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CHAPTER 1

NUTRITION DURING PREGNANCY

Neslihan ÖZDEMİR[1]

1. INTRODUCTION

Healthy nutrition involves consuming nutrients in amounts that meet the body's needs, taking into account factors such as age, sex, and physiological conditions (Başer & Özdemir, 2023). Pregnancy is a critical period marked by increased nutritional requirements. Inadequate or unbalanced nutrition during this time can negatively affect both maternal and fetal health (Kangalgil et al., 2018). The nutrients consumed during pregnancy can have significant and lasting effects on the health of both the mother and the newborn. It is believed that the physiological changes occurring during pregnancy, as well as insufficient or excessive intake of nutrients, can lead to epigenetic modifications in the fetus (Güler et al., 2019).

Fetal brain development is shaped by a combination of environmental factors in the womb and genetic interactions. Maternal nutrition influences the genetic structure of the fetus, guiding placental development and nutrient transfer. Brain development begins early in pregnancy and continues through crucial processes such as neurulation, cell migration, differentiation, and synapse formation. During this developmental phase, the fetal brain is highly sensitive to environmental influences and potential damage. As Özdemir et al. (2017) have pointed out, health is closely related to the foods individuals consume and the environmental factors to which they are exposed. Nutritional deficiencies or excesses during pregnancy can adversely affect fetal brain development. Nutrients—especially micro and macronutrients—directly influence this development and may lead to changes in the neurodevelopment of the fetus (Cortés-Albornoz et al., 2021).

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Therefore, the requirements for energy, macro-, and micronutrients should be carefully met throughout pregnancy (Naik et al., 2022). Additionally, attention should be paid to the traditional use of certain herbal products due to their potential impact on both maternal and fetal health (Ulcay & Şenel, 2020; Ulcay & Şenel, 2024; Ulcay, 2024).

2. Energy and Micro-Macro Nutrients During Pregnancy

2.1. Energy Needs

Since fetal growth is added to the normal metabolic regulation of the woman during pregnancy, it becomes one of the most critical periods for nutrition in human life. In a healthy pregnancy, weight gain in the first trimester can be approximately 1–2 kg. During the early stages of pregnancy, mild weight loss may occur as a result of nausea and vomiting. After the first trimester, weight increases regularly due to the accumulation of both fat and fat-free (lean) tissue (Öztürk, 2019).

Energy requirements during pregnancy vary depending on the trimester. While the increase in energy needs is minimal in the first trimester, it becomes more pronounced in the second and third trimesters (Sun et al., 2024). The energy requirement during pregnancy is higher compared to non-pregnant women. In the third trimester, basal metabolic rate increases by approximately 10–20% compared to non-pregnant individuals. In twin pregnancies, this increase is about 10% higher. These metabolic changes reflect physiological adaptations that occur to support fetal growth and development (Soykan & Güler, 2024).

2.2. Macronutrients

2.2.1.Carbohydrates

Carbohydrate consumption during pregnancy plays a critical role in both maternal health and fetal development. Carbohydrates are the primary source of energy during pregnancy and are an essential nutrient for fetal growth. The selection of carbohydrate sources is also of great importance. Low-glycemic index carbohydrates can help maintain more stable blood glucose levels and reduce the risk of gestational diabetes. Foods with a low glycemic index—such as whole grains, vegetables, and legumes—should be preferred. Additionally, adequate dietary fiber intake during pregnancy is necessary to maintain digestive health and prevent constipation (Ministry of Health, 2006).

According to a systematic meta-analysis conducted by O'Connor and colleagues, interventions aimed at improving dietary habits during pregnancy have been effective in enhancing the diet quality of expectant mothers. The study

found that pregnant women often tend to consume energy-dense but nutrient-poor foods. This tendency can lead to a decrease in the intake of fruits, vegetables, and whole grains, particularly during pregnancy and the postpartum period. The study emphasizes that interventions aimed at improving the quality of carbohydrate consumption are more effective when implemented by nutrition professionals (O'Connor et al., 2025).

2.2.2. Proteins

Proteins are critically important for fetal growth and tissue development (Na et al., 2024). Adequate protein intake during pregnancy is also essential for optimal birth weight in newborns (Güngör Tavşanlı & Büyükçanga, 2020). Protein consumption becomes especially significant in the third trimester to support maternal tissue formation, fetal growth, and increased protein synthesis. Protein sources include plant-based options such as legumes, grains, and nuts, as well as animal-based sources like meat and dairy products (Bala & Fakılı, 2024).

To meet the increased protein requirements during pregnancy, it is important to consume sufficient amounts of foods such as whole milk, cheese, yogurt, eggs, chicken, fish, red meat, and plant-based sources like broad beans, dry beans, chickpeas, and peas (Akdeniz Kudubeş & Zengin, 2023). According to a study by Switkowski and colleagues, protein intake during pregnancy has been shown to influence birth length and growth during childhood (Switkowski et al., 2016).

Protein intake during pregnancy is a key factor not only for fetal development but also for influencing the risk of obesity later in life. A study by Maslova and colleagues found that animal-based protein consumption during pregnancy was associated with a higher body mass index (BMI) in female offspring. The study indicated that replacing carbohydrates with animal protein during pregnancy increased the risk of overweight in girls (Maslova et al., 2014).

2.2.3. Fats

Fats play an important role in brain development and hormone production; in particular, omega-3 fatty acids have beneficial effects on neurodevelopment (Marriott, 2023). Omega-3 fatty acids are considered essential because they cannot be synthesized by the body and are required for feto-placental growth. To meet the needs of the fetus, maternal fat stores are utilized. Adequate intake of long-chain polyunsaturated fatty acids during pregnancy is associated with positive outcomes for both maternal and fetal health.

In the third trimester, the fetal demand for maternal fatty acids increases significantly due to rapid growth and fat tissue accumulation (Şener Özovalı et

al., 2022). During this period, the quantity and quality of fat intake are particularly important. Excessive fat consumption during pregnancy may elevate the levels of saturated fatty acids in cord blood, potentially leading to negative effects on fetal metabolism (Büyükuslu et al., 2018).

2.3. Micronutrients

The need for micronutrients during pregnancy can increase by 20% to 100% (Na et al., 2024). Micronutrients play a vital role in the healthy development of the fetus. Especially iron, folic acid, calcium and vitamin D deficiencies can lead to serious health problems (Kangaligil et al., 2018).

2.3.1 Vitamin D

During pregnancy, vitamin D plays a critical role in both maternal health and fetal growth and development. Throughout gestation, significant changes occur in maternal vitamin D and calcium metabolism to meet the calcium requirements for fetal bone mineralization. The fetus is entirely dependent on the mother for calcium and phosphorus, which are essential for bone development, tissue growth, and proper physiological function (Yeşiltepe Mutlu & Hatun, 2011).

Vitamin D deficiency can negatively impact bone development and reduce vitamin D levels in the infant's cord blood. Studies have shown that infants born to mothers who received vitamin D supplementation during pregnancy had higher cord blood vitamin D levels compared to those whose mothers did not receive supplementation. This finding highlights the crucial role of adequate maternal vitamin D intake in supporting healthy fetal growth and postnatal bone development.

Additionally, maternal vitamin D deficiency has been associated with adverse health outcomes in newborns, such as rickets, low birth weight, and abnormalities in skeletal development (Ayan et al., 2023).

2.3.2. Folic Acid

Folic acid intake during pregnancy plays an important role in terms of maternal health and pregnancy outcomes (Lassi et al., 2013). Folic acid has a critical role in preventing neural tube defects (Naik et al., 2022). Studies show that folic acid supplementation reduces the risk of neural tube defects by 72%. In addition, folic acid deficiency has been reported to be associated with pregnancy complications such as congenital heart disease, preeclampsia, intrauterine growth retardation, recurrent pregnancy loss, placental abruption and premature birth (Argyridis, 2019).

2.3.3. Vitamin B12

Vitamin B12 deficiency during pregnancy has been associated with adverse outcomes such as low birth weight, intrauterine growth restriction, preeclampsia, and gestational diabetes. Furthermore, B12 deficiency is linked to developmental disorders and impaired fetal growth. Maternal B12 status during pregnancy has been shown to have long-term effects on fetal development (Behere et al., 2021).

Studies have reported a decline in B12 levels as pregnancy progresses, with deficiency rates reaching up to 29% in the third trimester (Sukumar et al., 2016). In a randomized controlled trial conducted by Duggan and colleagues, pregnant women who received a daily supplement of 50 μg of vitamin B12 throughout pregnancy had higher B12 levels in both themselves and their infants. These women also showed lower homocysteine levels and improved metabolic and neurological development outcomes (Duggan et al., 2014).

Therefore, it is essential to regularly monitor B12 levels in pregnant women, particularly in those with low intake of animal-based foods, and provide supplementation when necessary (Duggan et al., 2014; Molly et al., 2008).

2.3.4. Vitamin A

Vitamin A plays a critical role during pregnancy in fetal development, immune function, and maternal health. Vitamin A deficiency has been associated with low vitamin A levels in newborns, which can lead to weakened immune function and an increased risk of infections (Azaïs-Braesco & Pascal, 2000). However, excessive intake of vitamin A can have teratogenic effects and may result in congenital abnormalities. A study by Dibley and Jeacocke reported that daily intake below 10,000 IU of vitamin A was not associated with birth defects, while intakes between 10,000 IU and 30,000 IU showed inconsistent outcomes. Therefore, intake exceeding 10,000 IU per day is not recommended during pregnancy (Dibley & Jeacocke, 2001).

A systematic review by Van den Broek and colleagues found that vitamin A supplementation reduced the risk of maternal anemia and night blindness, although it did not have a significant effect on reducing maternal or perinatal mortality. Vitamin A is essential for cellular differentiation, maintaining ocular integrity, and fetal skeletal development throughout pregnancy, as highlighted in a review by Maia and colleagues (Maia et al., 2019). Consequently, the need for vitamin A increases during pregnancy due to its crucial physiological roles.

3. Weight Gain During Pregnancy and Newborn Health

Maternal weight gain during pregnancy can have a direct impact on neonatal health. Inadequate weight gain may increase the risk of low birth weight and preterm birth (Akdeniz Kudubeş & Zengin, 2023). On the other hand, excessive weight gain can lead to macrosomia and increase the risk of birth complications (Na et al., 2024). Studies have shown that balanced nutrition during pregnancy improves neonatal birth weight and overall health outcomes (Kheirouri & Alizadeh, 2021).

Weight gain, as the first observed physiological change during pregnancy, is a critical factor for fetal growth and well-being. For individuals with a normal pre-pregnancy Body Mass Index (BMI) between 19–24 kg/m², the recommended weight gain is between 11 and 16 kilograms. This gain is associated with increased blood volume and extracellular fluid, as well as the development of the fetus, placenta, amniotic fluid, uterus, fat stores, and mammary glands (Bala & Fakılı, 2024).

In individuals with a high pre-pregnancy BMI (>30 kg/m²), excessive food intake may negatively affect fetal anthropometric development. According to a systematic review and meta-analysis by Stothard and colleagues, being overweight or obese before pregnancy is linked to an increased risk of various complications during pregnancy. Specifically, maternal obesity has been associated with gestational diabetes, hypertensive disorders, higher cesarean delivery rates, and wound infections (Stothard et al., 2009).

In a study by Kheirouri and Alizadeh, among 807 pregnant women examined, 46.2% gained weight within the recommended range, 29.4% gained insufficient weight, and 24.4% experienced excessive weight gain. The study indicated that factors such as pre-pregnancy BMI, folic acid intake, and mode of delivery were significantly associated with gestational weight gain. Moreover, weight gained during pregnancy was positively correlated with neonatal birth weight and length (Kheirouri & Alizadeh, 2021).

Nutritional habits during pregnancy are also associated with the development of colic in newborns. According to research, maternal dietary intake during pregnancy may influence the risk of colic in infants. Inadequate maternal nutrition has also been linked to neonatal colic (Mirzanoori et al., 2021).

Maternal body composition during pregnancy is crucial for both maternal health and fetal development. Bioelectrical Impedance Analysis (BIA) is considered a practical method for detecting potential complications and monitoring changes in body composition during pregnancy (Öçal, 2011). BIA measures differences in electrical conductivity between fat and lean tissue to assess body composition. Through this analysis, key parameters such as body fat percentage, lean body mass, total body water, basal metabolic rate, and body mass index can be obtained (Dalgalı & Ulcay, 2024).

4. Nutritional Behaviors and Habits During Pregnancy

Adopting healthy eating behaviors throughout pregnancy is critically important for both maternal and infant health. Nutritional education and individualized counseling services can positively influence pregnant women's dietary habits (Sun et al., 2024). In particular, dietary diversity helps expectant mothers meet their nutrient requirements and can reduce the risk of low birth weight (Kheirouri & Alizadeh, 2021).

Eating disorders such as pregorexia, which can occur during pregnancy, adversely affect the health of both the mother and the baby and require clinical monitoring (Puşuroğlu & Hocaoğlu, 2023). During pregnancy, when nutritional needs increase, inadequate and unbalanced nutrition negatively impacts maternal and fetal health, and severe maternal malnutrition is associated with poor pregnancy outcomes.

Therefore, assessing dietary habits and promoting informed food choices are essential for a healthy pregnancy (Pekşen et al., 2016).

4.1. Caffeine Consumption During Pregnancy

Consumption of caffeine-containing beverages during pregnancy has been strongly associated with increased risks of fetal mortality, intrauterine growth restriction, and low birth weight. Research indicates that caffeine metabolism in pregnant women is slower compared to non-pregnant individuals, requiring 1.5 to 3.5 times longer half-life for elimination from the body. The presence of caffeine in amniotic fluid, umbilical cord blood, urine, and fetal plasma demonstrates that this compound easily crosses the placental barrier and can directly affect the fetus (Demirci & Yılmaz, 2023).

Fetal caffeine exposure increases circulating catecholamine levels, causing vasoconstriction in placental blood vessels and reducing oxygen delivery to the fetus. This condition may adversely affect fetal growth and development. Furthermore, high caffeine intake has been linked to increased risks of

miscarriage, fetal death, intrauterine growth restriction, low birth weight, and preterm birth (Deniz et al., 2015).

A meta-analysis by Soltani and colleagues found that each 100 mg increase in daily caffeine intake during pregnancy raises the risk of low birth weight by 12%. For these reasons, it is recommended to limit caffeine consumption during pregnancy and to keep daily intake below 200 mg (Soltani et al., 2023).

4.2. Smoking During Pregnancy

Smoking during pregnancy has serious adverse effects on both maternal and fetal health. According to a comprehensive review by Avşar and colleagues, smoking during pregnancy is associated with numerous health issues, including low birth weight, preterm birth, sudden infant death syndrome (SIDS), and obesity. The study found that the negative effects of smoking are directly related to the quantity of cigarettes consumed and that the harmful impact on the fetus increases with longer duration of smoking. Additionally, maternal smoking has been linked to congenital abnormalities, respiratory diseases such as asthma, and neurodevelopmental disorders (Avşar et al., 2021).

A review by Nakamura and colleagues concluded that exposure to tobacco smoke during pregnancy can lead to birth complications and developmental disorders during childhood. Furthermore, these epigenetic changes may persist throughout childhood and potentially be transmitted across generations (Nakamura et al., 2021).

In a large-scale 10-year study by Tarasi and colleagues, smoking during pregnancy was found to significantly increase the risks of low birth weight (<2500 g), preterm birth, intrauterine growth restriction, and neonatal intensive care unit admission. Moreover, pregnant women who smoked more than 20 cigarettes per day exhibited a markedly higher risk of intrauterine death and neonatal infections (Tarasi et al., 2022).

Although e-cigarettes are often perceived as safer than traditional cigarettes, increased risks of fetal growth restriction and low birth weight have also been observed in pregnant e-cigarette users. These findings highlight that both cigarette and e-cigarette use during pregnancy pose serious health risks to the fetus, emphasizing that complete cessation is the healthiest choice (Regan & Pereira, 2021).

4.3. Alcohol Consumption During Pregnancy

Alcohol consumption during pregnancy can lead to serious neurodevelopmental and physical health problems in the fetus. One study

reported that alcohol use is often not completely discontinued during pregnancy, which is associated with addictive effects (Çelik and Akdeniz, 2020). Another study emphasized that alcohol consumption is linked to spontaneous miscarriage, preterm birth, and intrauterine growth restriction during pregnancy. Additionally, prenatal alcohol exposure has been shown to negatively affect fetal nervous system development, potentially resulting in cognitive impairments and learning difficulties later in life (Deniz et al., 2016).

5. Conclusion

In conclusion, nutritional habits during pregnancy are among the important factors affecting infant health both in the short term and the postnatal period. Adequate and balanced intake of nutrients supports a healthy pregnancy process, while avoiding harmful habits leads to positive health outcomes for both mother and baby. During this period, pregnant women can be provided with education on topics such as nutrition during pregnancy, the negative effects of smoking and alcohol consumption, and adequate intake of micro and macronutrients. Additionally, awareness can be supported through regular follow-up and individualized nutritional counseling by healthcare professionals. Public health programs can offer seminars and informative materials that promote healthy lifestyle habits throughout pregnancy. Such preventive and awareness-raising efforts will positively impact the health of both mother and baby, contributing to the upbringing of healthy generations.

6. REFERENCES

Akdeniz-Kudubeş, A., & Zengin, H. (2023). Yaşamın ilk 1000 gününde beslenmenin önemi. Bilecik Şeyh Edebali Üniversitesi Sağlık Bilimleri Fakültesi Dergisi, 1(1), 19-26.

Argyridis, S. (2019). Folic acid in pregnancy. Obstetrics, Gynaecology and Reproductive Medicine, 29(4), 118-120. https://doi.org/10.1016/j.ogrm.2019.01.008

Avşar, T. S., McLeod, H., & Jackson, L. (2021). Health outcomes of smoking during pregnancy and the postpartum period: An umbrella review. BMC Pregnancy and Childbirth, 21, 254. https://doi.org/10.1186/s12884-021-03729-1

Ayan, M. T., Özdemir, M., & Haskul, İ. (2023). Gebelikte D vitamini kullanımının bebeklerin kordon kanında vitamin D düzeyine etkisi. Unika Sağlık Bilimleri Dergisi, 3(3), 566-577. https://doi.org/10.47327/unikasaglik.2023.54

Azaïs-Braesco, V., & Pascal, G. (2000). Vitamin A in pregnancy: Requirements and safety limits. The American Journal of Clinical Nutrition, 71(suppl), 1325S–1333S.

Bala, E., & Fakılı, F. E. (2024). Gebelik döneminde makro ve mikro besin öğelerinin tüketiminin önemi. Journal of Health Sciences and Clinical Research, 3(3), 158-173. https://doi.org/10.5281/zenodo.14576608.

Başer, S., & Özdemir, H. Ö. (2023). Öğrencilerin paketlenmiş hazır gıda tüketimi-sosyal medya kullanımı ve sağlıklı beslenme tutumları arasındaki ilişkiler, İmperial Yayın, Afyonkarahisar, ISBN: 978-605-72560-2-7.

Behere, R. V., Deshmukh, A. S., Otiv, S., Gupte, M. D., & Yajnik, C. S. (2021). Maternal vitamin B12 status during pregnancy and its association with outcomes of pregnancy and health of the offspring: A systematic review and implications for policy in India. Frontiers in Endocrinology, 12, 619176. https://doi.org/10.3389/fendo.2021.619176.

Büyükuslu, N., Bilgi, Z. Z., Yoldaş İlktaç, H., & Garipağaoğlu, M. (2018). Gebelikte beslenmenin kordon kanı yağ asidi düzeylerine etkisi. Anadolu Klinikleri Tıp Bilimleri Dergisi, 24(1), 15-20.

Cortés-Albornoz, M. C., García-Guáqueta, D. P., Velez-van-Meerbeke, A., & Talero-Gutiérrez, C. (2021). Maternal nutrition and neurodevelopment: a scoping review. Nutrients, 13(10), 3530.

Çelik, A. A., & Akdeniz, G. (2020). Kadının anneliğe geçişi ile tüketim alışkanlıklarındaki değişime kuramsal bir bakış. Tüketici ve Tüketim Araştırmaları Dergisi, 12(2), 367-402.

Dalgalı, Ü., & Ulcay, T. (2024). Biyoelektriksel empedans analizinin sağlık alanındaki kullanımı. Şahna E. & Z. Selamoğlu (Ed.), Anatomi Alanında Uluslararası Araştırma ve Değerlendirmeler (ss. 11-24). Ankara: Serüven Yayınevi.

Demirci, Z., & Yılmaz, H. Ö. (2023).Gebelikte Kafein Tüketiminin Anne Ve Fetüs Üzerine Etkileri. Sağlık ve Toplum, 33(1), 52-58.

Deniz, A., Taş, F., Tomur, A., & Koç, A. (2015). The toxic effects of caffeine in pregnancy. Ibni Sina Journal of Medical Sciences, 1(3), 59-63.

Deniz, R., Baykuş, Y., & Kavak, E. Ç. (2016). Tekrarlayan erken gebelik kayıplarına yaklaşım. Kafkas Journal of Medical Sciences, 6(2), 130-137. https://doi.org/10.5505/kjms.2016.15010

Dibley, M. J., & Jeacocke, D. A. (2001). Safety and toxicity of vitamin A supplements in pregnancy. Food and Nutrition Bulletin, 22(3), 248-256.

Duggan, C., Srinivasan, K., Thomas, T., Samuel, T., Rajendran, R., Muthayya, S., ... & Kurpad, A. V. (2014). Vitamin B-12 supplementation during pregnancy and early lactation increases maternal, breast milk, and infant measures of vitamin B-12 status. The Journal of Nutrition, 144(5), 758-764. https://doi.org/10.3945/jn.113.187278

Güler, B., Bilgiç, D., Okumuş, H., & Yağcan, H. (2019). Gebelikte beslenme desteğine ilişkin güncel rehberlerin incelenmesi. *Dokuz Eylül Üniversitesi Hemşirelik Fakültesi Elektronik Dergisi, 12*(2), 143-151.

Güngör Tavşanlı, N., & Büyükçanga, T. (2020). Gebelikte alınan proteinin yenidoğan doğum ağırlığı üzerine etkisi. CBU-SBED, 7(2), 162-166.

Kangalgil, M., Acar, A. N., & Yardımcı, H. (2018). Gebelikte kazanılan vücut ağırlığı ile yenidoğanın bazı özellikleri arasındaki ilişkiyi inceleyen bir araştırma. Journal of Maternal-Fetal & Neonatal Medicine, 27(1), 20-26.

Kangalgil, M., Kahve, A. N., & Yardımcı, H. (2018). Gebelikte kazanılan vücut ağırlığı ile yenidoğanın bazı özellikleri arasındaki ilişkiyi inceleyen bir araştırma. *STED / Sürekli Tıp Eğitimi Dergisi, 27*(1), 20-26.

Kheirouri, S., & Alizadeh, M. (2021). Maternal dietary diversity during pregnancy and risk of low birth weight in newborns: A systematic review. Public Health Nutrition, 24(14), 4671-4681.

Lassi, Z. S., Salam, R. A., Haider, B. A., & Bhutta, Z. A. (2013). Folic acid supplementation during pregnancy for maternal health and pregnancy outcomes. Cochrane Database of Systematic Reviews, 2013(3), CD006896. https://doi.org/10.1002/14651858.CD006896.pub2

Maia, S. B., Souza, A. S. R., Caminha, M. F. C., Silva, S. L., Cruz, R. S. B. L. C., Santos, C. C., & Filho, M. B. (2019). Vitamin A and pregnancy: A narrative review. Nutrients, 11(3), 681. https://doi.org/10.3390/nu11030681

Marriott, B. P. (2023). Dietary supplements during pregnancy: A conundrum. The American Journal of Clinical Nutrition, 117(6), 643-644.

Maslova, E., Rytter, D., Bech, B. H., Henriksen, T. B., Rasmussen, M. A., Olsen, S. F., & Halldorsson, T. I. (2014). Maternal protein intake during pregnancy and offspring overweight 20 years later. American Journal of Clinical Nutrition, 100(4), 1139–1148. https://doi.org/10.3945/ajcn.113.082222

Mirzanoori, M., Rezaei, M., Amini, S., & Molavi, A. (2021). Did the diet during pregnancy of mothers of infants with colic differ from that of control infants? Nutrition Today, 56(6), 311-316.

Ministry of Health. (2006). Food and nutrition guidelines for healthy pregnant and breastfeeding women: A background paper. Wellington: Ministry of Health.

Molloy, A. M., Kirke, P. N., Brody, L. C., Scott, J. M., & Mills, J. L. (2008). Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. Food and Nutrition Bulletin, 29(2), S101-S111.

Na, X., Mackean, P. P., Cape, G. A., Johnson, J. W., & Ou, X. (2024). Maternal nutrition during pregnancy and offspring brain development: Insights from neuroimaging. Nutrients, 16(3337), 1-21.

Naik, V. D., Lee, J., Wu, G., Washburn, S., & Ramadoss, J. (2022). Effects of nutrition and gestational alcohol consumption on fetal growth and development. Nutrition Reviews, 80(6), 1568-1579. https://doi.org/10.1093/nutrit/nuab119

Nakamura, A., François, O., & Lepeule, J. (2021). Epigenetic alterations of maternal tobacco smoking during pregnancy: A narrative review. International Journal of Environmental Research and Public Health, 18(10), 5083. https://doi.org/10.3390/ijerph18105083

O'Connor, H., Meloncelli, N., Wilkinson, S. A., Scott, A. M., Vincze, L., Rushton, A., ... & de Jersey, S. (2025). Effective dietary interventions during pregnancy: A systematic review and meta-analysis of behavior change techniques to promote healthy eating. BMC Pregnancy and Childbirth, 25(112). https://doi.org/10.1186/s12884-025-07185-z

Öcal, Ç. (2011). Tüm gebelik süresince vücut kompozisyon karakteristiklerinin biyoelektriksel impedans yöntemi ile araştırılması (Uzmanlık tezi).Harran Üniversitesi, Tıp Fakültesi, Şanlıurfa.

Özdemir, H. Ö., Acar, N., Çizmeci, B., & Kahvecioğlu, R. (2017). Tüketicilerin organik ürünlere yönelik değerlendirmeleri: Kırşehir ilinde bir uygulama. *International Journal of Social Science, 55*, 493-505.

Öztürk, H. N. O. (2019). Gebe ve emzikli kadınların diyet kalite indekslerinin karşılaştırılması (Yüksek Lisans Tezi). Gazi Üniversitesi, Sağlık Bilimleri Enstitüsü, Ankara.

Pekşen Akça, R., Akgül, H., & Tekgöz, M. (2016). Gebe kadınların beslenme alışkanlıklarının belirlenmesi. SOBİDER: Sosyal Bilimler Dergisi, 3(9), 332-339.

Puşuroğlu, M., & Hocaoğlu, Ç. (2023). Pregorexia: Eating disorder in pregnancy. Psikiyatride Güncel Yaklaşımlar, 15(2), 251-256 https://doi.org/10.18863/pgy.1068128

Regan, A. K., & Pereira, G. (2021). Patterns of combustible and electronic cigarette use during pregnancy and associated pregnancy outcomes. Scientific Reports, 11, 13508. https://doi.org/10.1038/s41598-021-92930-5

Soykan, Y., & Güler, İ. (2024). Gebelikte beslenme ve destek tedavileri. Gazi Üniversitesi Tıp Fakültesi Yayınları.

Soltani, S., Salari-Moghaddam, A., Saneei, P., Askari, M., Larijani, B., Azadbakht, L., & Esmaillzadeh, A. (2023). Maternal caffeine consumption during pregnancy and risk of low birth weight: A dose–response meta-analysis of cohort studies. Critical Reviews in Food Science and Nutrition, 63(2), 224-233. https://doi.org/10.1080/10408398.2021.1945532

Stothard, K. J., Tennant, P. W. G., Bell, R., & Rankin, J. (2009). Maternal overweight and obesity and the risk of congenital anomalies: A systematic review and meta-analysis. JAMA, 301(6), 636-650. https://doi.org/10.1001/jama.2009.11

Sukumar, N., Rafnsson, S. B., Kandala, N. B., Bhopal, R., Yajnik, C. S., & Saravanan, P. (2016). Prevalence of vitamin B-12 insufficiency during pregnancy and its effect on offspring birth weight: A systematic review and meta-analysis. The American Journal of Clinical Nutrition, 103(4), 1232-1251. https://doi.org/10.3945/ajcn.115.123083

Sun, H., Chen, M., Liao, J., He, L., Wan, B., Yin, J., & Zhang, X. (2024). The maternal lifestyle in pregnancy: Implications for foetal skeletal muscle development. Journal of Cachexia, Sarcopenia and Muscle, 15(1641), 1-10. https://doi.org/10.1002/jcsm.13556

Switkowski, K. M., Jacques, P. F., Must, A., Kleinman, K. P., Gillman, M. W., & Oken, E. (2016). Maternal protein intake during pregnancy and linear growth in the offspring. American Journal of Clinical Nutrition, 104(5), 1128–1136. https://doi.org/10.3945/ajcn.115.128421

Şener-Özovalı, N., Yılmaz, T., & Dinç-Kaya, H. (2022). Gebelik ve doğum sonrası dönemde omega-3 kullanımı: Güncel literatürün gözden geçirilmesi. Sağlık

Akademisyenleri Dergisi, 9(2), 186-193. https://doi.org/10.52880/sagakaderg.1038308

Tarasi, B., Cornuz, J., Clair, C., & Baud, D. (2022). Cigarette smoking during pregnancy and adverse perinatal outcomes: A cross-sectional study over 10 years. BMC Public Health, 22, 2403. https://doi.org/10.1186/s12889-022-14881-4

Ulcay, S., & Şenel, G. (2020). Tokat çevresinde yayılış gösteren bazı tıbbi ve yenilebilir bitkilerin etnobotanik özelliklerinin belirlenmesi üzerine bir araştırma. Academic Platform-Journal of Engineering and Science, 8(1), 62-69.

Ulcay, S., & Senel, G. (2024). Plants used in traditional therapy in Pazar (Tokat-Türkiye) and their ethnobotanical properties. Pak. J. Bot, 56(1), 207-217.

Ulcay, S. (2024). An ethnobotanical study of medicinal and wild food plants in Kırşehir (Türkiye). In Anales del Jardín Botánico de Madrid (Vol. 81, No. 1, p. 3). Real Jardín Botánico.

Van den Broek, N., Dou, L., Othman, M., Neilson, J. P., Gates, S., & Gülmezoglu, A. M. (2011). Vitamin A supplementation during pregnancy for maternal and newborn outcomes. Cochrane Database of Systematic Reviews, 2011(3), CD008666. https://doi.org/10.1002/14651858.CD008666.pub2.

Yeşiltepe-Mutlu, G., & Hatun, Ş. (2011). Perinatal D vitamini yetersizliği. Çocuk Sağlığı ve Hastalıkları Dergisi, 54(2), 87-98.

CHAPTER 2

THE ROLE OF PROBIOTICS IN ALZHEIMER'S DISEASE

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1. INTRODUCTION

The most prevalent neurodegenerative disease, Alzheimer's disease (AD), mainly affects the elderly. One of the best ways to reduce cognitive deterioration in AD is to take probiotics. Selected bacterial strains have been shown in numerous in vivo investigations and current clinical trials to be useful in delaying the course of AD. Probiotics have been demonstrated to modify the gut microbiota, prevent oxidative stress, and control the inflammatory process. Thus, this study enumerates the available data, the variety of bacterial strains, the gut-brain axis abnormalities in AD, the pathogenic bacteria linked to AD, and the way probiotics work to prevent AD. The findings of this study contribute to the clear demonstration of the connection between AD and probiotic supplementation. Since probiotics have no negative effects on the human body, this review will aid in the development of future treatment plans. Since probiotics have no negative effects on the human body, this review will aid in the development of future treatment plans.

2. Probiotics

Probiotics are live bacteria that have positive health effects when taken in sufficient quantities. Probiotics have many health benefits, including preserving intestinal integrity, controlling pH, defending the gut microbiota from harmful bacteria, functioning as antibiotics, and boosting brain-derived neurotrophic factor (Larroya et al., 2019). These neurotrophic factors are made up of a particular kind of brain protein that promotes neuronal survival and differentiation. These elements are essential for neurological development, and deficiencies in them can result in a number of issues, including memory loss and learning disorders (Bathina et al., 2015). By directly modifying brain biochemical components like serotonin, γ-aminobutyric acid (GABA), and dopamine, probiotics also have a promising prognosis for treating mental diseases and memory impairments (Wang et al., 2016). It has been demonstrated that

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probiotics, which are very successful in reestablishing the intestinal microbiota's equilibrium, can slow the progression of Alzheimer's disease (AD), particularly oxidative stress and inflammatory responses, hence reducing cognitive loss (Wong et al., 2018). The *Lactobacillus* and *Bifidobacterium* genera are the most researched bacterial groups in the current studies on the effects of probiotics, prebiotics, and synbiotics on AD (Ale et al., 2021).

3. Alzheimer's Disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease that accounts for 80% of dementias worldwide, especially in older adults over 60 years of age (Deture et al., 2019). According to a global systematic research conducted in 2016, 43.8 million people worldwide suffer with AD (Dudek-Wicher at al., 2020). One of the biggest upcoming worldwide health issues, AD is expected to affect over 131 million people by 2050, according to the 2016 world AD estimate (Prince et al., 2016). Significant memory, cognitive, and motor deficits are hallmarks of AD.

Neurofibrillary tangles (NFTs) are intracellular clumps of hyperphosphorylated tau protein, known as the primary biomarker of Alzheimer's disease. The development of beta amyloid (Aβ) plaque deposits outside of neurons is another way that they contribute to AD. Neuroinflammation, vascular degeneration, disturbed calcium homeostasis, and ultimately neuronal death can result from this. AD is closely linked to neuropil filaments, synaptic dysfunction, and neuronal death (Guo et al., 2020).

The precise pathophysiology of AD is still unknown despite a great deal of research, but there is growing evidence that neuroinflammation plays a key role in the disease's development (Guo et al., 2020). Apoptosis, oxidative stress, tau protein hyperphosphorylation, Aβ aggregation, proinflammatory cytokines released by reactive glial cells, cerebrovascular disease, and alterations in the gut microbiome are the main theories put up about the pathophysiology of AD. Each of these primary pathologies can ultimately exacerbate and contribute to the burden of other significant AD pathologies. These pathologies can eventually combine to lead to neuronal malnutrition, synaptic deterioration, and death, resulting in neurodegeneration that manifests clinically as cognitive decline (Yang et al., 2024).

3.1. Changes in Gut Microbiota in the Etiology of Alzheimer's Disease

So far, the exact pathogenesis of AD remains unclear, although evidence is increasing to suggest a role for the gut microbiota in AD neuropathology. The gut microbiota of AD patients (Emery et al., 2017) and related animal models (Sun et al., 2019) differs significantly from that of healthy animals, according to recent investigations. According to these findings, changes that show a significant shift in the gut microbiome, like reduced microbial diversity, aberrant bacterial group proliferation or depletion, reduced probiotic abundance, and variations in microbial metabolites, may play a significant role in the pathophysiology of AD (Li et al., 2019).

An important factor in the pathophysiology of AD is neuroinflammation. The aberrant buildup of inflammatory chemicals through the stimulation of innate and adaptive immunity causes inflammation in the brain. Interactions between the gut microbiota and the expression of immune cells engaged in the central nervous system have been documented in a number of experimental investigations (Erny et al., 2015). The transit of Aβ oligomers from the stomach to the brain may result from these alterations in the gut microbiota, which may also enhance intestinal permeability and activate proinflammatory cytokines. After injecting Aβ1-42 oligomers into the stomach wall of mice in 2020, Sun and colleagues found that amyloid plays a role in AD and neuroinflammation (Sun et al., 2020). Through proinflammatory cytokines produced by systemic inflammation, this inflammatory environment may increase neuroinflammation and brain dysfunction.

One of the most significant pathogenic mechanisms in AD is the accumulation of Aβ in the brain. According to studies, the variety of bacteria in the gut microbiota can create Aβ and LPS, which will impact the neurological system and host immunity (Pistollato et al., 2016). LPS produced by gut bacteria interacts with the toll-like receptor 4 (TLR4) signaling pathway to activate immune cells when intestinal barriers are disrupted (Ghosh et al., 2020).

In neurodegenerative diseases, neurotransmitters such as acetylcholine (Ach), GABA, dopamine, histamine, noradrenaline, and serotonin (5-HT) might alter immune system pathways that affect behavior, memory, and learning. In fact, it has been discovered that intestinal bacteria may create neurotransmitters and are crucial in regulating the gut-brain axis. Recent postmortem brain scans of AD patients revealed markedly lower levels of the neurotransmitters glutamate and GABA, indicating impaired neuronal transmission and synaptic function in AD (Govindpani et al., 2020). Elevated GABA, a byproduct of Blautia-dependent arginine metabolism, has been linked in another study to a decreased risk of AD

[20]. The *Bifidobacterium*, *Lactobacillus*, and *Streptococcus* groups also generate GABA, the primary CNS inhibitory neurotransmitter (Strandwitz et al., 2018).

Over the course of the disease, AD patients exhibit increased brain oxidation. By disrupting the antioxidant system or reactive oxygen species (ROS) levels, the gut microbiota may affect the oxidative status in AD. Under oxidative stress, nitrate and nitrite can be converted by intestinal lactobacilli and bifidobacteria into nitric oxide (NO), which is toxic. Toxic substances called ROS are produced by the oxidative-reductive reaction of NO and are linked to both neuronal death and mitochondrial malfunction (Manoharan et al., 2016). In addition to hastening the buildup of Aβ, oxidative stress can initiate the oxidative process (Nam et al., 2018). In a double transgenic mouse model of AD, one study found elevated levels of oxidative stress and Aβ buildup (Kanamaru et al., 2015). By raising Aβ, tau hyperphosphorylation, and neuronal death, this oxidation is thought to be a pathogenic indicator of the development of AD in patients (Sharma et al., 2021).

3.2. Probiotic Deficiency in Alzheimer's Disease

Changes in probiotics have a big impact on how AD develops. In this instance, bacteria from the taxa Verrucomicrobia, Actinobacteria, Firmicutes, and Proteobacteria have been shown to significantly decline in AD. An rise in the genera Tenericutes and Bacteroidetes is also frequently observed (Rinninella et al., 2019). Cerebral Aβ accumulation is increased by this altered microbial makeup (Kowalski et al., 2019). According to a clinical investigation, AD patients' gut microflora composition had a lower microbial diversity, with higher levels of Bacteroidetes and a lower level of Firmicutes and Bifidobacterium (Rinninella et al., 2019). Probiotics' precise mode of action in AD is still unknown, though. The brain-gut-microbiota axis mechanisms may be the cause of this, according to research (Kowalski et al., 2019).

Any infection will cause intestinal permeability to rise, upsetting the gut-brain axis process because alterations in the intestinal microbiota might result in the colonization of harmful bacteria. By boosting the recruitment of immunological hemocytes to the brain, enterobacteria in the gut microbiota further regulates the course of AD (Wu et al., 2017).

3.3. Alzheimer's disease-related abnormalities in the gut-brain axis

The dynamic and reciprocal relationship between the central nervous system (CNS) and the gut microbiota is referred to as the gut-brain axis. Numerous neuroimmune and endocrine mediators connect gut functions to emotional and

cognitive brain regions, facilitating communication between the gut and the central nervous system.

Hormones like 5HT generated by the vagus nerve or endocrine cells facilitate communication between the gut and the central nervous system (Ma et al., 2019). The blood-brain barrier serves as a barrier of defense in both ways, keeping blood-borne infections out of the central nervous system. Soluble Aβ is normally removed by low-density lipoprotein receptors and delivered from the blood to the brain by the advanced glycation end-products receptor. The most significant genetic risk factor for late-onset Alzheimer's disease is the 4th allele of the apolipoprotein E gene, which causes increased pericyte destruction and is linked to breakdown of the blood-brain barrier in Alzheimer's disease (Kesika et al., 2020).

Another clinical observation in Alzheimer's disease (AD) is the presence of amyloid protein (α-synuclein), specifically in intestinal wall myenteric neurons. Through dendritic cells and epithelial microfold cells in Peyer's patches in the small intestine, this protein can move from the intestinal lumen to neuronal cells (Friedland et al., 2017). The buildup of α-synuclein in AD causes significant damage to the vagus nerve (Braak et al., 2016). Misfolded proteins (α-synuclein) frequently spread along the gut-brain axis. When these misfolded proteins aggregate, exocytosis releases them, which causes neuronal death in AD (Vasili et al., 2019).

These proteins can then be absorbed by other neurons, causing their susceptibility proteins to undergo programmed conformational changes. Through synapses, this process can spread throughout the network of neurons. These proteins can build up inside neurons in addition to extracellular Aβ buildup. As a result, neurofibrillary tangles (NFTs) are formed and extracellular Aβ buildup persists (Kowalski et al., 2019).

4. Probiotics affect brain functions through three main mechanisms:

Essential metabolites called short-chain fatty acids (SCFAs), which are produced when the gut microbiota ferments, increase the production of anti-inflammatory mediators while suppressing pro-inflammatory mediators. Probiotics work by activating the hypothalamic-pituitary-adrenal (HPA) axis and stimulating the adrenal glands to release cortisol, one of the strongest anti-inflammatory chemicals, through endocrine mechanisms. Additionally, probiotics stimulate the intestinal enteroendocrine L cells (EEC), which increases the synthesis of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1). Additionally, they use enterochromaffin cells (EC) to either directly release

particular neurotransmitters, like glutamate (GLU), or control neurotransmitters, like serotonin (5-HT). Together, these neurotransmitters and neuroactive metabolites give nerve cells a neuroprotective action that stops neurons from dying.

Saturated fatty acids, or SCFAs, are created in the colon as a result of eating fiber. *Bacteroides*, *Clostridium*, *Lactobacillus*, *Bifidobacterium*, and *Eubacterium* species ferment these metabolites, which are made up of acetate, butyrate, and propionate (Verbeke et al., 2015). Three primary mechanisms—immune modulation, endocrine routes, and neuronal processes are how SCFAs affect brain function. SCFAs support the intestinal mucosa's barrier function and immune response by preserving mucus production and fortifying the intestinal barrier through immunological regulation. By controlling the release of cytokines that promote the growth and differentiation of immune cells, SCFAs have immunomodulatory effects at the systemic level (Corrêa-Oliveira et al., 2016).

By inhibiting pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6, this interaction sets off an anti-inflammatory reaction (Vijav et al., 2014). SCFAs regulate neuroinflammation and prevent neuronal death in the central nervous system (CNS) via modifying the makeup and activity of microglial cells. SCFAs also function as endocrine signaling molecules by regulating the release of intestinal hormones. Studies in mouse colon cultures have shown that acetate and propionate significantly increase the synthesis of peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) via G-protein-coupled receptors (Psichas et al., 2014). GLP-1 is produced and secreted by intestinal enteroendocrine L cells and neurons in the brainstem's solitary tract nucleus. GLP-1 functions as a neuroprotective agent in the brain, preventing cell death and neuronal apoptosis (Sharma et al., 2014).

4.2. Probiotics as Therapeutic Targets in Alzheimer's Disease

According to results from human and animal research, using probiotics may help people with Alzheimer's disease (AD). It is impossible to completely rule out the possibility of publication bias with relation to these encouraging findings. According to analyses, *Bifidobacterium* and *Lactobacillus* species were the basis for almost 90% of the investigations. As a result, nearly all (100%) of the results show that Alzheimer's disease has comparable impacts, like enhanced memory and cognitive performance. *Streptococcus* and *Clostridium* species were the subject of only 10% of the research. Doses of 10^9 and 10^{10} CFU were typically utilized in studies looking at behavioral impacts. It has been demonstrated that

giving probiotics for two weeks in animal models and four weeks in human research is enough to have a noticeable impact.

Bifidobacteria infantis, *Bifidobacteria longum*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei*, in one or more strains for animal models, are the most often utilized probiotic formulations in Alzheimer's disease (AD). These probiotics are all regarded as "good" bacteria that may boost immunity and prevent the growth of dangerous bacteria. Probiotics boost intestine and brain functions and are essential for the gut-brain axis to function. However, because clinical research on AD patients only uses psychological questionnaires, care should be used when interpreting the results because they are subjective evaluations (Naomi et al., 2021).

5. Clinical Trial Effectiveness

The beneficial effects of probiotics have been well studied in a variety of fields. However, little is known about how probiotics regulate the disease, therefore it is unclear how they affect the early signs and progression of Alzheimer's disease (AD). The four clinical trials that have been conducted on AD patients thus far are compiled in Table 2. For example, a study by Akbari et al. (2016) found that probiotic therapy improved metabolic and cognitive functions in AD patients (Akbari et al., 2016).

In this trial, for 12 weeks, 30 Alzheimer's patients received 200 mL of probiotic-enriched milk (2×10^9 CFU/g each). The Mini Mental State Examination (MMSE) scores improved, insulin tolerance rose, serum MDA and hs-CRP levels dropped, and metabolic state stabilized by week 12 when compared to the control group. Nevertheless, there was no discernible shift in the levels of oxidative stress [39]. Comparable results were also found in a study by Agahi et al. (2018). For 12 weeks, probiotics at a daily dose of 3×10^9 CFU were given to 25 Alzheimer's patients. By the end of the twelve week, the patients' TYM scores and cognitive abilities had improved, their serum GSH levels had gone up, and their levels of 8-OHdG had dropped. Nevertheless, there was no discernible impact on overall antioxidant capacity (Agahi et al., 2018).

In this study, 25 Alzheimer's patients were administered probiotics at a daily dose of 3×10^9 CFU for 12 weeks. At the end of the twelfth week, the researchers found improvements in TYM scores and cognitive functions, increased serum GSH levels, and decreased 8-OHdG levels in the patients' serum. However, no significant effect on total antioxidant capacity was observed [40].

6. Conclusions and Future Perspectives

In conclusion, this review provides a wealth of evidence-based research on probiotics' potential to decrease the course of AD. been shown to be a useful aid in the therapeutic management of AD both in vivo and clinical investigations. In this case, probiotic treatment in AD patients has not been linked to any negative side effects. Additional clinical research is necessary to detect gut microbiome alterations unique to AD, since this could offer fresh insights into the assessment of probiotics as a potential therapeutic target. Large-scale research investigating the connection between microbial diversity and cognitive function as well as the course of AD may also yield important predictive data. Strategic advancements in the treatment and prevention of AD may be made possible by interdisciplinary approaches that investigate the connections between the host and microbiome.

7. REFERENCES

Agahi A., Hamidi G.A., Daneshvar R., Hamdieh M., Soheili M., Alinaghipour A., Taba S.M.E., & Salami M. (2018). Does severity of Alzheimer's disease contribute to its responsiveness to modifying gut microbiota? A double blind clinical trial. *Front. Neurol*, 2018;9:1–9. https://doi.10.3389/fneur.2018.00662.

Akbari E., Asemi Z., Kakhaki R.D., Bahmani F., Kouchaki E., Tamtaji O.R., Hamidi G.A., & Salami M. (2016). Effect of Probiotic Supplementation on Cognitive Function and Metabolic Status in Alzheimer's Disease: A Randomized, Double-Blind and Controlled Trial. *Front. Aging Neurosci*, 8:256. https://doi.10.3389/fnagi.2016.00256.

Ale E.C., & Binetti A.G. (2021). Role of Probiotics, Prebiotics, and Synbiotics in the Elderly: Insights into Their Applications. *Front. Microbiol*, 12:631254. https://doi.10.3389/fmicb.2021.631254.

Bathina S., & Das U.N. (2015). Brain-derived neurotrophic factor and its clinical Implications. Arch. Med. Sci, 11:1164–1178. https://doi.10.5114/aoms.2015.56342.

Braak H., & Del Tredici K. (2016). Potential pathways of abnormal tau and α-synuclein dissemination in sporadic Alzheimer's and Parkinson's diseases. *Cold Spring Harb. Perspect. Biol*. 8:a023630. https://doi.10.1101/cshperspect.a023630.

Corrêa-Oliveira R., Fachi J.L., Vieira A., Sato F.T., & Vinolo M.A.R. (2016). Regulation of immune cell function by short-chain fatty acids. *Clin. Transl. Immunol*, 5:e73. https://doi.10.1038/cti.2016.17.

Deture M.A., & Dickson D.W. (2019). The neuropathological diagnosis of Alzheimer's disease. *Mol. Neurodegener.* 14:1–18. https://doi.10.1186/s13024-019-0333-5.

Dudek-Wicher R., Junka A., Paleczny J., & Bartoszewicz M. (2020). Clinical Trials of Probiotic Strains in Selected Disease Entities. *Int. J. Microbiol*, 2020:1–8. https://doi.10.1155/2020/8854119.

Emery D. C., Shoemark D. K., Batstone T. E., et al., (2017). 16S rRNA Next Generation Sequencing Analysis Shows Bacteria in Alzheimer's Post-Mortem Brain. *Frontiers in Aging Neuroscience* 9: 195.

Erny D., Hrabě de Angelis A.L., Jaitin D., Wieghofer P., Staszewski O., David E., Keren-Shaul H., Mahlakoiv T., Jakobshagen K., Buch T., et al. (2015). Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci*. 18:965–977. https://doi.10.1038/nn.4030.

Friedland R.P., & Chapman M.R. (2017). The role of microbial amyloid in neurodegeneration. *PLoS Pathog*. 13:e1006654. https://doi.10.1371/journal.ppat.1006654.

Ghosh S.S., Wang J., Yannie P.J., & Ghosh S. (2020). Intestinal Barrier Dysfunction, LPS Translocation, and Disease Development. *J. Endocr. Soc*. 4:bvz039. https://doi.10.1210/jendso/bvz039.

Govindpani K., Turner C., Waldvogel H.J., Faull R.L.M., & Kwakowsky A. (2020). Impaired Expression of GABA Signaling Components in the Alzheimer's Disease Middle Temporal Gyrus. *Int. J. Mol. Sci.* 21:8704. https://doi.10.3390/ijms21228704.

Guo T., Zhang D., Zeng Y., Huang T.Y., Xu H., & Zhao Y. (2020). Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. *Mol. Neurodegener.* 15:40. https://doi.10.1186/s13024-020-00391-7.

Kanamaru T., Kamimura N., Yokota T., Iuchi K., Nishimaki K., Takami S., Akashiba H., Shitaka Y., Katsura K.-I., Kimura K., et al. (2015). Oxidative stress accelerates amyloid deposition and memory impairment in a double-transgenic mouse model of Alzheimer's disease. *Neurosci. Lett*. 587:126–131. https://doi.10.1016/j.neulet.2014.12.033.

Kesika P., Suganthy N., Sivamaruthi B.S., & Chaiyasut C. (2020). Role of gut-brain axis, gut microbial composition, and probiotic intervention in Alzheimer's disease. *Life Sci.* 2020:118627. https://doi.10.1016/j.lfs.2020.118627.

Kowalski K., & Mulak A. (2019). Brain-gut-microbiota axis in Alzheimer's disease. *J. Neurogastroenterol. Motil*, 25:48–60. https://doi.10.5056/jnm18087.

Larroya-García A., Navas-Carrillo D., & Orenes-Piñero E. (2019). Impact of gut microbiota on neurological diseases: Diet composition and novel treatments. *Crit. Rev. Food Sci. Nutr.* 59:3102–3116. https://doi.10.1080/10408398.2018.1484340.

Li B., He Y., Ma J., et al., (2019). Mild Cognitive Impairment Has Similar Alterations as Alzheimer's Disease in Gut Microbiota. *Alzheimers Dement* 15:1357–1366.

Ma Q., Xing C., Long W., Wang H.Y., Liu Q., & Wang R.F. (2019). Impact of microbiota on central nervous system and neurological diseases: The gut-brain axis. *J. Neuroinflamm*, 16:1–14. https://doi.10.1186/s12974-019-1434-3.

Manoharan S., Guillemin G.J., Abiramasundari R.S., Essa M.M., Akbar M., & Akbar M.D. (2016). The Role of Reactive Oxygen Species in the Pathogenesis of Alzheimer's Disease, Parkinson's Disease, and Huntington's Disease: A

Mini Review. Oxid. Med. Cell. Longev, 8590578. https://doi.10.1155/2016/8590578.

Nam E., Derrick J.S., Lee S., Kang J., Han J., Lee S.J.C., Chung S.W., & Lim M.H. (2018). Regulatory Activities of Dopamine and Its Derivatives toward Metal-Free and Metal-Induced Amyloid-β Aggregation, Oxidative Stress, and Inflammation in Alzheimer's Disease. *ACS Chem. Neurosci.* 9:2655–2666. https://doi10.1021/acschemneuro.8b00122.

Naomi R., Embong H., Othman F., Faisal Ghazi H., Maruthey N., & Bahari H. (2021). Probiotics for Alzheimer's Disease: A Systematic Review *Nutrients.* 22:14(1):20. https://doi.10.3390/nu14010020.

Pistollato F., Cano S.S., Elio I., Vergara M.M., Giampieri F., & Battino M. (2016). Role of gut microbiota and nutrients in amyloid formation and pathogenesis of Alzheimer disease. *Nutr. Rev.*74:624–634. https://doi.10.1093/nutrit/nuw023.

Prince M., Comas-Herrera A., Knapp M., Guerchet M., & Karagiannidou M. Improving Healthcare for People Living with Dementia Coverage, QualIty and Costs Now and In the Future. World Alzheimer Report 2016; King's College London; London, UK: 2016.

Psichas A., Sleeth M.L., Murphy K.G., Brooks L., Bewick G.A., Hanyaloglu A.C., Ghatei M.A., Bloom S.R., & Frost G. (2014). The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int. J. Obes*. 39:424–429. https://doi.038/ijo.2014.153.

Rinninella E., Raoul P., Cintoni M., Franceschi F., Miggiano G.A.D., Gasbarrini A., & Mele M.C. (2019). What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms*, 7:14. https://doi.10.3390/microorganisms7010014.

Sharma M.K., Jalewa J., & Hölscher C. (2014). Neuroprotective and anti-apoptotic effects of liraglutide on SH-SY5Y cells exposed to methylglyoxal stress. *J. Neurochem,* 128:459–471. https://doi.10.1111/jnc.12469.

Sharma C., & Kim S.R. (2021).Linking Oxidative Stress and Proteinopathy in Alzheimer's Disease. Antioxidants. 10:1231. https://doi.10.3390/antiox10081231.

Strandwitz P. (2018). Neurotransmitter modulation by the gut microbiota. *Brain Res.*1693:128–133. https://doi.10.1016/j.brainres.2018.03.015.

Sun J., Xu J., Ling Y., et al., (2019). Fecal Microbiota Transplantation Alleviated Alzheimer's Disease-Like Pathogenesis in APP/PS1 Transgenic Mice, *Translational Psychiatry* 9 189.

Sun J., Xu J., Yang B., Chen K., Kong Y., Fang N., Gong T., Wang F., Ling Z., & Liu J. (2020). Effect of *Clostridium butyricu*m against Microglia-Mediated Neuroinflammation in Alzheimer's Disease via Regulating Gut Microbiota and Metabolites Butyrate. *Mol. Nutr. Food Res.* 64:1900636. https://doi.10.1002/mnfr.201900636.

Vasili E., Dominguez-Meijide A., & Outeiro T.F. (2019). Spreading of α-synuclein and tau: A systematic comparison of the mechanisms involved. *Front. Mol. Neurosci*. 12:107. https://doi.10.3389/fnmol.2019.00107.

Verbeke K.A., Boobis A.R., Chiodini A., Edwards C.A., Franck A., Kleerebezem M., Nauta A., Raes J., Tol E.A.F., & van Tuohy K.M. Towards microbial fermentation metabolites as markers for health benefits of prebiotics. Nutr. Res. Rev. 2015;28:66. https://doi.10.1017/S0954422415000037.

Vijay N., & Morris M.E. (2014). Role of monocarboxylate transporters in drug delivery to the brain. *Curr. Pharm. Des*. 20:1487–1498. https://doi.10.2174/13816128113199990462.

Wang H., Lee I.S., Braun C., & Enck P. (2016). Effect of probiotics on central nervous system functions in animals and humans: A systematic review. *J. Neurogastroenterol. Motil.* 22:589–605. https://doi.10.5056/jnm16018.

Wong C.B., Kobayashi Y., & Xiao J. Probiotics for Preventing Cognitive Impairment in Alzheimer's Disease. In: Evrensel A., Ünsalver B.Ö., editors. Gut Microbiota Brain Axis. IntechOpen; Zama, Japan: 2018.

Wu S.C., Cao Z.S., Chang K.M., & Juang J.L. (2017). Intestinal microbial dysbiosis aggravates the progression of Alzheimer's disease in Drosophila. *Nat. Commun*, 8:24. https://doi.10.1038/s41467-017-00040-6.

Yang J., Liang J., Hu N., He N., Liu B., Liu G., & Qin Y. (2024). The Gut Microbiota Modulates Neuroinflammation in Alzheimer's Disease: Elucidating Crucial Factors and Mechanistic Underpinnings. *CNS Neurosci Ther*. 26;30(10):e70091. https://doi.10.1111/cns.70091.

Zhuang Z., Yang R., Wang W., Qi L., & Huang T. (2020). Associations between gut microbiota and Alzheimer's disease, major depressive disorder, and schizophrenia. *J. Neuroinflam*, 17:1–9. https://doi.10.1186/s12974-020-01961-8.

CHAPTER 3

EFFECTS OF SELENIUM ON HUMAN HEALTH

Ebru CÖTELİ[1]

1. INTRODUCTION

The word "selenium" comes from the ancient Greek moon goddess Selene. It was first discovered by the Swedish Jöns Jakob Berzelius. However, its functions as a trace element were discovered a century later (Köhrle, 1999). It is toxic in high concentrations, but it is essential to use this nonmetal in low concentrations as a trace element. This metal is essential. It is especially abundant in liver, eggs, and seafood. This element is a cofactor for many enzymes in our body. It is also an essential trace element known for its antioxidant function (Köhrle & Jakob, 2005). Selenium is a nonmetal. Its properties are similar to those of the element sulfur (S). It exists in nature in two forms: organic and inorganic. The organic form is more easily accessible to the human body than the inorganic form. The most important forms of this element are selenous acid (IV), selenium dioxide, sodium selenite (IV), dihydrogen selenide, selenic acid (VI), and sodium selenate (VI). Organic selenium compounds such as methylselenomethionine, selenourea, selenobetaine, selenocysteine, seleniocholine, methylselenocysteine, and selenomethionine are particularly important in living metabolism (Wesolowski & Ulewicz, 2000).

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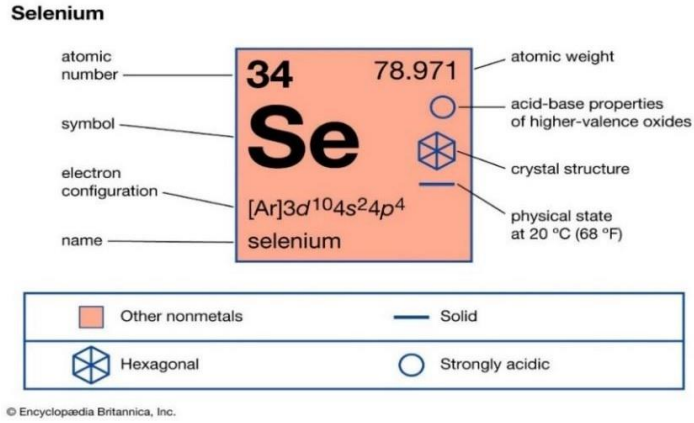

Figure 1. Selenium element and its properties

Selenium is widely distributed in nature. It penetrates the atmosphere and into lakes, seas, and oceans. Studies have isolated more than 30 specific selenoproteins, but they have only identified 15 of them (Rocha et al., 2017). It is then taken up by plants and passed on to animals and humans through the food chain (Wachowicz, 1993; Wesolowski & Ulewicz, 2000).

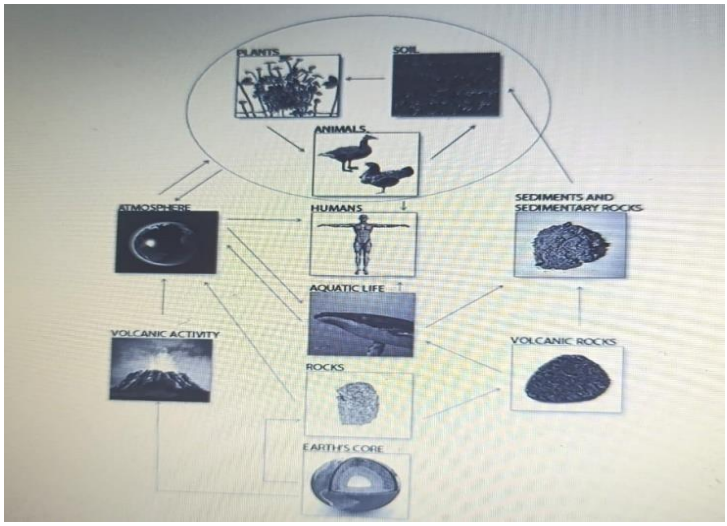

Figure 2. Cycle of the element selenium

2. Functions of Selenıum In Metabolism

Selenium has many functions in metabolism. It is a bioelement essential for living organisms (Ying & Zhang, 2019).

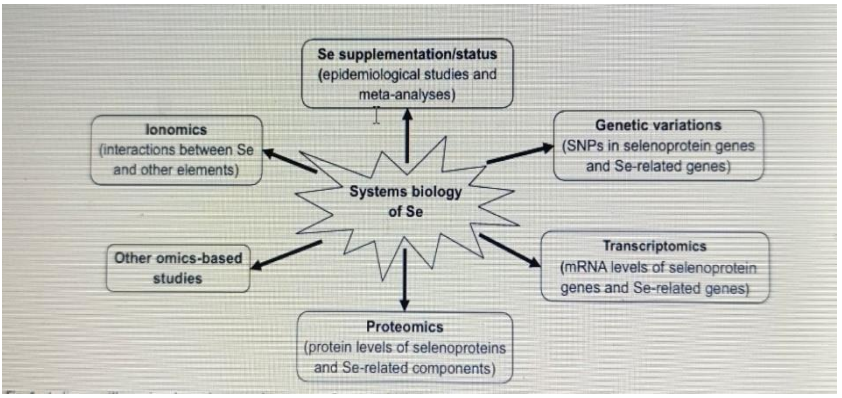

Figure 2. Biology of the Se element The functions of the selenium element in metabolism are as follows:

2.1. Antioxidant Properties of Selenium

Selenium has antioxidant properties due to its involvement in various bodily functions and biological effects. It is particularly closely associated with the element sulfur, a stable component of tissues. Selenium, in particular, has the ability to replace the sulfur element in the structure of amino acids such as cystine and methionine. For this reason, sulfur-containing amino acids gain a naturally occurring activity in their pure form (Johansson et al., 2012). The biochemical functions of selenium are regulated by selenium proteins containing selenocysteine residues (Hatfield & Gladyshev, 2002). Selenium, which exists in various forms, prevents the growth of tumor cells in vivo and in vitro (Chou et al., 1999; Ip et al., 2000; Menter et al., 2000; Tanaka et al., 2000). Studies have reported that the incidence and mortality rates of many types of cancer, including prostate, lung, and colorectal cancers, are reduced in people taking 200 µg Se per day (Clark et al., 1996). Glutathione Peroxidase (GSH-Px1) enzyme activity depends on the selenium content of the diet (Pilarczyk et al., 2012; Suchý et al., 2014). There is a relationship between vitamin E and selenium. Selenium, in particular, has the ability to perform many of the functions of vitamin E. Vitamin E and selenium have synergistic antioxidant functions. Selenium specifically protects the cell membrane and cytoplasm against oxidants, while vitamin E protects the cell membrane of unsaturated fatty acids from oxidative damage. In this process, selenium contributes to the antioxidant activity of vitamin E (Huberuk et al., 2017; Khariv & Hutyi, 2017).

2.2. Functions of Selenium in the Reproductive System

Scientific studies have shown that selenium has roles in reproductive function in animals (Ras-solov et al., 2009). It has been reported that oogenesis begins, ovarian activity increases, and secretion begins in the oviducts and uterus in female animals fed a selenium-rich diet. Selenium also plays a role in regulating ovaries in females through FSH (follicle-stimulating hormone) (Basini & Tamanini, 2000). In addition, selenium deficiency in men and poultry leads to decreased sexual activity and deterioration of steroid hormones and sperm quality (El-Sharawy et al., 2017).

2.3. Antiviral Function of Selenium

Selenium protects poultry against viral infections. Furthermore, animals fed a selenium diet are resistant to pathogenic microorganisms because selenium disrupts the genetic structure of pathogenic microorganisms. It also protects against new pathogenic microorganisms by enhancing the animals' immunity (Fisinin et al., 2007).

2.4. Relationship between Selenium and Osteogenesis

Studies have shown that selenium has significant effects on the activation and proliferation of bone tissue cells (osteoblasts) (Zeng et al., 2013). Selenium is the most important auxiliary of sulfotransferases, which function in cartilage metabolism. Sulfotransferases are responsible for transferring sulfur residues to the glycosaminoglycan molecule. Selenium is integral to these mechanisms. Experimental studies have shown that selenium deficiency in animal nutrition causes growth retardation, decreased blood calcium levels, and disorders in the absorption and excretion of calcium from the kidneys (Moreno-Reyes et al., 2001).

2.5. Anti-carcinogenic Functions of Selenium

Selenium has anticancer effects. Its anticancer effects have been reported to be due to multiple mechanisms (Ognerubova et al., 2009). The mechanism by which selenium destroys tumor cells stems from its accumulation and toxic effects on them (Wallenberg et al., 2014). It has been reported that high selenium supplementation stimulates damaged carcinogenic DNA. Selenium primarily protects cell components against oxidative radicals. It also prevents the formation of malignant tumors and eliminates the risk of frequent DNA chain breaks (Schlicht et al., 2004). In particular, selenium provides inhibition of the mechanism of pro-carcinogens and carcinogenesis (Baraboj, 2004).

2.6. Selenium's Free Radical Scavenging Function

There are studies showing that different forms of selenium (organic and inorganic) eliminate the harmful depressive effects of mycotoxins on animals. Selenium, in particular, has the ability to clean and eliminate aflatoxin-producing molds (Chena et al., 2014). Selenium, which manages the glutathione system, protects cell membranes from oxidative damage by capturing superoxide radicals (Uysal et al., 2005). Selenium, in particular, reduces the time required for the clearance and elimination of mycotoxins in the metabolism (Mubarak et al., 2009). Selenium reacts with heavy metal salts to form insoluble complexes. This acts as an antidote, helping to remove heavy metals from the body (Bjorklund, 2015). Selenium, in particular, prevents these heavy metals from causing lipid peroxidation thanks to its antioxidant properties (Perepelkinai et al., 2010).

3. CONCLUSIONS

Selenium has a biological role in human and animal metabolism. Selenium is thought to be important in many processes occurring in living organisms' metabolism and in chemical reactions whose structures have not yet been elucidated. More human experimental studies are needed to better understand the roles of selenium in biological processes. Due to the biological activity of the selenium element, it is vital to elucidate the metabolism of this element in biological systems. The society needs to be informed about the selenium element and its functions.

4. REFERENCES

Baraboj, V.A. (2004). Biologicheskie funkcii, metabolizm i mehanizm dejstvija selena. *Uspehi sovremennoj biologii*. 124(2), 157–168 (in Russian).

Basini, G., & Tamanini, C. (2000). Selenium stimulates estradiol production in bovine granulosa cells: possible involvement of nitric oxide. *Domest Anim Endocrinol*., 18(1), 1–17.

Bjorklund, G. (2015). Selenium as an antidote in the treatment of mercury intoxication. *Biometals*, 28(4), 605–614.

Chena, K., Fanga, J., Penga, X., Cuia, H., Chena, J., Wanga, F.,Chena, Z., Zuoa, Z., Denga, J., Laia, W., & Zhoub, Y. (2014). Effect of selenium supplementation on aflatoxin B1-induced histopathological lesions and apoptosis in bursa of Fabricius in broilers. *Food and Chemical Toxicology*, 74, 91–97.

Cho, D.Y., Jungand, U., Chung, A.S. (1999). Induction ofapoptosis byselenite and selenodiglutathione in HL-60 cells: correlation with cytotoxicity, *Biochemistry and Molecular Biology International*, 47, 781–793.

Clark, L.C., Combs, G.F., Turnbull, B.W., Slate, E.H., Chalker, D.K., Chow, J., Davis, L.S., Clover, R.A., Graham, G.F., Gross, E.G., Krongrad, A., Lesher, J.L., Park, H.K., Sanders, B.B., Smith, C.L., & Taylor, J.R. (1996). Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. *J. Am. Med. Assoc*., 276, 1957–1963.

El-Sharawy, M., Eid, E., Darwish, S,. Abdel-Razek, I., Islam, M.R., Kubota, K., Yamauchi, N., & El-Shamaa, I. (2017). Effect of organic and inorganic selenium supplementation on semen quality and blood enzymes in buffalo bulls. *Animal Science Journal*., 88(7), 999–1005.

Fisinin, V.I., Suraj, P.F., & Papazjan, G.T. (2007). Kakaja svjaz' mezhdu selenom i ptich'im grippom. *Efektivne ptahivnictvo*. 4, 21 25 (in Russian).

Hatfield, D.L., & Gladyshev, V.N. (2002). How selenium has altered our understanding of the genetic code. *Molecular and Cellular Biology*, 22(11), 3565–3576.

Huberuk, V., Gutyj, B., Gufriy, D., Binkevych, V., Hariv, I., Binkevych, O., & Salata, R. (2017). Impact of antioxidants on enzym activities of glutatione system of bulls bodies antioxidant defense under acute nitrate and nitrite toxicity. *Scientific Messenger LNUVMBT named after S.Z. Gzhytskyj*. 19(77), 220–224.

Ip, C., Thompson, H.J., Zhu, Z., & Ganther, H.E. (2000). In vitro and in vivo studies of methylseleninic acid: Evidence that a monomethylated selenium metabolite is critical for cancer chemoprevention. *Cancer Res*., 60, 2882–2886.

Johansson, A.L., Collins, R., Arnér, E.S., Brzezinski, P., & Högb, M. (2012). Biochemical discrimination between selenium and sulfur 2: mechanistic investigation of the selenium specificity of human selenocysteine lyase. *PLoS One*, 7(1).

Khariv, M.I., & Hutyi, B.V. (2017). Dynamika fahotsytarnoi aktyvnosti neitrofiliv u shchuriv za umov oksydatsiinoho stresu ta dii liposomalnoho preparatu. *Biolohiia tvaryn*. 19(1), 119-124 (in Ukrainian).

Köhrle, J. (1999). The trace element selenium and the thyroid gland. *Biochimie*, 81, 527-533.

Köhrle, J., Jakob, F., Contempré, B., & Dumont, J.E. (2005). Selenium, the thyroid, and the endocrine system. *Endocr Rev*., 26, 944-984.

Menter, D.G., A.L.Sabichiand, A.L., & Lippman, S.M. (2000). Selenium effects on prostate cell growth. *Cancer Epidemiology, Biomarkers &Prevention*, 9, 1171–1182.

Moreno-Reyes, R., Egrise, D., Nève, J., Pasteels, J.L., & Schoutens, A. (2001). Selenium deficiency – induced growth retardation is associated, with an impaired bone metabolism and osteopenia. *Bone and Mineral Research*, 16(8), 1556–1563.

Mubarak, A., Rashid, A., Khan, I., & Hussain, A. (2009). Effect of vitamin E and selenium as immunomodulators on induced aflatoxicosis in broiler birds. *Pakistan Journal of Social Science*, 7, 31–34.

Ognerubova, I.N., & Poddubnaja, I.V. (2009). Primenenie selena v onkologii. *Sovremennaja onkologija*, 11(2), 56–58 (in Russian).

Perepelkinai, L.I., & Lenchevskij, S.A. (2010). Rol' selena v jekologicheskom obosnovanii vyvedenija tjazhelyh metallov iz organizma zhivotnyh. *Dal'nevostochnyj agrarnyj vestnik*, 4, 24–27 (in Russian).

Pilarczyk, B., Jankowiak, D., Tomza-Marciniak, A., Pilarczyk, R., Sablik, P., Drozd, R., Tylkowska, A., & Skólmowska, M. (2012). Selenium Concentration and Glutathione Peroxidase (GSH-Px) Activity in Serum of Cows at Different Stages of Lactation. *Biological Trace Element Research*, 147(1–3), 91–96.

Rassolov, S.N., Eranov, A.M., & Zubova, T.V. (2009). Vlijanie preparata E-selena na vosproizvoditel'nuju funkciju korov. *Sibirskij vestnik sel'skohozjajstvennoj nauki*, 7, 113–115 (in Russian).

Rocha, João B.T., Piccoli, B.C., & Oliveira, C.S. (2017). Oliveirab Biological and chemical interest in selenium: a brief historical account. *The Free Internet Journal for Organic Chemistry*, 457–491.

Schlicht, M., Matysiak, B., Brodreller, B., Wen, X., Liu, H., Zhou, G., Dhir, R., Hessner, M.J., Tonellato, P., Suckow, M., Pollard, M., & Datta, M.W. (2004). Gross-species global and subset gene expression profiling identifies genes involved in prostate cancer response to selenium. *BMC Genomics*, 5, 58.

Suchý, P., Straková, E., & Herzig, I. (2014). Selenium in poultry nutrition: a review. *Czech Journal of Animal Science*, 59(11), 495-503.

Tanaka, T., Kohno, H., Murakami, M.,Kagami, S., & El-Bayoumy, K. (2000). Suppressing effects of dietary supplementation of the organoselenium 1,4-phenylenebis (methylene) selenocyanate and the citrus antioxidant auraptene on lung metastasis of melanoma cells in mice. *Cancer Res*., 60, 3713–3716.

Uysal, H., & Agar, G. (2005). Selenium protective activity against aflatoxin B1 adverse affects on Drosophila melanogaster. *Brazilian Archives of Biology and Technology*, 48(2), 227–233.

Wachowicz, B. (1993). Selenium in plants. *Wiad. Bot*., 37, 87-89.

Wallenberg, M., Misra, S., Wasik, A.M., Marzano, C., Björnstedt, M., Gandin, V., & Fernandesa, A.P. (2014). Selenium induces a multi-targeted cell death process in addition to ROS formation. *Journal of Cellular and Molecular Medicine*, 18(4), 671–684.

Wesolowski, M., & Ulewicz, B. (2000). Selenium – a trace element essential for humans, its presence, biological role and toxicity. *Farm. Pol*., 56, 1004-1019.

Ying, H., & Zhang, Y. (2019). Systems Biology of Selenium and Complex Disease. *Biological Trace Element Research*, 191, 38–50.

Zeng, H., Cao, J.J., & Combs, G.F. (2013). Selenium in Bone Health: Roles in Antioxidant Protection and Cell Proliferation. *Nutrients*, 5(1), 97–110.

CHAPTER 4

POISONINGS AND FIRST AID IN POISONINGS

Murat ÇINARLI[1] & Vugar Ali TURKSOY[2] & Esra ÇINARLI[3]

1. INTRODUCTION

Poisoning is defined as the disruption of normal physiological functions resulting from the ingestion of a toxic substance via different pathways (Ellenhorn & Barceloux, 1997). According to data from the World Health Organization (WHO), millions of people seek emergency treatment each year due to acute poisoning. Children, the elderly and individuals with chronic diseases are particularly at risk (WHO, 2021). Poisoning can be caused by drugs, food, chemicals, industrial toxins, gases such as carbon monoxide, and animal-derived toxins. The consequences of poisoning vary depending on the type and amount of substance ingested, the route of exposure and the individual's physiological characteristics. The clinical spectrum ranges from mild nausea and headache to multiple organ failure and death (Nelson et al., 2019).

Acute poisoning poses a significant public health problem, particularly among children and adolescents under the age of 20. Each year, approximately 45,000 deaths in this age group are caused by poisoning. Globally, the poisoning mortality rate in this age group is reported to be 1.8 per 100,000. There are also significant differences by income level: while the mortality rate is 0.5 per 100,000 in high-income countries, it is approximately four times higher at 2.0 per 100,000 in low- and middle-income countries. These differences are directly related to the strength of health systems, emergency response capacity and access to preventive measures. When evaluated by WHO regions, mortality rates are relatively low, not exceeding 4 per 100,000. However, the highest rates of poisoning-related deaths are found in Africa, low- and middle-income countries of Europe, and the Western Pacific region (Peden et al., 2008).

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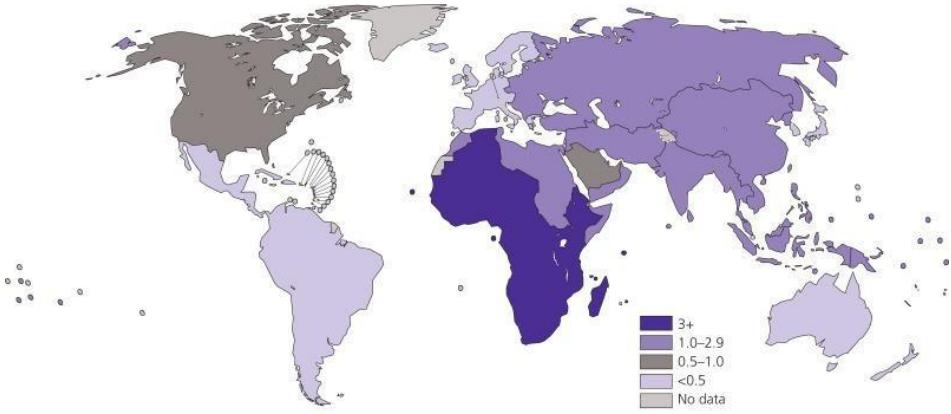

Figure 1 WHO poisoning mortality rates per 100,000 children by region and country income level

Poisonings can be classified in various ways, depending on the route by which the toxic substance enters the body, how it acts, and where it comes from. This classification system helps with both clinical diagnosis and treatment approaches, as well as the evaluation of epidemiological data.

2. POISONINGS

2.1. Poisoning by Route of Ingestion

2.1.1. Oral Poisoning

The most common type of poisoning is the oral ingestion of toxic substances. Notable examples include drug overdoses (e.g. analgesics, antidepressants and sedative-hypnotics), foodborne toxins, mushroom poisonings and the accidental ingestion of household chemicals. Although accidental ingestion is more prevalent among children, drug overdoses resulting from suicide attempts are a common cause among adolescents and young adults (Mowry et al., 2016).

2.1.2. Inhalation poisoning

Inhaling toxic substances through the respiratory tract is one of the most significant and often fatal types of poisoning. Prominent examples of such substances include carbon monoxide, methane, cyanide and agricultural pesticides. Exposure to carbon monoxide, particularly in enclosed spaces, is the most common cause of fatal inhalation poisoning worldwide. Its colourless, odourless and non-irritating nature makes exposure difficult to detect, thereby increasing the risk of mortality (Raub & Vosk, 2008).

2.1.3. Cutaneous poisonings

Dermal poisoning primarily occurs through chemical burns resulting from the absorption of chemicals through the skin or from direct contact. Organophosphate pesticides are among the most significant agents in this group. Due to their lipophilic properties, these substances can be rapidly absorbed through the skin, leading to systemic toxicity and serious poisoning. Industrial solvents, pesticides and strong acid-base chemicals can also cause dermal poisoning (Peter & Cherian, 2000).

2.1.4. Poisoning by parenteral route (injection, animal sting/bite)

Parenteral poisoning occurs as a result of poisonous animal stings or the direct introduction of a toxic substance into the bloodstream via injection. Noteworthy examples in this group include snake and scorpion stings, bee sting poisoning, and incorrect drug dosage or administration. Depending on the severity of the envenomation, animal poisoning can lead to serious clinical conditions ranging from local tissue damage to systemic organ failure. Improper injections, particularly of high doses of cardiovascular, neurological or analgesic drugs, can be life-threatening.

2.2. Poisonings by Source

2.2.1. Drug-related Poisonings

Analgesics, cardiovascular drugs, antidepressants and sedatives are among the most commonly encountered drug-derived toxins today. Due to their widespread use, incorrect dosages of these drugs — including intentional or accidental overdoses — pose a significant public health problem. Analgesics such as paracetamol and salicylates can damage the liver and kidneys at high doses, while cardiovascular drugs can cause serious arrhythmias and hypotension. Misuse of antidepressants can cause cardiac conduction disturbances and neurological symptoms, while sedatives can lead to severe conditions such as respiratory depression and altered consciousness. The toxic effects of these drug groups are evaluated in the context of both acute poisonings and chronic exposure, constituting a significant proportion of emergency department visits (Mowry et al., 2019).

2.2.2. Food poisoning

Bacterial toxins and some naturally occurring poisons also play a significant role in cases of food poisoning. Toxins produced by bacteria such as Salmonella and Staphylococcus aureus are among the main causes of food poisoning and typically cause gastrointestinal symptoms. Botulinum toxin, produced by

Clostridium botulinum, is one of the most potent biological toxins. It disrupts neuromuscular transmission, causing muscle paralysis and respiratory failure. Poisonous mushrooms, which are commonly found in nature, can also cause serious poisoning, particularly when consumed improperly, and they carry a high mortality risk due to their hepatotoxic and nephrotoxic effects. The conditions caused by these toxins require immediate medical attention and have widespread public health implications (Him et al., 2025).

2.2.3. Chemical poisoning

Household cleaning products, industrial solvents, pesticides and heavy metals are among the main causes of chemical poisoning. Misuse or accidental exposure to cleaning products such as bleach and hydrochloric acid can cause serious damage to the gastrointestinal and respiratory tracts. Industrial solvents, especially when inhaled in enclosed spaces, can depress the central nervous system and damage organs. Pesticides commonly used in agriculture can pose risks of acute and chronic poisoning when they enter the body through the skin, by inhalation or ingestion, and can cause neurological and systemic effects. Furthermore, heavy metals such as lead, mercury and arsenic build up in the body over time and can cause permanent damage to the nervous system, kidneys and liver. These chemicals are of particular concern because they pose serious risks to individual and environmental health.

2.2.4. Gas and vapor poisoning

Poisoning from gas poses serious health risks, particularly in confined spaces. The most prominent examples of these poisonings are carbon monoxide, carbon dioxide, cyanide and various industrial gases. Carbon monoxide binds to hemoglobin, inhibiting oxygen transport and rapidly leading to hypoxia. Breathing high concentrations of carbon dioxide can cause life-threatening conditions such as respiratory depression and acidosis. Cyanide, on the other hand, can block oxidative phosphorylation at the cellular level, leading to multiple organ failure. Depending on the type, industrial gases can exhibit various toxic effects, including respiratory irritation, central nervous system depression, and cardiac complications. Therefore, gas poisonings are considered critical toxic events requiring rapid diagnosis and emergency intervention.

2.2.5. Animal-derived poisonings

Animal-derived poisonings encompass toxic events resulting from stings or bites. Serious poisoning can result from stings or bites from land animals such as snakes, scorpions and bees, as well as from some marine animals such as

stonefish and jellyfish. Such poisonings generally result from direct entry of toxins into the bloodstream, and depending on the type of toxin, the clinical picture can range from local pain, swelling, and skin reactions to systemic neurological symptoms, cardiovascular complications, and multiorgan failure. Animal-derived toxins pose a significant public health risk because they require immediate medical attention and can be potentially fatal.

Alcohol and drug-related poisonings

Poisoning from alcohol and drugs is a significant toxicity resulting from the misuse of both legal and illicit substances. Ethanol, a widely consumed form of alcohol, can lead to central nervous system depression and respiratory and cardiac complications if consumed in excess. Toxic alcohols such as methanol and ethylene glycol produce serious systemic effects such as metabolic acidosis, visual disturbances, and renal failure. Volatile solvents, especially when inhaled, can have neurotoxic and cardiotoxic effects. Illicit substances, such as opioids, cocaine, and amphetamines, can cause life-threatening conditions such as respiratory depression, arrhythmia, neurological disorders, and psychiatric complications, depending on the route and dose of use. Therefore, alcohol and drug-related poisonings are a significant public health problem requiring immediate medical intervention and toxicological evaluation.

2.3. Poisonings According to Clinical Course

2.3.1. Acute poisonings

Acute poisoning is a clinical condition resulting from single or multiple exposures to toxic doses of drugs or chemicals over a short period of time (usually 24 hours). In this type of poisoning, toxic effects develop rapidly, and symptoms appear quickly. As the time available for intervention is limited, acute poisonings require immediate diagnosis and prompt treatment. Early diagnosis and appropriate medical intervention are therefore crucial for reducing morbidity and mortality (Hodgson, 2011).

2.3.2. Chronic Poisoning

Chronic poisoning is a toxic condition that develops as a result of repeated or continuous exposure over a long period (usually longer than three months). It is particularly common in occupational groups such as industrial battery workers, tinsmith apprentices, chimney sweeps, and shoemaking workers. While chronic exposures generally cause symptoms to appear after a long period of time, in some cases, symptoms similar to acute poisoning can occur after each exposure (Roberts et al., 2022).

2.3.3. Subacute Poisoning

Subacute poisoning is a clinical condition that results from the repeated introduction of toxic doses of chemical substances into the body over a short period of time (approximately one week). Similar to acute poisoning, symptoms develop rapidly; however, the exposure period is typically longer, ranging from a few days to one month. This type of poisoning is particularly common with pesticides and insecticides. Clinical findings are similar to acute poisoning, and early diagnosis and appropriate intervention are crucial to prevent potential complications.

2.4. Poisoning by Accident, Abuse, and Intent

2.4.1. Actual Accidental Poisonings

These poisonings occur as a result of carelessness, absent-mindedness, lack of precautions, or unconsciousness. They are the most common type of poisoning, especially in children (Lamireau et al., 1997).

Some examples of accidental poisonings include:

• Poisoning caused by carelessly leaving household appliances (gas, bottled gas, barbecues, fireplaces, stoves, etc.) on or by carbon monoxide (CO) leaking from faulty appliances;

• Poisoning caused by mistakenly drinking highly toxic liquids (washing soda, javelin, hydrochloric acid, etc.) mistakenly for beverages;

• Inadvertent consumption of poisonous plants or mushrooms;

• Inadvertent consumption of poisonous animals (e.g., some fish species or defectively produced honey).

These types of accidents are of great public health importance because they can be prevented, and serious consequences can be avoided through early intervention.

2.4.2. Medication Poisonings

Acute drug poisoning can result from incorrect dosages, changes in pharmaceutical formulations or drug interactions. The accumulation of unnecessary medications at home and unnecessary prescriptions by physicians are among the primary causes of accidental poisonings. Furthermore, misunderstanding dosage instructions or drug names on illegible prescriptions can also lead to poisonings related to treatment (Philips et al., 2001). Therefore,

correct and informed use of medications, clear and understandable prescribing, and patient education are crucial in preventing treatment-related poisonings.

2.4.3. Occupational Poisonings

It can cause both acute and chronic poisoning in individuals working in agriculture and industry. Workers can ingest toxic substances through inhalation, skin contact, or accidental ingestion, particularly during agricultural spraying without protective clothing and masks. Similarly, workers working in industrial facilities that handle toxic substances without appropriate protective measures are at serious risk of exposure (Landrigan & Baker, 1991). If precautions are not taken, such occupational poisonings can cause both short-term acute effects and long-term chronic health problems.

2.4.4. Suicidal Poisoning

It is the most common cause of death from poisoning. This situation poses a significant social problem worldwide. The easy availability of toxic chemicals and their accessibility to individuals intent on suicide have increased the frequency of suicidal poisonings. While cyanide, arsenic, and other toxic substances are rare, the most common poisons in suicide cases are prescription drugs. Individuals with depression and other psychiatric disorders have easy access to prescribed medications, which can be lethal if taken excessively. Pesticides and insecticides are used by agricultural workers, and aspirin and paracetamol in urban areas are sometimes used in suicide attempts, either accidentally or intentionally ingesting strong acids or bases (Hawton, 2009).

2.4.5. Criminal Poisonings:

It is a significant topic in the field of forensic medicine. The fundamental tasks of forensic toxicology include determining the type, quantity and characteristics of the poison used for lethal purposes, and presenting this information to the judiciary. Drugs and chemicals found at the scene must be meticulously collected, properly preserved, and analyzed. While the number of poisons used for criminal purposes is generally limited, such cases have serious forensic and legal implications. These classifications are critical for understanding the etiology of poisonings and determining appropriate treatment and intervention strategies (Trestrail, 2007).

3. Clinical Findings and Diagnosis

As poisoning cases can present with a wide variety of clinical findings, a systematic approach is necessary for physicians. Due to changes in consciousness and difficulty in cooperation, the history must often be obtained from additional sources such as relatives, witnesses, or official records. Important clues to consider include the patient's occupation, crime scene findings, empty medication containers, a distinctive odor in the environment, and the suspicion of suicide. Preventing secondary exposure is critical, particularly in inhalation poisonings. Accurate identification of the substance of exposure and access to reliable product information are essential for the effectiveness of treatment.

3.1. General Clinical Findings

Depending on the substance, dose and route of exposure, poisonings can present with a variety of clinical findings affecting different systems. (Vale & Meredith, 2012; Nelson et al., 2019). The most common findings are summarized by system below:

- Gastrointestinal system: Nausea, vomiting, abdominal pain, diarrhea, burning sensation in the mouth and throat.
- Central nervous system: Dizziness, headache, confusion, agitation, seizures, and in advanced cases, coma.
- Respiratory system: Dyspnea, hyperventilation, bronchospasm, cyanosis, and respiratory depression.
- Cardiovascular system: Tachycardia, bradycardia, arrhythmia, hypotension or hypertension, circulatory collapse.
- Skin and mucosa: Pallor, redness, sweating, dryness, burning, or rashes.
- Other findings: Renal failure, hepatotoxicity, metabolic acidosis, or alkalosis.

3.2. Specific Poisoning Findings

Some types of poisoning can be identified by their distinctive clinical symptoms. Knowing these findings is important for rapid diagnosis and initiation of appropriate treatment:

- Carbon monoxide poisoning: Headache, weakness, confusion; classic but rare cherry-red skin color (Weaver, 2009).

- Organophosphate/pesticide poisoning: Miosis (pinpoint pupil), bronchosurgery, bradycardia, muscle fasciculations (Eddleston, 2008).
- Opioid poisoning: Loss of consciousness, miosis, respiratory depression (Kim & Nelson, 2016).
- Paracetamol (acetaminophen) poisoning: Nausea and vomiting in the first 24 hours; signs of hepatic failure within 2–3 days (Prescott, 2000).
- Salicylate poisoning: Tinnitus, hyperventilation, metabolic acidosis (Chyka et al., 2007).
- Mushroom poisoning (Amanita phalloides): Delayed nausea, vomiting, and diarrhea; later hepatic and renal failure.
- Alcohol poisoning:
  - Ethanol: Confusion, loss of coordination.
  - Methanol: Visual disturbances, metabolic acidosis.
  - Ethylene glycol: Renal failure.

3.3. Diagnostic Approach

A systematic approach should be taken to ensure an accurate diagnosis of poisoning. (Nelson et al., 2019; Tintinalli, 2020; Hoffman, 2007).

3.3.1. Crime scene assessment

- The environment where the exposure occurred must be examined.
- Chemical containers, medication containers, and food and beverage residue should be preserved if possible and delivered to the medical team.
- In cases of animal bites/stings, the animal species or specimen (if available) is important for diagnosis and treatment.
- This information plays a critical role in correctly identifying the toxic substance and selecting the appropriate antidote/treatment.

3.3.2. History

- The type of substance ingested, the amount, the time of ingestion, and the route of exposure (oral, inhalation, dermal, injection) should be questioned in detail.
- Assessing whether this was a suicide attempt or an accident is important for both diagnosis and subsequent treatment/psychiatric support.
- The patient's other medications, chronic diseases, and allergy history should be asked.
- Chemical containers, medication containers, food and beverage residue, or animal samples found at the scene should be preserved, if possible, and delivered to the healthcare team.

3.3.3. Physical examination

- Vital signs (ABC: Airway, Breathing, Circulation) should be assessed first.
- Vital signs (temperature, pulse, blood pressure, respiratory rate, O_2 saturation) should be recorded.
- Pupillary changes (miosis, mydriasis, anisocoria),
- Skin findings (sweating, dryness, redness, rash, color changes),
- Neurological examination (level of consciousness, confusion, coma, seizures),
- Respiratory patterns (hyperventilation, respiratory depression, Kussmaul breathing) may provide specific clues to poisoning.

3.3.4. Laboratory investigations

- Blood chemistry, complete blood count, and liver and kidney function tests should be performed.
- Acidosis/alkalosis should be assessed with blood gas analysis.
- Toxicology screenings should be performed:
- Cardiac arrhythmias or QT prolongation should be investigated by ECG when necessary.

3.3.5. Imaging

- Chest X-ray: Should be obtained for evaluation in cases of suspected aspiration pneumonia, pulmonary edema, or foreign body aspiration.
- CT or MRI: Should be preferred in cases of central nervous system involvement, head trauma, or focal neurological findings.

3.4. Toxidromes

A 'toxidrome' is a set of typical clinical findings that are specific to certain groups of toxins. Prompt diagnosis and rapid intervention are crucial (Woolf et al., 2007; Tintinalli, 2020; Olson et al., 2007).

- Cholinergic toxidry (e.g., organophosphates): Increased salivation, lacrimation, urination, and stool, nausea, bronchorhea (SLUDGE syndrome).
- Anticholinergic toxidry (e.g., atropine, antihistamines, tricyclic antidepressants): Dry skin, dilated pupils, tachycardia, hallucinations, and urinary retention.
- Opioid toxidry: Miosis, respiratory depression, and loss of consciousness.
- Sympathomimetic toxidry (e.g., cocaine, amphetamines): Mydriasis, hypertension, tachycardia, agitation, and sweating.
- Sedative-hypnotic toxidry (e.g., benzodiazepines, barbiturates): Drowsiness, ataxia, and respiratory depression.

4. FIRST AID APPROACH

In cases of poisoning, first aid plays a critical role in saving the patient's life and preventing complications. While first aid practices vary depending on the route of exposure, the primary goals are to preserve vital functions, reduce toxin absorption, and refer the patient to professional healthcare. Improper administration can worsen the patient's condition.

4.1. General Prinsiples

4.1.1. Security

The most important step in first aid is ensuring the safety of the first-aider and those around them. In cases of poisoning, the environment may still contain toxic gases, smoke, chemicals or contaminated surfaces. Therefore, the patient should not be treated without first removing them from the toxic environment and taking

the necessary protective measures. The environment should be made safe and well ventilated if possible, and personal protective equipment such as gloves and masks should be worn. This will prevent exposure to both the first aider and others.

4.1.2. Evaluation of vital signs (ABCDE)

4.1.2.1. Airway

The patient's airway should be assessed promptly and any obstructions, such as dentures, tablet fragments or secretions, should be removed. If the patient is having difficulty breathing, the priority should be to establish an open airway using simple manoeuvres such as the head-chin position or the jaw thrust manoeuvre, or by using appropriate airway aids. Patients with a Glasgow Coma Scale (GCS) of 8 or less should undergo definitive airway security with intubation and mechanical ventilation. Furthermore, if corrosive substances have been ingested, careful assessment should be made due to the risk of oropharyngeal edema, and maintaining a patent airway should be a priority (Avva et al., 2023; Morrison & Sandilands, 2024).

4.1.2.2. Breathing

The presence, rate and quality of breathing should be assessed (Thim et al., 2012; Morrison & Sandilands, 2024).

- **Evaluation**

  - Respiratory rate, depth, and pattern should be measured.
  - Oxygen saturation should be monitored with pulse oximetry; however, it can be misleading when saturation is below 70% or in the presence of carboxyhemoglobin/methemoglobinemia.
  - If there is clinical suspicion, confirmation should be made with arterial blood gas (ABG) analysis, and targeted therapy should be planned.

- **Reversible causes:**

  - If hypoventilation is detected, sedative toxicity (especially opioids, benzodiazepines, GHB, baclofen) should be considered.
  - Opiate intoxication: This presents with miosis, altered consciousness, and respiratory depression. In this case, the primary treatment is naloxone.

- Naloxone should preferably be administered IV, and the dose and repeat administrations should be adjusted according to the clinical situation (e.g., impending respiratory arrest, suspicion of opioid dependence).
- It should be noted that repeated doses may be required because naloxone has a shorter half-life than most opioids.
- Therefore, close respiratory monitoring is mandatory.

➢ Benzodiazepine poisoning: Flumazenil can be used in some cases.

- However, its routine diagnostic use is not recommended due to the risk of seizures.
- It is also contraindicated in patients with proconvulsant drug or unknown substance ingestion, sodium channel blockade, or signs of stimulant toxidrome (prolonged QRS, tachycardia, fever, mydriasis).

- **Special situations:**

➢ Carboxyhemoglobin and methemoglobin levels should be measured in all patients with respiratory failure, and treatment (e.g., hyperbaric O_2, methylene blue) should be administered if necessary.

➢ If respiratory failure does not improve with antidote, emergency intubation and ventilation should be provided.

4.1.2.3. Circulation

- In all toxicology cases, vital signs (pulse, blood pressure, respiratory rate, temperature, oxygen saturation) must be checked and monitored at regular intervals.
- An ECG should be obtained immediately in patients with moderate or severe poisoning or who have ingested cardiotoxic substances.
- Continuous cardiac monitoring should be implemented when necessary; this allows for real-time monitoring of arrhythmia risk and assessment of the effectiveness of treatment.

Reversible causes and approach:

➢ Hypotension: IV fluid replacement is the first step; if there is no response, vasopressor therapy is considered.

- Arrhythmias:
  - QRS widening due to sodium channel blocking drugs → IV sodium bicarbonate.
  - Beta-blocker/calcium channel blocker toxicity → high-dose insulin, glucagon, or calcium.
  - Digoxin poisoning → antidigoxin antibodies.
- Hypertension: If it is due to sympathomimetics (cocaine, amphetamine), benzodiazepines should be the first choice; beta-blockers should not be given alone.

4.1.2.4. Disability

- **General evaluation:**
  - Impaired consciousness is a common clinical presentation in poisonings. Therefore, careful and repeated assessment is mandatory to determine the need for intervention.
  - The Glasgow Coma Scale (GCS) should be recorded and regularly monitored.
  - Pupil size, shape, and light reflexes should be assessed. (Miosis → opioid; mydriasis → anticholinergic/stimulant toxidrome may be clues.)
  - Identifying a cluster of symptoms can help diagnose toxic syndromes (toxidromes) or specific poisons.
  - Hypoglycemia and head trauma must be excluded in all patients who present unconscious.
- **Hypoglycemia:**
  - It should be noted that many poisons (e.g., salicylates, ethanol, insulin, oral antidiabetic agents) can cause hypoglycemia.
  - Capillary blood glucose should be measured in all patients presenting with poisoning, followed by official laboratory glucose levels.
  - If hypoglycemia is detected, IV glucose therapy should be initiated immediately.
- **Other neurological findings:**
  - Seizures: May be seen with agents such as TCAs, tramadol, cocaine, theophylline, etc. → Benzodiazepines are the first-line treatment.

- Agitation/Delirium: Often seen with sympathomimetic or anticholinergic poisoning → Benzodiazepines are preferred for treatment.
- Coma (GCS ≤ 8): Intubation should be considered for airway security.

4.1.2.5. Disorders in Electrolyte and Acid-Base Balance

Electrolyte and acid-base imbalances are associated with significant morbidity and mortality. Therefore, it is crucial to recognise them early and initiate appropriate treatment. Changes in potassium, sodium and glucose levels are among the most common complications of poisoning.

4.1.3. History

In cases of poisoning, the patient's medical history is critical to the diagnosis and treatment process. Information obtained from the patient or their relatives is key to guiding the clinical approach (Aki & Alessai, 2019).

The following points should be specifically questioned when taking the history:

- Substance ingested: The name and content of the substance that caused the poisoning.
- Amount: The estimated or exact amount of the substance ingested.
- Time: When the exposure occurred.
- Route of administration: Oral, inhaled, dermal, or intravenous routes.

Additionally:

- Materials such as medication containers, pesticide bottles, and cleaning or chemical containers should be brought with the patient for examination, if possible.
- Information obtained from witnesses can be particularly valuable in patients with altered consciousness.

4.1.4. Physical Examination

In cases of poisoning, the physical examination guides the diagnosis and treatment process, beginning with the assessment of vital signs. Furthermore, specific poisoning syndromes (toxidromes) should also be investigated (Aki & Alessai, 2019).

Key points to consider during the examination:

- Vital signs: Temperature, pulse, blood pressure, respiratory rate, and oxygen saturation are assessed.
- Specific toxidromes:
  - E.g., miosis + bronchorexia + bradycardia → organophosphate poisoning
  - mydriasis + tachycardia + dry skin → anticholinergic poisoning
  - hypothermia + bradycardia + hypoventilation → opioid poisoning
- Skin findings: Sweating, flushing, cyanosis, rash, or burns may provide clues to exposure.
- Pupillary changes: Miosis, mydriasis, or changes in light reflexes suggest a toxic etiology.
- Neurologic status: Level of consciousness (Glasgow Coma Scale), agitation, convulsions, motor abnormalities, or coma are assessed.
- Respiratory and cardiac findings: Respiratory depression, wheezing, arrhythmias, and conduction disturbances are investigated.

4.1.5. Laboratory and Imaging

In cases of poisoning, laboratory and imaging tests are of critical importance for both confirming the diagnosis and early detection of complications.

Basic examinations:

- Blood glucose: Hypoglycemia or hyperglycemia is assessed.
- Blood gas: Provides information about acid-base status, hypoxia, and lactate levels.
- ECG: Cardiac conduction disturbances, arrhythmias, and QT prolongation may be detected.
- Electrolytes: Especially sodium, potassium, calcium, and magnesium.
- Kidney and liver function tests: Creatinine, urea, AST, ALT, and bilirubin.

Specific toxin levels (if available):

- Paracetamol
- Salicylate
- Alcohol (ethanol, methanol, ethylene glycol, isopropanol)
- Carboxyhemoglobin / Methemoglobin
- Therapeutic drug levels such as lithium, digoxin, theophylline

Imaging methods:

- Chest X-ray: To evaluate aspiration pneumonia, pulmonary edema, or chemical pneumonia.
- CT/MRI: In cases where trauma, intracranial hemorrhage, or toxin-related encephalopathy is suspected.
- Ultrasonography: May contribute to kidney function, bladder filling, and fluid and electrolyte balance.

4.2. First Aid According to Poisoning Route

The first aid required for poisoning varies depending on how the toxin entered the body. Choosing the right approach is critical for the patient's prognosis (Mayo Clinic Staff, n.d.; Missouri Poison Center, n.d.).

4.2.1. Oral poisonings

What to do:

- The patient should never be induced to vomit. Vomiting, especially when caustic or corrosive substances (acid, alkali, bleach, etc.) are ingested, can cause additional burns to the esophageal and gastric mucosa. Furthermore, the risk of aspiration can damage the respiratory tract.
- A conscious patient's mouth can be rinsed with water. However, the patient should not swallow the water; they should simply rinse their mouth and spit it out.
- The substance causing the poisoning should be identified, if possible, and the packaging should be provided to the healthcare team.
- The patient should be transported to a healthcare facility as quickly as possible.

Things Not to Do:

- Traditional methods such as milk, yogurt, salt water, and vinegar should never be used. Such practices may increase the effects of the toxic substance or lead to delayed complications.
- Nothing should be given orally to anyone who is unconscious, experiencing a seizure, or whose swallowing reflexes are impaired.

4.2.2. Inhalation poisoning

Inhalation poisoning occurs when gases, vapours or aerosols such as carbon monoxide, methane, chlorine, ammonia, pesticides and smoke from fires are breathed in. In this type of poisoning, the toxic substance passes rapidly from the lungs into the bloodstream, causing systemic effects quickly.

What to do:

- First and foremost, the first aider must ensure their own safety; protective measures should be taken when entering a toxic environment.
- The patient should be immediately removed to fresh air or the area should be ventilated.
- If the patient has stopped breathing or is breathing inadequate, artificial respiration and basic life support should be initiated.
- It is beneficial to administer oxygen to a conscious patient (by the healthcare team).
- The patient should be transported quickly to a healthcare facility.

Things Not to Do:

- First aiders should never enter a toxic environment without protective equipment.
- The patient should not be given any liquids or food by mouth.
- Do not attempt to move the patient in hopes of "recovering"; movement can increase the effects of the toxin.

4.2.3. Cutaneous poisonings

Dermal poisoning occurs when the skin comes into contact with pesticides, industrial chemicals, acids, bases, petroleum derivatives and certain toxic plant or animal secretions. These substances can be absorbed through the skin, resulting in either systemic effects or local chemical burns.

What to do:

- The first aider must ensure their own protection and use barrier methods such as gloves.
- Contaminated clothing should be carefully removed from the patient, and areas in contact with the skin should be washed with plenty of soap and water for at least 15–20 minutes.
- Neutralization should not be achieved by prolonged rinsing of skin areas exposed to acids or bases; only rinsing with plenty of water should be performed.
- If there is a large burn or irritation, the patient should be covered with a sterile drape and referred to a healthcare facility.

Things Not to Do:

- Additional chemicals, such as vinegar, alcohol and detergent, should not be applied to the skin as this can increase tissue damage.damage.
- Forcibly scraping or rubbing the substance stuck to the skin is inappropriate; this can cause further absorption of the toxin.
- Traditional methods (e.g., applying oil or dusting with flour) should not be used.

4.2.4. Eye poisoning

Eye poisoning occurs when the eyes come into direct contact with cleaning products, acids, bases, pesticides, industrial chemicals or gases. Such exposure can cause serious damage to the cornea and conjunctiva, leading to complications that may result in vision loss.

What to do:

- The first aider should ensure their own protection (e.g., wearing gloves).

- The eye should be immediately flushed with plenty of clean water for at least 15–20 minutes. The eyelids should be held open during flushing, and the water should be applied so that it flows outward from the eye.
- Contact lenses, if present, should be removed.
- The type of chemical (acid, base, detergent, etc.) should be determined, if possible, and the healthcare team should be informed.
- The patient should be referred to an ophthalmologist.

Things Not to Do:

- Eyes should never be rubbed; this increases tissue damage.
- Milk, oil, drops, or other chemicals should not be placed in the eyes.
- Rinsing time should not be short; inadequate rinsing can cause permanent damage.

Animal bites and stings:

Animal bites and stings are a significant source of contact that can lead to poisoning. Stings from venomous animals, such as snakes, scorpions, bees and spiders, as well as bites from mammals, such as dogs and cats, can have serious local and systemic effects. In such cases, the effects of the venom and the risk of infection must both be considered.

What to do:

- First, the patient should be calmed and encouraged to avoid unnecessary movements, as movement causes the venom to spread more rapidly into the bloodstream.
- The bite or sting site should be gently cleaned with soap and water and covered with a sterile dressing.
- In snakebites, the bitten limb should be kept immobile and slightly below heart level.
- In venomous animal bites, a tourniquet should not be applied, and the wound should not be cut or suctioned.
- If signs of an allergic reaction (especially anaphylaxis for bee stings) are observed, emergency medical attention should be sought immediately.

- In animal bites that carry a risk of rabies, the patient should be referred to a healthcare facility after wound cleaning, and rabies vaccination and tetanus prophylaxis should be evaluated.

Things Not to Do:

- Cutting, suctioning, burning, or chemical application to the wound should be avoided.
- Ice should not be applied directly to the sting area (this may increase tissue damage).
- The commonly known "sucking out the poison" method should never be used.

5. HOSPITAL TREATMENT AND ANTIDOTES

The primary goals when treating cases of poisoning in a hospital setting are to preserve vital functions, prevent or reduce the absorption of the toxin, and eliminate it from the body. If necessary, specific antidote therapy will also be administered. The treatment process consists of the following steps:

5.1. Supportive Treatment (General Approach)

In cases of poisoning, the first and most important step is to stabilise the patient's vital functions before initiating specific treatment. Supportive care encompasses basic approaches that should be applied to all types of poisoning, significantly reducing mortality (Chandran & Krishna, 2019).

5.1.1. Airway, Breathing, and Circulation (ABC) Assessment

- Airway: In patients with decreased consciousness, the airway should be secured to prevent the risk of aspiration. Intubation is performed if necessary.
- Breathing: Assess for hypoxia and respiratory depression. Oxygen and ventilatory support may be provided.
- Circulation: Pulse, blood pressure, and capillary refill are assessed. Intravenous fluid replacement is initiated in cases of hypotension; vasopressor agents may be used in resistant cases.

5.1.2. Monitoring of Vital Signs

- Pulse, respiratory rate, blood pressure, oxygen saturation, and level of consciousness should be monitored regularly.

- Cardiac monitoring should be performed, and the patient should be monitored for rhythm disturbances.

5.1.3. Evaluation of Neurological Status

- Level of consciousness, pupillary reactions, and motor responses should be monitored.
- Benzodiazepines (e.g., diazepam) may be used in the presence of seizures.

5.1.4. Fluid-Electrolyte and Acid-Base Balance

- Dehydration, electrolyte disturbances, and metabolic acidosis are common in poisonings.
- Appropriate intravenous fluid therapy and acid-base adjustment should be administered when necessary.

5.1.5. Body Temperature Control

- Depending on the type of poisoning, hyperthermia or hypothermia may develop.
- Body temperature should be maintained within normal limits with active cooling or warming methods.

5.1.6. Symptomatic Treatment

- Nausea, vomiting, agitation, pain and other symptoms should be controlled with appropriate pharmacological agents.

5.2. Reducing Toxin Absorption

One of the key steps in treating poisoning is to minimise the absorption of the ingested toxic substance into the bloodstream. This approach is particularly effective in patients who present early, i.e. within the first hour. The methods used vary depending on the type and dose of toxin ingested, how much time has elapsed, and the patient's clinical condition (Morrison & Sandilands, 2024; Ünaldı, 2015).

5.2.1. Activated Charcoal

- It is used to prevent the gastrointestinal absorption of many drugs and chemicals.

- It is most effective in the first 60 minutes after ingestion; however, it may also be beneficial later in the course of some drugs (e.g., slow-release preparations, theophylline, carbamazepine).
- A single dose (usually 1 g/kg) is administered. Multiple doses may be administered in selected cases.
- Contraindications: Should not be used in patients with caustic (acid, base) or hydrocarbon poisoning, or in unconscious patients (unless the airway is secured).

5.2.2. Gastric Lavage (Stomach Washing)

- It is particularly used for life-threatening toxins that have been ingested recently (usually <1 hour).
- It is administered through a large-bore orogastric tube.
- Risks: Aspiration pneumonia, mechanical injury, electrolyte disturbances.
- It can only be administered after intubation in unconscious patients.

5.2.3. Cathartics

- It supports toxin elimination by accelerating intestinal passage.
- Osmotic laxatives such as magnesium citrate and sodium sulfate can be used.
- Their use alone is not recommended; they are usually administered in conjunction with activated charcoal.

5.2.4. Complete Bowel Irrigation

- It is based on the principle of bowel cleansing with a polyethylene glycol solution.
- It can be used with slow-release medications, iron tablets, lithium, and packaged medications.
- However, it is contraindicated in patients with cardiopulmonary instability or bowel obstruction.

5.3. Increasing Elimination

It is crucial to rapidly remove the ingested toxic substance from the body after it enters the systemic circulation in cases of poisoning. These methods are particularly applicable in cases where the toxin causes severe symptoms and the

body is unable to eliminate it naturally. (Morrison & Sandilands, 2024; Ünaldı, 2015).

5.3.1. Forced Diuresis

- The goal is to increase urine output by administering intravenous fluids.
- It can be used especially for toxins that are eliminated by the kidneys (e.g., some heavy metals, salicylates).
- However, it should be administered with caution due to the risk of fluid overload, electrolyte disturbances, and heart failure.

5.3.2. Urinary Alkalinization

- Urine pH is increased to 7.5–8.0 with sodium bicarbonate infusion.
- It accelerates the elimination of weakly acidic drugs (salicylates, phenobarbital).
- Electrolyte and acid-base balance should be closely monitored.

5.3.3. Hemodialysis

- It is effective when the toxin is low molecular weight, water-soluble, poorly protein-bound, and has a small volume of distribution.
- It is used in poisonings caused by methanol, ethylene glycol, lithium, salicylate, and phenobarbital.
- It is also preferred for severe metabolic acidosis and fluid-electrolyte imbalances.

5.3.4. Hemoperfusion

- It is based on the principle of passing blood through cartridges containing activated charcoal or resin.
- It can be particularly useful for toxins that are more protein-bound (e.g., theophylline, carbamazepine).

5.3.5. Other Techniques

- Hemodiafiltration: A combination of hemodialysis and hemofiltration; it offers advantages for some toxins.
- Plasmapheresis: Used to remove toxins that bind strongly to plasma proteins.

5.4. Antidotes

An antidote is a specific treatment that neutralises the effects of a toxin, blocks its action at the receptor level or speeds up its elimination by altering its metabolism. Antidotes can be lifesaving in cases of poisoning. However, as antidotes are not available for every toxin, supportive care is the primary approach in most cases (Chandran & Krishna, 2019; Morrison & Sandilands, 2024; Ünaldı, 2015; Thompson, et al., 2014).

5.4.1. Mechanisms of Action of Antidotes

- Direct chemical neutralization: Inactivating the toxic substance by binding to it (e.g., chelators – deferoxamine, dimercaprol).
- Pharmacological antagonism: Blocking the effect of the toxin at the receptor (e.g., naloxone – opioid receptor antagonist).
- Preventing toxic metabolite formation: Preventing the formation of toxic metabolites (e.g., fomepizole – alcohol dehydrogenase inhibitor).
- Physiological antagonism: Producing a pharmacological effect opposite to the effect of the toxin (e.g., atropine – counteracting excessive parasympathetic activity in organophosphate poisoning).

5.4.2. Basic Principles and Commonly Used Antidotes in Antidote Use

- Antidotes should only be used for the correct indication and at the appropriate dose.
- Identifying the substance causing the poisoning before administration is essential.
- The risk of complications can be high if some antidotes (e.g., flumazenil) are used incorrectly.
- It is vital that antidote stocks are kept adequate and up-to-date in healthcare facilities.

Table 1. Some examples of poisons and antidotes

Poison/Toxin	Antidote	Description
Opioids	Naloxone	It rapidly reverses respiratory depression.
Benzodiazepines	Flumazenil	It is used in selected cases; caution should be exercised in those with a history of epilepsy.
Organophosphates	Atropine + Pralidoxime (2-PAM)	Atropine provides muscarinic effects, while pralidoxime causes acetylcholinesterase reactivation.
Paracetamol	N-acetylcysteine (NAC)	It prevents liver necrosis and is most effective when administered within the first 8–10 hours.
Iron	Deferoxamine	It provides iron chelation.
Lead, mercury, arsenic	Dimercaptopropanesulfonate (DMPS), EDTA, BAL (Dimercaprol)	It is used in heavy metal poisoning.
Methanol, ethylene glycol	Fomepizole or Ethanol	It inhibits alcohol dehydrogenase, preventing the formation of toxic metabolites.
Carbon monoxide	100% Oxygen, Hyperbaric Oxygen	It rapidly reduces carboxyhemoglobin.
Cyanide	Hydroxocobalamin, Sodium thiosulfate	It provides detoxification by binding cyanide.

6. REFERENCES

Aki, E. S., & Alessai, J. (2019). General Approach to Poisoned. Poisoning in the Modern World: New Tricks for an Old Dog?, 3.

Avva, U., Lata, J. M., & Kiel, J. (2023). Airway management. In StatPearls [Internet]. StatPearls Publishing.

Chandran, J., & Krishna, B. (2019). Initial management of poisoned patient. Indian journal of critical care medicine: peer-reviewed, official publication of Indian Society of Critical Care Medicine, 23(Suppl 4), S234.

Chyka, P. A., Erdman, A. R., Christianson, G., Wax, P. M., Booze, L. L., Manoguerra, A. S., ... & Troutman, W. G. (2007). Salicylate poisoning: an evidence-based consensus guideline for out-of-hospital management. Clinical toxicology, 45(2), 95-131.

Eddleston, M., Buckley, N. A., Eyer, P., & Dawson, A. H. (2008). Management of acute organophosphorus pesticide poisoning. The Lancet, 371(9612), 597-607.

Ellenhorn MJ, Barceloux DG. *Medical Toxicology: Diagnosis and Treatment of Human Poisoning*. New York: Elsevier; 1997.

Hawton, K. (2009). van HK. Suicide. Lancet, 373(9672), 1372-81.

Him, T., Topçuoğlu, S., & Muratoğlu, K. (2025). Gıda Kaynaklı "Büyük Altı" Tehlikesi. Dicle Üniversitesi Veteriner Fakültesi Dergisi, 18(1), 79-85.

Hodgson, E. (Ed.). (2011). A textbook of modern toxicology. John Wiley & Sons.

Hoffman RS, Nelson LS, Howland MA. *Manual of Emergency and Critical Care Toxicology*. Cambridge University Press; 2007.

Hoffman, R. S. (2007). Understanding the limitations of retrospective analyses of poison center data. Clinical Toxicology, 45(8), 943-945.

Kim, H. K., & Nelson, L. S. (2016). Reversal of opioid-induced ventilatory depression using low-dose naloxone (0.04 mg): a case series. Journal of Medical Toxicology, 12(1), 107-110.

Lamireau, T., Llanas, B., Deprez, C., El Hammar, F., Vergnes, P., Demarquez, J. L., & Favarel-Garrigues, J. C. (1997). Severity of ingestion of caustic substance in children. Archives de Pediatrie: Organe Officiel de la Societe Francaise de Pediatrie, 4(6), 529-534.

Landrigan, P. J., & Baker, D. B. (1991). The recognition and control of occupational disease. Jama, 266(5), 676-680.

Mayo Clinic Staff. (t.y.). Poisoning: first aid. Mayo Clinic. Erişim adresi: **https://www.mayoclinic.org/first-aid/first-aid-poisoning/basics/art-20056657**

Missouri Poison Center. (t.y.). Skin exposure and inhaled poison first aid. Missouri Poison Center. Erişim adresi: https://missouripoisoncenter.org/first-aid-poisoning/

Morrison, E. E., & Sandilands, E. A. (2024). Principles of management of the poisoned patient. Medicine, 52(6), 334-339.

Mowry JB, Spyker DA, Brooks DE, Zimmerman A, Schauben JL. 2018 Annual Report of the American Association of Poison Control Centers' National Poison Data System (NPDS): 36th Annual Report. *Clin Toxicol*. 2019;57(12):1220–1413.

Mowry, J. B., Spyker, D. A., Brooks, D. E., Zimmerman, A., & Schauben, J. L. (2016). 2015 Annual report of the American association of poison control centers' National Poison Data System (NPDS): 33rd Annual Report. Clinical toxicology, 54(10), 924-1109.

Nelson LS, Howland MA, Lewin NA, Smith SW, Goldfrank LR, Hoffman RS (eds). *Goldfrank's Toxicologic Emergencies*. 11th ed. New York: McGraw-Hill; 2019.

Olson, K. R., Anderson, I. B., Benowitz, N. L., Blanc, P. D., Clark, R. F., Kearney, T. E., ... & Wu, A. H. (Eds.). (2007). Poisoning & drug overdose (Vol. 13). Lange Medical Books/McGraw-Hill.

Peden, M., Oyegbite, K., Ozanne-Smith, J., Hyder, A. A., Branche, C., Rahman, A. F., ... & Bartolomeos, K. (2008). Poisoning. In World Report on Child Injury Prevention. World Health Organization.

Peter, J. V., & Cherian, A. M. (2000). Organic insecticides. Anaesthesia and intensive care, 28(1), 11-21.

Phillips, J., Beam, S., Brinker, A., Holquist, C., Honig, P., Lee, L. Y., & Pamer, C. (2001). Retrospective analysis of mortalities associated with medication errors. American journal of health-system pharmacy, 58(19), 1835-1841.

Prescott, L. F. (2000). Paracetamol: past, present, and future. American journal of therapeutics, 7(2), 143-148.

Raub JA, Vosk T. Carbon monoxide poisoning—a public health perspective. *Toxicology*. 2008;145(1):1–14.

Roberts, S. M., James, R. C., & Williams, P. L. (Eds.). (2022). Principles of toxicology: environmental and industrial applications. John Wiley & Sons.

Thim, T., Krarup, N. H. V., Grove, E. L., Rohde, C. V., & Løfgren, B. (2012). Initial assessment and treatment with the Airway, Breathing, Circulation, Disability, Exposure (ABCDE) approach. International journal of general medicine, 117-121.

Thompson, T. M., Theobald, J., Lu, J., & Erickson, T. B. (2014). The general approach to the poisoned patient. Disease-a-Month, 60(11), 509-524.

Tintinalli JE. *Tintinalli's Emergency Medicine: A Comprehensive Study Guide*. 9th ed. McGraw-Hill; 2020.

Trestrail III, J. H. (2007). Criminal poisoning: Investigational guide for law enforcement, toxicologists, forensic scientists, and attorneys. Totowa, NJ: Humana Press.

Ünaldı, Y. C. (2015). Acil Servise Başvuran Zehirlenme Olgularının Değerlendirilmesi, Çukurova Üniversitesi Tıp Fakültesi Acil Tıp Anabilim Dalı, Uzmanlık Tezi.

Vale, J. A., & Meredith, T. J. (Eds.). (2012). Poisoning diagnosis and treatment. Springer Science & Business Media.

Weaver, L. K. (2009). Carbon monoxide poisoning. New England Journal of Medicine, 360(12), 1217-1225.

Woolf, A. D., Erdman, A. R., Nelson, L. S., Caravati, E. M., Cobaugh, D. J., Booze, L. L., ... & Troutman, W. G. (2007). Tricyclic antidepressant poisoning: an evidence-based consensus guideline for out-of-hospital management. Clinical Toxicology, 45(3), 203-233.

World Health Organization (WHO). Poisoning prevention and management. 2021.

CHAPTER 5

ADVANCES IN MICROBIAL METABOLOMICS FOR HEALTH SCIENCES

Belgin ERDEM[1]

1. INTRODUCTION

These days, microorganisms are great for biological research because of their ease of handling and significance for human health and the biosphere. Researchers can learn more about complicated biological processes and come up with new ways to fight diseases by studying microorganisms. They are useful models for learning about complicated biological processes because they are small, grow quickly, and can live in many different places (Ma'ayan, 2017). Metabolomics involves the systematic detection and measurement of metabolites, which are generally low molecular weight compounds with diverse chemical properties, in biological materials (Madsen, 2005). Metabolites are classified into hydrophilic polar compounds, such as sugars and amino acids, and hydrophobic non-polar compounds, like fatty acids (Hu, et al., 2020).

Endogenous metabolites are produced by the organism's enzymes, while exogenous metabolites originate from external sources like food and pollutants. Unlike other omics disciplines, metabolomics serves as direct biomarkers of biochemical activity, providing a clearer association with phenotypes (Pavarini et al., 2012). Metabolomics, which catalogs the metabolic activity of cells and tissues, utilizes various materials based on the organism studied. For humans and animals, plasma, urine, saliva, muscle or epithelial tissue, and cerebrospinal fluid may be used. In plants, roots, leaves, or flower petals are analyzed, while microorganisms utilize cell pellets or growth media (Wishart, 2019). This technique can reveal how internal and external factors like age, race, obesity, stress, and medication influence cellular function.

Metabolomics has emerged as a vital tool for developing biomarkers for non-obstetric disorders like cancer and Alzheimer's disease. Alongside other "omics" technologies, it enhances diagnosis and treatment by improving the understanding of disease mechanisms (Vo & Trinh 2024). Defined as the systematic study of metabolic profiles in biological samples, metabolomics examines molecules that are byproducts of metabolic reactions and cellular

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processes, providing insights into the physiological states of cells and their environmental responses (Belhaj et al., 2021). Microbial metabolomics, which studies metabolites and interactions with the environment in microorganisms, is essential to systems biology. It helps identify pathogens, screen for mutants, and ensure food safety. It advances our understanding of microbial functions, cancer, diabetes, and interactions between microbes, which benefits environmental science, agriculture, and medicine (Douglas, 2020). The metabolic processes of bacteria and their applications in a variety of fields are better understood thanks to this research. Additionally, metabolomics studies may lead to new diagnostic and treatment methods for bacterial infections. An example is a method developed by Oliver and colleagues (Olivier & Loots 2012), to distinguish *Mycobacterium tuberculosis* from other *Mycobacterium* strains and *Pseudomonas aeruginosa*, a non-tuberculous lung infection pathogen. Additionally, research on potential tuberculosis biomarkers was conducted by Lau et al. 2015.

This chapter reviews microbial metabolomics, its challenges, technological developments, applications, and future prospects, highlighting its significance in capturing molecular signatures across disease progression

2. SAMPLE PREPARATION METHODS

Procedures for preparing microbial samples for metabolomics depend on the chosen strategy, with extracellular metabolite analysis often requiring fewer steps than the more complex procedures needed for intracellular metabolites. Microbial samples are diverse, necessitating a carefully tailored research methodology for each bacterial strain, which includes not only the choice of analytical technique but essential sample processing **(**Boness et al., 2023).

Rapidly quenching enzyme activity, separating exo- and endo-metabolomes, and fully extracting metabolites are important sample preparation procedures (Figure 1). Even though intracellular metabolism can still be impacted after sampling, it is essential to maintain low temperatures when separating extracellular metabolites to prevent negative effects.

Figure 1 shows the general procedure for "-omics" investigations that can be carried out in bacterial culture.

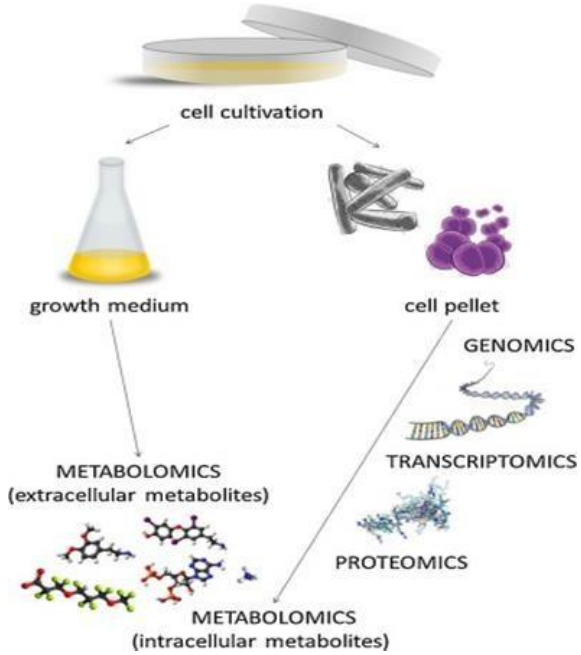

Figure 1. illustrates the workflow for bacterial culture analysis at the "omics" stage, encompassing the separation of the cell pellet from the growth medium, analysis of metabolites in the broth, and cell wall lysis. This is followed by the analysis of the bacterial genome, transcriptome, or metabolome using suitable extraction methods (Patejko et al., 2017).

3. APPLICATION AREAS OF MICROBIAL METABOLOMICS ANALYSES

Microbial metabolomics studies microbial metabolism, reflecting the physiological state of microorganisms through analyses of metabolites and metabolic pathways. It plays a crucial role in identifying biomarkers associated with various states, applicable in systems biology. Techniques can be targeted or untargeted based on research goals. Recent technological advancements facilitate accessing diverse natural products, leading to wide applications in diagnostics, drug development, agriculture, and bioremediation (Tang, 2011; Kaur, 2025). Microbial metabolomics has gained significant attention due to the importance of microbes as model organisms. Elmroth et al., (1992) conducted the initial study on bacterial contamination using GC-MS. Metabolomics is increasingly being used in microbiological fields for metabolic pathway analysis and identification, despite the complexity of microbial metabolites and genomic data availability (Villas-Boas & Bruheim 2007). Microbial metabolomics has recently produced several notable reviews, highlighting unresolved technical issues in areas such as

sample preparation, biomarker identification, and mechanism interpretation (Baidoo et al., 2012; Winder et al., 2011).

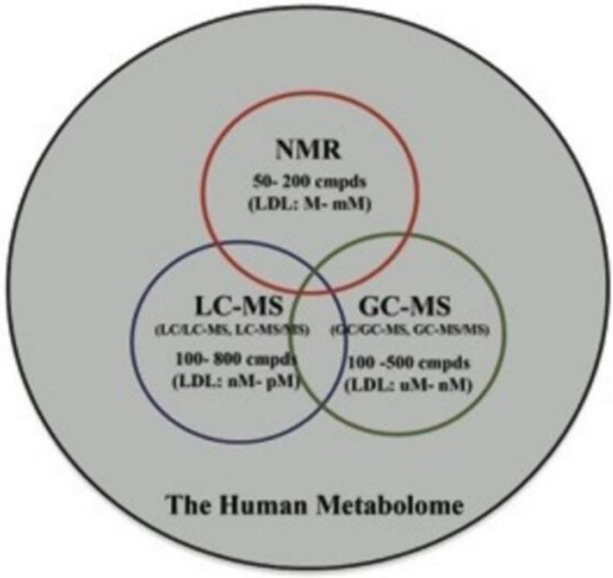

Figure 2. The most commonly used analytical platforms in metabolomics (Banoei et al., 2014)

3.1.1. Metabolomics Using Mass Spectrometry (MS)

Mass spectrometry (MS) is widely used in microbial metabolomics due to its high sensitivity and specificity. Gas Chromatography-Mass Spectrometry (GC-MS) utilizes a standard library of metabolite spectra to analyze multiple metabolites simultaneously, including organic acids, amino acids, and carbohydrates (Migne et al., 2018; Wu et al., 2018). While GC-MS can analyse metabolites quickly and accurately, it requires sample derivatisation. Recent studies have focused on microbial metabolomics utilising GC-MS (Kopka, 2006). Chloroformate derivatization of filamentous fungi was applied to obtain amino acid profiles, while microwave derivatization analyzed fatty acids in yeast samples. GC-MS technology, particularly GC-GC-MS, has enhanced sensitivity and separation for complex microbial metabolomics. Examination of *Pseudomonas aeruginosa* volatile metabolites identified new compounds, including aromatic molecules and alcohols (Smith et al., 2013).

The use of MS technology has expanded with advanced high-throughput separation techniques like GC-MS, LC-MS, and capillary electrophoresis-MS (CE-MS). GC-MS is a comprehensive technology for analyzing fatty acids,

sugars, sugar alcohols, aromatic amines, organic acids, and most amino acids simultaneously, established as a mature platform in microbial metabolomics. The Nielsen group has conducted several studies utilizing GC-MS, including the development of chloroformate derivatives for amino acid analysis in filamentous fungi, (Villas-Boas et al., 2003) a microwave derivatization method for yeast fatty acid analysis (Khoomrung et al., 2012), and a comparison of metabolic footprints of yeast mutants using GC-MS and direct infusion-MS (Mas et. al., 2007). However, GC-MS analysis necessitates a derivatization reaction.

Experimentally derived variations and the origins of chromatographic peaks are two key issues that can directly affect GC-MS analysis (Xu et al., 2010), so efficient, reproducible derivatization methods are critical elements for GC-MS metabolomics analysis and can help minimize experimental variations. The metabolomics community uses gas chromatography-mass spectrometry (GC-MS) extensively because it is a reliable and effective analytical (Beale et al., 2018).

When analysing unstable, non-volatile, and non-polar compounds without sample derivatization (Dodda et al., 2018; Zhao & Li 2018). Liquid chromatography-mass spectrometry (LC-MS) is crucial for high-throughput analysis of polar and non-polar metabolites, including coenzyme A esters and nucleic acids. However, high salt concentrations, which lower electrospray ionization efficiency, pose challenges for LC-MS in microbial metabolomics due to its limitations (Teleki & Takors, 2019). The rapid analysis provided by capillary electrophoresis-mass spectrometry (CE-MS) with minimal sample size and reagent use has been used to analyse a variety of metabolites in strains of *E. coli* and *Bacillus subtilis*, advancing our understanding of metabolism during sporogenesis and gene function investigations.

3.1.2. Nuclear Magnetic Resonance Spectroscopy (NMR) for Metabolomics

A vital technique for identifying organic compounds, NMR spectroscopy provides a thorough understanding of metabolites, despite its drawbacks, including low sensitivity and spectral resolution. The application of microbial metabolomics in microbiology is hampered by the intricacy and variability of intracellular metabolites. Despite these drawbacks, NMR methods are still popular for researching microorganisms, as shown by Boroujerdi et al., (2009) using *Vibrio coralliilyticus.* NMR allows for high-throughput analysis of biological samples and can map metabolic changes, such as during liquor

fermentation. However, its limited sensitivity complicates the detection of metabolites with diverse concentrations, restricting its broader use in the field (Barrilero et al., 2018).

4. ANALYSIS AND PROCESSING OF DATA

In recent years, common input preprocessing techniques used in metabolic analysis have been reviewed, focusing on baseline correction, normalization, scaling, peak alignment, detection, and quantification (Vettukattil, 2015). This preprocessing is made easier by a variety of computure program tools, such as MZ mine (a modular open-source software for mass spectrometry data processing), XCMS, (eXtensible Chromatography Mass-Spectrometry) and MET-IDEA (Metabolomics Ion-based Data Extraction Algorithm) (Myers et al., 2017), as well as proprietary computer software from businesses like Waters and ThermoFisher. Despite progress, microbial metabolomics still has problems with data analysis and signal processing, which calls for the creation of new techniques to optimise signalling systems and customise data analysis plans for various omics investigations (Fu et al., 2021).

5. MICROBIAL METABOLOMICS AND APPLICATIONS

Microbial metabolomics studies microorganisms' metabolic activities and interactions, aiding in biotechnology, agriculture, and pharmaceuticals. Mass spectrometry evaluates metabolism impact, enabling new methods for managing microbial populations and promoting beneficial interactions. Food microbiology, probiotic research, industrial microbiology, environmental microbiology, the development of antibiotics and anticancer drugs, diagnostic biomarkers, and the comprehension of disease mechanisms all depend on microbial metabolomics (Tang, 2011; Xu, et al., 2014). (Figure 3).

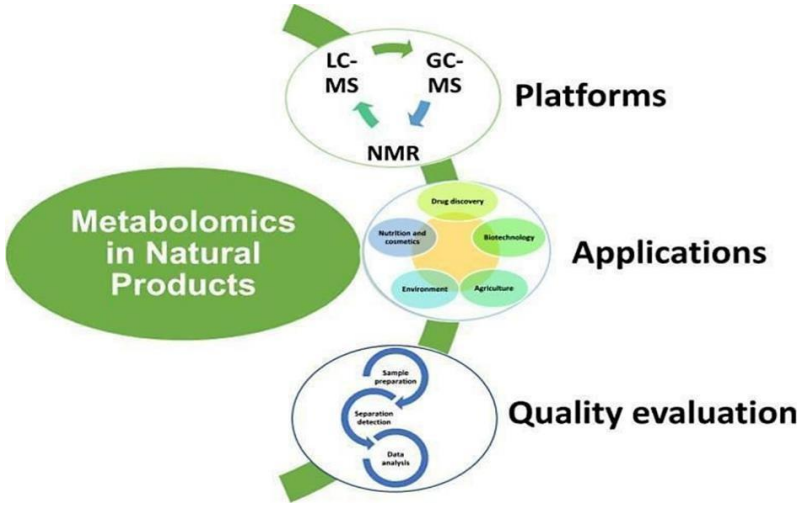

Figure 3. Applications of Metabolomics in Natural Products (Adeeyo et al., 2024)

5.1. Gut Microbiota Interactions

An important illustration of the interactions between the metabolomes of mammals and microbes, the gut microbiota is pivotal to systems biology and metabolomics (Zmora et al., 2019). Gut microbiome offers host benefits like nutrient scavenging, intestinal epithelial cell regeneration, pathogen defense, immunological support, and energy production. Metabolite analysis from co-metabolism helps understand its metabolic functions, impacting bile acid metabolism and critical host metabolic processes (Martin et al., 2012)**.** Intestinal flora, a significant contributor to human health and illness, regulate metabolic phenotypes, immunity, energy production, and infection prevention. Inadequate flora can lead to gastrointestinal and metabolic disorders. Microbial metabolomics can help researchers assess the relationship between flora and host health, as studies have linked specific changes to conditions like non-alcoholic fatty liver disease and irritable bowel syndrome (Oliphant et al., 2019).

5.2. Microbial Metabolomics, Nutrition, and Food

Metabolomics is a crucial field in food science, combining food and nutrition. It helps identify contaminants, pathogens, and byproducts of microbial degradation in food, ensuring food safety. Techniques like GC-MS and NMR are used to profile toxins, including those from GMOs. Microbial metabolomics also aids in evaluating dietary effects and monitoring toxins linked to foodborne illnesses. Recent research has shown the importance of microbial metabolomics in evaluating food safety and quality control (Qian et al., 2015).

5.3. Phytopathogens and Entomopathogens

Metabolomics is increasingly used to study plant pathogens and their diseases. The most popular method is MS, with secondary metabolites detected using GC-MS and LC-MS. MS-based metabolomics is reliable, sensitive, and repeatable. It uses separation techniques like GC and LC-MS, with LC-MS being the most popular due to its high metabolite coverage, reproducibility, specificity, and sensitivity. However, GC-MS can only detect volatile and low molecular weight substances. NMR spectroscopy is less sensitive than MS for lipid identification (Belhaj et al., 2021).

5.4. Infectious diseases

Microbial metabolomics, a technique using gas chromatography-mass spectrometry (GC-MS) and mass spectrometry (MS) to analyze secondary metabolites and bacterial mutations, is increasingly used to study pathogenic bacteria. This method identifies different bacterial species based on their metabolites and differentiates those causing diarrhea. However, issues like sample processing requirements, ion suppression, matrix effects, spectral interference, and technical complexity can hinder accurate and consistent analysis of metabolic patterns (Oyedeji et al., 2021).

5.5. Flora in the mouth

Microbial metabolomics is increasingly being used to study oral health conditions like carious teeth, periodontal diseases, cleft lip and palate, and oral tumors. PCA techniques can quickly identify bacteria like *Streptococcus mutans* and *Actinomyces viscosus*. Research shows that xylitol doesn't significantly affect dental plaque's glycolysis, while fluoride inhibits it. Salivary metabolite analysis highlights the arginine-proline pathway's importance in children with dental cavities. Microbial metabonomics is crucial for understanding dental caries communities and their impact on oral flora (Kikuchi et al., 2022).

6. Current Patterns

Microbial metabolomics has made significant progress in the past 20 years, providing insights into regulatory mechanisms and metabolic alterations. However, challenges remain, such as improved extraction techniques and standardized procedures for quenching metabolic activity. Current methods are limited to specific organisms and often fail to prevent metabolite leakage. Databases lack adequate coverage of various organisms, mainly focusing on microbes like yeast and *E. coli*. Integrated systems microbiology approaches are needed for deeper investigation of metabolic pathways and interactions. Key

metabolites identified could improve the diagnosis and treatment of inflammatory-associated diseases (Pietzner et al., 2017).

7. Conclusion

Microbial metabolomics is a fast-growing field that has significantly advanced systems microbiology. It has the potential to become a major technology platform and essential component in this field of microbiological research. The intricate relationships that exist between microorganisms and their surroundings can be studied with the help of microbial metabolomics. It could completely change how we think about microbial communities by shedding light on cellular functions and metabolic pathways. Additionally, metabolomics will improve our capacity to decipher the intricate networks found in microbial systems by integrating with other omics technologies like proteomics and genomics. This interdisciplinary approach has the potential to significantly improve our knowledge of biotechnology and microbial ecology. We as scientists have a lot of work to do in the next decade by analyzing genomics, proteomics, metabolomics, transcriptomics and microbial features using systems biology.

8. REFERENCES

Adeeyo, A. O., Amao, I. O., Ngandjui, Y. A. T., Alabi, M. A., & Msagati, T. A. M. (2024). *Metabolic profiling of plant and other natural products: Platforms, applications and quality evaluation*. *Microchemical Journal, 207*, 111779.

Baidoo, E. E., Benke, P. I., & Keasling, J. D. (2012). Mass spectrometry based microbial metabolomics. In Methods in Molecular Biology (Vol. 881, pp. 215–278).

Banoei, M.M.; Donnelly, S.J.; Mickiewicz, B.; Weljie, A.; Vogel, H.J. &Winston, B.W. (2014). Metabolomics in critical care medicine: a new approach to biomarker discovery. Clinical and Investigative Medicine, 37, E363-76.

Barrilero, R., Gil, M., Amigo, N., Dias, C. B., Wood, L. G., Garg, M. L., Ribalta, J., & Correig, X. (2018). LipSpin: A new bioinformatics tool for quantitative ^{1}H NMR lipid profiling. Analytical Chemistry, 90(3), 2031–2040.

Beale, D. J., Pinu, F. R., Kouremenos, K. A., Poojary, M. M., Narayana, V. K., Boughton, B. A., Kanojia, K., & Dias, D. A. (2018). Review of recent developments in GC–MS approaches to metabolomics based research. Metabolomics, 14(11), 1–31.

Baidoo, E. E., Benke, P. I., & Keasling, J. D. (2012). Mass spectrometry-based microbial metabolomics. In Methods in Molecular Biology (Vol. 881, pp. 215–278).

Barrilero, R., Gil, M., Amigo, N., Dias, C. B., Wood, L. G., Garg, M. L., Ribalta, J., Heras, M., Vinaixa, M., & Correig, X. (2018). LipSpin: A new bioinformatics tool for quantitative ^{1}H NMR lipid profiling. Analytical Chemistry,90(3),2031–2040.

Beale, D. J., Pinu, F. R., Kouremenos, K. A., Poojary, M. M., Narayana, V. K., Boughton, B. A., Kanojia, K., Dayalan, S., Jones, O. A. H., & Dias, D. A. (2018). Review of recent developments in GC–MS approaches to metabolomics-based research. Metabolomics, 14(11), 1–31.

Belhaj, M. R., Lawler, N. G., & Hoffman, N. J. (2021). Metabolomics and lipidomics: Expanding the molecular landscape of exercise biology. Metabolites, 11(3).

Boness, H. V. M., de Sá, H. C., Dos Santos, E. K. P., & Canuto, G. A. B. (2023). Sample preparation in microbial metabolomics: Advances and challenges. Advances in Experimental Medicine and Biology, 1439, 149–183.

Boroujerdi, A. F., Vizcaino, M. I., Meyers, A., Pollock, E. C., Huynh, S. L., Schock, T. B., Morris, P. J., & Bearden, D. W. (2009). NMR-based microbial

metabolomics and the temperature-dependent coral pathogen Vibrio coralliilyticus. Environmental Science & Technology, 43(20), 7658–7664.

Dodda S, Makula A, Polagani SR, & Kandhagatla RN (2018). High sensitive LC-MS/MS method for estimation of eluxadoline in human plasma and its application to pharmacokinetic study. Journal of Pharmaceutical and Biomedical Analysis165: 65–72.

Douglas, A. E. (2020). The microbial exometabolome: ecological resource and architect of microbial communities. Philosophical Transactions of the Royal Society B: Biological Sciences, 375(1798), 20190250.

Elmroth, I., Sundin, P., Valeur, A., Larsson, L., & Odham, G. (1992). Evaluation of chromatographic methods for the detection of bacterial contamination in biotechnical processes. Journal of Microbiological Methods, 15(1), 3.

Fu, J. B., Zhang, Y., Wang, Y. X., Zhang, H. N., & Liu, J. (2021). Optimization of metabolomic data processing using NOREVA. Nature Protocols, 17, 129–151.

Hu, Q., Tang, H., & Wang, Y. (2020). Challenges in analysis of hydrophilic metabolites using chromatography coupled with mass spectrometry. Journal of Analytical Testing, 4, 140–162.

Kaur, S., Dhiman, S., & Tripathi, M. (Eds.). (2025). Microbial metabolomics: Recent developments, challenges and future opportunities. Springer. 96-4824-5

Khoomrung, S., Chumnanpuen, P., Jansa-ard, S., Nookaew, I., & Nielsen, J. (2012). Fast and accurate preparation of fatty acid methyl esters by microwave-assisted derivatization in the yeast Saccharomyces cerevisiae. Applied Microbiology and Biotechnology, 94(6), 1637–1646.

Kikuchi, T., Hayashi, J.-i., & Mitani, A. (2022). Next-generation examination, diagnosis, and personalized medicine in periodontal disease. Journal of Personalized Medicine, 12(10), 1743.

Kopka, J. (2006). Current challenges and developments in GC-MS based metabolite profiling technology. Journal of Biotechnology, 124(1), 312–322.

Lau, S. K. P., Lam, C.-W., Curreem, S. O. T., Lee, K.-C., Lau, C. C. Y., Chow, W.-N., Ngan, A. H. Y., To, K. K. W., Chan, J. F. W., Hung, I. F. N., Yam, W.-C., Yuen, K.-Y., & Woo, P. C. Y. (2015). Identification of specific metabolites in culture supernatant of Mycobacterium tuberculosis using metabolomics: Exploration of potential biomarkers. Emerging Microbes & Infections, 4, e6.

Ma'ayan, A. (2017). Complex systems biology. Journal of the Royal Society Interface, 14(134), 20170391.

Madsen, E. (2005). Identifying microorganisms responsible for ecologically significant biogeochemical processes. Nature Reviews Microbiology, 3, 439–446.

Mas, S., Villas-Boas, S. G., Hansen, M. E., Akesson, M., & Nielsen, J. (2007). A comparison of direct infusion MS and GC-MS for metabolic footprinting of yeast mutants. Biotechnology and Bioengineering, 96(5), 1014–1022.

Martin, F.-P. J., Collino, S., & Rezzi, S. (2012). ^{1}H NMR-based metabonomic applications to decipher gut microbial metabolic influence on mammalian health. Magnetic Resonance in Chemistry, 50(8), 547–553. https://doi.org/10.1002/mrc.2810

Migne, C., Durand, S., & Pujos-Guillot, E. (2018). Exploratory GC/MS-based metabolomics of body fluids. In Methods in Molecular Biology, 1730 (pp. 239–246).

Myers, O. D., Sumner, S. J., Li, S., Barnes, S., & Du, X. (2017). Detailed investigation and comparison of the XCMS and MZmine 2 chromatogram construction and chromatographic peak detection methods for preprocessing mass spectrometry metabolomics data. Analytical Chemistry, 89(17), 8689–8695.

Oliphant, K., & Allen-Vercoe, E. (2019). Macronutrient metabolism by the human gut microbiome: Major fermentation by-products and their impact on host health. Microbiome, 7, 91.

Oyedeji, A. B., Green, E., Adebiyi, J. A., Ogundele, O. M., Gbashi, S., Adefisoye, M. A., Oyeyinka, S. A., & Adebo, O. A. (2021). Metabolomic approaches for the determination of metabolites from pathogenic microorganisms: A review. Food Research International, 140, Article 110042.

Patejko, M., Jacyna, J., & Markuszewski, M. J. (2017). Sample preparation procedures utilized in microbial metabolomics: An overview. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1043, 150-157.

Pavarini, D. P., Pavarini, S. P., Niehues, M., & Lopes, N. P. (2012). Exogenous influences on plant secondary metabolite levels. Animal Feed Science and Technology, 176(1–4), 5–16.

Pietzner, M., Kaul, A., Henning, A. K., Kastenmüller, G., Artati, A., Lerch, M. M., Adamski, J., Nauck, M., & Friedrich, N. (2017). Comprehensive metabolic profiling of chronic low-grade inflammation among generally healthy individuals. BMC Medicine, 15, 210.

Qian M, Zhang H, Wu L, Jin N, Wang J, & Jiang K (2015). Simultaneous determination of zearalenone and its derivatives in edible vegetable oil by

gel permeation chromatography and gas chromatography-triple quadrupole mass spectrometry. Food Chemistry 166: 23–28.

Smith D, Španěl P, Gilchrist FJ, & Lenney W (2013). Hydrogen cyanide, a volatile biomarker of Pseudomonas aeruginosa infection. J Breath Res. 7:44001.

Tang, J. (2011). Microbial metabolomics. Current Genomics, 12(5), 391-403.

Teleki A, Takors R (2019). Quantitative profiling of endogenous metabolites using hydrophilic interaction liquid chromatography-tandem mass spectrometry (HILIC-MS/MS). Methods in Molecular Biology 1859: 185–207.

Vettukattil, R. (2015). Preprocessing of raw metabonomic data. In J. Bjerrum (Ed.), Metabonomics (Methods in Molecular Biology, vol. 1277). Humana Press. https://doi.org/10.1007/978-1-4939-2377-9_10

Villas-Boas, S. G., & Bruheim, P. (2007). Cold glycerol-saline: The promising quenching solution for accurate intracellular metabolite analysis of microbial cells. Analytical Biochemistry, 370(1), 87-97.

Villas-Boas, S. G., Delicado, D. G., & Akesson M (2003). Simultaneous analysis of amino and non-amino organic acids as methyl chloroformate derivatives using gas chromatography-mass spectrometry. Analytical Biochemistry, 322(1), 134-138.

Vo, D. K., & Trinh, K T L (2024). Emerging biomarkers in metabolomics: Advancements in precision health and disease diagnosis. International Journal of Molecular Sciences, 25(23), 13190.

Ye, D., Li, X., Shen, J., & Xia X (2022). Microbial metabolomics: From novel technologies to diversified applications. TrAC Trends in Analytical Chemistry, 148, Article 116540.

Want, E. J., Masson, P., Michopoulos, F., & Wilson, I D Theodoridis, G., Plumb, R. S., Shockcor, J., Loftus, N., Holmes, E., & Nicholson, J K (2013). Global metabolic profiling of animal and human tissues via UPLC-MS. Nature Protocols, 8(1), 17-32.

Winder, C. L., Dunn, W. B., & Goodacre R (2011). TARDIS-based microbial metabolomics: Time and relative differences in systems. Trends in Microbiology, 19(7), 315-322.

Wishart, D S (2019). Metabolomics for investigating physiological and pathophysiological processes. Physiological Reviews, 99(4), 1819-1875.

Wu Z, Lu X, Chen F, Dai X, Ye Y, Yan Y & Liao L (2018). Estimation of early postmortem interval in rats by GC-MS-based metabolomics. Legal Medicine 31: 42–48.

Xia, J., & Wishart, D S (2011). Web-based inference of biological patterns, functions and pathways from metabolomic data using MetaboAnalyst. Nature Protocols, 6(5), 743-760.

Xu, F., Zou, L., & Ong C N (2010). Experiment-originated variations, and multi-peak and multi-origination phenomena in derivatization-based GC-MS metabolomics. Trends in Analytical Chemistry, 29(3), 269-280.

Xu, Y. J., Wang, C., Ho, W. E., & Ong C N (2014) Recent developments and applications of metabolomics in microbiological investigations. TrAC Trends in Analytical Chemistry, 56, 37-48. https://doi.org/10.1016/j.trac.2013.12.009

Zhao S, &Li L (2018). Dansylhydrazine isotope labeling LC-MS for comprehensive carboxylic acid submetabolome profiling. Analytical Chemistry 90: 13514–13522.

Zmora, N., Suez, J., & Elinav E (2019). You are what you eat: Diet, health and the gut microbiota. Nature Reviews Gastroenterology & Hepatology, 16, 35-56.

CHAPTER 6

EFFECTS OF THE HUMAN MICROBIOTA ON EPIGENETICS

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1. INTRODUCTION

The human microbiota, comprising trillions of microorganisms inhabiting the gut, skin, and other body sites, plays a crucial role in maintaining host homeostasis. Beyond its established functions in digestion and immune modulation, emerging evidence highlights its capacity to influence host gene expression through epigenetic mechanisms. Microbial metabolites, such as short-chain fatty acids, vitamins, and bile acids, can modulate DNA methylation, histone modifications, and non-coding RNA activity, thereby affecting metabolic, immunological, and neurological processes. Understanding these microbiota-epigenetic interactions offers novel insights into disease etiology and holds potential for developing targeted therapeutic interventions.

1.1. The Human Microbiota: Definition, Components, and Physiological Significance

The human microbiota is a complex ecosystem composed of bacteria, fungi, viruses, archaea, and other microorganisms that inhabit specific regions of the host organism (especially the gastrointestinal tract, skin, respiratory tract, and urogenital tract) (Lynch & Pedersen, 2016). This community has decisive influences not only on digestive and nutrient metabolism processes but also on immune system development, mucosal barrier integrity, neurological functions, and endocrine regulation (Cho & Blaser, 2012; Thursby & Juge, 2017).

The gut microbiota is the most densely populated and most studied part of this system. It has been shown that the human intestine contains approximately 10^{14} microorganisms, and their genomic content carries 150 times more genetic material than the human genome (Human Microbiome Project Consortium, 2012). The composition of the microbiota varies among individuals due to variables such as genetic factors, diet, mode of birth, antibiotic use, and environmental exposures (Lynch & Pedersen, 2016). While a healthy microbial

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balance is critical for maintaining host homeostasis, disruption of this balance (dysbiosis) has been linked to obesity, diabetes, inflammatory bowel diseases, and cardiovascular and neurological disorders (Fan & Pedersen, 2021).

1.2. The Medical Significance of Epigenetic Regulation

The human microbiota plays a critical role in epigenetic regulation processes. Microbiota-derived metabolites, particularly short-chain fatty acids (SCFAs), folate derivatives, and bile acids, influence host gene expression through various epigenetic mechanisms (Paul et al., 2015; Morović, 2021). These mechanisms include:

1. DNA Methylation: Folate and other methyl-providing metabolites produced by the microbiota promote the methylation of cytosine bases at CpG islands via DNA methyltransferases. These modifications lead to gene silencing or activation at gene promoters, resulting in long-term effects on immune responses, metabolic processes, and cellular differentiation. For example, inadequate or excessive influences on the gut microbiota early in development may increase the risk of metabolic syndrome and inflammatory diseases through DNA methylation (Chandrasekaran et al., 2024).
2. Histone Modifications: SCFAs and other microbiota metabolites regulate chromatin structure by affecting histone acetylase and deacetylase activity. Histone acetylation increases gene expression, while methylation or phosphorylation can lead to gene silencing. This mechanism plays a central role in both intestinal barrier function and the control of systemic inflammation. Microbiota-supported histone modifications are particularly important in shaping the immune response and regulating neuroendocrine axes (Cavalli & Heard, 2019).
3. Non-coding RNAs (ncRNAs): ncRNAs, such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), post-transcriptionally regulate gene expression via microbiota-derived signals. For example, signals from the gut microbiota alter the expression of specific miRNAs, thereby suppressing or activating inflammatory genes. LncRNAs, in turn, mediate chromatin remodeling and histone modifications, enabling long-term epigenetic control of genetic programs (Feinberg et al., 2016).

Influenced by factors such as birth type, breastfeeding, and antibiotic use, microbiota development in early life can determine epigenetic programming and have lasting effects on metabolic, neurological, and immunological health in the long term (Zhang et al., 2023). Therefore, microbiota-epigenetic interactions are gaining increasing clinical importance. This axis offers potential biomarkers and targets for both diagnostic and therapeutic strategies in metabolic diseases, cardiovascular disorders, cancer, and neuropsychiatric disorders (Rubas et al., 2025; Tu et al., 2025).

1.3. Clinical Importance of Microbiota-Epigenetic Interaction

The bidirectional relationship between microbiota and epigenetics has created a new paradigm for understanding the molecular effects of environmental factors on human health (Krautkramer et al., 2016). Microbiota-derived metabolites—especially short-chain fatty acids (SCFAs: acetate, propionate, butyrate)—can act as histone deacetylase (HDAC) inhibitors, loosening chromatin structure and altering gene expression (Donohoe et al., 2011). Furthermore, bacterial folate and B12 production indirectly regulate DNA methylation patterns by influencing the methylation cycle (Leung et al., 2016).

The clinical implications of these interactions are broad: dysbiosis-associated epigenetic changes have been implicated in the development of obesity, insulin resistance, inflammatory diseases, colorectal cancer, and neuropsychiatric disorders (Krautkramer et al., 2021; Avila Cobos et al., 2022). Therefore, the microbiota-epigenetics axis has become a key concept at the environment-genome interface, both in understanding the pathogenesis of human diseases and in developing targeted therapeutic approaches.

2. The Effects of the Human Microbiota on Epigenetics

The human microbiota is an ecosystem that shapes the physiological functions of host cells and mediates multilayered interactions, particularly at the epigenetic level. Microbial metabolites play a decisive role in epigenetic mechanisms that directly or indirectly regulate gene expression in the host cell genome (Cani & Jordan, 2018). These interactions are underpinned by the impact of metabolites produced by the microbiota, such as short-chain fatty acids (SCFAs), bile acids, and folate derivatives, on the host epigenome.

2.1 Epigenetic Effects of Microbiota-Derived Metabolites

The gut microbiota influences epigenetic regulation, particularly through the production of short-chain fatty acids such as butyrate, propionate, and acetate. Butyrate acts as a histone deacetylase (HDAC) inhibitor, increasing histone

acetylation and stimulating gene expression (Silva et al., 2020). This mechanism plays a critical role in maintaining intestinal epithelial integrity and regulating inflammation (Baxter et al., 2019). Propionate and acetate can alter DNA methylation profiles in genes associated with energy metabolism and immune responses (Krautkramer et al., 2016).

Bile acids are also important mediators in the microbiota-epigenetic axis. The microbiota converts primary bile acids into secondary derivatives, thereby affecting gene expression at the epigenetic level through nuclear receptors (e.g., FXR and PXR) (Wahlström et al., 2016). Furthermore, folate derivatives produced by the microbiota contribute to the direct regulation of DNA methylation by affecting S-adenosylmethionine (SAM) levels, which are involved in the methylation cycle (Selhub, 2002).

2.2 Epigenetic Regulatory Pathways: DNA Methylation, Histone Modifications, and ncRNAs

Epigenetic regulation is mediated through the interaction of DNA methylation, histone modifications, and non-coding RNAs (ncRNAs). The effects of microbial products on these pathways are multifaceted. For example, butyrate has been shown to suppress DNA methyltransferase (DNMT) activity, leading to hypomethylation (Krautkramer et al., 2016). At the same time, the microbiota modulates inflammatory gene expression by altering histone acetylation balance (Arpaia et al., 2013).

Non-coding RNAs are also one of the epigenetic reflections of microbiota influence. MicroRNAs (miRNAs), in particular, are expressed at different levels depending on the composition of the gut microbiota and play a role in the epigenetic control of host genes (Dalmasso et al., 2011). This reveals how microbiota changes reshape the epigenetic profile.

2.3 Microbiota-Epigenetic Interaction Networks: Gut-Brain, Gut-Liver, and Gut-Immune Axis

Microbiota-epigenetic interactions are effective not only in the intestinal epithelium but also at the systemic level. The gut-brain axis is associated with the epigenetic regulation of neurotransmitter synthesis and synaptic plasticity genes by short-chain fatty acids (Stilling et al., 2016). This mechanism explains the epigenetic effects of the microbiota on neuropsychiatric disorders such as depression and autism.

The gut-liver axis plays a key role in the epigenetic control of bile acid cycling and lipid metabolism. Microbiota can influence hepatic energy homeostasis by

altering histone acetylation patterns in genes associated with lipid metabolism in the liver (Dumas et al., 2018).

Finally, the gut-immune axis is implicated in the epigenetic regulation of immune cell differentiation and cytokine expression. Metabolites such as butyrate promote immune tolerance by increasing histone acetylation in the Foxp3 gene promoter region of T reg cells (Arpaia et al., 2013). Thus, the microbiota is becoming a key environmental factor in determining the direction of immune responses through epigenetic pathways.

3. Microbiota and Epigenetic Programming in Early Life

Early life is one of the periods when epigenetic programming occurs most dynamically. During this period, environmental factors, particularly birth type, diet, and medication use, can determine both the composition of the microbiota and the persistence of epigenetic marks (Robertson, 2005). These early differences in microbiota development can influence an individual's susceptibility to metabolic, immunological, and neurological diseases later in life (Mueller et al., 2015).

3.1 Mode of Delivery (Vaginal Delivery vs. Cesarean Section) and Epigenetic Consequences

The mode of delivery is a determining factor in a baby's initial microbiota colonization. Babies born via vaginal delivery acquire a rich microbial diversity from the mother's vaginal and intestinal flora, while babies born via cesarean section are generally colonized with microorganisms derived from the skin and specific to the hospital environment (Dominguez-Bello et al., 2010). These differences can alter the maturation process of the immune system by affecting the methylation patterns of immune genes at the epigenetic level (Al Nabhani & Eberl, 2020). For example, hypermethylation of the FOXP3 and IFNG genes has been reported in babies delivered via cesarean section, leading to disruptions in T cell regulation (Mueller et al., 2015).

3.2 Breast Milk, Nutrition, and Microbiota Development

Breast milk, with its prebiotic and probiotic components, is the most important nutritional source shaping the baby's gut microbiota. Human milk oligosaccharides (HMOs) promote the growth of beneficial bacteria, particularly Bifidobacterium longum, thus maintaining a healthy microbial balance (Zivkovic & Barile, 2011). This process is also critical from an epigenetic perspective; global DNA methylation levels have been shown to be more balanced in breast-fed infants, and inflammation-related genes are epigenetically suppressed

(Hartwig et al., 2020). In formula-fed infants, microbial diversity decreases, which may lead to epigenetic reprogramming of immune-related genes (LeDoare et al., 2018). Therefore, diet is a critical environmental regulator of the early-life epigenome.

3.3 Antibiotic Use and Epigenetic Reshaping of the Immune System

Antibiotic use in early life can cause permanent disruptions in the gut microbiota, leading to epigenetic reshaping of the immune system (Cox et al., 2014). Loss of microbiota leads to a decrease in short-chain fatty acids (especially butyrate) and, consequently, to a weakening of epigenetic mechanisms regulating T reg cell differentiation (Arpaia et al., 2013).

Furthermore, exposure to antibiotics can increase susceptibility to allergic and autoimmune diseases by altering DNA methylation patterns (Korpela et al., 2020). For example, neonatal antibiotic treatment has been reported in mouse models to induce hypomethylation of inflammatory cytokine genes, which is associated with an increased inflammatory response later in life (Tamburini et al., 2016).

In conclusion, early life microbiota-epigenetic interactions appear to play a fundamental role in the permanent programming of the immune system and determining disease susceptibility.

4. Microbiota–Epigenetic Interactions and Human Diseases

Microbiota–epigenetic interactions are increasingly important as a multilayered regulatory mechanism in human health and disease development. The gut microbiota can directly influence epigenetic regulation through biologically active molecules such as short-chain fatty acids (SCFAs), secondary bile acids, vitamins, and polyphenol metabolites (Liu et al., 2022). These interactions alter gene function through DNA methylation, histone modifications, and microRNA expression, reprogramming metabolic, cardiovascular, neurological, immune, and neoplastic processes (Marzano et al., 2017).

4.1 Metabolic Diseases: Obesity, Type 2 Diabetes, and Insulin Resistance

Metabolic diseases such as obesity and type 2 diabetes are among the important consequences of epigenetic changes associated with dysbiosis in the microbiota. Microbiota-derived SCFAs, such as butyrate and propionate, act as histone deacetylase (HDAC) inhibitors and increase the expression of genes involved in energy metabolism (Canfora et al., 2019). However, in dysbiosis, a decrease in these beneficial metabolites can lead to hypermethylation of genes that regulate insulin sensitivity, such as PPAR-γ and adiponectin (Biernacka et

al., 2021). This epigenetic reprogramming disrupts glucose homeostasis and develops insulin resistance. Furthermore, an increase in the Firmicutes/Bacteroidetes ratio has been shown to contribute to obesity by triggering epigenetic activation of lipogenesis genes (Kasai et al., 2015).

4.2 Cardiovascular Diseases: Atherosclerosis and Hypertension

The microbiota-epigenetic axis also plays an important role in cardiovascular diseases. Microbiota-derived metabolites such as trimethylamine-N-oxide (TMAO) have been shown to alter DNA methylation in atherosclerotic plaque formation (Koeth et al., 2013). Hypermethylation of endothelial nitric oxide synthase (eNOS) and vascular adhesion molecule genes, in particular, increases vascular inflammation and increases the risk of atherosclerosis (Jie et al., 2017).

In the development of hypertension, short-chain fatty acids such as acetate and butyrate produced by the gut microbiota have been reported to modulate renin-angiotensin system (RAS) genes by regulating histone acetylation (Kim et al., 2018). This suggests that the gut microbiota constitutes an epigenetic "remote control" mechanism on vascular tone.

4.3 Microbiota-Induced Epigenetic Alterations in Tumor Development

The epigenetic effects of the microbiota on cancer development are mediated primarily through the disruption of DNA methylation patterns and the dysregulation of histone modifications (Yu et al., 2020). For example, Helicobacter pylori infection triggers tumor development by inducing hypermethylation of tumor suppressor genes (e.g., CDH1, p16INK4a) in gastric cancer cells (Tahara et al., 2009). Similarly, Fusobacterium nucleatum induces epigenetic mechanisms that activate the β-catenin signaling pathway in colon cancer (Yang et al., 2017). Butyrate, produced by the microbiota, acts as an HDAC inhibitor and increases apoptosis in some cancer cells, while at low concentrations it can promote tumor growth (Donohoe et al., 2012). Therefore, the epigenetic effects of microbial metabolites play a dual role, depending on the tumor microenvironment.

4.4 Neurological and Psychiatric Diseases: The Gut–Brain Axis

The gut–brain axis serves as a critical bridge in the epigenetic regulation of neurological and psychiatric diseases. Microbiota composition alters neurotransmitter balance by affecting tryptophan metabolism and serotonin production (Clarke et al., 2013). In this process, short-chain fatty acids such as butyrate increase histone acetylation in the nervous system, modulating the expression of genes associated with neuroplasticity (Stilling et al., 2016).

In models of depression, increased hypermethylation of the brain-derived neurotrophic factor (BDNF) gene has been reported with dysbiosis (Kelly et al., 2016). In autism spectrum disorder, microbiota-derived metabolites (e.g., p-cresol) have been shown to negatively affect synaptic gene expression by disrupting histone modifications (Hsiao et al., 2013). 4.5 Autoimmune and Inflammatory Diseases: Crohn's Disease, Ulcerative Colitis, Rheumatoid Arthritis

In autoimmune and inflammatory diseases, the microbiota-epigenetic interaction involves the functional reprogramming of immune cells (Kamada et al., 2013). In Crohn's disease, a decrease in anti-inflammatory bacteria such as Faecalibacterium prausnitzii leads to hypermethylation of the IL-10 gene and suppression of regulatory T cell activity (Sokol et al., 2008). In ulcerative colitis, a decrease in histone H3K27 acetylation in the colonic epithelium enhances the inflammatory response (Ventham et al., 2016). In rheumatoid arthritis, the dysbiotic microbiota increases pro-inflammatory cytokine production through epigenetic changes that promote Th17 cell differentiation (Zhang et al., 2015). These findings suggest that epigenetic treatment strategies can be combined with microbiota-targeted approaches.

5. Microbiota-Based and Epigenetically Guided Therapeutic Approaches

This section discusses how microbiota-targeted interventions impact the epigenome; which strategies (probiotic/prebiotic/symbiotic, fecal microbiota transplantation, dietary interventions) hold promise based on clinical and experimental data; and the interaction between epigenetic drugs and the microbiota.

5.1 Epigenetic Effects of Probiotics, Prebiotics, and Symbiotics

Probiotics and prebiotics indirectly affect the host epigenome by altering microbial metabolite production. In particular, short-chain fatty acids (SCFAs; e.g., butyrate), which increase as a result of fermentation of probiotic bacterial strains and prebiotic fibers, exhibit HDAC inhibitory activity, increasing histone acetylation and contributing to the suppression of inflammatory genes (Paul et al., 2015; Morović, 2021). Clinical studies have shown that probiotic/symbiotic administration significantly alters DNA methylation profiles in the intestinal epithelium and peripheral tissues; these changes correlate with immune regulation and metabolic responses (Chandrasekaran et al., 2024). However, the effect is both species-specific and dose/treatment duration dependent; generalization should be made with caution due to heterogeneity in human studies (Miro-Blanch & Castillo, 2019).

5.2 Fecal Microbiota Transplantation (FMT) and Epigenetic Rearrangement

FMT is an approach that can radically alter the microbiota composition in a short period of time. RCTs and observational studies have reported significant changes in the host's metabolome and immune profiles after FMT, while some studies have observed reprogramming of DNA methylation patterns in peripheral cells (van der Vossen et al., 2021). Animal models and limited human data suggest that FMT can affect epigenetic signatures in the liver, immune system, and brain (Zhang et al., 2023; Lu et al., 2024). However, the effects of FMT vary depending on the recipient-donor interaction, the recipient's initial epigenome status, and the duration of follow-up; therefore, applications of FMT for epigenetic targeting are still experimental.

5.3 Diet-Based Microbiota Interventions (e.g., Fiber, Polyphenols, Ketogenic Diet)

Diet is the most powerful and sustainable intervention in the microbiota-epigenome axis. High-fiber diets provide HDAC inhibitory effects by increasing the production of butyrate and other SCFAs; this mechanism improves intestinal barrier integrity, reduces inflammation, and leads to beneficial epigenetic reprogramming in some tissues (Shock et al., 2021; Meiners et al., 2025). Polyphenols (e.g., green tea catechins, grape resveratrol) can both directly target epigenetic enzymes and influence DNMT and HDAC activity by inducing metabolites produced by the microbiota (Paul et al., 2015; Maiuolo et al., 2024).

Radical dietary interventions, such as the ketogenic diet, significantly alter the microbiota composition; Some studies have suggested that the ketogenic diet may be effective through epigenetic regulation (e.g., histone acetylation) while providing benefits in neurological diseases, but long-term effects and possible negative epigenetic consequences need further study (D. Li, 2022).

5.4 Epigenetic Drug Interactions with Microbiota

Epigenetic drugs (e.g., DNMT inhibitors; azacitidine/decitabine; HDAC inhibitors; vorinostat, romidepsin) directly affect the host epigenome and exhibit bidirectional interactions with the microbiota. On the one hand, the microbiota can influence the bioavailability and metabolism of some drugs, altering their efficacy and toxicity; on the other hand, epigenetic drugs can indirectly reshape the microbiota composition by altering host immunity and epithelial integrity (Weersma et al., 2020; Martínez-Montoro et al., 2024). Preliminary clinical and clinical evidence has demonstrated the potential for epigenetic drugs to increase

chemotherapy sensitivity through microbiota modulation; the combination of DNMT/HDAC inhibitors and microbiota interventions has shown promise in some lymphoma and solid tumor models (Tu et al., 2025).

6. Current Findings and Future Perspectives in Clinical Research

This section discusses the current status of the microbiota and epigenetic interaction in light of human studies, the technologies used, the perspective of personalized medicine, and the limitations in transitioning to clinical practice.

6.1 New Technologies Used in Microbiota and Epigenome Analysis

Currently, high-resolution and multi-omics approaches are used to assess microbiota and epigenetic interactions. Metagenomic analyses determine the species and gene function profiles of the gut microbiota, while methylomic analyses reveal DNA methylation patterns in detail (Zhang et al., 2023). Furthermore, RNA-seq and ncRNA profiling are important in assessing epigenetic regulatory pathways. Multi-omics integration (metagenomics + metabolomics + epigenomics + proteomics) allows for more reliable analysis of the epigenetic effects of microbiota-derived metabolites and their clinical implications (Li et al., 2024; Tu et al., 2025).

6.2 Use of Microbiota-Epigenetic Interactions in Personalized Medicine

From a personalized medicine perspective, targeted interventions are planned by considering individuals' baseline microbiota composition and epigenetic profiles. For example, DNA methylation and SCFA levels can be monitored before and after diet, probiotics, or FMT in individuals with metabolic syndrome to develop personalized therapy strategies (Chandrasekaran et al., 2024; Rubas et al., 2025). Furthermore, studies are increasingly focusing on enhancing treatment response through combinations of microbiota modulation and epigenetic drugs in cancer and neurological diseases (Weersma et al., 2020).

6.3 Current Limitations and Research Needs in the Transition to Clinical Practice

The most important limitations in the transition to clinical practice are as follows:

1. Human studies are still limited in number and short in duration, leading to methodological heterogeneity.

2. The target tissue-specific and long-term effects of microbiota and epigenetic interactions are not fully known.

3. The lack of multi-omics data analysis and standardization makes generalization of findings difficult.

Therefore, randomized controlled trials, long-term follow-up, and multi-omics integration are required in the future. Furthermore, the efficacy and safety of personalized microbiota-based interventions with individual-based metabolome and epigenome monitoring should be systematically evaluated, and biomarker-based guidelines should be developed (Meiners et al., 2025; Li et al., 2024).

7. CONCLUSION

The human microbiota plays a critical role in epigenetic regulation processes. Microbiota-derived metabolites, particularly short-chain fatty acids, folate derivatives, and bile acids, shape physiological homeostasis and immune responses by influencing host gene expression through DNA methylation, histone modifications, and non-coding RNAs (Paul et al., 2015; Morović, 2021). Early-life microbiota development, influenced by factors such as mode of birth, breastfeeding, and antibiotic use, can determine epigenetic programming and have lasting effects on metabolic, neurological, and immunological health in the long term (Chandrasekaran et al., 2024; Zhang et al., 2023).

The clinical importance of microbiota-epigenetic interactions is increasing. The role of this axis in metabolic diseases, cardiovascular disorders, cancer, and neuropsychiatric disorders offers potential biomarkers and targets for both diagnostic and therapeutic strategies (Rubas et al., 2025; Tu et al., 2025). The combination of microbiota-based interventions (such as probiotic/prebiotic applications, dietary modification, fecal microbiota transplantation) and epigenetic drugs is emerging as new strategies in personalized medicine (Weersma et al., 2020; Maiuolo et al., 2024). In the future, individualized analyses using multi-omics approaches will allow for more precise assessment of microbiota-epigenetic interactions, thereby increasing the efficacy and reliability of diagnostic, therapeutic, and preventive strategies. However, given the short follow-up times, methodological heterogeneity, and uncertainty about long-term effects in human studies, larger, randomized, and mechanistic studies are necessary. The integration of the microbiota and epigenetics axis into medical applications carries significant potential in the personalized medicine of the future.

8. REFERENCES

Al Nabhani, Z., & Eberl, G. (2020). Imprinting of the immune system by the microbiota early in life. *Mucosal Immunology, 13*(2), 183–189. https://doi.org/10.1038/s41385-019-0228-5

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J. R., Pfeffer, K., Coffer, P. J., & Rudensky, A. Y. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature, 504*(7480), 451–455. https://doi.org/10.1038/nature12726

Avila Cobos, F., Kuemmerle, L. B., Volders, P.-J., & Mestdagh, P. (2022). Microbiota–Epigenome interactions and their implications for human health and disease. *Nature Reviews Genetics, 23*(8), 532–548. https://doi.org/10.1038/s41576-022-00473-1

Baxter, N. T., Schmidt, A. W., Venkataraman, A., Kim, K. S., Waldron, C., & Schmidt, T. M. (2019). Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions. *mSystems, 4*(2), e00087-19. https://doi.org/10.1128/mSystems.00087-19

Biernacka, K. M., Clark, A. J., & Finucane, F. M. (2021). Microbiome-mediated epigenetic regulation in metabolic diseases. *Nutrients, 13*(4), 1217. https://doi.org/10.3390/nu13041217

Cani, P. D., & Jordan, B. F. (2018). Gut microbiota–mediated inflammation in obesity: A link with gastrointestinal cancer. *Nature Reviews Gastroenterology & Hepatology, 15*(11), 671–682. https://doi.org/10.1038/s41575-018-0034-1

Cavalli, G., & Heard, E. (2019). Advances in epigenetics link genetics to the environment and disease. *Nature, 571*(7766), 489–499. https://doi.org/10.1038/s41586-019-1411-0

Chandrasekaran, P., Sachdeva, N., & diğerleri. (2024). Effects of Probiotics on Gut Microbiota: An Overview. *International Journal of Molecular Sciences, 25*(11), 6022. https://doi.org/10.3390/ijms25116022

Cho, I., & Blaser, M. J. (2012). The human microbiome: At the interface of health and disease. *Nature Reviews Genetics, 13*(4), 260–270. https://doi.org/10.1038/nrg3182

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R. D., Shanahan, F., Dinan, T. G., & Cryan, J. F. (2013). The microbiome–gut–brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Molecular Psychiatry, 18*(6), 666–673. https://doi.org/10.1038/mp.2012.77

Cox, L. M., Yamanishi, S., Sohn, J., Alekseyenko, A. V., Leung, J. M., Cho, I., ... & Blaser, M. J. (2014). Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. *Cell, 158*(4), 705–721. https://doi.org/10.1016/j.cell.2014.05.052

Dalmasso, G., Nguyen, H. T. T., Yan, Y., Laroui, H., Charania, M. A., Ayyadurai, S., ... & Merlin, D. (2011). Microbiota modulate host gene expression via microRNAs. *PLoS One, 6*(4), e19293. https://doi.org/10.1371/journal.pone.0019293

D. Li. (2022). Diet–gut microbiota–epigenetics in metabolic diseases. *Progress in Biophysics & Molecular Biology, 172*, 40–55. https://doi.org/10.1016/j.pbiomolbio.2022.01.006

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proceedings of the National Academy of Sciences, 107*(26), 11971–11975. https://doi.org/10.1073/pnas.1002601107

Donohoe, D. R., Garge, N., Zhang, X., Sun, W., O'Connell, T. M., Bunger, M. K., & Bultman, S. J. (2011). The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. *Cell Metabolism, 13*(5), 517–526. https://doi.org/10.1016/j.cmet.2011.02.018

Donohoe, D. R., Holley, D., Collins, L. B., Montgomery, S. A., Whitmore, A. C., Hillhouse, A., Curry, K. P., Renner, S. W., Greenwalt, A., Ryan, E. P., Godfrey, V., Heise, M. T., & Bultman, S. J. (2012). A gnotobiotic mouse model demonstrates that dietary fiber protects against colorectal tumorigenesis in a microbiota- and butyrate-dependent manner. *Cancer Discovery, 4*(12), 1374–1386. https://doi.org/10.1158/2159-8290.CD-12-0059

Dumas, M. E., Barton, R. H., Toye, A., Cloarec, O., Blancher, C., Rothwell, A., ... & Holmes, E. (2018). Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proceedings of the National Academy of Sciences, 103*(33), 12511–12516. https://doi.org/10.1073/pnas.0601056103

Fan, Y., & Pedersen, O. (2021). Gut microbiota in human metabolic health and disease. *Nature Reviews Microbiology, 19*(1), 55–71. https://doi.org/10.1038/s41579-020-0433-9

Feinberg, A. P. (2018). The key role of epigenetics in human disease prevention and mitigation. *New England Journal of Medicine, 378*(14), 1323–1334. https://doi.org/10.1056/NEJMra1402513

Feinberg, A. P., Koldobskiy, M. A., & Göndör, A. (2016). Epigenetic modulators, modifiers and mediators in cancer aetiology and progression. *Nature Reviews Genetics, 17*(5), 284–299. https://doi.org/10.1038/nrg.2016.13

Hartwig, F. P., Loret de Mola, C., Davies, N. M., Victora, C. G., Relton, C. L., & Barros, F. C. (2020). Breastfeeding and DNA methylation in epigenome-wide association studies: A systematic review. *Environmental Epigenetics, 6*(1), dvaa002. https://doi.org/10.1093/eep/dvaa002

Hsiao, E. Y., McBride, S. W., Hsien, S., Sharon, G., Hyde, E. R., McCue, T., ... & Mazmanian, S. K. (2013). Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell, 155*(7), 1451–1463. https://doi.org/10.1016/j.cell.2013.11.024

Human Microbiome Project Consortium. (2012). Structure, function and diversity of the healthy human microbiome. *Nature, 486*, 207–214. https://doi.org/10.1038/nature11234

Jaenisch, R., & Bird, A. (2003). Epigenetic regulation of gene expression: How the genome integrates intrinsic and environmental signals. *Nature Genetics, 33*(3), 245–254. https://doi.org/10.1038/ng1089

Kamada, N., Seo, S. U., Chen, G. Y., & Núñez, G. (2013). Role of the gut microbiota in immunity and inflammatory disease. *Nature Reviews Immunology, 13*(5), 321–335. https://doi.org/10.1038/nri3430

Kasai, C., Sugimoto, K., Moritani, I., Tanaka, J., Oya, Y., Inoue, H., & Oka, M. (2015). Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population. *BMC Gastroenterology, 15*(1), 100. https://doi.org/10.1186/s12876-015-0330-2

Kelly, J. R., Borre, Y., O'Brien, C., Patterson, E., El Aidy, S., Deane, J., ... & Dinan, T. G. (2016). Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *Journal of Psychiatric Research, 82*, 109–118. https://doi.org/10.1016/j.jpsychires.2016.07.019

Kim, S., Goel, R., Kumar, A., Qi, Y., Lobaton, G., Hosaka, K., ... & Raizada, M. K. (2018). Imbalance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure. *Hypertension, 72*(4), 1004–1012. https://doi.org/10.1161/HYPERTENSIONAHA.118.11688

Koeth, R. A., Wang, Z., Levison, B. S., Buffa, J. A., Org, E., Sheehy, B. T., ... & Hazen, S. L. (2013). Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature Medicine, 19*(5), 576–585. https://doi.org/10.1038/nm.3145

Korpela, K., Costea, P., Coelho, L. P., Kandels-Lewis, S., Willemsen, G., Boomsma, D. I., ... & Bork, P. (2020). Selective maternal seeding and environment

shape the human gut microbiome. *Genome Research, 28*(4), 561–568. https://doi.org/10.1101/gr.233940.117

Krautkramer, K. A., Fan, J., & Bäckhed, F. (2021). Interactions between the gut microbiota and host epigenome in health and disease. *Nature Reviews Gastroenterology & Hepatology, 18*(7), 442–456. https://doi.org/10.1038/s41575-021-00459-3

Krautkramer, K. A., Kreznar, J. H., Romano, K. A., Vivas, E. I., Barrett-Wilt, G. A., Rabaglia, M. E., ... & Denu, J. M. (2016). Diet–microbiota interactions mediate global epigenetic programming in multiple host tissues. *Molecular Cell, 64*(5), 982–992. https://doi.org/10.1016/j.molcel.2016.10.025

LeDoare, K., Holder, B., Bassett, A., & Pannaraj, P. S. (2018). Mother's milk: A purposeful contribution to the development of the infant microbiota and immunity. *Frontiers in Immunology, 9*, 361. https://doi.org/10.3389/fimmu.2018.00361

Liu, H., Wang, J., He, T., Becker, S., Zhang, G., Li, D., & Ma, X. (2022). Butyrate: A double-edged sword for health? *Advances in Nutrition, 13*(1), 121–131. https://doi.org/10.1093/advances/nmab063

Lu, X., et al. (2024). Epigenetic programming mediates abnormal gut-liver axis in cholestatic disease: evidence from FMT experiments. *Engineering in Life Sciences, 24*(3), 145–160. https://doi.org/10.1002/elsc.202400016

Maiuolo, J., & diğerleri. (2024). The postbiotic properties of butyrate in modulation of host physiology. *International Journal of Molecular Sciences, 25*(13), 6971. https://doi.org/10.3390/ijms25136971

Miro-Blanch, J., & Castillo, M. (2019). Epigenetic Regulation at the Interplay Between Gut Microbiota and Host: A Focus on Diet and Disease. *Frontiers in Genetics, 10*, 638. https://doi.org/10.3389/fgene.2019.00638

Mueller, N. T., Bakacs, E., Combellick, J., Grigoryan, Z., & Dominguez-Bello, M. G. (2015). The infant microbiome development: Mom matters. *Trends in Molecular Medicine, 21*(2), 109–117. https://doi.org/10.1016/j.molmed.2014.12.002

Morović, W. (2021). Epigenetics: A New Frontier in Probiotic Research. *Trends in Microbiology, 29*(6), 487–496. https://doi.org/10.1016/j.tim.2021.02.008

Paul, B., et al. (2015). Gut microbiota and host epigenetics: Mechanisms and clinical implications. *Clinical Epigenetics, 7*(1), 64. https://doi.org/10.1186/s13148-015-0120-0

Robertson, K. D. (2005). DNA methylation and human disease. *Nature Reviews Genetics, 6*(8), 597–610. https://doi.org/10.1038/nrg1655

Rubas, N. C., et al. (2025). The gut microbiome and epigenomic reprogramming: therapeutic perspectives. *International Journal of Molecular Sciences, 26*(17), 8658. https://doi.org/10.3390/ijms26178658

Selhub, J. (2002). Folate, vitamin B12 and vitamin B6 and one carbon metabolism. *The Journal of Nutrition, 132*(8), 2333S–2336S. https://doi.org/10.1093/jn/132.8.2333S

Silva, Y. P., Bernardi, A., & Frozza, R. L. (2020). The role of short-chain fatty acids from gut microbiota in gut–brain communication. *Frontiers in Endocrinology, 11*, 25. https://doi.org/10.3389/fendo.2020.00025

Sokol, H., Pigneur, B., Watterlot, L., Lakhdari, O., Bermúdez-Humarán, L. G., Gratadoux, J. J., ... & Langella, P. (2008). Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proceedings of the National Academy of Sciences, 105*(43), 16731–16736. https://doi.org/10.1073/pnas.0804812105

Stilling, R. M., Ryan, F. J., Hoban, A. E., Shanahan, F., Clarke, G., Claesson, M. J., & Dinan, T. G. (2016). Microbes & neurodevelopment – Absence of microbiota during early life increases activity-related transcriptional pathways in the amygdala. *Brain, Behavior, and Immunity, 50*, 209–220. https://doi.org/10.1016/j.bbi.2015.07.009

Stilling, R. M., Ryan, F. J., Hoban, A. E., Shanahan, F., Clarke, G., Claesson, M. J., Dinan, T. G., & Cryan, J. F. (2016). Microbes and neuroepigenetics: An emerging field linking the microbiome and brain epigenome. *Nature Reviews Neuroscience, 17*(11), 647–659. https://doi.org/10.1038/nrn.2016.99

Tahara, T., Shibata, T., Okubo, M., Ishizuka, T., Nakamura, M., Nagasaka, M., & Arisawa, T. (2009). DNA methylation status in gastric mucosa as a biomarker for gastric cancer risk: A multicenter study. *PLoS One, 8*(7), e68999. https://doi.org/10.1371/journal.pone.0068999

Tamburini, S., Shen, N., Wu, H. C., & Clemente, J. C. (2016). The microbiome in early life: Implications for health outcomes. *Nature Medicine, 22*(7), 713–722. https://doi.org/10.1038/nm.4142

Tu, J., et al. (2025). Influence of the gut microbiome on lymphoma treatment: synergy with epigenetic therapy. *Clinical Cancer Research*. Advance online publication. https://doi.org/10.1158/1078-0432.CCR-24-XXXX

Ventham, N. T., Kennedy, N. A., Adams, A. T., Kalla, R., Heath, S., O'Leary, K. R., ... & Satsangi, J. (2016). Integrative epigenome-wide analysis demonstrates that DNA methylation may mediate genetic risk in inflammatory bowel disease. *Nature Communications, 7*, 13507. https://doi.org/10.1038/ncomms13507

Wahlström, A., Sayin, S. I., Marschall, H. U., & Bäckhed, F. (2016). Intestinal crosstalk between bile acids and microbiota and its impact on host metabolism. *Cell Metabolism, 24*(1), 41–50. https://doi.org/10.1016/j.cmet.2016.05.005

Weersma, R. K., et al. (2020). Interaction between drugs and the gut microbiome. *Gut Microbes*. https://doi.org/10.1080/19490976.2020.1740420

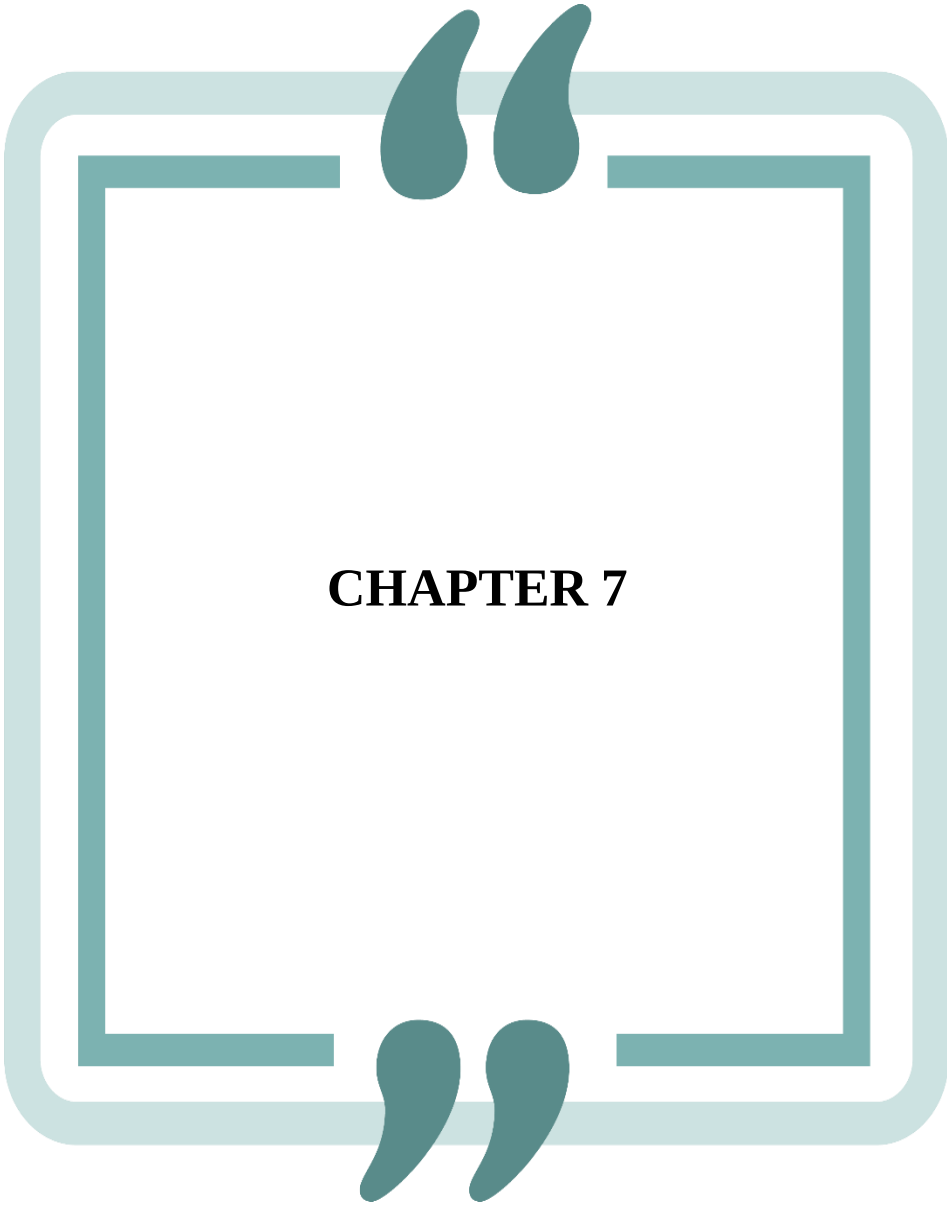
Yang, Y., Weng, W., Peng, J., Hong, L., Yang, L., Toiyama, Y., ... & Cai, S. (2017). Fusobacterium nucleatum increases proliferation of colorectal cancer cells and tumor development in mice by activating TLR4 signaling to nuclear factor–κB, and up-regulating miR-21 expression. *Gastroenterology, 152*(4), 851–866. https://doi.org/10.1053/j.gastro.2016.11.018

Yu, L., Lv, J., Zeng, H., Ma, G., & Zhong, Y. (2020). Microbiota and cancer: Epigenetic regulation and therapeutic implications. *Frontiers in Oncology, 10*, 948. https://doi.org/10.3389/fonc.2020.00948

Zhang, X., Zhang, D., Jia, H., Feng, Q., Wang, D., Liang, D., ... & Wang, J. (2015). The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nature Medicine, 21*(8), 895–905. https://doi.org/10.1038/nm.3914

Zhang, Y., et al. (2023). Multi-omics approaches to study gut microbiota-host epigenome interactions. *Trends in Molecular Medicine, 29*(8), 702–718. https://doi.org/10.1016/j.molmed.2023.04.004

Zivkovic, A. M., & Barile, D. (2011). Bovine milk as a source of functional oligosaccharides for improving human health. *Advances in Nutrition, 2*(3), 284–289. https://doi.org/10.3945/an.111.000455



CHAPTER 7

PREGNANCY AND MICROBIOTA

Adil Yüksel TOGAY[1]

1. INTRODUCTION

Pregnancy is a critical period for fetal growth and development, characterized by extensive changes in maternal physiology and immune responses. Recent research has highlighted the microbiota as an important factor that directly impacts maternal and fetal health (Nuriel-Ohayon, Neuman, & Koren, 2016). The microbiota is defined as the ecosystem of microorganisms living in the body and plays a role in regulating metabolic, immunological, and endocrine functions (Gomaa, 2020; Zmora, Suez, & Elinav, 2019). Throughout pregnancy, significant changes are observed, particularly in the composition of the intestinal and vaginal microbiota; these changes may impact both maternal metabolism and fetal immune system development (Koren et al., 2012; Gomez de Aguero et al., 2016). Furthermore, microbiota imbalances (dysbiosis) have been associated with obstetric complications for instance gestational diabetes, preeclampsia, and preterm birth (Crusell et al., 2018; Liu et al., 2019). Therefore, studies of the microbiota during pregnancy are increasingly important for developing potential therapeutic strategies to optimize maternal and child health.

1.1 The Importance of Maternal and Fetal Health During Pregnancy

Pregnancy is a unique "window of opportunity" where the mother's biopsychosocial status directly impacts pregnancy outcomes, newborn health, and the child's lifelong risk of illness. Globally, this issue carries significant weight: In 2023, approximately 260,000 women worldwide died from pregnancy-related causes, and 2.3 million babies were lost in the first 28 days of life. These indicators clearly demonstrate that accessibility to quality prenatal care, antenatal monitoring, and delivery services is a critical priority for health systems (UNICEF, 2025; WHO/UNICEF/UNFPA/World Bank/UN DESA, 2025).

Quality antenatal care is one of the most effective approaches to reducing maternal morbidity and mortality and improving perinatal outcomes. Current guidelines emphasize individualizing care (adapting visit frequency and content based on risk level, social needs, and available resources), evidence-based screening, and education and nutritional counseling. Group antenatal care models

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can benefit outcomes for instance patient education, breastfeeding initiation, and satisfaction (ACOG, 2025; ACOG, 2018).

The link between maternal health and fetal short- and long-term health has been comprehensively defined by the Developmental Origins of Health and Disease (DOHaD) framework. Nutrition, endocrine-metabolic status, stress, and environmental exposures during pregnancy can shape cardiometabolic and neurodevelopmental risks in childhood and adulthood through placental function, epigenetic regulation, and organogenesis (Hoffman, Reynolds, & Hardy, 2017; Gluckman & Hanson, 2013).

Concrete examples reinforce the clinical importance of this relationship. The HAPO Study showed that gradual increases in gestational glycemia are linearly associated with adverse pregnancy outcomes such as large babies, neonatal hypoglycemia, shoulder dystocia and cesarean section rates. These findings support the importance of glycemic screening and management (HAPO Study Cooperative Research Group, 2008). Furthermore, women with preeclampsia have a significantly increased long-term risk of cardiovascular disease after delivery; therefore, maternal cardiometabolic risk assessment and lifestyle interventions should be recommended in the postpartum period (Haßdenteufel et al., 2022).

Among preventive approaches, folic acid is one of the most robustly evidence-based interventions. A recent 2023 systematic review presented new data consistent with previous evidence that folic acid use before conception and in early pregnancy reduces the risk of neural tube defects; it found no additional risk for adverse outcomes such as multiple gestation, autism, or maternal cancer (Viswanathan et al., 2023).

Conclusion: Prioritizing maternal and fetal health during pregnancy; In addition to reducing mortality and morbidity, it also means investing in the lifelong health of the child and mother. Individualized antenatal care, effective management of metabolic and hypertensive disorders, and evidence-based preventive interventions such as folic acid are the cornerstones for safe motherhood and healthy generations (ACOG, 2025; UNICEF, 2025).

1.2. The concept of microbiota: definition and general characteristics

Microbiota refers to all microorganisms such as fungi, bacteria, viruses, and archaea that inhabit various parts of the human body (Lloyd-Price, Abu-Ali, & Huttenhower, 2016). The entire genetic material of these communities is called

the microbiome. The human microbiota exists in mutually beneficial symbiotic relationships with the host and plays a role in numerous vital functions,

digestion, immune system development, vitamin synthesis, and metabolic processes (Marchesi & Ravel, 2015).

For many years, the concept of microbiota was addressed solely in terms of pathogenic microorganisms. However, with the launch of the Human Microbiome Project (HMP) in 2007, microbial diversity and ecological balance in healthy individuals began to be comprehensively defined (Turnbaugh et al., 2007). This project demonstrated that the human microbiota is not merely a "passive passenger" but an active regulator of metabolic and immune homeostasis.

Microbiota composition is influenced by factors such as mode of birth, antibiotic use nutrition, environmental exposures, and age (Gomaa, 2020). While a healthy microbiota is characterized by high species diversity and balance, states of imbalance, called dysbiosis, have been linked to metabolic disorders, inflammatory diseases, and even neuropsychiatric conditions (Belizário & Napolitano, 2015).

Therefore, the microbiota is not merely a biological concept but has become a central research area for protecting human health, preventing disease, and applying personalized medicine.

1.3. Increasing Interest in Microbiota Research During Pregnancy

Interest in microbiota research during pregnancy has rapidly increased in recent years. One of the main reasons for this increase is the discovery of the direct relationship between fetal and maternal health and the microbiota (Aagaard et al., 2014). While the placenta and fetal environment were previously thought to be sterile during healthy pregnancies, current data indicate that low levels of bacterial DNA can be detected in these tissues. This finding has opened up new research areas in understanding the immune and metabolic adaptation processes of pregnancy through the microbiota (Fouhy et al., 2019).

Studies of the microbiota during pregnancy focus particularly on the relationship between the vaginal and intestinal microbiota and pregnancy complications. Changes in microbial composition in conditions including preterm birth, preeclampsia, gestational diabetes, and fetal growth restriction are important for understanding both pathophysiological mechanisms and potential therapeutic interventions (Nuriel-Ohayon, Neuman, & Koren, 2016).

Technological advances have also accelerated this research. 16S rRNA sequencing, metagenomics, and metatranscriptomics analyses reveal microbiota diversity and functionality with high resolution. These methods allow us to better understand the dynamic changes in the microbial ecosystem during pregnancy, individual differences, and the impact of environmental factors (Koren et al., 2012).

2. General Dynamics of the Microbiota During Pregnancy

2.1. Changes in the Gut Microbiota During Normal Pregnancy

Dynamic changes are observed in the gut microbiota during different stages of pregnancy. In healthy pregnancies, the composition and diversity of the gut microbiota vary across trimesters (Koren et al., 2012). During the first trimester, the gut microbiota is generally characterized by high species diversity and balance; during this period, the maternal immune system is more tolerant, and the metabolic profile is within normal limits.

In the second and especially the third trimester, significant changes occur in the gut microbiota. Increases in the proportions of Firmicutes and Proteobacteria and decreases in the proportion of Bacteroidetes have been observed (DiGiulio et al., 2015). These changes are interpreted as adaptations that optimize maternal energy storage and fat metabolism. In fact, these microbiota changes support maternal energy reserves and fetal growth throughout pregnancy (Koren et al., 2012).

Furthermore, these adaptive changes in the gut microbiota during normal pregnancy also regulate inflammatory responses. An increase in Proteobacteria and Actinobacteria during the third trimester promotes metabolic adaptation by triggering low-level inflammation, which is important for prenatal energy balance and immune tolerance (DiGiulio et al., 2015; Koren et al., 2012).

In conclusion, during a healthy pregnancy, the gut microbiota exhibits trimester-specific changes to meet both the mother's metabolic needs and support fetal development. These natural changes provide a reference base for understanding the microbiota balance throughout pregnancy and for early detection of potential complications.

2.2 Vaginal Microbiota and Its Role in Pregnancy

The vaginal microbiota plays a critical role in maternal and fetal health during pregnancy. In healthy women, the vaginal microbiota is predominantly Lactobacillus species, and low pH and lactic acid production prevent the colonization of pathogenic microorganisms (Ravel et al., 2011). Maintaining this

balance throughout pregnancy reduces the risk of miscarriage, preterm birth, and intrauterine infection.

Changes in the vaginal microbiota can be observed later in pregnancy. Lactobacillus dominance increases, particularly in the third trimester; this is interpreted as an adaptive mechanism to reduce the risk of maternal and neonatal infection during labor (Romero et al., 2014). Conversely, dysbiotic conditions, such as bacterial vaginosis or low Lactobacillus diversity, have been associated with preterm birth and low birth weight (DiGiulio et al., 2015).

The vaginal microbiota also plays a role in shaping the neonatal gut microbiota. During vaginal birth, the baby's first exposure to microorganisms comes from the mother, and this initial colonization is critical for immune system development and metabolic adaptation (Fouhy et al., 2019). Cesarean delivery alters this natural colonization, and temporary or permanent changes in the gut microbiota may occur (Dominguez-Bello et al., 2010).

Consequently, the vaginal microbiota plays a central role in maternal-fetal health throughout pregnancy. Maintaining Lactobacillus dominance and microbial diversity is vital for both reducing the risk of infection and developing the neonatal microbiota.

2.3. The Impact of Oral Microbiota and Periodontal Health on Pregnancy Complications

The oral microbiota encompasses all bacteria, fungi, and viruses found in the oral cavity and plays a critical role in maternal-fetal health during pregnancy. By maintaining healthy teeth and gum tissue, the oral microbiota prevents the overgrowth of pathogenic microorganisms. Hormonal changes (estrogen and progesterone increases) and immune modulation during pregnancy can lead to gingival inflammation and disruption of the microbiota balance (Offenbacher et al., 1996).

Periodontal disease has been identified as a significant factor increasing the risk of miscarriage, preterm birth, and preeclampsia during pregnancy (Polyzos et al., 2010). The transmission of oral pathogens to the systemic circulation can disrupt placental function by triggering inflammatory cytokine responses. For example, periodontal pathogens such as Porphyromonas gingivalis and Fusobacterium nucleatum can reach the placenta and affect fetal development (Han et al., 2004).

Furthermore, maintaining oral hygiene during pregnancy not only supports maternal periodontal health but can also influence the initial profile of the

neonatal oral and intestinal microbiota. Regular dental checkups, plaque control, and dietary management help protect the health of both mother and fetus by reducing periodontal inflammation (Xiong et al., 2006).

Consequently, oral microbiota and periodontal health should be considered critical biological and clinical targets for preventing pregnancy complications and protecting maternal-fetal health.

2.4. Is placental microbiota real or contamination?

Traditionally, the placenta and fetal environment have been considered sterile. However, some recent studies have suggested that low levels of bacterial DNA can be detected in the placenta and have introduced the concept of "placental microbiota" (Aagaard et al., 2014). These studies have hypothesized that the fetus may be exposed to microorganisms before birth.

Conversely, some researchers emphasize that placental microbiota findings may be due to laboratory contamination. In particular, the low biological signal and the risk of environmental contamination during sampling have led to debate about the authenticity of the placental microbiota (Lauder et al., 2016). Various metagenomic and 16S rRNA analyses have shown that the microorganism profiles detected in placenta samples are mostly consistent with laboratory and reagent contaminants.

Recent systematic reviews reveal that evidence for the existence of the placental microbiota is limited and controversial (Fouhy et al., 2019). Despite this, the detection of certain pathogens in the placenta (e.g., Fusobacterium nucleatum) has been associated with intrauterine infection and pregnancy complications. Therefore, research on the existence and role of the placental microbiota continues, both in terms of methodological sensitivity and clinical relevance.

3. Microbiota Modulation: Prebiotics, Probiotics, and Nutritional Strategies

Maintaining microbiota health during pregnancy is critical for both maternal and fetal health. Modulation of the microbiota is possible through probiotics, prebiotics, and dietary strategies. Probiotics, as live microorganisms, balance the intestinal, vaginal, or oral microbiota, preventing pathogen overgrowth and regulating immune responses (Hanson et al., 2022).

Prebiotics, on the other hand, support the growth of beneficial bacteria by providing a nutritional source for intestinal bacteria. Fructooligosaccharides and galactooligosaccharides, in particular, promote the growth of Lactobacillus and

Bifidobacterium species (Roberfroid, 2007). This approach may be effective in reducing pregnancy risks such as gestational diabetes and inflammatory complications.

Nutritional strategies are also important for microbiota modulation. Dietary fiber, fermented foods, omega-3 fatty acids, and polyphenols support gut microbiota diversity and metabolic function. For example, a fiber-rich diet reduces inflammation and improves glucose metabolism by increasing the production of short-chain fatty acids (SCFAs) (Koren et al., 2012).

Studies on the safety of probiotic and prebiotic use during pregnancy have largely shown that Lactobacillus and Bifidobacterium species are safe. However, dose and species selection should be planned considering the gestational age and individual health status (Martínez et al., 2020).

In conclusion, microbiota modulation stands out as a strategy that both reduces the risk of complications during pregnancy and supports maternal-fetal health. Probiotic, prebiotic, and nutrition-focused approaches may play an important role in personalized pregnancy care in the future.

4. Future research areas and microbiota-focused pregnancy applications

Microbiota research during pregnancy is rapidly evolving, both in terms of basic science and clinical practice. Future studies will focus on the development of microbiota-based diagnostic, risk assessment, and treatment strategies. In particular, advanced metagenomics and metabolomics analyses of gut, vaginal, and oral microbiota profiles in relation to pregnancy complications will pave the way for personalized medicine applications (Nuriel-Ohayon, Neuman, & Koren, 2016).

Research on microbiota modulation will focus on the safety of probiotic and prebiotic applications during pregnancy, optimal dosage, and strain selection. Furthermore, dietary interventions, functional foods, and microbiota-friendly lifestyle strategies can support maternal-fetal health (Hanson et al., 2022).

In the future, early detection of complications such as preterm birth, preeclampsia, and gestational diabetes may be possible through non-invasive microbiota-based biomarkers. Additionally, artificial intelligence and machine learning techniques can be integrated into clinical decision support systems by creating risk profiles in the analysis of microbiota data (Vatanen et al., 2019).

5. The Relationship Between Pregnancy Complications and the Microbiota

Microbiota changes during pregnancy can play an important role in the development of various obstetric complications. The relationships between gut, vaginal, and oral microbiota profiles and conditions such as preterm birth, preeclampsia, and gestational diabetes are becoming increasingly clear (Nuriel-Ohayon, Neuman, & Koren, 2016).

Preterm birth has been associated with an imbalance in the vaginal microbiota. Low Lactobacillus dominance and increased pathogenic anaerobes can trigger inflammatory cytokine production, thereby initiating premature cervical ripening and uterine contractions (Romero et al., 2014).

In preeclampsia and other hypertensive pregnancy disorders, changes in the Firmicutes/Bacteroidetes ratio in the gut microbiota and increased lipopolysaccharide levels may trigger metabolic inflammation. This may contribute to the development of preeclampsia by affecting maternal vascular and endothelial functions (Amabebe & Anumba, 2018). In gestational diabetes, increased Proteobacteria and Actinobacteria in the gut microbiota have been associated with insulin resistance and maternal metabolic disorders. Monitoring microbiota profiles and, when necessary, probiotic or strategic nutritional interventions are considered a potential way to reduce the risk of gestational diabetes (Kuang et al., 2020).

Additionally, oral microbiota and periodontal disease have been associated with both preterm birth and low birth weight. Pathogens such as Fusobacterium nucleatum and Porphyromonas gingivalis can disrupt placental function by increasing systemic inflammation (Han et al., 2004).

5.1 Gestational Diabetes and Gut Microbiota

Gestational diabetes (GDM) is a metabolic disorder characterized by glucose intolerance that begins during pregnancy and generally resolves postpartum. Recent research has shown that the gut microbiota plays a significant role in the development of GDM. While in normal pregnancy, the gut microbiota exhibits adaptive changes that support energy metabolism and immune functions, this balance is disrupted in GDM (Kuang et al., 2020).

In women with GDM, an increase in certain bacterial species, such as Proteobacteria and Actinobacteria, changes in the Firmicutes/Bacteroidetes ratio, and changes in short-chain fatty acid (SCFA) production have been observed in

the gut microbiota. These changes may increase maternal insulin resistance and negatively impact glucose metabolism (Crusell et al., 2018).

Furthermore, the impact of GDM on the gut microbiota may also affect fetal metabolism. Disruptions in the maternal gut microbiota can have lasting effects on the fetal postnatal metabolic profile and increase the risk of obesity and type 2 diabetes (Collado et al., 2008).

Microbiota-based interventions are considered promising strategies for reducing the risk of GDM. Probiotic and prebiotic applications can reduce inflammation and improve glucose metabolism by promoting the growth of beneficial bacteria (Lindsay et al., 2015). Furthermore, dietary modifications and increased fiber intake may prevent the development of GDM by balancing the gut microbiota.

Preterm birth, that is, births occurring before 37 weeks of gestation, is a significant risk factor for neonatal morbidity and mortality. Recent research suggests that an imbalance in the vaginal microbiota may increase the risk of preterm birth (Romero et al., 2014).

A healthy vaginal microbiota is generally Lactobacillus-dominant, and low pH inhibits the proliferation of pathogenic bacteria. However, a decrease in Lactobacillus species and an increase in pathogenic anaerobic bacteria (e.g., Gardnerella vaginalis, Atopobium vaginae, Prevotella species) may trigger inflammatory cytokine production, leading to premature cervical ripening and uterine contractions (DiGiulio et al., 2015). Furthermore, the vaginal microbiota profile is being evaluated as a potential biomarker for predicting the risk of preterm birth. It has been suggested that this risk can be reduced through microbiota modulation and probiotic or prebiotic applications (Brown et al., 2019). Regulation of the vaginal microbiota during pregnancy may support both maternal and neonatal health.

5.2 Microbiota–Fetal Immune System Interactions in the Intrauterine Period

The fetal immune system is shaped by maternal and environmental factors during the intrauterine period. The microbiota can play a critical role in this process. Metabolites and molecules from microorganisms in the intestinal and vaginal microbiota can interact with the fetus through the placenta and influence immune system development (Gomez de Aguero et al., 2016).

In the intrauterine period, the fetal immune system is in the maturation process of immune components such as T cells, B cells, and natural killer cells.

Microbiota-derived short-chain fatty acids (SCFAs) and other metabolites modulate fetal immune responses, balancing inflammation and tolerance mechanisms (Dahl et al., 2018).

Furthermore, disruptions in the maternal gut microbiota can lead to increased inflammatory signals, which can impact fetal immune system development and shape postnatal immune responses and metabolic profiles (Collado et al., 2016). These interactions may influence the risk of allergies, autoimmune diseases, and metabolic disorders.

5.3 Effects of Delivery Mode (Vaginal vs. Cesarean Section) on the Neonatal Microbiota

The mode of delivery is a critical factor in the formation and development of the neonatal microbiota. During vaginal delivery, the newborn acquires microbial colonization from the mother's vaginal and intestinal microbiota. This supports the maturation of the neonatal immune system and long-term metabolic health (Dominguez-Bello et al., 2010).

In contrast, during Cesarean delivery, the infant acquires less microbial colonization from the maternal vaginal microbiota and more from the skin and hospital environment. This is associated with decreased Bacteroides and Bifidobacterium diversity and increased pathogenic bacterial counts in the gut microbiota (Biasucci et al., 2010). This differential colonization pattern can influence the formation of immune tolerance and the inflammatory response and has been linked to an increased risk of allergy, asthma, and obesity in some studies (Azad et al., 2013).

In contrast, some studies suggest that strategies such as maternal vaginal fluid transfer for microbiota “restoration” in infants born by cesarean section may restore neonatal microbiota development to levels close to those of vaginal birth (Dominguez-Bello et al., 2016).

6. Use of Prebiotics and Probiotics During Pregnancy

Probiotics, as live microorganisms, help regulate immune responses by maintaining the balance of the intestinal, vaginal, and oral microbiota, thereby preventing the overgrowth of pathogenic species (Hanson et al., 2022). During pregnancy, probiotic supplementation can benefit both maternal and fetal health, particularly by preserving intestinal microbiota balance and reducing inflammation.

Prebiotics, on the other hand, are nutritional components that promote the proliferation of beneficial bacteria in the gut. Fructooligosaccharides,

galactooligosaccharides, and other fibrous components contribute to the regulation of energy metabolism and the immune response by increasing the diversity of the intestinal microbiota (Roberfroid, 2007).

Some benefits of using probiotics and prebiotics during pregnancy include:

Reducing the risk of gestational diabetes: Probiotics can increase insulin sensitivity by supporting the proliferation of beneficial bacteria in the gut (Lindsay et al., 2015).

Reducing the risk of preeclampsia and hypertension: Microbiota modulation can reduce the inflammatory response and support endothelial function (Mekonnen et al., 2021).

Reducing the risk of preterm birth: Balancing the vaginal and intestinal microbiota may reduce the risk of preterm birth (Brown et al., 2019).

Furthermore, probiotic and prebiotic applications support the development of the neonatal microbiota, contributing to the maturation of the immune system after birth. Product selection, dose, and duration of use are important for safety and effectiveness during pregnancy.

7. Future Perspectives and Clinical Implications

7.1 New Microbiota-Based Therapeutic Approaches

In recent years, with the understanding of the critical role of the microbiota in health, new microbiota-based therapeutic approaches aimed at supporting maternal and fetal health during pregnancy have emerged. One of the most notable of these treatments is fecal microbiota transplantation (FMT). FMT is based on the transfer of gut microbiota derived from a healthy donor to individuals experiencing microbiota imbalances. While proven effective in clinical practice, particularly in the treatment of Clostridioides difficile infection, FMT is still experimental in terms of its safety and efficacy in pregnancy (Allegretti et al., 2019). However, given the relationship between gut microbiota and pregnancy complications (gestational diabetes, preeclampsia, etc.), pregnancy-specific applications of these methods may be considered in the future.

In addition, experimental therapeutic approaches supported by probiotic and prebiotic supplements are also attracting attention. It is suggested that probiotics may reduce the risk of preterm birth by regulating the vaginal microbiota during pregnancy and support maternal and fetal health by modulating the immune response (Koren et al., 2012). Prebiotics, on the other hand, may have positive

effects on metabolic health during pregnancy by promoting the proliferation of beneficial bacteria.

Furthermore, postbiotics (metabolites of beneficial microorganisms) and microbiota-based biotechnological products are also being investigated. While there is currently insufficient evidence on the safety of these treatment strategies in pregnancy, positive effects have been reported in animal models and a limited number of human studies (Zmora et al., 2019).

In the future, microbiota-based therapies are anticipated to play a significant role in pregnancy management, in conjunction with personalized medicine. However, more randomized controlled trials and safety data are needed before these approaches can be implemented in routine clinical practice.

7.2 Use of Microbiota-Based Biomarkers in Obstetrics

In recent years, microbiota profiling has been evaluated as a potential biomarker for the early diagnosis of obstetric complications. It has been suggested that changes in the composition of the gut and vaginal microbiota may serve as biomarkers, particularly in predicting the risk of gestational diabetes, preeclampsia, and preterm birth (Koren et al., 2012; Dunlop et al., 2021). High-resolution metagenomic approaches are allowing clearer understanding of the association between specific bacterial species or microbial metabolites and diseases. However, standardization and large-scale cohort studies are needed to integrate these biomarkers into clinical practice (Davenport et al., 2017).

7.3 Microbiota Analysis in Pregnancy Monitoring

The use of microbiota analysis during pregnancy holds promise for the early diagnosis of complications that may affect both maternal and fetal health. Regular monitoring of changes in the gut, vaginal, and oral microbiota may enable more effective management of high-risk pregnancies (DiGiulio et al., 2015). However, several limitations prevent the introduction of microbiota analyses into clinical routine. These include cost, technical differences, lack of standardization in sample collection and storage conditions, and difficulties in clinical interpretation (Marchesi et al., 2016). Therefore, translational research is needed to integrate microbiota analyses into obstetric practice.

7.4 Personalized Medicine and Microbiota-Based Approaches

Personalized medicine aims to develop customized treatment and prevention strategies based on individuals' genetic, epigenetic, and microbiota profiles. Specifically, microbiota-based personalized approaches to pregnancy hold great potential for preventing complications and optimizing maternal and fetal health

(Ravel & Brotman, 2016). Specifically, tailoring probiotic, prebiotic, and postbiotic interventions to individual microbiota characteristics can increase treatment effectiveness. Furthermore, artificial intelligence– and bioinformatics–driven analyses are expected to facilitate the development of personalized, microbiota-based protocols for pregnancy monitoring (Knight et al., 2017).

8. CONCLUSION

Evidence accumulating over the last decade demonstrates that the gut, vaginal, and oral microbiota dynamically change in composition and function throughout pregnancy; These alterations may be linked to major obstetric outcomes, including preterm birth, preeclampsia, and gestational diabetes. Maintaining Lactobacillus-dominant profiles in the pregnant vagina is associated with a reduced risk of preterm birth, while maintaining the short-chain fatty acid (SCFA) ecosystem in the gut is associated with metabolic and immune homeostasis. While findings regarding the presence of a placental microbiota are methodologically controversial, there is evidence that certain pathogens can reach placental tissue. Overall, the findings indicate that the microbiota has a crucial influence on regulating inflammation, maintaining barrier integrity, and modulating metabolism within the maternal-fetal axis.

The most mature clinical approach is risk-based lifestyle and dietary modifications combined with targeted probiotic/prebiotic use. These strategies can be applied complementarily to reduce the risk of GDM, support vaginal eubiosis, and control periodontal inflammation. However, microbiota profiling (vaginal/gut) in routine care has not yet been standardized; guideline-level consensus is needed for sampling, analysis platforms, thresholds, and interpretation of results. Balancing benefit and risk for antibiotic indications is important to mitigate potential adverse effects on the microbiota; post-treatment rehabilitation (diet, probiotics/prebiotics) may be a reasonable adjunctive strategy. Approaches to promote neonatal colonization after cesarean section (e.g., nutritional interventions) may be considered in selected cases, but large-scale safety and efficacy data are awaited.

9. REFERENCES

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., & Versalovic, J. (2014). The placenta harbors a unique microbiome. Science Translational Medicine, 6(237), 237ra65. https://doi.org/10.1126/scitranslmed.3008599

Amabebe, E., & Anumba, D. O. C. (2018). The vaginal microenvironment: The physiologic role of Lactobacilli. Frontiers in Medicine, 5, 181. https://doi.org/10.3389/fmed.2018.00181

Allegretti, J. R., Mullish, B. H., Kelly, C., & Fischer, M. (2019). The evolution of the use of faecal microbiota transplantation and emerging therapeutic indications. The Lancet, 394(10196), 420–431. https://doi.org/10.1016/S0140-6736(19)31266-8

American College of Obstetricians and Gynecologists. (2018). Group prenatal care (Committee Opinion No. 731). Obstetrics & Gynecology, 131(3), e104–e109. https://doi.org/10.1097/AOG.0000000000002539

American College of Obstetricians and Gynecologists. (2025). Tailored prenatal care delivery for pregnant individuals (ACOG Clinical Consensus). Retrieved from https://www.acog.org

Azad, M. B., Konya, T., Maughan, H., et al. (2013). Gut microbiota of healthy Canadian infants: Profiles by mode of delivery and infant diet at 4 months. CMAJ, 185(5), 385–394. https://doi.org/10.1503/cmaj.121189

Belizário, J. E., & Napolitano, M. (2015). Human microbiomes and their roles in dysbiosis, common diseases, and novel therapeutic approaches. Frontiers in Microbiology, 6, 1050. https://doi.org/10.3389/fmicb.2015.01050

Biasucci, G., Benenati, B., Morelli, L., Bessi, E., & Boehm, G. (2010). Cesarean delivery may affect the early biodiversity of intestinal bacteria. The Journal of Nutrition, 140(9), 1796S–1800S. https://doi.org/10.3945/jn.110.124382

Brown, R. G., Al-Memar, M., Marchesi, J. R., et al. (2019). Establishment of vaginal microbiota composition in early pregnancy and its association with subsequent preterm prelabor rupture of membranes. Microbiome, 7, 9. https://doi.org/10.1186/s40168-019-0610-9

Collado, M. C., Isolauri, E., Laitinen, K., & Salminen, S. (2008). Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. The American Journal of Clinical Nutrition, 88(4), 894–899. https://doi.org/10.1093/ajcn/88.4.894

Collado, M. C., Rautava, S., Aakko, J., Isolauri, E., & Salminen, S. (2016). Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Scientific Reports, 6, 23129. https://doi.org/10.1038/srep23129

Crusell, M. K. W., Hansen, T. H., Nielsen, T., Allin, K. H., Ruhlemann, M. C., Damm, P., Vestergaard, H., Hansen, L. B., Pedersen, O., & Hollegaard, M. V. (2018). Gestational diabetes is associated with changes in the gut microbiota composition in third trimester of pregnancy and postpartum. Microbiome, 6, 89. https://doi.org/10.1186/s40168-018-0485-9

Dahl, C. P., Svingen, T., & Skogstrand, K. (2018). Maternal gut microbiota and fetal immune development. Current Opinion in Endocrine and Metabolic Research, 3, 1–8. https://doi.org/10.1016/j.coemr.2018.03.001

Davenport, E. R., Sanders, J. G., Song, S. J., Amato, K. R., Clark, A. G., & Knight, R. (2017). The human microbiome in evolution. BMC Biology, 15(1), 127. https://doi.org/10.1186/s12915-017-0454-7

DiGiulio, D. B., Callahan, B. J., McMurdie, P. J., Costello, E. K., Lyell, D. J., Robaczewska, A., Sun, C. L., Goltsman, D. S., Wong, R. J., Shaw, G., Stevenson, D. K., Holmes, S. P., Relman, D. A., & HMP Pregnancy Study. (2015). Temporal and spatial variation of the human microbiota during pregnancy. Proceedings of the National Academy of Sciences, 112(35), 11060–11065. https://doi.org/10.1073/pnas.1502875112

Dominguez-Bello, M. G., Costello, E. K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., & Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences, 107(26), 11971–11975. https://doi.org/10.1073/pnas.1002601107

Dominguez-Bello, M. G., De Jesus-Laboy, K. M., Shen, N., et al. (2016). Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. Nature Medicine, 22(3), 250–253. https://doi.org/10.1038/nm.4039

Fouhy, F., Guinane, C. M., Hussey, S., Wall, R., Ryan, C. A., Dempsey, E. M., Murphy, B., Ross, R. P., & Stanton, C. (2019). High-throughput sequencing reveals the incomplete, variable, and disrupted nature of the human placental microbiome. Microbiome, 7, 152. https://doi.org/10.1186/s40168-019-0751-7

Gluckman, P. D., & Hanson, M. A. (2013). Early developmental conditioning of later health and disease: Physiology or pathophysiology? Physiological Reviews, 93(3), 1089–1116. https://doi.org/10.1152/physrev.00029.2012

Gomaa, E. Z. (2020). Human gut microbiota/microbiome in health and diseases: A review. Antonie van Leeuwenhoek, 113(12), 2019–2040. https://doi.org/10.1007/s10482-020-01474-7

Gomez de Aguero, M., Ganal-Vonarburg, S. C., Fuhrer, T., et al. (2016). The maternal microbiota drives early postnatal innate immune development. Science, 351(6279), 1296–1302. https://doi.org/10.1126/science.aad2571

Han, Y. W., Ikegami, A., Bissada, N. F., & Li, M. (2004). Fusobacterium nucleatum induces premature and term stillbirths in pregnant mice: Implications for human pregnancy outcomes. Infection and Immunity, 72(4), 2272–2279. https://doi.org/10.1128/IAI.72.4.2272-2279.2004

HAPO Study Cooperative Research Group. (2008). Hyperglycemia and adverse pregnancy outcomes. The New England Journal of Medicine, 358(19), 1991–2002. https://doi.org/10.1056/NEJMoa0707943

Haßdenteufel, K., Müller, M., Gutsfeld, R., Goetz, M., Bauer, A., & Wallwiener, M. (2022). Long-term effects of preeclampsia on maternal cardiovascular health. Journal of Hypertension and Management, 8, 054. https://doi.org/10.23937/2474-3690/1510054

Hanson, M. A., Gluckman, P. D., & Gardner, D. S. (2022). Maternal nutrition, the microbiome, and offspring health. Nature Reviews Endocrinology, 18(12), 753–769. https://doi.org/10.1038/s41574-022-00748-0

Hoffman, D. J., Reynolds, R. M., & Hardy, D. B. (2017). Developmental origins of health and disease: Current knowledge and potential mechanisms. Nutrition Reviews, 75(12), 951–970. https://doi.org/10.1093/nutrit/nux053

Knight, R., Vrbanac, A., Taylor, B. C., Aksenov, A., Callewaert, C., Debelius, J., ... & Dorrestein, P. C. (2017). Best practices for analysing microbiomes. Nature Reviews Microbiology, 16(7), 410–422. https://doi.org/10.1038/s41579-018-0029-9

Koren, O., Goodrich, J. K., Cullender, T. C., Spor, A., Laitinen, K., Bäckhed, H. K., Gonzalez, A., Werner, J. J., Angenent, L. T., Knight, R., Bäckhed, F., & Ley, R. E. (2012). Host remodeling of the gut microbiome and metabolic changes during pregnancy. Cell, 150(3), 470–480. https://doi.org/10.1016/j.cell.2012.07.008

Korpela, K., Salonen, A., Virta, L. J., et al. (2016). Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. Nature Communications, 7, 10410. https://doi.org/10.1038/ncomms10410

Kuang, Y., Li, J., Wang, Y., Zhang, Y., Song, Y., & Zhao, W. (2020). Gut microbiota in gestational diabetes mellitus: A review. Nutrition & Metabolism, 17, 51. https://doi.org/10.1186/s12986-020-00467-3

Lauder, A. P., Roche, A. M., Sherrill-Mix, S., Bailey, A., Laughlin, A., Bittinger, K., Leite, R., Elovitz, M. A., Parry, S., & Bushman, F. D. (2016). Comparison of placenta samples with contamination controls does not provide evidence for a distinct placenta microbiota. Microbiome, 4, 29. https://doi.org/10.1186/s40168-016-0172-3

Lindsay, K. L., Kennelly, M., Culliton, M., Kennedy, R., & McAuliffe, F. M. (2015). Effect of probiotics in prevention of gestational diabetes mellitus in overweight and obese women: A randomised controlled trial. BMJ Open, 5(10), e008676. https://doi.org/10.1136/bmjopen-2015-008676

Liu, Y., Wang, Z., Li, X., Shi, Y., & Li, Y. (2019). Gut microbiota and preeclampsia: Current evidence and potential mechanisms. Frontiers in Cellular and Infection Microbiology, 9, 423. https://doi.org/10.3389/fcimb.2019.00423

Marchesi, J. R., & Ravel, J. (2015). The vocabulary of microbiome research: A proposal. Microbiome, 3, 31. https://doi.org/10.1186/s40168-015-0094-5

Martínez, I., Lattimer, J. M., Hubach, K. L., Case, J. A., Yang, J., Weber, C. G., Louk, J. A., Rose, D. J., Kyureghian, G., Peterson, D. A., & Haub, M. D. (2020). Gut microbiome composition is linked to whole grain-induced immunological improvements. The ISME Journal, 14, 325–339. https://doi.org/10.1038/s41396-019-0532-3

Mekonnen, T. H., Gedamu, G., & Gebreyes, A. (2021). Probiotics and prebiotics for prevention of preeclampsia: A review. International Journal of Women's Health, 13, 723–733. https://doi.org/10.2147/IJWH.S300065

Nogacka, A., Salazar, N., Suárez, M., et al. (2018). Impact of early life antibiotics on gut microbiota, immune development and metabolism. Current Opinion in Gastroenterology, 34(2), 109–116. https://doi.org/10.1097/MOG.0000000000000437

Nuriel-Ohayon, M., Neuman, H., & Koren, O. (2016). Microbial changes during pregnancy, birth, and infancy. Frontiers in Microbiology, 7, 1031. https://doi.org/10.3389/fmicb.2016.01031

Offenbacher, S., Beck, J., Jared, H., & Williams, R. (1996). Periodontal infection as a possible risk factor for preterm low birth weight. Journal of Periodontology, 67(10s), 1103–1113. https://doi.org/10.1902/jop.1996.67.10s.1103

Polyzos, N. P., Polyzos, I. P., Zavos, A., & Falagas, M. E. (2010). The role of periodontal disease in adverse pregnancy outcomes: A systematic review. American Journal of Obstetrics and Gynecology, 202(4), 321–332. https://doi.org/10.1016/j.ajog.2009.12.052

Ravel, J., Gajer, P., Abdo, Z., Schneider, G. M., Koenig, S. S. K., McCulle, S. L., Karlebach, S., Gorle, R., Russell, J., Tacket, C. O., Brotman, R. M., Davis, C. C., Ault, K., Peralta, L., & Forney, L. J. (2011). Vaginal microbiome of reproductive-age women. Proceedings of the National Academy of Sciences, 108(Supplement 1), 4680–4687. https://doi.org/10.1073/pnas.1002611107

Roberfroid, M. B. (2007). Prebiotics: The concept revisited. The Journal of Nutrition, 137(3 Suppl 2), 830S–837S. https://doi.org/10.1093/jn/137.3.830S

Romero, R., Hassan, S. S., Gajer, P., Tarca, A. L., Fadrosh, D. W., Nikita, L., Galuppi, M., Lamont, R. F., Chaemsaithong, P., Miranda, J., Chaiworapongsa, T., & Ravel, J. (2014). The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome, 2, 4. https://doi.org/10.1186/2049-2618-2-4

Turnbaugh, P. J., Ley, R. E., Hamady, M., Fraser-Liggett, C. M., Knight, R., & Gordon, J. I. (2007). The human microbiome project. Nature, 449(7164), 804–810. https://doi.org/10.1038/nature06244

United Nations Children's Fund (UNICEF). (2025, March). Neonatal mortality (Indicator profile). Retrieved from https://data.unicef.org

Vatanen, T., Franzosa, E. A., Schwager, R., Tripathi, S., Arthur, T. D., Vehik, K., Lernmark, Å., Hagopian, W., Rewers, M., She, J. X., Toppari, J., Ziegler, A. G., Akolkar, B., Krischer, J. P., Stewart, C. J., Ajami, N. J., Petrosino, J. F., & Xavier, R. J. (2019). The human gut microbiome in early-onset type 1 diabetes from the TEDDY study. Nature, 562(7728), 589–594. https://doi.org/10.1038/s41586-018-0850-x

Viswanathan, M., Urrutia, R. P., Hudson, K. N., Middleton, J. C., & Kahwati, L. C. (2023). Folic acid supplementation to prevent neural tube defects: Updated evidence report and systematic review for the U.S. Preventive Services Task Force. JAMA, 330(5), 454–459. https://doi.org/10.1001/jama.2023.12345

World Health Organization, United Nations Children's Fund (UNICEF), United Nations Population Fund (UNFPA), World Bank Group, & United Nations Department of Economic and Social Affairs, Population Division. (2025, April). Trends in maternal mortality 2000 to 2023: Estimates by the UN Maternal Mortality Estimation Inter-Agency Group. Retrieved from https://www.who.int

Xiong, X., Buekens, P., Fraser, W. D., Beck, J., & Offenbacher, S. (2006). Periodontal disease and adverse pregnancy outcomes: A systematic review. BJOG: An International Journal of Obstetrics & Gynaecology, 113(2), 135–143. https://doi.org/10.1111/j.1471-0528.2006.00896.x

Yang, H., Zhao, Y., Zhang, L., & Li, J. (2020). Gut microbiota and preeclampsia: Exploring the relationship. Journal of Maternal-Fetal & Neonatal Medicine, 33(21), 3649–3657. https://doi.org/10.1080/14767058.2019.1616332

Zmora, N., Suez, J., & Elinav, E. (2019). You are what you eat: diet, health and the gut microbiota. Nature Reviews Gastroenterology & Hepatology, 16(1), 35–56. https://doi.org/10.1038/s41575-018-0061-2

CHAPTER 8

INNOVATIVE PHYSIOTHERAPY APPROACHES IN PELVIC FLOOR DYSFUNCTION

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1. INTRODUCTION

The pelvis, which supports the weight of the upper body, is a central structure of the human body situated between the lumbar region of the abdomen above and the thighs below. The pelvic cavity refers to the space enclosed by the pelvic bones. Superiorly, it is continuous with the abdominal cavity, while inferiorly it is bounded by the pelvic floor. This cavity is divided into two regions: the greater pelvis and the lesser pelvis. The greater pelvis, considered a part of the abdominal cavity, is also known as the false pelvis. In contrast, the lesser pelvis belongs to the true pelvic region and is referred to as the true pelvis. Posteriorly, the pelvic cavity is bordered by the sacrum and the coccyx. Functionally, the pelvic cavity serves as a housing space for the urinary bladder, pelvic colon, internal reproductive organs, and the rectum. Additionally, it contains various internal structures and tissues, including muscles, arteries, veins, nerves, and pelvic connective tissue (1). The pelvic floor is a group of muscles that provide support for the organs within the pelvis. It consists of the pelvic diaphragm, which stretches from the pubic symphysis at the front to the coccyx at the back, forming a hammock-like structure that upholds the pelvic organs. The muscles of the pelvic floor include the levator ani group — composed of the puborectalis, pubococcygeus, and iliococcygeus muscles — along with the coccygeus muscle. The levator ani muscles play a crucial role in maintaining pelvic organ support and are innervated by the fourth sacral nerve (2). The pelvic floor structures receive their main nerve supply from the sacral nerves S3 and S4 via the pudendal nerve, and their primary blood supply comes from the parietal branches of the internal iliac artery. The muscles of the pelvic floor support the pelvic organs — including the bladder, urethra, prostate (in males), vagina and uterus (in females), anus, and rectum — as well as the intra-abdominal contents, help maintain urinary and fecal continence, and contribute to sexual functions such as arousal and orgasm (3). The pelvic floor is crucial due to its association with various

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important functions. The pelvic floor, operates in close coordination with the diaphragm and plays an essential role in the respiratory process. During inhalation, both the diaphragm and the PF descend caudally, whereas during exhalation, the diaphragm ascends cephalically as it relaxes, and the pelvic floor contracts. This synchronous movement contributes to maintaining optimal intra-abdominal pressure. Any disruption in the coordination between the diaphragm and the pelvic floor may lead to pressure imbalances, which can consequently cause dysfunctions in other physiological systems, such as impaired peritoneal fluid drainage or reduced postural stability. The pelvic floor is a key component in maintaining postural stability and overall body alignment. Together with core and postural muscles (abdominal, gluteal, and multifidus muscles) the pelvic floor contributes to proper stabilization of the trunk and pelvis. The activity of the pelvic floor is modulated by the tension within these associated core muscles. The proper functioning of the pelvic floor is also influenced by its myofascial connections with the lower extremities. The pelvic floor is linked to the lower limb through fascial continuities involving the gluteal muscles and the internal obturator muscle, both of which regulate hip joint mechanics and lower limb biomechanic. Altered tension or dysfunction in the gluteal region may disturb hip joint mobility, modify mechanical loading patterns, and consequently affect locomotion. Similarly, dysfunctions of the hip joint or lower limb can predispose individuals to pelvic floor -related disorders. The pelvic floor is also integrated with the upper limb complex, cervical spine, and craniofacial structures through fascial connections, including the transversalis, mediastinal, and cervical fascia. Alterations or dysfunctions within this fascial network may manifest as upper limb disorders, diaphragmatic dysfunction, or even parafunctional conditions such as bruxism (4).

Pelvic floor dysfunction (PFD) encompasses a broad spectrum of symptoms, anatomical alterations, and functional disorders resulting from abnormal activity of the pelvic floor muscles. This dysfunction may present as increased muscle tone (hypertonicity), reduced muscle tone (hypotonicity), or impaired coordination of the pelvic floor musculature. Structural changes affecting the support of pelvic organs are also included within this spectrum and are referred to as pelvic organ prolapse (POP). PFD include pelvic organ prolapse, urodynamic stress urinary incontinence, detrusor overactivity, bladder oversensitivity, and voiding dysfunction, as well as associated symptoms such as anal incontinence, dyspareunia, and perineal or pelvic pain (5,6).

Pelvic organ prolapse is a pathological condition characterized by the descent of one or more pelvic organs—including the uterus, bladder, and rectum—from

their normal anatomical positions into the vaginal canal, predominantly resulting from the attenuation or failure of the pelvic floor support mechanisms. Although not life-threatening, pelvic organ prolapse can profoundly compromise a patient's quality of life and is frequently associated with considerable psychosocial and functional morbidity (7). Stress urinary incontinence is defined as the involuntary loss of urine through the external urethral orifice that occurs when an increase in intra-abdominal pressure is produced by activities such as sneezing, coughing, or laughing (8). Although not a life-threatening condition, urinary incontinence has been correlated with significant psychosocial consequences, including social withdrawal, anxiety, depressive symptoms, occupational impairment, and sexual dysfunction (9). According to the ICS, detrusor overactivity is defined as "the occurrence of detrusor contraction(s) during filling cystometry". It represents a urodynamic observation characterized by involuntary contractions of the detrusor muscle, which may occur spontaneously or be provoked during bladder filling. These contractions generate waveforms of variable amplitude and duration on the cystometrogram. Epidemiological data indicate that detrusor overactivity is present in approximately 10% of the general population and in nearly 80% of elderly individuals undergoing urodynamic evaluation. Clinically, detrusor overactivity may present with symptoms such as urgency, frequency, and urge urinary incontinence, resulting in a substantial deterioration of the affected individuals' quality of life (10). According to the ICS, bladder oversensitivity is defined as an increased perception of bladder sensation during the filling phase, characterized by an early desire to void in the absence of any corresponding rise in detrusor pressure on cystometric evaluation (11). The ICS defines female voiding dysfunction as abnormally slow and/or incomplete bladder emptying, characterized by reduced urinary flow rates and/or elevated post-void residual volumes, preferably confirmed through repeated measurements to ensure diagnostic accuracy (12). Anal incontinence is defined as the involuntary loss of fecal matter or flatus, resulting from a failure of the normal mechanisms responsible for maintaining continence (13). Dyspareunia is defined as genital pain occurring before, during, or after penile–vaginal sexual intercourse (14). Pelvic pain is defined as pain localized to the lower abdominal region below the umbilicus, encompassing the pelvis, pelvic organs, and genital structures. It is associated with a substantial individual burden, including diminished physical functioning, adverse psychological outcomes, reduced social engagement, impaired sexual function, and negative effects on occupational performance (15).

2. ASSESSMENT METHODS IN PELVIC FLOOR DYSFUNCTION

Various methods (including clinical examination techniques, imaging methods, functional tests, questionnaires and self-report measurement tools, and current technologies) are used in the assessment of pelvic floor dysfunction. These approaches enable accurate evaluation of patients' symptoms and facilitate the development of an appropriate treatment plan (16).

2.1. Clinical Assessment Method

2.1.1. Digital Palpation Method

With the digital palpation method, pelvic floor muscle contraction is assessed through a vaginal examination. The individual squeezes the index and middle fingers placed inside the vagina. The strength of the contraction is generally evaluated using the Modified Oxford Scale (MOS). According to this scale: 0 = no contraction, 1 = flicker/tremor, 2 = weak, 3 = moderate, 4 = good, 5 = strong contraction (16). In addition to muscle strength, other parameters such as power, endurance, repetitions, fast contractions, and timing of each contraction can be standardized using the PERFECT scheme. This method is frequently used, particularly in women, to quickly screen pelvic floor muscle function and to teach exercises (17). Digital vaginal examination is practical and economical since it requires no equipment; it also allows direct feedback from the clinician or physiotherapist. When performed correctly, it demonstrates good reproducibility among experienced evaluators and provides patients with the opportunity to learn correct muscle activation. However, it is a subjective method and depends on the evaluator. Its reliability is limited when not performed by experienced practitioners (18,19).

2.2. Pelvic Organ Prolapse Quantification System (POP-Q)

The Pelvic Organ Prolapse Quantification System (POP-Q) is an internationally standardized method used to determine the severity of pelvic organ prolapse (13). During vaginal examination, nine anatomical reference points are evaluated, and the degree of prolapse is classified into stages 0 to 4. According to the standardized POP-Q assessment, Stage 0 indicates the absence of prolapse; Stage 1 refers to a prolapse point located more than 1 cm above the hymen; Stage 2 indicates the prolapse point lies within ±1 cm of the hymenal plane, either inside or outside; Stage 3 is defined as the most distal prolapse point being more than 1 cm below the hymen but less than 2 cm short of the total vaginal length; and Stage 4 represents complete prolapse, in which the vagina is entirely descended outside (20).

2.3. Ultrasonography (USG)

Translabial/transperineal 2D and 3D/4D ultrasonography enables dynamic imaging of pelvic floor structures. Bladder neck mobility, bladder wall thickness, urethral integrity, pelvic compartment prolapses, and levator ani anatomy/function can be examined using this method (15). Ultrasonography is a non-invasive, well-tolerated, and repeatable technique. As it provides real-time images, organ movement can be observed instantly during patient manoeuvres such as straining, coughing, or contracting. With 3D/4D ultrasound technology, the levator muscle complex, urethra, bladder, and rectum can be visualised simultaneously (16). Artificial intelligence-supported analysis algorithms enable automatic measurements such as levator ani muscle and hiatus segmentation, thereby reducing operator dependency and increasing the reproducibility and reliability of the assessment (17). Furthermore, technologies such as tactile imaging can be used to obtain a three-dimensional map of the pressure distribution in pelvic floor tissues and to quantify tissue elasticity (18).

2.4. Magnetic Resonance Imaging (MRI)

Magnetic Resonance Imaging is a method that allows for detailed assessment of the contrast resolution of soft tissues. Dynamic MRI defecography is particularly preferred for the assessment of posterior compartment prolapse and defecation disorders. Radiation-free multiplanar images can be obtained, allowing all pelvic compartments to be evaluated simultaneously (19).

2.5. Defecography

Defecography is a traditional method, particularly for evaluating posterior compartment (rectum and anal canal) dysfunction. Classically, a barium contrast medium is administered into the patient's rectum, and the defecation act is imaged under fluoroscopy on a special toilet chair. During this procedure, which is recorded on video, pathologies such as rectocele (rectal wall hernia), rectal prolapse, rectal intussusception (inward folding of the rectum), and paradoxical contraction of the puborectalis muscle (anismus) can be detected (20).

2.6. Mobile Applications and Sensor-Equipped Devices

Mobile health solutions that facilitate remote assessment are becoming increasingly widespread. For example, many mobile applications guide women through pelvic floor exercises, while others provide real-time biofeedback through integrated sensors (e.g., pressure probes, accelerometers) (21).

2.7. Questionnaires

In assessing pelvic floor dysfunction, it is crucial to understand patients' symptoms and the impact of these symptoms on their quality of life. Objective measurements alone may not reflect the severity of the discomfort experienced by the patient. Therefore, standard questionnaire forms and scales based on patient self-report have been developed. The Pelvic Floor Distress Inventory (PFDI-20), Pelvic Floor Impact Questionnaire (PFIQ-7) and International Consultation on Incontinence Questionnaires (ICIQ) are prominent examples. The PFDI-20 determines symptom burden by assessing urinary incontinence (UDI-6), prolapse (POPDI-6) and anorectal symptoms (CRADI-8) through its 20-item structure. The PFIQ-7, on the other hand, assesses the impact of pelvic floor disorders on daily life and emotional health, focusing on the quality of life dimension. ICIQ forms (particularly the ICIQ-UI short form) standardise the assessment of urinary incontinence frequency, volume, and impact on quality of life; they also have subtypes for overactive bladder (ICIQ-OAB) and vaginal symptoms (ICIQ-VS) (22). For urinary/faecal incontinence, the short form questionnaires of the International Continence Advisory Committee (ICIQ) are preferred (23).

3. INNOVATIVE PHYSIOTHERAPY APPROACHES

In pelvic floor rehabilitation, alongside traditional approaches (such as pelvic floor muscle training, manual therapy techniques, behavioural and lifestyle changes, and functional exercise training), technology-assisted innovative treatment methods have gained increasing importance in recent years. Methods such as virtual reality applications, biofeedback, and tele-rehabilitation applications have been developed or adapted to existing systems to increase patient participation and motivation and, in some cases, to provide remote access (24).

3.1. Pelvic floor muscle training (PFMT)

Pelvic floor muscle training programmes aim to strengthen the core pelvic floor muscles, increase their endurance, and enable them to contract at the right time. The most well-known PFMT is the Kegel exercise. It has been reported that women who performed PFMT achieved a higher rate of urinary control and a significant reduction in symptom severity compared to the control group (25).

According to the NICE guidelines, PFMT is recommended for at least 4 months for pelvic organ prolapse and at least 3 months for faecal incontinence, while the AUA guidelines also indicate PFMT (with or without biofeedback) as

a low-risk first-line treatment option (26,27). Patient adherence is important. A study that followed women with stress incontinence for 15 years reported that only 28% continued with weekly exercises (28). PFMT is non-invasive, safe, and low-cost. It improves symptom control by increasing pelvic floor muscle strength and endurance. However, since targeting the correct muscle groups is critical for treatment effectiveness, it is essential that the programme be performed under specialist supervision.

3.1.1 Fundamental Mechanisms for PFMT

Increasing muscle strength: Through repeated contractions, the thickness and strength of the pelvic floor muscles are enhanced, thereby strengthening the area around the urethra and rectum. The levator ani muscle is specifically targeted.

Timing awareness: Training the patient to reflexively contract the pelvic floor muscles during moments of strain. This strategy ensures urethral closure during increases in intra-abdominal pressure.

Core muscle training: Strengthening the core muscles (particularly the m. transversus abdominis) facilitates the reflexive co-contraction of the pelvic floor muscles. This promotes overall body stabilisation and provides additional support to the pelvic floor (25).

3.2. Virtual Reality-Assisted Rehabilitation Applications

Virtual reality environments are simulated environments designed in three dimensions using computer hardware, which give the user the feeling of being present in that environment and appeal to interactive and sensory inputs. In recent years, the use of virtual reality in healthcare and physiotherapy has been increasing, offering significant innovations in the rehabilitation process (29,30). Virtual reality enables movements to be performed in a goal-oriented, intensive, repetitive, and enjoyable manner by providing visual feedback and is particularly preferred in chronic, long-term rehabilitation processes (31,32).

Additionally, VR ensures that patients participate in treatment with greater motivation and makes the rehabilitation process more engaging. However, there are also some disadvantages. Side effects such as dizziness, nausea, and blurred vision may occur during virtual reality applications. High costs and difficulties in accessing equipment also limit the widespread use of VR. Moreover, the effects of long-term use are not yet fully understood, creating uncertainty regarding potential risks (33).

The use of virtual reality applications in pelvic floor rehabilitation is becoming increasingly widespread. In VR-based treatments, patients exercise their pelvic floor muscles by performing movements in a simulated environment using VR goggles or a screen. According to studies, VR games have been shown to improve pelvic floor muscle strength and patients' quality of life (34).

In a randomised controlled trial, Lin et al. (2022) compared the effectiveness of pelvic movement training in medical students using traditional methods versus a VR-supported Wii Fit application. A total of forty-four students without prior pelvic movement training were randomly assigned to two groups. The control group received only conventional lessons, whereas the experimental group attended additional VR-supported sessions alongside the traditional curriculum. Both groups exhibited significant gains in knowledge and practical skills following the training. However, while performance in written examinations was comparable, the VR group achieved markedly higher scores in practical assessments. At the two-week follow-up, knowledge levels were maintained, whereas a partial decline in practical skills was noted. The vast majority of students reported that VR facilitated learning, was motivating, and enjoyable. This study highlights that VR can make a valuable contribution to traditional education, particularly in enhancing practical skills and increasing student motivation (35).

A randomised controlled trial examining the effect of VR-assisted pelvic floor exercises on urinary incontinence demonstrated that an 8-week VR-assisted exercise programme improved pelvic floor muscle function and quality of life, but this improvement was not statistically different from that of a traditional exercise programme (34). Back pain and pelvic pain during pregnancy are common problems affecting approximately 50% of women. In a study conducted in the Spanish cities of Seville and Malaga, 66 pregnant women (in the 2nd and 3rd trimesters, with a pain VAS score ≥ 4) were included in a physiotherapy programme consisting of 3 sessions per week for 4 weeks. The control group received a standard physiotherapy programme (daily health checks, thermotherapy, TENS, therapeutic massage and breathing, thoracic, lumbar and pelvic mobilisation, breathing and stretching exercises), the experimental group received virtual reality breathing exercises and free movement and relaxation exercises in a virtual nature setting in addition to standard physiotherapy. The study suggests that VR technology can reduce pain perception through its distracting effect, improve psychological well-being by promoting relaxation, and enhance compliance with physiotherapy (14).

3.3. The Role of Telerehabilitation in Pelvic Floor Dysfunction (Remote Rehabilitation)

Telemedicine is defined as the application of information and communication technologies by healthcare professionals to support the diagnosis, management, and prevention of diseases and injuries, to facilitate the exchange of health-related information, to conduct research and evaluation, to provide training for healthcare providers, and to enhance both individual and community health (36).

In pelvic floor dysfunction, tele-rehabilitation services can enable patients to undergo treatment at home via online platforms with the guidance of physiotherapists. This service can provide access for women living in rural or remote areas who are reluctant to visit the clinic due to urogenital problems or who have difficulty accessing pelvic floor rehabilitation services.

The 6-month results of online group-based pelvic floor muscle training in women over 65 years of age were evaluated. Improvements were maintained after the 12-week programme, urinary incontinence decreased by 73%, and quality of life and self-efficacy increased. Most participants adhered to the exercises and reported high satisfaction. The findings indicate that telerehabilitation is effective and sustainable in the treatment of urinary incontinence in older women (37).

Of the 34 women aged 65 and over with urinary incontinence who participated in the online group-based pelvic floor muscle training programme, 33 completed the programme, demonstrating a high retention rate (97%). The majority of participants (72%) were completely satisfied with the treatment outcomes, 25% were partially satisfied, and only one woman (3%) reported dissatisfaction. The results indicated that online group-based PFMT is a feasible, safe, and effective alternative for both participants and clinicians (38).

In a study conducted by Santiago and colleagues, women who underwent pelvic floor muscle training online at home were compared with women who received face-to-face training at a clinic. The study found that both groups showed significant improvements in quality of life, symptom severity, pad test, and daily pad usage. However, there was no significant difference in the magnitude of improvement between the groups. Both groups adhered to home exercises and sessions at a high rate, with satisfaction levels reported as an average of 9/10. Improvements were also seen in sexual function and anxiety-depression scores, but no differences were found between the groups (39). This research suggest that remote training can be at least as effective as traditional

methods. Therefore, it is an attractive option for patients who cannot find the time to come to the clinic or who have barriers to access. In conclusion, telerehabilitation has emerged as an innovative and powerful tool in pelvic floor physiotherapy. With the widespread adoption of this approach, particularly in the long term, more patients will be able to access conservative treatments, clinical outcomes will improve, and there may be cost-saving benefits for the healthcare system.

3.4. Electrotherapy

Electrotherapy methods (Functional Electrical Stimulation (FES), Neuromuscular Electrical Stimulation (NMES), Transcutaneous Electrical Nerve Stimulation (TENS) aim to alleviate incontinence and overactive bladder symptoms by activating the pelvic floor muscles and related nerves through electrical stimulation.

FES/NMES: These approaches, typically delivered via vaginal or rectal probes, aim to treat stress and mixed incontinence by directly activating the pelvic muscles. In a meta-analysis by Huang et al. (2024), combining FES/NMES with pelvic floor exercises significantly reduced the severity of pelvic floor dysfunction and increased muscle strength but did not significantly change quality of life (40).

Transcutaneous Nerve Stimulation: A systematic review indicates that transcutaneous nerve stimulation applied via the tibial or sacral nerve is effective in improving overactive bladder symptoms (41).

3.5. Behavioural Changes and Lifestyle Modifications

Bladder training is the first-line treatment for pelvic floor dysfunction. A 6-week plan to reduce toilet frequency (e.g., gradually increasing the interval between urination) is recommended. Keeping a voiding diary, recording urine volumes and times, is beneficial during this process. The following general recommendations are given regarding fluid intake, diet, and toilet habits during bladder training:

Diet and Fluid Management: The type and amount of daily beverages affect symptoms. Caffeine intake (coffee, tea, cola drinks) can particularly increase irritable bladder symptoms. A 2023 systematic review reported that caffeine restriction reduced urgency, voiding frequency, and nocturia; conversely, high fluid intake worsened symptoms. Participants who restricted caffeine showed significant improvement in urinary urgency and incontinence episodes (42).

Weight Control: Systematic reviews have shown that weight loss significantly reduces incontinence symptoms. In their meta-analysis, Sheridan et al. (2021) noted that weight loss initiatives reduced the risk of incontinence in women and decreased the prevalence of both stress incontinence and urge incontinence (43).

Smoking Cessation: Smoking increases the risk of stress incontinence through chronic coughing and may worsen overactive bladder symptoms through its vascular effects. Studies show that women who smoke have significantly higher overactive bladder and incontinence scores than non-smokers (44). A study conducted on young adults showed a marked improvement in urinary frequency after quitting smoking (45).

4. CONCLUSION

Traditional approaches for evaluating and managing pelvic floor dysfunction include clinical examination; imaging modalities such as ultrasound, MRI, and defecography; objective techniques like perineometry and electromyography; as well as standardized questionnaires. However, innovative methods such as artificial intelligence-based analyses, virtual reality applications, mobile health solutions, and tele-rehabilitation are now coming to the fore. These modern approaches increase patient compliance, facilitate accessibility, and contribute to the individualisation of treatment processes. Therefore, current approaches demonstrate that a multidisciplinary, technological, and evidence-based perspective is becoming increasingly important in the management of pelvic floor dysfunctions.

5. REFERENCES

1. Chaudhry SR, Nahian A, Chaudhry K. Anatomy, Abdomen and Pelvis, Pelvis. [Updated 2023 Jul 25]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482258/

2. Cho ST, Kim KH. Pelvic floor muscle exercise and training for coping with urinary incontinence. J Exerc Rehabil. 2021 Dec 27;17(6):379-387. doi: 10.12965/jer.2142666.333. PMID: 35036386; PMCID: PMC8743604.

3. Faubion SS, Shuster LT, Bharucha AE. Recognition and management of nonrelaxing pelvic floor dysfunction. Mayo Clin Proc. 2012 Feb;87(2):187-93.

4. Tim S, Mazur-Bialy AI. The Most Common Functional Disorders and Factors Affecting Female Pelvic Floor. Life (Basel). 2021 Dec 14;11(12):1397. doi: 10.3390/life11121397. PMID: 34947928; PMCID: PMC8704638.

5. Grimes WR, Stratton M. Pelvic Floor Dysfunction. [Updated 2023 Jun 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559246/

6. Romeikienė, K. E., & Bartkevičienė, D. (2021). Pelvic-Floor Dysfunction Prevention in Prepartum and Postpartum Periods. Medicina, 57(4), 387. https://doi.org/10.3390/medicina57040387

7. Gao, J., Li, Y., Hou, J., & Wang, Y. (2025). Unveiling the depths of pelvic organ prolapse: From risk factors to therapeutic methods (Review). Experimental and Therapeutic Medicine, 29, 11. https://doi.org/10.3892/etm.2024.12761

8. Yang, X., Wang, X., Gao, Z., Li, L., Lin, H., Wang, H., Zhou, H., Tian, D., Zhang, Q., & Shen, J. (2023). The Anatomical Pathogenesis of Stress Urinary Incontinence in Women. Medicina, 59(1), 5. https://doi.org/10.3390/medicina59010005

9. Evaluation and Management of Female Stress Urinary Incontinence F. A. Jefferson and B. J. Linder Mayo Clinic Proceedings 2024 Vol. 99 Issue 11 Pages 1802-1814 DOI: 10.1016/j.mayocp.2024.07.003

10. Teji M, Raison N, Faure-Walker N. The Clinical Significance of the Subtypes of Detrusor Overactivity: A Systematic Review. Neurourol Urodyn. 2025 Sep;44(7):1484-1490. doi: 10.1002/nau.70110. Epub 2025 Jul 3. PMID: 40605566; PMCID: PMC12319479.

11. Verhovsky G, Baberashvili I, Rappaport YH, Zilberman DE, Neheman A, Gal J, Zisman A, Stav K. Bladder Oversensitivity Is Associated with Bladder

Outlet Obstruction in Men. J Pers Med. 2022 Oct 8;12(10):1675. doi: 10.3390/jpm12101675. PMID: 36294814; PMCID: PMC9605007.

12. Sanjay Sinha, Claire C. Yang, Salvador Arlandis, Howard B. Goldman, Female voiding dysfunction: A review of clinical presentation, urodynamic diagnosis and management, Continence, Volume 6, 2023, 100578,https://doi.org/10.1016/j.cont.2023.100578.

13. Abdissa K, Mesfin E, Tesfaye K. Prevalence and Associated Factors of Anal Incontinence at Six Weeks after Vaginal Delivery: A Cross-sectional Study at Three Teaching Hospitals in Addis Ababa, Ethiopia. Ethiop J Health Sci. 2024 May;34(3):211-220. doi: 10.4314/ejhs.v34i3.6. PMID: 40438447; PMCID: PMC12110197.

14. Hurt K, Zahalka F, Zikan M, Rackova J, Rakovicova I, Rakovic J, Halad M. Hypoxia as a potential cause of dyspareunia. PLoS One. 2023 Apr 17;18(4):e0281268. doi: 10.1371/journal.pone.0281268. PMID: 37068077; PMCID: PMC10109496.

15. Beales D, Asinelli R, Klokset M, O'Kane L, Urstad T, Wise E, Zabatiero J, Thompson J, Pontre J, Waller R. Association between pelvic pain bothersomeness and pain sensitivity: A community-based cross-sectional study of young adult females in the Raine Study. BJOG. 2022 Nov;129(12):1981-1991. doi: 10.1111/1471-0528.17232.

16. Laycock, J. (1994). Clinical evaluation of the pelvic floor. In B. Schussler, J. Laycock, P. Norton, & S. Stanton (Eds.), *Pelvic floor re-education* (pp. 42–48). Springer.

17. Laycock, J., & Jerwood, D. (2001). Pelvic floor muscle assessment: The PERFECT scheme. *Physiotherapy, 87*(12), 631–642. https://doi.org/10.1016/S0031-9406(05)61108-X

18. El-Sayegh, B., Dumoulin, C., Leduc-Primeau, F., & Sawan, M. (2024). Improving pelvic floor muscle training with AI: A novel quality assessment system for pelvic floor dysfunction. *Sensors, 24*(21), 6937. https://doi.org/10.3390/s24216937

19. Abe-Takahashi, Y., Kitta, T., Ouchi, M., Okayauchi, M., Chiba, H., Higuchi, M., & Shinohara, N. (2020). Reliability and validity of pelvic floor muscle strength assessment using the MizCure perineometer. *BMC Women's Health, 20*(1), 257. https://doi.org/10.1186/s12905-020-01127-x

20. Persu C, Chapple CR, Cauni V, Gutue S, Geavlete P. Pelvic Organ Prolapse Quantification System (POP-Q) - a new era in pelvic prolapse staging. J Med Life. 2011 Jan-Mar;4(1):75-81. Epub 2011 Feb 25. PMID: 21505577; PMCID: PMC3056425.

21. García-Mejido, J. A., Hurtado-Guijosa, A., Fernández-Gomez, A., Fernández-Palacín, F., Lao-Peña, C., & Sainz-Bueno, J. A. (2024). Influence of transperineal ultrasound on the POP-Q system in the surgical indication of symptomatic pelvic organ prolapse. *Journal of Clinical Medicine, 13*(20), 6224. https://doi.org/10.3390/jcm13206224

22. Dietz, H. P. (2017). Pelvic floor ultrasound: A review. *Clinical Obstetrics and Gynecology, 60*(1), 58–81. https://doi.org/10.1097/GRF.0000000000000264

23. De Vicari, D., Barba, M., Cola, A., Costa, C., Palucci, M., & Frigerio, M. (2025). AI-enhanced 3D transperineal ultrasound: Advancing biometric measurements for precise prolapse severity assessment. *Bioengineering (Basel), 12*(7), 754. https://doi.org/10.3390/bioengineering12070754

24. Huang, X., Wang, D., Li, S., Yang, L., Zhao, J., & Guo, D. (2024). Advancements in artificial intelligence for pelvic floor ultrasound analysis. *American Journal of Translational Research, 16*(4), 1037–1047.

25. Egorov, V. (2024). Digital twin of the female pelvic floor. *Open Journal of Obstetrics and Gynecology, 14*(11), 1687–1694. https://doi.org/10.4236/ojog.2024.1411138

26. Welch, E. K., Ross, W., Dengler, K. L., Gruber, D. D., & Lamb, S. (2024). The "ins and outs" of dynamic magnetic resonance imaging for female pelvic organ prolapse. *International Urogynecology Journal, 35*(11), 2223–2225. https://doi.org/10.1007/s00192-024-05935-9

27. Grimes, W. R., & Stratton, M. (2023). *Pelvic floor dysfunction*. In StatPearls. StatPearls Publishing.

28. Woodley, S. J., Moller, B., Clark, A. R., Bussey, M. D., Sangelaji, B., Perry, M., & Kruger, J. (2023). Digital technologies for women's pelvic floor muscle training to manage urinary incontinence across their life course: Scoping review. *JMIR mHealth and uHealth, 11*, e44929. https://doi.org/10.2196/44929

29. PhysioPedia. (n.d.). *Pelvic floor distress inventory (PFDI-20).* Retrieved August 31, 2025, from https://www.physio-pedia.com/Pelvic_Floor_Distress_Inventory

30. Förstl, N., Adler, I., Süß, F., & Dendorfer, S. (2024). Technologies for evaluation of pelvic floor functionality: A systematic review. *Sensors, 24*(12), 4001. https://doi.org/10.3390/s24124001

31. Çelik, M., & Ersin, A. (2022). Pelvik taban disfonksiyonunda EMG-biofeedback etkinliği. *Atlas Journal of Medicine, 2*(4), 27–37.

32. Sheng, Y., Carpenter, J. S., Ashton-Miller, J. A., & Miller, J. M. (2022). Mechanisms of pelvic floor muscle training for managing urinary incontinence in women: A scoping review. *BMC Women's Health, 22*(1), 161. https://doi.org/10.1186/s12905-022-01742-w

33. National Institute for Health and Care Excellence (NICE). (2021). *Pelvic floor dysfunction: Prevention and non-surgical management (NICE Guideline NG210).* https://www.nice.org.uk/guidance/ng210

34. Kobashi, K. C., Albo, M. E., Dmochowski, R. R., Ginsberg, D. A., Goldman, H. B., Gomelsky, A., ... & Lemack, G. E. (2017). Surgical treatment of female stress urinary incontinence: AUA/SUFU guideline. *Journal of Urology, 198*(4), 875–883. https://doi.org/10.1016/j.juro.2017.06.061

35. Bø, K., Kvarstein, B., & Nygaard, I. (2005). Lower urinary tract symptoms and pelvic floor muscle exercise adherence after 15 years. *Obstetrics & Gynecology, 105*(5 Pt 1), 999–1005. https://doi.org/10.1097/01.AOG.0000157207.95680.6d

36. Hayre, C. M., Muller, D. J., & Scherer, M. J. (2020). *Virtual reality in health and rehabilitation* (1st ed.). CRC Press.

37. Renganayagalu, S. K., Mallam, S. C., & Nazir, S. (2021). Effectiveness of VR head-mounted displays in professional training: A systematic review. *Technology, Knowledge and Learning, 26*(1), 1–43. https://doi.org/10.1007/s10758-020-09489-9

38. Montoro-Cárdenas, D., Cortés-Pérez, I., Zagalaz-Anula, N., Osuna-Pérez, M. C., Obrero-Gaitán, E., & Lomas-Vega, R. (2021). Nintendo Wii Balance Board therapy for postural control in children with cerebral palsy: A systematic review and meta-analysis. *Developmental Medicine & Child Neurology, 63*(11), 1262–1275. https://doi.org/10.1111/dmcn.14947

39. Montoro-Cárdenas, D., Cortés-Pérez, I., Ibancos-Losada, M. D. R., Zagalaz-Anula, N., Obrero-Gaitán, E., & Osuna-Pérez, M. C. (2022). Nintendo Wii therapy improves upper extremity motor function in children with cerebral palsy: A systematic review with meta-analysis. *International Journal of Environmental Research and Public Health, 19*(19), 12343. https://doi.org/10.3390/ijerph191912343

40. Zhang, H., Xu, H., Zhang, Z. X., & Zhang, Q. (2022). Efficacy of virtual reality-based interventions for patients with breast cancer symptom and rehabilitation management: A systematic review and meta-analysis. *BMJ Open, 12*(3), e051808. https://doi.org/10.1136/bmjopen-2021-051808

41. Rutkowska, A., Salvalaggio, S., Rutkowski, S., & Turolla, A. (2022). Use of virtual reality-based therapy in patients with urinary incontinence: A systematic review with meta-analysis. *International Journal of*

Environmental Research and Public Health, 19(10), 6155. https://doi.org/10.3390/ijerph19106155

42. Lin, H. T., Tsai, H. J., Li, Y. I., & Hu, W. P. (2022). Benefits of applying virtual reality in pelvic movement training through a Wii Fit: A randomized controlled trial. *BMC Medical Education, 22*(1), 47. https://doi.org/10.1186/s12909-022-03109-z

43. García-López, F. J., Pastora-Bernal, J. M., Moreno-Morales, N., Estebanez-Pérez, M. J., Liñán-González, A., & Martín-Valero, R. (2023). Virtual reality to improve low-back pain and pelvic pain during pregnancy: A pilot RCT for a multicenter randomized controlled trial. *Frontiers in Medicine, 10,* 1206799. https://doi.org/10.3389/fmed.2023.1206799

44. Arık, Y. (2023). Tele-tıp uygulamalarının sağlık hizmetleri pazarlaması kapsamında değerlendirilmesi. *Hacettepe Sağlık İdaresi Dergisi, 26*(2), 511–534.

45. Le Berre, M., Filiatrault, J., Reichetzer, B., Kairy, D., Lachance, C., & Dumoulin, C. (2025). Group-based pelvic floor telerehabilitation for urinary incontinence in older women: A six-month follow-up pilot study. *Maturitas.* Advance online publication. https://doi.org/10.1016/j.maturitas.2025.108621

46. Le Berre, M., Filiatrault, J., Reichetzer, B., & Dumoulin, C. (2023). Group-based pelvic floor telerehabilitation to treat urinary incontinence in older women: A feasibility study. *International Journal of Environmental Research and Public Health, 20*(10), 5791. https://doi.org/10.3390/ijerph20105791

47. Santiago, M., Cardoso-Teixeira, P., Pereira, S., Firmino-Machado, J., & Moreira, S. (2023). A hybrid-telerehabilitation versus a conventional program for urinary incontinence: A randomized trial during COVID-19 pandemic. *International Urogynecology Journal, 34*(3), 717–727. https://doi.org/10.1007/s00192-022-05108-6

48. Huang, Y., Huang, Z., Ou, Y., et al. (2024). Meta-analysis of the therapeutic effect of electrical stimulation combined with pelvic floor muscle exercise on female pelvic floor dysfunction. *European Journal of Medical Research, 29,* 380. https://doi.org/10.1186/s40001-024-01979-1

49. Booth, J., Connelly, L., Dickson, S., Duncan, F., & Lawrence, M. (2018). The effectiveness of transcutaneous tibial nerve stimulation (TTNS) for adults with overactive bladder syndrome: A systematic review. *Neurourology and Urodynamics, 37*(2), 528–541. https://doi.org/10.1002/nau.23351

50. Park, J., Lee, H., Kim, Y., Norton, C., Woodward, S., & Lee, S. (2023). Effectiveness of fluid and caffeine modifications on symptoms in adults with overactive bladder: A systematic review. *International Neurourology Journal, 27*(1), 23–35. https://doi.org/10.5213/inj.2346014.007

51. Sheridan, W., Da Silva, A. S., Leca, B. M., et al. (2021). Weight loss with bariatric surgery or behaviour modification and the impact on female obesity-related urine incontinence: A comprehensive systematic review and meta-analysis. *Clinical Obesity, 11*(4), e12450. https://doi.org/10.1111/cob.12450

52. Kawahara, T., Ito, H., Yao, M., & Uemura, H. (2020). Impact of smoking habit on overactive bladder symptoms and incontinence in women. *International Journal of Urology, 27*(12), 1078–1086. https://doi.org/10.1111/iju.14357

53. Madhu, C., Enki, D., Drake, M. J., & Hashim, H. (2015). The functional effects of cigarette smoking in women on the lower urinary tract. *Urologia Internationalis, 95*(4), 478–482. https://doi.org/10.1159/000438928