Chapter 12

PROBIOTIC USE IN OBESITY AND METABOLIC SYNDROME

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ABSTRACT

Today, obesity is becoming endemic; around 1.7 billion people across the planet are overweight. The World Health Organization has declared obesity a global epidemic and taken it under control. Obesity causes a number of diseases, namely, cardiovascular diseases, type 2 diabetes, dyslipidemia, premature death, hepatobiliary disease (non-alcoholic fatty liver disease, gallbladder dyskinesia, cholelithiasis) and number of tumor sites, including lung cancer, breast cancer, uterine cancer and ovarian cancer. The constantly increasing cohort of patients with obesity-related diseases demands an urgent change of paradigm from interventional measures to predictive, preventive, and personalized medicine.

Childhood obesity is a predisposing factor for chronic diseases both in the adolescent and into adulthood. Current research efforts have focused on host and environmental factors that may affect energy balance. This has led to a plausible, biological postulation that an obese microbiota profile may exist and may demonstrate increased energy yielding behaviour by such bacteria. Consequently, the gut microbiota is gaining significant research interest in relation to obesity in an attempt to better understand the aetiology of obesity and potentially new methods of its prevention and treatment.

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Several studies have revealed the positive effects of probiotics use under the conditions of experimental obesity. In our work we have shown that probiotics mixture of lyophilized strains *Lactobacillus* (*L.* 3.2×10^{10} CFU/kg) and *Bifidobacterium* (*B.* ) at least partially prevent the MSG-induced obesity in rats. However, studies have shown that multistrain alive probiotics are more effective than monostrain probiotics. Also, it is interesting to compare the influence of the lyophilized and alive strains on obesity and reveal the gender-specific differences in obesity development. We studied the effectiveness of short-term periodic consumption of multiprobiotic from childhood on metabolic profile in adult rats with a monosodium glutamate (MSG)-induced model of obesity. Daily oral administration of 140 mg/kg (1.4×10^{10} CFU/kg) of alive multiprobiotic “Symbiter” containing concentrated biomass of 14 probiotic bacteria of *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, and *Propionibacterium* genera to neonatal MSG-treated rats by 2-weeks courses led to significant reduction of total body and VAT weight, together with improvement in insulin sensitivity and prevention of NAFLD development.

We have confirmed these experimental data in clinical studies. We demonstrated that administration of probiotics leads to statistically significant reduction of proinflammatory cytokines (IL-6, IL-8, TNF-α, IL-1β, IFN-γ) in patients with type 2 diabetes and NAFLD.

In this chapter we discuss connections between gut microbiota, energy homeostasis, and inflammation and its role in the pathogenesis of obesity-related disorders and possible ways of their prevention.

**Keywords:** obesity, probiotic, multistrain, monostrain, prevention, gut microbiota, intestinal permeability, innate immunity, metabolic inflammation, endocannabinoid system, bile acid metabolism, Farnesoid X-Receptor (FXR), short-chain fatty acids, Toll Like Receptors (TLRs), Fasting-induced adipose factor (FIAF)

### INTRODUCTION

Today, obesity is becoming endemic. Approximately 1.7 billion people on the planet are overweight. Approximately 20 million of children under 8 years of age have problems with excess weight. World Health Organization has declared obesity a global epidemic and taken steps to bring it under control. The obesity epidemic is recognized to be a result of changes in energy intake and/or energy expenditure that have led to energy imbalance in a large portion of the population [1].

Environmental changes lead to obesity through behaviors related to energy intake and expenditure; thus, researchers have turned their attention to determinants of eating patterns, physical activity, and sedentary behaviors. However, these may differ by population group, including gender and age groups. Different dietary regimens can affect body weight, e.g., increased fruit and vegetable intake results in greater reduction of weight than limited intake of high-fat, low-nutrient dense foods with controlled physical activity [2]. Environmental factors such as food availability on the one hand, and psychosocial factors, including eating traditions and beliefs regarding food taste and health impact on the other, may also contribute to the country-specific eating patterns responsible for lower or higher risk of developing obesity in a particular population. Apart from traditional patterns, healthy or unhealthy eating practices as well as level of individuals’ physical activity could be affected by policy-related factors, such as promotion and availability of high-energy food or implementation of
population-based programs aimed to change lifestyle. Social and environmental factors considered at four levels – international, national/regional, community/locality, and work/school/home – might have a more profound effect on body weight status than do individuals’ characteristics [3, 4]. Identification of such factors is necessary in order to inform healthy public policies.

Childhood obesity is a major problem across the world. The United States takes first place in the global rankings for prevalence of obesity. Childhood obesity in the US has more than doubled in children and quadrupled in adolescents in the past 30 years [5]. The percentage of children aged 6-11 years in the US classed as obese increased from 7% in 1980 to nearly 18% in 2012. Similarly, the percentage of adolescents aged 12-19 years classed as obese increased from 5% to nearly 21% over the same period [6]. In the US, the main factors contributing to this weight gain are the widespread network of fast food restaurants and hypodynamic.

The increase in overweight is particularly significant among African-American and Latin American children, and especially among Mexican school-age children, among whom the prevalence of obesity has increased over recent years and now affects approximately one out of every three school-age children [7, 8]. In Mexico, the “obesogenic environment” is also a prevalent characteristic of schools. A number of studies conducted in elementary schools indicate that school-age children have opportunities for food consumption as many as five times within a 4.5-hour period during school hours, which may be equivalent to 50% of the total daily requirements (840-1259 kcal). In addition, while there is a high availability of energy-dense products, access to both systematic and recreational physical activity is limited [9].

Being overweight is in danger of becoming the new norm for children as well as adults in Europe, the World Health Organization warns, issuing figures showing that up to a third of 11-year-olds across the region are too heavy. Over half of adults living in the European Union countries are now overweight or obese. The rate of obesity has more than doubled over the past 20 years in most EU member states, international experts say. The UK comes out worst, closely followed by Ireland and Malta, where a quarter of the population is obese. The European Commission and the Organisation for Economic Co-operation and Development (OECD), which compiled the Health at a Glance: Europe 2010 report, believe the key to success is encouraging children to adopt healthy habits. Currently, one in seven children in the EU is overweight or obese, and the figures are set to rise even further.

According to a recent report published by the WHO Regional Office for Europe, being overweight is so common that it risks becoming a new norm in the WHO European Region [10]. For example, up to 27% of 13-year-olds and 33% of 11-year olds are overweight. “Our perception of what is normal has shifted; being overweight is now more common than unusual. We must not let another generation grow up with obesity as the new norm,” said the WHO Regional Director for Europe, Zsuzsanna Jakab. “Physical inactivity – coupled with a culture that promotes cheap, convenient foods high in fats, salt and sugars – is deadly.”

The country profiles compiled by the Regional Office give a bleak picture of nutrition, obesity and physical inactivity in the European Region’s 53 Member States [10]. The profiles were launched at a conference, Physical Inactivity: Part of the Problem, in Athens, Greece, at the opening event for the Greek Presidency of the European Union (EU). Among 11-year-old boys and girls, the prevalence of overweight was highest in Greece (33%), Portugal (32%), Ireland (30%), and Spain (30%), and lowest in the Netherlands (13%) and Switzerland (11%).

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In 23 out of 36 countries, more than 30% of boys and girls aged 15 years and over are not getting enough physical activity. Among adults, women’s rates of insufficient physical activity range from 16% in Greece and 17% in Estonia to 71% in Malta and 76% in Serbia. “We need to create environments where physical activity is encouraged and the healthy food choice is the default choice, regardless of social group. Physical activity and healthy food choices should be taken very seriously in all environments – schools, hospitals, cities, towns and workplaces. As well as the food industry, the urban planning sector can make a difference,” said Joao Breda, Programme Manager, Nutrition, Physical Activity and Obesity at the Regional Office [11].

In Ukraine, no figures are available for the prevalence of overweight and obesity in schoolchildren (children 0-9 years) based on measured intercountry comparable data. Ukraine is not yet participating in the WHO European Childhood Obesity Surveillance Initiative (COSI). In terms of the prevalence of overweight and obesity in adolescents, up to 22% of boys and 12% of girls among 11-year-olds were overweight, according to data from the Health Behaviour in School-aged Children (HBSC) survey (2009/2010). Among 13-year-olds, the corresponding figures were 21% for boys and 9% for girls, and among 15-year-olds, 17% and 8%, respectively [12].

What are countries doing right in terms of efforts to stem the epidemic of obesity? Some countries have managed to contain the epidemic. France and some Scandinavian countries are at least keeping it at a stable level. These countries have implemented policies through a whole-of-government approach and intersectoral initiatives in line with Health 2020, the WHO policy framework for health. The palette of actions includes the promotion of vegetable and fruit consumption in school, along with school lunch initiatives, taxes on foods to reduce intake, tighter controls of advertising, robust systems for surveillance and monitoring, and action to promote physical activity, especially among children. The WHO recommends the following action at different levels. National governments can enforce legislation and insist on informative labelling, nutrient profiling, and regulated marketing of food products, requiring the food industry to take responsibility. Local governments can make healthy foods available, and insist on town planning and infrastructure that encourage healthy lifestyles and make the healthy choices easy choices. At the individual level, consumers can be empowered to make informed choices through having access to adequate information [13].

Overweight and obesity cause a number of diseases, namely, cardiovascular diseases, type 2 diabetes, dyslipidemia, premature death, hepatobiliary disease (non-alcoholic fatty liver disease, gallbladder dyskinesia, cholelithiasis) and a number of tumor sites, including lung cancer, breast cancer, uterine cancer and ovarian cancer. The permanently growing cohort of patients with obesity-related diseases requires an urgent change of paradigm from interventional measures to predictive, preventive, and personalized medicine. Evidence suggests that even if excess childhood weight is lost, adults who were obese children retain an increased risk of cardiovascular problems. And although dieting can combat obesity, children who diet are at a greater risk of putting on weight following periods of dieting. Eating disorders, symptoms of stress and postponed physical development can also be products of dieting (WHO Europe, 2009).

Childhood obesity is a predisposing factor for chronic diseases, both in the adolescent and into adulthood. Children who are overweight or obese are at greater risk of poor health in adolescence and also in adulthood. Among young people, orthopedic problems and psychosocial problems, such as low self-image, depression, and impaired quality of life, can

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result from overweight. Excess weight problems in childhood are associated with an increased risk of being an obese adult, at which point cardiovascular disease, diabetes, certain forms of cancer, osteoarthritis, a reduced quality of life, and premature death become health concerns [14].

Obese children and adolescents have been observed to have a more unfavourable lipid profile than children and adolescents with normal body weight [15, 16]. Researchers have shown that obese adolescents had an abnormal "atherogenic" lipid profile consisting of elevated LDL cholesterol and TG and low HDL cholesterol compared to normal-weight children [15-17]. Although the pathophysiology underlying the development of dyslipidemia in obese children is multifactorial and not yet completely defined, insulin resistance has been hypothesized as playing a major role in the relationship between dyslipidemia and obesity [18]. Several studies have demonstrated that dyslipidemia is associated with hyperinsulinemia/insulin resistance [19, 20] and type 2 diabetes [21].

Childhood obesity, when associated with serum lipoprotein changes, triggers atherosclerosis. Evidence suggests that the atherosclerotic process begins in childhood, and that the extent of early atherosclerosis of the aorta and coronary arteries can be associated with lipoprotein levels and obesity. Furthermore, many studies in childhood demonstrate an important relationship between parameters of insulin sensitivity, body fat distribution, and the development of lipid abnormalities [22].

Pediatric nonalcoholic fatty liver disease (NAFLD) is becoming the most frequent chronic liver disease in obese children and adolescents. A growing body of evidence from epidemiologic studies in both adults and children has established NAFLD as an independent predictor for development of metabolic syndrome, type 2 diabetes (T2D), and cardiovascular disease [23, 24]. Similarly to adults, children with NAFLD have a higher prevalence of atherosclerosis when compared to control subjects [25, 26].

NORMAL FLORA: ROLE AND FUNCTIONS

The human body is not only a complex group of organs and systems, but also contains more than 500 different species of microorganisms that accompany the human being from birth to death [27]. The human biological entity is a stable symbiosis of two equal autonomous systems: the macroorganism (host) and a system of symbiotic microorganisms which are evolutionarily adapted to life in relatively opened human organs on the basis of mutually beneficial relations [28, 29]. During phylogenesis the symbiosis of the human being and microflora steadily improved, resulting in the transformation of host microbiota into a kind of vital regulatory body [30], consisting of a large number of microbial cells, 1-3 times higher in number than the number of the host's own human cells [31, 32, 33]. This "organ" performs a wide range of functions, and its disorder provokes the loss of the human body's vitality. Microorganisms that are constant in healthy people belong to the normal microbiota, regarded as a set of populations of microbes in individual organs and systems in a certain qualitative and quantitative ratio, supporting the biochemical, metabolic, and immunological balance of the host organism that is necessary to maintain health [34].

The human microbiota includes hundreds of different species with a total number of cells over $10^{11} - 10^{13}$. Moreover, the species composition of the microorganisms depends on the
inhabited organs [35]. The largest number of microorganisms exists in the habitats of the digestive tract. Each part of the digestive system is characterized by a different composition of microbial flora (Table 1).

The largest variety of microorganisms is found in the colon [31, 36]. The dominant species of obligate microflora are asporogenous gram-positive and gram-negative saccharolytic anaerobes: *Bifidobacterium*, *Lactobacillus*, *Propionibacterium*, and *Bacteroides*. *Bifidobacteria* and *Bacteroides* compose 85-98% of intestinal microflora [37].

Analysis of the literature reveals the four basic physiological functions of normal microflora of the human body, proven by a number of researches: 1) immune defense (colonization resistance) [38-42]; 2) biosynthetic [43]; 3) digestive [44]; 4) detoxification [45].

Implementation of colonization resistance occurs through the synthesis of antimicrobial metabolites, hydrogen peroxide, bactericins, lysozyme, and short-chain fatty acids (SCFA) providing antagonistic activity against pathogens [46]. Some bacterial strains, such as *Akkermansia muciniphila*, enhance mucosal defense against pathogenic microorganisms increasing mucin production and secretion of antimicrobial peptide regenerating islet-derived 3-γ (RegIII-γ). This substance is significantly decreased due to the high growth rate symbiotic bacteria effectively competing for food and adhesion sites.

**Table 1. The content of microflora in the different part of human digestive tract**

<table>
<thead>
<tr>
<th>The habitats of digestive tract</th>
<th>The number of microorganism cells per 1 g of content</th>
<th>Lumen microflora</th>
<th>Surface microflora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouth</td>
<td>10^8-10^9</td>
<td>10^11-10^12</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>10^2-10^3</td>
<td>10^5</td>
<td></td>
</tr>
<tr>
<td>Proximal small intestine</td>
<td>10^3-10^4</td>
<td>10^11-10^14</td>
<td></td>
</tr>
<tr>
<td>Distal small intestine</td>
<td>10^5-10^10</td>
<td>10^10-10^12</td>
<td></td>
</tr>
<tr>
<td>Colon</td>
<td>10^11-10^12</td>
<td>10^10-10^12</td>
<td></td>
</tr>
</tbody>
</table>

Another mechanism of colonization resistance is immune-modulatory effect and increase of the immune system activity of the host by microflora [38, 47]. In particular, it has been established that some strains of lactobacilli and bifidobacteria have the capacity to increase phagocytic activity of macrophages, natural killer cells, to regulate the synthesis of interleukin (IL), tumor necrosis factor α (TNF-α), and interferon (IFN), to increase production of immunoglobulins (Ig), and stimulate the reaction T cellular immunity [48]. The host symbiotic bacteria realize the effect on the immune system through the interaction between their pathogen-associated microbial patterns (PAMP) (including lipopolysaccharide (LPS) and lipoteichoic acids (LTK) of cell walls of bacteria) and specific toll-like receptors (TLR) of epithelial and dendritic cells (DC) of the digestive tract [49, 50] [51]. Bacterial cells are recognized by the host in three ways: interaction with TLR on DC projection on the surface of the mucosa; interaction with TLR on DC in the subepithelial layer after the translocation of bacteria through M cells in lymph plaques without degradation; enterocyte way through
binding to enterocyte receptor and subsequent PAMP presentation to DC. Binding of PAMP leads to connection of the adaptor protein, the myeloid differentiation primary response gene (MYD88), to TLR. Another domain of this protein interacts with the interleukin 1 receptor associated kinases (IRAK): IRAK1 and IRAK4. IRAK4 phosphorlates IRAK1, which allows the joining of another adaptor protein, TNF receptor-associated factor 6 (TRAF6). TRAF6 is associated with mitogen-activated protein kinase (MAP2K), transforming growth factor (TGF)-β-activated kinase (TAK) 1, TAK-binding protein 1 (TAB1), or NF-κB-inducing kinase (NIK). As a result, the phosphorylation and activation of IκB kinase (IKK), the phosphorlates inhibitor of NF-κB IκB, takes place. This provides the release of NF-κB, which migrates to the nucleus and triggers the transcription of various cytokines, chemokines, adhesion molecules, and acute phase proteins, for instance IL-1β, IL-6, and IL-8. In most cells, the activation of NF-κB inhibits apoptosis [50]. In addition, TLR stimulation by LPS can cause the activation of the MyD88-independent signaling pathway, which leads to early induction of IFN-β, as well as activation of IFN-induced genes such as iNOS [50].

It should be noted that commensal bacteria generally do not cause inflammation through hyperactivation of the immune system. This is due, firstly, to lack of PAMP produced by microflora, and secondly, to normal expression of TLR3 and TLR5 by enterocytes and poor expression of TLR2 and TLR4 in the normal state. Lack of TLR2 and TLR4 is a possible explanation for the insensitivity of intestinal cells to LPS of commensal bacteria, but the presence of TLR3 and TLR5 causes sensitivity of the epithelium to infection mediated by flagellated bacteria or components of enteropathogenic bacteria [52, 53]. In support of this, Furrie et al. showed that TLR2 and TLR4 expression was observed only in the crypts, and as epithelial cells become mature and move to the villi surface the decreased expression of these receptors was noted [54]. In addition, intestinal epithelial cells express a large quantity of peptide inhibiting TLR (TOLLIP), which inhibits TLR2 and TLR4 mediated ways and thus protects the host organism from a chronic inflammatory response to commensal bacteria [55]. Another mechanism for maintaining tolerance to symbiotic bacteria is their ability to reduce ubiquitination of IκB, which reduces its destruction and prevents NF-κB translocation to the nucleus and consequent activation of pro-inflammatory genes [56].

Colonization resistance is also realized by the strengthening of the mucosal protective barrier of the digestive tract by commensal microorganisms. The result of the interaction of epithelial cells with symbiotic physiological microflora is the formation of pre-epithelial film, consisting of a layer of molecules of mucus secretory Ig A, immune cells, microcolonies of obligate bacteria, enzymes and metabolites of microorganisms and the host [57]. This barrier closes the way to specific receptors on the epithelium for the living cells of harmful microflora and its toxins.

An important role of intestinal microbiota is the synthesis of various biomolecules. For instance, microflora produces a wide range of vitamins (C and B, folate and niacin) and essential amino acids and facilitates their absorption [58]. Flora promotes better absorption of calcium and vitamin D [59]. SCFA (lactate, acetate, propionate, butyrate, etc.), synthesized by saccharolytic flora, play an important role in the energy supply and nutrition of intestinal epithelium, enhance the intestinal barrier, and help to eliminate potential pathogens [60]. Anaerobic bacteria synthesize biologically active substances: β-alanine, 5-aminovaleric and γ-aminobutyric acid [61, 62].

Normal flora of the human body is involved in the metabolism of proteins, carbohydrates, lipids, and nucleic acids; it breaks down cellulose, provides the epithelium with substrates of
gluconeogenesis and lipogenesis, and stimulates intestinal motility [44]. Intestinal bacteria facilitate hydrolysis of casein by synthesis of phosphoprotein phosphatase and promote the metabolism of lactose by synthesis of bacterial \(\beta\)-galactosidase. The microflora regulates the recirculation of bile acids (BA) and intestinal glands’ secretion. In the distal ileum and colon, part of the salts of primary bile acids (cholic and chenodeoxycholic acid) is converted into secondary (lithocholic and deoxycholic) by dehydroxylation under the action of bacteria enzymes. Dehydroxylation and splitting of bile conjugates increases the solubility of bile acids in the plasma membrane lipids, resulting in their easier absorption [63]. An important function of the microflora is to convert bilirubin to urobilinogen, which is partially absorbed and partially excreted with the urine and the feces [45].

The detoxification function of saccharolytic bacteria is explained as acidification of the intestine, inhibition of the growth of pathogenic microorganisms and decrease of the synthesis and activity of harmful microbial enzymes. Lactobacilli form endogenous antibiotic substances lactolin, lactocidine, acidophilin when ferment lactic acid. The normal microflora also inhibits the decarboxylation process of food histidine, and thus the synthesis of histamine, which reduces the risk of food allergy [31].

In addition to the abovementioned functions, gut microbiota is involved in thermoregulation (maintaining an optimal temperature in the gut), maintaining an optimal pH of the pre-epithelial zone, redox potential, ion composition, viscosity of glycocalyx, regulation of apoptosis, differentiation and regeneration of tissues (especially epithelial), regulation of gas composition of lumen, and increasing resistance of epithelial cells to mutagens (carcinogens) [64, 65].

Taking in account the above data, we can therefore conclude the critical role of normal microflora in the functioning of the digestive tract and the organism as a whole. Current research efforts have focused on host and environmental factors that may affect energy balance. This has led to a plausible, biological postulation that an obese microbiota profile may exist and may demonstrate increased energy-yielding behaviour by such bacteria. Consequently, the gut microbiota is gaining significant research interest in relation to obesity in an attempt to better understand the etiology of obesity and potentially new methods of its prevention and treatment.

**The Gut Microbiota as an Environmental Factor That Prevents Obesity**

Prevention and management of obesity is proposed to begin in childhood when environmental factors exert a long-term effect on the risk of obesity in adulthood. Thus, identifying modifiable factors may help to reduce this risk. Therefore, the search for new non-toxic means of obesity prevention is the urgent challenge of modern science. Recent evidence suggests that gut microbiota is involved in the control of body weight, energy homeostasis and inflammation, and thus plays a role in the pathophysiology of obesity. Prebiotics and probiotics are of interest, as they have been shown to alter the composition of gut microbiota and to affect food intake and appetite, body weight, and composition and metabolic functions through gastrointestinal pathways and modulation of the gut bacterial community [66].

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Today, the question of probiotics' influence on lipid metabolism and obesity is actively debated in the scientific literature [67-69]. Bäckhed et al. were the pioneers in research into the role of colon microflora in metabolism regulation [70]. Their findings were the impetus for the research in this field. Further studies have shown the alteration of intestinal microbiota composition in overweight people.

Thus, intestine microbiocenosis can be considered the environmental factor that modulates the development of obesity. It was demonstrated that prolonged exposure to a high fat diet significantly changed the composition of the colon microflora in mice, having reduced the level of Bifidobacterium and Lactobacillus that are known to produce many positive physiological effects, e.g., improving the barrier function of the intestinal mucosa and having increased levels of Firmicutes and Proteobacteria, which produce a lot of toxic substances [71, 72]. It was found that the oligofructose prebiotic, which is a supplement to a high fat diet, resulted in the recovery of the normal composition of bifidoflora, hence elimination endotoxemia and reduction of the obesity development.

These data suggest that bifidoflora may reduce intestinal permeability and the level of circulating endotoxin. In addition, the growth of bifidobacteria improved the sensitivity to glucose and decreased the body weight gain and production of pro-inflammatory mediators [73-75]. Recently, the beneficial effects of probiotic bacteria on obesity development have been established. For example, the use of L. gasseri SBT2055 and L. paracasei ssp. paracasei F19 prevented the development of diet-induced obesity [74, 76].

The most important cause of obesity is the excessive consumption of fat and easily digestible carbohydrates, but an accumulation of data has led scientists to believe that the uncontrolled use of food additives such as MSG taste enhancer can also lead to obesity [77]. In our experiments we aimed to investigate obesity in 4-month adult rats treated neonatally with MSG and the preventive effects of short-term probiotic introduction on the development of the obesity. Our work included two series of experiments in which the antiobesity effects of lyophilized 3-strain probiotic and alive multistrain probiotic were studied.

**EFFECT OF SHORT-TERM PERIODIC CONSUMPTION OF LYOPHILIZED PROBIOTICS ON MSG-INDUCED OBESITY OF RATS**

In the first series of experiments we investigated effects of lyophilized probiotic mixture on the MSG-induced obesity of adult male rats [78]. According to this model of obesity, 4 mg/g of MSG is injected to newborn rats at 2nd, 4th, 6th, 8th, and 10th days of life in the volume of 8 µl/g subcutaneously. An aqueous solution of a mixture of strains contained L. casei IMVB-7280, B. animalis VKL, and B. animalis VKB in proportion of 2:1:1. Probiotic was administered at a dose of 5*10⁹ CFU/kg per os. Administration of probiotics was started at the age of 4 weeks, just after wean, and continued for 3 month intermittently alternating two-week course of introduction with two-week course of break. Generally we included 45 rats in these experiments, divided into three groups: intact rats, rats injected with MSG (MSG-group), and rats injected with MSG and treated with probiotic (MSG+probiotic). All animals were on a standard diet. In 4-month-old rats we measured anthropometrical parameters (body length, body mass index (BMI) and Lee obesity index) and biochemical indicators (cholesterol, triglycerides, high density lipoproteins cholesterol (HDL-cholesterol), low

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density lipoproteins cholesterol (LDL-cholesterol), very low density lipoproteins cholesterol (VLDL-cholesterol), bilirubin, and activity of alanine and aspartate aminotransferase, leptin and adiponectin).

Administration of MSG in the neonatal period led to the development of obesity in 4-month old rats. Table 2 shows the anthropometric parameters in 3 groups of rats.

Table 2. Anthropometric parameters of male rats with MSG-induced obesity under treatment with lyophilized probiotic strains

<table>
<thead>
<tr>
<th></th>
<th>Intact rats (n=15)</th>
<th>MSG-induced obesity</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSG-group (n=15)</td>
<td>MSG+probiotic group (n=15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, g</td>
<td>241.9±25.5</td>
<td>261.0±17.8</td>
<td>256.7±27.7</td>
<td>0.016</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Body length, cm</td>
<td>21.40±0.90</td>
<td>20.30±1.60</td>
<td>21.50±0.60</td>
<td>0.023</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>BMI</td>
<td>0.53±0.05</td>
<td>0.64±0.08</td>
<td>0.56±0.07</td>
<td>0.002</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Lee Index</td>
<td>0.29±0.01</td>
<td>0.32±0.02</td>
<td>0.30±0.01</td>
<td>0.013</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Visceral fat mass, g</td>
<td>2.53±0.78</td>
<td>17.31±5.69</td>
<td>10.65±3.89</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as the M ± SD. p1 – group I vs group II. p2 - group I vs group III. p3 - group II vs group III. Significant p-value – <0.05.

Table 3. Biochemical indicators of liver in blood serum of rats with MSG-induced obesity under treatment with lyophilized probiotic strains

|                                | Intact rats (n=15) | MSG-induced obesity | p1     | p2     | p3     |
|                                | MSG-group (n=15)   | MSG+probiotic group (n=15) |        |        |        |
| Alaninamino-transferase, µkat/l| 0.228±0.033        | 0.211±0.031          | 0.221±0.034 | >0.05  | >0.05  | >0.05  |
| Aspartate transaminase, µkat/l | 0.389±0.034        | 0.377±0.041          | 0.392±0.044 | >0.05  | >0.05  | >0.05  |
| Total albumin, µmol/l          | 12.4±2.1           | 12.7±1.5             | 12.3±2.0  | >0.05  | >0.05  | >0.05  |
| Indirect bilirubin, µmol/l     | 7.9±1.7            | 8.1±1.1              | 7.7±1.4   | >0.05  | >0.05  | >0.05  |
| Direct bilirubin, mmol/l       | 4.4±0.9            | 4.6±0.9              | 4.6±1.0   | >0.05  | >0.05  | >0.05  |

Data are presented as the M ± SD. p1 – group I vs group II. p2 - group I vs group III. p3 - group II vs group III. Significant p-value – <0.05.

It was found that body weight of 4-month-old rats of group II exceeded benchmarks of group I by 7.9% (p<0.05). Body length in group II was decreased by MSG introduction by 5.4% (p<0.05) compared to control. The calculation of body mass index and Lee index suggested the development of obesity in group II. Also observed was the significant increase of visceral adipose tissue mass in animals injected with MSG by 583% (p<0.001) in comparison with control (Table 2). These data confirm the results of other researchers, who found that the introduction of MSG to newborn rats induces the development of visceral obesity in adult animals and may be used as one of the models of obesity in rodents [22].
While development of MSG-induced obesity was registered, there were no functional changes in the liver. This was confirmed by determination of bilirubin and albumin concentration and activity of alanine and aspartate aminotransferase in blood serum (Table 3).

However, in the blood of animals injected with MSG, the lipid metabolism changes that are characteristic of metabolic syndrome were observed. Under the neonatal injection of MSG the concentration of total cholesterol, triglycerides, VLDL-cholesterol, and LDL-cholesterol significantly increased by 55% (p<0.001), 210% (p<0.001), 210% (p<0.001) and 83% (p<0.001) correspondingly compared to control (Table 4). In addition, it was found that MSG introduction influenced HDL-cholesterol concentration, decreasing it by 33.1% (p<0.001) (Table 4) [79].

Table 4. Biochemical parameters of lipid metabolism in serum of rats with MSG-induced obesity under treatment with lyophilized probiotic strains

<table>
<thead>
<tr>
<th></th>
<th>Intact rats (n=15)</th>
<th>MSG-induced obesity</th>
<th>p1</th>
<th>p2</th>
<th>p3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MSG-group (n=15)</td>
<td>MSG+probiotic group (n=15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.15±0.27</td>
<td>3.53±0.57</td>
<td>2.91±0.72</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.53±0.34</td>
<td>7.04±0.26</td>
<td>4.72±0.37</td>
<td>&lt;0.001</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>VLDL-cholesterol, mmol/l</td>
<td>0.51±0.12</td>
<td>1.58±0.26</td>
<td>1.07±0.41</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/l</td>
<td>1.63±0.14</td>
<td>1.09±0.19</td>
<td>1.37±0.11</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/l</td>
<td>2.37±0.22</td>
<td>4.35±0.29</td>
<td>3.02±0.49</td>
<td>&lt;0.001</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Data are presented as the M ± SD. p1 – group I vs group II. p2 - group I vs group III. p3 - group II vs group III. Significant p-value – <0.05.

Taking into account the literature data which show that adipose tissue is an active secretory organ and might influence obesity development, we investigated the concentration of adipose-derived hormones in rats of all groups. In rats with MSG-caused obesity, changes in the concentration of adipose-derived hormones were observed. The serum level of adiponectin of rats with MSG-induced obesity decreased by 58.8% (p<0.01) compared to control (Figure 1) [78]. Such results also have been represented in the literature, which has demonstrated the decreased level of adiponectin in obese humans and people with insulin resistance.

Investigations conducted on obese rhesus monkeys with T2D have confirmed that adiponectin levels decrease simultaneously with pathologies development [80, 81]. Scherer Lab set a line of transgenic mice with adiponectin levels in serum increased by 3 times [82]. In this model, sensitivity of peripheral tissues to insulin was improved due to the activation of carbohydrate and lipid metabolism associated with increased activation of 5’ AMP-activated protein kinase (AMPK) in the liver and the expression of peroxisome proliferator-activated receptors γ (PPAR-γ) in visceral adipose tissue. These animals were resistant to the development of insulin insensitivity induced by consumption of a high fat diet [83]. Treatment of obese animals with recombinant adiponectin leads to hyperglycemia and decrease of free fatty acids (FFA) in plasma, and improves insulin sensitivity [84]. Activation of PPARγ in vivo increases adiponectin levels [85]. In adiponectin-deficient mice, hepatic insulin resistance was observed in connection with the decrease of therapeutic response to
agonists of PPAR-γ. This indicates that adiponectin is an important factor enhancing PPARγ-mediated improvement in insulin sensitivity [86].

**Figure 1.** Adiponectin level in serum of 4-month-old obese rats in the conditions of short-term periodic consumption of lyophilized probiotic mixture (M±m, n=10 in each group): * - p<0.05 compared to intact rats.

The physiological function of leptin is to prevent obesity caused by excess flow of food into the body. Reduced leptin secretion during fasting is a kind of signal to increase energy absorption. Excessive consumption of food increases thermogenesis through activation of energy formation in brown fat. Energy formation occurs due to the induction of the genes expression responsible for the synthesis of mitochondrial proteins of type 1, 2, and 3. These proteins dissociate oxidative phosphorylation and regulate the rate of thermogenesis in the body [87]. The leptin concentration in visceral adipose tissue of rats with MSG-induced obesity increased by 74.7% (p<0.01) compared to control (Figure 2). In case of obesity the leptin increase is observed due to the resistance of the hypothalamus to lipocytokine’s central action. However, the effect of leptin on peripheral tissues persisted, so we can suspect the presence of selective leptin resistance. Tissues' resistance to leptin develops gradually, activating the growth of adipose tissue [87]. It was shown that the introduction of MSG causes lesions in the arches and ventromedial nuclei of the hypothalamus, causing insensitivity to leptin and insulin in this region. This results in the development of hyperleptinemia and hyperinsulinemia [88].

Administration of lyophilized probiotics to animals that received MSG (4 mg/g) at 2nd, 4th, 6th, 8th, and 10th days of life prevented the development of obesity in rats. Thus, in the MSG+probiotic group, body length was increased by 6.1% (p<0.05) compared to the MSG-group, and did not differ from control. Obesity prevention was confirmed by significant reduction of Lee index and visceral adipose tissue mass. Although there was no difference between Lee index in MSG- and MSG+probiotic groups, probiotic restored this parameter to control value. Visceral adipose tissue mass was reduced by 38.5% (p<0.001) in rats treated with probiotics (Table 2).

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Periodic administration of probiotic led to restoration of lipid metabolism in rats. The probiotic strains influenced the cholesterol concentration, which was restored to the level of control. In the organism of animals injected with probiotic strains, the VLDL-cholesterol concentration decreased by 32.3% (p<0.05) and LDL-cholesterol by 30.6% (p<0.05), and HDL-cholesterol increased by 25.7% (p<0.05) compared with group II. These values did not reach the level of control (Table 3). Other works have also shown the improvement of lipid metabolism by probiotics in obese rats [89]. Decrease of cholesterol concentration in mice with high fat diet-caused hypercholesterolemia under the influence of Lactobacillus and Bifidobacterium, especially L. acidophilus IMB B-7279, B. animalis VKB and B. animalis VKL [90], for example, has been established. It was also demonstrated that modulation of gut microbiota, e.g., dietary intervention with oligofructoses, reduced metabolic endotoxemia and the cecal content of LPS, improved glucose intolerance and insulin sensitivity, and decreased body weight gain in both high-fat fed and ob/ob mice [73, 91]. In models of diabetes, probiotic intervention has been examined for its ability to impact on metabolic biomarkers of disease. Tabuchi et al. showed that L. rhamnosus GG improved glucose tolerance in the streptozotocin-induced rat model of diabetes, possibly due to the prevention of insulin secretion decrease [92]. Studies using the traditional Indian yogurt, dahi, supplemented with probiotic strains of L. acidophilus and L. casei, have shown that this product can improve markers of diabetes, including hyperglycemia and hyperinsulinemia in high-fructose induced rat models of diabetes [93, 94].

In our study we found that probiotic introduction led to the normal hormonal activity of adipose tissue. Thus, it was observed the increase of the adiponectin level and the decrease of the leptin concentration in adipose tissue of MSG+probiotic group.

Summing up the above data, in our experiments we are the first to show that the short-term introduction of lyophilized probiotics restored the anthropometric parameters, lipid metabolism, and hormonal activity of adipose tissue in rat model with MSG-induced obesity. (Table 2, 3; Figure 1, 2) [78]. These results are consistent with other studies and show the
effectiveness of probiotics in obesity prevention. Indeed, the gut microflora has an impact on the energy metabolism and fat storage, and numerous studies have confirmed the destruction of gut microbiota composition in obese, overweight, and/or type 2 diabetic subjects [33, 95, 96]. Therefore, the altered microflora is believed to be one of the pathogenetic factors in these metabolic disorders. One of the mechanisms of regulation of energy, glucose, and lipid metabolism of the host organism by microflora is farnesoid X receptor (FXR)-dependent. FXR is nuclear receptor sensitive to bile acids. Activated by physiological concentrations of bile acids, FXR regulates expression of numerous bile acid-responsive genes, mainly in the liver and intestine, involved in cholesterol and bile acid homeostasis [97]. Many microorganisms can activate FXR in intestine, which results in decrease of body weight gain, plasma cholesterol levels, and liver triglycerides. The exact process of microflora-induced activation of FXR includes several steps. Symbiotic bacteria possessed bile salt hydrolase activity decreases the abundance of tauro-bmuricholic acid, which is an antagonist of the host bile receptor FXR. The deblocking of FXR in enterocytes leads to secretion of fibroblast growth factor (FGF) 15, which translocates through the portal vein to the liver and represses the hepatocyte gene of cholesterol 7 α-hydroxylase (CYP7A1). The consequence of this is the inhibition of bile acid synthesis, improvement of energy metabolism, and, in obese individuals, reduction of the severity of obesity.

However, the exact composition of symbiotic bacteria that can contribute to restoration of the gut microbiota in obese individuals remains debatable [98]. In particular, different Lactobacillus and Bifidobacterium strains have specific effects on the markers of obesity in rodent models. Analysis of more than 20 articles from 2013 to July 2014 by Cani et al. shows that at least 15 different strains of Lactobacillus and two strains of Bifidobacterium do not equally influence body weight, fat mass, glucose metabolism, inflammatory markers, plasma and hepatic lipids and plasma cholesterol levels [98]. Furthermore, there was no individual strain that had all of these effects on different models of obesity in rats. In our research, the combination of two Bifidobacterium and one Lactobacillus lyophilized strains only decreased body length, but did not impact on body mass index and Lee index, strongly reduced the fat mass and serum lipids and improved hormonal activity of adipose tissue, thus demonstrating the more pronounced effect on obesity compared with effects of single strains described in the abovementioned article [98].

In order to compare the effects of three-strain vs. multistrain and lyophilized vs. alive probiotic we performed a second series of experiments designed to investigate the antiobesity activity of alive multiprobiotic — Sy mbiter” on the model of MSG-induced obesity of rats [99].

**EFFECT OF SHORT-TERM PERIODIC CONSUMPTION OF ALIVE MULTIPROBIOTIC ON MSG-INDUCED OBESITY OF RATS**

To study the antiobesity effects of alive multiprobiotic — Sy mbiter” we used the same model of obesity described above. MSG was administered to rats at a dose of 140 mg/kg (1.4×10^{10} CFU/kg) per os. It contains 14 probiotic strains of Lactobacillus + Lactococcus (6×10^{10} CFU/g), Bifidobacterium (1×10^{10}/g), Propionibacterium (3×10^{10}/g) and Acetobacter (1×10^{6}/g) genera.
Figure 3. Body weight changes in rats from birth to the age of 4-months in the conditions of short-term periodic consumption of alive multistrain probiotic (M±m, n=10 in each group): * - p<0.05 compared to intact rats, # - p<0.05 compared to MSG-group.

Figure 4. Body weight of 4-month-old rats in the conditions of short-term periodic consumption of alive multistrain probiotic (M±SD, n=10 in each group): * - p<0.05 compared to intact rats, # - p<0.05 compared to MSG-group.

Figure 3 shows sex specific weight gain from 30 to 120 days of age in all experimental rats groups. In male rats at the 30-day age we did not find significant changes in body weight at all experimental groups. Beginning from day 60 we established significant weight gain in the MSG-group comparative to other groups, and at day 120 the rats’ average body mass was 263.4±26.9 g. Specifically, the additional diet correction with multiprobiotic —"Symbiter"— led to weight reduction by 14.5% (p=0.005) compared to the value obtained in MSG-rats after 3 months of feeding (Figure 3). Unlike in the male rats, in female rats, on day 30, 60 and 90, we...
observed significantly higher body weight in the MSG-group compared with intact control. But on day 120 the weight did not differ between intact and MSG-groups (p=0.914), which may suggest the delay of growth. Short-term periodic administration of probiotic beginning from the age of 30 days induced weight reduction by 17.3% (p=0.001) at 120 days in MSG-probiotic female rats compared to the MSG-group (Figure 3). Also, the body weight in this group was significantly lower (14.3% (p=0.001)) compared to intact rats (Figure 1A). The weight of 4-month-old animals is shown in Figure 4.

Neonatal treatment with MSG caused stunted growth in both MSG-groups, which manifested in significantly smaller naso-anal length compared to intact rats. This is why, despite lower weight in both sexes, after probiotic administration, we stated the development of obesity in 50% of female and 40% of male animals from the MSG-probiotic group compared to intact rats, which was totally confirmed by Lee index higher than 0.300 (Figure 5). However, the incidence of obesity was higher in the MSG-group than the probiotic group – in females (90% vs 50%, p=0.051) and, respectively, in males (90% vs 40%, p=0.019). Therefore, short-term periodic consumption of multiprobiotic had a preventive effect on glutamate-induced obesity. In particular, we observed significantly lower Lee index after probiotic correction in females (0.301±0.01 vs 0.313±0.01, p=0.024) and males (0.299±0.007 vs 0.319±0.01, p=0.004) compared to the MSG-group (Figure 5).

Figure 5. Lee index in 4-month-old rats in the conditions of short-term periodic consumption of alive multistrain probiotic (M±SD, n=10 in each group): * - p<0.05 compared to intact rats, # - p<0.05 compared to MSG-group.

We observed a 5-7 times increase of total weight of VAT in rats with MSG-group compared to intact control (Figure 6). Gender specific analysis showed the development of more pronounced visceral obesity in males, because of significant higher deposition of VAT in MSG-rats (18.72±5.46g vs 14.1±2.89g; p=0.036). Short-term concomitant administration of probiotic bacteria led to a decrease of VAT weight by 52.43% (p=0.001) in females and respectively by 58.86% (p=0.001) versus MSG-group (Figure 6), although its level did not reach the control values.

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To establish the alteration of eating behavior under conditions of MSG-induced obesity we examined food intake in one, two-, three- and four-month-old rats of all groups. A slight age-dependent increase in food intake was demonstrated both in male and female rats. It should be noted that there was no significant difference in food consumption in the experimental group to suggest that MSG-obesity is not a result of excessive caloric intake but associated with the metabolic disorder (Figure 7).

Figure 6. Visceral adipose tissue weight in 4-month-old rats in the conditions of short-term periodic consumption of alive multistrain probiotic (M±SD, n=10 in each group): * – p<0.05 compared to intact rats, # - p<0.05 compared to MSG-group.

Figure 7. Dynamics of food intake changes in rats from 1-month to 4-month age in the conditions of short-term periodic consumption of alive multistrain probiotic (M±SD, n=10 in each group).
Zhang et al. analysed the composition of microflora in patients with type 2 diabetes mellitus (T2DM) and found significant shifts in bacterial abundance in the gut [100]. They proposed *Verrucomicrobiae* as a potential marker of T2D due to its large decrease in diabetic individuals. The authors also revealed the lower abundance of *Bacteroides*, *Streptococcus*, *Haemophilus*, *Megamonas*, *Roseburia*, *Akkermansia muciniphila* ATCCBAA-835, and *Faecalibacterium prausnitzii* L2-6 in the gut of patients with normal glucose tolerance. On the other hand, T2DM patients had altered microbiota, with an increase of *Subdoligranulum*, *Clostridiales*, *Lachnospiraceae*, *Eubacterium*, *Sporobacter*, *Abiotrophia* and *Peptostreptococcus* due to operational taxonomic unit analysis [100]. Thus, the association between microbiota and glucose tolerance was established as key to the search for reliable markers of T2D, opening the field for research of probiotic correction of patients with T2D microflora.

In the current study we showed that insulin resistance and glucose intolerance develop with MSG-induced obesity. The fasting blood glucose of MSG-rats was significantly higher than that of intact animals of both sexes (6.11±1.15 vs 4.62±0.42 mmol/L, p=0.003 – in females; 6.17±0.64 vs 4.59±0.67 mmol/L, p=0.001 – respectively in males). Female rats from the MSG-probiotic group presented significant decrease of fasting glucose level by 17.34% (p=0.024), and males by 16.04% (p=0.010) compared to the MSG-group (Figure 8). There was therefore a significant improvement of glucose tolerance in the conditions of probiotic intervention. Analysis of the HOMA-IR and serum insulin concentrations showed that under conditions of MSG-induced obesity rats became insulin resistant. Both the HOMA-IR index (2.3±1.2 vs 0.46±0.21 (p=0.001) in females and 2.77±0.92 vs 0.66±0.34 (p=0.001) in males)

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and serum insulin concentration (8.19±2.90 vs 2.33±1.16 (p=0.001) in females, 10.02±2.62 vs 3.21±1.53 (p=0.001) in males) were significant higher in rats with MSG-induced obesity compared to control (Figure 9, 10).

Figure 9. Insulin level in serum of 4-month-old rats in the conditions of short-term periodic consumption of alive multistrain probiotic (M±SD, n=10 in each group): * - p<0.05 compared to intact rats, # - p<0.05 compared to MSG-group.

Figure 10. HOMA-IR of 4-month-old rats in the conditions of short-term periodic consumption of alive multistrain probiotic (M±SD, n=10 in each group): * - p<0.05 compared to intact rats, # - p<0.05 compared to MSG-group.

The 2-week periodic multiprobiotic courses improved the insulin sensitivity, which manifested in significant 2-fold decrease of HOMA-IR and fasting insulinemia in comparison to the MSG-group. After treatment with probiotic the HOMA-IR index did not exceed the normal range, but was not significantly higher compared to intact control rats (Figure 10).
These data indicate the restoration of the normal insulin sensitivity in obese rats under the influence of multistrain probiotic. Similar results were obtained by De Vrieze et al. in a study of patients with metabolic syndrome, which observed the decrease of the insulin resistance by transfer of microbiota from lean individuals to obese [101].

**Impact of Short-Term Probiotic Courses on Adipocytokine Levels in Obese-Induced Rats**

Analysis of the secretory function of adipose tissue showed a change in the concentration of adipose-derived hormones in rats with experimental obesity. Thus, the level of adiponectin in the serum of rats with MSG-induced obesity decreased by 2.43 times ($p=0.001$) in males and 1.75 ($p=0.020$) in females (Figure 11). Concentrations of leptin in adipose tissue were higher by 45.9% ($p=0.019$) and 61.2% ($p=0.009$) respectively in males and females in comparison with the intact control group (Figure 12). In serum, leptin level was less than in the VAT. MSG increased the leptin level by 93.3% ($p<0.05$) and 83.6% ($p<0.01$) respectively both in males and females (Figure 13). Gender specific analysis did not confirm changes of leptin concentrations in adipose tissue, but all experimental groups represented significantly lower serum adiponectin concentrations in males compared to females (intact – 4.29±1.67 vs 6.5±1.99 µg/ml, $p=0.001$; MSG – 1.73±0.56 vs 3.77±1.64 µg/ml, $p=0.030$).

![Figure 11. Adiponectin level in serum of 4-month-old rats in the conditions of short-term periodic consumption of alive multistrain probiotic (M±SD, n=10 in each group): * - $p<0.05$ compared to intact rats, # - $p<0.05$ compared to MSG-group.](image)

A significant increase of serum adiponectin after probiotic administration was found in males by 89% ($p=0.028$) compared to MSG-group females by 38.2% ($p=0.039$) compared to MSG-group (Figure 11). The VAT leptin in males decreased by 14.3% ($p=0.047$) compared to the MSG-group, and in females by 14.9% ($p=0.044$) compared to the MSG-group (Figure 12). The serum leptin in the MSG-probiotic group decreased insignificantly (Fig 13). The
results also showed that the concentration of adiponectin and leptin in the case of probiotics rationing did not differ from the level of intact rats (Figure 11, 12).

Figure 12. Leptin level in adipose tissue of 4-month-old rats in the conditions of short-term periodic consumption of alive multistrain probiotic (M±SD, n=10 in each group): * - p<0.05 compared to intact rats.

Figure 13. Leptin level in serum of 4-month-old rats in the conditions of short-term periodic consumption of alive multistrain probiotic (M±SD, n=10 in each group): * - p<0.05 compared to intact rats.

Thus, intermittent administration of probiotics for two-week courses restored the hormonal activity of adipose tissue.

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Morphological Changes in Male Rat Liver under the Conditions of Obesity and Probiotic Administration

One of the complications of obesity is the liver pathology. In our work we confirmed the development of NAFLD in conditions of neonatal injection of MSG. We assessed the histological structure of liver tissue of all experimental groups of rats, and found evidence of steatosis, lobular inflammation and ballooning degeneration in the MSG-group (Table 5). The original microscopic photos show the pronounced total granular dystrophy of hepatocytes, perivascular leukocyte infiltration, and focal necrosis of hepatocytes (Figure 14).

Table 5. Morphological changes in male rat liver assessed by NAFLD activity score (NAS) in the conditions of short-term periodic consumption of alive multistrain probiotic

<table>
<thead>
<tr>
<th></th>
<th>Intact group (n=20)</th>
<th>MSG-obesity (n=20)</th>
<th>MSG-probiotic (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steatosis (0–3)</td>
<td>0.10±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.15±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85±0.13&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lobular inflammation (0–2)</td>
<td>0.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.95±0.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.1±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ballooning degeneration (0–2)</td>
<td>0.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.15±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0±0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total NAS (0–8)</td>
<td>0.10±0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.95±0.15&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as the M ± SEM. One-way ANOVA with post hoc comparisons by Fisher LSD test were performed for data analysis. a, b, c: shows significant differences in the same row (p<0.05).

Figure 14. Light microscopic micrographs of the liver tissue of studied rats in the conditions of short-term periodic consumption of alive multistrain probiotic, stained with hematoxylin and eosin, ×400.
We found significantly lower total score (0.95±0.15 vs 3.3±0.28, p<0.001), degree of steatosis (0.85±0.13 vs 2.15±0.16, p<0.001), and manifestation of lobular inflammation (0.1±0.06 vs 0.95±0.15, p<0.001) due to NAFLD activity score in MSG-probiotic group compared to MSG-obesity. NASH we confirmed only in 20% of rats with MSG-obesity (p=0.035) (Table 5, Figure 14).

Moreover, the improvement of lipid metabolism that was impaired in the conditions of obesity and NAFLD development was observed in the probiotic group. Probiotic administration reduced total hepatic lipids (by 20.9%, p<0.001) and triglycerides (by 25%, p<0.001) content compared to the MSG-group [102].

Integrating all of the above results we can conclude the significant preventive effect of probiotic consumption on the MSG-induced obesity of rats, confirmed with improvement of anthropological parameters, lipid and glucose metabolism, hormonal activity of adipose tissue and the decrease of NAFLD development [78, 99]. Also, comparing the effects of lyophilized 3-strain and alive multistrain probiotic, we found more pronounced prophylactic properties of multistrain probiotic, possibly attributable to its more complex action on the host organism and synergetic action of different probiotic bacteria.

MECHANISM LINKING THE INTESTINAL MICROBIOTA AND OBESITY-ASSOCIATED DISEASE

The mechanisms of obesity development and the influence of microbiota thereon are subjects to which scientists are paying close attention. The most frequent cause which leads to the development of obesity is a disbalance between energy intake and energy expenditure. In this complex process, genetic susceptibility, environmental and lifestyle factors are involved. Recent advances in next generation sequencing technology and mechanistic testing in gnotobiotic mice have identified the gut microbiota as an environmental factor which influences whole-body metabolism by affecting energy balance but also inflammation and gut barrier function, integrating peripheral and central food intake regulatory signals and thereby increasing body weight. Underlying mechanisms whereby the gut microbiota contributes to host metabolism have been revealed in studies on germ-free mice which were protected against developing diet-induced obesity.

Fasting-induced adipose factor (FIAF). One of the key mechanisms by which germ-free animals seem protected from diet-induced obesity is elevated levels of fasting-induced adipose factor (FIAF), also known as angiopoietin-like protein 4. FIAF is a circulating lipoprotein lipase (Lpl) inhibitor produced by the intestine, liver, and adipose tissue [103]. Conventionalization of germ-free mice suppresses expression of FIAF in gut epithelial cells [70], thereby leading to higher adipocyte Lpl activity, which results in increased cellular uptake of fatty acids, adipocyte triglyceride accumulation and greater fat storage (Figure 15). Germ-free Fiaf−/− mice are similarly obese to their conventionally reared counterparts. After conventionalization, germ-free Fiaf−/− mice produced higher on 57% total body fat as observed in wild-type littermates [70]. Consistently, germ-free Fiaf−/− mice fed a high-fat, high-carbohydrate diet were not protected from diet-induced obesity, suggesting that FIAF is a mediator of microbial regulation of energy storage [104].

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In contrast, mice fed a high-fat diet complemented with *Lactobacillus paracasei* exhibited significantly reduced body fat, which was paralleled by increased circulating levels of FIAF [105]. Fleissner et al. showed that germ-free mice on high-fat diet showed increased intestinal mRNA expression of FIAF with no major changes in circulating FIAF compared with conventionalised mice, suggesting that the FIAF mechanism is not universally associated with gut microbiota-related fat mass development [106].

**AMP-activated protein kinase (AMPK).** Furthermore, Bäckhed and colleagues have also demonstrated that germ-free mice have increased levels of phosphorylated AMPK in muscle and liver. AMPK is a key enzyme that controls cellular energy status which activates key enzymes of mitochondrial fatty acid oxidation, including acetyl-CoA carboxylase (ACC) and carnitine-palmitoyltransferase I (CTP1), indicative of increased energy expenditure (Figure 15). The exact pathway whereby the microbiota signals to liver or skeletal muscle AMPK is unclear, but appears to be independent from FIAF [104].

**Intestinal microbiota, short-chain fatty acids and energy harvest from the diet.** Another mechanism is related to energy extraction from undigested food components. The gut microbiota that digests complex dietary carbohydrates produces many monosaccharides and SCFAs, such as acetate, propionate, and butyrate [104], which represent an important energy source. Conventionalization of germ-free mice doubled the density of small intestinal villi capillaries [107] and enhanced uptake of these components from the gut into the portal blood, where they eventually participate in hepatic *de novo* lipogenesis promoting fat accumulation in the liver and adipose tissue [104]. This reaction is controlled by carbohydrate responsive element binding protein (ChREBP) and sterol responsive element binding protein (SREBP-1).
Furthermore, monosaccharides produced by microbial fermentation absorb and transfer to the liver via portal vein, and activate ChREBP, which increases the transcription of several proteins involved in hepatic de novo lipogenesis, thus contributing to hepatic steatosis [109].

SCFAs act in the gut as signaling molecules, and are specific ligands for at least two G protein-coupled receptors, GPR41 and GPR43, mainly expressed by intestinal epithelial cells [109, 110]. Samuel et al. have demonstrated that conventionally raised Gpr41−/− mice and germ-free Gpr41−/− mice colonized with only Bacteroides thetaiotaomicron and Methanobrevibacter smithii are significantly leaner than wild-type littermates, while there are no differences between wild-type or Gpr41−/− germ-free mice [111]. Gpr41, which is produced by enteroendocrine cells, might be a regulator of host energy balance through effects that are dependent upon the gut microbiota (Figure 15). Activation of GPR41 increases production of peptide YY (PYY), an enteroendocrine cell hormone that normally inhibits gut motility, increases intestinal transit rate, and reduces extraction of energy from the diet, thus affecting peripheral glucose utilisation [111]. A recent study has shown that Gpr43−/− mice are resistant to diet-induced obesity and insulin resistance, at least partly due to Gpr43-regulated energy expenditure [112].

Increased intestinal permeability, innate immunity and metabolic inflammation. There is also growing interest in gut microbiota and intestinal mucus layer interlinks in the context of obesity and the diseases associated with it. Several studies have confirmed this interaction, including a recent one showing that TM-IEC C1galt(−/−) mice with altered intestinal architecture have impaired gut microbiota composition with inverse shifts in the abundance of the phyla Bacteroidetes and Firmicutes. These knockout mice, due to impaired mucus glycosylation, had an elongated gastrointestinal tract with deeper ileal crypts, a small increase in the number of proliferative epithelial cells, and thicker circular muscle layers in both the ileum and colon [113]. Kashyap et al. [114] mentioned that modification of carbohydrate landscape of the distal gut in Fut2(−/−) mice that lack fucosylated host glycans can alter the fecal composition and function of resident microbes as compared to Fut2(+) control mice. Thus, the mucus layer plays a role in the regulation of gut microbiota composition, balancing intestinal inflammation, and affects gut architecture. Nevertheless, key mechanisms linking intestinal mucus and gut microbiota are not fully elucidated.

The intestinal epithelium is the largest surface of cross-talks with gut microbes. The innate immune system of the intestine is one of the most important factors involved in the interaction between microflora and the host. This symbiosis can, on the one hand, lead to the destruction of pathogenic microorganisms, and the other, on the contrary, promote tolerance to commensal bacteria, creating ecological niches for useful gut microorganisms [49, 115]. TLRs are a family of integral membrane pattern-recognition receptors which have a crucial role in the innate immune system and are important for maintaining this balance [116]. TLR4 mainly recognizes LPS, whilst other TLRs are activated by microorganism-derived ligands such as flagellin and double- and single-stranded RNA and DNA [117].

Some lines of experimental evidence suggest that HFD may affect epithelial integrity due to changes in the distribution and localisation of zonula occludens-1 (ZO-1) and occludin (two tight junction proteins) in intestinal tissue and hence lead to impaired gut permeability, and consequently to low-grade systemic inflammation [118-120]. A recent study showed that HFD mice, as compared to a control diet, had reduced trans-epithelial resistance and mRNA expression of zona occludens-1 by 38% (P<0.001) and 40% (P=0.025) respectively. Parallel
to alteration of intestinal permeability, a 6.6-fold elevation of TNF-α mRNA (P=0.037) expression in the proximal colon was observed [121]. Cani et al. demonstrated that bacterial LPS, continuously produced in the gut through lysis of gram-negative bacteria, is a microbiota-related factor which can trigger the inflammatory process by binding to the CD14/TLR-4 complex at the surface of innate immune cells [120]. The authors mention that, after four weeks of high-fat feeding, mice exhibited an obese phenotype accompanied by a change in gut microbiota composition (the reduction of *Bifidobacteria* and *Eubacteria spp.* and a 2-3-fold increase in circulating LPS levels, which they called “metabolic endotoxemia,” since LPS plasma concentrations were much lower than those observed during septic shock [122]. In fact, in this study, continuous subcutaneous low-rate infusion of LPS led to excessive weight gain and insulin resistance in mice. Moreover, LPS receptor *Cd14-/-* mice tend to be resistant to this chronic inflammatory state and were hypersensitive to insulin even when they were fed a normal diet, suggesting that CD14 may modulate insulin sensitivity in physiological conditions [123]. Deletion of TLR-4 prevented the high-fat diet-induced insulin resistance [124]. Molecular links by which TLR4 induced insulin resistance are not fully elucidated, but some studies mention that TLR4 signaling interferes with insulin signaling. Furthermore, stimulation of TLR4 by fatty acid can lead to recruitment of pro-inflammatory macrophages to adipose tissue [125, 126]. Cross-talk between macrophages and adipocytes in adipose tissue involves activation of NF-κB and JNK by TLR signaling and mediates insulin resistance by phosphorylation of IRS-1 [127, 128].

In a recent study, Csak et al. demonstrated that knockout of *Tlr4* protected mice from fibrosis development and led to significant attenuation of steatohepatitis, serum alanine transaminase levels, and oxidative stress [129].

Another member of the pattern-recognition receptors family, TLR5, may be attributed to an altered gut microbiota metabolic changes development in host. TLR5-deficient mice exhibit hyperphagia and develop hallmark features of metabolic syndrome, including hyperlipidemia, hypertension, insulin resistance, and increased adiposity, and these phenotypes associated with altered gut microbiota composition. Furthermore, transplantation of microbiome from TLR5-deficient mice to WT germ-free mice donated many features of metabolic syndrome to the recipients [130].

TLR-2 recognizes components of gram-positive bacteria cell wall, such as peptidoglycan and lipoteichoic acid. In a methionine-choline deficient (MCD) diet-induced model of NASH, the role of TLR-2 was examined. TLR-2-deficient mice demonstrated a significantly higher steatosis, inflammation, and necrosis histological score as compared with WT littermates, as well as an increase in liver injury associated with approximately 3-fold elevation of TNF-α mRNA expression. It is possible that the TLR-2 deficiency exacerbates NASH by altering signaling via the TLR-4 pathway due to their polymorphism [131, 132].

TLR-9 is a pattern recognition receptor that recognizes bacteria-derived cytosine phosphate guanine (CpG)-containing DNA, and can be involved in the pathogenesis of NAFLD. TLR9 or MyD88 (adaptor molecule for TLR9) deficient mice had significantly lower insulin resistance and showed less steatohepatitis and liver fibrosis histological pattern than WT mice. TLR9 signaling induces production of IL-1β by Kupffer cells and therefore increased lipid accumulation in hepatocytes, which leads to NF-kB inactivation, resulting in cell death [133].

It was also demonstrated that modulation of gut microbiota, e.g., by antibiotic treatment or dietary intervention with oligofructoses, reduced metabolic endotoxemia and the cecal
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content of LPS, improved glucose intolerance, insulin sensitivity, and decreased body weight gain in both HFD and ob/ob mice [73, 91]. These findings directly demonstrate that modulation of the immune system integrated with pathogen-sensing systems (e.g., TLRs), and support the emerging view that the gut microbiota contributes to inflammation and metabolic disease (Figure 16).

Figure 16. Interaction between gut microbiota, host innate immunity and metabolic inflammation.

A recent study [134] examined the possibility that MyD88 – the central adaptor molecule for the majority of TLRs – acts as a sensor involved in the interaction between gut microbes and the host in obesity. Specific MyD88 deletion in the intestinal epithelial cell by tamoxifen protects against diet-induced obesity, and is associated with increased energy expenditure, improved glucose homeostasis, reduced hepatic steatosis, and reduction in whole-body fat mass by 30%. MYD88 deletion protects mice against HFD-induced metabolic endotoxemia, thereby supporting the hypothesis that the deletion improves metabolic inflammation. Gut microbiota transplantation from MyD88-KO HFD mice into germ-free recipient mice fed a HFD or intestinal deletion after the onset of obesity reduced body weight gain, fat mass development and adipose tissue inflammation, indicating that targeting intestinal epithelial MyD88 constitutes a putative therapeutic approach for obesity and associated disorders.

Kleinridders et al. demonstrated that mice with MyD88 deletion in the central nervous system are protected from HFD-induced weight gain, leptin resistance, and the induction of leptin resistance by acute central application of palmitate [135]. Conversely, Everard’s study showed that a key mechanism leading to protection against diet-induced obesity was changes in food intake independent from similar energy intake and energy absorption. These data suggest a tissue-dependent impact of MyD88 deletion on energy intake relative in the central nervous system with fatty acids signalling through MyD88 controls leptin sensitivity and
appetite, whereas in the intestine MyD88 controls energy metabolism via cross-talks with gut microbes [134].

Altered bile acid metabolism and BSH activity. BAs act as signaling molecules and activate nuclear BA receptor, called FXR, and the G-protein coupled receptor TGR5, and thereby regulate energy, hepatic lipid, and glucose metabolism [136, 137]. The FXR is strongly expressed at bile acid excretion (liver) and absorption (intestine) regions. Activation of FXR induces expression of small heterodimer binding partner (SHP) and inhibits its activation of the CYP7A1 – the first and rate-limiting enzyme of BA synthesis [138]. The FXR-induced FGF15 (the human ortholog FGF 19), originating in the small intestine, represses hepatic bile acid synthesis through FGF receptor 4 (FGFR4), expressed in the liver or alternatively by activation of SHP [139]. The FXR-dependent FGF15/FGFR4 gut-liver signaling pathway that cooperates with hepatic SHP maintains bile acid's own synthesis and entero-hepatic circulation, but also plays a key role in the control of hepatic de novo lipogenesis, VLDL triglyceride export and plasma triglyceride turnover [140].

A recent study discussed the impact of gut microbiota modulation on BA synthesis. Administration of VSL#3 probiotics promotes ileal BA deconjugation with subsequent fecal BA excretion in mice. These events are associated with changes in ileal BA absorption, and increased hepatic BA neosynthesis via downregulation of the gut-liver FXR-FGF15 axis (Figure 17). Treatment with a FXR agonist normalized fecal BA levels in probiotic-administered mice, whereas probiotic-induced alterations in BA metabolism are abolished upon FXR and FGF15 deficiency [141].

This study showed that the principal site of protective bile acid signaling against lipid accumulation is located in the liver, not the intestine. Using organ-specific FXR knockout mice, which were fed a 1% cholesterol diet for 28 days, the authors observed elevated triglycerides and bile acid levels with strong lipid accumulation, characterized by larger vacuoles in hepatic Fxr -/- sections, while intestinal Fxr -/- mice show no histological difference, maintaining normal serum cholesterol and bile acid levels as compared to WT controls [142].

Several recent studies in mice mention that alteration of the gut microbiota changes host bile acid composition. It has been found that, in germ-free mice, a large proportion of the bile acid profile is constituted by tauro-b-muricholic acid (TβMCA) (34.5% vs 1.8% of the plasma profile in conventionally raised) [143]. Bacterial suppression through antibiotic treatment induced a similar shift with taurine-conjugated bile acids increasing in the tissue bile acid profiles and notably can antagonize the intestinal FXR/ FGF15 [144, 145].

In a recent animal study, reduction of BSH activity by antibiotic or tempol treatment of HFD-fed mice prevented NAFLD as a result of modulation of the gut microbiota, altering the metabolism of bile acids, with a notable increase of the FXR antagonist T-β-MCA which inhibited FXR signaling in the intestine. Compared with control mice, animals with intestine-specific FXR disruption had hepatic triglyceride accumulation reduced by 50% in response to a HFD. The inhibition of intestinal FXR signaling elicits an improvement in mitochondrial function and results in decreased serum ceramide levels, which down-regulate hepatic SREBP1c and CIDEA expression, resulting in decreased hepatic steatosis. It is interesting that administration of C16:0 ceramide to antibiotic-treated mice fed a HFD reversed hepatic steatosis [146].

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Joyce et al., using a controlled expression system, showed that bacterial BSH mediates a microbe-host dialogue that regulates lipid metabolism and weight gain in the host. The colonization of the gastrointestinal tract by E. coli MG1655 as a delivery vector capable of expressing cloned BSH genes led to significantly altered plasma bile acid composition and regulated transcription of key genes involved in lipid metabolism (Ppar-γ, Angptl4) and gastrointestinal homeostasis (RegIIIγ) in mice. High-level expression of BSH in conventionally raised mice resulted in a significant reduction in host weight gain, plasma cholesterol, and liver triglycerides [147]. Because numerous well-known probiotics exhibit BSH activity [148], this may partially account for their metabolic effects.

TGR5 (also known as GPBAR1, M-BAR and BG37) is a G-protein coupled receptor expressed in brown adipose tissue and muscle, where its activation by secondary lithocholic bile acids with subsequent induction of the enzyme 2-iodothyronine deiodinase triggers an increase in energy expenditure. This enzyme converts inactive thyroxine (T4) to tri-iodothyronine (T3), resulting in an increase in metabolic rate and energy expenditure. Stimulation of TGR5 attenuates diet-induced obesity [137]. Thomas et al. demonstrated that TGR5 is expressed in L-cells, and that its activation induces intestinal GLP-1 release, leading to improved liver and endocrine pancreatic function and enhanced glucose tolerance in HFD mice. These data show that the TGR5 signaling pathway is critical in regulating intestinal GLP-1 secretion in vivo, and suggest that pharmacological targeting of TGR5 may constitute a promising treatment of metabolic disorders [149].
In conclusion, bile acids have a bacteriostatic activity, and diet enriched with fats changes the bile acid composition, which influences the conditions of the gut microbial environment and causes dysbiosis [150]. On the other hand, by modifying bile acid metabolism and FXR/TGR5 signaling, gut flora could therefore contribute indirectly to the pathogenesis of metabolic syndrome, and manipulation of its composition could be a promising novel drug target for treatment of obesity associated disease.

We first studied the influence of probiotic administration on insulin sensitivity in a rat model of MSG-induced obesity. Nagata et al. found that mice, after neonatal treatment with MSG, were observed to be obese but had no polyphagia, and were glycosuric by 29 weeks of age. The pathological study showed hypertrophy of the pancreatic islet, with elevation of glucose and insulin serum concentrations at 29 and 54 weeks of age compared to control mice [151]. On the other hand, in the early phase of obesity the level of plasma glucose can be normal but associated with hyperinsulinemia [152], which indicates that insulin resistance is present, and high insulin levels may be compensatory due to direct hypersecretion of β-cells [153].

Our data is in agreement with recently reported studies [93, 94], and showed that, in addition to reduction in total body weight, administration of multiprobiotic by short courses led to improvement of insulin sensitivity, which was confirmed by significant decreasing of hyperinsulinemia and HOMA-IR in the MSG-probiotic group compared to MSG-animals. On the other hand, we observed significant increase of serum adiponectin, defined as one of the main regulators of peripheral tissues’ sensitivity to insulin [83], only in females, compared to the MSG-group.

As previously reported for MSG rats and mice, leptin mRNA expression levels and serum levels in MSG-treated mice were significantly higher than those in normal controls [154, 155]. The increased leptin production in adipose tissues due to pancreatic hypertrophy and hyperinsulinaemia has been reported to induce the development of leptin resistance [156]. We mentioned that probiotics helped to restore the hormonal activity of adipose tissue. Thus, concentration of leptin under the proiotics admistration did not differ from the level in intact rats.

In summary, we studied the effectiveness of short-term periodic consumption of multiprobiotics from childhood on metabolic profile in adult rats with an MSG-induced model of obesity. Subcutaneous neonatal injection of MSG is able to induce obesity without hyperphagia, which is diagnosed by high Lee index and characterized by small corporal weight and naso-anal length. Obesity in rats is caused by alterations in hypothalamic arcuate nucleus and impairs leptin and insulin signaling in this region, resulting in hyperleptinemia and hyperinsulinemia.

Our study has indicated that daily oral administration of 2.5 ml/kg of alive multiprobiotic —“ Symbiter,” containing concentrated biomass of 14 probiotic bacteria of Bibidobacterium, Lactobacillus, Lactococcus, and Propionibacterium genera to neonatal, MSG-treated rats by 2-weeks courses led to significant reduction of total body and VAT weight, together with improvement in insulin sensitivity and prevention of NAFLD development. Our study has shown that multistrain alive probiotics are more effective than a mixture of three lyophilized strains of Bibidobacterium and Lactobacillus. In addition, gender-specific differences in susceptibility to neonatal injection of MSG were established. We found the more pronounced obesity development in male rats, accompanied by stronger weight gain, accumulation of adipose tissue, hormonal disbalance, and insulin insensitivity.

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Most medications for the treatment of obesity are taken out production because of their adverse effects. Orlistat is the only drug that can be taken by patients over a prolonged time period. However, little attention is paid to the search for means of obesity prophylaxis. In current scientific literature, there is an abundance of studies which confirm beneficial effects of probiotics on the human organism. The question of the impact of probiotics on fat metabolism and obesity is one which is being actively debated in the scientific literature. The gut microbiota has recently been proposed to be an environmental factor involved in the control of body weight and energy homeostasis [157, 158]. This "exteriorized organ" contributes to human homeostasis via multiple metabolic functions and diverse control mechanisms.

The experimental data obtained from the animal model were confirmed by our team in clinical studies [159]. We examined 72 patients with T2D and NAFLD. Patients were divided into two groups, each group receiving a different mode of therapy. The main group (n = 45) received oral antidiabetic therapy and multistrain probiotic “Syntibiter” within 30 days. Patients of the comparison group (n = 27) received only hypoglycemic drugs. Also, in each group we identified patients with normal and elevated level of transaminases.

![Figure 18. Cytokine profile changes in patients with NAFLD and normal transaminases.](image)

We observed a 1.5-2x increase of cytokines in patients with NAFLD and elevated transaminase levels compared to patients with normal transaminase levels (Figure 18, 19). We noted a statistically significant reduction of proinflammatory cytokines in plasma after 30 days of therapy with multistrain probiotic in patients with elevated levels of transaminases.

In particular, the level of interleukin (IL)-6 decreased on 40% (p=0.041), IL-8 - 26.54% (p <0.001), tumor necrosis factor (TNF)-α - 20.83% (p <0.001), IL-1β - 17.7% (p<0.001) and interferon (IFN)-γ on 21.84% (p <0.001) respectively. In patients with normal levels...
transaminases and NAFLD were significantly decreased only IL-6 on 17.1% (p = 0.041), IL-8 - 21.4% (p < 0.001) and TNF-α on 13.8% (p = 0.008). Significant changes in cytokines levels in patients of the comparative group were not observed.

This brief and thorough clinical analysis confirmed the benefit of multistrain probiotics in management of obesity and its consequences. Thus, probiotics can be recommended for use in patients with different stages of NAFLD and T2D as an adjunct to standard treatment regimens, since they decrease manifestations of low-grade systemic inflammatory response.

![Figure 19. Cytokine profile changes in patients with NAFLD and elevated transaminases.](image)

**CONCLUSION**

In this chapter we have discussed the connections between gut microbiota, energy homeostasis, and inflammation, and the role of gut microbiota in the pathogenesis of obesity-related disorders and possible methods for their prevention. Prebiotics and probiotics have physiological functions that contribute to the health of gut microbiota, maintenance of a healthy body weight, and control of factors associated with obesity through their effects on mechanisms controlling food intake, body weight, gut microbiota, and inflammatory processes. Our study has shown more expressed antiobesity properties of alive multistrain probiotic as compared to lyophilized 3-strain probiotic with *Bibidobacterium* and *Lactobacillus*.

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