GENERAL REVIEW

UDC: 616.379-008.64-02
https://doi.org/10.2298/VSP151216028N

Diabetes mellitus and obesity as a result of a disrupted homeostatic microbiome. New data on etiopathogenesis of diabetes mellitus

Dijabetes i gojaznost kao rezultat narušavanja homeostatskog mikrobioma. Novi podaci o etiopatogenezi dijabetesa

Dragan M. Nikolić

University of Belgrade, School of Medicine, Clinic for Endocrinology, Diabetes and Metabolic Diseases – Laboratory for Human Pancreatic Islets Culture, Belgrade, Serbia

Key words:
microbiota; diabetes mellitus; obesity; pancreas; digestive system.

Ključne reči:
mikrobiota; dijabetes melitus; gojaznost; pankreas; gastrointestinalni sistem.

Introduction

Diabetes mellitus is a disease in expansion. According to International Diabetes Federation in 2014 an estimated 387 million people in the world have diabetes mellitus (DM), with expected increase of 200 million in 20 years [1]. Diabetes mellitus is characterized by metabolic disorder and hyperglycemia and results from insulin deficiency or decreased effects of insulin on the target tissues. The etiology of DM is reasonably well known as well as the mechanism of its pathogenesis.

Diabetes mellitus type 1 is characterized by absolute lack of insulin and physiological destruction of β cells of pancreas. Autoimmune damage of pancreatic islets is a long-term process and clinical manifestation of the disease occurs when more than 80% of the β cells are irreversibly damaged.

Diabetes mellitus type 2 is a metabolic disease caused by defective insulin secretion and insulin resistance [2]. It is believed that many factors influence the onset of the disease: genetic factors, reduced physical activity, overweight, malnutrition in fetal and prenatal period, certain drugs (steroids, diuretics, anti-hypersensitive). Adipose tissue produces leptin, tumor necrosis factor (TNF) alpha, resistin, adiponectin and interleukin (IL)-6 thereby affecting insulin resistance and possible dysfunction of pancreatic β cells [3–5]. New possible factors in etiopathogenesis of DM include microbiological agents, such as disrupted saprophytic flora (the homeostatic microbiome – HM) and infections of the pancreas. Some studies [6, 7] showed that certain types of bacteria and fungi could cause increased or decreased insulin secretion in the body thereby causing insulin resistance and the development of diabetes mellitus and obesity. This leads to the question how microorganisms affect insulin secretion and the occurrence of diabetes mellitus. Microorganisms in the pancreas could lead to destruction of β cells due to activation of immune system as a response to infections inducing in this way DM type 1. In interaction with pancreatic cells, microorganisms could lead to increased (*Candida*) or decreased (bacteria) insulin secretion thereby increasing chances for the onset of DM type 2. Saprophytes in the intestinal tract and other organs lined with mucosa secretory products are able to pass via the bloodstream to the pancreas thus indirectly affecting insulin secretion. This report suggests that the total body load of microbes (the microbiome) is an important physiological factor, and that maintenance of this HM is important for human health. A speculation that can be derived from this is that disruption in the HM may contribute to DM and obesity. There are several lines of evidence that support this hypothesis.

Infections of the pancreas

One of the important factors that might affect insulin secretion is an infection of pancreas. It is well known that certain pathological conditions such as acute pancreatitis, necrotic pancreatitis and cysts are caused by following microorganisms: Gram (+) bacteria, 74%, Gram (–) bacteria, 21% (*Enterobacter*, up to 58%), *Candida albicans*, 5–24% [6, 8]. These microorganisms are mostly saprophytic and they are part of the normal gastrointestinal flora [6–9]. However, the extent of pancreas infection and effects and types of the infection are unclear. For example, severe cases of acute pan-

Correspondence to: Dragan M Nikolić, University of Belgrade, School of Medicine, Clinic for Endocrinology, Diabetes and Metabolic Diseases – Laboratory for Human Pancreatic Islets Culture, Dr Subotića 13, 11 000 Belgrade, Serbia. E-mail: dragannikolic8@yahoo.com

creatitis might lead to numerous complications such as DM, and a damaged pancreas could be infected by bacteria from the small intestine. Symptoms of inflammation include fever, increased number of leukocytes, and, in severe cases, organ failure may also adversely effect the pancreas.

Possible mechanisms of pancreatic infection

Microorganisms from the intestine may enter the pancreas in three different ways (Figure 1): directly from the duodenum through the pancreatic duct (*ductus pancreaticus*) [10, 11], penetration into body cavity due to injuries and via the blood. Certain pathological conditions that can facilitate direct penetration of microorganisms from the intestine to the pancreas include reduced secretion of pancreatic juice (in patients with acute and chronic pancreatitis), weakening of the sphincter of Oddi (sphincter muscles get weaker with age, a major risk factor for the onset of DM type 2), anatomical changes in intestinal tract, tumors, and excessive and frequent food intake that can cause distension of the stomach and intestine, disrupting the sphincter of Oddi so that it remains open or to expand the lumen of the sphincter.

In support of this hypothesis, there is the observation that the microorganisms found in the pancreas originate mainly from the small intestine and that they have colonized the pancreas though the sphincter of Oddi [6, 7]. Among tested samples, 31% were contaminated by bacteria (*Pseudomonas aeruginosa*, *Enterobacter* spp. and *Staphylococcus* spp.), 24% were contaminated with *Candida albicans* and 45% were sterile [6, 7].

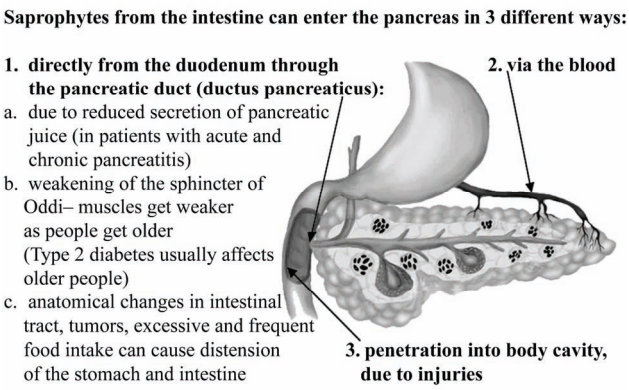

Fig. 1 – Possible ways of pancreatic infection.

Effects of infections *in vivo* on insulin secretion

Infections in the body can influence insulin secretion and glucose metabolism. Infection causes marked changes in whole body glucose metabolism as a result of acceleration in endogenous glucose production due to increased gluconeogenesis [12]. It was shown that acute infection in humans causes insulin resistance and glucose intolerance [13]. Endotoxin or lipopolysaccharide (LPS) is a potent stimulator of inducible nitric oxide synthase (iNOS). LPS injection lead to hyperglycemia, insulin resistance and increased iNOS protein expression and activity [14]. One study with rats infected with *E. coli* demonstrated that infected animals were hyperthermic and showed the increased rates of glucose metabolism as well as mild hyperlacticacidemia. Plasma catecholamine concentrations were increased by 50%–70% [15]. Results of experiment with male rats treated with bacterial endotoxin (*Salmonella enteritidis*) suggest that variations in an individual's early life bacterial environment may contribute to differences in glucose homeostasis, insulin action and disease susceptibility later in life [16]. The likelihood of elevated C-reactive protein (CRP) concentrations increase with increasing of HbA1c levels. Exposure to multiple pathogens could cause a chronic low-grade inflammation, resulting in insulin resistance [17].

The homeostatic microbiome

The HM consists of all microorganisms that normally inhabit the human body (gastrointestinal system, urogenital system, skin and all cavities lined with mucosa) and it is necessary to maintain normal homeostasis and health. It follows that the HM (i.e., saprophytic microorganisms) is an important contributor to physiology. Since there is a mutual interaction on genetic and biochemical levels between microorganisms that inhabit different places in human body, so there is their communication with other physiological systems that participate in the homeostasis of the human body – the law of connected vessels (Figure 2).

Fig. 2 – Connection of the homeostatic microbiome with other physiological systems involved in maintenance of homeostasis in organism (endocrine, nervous, muscular, integumentary, skeletal, female and male reproductive, urinary, digestive, respiratory, cardiovascular and lymphatic system).

After birth, many microorganisms rapidly colonize the intestinal tract and remain there throughout life. After the death they contribute to the decay of the organism. Homeostatic microorganisms in the body may have two effects: primary, being involved in the digestion of food (food as nutrient medium for their growth and development), and secondary, the production of metabolites that are absorbed by the blood thereby affecting the me-

tabolism and physiological state of the organism. Factors that cause disorder of saprophytic flora are improper diet, too cold or too hot food and beverages (growth of *Candida albicans* may be very invasive at 25°C), immunological insufficiency and long-term treatment with antibiotics, corticosteroids and immunosuppressive drugs [7]. Disruption of the HM may lead to intensive growth of some microorganisms. When these organisms enter other organs they can cause severe diseases and even death of the host.

The HM microorganisms can flourish and increase their numbers primarily in the gastrointestinal tract (GIT), and in the urogenital system, and could have indirect effects on insulin secretion. Until now, most attention is focused on one part of the HM – the gut microbial community. A healthy adult human harbors some 100 trillion bacteria in gut alone [18]. The human body contains a total of 10 times more microbes than the number of somatic cells and at least 100 times more genes in relation to the complete human genome [19].

Microbiome should be considered as an organ since it weighs 1–2 kg. Metagenomic analyzes of human mucosal and fecal samples established that phyla *Bacteroidetes* and *Firmicutes* constitute 90% of the microbiota and the rest (10%) are *Actinobacteria, Proteobacteria* and *Fusobacteria* [19, 20]. *Pseudomonas aeruginosa* and *Enterobacter* belong to the group of *Proteobacteria*, while *Staphylococcus* belong to the largest group, *Firmicutes* (Figure 3).

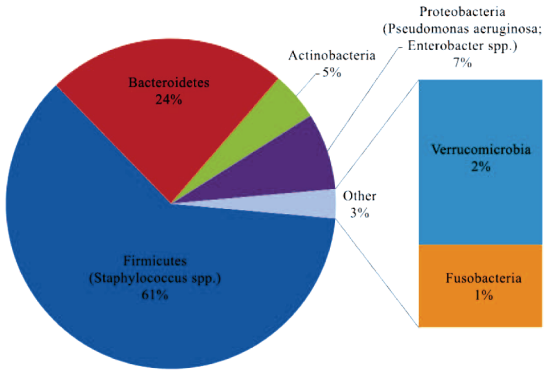

Fig. 3 – Percentage of specific phyla obtained by metagenomic analyses of human mucosal and fecal samples. *Pseudomonas aeruginosa* and *Enterobacter* belong to the group *Proteobacteria* while *Staphylococcus* belong to the largest group, *Firmicutes,* represented by 90%.

Microbiome researches are relating primarily to bacteria, not fungi. However, opportunistic infections caused by *Candida* are obvious example of disrupt homeostatic microbiome. The yeast *Candida albicans* is a commensal and a constituent of the normal microflora in 80% of human population where predominately colonize mucosal surface of GIT, but also urogenital tract and to a lesser extent the skin [21, 22]. It can cause infections that range from superficial to systemic and potentially life-threatening diseases, such as in patients with weakened immune system, i.e., patients with human immunodeficiency virus (HIV) or cancer [23] and in patients after long-term antibiotic therapy [24], as the growth of intestinal saprophytes (bacteria) is normally kept under control.

The growth of *Candida* is normally limited by immune system and other microorganism occupying the same location of the body. Immune response to *Candida* infections in human is not well known. I suppose it is not likely that the yeast growth is directly correlated with activation of gut associated lymphoid tissue (GALT). It is more likely that saprophytic bacteria normally present in intestinal tract have substantial influence on *Candida* growth control and antibiotic therapies could disrupt this relation leading to persistent *Candida* infections. Each microorganism in the human body occupies a particular ecological niche. They overlap and act as a system of connected vessels. Reduction in the number of one type of microorganism leads to an increase in the number and spread of other types. Microorganisms in a certain amount are an essential factor of every human HM. Due to increased number, some microorganisms may become pathogenic and threaten the health of the host. If the presence of all microorganisms in the HM is presented as 100%, it is not determined what percentage occupies each microorganism. Determining their ideal relationship, it would be easier to predict and prevent certain diseases in humans. The HM plays an important role in health preserving of the host. Disorder in the HM leads to insulin resistance, obesity and diabetes mellitus, but it is also assumed that disorder in the HM can provoke other diseases.

The HM may affect heart disease. Amount of formic acid in urine is inversely proportional to blood pressure which is a risk factor for heart disease. Important source of formic acid is microbiome in intestine [25]. It is known that microorganisms by mimicry can provoke an autoimmune attack and the onset of diabetes mellitus or multiple sclerosis [26]. There is a possible connection between microbiome and autism [27]. It is known that typhus, caused by infection of GIT, is characterized by headaches, constipation, and at a later stage, by diarrhea and disturbance of consciousness (neurological symptoms) [28]. Some data suggest the impact of microbiota on the incidence of cancer [29]. Formation of the HM is shown in Figure 4 (Ring of Life) and takes place in several stages: pregnancy and childbirth, breastfeeding, contact with family and wider environment, nutrition and sexual contacts.

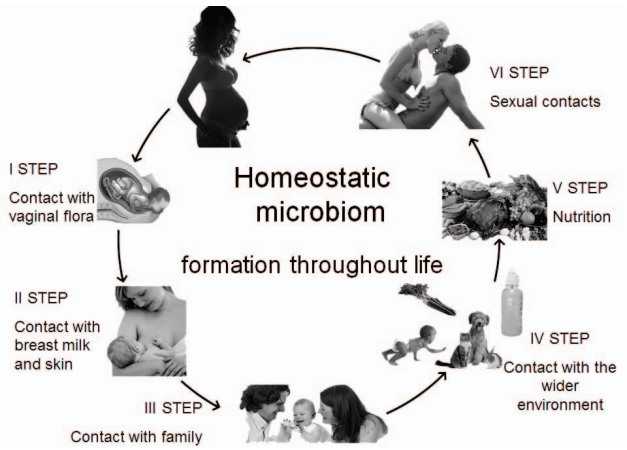

Fig. 4 – Ring of life. Steps important in formation of homeostatic microbiome throughout life.

Pregnancy

Microbial abundance and diversity might differ in pregnancy. Metagenomic analysis of DNA that was isolated from the vagina showed that the diversity of microbiota was reduced in pregnancy with dominance of *Lactobacillus* species and the orders *Lactobacillales* (and *Lactobacillaceae* family), *Clostridiales*, *Bacteroidales*, and *Actinomycetales* [30]. Thus, during pregnancy, the first group of the HM organisms is present which will come into contact with the baby during birth.

Childbirth

At a delivery, the child comes into contact with the HM of its mother (microflora of the urogenital and GIT). Several lines of evidence suggest that this can influence the likelihood in later life of overweight and obesity. For example, the composition and development of infant gut microbiota are influenced by weight and weight gain of mothers during pregnancy. Fecal microbial composition showed significantly higher concentrations of *Bacteroides* and *Staphyloccocus* and lower concentrations of *Bifidobacterium* group in infants of overweight mothers [31]. Kalliomaki et al. [32] reported that numbers of the genus *Bifidobacterium* was higher in children with normal weight than in children developing overweight. In addition, number of fecal *S. aureus* organisms was lower in children with normal weight than in obese children. They suggest that *S. aureus* may act as a trigger of low-grade inflammation, contributing to the development of obesity [33]. *In vitro* studies showed that *Staphyloccocus* spp. drastically reduce insulin secretion of human pancreatic islets leading to insulin resistance and obesity [6, 7].

Breastfeeding

Infants receive their first microbial "package" at the time of the birth. These bacteria reflect the microbiota of maternal vagina and GIT. Further intestinal colonization takes place during breastfeeding by bacteria in breast milk and breast skin. This finding begs the question as to what role this community plays in colonization of the infant GIT and maintaining mammary health [34]. Bacteria are better for extracting nutrition from mothers' milk since induce glycoside hydrolases which converts carbohydrate glycans, which are abundant in milk, into usable sugars. The HM has the capacity to break down complex, indigestible carbohydrates and creates small fatty-acid molecules as waste products, particularly formic acid, acetic acid and butyric acid that can pass through the gut wall into the bloodstream [19, 20]. Breastfeeding protects infants from diarrheal and respiratory diseases and it is associated with a reduced risk of developing obesity [35, 36].

Culture-dependent methods confirmed the presence of *Staphylococcus* and *Streptococcus* (most abundant species) in human milk [37]. Techniques based on amplification of bacterial 16S rRNA in human milk detected several genera of bacteria, including *Lactobacillus* and *Bifidobacterium* (relative abundance 2%–3%). *Staphylococcus* was represented by 22%–59%. Previous studies showed that the microbiota present in the lower GIT [38], vagina [39], oral cavity [40], and more importantly, the differential composition of these communities in healthy versus diseased states, are related to the health of the human host. Human milk contains oligosaccharides with probiotic properties that promotes the growth of bifidobacteria after birth [33, 41, 42].

Contact with family

Contact with family is essential for the formation of a functioning HM. Analyzes of the genome of *Methanobrevibacter smitthii* and *Bacteroides thetaiotaomicron* strains of close relatives who live together showed it is identical in 96% [42]. Thus, early gut colonizers, such as those acquired from parents and siblings, may remain in the intestine throughout the life of the individual.

Recent studies [43] have shown during five-year monitoring of 37 patients, by using a method for bacterial 16S rRNA amplicon sequencing, that individual microbiota was remarkably stable, with 60% strains (approximately 100 species) remaining over this period. This suggests that most strains are intestinal residents for decades. Members of *Bacteroides* and *Actinobacteria* are significantly more stable components than the population average.

Contact with the wider environment (ecological niche)

A child with its microbiome represents an entity which occupies one ecological niche. This niche will inevitably overlap with other spatial niches, animal, plant and ecological niches of other children, which could also lead to the formation of specific microbiome.

Sexual contacts

There are indications that human microbiota have a protective role in the prevention of sexually transmitted diseases (bacterial vaginosis) [44]. In my opinion, sexual contact is also important in formation and maintenance of the HM. Sexual contact inevitably leads to the exchange between the microbiome of partners. This contact is an essential and useful for individual to maintain social contact with other person, but it can also be harmful to the HM leading to its disruption if microbiome of two individuals is not compatible. Incompatibility of two microbiome may lead to sterile marriages. Promiscuous persons are subjected to frequent distortions of their HM. Probably microbiome of reproductive system affects sperm motility and thus indirectly affects the sex of the child. This subject is not sufficiently explored.

Nutrition

Nutrition is one of the key factors in the early development and long-term maintenance of homeostatic microbiome. The question is how diet affects obesity and whether it is the main cause of obesity and diabetes mellitus. Nutritionists recommend for healthy diet the following: 20% of

protein intake, to 50%–60% of carbohydrate intake and lipid intake less than 30% [45]. It has long been known that carbohydrates are necessary for the health preservation. One of the most ancient data is written in the Bible [46]. During the seven years of hunger, Jacob sent his sons in Miser for wheat: "I hear there is wheat in Miser. Go there and buy it so we stay alive and don`t die of hunger". Back then, they knew they would not survive without wheat, and they had cattle, sheep, goats, and camels. In contrast to this recommendation [45], a nation from the north, the Inuit, does not have carbohydrates in their thousand-year-long traditional diet. They mostly eat fishes, marine mammals and caribous. Inuit are not prone to diabetes mellitus and cardiovascular diseases. What sets them apart from other nations? The answer is the composition of the HM.

A research on twins shown that genetic factors do not have a decisive influence on obesity [47]. He found out that twins of which one is nourished and the other is malnourished had different microbiome. Recent studies (rodent model and in human) have shown that changes in gut microbiota may play an important role in the development of obesity [48]. Genetically obese mice had 50% reduction in *Bacteroidetes* and a proportional increase in *Firmicutes* compared to lean mice. Comparing distal gut microbiota of obese and lean human subject, Ley et al. [49] demonstrated that obese people had lower *Bacteroidetes* and more *Firmicutes* than lean subjects. After a fat restricted or a carbohydrate restricted low calorie diet, the ratio of *Bacteroidetes* to *Firmicutes* approaches a lean type.

In a series of experiments, Bäckhed et al. [45] showed that conventionally reared mice had 40% higher body fat content than germ-free mice, even they consumed less food. After conventionalization (transplantation of distal gut microbiota from normal mice to germ-free mice) body fat content increase 60% after 2 weeks without increase in food consumption. Cani et al. [50, 51] proposed that metabolic endotoxemia may provoke metabolic diseases such as diabetes mellitus and obesity in response to a high-fat diet. They hypothesized that bacterial lipopolysaccharide (LPS) from Gram-negative intestinal bacteria can be a triggering factor.

There are attempts of surgical interventions (Roux-en-Y gastric bypass) to reduce obesity and insulin resistance [52]. Approximately 80% of the patients with diabetes type 2 experience complete remission, defined as normoglycemia which can be explained by a reduction in body weight. It has also an impact on the food flow. Taken food bypasses the duodenum and directly goes to jejunum. Since there are no nutrients in the duodenum, microflora can not be held. Therefore, the possibility of penetration of the conditional pathogens through sphincter of Oddi into the pancreas is significantly reduced. Their impact and penetration into the pancreas is previously discussed [6, 8]. Metagenomic analysis of samples from the jejunum reveals that most represented bacteria is of genus *Streptococcus*, whereas dominant bacteria in the distal ileum, colon and rectum are *Bacteroidetes* and *Firmicutes*. This is anatomically very dangerous operation; it bypasses most of the stomach and the upper part of the small intestine and reduces the metabolic and exogenous effects of the pancreas and liver.

Obese and lean people have a different composition of the microflora, so it can play a role in the development of obesity. High-fat and low-fiber diet changes microbiome, leading to metabolic endotoxemia, increased depositing of lipids and decreased sensitivity to insulin resistance [50, 51].

Reduced number of *Bifidobacterium* and increased number of *Firmicutes* spp (274 genera, including *Staphylococcus*, *Enterococcus* and *Streptococcus*) lead to increased permeability of the intestinal epithelium and elevated levels of fatty acid and endotoxin in the blood and at the same time reduce the concentration of ghrelin, angiopoietins (angiopoietin-like protein 4, that inhibits the absorption of fatty acids from circulating triglycerides in adipose and muscle tissue, a potent inhibitor of lipoprotein lipase), and in turn, lead to an increase lipogenesis in the liver and adipose tissue, number of B-cells and insulin secretion in pancreas decrease and insulin resistance occurs in muscle (reduced insulin sensitivity) [53].

To restore normal relationship within the microbiome that inhabits GIT it is recommended to take prebiotics in the diet. Probiotics may change the composition of the microflora only briefly because without intake of probiotics comes to normalization and the establishment of the previous microflora in GIT.

A prebiotic is a non-digestible food ingredient, particularly oligosaccharides, that selectively stimulates the growth of beneficial commensal colonic microbiota (e.g., *Bifidobacterium* and *Lactobacillus* species) and thus improves host health [54]. Among the natural non-digestible oligosaccharides that fulfill the criteria of a colonic food are fructo-oligosaccharides that are fermented by a number of colonic bacteria to modulate the growth of beneficial bacteria [55]. The number of *Bifidobacteria* increases in the presence of inulin-type fructans. This increase occurs within a few days, but rapidly disappears upon withdrawal of prebiotics, after one week [56]. The extent of increase in number of *Bifidobacteria* depends on their initial number.

Dietary fructans, present in various fruits and vegetables or as food additives, are used as an energy substrate by bacteria, including *Bifidobacterium* spp, that express β-fructofuranosidase, which promotes their growth in the gut. The number of *Bifidobacterium* spp in mice with diet-induced or genetically determined obesity increase with intake of inulin-type fructans [57]. The number of *Bifidobacteria* was inversely correlated with the development of fat mass, glucose intolerance and lipopolysaccharide (LPS) level [58].

Summary-impact of contemporary nutrition on the homeostatic microbione

Food with long-term preservation is present on today's market. This means that such food produced on farms does not contain the necessary and essential microbial agents. Herbal food is produced on large surfaces. In such large plantations it is necessary to treat the surface with insecticides, herbicides and particularly fungicides that directly destroy the natural microorganisms normally inhabiting the surface of fruits and vegetables.

It is the same case with animal food. Animals are mostly grown on large farms and treated with antibiotics.

The food that they eat is also obtained by modern farm growing. Animals (cows, pigs and poultry) generally lead sedentary lifestyle, which means that they have no contact with the microbiome of the soil and the environment. Such food is usually represented in highly developed countries where the highest percentage of diabetes mellitus and obese people is presented. Organically produced food was grown in the villages and individual farmers (e.g., in Serbia), the food was produced in small quantities and was not treated with chemical preparations and originally contained a certain percentage of indigenous bacteria and fungi, and that is the advantage of such a diet. One of the factors that accompanies the onset of diabetes mellitus and obesity is the migration of the population. A person grew up in one ecological system and had contact with one microbiome through diet and direct contact, and once moving, for example, from Europe to America comes to an entirely different surroundings. This is direct evidence that the homeostatic saprophyte flora acquired by a healthy diet for years must be maintained throughout life and without a big attack by intake of unknown and new types of microorganisms. To understand better the impact of local microbes on food, it is enough to mention the original production of cheeses that are very different from place to place and have different tastes and odors, originating mostly from local microbiological agents. All listed above indicate that food should be considered not only as a source of essential nutrients but also as a necessary source of microbiological agents. Many harmful microbiological agents if present normal amounts in the HM represent the real source of essential metabolites and enzymes that contribute to maintaining the host health, but if their numbers begin to grow without control, they can cause severe illness and death of the host. Example of the symbiosis of the organism and the microbiome is peristalsis of GIT. It is well known how dangerous it is to interrupt normal movement of food and bowel motion as well as the consequences of persistent diarrhea for the body. *E. coli* causes diarrhea, but the question is whether its presence in allowed quantity is necessary for the regular emptying of the bowel.

The question is which is the favorable ratio of microbes in the body, particularly in the intestine. Their percentage in healthy people and in members of the same population should be determined and their relationship and tolerance to change of amount should be expressed in mathematical formulas. By establishing a mathematical model for the HM, it is easy to determine disorders and predisposition for certain diseases, in this case diabetes mellitus. Why food rich in sugar is often accompanied by overgrowth of the fungus *Candida*. According to our results *Candida* increases insulin secretion of pancreatic islets, thus helping the elimination of high glucose doses in the blood and prevents glucotoxicity. It is obvious example of the necessity to take food and associated microbiome together – *Candida* is the most present in the sugar-rich food. Intake of probiotics is desirable, but in the disrupted HM they cannot compensate all microbial agents that are necessary for the preservation of human health. It is known that certain members of the HM regulate and affect the insulin secretion.

Our research results demonstrated that microorganisms can influence on pancreatic islets insulin secretion. Namely, they perform their impact directly (when present in pancreas) [59] and indirectly, by secreting their metabolites which have influence on pancreas islets trough the blood vessels - as a consequence of the increase in their number in human body, disorder of the HM emerged [60] (Figure 5).

Bacterial agents (*Enterobacter* spp, *Pseudomonas aeruginosa*, *Staphyloccocus* spp.) reduce insulin secretion leading to postprandial hyperglycemia.

Fungal agents (e.t., *Candida albicans*) increase insulin secretion causing postprandial hypoglycemia and insulin resistance. It is known that increased insulin secretion is frequent in obese persons.

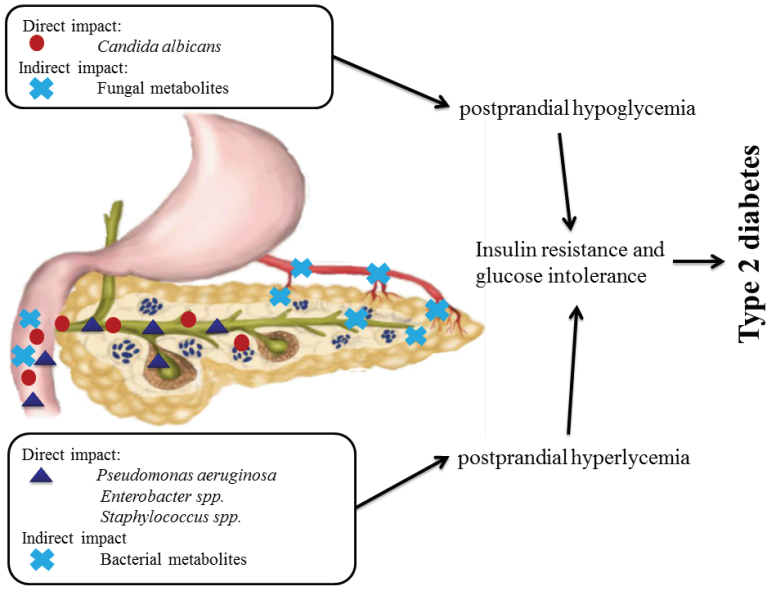

Fig. 5 – Direct and indirect influence of Candida and bacteria on the development of diabetes mellitus type 2.

Both cases lead to glucose intolerance and insulin resistance and in some cases the development of the DM type 2 and obesity.

In a healthy body, microorganisms are part of the homeostatic microbiome and play a key role in maintaining health, digestion and metabolism. Formation of the HM (Ring of Life) takes place in several stages: pregnancy and childbirth, breastfeeding, contact with family and wider environment, nutrition and sexual contacts. Many internal and environmental factors can lead to disorders of homeostatic microbiome, which leads to certain diseases, including disorder of glucose homeostasis.

Conclusion

We can conclude that infection of pancreas and change (disrupt) of the HM play an important role in etiopathogenesis of diabetes mellitus and obesity.

The HM is a great enigma and a challenge for further scientific research. The goal is to initiate extensive researches – interactive collaboration of scientific community in various fields, researches linking the man as an individual in a sustainable ecosystem, but they should be strictly controlled because the knowledge and the results can be manipulative, used not only for treatment but also for causing the disease. Complete understanding of the human microbiome could easily compromise moral, ethical and religious principles of understanding life and it is a serious subject for another debate and discussion.

Acknowledgment

This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grant No. 41002).

R E F E R E N C E S

1. International Diabetes mellitus Federation, 2014. Available from: http://www.idf.org/diabetes mellitusatlas
2. *Saltiel AR, Kahn CR.* Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001; 414(6865): 799–806.
3. *Bloomgarden ZT.* The 1st World Congress on the Insulin Resistance Syndrome. Diabetes Care 2004; 27(2): 602–9.
4. *Nesto RW.* The relation of insulin resistance syndromes to risk of cardiovascular disease. Rev Cardiovasc Med 2003; 4 Suppl 6: S11–8.
5. *Scheen AJ.* Pathophysiology of type 2 diabetes. Acta Clin Belg 2003; 58(6): 335–41.
6. *Nikolić DM.* Effects of bacterial infection on insulin secretory capacity of human adult pancreatic islets. Br J Biomed Sci 2011; 68(4): 181–4.
7. Nikolic DM. Effects of Candida on insulin secretion of human adult pancreatic islets and possible onset of diabetes. Br J Biomed Sci 2014; 71(2): 73–8.
8. *Slavov E, Georgiev IP, Dzhelebov P, Kanelov I, Andonova M, Mircheva Georgieva T,* et al. High-fat feeding and Staphylococcus intermedius infection impair beta cell function and insulin sensitivity in mongrel dogs. Vet Res Commun 2010; 34(3): 205–15.
9. *Gloor B, Müller CA, Worni M, Stahel PF, Redaelli C, Uhl W*, et al. Pancreatic infection in severe pancreatitis: the role of fungus and multiresistant organisms. Arch Surg 2001; 136(5): 592–6.
10. *Chung RT, Schapiro RH, Warshaw AL.* Intraluminal pancreatic candidiasis presenting as recurrent pancreatitis. Gastroenterology 1993; 104(5): 1532–53.
11. *Bonatti H, Steurer W, Konigsrainer A, Allerberger F, Margreiter R.* Infection of the pancreatic duct following pancreas transplantation with bladder drainage. J Chemother 1995; 7(5): 442–5.
12. *McGuinness OP, Donmoyer C, Ejiofor J, McElligott S, Lacy DB.* Hepatic and muscle glucose metabolism during total parenteral nutrition: impact of infection. Am J Physiol 1998; 275(5 Pt 1): E763–9.
13. *Sammalkorpi K.* Glucose intolerance in acute infections. J Intern Med 1989; 225(1): 15–9.
14. *Sugita H, Kaneki M, Tokunaga E, Sugita M, Koike C, Yasuhara S*, et al. Inducible nitric oxide synthase plays a role in LPS-induced hyperglycemia and insulin resistance. Am J Physiol Endocrinol Metab 2002; 282(2): E386–94.
15. *Hargrove DM, Bagby GJ, Lang CH, Spitzer JJ.* Adrenergic blockade does not abolish elevated glucose turnover during bacterial infection. Am J Physiol 1988; 254(1 Pt 1): E16–22.
16. *Walker FR, Owens J, Ali S, Hodgson DM.* Individual differences in glucose homeostasis: Do our early life interactions with bacteria matter? Brain Behav Immun 2006; 20(4): 401–9.
17. *Fernández-Real JM, López-Bermejo A, Vendrell J, Ferri MJ, Recasens M, Ricart, W.* Burden of infection and insulin resistance in healthy middle-aged men. Diabetes Care 2006; 29(5): 1058–64.
18. *Devaraj S, Hemarajata, Versalovic J.* The human gut microbiome and body metabolism: Implications for obesity and diabetes mellitus. Clin Chem 2013; 59(4): 617–28.
19. *Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS,* et al. Metagenomic analysis of the human distal gut microbiome. Science 2006; 312(5778): 1355–9.
20. *Candela M, Maccaferri S, Turroni S, Carnevali P & Brigidi P.* Functional intestinal microbiome, new frontiers in prebiotic design. Int J Food Microbiol 2010; 140(2–3): 93–101
21. *Staib P, Kretschmar M, Nichterlein T, Hof H, Morschhäuser J.* Differential activation of a Candida albicans virulence gene family during infection. Proc Natl Acad Sci USA 2000; 97(11): 6102–7.
22. *Berman J, Sudbery PE.* Candida albicans: a molecular revolution built on lessons from budding yeast. Nat Rev Genet 2002; 3(12): 918–30.
23. *Miller LG, Hajjeh RA, Edwards JE Jr.* Estimating the cost of nosocomial candidemia in the United States. Clin Infect Dis 2001; 32(7): 1110.
24. *Biswas S, Van Dijck P, Datta A.* Environmental sensing and signal transduction pathways regulating morphopathogenic determinants of Candida albicans. Microbiol Mol Biol Rev 2007; 71(2): 348–76.
25. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, Pettersson S. Host-gut microbiota metabolic interactions. Science 2012; 336(6086): 1262–7.
26. *Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W,* et al. Host-gut microbiota metabolic interactions. Science 2012; 336(6086): 1262–7.
27. *Louis P.* Does the human gut microbiota contribute to the etiology of autism spectrum disorders? Dig Dis Sci 2012; 57(8): 1987–9.
28. *Helmick CG, Bernard KW, D'Angelo LJ.* Rocky Mountain spotted fever: clinical, laboratory, and epidemiological features of 262 cases. J Infect Dis 1984; 150(4): 480–8.
29. *Uronis JM, Mühlbauer M, Herfarth HH, Rubinas TC, Jones GS, Jobin C.* Modulation of the intestinal microbiota alters colitis-as-

sociated colorectal cancer susceptibility. PLoS One 2009; 4(6): e6026.
30. *Aagaard K, Riehle K, Ma J, Segata N, Mistretta TA, Coarfa C,* et al. A metagenomic approach to characterization of the vaginal microbiome signature in pregnancy. PLoS One 2012; 7(6): e36466.
31. *Collado MC, Isolauri E, Laitinen K, Salminen S*. Effect of mother's weight on infant's microbiota acquisition, composition, and activity during early infancy: a prospective follow-up study initiated in early pregnancy. Am J Clin Nutr 2010; 92(5): 1023–30.
32. *Kalliomäki M, Collado MC, Salminen S, Isolauri E*. Early differences in fecal microbiota composition in children may predict overweight. Am J Clin Nutr 2008; 87(3): 534–8.
33. *Marik PE*. Colonic flora, probiotics, obesity and diabetes mellitus. Front Endocrinol (Lausanne) 2012; 3: 87.
34. *Hunt KM, Foster JA, Forney LJ, Schütte UM, Beck DL, Abdo Z*, et al. Characterization of the diversity and temporal stability of bacterial communities in human milk. PLoS One 2011; 6(6): e21313.
35. *von Kries R, Koletzko B, Sauerwald T, von Mutius E, Barnert D, Grunert V,* et al. Breast feeding and obesity: cross sectional study. BMJ 1999; 319(7203): 147–50.
36. *Gillman MW, Rifas-Shiman SL, Camargo CA Jr, Berkey CS, Frazier AL, Rockett HR* et al. Risk of overweight among adolescents who were breastfed as infants. JAMA 2001; 285(19): 2461–7.
37. *Heikkila MP, Saris PE*. Inhibition of Streptococcus aureus by the commensal bacteria of human milk. J Appl Microbiol. 2003; 95(3): 471–8.
38. *Ott SJ, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Fölsch UR,* et al. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. Gut 2004; 53(5): 685–93.
39. *Schwebke JR*. Abnormal vaginal flora as a biological risk factor for acquisition of HIV infection and sexually transmitted disease. J Infect Dis 2005; 192(8): 1315–7.
40. *Colombo AP, Boches SK, Cotton SL, Goodson JM, Kent R, Haffajee AD*, et al. Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis and periodontal health using the human oral microbe identification microarray. J Periodontol 2009; 80(9): 1421–32.
41. *Bode L*. Human milk oligosaccharides: prebiotics and beyond. Nutr Rev 2009; 67(Suppl 2): S183–91.
42. *Chichlowski M, German JB, Lebrilla CB,Mills DA*. The influence of milk oligosaccharides on microbiota of infants: opportunities for formulas. Annu Rev Food Sci Technol 2011; 2: 331–51.
43. *Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL,* et al. The long-term stability of the human gut microbota. Science 2013; 341(6141): 1237439.
44. *Brotman RM*. Vaginal microbiome and sexually transmitted infections: an epidemiologic perspective. J Clin Invest 2011; 121(12): 4610–7.
45. *Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A* et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 2004; 101(44): 15718–23.
46. Serbian Bible. Belgrade: British and Foreign Biblical Society Genesis 1981. 42; 2–3. (Serbian)
47. *Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE*, et al. A core gut microbiome in obese and lean twins. Nature 2009; 457(7228): 480–4.
48. *Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G,* et al. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA 2004; 292(12): 1440–6.
49. *Ley RE, Turnbaugh PJ, Klein S, Gordon JI*. Microbial ecology: human gut microbes associated with obesity. Nature 2006; 444(7122): 1022–3.
50. *Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D,* et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007; 56(7): 1761–72.
51. *Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O,* et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009; 58(8): 1091–103.
52. *Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Leccesi L,* et al. Bariatric surgery versus conventional medical therapy for type 2 diabetes. N Engl J Med 2012; 366(17): 1577–85.
53. *DiBaise JK, Zhang H, Crowell MD, Krajmalnik-Brown R, Decker GA, Rittmann BE*. Gut microbiota and its possible relationship with obesity. Mayo Clin Proc 2008; 83(4): 460–9.
54. *Cani PD, Delzenne NM*. The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des 2009; 15(13): 1546–58.
55. *Xiao S, Fei N, Pang X, Shen J, Wang L, Zhang B,* et al. A gut microbiota-targeted dietary intervention for amelioration of chronic inflammation underlying metabolic syndrome. FEMS Microbiol Ecol 2014; 87(2): 357–67.
56. *Schrezenmeir J, de Vrese M*. Probiotics, prebiotics, and synbiotics - approaching a definition. Am J Clin Nutr 2001; 73(2 Suppl): 361S–4S.
57. *Roberfroid MB*. Functional foods: concepts and application to inulin and oligofructose. Br J Nutr 2002; 87 Suppl 2: S139–43.
58. *Gibson GR, Roberfroid MB*. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995; 125(6): 1401–12.
59. *Nikolic DM*. Effects of pancreatic infection on insulin secretion and posible onset of diabetes. In Proceedings of the International Conference on Clinical Microbiology and Microbial Genomics; San Antonio USA; 2012 November 12–14. J Microbial Biochem Technol 2012; 4(5): 39.
60. *Nikolic DM*. The Influence of Microorganisms (Microbiom) on Insulin Secretion of Human Pancreatic Islets. New Data on the Etiopathogenesis of Type 2 Diabetes mellitus. Proceedings of the BITs 5th Annual World DNA and Genome Day; 2014 April 25–28. Dalian, China; 2014. p. 130.

Received on December 16, 2015.
Revised on January 17, 2017.
Accepted on February 14, 2017.
Online First March, 2017.