MICROBIOTA OF HUMAN GASTROINTESTINAL TRACT

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The human intestinal microbiota contains members of all three domains which makes it one of the most complex ecosystems on earth. Gut microorganisms play very important role in functioning of human organisms. The article below is a review of literature that focuses on composition and functions of the intestinal microbiota. It also contains the results of many researches connected with correlation between changes in gut microbiota and obesity of the host organism.

Introduction

The human gastrointestinal tract (GIT) is colonized within the first days of life by microbes that contribute the digestion and immune system among others [1, 2]. It is one of the most complex ecosystems on earth and contains members of all tree domains: Archaea, Eukarya and Bacteria, which predominate in this environment [3, 4]. The vast arrays of bacterial cells in the human GIT are ten times more numerous than number of the body cells in total [5, 6]. Nine of twenty five known Bacteria types were found within the human gastrointestinal tract microbiota. Predominating are: Firmicutes (46-60%), Proteobacteria (10-30%), Bacteroidetes and Actinobacteria (8-28%) [3, 7]. Gut microbiota can be divided into two groups – residents and travelers. Residents are autochthonous components, often symbiotic with human organism while travelers are allochthonous that are result of diet and other environmental factors. Travelers compete with residents for settlement [4, 8]. However, 70% of GIT microbiota is considered to be inconstant and its abundance depends on human organism. Composition of microbiota in various parts of gastrointestinal tract differs due to variable environmental condition [7].
The majority of GIT microbiota reside in colon – their abundance reach $10^{12}$ cells/g of luminal content [5]. Population of upper parts of the human gastrointestinal tract (mouth, esophagus, stomach) is less differential and numerous. Mainly due to leaching of substantial part of bacterial by the flow of gastrointestinal reflux and secretory activity of the stomach, duodenum, liver and salivary [9, 10].

**Mouth, stomach and duodenum microbiota**

Human mouth is resided by members of 9 Bacteria types, 1 Archaea type and is considered to be the major population of upper GIT ($10^8$ cfu/g) [10]. The growth of large microbial population is favored here by diversity of surface (with different physicochemical and biological characteristics), a relatively constant temperature (35-37°C), neutral pH and the presence of a substance required for bacterial growth [8]. In addition, the availability of oxygen varied in different parts of the mouth which allows the growth of aerobic and anaerobic bacteria [10] – ratio of anaerobic to aerobic bacteria is 10:1 [8]. 700 bacterial species were isolated from human mouth. Majority of them belong to *Streptococcus* sp. (type *Firmicutes*) which are characterized with the ability of adherence to various surfaces such as teeth, oral mucosa or tongue. This feature facilitate his existence in this environment. Other bacterial species isolated from mouth belong to: *Peptococcus, Staphyloccocus, Bifidobacterium, Lactobacillus* and *Fusobacterium* [10]. In addition to bacteria, the oral cavity is colonized by yeasts such as *Candida albicans*, which, during treatment with antibiotics, or in immunocompromised people can cause thrush, and even penetrate into the body, causing systemic candidiasis [8, 10]. The microbiota of the esophagus is transitory. This means that mainly consists of the same bacteria.
as those found in the mouth – mostly *Streptococcus sp.*, *Lactococcus sp.* and *Peptococcus sp.* [10]. However, the diversity is smaller and the total number of bacterial species that reside esophagus is 95 (belonging to 6 Bacteria types) [7].

As it was mentioned, the secretory activity of the stomach and duodenum affects significantly the number of microorganisms in these parts of the gastrointestinal tract. Similar effect is shown by low gastric pH. In both stomach and duodenum, the abundance of microorganisms is less than $10^4$ cfu/g. *Helicobacter pylori*, *Lactobacillus* sp., *Veillonella* sp. and *Clostridium* sp., as the microorganisms colonizing acidic environment, are the main residents of the stomach and duodenum [9, 10, 11]. Moreover, 128 bacterial species (belonging to 8 Bacteria types) and few yeast species, such as *Candida albicans*, were isolated from these parts of gastrointestinal tract [11].

**Intestinal microbiota**

Intestinal microbiota includes microorganisms colonizing such parts of gastrointestinal tract as the small intestine (jejunum and ileum), colon and rectum. Microbiota of these parts of the digestive system is much more diverse and larger than the population of upper parts of GIT. This complex ecosystem consists of 17 families, 45 genera and over 1,000 species of microorganisms [1]. Slower movement of gastric contents and significantly increased pH (gastric pH is 1.0-2.0 unit while in the mucosa of the small intestine may be even 8.0) positively affect the development of diverse biocenosis [8]. Majority of gut bacteria is anaerobic (facultative or obligate), however in the intestinal mucus environment favorable for growth of microaerophiles exists [7].

In the adult human gut microbiota *Bifidobacterium*, *Bacteroides*, *Clostridium* and *Eubacterium* dominate, less from the genus *Lactobacillus*, *Escherichia*, *Enterobacter*, *Streptococcus* or *Klebsiella*. In the case of breast-fed infants 60-90% of the bacteria belong to genus *Bifidobacterium*, less to *Bacteroides* and *Lactobacillus* (below 1%). Predominant bacterial species are only 30% of microorganisms present in the gut of every human being, the remaining 70% are unique micro-organisms [7]. In addition to bacteria, the second fairly large group are viruses. From the human fecal 1200 viral genotypes were isolated and their number reaches up to $10^9$ virions per gram of dry weight [12].

Microbiota of the jejunum resembles the microbiota of the duodenum. There are bacteria of the genera *Bacteroides*, *Lactobacillus* and *Streptococcus* and the yeast *Candida albicans*. However, the abundance is higher and is up to $10^7$ cfu/g [3]. Similarly is in the ileum, although except from *Bacteroides* and *Lactobacillus*, bacteria belonging to genus *Clostridium*, *Enterococcus*, and *Veillonella*, and the family *Enterobacteriaceae* are also predominant [7].

The population of microorganisms of colon and rectum is the biggest in number [7]. In the large intestine, abundance of microorganisms is $10^{11}$ cfu/g, while in rectum can reach even $10^{12}$ cfu/g, which represents approximately 30% of
its content [8]. Large intestine is inhabited by 800 species belonging to 9 Bacteria
types and one type of Archaea. Among 9 Bacteria types, 2 types predominate –
**Firmicutes** (46-60%) and **Bacteroidetes** (with **Actinobacteria** 8-28%) [3, 8]. More
than 270 species out of 800 that can be found in colon, belong to those 2 Bacteria
types. Both **Firmicutes** and **Bacteroidetes**, characterize in high fermentation
activity. Main representatives of type **Firmicutes** in colon belong to classes **Bacilli**
and **Clostridia**. The first produces lactic acid and acetic acid as a result of the
saccharide fermentation. The latter (represented mostly by bacteria of the genus **Clostridium** and **Eubacterium**) characterizes in proteolytic and saccharolytic
ability, that results in production of organic acids (such as butyric, lactic, acetic,
formic). The fermentation activity of bacteria belonging to **Bacteroidetes** type is
also very strong. These bacteria (mainly the genus **Bacteroides**) produce many
organic acids such as acetic, succinic, lactic, formic, propionic, and in smaller
quantities acids: butyric, isobutyric and isovaleric [3].

As previously mentioned, the neonatal gastrointestinal tract is sterile, and its
colonization occurs within the first few days of life [1, 2]. The formation of the
intestinal microbiota of infant is combined with maternal vaginal microbiota, but
only within the first day after birth [4]. Intestinal microflora of children born by
Caesarean section will therefore differ from the microflora of children born
vaginally [1]. However, the research team of Palmer [6] showed in their studies
that there is no correlation between breast milk microbiota and the intestinal
microflora of the child, and the previous reports confirming this relationship may
have resulted from contamination of milk with the microorganisms occurring on
the skin of the mother [4, 6]. External factors affecting the development of the
human intestinal microbial group include antibiotic therapy and diet (eg. vegetarian).
Studies conducted in 2002 by a team of Hayashi, Sakamoto and Benno [13] have
shown that the permanent elimination of meat from the daily diet may lead to
greater variety of bacteria of the genus **Clostridium**. Several species of **Clostridium**
(eg. **Clostridium ramnosum**), which do not occur in the digestive system of people
using a traditional diet, were isolated from the stool of vegetarians. What is more,
**Fusobacterium prausnitzii** – commonly occurring bacteria in the colon of non-
limiting consumption of meat – was not isolated from the faeces vegetarians [13].
Significant differences in the qualitative and quantitative composition of intestinal
microbiota can also be seen in people in different age groups. Digestive system of
children is inhabited by larger amounts of bacteria **Bifidobacterium** and **Clostridium**
than gastrointestinal tract of adults. Moreover, the intestinal microbiota of children
is much less complex [14].

Recent studies [15], published at the beginning of March 2010 reported that
the intestinal microbiota of mothers who took on much weight during pregnancy is
richer in **Escherichia coli** and other bacteria from family **Enterobacteriaceae** and
bacteria of the genus **Staphylococcus**, and fewer bacteria from genus **Bifidobacterium**
and **Bacteroides**. Bacteria of the genus **Bacteroides** are associated with higher
levels of HDL-cholesterol and folic acid, whereas **Bifidobacterium** is associated
with increased levels of vitamin B [15].
Functions of the intestinal microbiota

The intestinal microbiota has three functions: metabolic, trophic and protective [1, 8]. The most important role is to build resistance to infection (protective function) by increasing the activity of the immune system and creating a natural barrier against colonization by exogenous pathogenic bacteria [2, 3, 7]. This barrier is based on the competition (the living space and nutrients) by the production of bacteriocins and organic acids, which by lowering the pH inhibit the growth of pathogenic microorganisms. The protective function has been confirmed by tests carried out on animals with a sterile digestive tract (germfree), which were more susceptible to infection [1, 7, 16]. Moreover, they suffered from reduced vasculature bodies, lower activities of digestive enzymes and lower levels of epithelial lymphocytes [16].

Table 1

Fermentable substrates that reach the human colon [19]

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Component</th>
<th>Amount (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>Resistant starch</td>
<td>5-35</td>
</tr>
<tr>
<td></td>
<td>Non-digestible polysaccharides</td>
<td>10-25</td>
</tr>
<tr>
<td></td>
<td>Oligosaccharides (FOS, GOS, inulin)</td>
<td>2-8</td>
</tr>
<tr>
<td></td>
<td>Monosaccharides (sugar alcohols)</td>
<td>2-5</td>
</tr>
<tr>
<td></td>
<td>Mucins</td>
<td>3-5</td>
</tr>
<tr>
<td></td>
<td>Synthetic carbohydrates (lactulose, polydextrose, modified cellulose)</td>
<td>Variable</td>
</tr>
<tr>
<td>Proteins</td>
<td>Of dietary origin</td>
<td>1-12</td>
</tr>
<tr>
<td></td>
<td>Of endogenous origin (pancreatic enzymes and other secretions)</td>
<td>4-8</td>
</tr>
<tr>
<td></td>
<td>Desquamated epithelial cells</td>
<td>30-50</td>
</tr>
<tr>
<td>Others</td>
<td>Non-protein nitrogen (urea, nitrate)</td>
<td>~0.5</td>
</tr>
<tr>
<td></td>
<td>Organic acids, lipids, bacterial recycling</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

Inoculation of germfree mice intestine with only one species of bacteria (*Bacteroides thetaiotaomicron*) already had affected positively metabolism, angiogenesis, protective barrier function and the development of the nervous system [17].

Secondly, intestinal microorganisms have a beneficial effect on metabolic activity of the organism [1, 3, 8]. They are important in proper functioning of the whole organism by carrying out fermentation of undigested debris (such as "resistant" starch among others) in the large intestine (Tab.1). The metabolic activity leads to the acquisition of energy and absorbable substrates for the host organism and to provide energy and nutrients needed for growth of bacteria. Fermentation of saccharides is the main source of energy for intestinal epithelial cells [1]. The products of metabolism are short chain fatty acids (SCFA) (Fig. 2). SCFA stimulate cell proliferation, differentiation of intestinal enterocytes, affect
mineral balance and regulate the metabolism of glucose and lipids [7]. Many tissues in the human body is characterized by the ability to SCFA oxidation to obtain energy [18]. Intestinal microorganisms also take part in the synthesis of vitamin and absorption of calcium, magnesium and iron [1].

![Diagram](image)

**Fig. 2. Metabolism of saccharides in human colon [3]**

Intestinal microorganisms have also trophic function. Produced in the process of the saccharides fermentation, SCFA stimulate proliferation and differentiation of intestinal epithelial cells thereby ensuring the control of continuity of the small and large intestine epithelium [1, 8]. Studies conducted on rats have shown that cells of the small intestine epithelium of germfree rats were less numerous than cells in rats with conventional intestinal microbiota [20]. The intestinal microorganisms have also beneficial effect on immune homeostasis (constancy of internal environment of human body) [8].

Not all microorganisms colonizing the gastrointestinal tract contributes positively to the health of the host. Pathogens and microorganisms producing toxins can be also found there. They have harmful impact on host organism. Pathogens resident in human intestine include some species of the genus *Enterococcus*, *Streptococcus* and *Escherichia coli* strains, which naturally exists in the human gastrointestinal tract, however can have a negative impact when they dominant in this environment. Changes in the composition of intestinal microbiota can be caused by many factors, which include: gastrointestinal surgery, lesions of the colon, kidney and liver cancers, impaired immune system, antibiotic treatment and radiological, aging, poor diet and stress. Such an excessive multiplication of certain microorganisms
can lead to food poisoning, diarrhea (e.g., enterotoxigenic strains of *Escherichia coli* can cause the so-called ‘Travellers diarrhea’), and the spread of microorganisms outside the gastrointestinal tract (most commonly *Escherichia*, *Klebsiella* and *Proteus*) [1, 7].

Serious threat to the health of the host is not only excessive multiplication of microorganisms, but also their translocation from the gastrointestinal tract into other parts of the body. The displacement can be caused by dysfunction of intestinal mucosal barrier. Microorganisms can enter the lymph nodes, liver or spleen and cause sepsis, shock, organ failure and even death of the human [21].

Intestinal microorganisms substantially may be responsible for tumors in the kidneys, liver, ovary, and gastrointestinal tract (especially the colon). A diet rich in fats and high intakes of red meat leads to changes in the composition of intestinal microorganisms, and thus even to the tumor [22]. Studies on germfree rats (with a sterile digestive tract) have shown that they have lower levels of carcinogens in the tissues than the rats with a standard intestinal microbiota. In both cases, rats were fed with food that people eats. Intestinal microorganisms may play an important role in neoplasia through the production and activation of carcinogens and genotoxic compounds [7, 23].

Changes in human intestinal microbiota may be caused by diseases. Number of bacteria of the genus *Bifidobacterium* and *Lactobacillus* is significantly reduced in the case of faecal pouchitis, while the number of *Clostridium* sp. increases. This causes the reduction of the concentrations of protein metabolism products and increase of the pH of intestine content. In the case of ulcerative colitis also the number of *Bifidobacterium* sp. is reduced, and the number of *Enterobacteriaceae* increases. Such changes induce abnormal immune response to external antigens (allergens and pathogens), and even their own intestinal bacteria [7].

**Metabolic syndrome**

Metabolic syndrome, also known as insulin resistance syndrome, is a set of interrelated factors leading to the atherosclerosis, type 2 diabetes and vascular complications [24]. The most important anomaly to detect the presence of metabolic syndrome is abdominal obesity (waist circumference in women > 89 cm, men > 102 cm) that occurs along with two of the deviations: hypertension, low HDL cholesterol, and glucose hypertriglyceridemia [24, 25]. Insulin resistance syndrome may have both, genetic background and be caused by a ‘sedentary’ lifestyle and caloric and unvaried diet. The risk of the metabolic syndrome increases with age. This disease affects less than 10% of people 20 years of age and 40% of people over 60 years of age, but its syndromes may also be detected in early childhood. It was also found that Hispanics and Asians are at higher risk of developing metabolic syndrome [26].
Obesity and microbiota

For many years, several groups of scientists focused their research on the relation between the composition of intestinal microbiota and obesity. As the first, in 2004, Dr. Jeff Gordon of the University of St. Louis suggested that intestinal microorganisms may play a role in the controlling of body weight [3, 27, 28]. According to him, some bacteria are able to generate energy more effectively than others, so people with microbiota more efficient in the mobilization of energy absorbs more calories and gain weight more easily, and hence are vulnerable to obesity [27, 29].

Two research groups: Backhed et al [30] and Ley et al [27] demonstrated that the share of the type of Bacteroidetes bacteria is higher in individuals with normal weight. The first team has observed this phenomenon in mice, in which the percentage of these bacteria in lean subjects was 40% (Fig. 3) [3, 30]. The second team focused their research on a group of people (here Bacteroidetes share in subjects with normal weight was 20%) [3, 27].

![Fig. 3. The percentage of Bacteroidetes bacteria in lean and obese mice [30]](image)

Ley et al [27] by examining two groups of obese people who received a diet with limited fat (FAT-R group) and saccharides (group CARB-R) not only observed a reduction in body weight of the latter by up to 12 kg during the year, but also that during the diet the share of type Bacteroidetes increased and Firmicutes decreased (Fig. 4).

Ratio of the correlation between the increase in the number of Bacteroidetes and the percentage of weight loss was higher in group CARB-R [27]. In obese people the percentage of Firmicutes is greater than in lean, and thus, their intestinal microbiota is characterized by a higher fermentation activity and better efficiency of digestion of food intake [3].
Fig. 4. Changes in the percentage of Firmicutes and Bacteroides bacteria during diet [27]

Greater share of Firmicutes in mice genetically modified in the direction of obesity was also confirmed by Backheda et al [30]. They found that the population of intestinal bacteria in such mice have more genes encoding enzymes causing better utilization of undigested fiber. Increased efficiency of metabolism of intestinal microbiota of obese mice was also confirmed by monitoring food and calorie intake of faecal and metagenomic and biochemical analysis of feaces. This shows that the bacteria of the type Bacteroidetes may affect the metabolic potential of intestinal microorganisms. The last stage of this study was to analyze mice with a sterile gut – germfree. These mice were very lean and only inoculation of microbiota into their digestive gut caused the weight gain. The explanation of the phenomenon was that intestinal microbes may influence a signal involved in the regulation of body weight on the molecular level. As evidence, the impact of the starvation diet on adipose factor (FIAF – protein, whose concentration increases during starvation), whose expression was inhibited by intestinal microbiota was studied. Lack of expression of this protein contributes to the higher activity of lipoprotein lipase and increased triglyceride accumulation in adipocytes and, thus, to increase body weight. Lack of intestinal microbiota is equivalent to an unlimited expression of FIAF [29, 30].

So far, all research showed that the composition of the gut microbiota changes with a change in body weight. However, recent reports confirm that intestinal microorganisms affects the metabolic syndrome. Aim of research led by Vijay-Kumar et al [31] were mice with the genome lacking the gene coding the Toll-like receptor 5 (TLR5). This receptor is a protein comprising the innate immune system, whose expression occurs in the intestinal mucosa and is involved in protecting the body against infections. Mice lacking the receptor TLR5 (T5KO) underwent the 12-week antibiotics treatment, which resulted in reduced numbers of intestinal microorganisms by 90%. Then the remaining microbiota was transplanted into germfree wild-type mice intestine. After some time symptoms of metabolic
syndrome occurred, namely obesity (Fig. 5), hypertension, insulin resistance and hipertriglicerymedia [31].

These studies suggest that one of the ways of preventing and treating obesity can be control and administration of the composition of intestinal microbiota.

**Conclusions**

Intestinal microorganisms play an important role in building resilience and metabolism of the human body. It has been proved that they affect the development of metabolic syndrome. Bacteria of the type *Firmicutes* show strong metabolic activity, which may significantly affects obesity, so it is important to analyze carefully the qualitative and quantitative composition of bacterial populations of this type inhabiting the intestine of healthy and lean, as well as people suffering from metabolic syndrome, mainly obese. In this way, the microbial background metabolic syndrome can be specified, and reflect on the prevention and treatment of this disease.

**References**


Mikrobiota przewodu pokarmowego człowieka

Streszczenie

Zespół mikroorganizmów zasiedlających układ pokarmowy człowieka zasiedlany jest przez przedstawicieli wszystkich trzech domen życia i tym samym zaliczany do jednych z najlepiej rozwiniętych ekosystemów na ziemi. Drobnoустroje układu pokarmowego pełnią bardzo ważne funkcje. Powyższy tekst przedstawia przegląd literatury dotyczącej składu i funkcji tej mikroflory, jak również zawiera wyniki badań skupiających się na związku między zmianami w jej składzie a otyłością gospodarza.