

Patrycja Pokrzywnicka¹, Janusz Gumprecht²

¹Municipal Hospital in Zabrze

²Department of Internal Diseases, Diabetology and Nephrology in Zabrze

Intestinal microbiota and its relationship with diabetes and obesity

ABSTRACT

The number of people who are obese and who suffer from type 2 diabetes is one of the most prominent health problems of our time. Among commonly known reasons we may distinguish excess of food in relation to how much food energy our organism really needs (change in life style and diet), genetic predisposition, endocrine disorders, and use of medicines. However, according to latest reports, intestinal flora plays a significant part in aetiology of these medical conditions. The fact that intestinal microflora may affect body weight, sensitivity to insulin, metabolism of sugars and lipids leads to a conclusion that any change within intestinal microflora may be the reason for pathogenesis of obesity and diabetes. Moreover, any attempt to modify it may cause decrease or limitation of the intensity of the medical conditions mentioned above. Intestinal microbiota is now one of the most developing subjects for research. Many of the world's medical projects including MetaHIT (UE and China), MicroBES (France), Human Microbiome Project — HMP (USA) focus on research on the role of intestinal bacteria for people's health. Scientists are particularly interested in the possibility of modification of the intestinal microorganisms in order to treat or prevent many conditions including obesity and other diseases of affluence. (Clin Diabetol 2016; 5, 5: 164–172)

Key words: obesity, intestinal microbiota, diabetes mellitus, short chain fatty acids, prebiotics, probiotics

Address for correspondence:

lek. Patrycja Pokrzywnicka

Szpital Miejski w Zabrzu

e-mail: patpok2510@gmail.com

Translation: lek. Małgorzata Kamińska

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Microbiota — another organ of the human body

Human gastrointestinal tract, and particularly large intestine, is colonized by a number of microorganisms. It is estimated that total weight of these microorganisms is 1.5 kg and that their genome includes 100-fold more genes than the genome of the human. Until recently, it has been stated that the number of microbial cell is 10-fold higher than the number of cells in the human body. However, recent studies show that these figures are overestimated. This tightly packed ecosystem has metabolic potential comparable with the metabolic activity of the liver. The role of the microorganisms in the maintenance of health is enormous, and yet constantly discovered. Most of the isolated microorganisms (94–98%) fall into four basic groups of bacteria: *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria* (8%) and *Actinobacteria* (3%). The remaining, though few, are very diverse taxonomically [1–3]. The number of bacteria and their functions differ among specific parts of the gastrointestinal tract and depend on many factors, such as environmental pH, oxygen availability and also the kind of food ingested. Ecosystem has many important functions, and therefore human organism could not survive without inhabiting microbes. Hence, microbiom is considered as another organ of human organism. The composition of intestinal flora is different in every human — it is unique as a fingerprint and can be identified (so far imperfectly) using molecular tests. Importantly, conventional microbiological tests, consisting in *in vitro* cultures, are practically useless in intestinal flora identification. Data collected in “clone libraries”, DNA identification and phylogenetic trees only confirm the complexity of this issue [4].

Development of intestinal microflora

Foetal gastrointestinal tract is sterile, and first colonization occurs at birth. Type of delivery significantly

influences the composition of microflora. Contact of the child with bacterial flora of the mother's vagina and faeces as well as the presence of microorganisms in surrounding environment immediately after natural delivery or absence of bacteria after caesarean delivery determines the type of first acquired microorganism. Intensive phase of the development of bacterial colonies ends at the age of two [5]. In the intestines of children born naturally, bacteria of the phylum *Bifidobacterium* and *Lactobacillus* appear immediately, whereas in children delivered by Caesarean section, these microorganisms appear in the intestines after about 30 days [6]. Another factor affecting intestinal microflora in infants is the diet, i.e. the type of milk consumed. It has been shown that in breast-fed infants' bacteria of the phylum *Bifidobacterium* appear earlier than in children fed with synthetic milk. *Bifidobacteria* provide protection against pathogenic strains by producing acetic and lactic acids. Bacterial colonization depends also on many other factors, including hygiene level and medication [6]. The study of identical twins found great similarity in the composition of intestinal microflora immediately after birth and its analogous changes throughout life, which confirms that the main factors responsible for the composition of microflora might be of genetic and environmental nature [7]. Considering variability of the microflora during lifetime, microbiological profiles of intestinal flora of infants and adults may be created. The microflora of infants is composed of four main groups of bacteria: *Bifidobacterium*, *Staphylococcus*, *Streptococcus* and *Enterobacteriaceae*, whereas intestinal microflora in adults includes mainly two types of bacteria: *Bacteroidetes* and *Firmicutes*.

The impact of various factors on the composition of intestinal microflora

The study by De Filippo et al. which compared 6-year-old children living in Africa and in West Europe (in the vicinity of Florence, Italy) showed that the composition and proportions of the microbiota depend on the diet. The significant fundamental difference reported by the authors in the conclusions was different proportion of *Bacteroidetes* versus *Firmicutes* and *Proteobacteria*. The researchers found that the intestinal flora of children from Burkina Faso in Africa, where the diet was low in meat and contained a lot of fibre, vegetables and starch, contained considerably more bacteria of the phylum *Bacteroidetes* and correspondingly less bacteria of the phylum *Firmicutes* compared to children from West Europe whose diet contained mainly meat and was rich in animal fat and monosaccharides. At the same time, examinations of stool samples from African children showed

the presence of bacteria of the genera *Prevotella* and *Xylanibacter*, microorganisms able to produce the enzymes hydrolysing cellulose and xylan, as well as higher content of short-chain fatty acids, which are additional source of energy. Based on these observations, the researches have proved that the diet is closely related to the composition of the microflora, and that particular microorganisms appear in the microflora depending on the composition of food [8]. Differences in the proportions of bacteria translate into differences in quantity and quality of the final products of bacterial metabolism — short-chain fatty acids (SCFAs). The above-described relationship is confirmed by a study of intestinal microflora in subjects on a vegetarian diet. The study demonstrated the decrease in the number and changes in the diversity of bacteria of the genus *Clostridium* and the increase in the number of *Bacteroidetes* [9]. However, researchers' opinions are divergent and not all studies have found similar correlations. Ley et al. showed identical contents of *Bacteroidetes* and *Firmicutes* in obese subjects who were on high-fat diet and in those who had switched to a low-calorie diet [10]. There are also studies suggesting complete lack of associations between *Bacteroidetes/Firmicutes* ratio and diet or BMI [11]. However, this topic is still developing and requires a lot of research and analyses taking into account all the possible factors affecting the reliability of the results [12]. There are also other factors, besides the diet, that affect the composition of intestinal microflora, such as genotype, age, gender, and environmental conditions. The intestinal microflora changes with age, which has been demonstrated by comparing the flora of children and adults and the elderly [13, 14]. In newborns the most prevalent are the bacteria of the phylum *Bifidobacterium*, but during the life the intestinal flora undergoes dynamic changes, eventually forming a very complex ecosystem [7]. Comparison of the intestinal flora of adolescents and adults showed increased content of *Bifidobacterium* and *Clostridium* in the microflora of adolescents [20]. The intestinal microflora of elderly people is characterised by the increased number of bacteria of the phylum *Bacteroidetes* and less variety of microorganisms [16].

Functions of intestinal microbiota

Intestinal microbiota has trophic, protective and metabolic functions:

- trophic function — microflora significantly influences proliferation and differentiation of colonic epithelium and proliferation of endothelial lymphocytes. It also affects the development of enterocytes in small intestine. Enterocytes are cells responsible for absorption of substances in

small intestine. It has been observed that in areas with a large number of bacteria the number and length of intestinal villi is increased compared to less intensely colonized areas. This association confirms the hypothesis that intestinal microflora stimulates the maturation of enterocytes;

- protective function — microflora forms a barrier against colonization by pathogenic bacteria. It stimulates the production of substances that inhibit the adhesion of pathogens to intestinal epithelium. Moreover, it lowers the pH of chyme by the production of organic acids;
- metabolic function — it is believed that the most important metabolic process is degradation of debris by fermentation resulting in fatty acids production. Additionally, microflora is involved in the synthesis of vitamins B and K and increases the bioavailability of minerals.

Microbiota functions, summarized above, are associated with both the digestive and immune systems. The enterotype is also responsible for the proper course and control of many metabolic processes, and impairment of its function may lead to problems in maintaining homeostasis [17]. The enterotype determines the products of metabolism which then have a beneficial or detrimental effect on human organism [18]. Another important role of microflora is its impact on the structure of the final section of the digestive tract. It also influences bowel motor function [16]. So, what composition of the flora would be most appropriate to preserve the balance of the metabolic processes? It seems that the basic criterion is the variety, because it affects favourably the metabolic processes.

Dysbiosis

As it has been proven above, there are many factors that can disturb the microflora balance and cause dysbiosis. The most important of these factors is high-fat, high-protein diet [19]. Dysbiosis may be also caused by some drugs, such as antibiotics, proton pump inhibitors (PPI), nonsteroidal anti-inflammatory drugs (NSAIDs), iron, methotrexate, and metformin. Additionally, chronic stress and gastrointestinal tract infections may change the proportions of two main phyla, *Bacteroides* and *Firmicutes* [20].

Disturbed *Bacteroidetes/Firmicutes* ratio is observed in overweight people [17]. High-fat and low-carbohydrate diet leads to deficiency of *Bifidobacteria* [18]. In the gastrointestinal tract of obese individuals the most prevalent becomes a type of Gram-positive bacteria, *Mollicutes*, which very efficiently generate energy from food.

Impact of microbiota on energy homeostasis and fat storage

The impact of intestinal microflora on energy homeostasis and fat storage in host organism are still poorly understood. Stimulation or inhibition of metabolic pathways correlates with changes in the qualitative or quantitative composition of bacterial enterotype. A study on mice showed that the treatment with antibiotics, norfloxacin and ampicillin, caused changes in the composition of intestinal microflora, leading to improvement in fasting glycaemia, glucose tolerance and insulin sensitivity compared to untreated control group [21].

Leaky gut syndrome

Dysbiosis leads, among others, to disorders of the structure and function of the intestinal barrier resulting in a condition called "leaky gut syndrome". In the future, this syndrome might prove to be the most important consequence of dysbiosis. Damage to the barrier enables the penetration into the body of microorganisms and their metabolites causing chronic inflammation. Tight junctions, gap junctions and desmosomes ensure the integrity of the enterocytes. Dysbiosis impairs the synthesis of zonulin 1 and occludin (important elements of the tight junction) causing functional damage to the integrity of the mucosa and, subsequently, increasing its permeability to many harmful substances and antigens. Besides the SCFAs, also very toxic bacterial lipopolysaccharide (LPS) can enter the organism. This endotoxin is a component of the outer membrane of the cell wall of Gram-negative bacteria and cyanobacteria inhabiting the gastrointestinal tract [22].

The development of obesity is accompanied by systemic inflammatory response. Inflammation is associated with the release of tumour necrosis factor (TNF-alpha) and IL-6 from both macrophages and adipocytes. Inflammation in the intestinal mucosa causes a loss of intestinal barrier integrity, which increases the permeability of the intestinal wall to bacteria, bacterial lipopolysaccharide (LPS) or other bacterial particles [28].

Recently, there has been a discussion on the role of the intestinal barrier permeability to dietary proteins and the possibility of evoking the so-called IgG-mediated allergy, which may result in obesity and metabolic diseases [23]. Some studies suggest potential pathological effect of anti-IgG and resulting chronic inflammation on the development of obesity.

The intestinal microflora by synthesis and secretion of many chemicals may cause two-fold increase in the capillary density in the epithelium of the small intestine

resulting in higher absorption of monosaccharides within this part of the intestinal tract [24].

Intestinal bacteria synthesize glycosidic hydrolases — enzymes required to break down complex plant polysaccharides (starch, cellulose, pectin, xylan, inulin) contained in those products consumed that cannot be digested by human enzymes [25]. Thanks to symbiosis between human organism and intestinal bacteria, it is possible to obtain energy from SCFAs — compounds that cannot be degraded by digestive enzymes, but can enter the blood stream as a result of fermentation carried out by the microflora. It is possible to provide this way 80–200 calories a day, which is ~ 4–10% of the daily requirement of an adult [26]. Although the amount of energy seems insignificant, in the long term this can have a considerable impact on body weight. The main compounds generated as a result of the above-mentioned symbiosis are short-chain fatty acids (SCFAs) which include i.a. acetate, propionate and butyrate, wherein the acetic acid is the dominant type of SCFAs [27]. The only one SCFA constituting a source of energy for the body is propionic acid, which can be used in the synthesis of glucose and lipids [28].

Metabolic functions of SCFAs play significant roles in energy homeostasis of the body and in the development of obesity and type 2 diabetes: they activate G protein-coupled receptor 41 (GPR41) by stimulating the secretion of peptide YY (PYY) [29]. PYY is a hormone that slows intestinal transit and thus increases the absorption of nutrients and production of energy. Its influence is currently being explored, as it may affect the development of obesity [19]. SCFAs are also signalling molecules. Propionic, acetic and butyric acids are ligands for receptors coupled to G proteins, GPR41 and GPR43, belonging to a group of cell receptors — G-protein coupled receptors (GPCRs). Both are epithelial receptors. Some studies suggest that GPR41 controls energy balance of the body via the interaction with metabolites produced by the microflora. GPR43 is proposed as a “molecular connector” between diet, gut microflora, immunity and inflammatory response. It was shown that mice lacking GPR41 and GPR43 have lower weight than their wild-type animals [16].

Butyric acid-dependent stimulation of leptin production in adipocytes and induction of glucagon-like peptide 1 (GLP-1) secretion by the intestinal L cells [30] as well as increase in thermogenesis, fatty acid oxidation and mitochondrial activity within the muscle and brown adipose tissue [31] are other examples of the impact of SCFAs on the metabolism of the host.

SCFAs’ roles are, however, diverse and they depend on existing conditions. Butyric acid may exhibit anti-inflammatory activity, reducing the release of cytokines

and chemokines [30]. In a study on obese mice fed with a high-fat diet enriched with butyrate, reduction and even normalization of insulin resistance was observed [31]. Another study showed that eating a diet low in carbohydrates results in reduced concentration of butyric acid in stool samples and a decrease in the number of bacteria responsible for its production [32]. Based on this findings, it can be hypothesized that butyrate has a positive effect on metabolism in pathological states, but does not play a major role in normal conditions [12].

A mechanism promoting the development of obesity and fat storage is reduced expression of fasting-induced adipocyte factor (FIAF). FIAF, also known as angiopoietin-like protein 4, inhibits the lipoprotein lipase (LPL), an enzyme responsible for the storage of energy as fat. Since FIAF facilitates the release of fatty acids from lipoprotein triglycerides, reduced expression of FIAF is associated with higher LPL activity in adipocytes and increased energy storage.

The intestinal microflora may also affect lipid metabolism of the host by inhibiting adenosine monophosphate-activated protein kinase (AMPK) [33]. AMPK is an enzyme that controls energy status at the cellular level [34]. “Germ-free” (GF) mice (the mice of sterile environment), even when fed with Western diet containing high amounts of sugar and fat, are protected against obesity. This is possible due to the high activity of the phosphorylated form of AMPK in the liver and skeletal muscles of these animals, and thereby high-efficiency oxidation of fatty acids in these two organs [33]. Active AMPK phosphorylates acetyl-CoA carboxylase, which leads to a decrease of malonyl-CoA. The compound inhibits carnitine palmitoyltransferase, an enzyme involved in the transfer of long-chain fatty acids into the mitochondria. This activates the process of fatty acids’ oxidation [16].

Bile acids

It has been found that the intestinal microflora affects the bile acid synthesis and metabolism. Bile acids can activate signalling pathways by both nuclear receptors and previously described GPCRs, the receptors present on the cell surface. Farnesoid X receptor (FXR) was the first identified nuclear receptor that is activated by bile acids. In the blood of mice lacking this receptor (FXR^{-/-}) elevated levels of triglycerides and glucose have been observed. This indicates that it may be involved in glucose and lipid metabolic pathways [35, 36]. Activation by bile acids of TGR5 membrane receptor that is expressed in the brown adipose tissue and large intestine results in increased energy utilization in these tissues [37], and thus might prevent insulin resistance and obesity.

Changes in intestinal microflora associated with obesity

Studies on germ-free mice that underwent transplantation of intestinal bacteria from obese or normal-weight mice showed an association between obesity and intestinal microflora. It was observed that, although both groups were fed with the same diet, GF mice that receive the microflora from mice with excess body weight also became obese [38]. Observations of people in a homogenous population who, despite the use of similar diets were more or less likely to gain weight and develop metabolic disorders, suggested the possible association of the intestinal microbiota with the aetiology of obesity [39, 40]. The predominance of two types of bacteria and, more specifically, their proportions were reported repeatedly in the conclusions of many studies.

In obese subjects *Bacteroidetes* and *Firmicutes* ratio was shifted in favour of *Firmicutes*. Weight reduction was associated with proportional increase in the number of *Bacteroidetes* [41]. It has been proven that the increase in the number of *Firmicutes* by 20% and a similar decrease in the number of *Bacteroidetes* results in the raise in the amount of energy obtained from the food by 150 kcal [42]. Furthermore, researchers found the increase in the number of *Bacteroides* as a result of bariatric surgery (Roux-en-Y gastric bypass, RYGB) leading to weight loss in obese people. On the other hand, it should be mentioned once again that not all research supports such significant role of the intestinal microbiota in the pathogenesis of obesity. Some publications report the lack of correlation of the number of *Bacteroidetes* and *Firmicutes* with body weight [26]. It is not entirely clear whether changes in the microbiota in obese subjects are the result or the cause of obesity and certainly further studies are needed. In another experiment, the researchers compared genetically modified, leptin-deficient obese mice (*ob/ob*) and lean mice (*ob/+* and *+/+*) [43]. Leptin was chosen as a differentiating parameter, because it controls the appetite [44]. It has been shown that the microorganisms inhabiting the intestines of *ob/ob* mice have enzymes that enable degradation of otherwise indigestible polysaccharides contained in the food. Analyses of stools samples from obese individuals have also demonstrated a higher amount of final product of fermentation, such as acetic and butyric acids, and lower calorie content. In addition, it was observed that mice that are prone to insulin resistance and steatohepatitis, the most common complications of obesity, had abnormal levels of metabolites associated with the changes of phosphatidylcholine in blood and urine. When feeding the mice with a high fat diet, their

microflora begins to transform food-derived choline into hepatotoxic methylamine. Choline is necessary for the secretion of very low density lipoprotein (VLDL). VLDL is synthesized in the liver, and its main function is to transport lipids from the liver to fat tissue cells — adipocytes. Intestinal microflora may participate in the pathogenesis of insulin resistance and steatohepatitis by reducing the bioavailability of choline. It can also initiate lipid peroxidation in the host organism [45]. Another difference observed in the microflora of obese individuals is increased number of methanogenic bacteria, which remove the harmful excess of H₂ from the environment and thereby improve fermentation processes performed by the bacteria [43].

Dysbiosis, obesity, insulin resistance and the development of type 2 diabetes are associated with chronic systemic inflammation within adipose tissue. Gram-negative bacteria include LPS (described above) and peptidoglycans of inflammatory properties. The colonization of germ-free mice with *Escherichia coli* results in increased infiltration of adipose tissue by macrophages and enhanced secretion of cytokines. A diet high in fat results in elevated levels of LPS in human serum [46, 47]. LPS enters the blood in chylomicrons or, according to another theory, as a result of increased permeability of the gut [48].

LPS molecules have an affinity for Toll-like receptor 4 (TLR4). TLR4 is a pattern recognition receptor (PRR) that binds to molecules of microorganisms. These receptors belong to the innate immune system and their stimulation activates pro-inflammatory signalling cascade [49, 50].

The level of LPS, which is one of pathogen-associated molecular patterns (PAMPs), is constantly monitored by TLR4 [50]. In rats susceptible to the occurrence of obesity, increased intestinal permeability and elevated levels of LPS in the blood have been observed [16]. Increased intestinal permeability has been noted in mice fed with a high-fat diet [51]. To sum up, elevated PAMP level and activation of PRRs induce inflammation, which in turn is associated with the development of metabolic disorders, such as insulin resistance or diseases of the cardiovascular system.

Intestinal microflora in type 2 diabetes

A number of studies have been performed on the correlation between the composition of the intestinal microflora and type 2 diabetes (T2D). It is believed that both insulin resistance and dysfunction of insulin-producing beta-cells arise from the interaction of many environmental and genetic factors. Also, some relationship was found between qualitative composition of intestinal microflora and T2D. A study including

30 obese people, of whom seven were suffering from T2D, showed that patients with diabetes had reduced number of *Faecalibacterium prausnitzii* — bacteria belonging to the phylum *Firmicutes* that are normally found in the intestinal microflora. The study group underwent bariatric surgery, after which in patients with T2D the number of *F. prausnitzii* increased, but was still lower than in subjects without diabetes. At the same time, reduced glucose and insulin concentration, and glycosylated haemoglobin in the blood were also found, as well as improvement in insulin resistance with the estimation based on the test results of homeostasis model assessment of insulin resistance (HOMA-IR). In addition, a decrease in markers of inflammation, i.e. CRP and IL-6, was also recorded [52]. Another study performed by Larsen et al. also concerned the phylum of *Firmicutes*. The study included 36 male adults, both with normal weight and obese, of whom 18 subjects had type 2 diabetes. The analysis of intestinal microflora showed reduced proportions of the phylum *Firmicutes* and class *Clostridia* in diabetic patients. It was also noted that the ratio of *Bacteroidetes* to *Firmicutes* correlated with plasma glucose concentration, similarly as in the above-described experiment, but there was no relationship to BMI, which certainly requires further analysis [53]. Data about ratios of *Bacteroidetes* to *Firmicutes*, i.e. Gram-negative to Gram-positive bacteria, in patients with both obesity and type 2 diabetes are not completely clear-cut; however, a positive correlation has been shown in mice between the plasma concentration of lipopolysaccharide (LPS) and weight gain, triglyceride accumulation, insulin resistance and type 2 diabetes. LPS, above-described component of cell membranes of Gram-negative bacteria, may be involved in the development of inflammation associated with type 2 diabetes. The results of animal studies have been confirmed by measurements of plasma LPS in healthy subjects and in patients with type 2 diabetes. It has been shown that lipopolysaccharide concentration was higher in diabetic patients than in healthy subjects. This association may suggest that LPS is involved in the pathogenesis of type 2 diabetes [54]. What is more, administration of polymyxin B to rats results in decreased plasma concentration of LPS and reduces the incidence of steatohepatitis and other above-mentioned diseases.

Do available data allow the development of specific concepts to support the treatment of obesity and diabetes by modifying microflora of the patient? Changes in the ecosystem of the gut as part of the therapy and prevention of obesity and type 2 diabetes? Although the exact role of microorganisms belonging to the intestinal microflora is still under research, the information

gathered so far allow for starting the research aimed at introducing changes within the intestinal ecosystem and using them as a part of the therapy. Currently available pharmacological tools include antimicrobials, prebiotics and probiotics. Targeted use of these drugs changes the composition of the intestinal microflora and is at least partially effective in the preventing or treating metabolic diseases.

Antimicrobial drugs

It has been shown that the antimicrobial therapy reduces morbidity and delays the onset of type 1 diabetes (T1D). Brugman et al. conducted a study on rat models of T1D — BB-DP (Bio-breeding diabetes prone). The intestinal microflora of rats that did not develop T1D was characterized by a reduced content of *Bacteroidetes*. The researchers then analysed the effect of antibiotics on the incidence of T1D. Antibiotic treatment in BB-DP rats caused changes in the intestinal microflora and reduced the incidence of T1D or delayed the symptoms' onset. These results suggest that intestinal microflora may be involved in the pathogenesis of type 1 diabetes. Furthermore, factors that can modify the composition of the intestinal flora, e.g. analysed antibiotics, may be part of a therapeutic intervention [55]. Also in type 2 diabetes (ob/ob mice) antibiotic treatment (ampicillin and norfloxacin) resulted in a significant improvement in glucose tolerance. These animals had reduced concentration of triglycerides in the liver and LPS in the blood, as well as increased levels of glycogen in the liver and adiponectin in blood.

Prebiotics

Prebiotics are non-digestible compounds, such as inulin and fructooligosaccharides, that stimulate the growth and activity of the intestinal bacterial strains; they are also a source of energy for intestinal epithelial cells and probiotic bacteria of the gastrointestinal tract. They accelerate the growth of beneficial commensal microorganisms such as *Lactobacillus* and *Bifidobacterium*. Studies with oligofructose provided very interesting conclusions of practical value. The first study demonstrated that the oligofructose added to high-fat diet increases the levels of insulin, decreases blood glucose levels and reduces both the amount of energy obtained and weight gain. It should be noted that this was achieved by an increased concentration of incretins, intestinal hormones that influence postprandial secretion of insulin by the beta-cells of pancreatic islets, and thus are indirectly involved in the regulation of appetite and body weight [56, 57]. Another study found a positive correlation between *Bifidobacterium* and improved glucose tolerance, insulin secretion in

response to increased blood glucose levels and normalization of inflammatory factors [58].

Reduction of fat absorption was observed in individuals who consumed food with prebiotics [59]. It was also shown that administration of oligofructose resulted in earlier satiety after breakfast and dinner, and significantly mitigated hunger. The above-described relationships and observations seem to imply an important role of prebiotic supplementation and provide the background for further research on the use of these compounds to modify the intestinal microflora and, consequently, aid in the treatment of obesity, overweight and type 2 diabetes. In summary, prebiotics biochemically reduce the activity of the intestinal endocannabinoid system, increasing the concentration of GLP-2 that stimulates the synthesis of proteins forming the tight junction (zonula occludens 1 and 2, occludin); increase the number of *Lactobacillus* and *Bifidobacterium*; contribute to improving the functions and integrity of the intestinal barrier; contribute to reduction of endotoxaemia; decrease proinflammatory cytokines; and reduce oxidative stress [60].

Probiotics

Probiotics, i.e. live bacteria which have a beneficial effect on human health, provide a diverse, well-functioning intestinal microflora, which guarantees optimal generation of energy from food and its storage in the body.

Administration of probiotics modulates intestinal flora by increasing the number of *Bifidobacteria* and *Lactobacilli*. This results in improved function and integrity of the intestinal barrier, which translates into a reduction of internal toxemia and inflammatory response and, consequently, improvement in insulin sensitivity and glucose or lipid metabolism [61]. Effect of probiotics is strain-specific and their use should be preceded by relevant clinical trials. The most metabolically efficient bacterial species include: *Lactobacillus salivarius*, *Lactobacillus paracasei*, *Lactobacillus reuteri*, *Lactobacillus plantarum*, *Lactobacillus gasei*, and *Bifidobacterium lactis*. So far, there were only two randomized clinical trials published that evaluated the efficacy of probiotics in the treatment of glucose intolerance and/or diabetes [62] and visceral obesity and overweight [63]. The first study assessed the effect of administration of *Lactobacillus acidophilus* NCFM on insulin sensitivity and the response to LPS endotoxin in 45 individuals with normal glucose metabolism or with impaired glucose tolerance or type 2 diabetes [62]. This double-blind, randomized, placebo-controlled study lasted 4 weeks. Administration of 1010 CFU of probiotic

bacteria daily improved insulin sensitivity and did not affect systemic inflammatory response. The second study [63] assessed the effect of *Lactobacillus gasei* SBT2055 (1010 CFU/day) on anthropometric parameters in 87 patients with a BMI of 24.2–37.0 kg/m² and visceral obesity. The study lasted 12 weeks and met the requirements of evidence-based medicine (EBM). It was found that the administration of probiotic lowers body weight, BMI, waist circumference and hip circumference and reduces visceral fat and subcutaneous tissue. Not without significance are also the form in which probiotic bacteria are administered. The best known probiotics' carriers are dairy products (yoghurts). However, due to short expiration term and the requirement to store these products in the refrigerator, it is difficult to provide the optimum number of health-beneficial bacteria. It seems, therefore, that the optimal and safe form of administration of probiotics are freeze-dried products available in the form of capsules or sachets.

Intestinal microflora transplantation

This treatment method has gained a lot of interest of the medical community; however, due to hygienic, aesthetic and cultural aspects is less accepted by the patients. Very promising results of experimental studies on animals encouraged researchers to use intestinal microflora transplantation (IMT) in the treatment of metabolic disorders and obesity. So far, a single study demonstrated increased sensitivity to insulin in patients six weeks after IMT. The method consists of introducing the donor stool, after its homogenization or dilution, into the duodenum (in patients with metabolic diseases) or into large intestine (in *Clostridium* infections) using endoscopic technique. The centres with the greatest experience are the Centre for Digestive Diseases in Sydney (> 3000 transplantations) and the Academic Medical Centre in Amsterdam (> 200 transplantations). It should be emphasized that no serious adverse events were observed [64].

Summary

There is no doubt that intestinal bacteria play a very important role in the pathogenesis of obesity and type 2 diabetes. However, application of this knowledge in clinical practice is still insufficient. It is obvious that by modulating microflora, we can favourably affect the metabolism. There are a number of tools available, including prebiotics, probiotics and antimicrobial drugs (such as rifaximin — an antibiotic widely used in gastroenterology). Furthermore, increasingly bold and more advanced attempts of microflora transplantation also seem to be a promising treatment method.

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