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OPIS PRZYPADKU
CASE REPORT

Individual gut microbiological signature in obese diabetic spouses – case report and literature review

Analiza mikrobiomu jelitowego otyłych małżonków chorujących na cukrzycę – opis przypadku i przegląd literatury

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ABSTRACT

INTRODUCTION: Due to the fact that the gut microbiome signature becomes more pronounced in type 2 diabetes, a better understanding of the role of microflora in diabetes (existing dysbiosis) provides new insight into the pathophysiology of this disorder. This study focused on the gut microbiome profiles of a married couple with type 2 diabetes and obesity living for last 35 years in a shared household in terms of their nutritional status, lifestyle and diabetes treatment methods. At the same time, an attempt was made to answer the question of which factors have the most significant impact on the intestinal microbiome.

MATERIAL AND METHODS: Medical interviews of subjects, anthropometric measurements, body composition, 24-hour nutritional interviews, glycemic control, and stool samples were analyzed. The quantitative and qualitative examination of the fecal intestinal flora was performed by the next-generation sequencing method.

RESULTS: There were no significant differences in the study of the gut microbiome between the two subjects. The dominant bacterial phyla were *Firmicutes* and *Actinobacteria*, while *Bacteroidetes* and *Proteobacteria* shared smaller proportions, between 2 and 7%. Phylum *Firmicutes* was presented by the dominant *Lachnospiraceae* family (29–31%), *Ruminococcaceae* (16–19%), and *Streptococcaceae* (3–11%). The *Actinobacteria* phylum was proportionally less abundant and mainly represented by *Bifidobacteriaceae* (6–12%).

CONCLUSIONS: May be the common living conditions have a significant influence on gut microbiota composition of diabetic spouses, despite differences in gender, comorbidities, diabetes therapy, diet and behaviors.

KEYWORDS

gut microbiome, spouses, obesity, type 2 diabetes

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STRESZCZENIE

WSTĘP: Zmiany składu mikrobiomu jelitowego odgrywają istotną rolę w patofizjologii wielu schorzeń, w tym cukrzycy typu 2. Celem pracy była ocena profilu mikrobiomu jelitowego małżeństwa z otyłością i cukrzycą typu 2, mieszkającego 35 lat we wspólnym gospodarstwie domowym, pod względem stanu odżywienia, stylu życia i metod leczenia cukrzycy. Jednocześnie podjęto próbę odpowiedzi na pytanie, które czynniki mają największy wpływ na ewentualną dysbiozę jelitową.

MATERIAŁ I METODY: Analizie poddano wyniki pomiarów antropometrycznych pacjentów, skład ich ciała, 24-godzinny wywiad żywieniowy, profil glikemii i próbki kału. Do ilościowego i jakościowego badania flory jelitowej w kale zastosowano metodę sekwencjonowania nowej generacji.

WYNIKI: Nie stwierdzono znaczących różnic w badaniu mikrobiomu jelitowego pomiędzy małżonkami. Dominującymi gromadami bakterii były *Firmicutes* i *Actinobacteria*, podczas gdy *Bacteroidetes* i *Proteobacteria* występowały w proporcjach od 2 do 7%. Gromada *Firmicutes* była reprezentowana przez dominującą rodzinę *Lachnospiraceae* (29–31%), *Ruminococcaceae* (16–19%) i *Streptococcaceae* (3–11%). Gromada *Actinobacteria* była proporcjonalnie mniej liczna i reprezentowana głównie przez *Bifidobacteriaceae* (6–12%).

WNIOSKI: Być może wspólne warunki życia mają najbardziej istotny wpływ na skład mikroflory jelitowej małżonków chorych na cukrzycę, mimo różnic dotyczących płci, schorzeń współistniejących, terapii cukrzycy, stosowanej diety i zachowań zdrowotnych.

SŁOWA KLUCZOWE

mikrobiom jelitowy, małżeństwo, otyłość, cukrzyca typu 2

INTRODUCTION

Intestinal bacteria play a key role in the maintenance of systemic and intestinal immune and metabolic homeostasis, influencing nutrient absorption and the development of the immune system as well as its functions [1].

It is estimated that the number of microbial cells in the human body is fifteen times greater than that of our own somatic and sex cells, with their greatest and most established ecosystem in our intestines. Metagenomic analyses of the human microbiome have shown that the gut has 3.3 million unique genes, 150 times more than our own genome, and the diversity of gut bacteria is estimated at over 1000 species [2].

The large intestine is inhabited by 1014 microorganisms (belonging to over a thousand species), which make up 50% of the intestinal content, and 1 g of human stool contains 1012 bacterial cells. The intestinal microflora consists of 17 families, 50 genera and over 1000 species of bacteria [3]. Depending on health, age and gender, the most common are the bacteria of the *Firmicutes* (80–64%), *Bacteroidetes* (23–17%), *Proteobacteria* (8–1%), and *Acinetobacteria* (2,5–1%) phylum [4,5].

Although the composition of microbes is relatively stable in healthy adults (defined as eubiosis), environmental changes and/or medical interventions can cause a constant change in the diversity and/or abundance of individual taxonomic groups of bacteria, leading to dysbiosis in the human body. Dysbiosis is an important factor in the development of several pathological conditions, such as aging, obesity, cardiovascular diseases, diabetes, chronic liver diseases, neurodegenerative disease (Parkinson's disease, Alzheimer's disease, etc.), and some cancers [5,6,7].

Studies using animal and human organisms indicate that age-related gut dysbiosis may contribute to unhealthy aging and reduced longevity by triggering the innate immune response and chronic low-grade inflammation, leading to many age-related degenerative pathologies and unhealthy aging [8,9].

Due to the fact that the "gut microbiome signature" becomes more pronounced in type 2 diabetes, a better understanding of the role of microflora in diabetes (existing dysbiosis) provides new insight into the pathophysiology of this disorder. At the same time, establishing the pathogenetic relationship between the intestinal microbiome and diabetes management may lead to the development of integrated therapy in the context of nutrition, aging and pharmacological advices [10,11].

This study focused on the gut microbiome profiles of a married couple (both diagnosed with type 2 diabetes) who lived for last 35 years in a shared household.

At the same time, an attempt was made to answer the question of which factors (in the terms of their lifestyle, diet, nutritional status, and diabetes treatment methods) have the most significant impact on the intestinal microbiome.

MATERIAL AND METHODS

Material

The diabetic participants, a married woman (W) aged 66 and man (M) aged 67, are pensioners, and their place of residence is Polish city up to 200,000 inhabitants. The woman was diagnosed with type 2 diabetes 9 years ago and was treated with diet and oral medications (metformin XR 2 × 1000). Coexisting diseases: arterial hypertension and chronic coronary artery disease. Daily number of meals: 3 meals and 2 snacks.

Stimulants: 2–3 cups of coffee a day. Physical activity: housework and daily walks of about 1 km long.
The man was diagnosed with type 2 diabetes diagnosed 7 years ago and was initially treated with diet and oral medications (metformin XR 2 × 1000) and insulin therapy (high-mix analogue insulins twice a day in a total daily dose of 36–40 j), while metformin XR 2 × 1000 has been used for the last 3 years. Coexisting diseases: arterial hypertension, chronic coronary artery disease, chronic obstructive pulmonary disease and degenerative disease of the spine. Daily number of meals: 3 meals and 3 snacks. Stimulants: 2 cups of coffee a day, a bottle of beer 1–2 times a week; 10 cigarettes a day for 20 years. Physical activity: housework and daily walks of about 1 km long.

Methods

Anthropometric measurements and body composition analysis

Anthropometric measurements, including measurements of body weight, height, and waist and hip circumferences, were collected. Body height was measured to the nearest 0.1 cm using a non-metallic and non-stretchable tape. The weight and body composition analyses were carried out with electrical bioimpedance using the Tanita MC-780 multifrequency segmental Body Composition Analyzer (Tanita Corporation, Tokyo) [12].

Assessment of the diet by means of a 24-hour nutritional interview

Assessment of the diet was made based on a 24-hour nutritional interview concerning the consumption of dishes and products during a period of three days (two weekdays and one holiday). All products, meals, and drinks, as well as the dietary supplements, that were part of basic meals and consumed in the form of snacking between meals, both at home and outside, were analyzed. The obtained results were interpreted individually for each of the spouses. In order to estimate the quantitative food ration, the "Album of photographs of food products and dishes" was used [13]. The studies of the two subjects were carried out individually by a qualified dietitian. The content of vitamins and minerals in daily food rations was compared with the standards of recommended dietary allowances (RDA). In the case of sodium, potassium, and vitamins D and E, the level of adequate intake (AI) was used as the norm for comparison. Nutrition standards for the Polish population were taken into account [14].
The content of energy and basic nutrients, vitamins, and minerals in the daily food ration was calculated with using the "Dietetyk 2" computer program. The loss coefficient included in the computer program was assumed, i.e., vitamins C–55%, vitamins B1–20%, vitamins B2–15%, vitamin E–30%, vitamins A–25%, and folates–40%. For the remaining nutrients, losses of 10% were assumed [15].

Diet quality assessment

Each subject separately filled out the Questionnaire Eating Behavior developed by the Team of Behavioral Determinants of Nutrition, Committee for the Science of Human Nutrition of the Polish Academy of Sciences. This questionnaire allows for the collection of information about the usual frequency of consumption of foods from 16 food groups. The respondents selected six categories of consumption frequency: from "never" to "several times a day", which were then converted into a daily frequency (times per day). Taking into account the frequency of consumption of products, dishes and drinks, two indicators were calculated and used to assess the quality of the diet. The first of these indicators, the Pro-Health Diet Index (pHDI-8), gathered 8 food groups with potentially beneficial effects on health: vegetables, fruits, wholemeal and bran bread, milk (including flavored milk), fermented milk drinks (yoghurt, kefir, etc.), cottage cheese (including homogenized cheese), fish preserves and other fish dishes, legumes and dishes containing seeds. The second – Unhealthy Diet Index (nHDI-8); covering 8 food groups with potentially adverse health effects: sweets and confectionery, fried foods, alcohol and alcoholic beverages, sweetened carbonated drinks, canned meat, fish and vegetables, instant soups, fast-food, hard cheeses and cheese spreads.
The indices were calculated by summing up the frequency of consumption, previously expressed as times per day, and then converting it to a 100-point scale [7]. On the basis of this scale, the intensity of dietary features was determined, whether favorable or unfavorable to health, and the intensity of the features was described as either low (0–25 points), moderate (26–75 points) or high (76–100 points).
The questionnaires were completed within 30–35 minutes.

Glycemic control

Classic self-monitoring of glycaemia

Seven-day daily glycemic control was analyzed, which consisted of determining the concentration of glucose in capillary blood using dry strip tests with a glucometer according procedures. The respondents kept self-control notebooks in which they wrote down the time and the measurement results, the dose of anti-diabetic drugs, the size and composition of meals, and additional information on physical exertion and stressful situations.

Determination of glycated hemoglobin

HbA1c in whole blood was measured by National Glycohemoglobin Standardization Program (NGSP), a certified method using high-performance liquid chromatography (HPLC) on D-10 equipment of Bio--Rad (Hercules, CA, USA). The sensitivity was 0.05% with an intra-assay coefficient of variation (CV) of 2.05% and an inter-assay CV of 3.66%.

Analysis of stool samples

During the last month before stool collection, the investigated participants were not treated with antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), metamizole, paracetamol, steroids, iron preparations, or other drugs used in gastrointestinal diseases (including proton pump inhibitors). They also did not take probiotics or prebiotics and/or symbiotics. Stool samples (2 g) were collected by individuals in sterile containers and were delivered within two hours to the laboratory, where they were frozen at -80°C. Then, the samples were transferred in a subjected manner to the molecular laboratory.

Qualitative and quantitative analysis of the intestinal microbiome by next-generation sequencing

The quantitative and qualitative examination of the fecal intestinal flora was performed by the next--generation sequencing (NGS) method of A&A Biotechnology (Gdynia, Poland) in cooperation with Macrogene inc (Korea) based on studies by Klindworth et al. [16]. The analysis was carried out in the following stages:

- DNA isolation from frozen human stool samples. Isolation using mechanical and enzymatic lysis and purification on ion-exchange membranes. DNA eluate parameters: minimum concentration 0.1ng/µl in a minimum volume of 20 µl in each sample
- Illumina SBS DNA sequencing technology. The Amplicon library was prepared from supplied DNA by amplifying the V3–V4 region using a pair of primers and incorporating illumina adapters with indexes. Due to the expected repeatability of the tests and the need for comparison with other results, the required DNA sequencing procedure is based on the studies of Klindworth et al. [16]
- Testing the input material in terms of quality (electrophoresis) and quantity with the aid of Victor 3 fluorometry using picrogreen, as well as checking the quality of the library itself
- Sequencing by means of the original Illumina kits (Herculase II Fusion DNA Plymerase Nextera XT Index Kit V2) on the MISec platform in 2 × 300 bp paired reading mode (v3 chemistry = 600 cycle), with up to 100 K readings per sample
- The open-source Statistical Analysis of Metagenomic Profiles (STAMP (v 2.1.3)) according to [17].

RESULTS

Anthropometric characteristics and nutritional status

Both spouses are obese to the 1st degree with a body mass index (BMI) of 32.85 kg/m^2 (W) and 32.81 kg/m^2 (M), respectively, and bioelectrical impedance analysis (BIA) showed that body fat was 42% (N: 24––35.99%) for W and 40.8% (N: 12–25%) for M. Total body water (TBW) was insufficient, at 41% for W (norm: 45–60%) and 42.1% for M (norm 50–65%; Table I).

Table I. Anthropometric characteristics and the nutritional status of the respondents

Parameters	Woman	Man
Height (cm)	162	167
Weight (kg)	86.2	91.5
BMI (kg/m^2)	32.85	32.81
Fat mass	42%	40.8%
TBW (%)	41%	42.1%
Muscle mass	47.5 kg	51.5 kg
PHY	3	3
BMR	1523 kcal	1639 kcal

BMI – body mass index; TBW – total body water; PHY – physique rating; BMR – basal metabolic rate.

The proportion of saturated fatty acids exceeded the recommended 10% energy value, and the consumption of cholesterol was higher than 300 mg/day. At the same time, the spouses consumed too little dietary fiber (W–12.5 g, M–14 g, and norm–25 g) [13,14].

The average intake of vitamins and minerals found in meals was exceeded with regard to vitamins A, E, B2, and B12 and macro- and micro-elements sodium, iron, phosphorus, and copper. The diet of the respondents was poor in magnesium; calcium; vitamins D, B1, and C; and folates (Table II).

Table II. Content of selected minerals and vitamins in food rations

Nutrient content	Woman		Man	
	consumption amount	recommended norm %	consumption amount	recommended norm %
Sodium (mg)	1470	105	1936	138
Potassium (mg)	3002	64	3347.9	72
Calcium (mg)	54.2	45	56.9	52
Phosphorus (mg)	1022.9	146	1217	173
Magnesium (mg)	246.8	77	282	67
Iron (mg)	10.62	106	14.1	142
Zinc (mg)	8.69	108	10.92	99
Copper (mg)	1.06	117	1.43	152
Vit. A (μg)	1259	140	1223.4	174
Vit. D (μg)	3.81	38	3.45	34
Vit. E (mg)	13.82	138	11.84	148
Thiamine (mg)	1.22	96	0.95	86
Riboflavin (mg)	1.57	120	1.34	122
Niacin (mg)	17.77	111	13.79	98
Vit. B6 (mg)	1.97	116	155	103
Folic acid (μg)	278.31	79	221.38	55
Vit. B12 (μg)	4.43	184	3.7	154
Vit. C (mg)	68.11	75	69.9	92

Diet quality

There were differences between the two respondents with regard to a healthy and unhealthy diet (Table III). But similar values of the pHDI-8 and nHDI-8 for the respondents may indicate that they prefer a similar diet, while making various dietary mistakes.

Table III. Diet quality of participants

Diet quality index (pts)	Woman	Man
pHDI-8	53	51
nHDI-8	21	24

The intensity of features: small 0–33 pts; moderate 34–66 pts; large 67––100 pts. pHDI-8 – Pro-Health Diet Index; nHDI-8 – Unhealthy Diet Index.

The man more often than the woman consumed unhealthy products for example: red meat, white bread, instant and fast foods, as well as alcoholic beverages. The woman consumed hard cheeses and cheese spreads, canned fish. They ate sweets several times a week. Dark bread, fruit, vegetables and legumes were more often consumed by women.

Glycemic control

Each patient performed 28 blood glucose measurements using classic self-monitoring. The mean values of the measurements were as follows: W – on an empty stomach, 104 ± 12 mg/dl range: 112–189 mg/dl, no episodes of hypoglycemia; M – on an empty stomach, 115 ± 24 mg/dl range: 123–229 mg/dl, no episodes of hypoglycemia. Glycated hemoglobin values were 7.2% (W) and 7.7% (M).
The results of the research indicate that diabetes is not controlled in the analyzed patients.

Fecal microbiota composition

There were no significant differences in the study of the gut microbiome between spouses. The dominant bacterial phyla were *Firmicutes* and *Actinobacteria*, while *Bacteroidetes* and *Proteobacteria* shared smaller proportions, between 2 and 7% (Figure 1).
The dominant *Lachnospiraceae* family (29–31%) is a phylogenetically and morphologically heterogeneous taxon belonging to the clostridial cluster XIVa of the phylum *Firmicutes* [18]. Phylum *Firmicutes* was also presented by *Ruminococcaceae* (16–19%) and *Streptococcaceae* (3–11%). The *Actinobacteria* phylum was proportionally less abundant and mainly represented by *Bifidobacteriaceae* (6–12%; Figure 2).

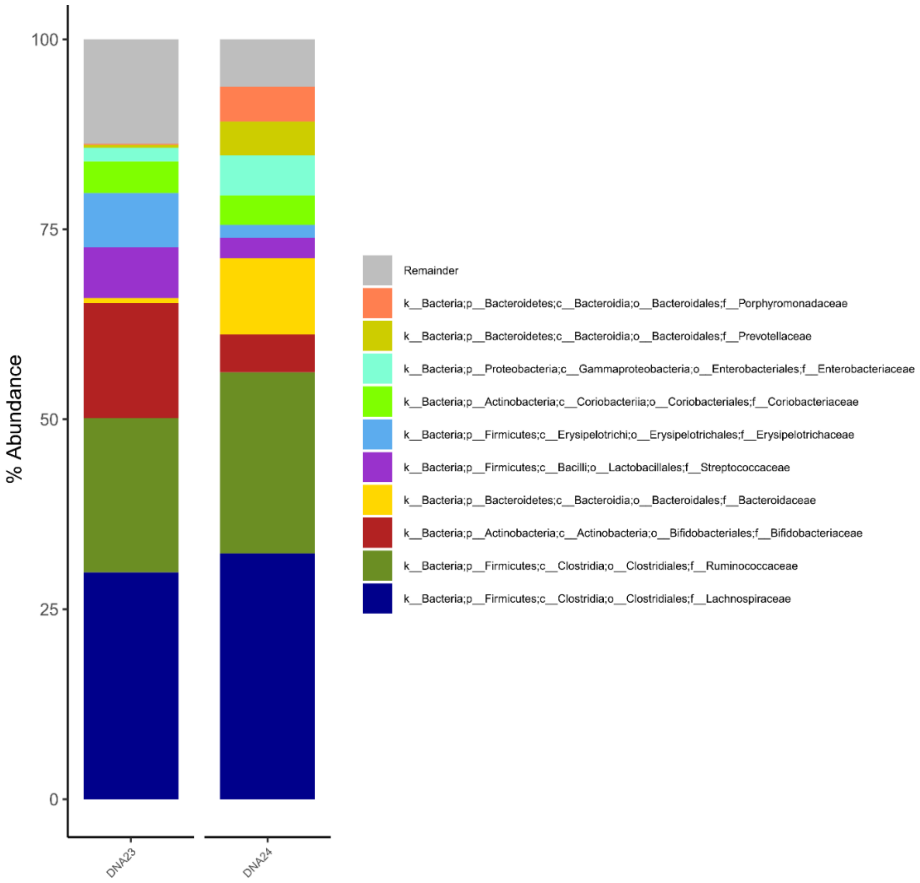

Fig. 1. Microbiota profile of fecal samples from the spouses at the phylum level. Taxonomic differences of fecal microbiota between spouses: DNA23–W; DNA24–M.

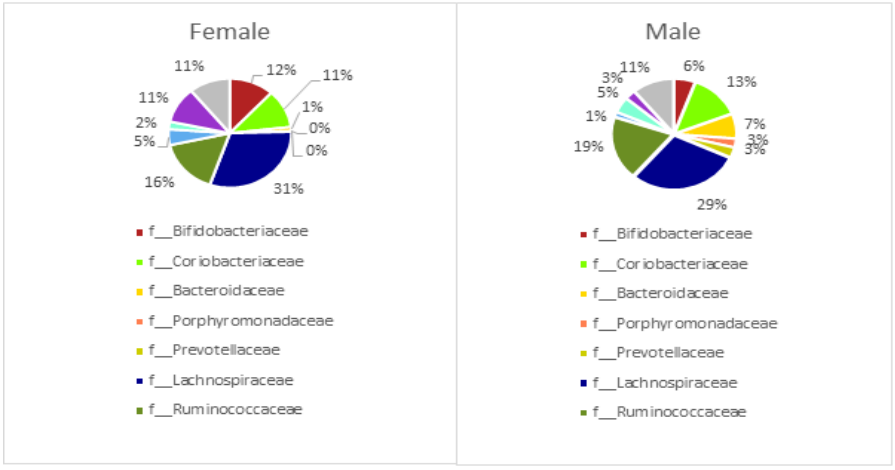

Fig. 2. Mean relative abundance (%) of bacterial genera in fecal samples collected from two patients.

DISCUSSION

Gut microbiota composition is shaped by hundreds of factors, including host genetics, gender, age, height, weight, diet, immune system, gastrointestinal secretions, blood levels of various molecules or red blood cell counts, stool consistency, sleep, medical history, ethno-geographical and socio-economic conditions, sanitary conditions, smoking, antibiotics and antibiotic-like substances, and laxatives and less intuitive drugs (e.g., antihistamines, antidepressants, and metformin) [19].

To the best of our knowledge, this study is the first concerning the analysis of the microbiome of people living in a common household for many years. The

couple are of the same ethnicity, of a similar age and physical activity and have been suffering from type 2 diabetes and hypertension for about 8 years. Anthropometric measurements and body composition analysis showed both obesity of the first degree, excess body fat, and low water content. They follow a die, with excessive amounts of fats and a deficiency of carbohydrates, fiber, and certain vitamins and minerals. The spouses differ in sex, therapy and glycemic control of type 2 diabetes, comorbidities, and stimulants. However, it should be noted that although the woman declared that she does not smoke cigarettes, we must have in mind that her spouse smokes 10 cigarettes a day for last 20 years which shows that she was a passive smoker. The couple did not differ in their physical activity, both in the past and now.

In the stool test, *Firmicutes* and *Actinobacteria* phyla constitute approx. 80% of the intestinal microbiome, and *Lachnospiraceae, Ruminococcaceae,* and *Bifidobacteriaceae* are the three dominant bacterial families in two type 2 diabetes obese patients analyzed. In healthy people, the dominant gut microbial phyla are *Firmicutes, Bacteroidetes, Actinobacteria*, *Proteobacteria, Fusobacteria,* and *Verrucomicrobia*, with the two phyla *Firmicutes* and *Bacteroidetes* representing 90% of gut microbiota [19,20]. *Clostridium* genera represent 95% of the *Firmicutes* phyla. The others phyla, including *Actinobacteria* (mainly *Bifidobacterium*), *Proteobacteria, Verrucomicrobia,* and *Euryarchaeota*, are represented in lower concentrations [11].

Ley et al. [21] found an increase in *Firmicutes* abundance ($p = 0.002$) and a corresponding decrease in *Bacteroidetes* ($p < 0.001$) associated with a high BMI. Within *Firmicutes*, high abundances of *Lachnospiraceae* were positively correlated with glucose and/or lipid metabolism, indicating metabolic disturbance [22,23]. Zeng et al. [24], administering 36 weeks of a high-fat diet to mice, found increased amounts of *Firmicutes* compared to mice fed with a low-fat diet, particularly *Lachnospiraceae*. Among *Firmicutes*, the *Lachnospiraceae*, *Lactobacillaceae*, and *Ruminococcaceae* species hydrolyze starch and other sugars to produce propionate, butyrate, and other short-chain fatty acids [22,25]. Genomic analysis of *Lachnospiraceae* revealed a considerable capacity to utilize diet-derived polysaccharides, including starch, inulin, and arabinoxylan, with substantial variability among species and strains.

In agreement with our results, Takagi et al. [26] demonstrated that the levels of the *Actinobacteria* phylum were significantly increased in patients with hypertension, hyperlipidemia, and type 2 diabetes, and this increase was reflected in the increased abundance of the *Bifidobacterium* genus. Phylum *Actinobacteria* is involved in lipid metabolism [27]. Hashimoto et al. [28] also found that the *Actinobacteria* phylum was highly abundant in patients with type 2 diabetes, whereas the *Bacteroidetes* phylum was less abundant. The levels of *Bifidobacterium* were negatively correlated with carbohydrate intake [29].

Studies on humans have indicated that a lower proportion of *Bacteroidetes* and a higher proportion of *Firmicutes* are associated with obesity and insulin resistance [23,30]. A higher *Firmicutes/Bacteroidetes* ratio and a higher relative abundance of *Lachnospira* and *Roseburia* were found in pre-menopausal women than in post-menopausal women who had similar levels to men by Santos-Marcos et al. [31]. In our investigation, the female was post-menopausal, which may explain the similar gut microbiota profile in comparison with her spouse.

In contrast, Larsen et al. [32] demonstrated that in diabetics, the relative abundance of *Firmicutes* was significantly low while the proportion of *Bacteroidetes* and *Proteobacteria* was considerably high.

In the treatment of diabetes, the female patient is treated with metformin, and her husband is treated with metformin and insulin.

The number of publications on the effect of metformin on the gut microbiome is steadily increasing [33,34], while there are few reports on the effect of insulin or insulin/metformin treatment on the gut microbiome.

Metformin, which is used in the treatment of diabetes mellitus for both spouses, can improve glucose homeostasis and exert hypoglycemic effects by affecting the gut microbiota (significantly increasing the abundance of the phylum *Verrucomicrobia,* genus *Akkermansia*, and species *Akkermansia muciniphila*), through which it maintains the intestinal barrier function, increases the production of short-chain fatty acids, regulates bile acid metabolism, and affects glucose homeostasis [34]. However, in the available literature, there are no reports on the effect of insulin therapy on the intestinal microbiome. Wang et al. [35] indicate that intensive insulin therapy recovers diabetes-associated gut structural abnormalities and restores the microbiome landscape.

Epidemiological studies on the association between cigarette smoking and gut microbiome measured in stool samples is limited. In these studies, *Proteobacteria* and *Bacteroidetes* phyla were increased, as well as the genera of *Clostridium, Bacteroides* and *Prevotella*. Decreased *Actinobacteria* and *Firmicutes* phyla [36,37]. The number of cigarettes smoked per day was not associated with any bacterial taxa and phylum level, but among current smokers, relative abundances of the phylum *Actinobacteria* were inversely associated with pack-years of smoking [37]. According Lee et al. [38] current smokers had an increased proportion of the phylum *Bacteroidetes* with decreased *Firmicutes* and *Proteobacteria* compared with never smokers, whereas there were no differences between former and never smokers.

A relationship between diet and gut microbiota composition has been documented by various researchers [20,24,39,40]. The diets of the two individuals contains too much fat, sodium and certain vitamins and micronutrients and is poor in fiber; magnesium; calcium; vitamins D, B1, and C; and folates. Favorable eating behavior was mainly related to the number of meals, vegetables and fruits consumed; negative – snacking between meals, consumption of sweets, processed foods and alcohol. The values of the indexes describing the quality of the diet were similar.

For each of the eight B-vitamins, Magnúsdóttir et al. [41] described the known biosynthesis pathways and presented the frequency of the respective functional roles of each in their analyzed taxonomic groups. Synthesis of thiamin monophosphate (Vit. B1) is present in most phyla, except for *Firmicutes*, and this synthesis is most prevalent in *Bacteroidetes* and *Fusobacteria*, similar to folate biosynthesis pathway.

A high sodium diet in mice is associated with a decreased abundance of *Lactobacillus* spp., *Oscillibacter*, *Pseudoflavonifractor*, *Clostridium* XIVa, *Johnsonella*, and *Rothia* and an increased abundance of *Parasutterella* spp., *Erwinia genus*, *Christensenellaceae*, *Corynebacteriaceae*, *Lachnospiraceae*, and *Ruminococcus* [20]. This is in line with our findings, where the feces of the subjects are dominated by *Lachnospiraceae*, *Ruminococcus*, and *Bifidobacteriaceae.*

Poor dietary fiber consumption, as is the case with this married couple, reduces bacteria diversity in the gut but also reduces production short-chain fatty acids (SCFA) and a shift towards the utilization of less favorable substrates, such as dietary and endogenous protein sources, by the gut microbiota [42,43]. The fermentation of proteins and amino acids by the microorganisms in the gut increased production of cytotoxic and proinflammatory metabolites that contribute to the development of chronic diseases, including type 2 diabetes [44].

Limitation of the study

Only two people living in a shared household who suffer from the same disease and are treated with metformin (together with insulin for the male subject) participated in the study. It is necessary to increase this group in terms of its size, taking into account the statistical analysis of all data. Research and the results obtained should be treated as proof of concept and perhaps constitute a reference point for further research.

CONCLUSIONS

This clinical case may be opening a new scenario for the treatment of elderly diabetic patients, identifying new pathway to be further impacted by dieticians and diabetologists.

In the available literature, the authors did not find similar studies on the analysis of the gut microbiota of type 2 diabetes mellitus spouses in relation to the anti--diabetic medications taken, comorbidities, diet, lifestyle, etc.

Our study, although involving only one couple of diabetic spouses, suggests that the similar diet, common living conditions, and similar physical activity during the life have a significant influence on gut microbiota composition. Gender, medications used, comorbidities, and even stimulants seem to be of less importance.

As a result of integrating microbiota data into the 60-year-old Wisconsin Longitudinal Study, Herd et al. [45] and Dill-McFarland et al. [46] found that common living conditions (socialness) with family and friends is associated with differences in the human fecal microbiota. Authors concluded that spouses have more similar microbiota and more bacterial taxa in common than siblings do. Moreover, married individuals harbor microbial communities of greater diversity and richness compared to those living alone, with greatest diversity among couples reporting close relationships.

There are many reports indicating that diet largely influences gut microbiota composition and function of the intestinal microflora. Hence, the correct composition of flora in the human digestive tract may be inextricably linked to human nutrition.

A rapid increase in the prevalence of obesity and type 2 diabetes mellitus worldwide is related to changes in the environment, which have a negative impact on the risk factors for diabetes [47]. These environmental changes include most specifically changes in dietary habits, which modulate gut microbiome composition largely by regulating excessive biological functions [38,39,48].

Hippocrates' notion "Let food be thy medicine and medicine be thy food" remains highly relevant millennia later [43].

Therefore, sensible dietary intervention can improve the modified microflora, contributing to the prevention and treatment of obesity, diabetes, cardiovascular disease, and other nutritional diseases.

Author's contribution

Study design – M. Piłot, S. Dzięgielewska-Gęsiak
Data collection – M. Piłot
Data interpretation – S. Dzięgielewska-Gęsiak, M. Muc-Wierzgoń
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