acids; [3] CHO: Carbohydrates. Supplements are grouped based on their interaction with the gut microbiota: Strong (green), moderate/emerging (yellow), and lack of evidence (orange).

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

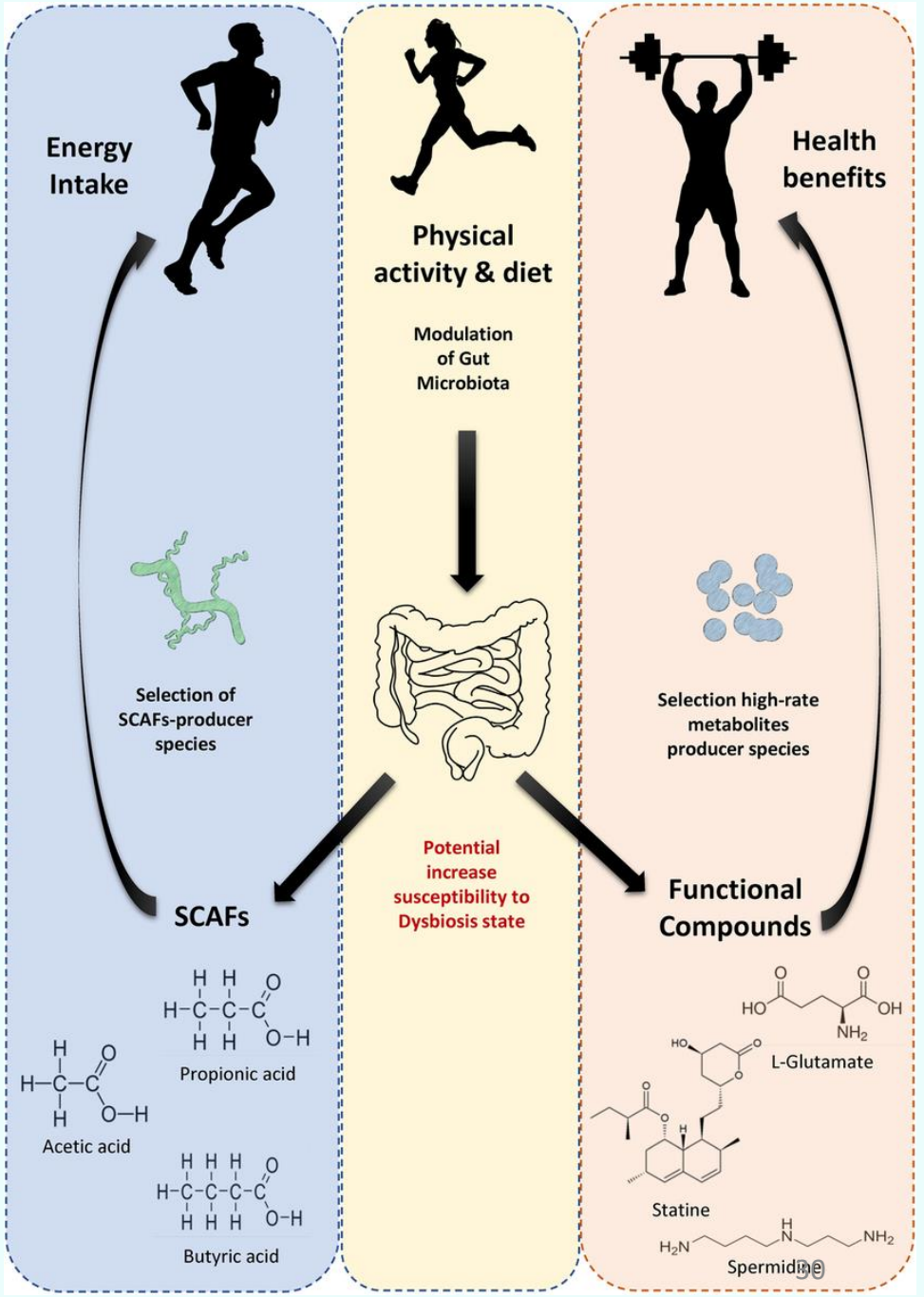
Micronutrients: III. Supplements

(Zhang *et al.*, 2024)

Intervention	Exercise Type	Study Model	Duration	Results	Reference
Lactobacillus plantarum PS128	Triathlon championship	Triathletes	3 weeks	TNF-α, IFN-γ, IL-8, and IL-6 cytokine levels ↓ IL-10 cytokines levels ↑ Oxidative stress ↓ Thioredoxin and component 5a ↑ Free amino acid content ↑	Huang et al., 2019
Lactobacillus plantarum PS128	Usual training	Triathletes	4 weeks	Acetic acid, propionic acid, and butyric acid ↑ Endurance capacity ↑ No effect on VO2max	Huang et al., 2020
Bifidobacterium longum subsp. longum Olympic No. 1 (OLP-01)	Usual training	Well-trained runners	5 weeks	12-min Cooper's test running distance ↑ No change in the abundance of the gut microbiota.	Lin et al., 2020
Commercially available probiotic (PRO)	Usual training	Male elite cyclists	90 days	The incidence and severity of gastrointestinal tract symptoms ↓ No significant changes in VO2max, mean levels of IL-6, or TNF-α.	Pugh et al., 2020
Lactobacillus casei	Usual training	Male badminton players	6 weeks	Anxiety and stress levels ↓ Aerobic capacity ↑ No significant difference in energy intake and macronutrients.	Salleh et al., 2021
Probiotic blend	Usual training	Male elite cyclists	6 weeks	Incidence of gastrointestinal Symptoms ↓ No significant changes in IL-6 and TNF-α levels.	Schreiber et al., 2021
Probiotic (Ultra biotic 60TM) and *Saccharomyces boulardii*	During competition season	Elite male rugby athletes	17 weeks	Muscle soreness and leg heaviness scores ↓ Sleep quantity, quality, and motivation scores ↑	Harnett et al., 2021
Probiotic (SANPROBI BARRIER)	During a marathon	Runners	3 months	Incidence of constipation ↓ Sodium, potassium, iron concentration, and HDL cholesterol ↑ LDL cholesterol and triglyceride concentration ↓	Smarkusz-Zarzecka et al., 2022
Yogurt with *Bifidobacterium animalis* ssp. lactis BB-12	Usual training	Female taekwondo athletes	8 weeks	L-arginine biosynthesis I, fatty acid biosynthesis and oxidation, and L-isoleucine biosynthesis III pathways ↑ Tyrosine degradation I ↓	Zhu et al., 2023
Heat-killed *Lactiplantibacillus plantarum* TWK10	Fixed-intensity exercise challenge	Healthy males	6 weeks	Exercise endurance time ↑ Muscle weight and muscle strength ↑ Serum lactate and ammonia levels during exercise ↓	Cheng et al., 2023

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Dietary Pattern and Performance:



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

D. Future Direction for Research:

BioArte Analysis technology analyses microbial biology and physiology to provide personalized recommendations for optimal body function, potentially aiding in chronic disease treatment and sports performance enhancement.

Probiotic Strains:

- Research is needed to identify specific probiotics that enhance athletic performance and recovery, focusing on **optimal strains and dosages** for different sports.

Microbiome Interactions

- Further studies are essential to explore how **microbiota may synergize with some drugs** and modulate their efficacy in chronic diseases. response to a drug.

Personalized Nutrition

- Establish personalized nutrition plans that **consider individual microbiome profiles**, optimizing dietary recommendations for athletes based on gut composition.

Focused Research Approach

- Longitudinal studies are necessary to investigate the **long-term effects of specific sports and diet** on gut health, performance, and overall well-being in athletes.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Key Takeaways:

The Gut Microbiome

Including *Veillonella atypica,* plays a crucial role in converting food into fuel and maintaining metabolic processes during exercise and recovery, potentially **optimizing overall athletic performance.**

Exercise

Has been found to **alter the composition of gut microbiota**, as evidenced by studies using different training and hypoactivity models.

Nutritional interventions

Promote physical activity and synergize with gut microbiota, highlighting the importance of **balancing nutrition** for optimal health and physical performance.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

1. Scheiman, J., Luber, J.M., Chavkin, T.A., MacDonald, T., Tung, A., Pham, L.D., Wibowo, M.C., Wurth, R.C., Punthambaker, S., Tierney, B.T. and Yang, Z., 2019. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. *Nature medicine*, *25*(7), pp.1104-1109.
2. Clark, A. and Mach, N., 2016. Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes. *Journal of the International Society of Sports Nutrition*, *13*, pp.1-21.
3. Humińska-Lisowska, K., Zielińska, K., Mieszkowski, J., Michałowska-Sawczyn, M., Cięszczyk, P., Łabaj, P.P., Wasąg, B., Frączek, B., Grzywacz, A., Kochanowicz, A. and Kosciolek, T., 2024. Microbiome features associated with performance measures in athletic and non-athletic individuals: A case-control study. *Plos one*, *19*(2), p.e0297858.
4. Aya, V., Flórez, A., Perez, L. and Ramírez, J.D., 2021. Association between physical activity and changes in intestinal microbiota composition: A systematic review. *PLoS One*, *16*(2), p.e0247039.
5. Donati Zeppa, S., Agostini, D., Gervasi, M., Annibalini, G., Amatori, S., Ferrini, F., Sisti, D., Piccoli, G., Barbieri, E., Sestili, P. and Stocchi, V., 2019. Mutual interactions among exercise, sport supplements and microbiota. *Nutrients*, *12*(1), p.17.
6. Sales, K.M. and Reimer, R.A., 2023. Unlocking a novel determinant of athletic performance: The role of the gut microbiota, short-chain fatty acids, and “biotics” in exercise. *Journal of Sport and Health Science*, *12*(1), pp.36-44.
7. Boytar, A.N., Skinner, T.L., Wallen, R.E., Jenkins, D.G. and Dekker Nitert, M., 2023. The effect of exercise prescription on the human gut microbiota and comparison between clinical and apparently healthy populations: a systematic review. *Nutrients*, *15*(6), p.1534.
8. Boisseau, N., Barnich, N. and Koechlin-Ramonatxo, C., 2022. The nutrition-microbiota-physical activity triad: an inspiring new concept for health and sports performance. *Nutrients*, *14*(5), p.924.
9. Gizard, F., Fernandez, A. and De Vadder, F., 2020. Interactions between gut microbiota and skeletal muscle. *Nutrition and Metabolic Insights*, *13*, p.1178638820980490.

Further readings:

10. Lensu, S. and Pekkala, S., 2021. Gut microbiota, microbial metabolites and human physical performance. *Metabolites*, *11*(11), p.716.

11. Zhang, L., Li, H., Song, Z., Liu, Y. and Zhang, X., 2024. Dietary Strategies to Improve Exercise Performance by Modulating the Gut Microbiota. *Foods, 13*(11), p.1680

12. Bessa, C., Francisco, T., Dias, R., Mateus, N., Freitas, V.D. and Pérez-Gregorio, R., 2021. Use of polyphenols as modulators of food allergies. From chemistry to biological implications. *Frontiers in Sustainable Food Systems*, *5*, p.623611.

13. Ticinesi, A., Nouvenne, A., Cerundolo, N., Catania, P., Prati, B., Tana, C. and Meschi, T., 2019. Gut microbiota, muscle mass and function in aging: a focus on physical frailty and sarcopenia. Nutrients, 11(7), p.1633.

14. Puértolas-Balint, F. and Schroeder, B.O., 2020. Does an apple a day also keep the microbes away? The interplay between diet, microbiota, and host defense peptides at the intestinal mucosal barrier. Frontiers in immunology, 11, p.1164.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

Exploring the Connection Between Sports, Gut Microbiome and Nutrition:
Impact on Health and Performance.

Key Points:

This presentation provides a brief overview of the microbiota, sports, and nutrition triangles in order to better understand their relationship to improved overall health and athletic performance.

- Gut microbiota composition plays a key role in the health and athletic performance
- Analysis of literature data shows that athletes' gut microbiota exhibits greater diversity and is influenced by exercise, highlighting the impact of athletic performance on microbiota modulation.
- The role of diet in enriching microbiota composition and enhancing sports performance.

A. Gut Microbiota

- The gut microbiota as a real “orchestra conductor” in the host’s physio(patho)logy due to its implications in many aspects of health and disease.

- Technological advancements in sequencing, bioinformatics, and quantification have enabled population-level studies on human microbiota, aiding in understanding its role in physiology and functional imbalance in chronic pathologies.

- The human gut microbiota primarily consists of two major phyla: *Bacteroidetes* and *Firmicutes*, along with less prevalent phyla such as *Actinobacteria* and *Verrucomicrobia* (5)

  ⇨ Here is a quick overview of the role of microbiota:

Physiological Roles

I. Intestinal Homeostasis:

- **Barrier Function**: for protecting against pathogens and maintaining overall gut health and immune function.(8)
- **Nutrient Absorption:** The gut microbiota aids in the absorption of nutrients, which is essential for recovery and performance. A diverse and balanced microbiome can enhance the bioavailability of nutrients from food, supporting energy levels and recovery processes (2)

II. **Metabolism:**

The gut microbiota plays a critical role in controlling oxidative stress, inflammatory responses, metabolism, and energy expenditure especially during intense exercise (6)

Pathological Roles

I. **Dysbiosis**:

Dysbiosis refers to the qualitative and/or functional alteration of the intestinal microbiota. It is implicated in the chronicity of various diseases such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), metabolic diseases (e.g., type 2 diabetes, obesity), depression, **Microbial Disruptions demonstrated to affect skeletal muscle function and limit exercise capacity. (7)**

II. **Inflammation:**

The gut microbiota plays a role in natural inflammation observed in the digestive tract. While a low level of inflammation is essential for immune activation and microbiota control, significant inflammatory reactions can be triggered by pathogenic species or strenuous exercise (8)

III. **Immune function:**

The gut microbiota can program the mucosal immune system, which is vital for athletes who are often exposed to physical stressors that can compromise immune function (2). Affecting susceptibility to respiratory tract infections (URTI), which are common in athletes during intense training or competition (6)

The Gut-Brain Axis:

- Microbiota metabolites can diffuse through the intestinal wall, directly modulate the ENS, and indirectly affect the CNS.
- Intestinal dysbiosis in neurodevelopmental disorders and neurodegenerative diseases may contribute to both digestive and neurological symptoms. (8)
- **Stress Response Modulation**: The gut microbiota interacts with the gut-brain axis, influencing the body's stress response. Changes in microbiota composition due to exercise-induced stress can affect hormone release, mood, and overall mental health, which are critical for optimal performance (2)

The Gut-Muscle Axis:

Some scholars studied the Gut Microbiota and Skeletal Muscle mechanisms of Interaction:

1 - Gut bacteria influence skeletal muscle through various pathways, including the regulation of inflammatory and redox pathways, nutrient availability, metabolite production, anabolic and catabolic processes, mitochondrial biogenesis, and immune system regulation. (9)

2 - The first comprehensive review linking intestinal microbiota to muscle atrophy was published by Bindels and Delzenne's 2020, linked intestinal microbiota to muscle atrophy, with subsequent studies revealing functional connections between gut microbiota and skeletal muscles, such as muscle lipogenesis and structure changes in mice.(8)

3 - Gut microbiota depletion (e.g., via antibiotic treatment) negatively impacts muscle endurance and glucose homeostasis, Mice treated with broad-spectrum antibiotics have lower treadmill run times during time-to-exhaustion (TTE) trials.(6) effects which can be reversed by reintroducing natural microbiota. (8)

4 - One possible explanation for the association between butyrate-producing bacteria and increased power output during high-intensity exercise is that

butyrate, a SCFA, is known to induce positive changes in skeletal tissues. (3) Examples butyrate-producing bacteria include:

- **Blautia wexlerae** - shown positive correlations with performance measures
- **Eubacterium rectale** - associated with beneficial effects on gut health and metabolism
- **Intestinimonas timonensis**
- **Roseburia hominis** - linked to anti-inflammatory properties and is enriched in the microbiomes of endurance athletes

Understanding the link between muscle and microbiota will lead us to discuss the role of the microbiota in exercise.
The relationship between gut microbiota and exercise appears to be bidirectional. a well-balanced gut microbiota can enhance exercise performance by improving metabolic processes crucial for endurance and overall physical capacity.

A -Role of Microbiota in Exercise

Performance Enhancement (5) The status of gut microbiota can influence exercise performance through various physiological and metabolic pathways. mechanisms:

1. Antioxidant Activity: Enhanced performance has been related to the antioxidant enzyme system, which is regulated by gut bacteria**. Polyphenol Metabolism:** The gut bacteria metabolises non-extractable polyphenols, which are not readily absorbable in their natural state, into bioactive chemicals. These metabolites have anti-inflammatory and free radical-scavenging properties, which contribute to the host's antioxidant capacity **(5).**

2- Short-Chain Fatty Acids (SCFAs): Gut bacteria produce short-chain fatty acids (SCFAs) that regulate metabolism in organs like skeletal muscle and enhance endurance exercise ability.

- Studies show that SCFAs, particularly butyrate, aid in endurance sports. help maintain energy levels after prolonged physical exertion. **(3)**
- SCFAs, produced by Specific strains such as Bifidobacterium and Lactobacillus can improve exercise performance by improving endurance via reducing serum lactate levels. For example **Bifidobacterium adolescentis**, probiotic-like bacteria, are linked to VO2 max, suggesting a connection between these bacteria and enhanced aerobic performance. (6)

3- Metabolic Conversion: The study shows a natural, microbiome-encoded enzymatic pathway that improves athletic performance by converting lactate to propionate in the presence of **Veillonella atypica**,. Propionate offers various advantages, including acting as an energy source for colon cells, influencing immunological responses, and perhaps boosting exercise performance by increasing metabolic efficiency. This reveals a symbiotic interaction between the microbiota and the host, especially among physically active people. ***Veillonella atypica**: This bacterium has been demonstrated to enhance exhausting treadmill run duration in mice by converting exercise-induced lactate to propionate, a beneficial short-chain fatty acid**. (1)**

4- Glycogen Storage:
Bifidobacterium longum and Lactobacillus salivarius can improve exercise performance by increasing muscle and liver glycogen storage capacity and GLUT4 glucose transporter type 4 expression in skeletal muscle, which supports energy demands during exercise via gluconeogenesis. (6)

- Vice versa, exercise can positively influence gut microbiota composition, leading to a healthier gut environment

B. <u>Athletic performance impact on Gut microbiota:</u>
The analysis of literature data on this topic shows that the gut microbiota of athletes is different from that of other populations and displays higher microbial diversity (Table 1). (Table 2) **(8)**

<u>Effects of exercise on gut microbiota modulation:</u>
Exercise has a significant impact on gut microbiota composition, affecting various

aspects of microbial diversity and richness. Here are the key findings from recent studies: (2)

1. **Impact on Diversity**:
   Training Modalities and Specific Microbial Changes:
   Different training modalities, such as spontaneous exercise, high-intensity interval training (HIIT), and moderate-intensity continuous training (MICT), induce distinct changes in gut microbiota
   - **HIIT** tends to increase the Bacteroidetes/Firmicutes ratio, which is often associated with improved metabolic health. It also raises the levels of bacterial families such as Clostridiaceae, Lachnospiraceae, and Ruminococcaceae
   - **MICT** tends to affect different bacterial taxa compared to HIIT, such as increasing Parasutterella excrementihominis and Lactobacillus johnsonii (8)

2. **Enrichment of Beneficial species**: studies suggest that athletes exhibit higher levels of alpha diversity. For instance, aerobic exercise has been shown to increase the abundance of ***Bacteroides*** in healthy elderly women thought to be beneficial for overall health and performance. (3)
   Professional athletes, such as rugby players, have been observed to have higher alpha diversity in their gut microbiota compared to sedentary individuals. This increased diversity is associated with better gut health (5)

3. **Influence on Inflammation**: changes in microbial composition due to exercise can influence the levels of pro-inflammatory and anti-inflammatory cytokines, impacting recovery and performance.

4. **Gut-Brain Axis Interaction**: Exercise-induced changes in gut microbiota can affect neurotransmitter production (e.g., endorphines released by PA, serotonin, and dopamine), which may help mitigate stress and anxiety in athletes. (2)

5. **Nutrient Utilization**: Nutrient flow critical for energy production and recovery in athletes, is maintained by exercise which improves the gut microbiota's bioavailability (ability to absorb and utilise nutrients). (4)

Mechanisms of Modulation: (5)

Exercise has the potential to alter the composition of the gut microbiota through:

1- Altered Gut Transit Time

2- Modify Bile Acids Profile

3- SCFA Production

4- Immune Modulation: by increasing immunoglobulin A (IgA) levels, as well as the number of B and CD4+ T cells

5- Heat Shock Proteins: Regular exercise reduces the response to heat stress, preventing the breakdown of tight junction proteins between intestinal epithelial cells, thereby enhancing gut barrier resilience

Risks of Overtraining: based on the systematic review and related literature: (**7**) moderate to high-intensity exercise can be beneficial, but excessive or overtraining can negatively impact gut microbiota and overall performance via the following mechanisms:

1. **Disturbance of Gut Microbiota**: High-intensity exercise can disrupt the gut microbiota, potentially reducing beneficial bacteria and increasing harmful ones, potentially affecting gut health and overall well-being.
2. **Increased Intestinal Permeability**: Overtraining can lead to increased intestinal permeability, often referred to as "leaky gut." This condition allows toxins and bacteria to enter the bloodstream, potentially leading to systemic inflammation and other health issues
3. **Immunosuppression**: Intense and prolonged training can suppress the immune system, making the body more susceptible to infections. This immunosuppression can also affect the gut microbiota balance
4. **Altered Metabolic Function**: Overtraining may disrupt the metabolic functions of gut bacteria, which can impact nutrient absorption and energy

metabolism. This disruption can lead to fatigue, decreased performance, and metabolic issues

5. **Psychological Effects**: has been shown to affect gut microbiota composition, potentially leading to a cycle of negative health outcomes.

Need for Balance: The review emphasizes the importance of finding a balance in exercise intensity and volume.

C. Nutrition: (8) (5)

The impact of diet on performance and gut microbiota is significant, as dietary choices can influence both. Key points from the review regarding this relationship include: (2)

1. **Microbiota Composition**: high-fiber diets promoting beneficial bacteria growth.
2. **Inflammation and Recovery:** anti-inflammatory foods promoting faster recovery and better performance, while high-fat, sugar-rich diets may worsen inflammation.
3. **Gut-Brain Axis:** Dietary choices that support a healthy microbiome can enhance mental well-being, reduce anxiety, and improve focus, all of which are important for optimal athletic performance

- The gut produces hormones like GABA and neuropeptide Y, while microbiota secretes tryptophan, modulating neurotransmitters. (5)
- Dopamine production in the GI tract during exercise-induced stress is influenced by factors like tyrosine levels, stress type, sex, and enteric bacteria. (5)

Influence of Macro- and Micro-nutrients on Gut Microbiota Composition

The composition of gut microbiota is significantly influenced by the consumption of various macro- and micro-nutrients. Here's a detailed overview based on the referenced studies:

Macronutrients

1. **Fats**
    - **High-Fat Diet (HFD):** Consuming a high-fat diet significantly reduces the fecal concentration of short-chain fatty acids (SCFA), such as butyrate, and decreases the population of **Bifidobacteria** and increase the total anaerobic microflora and **Bacteroides**
    - **Fat Quality:** The type of fat also plays a crucial role. n-3 PUFAs have anti-inflammatory properties and beneficially modulate gut microbiota
2. **Proteins**
    - **Diversity and Specific Strains:** Protein consumption is positively correlated with overall microbial diversity. For example, whey and glycated pea protein supplementation can increase beneficial bacteria like **Bifidobacterium** and **Lactobacillus** while decreasing pathogenic bacteria such as Bacteroides fragilis and Clostridium perfringens
    - **SCFA Production:** Pea protein consumption stimulates SCFA production, which aids in maintaining the mucosal barrier
3. **Carbohydrates**
    - **Digestible Carbohydrates:** These include starches and sugars (glucose) that are broken down in the small intestine. Anaerobic metabolism uses **glucose** as its only source of **fuel**
    - **Non-Digestible Carbohydrates:** They serve as the main energy source for gut bacteria, influencing gut microbiota composition and function. Fibers are considered **prebiotics** because they selectively stimulate the growth or activity of beneficial microorganisms.
        - A fiber and whole-grain-rich diet for six weeks increased **Prevotella** abundance, which is predictive of weight loss (5)

Micro-nutrients

While the referenced clinical studies do not clearly focus on micro-nutrients impact, it is understood that supplements and vitamins also play a key role in gut microbiota composition by supporting various metabolic functions and maintaining overall gut health.

1- **Prebiotics and Probiotics**: (2)
   - **Prebiotics** Regular exercise may enhance the effectiveness of by promoting a more favorable gut environment for beneficial bacteria to thrive. This interaction can lead to improved metabolic health and better recovery from exercise. The modulation of gut microbiota through prebiotics may improve the stress response in athletes, potentially leading to better performance and recovery.
   - **Probiotics** Regular consumption can enhance immune function, reduce infections, and support recovery from stress. Gut health can also influence mood and mental well-being, alleviating anxiety and depression symptoms. Probiotic supplementation can reduce gastrointestinal complaints, especially during intense training or competition. However, the effects of probiotics can vary significantly, necessitating personalized approaches for optimal benefits. **Dietary Recommendations**: Specific recommendations for dosages and strains of probiotics for athletes are still being established.
   - **Synergistic Benefits:** The combination of prebiotics and probiotics (often referred to as synbiotics) can have synergistic effects, enhancing the survival and colonization of probiotics in the gut and promoting a healthier microbiome overall.
2- **Vitamins: (7)**

   - **Vitamins A, D, E, and K** are essential for gut health, influencing gut microbiota composition, nutrient absorption, and immune function.
   - **Vitamin D** plays a crucial role in immune response modulation and maintaining a healthy gut microbiome, promoting better health outcomes through a diverse gut microbiome.
   - **B Vitamins**: B vitamins, including B6, B12, folate, and riboflavin, are crucial for energy metabolism, exercise performance, neurotransmitter synthesis, and gut health by supporting beneficial bacteria growth.

- **Antioxidant Vitamins**: Vitamins C and E, with their antioxidant properties, can mitigate oxidative stress linked to high-intensity exercise, potentially safeguarding gut microbiota from potential damage.

Supplementation Considerations: Excessive vitamin supplementation can cause gut microbiota imbalances, potentially leading to dysbiosis, as certain vitamins may promote the growth of certain bacterial strains at the expense of others.

Dietary Pattern and Performance:

Dietary Sources: A balanced diet, including fruits, vegetables, whole grains, and lean proteins, is crucial for athletes for optimal nutrition during training and recovery.

Most studies emphasize the importance of individual dietary patterns in relation to specific sports. For example, endurance athletes may benefit from higher carbohydrate intake to support energy needs, while strength athletes may require more protein for muscle repair and growth. (3)

Personalized Nutrition: Tailoring dietary interventions to support a healthy microbiome may enhance performance, recovery, and overall health.

Research and Future Directions

1. **Research Needs**:

- There is still a need for more specific dietary recommendations regarding dosages and strains of probiotics for athletes, as current guidelines are not well established
- Microbiota may synergize with some drugs and modulate their efficacy in chronic diseases. Future microbiota-based tests to predict each patient's response to a drug.

2. **Futuristic Approach:**

- Potential for Health Interventions: Understanding how exercise influences the microbiome opens avenues for potential health interventions, such as using structured PA as a strategy to improve gut health and manage conditions like IBS, obesity and metabolic diseases (3)

- *BioArte* Analysis technology analyzes microbial biology and physiology, providing personalized recommendations tailored for optimal body function, potentially aiding in chronic disease treatment and sports performance optimization.

Key Takeaways

1. **The gut microbiome** is crucial for converting food into fuel for athletes and maintaining metabolic process throughout exercise and recovery. Ex: Veillonella atypica, is believed to contribute to gut health by producing SCFAs with anti-inflammatory properties, potentially optimizing athletic performance and metabolic health
2. **Exercise** modulates gut microbiota composition. Studies using both training and hypoactivity models have shown changes in gut bacteria due to physical activity
3. **Nutritional interventions** physical activity and have synergistic effects on gut microbiota. **Balancing Nutrition** is a crucial element to maintain optimal health and physical performance

The ultimate goal is to determine which combination of these three components works best to improve sports performance, postpone the start of illness, or

optimize treatment outcomes. The numerous opportunities provided by biotic supplements and training methods create new directions for investigation in the future.

References:

1. Scheiman, J., Luber, J.M., Chavkin, T.A., MacDonald, T., Tung, A., Pham, L.D., Wibowo, M.C., Wurth, R.C., Punthambaker, S., Tierney, B.T. and Yang, Z., 2019. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. *Nature medicine*, *25*(7), pp.1104-1109.
2. Clark, A. and Mach, N., 2016. Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes. *Journal of the International Society of Sports Nutrition*, *13*, pp.1-21.
3. Humińska-Lisowska, K., Zielińska, K., Mieszkowski, J., Michałowska-Sawczyn, M., Cięszczyk, P., Łabaj, P.P., Wasąg, B., Frączek, B., Grzywacz, A., Kochanowicz, A. and Kosciolek, T., 2024. Microbiome features associated with performance measures in athletic and non-athletic individuals: A case-control study. *Plos one*, *19*(2), p.e0297858.
4. Aya, V., Flórez, A., Perez, L. and Ramírez, J.D., 2021. Association between physical activity and changes in intestinal microbiota composition: A systematic review. *PLoS One*, *16*(2), p.e0247039.
5. Donati Zeppa, S., Agostini, D., Gervasi, M., Annibalini, G., Amatori, S., Ferrini, F., Sisti, D., Piccoli, G., Barbieri, E., Sestili, P. and Stocchi, V., 2019. Mutual interactions among exercise, sport supplements and microbiota. *Nutrients*, *12*(1), p.17.
6. Sales, K.M. and Reimer, R.A., 2023. Unlocking a novel determinant of athletic performance: The role of the gut microbiota, short-chain fatty acids, and “biotics” in exercise. *Journal of Sport and Health Science*, *12*(1), pp.36-44.
7. Boytar, A.N., Skinner, T.L., Wallen, R.E., Jenkins, D.G. and Dekker Nitert, M., 2023. The effect of exercise prescription on the human gut microbiota and comparison between clinical and apparently healthy populations: a systematic review. *Nutrients*, *15*(6), p.1534.

8. Boisseau, N., Barnich, N. and Koechlin-Ramonatxo, C., 2022. The nutrition-microbiota-physical activity triad: an inspiring new concept for health and sports performance. *Nutrients*, *14*(5), p.924.

Further readings:

https://www.mysportscience.com/post/gut-microbiota-and-exercise-performance

9. Gizard, F., Fernandez, A. and De Vadder, F., 2020. Interactions between gut microbiota and skeletal muscle. *Nutrition and Metabolic Insights*, *13*, p.1178638820980490.

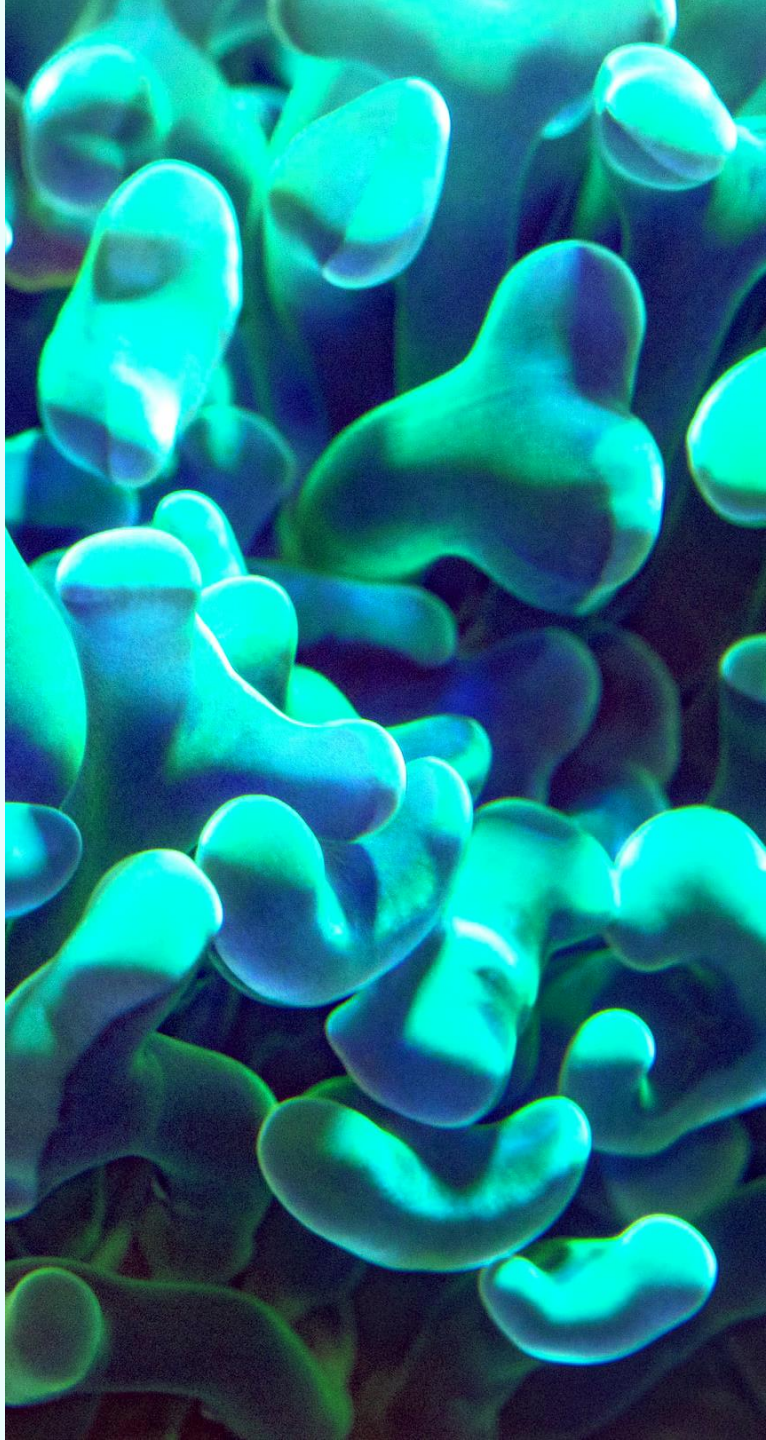
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Post-graduate certificate level 7 (30 ECTS)

Nutrition-Micorbiome Interplay in AUTISM

Eman Salah Zaky
MSc., MBBCH., RD
Clinical Nutrition and Health Consultant

Malta Further & Higher Education Authority



Exploring therapeutic interventions and nutritional plans that may impact the symptoms of autism.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

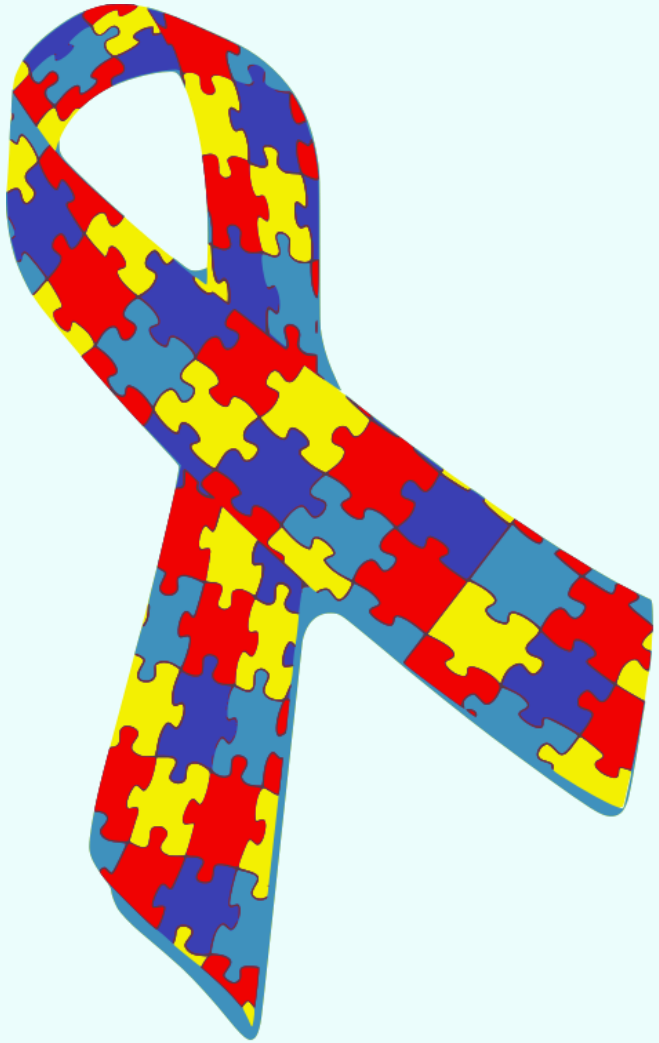
Content and Learning Objectives

1. Understanding the diagnostic criteria for Autism Spectrum Disorder (ASD) and the role microbiota in its etiology

2. Investigating how the gut microbiota affects the gut-brain axis and how it might affect Autistic people

3. Exploring Therapeutic interventions and nutritional plans that may influence the symptoms of autism.

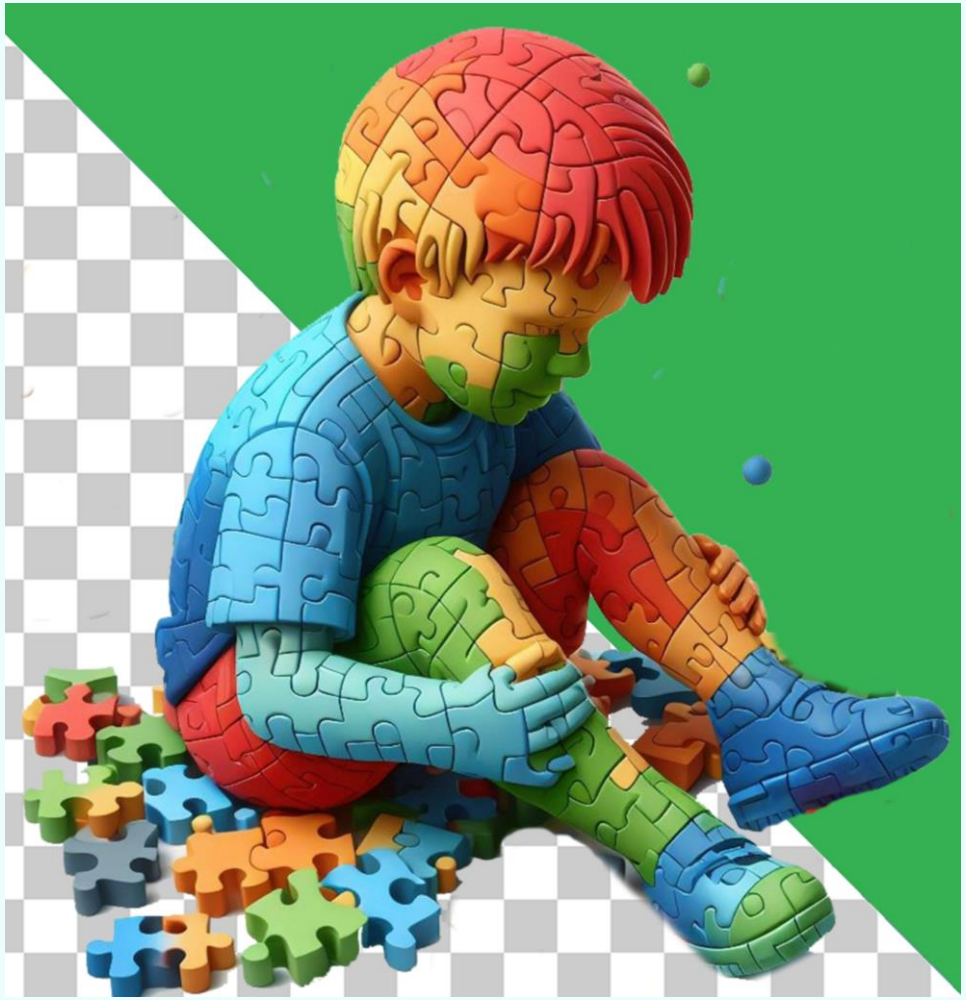
4. Considering implications for future Interventions based on personalised nutrition plans and microbiome analysis.

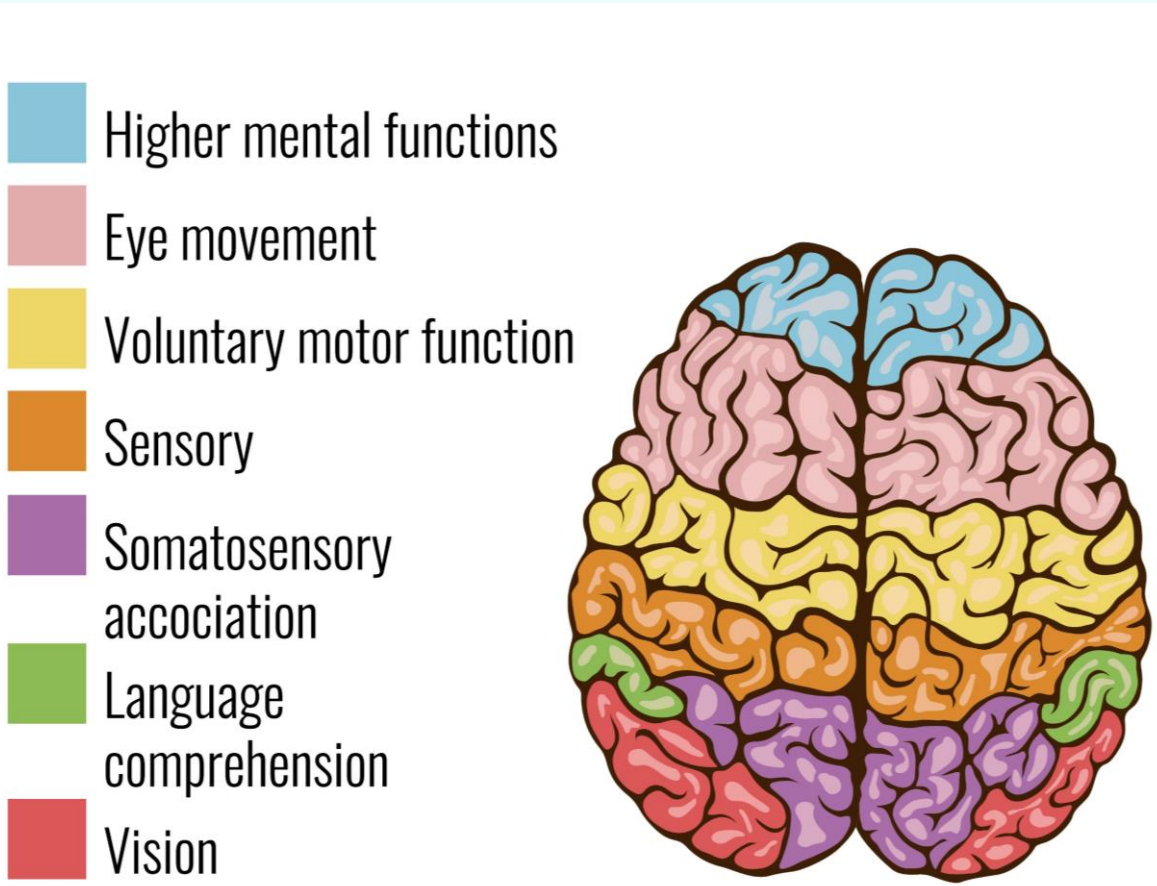


This Photo by Unknown Author is licensed under CC BY-SA

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Understanding Autism





INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Key Facts

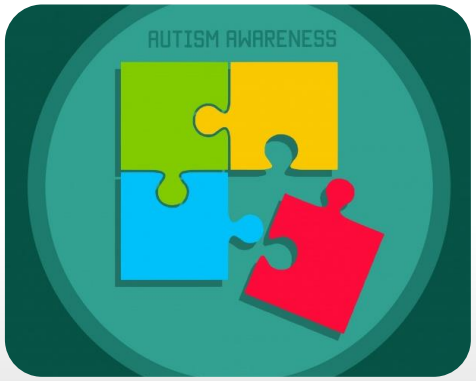
Autism, also known as autism spectrum disorder, is a brain-related condition affecting about **1 in 100** children.

Detection of symptoms can occur in early childhood, but **diagnosis** is often delayed.

Individuals with autism have varying **abilities** and needs, with some living independently and others requiring lifelong care.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
Core Clinical Features



Characteristics of Autism:

1. Impaired social interaction
2. Communication challenges
3. Restricted interests and repetitive behaviors
4. Sensory sensitivities

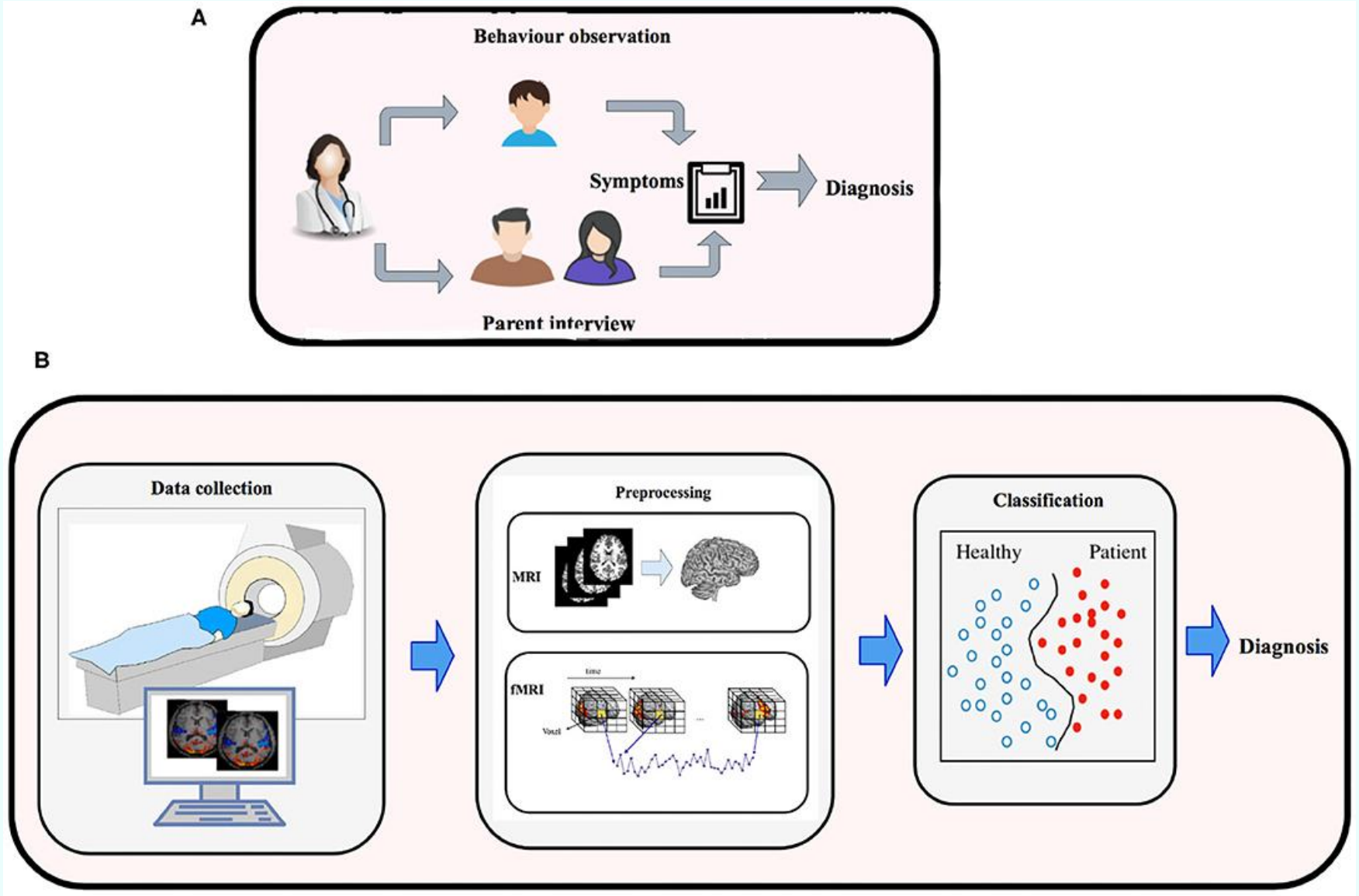
Medical conditions associated with autism:

- Food restriction
- Difficult eating behaviors
- Gastrointestinal disorders
- Selective eating habits
- Aversions to specific food characteristics

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Diagnosis



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Diagnosis

Nutritional Assessment for Children and Adults with ASD

By James B. Adams, Ph.D., Director ASU Autism/Asperger's Research Program, with assistance from the following nutritionists/physicians: Kelly Barnhill, Meg Breuning, Pamela Compart, Vicki Kobliner, Liz Lipski, Tom Malterre, and Julie Matthews, and nutritional biochemist Tapan Audhya.

Introduction

This assessment is intended to help families, nutritionists and researchers make a rough estimate of the quality of diet and nutritional supplementation of a person with autism.

To complete the assessment, estimate the average number of servings of each of the following foods and give them point values. (Alternatively you can take a 3-day diet history of all the foods you eat, and use that for scoring). Calculate the total score, and then you'll find a scale at the bottom of the assessment to help you rate the approximate dietary quality.

Assess Quality of your Diet

VEGETABLES: *Number of servings/day of vegetables (1 serving is half cup for teens/adults, ¼ cup for children ages 4-8; for leafy greens, the serving size is 1 cup for adults, ½ cup for children)*		
Most Vegetables. (except potatoes, corn – see below)	**Servings/Points** **2 points for each serving** **(only 1 point if deep-fried)**	**Points**
Potatoes (including sweet potatoes but not yams)	**1 point for each serving** **(zero if French fried)**	
Corn (actually a grain, not a vegetable, but listed here since many people think of it as a vegetable; most corn in the US is Genetically Modified to have a high sugar/starch content, except for Organic which is not genetically modified and is healthier)	**1 if organic and fresh/frozen;** **0 if conventional (GMO)**	
Bonus Points for Leafy Greens: Common leafy greens include: arugula, beet greens, cabbage, collard greens, kale, leaf lettuce, mustard greens, romaine lettuce, spinach, swiss chard, turnip greens Note: iceberg lettuce does not count for bonus points, as it has little nutritional value compared to other lettuces	**1 point for each serving, maximum 2 points**	
Bonus Points for Fresh or Frozen (not canned)	**Mostly fresh or frozen = 2** **Almost half fresh or frozen =1** **Little fresh/frozen = 0 points**	
	Subtotal of all points for vegetables (maximum of 16 points)	

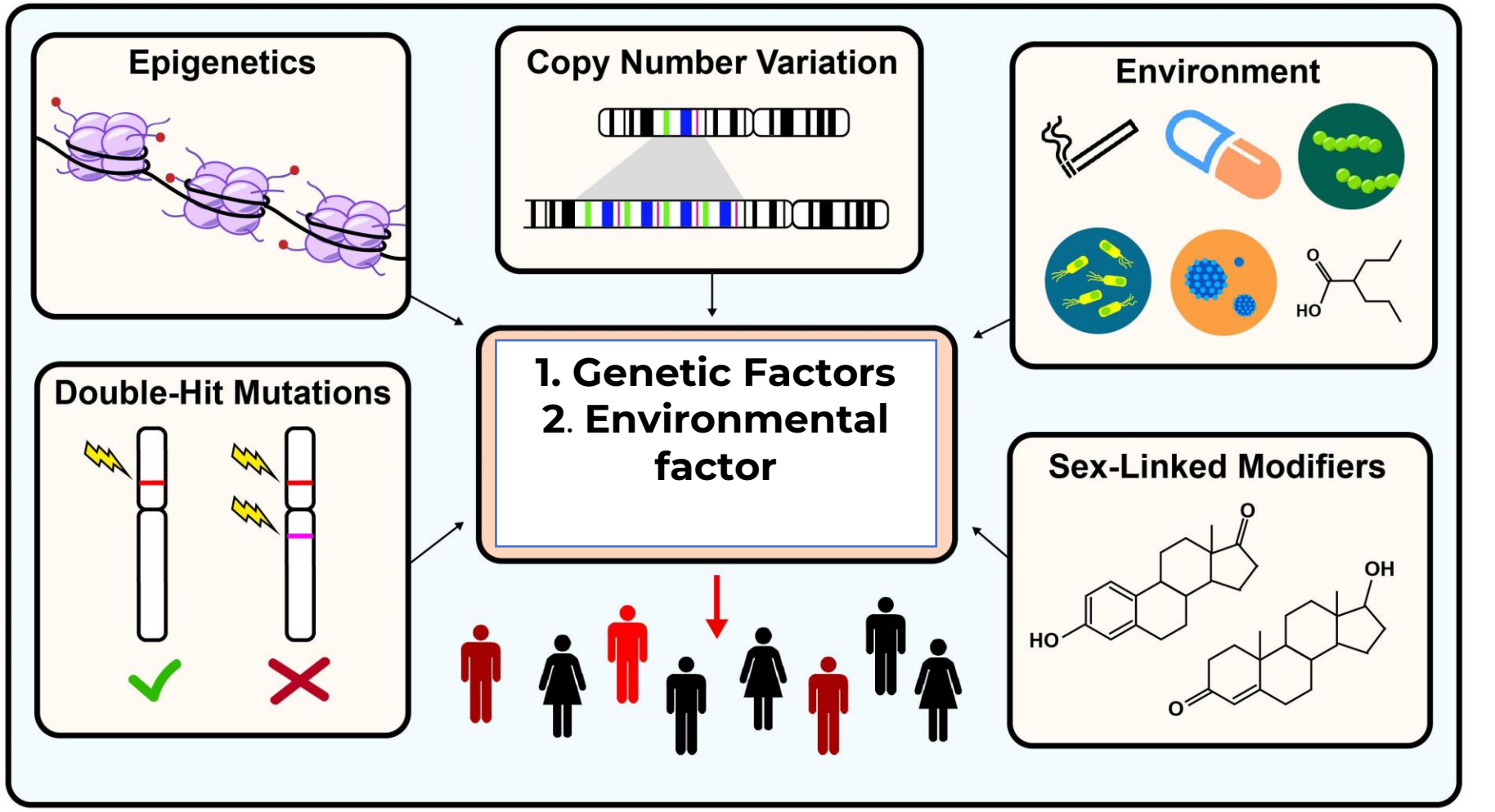
FRUIT: *Number of servings of fruit (1 serving = half cup for teens/adults, 1/3 cup for children ages 4-8). If fruit is dried use half that amount*		
Whole Fruit: fresh or frozen Common fruits include: apples, avocado ,banana , blackberries, blueberries, cherries, cantaloupes, cranberries, dates, figs, grapefruit, grapes, mango, nectarine, orange, papaya, peaches, pears, pineapple, raspberries, strawberries, watermelon	**2 points per serving, maximum 6 points**	
Dried Fruit: without added sugar Common dried fruits include: Apricots, blueberries, cherries, cranberries, raisins Dried fruit	**2 points per serving, included in total fruit points** **Max 2 points**	
Fruit Juice 100% juice only	**1 point per 4 oz serving,** **maximum 2 points**	
Bonus points: Avocado Berries: Fresh or frozen blackberries, blueberries, boysenberries, cranberries, raspberries, strawberries	**1 point for each serving, maximum 2 points**	
PROTEIN:		
Common protein foods: include: legumes, beef, chicken, eggs, fish, lamb, nuts , pork, tofu *(1 serving is 4 ounces animal protein, 1 cup legumes including tofu, or 2 ounces nuts for teens/adults, half that for children ages 4-8 years).* **Dairy Products** *Serving = 8 oz milk, 2 oz cheese, 6 oz kefir or yogurt for adults, and half that for children ages 4-8* **Note:** many children with ASD do not tolerate dairy products and should consider a trial of a dairy-free diet after consulting with their physician/nutritionist	**4 points each serving** **Max 12 points**	
Lunch Meats/Deli Meats Common ones include: bologna, deli turkey, ham, hot dogs	**-1 point per serving**	
GRAINS *Number of servings of grains (1 serving = ½ cup for teens/adults, 1/3 cup for children ages 4-8, or 1 slice of bread or equivalent # of crackers or pretzels, 2 medium sized cookies)*		
Whole Grains: Common whole grains include: amaranth, buckwheat, brown rice, bulgur, millet, oats, quinoa, teff, wild rice	**1 point per serving** **Maximum 4 points**	
Refined Grains: Common refined grains include: bread, cakes, cereal, cookies, crackers, noodles/pasta, pastries, pizza, pretzels, snack foods,	**-1 point per serving**	
FATS AND OILS *Oils: 1 serving = 1 tsp* *Nuts and seeds: 1 serving = 2 Tbsp*		
Nuts and Seeds: Common nuts and seeds include: almonds, cashews, coconut, hazelnuts, hemp seeds, nut butter, peanuts,	**1 point per serving** **Maximum 4 points** (can be counted both here and in protein section)	

https://cdn.shopify.com/s/files/1/0667/4219/0293/files/Nutritional_Assessment_for_ASD.pdf?v=1697942948
(Nutritional Assessment sheet)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

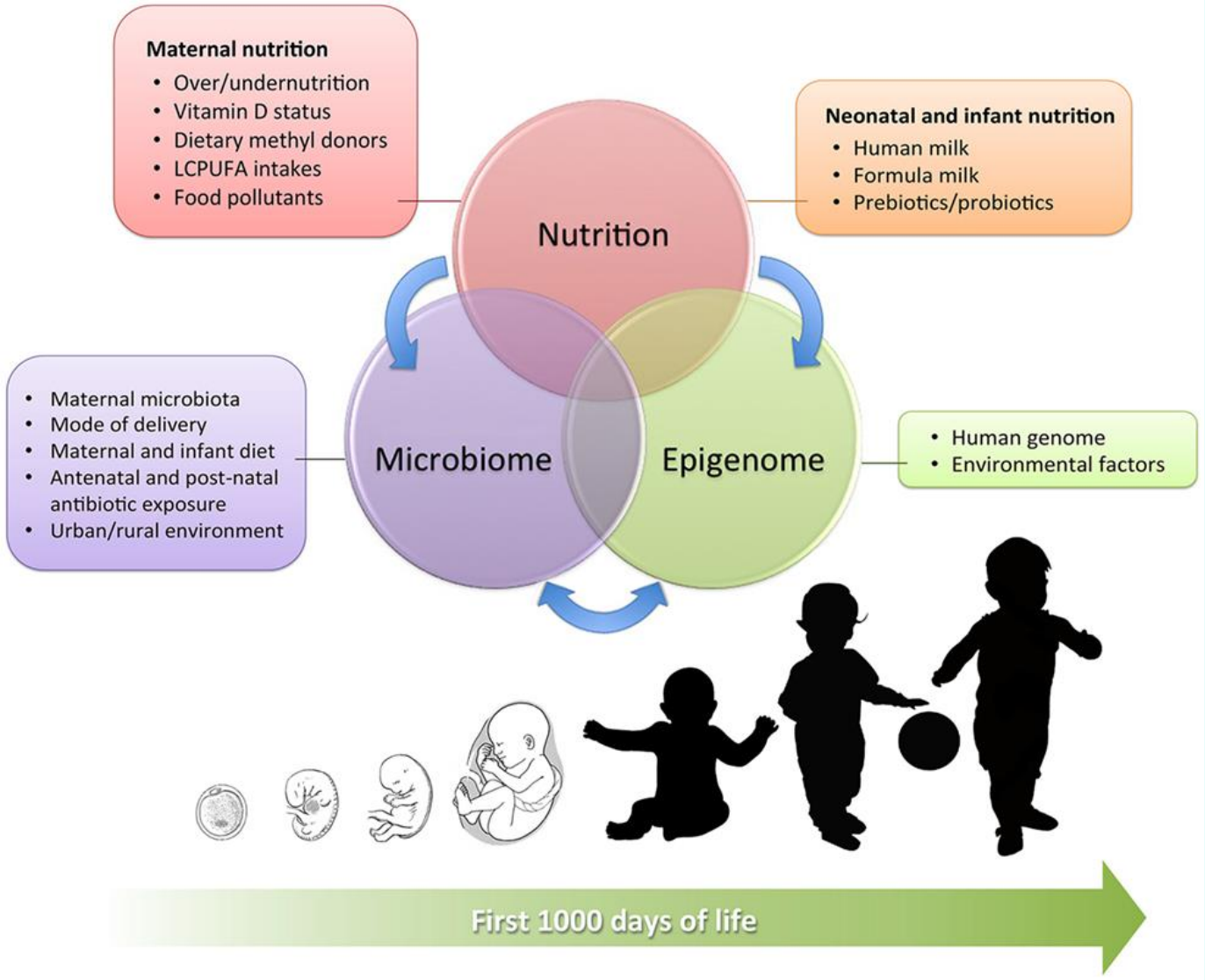
Pathogenesis



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Pathogenesis

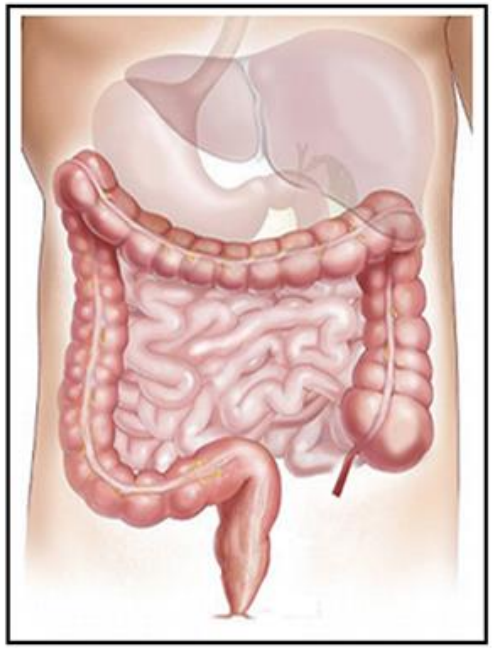


This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

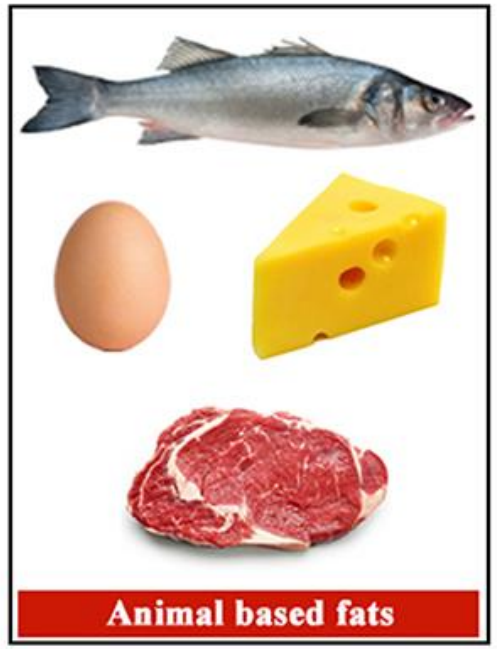
Pathogenesis



Human intestine

↑ *Bifidobacterium spp.*
Lactobbacillus spp.
SCFA producing bacteria ↓

↓ *Clostridium perfringens*
Ruminococcaceae family
Enterococcus ↑

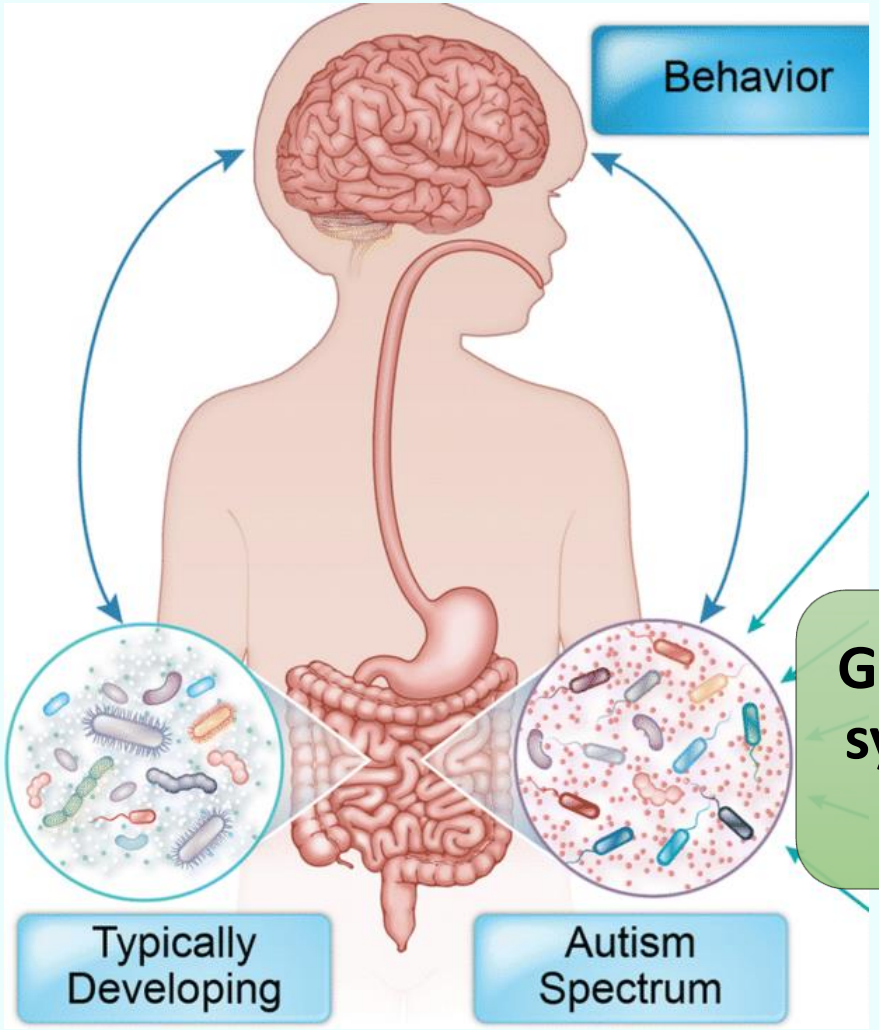


Gut-Microbiome and Nutrition

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Gut Connection



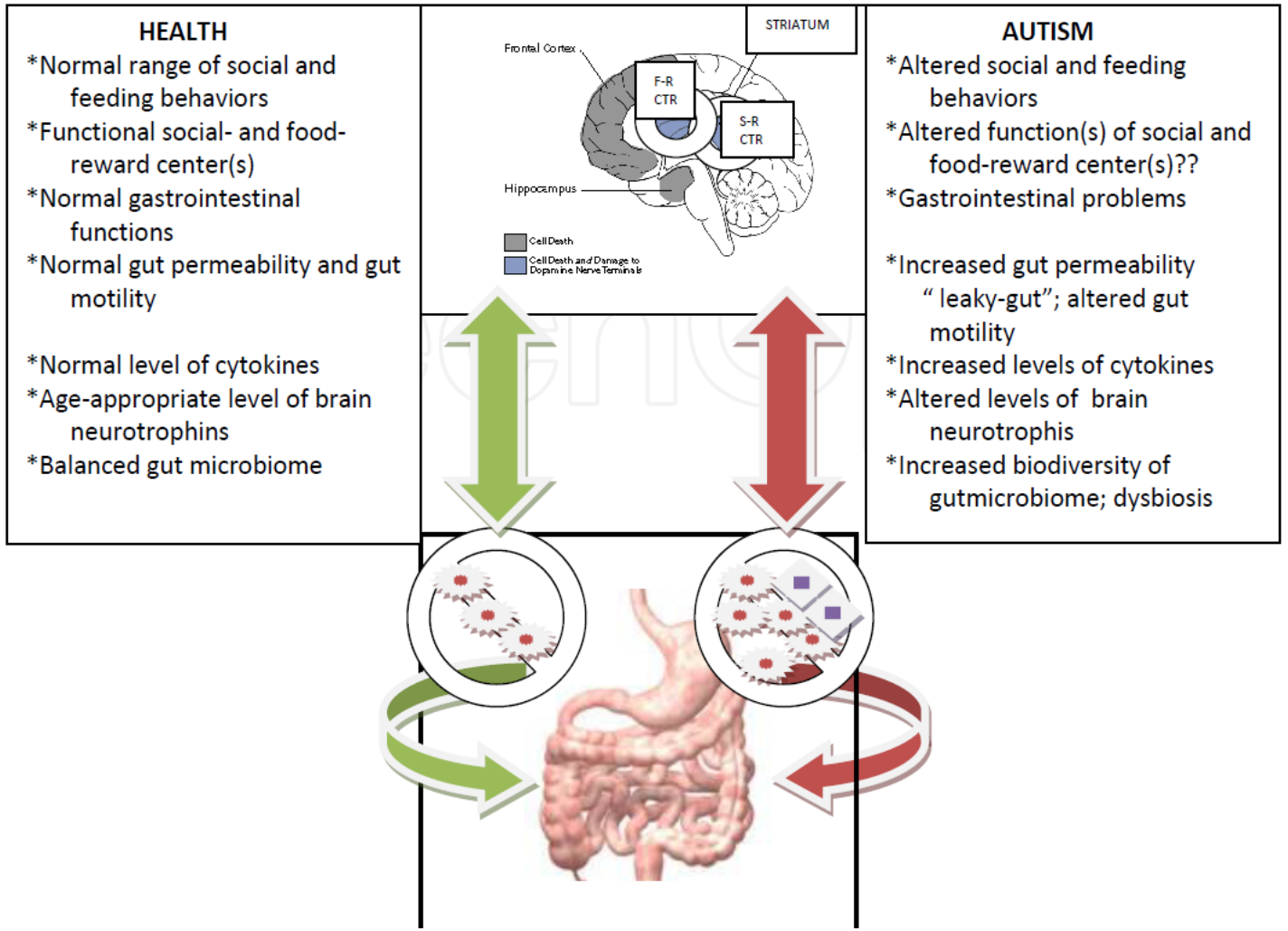
Gastrointestinal symptoms with ASD:

- 9% to 91% in children
- 9% to 70% in Adults

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Gut Connection



Sajdel-Sulkowska, E. M., & Zabielski, R. (2013). **Gut microbiome and brain-gut axis in autism—aberrant development of gut-brain communication and reward circuitry**. In *Recent Advances in Autism Spectrum Disorders-Volume I*. IntechOpen.

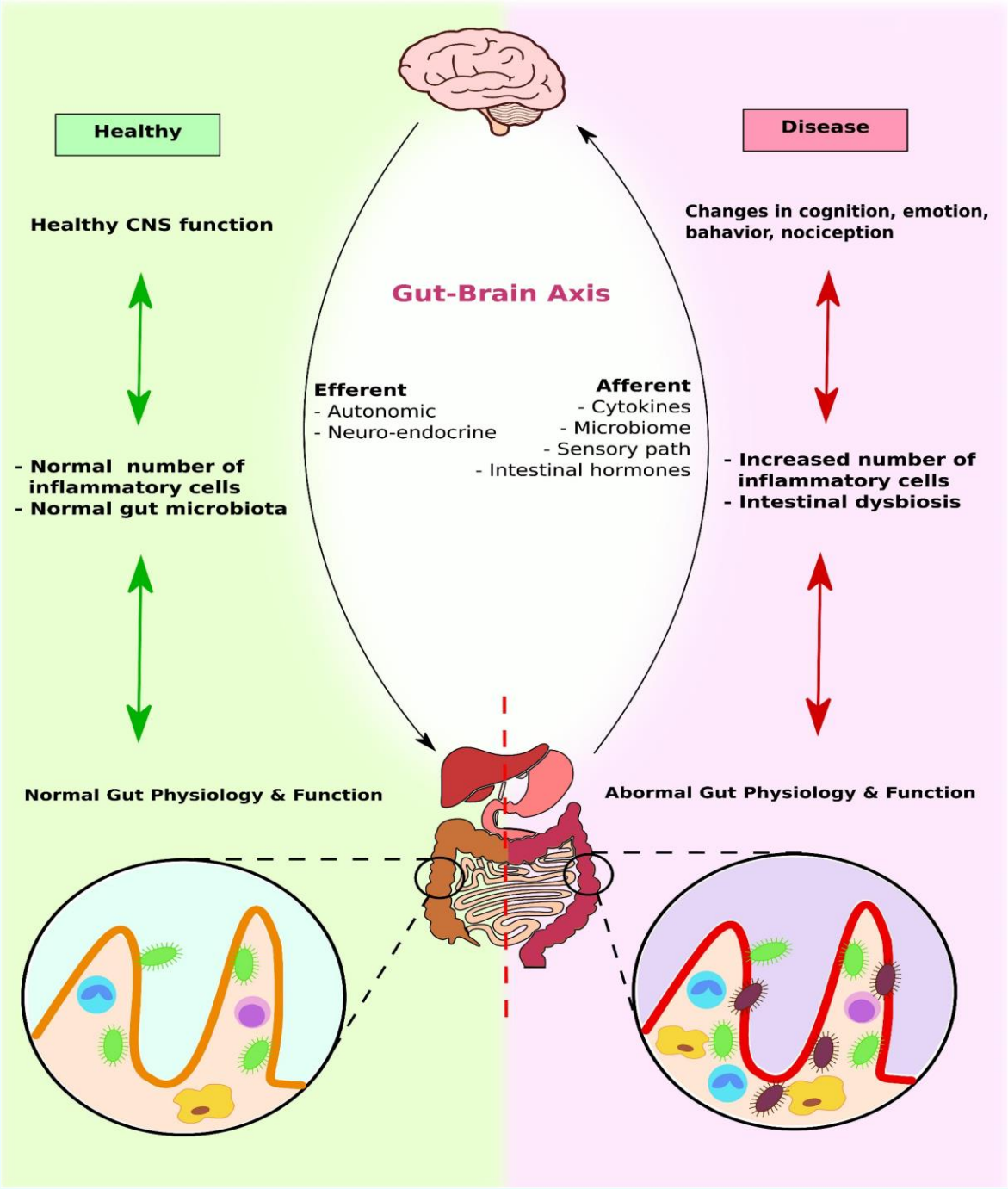
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Autism Spectrum Disorder (ASD)
Gut-Brain Axis

The Gut-brain Axis Is A Bidirectional Communication System

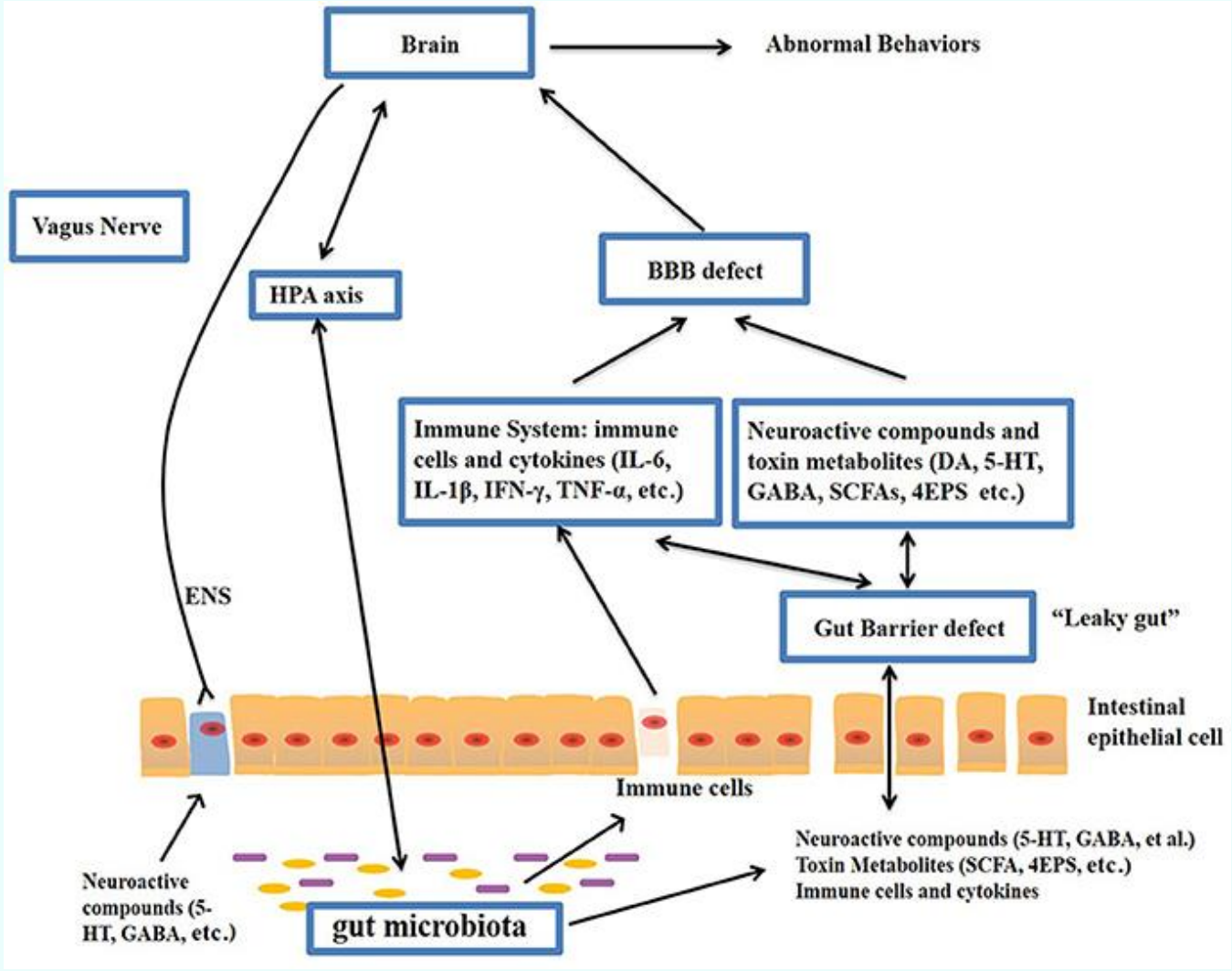
- The Gastrointestinal System
- The Central Nervous System

- Gut Microbiome
- Immune System
- Enteric Nervous System



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

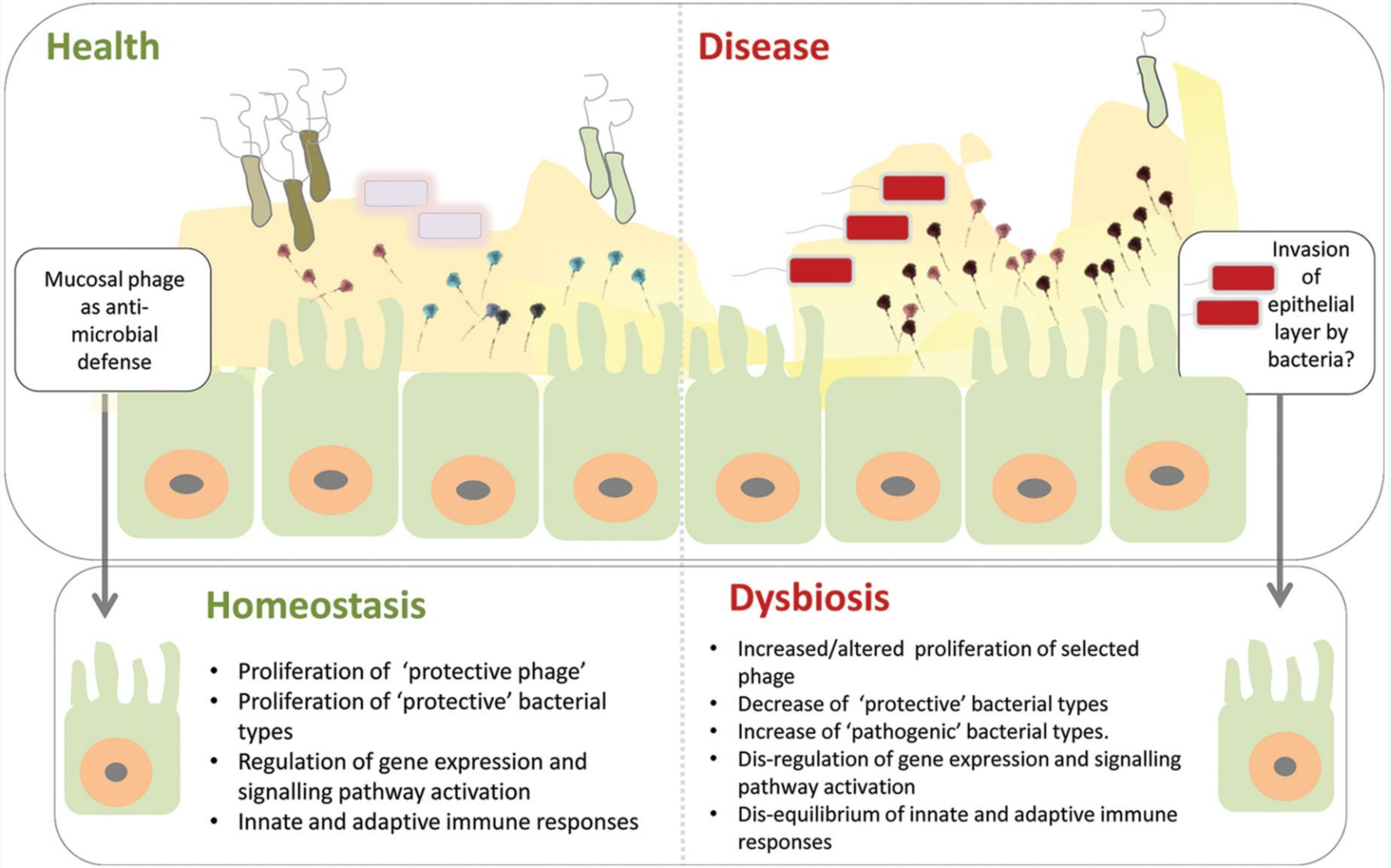
Autism Spectrum Disorder (ASD)
The Role of Microbiota



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Dysbiosis

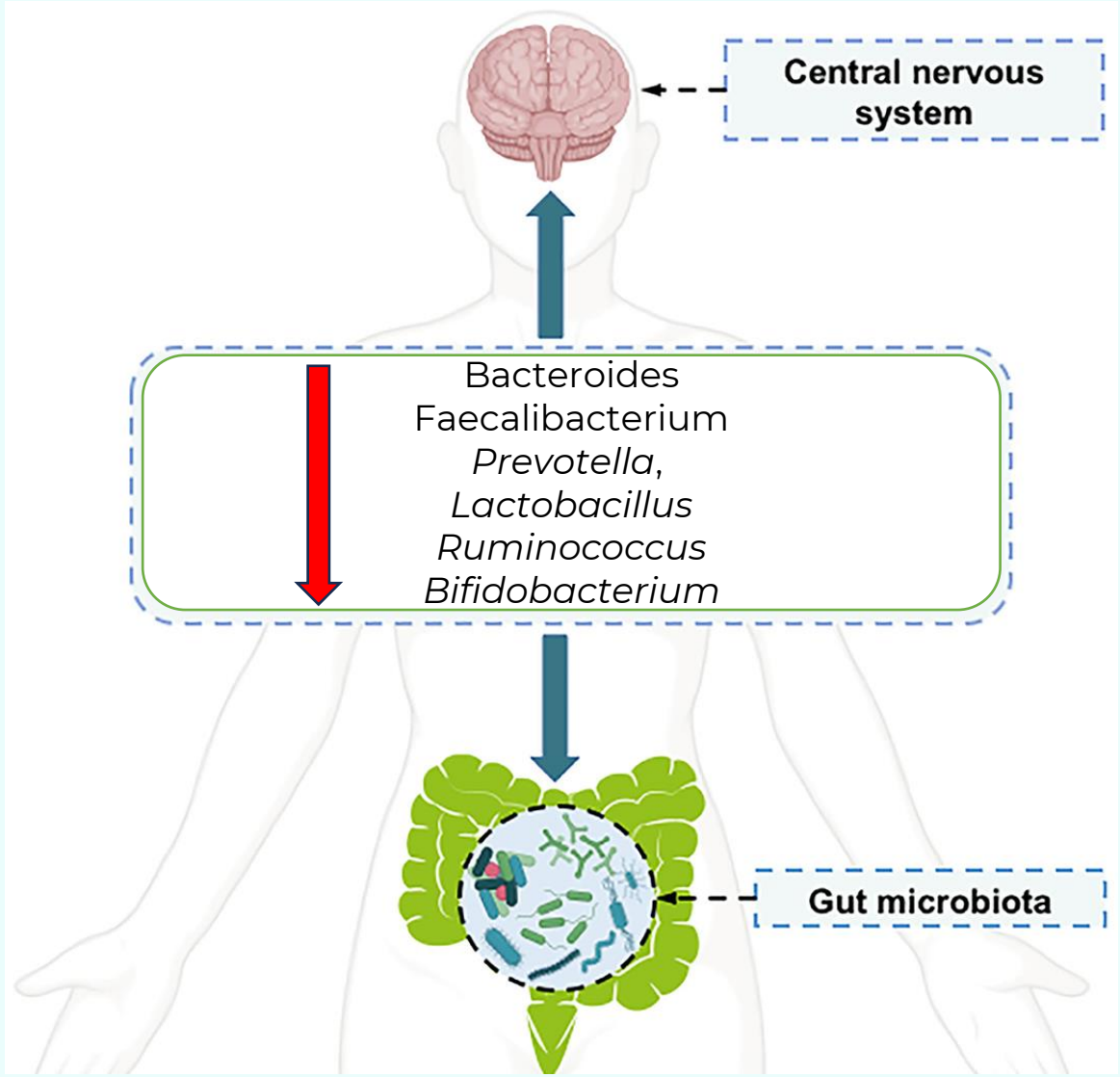


This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

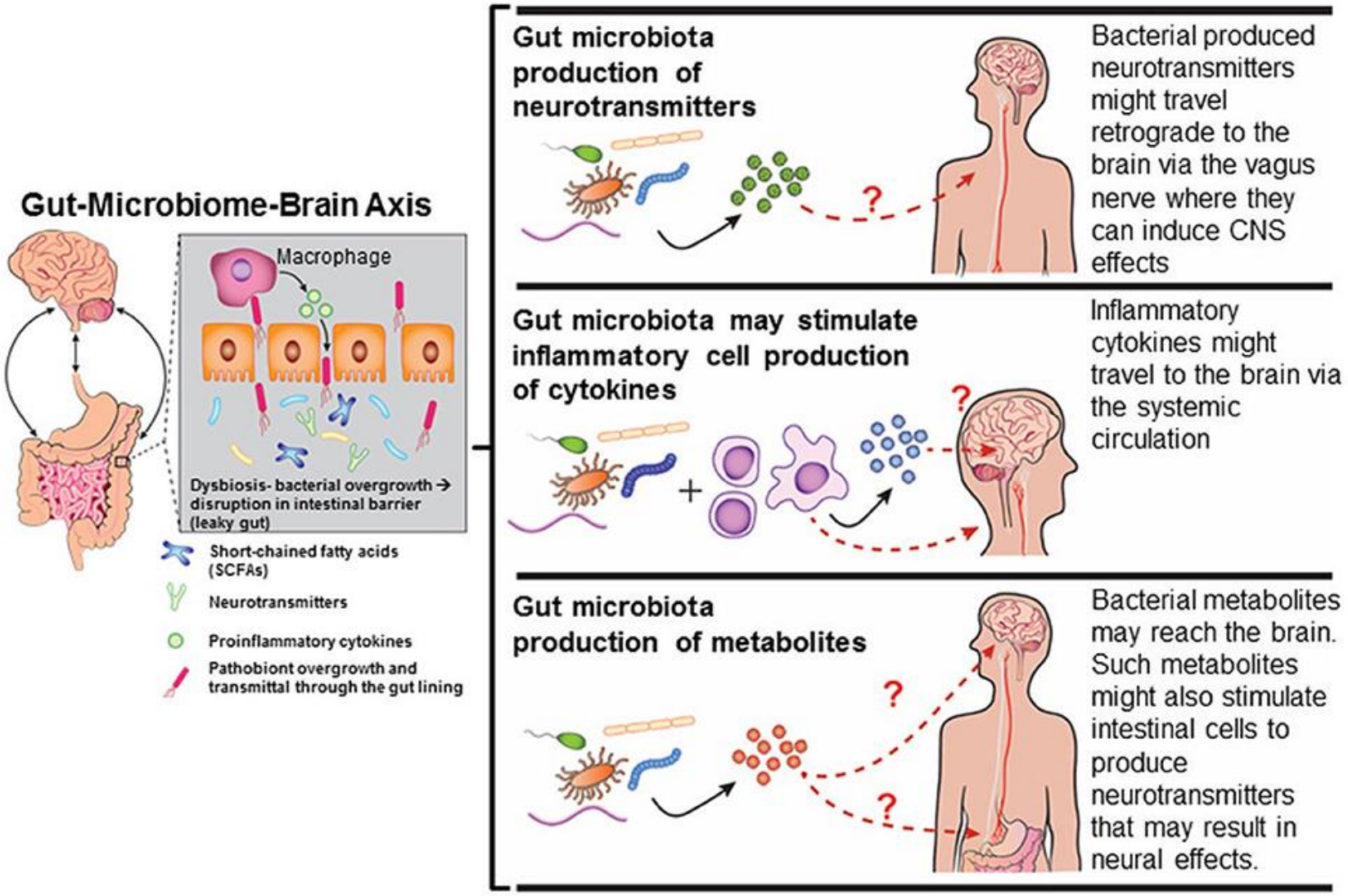
Dysbiosis



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

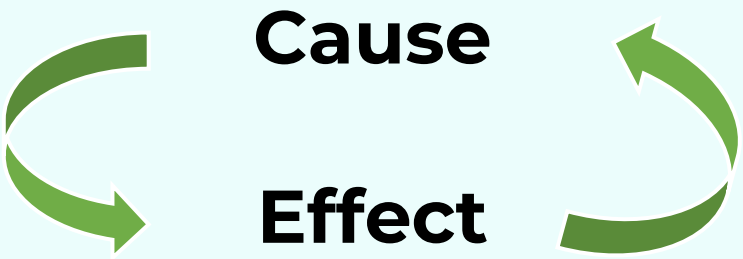
Autism Spectrum Disorder (ASD)

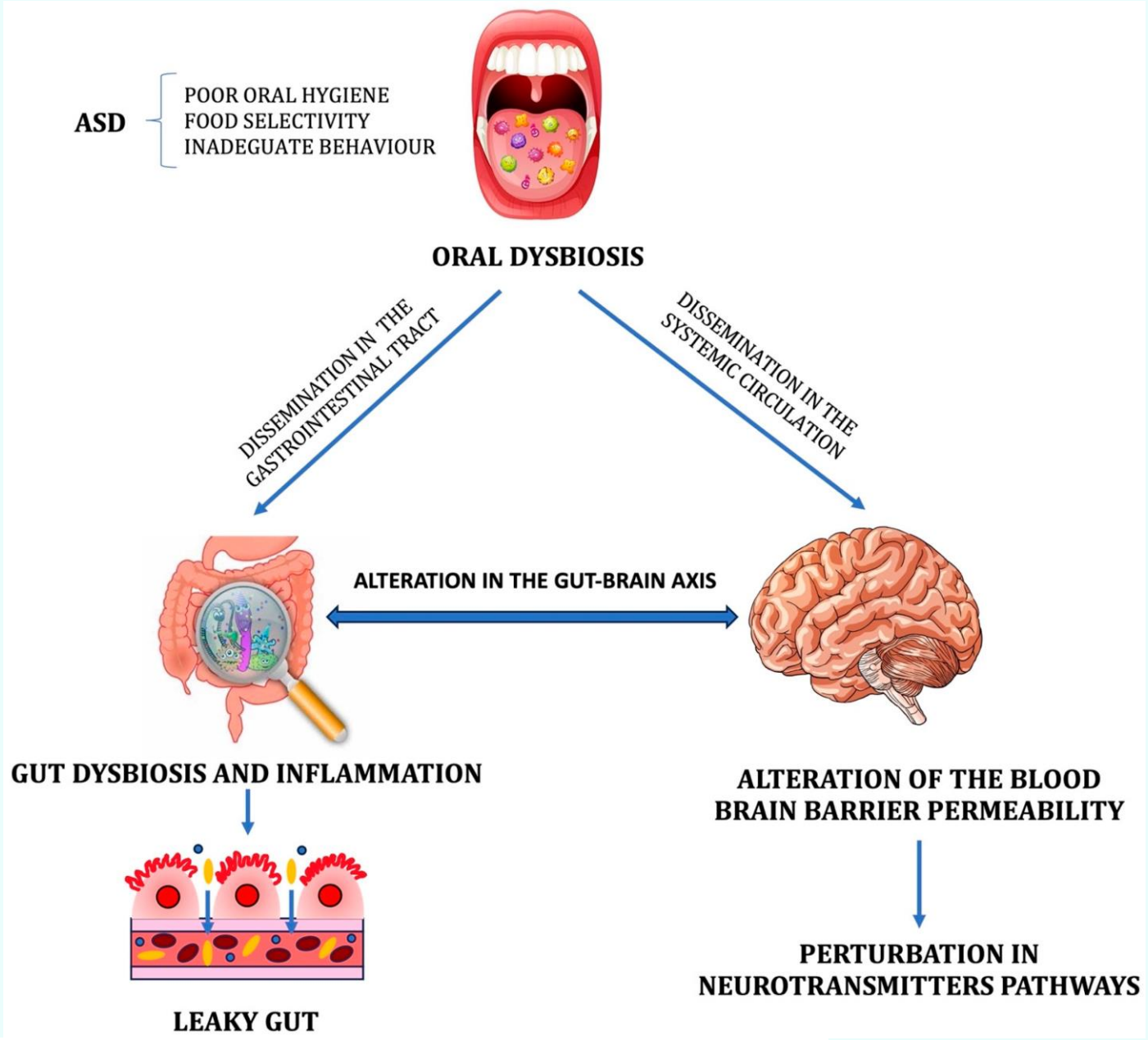
Dysbiosis



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder Is Dysbiosis a Cause or A Consequence Of Autism?



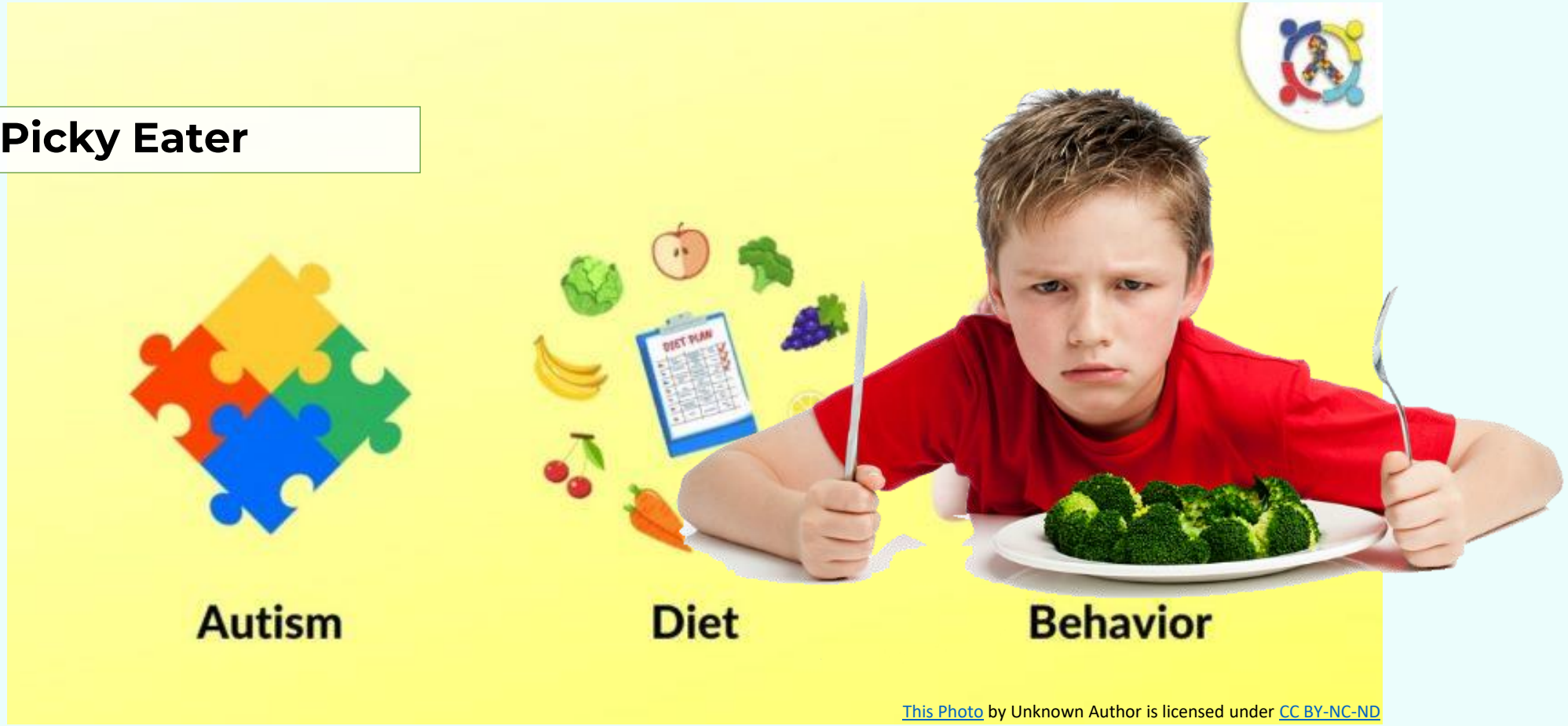


Mussap, M., Beretta, P., Esposito, E., & Fanos, V. (2023). Once upon a Time Oral Microbiota: A Cinderella or a Protagonist in Autism Spectrum Disorder?. *Metabolites*, *13*(12), 1183.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
Nutritional Status

Picky Eater



This Photo by Unknown Author is licensed under CC BY-NC-ND

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Impact of Nutritional Status on Microbiota

Gut bacteria	ASD group			ASD group ($n = 30$)	TD group ($n = 28$)	p^{**}
	ASD-diet ($n = 16$)	ASD-no diet ($n = 14$)	p^{*}			
Genus						
Akkermansia	1.39 ± 1.90	1.84 ± 2.74	0.618[a]	1.60 ± 2.30	1.45 ± 2.17	0.938[a]
Alistipes	3.61 ± 2.82	4.42 ± 3.61	0.494[b]	3.98 ± 3.18	4.30 ± 2.92	0.565[b]
Bacteroides	23.98 ± 11.66	21.36 ± 7.82	0.482[b]	22.75 ± 9.98	24.45 ± 10.14	0.554[b]
Bifidobacterium	1.44 ± 1.80	4.22 ± 3.62	**0.008**[a]	2.73 ± 3.09	3.53 ± 2.35	0.054[a]
Blautia	2.84 ± 1.11	2.34 ± 1.18	0.114[a]	2.60 ± 1.15	2.65 ± 1.23	0.803[a]
Clostridium	6.47 ± 3.16	7.60 ± 3.65	0.280[a]	6.99 ± 3.39	6.90 ± 3.35	0.901[a]
Coprococcus	0.85 ± 0.84	0.63 ± 0.48	0.760[a]	0.74 ± 0.63	0.77 ± 0.69	0.962[a]
Dialister	0.60 ± 1.10	1.36 ± 1.65	0.105[a]	0.94 ± 1.40	0.67 ± 0.78	0.871[a]
Enterococcus	0.56 ± 2.03	0.10 ± 0.10	0.427[a]	0.34 ± 1.48	0.33 ± 0.83	0.122[a]
Eubacterium	3.66 ± 2.38	2.54 ± 1.54	0.114[a]	3.13 ± 2.08	3.07 ± 2.05	0.988[a]
Faecalibacterium	16.34 ± 7.68	11.72 ± 4.62	0.060[b]	14.18 ± 6.75	15.13 ± 5.09	0.312[b]
Lachnoclostridium	1.18 ± 0.81	0.96 ± 1.09	0.561[a]	1.07 ± 0.96	0.82 ± 0.71	0.586[a]
Lactobacillus	0.64 ± 0.65	1.03 ± 2.28	0.786[a]	0.82 ± 1.61	0.67 ± 1.26	0.697[a]
Prevotella	12.93 ± 12.52	10.87 ± 11.72	0.739[a]	11.96 ± 11.99	6.89 ± 9.16	0.093[a]
Pseudomonas	0.65 ± 2.46	0.05 ± 0.05	0.683[a]	0.37 ± 1.79	0.06 ± 0.06	0.534[a]
Roseburia	3.47 ± 1.98	1.32 ± 0.99	**0.002**[a]	2.47 ± 1.92	2.89 ± 1.92	0.304[a]
Ruminococcus	2.55 ± 1.72	2.54 ± 1.23	0.618[a]	2.55 ± 1.48	2.81 ± 1.34	0.350[a]
Streptococcus	0.49 ± 0.33	2.27 ± 5.60	0.394[a]	1.32 ± 3.86	1.53 ± 1.71	0.058[a]
Sutterella	0.62 ± 1.22	2.13 ± 1.98	**0.015**[a]	1.32 ± 1.77	1.78 ± 1.76	0.119[a]
Turicibacter	0.80 ± 1.21	0.62 ± 0.65	0.868[a]	0.72 ± 0.98	0.56 ± 0.60	0.749[a]
Species						
Akkermansia muciniphila	1.19 ± 1.40	2.27 ± 3.30	0.269[a]	1.69 ± 2.49	1.75 ± 2.60	0.935[a]
Bacteroides frágilis	0.45 ± 0.47	1.21 ± 2.85	0.301[a]	0.80 ± 1.97	1.21 ± 1.32	0.075[a]
Bacteroides intestinalis	0.02 ± 0.03	0.13 ± 0.33	0.944[a]	0.07 ± 0.23	0.55 ± 2.43	0.453[a]
Bifidobacterium adolescentis	0.30 ± 0.47	1.25 ± 2.10	**0.046**[a]	0.74 ± 1.52	0.65 ± 1.12	0.463[a]
Bifidobacterium longum	0.23 ± 0.49	1.11 ± 2.67	**0.002**[a]	0.64 ± 1.87	1.21 ± 1.80	**0.002**[a]
Clostridium bartletti	0.42 ± 0.39	0.63 ± 0.63	0.647[a]	0.52 ± 0.09	0.22 ± 0.04	0.335[a]
Clostriduim glycolicum	0.42 ± 0.28	0.55 ± 0.49	0.678[a]	0.48 ± 0.39	0.31 ± 0.30	0.028[a]
Coprococcus comes	0.19 ± 0.20	0.37 ± 0.36	0.156[a]	0.28 ± 0.29	0.27 ± 0.25	0.767[a]
Eubacterium eligens	1.76 ± 1.83	1.48 ± 1.53	0.561[a]	1.68 ± 0.30	1.01 ± 1.01	0.171[a]
Eubacterium ventriosum	10.77 ± 9.20	8.60 ± 8.14	0.618[a]	9.76 ± 8.64	12.58 ± 8.89	0.101[a]
Faecalibacterium prausnitzii	6.60 ± 11.80	3.95 ± 5.60	0.982[a]	5.36 ± 9.37	3.99 ± 7.69	0.735[a]
Flavonifactor plautii	0.95 ± 2.00	0.76 ± 1.03	0.802[a]	0.86 ± 1.60	0.89 ± 0.89	0.111[a]
Lactobacillus reuteri	0.29 ± 0.44	0.18 ± 0.42	0.498[a]	0.24 ± 0.43	0.30 ± 0.41	0.350[a]
Lactobacillus salivarius	0.04 ± 0.07	0.52 ± 1.71	0.386[a]	0.27 ± 1.17	0.03 ± 0.08	0.182[a]
Roseburia hominis	0.37 ± 0.40	0.10 ± 0.17	**0.002**[a]	0.24 ± 0.34	0.49 ± 1.37	0.331[a]
Trabulsiella odonototermitis	0.63 ± 1.33	0.34 ± 0.55	0.483[a]	0.49 ± 1.03	0.50 ± 0.65	0.366[a]

The results are expressed as means ± SD. Statistically significant differences (indicated in bold): $p < 0.05$ ([a]Mann–Whitney test, [b]independent sample t-test); comparison between *ASD-diet and ASD-no diet, **ASD group and TD group. ASD, autism spectrum disorder; ASD-diet, ASD children with gluten- and casein-free diet; ASD-no diet, ASD children without restricted diet; TD, typical development.

Mendive Dubourdieu, P., & Guerendiain, M. (2023). **Understanding the link between gut microbiota, dietary intake, and nutritional status in children with autism and typical development.** *Frontiers in Nutrition*, *10*, 1202948.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Therapeutic Strategies

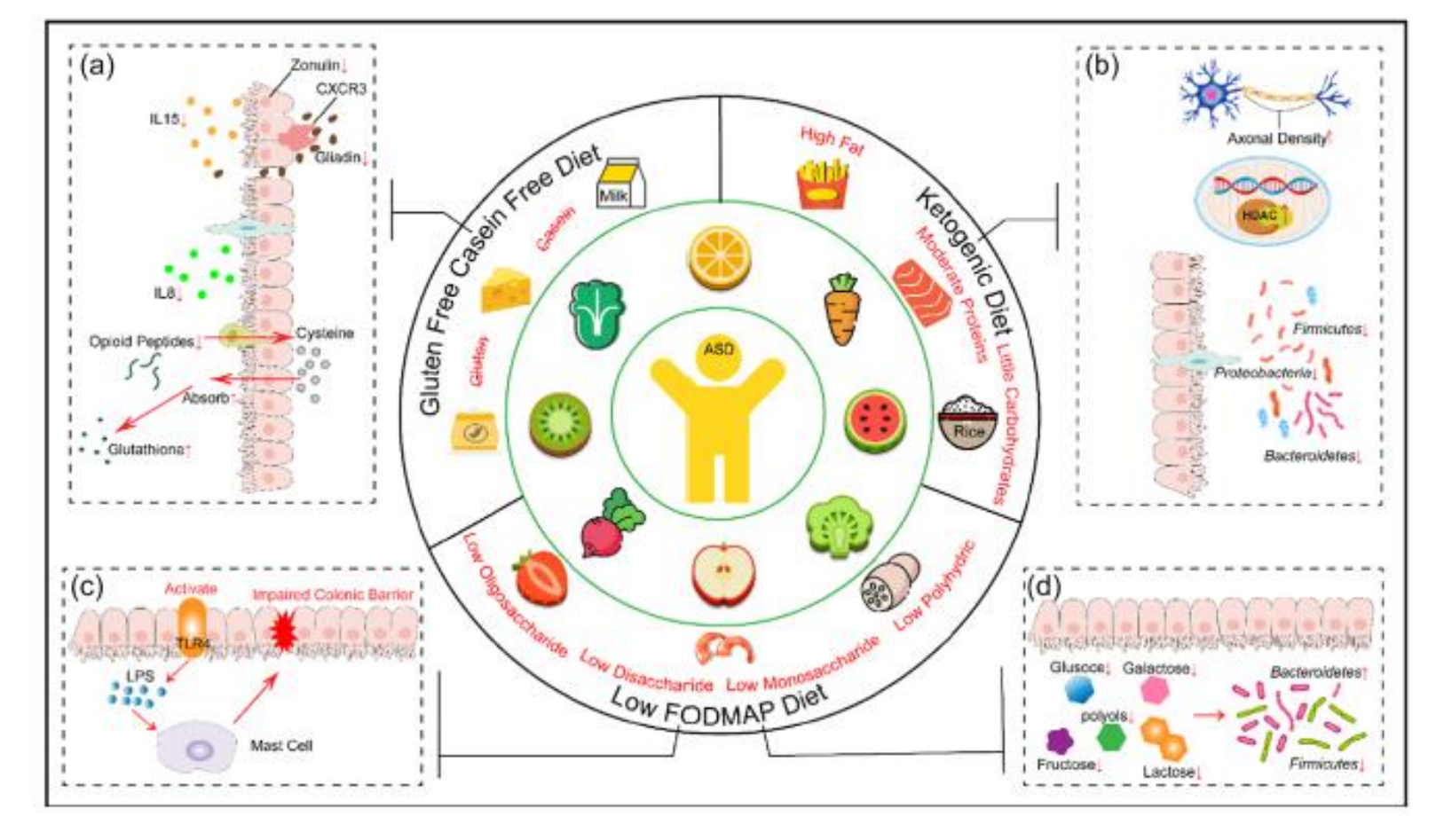
- **Therapeutic Strategies**
  - **1- Nutritional Intervention**
    - Gluten Free Diet
    - Ketogenic Diet
    - The Carbs Diet
    - Mediterranean Diet
    - Low-fodmaps Diet
    - Dietary Fibers
  - **2- Supplements**
  - **3- Pre and Probiotics**
  - **4- Antimicrobial Agents**
  - **5- Microbial Transfer Therapy**

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

1- Nutrition Intervention:

Optimizing Nutrition for ASD Management

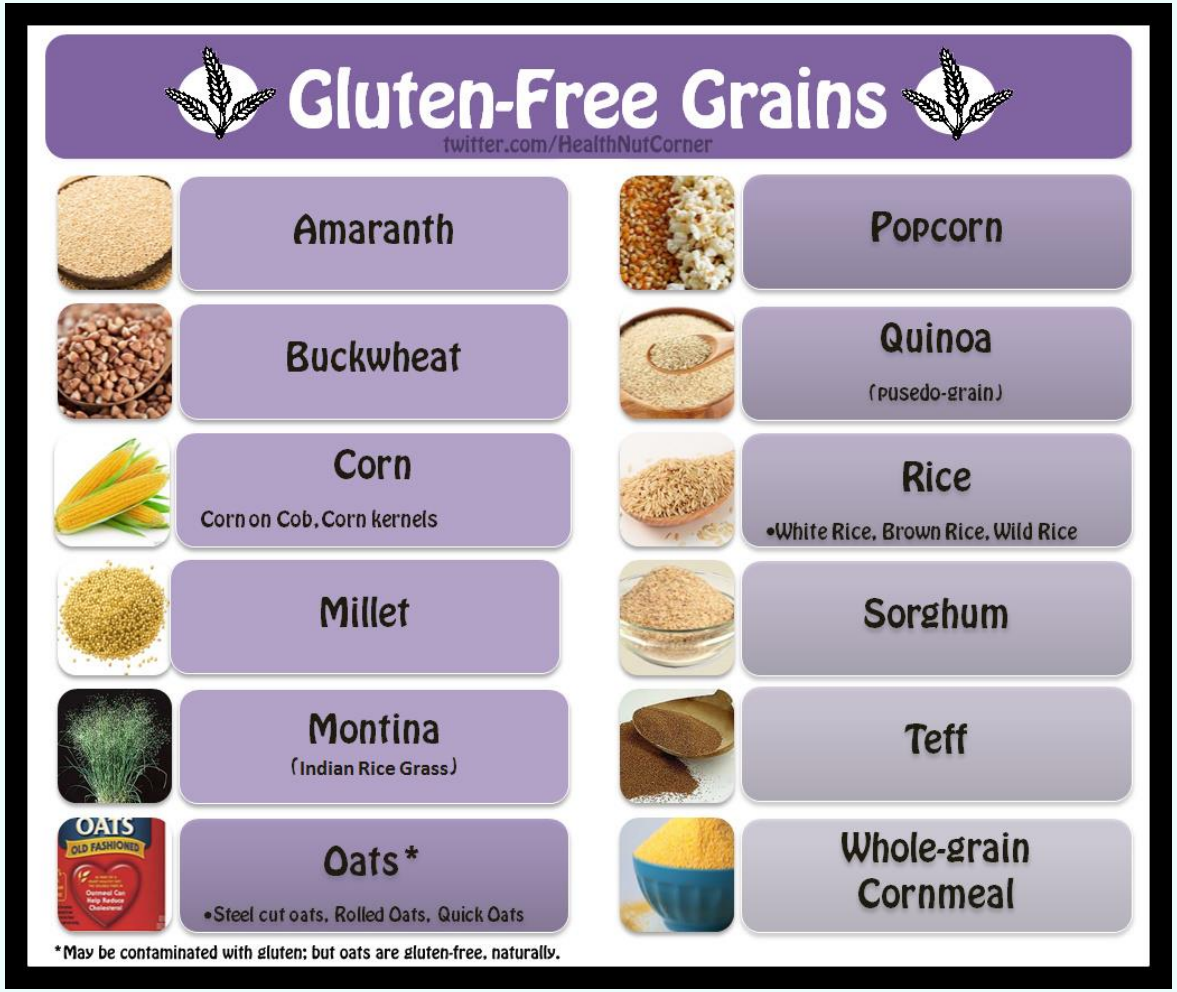


INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
Nutrition Intervention

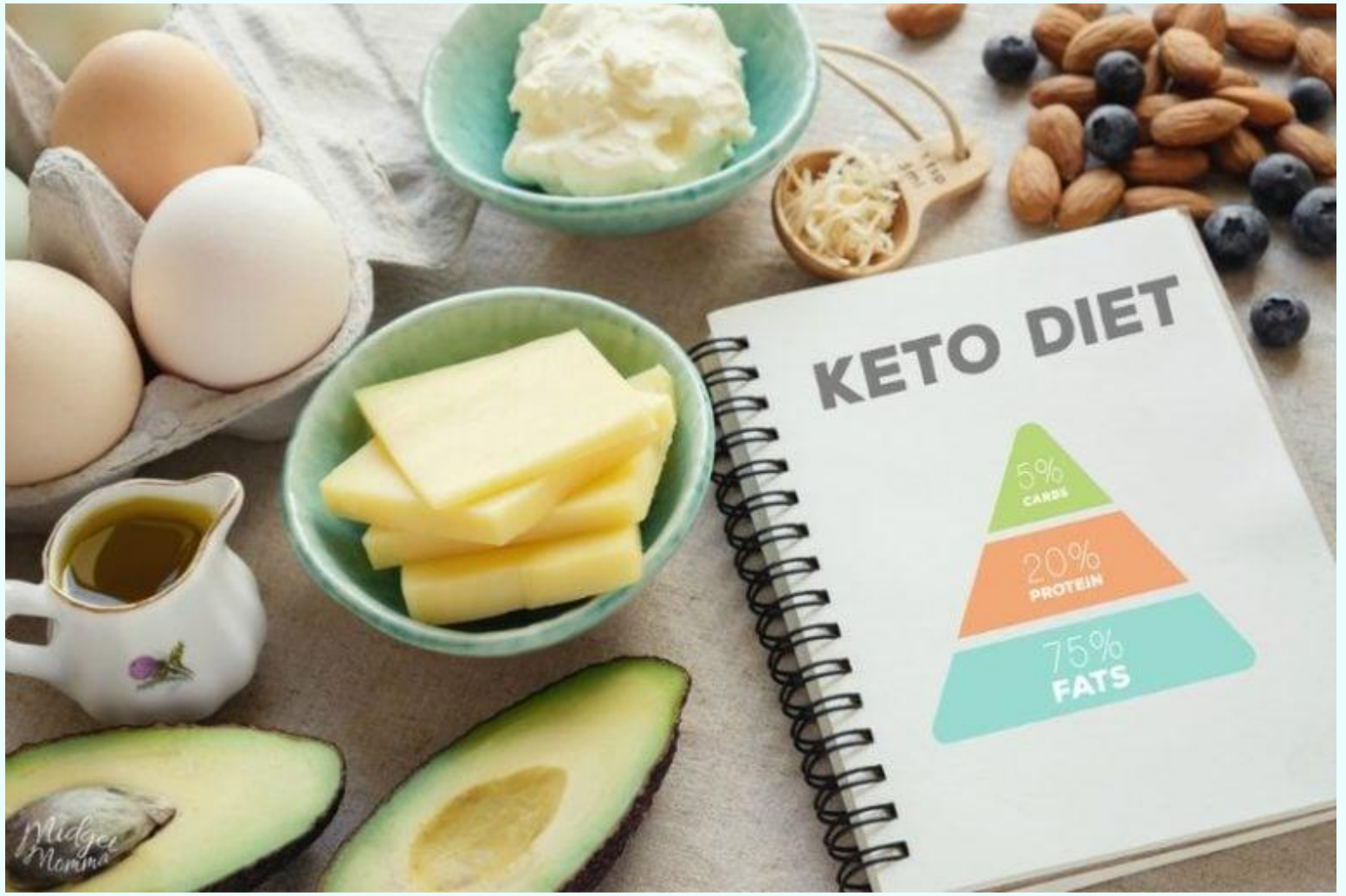
Gluten-free

Casein-free diet



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

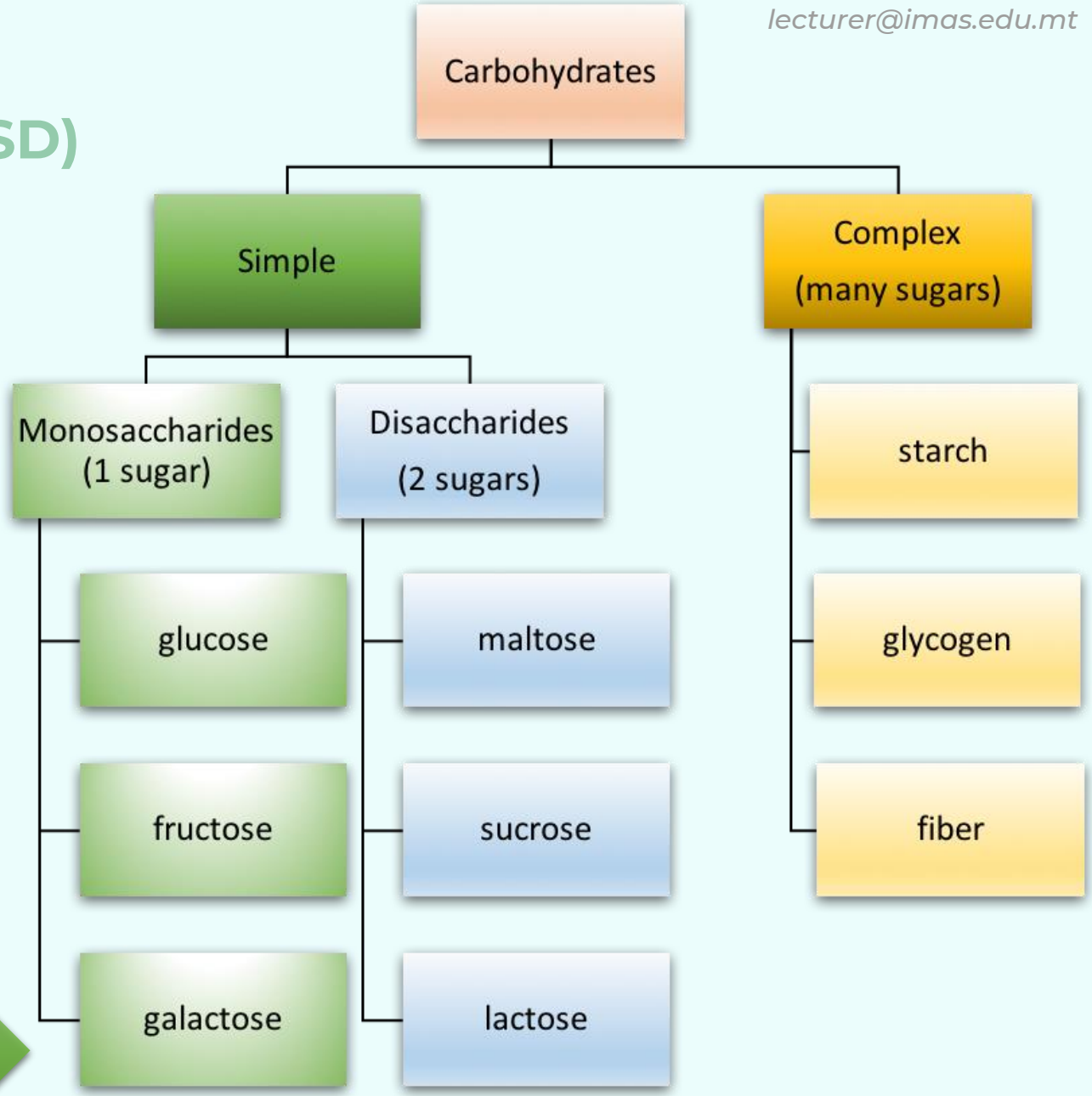
Autism Spectrum Disorder (ASD)
Nutritional Intervention



Ketogenic Diet

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

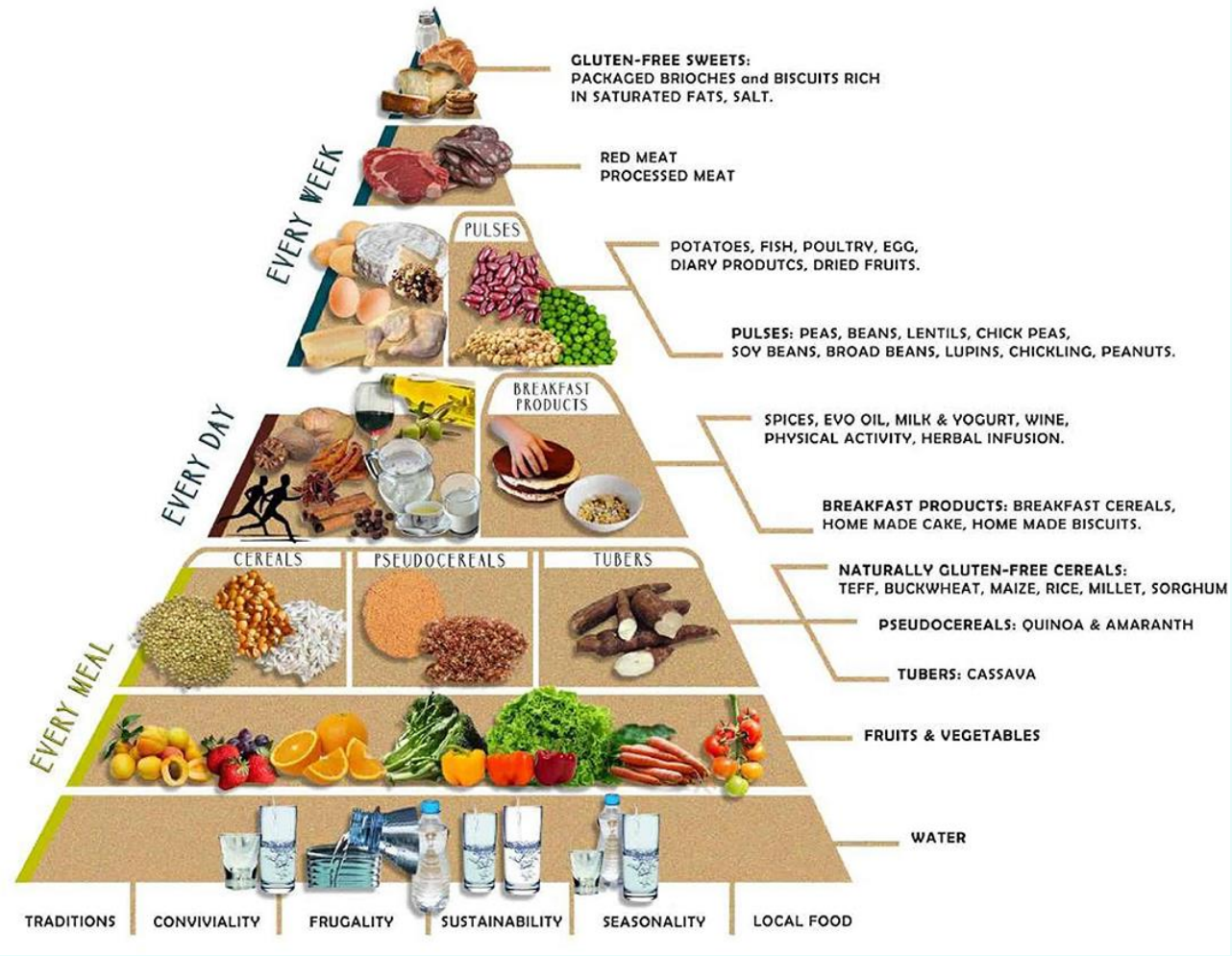
Autism Spectrum Disorder (ASD)
Nutritional Intervention

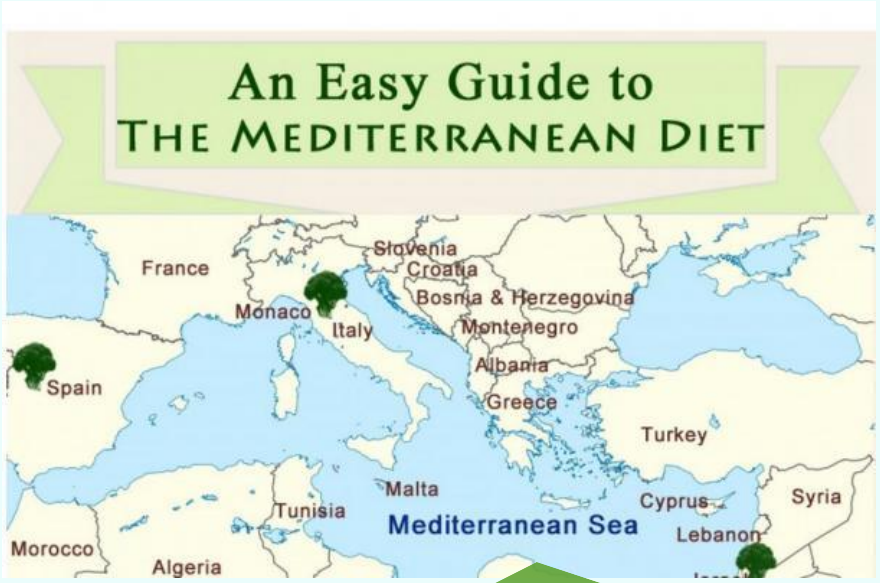


The Specific Carbohydrate Diet

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
Nutritional Intervention





The Mediterranean Diet

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
Nutritional Intervention

F ERMENTABLE
O LIGOSACCHARIDES
D ISACCHARIDES
M ONOSACCHARIDES
A ND
P OLYOLS

FODMAP FRIENDLY

Eliminate foods containing fodmaps

excess fructose	lactose	fructans	galactans	polyols
fruit apple, mango, nashi, pear, tinned fruit in natural juice, watermelon **sweeteners** fructose, high fructose corn syrup **large total fructose dose** concentrated fruit sources, large serves of fruit, dried fruit, fruit juice **honey** corn syrup, fruisana	**milk** milk from cows, goats or sheep, custard, ice cream, yoghurt **cheeses** soft unripened cheeses eg. cottage, cream, mascarpone, ricotta	**vegetables** asparagus, beetroot, broccoli, brussels sprouts, cabbage, eggplant, fennel, garlic, leek, okra, onion (all), shallots, spring onion **cereals** wheat and rye, in large amounts eg. bread, crackers, cookies, couscous, pasta **fruit** custard apple, persimmon, watermelon **miscellaneous** chicory, dandelion, inulin	**legumes** baked beans, chickpeas, kidney beans, lentils	**fruit** apple, apricot, avocado, blackberry, cherry, lychee, nashi, nectarine, peach, pear, plum, prune, watermelon **vegetables** cauliflower, green capsicum (bell pepper), mushroom, sweet corn **sweeteners** sorbitol (420) mannitol (421) isomalt (953) maltitol (965) xylitol (967)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
Nutritional Intervention

Arabinoxylan:

Streptococcus, Bifidobacteria → Acetate, propionate, butyrate

B-glucan:

Lactobacilli, Enterococcus, Bifidobacteria → Variable SCFA

B-fructans:

Bifidobacteria, Lactobacillus, Streptococcus, Flavobacterium → SCFA butyrate

Dietary Fibers

Pectin:

Bacteroides, Prevetella, Bacillus, Agrobacterium, Pseudomonas, Ralstonia, Dickeya → Variety of SCFA and gases, including CO_2, H_2, H_2S, CH_4.

Cellulose:

Bacteroides, Clostridium, Fibrobacter, Ruminococcus → Succinate, a variety of SCFS, predominantly acetate.

Lignan:

Rhodococcus, Pseudomonas, Sphingobacterium → Lipids

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

2- Supplementation

TABLE 3 Nutritional factors of food with autism spectrum disorder (ASD) mitigation effect.

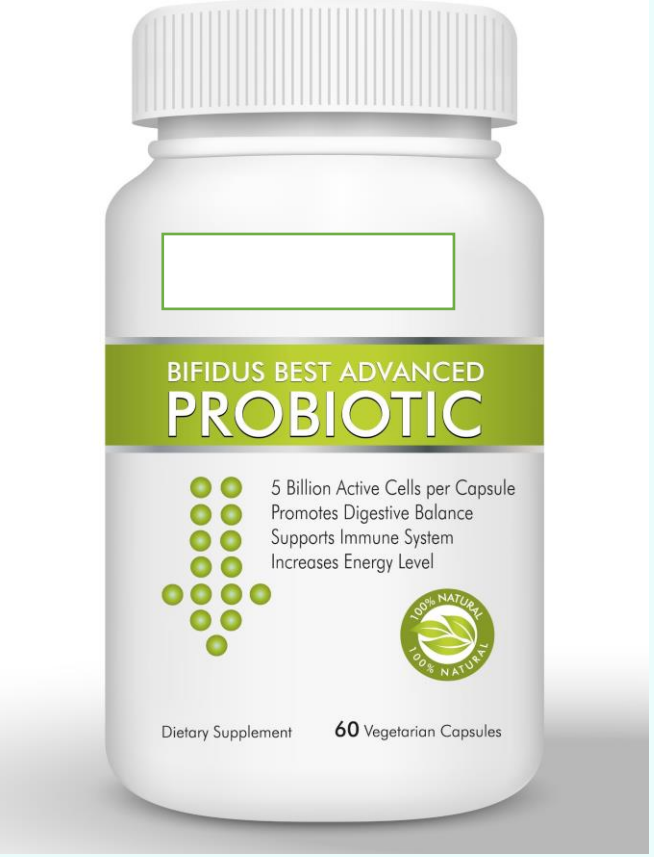
		Sort	Sources	Study type	Study object	Mechanism	Outcomes	Re...ces
Natural products	Vitamins	V_D	Oncorhynchus; cod liver oil; mushroom; egg yolk	Clinical study	ASD children	Increase the level of serum 25-OH-V_D	Improve scores on the Autism Assessment Scale and the Autism Treatment Assessment Scale	Javadfar et al. (2020)
		V_{B6}	Chicken; Soybean; Banana; Peanut	Rodent model	Eph B6-deficient C57BL/6J mice	Restore the balance of intestinal microecology and the excitation/inhibition balance of medial prefrontal cortex	Repair social behavioral deficits	Li, Luo et al. (2020)
Unsaturated fatty acid		$\omega-3$ and $\omega-6$ fatty acid	Seafood; nut; corn; soybean; beef	Clinical study	ASD children	Improve the fatty acid composition of erythrocyte membrane	Improve ASD-like phenotype	Parellada et al. (2017)
				Rodent model	Valproic acid exposure-induced rat	Inhibit the activation of STAT3 in the hippocampus by reducing pro-inflammatory cytokines	Slow the inflammatory response of ASD, repetitive behaviors, and social deficits	Jia et al. (2022)
						Restore the abundance of intestinal flora and normalize the level of Firmicutes	Ameliorate gut dysbiosis	Wang, Xu et al. (2020)

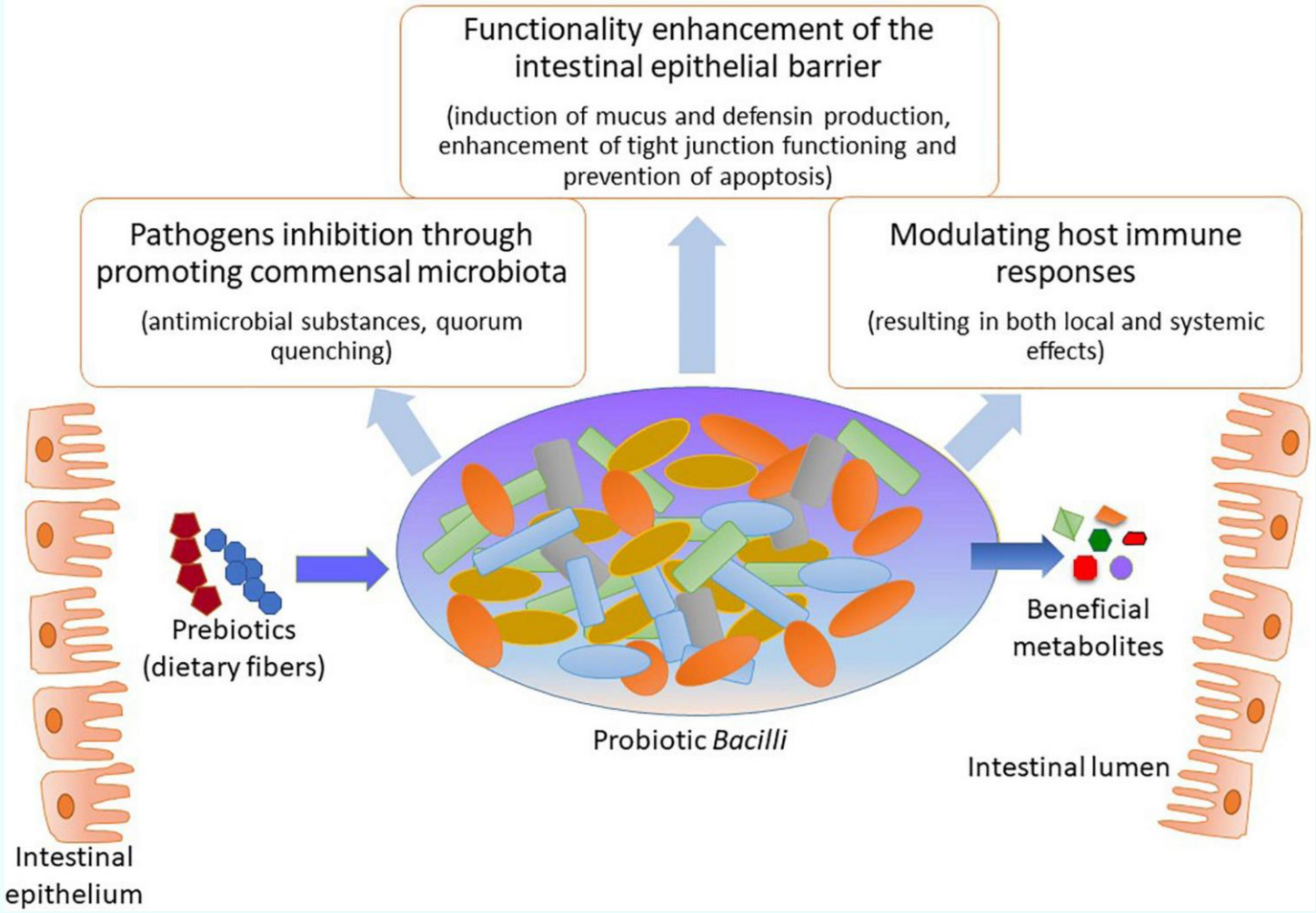
Li, W., Kong, Q., Tian, P., Wang, L., Jin, X., Zhao, J., ... & Wang, G. (2024**). Dietary nutrients that potentially mitigate autism spectrum disorder and dietary recommendations for children and pregnant women**. *Food Frontiers*.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

3- Pre & Probiotics

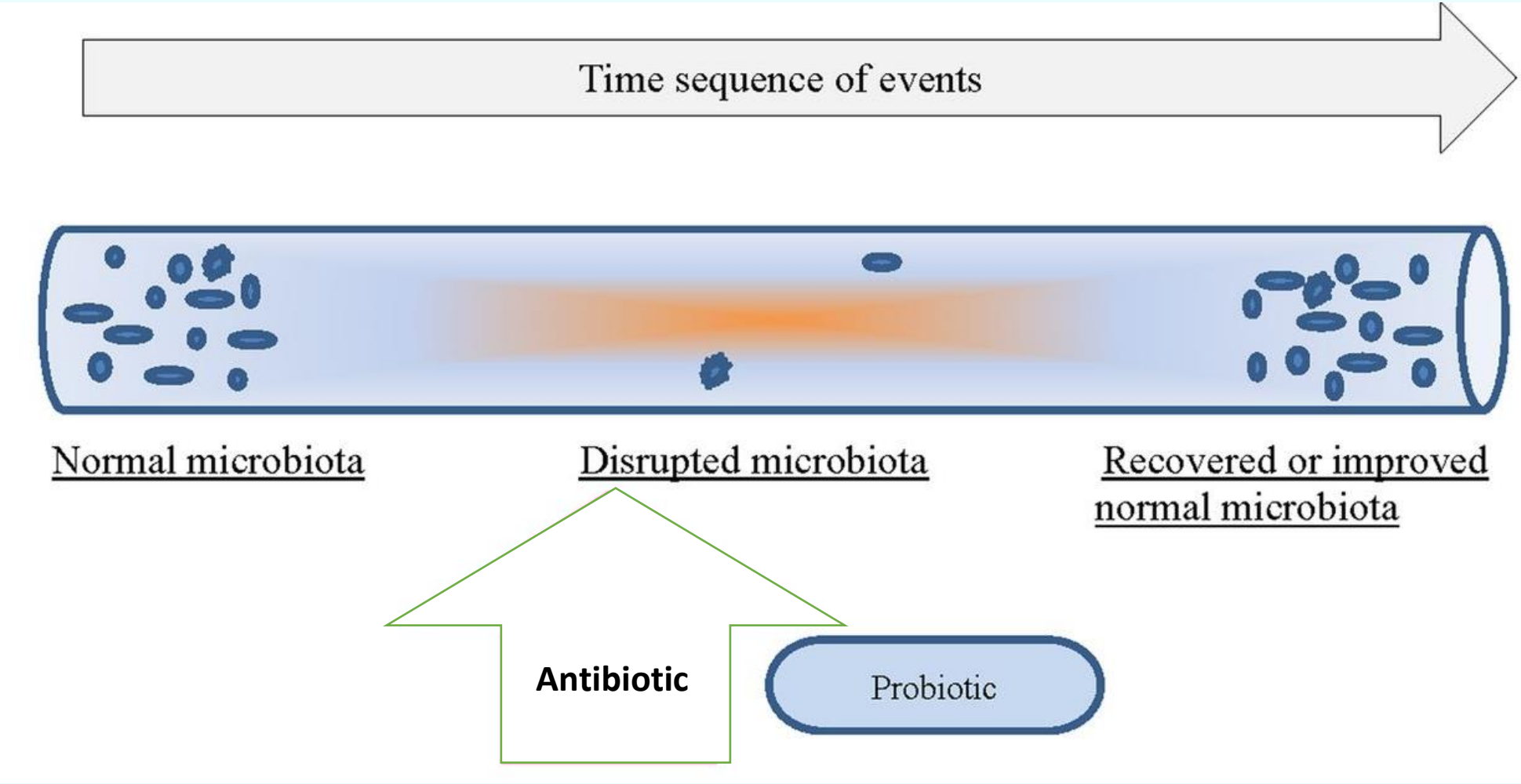




INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

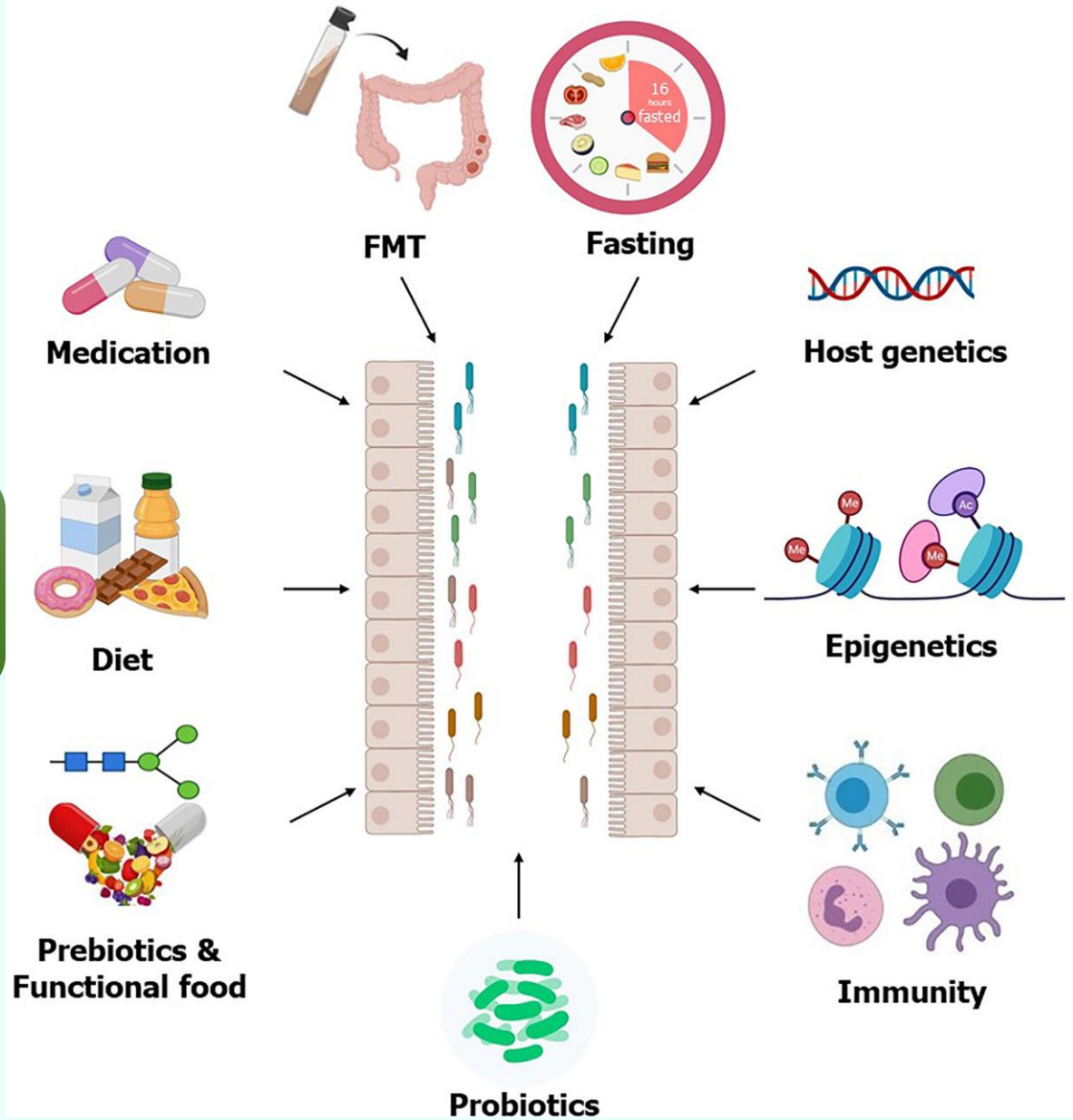
4- Antimicrobial Agents



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
5- Microbiota-Modulating Interventions

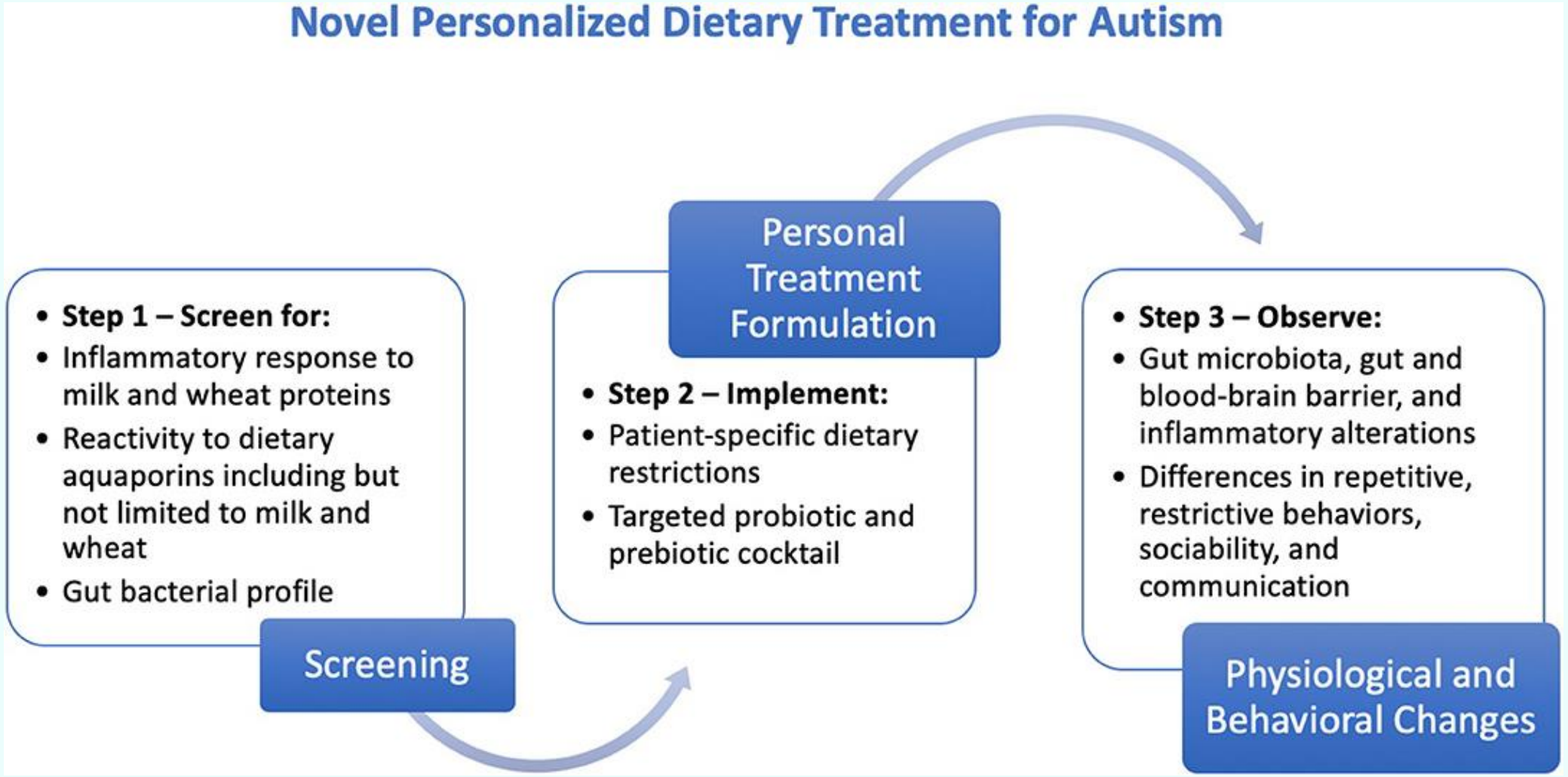
Microbiota Transfer Therapy or FMT has shown potential in treating autism-like symptoms by restoring a healthy gut microbiome composition



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

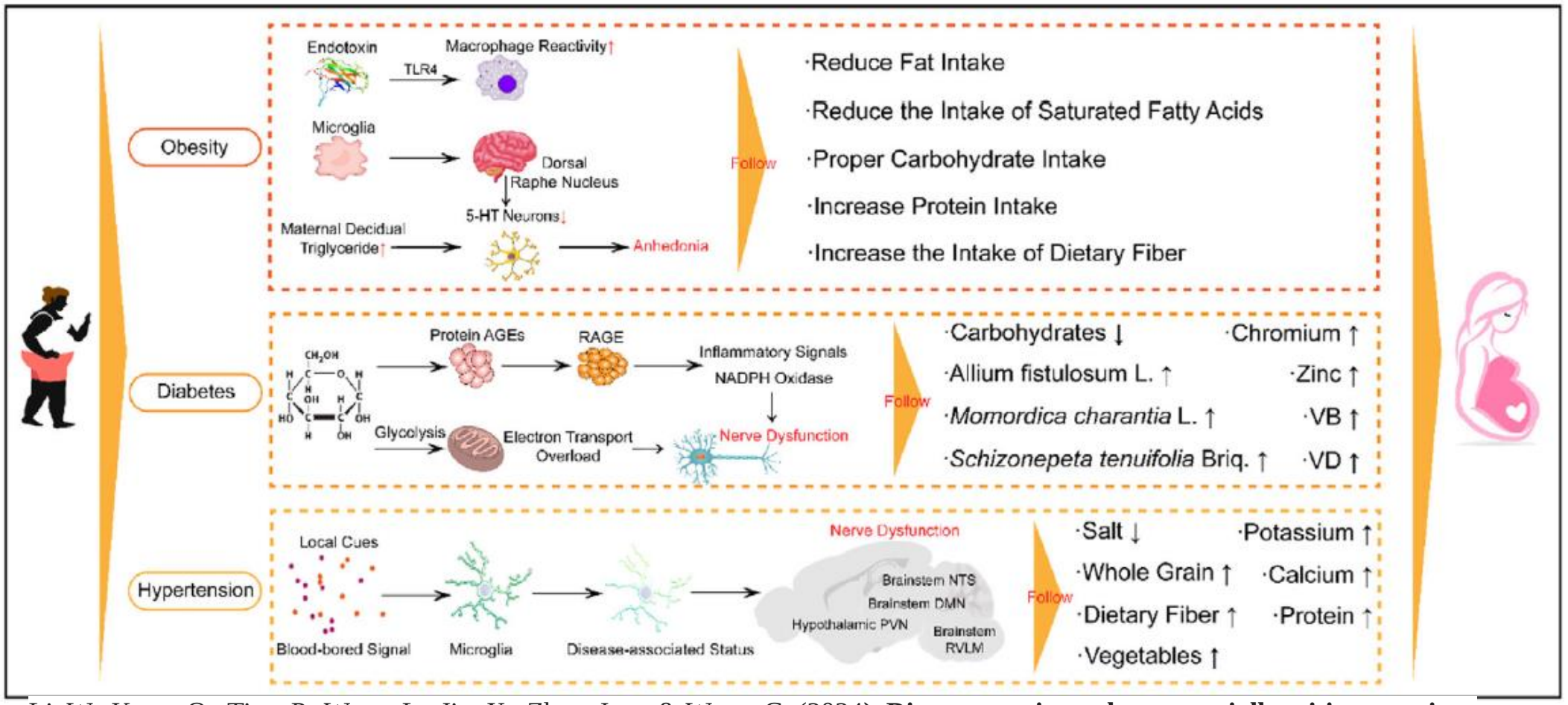
Personalized Intervention



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Maternal Role in Intervention

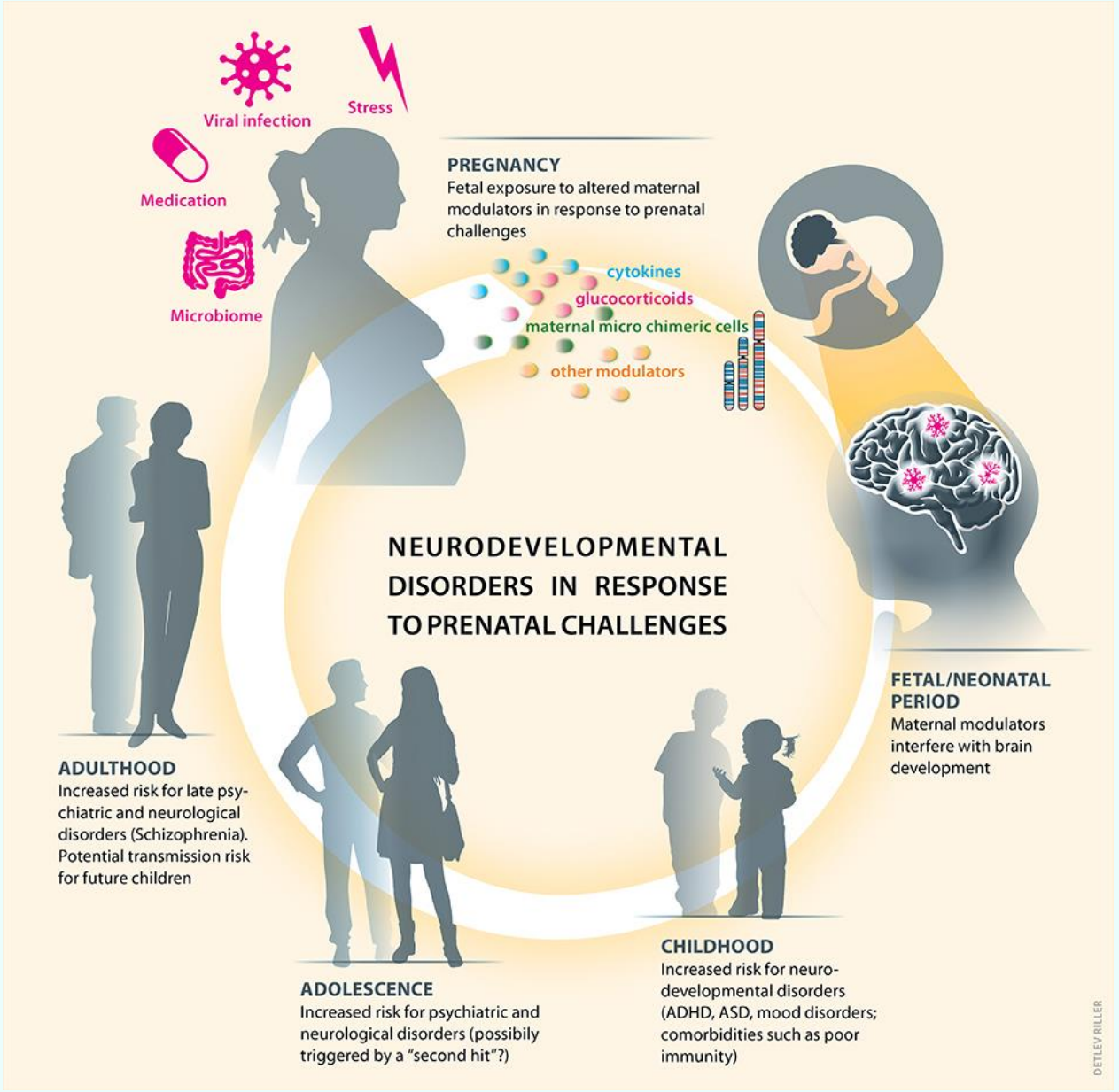


Li, W., Kong, Q., Tian, P., Wang, L., Jin, X., Zhao, J., ... & Wang, G. (2024**). Dietary nutrients that potentially mitigate autism spectrum disorder and dietary recommendations for children and pregnant women**. *Food Frontiers*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
Maternal Role in Intervention

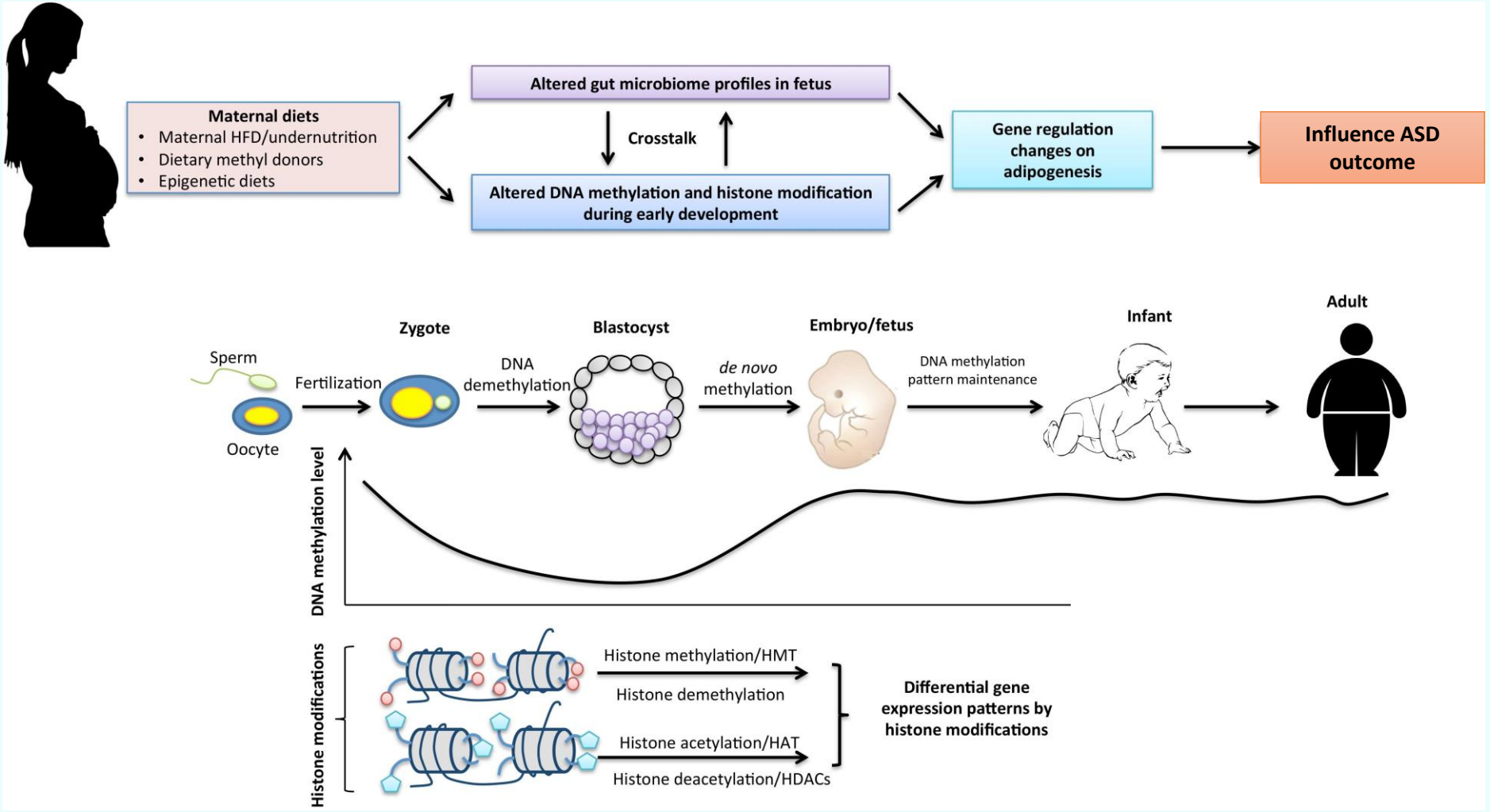
Maternal intervention during pregnancy and postnatal periods can influence a child's well-being, particularly in the context of **autism**, by reducing risk factors and supporting **neurodevelopment**.



This Photo by Unknown Author is licensed under CC BY

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
Maternal Role in Intervention



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

My Pregnancy Plate

Choose 2 to 3 servings of nonfat or 1% milk or yogurt (cow, soy or almond). A serving is 8 oz. Choose yogurt with less than 15 g of sugar per serving.

Drink mainly water, decaf tea or decaf coffee and avoid sugary beverages.

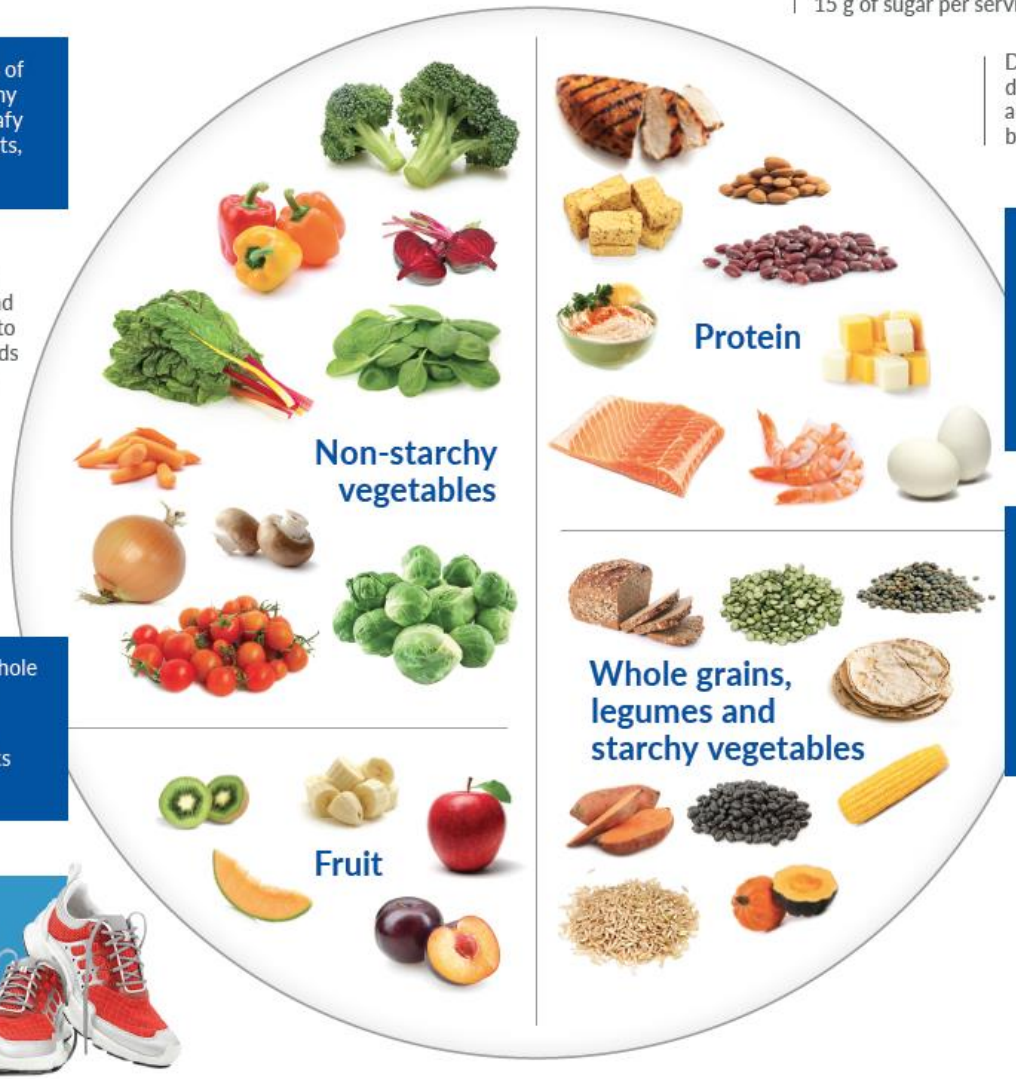
Choose large portions of a variety of non-starchy vegetables, such as leafy greens, broccoli, carrots, peppers or cabbage.

Choose small amounts of healthy oils (olive and canola) for cooking or to flavor foods. Nuts, seeds and avocados contain healthy fats.

Choose a variety of whole fruits. Limit juice and dried fruits.

Fruit is great for snacks and dessert, too.

Aim for at least 30 minutes of walking or another physical activity each day.



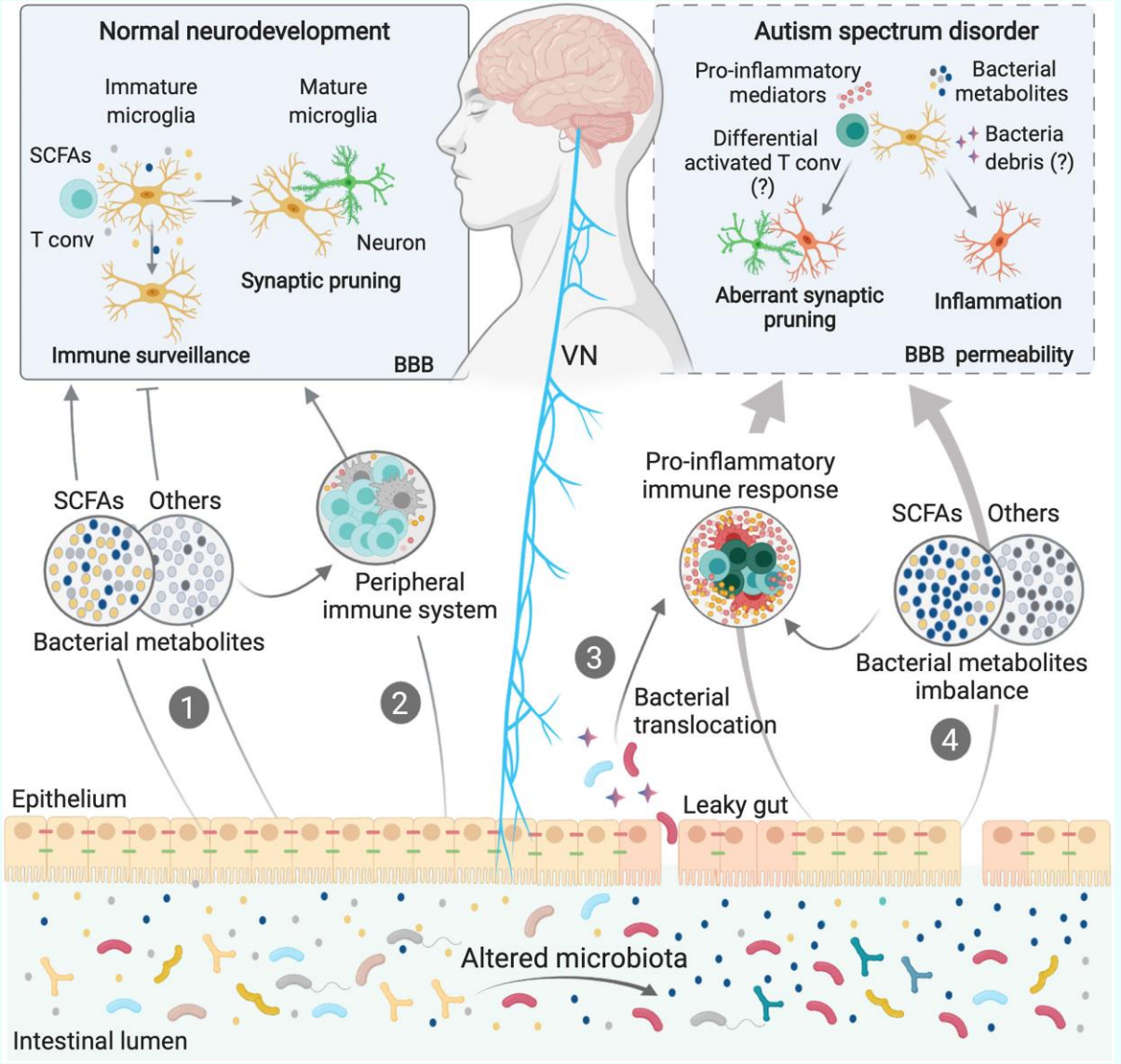
Choose protein sources such as poultry, beans, nuts, low-mercury seafood, eggs, tofu or low-fat cheese. Limit red meat and avoid cold cuts and other processed meats.

Choose whole grains, such as whole wheat bread or pasta, brown rice, quinoa or oats and other healthy starches like beans, lentils, sweet potatoes or acorn squash. Limit white bread, white rice and fried potatoes.

OHSU

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

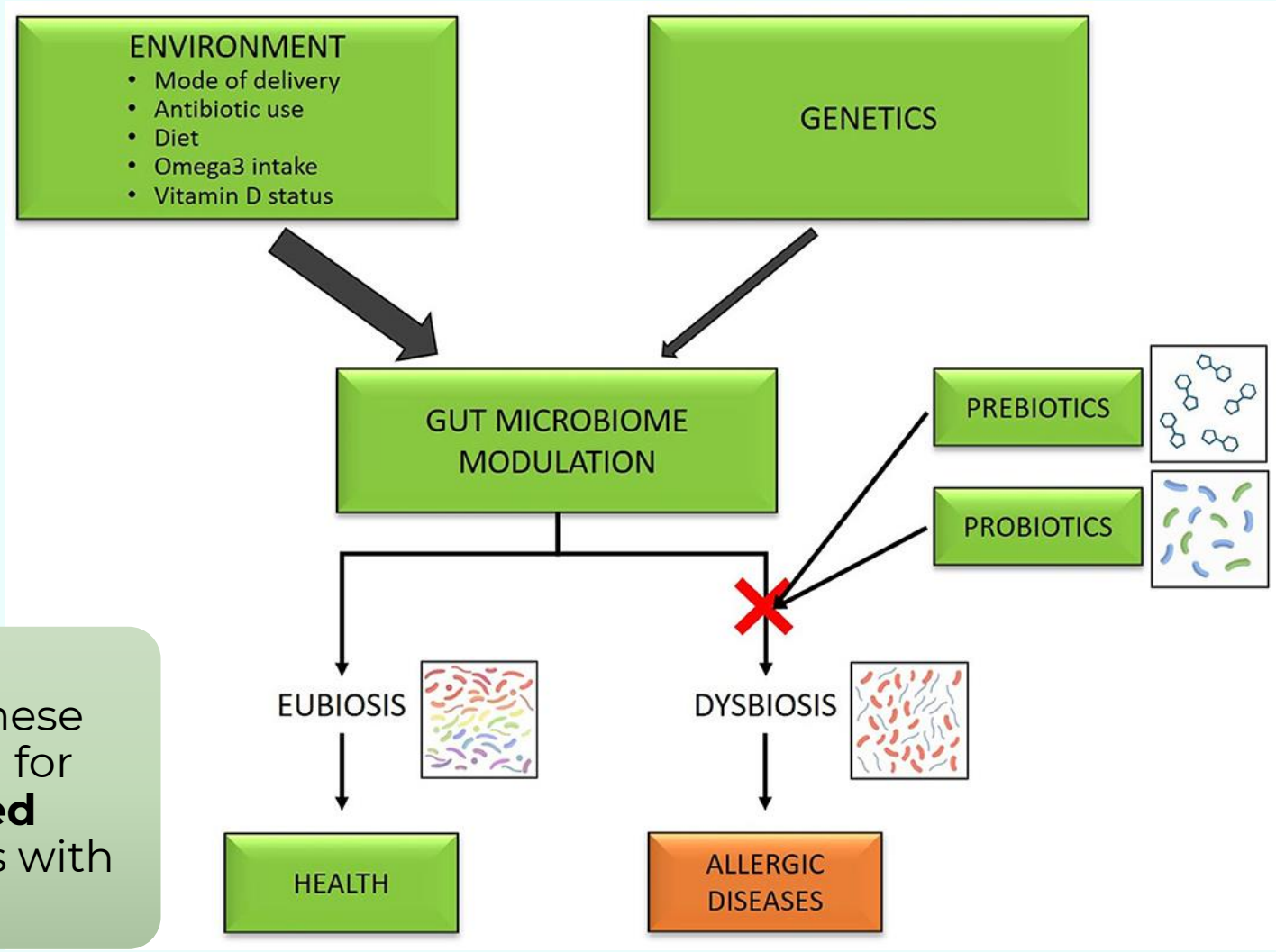
Autism Spectrum Disorder (ASD)
In A Nutshell



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)
Summary of Interventions

Various interventions aimed at **modulating** the gut **microbiota**, such as **probiotics, prebiotics**, and **dietary modifications,** have shown promise in improving ASD-related symptoms



Understanding the mechanisms behind these interventions is crucial for creating **personalized therapies** for individuals with ASD

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autism Spectrum Disorder (ASD)

Conclusion

Understanding the impact of nutrition and microbiota on autism spectrum disorders is crucial for developing **holistic treatments**

Tailoring interventions to address underlying biological mechanisms can lead to more effective and **personalized treatment strategies**

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

1- Taniya, M. A., Chung, H. J., Al Mamun, A., Alam, S., Aziz, M. A., Emon, N. U., ... & Xiao, J. (2022). Role of gut microbiome in autism spectrum disorder and its therapeutic regulation. Frontiers in cellular and infection microbiology, 12, 915701.

2- Mendive Dubourdieu, P., & Guerendiain, M. (2023). Understanding the link between gut microbiota, dietary intake, and nutritional status in children with autism and typical development. Frontiers in Nutrition, 10, 1202948.

3- Li, W., Kong, Q., Tian, P., Wang, L., Jin, X., Zhao, J., ... & Wang, G. (2024). Dietary nutrients that potentially mitigate autism spectrum disorder and dietary recommendations for children and pregnant women. Food Frontiers.

4- Sajdel-Sulkowska, E. M., & Zabielski, R. (2013). Gut microbiome and brain-gut axis in autism—aberrant development of gut-brain communication and reward circuitry. In Recent Advances in Autism Spectrum Disorders-Volume I. IntechOpen.

5- De Sales-Millán, A., Aguirre-Garrido, J. F., González-Cervantes, R. M., & Velázquez-Aragón, J. A. (2023). Microbiome–Gut–Mucosal–Immune–Brain Axis and Autism Spectrum Disorder (ASD): A Novel Proposal of the Role of the Gut Microbiome in ASD Aetiology. Behavioral Sciences, 13(7), 548.

6- Ristori, M. V., Quagliariello, A., Reddel, S., Ianiro, G., Vicari, S., Gasbarrini, A., & Putignani, L. (2019). Autism, gastrointestinal symptoms and modulation of gut microbiota by nutritional interventions. Nutrients, 11(11), 2812.

STUDY MATERIAL:

https://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders?gad_source=1&gclid=Cj0KCQjw_qexBhCoARIsAFgBleu2vAd59WUmhZPgGTUfq9_TMXnzw82x4swhcmpGnfTG4JiBi2rmWZUaAidwEALw_wcB

https://youtu.be/hczbp0-v4kI?si=6MbW8U6YzhTafeTA

https://autismnrc.org/

https://cdn.shopify.com/s/files/1/0667/4219/0293/files/Nutritional_Assessment_for_ASD.pdf?v=1697942948 (Nutritional Assessment sheet pdf)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

Learning Outcomes:

1. Understanding the diagnostic criteria for Autism Spectrum Disorder (ASD) and the role microbiota in its etiology
2. Investigating how the gut microbiota affects the gut-brain axis and how it might affect Autistic people
3. Exploring Therapeutic interventions and nutritional plans that may influence the symptoms of autism.
4. Considering implications for future Interventions based on personalised nutrition plans and microbiome analysis.

Understanding Autism :

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by core symptoms, including social deficits, language barriers, and repetitive stereotyped behavior. It was first defined by Professor Leo Kanner as a common neurodevelopmental disorder that primarily affects children. (**3**)

Introduction:

Key Facts:

- Autism, also known as autism spectrum disorder, is a brain-related condition affecting about 1 in 100 children.
- Detection of symptoms can occur in early childhood, but diagnosis is often delayed.
- Individuals with autism have varying abilities and needs, with some living independently and others requiring lifelong care.

Characteristics of Autism:(2)

The characteristics of autism can vary widely from person to person, but some common features include:

1. Impaired social interaction: Individuals with ASD may have difficulty initiating or maintaining social relationships, understanding social cues, and demonstrating appropriate emotional responses in social situations.

2. Communication challenges: Speech and language difficulties are common in individuals with ASD. Some individuals may have delayed language development or use repetitive and stereotyped language patterns. Others may have difficulty understanding and using nonverbal communication, such as gestures and facial expressions.

3. Restricted interests and repetitive behaviours: People with ASD often have intense and narrow interests, focusing on specific topics or objects. They may engage in repetitive behaviors, such as repetitive movements (e.g., hand flapping, rocking) or rigid adherence to routines.

Food restriction, difficult eating behaviours and GI disorders were easily recorded among medical conditions associated to autism. Children exhibit very selective eaters (“picky eaters”) and most of them show aversions to specific food colours, texture, smells, or other foods’ characteristics. (**6**)

4. Sensory sensitivities: Many individuals with ASD have sensory sensitivities, which can result in heightened or diminished response to sensory stimuli such as noise, lights, textures, or smells. This can lead to sensory-seeking or sensory-avoidant behaviours.

It's important to note that autism is a spectrum disorder, meaning that individuals may present with varying degrees of impairment and different combinations of these characteristics. Early diagnosis and intervention can help individuals with ASD to develop their strengths and overcome challenges.

Diagnosis

The diagnosis of autism spectrum disorder involves a comprehensive evaluation by healthcare professionals who specialize in neurodevelopmental disorders. While I can provide some general information, it's important to consult with a qualified healthcare professional for an accurate diagnosis.

Autism is typically diagnosed based on observations of an individual's behavior, developmental history, and standardized assessments. The evaluation process commonly includes the following components:

1. **Parent/Caregiver Interview**: Gathering detailed information from parents or caregivers about the individual's behavior, development, and current challenges.
2. **Developmental History**: Gathering information about early developmental milestones, including social interaction, communication skills, and repetitive behaviors.
3. **Behavioral Observation**: Observing the individual's behavior, social interactions, communication abilities, and specific patterns of play or interests. This evaluation may involve structured assessments like the Autism Diagnostic Observation Schedule (ADOS) or the Childhood Autism Rating Scale (CARS).
4. **Medical Evaluation**: Assessing the individual's overall health and ruling out any medical conditions or genetic syndromes that may be contributing to their symptoms.
5. **Psychological Classification**: Administering standardized tests and measures to assess cognitive abilities, language skills, adaptive functioning, and socio-emotional development.
6. **Nutritional Assessment:** This assessment is intended to help families, nutritionists and researchers make a rough estimate of the quality of diet and nutritional supplementation of a person with autism.

It's important to note that the diagnostic process may vary depending on the individual and the clinician. Early diagnosis and intervention play a vital role in supporting individuals with ASD, so seeking professional help is crucial if you suspect someone may have ASD. **(1)**

Pathogenesis: The pathology of ASD is complex and involves a combination of factors that can contribute to the development of the disorder. These factors include:

1. **Genetic Factors**
    a- Genetic variation and epigenetic modifications play a role in its development
    b- Genetic components, where heritability has been estimated to be from 60% to more than 80%
    c- with copy number variations and changes in gene expression being identified in some individuals with ASD. (6)
2. **Environmental factors** such as exposure to environmental chemical agents, air pollution, and pesticide residues on fruits and vegetables have also been linked to an increased risk of ASD.
3. **Maternal factors** significantly influence the gut microbiota of individuals with Autism. Antibiotic prophylaxis during pregnancy can alter the gut microbiota composition, especially during the first week after delivery. The mode of delivery, whether caesarean section or vaginal, also influences the type of microbiota colonizing the newborn's intestine. (5)
    a- **Maternal nutrition** can impact the risk of ASD in offspring. The type of diet, whether breast milk or formula, also shapes the infant's gut microbiota diversity. Dietary factors such as poor nutrition, high caffeine intake, high salt intake, high-energy, high-density diet, Western diet, PUFA intake and maternal obesity can increase the risk of ASD.

    b- **The gut microbiome** play a significant role in autism. Studies have shown that individuals with autism often present gastrointestinal (GI) symptoms, and alterations in the gut microbiome have been observed. The "bacterial theory" of autism suggests that changes in microbial composition may be involved in the pathogenesis or progression of autism. (4) Common findings include a decreasing trend in the ratio of Bacteroidetes to Firmicutes, as well as increased abundance of Clostridium in children with ASD (5).

It is important to note that while these factors may contribute to ASD, none of them can be considered an absolute cause of the disorder. The development of ASD is multifactorial, involving a combination of genetic, epigenetic, prenatal, environmental, and micorbiome factors.

Gut Connection: (5)

Gastrointestinal symptoms are prevalent in individuals with autism spectrum disorder (ASD), with estimates ranging from 9% to 91% in children and 9% to 70% in individuals overall. These symptoms can include abdominal pain, constipation, diarrhea, bloating, vomiting, and gas.

Research on the gut microbiota of people with Autism has revealed its impact on gastrointestinal disorders. Recent studies have investigated the impact of gut bacteria in memory, stress, mood, neurodevelopment, in autistic individuals.

The gut-microbiome-brain axis is a multidisciplinary field that is gaining global interest, with research demonstrating its importance for the behavioural and cognitive health of children with autism.

Gut brain Axis: (1)
The gut-brain axis is a bidirectional communication system between the gastrointestinal system and the central nervous system, involving the gut microbiome, immune system, and enteric nervous system. **The microbiota-gut-brain axis** connects the central nervous system and the enteric nervous system via Vagus nerves.

Role of Microbiome: The gut microbiome, comprising trillions of microorganisms, plays a significant role in this axis. Changes in the gut microbiota composition and disruption of this axis have been linked to neurological disorders, including autism spectrum disorder. **(5)**

The gut microbiota functions include:

- Nutrient metabolism and fermentation of dietary components, producing short-chain fatty acids that serve as an energy source and have effects on gut health and immune function.
- Interaction with the immune system, influencing its development and function, and playing a role in maintaining immune homeostasis.
- Production of neurotransmitters such as serotonin, dopamine, and GABA, which regulate mood, behavior, and cognitive function.
- Communication with the brain through the gut-brain axis, affecting brain function and behavior.
- Regulation of intestinal barrier function, maintaining gut health and preventing the entry of harmful substances into the bloodstream.

Examine the Microbiota relation with Autism (6)
The gut-brain axis plays a significant role in the etiology of Autism. Gut

microbiota bacteria can use food that escapes digestion to produce metabolites, such as SCFAs and 5′-HT, which can be used by the host. Increased 5′-HT production by the microbiota leads to tryptophan depletion and hyperserotonemia, which is associated with gastrointestinal symptoms.

Dysbiosis

Dysbiosis, or an imbalance in gut microbiota, has been linked to autism spectrum disorder (ASD) development.

It is characterised by decreased microbial diversity, metabolic abnormalities, and poor gut barrier function. The absorption of nutrients in the small intestine is low, leading to more monosaccharides and disaccharides reaching the large intestine, benefiting bacteria that ferment simple sugars and show greater growth. This can cause GIT symptoms like gas and bloating. (1)

Children with severe autism have a lower abundance of Bacteroides and Faecalibacterium, higher abundance of Clostridial, and higher levels of Erysipelotrichaceae.(5)
The lower abundance of bacteria types observed in children with Autism Spectrum Disorder (ASD) compared to healthy controls includes Prevotella, Coprococcus, Enterococcus, Lactobacillus, Streptococcus, Lactococcus, Staphylococcus, Ruminococcus, and Bifidobacterium species. Studies have also reported a higher abundance of Clostridia and Desulfovibrio bacteria in individuals with ASD. (6)

Dysbiosis, an altered gut microbial composition, can lead to increased intestinal permeability and neuroinflammatory responses, commonly referred to as "leaky gut." This leads to the passage of macromolecules, such as bacteria-derived lipopolysaccharide, into the bloodstream, triggering a systemic immune and inflammatory response. This, in turn, can lead to impaired social communication in individuals with Autism Spectrum Disorder (ASD). (6)

The gut microbiota can also influence brain function by modulating the vagus nerve activity, allowing bidirectional communication between the intestinal lumen and brainstem, affecting neurogenesis, neurodevelopment, mental homeostasis, emotions, and immune response modulation. (5)

So, is dysbiosis the cause of autism or the result of autistic eating behaviour?

The role of dysbiosis in ASD aetiology is yet to be confirmed by further research, but Yap's research suggests it may not be the primary cause but rather a result of changing dietary habits in autistic individuals. (5)

According to Sulkowska and colleague book, *Recent Advances in Autism Spectrum Disorders*, Autism-related gut microbiome changes may be both a cause and a result of autistic behaviour.
Disruptions in the gut microbiome can impair the normal operation of the reward circuitry, including social and eating behaviour. This disturbance is thought to be caused by abnormalities in the dopaminergic-oxytocinergic "wanting circuitry."
Also gut bacteria play a role in controlling brain serotonin levels, and abnormal blood serotonin levels are routinely identified in some children with autism. (4)

There is still much debate and research needed to determine the exact relationship between dysbiosis and autism. Further investigation is necessary to fully understand the complex interactions between gut health and neurological conditions like autism.

Nutrition Status:

Neurotypical children often exhibit a "picky eater" attitude towards certain foods, typically around the age of six. However, children with ASD experience intensified **food selectivity** from an early age, leading to malnutrition risk and negative health consequences. (6)

- Factors determining food selectivity include texture, appearance, taste, smell, and temperature, as well as reluctance to try new foods. A strong preference for starches, snacks, and processed foods, along with a rejection of fruits, vegetables, or protein, is common. (6)

- Autism spectrum is linked to nutritional deficiencies due to food selectivity. Children with ASD tend to have a limited variety of foods and inadequate intake of certain nutrients, including minerals like calcium, iron, zinc, potassium, and copper, as well as vitamins A, D, E, riboflavin, C, B-12, folic acid, and choline.
- Studies have shown decreased bone development and increased fracture risk in children with ASD due to a lack of calcium and vitamin D. Some cases of excessive beta carotene and vitamin C deficiency have also been documented.
- Increased consumption of snack foods and calorie-dense foods can lead to excessive weight gain, higher rates of obesity, and obesity-related complications. (3) They also consume excessive sodium, likely due to packaged foods. (6)

This can result in obesity or undernutrition. The DSM-5 emphasizes the importance of food regulation in children with ASD..

- Gut microbiota changes, such as altered short-chain fatty acid composition, energy homeostasis, and inflammation. Therefore, it's crucial to consider the impact of nutritional status on the intestinal microbiota. (6)
- A study comparing gut microbiota composition in children with autism spectrum disorder and typical development found significant differences, particularly in certain bacterial species. Excess weight children with ASD had lower levels of certain bacteria (Roseburia and Faecalibacterium prausnitzii), fewer Bifidobacterium longum, and higher Clostridium glycolicum. (2) Narrow interests in food intake can result in reduced α-diversity of the gut microbiota and a thinner fecal texture (3).

  Further research and studies would contribute to a broader understanding of the differences in bacterial species between children with autism and those with typical development.

Therapeutic Strategies:

The potential impact of nutritional deficits on the overall health and well-being of children with ASD, including the importance of addressing these issues through dietary interventions and supplementation.

Several therapeutic strategies target the gut-brain axis and the gut microbiome, including dietary restrictions, antimicrobial agents, probiotics, and supplementation with specific nutrients.

Understanding their unique needs is essential for developing tailored nutrition plans to support their overall health and well-being.

1- **Nutritional Intervention**, Nutritional therapy can play a significant role in managing ASD symptoms. By focusing on nutrient-dense foods and addressing gut health, it is possible to positively impact behavioral and cognitive function.

The most studied dietary approaches in ASD are the gluten-free/casein-free diet, ketogenic diet, specific carbohydrate diet, and Mediterranean diet. (6)

A. **Gluten-free or casein-free diet** which involves:

i. Exclusion of all food items containing wheat, oats, barley, or rye, which are all flours, bread, rusks, pasta, pastries, and other bakery products made with these cereals, while the elimination of casein means no intake of dairy products, including breast milk, yoghurt, cheese, butter, cream, or ice cream, among others.

ii. The diet has been shown to improve behaviour, decrease urine peptides, and alleviate gastrointestinal symptoms in individuals with ASD.

iii. The diet is recommended as it can improve intestinal tissue, absorption of nutrients, and prevent methylation inhibition

iv. However, evidence on healthy subjects showed that a gluten-free diet has been associated with the reduction of beneficial gut bacteria populations, an increase in opportunistic pathogens, and an immune-suppressive effect. Some studies suggest that this diet could decrease fibre intake, potentially worsening gastrointestinal issues.

v. The research on this diet's effect in autism is fragmented due to its quantity, quality, and methodological limitations, with some studies indicating positive effects and others indicating potential negative outcomes.

b. **The Ketogenic Diet** is a high fat and low-carbohydrate diet that has been shown to be effective in treating epileptic patients who fail to respond to anticonvulsant medications. It has also been suggested as a treatment for Autism.
    i. Animal studies have shown that keto can improve behavioral aspects, such as sociability, repetitive behaviors, and social communication. KD treatment of BTBR mice also improved deficiencies typical of Autism related to myelin formation and white matter development, acting on neurotransmitter signaling pathways.
    ii. Can optimize gut bacteria, regulate enzyme activity, and increase brain neuron density. (3)
    iii. However, Keto-diet is associated with higher inflammation and defective mitochondrial risk, and its side effects of constipation and reflux may worsen gastrointestinal comorbidities in autistic patients.

c. **The Specific Carbohydrate Diet** is a dietary protocol used to alleviate symptoms of malabsorption and prevent the growth of pathogenic gut microbiota. Developed in the 1930s, it is used to treat Crohn's disease, ulcerative colitis, diverticulitis, and chronic diarrhea.
    i. The diet recommends monosaccharides from fruit, vegetables, and honey, while restricting complex carbohydrates due to their longer digestibility and potential absorption difficulties.
    ii. A study examining the diet protocol in a child with autism found that the protocol was well tolerated, leading to improvements in growth status, gastrointestinal symptoms, and behaviors. Further research is needed to evaluate the SCD protocol in young children with Autism GI concerns.

d. **The Mediterranean Diet** is a diet characterized by high consumption of fruits, vegetables, legumes, nuts, cereals, and olive oil, moderate intake of fish, dairy products, and alcohol, and low intake of saturated lipids, sweets, and red and processed meat. This diet is popular among populations near the Mediterranean Sea.
    i. It has been shown to have beneficial effects against cardiovascular, metabolic, and mental diseases. However, no studies have reported the effect of it on Autistic patients.

ii. A study by Ríos-Hernández et al. found that low adherence to MD is associated with an increased likelihood of an ADHD diagnosis in children and adolescents. Individuals with ADHD missed a second serving of fruit daily, reduced intake of vegetables, pasta, and rice, and skipped breakfast more often than controls. High consumption of sugar, candy, cola beverages, and noncola soft drinks, as well as low consumption of fatty fish
iii. Further research is needed to investigate causality and determine if dietary manipulation could reverse ADHD symptoms. (6)

e. **A low-FODMAP diet**, containing fermented oligosaccharides, disaccharides, monosaccharides, and polyols.
    i. Limited research suggests a low-FODMAP diet may alleviate symptoms in children with ASD, but it reduces intestine-related bacteria, potentially impacting immune function.
    ii. Further modifications are needed to fully understand its benefits and drawbacks. (3)

f. **Dietary fiber,** found in plant-based foods essential for healthy diet. Soluble fibers, like pectin and beta-glucan, help lower cholesterol and regulate blood sugar levels. Insoluble fibers, like cellulose and lignin, add bulk to the stool, promoting bowel movements and preventing constipation.
    Significantly increase microbial diversity, with gastrointestinal bacteria producing short-chain fatty acids (SCFAs) like butyrate, acetate, and propionate.
    i. SCFAs also impact the central nervous system by altering metabolic and immune system function and regulating gene expression. Butyrate, one SCFA produced, can improve the CNS function by inhibiting histone deacetylases, which can lead to transcriptional dysfunction and potentially cause neurodegenerative diseases.
    ii. Studies show increased production of SCFAs in children with Autism, altering dietary fiber fermentation and abundance, potentially acting as a biomarker for neurodegenerative disorders.
    iii. Therefore, SCFAs can be both beneficial and detrimental to neurodegenerative disorders. (1)

2- Supplementation

a. Supplementation is crucial for individuals with Autism due to potential nutritional deficiencies and inadequate intake. Studies show lower intake of minerals like calcium, iron, zinc, potassium, copper, and vitamins. (4)
b. Vitamin C deficiency and scurvy have also been reported. Monitoring and addressing nutritional deficits through proper supplementation is essential for ensuring adequate nutrient intake and overall health in children with ASD. (6)
c. Vitamin D supplementation has been shown to improve ASD symptoms by increasing serum 25-OH-VD concentrations, as measured by the Childhood Autism Rating Scale and the Autism Treatment Evaluation Scale. However, the results can be inconsistent.
d. Folic acid and vitamin B12 supplementation are recommended for children with ASD to address deficiencies linked to poor adaptive behaviour. Therefore, including folic acid and VB12 supplementation in mitigation strategies for ASD is suggested.
e. Unsaturated fatty acid supplementation may alleviate ASD symptoms, but the mechanism and ideal intake remain unclear. (3)
f. Research suggests vitamins, natural products, and functional foods may also help, but further studies are needed to determine optimal dosages and formulations for children with ASD. (3)

3- Pre and Probiotics

Prebiotics and probiotics have been found to improve host health by promoting the metabolism and proliferation of beneficial bacteria.

- Prebiotics can alleviate symptoms by increasing the abundance of Lactobacillus reuteri, a bacteria producing serotonin and regulating intestinal microbiota.
- According to Williams et al. (2011) abnormalities in carbohydrate digestion and transporters, involving disaccharidases and hexose transporters, are linked to dysbiosis, with decreased Bactoroidetes and increased Firmicutes ratios. These abnormalities respond to probiotic and dietary responses, affecting microbiome composition, intestinal barrier function, and mucosal immune responses. (4)
- Probiotics, on the other hand, have limited Autistic prevention effects and need to be improved through improved biofilm formation, oral delivery systems, and nano-encapsulation materials. **(3)**

4- **Antimicrobial agents** have been used to reduce dysbiosis and control gut permeability. (6)
   - Antibiotics, which are antimicrobial agents, can have both positive and negative effects on the gut microbiome. They can cause dysregulation of the gut microbiota, leading to long-term effects.
   - Studies show that maternal antibiotic prophylaxis can affect Bifidobacterium spp. counts in human milk during the first week after delivery. The composition and diversity of the infant gut microbiota, including Actinobacteria, Bacteroidetes, Bifidobacterium, Lactobacillus, Clostridium, Proteobacteria, Firmicutes, and Enterococcus spp., can also be influenced by antibiotic use before and after delivery, as well as the mode of delivery. (5)

   - However, antibiotic treatment can be effective in certain clinical situations, such as recurrent Clostridioides difficile infections. They can inhibit certain bacteria growth and alter metabolic pathways, leading to neurodegenerative diseases and behavioral disorders. (1)

5- **Microbiota-Modulating Interventions**: It is important to consult with healthcare professionals and specialists to assess individual circumstances and determine the most appropriate maternal interventions for each specific case.

- Various interventions aimed at modulating the gut microbiota, such as probiotics, prebiotics, and dietary modifications, have shown promise in improving ASD-
- **Microbiota Transfer Therapy** has shown potential in treating autism-like symptoms by restoring a healthy gut microbiome composition. However, many studies have limitations in sample sizes, randomization, and control groups, reducing the validity of the results. Recent clinical trials have shown satisfactory results for MTT on ASD children, with minimal adverse effects. (1)

Personalised Intervention

Personalized treatments refer to healthcare approaches that are tailored to the specific needs, preferences, and characteristics of an individual. In the context of personalized treatments in relation to Autism. Interventions should take into account factors such as genetic predisposition, environmental influences, gut microbiota, and metabolic disturbances. These interventions aim to address the unique challenges and symptoms experienced by individuals with ASD through personalized and

individualized approaches. **(5)**

Maternal Role in ASD: The prenatal environment and maternal health have been found to influence the risk of ASD. Identified risk factors:

- **Maternal obesity** during pregnancy can activate macrophages and reduce 5-HT neurons in offspring, leading to ASD symptoms.
- **Gestational diabetes** can cause abnormalities in advanced glycation end product metabolism and glycolysis, resulting in neural developmental abnormalities in offspring.
- **Gestational hypertension** can contribute to neural developmental disorders by causing pathological changes in microglial cells in offspring. (3)

Maternal intervention refers to various actions or treatments that a mother can undertake during pregnancy or early postnatal periods to potentially influence the well-being and development of her child. In the context of autism, maternal intervention can include a range of strategies aimed at reducing risk factors or supporting the child's development. Some examples of maternal interventions in autism may include: (4)

1. Prenatal care: Receiving proper prenatal care, including regular check-ups, necessary vaccinations, and a healthy diet, can contribute to overall maternal health and the well-being of the developing fetus.
2. Avoidance of certain substances: Avoiding exposure to harmful substances such as tobacco smoke, alcohol, and illicit drugs during pregnancy is important, as these substances can potentially impact fetal development and increase the risk of various health issues, including neurodevelopmental disorders.
3. Dietary modifications: Following a balanced and nutritious diet during pregnancy can provide essential nutrients and support proper fetal growth and development.
4. Stress management: Engaging in stress-reducing activities, such as exercise, relaxation techniques, and seeking social support, may help manage stress levels during pregnancy. Chronic maternal stress has been associated with adverse developmental outcomes in children.
5. Genetic testing and counseling: Genetic testing and counseling can provide valuable information about genetic risk factors and guide decision-making regarding reproductive choices.

6. Microbiota testing in pregnant women assesses gut microbiota composition and activity, include maternal diet's impact on placenta-brain axis, and hypertension's impact on gut microbiota during pregnancy. (3)
   Understanding the mechanisms underlying these interventions is critical for developing targeted therapies for individuals with ASD.

References:

1- Taniya, M. A., Chung, H. J., Al Mamun, A., Alam, S., Aziz, M. A., Emon, N. U., ... & Xiao, J. (2022). **Role of gut microbiome in autism spectrum disorder and its therapeutic regulation.** *Frontiers in cellular and infection microbiology*, *12*, 915701.

2- Mendive Dubourdieu, P., & Guerendiain, M. (2023). **Understanding the link between gut microbiota, dietary intake, and nutritional status in children with autism and typical development.** *Frontiers in Nutrition*, *10*, 1202948.

3- Li, W., Kong, Q., Tian, P., Wang, L., Jin, X., Zhao, J., ... & Wang, G. (2024**). Dietary nutrients that potentially mitigate autism spectrum disorder and dietary recommendations for children and pregnant women**. *Food Frontiers*.

4- Sajdel-Sulkowska, E. M., & Zabielski, R. (2013). **Gut microbiome and brain-gut axis in autism—aberrant development of gut-brain communication and reward circuitry**. In *Recent Advances in Autism Spectrum Disorders-Volume I*. IntechOpen.

5- De Sales-Millán, A., Aguirre-Garrido, J. F., González-Cervantes, R. M., & Velázquez-Aragón, J. A. (2023). **Microbiome–Gut–Mucosal–Immune–Brain Axis and Autism Spectrum Disorder (ASD): A Novel Proposal of the Role of the Gut Microbiome in ASD Aetiology**. *Behavioral Sciences*, *13*(7), 548.

6- Ristori, M. V., Quagliariello, A., Reddel, S., Ianiro, G., Vicari, S., Gasbarrini, A., & Putignani, L. (2019). **Autism, gastrointestinal symptoms and modulation of gut microbiota by nutritional interventions.** *Nutrients*, *11(11), 2812*.

Study material:

https://www.who.int/news-room/fact-sheets/detail/autism-spectrum-disorders?gad_source=1&gclid=Cj0KCQjw_qexBhCoARIsAFgBleu2vAd59WUmhZPgGTUfq9_TMXnzw82x4swhcmpGnfTG4JiBi2rmWZUaAidwEALw_wcB

https://youtu.be/hczbp0-v4kI?si=6MbW8U6YzhTafeTA

https://autismnrc.org/

https://cdn.shopify.com/s/files/1/0667/4219/0293/files/Nutritional_Assessment_for_ASD.pdf?v=1697942948 (Nutritional Assessment sheet)

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

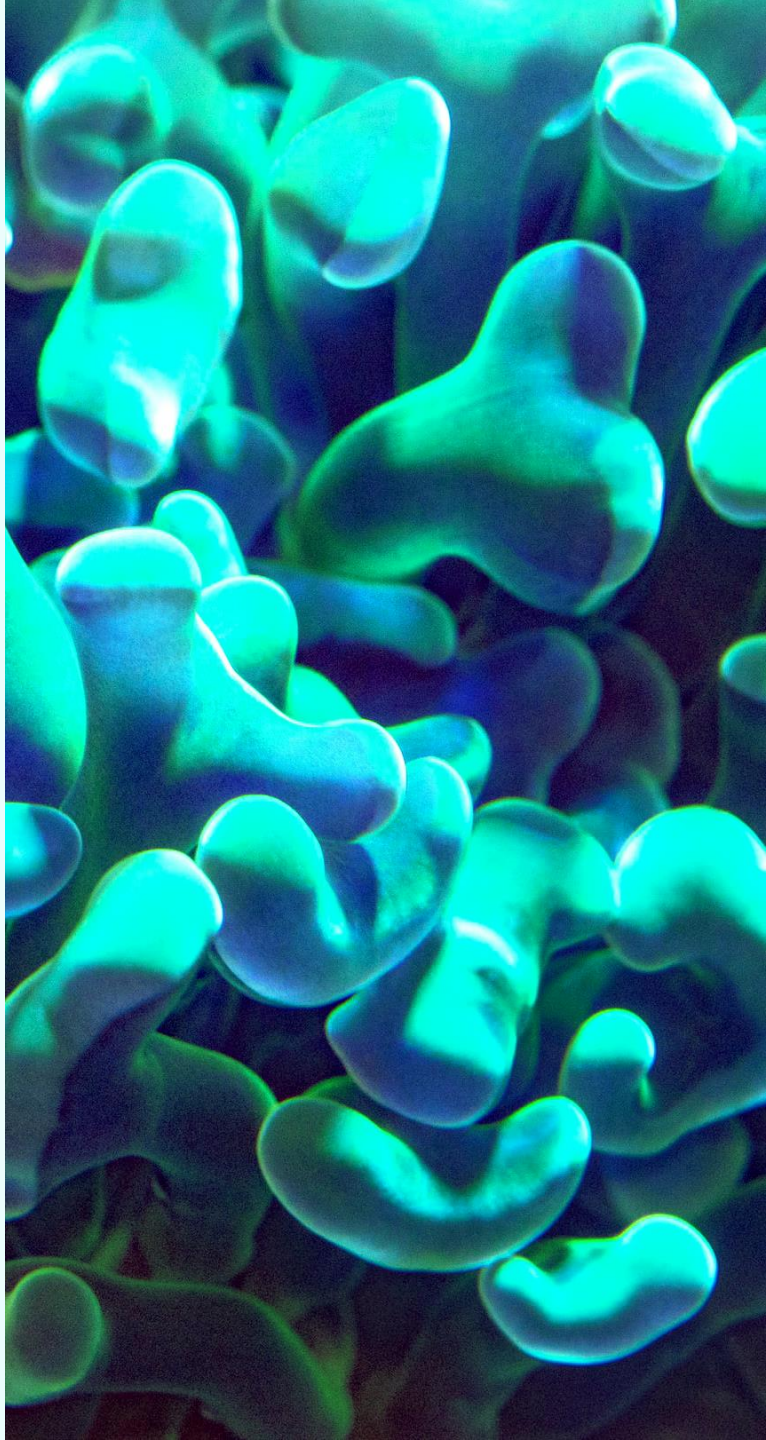
Post-graduate certificate level 7 (30 ECTS)

MICROBIOME-IMMUNE-BRAIN AXIS

Name of the lecturer
Qualification

lecturer@imas.edu.mt

Malta Further & Higher Education Authority



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The Microbiome-Immune-Brain Axis and its impact on Health

- The human host and microbe coevolution has established a mutualistic symbiosis.
- The human gastrointestinal (GI) tract is populated with a diverse microbial community.

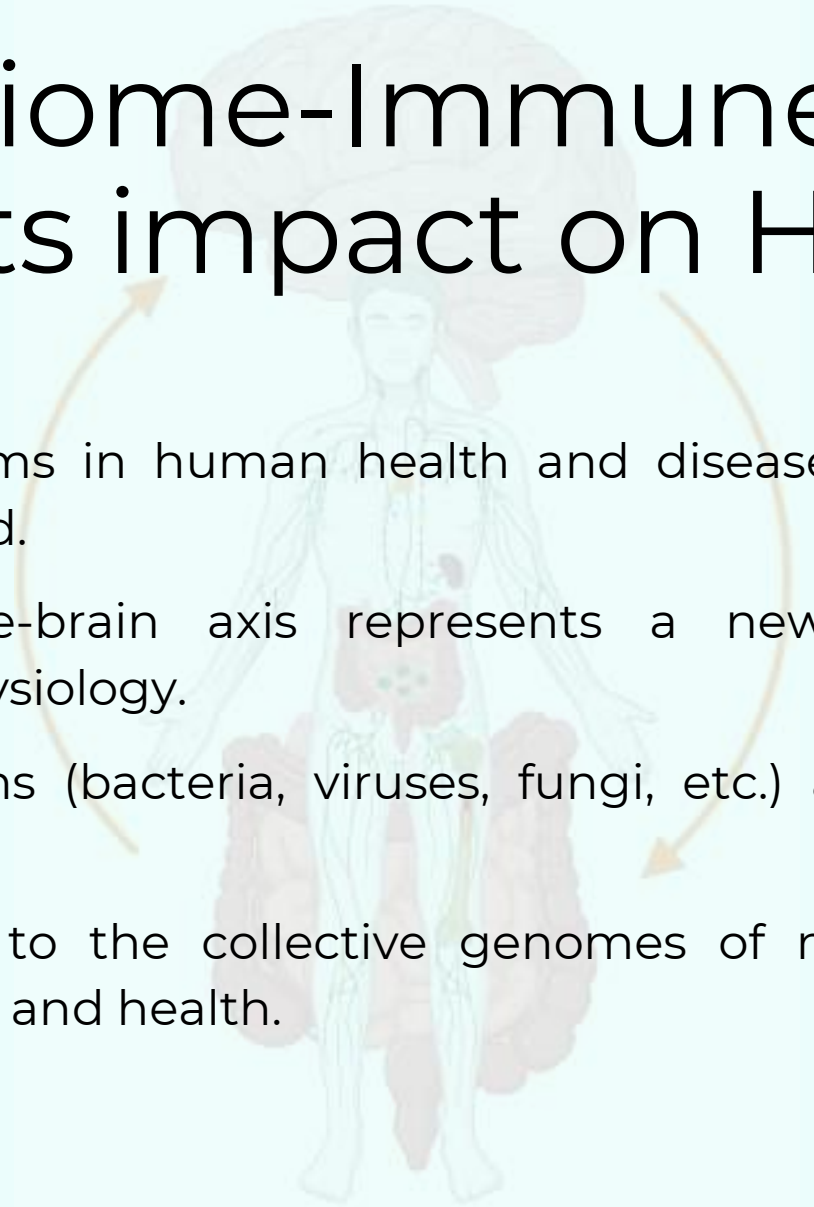
🔷 **Implications on:**
- Health maintenance
- Development
- Aging
- Disease

- New technologies unveiled the bidirectional immunomodulated communication between the gut microbiome and the central nervous system, referred to as the "**microbiome-immune-brain axis**".

Front. Microbiol. 2023, 14:1118529

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The Microbiome-Immune-Brain Axis and its impact on Health



- The role of microorganisms in human health and disease, including brain health, is increasingly acknowledged.
- The microbiome-immune-brain axis represents a newly recognized frontier in understanding human physiology.
- Trillions of microorganisms (bacteria, viruses, fungi, etc.) are crucial determinants of health and disease.
- **Gut microbiome**: Refers to the collective genomes of microorganisms in the gut, influencing bodily systems and health.

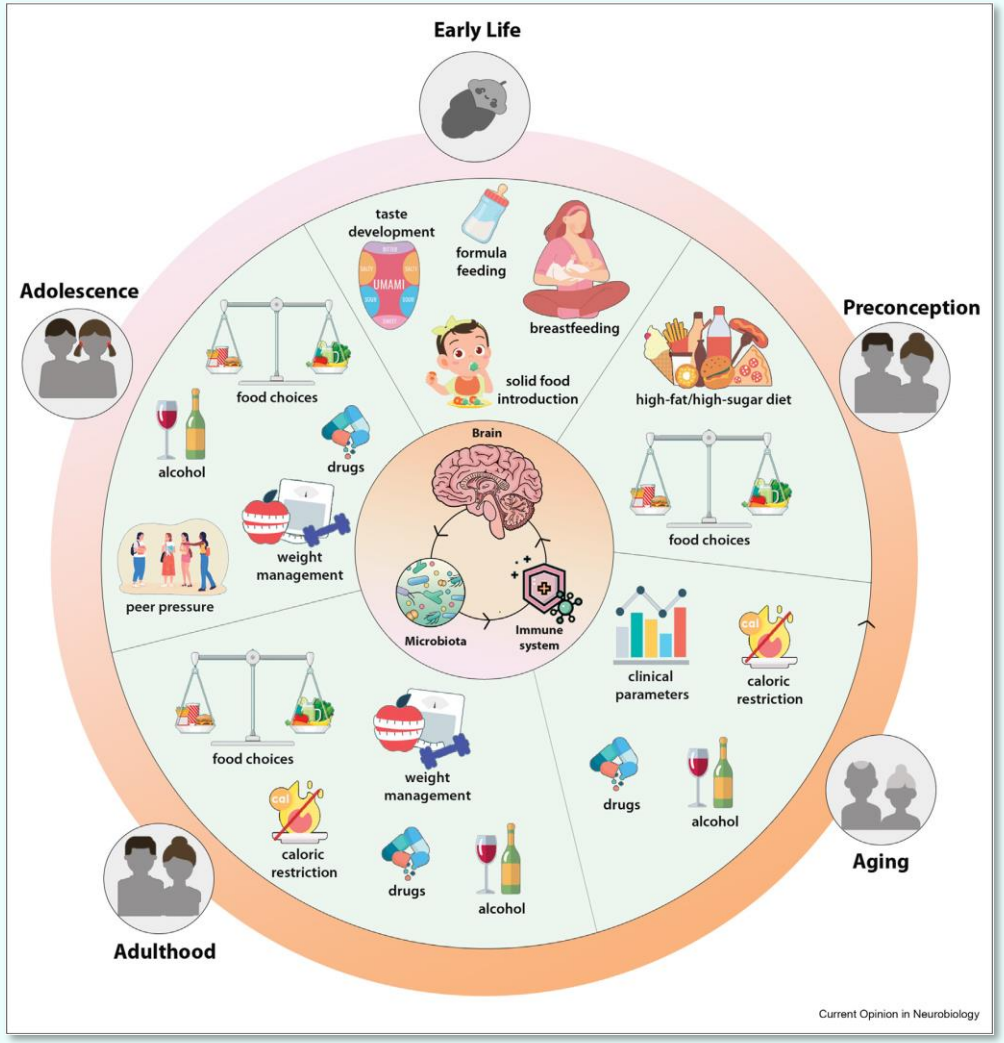
Front. Microbiol. 2023, 14:1118529

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiome, Brain, and Immunity: A Lifespan Perspective

◆ Parental diet and lifestyle influence microbiome composition, immune system, and cognitive development in offspring.

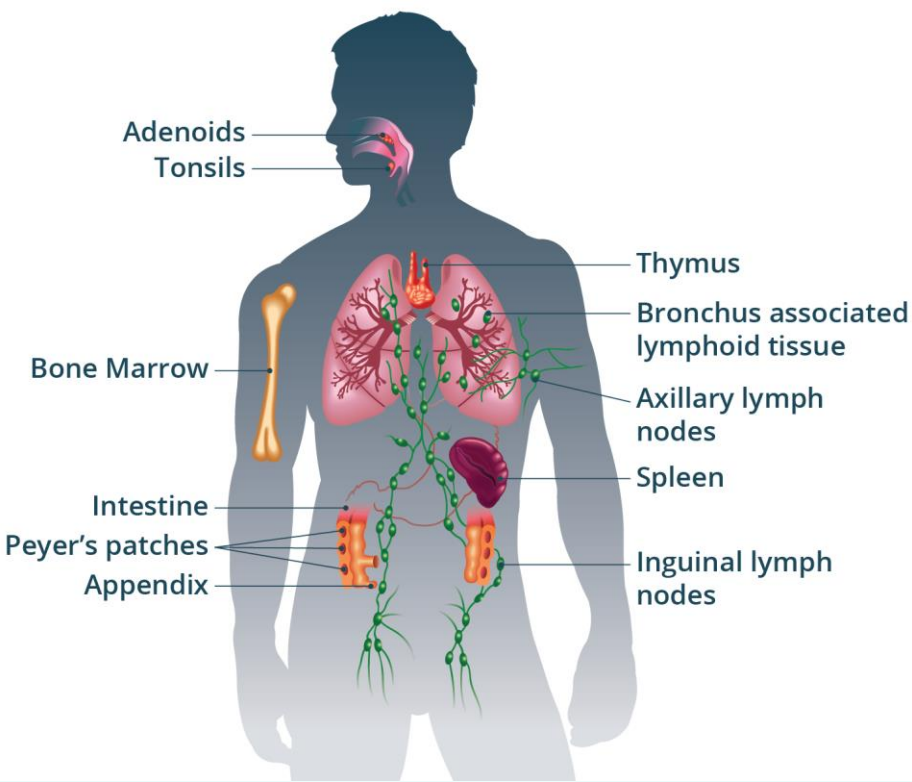
- **Early Life**: Infant nutrition modulates the microbiota, shaping brain and immune system development.
- **Adolescence**: Food choices, weight management, and social influence play a crucial role in microbiota health, affecting cognitive and immune functions.
- **Adulthood**: Diet, alcohol consumption, weight control, and caloric restriction impact the microbiota and can have long-term effects on brain and immune functions.
- **Aging**: Changes in microbiota composition are linked to frailty, chronic inflammation, and cognitive decline, influenced by clinical and lifestyle factors.



Current Opinion in Neurobiology 2023, 78:102652

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The immune system: an overview



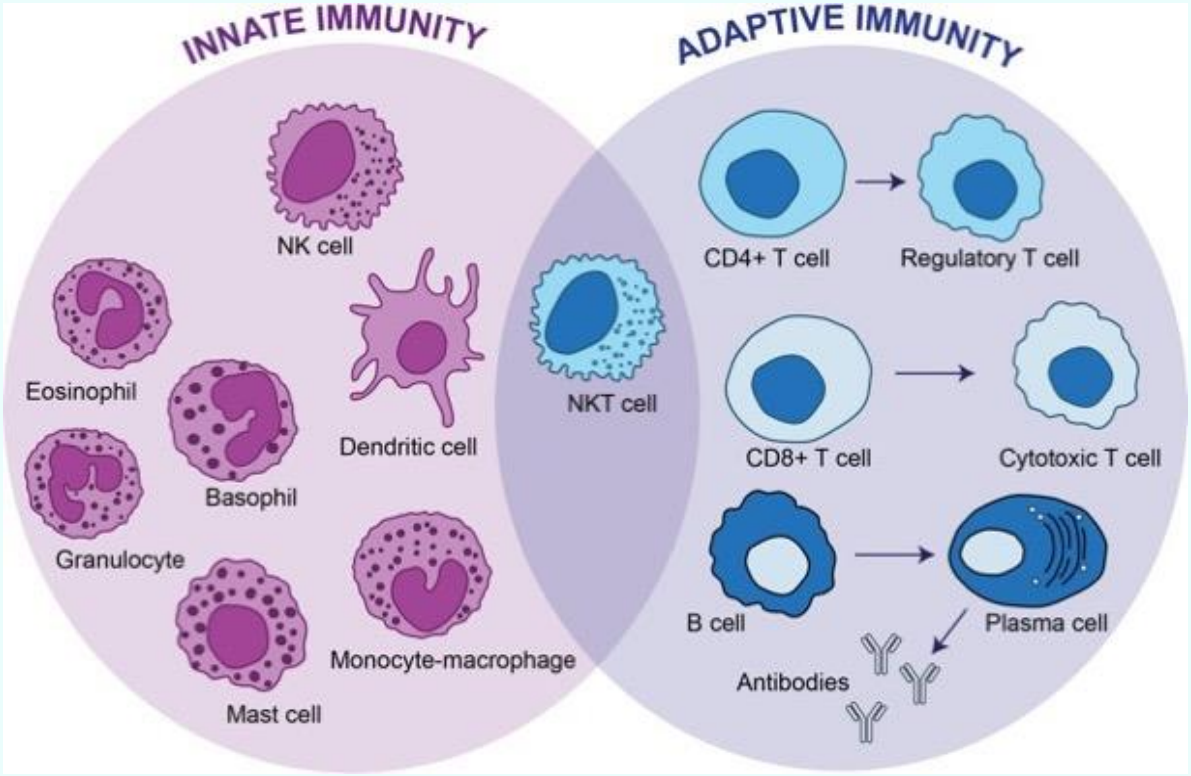
- A complex network of organs, immune cells, proteins, and chemicals.
- Defends against pathogens (bacteria, viruses, parasites) and abnormal cells (tumors, transplants).
- Innate immunity: present at birth, provides general defense, does not adapt to specific pathogens.
- Adaptive immunity: involves T and B cells that reorganize genetically to target specific antigens.

healthdirect.gov.au

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

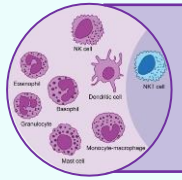
Innate and adaptive immunity

- **Innate immunity**: immediate, non-specific defense (NK cells, granulocytes, macrophages, etc.)
- **Adaptive immunity**: slower but highly specific (T cells and B cells, antibodies)
- Both systems work together to protect against infections and abnormal cells.



J Allergy Clin Immunol 2009, 125:24-32

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



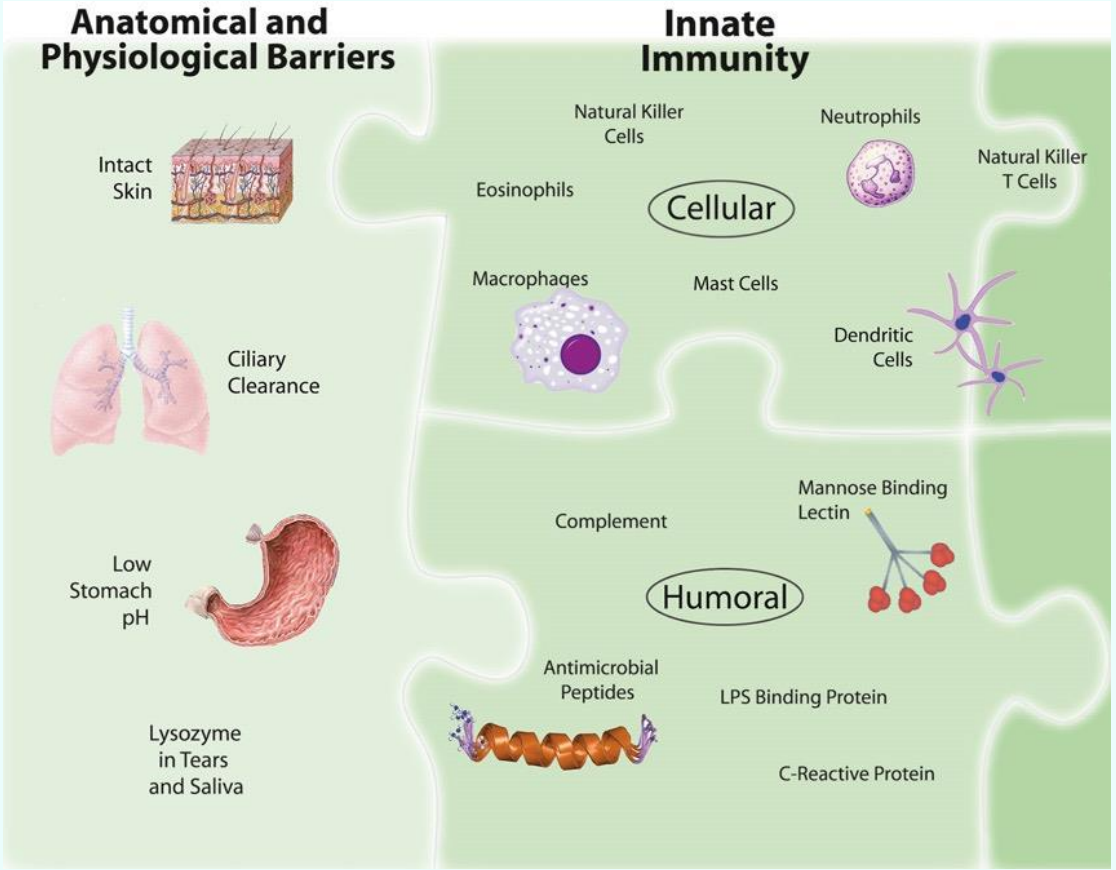
Innate immune system

First Line of Defense:

- Physical barriers: skin, mucosa, tight junctions
- Chemical barriers: sebum, saliva, tears
- **Microbiome**: competes with pathogens

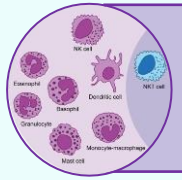
Key Cells and molecules:

- Cells: macrophages, neutrophils, dendritic cells, NK cells
- Soluble factors: complement system
- Cytokines and chemokines: drive inflammation, recruit cells



J Allergy Clin Immunol 2009, 125:24-32

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



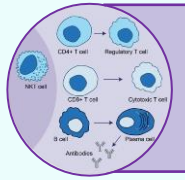
Innate immune system

- Foreign recognition through pattern recognition receptors (PRRs) that detect pathogen-associated (PAMPs) or damage-associated (DAMPs) molecular patterns
- Triggering an inflammatory response: increased vascular permeability, immune cell recruitment, and release of reactive oxygen species and inflammatory mediators
- Contain and eliminate infections and initiate tissue repair

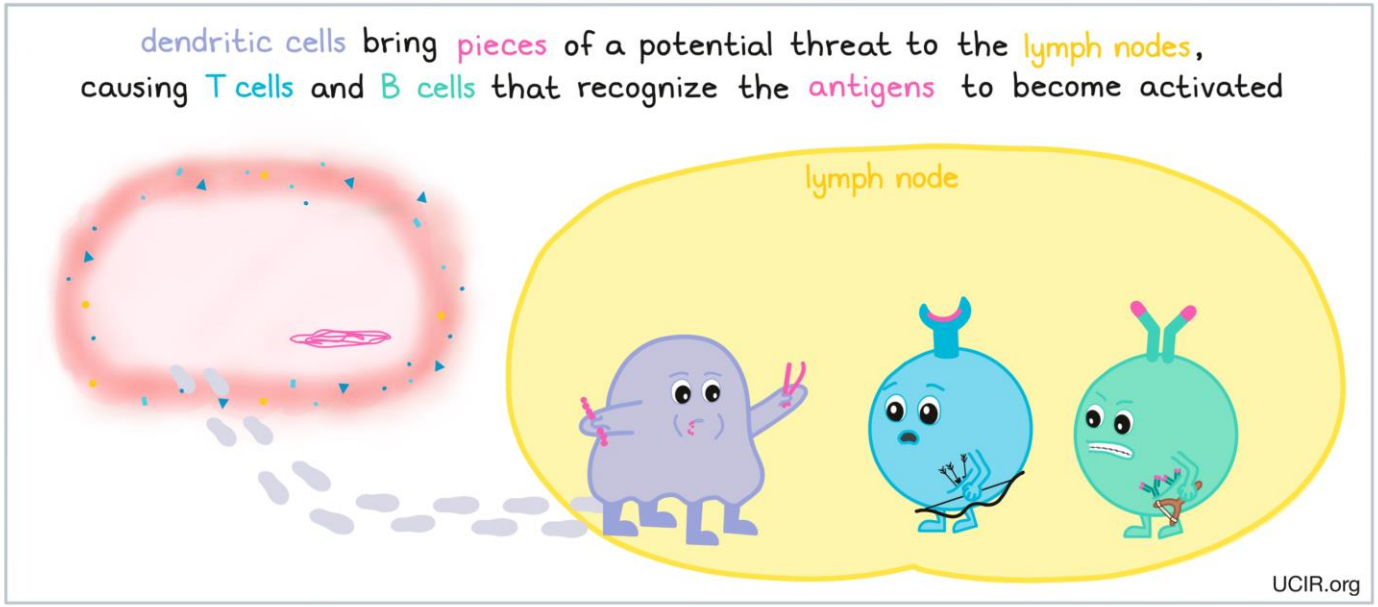
- Innate cells like macrophages and dendritic cells can undergo epigenetic and metabolic reprogramming after infections, resulting in a heightened response upon future encounters with pathogens
- **Trained immunity**: not antigen-specific but enhances the innate system's ability to fight diverse threats

J Allergy Clin Immunol 2009, 125:24-32

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



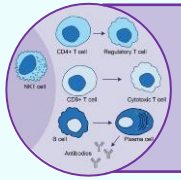
Adaptive immune system



- T cells: $CD4^+$ (helper), $CD8^+$ (killer)
- B cells: antibody production
- Highly specific, long-lasting defense

J Allergy Clin Immunol 2009, 125:24-32

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Adaptive immune system

T cell-mediated cellular immunity

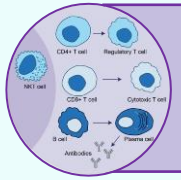
- The first signal comes from the T cell receptor (TCR) recognizing an antigen presented by MHC molecules on an antigen-presenting cell (APC).
- The second signal is delivered by costimulatory molecules (CD28 on T cells interacting with CD80/CD86 on APCs).
- The third signal involves cytokines secreted by the APC, driving T cell proliferation and differentiation into effector T cells, which orchestrate immune responses.

B cell-mediated humoral immunity

- B cells recognize antigens via their B cell receptors (BCRs),
- Fragments are internalized and presented on MHC class II to helper T cells. This interaction, along with signals from CD40-CD40L and cytokines like IL-4, IL-5, and IL-6, fully activates B cells.
- B cells then differentiate into plasma cells that secrete large amounts of antibodies, and into memory B cells (MBCs) that ensure a quicker, stronger response upon re-exposure to the same pathogen.

J Allergy Clin Immunol 2009, 125:24-32

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Adaptive immune system

Building immunological memory

A hallmark of adaptive immunity is its ability to “remember.” After infection, some T cells become memory T cells (TEM, TRM, TCM) that persist long-term and respond rapidly to future encounters. Similarly, memory B cells quickly transform into antibody-producing plasma cells upon re-infection. This immunological memory is the foundation for effective vaccines and sustained protection against recurrent infections.

J Allergy Clin Immunol 2009, 125:24-32

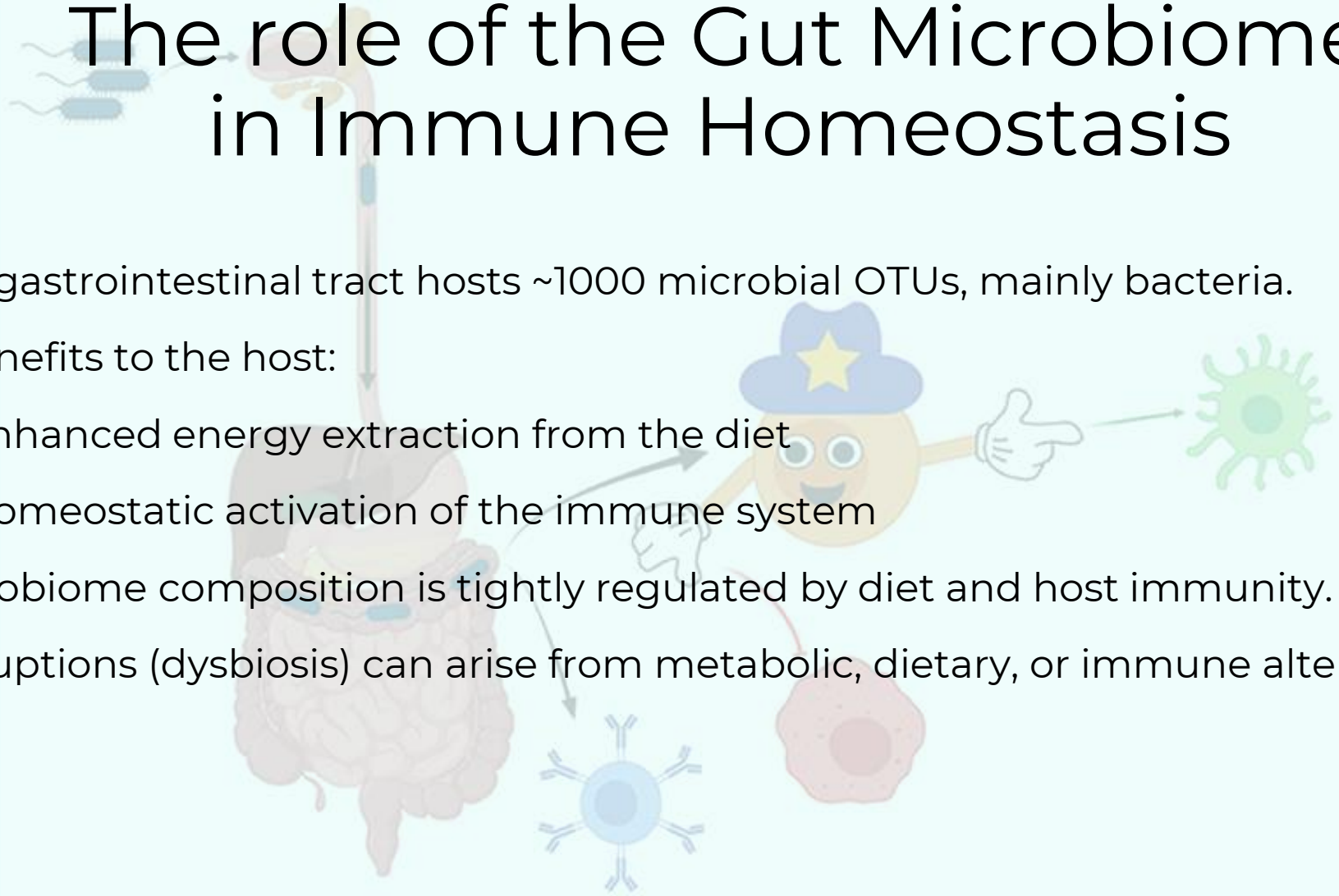
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The role of the Gut Microbiome in Immune Homeostasis

- The gastrointestinal tract hosts ~1000 microbial OTUs, mainly bacteria.

Key benefits to the host:

  - Enhanced energy extraction from the diet
  - Homeostatic activation of the immune system
- Microbiome composition is tightly regulated by diet and host immunity.
- Disruptions (dysbiosis) can arise from metabolic, dietary, or immune alterations.



Cell Host & Microbe. 2016 Aug 10;20(2):202-14

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

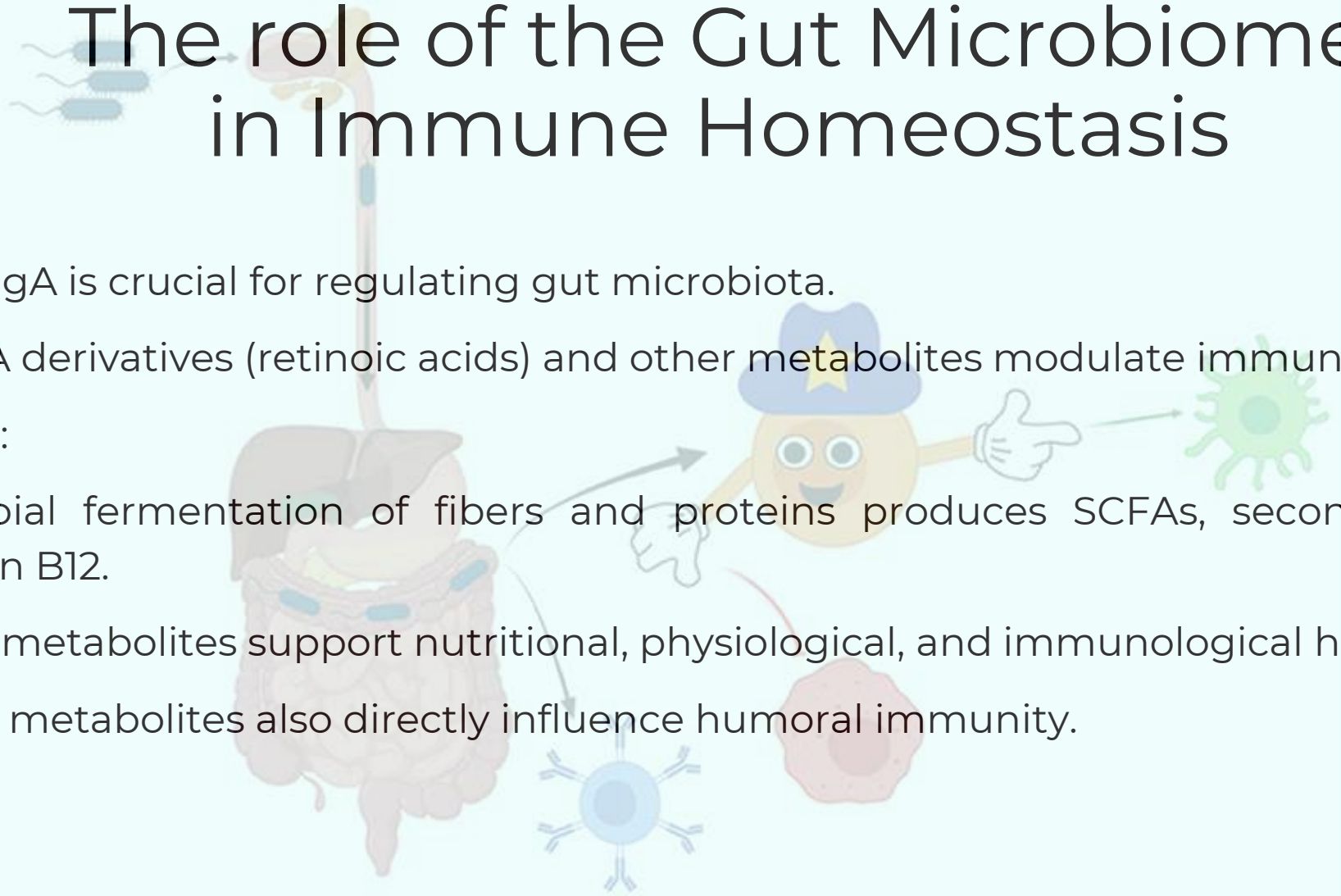
The role of the Gut Microbiome in Immune Homeostasis

- Mucosal IgA is crucial for regulating gut microbiota.
- Vitamin A derivatives (retinoic acids) and other metabolites modulate immune responses.

In the colon:

- Microbial fermentation of fibers and proteins produces SCFAs, secondary bile acids, vitamin B12.
- These metabolites support nutritional, physiological, and immunological homeostasis.

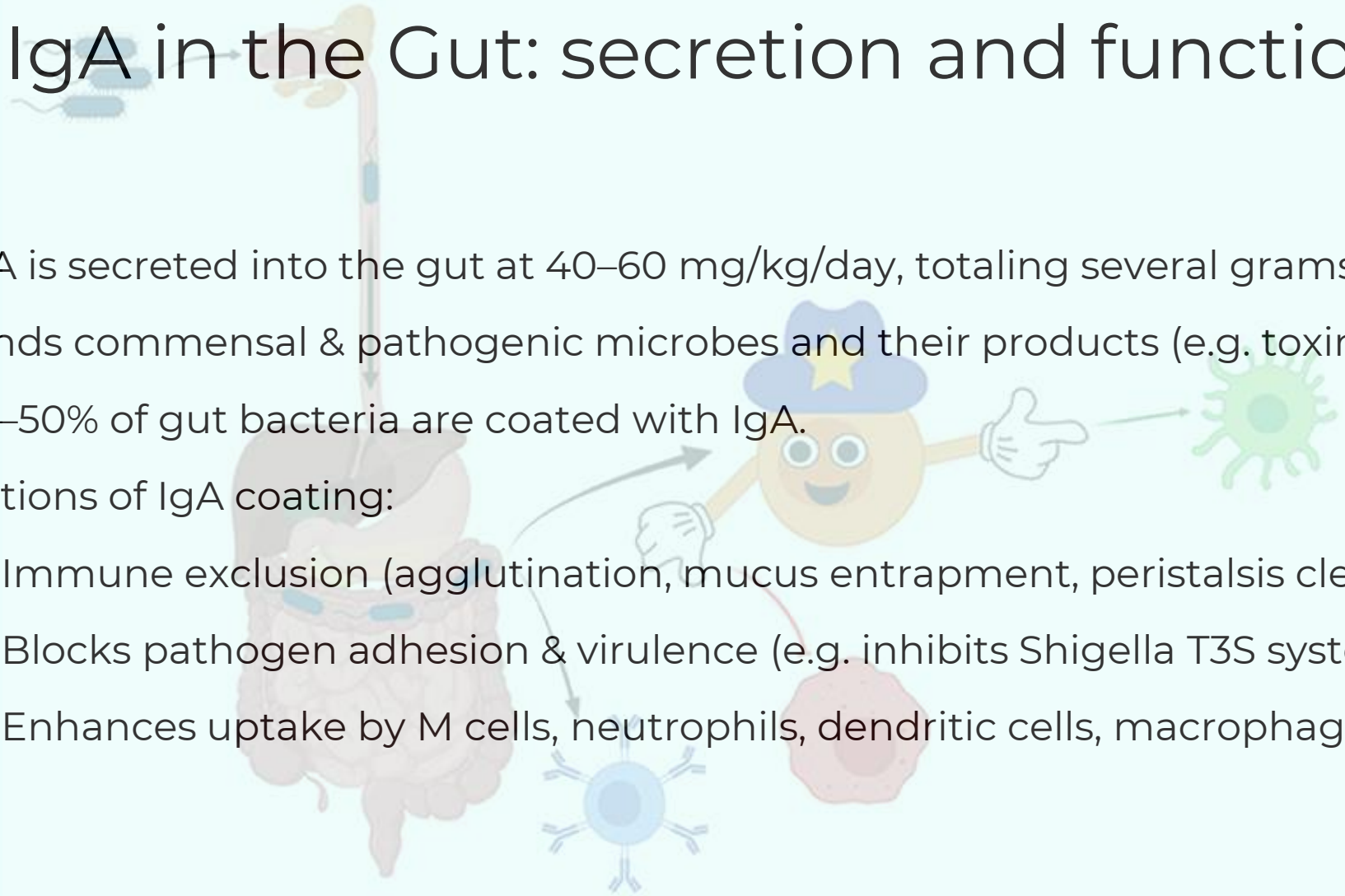
- Microbial metabolites also directly influence humoral immunity.



Cell Host & Microbe. 2016 Aug 10;20(2):202-14

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

IgA in the Gut: secretion and functions



- IgA is secreted into the gut at 40–60 mg/kg/day, totaling several grams daily.
- Binds commensal & pathogenic microbes and their products (e.g. toxins).
- 20–50% of gut bacteria are coated with IgA.

Functions of IgA coating:

- Immune exclusion (agglutination, mucus entrapment, peristalsis clearance)
- Blocks pathogen adhesion & virulence (e.g. inhibits Shigella T3S system)
- Enhances uptake by M cells, neutrophils, dendritic cells, macrophages.

Cell Host & Microbe. 2016 Aug 10;20(2):202-14

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiome-driven IgA response

- Gut microbiota induce GALT development (e.g. Peyer's patches, ILFs).

Needed for:

- IgM → IgA class switch recombination (CSR)
- Somatic hypermutation & IgA repertoire diversification

Germ-free or restricted flora mice show:

- Fewer IgA-producing plasma cells
- Altered B cell maturation (esp. marginal zone B cells)

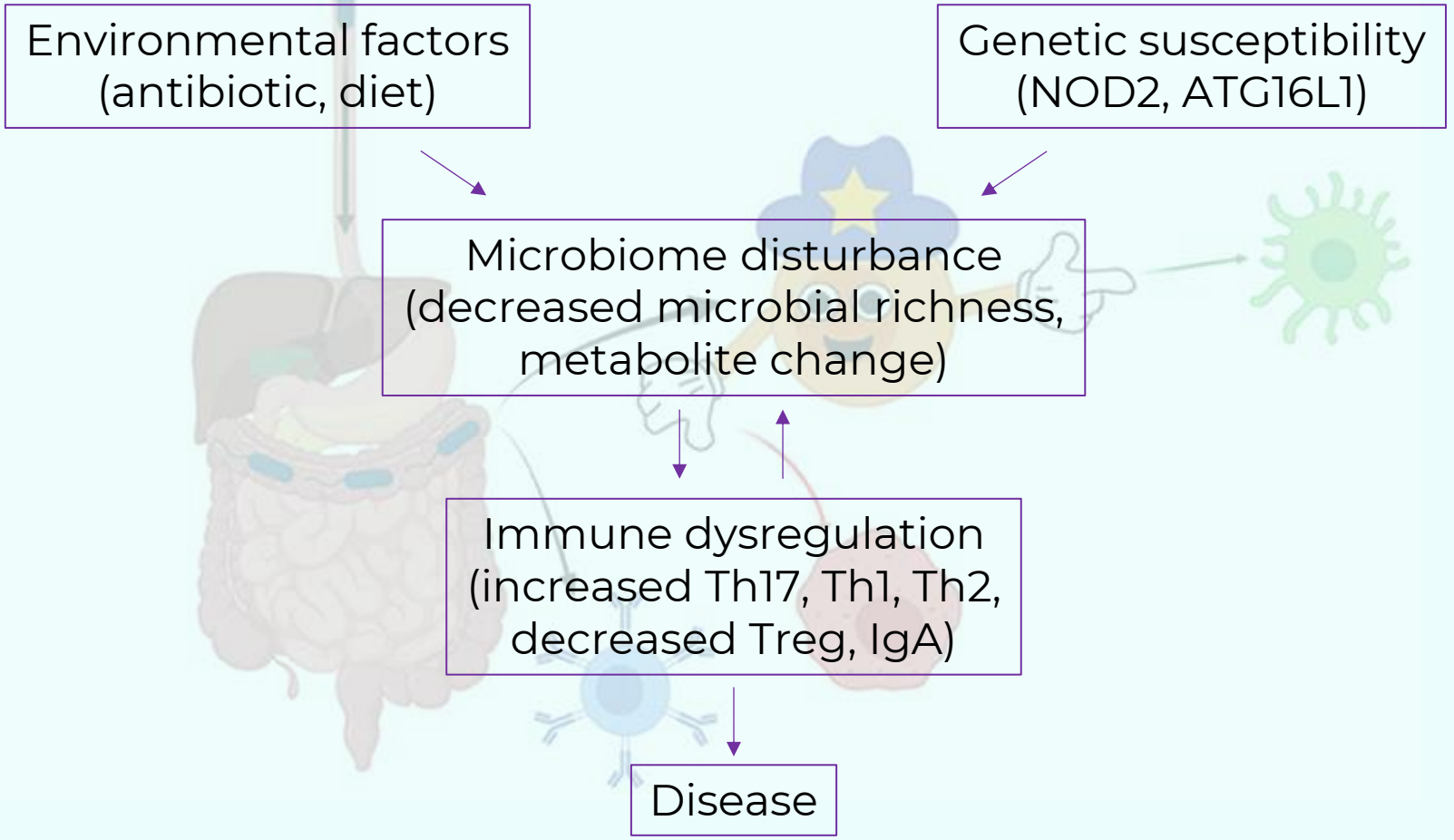
Restoration by colonization:

- Certain bacteria (e.g. E. coli, Bifidobacteria, SFB) enhance IgA responses.

Cell Host & Microbe. 2016 Aug 10;20(2):202-14

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiome perturbation and the Immune system



Cell Research (2020) 30:492 – 506

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiome perturbation and the Immune system

- Inflammatory bowel disease
- Systemic autoimmune diseases
- Cardiometabolic diseases
- Cancer

Cell Research (2020) 30:492 – 506

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Inflammatory Bowel disease (IBD)

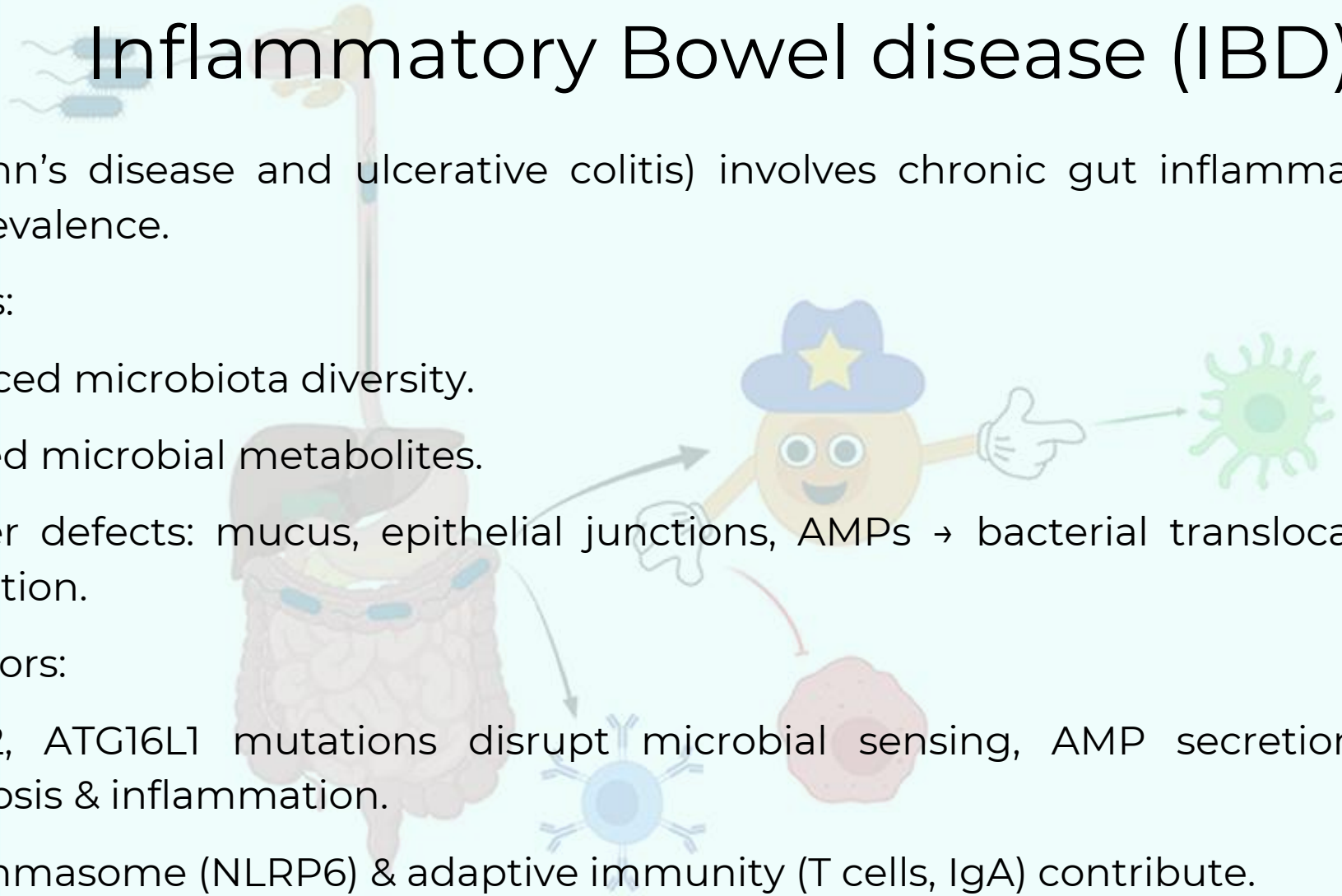
- IBD (Crohn's disease and ulcerative colitis) involves chronic gut inflammation with rising global prevalence.

Key features:

- Reduced microbiota diversity.
- Altered microbial metabolites.
- Barrier defects: mucus, epithelial junctions, AMPs → bacterial translocation & immune activation.

Genetic factors:

- NOD2, ATG16L1 mutations disrupt microbial sensing, AMP secretion, autophagy → dysbiosis & inflammation.
- Inflammasome (NLRP6) & adaptive immunity (T cells, IgA) contribute.



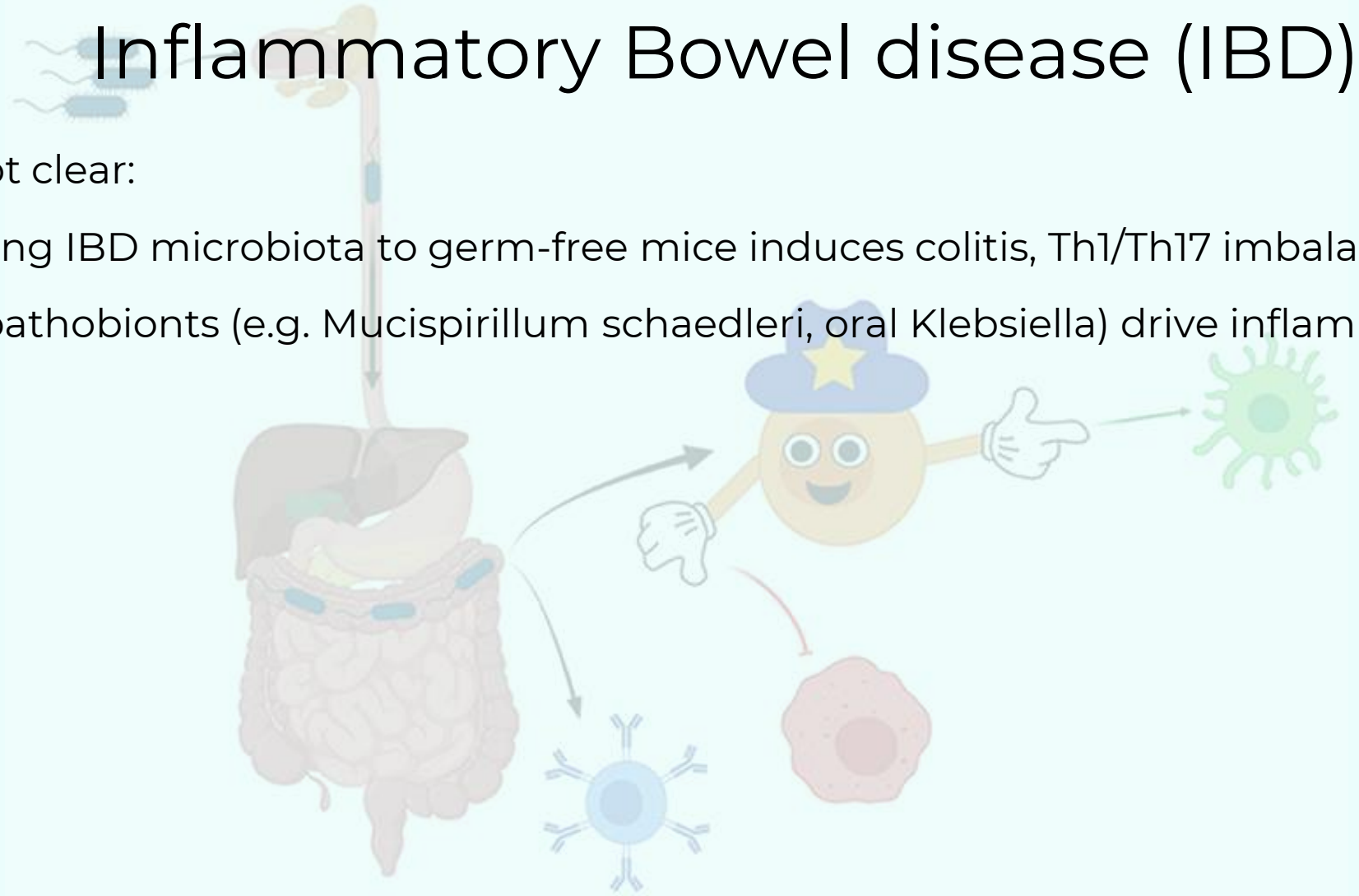
Cell Research (2020) 30:492 – 506

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Inflammatory Bowel disease (IBD)

Causality not clear:

- Transferring IBD microbiota to germ-free mice induces colitis, Th1/Th17 imbalances.
- Specific pathobionts (e.g. Mucispirillum schaedleri, oral Klebsiella) drive inflammation.



Cell Research (2020) 30:492 – 506

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Systemic autoimmune diseases

- Rheumatoid Arthritis (RA): systemic autoimmune disease → joint inflammation, cartilage and bone damage.

Risk factors:

  - Genetics (e.g. HLA-DRB1)
  - Microbiome and environment

Mechanisms:

  - Microbial ligands (TLR2/4) & SCFAs drive Th17/IL-17 responses.
  - SFB colonization or dysbiotic RA microbiota → Th17 activation & arthritis.
  - P. gingivalis promotes Th17 via TLR2 & IL-1, worsening arthritis.
- Impact of microbiome-targeted therapies on RA under investigation.

Cell Research (2020) 30:492 – 506

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Cardiometabolic diseases

- Chronic low-grade inflammation is a key driver of obesity, T2D, NAFLD/NASH, atherosclerosis

Gut-derived factors:

  - LPS → TLR4 activation → promotes liver & metabolic inflammation (NAFLD/NASH)
  - NLRP6 and NLRP3 inflammasomes → protect by shaping microbiota
  - T1D: GF NOD mice develop severe diabetes; microbiota colonization reduces it
  - Obesity:
    - Tryptophan metabolites + miR-181 → control adipose inflammation
    - NLRP12 → preserves SCFA producers (↓ obesity)

Atherosclerosis:

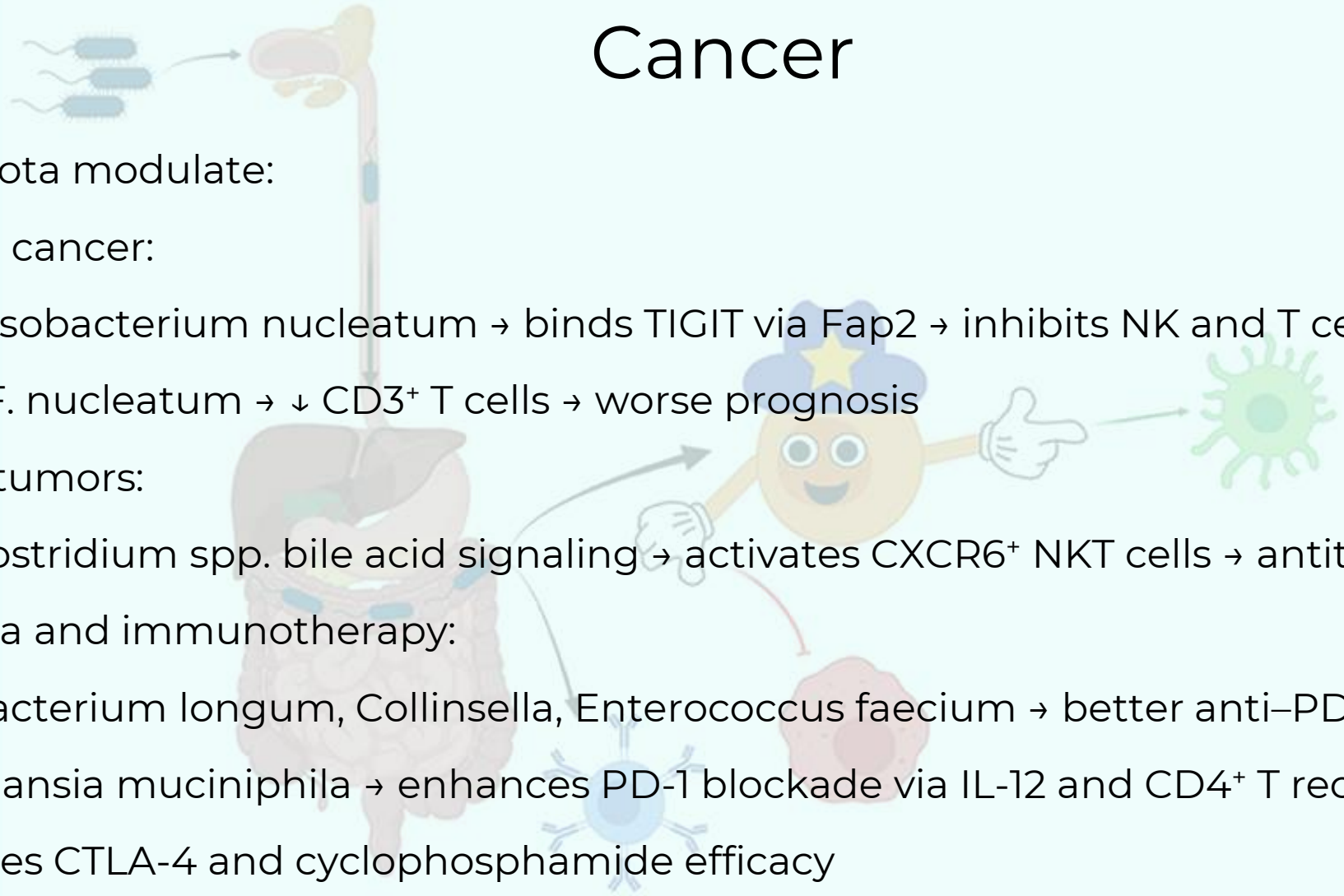
  - TMAO (from gut microbes) → ↑ CD36 and SR-A1 on macrophages → foam cell formation & plaque growth

- Microbiota → systemic immune modulation → cardiometabolic outcomes

Cell Research (2020) 30:492 – 506

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Cancer



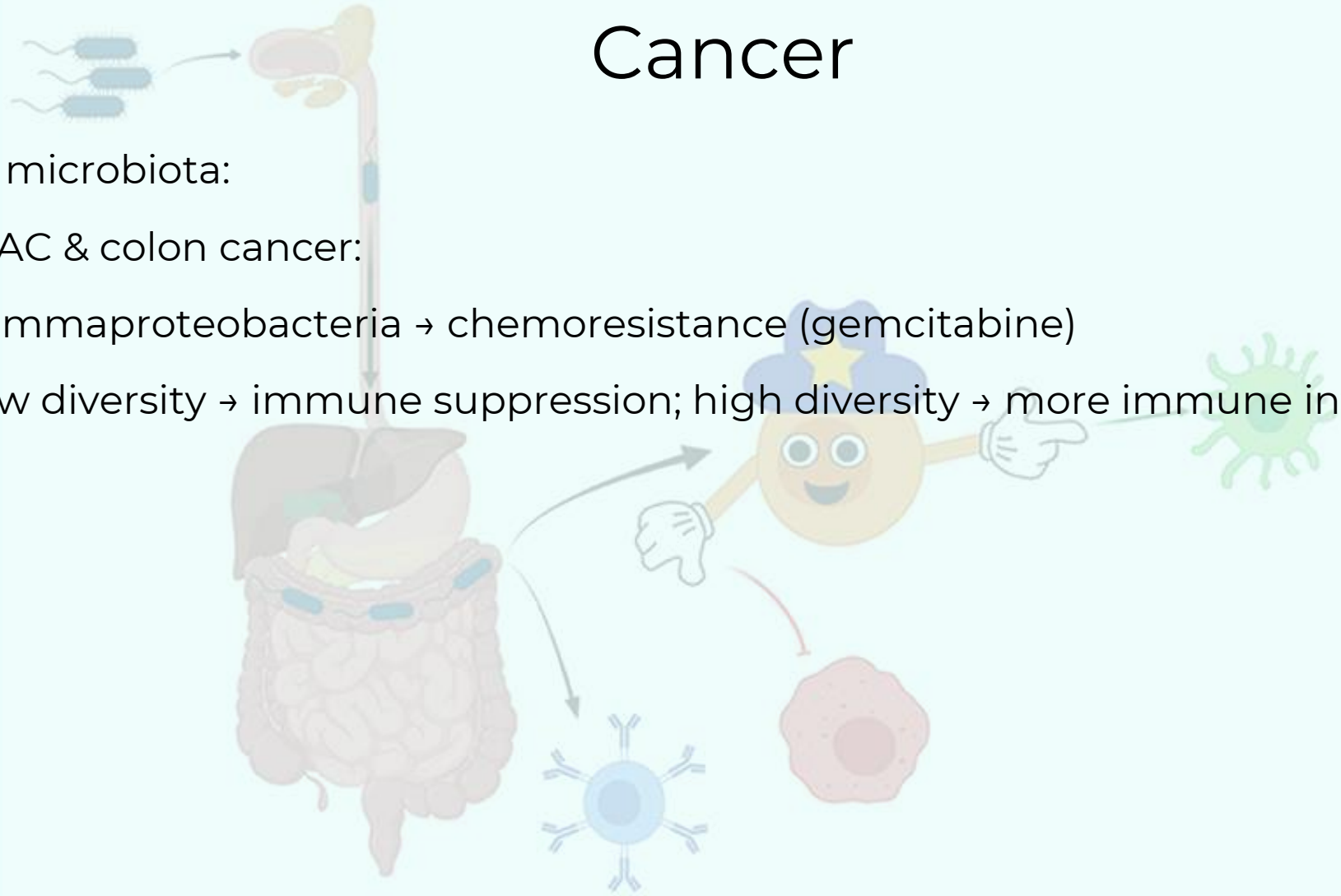
Gut microbiota modulate:

- Colon cancer:
  - Fusobacterium nucleatum → binds TIGIT via Fap2 → inhibits NK and T cells
  - ↑ F. nucleatum → ↓ $CD3^+$ T cells → worse prognosis
- Liver tumors:
  - Clostridium spp. bile acid signaling → activates $CXCR6^+$ NKT cells → antitumor effects
- Microbiota and immunotherapy:
- ↑ Bifidobacterium longum, Collinsella, Enterococcus faecium → better anti–PD-1 responses
- ↑ Akkermansia muciniphila → enhances PD-1 blockade via IL-12 and $CD4^+$ T recruitment
- Also shapes CTLA-4 and cyclophosphamide efficacy

Cell Research (2020) 30:492 – 506

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Cancer



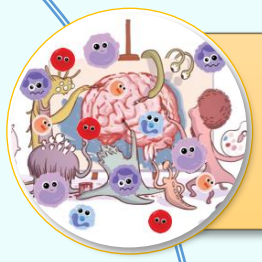
Intra-tumor microbiota:

- In PDAC & colon cancer:
  - Gammaproteobacteria → chemoresistance (gemcitabine)
  - Low diversity → immune suppression; high diversity → more immune infiltration

Cell Research (2020) 30:492 – 506

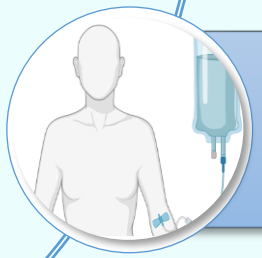
INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiome-Immune-Brain Axis: Key areas of focus



Microbiome-immune-brain interactions: the gut microbiota's impact on immune maturation, neuroinflammation, and neurobehavioral profiles.

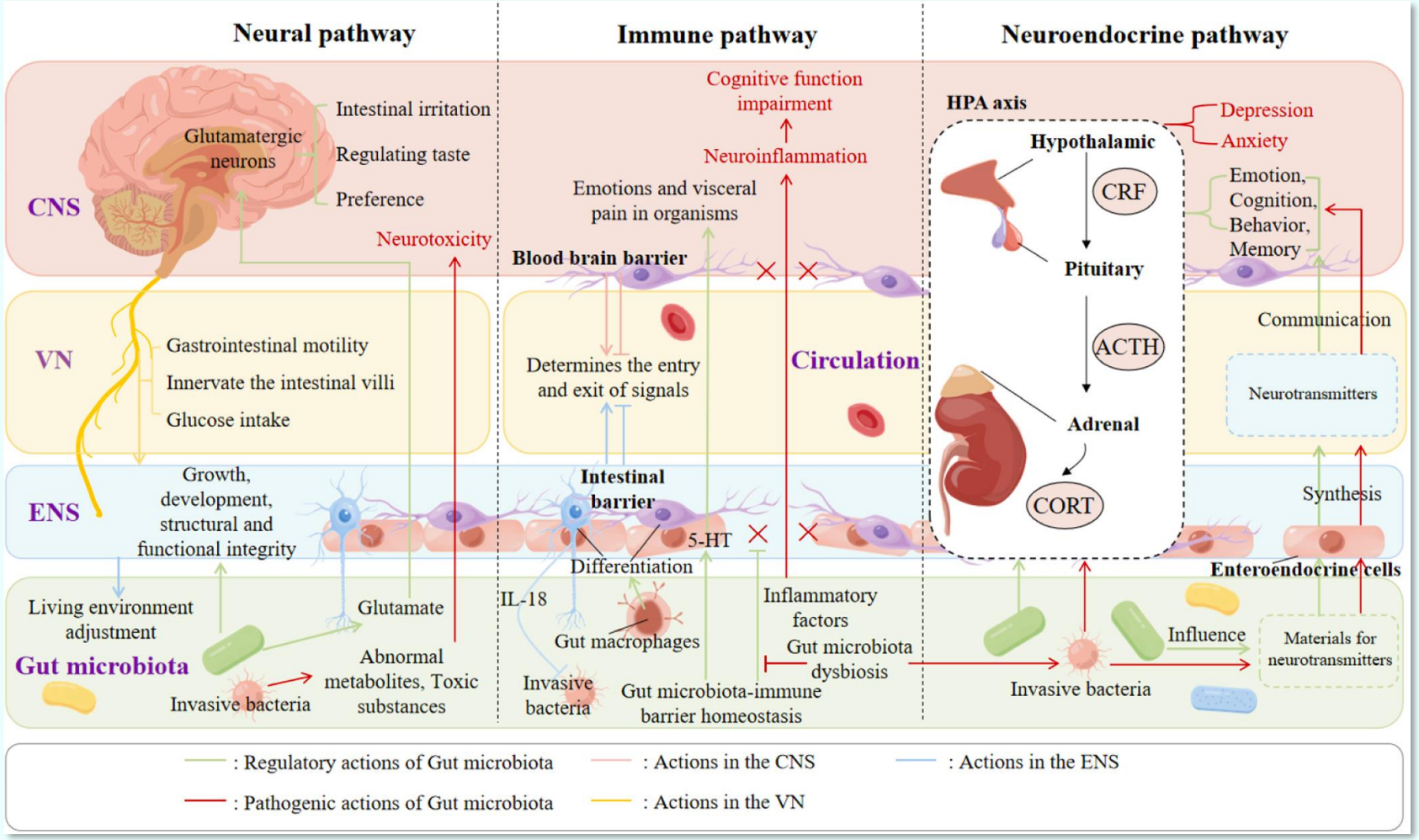
Implications for health: understanding immune system disruptions associated with gut-brain axis disorders.



Therapeutic potential: targeting the microbiome-gut-brain axis for treating neurodevelopmental, stress-related, and neurological disorders.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The gut-brain communication pathways: immunomodulatory mechanisms of communication

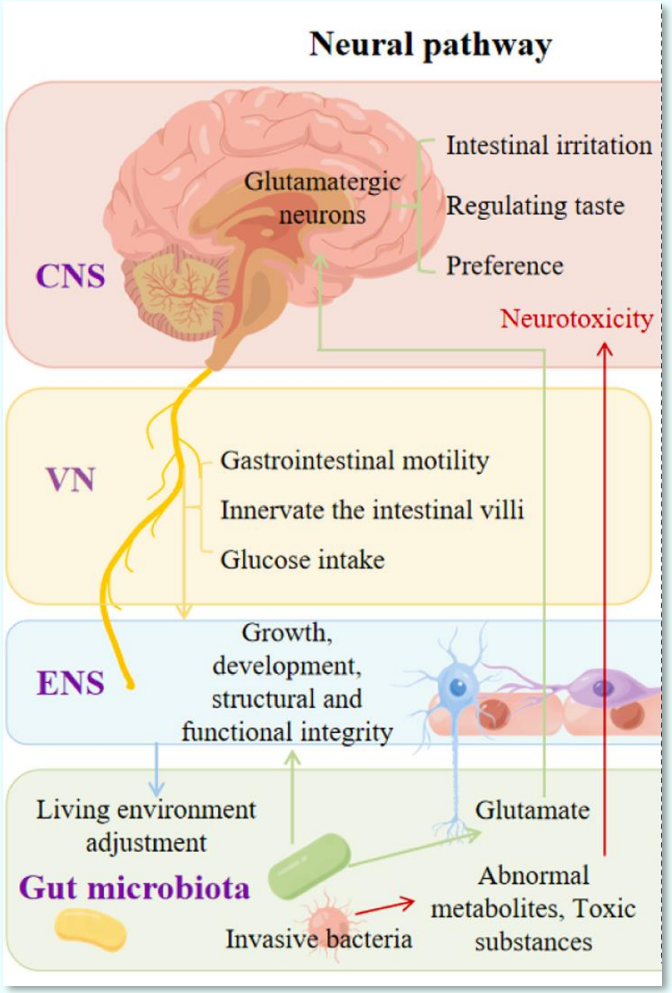


Microbiome-immune-brain interactions

Biomedicine & Pharmacotherapy 178 (2024) 117207

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The neural pathway

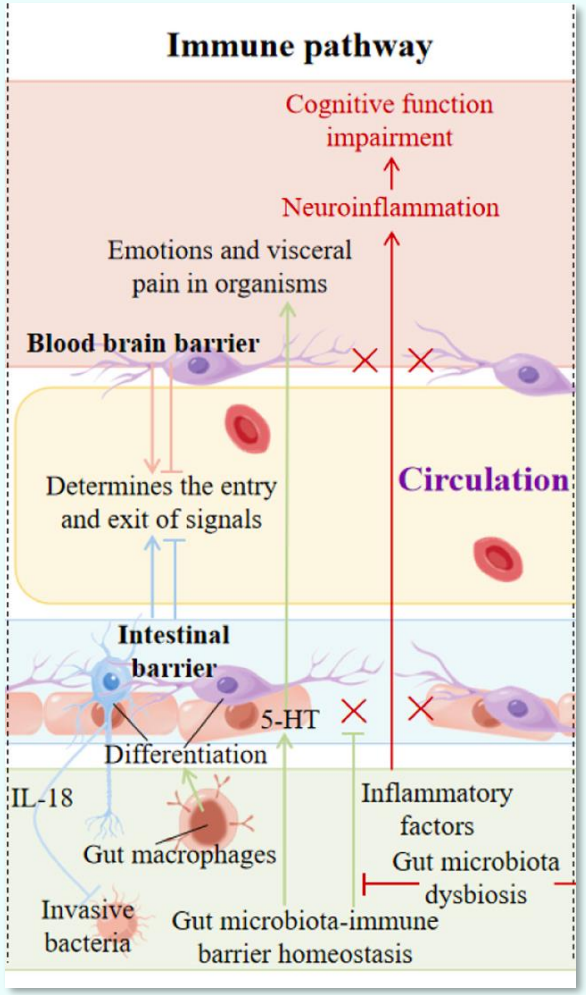


- The ENS, a network of neurons and glial cells within the gut, autonomously regulates GI function and mediates communication among the gut microbiota and the CNS.
- The ENS provides a supportive environment for the gut microbiota, while the microbiota is crucial for the proper growth, development, structure, and function of the ENS. Microbial depletion can negatively impact ENS integrity, a process partially reversible by microbial metabolites like LPS and SCFAs.
- Chemicals released by gut microbes and cells can activate ENS afferent neurons. These signals are then relayed to specific brain regions via the VN, influencing brain activity.
- The VN serves as a critical bridge, transmitting signals from the ENS to the CNS, including rapid glutamate-mediated signals and those influencing reward pathways via dopamine release.

Biomedicine & Pharmacotherapy 178 (2024) 117207

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The immune pathway



- Gut macrophages, influenced by the microbiota, support ENS development and function. This interaction, along with ENS regulation of gut immunity (via IL-18), contributes to gut barrier integrity

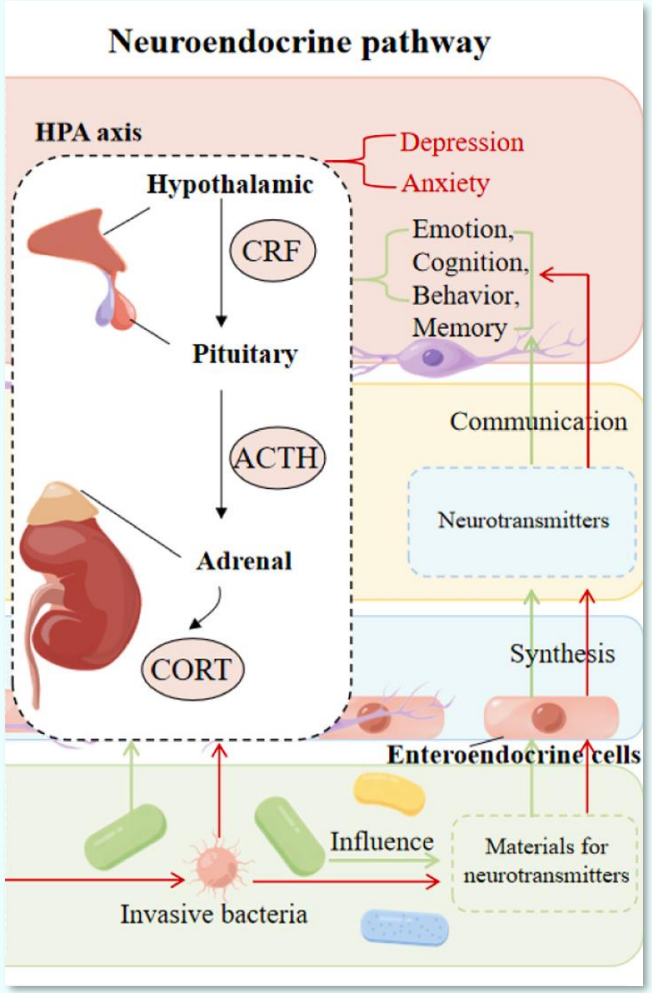
- A balanced interplay between the gut microbiota and immune system protects the brain from inflammation and significantly impacts the production of serotonin in the gut, which can then influence emotions and visceral pain through peripheral signaling.

- The integrity of the intestinal barrier and blood-brain barrier dictates the passage of signals between the gut, microbiota, and brain. Dysbiosis can compromise these barriers, leading to neuroinflammation and cognitive impairment.

Biomedicine & Pharmacotherapy 178 (2024) 117207

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The neuroendocrine pathway

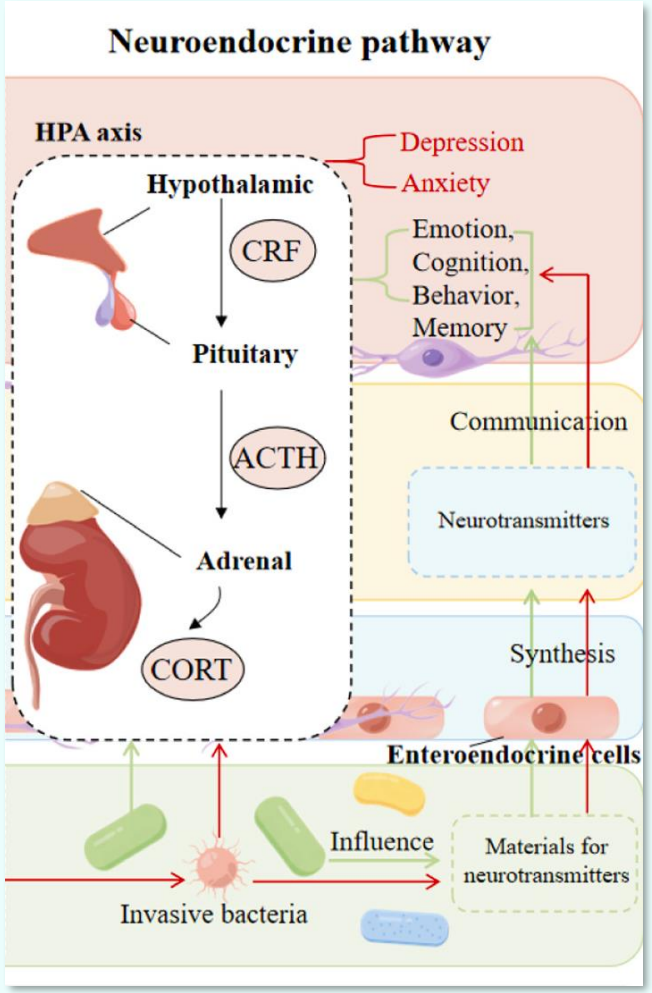


- The HPA axis is a primary endocrine communication pathway regulating the body's stress response and influencing sympathetic, parasympathetic, metabolic, immune, and CNS functions through the release of CRF from the hypothalamus.
- This pathway plays a crucial role in maintaining overall health, with early gut microbiota composition potentially influencing HPA axis development.
- Imbalances in the gut microbiota can trigger the pituitary gland, leading to the release of ACTH and subsequently cortisol from the adrenal glands, creating a negative feedback loop involving the brain.
- Inflammation or changes in gut microbiota can activate the HPA axis, leading to the release of stress hormones like cortisol, which can affect mood, cognition, and behavior. Abnormal CRF secretion is implicated in mood disorders.

Biomedicine & Pharmacotherapy 178 (2024) 117207

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The neuroendocrine pathway



- The gut is a significant source of neurotransmitters and hormones, including 5-HT and dopamine.
- Gut microorganisms regulate enteroendocrine cells, the primary source of peripheral 5-HT, that enters circulation and reaches brain regions involved in emotions, appetite, and sleep, also affecting gut motility and barrier permeability.
- Gut bacteria can metabolize precursors like tyrosine into neuroactive compounds, which can cross the BBB and influence brain development and behavior.
- The CNS communicates with gut bacteria through neurotransmission, influencing microbial adaptation. Conversely, gut-derived neurotransmitters and metabolites are linked to memory, cognition, and mood disorders, with interventions targeting these molecules showing potential therapeutic effects on anxiety-like behaviors.

Biomedicine & Pharmacotherapy 178 (2024) 117207

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

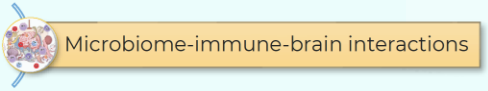
The Microbiome-Immune-Brain axis: A link between Immunity and Neurological Health

- The immune system was traditionally seen as a defense against pathogens but is now recognized as deeply interconnected with gut microbiota.

Commensal bacteria contribute to host health by:

  - Providing nutrients
  - Metabolizing indigestible compounds
  - Preventing pathogen colonization

- Host recognition of gut microbes occurs through pathways involving SCFAs, tryptophan metabolites, and bile acids. This interaction helps shape immune responses and overall systemic health.



Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The Microbiome-Immune-Brain Axis

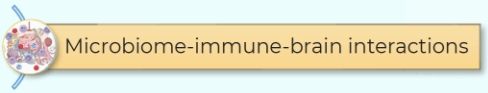
- The immune system impacts cognition, mood, and behavior.
- Neuroinflammation occurs when immune cells infiltrate the brain, leading to changes in function and structure.
- Cytokines and chemokines cross the blood-brain barrier and influence neuronal activity, synaptic transmission, and neurogenesis

The immune response to commensal bacteria shapes both innate and adaptive immunity, involving:

- B and T lymphocytes in Peyer's patches
- Plasma cells
- Cytokine differentiation

These interactions are still not fully understood, but they play a crucial role in health and disease.

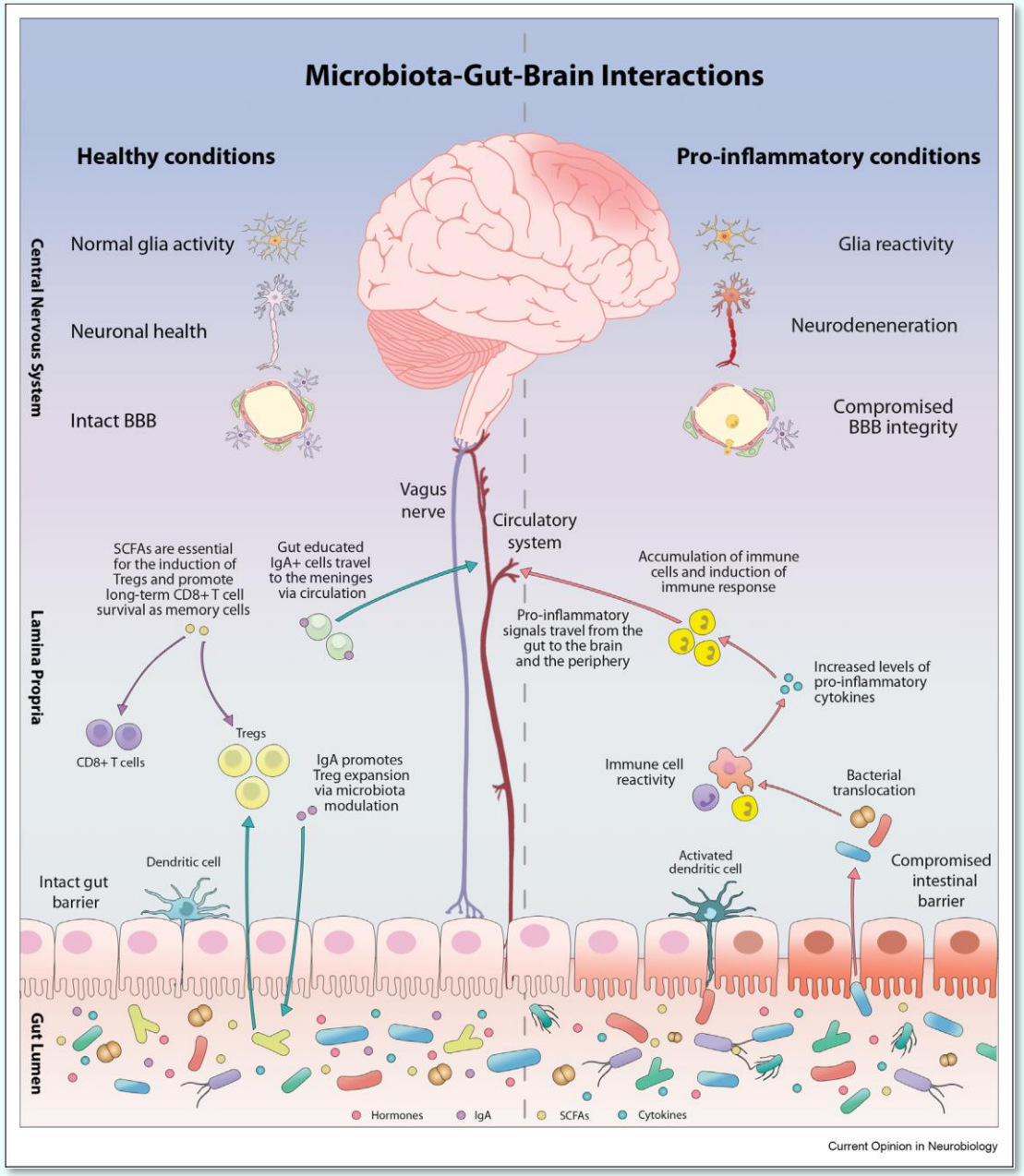
◆ *This growing field of research underscores how the **gut microbiota** is more than a passive component of digestion—it **actively shapes immune response and brain functions**.*



Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiota and Brain Health: A Two-Way Communication



◆ The gut microbiota plays a crucial role in maintaining brain and immune health.

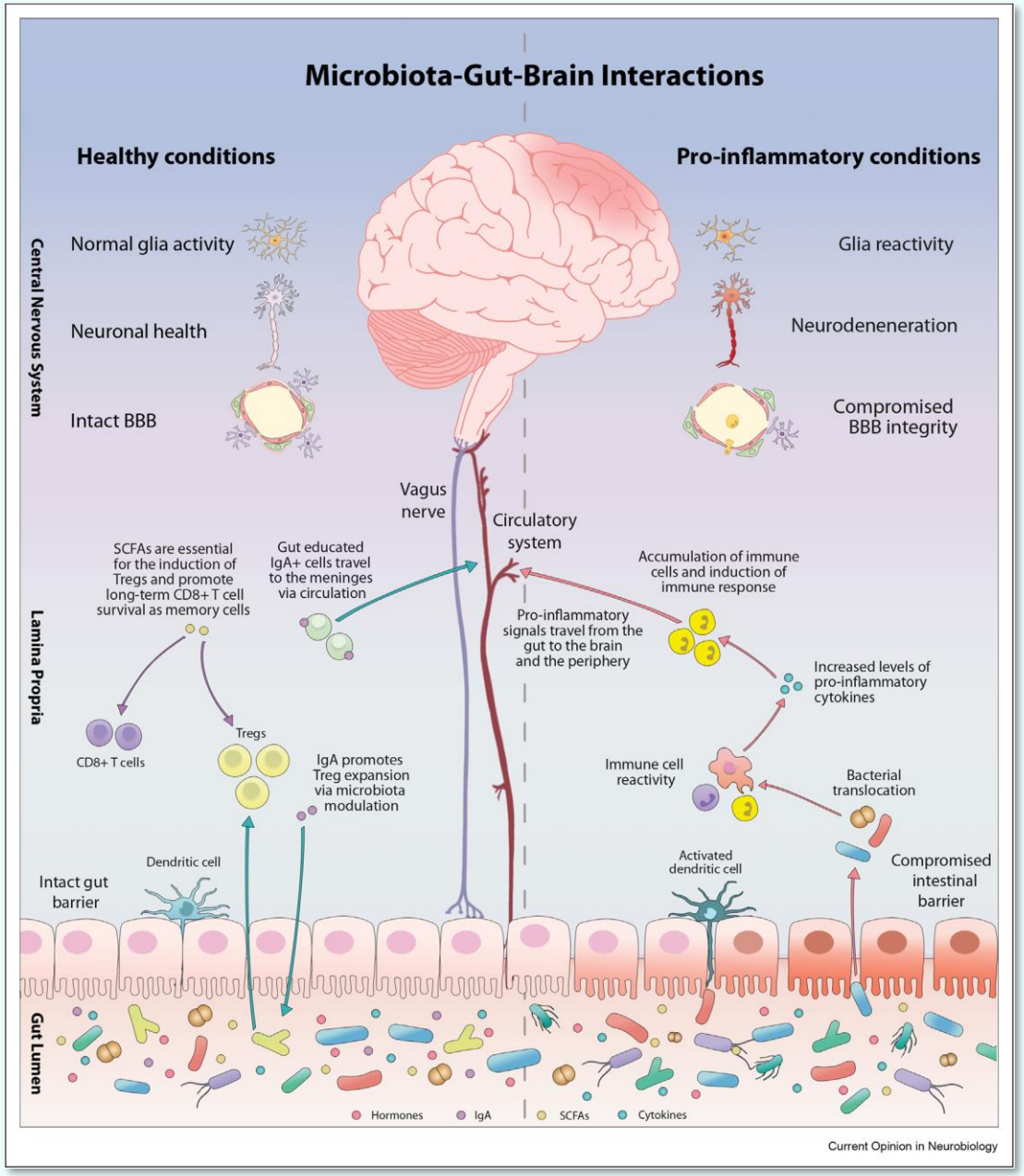
In **healthy conditions**:

- The gut barrier remains intact, preventing harmful bacterial translocation.
- Short-chain fatty acids (SCFAs) support regulatory T cells (Tregs) and long-term immune balance.
- The vagus nerve and circulatory system facilitate beneficial gut-brain communication.
- The brain exhibits normal glia activity, neuronal health, and an intact blood-brain barrier (BBB).

Current Opinion in Neurobiology 2023, 78:102652

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiota and Brain Health: A Two-Way Communication



Under **pro-inflammatory conditions**:

- A compromised gut barrier leads to bacterial translocation.
- Increased immune cell reactivity and accumulation of pro-inflammatory cytokines.
- This results in glia reactivity, neurodegeneration, and BBB impairment.

◆ **Implications:** The gut-immune-brain axis highlights how gut health directly influences neurological function and inflammation-related disorders.

Current Opinion in Neurobiology 2023, 78:102652

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

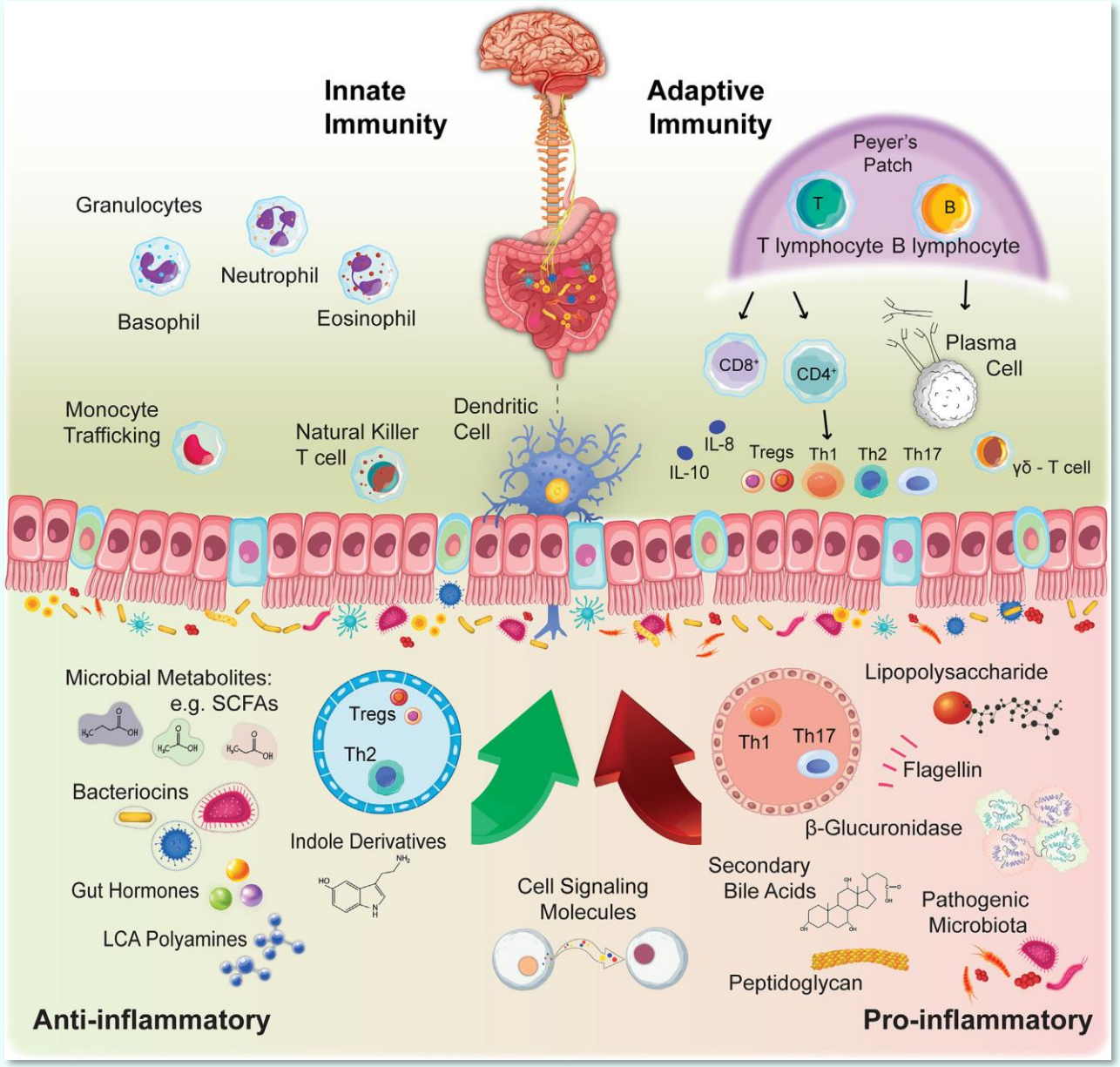
Innate vs Adaptive Immunity

- Microbes in the gastrointestinal tract are essential for the development and maturation of the immune system.
- The immune system also regulates the balance of commensal bacteria, maintaining gut homeostasis.

Gut microbes can be broadly classified as:

- **Anti-inflammatory** → Promote immune regulation (e.g., SCFAs, Tregs, IL-10).
- **Pro-inflammatory** → Activate immune responses (e.g., Th17 cells, IL-6, TNF-α).

Cell Reports Medicine (2025), 101982



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The gut microbiome, stress, and immune system: implications for brain disorders

- The gut microbiome plays a critical role in brain function and immune regulation.

- Cytokines and immune cells (monocytes, T and B lymphocytes, natural killers) can influence brain function.
- Microglia, the brain's immune cells, are influenced by gut microbiota and microbial metabolites.
- Imbalance in immune responses may contribute to neuroinflammatory and neuropsychiatric conditions.

Microbiome-immune-brain interactions

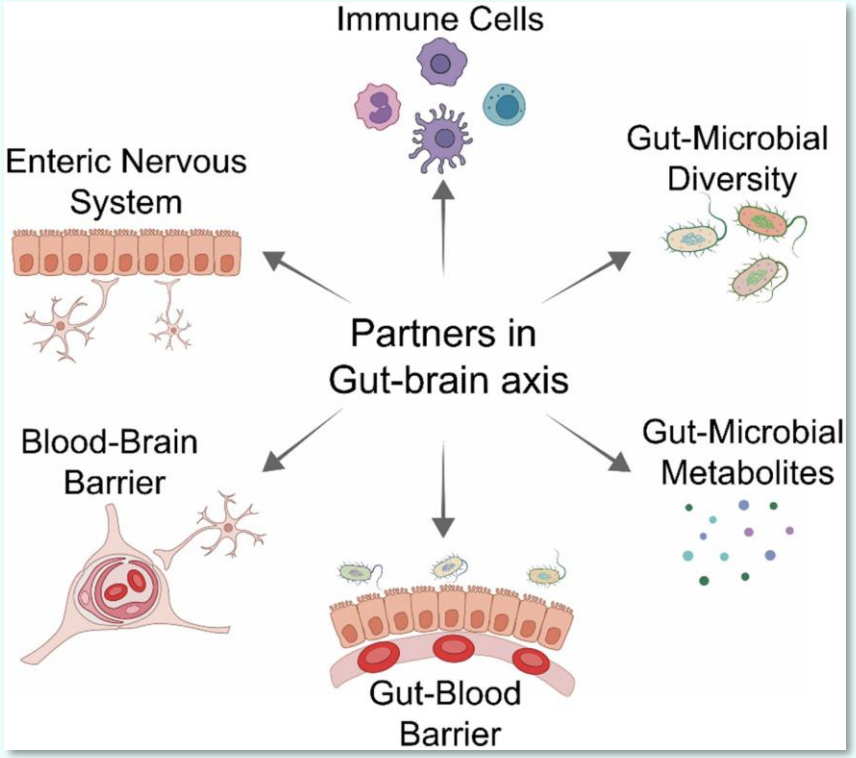
Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

The role of the gut microbiome in brain disorders

Immune cells: regulate inflammation and immune responses affecting brain function

Enteric nervous system: The "second brain" that communicates directly with the CNS, influencing mood and cognition



Gut-microbial diversity: A balanced microbiome supports neurological and immune health

Blood-brain barrier: Protects the brain but can be compromised by systemic inflammation and microbial imbalances

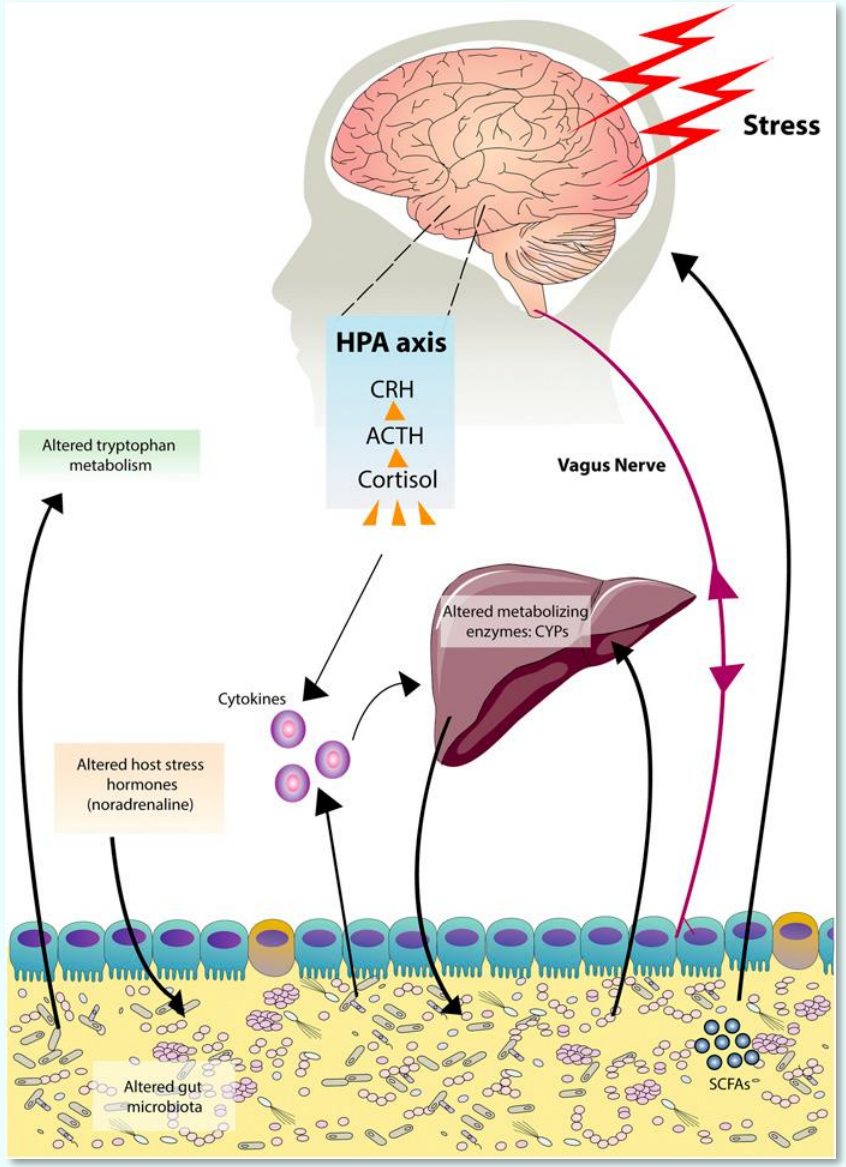
Gut-microbial metabolites: Microbial byproducts like SCFAs influence brain activity and immune signaling

Gut-blood barrier: Controls the passage of substances between the gut and bloodstream, preventing harmful compounds from reaching the brain

Gut Microbes, 16:1, 2422468

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Gut-Brain Axis and Stress Response



- Bidirectional communication via vagus nerve, immune signaling, and metabolic pathways.

HPA Axis Activation:
Stress triggers CRH → ACTH → Cortisol release

Cortisol effects:

- Alters gut microbiota composition.
- Affects neurotransmitter production (GABA, serotonin, dopamine).
- Modifies immune responses via cytokines.

Gut Microbiota and Brain Disorders:

- Dysbiosis contributes to neuroinflammation.
- Implicated in neuropsychiatric and neurodegenerative diseases.

Pharmacol Rev 71:198–224, 2019

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Stress-mediated microbiome-immune axis changes driving neuroinflammation and neurological diseases

- Stress disrupts gut microbiota, affecting tryptophan metabolism and immune responses.
- Altered microbiota increases cytokine production, leading to neuroinflammation.
- Microglia activation contributes to depression, anxiety, and neurodegeneration.
- SCFAs help regulate inflammation and microglial function.

Pharmacol Rev 71:198–224, 2019

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Neuropsychiatric conditions

Major depressive disorder (MDD)

- Chronic inflammation is a key factor in MDD.
- HPA axis dysregulation and immune activation contribute to symptoms.
- MDD patients show altered immune profiles (e.g., increased monocytes, granulocytes).

Social anxiety disorder (SAD): marked by intense fear of social judgment and avoidance of social interactions.

- SAD is Gut microbiota influences immune function and social fear sensitivity.
- Reported social fear in mice, altering immune responses.
- Immune changes (reduced IL-17A and altered T cell profiles in gut-associated lymphoid tissue) and microbiome alterations are reported.

Front. Neurol. 2021, 12:721126

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Neurodevelopmental disorders

- **Schizophrenia:** Neurodevelopmental disorder influenced by genes and environment.
  - Gut-brain axis involvement through inflammation, microbiome alterations, and immune response.

Risk factors:

- Increased intestinal permeability (biomarkers: soluble CD14, LBP).
- Gluten sensitivity and *Toxoplasma gondii* exposure.
- Altered gut microbiota (↑ *Eggerthella*, ↓ *Bacteroides*) linked to inflammation.

Immune system activation:

- ↑ Pro-inflammatory cytokines (TNF-α, IL-9).
- ↑ IgM/IgA responses to commensal bacteria (e.g., *Pseudomonas aeruginosa, Klebsiella pneumoniae*).
- Microbiota-immune interactions may play a role in gene-environment interplay and new therapeutic targets.

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Neurodevelopmental disorders

- **Autism Spectrum Disorders (ASDs):** Neurodevelopmental disorders with social, communication, and behavioral deficits.
  - Gut microbiome alterations linked to inflammation and immune activation.
  - ASD gut inflammation overlaps with inflammatory bowel disease.
  - ↑ Mucosa-associated cytokines (IL-5, IL-15, IL-17), zonulin → Leaky gut.
  - Infections, autoimmune disorders, or inflammation during pregnancy increase ASD risk.
  - Cytokines IL-6 and IL-17A disrupt fetal brain development.

Microbiota-targeted therapies:

- Probiotics improve social interaction and anxiety in ASD models.
- Prebiotics restore gut microbiota, reduce inflammation, and enhance immune balance.

Potential therapeutic targets:

- Modulating gut microbiota to mitigate ASD symptoms.

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Neurodevelopmental disorders

- **Attention deficit hyperactivity disorder (ADHD):** Neurodevelopmental disorder with inattention, hyperactivity, and impulsivity.

  - Western-style diet linked to microbiota alterations.
  - Elimination diets (Few-Foods Diet): 63% symptom improvement.
  - Microbiota alterations in ADHD → altered dopamine metabolism and inflammation.
  - Neurocognitive findings: increased precuneus activation correlates with symptom reduction.

Microbiome-based interventions:

- Probiotics (*L. rhamnosus*, *B. bifidum*) show positive effects on cognition and behavior.
- Synbiotics (prebiotics + probiotics) as potential future therapies.

Further research needed: influence of geography, age, and diet on ADHD-microbiome interactions.

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autoimmune disorders

- **Multiple sclerosis (MS):** Chronic autoimmune disease causing CNS myelin destruction.
  - Linked to gut microbiota alterations and inflammatory pathway activation.

Microbiota-immune comunication in MS

- Monocyte activation influenced by gut microbiome.
- Probiotics (*Lactobacillus, Bifidobacterium, Streptococcus*) reduce inflammation.
- Dendritic cells are key in T cell activation, regulated by SCFAs.
- IgA+ immune cells in the meninges increase in response to gut inflammation.

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Autoimmune disorders

- **Multiple sclerosis (MS):** Chronic autoimmune disease causing CNS myelin destruction.

Gut microbiota and MS pathogenesis

- Molecular mimicry: *Lactobacillus reuteri* produces peptides similar to myelin, exacerbating autoimmunity.
- FMT from MS patients worsens symptoms in mice, reducing IL-10+ Tregs and increasing anti-MOG antibodies.

Potential therapies:

- Probiotics reduce demyelination and intestinal inflammation.
- Antibiotic pre-treatment enhances regulatory T and B cells, reducing MS severity.
- Targeting IgA diversity in the gut may offer new therapeutic strategies.

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Neurodegenerative diseases

◆ **Parkinson's disease (PD):** Neurodegenerative disorder characterized by dopaminergic neuron loss and α-synuclein aggregation.

- Constipation is an early symptom, appearing years before diagnosis.
- α-synuclein is found in gut nerve fibers and may spread to the brain via the vagus nerve.

Microbiome and PD Pathology

- Gut microbiota influence α-synuclein aggregation and clearance.
- Vagus nerve involvement: Vagotomy reduces PD risk and slows disease progression.
- No consensus on a distinct PD-specific microbial pattern due to study limitations.

Immune System and Inflammation in PD

- Immune-related genes and cytokine signaling are linked to PD risk.
- Microglial activation and increased inflammation markers (complement, cytokines, inflammasomes) found in PD brain tissue.

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Neurodegenerative diseases

- **Parkinson's disease (PD):** Neurodegenerative disorder characterized by dopaminergic neuron loss and α-synuclein aggregation.

Microbiome Alterations in PD

- Reduced Prevotella across multiple populations (Germany, Finland, Russia, Japan).
- Increased *Akkermansia muciniphila* correlates with constipation in PD patients.
- SCFA-producing bacteria are altered, linking microbiome changes to neuroinflammation.
- Bacteroides associated with TNF-α and Verrucomicrobia with IFN-γ, reinforcing microbiota-immune interactions.

Future Research and Therapeutic Directions

- Understanding microbiome shifts at different PD stages (including prodromal phase).
- Targeting gut-immune interactions for potential immune-based therapies.

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Neurodegenerative diseases

◆ **Alzheimer disease (AD):** Most prevalent neurodegenerative disorder leading to dementia.

- Characterized by Aβ plaques, tau hyperphosphorylation, neurofibrillary tangles, neuronal loss, and neuroinflammation.
- Disease progresses from the transentorhinal cortex to the hippocampus and cortical areas.

Neuroinflammation and Immune Response

- Toll-like receptor activation by Aβ triggers inflammasome complexes and microglial-driven inflammation, accelerating cognitive decline.
- Misfolded tau disrupts protein turnover at synapses, leading to neuronal dysfunction.
- Debate remains: Is Aβ accumulation a dysregulated immune response or a direct driver of AD?

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Neurodegenerative diseases

- **Alzheimer disease (AD):** Most prevalent neurodegenerative disorder leading to dementia.

Microbiome Alterations in AD

- Elevated *Bacteroides* levels correlate with reduced microglial phagocytic activity, promoting Aβ accumulation.
- Reduced beneficial bacteria: *Eubacterium rectale*, *Bifidobacterium*, *Dialister*.
- Increased pathogenic bacteria: *Escherichia/Shigella*, *Bacteroides*, *Ruminococcus*.
- Pro-inflammatory cytokines (IL-1β, CXCL2) are elevated in AD, linking gut microbiota to systemic inflammation.

Pathogen Hypothesis & Microbiota Influence

- Aβ may function as an antimicrobial peptide, trapping pathogens.
- HSV-1 (herpes simplex virus-1) found in Aβ plaques and tau tangles.
- Microbiome changes modulate Aβ deposition and neuroinflammation.

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Neurodegenerative diseases

- **Alzheimer disease (AD):** Most prevalent neurodegenerative disorder leading to dementia.

Therapeutic Approaches

- Antibiotics and probiotics influence Aβ pathology.
- Fecal microbiota transplantation (FMT) from healthy donors reduced Aβ and tau abnormalities in mouse models.
- *L. plantarum* administration prevented cognitive decline, Aβ plaque formation, and tau hyperphosphorylation in AD models.
- Microbiota from AD patients induced cognitive deficits in animal models.

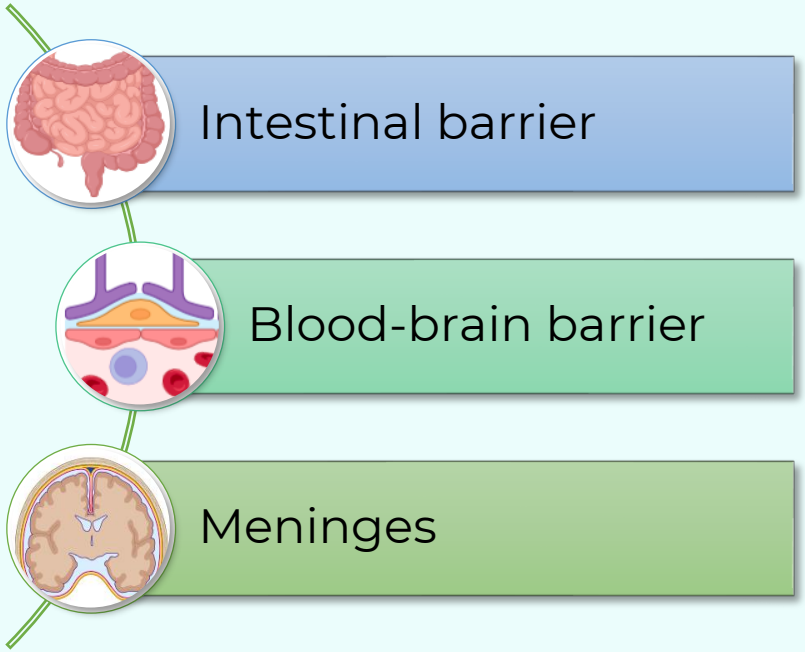
Future Directions

- Need for biomarkers for early detection.
- Further research on mechanisms of microbiome-targeted therapies: microbiome-based interventions hold potential as adjunct therapies to slow or halt AD progression.

Cell Reports Medicine (2025), 101982

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

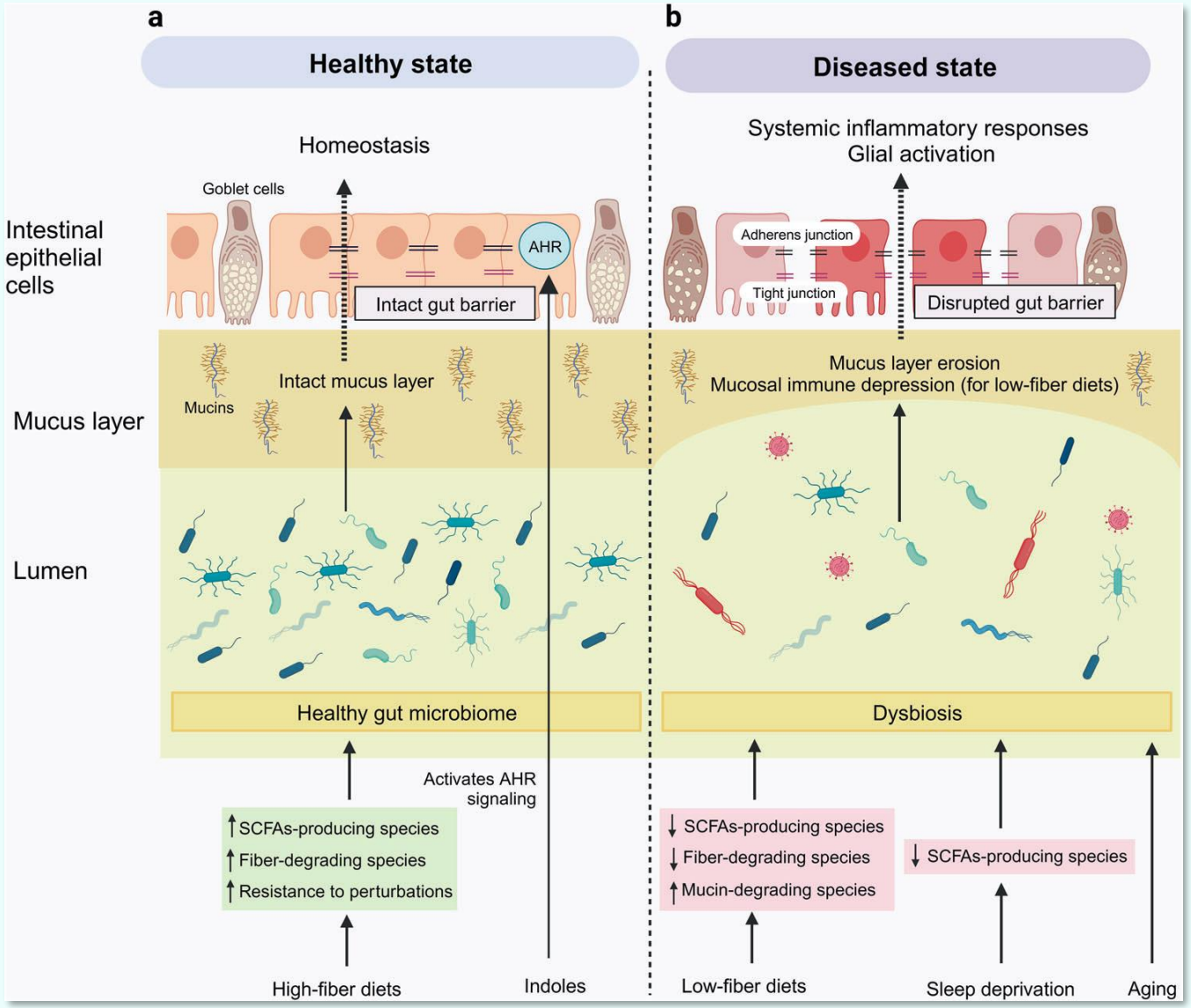
Points of intervention to improve Microbiome–Immune–Brain Axis



Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Intestinal barrier: microbial metabolites



Key components of the intestinal barrier:

- Mucus layer (mucins, goblet cells)
- Epithelial barrier
- Gut vascular barrier

Aging and barrier breakdown:

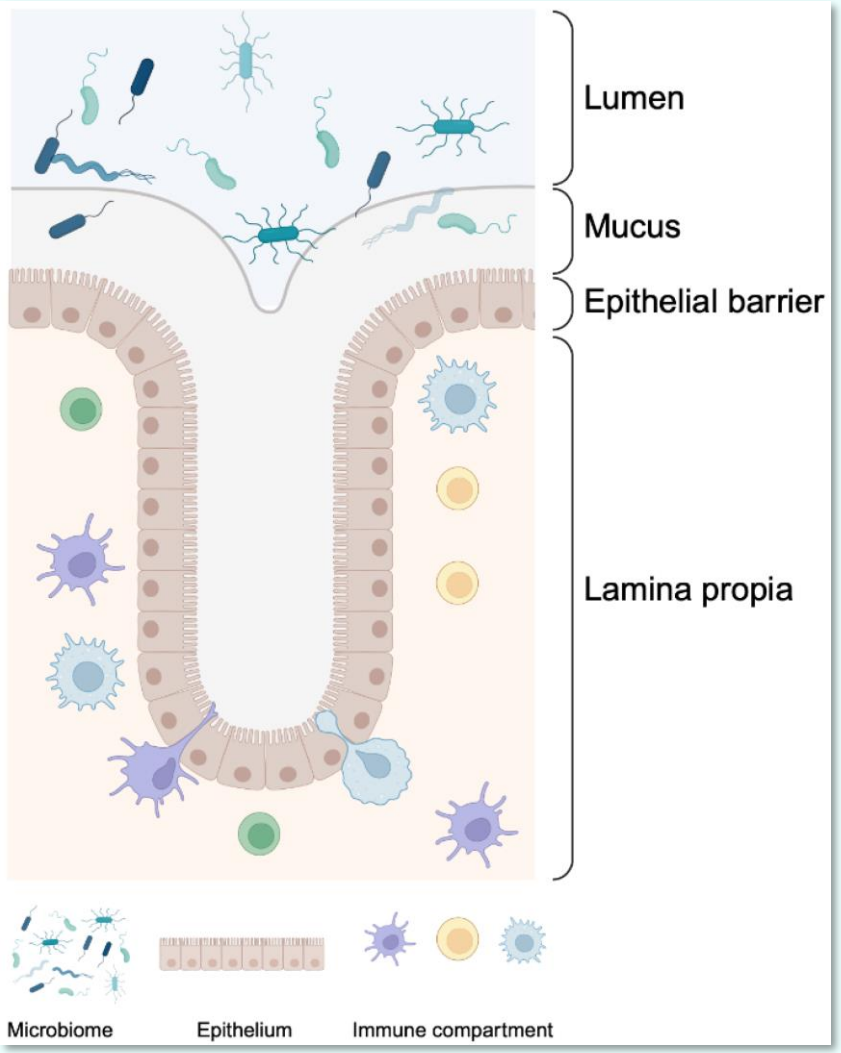
- Increased permeability → systemic inflammation → BBB impairment.
- Associated to CNS inflammation and neurodegeneration.

Therapeutic potential

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Intestinal barrier: microbiome's role



Gut microbiome regulates mucus layer homeostasis:

- Supports goblet cell function
- Prevents ER stress in mucus production

Aging disrupts microbiota balance

- Alters mucin production → weakens the barrier
- Allows harmful metabolites to enter circulation

Microbiome disruption → increased inflammatory metabolites

- Accumulate in the brain → microglial dysfunction → oxidative stress

Neurodegeneration Progression:

- Intestinal permeability increases
- Triggers systemic and CNS inflammation

Cells 2024, 13, 477

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Intestinal barrier restoration by microbial metabolites: Potential therapeutic strategies

Fecal Microbiota Transplantation (FMT)

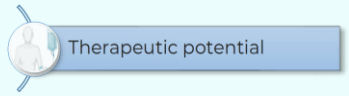
From young to aged mice: Restores gut barrier, reduces inflammation.

In AD models: Decreases Aβ plaques, tau aggregation, and microglial activation.

Microbiota-targeted therapies:

Probiotics, prebiotics, and dietary interventions to support gut integrity.

Regulating gut-brain immune interactions.

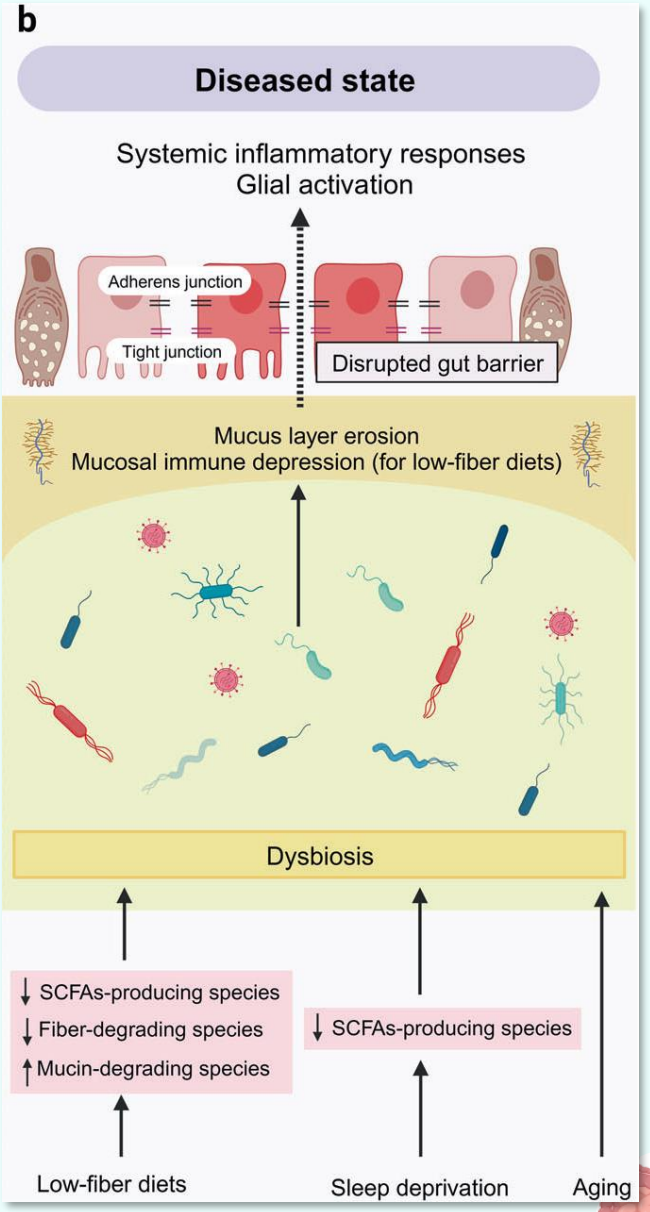


Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Intestinal barrier: short chain fatty acids (SCFAs)

- Gut microbiota-produced short-chain fatty acids (SCFAs) from dietary fiber fermentation enhance the mucus layer, maintaining intestinal barrier integrity. High-fiber diets promote SCFA production and mucus secretion.
- Conversely, low-fiber diets foster mucin-degrading bacteria, leading to mucus layer erosion and increased permeability. Western-style diets reduce SCFA production and increase mucus penetrability.
- Increased intestinal permeability facilitates bacterial translocation, triggering systemic inflammation, neuroinflammation, and neurodegeneration.



Therapeutic potential

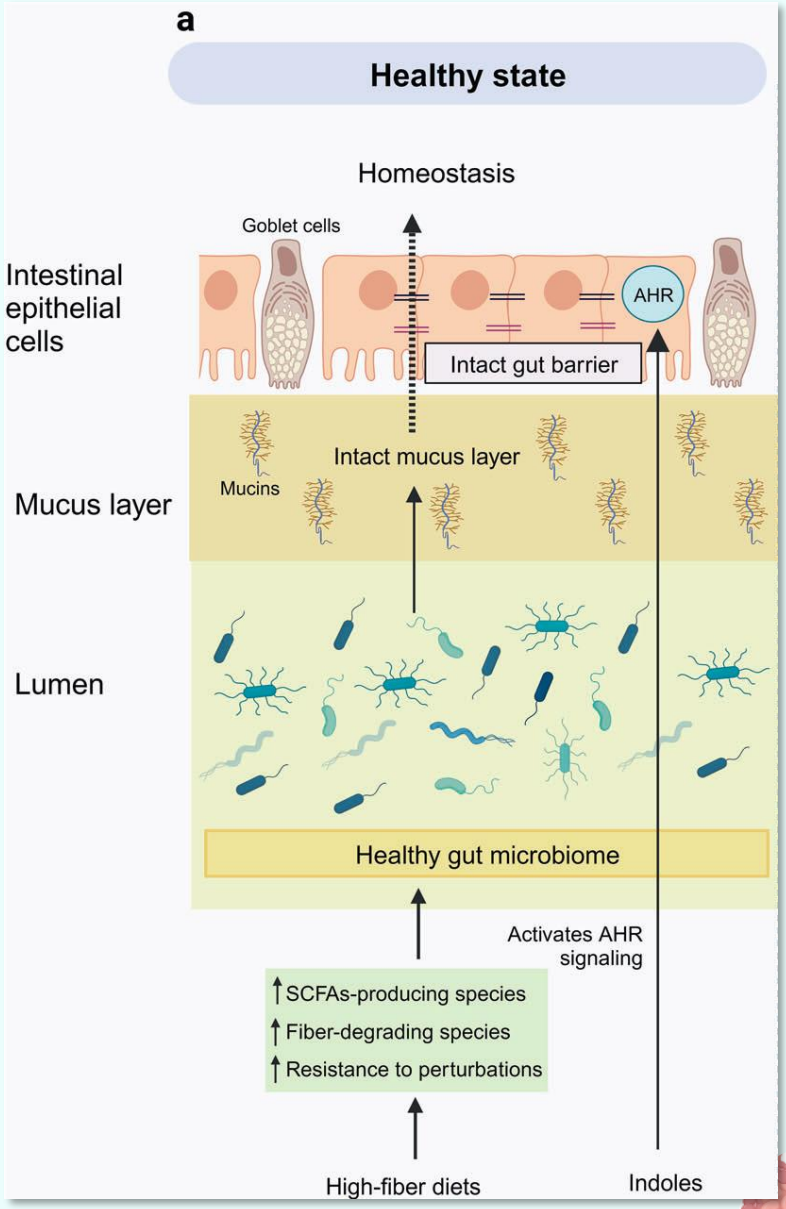
Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Intestinal barrier: Indole and its derivatives

- Indole and its derivatives are key metabolites produced by gut bacteria from dietary tryptophan fermentation, fundamentail in the maintainance of the intestinal barrier integrity.

- These compounds improve barrier function by activating the epithelial Aryl Hydrocarbon Receptor (AHR). AHR activation influences epithelial cell differentiation and crypt stem cell proliferation.

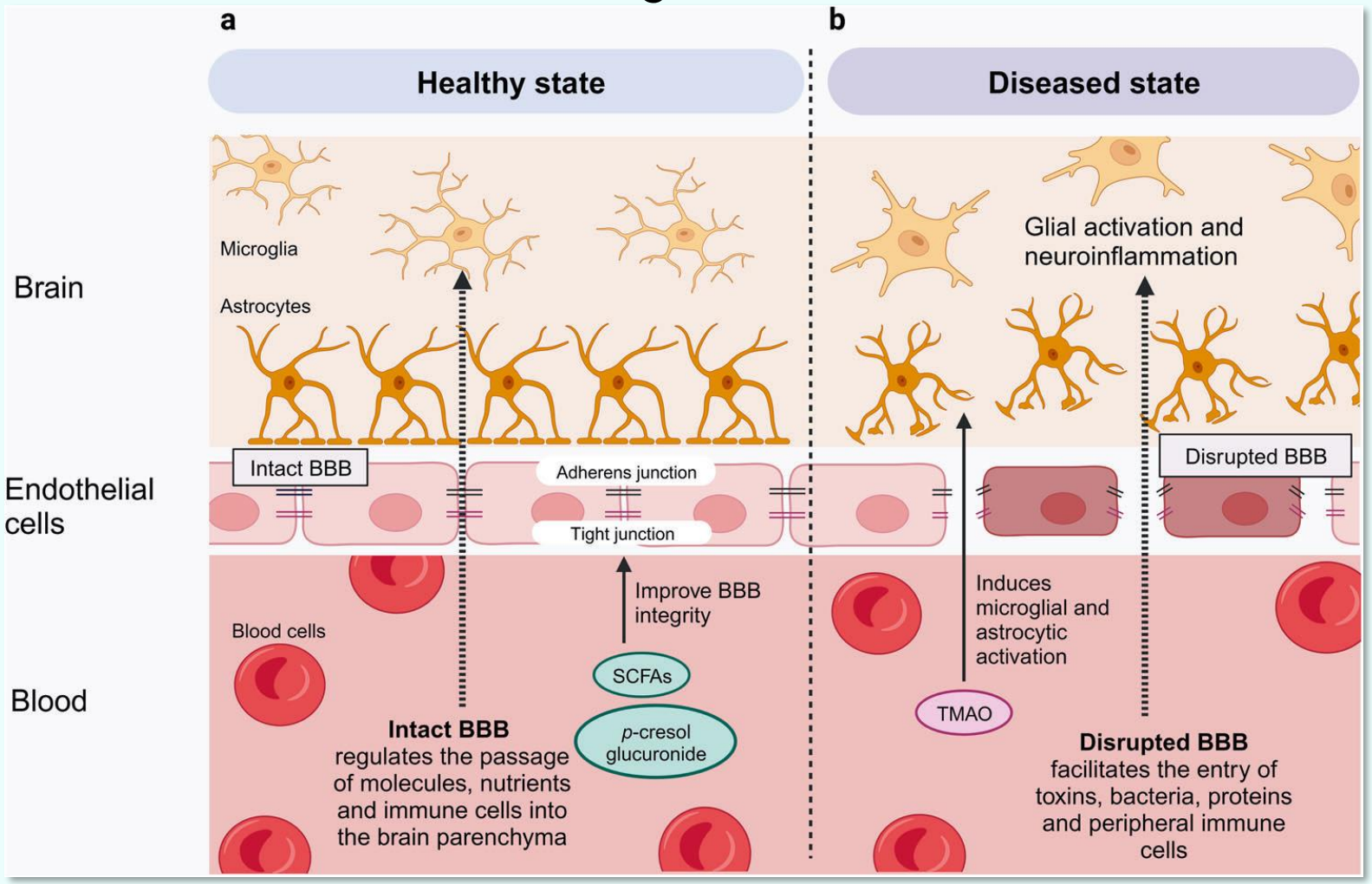


Therapeutic potential

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Blood–brain barrier (BBB) restoration by microbial metabolites



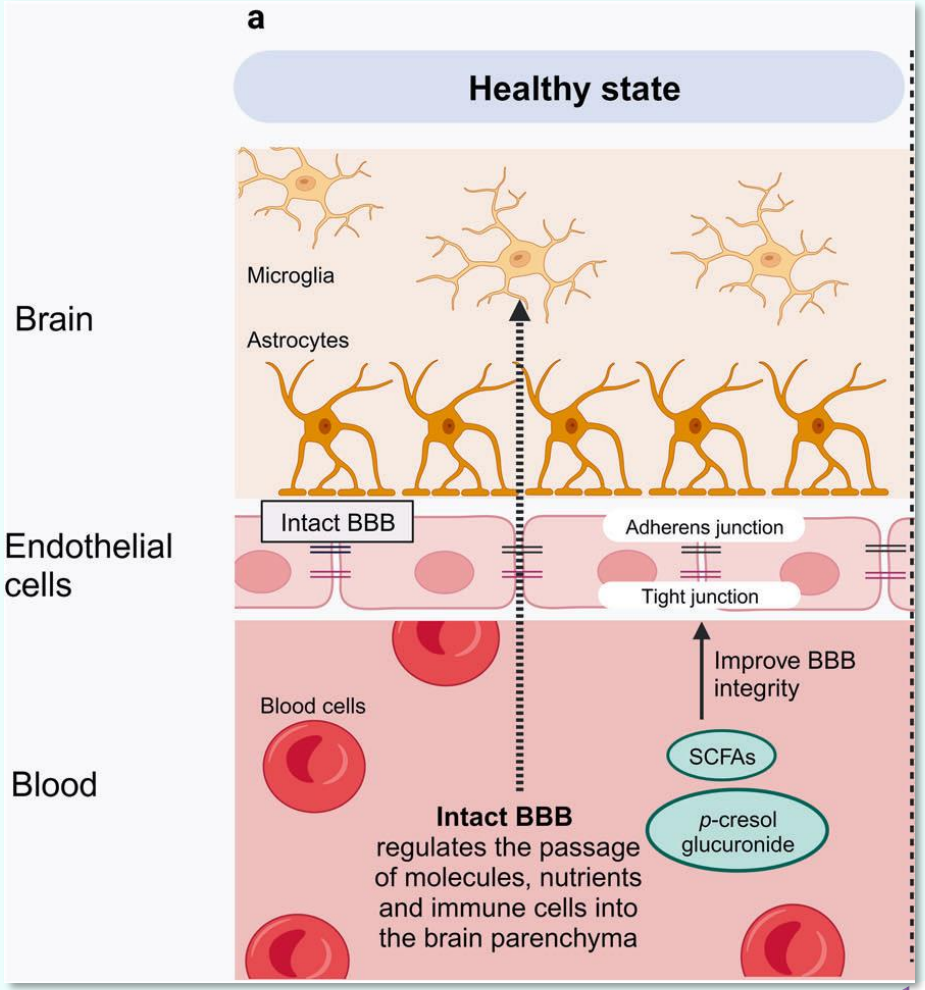
- The BBB is crucial for CNS homeostasis, regulating molecule passage, removing waste, and acting as an immunological barrier.
- It comprises tightly packed endothelial cells, tight junctions, and other cells forming the neurovascular unit.
- BBB integrity declines with age and is implicated in neurodegenerative diseases; gut microbiota metabolites may influence BBB function.

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Improve BBB: short chain fatty acids (SCFAs)

- Microbial-derived SCFAs play a significant role in regulating BBB integrity.
- Germ-free or antibiotic-treated mice show increased BBB permeability, indicating the importance of gut microbiota.
- SCFAs like propionate and butyrate enhance BBB integrity by reducing oxidative stress and regulating tight junction proteins.
- **Potential Therapeutic Role:** SCFAs hold promise for therapeutic strategies targeting BBB dysfunction, but further research is needed.



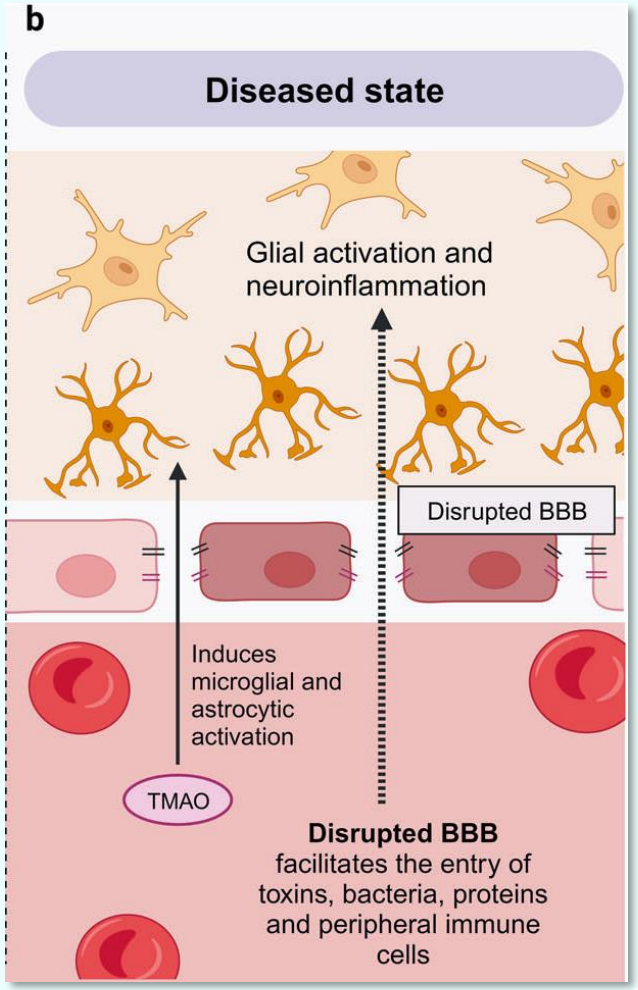
Therapeutic potential

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Trimethylamine N-oxide disrupts BBB integrity

- Trimethylamine N-oxide (TMAO) is a metabolite that influences BBB integrity. Increased TMAO levels are observed in the plasma and CSF of individuals with mild cognitive impairment, AD, and PD.
- Increased TMA production in the gut microbiome of PD patients leads to elevated TMAO.
- TMAO induces neuronal senescence, oxidative stress, alters synaptic plasticity, and contributes to neuroinflammation.



Therapeutic potential

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Meninges as an emerging target

- The meninges are a three-layered membrane (dura, arachnoid, pia mater) covering the brain and spinal cord.
- Meninges actively participate in neuro-immunological crosstalk with the brain, not just a structural barrier.
- The meninges host various immune cells, with changes occurring during aging towards a pro-inflammatory state.
- **Relevance to Disease:** Meningeal dysfunction contributes to neuroinflammation in neurodegenerative diseases and influences microglial activation and amyloid-beta deposition.

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Restoration of peripheral immune homeostasis

- **Immune dysregulation and neurodegeneration:** A key factor contributing to neurodegeneration is the dysregulation of the immune system, encompassing both innate and adaptive immune responses, which leads to chronic inflammation. While traditionally the focus has been on peripheral immune cells infiltrating the CNS, it's now clear they can also influence neurodegenerative processes outside the brain.

- **Bidirectional gut-immune communication and T cell balance:** The gut microbiome is a critical player in bidirectional communication with the immune system. The gut microbiome and its metabolites can finely tune the balance of T cell subsets, particularly TH17 cells, which are essential for maintaining gut barrier function and overall tissue homeostasis.

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Restoration of peripheral immune homeostasis

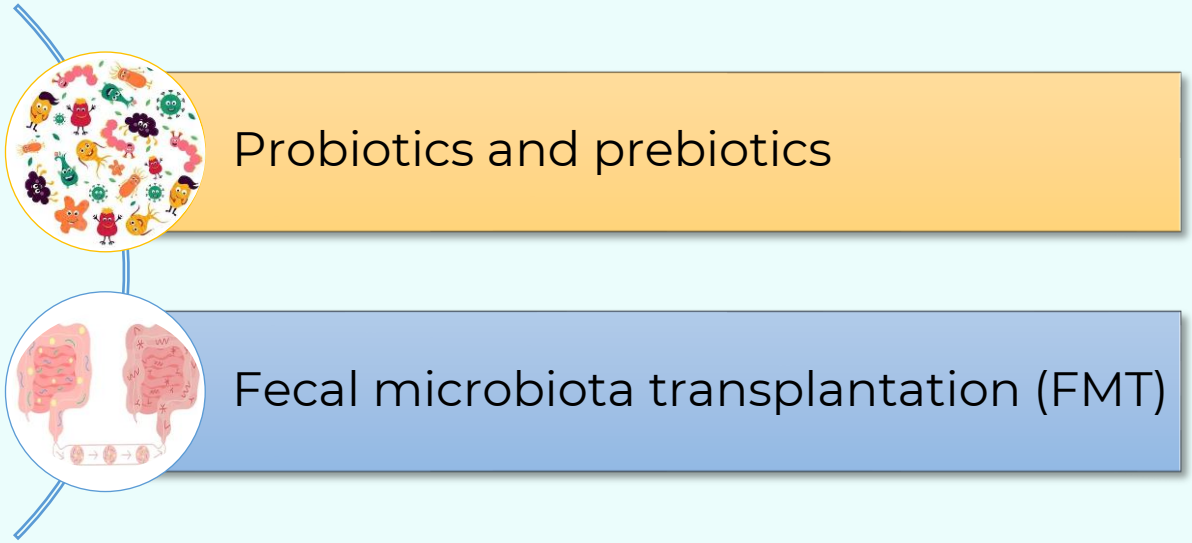
- **Dietary influence on gut microbiome and immune function:** Unhealthy dietary interventions, such as those high in fat, sugar, and salt, can negatively impact the gut microbiome and impair TH17 cell function. This disruption in the gut microbiome can contribute to imbalances in the ratio of Treg and TH17 cells, disrupting intestinal immunity and contributing to systemic inflammation.

- **Microbial metabolites as modulators of immune responses:** SCFAs produced by the gut microbiome from dietary fiber are potent modulators of various immune cells, including T cells and B cells. For example, butyrate promotes the development of regulatory T cells (Tregs) and supports regulatory B cell function, both of which are critical for controlling inflammation. However, a rapid shift to a low-fiber diet can dramatically reduce SCFA production and impair T cell function. It's also worth noting that some secondary bile acids can have anti-inflammatory effects by favoring Treg cell development and suppressing TH17 cell responses.

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Microbiome based therapeutics



- Alterations in the gut microbiome in neurodegenerative disease patients have prompted the exploration of clinical applications of microbiome-based therapeutics.

- The rationale for these therapies is supported by studies demonstrating that the human gut microbiome is a major determinant of plasma metabolome, potentially playing a more dominant role than genetics.

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

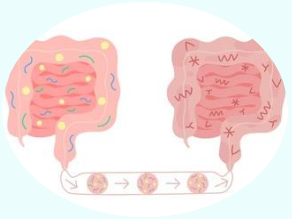
Probiotics and prebiotics

Probiotics are live microorganisms that, when administered adequately, provide a health benefit to the host. They aim to modulate the gut microbiome and increase beneficial metabolite production.

- **Benefits for Cognition:** Research, primarily in animal models, shows probiotics and prebiotics benefit cognitive functions. This is linked to enhanced intestinal and blood-brain barrier integrity.
- **Mechanism of action:** Probiotics and prebiotics re-establish beneficial gut microbiota (especially SCFA-producing species), increase SCFA levels, reduce systemic inflammation (by reducing LPS), and decrease glial activation.
- **Neurodegenerative impact:** These actions contribute to reducing neurodegenerative disease pathology and may help restore neurotransmitter systems, which are often compromised in these conditions.

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt



Fecal microbiota transplantation (FMT)

FMT involves transferring the gut microbial community from a healthy donor to a patient to treat conditions linked to gut dysbiosis.

- **Mechanism of action:** FMT aims to alter the patient's gut microbiome, but it's a broad modulation strategy, not a precise targeting of specific bacteria.
- **FMT Evolution:** FMT has evolved from a crude procedure to a developing mainstream therapy, with increased standardization and focus on safety.
- **Key Applications:** FMT has shown efficacy in treating *Clostridioides difficile* infection and shows promise in managing inflammatory bowel disease.

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Clinical challenges of microbiome-based therapeutics

Despite preclinical promise, clinical trials of microbiome-based therapeutics show mixed efficacy in improving cognitive/motor function. The main limitations are due to the lack comprehensive gut microbiome profiling for many clinical trials, hindering correlation of microbiome changes with clinical outcomes.

- **Probiotic challenges:** probiotics face limitations in survival (low gastric pH) and gut colonization, requiring improved delivery strategies.
- **Prebiotic challenges:** prebiotics have non-specific effects, making microbiome manipulation difficult to control, and their effectiveness depends on the existing gut microbiota.
- **FMT challenges:** FMT is limited by its variable degree of engraftment, limited scalability, and a lack of standardized protocols.

Signal Transduction and Targeted Therapy (2024) 9:37

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758
lecturer@imas.edu.mt

Take home messages

The microbiome-immune–brain axis is a promising therapeutic target for neurodegenerative diseases due to its influence on glial function.

Preclinical studies show that microbiome-based interventions can improve gut and brain barrier integrity and reduce glial activation and cognitive impairment.

Translating these findings to human therapies is challenging due to difficulties in establishing causality and the limitations of current animal models.

Advancements in technologies, such as novel sampling methods and single-cell analysis, are enhancing understanding of the complex interactions within microbiome-immune–brain axis.

The malleability of the gut microbiome offers exciting potential for developing personalized microbiome-based therapeutics for neurodegenerative conditions, requiring continued research.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

REFERENCES

- Biomedicine & Pharmacotherapy 2024, 178:117207
- Current Opinion in Neurobiology 2023, 78:102652
- Cell Reports Medicine 2025, 6:101982
- Gut Microbes 2024, 16:1 2422468
- Pharmacological Reviews 2019, 71:198–224
- Signal Transduction and Targeted Therapy 2024, 9:37
- Frontiers in Microbiology 2024, 14:1118529
- Frontiers in Neurology 2021, 12:721126
- Cells 2024, 13(6):477

STUDY MATERIAL

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Post-graduate certificate level 7 (30 ECTS)

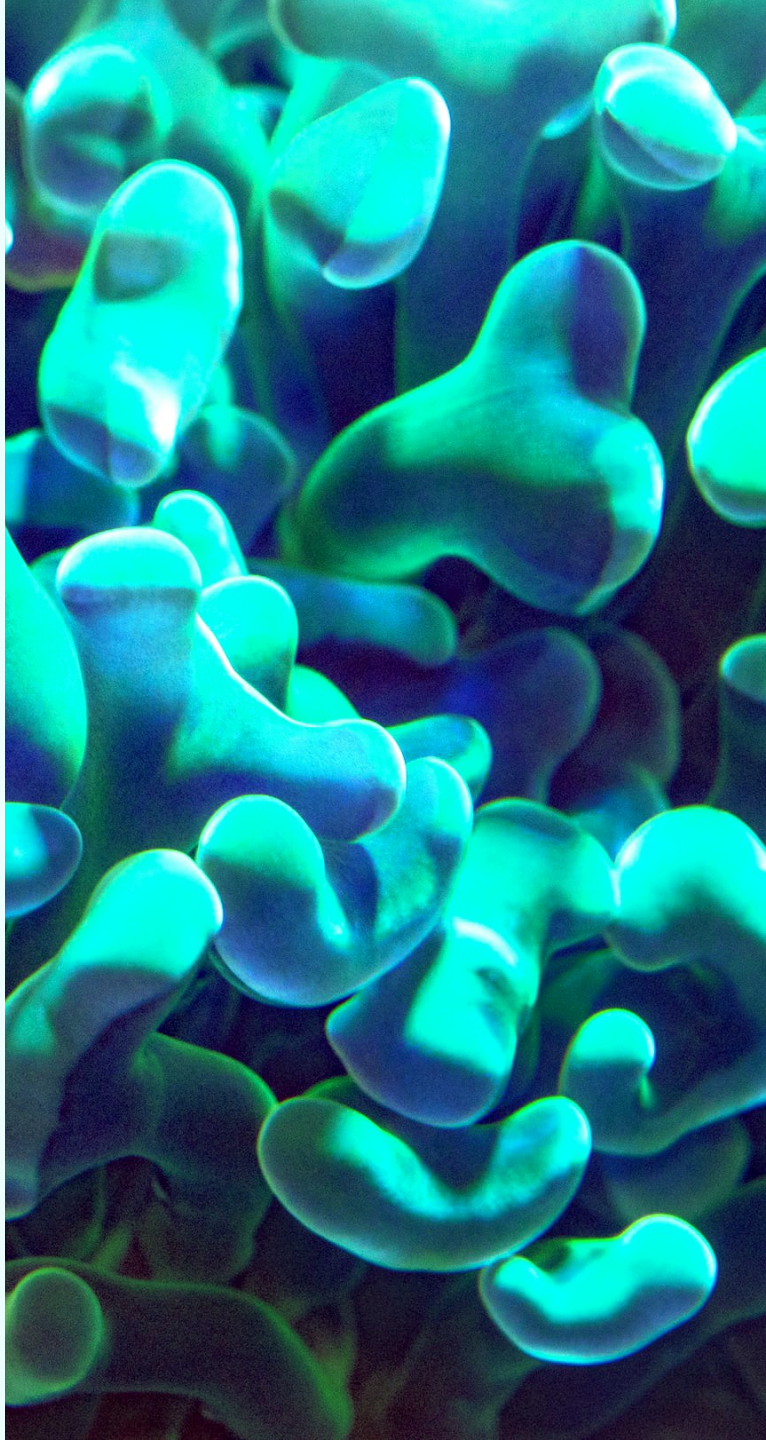
Dietary recipes for managing dysbiosis

A tailored approach to restoring gut balance

Adriana Chiarelli
PhD in Microbiology

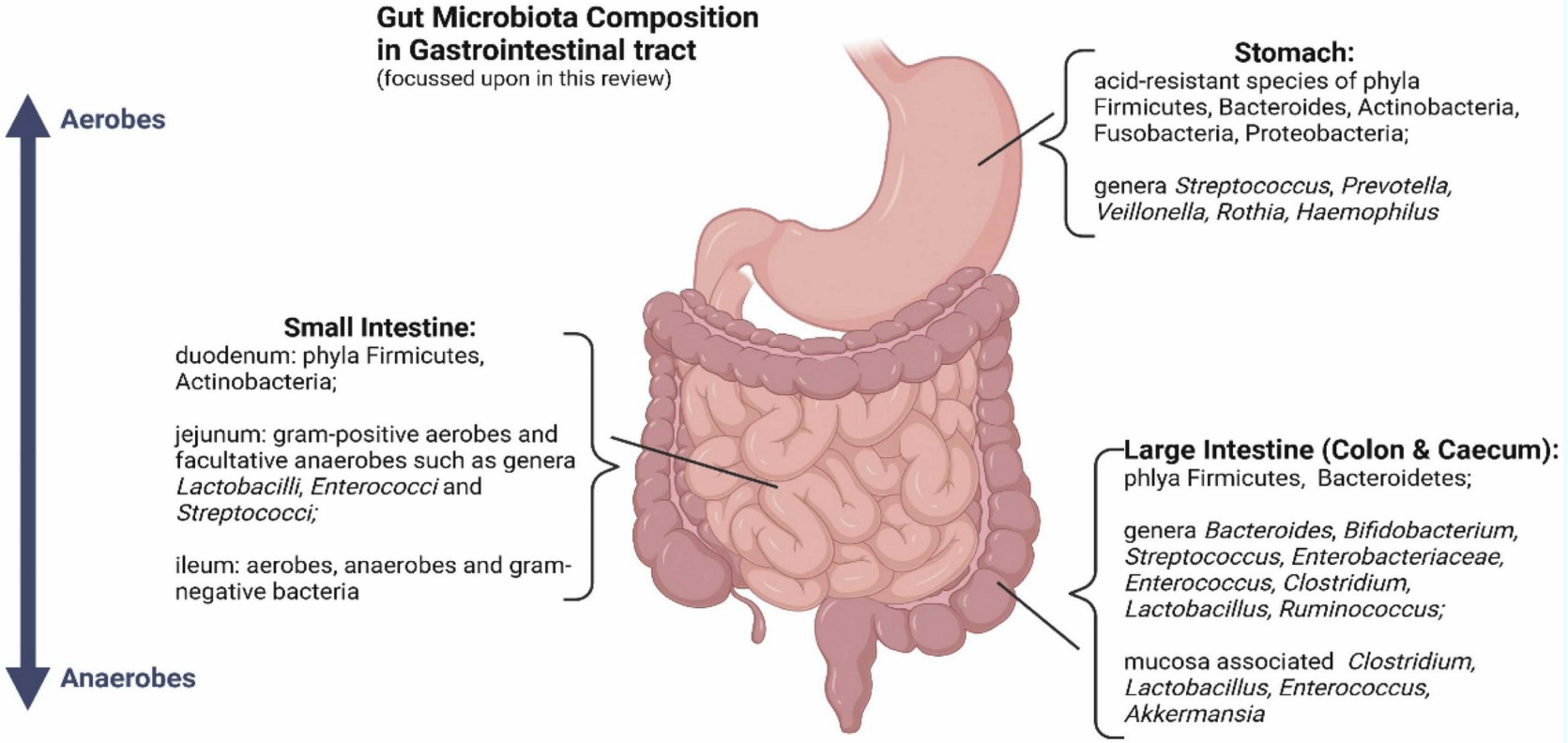
?@imas.edu.mt

Malta Further & Higher Education Authority



INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Overview of the gut microbiota in the GI tract



https://doi.org/10.1016/j.brainresbull.2024.110883

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

The «healthy» gut microbiota: composition and key features

Healthy gut = state of ***eubiosis***

> 90% of colonising species

Diversity

Stability and resistance

Resilience

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

What is microbial dysbiosis?

"All disease begins in the gut." – Hippocrates of Kos

Eubiosis
From Ancient Greek
εὖ (**"well, good"**) and βίωσις
(bíōsis, "way of living")

Dysbiosis
From Ancient Greek
δυσ- (dus-, **"bad"**) and βίωσις
(bíōsis, "way of living")

Any deviation from the normal structure of the gut microbiota (taxonomical and metagenomic function)
characterised by an **imbalance** in the composition and/or function of the microbial ecology

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Factors influencing gut health

Regulators of gut microbiota composition and function

Hrncir T. Microorganisms. 2022

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Diet and dysbiosis: rule of thumb

RESTORE GUT BALANCE

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

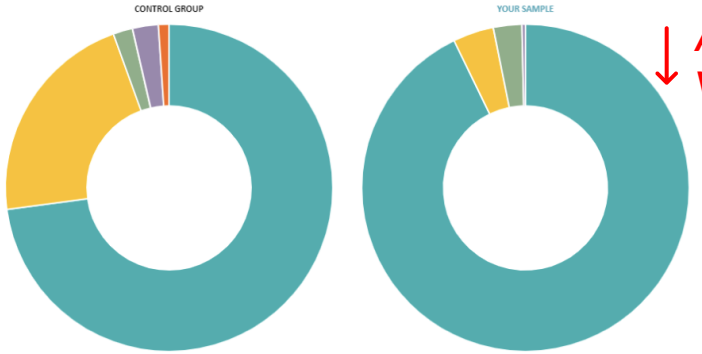
Patient 1: Lactose Intolerance, GERD, IBS, Hyperthyroidism

2. Variation of the main bacteria phyla

Phylum	Control Min (%)	Control Max (%)	Your Sample (%)
Firmicutes	65.569	79.822	92.754
Bacteroidetes	14.681	28.348	4.007
Proteobacteria	0.593	3.244	2.823
Actinobacteria	0.877	5.155	0.339
Verrucomicrobia	0.640	3.610	0.000

↑ *Firmicutes/Bacteroidetes*

↓ *Actinobacteria and Verrumicrobia*

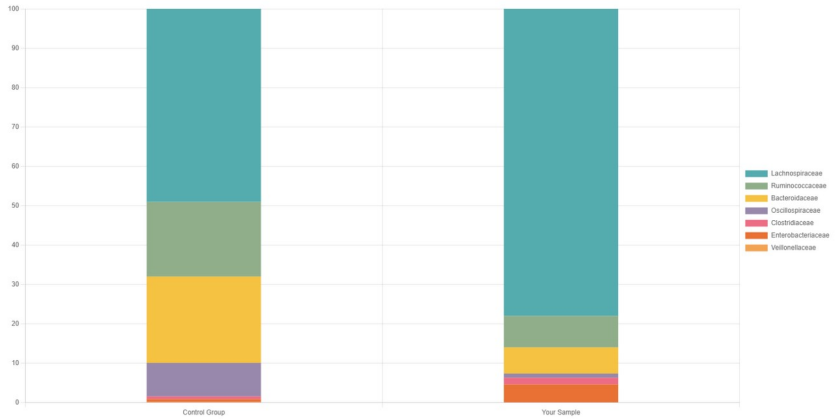


3. Families of interest

Family	Control Min (%)	Control Max (%)	Your Sample (%)
Bacteroidaceae	8.791	22.421	3.784
Clostridiaceae	0.263	1.271	0.988
Enterobacteriaceae	0.280	1.329	2.577
Lachnospiraceae	25.695	44.222	44.547
Oscillospiraceae	2.522	9.627	0.600
Ruminococcaceae	7.964	19.113	4.574
Veillonellaceae	0.040	0.298	0.047

++Enterobacteriaceae

++Oscillospiraceae



Pro-inflammatory bacteria

Genus	Control Min (%)	Control Max (%)	Your Sample (%)
Acinetobacter	0.000	0.000	0.000
Alistipes	1.334	3.246	0.052
Clostridium	0.742	1.592	1.556
Escherichia	0.306	1.057	1.913
Haemophilus	0.083	0.415	0.087
Helicobacter	0.000	0.000	0.000
Klebsiella	0.058	0.555	0.000
Prevotella	2.423	9.395	2.987
Salmonella	0.000	0.000	0.038
Streptococcus	0.774	4.078	0.985
Sutterella	0.274	0.765	0.137
Turicibacter	0.107	0.388	0.476

Anti-inflammatory bacteria

Genus	Control Min (%)	Control Max (%)	Your Sample (%)
Akkermansia	0.641	3.469	0.000
Bifidobacterium	1.894	4.520	0.302
Coprococcus	1.451	3.564	0.470
Enterococcus	0.237	1.490	0.190
Eubacterium	0.939	1.754	0.243
Faecalibacterium	7.055	12.470	3.600
Lactobacillus	0.015	0.099	0.081
Oxalobacter	0.019	0.059	0.000
Parabacteroides	1.113	2.095	0.032
Ruminococcus	3.422	6.044	0.362

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 1: Lactose Intolerance, GERD, IBS, Hyperthyroidism

VEGETABLES and LEGUMES:	**VEGETABLES and LEGUMES:**
Bell Peppers, Sweet Red, **Cooked**	Artichoke
Cabbage, green, Red	Asparagus
Chicory Green, Red	Beans, Large White
Chicory root	Mushrooms, White Button
Chicory, Sugarloaf	Spinach
Chicory, wild	Broccoli
Dandelion Greens	Cauliflower
Endive, Belgian, Red	Leeks
Endive, curly (Frisée)	
Endive, Escarole	
Garlic, black - oil	
Puntarelle (sprouts of Catalonian chicory)	
Radicchio (Italian chicory)	
Ramps	
FRUITS:	**FRUITS:**
Bergamot	Canned fruits
Blackberry	Grapefruit
Blueberry	Orange, sour
Cloudberry	Lime
Cranberry	Lemon
Grape, Red	
Guavas	
Mango	
Papaya	
Persimmon	
Watermelon	

GRAINS & PSEUDOGRAINS:	**GRAINS & PSEUDOGRAINS:**
Sorghum	Breakfast cereals
	Oat Bran
	Quinoa
	Rice Bran
MEAT:	**MEAT:**
Rabbit	Bacon
Turkey breast	Beef skirt steak
	Deli Meat
	Ham
	Hot Dogs
	Liver, Lamb
SEAFOOD (fish and shellfish):	**SEAFOOD (fish and shellfish):**
Clams	Cuttlefish
Mackerel	Oysters
DAIRY and EGGS	**DAIRY and EGGS**
Egg Yolk	Ice cream
	Butter
NUTS and SEEDS	**NUTS and SEEDS**
Chestnut	Cumin Seed
Fennel seeds	Pumpkin Seeds
Mustard Seeds	
SPICES and HERBS	**SPICES and HERBS**
Cinnamon	Chili pepper
Cloves	Coriander
Ginger	Peppermint
Nutmeg	Pepperwort
Oregano, Mediterranean	
Rosemary	
Sage	
Thyme	

CONDIMENTS and OTHERs:	**CONDIMENTS and OTHERs:**
Bee bread	Barbecue sauces
Coconut milk	Chewing gum
Coconut oil	Cocoa mix
Guarana	Diet soda
Herbal Tea, Heater (Erica manipuliflora)	Flavored coffee syrups
Herbal Tea, Lysimachia	Frozen desserts
Herbal tea, Milk thistle	Grapefruit juice
Herbal Tea, Tulsi leaves (Holy basil)	Gelatin
Tea, Green	Maple syrup alternatives
Thyme, oil	Salad dressings
	Sorbet
	Sucralose
	Sugar-free candy

GOAL: reduce the dysbiosis

IN GREEN: FOODS BENEFICIAL FOR THIS PATIENT

IN YELLOW: FOODS TO LIMIT FOR THIS PATIENT

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 1: example of personalised day meal plan

*Example of **breakfast**: spiced sorghum porridge*

Ingredients

Cooked sorghum
Water or almond milk
Spices: cinnamon, nutmeg, cloves, turmeric
Maple syrup (opt)

Instructions

Combine cooked sorghum with water (or almond milk), and the spices.
Stir occasionally until it thickens (about 5').
Serve warm

Spices like turmeric and cinnamon are anti-inflammatory and help with digestion. Turmeric helps with hyperthyroidism and is soothing for IBS and GERD.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 1: Lactose Intolerance, GERD, IBS, Hyperthyroidism

*Example of **LUNCH**: endive salad with walnuts and mackerel**

Ingredients

Endive heads
Mackerel in olive oil
Walnuts
Dried cranberries
Lactose-free cheese (ex. Ricotta)
EVO, Lemon juice, Salt & pepper
Toasted pumpkin seeds (opt)

Instructions

Prepare the dressing with oil, lemon juice, salt and pepper. Assemble all the ingredients and season the salad in a large bowl.

Rich in omega-3, high in fiber, antioxidants
Good source of vitamins A, C, and K, as well as folate

**VEGGIE OPTION: replace with firm tofu (press it to remove excess water then slice it into cubes and lightly grill in olive oil*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 1: Lactose Intolerance, GERD, IBS, Hyperthyroidism

*Example of **DINNER**: herb-roasted rabbit with chestnuts and radicchio*

Ingredients

Rabbit legs (or cutlets)
Chestnut
Radicchio
EVO, dried oregano, fresh thyme
Salt and pepper
Cooked sorghum

Instructions

Mix olive oil, oregano, thyme, salt, and pepper; rub on rabbit. Roast rabbit.
Boil chestnuts.
Cook radicchio with olive oil, salt, and pepper; add boiled chestnuts.
Serve rabbit with radicchio and chestnut mix together with sorghum.

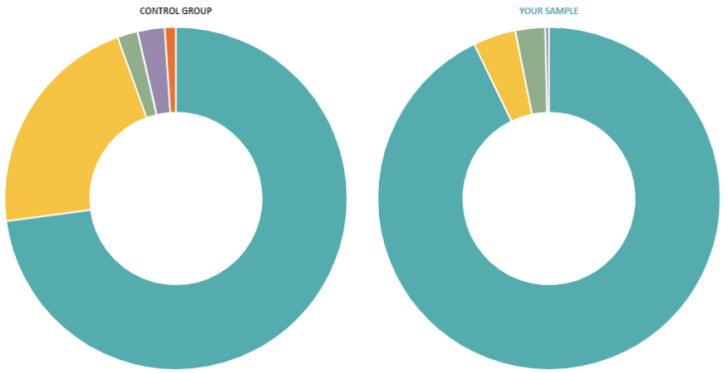
nutrient-dense, low-fat meal (lean protein), vitamins, and fiber, making it suitable for lactose intolerance, GERD, and IBS

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 1: expected **gut microbiota shifts** to dietary changes

2. Variation of the main bacteria phyla

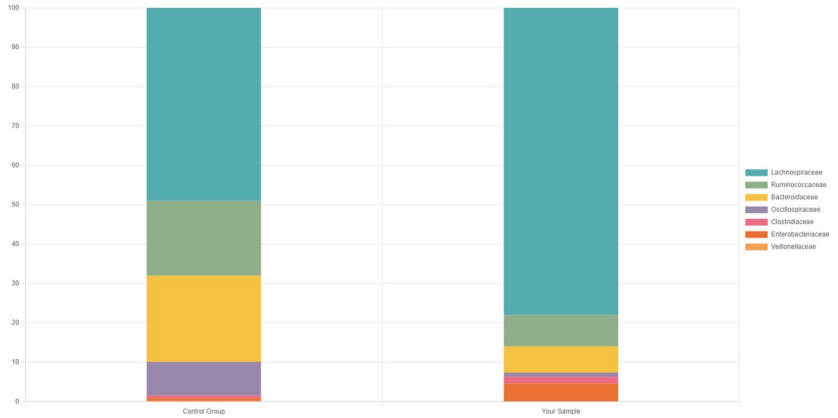
Phylum	Control Min (%)	Control Max (%)	Your Sample (%)
Firmicutes	65.569	79.822	92.754
Bacteroidetes	14.681	28.348	4.007
Proteobacteria	0.593	3.244	2.823
Actinobacteria	0.877	5.155	0.339
Verrucomicrobia	0.640	3.610	0.000



Bring Firmicutes/Bacteroidetes to 1

3. Families of interest

Family	Control Min (%)	Control Max (%)	Your Sample (%)
Bacteroidaceae	8.791	22.421	3.784
Clostridiaceae	0.263	1.271	0.988
Enterobacteriaceae	0.280	1.329	2.577
Lachnospiraceae	25.695	44.222	44.547
Oscillospiraceae	2.522	9.627	0.600
Ruminococcaceae	7.964	19.113	4.574
Veillonellaceae	0.040	0.298	0.047



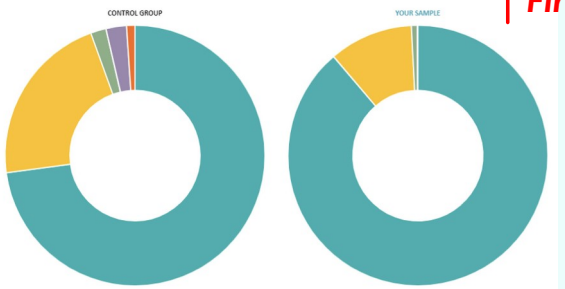
- *Reduce **Enterobacteriaceae (pro-inflammatory)***
- *Reduce **Oscillospiraceae***
- *Increase Actinobacteria and Verrumicrobia*
- *Enhance the % of ANTI-inflammatory bacteria (Akkermansia, Bifidobacterium, Faecalibacterium, Oxalobacter)*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 2: bloating, abdominal pain, flatulence, cramps, high cholesterol, high triglycerides

2. Variation of the main bacteria phyla

Phylum	Control Min (%)	Control Max (%)	Your Sample (%)
Firmicutes	65.569	79.822	88.557
Bacteroidetes	14.681	28.348	10.417
Proteobacteria	0.593	3.244	0.725
Actinobacteria	0.877	5.155	0.075
Verrucomicrobia	0.640	3.610	0.000



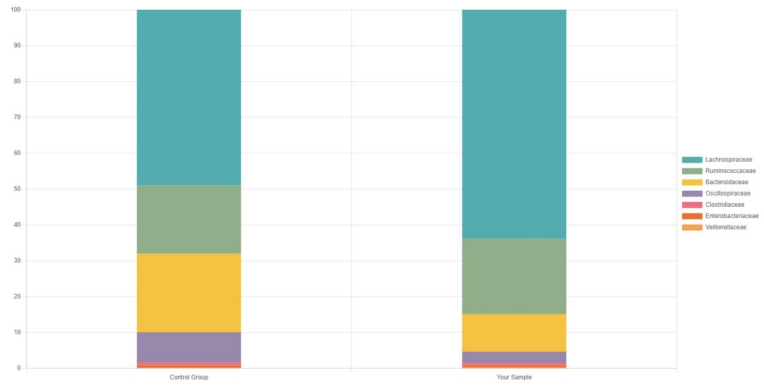
↑ *Firmicutes/Bacteroidetes*

↓ *Actinobacteria and Verrumicrobia*

3. Families of interest

Family	Control Min (%)	Control Max (%)	Your Sample (%)
Bacteroidaceae	8.791	22.421	9.009
Clostridiaceae	0.263	1.271	0.514
Enterobacteriaceae	0.280	1.329	0.545
Lachnospiraceae	25.695	44.222	55.164
Oscillospiraceae	2.522	9.627	2.761
Ruminococcaceae	7.964	19.113	18.198
Veillonellaceae	0.040	0.298	0.228

← *++Lachnospiraceae*



Pro-inflammatory bacteria

Genus	Control Min (%)	Control Max (%)	Your Sample (%)
Acinetobacter	0.000	0.000	0.000
Alistipes	1.334	3.246	0.256
Clostridium	0.742	1.592	0.855
Escherichia	0.306	1.057	0.465
Haemophilus	0.083	0.415	0.042
Helicobacter	0.000	0.000	0.000
Klebsiella	0.058	0.555	0.038
Prevotella	2.423	9.395	2.834
Salmonella	0.000	0.000	0.000
Streptococcus	0.774	4.078	1.045
Sutterella	0.274	0.765	0.000
Turicibacter	0.107	0.388	0.000

Anti-inflammatory bacteria

Genus	Control Min (%)	Control Max (%)	Your Sample (%)
Akkermansia	0.641	3.469	0.000
Bifidobacterium	1.894	4.520	0.028
Coprococcus	1.451	3.564	4.372
Enterococcus	0.237	1.490	0.000
Eubacterium	0.939	1.754	1.277
Faecalibacterium	7.055	12.470	10.971
Lactobacillus	0.015	0.099	0.103
Oxalobacter	0.019	0.059	0.000
Parabacteroides	1.113	2.095	0.329
Ruminococcus	3.422	6.044	3.860

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 2: bloating, abdominal pain, flatulence, cramps, high cholesterol, high triglycerides

FRUITS: Apricot Blueberry Coconut Cranberry Grape, Red Grapefruit Kiwi Mango Nectarines Papaya Persimmon, Fuyu Pomegranate Strawberry Watermelon	FRUITS: Apple (Red) Banana (Unripe) Cantaloupe Dried Fruit (Raisins) Passion Fruit Pear Pineapple Plantains Prunes
GRAINS & PSEUDOGRAINS: Cornmeal, Yellow Rice, White, cooked and chilled Sorghum	**GRAINS & PSEUDOGRAINS:** Pizza, frozen Wheat, refined products
MEAT: Liver, Beef Liver, Lamb	**MEAT:** Chicken, prepared Cold cuts (deli meats) Turkey, processed slices
SEAFOOD (fish and shellfish): Anchovies Carp Cuttlefish Grouper Halibut Herring, Atlantic Mackerel Perch Pike Salmon, Atlantic, Coho (Wild), Sockeye Sandsmelt Tench Trout, Rainbow Tuna, Bluefin Whitefish	**SEAFOOD (fish and shellfish):** Swordfish Sturgeon Fish Roe

DAIRY and EGGS Cheese, Goat Egg Yolk, poultry Ghee (Clarified Butter) Kefir	**DAIRY and EGGS** Cottage cheese Ice cream Sour Cream Whipping cream
NUTS and SEEDS Almond Chia seeds	**NUTS and SEEDS** Flaxseed
SPICES and HERBS Basil Bay Leaves Cilantro Coriander Dill Marjoram Parsley Peppermint Sage Spearmint Tarragon Thyme Triphala Turmeric	**SPICES and HERBS** Paprika
CONDIMENTS and OTHERs: Cod Liver Oil Herbal Tea, Angelica acutiloba Herbal Tea, Echinacea Kimchi Pickles Sauerkraut Seaweed, Laver Tea, Black Tempeh	**CONDIMENTS and OTHERs:** Almond milk Coconut milk Coconut oil Hemp milk Palm Oil Seaweed, Brown Seaweed, Red Soups, canned Soy milk Soy puddings Vitamins chewable

GOAL: reduce the dysbiosis

VEGETABLES and LEGUMES: Aubergine (Eggplant) Bell Peppers, Sweet Red, Cooked Cabbage, Chinese (Bok Choy) Cabbage, Red Cabbage, savoy Carrots Chicory Greens Chicory root Chives Endive, Escarole Fennel Garden Cress Garlic Lettuce, Red Leaf, Romaine Potatoes, cooked and chilled Snow Peas Squash, Butternut Sweet Potatoes, Backed Turnip, Greens Watercress Zucchini	**VEGETABLES and LEGUMES:** Asparagus Broccoli Collard greens Dandelion Greens Jerusalem Artichokes (Topinambur) Kale Mustard Greens Radish Ramps Spinach Swiss Chard

IN GREEN: FOODS BENEFICIAL FOR THIS PATIENT

IN YELLOW: FOODS TO LIMIT FOR THIS PATIENT

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 2: bloating, abdominal pain, flatulence, cramps, high cholesterol, high triglycerides

*Example of **breakfast**: Chia pudding with berries (water based)*

Ingredients

Chia seeds
Water
Honey or maple syrup (optional)
Vanilla extract (optional)
Mixed berries
Cinnamon (optional)

Instructions

Mix all ingredients.
Refrigerate overnight.
Serve with berries on top.

Low-acid, high in fiber but gentle on the stomach, lactose-free, provides steady energy for hyperthyroidism

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 2: bloating, abdominal pain, flatulence, cramps, high cholesterol, high triglycerides

*Example of **LUNCH**: Grilled mackerel with root vegetables*

Ingredients

Mackerel
Parsnip
Turnip
Carrot
Oregano, turmeric, olive oil
Lemon wedge

Instructions

Toss the parsnip, turnip, and carrot in olive oil, turmeric, oregano, salt, and pepper. Spread on a baking sheet and roast for 20-25 minutes, until tender.
Grill the mackerel and serve with veggies + lemon wedge

High in omega-3 fatty acids (anti-inflammatory).
Parsnip, turnip, and carrot provide fiber while being gentle on digestion, helping to reduce bloating and cramps.
Turmeric and oregano contribute to gut health by reducing inflammation.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 2: bloating, abdominal pain, flatulence, cramps, high cholesterol, high triglycerides

*Example of **DINNER**: sweet potato and pomegranate salad with feta and almonds*

Ingredients

Sweet potatoes
Olive oil, lemon juice
Salt and pepper
Feta cheese, crumbled
Pomegranate seeds
Almonds
Fresh mint (opt)

Instructions

Roast sweet potatoes for 25-30’
In a large bowl, combine roasted sweet potatoes feta cheese pomegranate seeds almonds and thyme.
Drizzle with fresh lemon juice, adjust with salt and pepper.

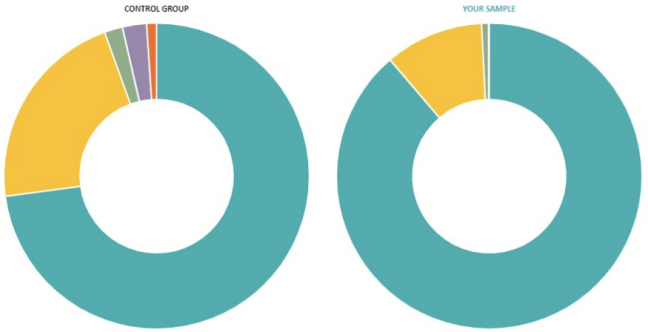
High in fiber, vitamins A and C, and antioxidant
Good for cholesterol management

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 2: expected **gut microbiota shifts** to dietary changes

2. Variation of the main bacteria phyla

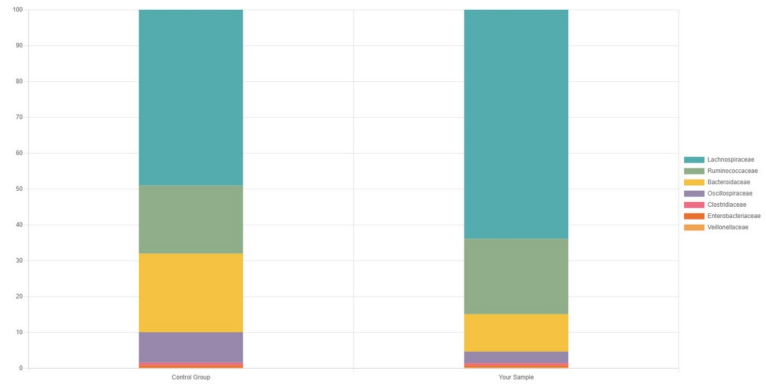
Phylum	Control Min (%)	Control Max (%)	Your Sample (%)
Firmicutes	65.569	79.822	88.557
Bacteroidetes	14.681	28.348	10.417
Proteobacteria	0.593	3.244	0.725
Actinobacteria	0.877	5.155	0.075
Verrucomicrobia	0.640	3.610	0.000



3. Families of interest

Family	Control Min (%)	Control Max (%)	Your Sample (%)
Bacteroidaceae	8.791	22.421	9.009
Clostridiaceae	0.263	1.271	0.514
Enterobacteriaceae	0.280	1.329	0.545
Lachnospiraceae	25.695	44.222	55.164
Oscillospiraceae	2.522	9.627	2.761
Ruminococcaceae	7.964	19.113	18.198
Veillonellaceae	0.040	0.298	0.228

++Lachnospiraceae



- ***Bring Firmicutes/Bacteroidetes to 1***
- ***Increase Actinobacteria and Verrumicrobia***
- ***Reduce Lachnospiraceae*** *(yogurt, fermented foods support that)*

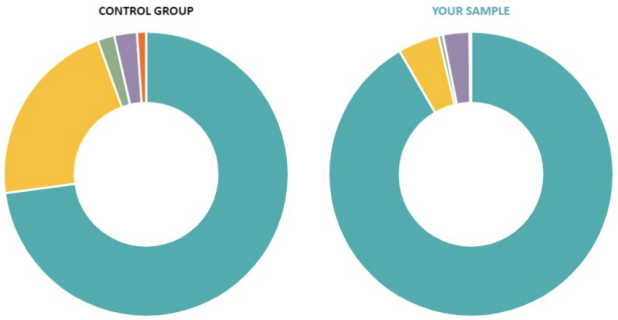
- ***Enhance the % of ANTI-inflammatory bacteria (Akkermansia, Bifidobacterium, Faecalibacterium, Oxalobacter)***

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 3: pediatric (8 years old) with lactose intolerance, gluten intolerance, IBS, elevated blood glucose

2. Variation of the main bacteria phyla

Phylum	Control Min (%)	Control Max (%)	Your Sample (%)
Firmicutes	65.569	79.822	91.570
Bacteroidetes	14.681	28.348	4.691
Proteobacteria	0.593	3.244	0.492
Actinobacteria	0.877	5.155	3.012
Verrucomicrobia	0.640	3.610	0.161



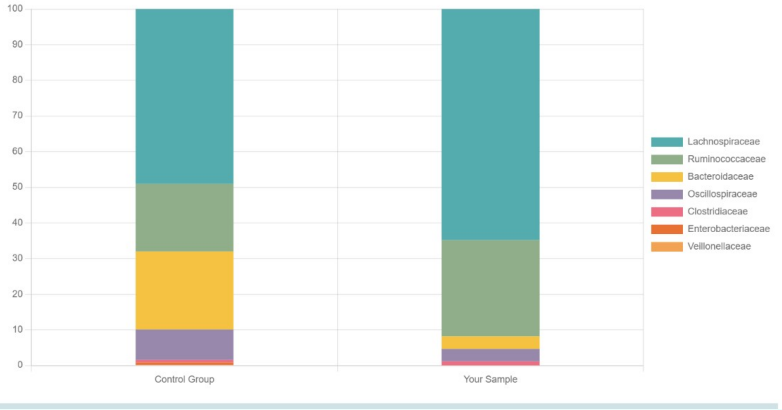
↑ ***Firmicutes/Bacteroidetes***

3. Families of interest

Family	Control Min (%)	Control Max (%)	Your Sample (%)
Bacteroidaceae	8.791	22.421	2.443
Clostridiaceae	0.263	1.271	0.754
Enterobacteriaceae	0.280	1.329	0.039
Lachnospiraceae	25.695	44.222	45.086
Oscillospiraceae	2.522	9.627	2.430
Ruminococcaceae	7.964	19.113	18.787
Veillonellaceae	0.040	0.298	0.000

← ***++Enterobacteriaceae***

← ***++Lachnospiraceae***



Pro-inflammatory bacteria

Genus	Control Min (%)	Control Max (%)	Your Sample (%)
Acinetobacter	0.000	0.000	0.000
Alistipes	1.334	3.246	0.242
Clostridium	0.742	1.592	0.807
Escherichia	0.306	1.057	0.039
Haemophilus	0.083	0.415	0.186
Helicobacter	0.000	0.000	0.000
Klebsiella	0.058	0.555	0.000
Prevotella	2.423	9.395	0.045
Salmonella	0.000	0.000	0.000
Streptococcus	0.774	4.078	0.962
Sutterella	0.274	0.765	0.000
Turicibacter	0.107	0.388	0.420

Anti-inflammatory bacteria

Genus	Control Min (%)	Control Max (%)	Your Sample (%)
Akkermansia	0.641	3.469	0.136
Bifidobacterium	1.894	4.520	2.691
Coprococcus	1.451	3.564	1.939
Enterococcus	0.237	1.490	0.378
Eubacterium	0.939	1.754	0.760
Faecalibacterium	7.055	12.470	5.671
Lactobacillus	0.015	0.099	0.000
Oxalobacter	0.019	0.059	0.000
Parabacteroides	1.113	2.095	0.838
Ruminococcus	3.422	6.044	5.391

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 3: pediatric (8 years old) with lactose intolerance, gluten intolerance, IBS, elevated blood glucose

MEAT: N/a	**MEAT**: Bacon Meat, cured Pork rinds Salami
SEAFOOD (fish and shellfish): Anchovies Halibut Herring, Atlantic Mackerel Perch Pike Salmon, Atlantic Salmon, Coho (Wild) Salmon, Sockeye Sardines Tench Trout, Rainbow Tuna, Bluefin Whitefish	**SEAFOOD (fish and shellfish):** Shark Swordfish King Mackerel Tilefish Albacore Tuna (white tuna) Farmed Fish
DAIRY and EGGS n/a	**DAIRY and EGGS** Cheese, cured
NUTS and SEEDS Brazil nuts	**NUTS and SEEDS** Peanuts Pine Nuts
SPICES and HERBS Turmeric	**SPICES and HERBS** Liquorice Root Black Pepper Nutmeg Ginseng

VEGETABLES and LEGUMES: Carrots Chicory root Chicory, Sugarloaf Chicory, wild Dandelion Greens Endive, Belgian, Red Endive, curly (Frisée) Endive, Escarole Leeks Mushrooms, Shiitake Mushrooms, White Button Potatoes, cooked and chilled Puntarelle (sprouts of Catalonian chicory) Radicchio (Italian chicory) Radish Tapioca Tomato	**VEGETABLES and LEGUMES**: Broccoli Cauliflower Brussels sprouts
FRUITS: Coconut Cranberry	**FRUITS:** Bananas Grapes Mangos Pineapples Dried fruits (raisins, apricots, etc.) Grapefruit
GRAINS & PSEUDOGRAINS: Amaranth Buckwheat Corn Rice flakes Rice, Brown	**GRAINS & PSEUDOGRAINS:** White bread White rice Pasta made from refined wheat flour Processed cereals

GOAL: reduce the dysbiosis

CONDIMENTS and OTHERs: Cod Liver Oil Herba Tea, Chlorella pyrenoidosa Herbal Tea, Angelica acutiloba Herbal Tea, Echinacea Konjac flour	**CONDIMENTS and OTHERs:** **High-Sodium Condiments**: • Soy sauce, teriyaki sauce, BBQ sauce, ketchup (in large amounts), processed salad dressings. **Sugar-Laden Condiments**: • Ketchup, sweet chili sauce, BBQ sauce, honey mustard, and some commercial salad dressings. **Artificial Sweeteners and Additives**: • Aspartame, sucralose, saccharin, and other artificial sweeteners • found in diet sodas, sugar-free products, and processed condiments. **Monosodium Glutamate (MSG):** • Found in many processed condiments, Asian sauces, and packaged foods. Mayonnaise (in large amounts) **Trans Fats and Hydrogenated Oils**: • Found in processed condiments, margarine, and some commercially baked goods. **Cream-Based Dressings and Sauces**: • Ranch, blue cheese dressing, Alfredo sauce.

IN GREEN: FOODS BENEFICIAL FOR THIS PATIENT

IN YELLOW: FOODS TO LIMIT FOR THIS PATIENT

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 3: pediatric (8 years old) with lactose intolerance, gluten intolerance, IBS, elevated blood glucose

*Example of **breakfast**: chia seed pudding with coconut milk and cranberries*

Ingredients

Chia seeds
coconut milk
cranberries
maple syrup (opt)
Cinnamon (opt)

Instructions

Combine chia seeds and milk in a bowl or jar, stir, and refrigerate overnight. Serve with cranberries.

Chia seeds are rich in omega-3 fatty acids, and its high fiber content aids digestion and is beneficial for IBS. Berries provide antioxidants and the low glycemic index helps manage blood sugar levels.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 3: pediatric (8 years old) with lactose intolerance, gluten intolerance, IBS, elevated blood glucose

*Example of **lunch**: Brown rice with mushrooms, endives and turmeric*

Ingredients

Brown rice
Mushrooms sliced
Endives sliced
Turmeric
Olive oil
Salt

Instructions

Cook the rice. In a skillet, add mushrooms to oil and cook until soft. Then add endives. Finally, add turmeric, salt.

Benefits:

Gluten-free, easy to digest, and balanced with protein and healthy fats.

**VEGGIE OPTION: replace with firm tofu (press it to remove excess water then slice it into cubes and lightly grill in olive oil*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 3: pediatric (8 years old) with lactose intolerance, gluten intolerance, IBS, elevated blood glucose

*Example of **dinner**: Grilled salmon with quinoa and steamed veggies*

Ingredients

Salmon fillet
Quinoa
Broccoli
Carrots
Salt and pepper
Lemon wedge

Instructions

Cook quinoa, season salmon with pepper and salt,
then grill in olive oil for 3-4 minutes
Steam broccoli and carrots until tender
Serve with lemon juice

Benefits:

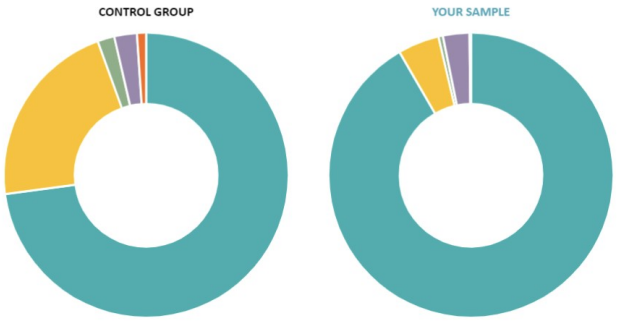
Rich in Omega-3s and lean protein. Gentle on digestion

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 3: expected **gut microbiota shifts** to dietary changes

2. Variation of the main bacteria phyla

Phylum	Control Min (%)	Control Max (%)	Your Sample (%)
Firmicutes	65.569	79.822	91.570
Bacteroidetes	14.681	28.348	4.691
Proteobacteria	0.593	3.244	0.492
Actinobacteria	0.877	5.155	3.012
Verrucomicrobia	0.640	3.610	0.161



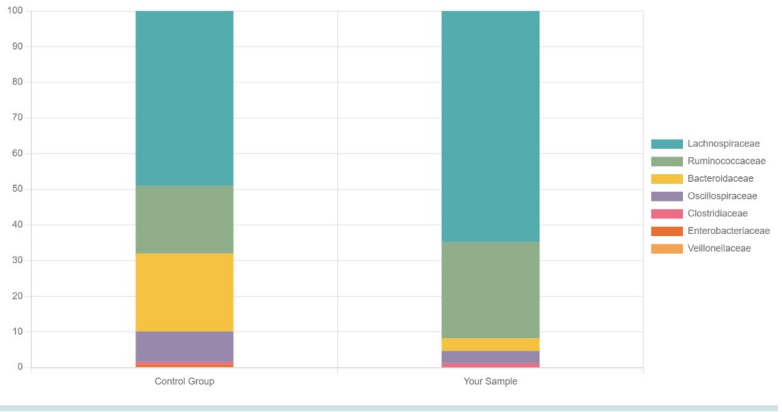
↑ ***Firmicutes/Bacteroidetes***

3. Families of interest

Family	Control Min (%)	Control Max (%)	Your Sample (%)
Bacteroidaceae	8.791	22.421	2.443
Clostridiaceae	0.263	1.271	0.754
Enterobacteriaceae	0.280	1.329	0.039
Lachnospiraceae	25.695	44.222	45.086
Oscillospiraceae	2.522	9.627	2.430
Ruminococcaceae	7.964	19.113	18.787
Veillonellaceae	0.040	0.298	0.000

← ***++Enterobacteriaceae***

← ***++Lachnospiraceae***



- ***Bring Firmicutes/Bacteroidetes close to 1***
- ***Increase Verrumicrobia***
- ***Reduce Enterobacteriaceae***

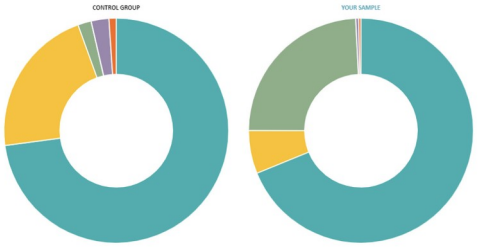
- ***Enhance the % of ANTI-inflammatory bacteria (Akkermansia, Bifidobacterium, Faecalibacterium, Oxalobacter)***

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 4: constipation, bloating, eczema, non-alcoholic fatty liver disease (NAFLD)

2. Variation of the main bacteria phyla

Phylum	Control Min (%)	Control Max (%)	Your Sample (%)
Firmicutes	65.569	79.822	68.758
Bacteroidetes	14.681	28.348	6.186
Proteobacteria	0.593	3.244	24.206
Actinobacteria	0.877	5.155	0.406
Verrucomicrobia	0.640	3.610	0.332



↓ *Bacteroidetes*

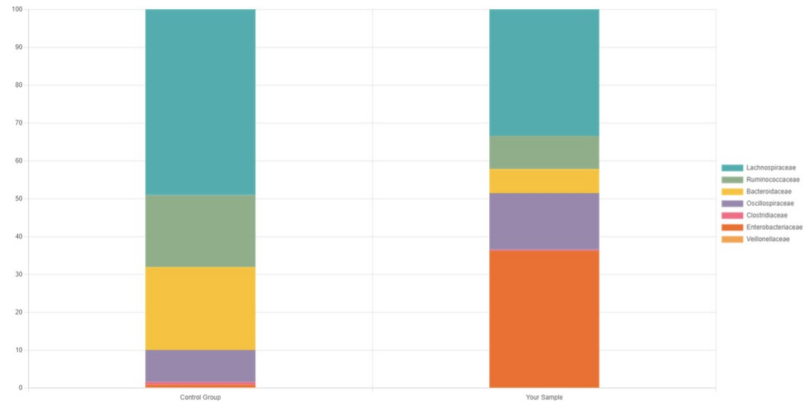
↑ *Proteobacteria*

↓ *Actinobacteria*

3. Families of interest

Family	Control Min (%)	Control Max (%)	Your Sample (%)
Bacteroidaceae	8.791	22.421	4.008
Clostridiaceae	0.263	1.271	0.212
Enterobacteriaceae	0.280	1.329	22.774
Lachnospiraceae	25.695	44.222	20.995
Oscillospiraceae	2.522	9.627	9.377
Ruminococcaceae	7.964	19.113	5.501
Veillonellaceae	0.040	0.298	0.000

++Enterobacteriaceae



Pro-inflammatory bacteria

Genus	Control Min (%)	Control Max (%)	Your Sample (%)
Acinetobacter	0.000	0.000	0.000
Alistipes	1.334	3.246	0.573
Clostridium	0.742	1.592	0.379
Escherichia	0.306	1.057	22.739
Haemophilus	0.083	0.415	0.000
Helicobacter	0.000	0.000	0.000
Klebsiella	0.058	0.555	0.034
Prevotella	2.423	9.395	0.183
Salmonella	0.000	0.000	0.000
Streptococcus	0.774	4.078	0.332
Sutterella	0.274	0.765	0.033
Turicibacter	0.107	0.388	0.074

Anti-inflammatory bacteria

Genus	Control Min (%)	Control Max (%)	Your Sample (%)
Akkermansia	0.641	3.469	0.029
Bifidobacterium	1.894	4.520	0.079
Coprococcus	1.451	3.564	1.144
Enterococcus	0.237	1.490	0.023
Eubacterium	0.939	1.754	0.409
Faecalibacterium	7.055	12.470	1.021
Lactobacillus	0.015	0.099	0.206
Oxalobacter	0.019	0.059	0.045
Parabacteroides	1.113	2.095	0.451
Ruminococcus	3.422	6.044	6.159

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 4: constipation, bloating, eczema, non-alcoholic fatty liver disease (NAFLD)

VEGETABLES and LEGUMES: Artichoke Chicory Green Chicory Red Chives Garlic Garlic, black Garlic, wild (Allium ursinum) Leeks Onion Ramps	**VEGETABLES and LEGUMES**: Beans, Large White Mushrooms, White Button Spinach
FRUITS: Blueberry Strawberry Apple Blackberry Cloudberry Cranberry Grape, Red Orange Pineapple	**FRUITS:** Canned fruits Dried Fruit
GRAINS & PSEUDOGRAINS: Buckwheat Sorghum, Whole Grain	**GRAINS & PSEUDOGRAINS:** Breakfast cereals Oat Bran Quinoa Rice Bran Sorghum, hulled
MEAT: Deer Ostrich Steak Rabbit Skirt Steak Top Blade Chuck Steak Turkey	**MEAT**: Bacon Chicken wing Beef skirt steak Deli Meat Ham Hot Dogs Liver, Beef Liver, Lamb

SEAFOOD (fish and shellfish): Clams Mackerel, Atlantic	**SEAFOOD (fish and shellfish):** Crab, Blue Cuttlefish Oysters Shrimp
DAIRY and EGGS Cheese, Brie Cheese, Feta Cheese, Fontina Cheese, Swiss Egg Yolk Milk, goat's Mozzarella	**DAIRY and EGGS** Ice cream
NUTS and SEEDS Chestnut Fennel seeds Hazelnut	**NUTS and SEEDS** Cumin Seed Pumpkin Seeds
SPICES and HERBS Chili pepper Cinnamon Cloves Coriander Ginger Oregano, Mediterranean Rosemary Sage	**SPICES and HERBS** -
CONDIMENTS and OTHERs: Tea, Green Dark Chocolate (>70-85% Cocoa) Herbal tea, Milk thistle	**CONDIMENTS and OTHERs:** Barbecue sauces Chewing gum Cocoa mix Diet soda Flavored coffee syrups Frozen desserts Gelatin Grape Juice Maple syrup alternatives Salad dressings Sodas Sorbet Sucralose Sugar-free candy Tea, Black Water, Tap

GOAL: reduce the dysbiosis

IN GREEN: FOODS BENEFICIAL FOR THIS PATIENT

IN YELLOW: FOODS TO LIMIT FOR THIS PATIENT

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 4: constipation, bloating, eczema, non-alcoholic fatty liver disease (NAFLD)

*Example of **breakfast**: buckwheat pancakes with blueberries and cinnamon*

Ingredients

Buckwheat flour
Baking powder
Cinnamon
Hazelnut milk
Coconut oil
Cinnamon
Blueberries

Instructions

Combine buckwheat flour, baking powder, cinnamon, and salt. In another bowl, combine milk, maple syrup (if using) and melted coconut oil. Combine both
Cook in a non-stick skillet with a light grease of oil. Pour ¼ of batter and cook until bubbles on the surface, then flip and cook 2'. Serve with blueberries on top.

A diet rich in fiber, antioxidants, and healthy fats can alleviate constipation and bloating, support skin health in eczema, and promote liver function in NAFLD.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 4: constipation, bloating, eczema, non-alcoholic fatty liver disease (NAFLD)

*Example of **lunch:** omelette with chicory and cheese*

Ingredients

Eggs white
Chicory (green)
Cheese (fontina, mozzarella, or feta)
Olive oil

Instructions

Cook an omelette with egg whites and cheese.
Meanwhile, boil chicory and season with oil.
Serve hot.

Rich in vitamins A, C, and K, as well as folate and antioxidants
Healthy fats and proteins of good quality (eggs)
Fibers

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 4: constipation, bloating, eczema, non-alcoholic fatty liver disease (NAFLD)

*Example of **dinner**: baked mackerel with buckwheat, roasted carrots, and turnips*

Ingredients

Mackerel fillet
Buckwheat
Roasted carrots
Turnips

Instructions

Bake the salmon and
roast the veggies.
Serve with cooked buckwheat.

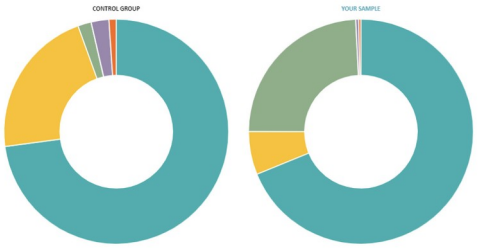
Omega-3s from fish are beneficial for liver health.
Buckwheat is a good source of fiber and can help with digestion.
Roasted carrots and turnips add more fiber and nutrients.

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Patient 4: expected gut microbiota shifts to dietary changes

2. Variation of the main bacteria phyla

Phylum	Control Min (%)	Control Max (%)	Your Sample (%)
Firmicutes	65.569	79.822	68.758
Bacteroidetes	14.681	28.348	6.186
Proteobacteria	0.593	3.244	24.206
Actinobacteria	0.877	5.155	0.406
Verrucomicrobia	0.640	3.610	0.332



↑ *Bacteroidetes*

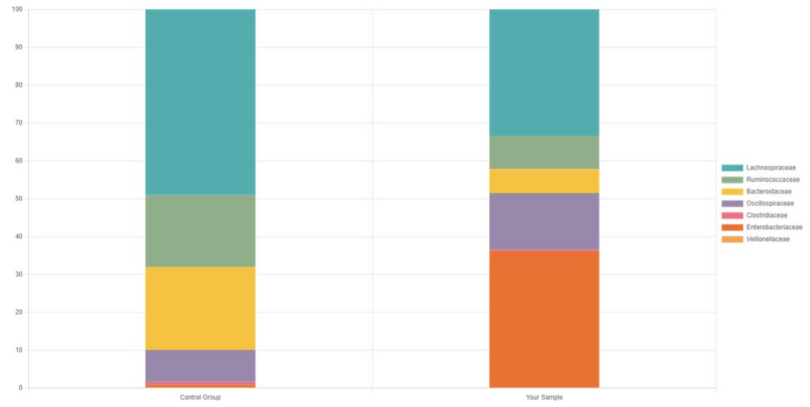
↓ *Proteobacteria*

↑ *Actinobacteria*

3. Families of interest

Family	Control Min (%)	Control Max (%)	Your Sample (%)
Bacteroidaceae	8.791	22.421	4.008
Clostridiaceae	0.263	1.271	0.212
Enterobacteriaceae	0.280	1.329	22.774
Lachnospiraceae	25.695	44.222	20.995
Oscillospiraceae	2.522	9.627	9.377
Ruminococcaceae	7.964	19.113	5.501
Veillonellaceae	0.040	0.298	0.000

++Enterobacteriaceae



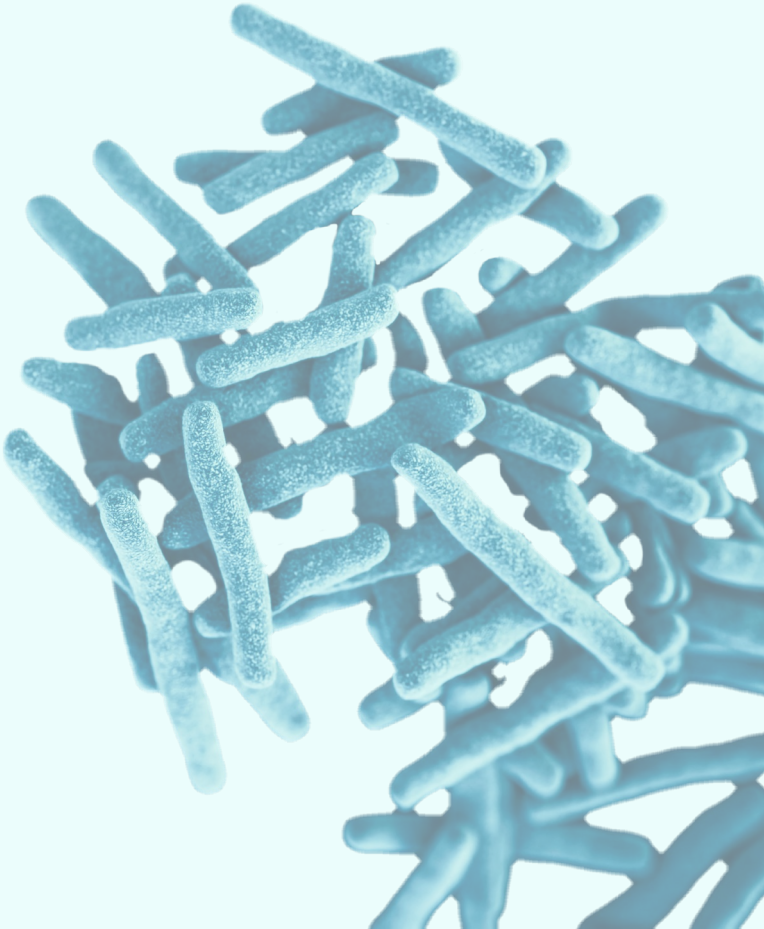
- *Bring Firmicutes/Bacteroidetes close to 1*
- *Increase Actinobacteria*
- *Reduce **Enterobacteriaceae** and **Proteobacteria***

- *Enhance the % of ANTI-inflammatory bacteria (Akkermansia, Bifidobacterium, Faecalibacterium, Oxalobacter)*

Take-home messages

- **Gut microbiota composition influences health**
- **Knowing its composition may drive towards more tailored and more efficient diet modifications**
- **Diet modifications can restore balance!**
- **Specific foods, based on microbiota's profile, promote healing**
- **Ongoing monitoring and adjustment are necessary**

Personalized nutrition *is key!*

INSTITUTE OF MICROBIOME AND APPLIED SCIENCES
contact@imas.edu.eu
+356 21383758

Thanks for your attention