

# Gut microbiota and its metabolic potential

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**Abstract. – OBJECTIVE:** The incidence of obesity and other metabolic-related diseases has been gradually increasing. Multiple genetic as well as environmental factors play a significant role in the pathogenesis of these entities. Currently, the involvement of gut microbiota in metabolic processes has been acknowledged. This paper focuses on obesity, type 2 diabetes, and nonalcoholic fatty liver disease regarding their link with microbiome structure and its function.

**MATERIALS AND METHODS:** We analyzed literature available in PubMed, Embase, and Google Scholar databases regarding a linkage of metabolic-associated diseases and gut microbiota

**RESULTS:** Gut microbiota plays a significant role in host metabolism. Depending on its composition; however, it may contribute to the development of metabolic-associated diseases. In this context, not only composition of gut microbiota is important, but also its activity. Short-chain fatty acids or lipopolysaccharides are crucial metabolites involved in maintaining metabolic balance.

**CONCLUSIONS:** Gut microbiota malfunctions might potentially induce obesity, type 2 diabetes, and nonalcoholic fatty liver disease.

*Key Words:*

Gut microbiota, Gut dysbiosis, Obesity, Metabolic-associated diseases, Nutrition, Short-chain fatty acids.

## Introduction

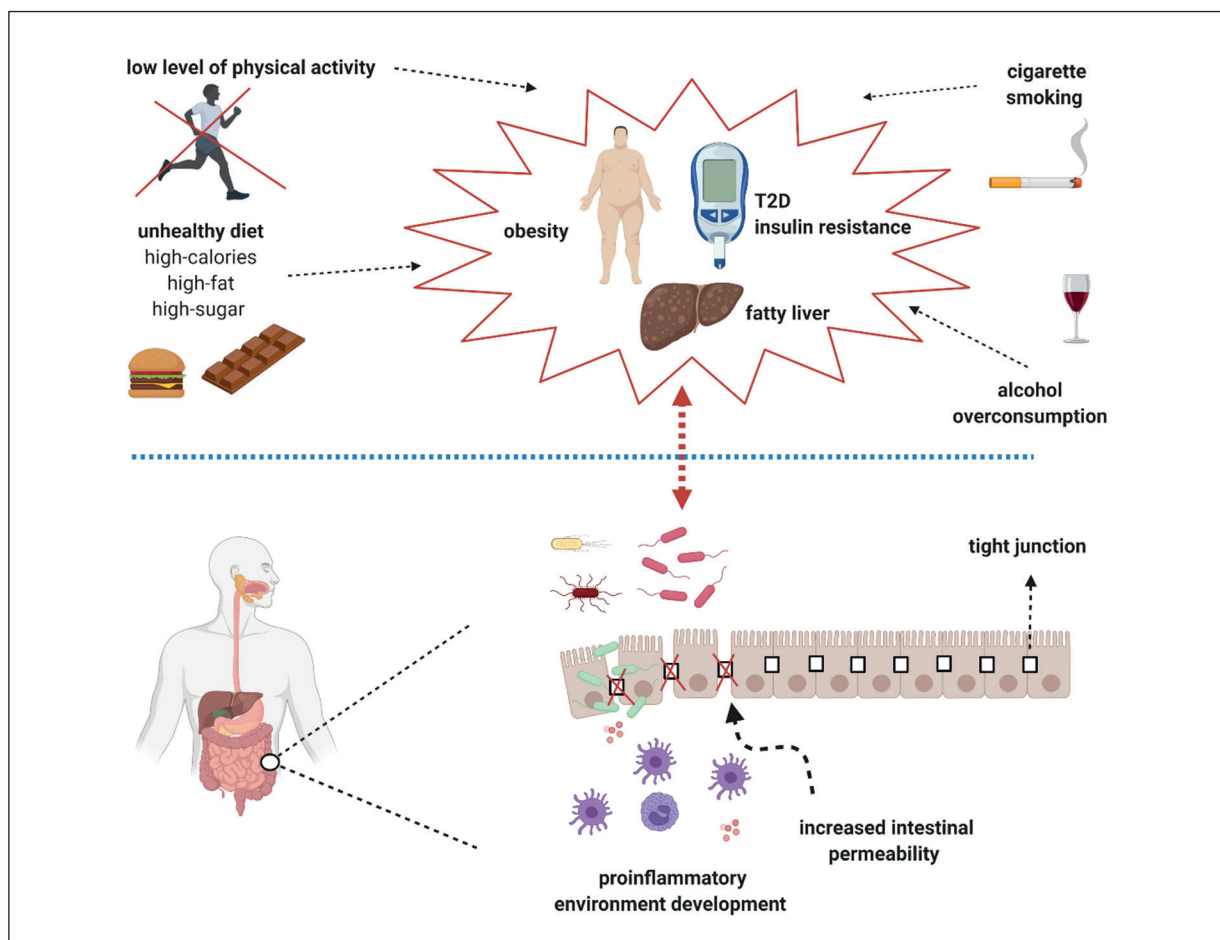
Metabolic diseases are growing problem of global public health<sup>1,2</sup>. Over the past 50 years, the frequency of obesity in modern world has grown to a pandemic<sup>3</sup>. If the upward trend continues, by 2025 there will be 18% of obese males and 21% of females worldwide. The imbalance in body composition, with increased fat con-

tent, contributes to elevated levels of free fatty acids in the blood as well as increased levels of inflammatory mediators, thus initiating the development of low-grade chronic inflammation<sup>4,5</sup>. Dyslipidemia, which is commonly observed in patients with obesity, in combination with chronic inflammation results in blood vessel dysfunction and as consequently heighten the risk of cardiovascular disease<sup>6</sup>. Chronic inflammation associated with obesity is a major contributor to the development of insulin resistance followed by type 2 diabetes (T2D)<sup>7</sup>. Effective treatment of obesity requires a systematic assessment of factors that may have potential impact on energy intake and expenditure, thus metabolism<sup>8</sup>. There is a close relationship between the gut microbiota skewed composition and development of selected metabolic-associated diseases, i.e. obesity, T2D, and non-alcoholic fatty liver disease (NAFLD) (Figure 1). It has been established that selected gut microbiota metrics, such as its diversity and abundance, might be reliable but ambiguous indicators of the gut microbiota thus host metabolism balance.

## The Activity of Gut Microbiota

The composition and activity of gut microbiota have been intensively analyzed during last several years. Analysis of the 16S rRNA gene, which dates back to the early 2000s, has largely identified multiple types of intestinal microorganisms<sup>9</sup>. Human microbiome consists of a variety of organisms, ranging from its dominant bacteria, through fungi, viruses, and *Archeae*<sup>9-12</sup>.

Notably, the maintenance of the gut microbiota balance affects the – so called -well-being<sup>13,14</sup>. Microorganisms living in the digestive tract perform multiple metabolic and immunosuppressive functions<sup>15</sup>. One of the main roles of gut microbiota is to break down undigested food and xenobiot-



**Figure 1.** The link between gut microbiota imbalance and development of selected metabolic-associated diseases. T2D – type 2 diabetes.

ics<sup>16,17</sup>. During fermentation in the large intestine, bacteria (mainly due to the metabolic activity of the Bacteroides and Firmicutes phyla) break down food-derived fiber to produce gases such as hydrogen, carbon dioxide and methane as well as short-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs). These fermentation products affect glucose and fat metabolism. The body uses up to 7-10% of its total energy from food. SCFAs also affect the proliferation of enterocytes, reduce the pH within the intestinal lumen, participate in the renewal of the intestinal epithelium (butyric acid is the main trophic mediator), stimulate the development of hepatocytes (propionic acid) and peripheral tissues (acetic acid)<sup>18-20</sup>. Moreover, SCFAs stimulate the absorption of calcium, magnesium, and iron from the large intestine<sup>18-20</sup>. Propionic acid is used in the process of hepatic gluconeogenesis and lipogenesis in adipose tissue. Additionally, acetic acid is

involved in the synthesis of cholesterol<sup>21</sup>. SCFAs also play a significant role in the gut-brain axis. However, the mechanisms by which SCFAs can affect affective processes and cognition are not fully explained<sup>22</sup>. Potential mechanisms include interaction with G-protein coupled receptors or histone deacetylases, humoral effects, hormonal and immune pathways as well as neural networks<sup>22</sup>.

### ***The Link Between Gut Microbiota and Energy Management and Fat Storage in Human Body***

The energy management mediated by intestinal bacteria is predominantly due to the content of lipopolysaccharide (LPS) and by the synthesis of SCFAs. LPS is an endotoxin of Gram-negative bacteria. Gut skewed composition in the obese has been observed in multiple studies<sup>23-26</sup>. It has been related to increased permeability of the

intestinal barrier thus loss of integrity of the gastrointestinal mucosa. In this case, LPS easily penetrates from the intestines into the bloodstream and from there to the adipocytes causing in situ inflammation<sup>27</sup>. LPS activity in adipose tissue with the participation of SCFAs, the increased amount of which is detected in obese individuals, leads to increased energy intake and excessive fat deposits<sup>28</sup>. SCFAs and LPS reduce the expression of FIAF tissue factor (Fasting-Induced Adipose Factor), stimulated by starvation, which helps to release fatty acids from triacylglycerols. Consequently, increased LPS activity in adipocytes and increased energy intake occur<sup>29</sup>.

Another negative effect of excess SCFAs and LPS action is blocking of AMPK (adenosine monophosphate activated protein kinase), which plays an important role in lipid metabolism. Blocking AMPK activity leads to a decrease in the oxidation of fatty acids in the liver and skeletal muscles<sup>30</sup>. Additionally, it has been shown that the interaction of SCFAs and LPS affect energy acquisition by activating G protein coupled receptor (GPR) 41 resulting in the secretion of YY peptide (PYY), which slows down intestinal motility, thus increases the absorption of nutrients<sup>29</sup>. SCFAs also contribute to the control of host appetite by stimulating glucagon-like peptide-1 (GLP-1) secretion and activation of the free fatty acid receptor 2 (FFAR2), which consequently affects the energy balance<sup>17,31,32</sup>. It has been also demonstrated observed that gut microbiota is involved in the synthesis of bile acids, which can affect the glucose and lipid metabolism pathways. The nuclear receptor FXR (Farnesoid X receptor) was discovered as the first of the receptors activated by bile acids. The elevated levels of triacylglycerols and glucose in mice lacking this receptor have been noted<sup>33,34</sup>. On the other hand, activation of the TGR5 (G-protein coupled receptor expressed in brown tissue) receptor present in brown adipose tissue and intestines seems to prevent insulin resistance and obesity through increased energy utilization in these tissues<sup>35</sup>.

## Gut Microbiota Imbalance in Selected Metabolic Diseases

### *Obesity*

The link between obesity and gut microbiota imbalance has been confirmed in multiple trials<sup>10,17,36-38</sup>. Pioneering studies were conducted in an animal model (gnotobiotic/germ-free) that

have never had contact with any of microorganisms earlier<sup>36</sup>. The rodents were divided into two groups: individuals from one group were transplanted with gut microbiota from genetically obese rodents, while the other received the gut microbiota from lean mice. Although both groups were fed the same diet, mice transplanted with faeces collected from obese donors had higher increase in body fat and elevated energy intake from food<sup>36</sup>. Another study was carried out comparing genetically modified obese mice lacking the leptin (*ob/ob*) gene with lean mice<sup>27</sup>. Leptin regulates appetite; therefore, it was considered as a differentiating factor<sup>39</sup>. Individuals with the *ob/ob* genotype had microbes producing enzymes that degrade undigested polysaccharides. Additionally, more fermentation products (i.e. acetic and butyric acid) and lower calories were detected in stool samples from obese mice<sup>36</sup>. Energy generated from compounds that are not digested, but fermented in the large intestine, can be 4-10% of additional energy, which is around 80-200 kcal/day<sup>40</sup>.

Other studies have shown that fermentation of SCFAs increases energy consumption by up to 150 kcal/day<sup>36,41</sup>. Therefore, gut microbiota in obese patients has significant impact on higher calorie intake from digested food, which promotes the development of overweight, and consequently leads to obesity. Additionally, the gut microbiota in mice fed with a high-fat diet converts choline from food into liver toxic trimethylamine (TMA); therefore, gut microbiota by reducing the availability of choline, may contribute to the occurrence of insulin resistance and fatty hepatitis<sup>10</sup>. Moreover, the trimethylamine oxides (TMAO) formed from these compounds are currently recognized as factors responsible for the development of atherosclerotic disease<sup>42</sup>.

Acetic acid (acetate) affects the homeostasis of the interaction between immune system and gut microbiome. Intraperitoneal injection of acetate causes loss of appetite, suggesting that it may cross the blood-brain barrier. Acetic acid accumulates in the hypothalamus, induces the activation of acetyl coenzyme A carboxylase and changes the expression profile of regulatory neuropeptides, thus causes suppression of appetite. The mechanism described above could potentially be used to treat obesity. Studies have also been conducted in mice on the difference in the composition of intestinal microorganisms in obese and lean individuals. By analyzing the 16S rRNA gene, it was noted that obese mice had

50% more bacteria belonging to the *Bacteroides* and proportionally more *Firmicutes* compared to lean individuals<sup>43</sup>. Similar results were obtained in Ley et al<sup>44</sup> study including 15 obese patients. Interestingly, Cani et al<sup>43</sup> have proven that lean subjects had more *Akkermansia muciniphila* compared to obese patients. The researcher concluded that they strengthen the intestinal epithelium and prevent the LPS molecule from entering the intestinal epithelium into the blood and thus do not cause inflammation of the adipose tissue, which leads to its growth. It was noted that mice lost weight and the serum LPS concentration was decreased<sup>47</sup>.

### **Nonalcoholic Fatty Liver Disease**

In developed countries there is a positive relationship between the obesity epidemic and the NAFLD occurrence<sup>48</sup>. NAFLD is divided into two pathological conditions: non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH)<sup>48</sup>. In NAFL, there is no damage to liver cells and inflammation; however, disturbed lipid metabolism occurs, which is a consequence of entities like insulin resistance, obesity, type 2 diabetes or dyslipidemia<sup>49-51</sup>. Gut microbiota seems to have a major impact on the spectrum of these metabolic changes. A study with germ-free mice has shown that microbiota can promote enhanced triglyceride synthesis and increased fatty acid accumulation in liver cells<sup>41</sup>. Another study has shown that gut microbiota modulation with norfloxacin and ampicillin enhanced glucose tolerance in mice. Consequently, glucose tolerance has significantly improved, TG levels and glycogen content increased in the liver, while LPS levels and adiponectin increased in the blood, which protected hepatocytes from excessive TG accumulation and increased fatty acid oxidation in the liver<sup>52</sup>. Moreover, it has been proven that adiponectin reduces body fat and improves insulin sensitivity<sup>53</sup>. Ethanol produced by intestinal microorganisms may be another cause of fatty liver formation. Most of this compound was present in the breath exhaled by ob/ob mice than by rodents with normal body weight. However, after antibiotic therapy, ob/ob mice already exhaled 50% less ethyl alcohol<sup>54</sup>. To add, metabolism of choline and fatty acids indicates the association of intestinal microorganisms with the occurrence of NAFLD<sup>34,55</sup>. A high-fat diet makes intestinal bacteria to transform choline from food into toxic to hepatocytes methylamine<sup>10</sup>. Consequently, the bioavailability of choline is reduced, which is

necessary for the production of very low-density lipoprotein (VLDL) that transports fat from the liver to adipocytes and increases the risk of NAFLD development.

NAFLD is a relatively mild entity, but in about 15-20% leads to NASH<sup>56</sup>. Inflammation and an oxidative stress, during which free radicals are formed, destroy hepatocytes and cause their necrosis<sup>57</sup>. Free radicals produce pro-inflammatory cytokines, which in turn are released by Kupffer cells and lead to liver hypersensitivity to the toxic effects of the TNF- $\alpha$  (tumour necrosis factor alpha) being produced, among others, by monocytes and macrophages<sup>53</sup>. Loss of intestinal mucosa integrity during dysbiosis increases intestinal permeability for LPS, which binds to specific receptors on Kupffer's cells in the liver and triggers a cascade of events leading to the production of proinflammatory cytokine, f.i. TNF- $\alpha$ <sup>44</sup>. After administration of polymyxin B to rats, a toxic agent to Gram-negative bacteria, plasma LPS levels decreased and the incidence of fatty hepatitis was also diminished<sup>58</sup>.

### **Type 2 Diabetes**

The pathogenesis of T2D includes genetic and environmental factors as well gut microbiota imbalance<sup>52</sup>. The concentration of endotoxins and inflammatory factors were decreased in mice that received ampicillin and neomycin<sup>45</sup>. Intravenous administration of toxic LPS to mice induced insulin resistance and initiated obesity<sup>45</sup>. On the other hand, increased levels of LPS are observed in humans who ingest high energy diet, which stimulates the growth of Gram-negative bacteria in the intestine<sup>17</sup>. It has been observed that patients with diabetes have significantly higher concentration of these compounds in blood serum compared to healthy subjects<sup>45</sup>.

The comparison of gut microbiota composition between patients with diabetes and healthy subjects revealed significant alterations. Jean-Pierre Furet et al<sup>59</sup> conducted a study in a group of 30 obese patients among whom 7 persons had T2D. The control group consisted of 13 slim people. An additional element differentiating the studied groups was the bariatric surgery on the obese by means of Roux-en-Y gastric bypass (RYGB)<sup>10</sup>. The composition of the microbiota was analyzed before bariatric surgery, 3 and 6 months after the intervention. A decrease in the number of *Faecalibacterium prausnitzii*, the main producer of butyric acid bacteria, was observed in patients with T2D. However, three months after RYGB

surgery, the number of these bacteria began to increase, and after six months their number stabilized<sup>60</sup>. After surgery, patients with diabetes had a decrease in glucose, insulin and glycated hemoglobin concentration, as well as improved their HOMA-IR (homeostasis model assessment of insulin resistance). In addition, an increase in the number of *F. prausnitzii* has contributed to a decrease in the concentration of inflammatory markers, including C-reactive protein and interleukin 6<sup>10</sup>. Zhang et al<sup>61</sup> analyzed the gut microbiota of 121 patients divided into 3 groups: patients with normal glucose tolerance, subjects with pre-diabetes (fasting glucose levels and impaired glucose tolerance) and persons with recently diagnosed diabetes. Comparative characteristics were based on the 16S rRNA gene analysis. It was noted that the numbers of *A. muciniphila* and *F. prausnitzii* were higher in healthy subjects compared to those diagnosed with prediabetes. In addition, the number of *Verrucomicrobiae* decreased in people with diabetes. Reduced colonization of the intestine with these bacteria leads to a decrease in insulin sensitivity. Therefore, the abundance of these microorganisms could potentially serve as a biomarker for the progression of glucose intolerance, and through targeted probiotic therapy as preventive agent from T2D. Another marker of T2D development may be bacteria belonging to *Proteobacteria*, which may constitute up to 85-90% of the microbiome in patients with early insulin resistance<sup>17</sup>. It was confirmed that the gut microbiota of patients with diabetes changed after faecal transplantation taken from healthy donors. The level of butyrate-producing bacteria, which improved tissue sensitivity to insulin, was increased<sup>56,62</sup>. Butyric acid has already been proven to play an important role in maintaining the integrity of the intestinal mucosa by participating in regulating the expression of genes encoding proteins that build tight junctions. An enhanced intestinal barrier integrity reduces intestinal permeability to proinflammatory agents and by limiting inflammation can be a gripping point in T2D diagnosis and therapy<sup>62</sup>.

## Conclusions

The composition and activity of gut microbiota is strongly associated with nutrition and metabolism. Gut dysbiosis is observed in metabolic-associated conditions, such as obesity, insulin resistance, type 2 diabetes, and NAFLD. However, it is

still uncertain whether gut microbiota imbalance is the cause or effect of these diseases. Modern state of art techniques including transcriptomics, proteomics and metabolomics with the latter one considered to be the closest to phenotype are of particular interests as the microbiota taxonomy differs significantly between individuals whilst the function is more stable across population. Further studies should therefore assess on how gut microbiota potency might prevent and/or treat microbial-linked entities.

## Conflict of Interest

The Authors declare that they have no conflict of interests.

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