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# GUT MICROBIOTA AND ITS CORRELATIONS WITH BODY MASS INDEX AND AGE IN PATIENTS WITH TYPE 2 DIABETES MELLITUS AND THYROID DYSFUNCTION

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*Modern studies show that it is the transit microflora, the percentage of which is negligible compared to the total volume of the intestinal microbiota, that can cause severe damage and cause the appearance of chronic and acute diseases not only of the intestines but also of the immune, nervous, endocrine systems, etc. Instead, the obligate intestinal microbiota and even the opportunistic microbiota exhibit a number of beneficial properties, ranging from the synthesis of short-chain fatty acids, butyrate, and acetate to promoting the synthesis of vitamins and hormones such as dopamine, serotonin, etc. At the same time, the adverse effects of the gut microbiota cannot be discounted.*

***The aim**. The study aims to find correlations between gut microbiota, body mass index, and age in patients with type 2 diabetes and thyroid dysfunction.*

***Materials and methods.** The study included 84 patients with type 2 diabetes, obesity, and thyroid dysfunction. To analyze the composition of the intestinal microbiota, fecal samples were taken, and the quantitative and qualitative composition was calculated using the principle of PCR sequencing. Statistical methods of comparison and correlation of variables were used.*

***Results.** In patients, several significant correlations were found between the gut microbiota and the parameters studied. A negative correlation was found between body mass index and Bifidobacterium spp. and Escherichia coli, and a positive correlation was found between body mass index and some opportunistic pathogens. Thus, with Shigella spp. and Staphylococcus aureus, there is a direct weak reliable relationship, while an inverse reliable relationship with Helicobacter pylori. Interesting correlations were found with a tendency to confidence with Salmonella spp. and Bacteroides thetaiotaomicron direct weak and a tendency to probable feedback between BMI and Faecalibacterium prausnitzii and Candida spp.*

***Conclusion.** We found that body mass index has a greater impact on the gut microbiota than age. Also, the data obtained indicate that obesity negatively affects the number of some beneficial bacteria. We can assume that, like metformin, one of the most common drugs for the treatment of type 2 diabetes mellitus, it can affect the composition of the intestinal microbiota*

***Keywords:** gut microbiota correlations, diabetes mellitus, thyroid, body mass index*

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## 1. Introduction

Diabetes mellitus (DM) is a chronic disease that requires constant monitoring of blood glucose levels and its regulation with insulin or other oral hypoglycemic drugs. As of June 1, 2020, the total number of people suffering from diabetes and receiving insulin who are registered at the dispensary is 214,400 people, which indicates the high prevalence of this disease among the population [1]. Since diabetes leads to the appearance of a wide list of complications and the absorption of oral drugs depends on the functional state of the gastrointestinal tract, the intestinal microbiota is also gradually gaining interest in the study of the pathogenesis of diabetes. However, there are still many unknowns in this direction. Diseases of the thyroid gland occupy the first place in the structure of endocrine pathology, exceeding diabetes. Among diseases affecting other organs and systems, endocrine pathology ranks third. The prevalence of primary hypothyroidism, the most common form, is estimated at 1.4 % to 12 % of the population. In women, subclinical primary hypothyroidism is observed in 7–10 % of cases and in men – between 2–3 % [2, 3]. It is believed that 3–5 % of cases of subclinical hypothyroidism turn into clinical hypothyroidism every year [4]. The presence of a high prevalence of hypothyroidism in Ukraine can create a risk of endocrinopathies, which are associated with the effect of an insufficient number of thyroid hormones on the body and can have a negative effect on the functioning of other organs and systems, including even retardation of mental development. [5].

For over a century, there has been scientific evidence supporting the relationship between thyroxine (T4), triiodothyronine (T3) and glucose metabolism. The effect of excess thyroid hormone on carbohydrate metabolism was first observed and documented, leading to the recognition of the link between hyperthyroidism and insulin resistance. Later, it was found that insulin resistance is present in people with hypothyroidism [6]. Patients with hypothyroidism are treated with hormone replacement therapy, while in Graves' disease, thyrostatics are used. The combination of these diseases and pathogenetic conditions associated with impaired carbohydrate metabolism leads to the appearance of metformin in this list of drugs. It is noted in the literature that metformin, as a first-line drug in the treatment of type 2 diabetes, affects the composition of the intestinal microbiota. However, the specific changes and their consequences for glucose metabolism are complex and only partially consistent across studies. Metformin has been associated with increased numbers of certain types of microbiota, such as *Bacteroidetes*, which may contribute to its hypoglycemic effects [7], so we set out to find a correlation between biochemical data and gut microbiota.

The main aim of our study was to evaluate the features of the intestinal microbiota and its correlation with body mass index, age in patients with type 2 diabetes and thyroid dysfunction.

**2. Materials and methods**

84 patients with diagnosed type 2 diabetes mellitus, obesity and thyroid dysfunction (autoimmune thyroiditis, hypothyroidism, Graves' disease) aged 38.0 ± 1.39 years old; among them, 28 men and 56 women at the Lviv Regional Endocrinological Institute took part in the study, centre during 2019–2022. The clinical study was conducted in accordance with the requirements of the current legislation of Ukraine and in accordance with the ethical principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine. The study design, information for the patient, and the form of informed consent for participation in the study were reviewed and approved by the Ethics Committee of Danylo Halytsky Lviv National Medical University, protocol No. 3-BP dated March 27, 2019. All patients signed informed consent for participation in this study.

Diagnosis of T2DM was carried out based on the criteria specified in the "Unified clinical protocol of primary and secondary (specialized) medical care. Diabetes mellitus type 2" (Order of the Ministry of Health of December 21, 2012, No. 1118), for the diagnosis of primary hypothyroidism and endemic goiter, the "Standards of diagnosis and treatment of endocrine diseases" were used. Patients received treatment according to the mentioned domestic and international protocols for providing medical care. All subjects were treated with an established diagnosis of T2DM – metformin in a dose of 2000 mg; patients with hypothyroidism – levothyroxine in an individually selected dose ranging from 50 to 125 μg; patients with Graves' disease – thiamazole in a dose of 30 mg per day.

The following studies were performed on the obtained faeces samples: sequencing of the faeces microbiome by quantifying the content of *Firmicutes, Bacteroidetes, Actinobacteria, Lactobacillus spp., Bifidobacterium spp., Escherichia coli, Bacteroides fragilis group, Bacteroides thetaiotaomicron, Akkermansia muciniphila, Faecalibacterium prausnitzii, Clostridium difficile, Clostridium perfringens, Klebsiella pneumoniae, Klebsiella oxytoca, Escherichia coli enteropathogenic, Enterococcus spp., Proteus spp., Enterobacter spp. / Citrobacter spp., Fusobacterium nucleatum, Parvimonas micra, Staphylococcus aureus, Salmonella spp., Shigella spp., Candida spp.* and qualitative analysis of the presence of *Candida albicans, Candida glabrata, Candida krusei, Helicobacter pylori*. The relationship between *Firmicutes* and *Bacteroidetes (F/B)* and *Bacteroides fragilis* group and *Faecalibacterium prausnitzii (B.fragilis/F.prausnitzii)* was also determined. To calculate the body mass index of patients, anthropometric measurements such as height and weight were performed. Glucose and insulin levels, HOMA-IR, HOMA-β, Caro index, thyrotropin (TSH), free thyroxine T4 (fT4) and free triiodothyronine (fT3) were also determined to assess carbohydrate metabolism and thyroid status.

Statistical data analysis was carried out using Microsoft Excel (USA) and STATISTICA 6.0 (StatSoft, USA) software, which was based on literature sources [8].

The normality of the distribution of the parametric variable in the sample was assessed using the Shapiro-Wilk test. To compare variables with a normal distribution, the t-test was used for dependent (comparison of indicators before and after treatment (exposure) in one group, assuming an equal number of cases) and independent variables for all other cases (comparison between groups, with unequal number of observations, etc.).

Correlation analysis using the Pearson method was conducted, which is recognized as the most accurate for studying the correlation between the studied parameters (biochemical and hormonal indicators, insulin resistance indices). The coefficient of linear correlation ($r$) and its reliability (p) were calculated, which are indicated accordingly in the tables (correlation matrices). The correlation coefficient was considered significant when $p<0.05$. Using the correlation coefficient $r$, we simultaneously obtain information about the direction of interaction (direct +, inverse -) and the strength of the connection (from 0 to 1). If $r=0$ is considered unrelated, then a range of 0 to 0.3 indicates a weak correlation, a range of 0.3 to 0.7 indicates a medium strength association, and a range of 0.7 to 1.0 confirms a strong correlation. Pearson's method is recognized as the most accurate for studying correlation.

The difference between the compared samples according to the relevant parameter was evaluated as reliable in the case when $p<0.05$. In the paper, the degrees of reliability $p<0.01$ and $p<0.001$ and the tendency to significant changes – $0.05 < p < 0.1$ [8] were also used to describe the obtained results.

**3. Results**

Several correlations were observed in the examined patients included in our study. Such interactions as insulin–HOMA-IR ($r=0.878$; $p<0.001$), glucose–fasting HbA1c ($r=0.642$; $p<0.001$), insulin–Caro ($r=-0.664$; $p<0.001$), insulin–HOMA -β ($r=0.590$; $p<0.001$), fT4–

fT3 ($r$=0.413; p<0.05) were characterized by a high degree of probability, were obvious and expected.

A weak direct correlation was found between the "Other" group and body mass index ($r$=0.268; p=0.046), but no relationship was found between the age of the patients.

During the analysis of correlations, it was found that age does not have a reliable connection with obligate representatives of the intestinal microbiota. However, significant correlations have been found between body mass index (BMI) and certain types of bacteria. Specifically, a weak inverse correlation was found between BMI and *Bifidobacterium spp.* ($r$=-0.284; p=0.034), as well as from *Escherichia coli* ($r$=-0.294; p=0.028). The correlation between BMI and *Bifidobacterium* suggests that metformin is able to affect these colonies, which is consistent with the results of other studies [1].

It is also worth noting the tendency towards a probable inverse relationship between BMI and *Faecalibacterium prausnitzii* ($r$=-0.244; p=0.070), as well as a possible direct relationship between BMI and *Bacteroides thetaiotaomicron* ($r$=0.237; p=0.079). When assessing the correlation between opportunistically pathogenic representatives of the intestinal microbiota, age and BMI in the examined patients, the relationship between BMI and *Shigella spp.* direct weak reliable ($r$=0.285, p=0.033), with *Staphylococcus aureus* direct weak reliable ($r$=0.275, p=0.040), *Salmonella spp*. direct weak with a trend towards reliability ($r$=0.250, p=0.063). Analysis of the correlation of opportunistic bacteria with the age of patients revealed no reliable relationships or trends to reliability.

Analyzing the correlation of age and BMI with fungi of the genus *Candida* and *H.pylori* DNA in the intestinal microbiota revealed a number of relationships shown in Table 1.

Table 1

Correlations between detected *Candida* fungi and *H.pylori* DNA in intestinal microbiota, age and BMI in examined patients

| Indicator | Degree of correlation $r$= | |
|---|---|---|
| | Age | BMI |
| Candida spp. | –0.275* p=0.040 | –0.233# p=0.085 |
| Candida krusei | 0.086 p=0.528 | 0.001 p=0.997 |
| Helicobacter pylori | –0.085 p=0.532 | –0.318* p=0.017 |

*Note: * – significant difference between average values (p<0.05); # – tendency to a reliable difference between the average values (0.05<p<0.1)*

The obtained results indicate that the patient's weight-to-height ratio has a greater influence on the gut microbiota than his age. It is noted that an increase in BMI negatively affects the number of certain types of bacteria, which are markers of health.

**4. Discussion of research results**

It is worth noting the trend towards a probable inverse relationship between BMI and *Faecalibacterium prausnitzii* and a direct relationship between BMI and *Bacteroides thetaiotaomicron*. When assessing the correlation between opportunistic pathogenic representatives of MK, age and BMI in the examined patients, a direct weak reliable relationship of BMI with *Shigella spp.*, with *Staphylococcus aureus*, direct weak with a tendency towards reliability with *Salmonella spp.* was found. Considering literary sources, we will find that there is the most inconsistency regarding BMI. The most recently published meta-analysis of 22 studies [9] concluded that six studies found significantly higher associations of BMI with the *Firmicutes phylum* and marginally lower associations with the *Bacteroidetes phylum*, leaving no clear answer. Therefore, the search for an unambiguous marker of obesity based on BMI continues.

Analyzing the correlation of age and BMI with fungi of the genus *Candida*, as well as with DNA of *H. pylori* in MK, a number of connections were revealed. Unexpectedly, an inverse significant association of *H. pylory* with BMI and a trend toward an inverse correlation between BMI and *Candida spp.* The question of the correlation of fungi *Candida spp.* quite contradictory with age. For example, a large study that identified and characterized four mycobiome (non-microbiome) enterotypes using ITS profiling of 3,363 samples from 16 cohorts found a direct association between *Candida* aerobic respiration and age [10]. At the same time, the authors of this study noted that the mycobiome of the European population was characterized by the expansion of fungi of the genera *Saccharomyces* and *Penicillium*, but the depletion of the genus *Candida*, while the mycobiome of populations from Asia contained relatively more *Candida* and less *Saccharomyces*. Despite this, the European population, according to these researchers, shows a relatively lower diversity of fungi in general compared to the population of other continents. Accordingly, in our study, where only 84 samples of Caucasians (residents of Lviv Oblast) were involved, age was inversely correlated (albeit to a weak degree) with Candida species. We assume that this may be related to the peculiarity of the diet of middle-aged people, more precisely, to the use of dairy products, and in addition, to the variety of MK. This assumption is confirmed by the data of the analysis, which included 1244 participants [11]. Summarizing the results of their study, the scientists found a positive relationship between the bacterial α-diversity of MK and *Saccharomyces* and a negative relationship with the genus *Candida*. The same relationship was recorded for the fact of consumption of dairy products, which, according to the authors, contributes to a healthier diet from the point of view of "intestinal eco-health, changing the relationships of intestinal microbes."

**Study limitations.** Given the small sample size, we cannot claim that the obtained results can be extrapolated to all patients with T2DM and thyroid dysfunction.

**Prospects for further research.** The obtained results expand the understanding of the influence of intestinal microbiota on the course of diseases associated with impaired carbohydrate metabolism. Further studies with the involvement of a wider cohort of patients will help to choose a method of therapeutic tactics that will be justified and effective, considering the peculiarities of the structural and functional state of the intestinal microbiota.

## 5. Conclusions

Our study aimed to find correlations between gut microbiota, body mass index, and age in patients with type 2 diabetes and thyroid dysfunction. Several significant correlations between gut microbiota and the studied parameters were found. An inverse correlation was found between body mass index and *Bifidobacterium spp.* and *Escherichia coli* and a direct correlation between body mass index and some opportunistic microorganisms, because reliable correlations were found between body mass index (BMI) and certain types of bacteria. So, *Shigella spp.* and *Staphylococcus aureus* have a direct, weakly significant association while inversely significant association with Helicobacter pylori. Correlations with a trend toward reliability with Salmonella spp. and *Bacteroides thetaiotaomicron* direct weak and a tendency towards a probable inverse relationship between BMI and *Faecalibacterium prausnitzii* and *Candida spp.*. When evaluating the correlation between the representatives of the intestinal microbiota and age in the examined patients, the only weak inverse reliable relationship with *Candida spp.*

We concluded that body mass index has a greater effect on the gut microbiota than age, and obesity has a negative effect on the abundance of some beneficial bacteria. We assume that metformin, as a common drug for the treatment of type 2 diabetes, can affect the composition of the intestinal microbiota. The obtained data reflect the importance of studying the role of intestinal microbiota in the pathogenesis and treatment of endocrine disorders.

**Conflict of interests**

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

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**Data availability**

Data will be provided upon reasonable request.

**Use of artificial intelligence**

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

**References**

1. Moskva, K., Kikhtyak, O., Lapovets, L., Urbanovych, A. (2022). Changes in the gut microbiota under the influence of metformin, pioglitazone, and levothyroxine in overweight patients with type 2 diabetes mellitus and hypothyroidism. Problems of Endocrine Pathology, 79 (4), 45–51. https://doi.org/10.21856/j-PEP.2022.4.06

2. Vatseba, T., Skrypnyk, N. (2013). The Method of the of Hypothyroidism in Iodine Deficiency Treatment Optimization by Acting on Insulin Resistance. Liky Ukrainy, 8 (174), 62–66.

3. Kapadia, K. B., Bhatt, P. A., Shah, J. S. (2012). Association between altered thyroid state and insulin resistance. Journal of Pharmacology and Pharmacotherapeutics, 3 (2), 156–160.

4. Biondi, B., Cooper, D. S. (2007). The Clinical Significance of Subclinical Thyroid Dysfunction. Endocrine Reviews, 29 (1), 76–131. https://doi.org/10.1210/er.2006-0043

5. Pankiv, V. (2021). Blood level of thyroid-stimulating hormone as a basic diagnostic marker and criterion of success in the treatment of thyroid diseases. International journal of endocrinology, 13 (2), 147–151. https://doi.org/10.22141/2224-0721.13.2.2017.100604

6. Brenta, G. (2011). Why Can Insulin Resistance Be a Natural Consequence of Thyroid Dysfunction? Journal of Thyroid Research, 2011, 1–9. https://doi.org/10.4061/2011/152850

7. Moskva, K., Kikhtyak, O., Lapovets, L., Lanyush, F. (2023). Comparison of changes in the gut microbiota influenced by combinations of liraglutide with metformin and pioglitazone with metformin in overweight patients with diabetes. 59th EASD Annual Meeting of the European Association for the Study of Diabetes. Diabetologia, 66 (Suppl 1), 331. https://doi.org/10.1007/s00125-023-05969-6

8. Thomas, A. (2006). Lang and Michelle Secic. How to report statistics in medicine: annotated guidelines for authors, editors, and reviewers, Philadelphia: American College of Physicians, 490.

9. Pinart, M., Dötsch, A., Schlicht, K., Laudes, M., Bouwman, J., Forslund, S. K. et al. (2021). Gut Microbiome Composition in Obese and Non-Obese Persons: A Systematic Review and Meta-Analysis. Nutrients, 14 (1), 12. https://doi.org/10.3390/nu14010012

10. Lai, S., Yan, Y., Pu, Y., Lin, S., Qiu, J.-G., Jiang, B.-H. et al. (2023). Enterotypes of the human gut mycobiome. Microbiome, 11 (1). https://doi.org/10.1186/s40168-023-01586-y

11. Shuai, M., Fu, Y., Zhong, H., Gou, W., Jiang, Z., Liang, Y. et al. (2022). Mapping the human gut mycobiome in middle-aged and elderly adults: multiomics insights and implications for host metabolic health. Gut, 71 (9), 1812–1820. https://doi.org/10.1136/gutjnl-2021-326298

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