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The influence of dietary compounds on gut microbiota

Vpliv prehrane na črevesno mikrobioto

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Abstract

The gut microbiota is a complex community composed of trillions of microbes that adapts to its host over the lifetime. Recently, the advances of the methods of high-throughput sequencing have allowed the identification of microbial species in a stool sample, and mass spectrometry identification of their metabolites, both of which together have enabled much of the relevant research in the field. It has became evident that gut microbiota plays an important role in human health and influences the risk of developing many chronic diseases, including obesity, inflammatory bowel disease, type 2 diabetes, cardiovascular disease, and cancer. The diverse ecosystem of the gut includes bacteria, viruses, phages, yeasts, archaea, fungi and protozoa. They are responsible for the production of bioactive metabolites, regulation of immune function, energy homeostasis and protection against pathogens. The mentioned functions are dependent on the diversity and abundance of the microbiota which is the reflection of the dietary habits and genetics of the host among other factors. As such, gut microbiota has significant interindividual variations. Diet and lifestyle changes present important determinants in microbiota shaping. The use of antibiotics, different sanitation measures, consumption of processed food and different diets are also reflected in the shifts of gut microbiota composition. Some of the dramatic dietary alterations can cause changes in gut microbiota composition already within 24 h and some of these changes may be difficult to reverse. Through modulation of gut microbiota composition, diet could offer a potential to manage the risk of developing disease and at the same time improving the quality of life and longevity. In this review we look at the role of diet, and specific dietary components, namely carbohydrates, proteins, fats and polyphenols on gut microbiota composition.

Izvleček

Črevesna mikrobiota je kompleksna skupnost, sestavljena iz milijarde mikroorganizmov, ki živijo z gostiteljem in se mu vse življenje prilagajajo. V zadnjem času je napredek metod sekvencioniranja DNK visoke zmogljivosti omogočil identificiranje posameznih vrst bakterij v vzorcu blata, metoda masne spektrometrije identifikacijo njihovih presnovkov, oboje pa veliko raziskav na tem področju. Postalo je očitno, da igra črevesna mikrobiota pomembno vlogo pri zdravju ljudi in vpliva na tveganje za razvoj številnih kroničnih bolezni, vključno z debelostjo, vnetno črevesno boleznijo, diabetesom tipa 2, srčno-žilnimi boleznimi in rakom. Raznolik ekosistem v črevesju zajema bakterije, viruse, fage, kvasovke, arheje, glive in protozoje. Odgovorni so za tvorbo bioaktivnih presnovkov, uravnavanje imunskega delovanja, energijsko homeostazo in zaščito pred patogenimi mikroorganizmi. Te funkcije so odvisne od raznolikosti in številčnosti mikrobiote, ki pa je med drugim tudi odraz prehranjevalnih navad in genetike gostitelja. Črevesna mikrobiota tako kaže pomembne razlike med posamezniki. Prehrana in življenjski slog sta pomembna dejavnika pri oblikovanju mikrobiote. Uporaba antibiotikov, različni sanitarni ukrepi, uživanje predelane hrane in različne diete se kažejo tudi v spremembah sestave mikrobiote črevesja. Nekatere dramatične prehranske spremembe lahko povzročijo hitre spremembe v sestavi črevesne

mikrobiote, in sicer že v 24 urah, nekatere od teh sprememb pa je težko povrniti v prvotno sestavo. Z moduliranjem sestave črevesne mikrobiote ponuja prehrana orodje za zmanjšanje tveganja za razvoj bolezni, hkrati pa izboljša kakovost življenja in vpliva na podaljšanje življenjske dobe. Namen preglednega članka je predstaviti dosedanje znanje o vplivu prehrane in posameznih sestavin hrane, in sicer ogljikovih hidratov, beljakovin, maščob in polifenolov na sestavo črevesne mikrobiote.

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1 Introduction

Scientists have been interested in gut microbiota for years but the inability to isolate and culture anaerobic microorganisms *in vitro* was holding back much of the relevant research in that field. With advances in the methods of high-throughput sequencing and mass-spectrometry in the last 15 years, it has finally become possible to identify microbial species and their function in a stool sample bypassing the traditional culture-dependent techniques for the isolation, identification and characterization of microorganisms (1,2). Nowadays, sequencing platforms allow analysis of all the genomes within an ecosystem (i.e. shotgun metagenomics), or a description of the taxa within a given community by sequencing conserved marker genes (i.e. 16S rRNA gene). On the other hand, mass spectrometry allowed detection of microbial derived products, including metabolites and proteins, which regulate numerous biological pathways and also facilitate interspecies interactions within the human host (1,3,4).

Gut microbiota consists of a diverse microbial community that encompasses 10^{14} microorganisms, including bacteria, viruses, phages, yeasts, archaea, fungi and protozoa, all of which are commensal and play a role in the complex interactions within the human gastrointestinal (GIT) tract (2,5). Within the host, it also varies taxonomically and functionally according to the intestine anatomical regions (3). The mutual synergy between the gut microbiota and its host is often being referred to as a »superorganism« or a host extra organ (6).

Merely in the colon, the density of bacterial cells has been estimated to be 10^{12} colony forming units (CFU) per gram content (5). The dominant phyla in GIT are Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. However, gram positive Firmicutes and gram negative Bacteroidetes represent 90% of gut microbiota (3,5). A useful method to stratify human gut microbiota is by separation of major taxa into clusters termed enterotypes. Three predominant variants (enterotypes) were identified by their enrichment in *Bacteroides* spp. (enterotype 1), *Prevotella* spp. (enterotype 2) and *Ruminococcus* spp. (enterotype 3). Taken into account that enterotypes are defined depending on gut microbiota

and gut microbiota changes rapidly in response to interventions, it would be easy to conclude that enterotypes are not a constant but rather a dynamic feature of individuals. However, even though enterotype is influenced by various factors, the alternations of microbiota composition during short intervals of time is insufficient to change the enterotype patterns due to the reversibility and relative stability of gut microbiota. Consequently, enterotypes are associated with long-term dietary habits and remain rather stable throughout the adulthood while at the same time are unrelated to nationality or host characteristics such as body mass index, age or gender (7). The gut microbiota is important for the health of its host by performing many immune and metabolic functions (3). It is involved in the extraction, synthesis, and absorption of many nutrients and metabolites (including bile acids, vitamins, amino acids) and it has a crucial role in preventing the colonization/invasion of pathogenic bacteria through the consumption of available nutrients, pH modification, competition for adhesion molecules and receptors, and/or producing antimicrobial substances such as acids, bacteriocins, and others (3,5). The most important metabolic activity of gut microbiota in the large intestine is the production of short chain fatty acids (SCFA) (acetate, propionate, and butyrate), mainly through fermentation of complex carbohydrates, such as oligosaccharides and resistant starch (6,8,9). Undigested proteins are also a substrate for SCFA production (9). Absorbable SCFAs are considered anti-inflammatory, an important modulators of gut health and immune function, intestinal hormone production, and lipogenesis (5,8,9). Furthermore, gut microbiota has a critical role in regulating the development, homeostasis and function of innate and adaptive immune cells (3).

The gut microbiota composition is formed in infancy, when individual is colonized by microorganisms from caregivers and the surrounding environment (10). The infant's microbiota is rather instable with low diversity of bacteria and is strongly influenced by the mode of delivery, birth gestational age, type of feeding and the introduction of complementary feeding. It changes through the exposure to a variety of environmental factors and eventually evolves toward a more stable adult-like composition by the age of 4 years (10-15). Furthermore, host genetics and dietary habits are responsible for the huge variability in microbiota composition and functionality among individuals. Likewise, body mass index, antibiotics, lifestyle, exercise frequency and ethnicity also influence the individual gut microbiota composition. Still, there is a similarity in microbiota composition among family members (3,11,16).

Diet is an important determinant of microbiota composition by modulating the abundance of specific species and their functions. These could in turn induce changes in host physiology, including disease development and progression (10-15). This review aims to provide the current knowledge of the role of diet (especially macronutrients) on gut microbiota composition but also presents in short other factors.

2 Influence of diet on the gut microbiota

In humans diet plays an important role in shaping the gut microbiota (17). It is the result of nutrient-induced selective pressures placed on the gut microbiota, favouring bacterial species enriched

in the genes required for specific substrate metabolism (18).

There were several fundamental changes in the lifestyle of humans throughout the history, which had an impact on the co-evolution of microbial species associated with the host. The last radical change of dietary habits occurred less than 200 years ago with the Industrial Revolution and the shift away from local and seasonal foods. Gut microbiota had to adapt to these profound changes in human diet. The modern diet is characterized by a high intake of animal products and sugars, low intake of plant-based foods (fruits, vegetables and wholegrain cereals) and the use of additives and preservatives (5).

Even though the gut microbiota of a healthy adult is relatively stable, the changes in gut microbiota composition and/or abundance occur frequently due to dietary choices. Fruits and nuts, whole grain products, vegetables and legumes, meat and diary products, as well as food constituents, such as fat, protein, phytochemicals and fibres, impact gut microbiota composition in humans (17). In general, plant-based diets increase luminal fibre and complex carbohydrate content selecting for species enriched in carbohydrate-active enzymes. On the other hand, animal-based diets that are rich in fats and proteins and low in fibre increase luminal bile acid content, favouring bile acid-resistant microbes enriched with genes for bile acid metabolism, such as bile salt hydrolases and sulfite reductase (18).

Particularly significant changes in diet (i.e. shifting from meat-based diets to strictly plant based) alter the composition and function of gut microbiota fast, already within 24 h (5,19). Furthermore, if the diet becomes less diverse or deficient in some nutrients, some species of the gut microbiota are cut off causing the disruption between the host and its intestinal microbiota. On contrary, a varied diet provides nutrients for a plethora of species of gut microbiota, which helps maintain homeostasis in the gut (11).

3 Carbohydrates and fibres

It has been estimated that the quantity of dietary carbohydrates that enter the colon each day is approximately 40 g and they belong to different categories such as: resistant starch, non-starch polysaccharides, oligosaccharides, as well as some di- and monosaccharides (4). The composition of gut microbiota is susceptible to both quality and quantity of ingested carbohydrates, which serve as the main carbon and energy source for the gut microbiota (11).

Upon degradation in the small intestine, starches and sugars (i.e. glucose, fructose, sucrose, and lactose) release glucose in the bloodstream and stimulate insulin response (5). Fibres may be of particular importance as a non-digestible carbohydrates, representing a primary energy source for many gut microorganisms and stimulating the growth and activity of beneficial gut microorganisms (17).

Excess intake of carbohydrates as part of a Western diet high in refined grains, starch, and added sugar and low intake of dietary fibres (only 15 g/day) negatively impacts gut microbiota and is associated with reduced diversity of gut microbiota (i.e. bifidobacteria increased while *Lactobacillus* spp., *Streptococcus* spp., and *Roseburia* spp. decreased) (20). In addition, lower amounts of SCFAs were found in individuals consuming a Western diet (20). On the contrary, people in traditional societies, with a high fibre intake (50–120 g/day) harbour a much

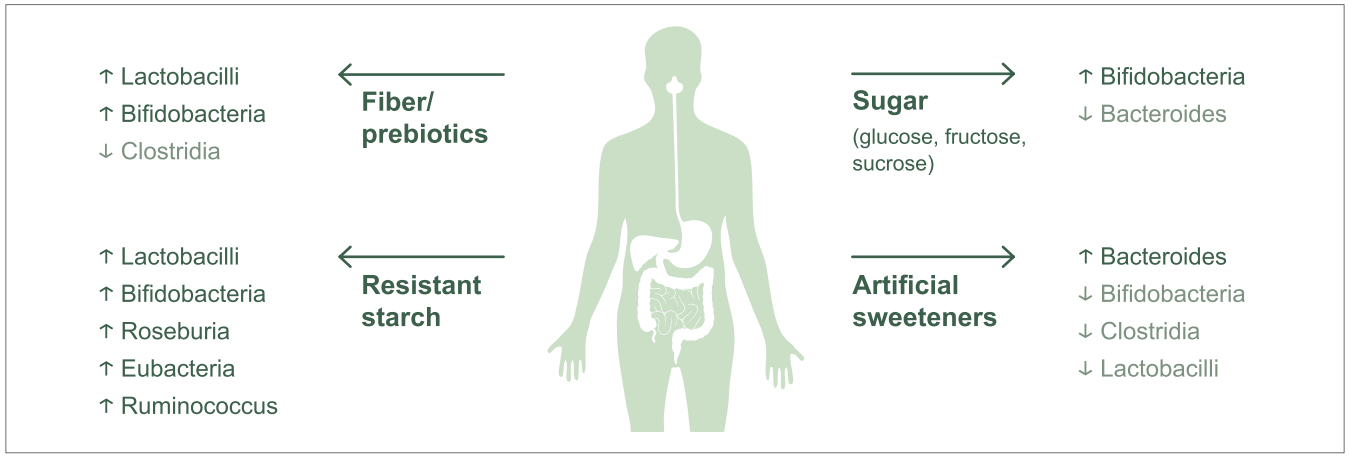

Figure 1: Impact of carbohydrates and fiber on gut microbiota (According to Singh et al., 2017) (22).

more diverse gut microbiota that is characterized by increasing good bacteria such as *Bifidobacteria* spp. and lactic acid bacteria and decreasing the counts of *Bacteroides* spp. (Figure 1). In addition, resistant starch and whole grain barley increased the abundance of *Ruminococcus* spp., *E. rectale*, and *Roseburia* spp. (4). Furthermore, as described in the study of Mills et al., people consuming high levels of simple sugars (glucose, fructose, and sucrose) in the form of date fruits had an increased abundance of bifidobacteria, with reduced *Bacteroides* spp. The addition of lactose to such diet caused the same bacterial shifts while also decreasing *Clostridia* spp. Also, lactose supplementation increased the fecal concentration of SCFAs (4).

Diet high in both sugar and fats resulted in gut microbiota dysbiosis characterised by a decrease in microbial diversity, an increase in clostridia and bacilli and a decrease in *Lactobacillus* spp. A noted increase in the Firmicutes:Bacteriodetes ratio was observed. Next, high sugar low fat diets caused an increase in two Proteobacteria members, namely *Sutterella* spp. and *Bilophila* spp. (4).

Artificial sweeteners (saccharin, sucralose, and aspartame) were originally marketed as a health-conscious, no-calorie alternative to natural sugar. However, recent work by Suez et al. showed that artificial sweeteners can lead to glucose intolerance faster than glucose or sucrose. The effects produced by artificial sweeteners were thought to be mediated through alteration of gut microbiota (i.e. increased abundance of *Bacteroides* spp. and decreased *Lactobacillus reuteri* (21).

4 Dietary protein

Dietary proteins have been reported to influence the composition of gut microbiota since 1977 (6) and the advances of 16S rRNA sequencing enabled the assessment of the impact of dietary protein on the composition of gut microbiota in much more detail.

Protein serves as an important nitrogen source for the gut microbiota (22). The undigested nitrogenous compounds that are not absorbed in the small intestine are transferred to the large intestine where they are metabolized by the resident gut microbiota. About 25 g of protein, peptides and free amino acids enter the colon on daily basis (4). The initial step of bacterial protein catabolism includes the extracellular hydrolysis of

proteins with hundreds of different bacterial proteases. Both the amount and kind of metabolites are directly influenced by the intake amount and source of dietary protein (8).

Bacteria involved in the protein metabolism in the small intestine include *E.coli*, *Streptococcus* spp., *Succinivibrio dextrinosolvens*, *Mitsuokella* spp., etc. (5,8). In the large intestine, the concentration of bacteria is much higher and the transit time longer than in the small intestine. Proteolytic activity in the large intestine has been associated with the genera *Bacteroides*, *Propionibacterium*, *Streptococcus*, *Fusobacterium*, *Clostridium*, and *Lactobacillus* (8,23). Gut microbiota and residual pancreatic proteases digest proteins and peptides that escape digestion in the small intestine, resulting in the production of numerous microbial metabolites (i.e. SCFAs, ammonia, polyamines, hydrogen sulfide, phenolic, indolic compounds, etc.). Some of these microbial metabolites (i.e. hydrogen sulfide, ammonia, and indolic compounds) have potentially negative effects on the host health. Others are bioactive molecules involved in various physiological processes in the host (8).

Factors, such as protein source, concentration and amino acid composition, can all affect gut microbiota and changes of gut microbiota composition can affect protein metabolism as well as the content of microbial metabolites produced. Both of the latter are closely associated with the health of the host (8).

The protein sources are mainly classified as of animal or plant origin. Consumption of plant protein positively correlates with overall microbial diversity due to its lower digestibility when compared to animal protein (4,5,8).

Consumption of soybean and peanut proteins positively modulates the beneficial bacterial composition in the GIT. A diet enriched with peanut protein or whey altered gut microbiota diversity with an increase of commensal *Bifidobacterium* spp. and *Lactobacillus* spp., and a reduction of *Enterobacteria* spp., *Bacteroides fragilis* and *Clostridium perfringens* (5,8). Increased numbers of *Bifidobacterium* spp. contribute to generation of more microbial metabolites, including SCFAs and lactic acid, resulting in a lower pH in GIT that inhibits toxic metabolites, such as amine and benzpyrole. Intake of soybean can alter gut microbiota composition with increased communities of genera *Escherichia* and *Propionibacterium* (8).

Compared to plant proteins, animal proteins are highly digestible, however, animal-based diets are often also high in fat in addition to protein (5). Increased numbers of bile-tolerant anaerobes (genera *Bacteroides*, *Alistipes* and *Bilophila*) were found in the microbiota of individuals consuming animal-based proteins (5). Casein has been shown to increase the counts of lactobacilli and bifidobacteria, while on the other hand decreasing the counts of *Staphylococcus* spp., coliforms, and *Streptococcus* spp. in GIT (8). Moreover, animal protein is characterized by a reduction of SCFA and an increase of both gut pH and ammonia concentration (8).

The individuals consuming a diet rich in beef had high levels of *Bacteroides* spp. and *Clostridium* spp. and at the same time low levels in *Bifidobacterium adolescentis* when compared to their vegetarian counterparts (5).

When protein intake is increased, the amount of undigested protein in the large intestine increases. Consequently, more substrate for gut microbiota is available. High concentration of protein increased the abundance of bile-tolerant

microorganisms (genera *Alistipes*, *Bilophila* and *Bacteroides*) while decreasing the levels of Firmicutes that metabolize plant polysaccharides (genera *Roseburia*, *Eubacterium rectale* and *Ruminococcus*) (24). In addition, high-protein diet led to a decrease in abundance of genera *Ruminococcus* and *Akkermansia* (4). High concentrations of protein intake can result in increases in counts of potential pathogens due to disruption in the homeostasis of the gut micro-ecosystem with reductions of beneficial microbes (8). Similarly, when the concentration of protein in the diet is too low to meet the basic requirement of the host, it can increase the abundance of potential pathogens (i.e. coliforms) and decrease the population of beneficial bacteria in the gut (i.e. lactobacilli). Furthermore, lower concentrations of dietary protein decreased butyrate-producing bacteria including lactobacilli and bifidobacteria, which have a protective, anti-inflammatory activity against carcinogenesis and intestinal disorders (25,26).

5 Fats

Dietary fat vary greatly with respect to its structure and composition of fatty acids. The latter can be short (i.e. 6 carbon atoms) or long (up to 24 carbon atoms), and can also contain double bonds (4). Besides the amount, fatty acid composition of the fat source is important when analyzing its impact on gut microbiota composition and function. The typical Western diet is both high in saturated and trans fats while low in mono- and polyunsaturated fats (5).

Animal studies have shown that a high fat diet leads to the establishment of microbiota low in *Lactobacillus intestinalis* and high in *Clostridiales*, *Bacteroidales*, and *Enterobacteriales* (27). In addition, lard-fed mice showed increased numbers of *Bacteroides* spp. and *Bilophila* spp. and reduced levels of *Desulfovibrio* spp., while those fed with fish oil had increased lactic acid bacteria (*Lactobacillus* spp. and *Streptococcus* spp.), genera *Verrucomicrobia* (*A. muciniphila*), and *Actinobacteria* (*Bifidobacterium* spp. and *Adlercreutzia* spp.) and mice fed with a diet rich in milk fat showed increased levels of *Bilophila wadsworthia* (27).

In humans, rapid and profound changes of gut microbiota composition caused by consumption of high-fat diets consisting solely of animal-based foods (i.e. meat and cheese) were observed in the study of David et al. The particular study showed that humans given an animal-based diet, consisting of 69.5% kcal from fat, 30.1% kcal from protein and nearly 0 g of fiber, altered the composition of gut microbiota within 48 hours of diet initiation (28). The growth of bile-tolerant and pathogenic hydrogen-sulfide producing bacteria such as *Bilophila wadsworthia* was observed in the same study (28). A recent interventional study has shown that a high-fat diet in healthy adults is associated with increased levels of *Alistipes* spp. and *Bacteroides* spp. and a decrease in *Faecalibacterium* spp. (29). Consumption of a low fat diet leads to the over-abundance of bifidobacteria and a reduction of fasting glucose and total cholesterol, while a high saturated fat diet increased the relative proportion of *Faecalibacterium prausnitzii* (30). High-fat diets also enrich the abundance of *Bacteroides* spp. as well as of total anaerobic microorganisms (Figure 2) (30,31).

When monounsaturated fat intake was high, no shifts in relative abundance of any bacterial genera were observed. However, such diet resulted in a reduced total bacterial load and plasma

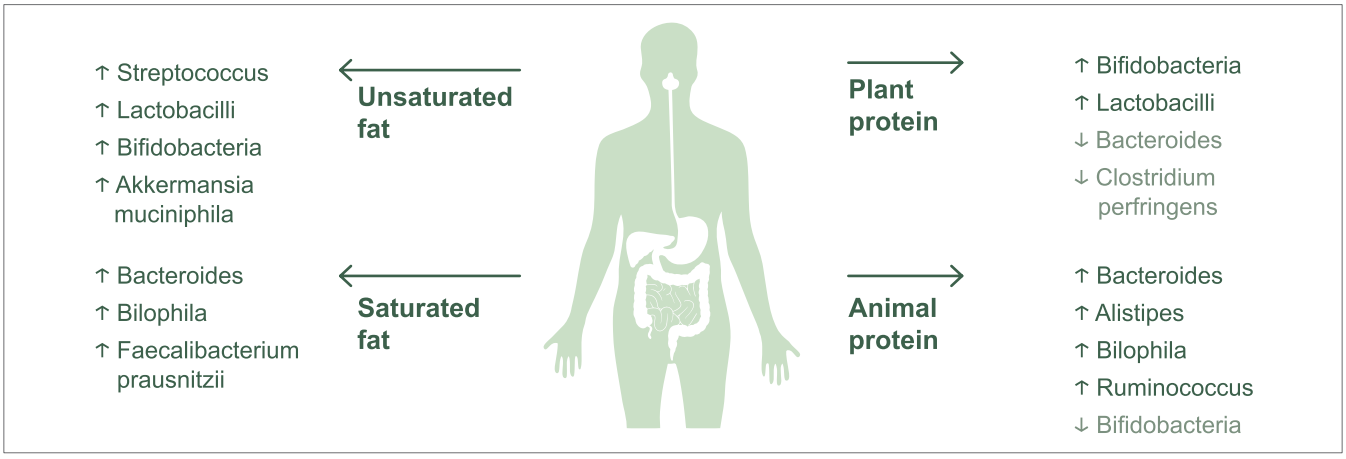

Figure 2: Impact of protein and fat on gut microbiota (According to Singh et al., 2017) (22).

total- and LDL-cholesterol (30). Likewise, consumption of salmon (that is high in mono- and polyunsaturated fats) was not noted to alter faecal microbiota composition in the study of Urwin et al. (32). A study comparing saturated fat (lard) to long-chain polyunsaturated fatty acids (PUFA, from fish oil) detected significant differences in the gut microbiota composition and host adiposity (33). High-saturated fat, such as palm oil, reduced microbial diversity and increased Firmicutes:Bacteroidetes ratio (4). In the study of Martinez et al., diet rich in fish oil prevented against negative shifts in gut microbes and resulted in a decrease in host obesity-associated inflammation (33). Next, Huang et al. showed that a diet rich in omega-6 PUFA (from safflower oil) resulted in altered gut microbiota composition compared with diets rich in saturated milk fat or lard (34). Prieto et al. found that a diet enriched with extra virgin olive oil had a different effect on the gut microbiota in comparison with an enriched butter diet (35). In people taking omega-3 PUFA supplementation a decrease in *Faecalibacterium* spp., often associated with an increase in the *Bacteroidetes* spp. and butyrate-producing bacteria belonging to the *Lachnospiraceae* family, has been observed (36).

6 Gut microbiota and polyphenols

Dietary polyphenols include flavonols, flavones, anthocyanins, proanthocyanidins, phenolic acids, catechins, etc. Most of them were studied for their antioxidant properties and as inhibitors of pathogenic microorganism growth (5). Polyphenol intake is affected by several factors including geographical area, the population characteristics (i.e. age, gender and socio-cultural factors) and most importantly their dietary habits. The intake of total polyphenols is comparable in European countries and North and South America (about 900 mg/day and 800 mg/day respectively). However, within Europe, the intake of polyphenols varies greatly (i.e. Poland and France have both intake of above 1000 mg/day, while Italy has around 650 mg/day and Spain about 300 mg/day) (37). A majority of polyphenols enter the large intestine without being absorbed in the small intestine (38,49). There the polyphenols are degraded by the resident microbiota, including *Bacteroides*

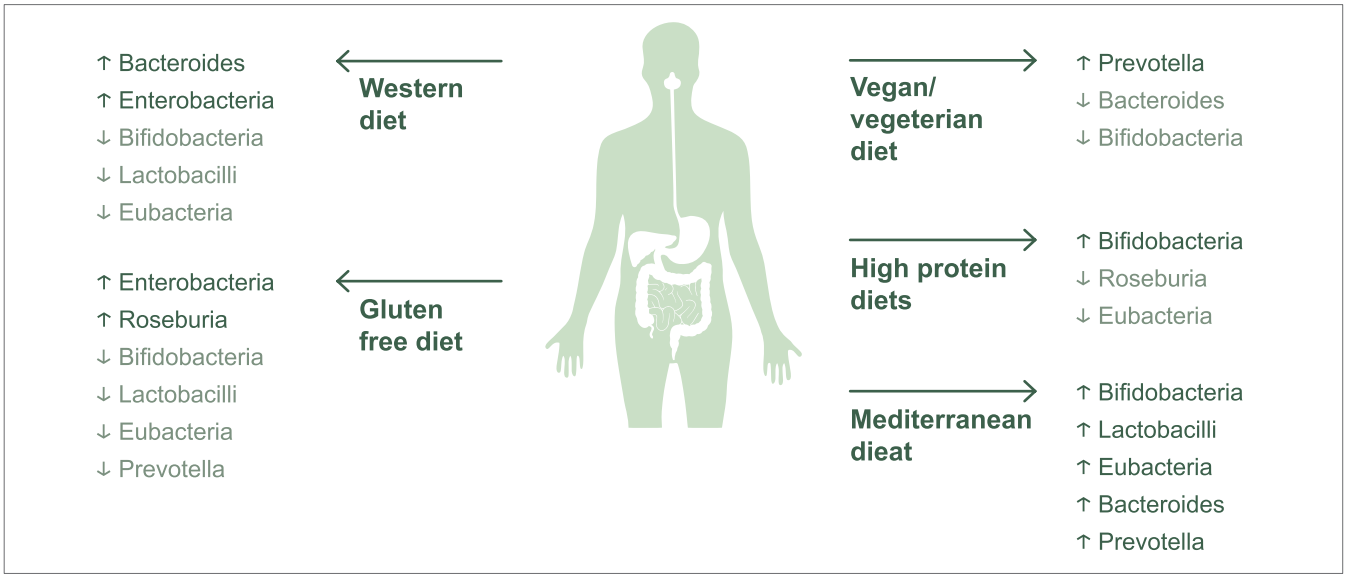

Figure 3: Impact of different diets on gut microbiota (According to Singh et al., 2017) (22).

diastasonis, *Bacteroides ovatus*, *Bacteroides uniformis*, *Enterococcus casseliflavus*, *Eubacterium cellulosolvens*, *Eubacterium ramulus* and *Lachnospiraceae* CG191 (11,38). After the initial hydrolysis, the resultant monomers and the aglycones are metabolized via decarboxylation and ring-cleavage to form simpler forms such as hydroxyphenyl propionic acid and hydroxyphenyl acetic acids (39).

Polyphenols can stimulate the growth of commensal and beneficial microbiota while inhibiting pathogenic bacteria. Coffee, tea and red wine are all rich sources of polyphenols associated with prebiotic and bifidogenic activity. For example, the growth of *Clostridium perfringens*, *C. difficile* and *Bacteroides* spp. was significantly inhibited by tea phenolics and their derivatives, while *Bifidobacterium* spp. and *Lactobacillus* spp. were less affected (40). Moreover, reductions in pathogenic *C. perfringens* and *C. histolyticum* spp. have been observed after consumption of fruit, seed, wine and tea polyphenols (40-44). Next, the study of Queipo-Ortuno et al. (45) showed that daily consumption of red wine polyphenols increased abundance of *Bifidobacterium* spp., *Prevotella* spp., *Bacteroides* spp., *Enterococcus* spp., *Bacteroides uniformis*, and *Blautia coccoides-Eubacterium rectale*.

7 Different diets

There are many popular diets including Western, gluten-free, vegan or vegetarian, Mediterranean, etc. Most of these diets due to different composition affect selective growth of different bacteria in the gut and have been linked to different microbiome profiles. For instance, Western diet can cause dysbiosis by increasing the counts of *Clostridium innocuum*, *Eubacterium dolichum*, *Catenibacterium mitsuokai* and *Enterococcus* spp. and at the same time decreasing *Bifidobacteria* spp., *Eubacteria* spp. and *Bacteroidetes* spp. (11).

Wu et al. (46) showed that the *Bacteroides* enterotype were highly associated with a diet rich in animal protein, particular types of amino acids and saturated fats (as in Western diet) (Figure 3). On the contrary, the *Prevotella* enterotype

was associated with high intake of carbohydrates and simple sugars (46,47). Furthermore, SCFAs are found in lower amounts in individuals consuming a Western diet due to lower intake of dietary fibre. Consumption of Western diet has also been linked to production of cancer-promoting nitrosamines (5). Scott et al. demonstrated that aerobic genera, such as *Escherichia*, *Pseudomonas*, *Proteus*, and *Klebsiella*, were able to produce nitrosamines (48).

A gluten free diet (GFD) is a diet used for the treatment of celiac disease. However, it has also become very popular among healthy individuals. It has been shown that a GFD could lead to modifications of the composition of gut microbiota and consequently immune properties (4). In studies of the intestinal microbiota in humans with celiac disease, the counts of aerobic *Staphylococcus* spp., as well as anaerobic *Clostridium* and *Bacteroides* spp., were higher when compared with healthy adults. Furthermore, the gut microbiota of individuals adhering to GFD is characterized by low *Bifidobacterium* spp. and *Lactobacillus* spp., whereas potentially pathogenic bacteria, such as *E. coli* and total *Enterobacteriaceae* family, increased proportionally with the reduction in polysaccharide/fibres intake after the introduction of GFD (5,11). Even a short-term GFD leads to reduced levels of *Ruminococcus bromii* and *Roseburia faecis* and increased *Victivallaceae* and *Clostridiaceae* spp. (5,11). It is therfore important to include prebiotic-rich foods in the GFD diet, such as fructan type resistant starches (i.e. oligofructose and inulin), to avoid adverse effects of GFD on the gut microbiota and to promote the growth of beneficial species in the GIT (11).

Vegan and vegetarian diets are rich in fermentable plant-based foods. The high amounts of fibre consumed can modulate the composition of gut microbiota, and those individuals that adhere to vegan or vegetarian diet were reported to have a microbiota characterized by a lower abundance of *Bacteroides* spp. and *Bifidobacterium* spp. (5). High amounts of fibre intake can result in increased SCFA production, which can decrease intestinal pH. Individuals consuming a vegan or vegetarian diet showed lower intestinal and stool pH, which prevented the growth of potentially pathogenic bacteria such as *E. coli* and other members of the *Enterobacteriaceae* family (47). Also, enrichment of genus *Prevotella* versus *Bacteroides* was shown for vegetarians and individuals who consume a high proportion of fruit and vegetables and a low proportion of meat (11).

The Mediterranean diet is often regarded as a healthy balanced diet. It is characterized by high intake of vegetables, moderate consumption of poultry, olive oil, cereals, legumes, wine, nuts, fish and a low amount of red meat, dairy products, and refined sugars. It is beneficial due to higher content of mono-unsaturated and poly-unsaturated fatty acids, as well as high levels of antioxidants, fibres and vegetable protein content (5). The gut microbiota in individuals consuming Mediterranean diet showed increased levels of *Lactobacillus* spp., *Bifidobacterium* spp., and *Prevotella* spp., and low levels of *Clostridium* spp. (5,11). The positive effect of Mediterranean diet is associated with weight loss, improvement of the lipid profile and decreased inflammation, which might be the result of diet-derived changes in the composition of gut microbiota (5).

The dietary protein intake in humans differs greatly according to the food availability and cultural dietary habits (46). While the daily protein intake in

developing countries presents a persistent problem, the avarage protein intake in developed countries is usually higher than the recommended dietary intake of 0.83 g of protein/kg/day (49). High protein diets are mainly characterized by a higher proportion of protein (25 – 30% of total energy intake) when compared to the usual macronutrient proportion (24). Such diets with caloric restriction may facilitate body weight reduction while increasing satiety but are also associated with potentially deleterious health effects in the long-term (23). The ratio of available carbohydrates to protein determines substrate utilization by the gut microbiota, and the availability of complex carbohydrates generally lowers protein fermentation (23).

In individuals consuming a high-protein diet counts of *Bifidobacterium* spp. and the butyrate producing bacteria *Roseburia/Eubacterium rectale* were reduced (23,24).

The ingestion of resistant starch has been positively associated with both *Bifidobacterium* and *Eubacterium* spp., and reduced intake of carbohydrates led do a decline of both genera (24).

8 Prebiotics and probiotics

Prebiotics are foods or dietary supplements that encourage the growth of saccharolytic bacteria that metabolize non-digestible carbohydrates such as inulin and fructose-rich oligosaccharides (FOS). In order to be considered a prebiotic, the product must be resistant to gastric acidity, non-digestible by the host in the small intestine, fermentable by bacteria, and promote the abundance of beneficial bacteria (49). Mechanisms behind the beneficial effects of dietary fibre include SCFA production, stimulation of intestinal gluconeogenesis, increased epithelial integrity, release of peptide YY (PYY) and of glucagon-like neuropeptide-1 (GLP-1) to promote satiety and insulin sensitivity, increased expression of antimicrobial peptides, and alteration of gut microbiota composition (35). Inclusion of 10% (w/w) short chain FOS in high-fat diets (60% kcal from fat) altered gut microbiota composition and function as measured by changes in metabolic by-products of the gut microbiota (50). Also, inclusion of fibres improved insulin sensitivity in humans (50). Consumption of galacto- oligosaccharides (GOS) and FOS has shown to improve gut microbiota composition by increasing bifidobacteria and decreasing *E. coli* (50).

Nowadays, probiotics are highly investigated for their effects on host health. They also represent one of the most widely consumed dietary supplements (10). Lactic acid bacteria can be found in fermented food such as yogurt and represent microorganisms that may beneficially regulate intestinal health through their effect on the gut microbiota composition and production of anti-inflammatory cytokine IL-10 (5).

Probiotics are thought to have anti-inflammatory, hypoglycaemic, insulinotropic, antioxidative, and satietogenic properties (10). However, studies investigating the effects of probiotics on the human gut microbiota have inconclusive results which might be a consequence of variations in individual responses to probiotics and probiotic colonization (51-55).

A clinical study of Ferrario et al. that included healthy adults who were given the probiotic strain *L. paracasei* DG revealed that the changes observed in the gut microbiota composition depended on an individual's starting microbial profile (53).

Mixed strain probiotics or synbiotics (combination of prebiotics and probiotics) seem to be more efficient than single microbial isolate alone (35). A randomized placebo-controlled trial of Rajkumar et al. included 60 overweight healthy adults who were given probiotic mixture containing three strains of *Bifidobacterium*, four strains of *Lactobacillus*, and one strain of *Streptococcus* (VSL #3) genera. Significant increases in the concentration of *Lactobacillus* spp., *Bifidobacteria* spp., and *Streptococcus* spp. were found when compared to placebo (56). The adults from the study also had fewer total coliforms and *E. coli*, as well as reduced triglycerides, total cholesterol, LDL-cholesterol, VLDL-cholesterol, and high-sensitivity C-reactive protein. Probiotic-containing yogurt has also been shown to significantly reduce counts of the enteropathogens *E. coli* and *Helicobacter pylori* (57).

Even though probiotics present a promising therapy, more evidence is needed. The assessment of probiotic colonization ability, including their load in the lumen or mucosa, position throughout the GIT and how long they remain in GIT after supplementation ceases needs further research. Also, the efficacy of probiotics in the modulation of gut microbiota composition needs further investigation, given the great inter- individual variation in microbiota composition (10,35).

9 Conclusion

The gut microbiota is a complex ecosystem that undergoes variations and adapts to its host over lifetime due to many factors. Increasingly recognized is the influence of diet on gut microbiota composition. Digestible and non-digestible carbohydrates, protein, fats, polyphenols, pre-and probiotics, as well as different dietary regimes all induce shifts in the gut microbiota with consequent modulation of the host immunologic and metabolic markers. Gut microbiota has been associated with the occurrence of diseases such as chronic gastrointestinal diseases, obesity, autism, diabetes, chronic inflammation, etc. Considering this close relationship between the diet, gut microbiota and health, it might be possible to improve our health through diet modulation. Changing the gut microbiota through diet, probiotics, prebiotics, and even antibiotics might offer a powerful route to preventing many of 'Western-associated' diseases.

Long-term dietary habits have the most profound impact on the gut microbiota. For that reason, healthy eating patterns with adequate intake of fruits and vegetables, ensuring a rich source of dietary fibre, together with healthy fats (mono- and poly-unsaturated fatty acids) and a trend towards more plant-derived proteins could help promote gut microbiota diversity and functionality enabling it to benefit its host.

References

1. Durack J, Lynch SV. The gut microbiome: relationships with disease and opportunities for therapy. J Exp Med. 2019;216(1):20-40. DOI: 10.1084/jem.20180448 PMID: 30322864
2. Cani PD. Human gut microbiome: hopes, threats and promises. Gut. 2018;67(9):1716-25. DOI: 10.1136/gutjnl-2018-316723 PMID: 29934437
3. Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GA, Gasbarrini A, et al. What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms. 2019;7(1):14. DOI: 10.3390/microorganisms7010014 PMID: 30634578

4. Mills S, Stanton C, Lane JA, Smith GJ, Ross RP. Precision Nutrition and the Microbiome, Part I: Current State of the Science. Nutrients. 2019;11(4):923. DOI: 10.3390/nu11040923 PMID: 31022973
5. Singh RK, Chang HW, Yan D, Lee KM, Ucmak D, Wong K, et al. Influence of diet on the gut microbiome and implications for human health. J Transl Med. 2017;15(1):73. DOI: 10.1186/s12967-017-1175-y PMID: 28388917
6. Lazar V, Ditu LM, Pircalabioru GG, Picu A, Petcu L, Cucu N, et al. Gut Microbiota, Host Organism, and Diet Trialogue in Diabetes and Obesity. Front Nutr. 2019;6:21. DOI: 10.3389/fnut.2019.00021 PMID: 30931309
7. Costea PI, Hildebrand F, Arumugam M, Bäckhed F, Blaser MJ, Bushman FD, et al. Enterotypes in the landscape of gut microbial community composition. Nat Microbiol. 2018;3(1):8-16. DOI: 10.1038/s41564-017-0072-8 PMID: 29255284
8. Zhao J, Zhang X, Liu H, Brown MA, Qiao S. Dietary Protein and Gut Microbiota Composition and Function. Curr Protein Pept Sci. 2019;20(2):145-54. DOI: 10.2174/1389203719666180514145437 PMID: 29756574
9. Diether NE, Willing BP. Microbial Fermentation of Dietary Protein: An Important Factor in Diet–Microbe–Host Interaction. Microorganisms. 2019;7(1):19. DOI: 10.3390/microorganisms/7010019 PMID: 30642098
10. Kolodziejczyk AA, Zheng D, Elinav E. Diet-microbiota interactions and personalized nutrition. Nat Rev Microbiol. 2019;17(12):742-53. DOI: 10.1038/s41579-019-0256-8 PMID: 31541197
11. Duda-Chodak A, Tarko T, Satora P, Sroka P. Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. Eur J Nutr. 2015;54(3):325-41. DOI: 10.1007/s00394-015-0852-y PMID: 25672526
12. Takiishi T, Fenero CI, Câmara NO. Intestinal barrier and gut microbiota: shaping our immune responses throughout life. Tissue Barriers. 2017;5(4):e1373208. DOI: 10.1080/21688370.2017.1373208 PMID: 28956703
13. Henderickx JG, Zwittink RD, van Lingen RA, Knol J, Belzer C. The Preterm Gut Microbiota: An Inconspicuous Challenge in Nutritional Neonatal Care. Front Cell Infect Microbiol. 2019;9:85. DOI: 10.3389/fcimb.2019.00085 PMID: 31001489
14. Tauchi H, Yahagi K, Yamauchi T, Hara T, Yamaoka R, Tsukuda N, et al. Gut microbiota development of preterm infants hospitalised in intensive care units. Benef Microbes. 2019;10(6):641-51. DOI: 10.3920/BM2019.0003 PMID: 31179713
15. Catinean A, Neag MA, Mitre AO, Bocsan CI, Buzoianu AD. Microbiota and Immune-Mediated Skin Diseases-An Overview. Microorganisms. 2019;7(9):E279. DOI: 10.3390/microorganisms7090279 PMID: 31438634
16. Iizumi T, Battaglia T, Ruiz V, Perez Perez GI. Gut microbiome and antibiotics. Arch Med Res. 2017;48(8):727-34. DOI: 10.1016/j.arcmed.2017.11.004 PMID: 29221800
17. Madsen L, Myrmel LS, Fjære E, Liaset B, Kristiansen K. Links between Dietary Protein Sources, the Gut Microbiota, and Obesity. Front Physiol. 2017;8:1047. DOI: 10.3389/fphys.2017.01047 PMID: 29311977
18. Martin AM, Sun EW, Rogers GB, Keating DJ. The Influence of the Gut Microbiome on Host Metabolism Through the Regulation of Gut Hormone Release. Front Physiol. 2019;10:428. DOI: 10.3389/fphys.2019.00428 PMID: 31057420
19. Sonnenburg JL, Bäckhed F. Diet-microbiota interactions as moderators of human metabolism. Nature. 2016;535(7610):56-64. DOI: 10.1038/nature18846 PMID: 27383980
20. Hills RD, Pontefract BA, Mishcon HR, Black CA, Sutton SC, Theberge CR. Gut Microbiome: Profound Implications for Diet and Disease. Nutrients. 2019;11(7):1613. DOI: 10.3390/nu11071613 PMID: 31315227
21. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature. 2014;514(7521):181-6. DOI: 10.1038/nature13793 PMID: 25231862
22. Lang JM, Pan C, Cantor RM, Tang WH, Garcia-Garcia JC, Kurtz I, et al. Impact of Individual Traits, Saturated Fat, and Protein Source on the Gut Microbiome. MBio. 2018;9(6):e01604-18. DOI: 10.1128/mBio.01604-18 PMID: 30538180
23. Portune KJ, Beaumont M, Davila A-M, Tom D, Blachier F, Sanz Y. Gut microbiota role in dietary protein metabolism and health-related outcomes: The two sides of the coin. Trends Food Sci Technol. 2016;57 Part B:213-32. DOI: 10.1016/j.tifs.2016.08.011
24. Blachier F, Beaumont M, Portune KJ, Steuer N, Lan A, Audebert M, et al. High-protein diets for weight management: interactions with the intestinal microbiota and consequences for gut health. A position paper by the my new gut study group. Clin Nutr. 2019;38(3):1012-22. DOI: 10.1016/j.clnu.2018.09.016 PMID: 30274898
25. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci USA. 2008;105(43):16731-6. DOI: 10.1073/pnas.0804812105 PMID: 18936492
26. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nat Rev Microbiol. 2014;12(10):661-72. DOI: 10.1038/nrmicro3344 PMID: 25198138

27. Lecomte V, Kaakoush NO, Maloney CA, Raipuria M, Huinao KD, Mitchell HM, et al. Changes in gut microbiota in rats fed a high fat diet correlate with obesity-associated metabolic parameters. PLoS One. 2015;10(5):e0126931. DOI: 10.1371/journal.pone.0126931 PMID: 25992554
28. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. 2014;505(7484):559,63. DOI: 10.1038/nature12820 PMID: 24336217
29. Wan Y, Wang F, Yuan J, Li J, Jiang D, Zhang J, et al. Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. Gut. 2019;68(8):1417-29. DOI: 10.1136/gutjnl-2018-317609 PMID: 30782617
30. Fava F, Gitau R, Griffin BA, Gibson GR, Tuohy KM, Lovegrove JA. The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome 'at-risk' population. Int J Obes. 2013;37(2):216-23. DOI: 10.1038/ijo.2012.33 PMID: 22410962
31. Drasar BS, Crowther JS, Goddard P, Hawksworth G, Hill MJ, Peach S, et al. The relation between diet and the gut microflora in man. Proc Nutr Soc. 1973;32(2):49-52. DOI: 10.1079/PNS19730014 PMID: 4791056
32. Urwin HJ, Miles EA, Noakes PS, Kremmyda LS, Vlachava M, Diaper ND, et al. Effect of salmon consumption during pregnancy on maternal and infant faecal microbiota, secretory IgA and calprotectin. Br J Nutr. 2014;111(5):773-84. DOI: 10.1017/S0007114513003097 PMID: 24128654
33. Martinez KB, Leone V, Chang EB. Western diets, gut dysbiosis, and metabolic diseases: are they linked? Gut Microbes. 2017;8(2):130-42. DOI: 10.1080/19490976.2016.1270811 PMID: 28059614
34. Huang EY, Leone VA, Devkota S, Wang Y, Brady MJ, Chang EB. Composition of dietary fat source shapes gut microbiota architecture and alters host inflammatory mediators in mouse adipose tissue. JPEN J Parenter Enteral Nutr. 2013;37(6):746-54. DOI: 10.1177/0148607113486931 PMID: 23639897
35. Prieto I, Hidalgo M, Segarra AB, Martínez-Rodríguez AM, Cobo A, Ramírez M, et al. Influence of a diet enriched with virgin olive oil or butter on mouse gut microbiota and its correlation to physiological and biochemical parameters related to metabolic syndrome. PLoS One. 2018;13(1):e0190368. DOI: 10.1371/journal.pone.0190368 PMID: 29293629
36. Costantini L, Molinari R, Farinon B, Merendino N. Impact of Omega-3 Fatty Acids on the Gut Microbiota. Int J Mol Sci. 2017;18(12):2645. DOI: 10.3390/ijms18122645 PMID: 29215589
37. Del Bo' C, Bernardi S, Marino M, Porrini M, Tucci M, Guglielmetti S, et al. Systematic Review on Polyphenol Intake and Health Outcomes: Is there Sufficient Evidence to Define a Health-Promoting Polyphenol-Rich Dietary Pattern? Nutrients. 2019;11(6):1355. DOI: 10.3390/nu11061355 PMID: 31208133
38. Braune A, Blaut M. Bacterial species involved in the conversion of dietary flavonoids in the human gut. Gut Microbes. 2016;7(3):216-34. DOI: 10.1080/19490976.2016.1158395 PMID: 26963713
39. Yadav M, Verma MK, Chauhan NS. A review of metabolic potential of human gut microbiome in human nutrition. Arch Microbiol. 2018;200(2):203-17. DOI: 10.1007/s00203-017-1459-x PMID: 29188341
40. Lee HC, Jenner AM, Low CS, Lee YK. Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. Res Microbiol. 2006;157(9):876-84. DOI: 10.1016/j.resmic.2006.07.004 PMID: 16962743
41. Cuervo A, Valdés L, Salazar N, de los Reyes-Gavilán CG, Ruas-Madiedo P, Gueimonde M, et al. Pilot study of diet and microbiota: interactive associations of fibers and polyphenols with human intestinal bacteria. J Agric Food Chem. 2014;62(23):5330-6. DOI: 10.1021/jf501546a PMID: 24877654
42. Tzounis X, Rodriguez-Mateos A, Vulevic J, Gibson GR, Kwik-Uribe C, Spencer JP. Prebiotic evaluation of cocoa-derived flavanols in healthy humans by using a randomized, controlled, double-blind, crossover intervention study. Am J Clin Nutr. 2011;93(1):62-72. DOI: 10.3945/ajcn.110.000075 PMID: 21068351
43. Cueva C, Sánchez-Patán F, Monagas M, Walton GE, Gibson GR, Martín-Álvarez PJ, et al. In vitro fermentation of grape seed flavan-3-ol fractions by human faecal microbiota: changes in microbial groups and phenolic metabolites. FEMS Microbiol Ecol. 2013;83(3):792-805. DOI: 10.1111/1574-6941.12037 PMID: 23121387
44. Sánchez-Patán F, Cueva C, Monagas M, Walton GE, Gibson GR, Quintanilla-López JE, et al. In vitro fermentation of a red wine extract by human gut microbiota: changes in microbial groups and formation of phenolic metabolites. J Agric Food Chem. 2012;60(9):2136-47. DOI: 10.1021/jf2040115 PMID: 22313337
45. Queipo-Ortuño MI, Boto-Ordóñez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo M, Estruch R, et al. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. Am J Clin Nutr. 2012;95(6):1323-34. DOI: 10.3945/ajcn.111.027847 PMID: 22552027
46. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, et al. Linking long-term dietary patterns with gut microbial enterotypes. Science. 2011;334(6052):105-8. DOI: 10.1126/science.1208344 PMID: 21885731
47. Zimmer J, Lange B, Frick JS, Sauer H, Zimmermann K, Schwiertz A, et al. A vegan or vegetarian diet substantially alters the human colonic faecal microbiota. Eur J Clin Nutr. 2012;66(1):53-60. DOI: 10.1038/ejcn.2011.141 PMID: 21811294
48. Scott KP, Gratz SW, Sheridan PO, Flint HJ, Duncan SH. The influence of diet on the gut microbiota. Pharmacol Res. 2013;69(1):52-60. DOI: 10.1016/j.phrs.2012.10.020 PMID: 23147033

49. Zaman SA, Sarbini SR. The potential of resistant starch as a prebiotic. Crit Rev Biotechnol. 2016;36:578-84. DOI: 10.3109/07388551.2014.993590 PMID: 25582732
50. Respondek F, Gerard P, Bossis M, Boschat L, Bruneau A, Rabot S, et al. Short-chain fructo-oligosaccharides modulate intestinal microbiota and metabolic parameters of humanized gnotobiotic diet induced obesity mice. PLoS One. 2013;8(8):e71026. DOI: 10.1371/journal.pone.0071026 PMID: 23951074
51. 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. DOI: 10.1111/1574-6941.12228 PMID: 24117923
52. Markowiak P, Śliżewska K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. Nutrients. 2017;9(9):9. DOI: 10.3390/nu9091021 PMID: 28914794
53. Ferrario C, Taverniti V, Milani C, Fiore W, Laureati M, De Noni I, et al. Modulation of fecal Clostridiales bacteria and butyrate by probiotic intervention with Lactobacillus paracasei DG varies among healthy adults. J Nutr. 2014;144(11):1787-96. DOI: 10.3945/jn.114.197723 PMID: 25332478
54. Kristensen NB, Bryrup T, Allin KH, Nielsen T, Hansen TH, Pedersen O. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. Genome Med. 2016;8(1):52. DOI: 10.1186/s13073-016-0300-5 PMID: 27159972
55. Laursen MF, Laursen RP, Larnkjær A, Michaelsen KF, Bahl MI, Licht TR. Administration of two probiotic strains during early childhood does not affect the endogenous gut microbiota composition despite probiotic proliferation. BMC Microbiol. 2017;17(1):175. DOI: 10.1186/s12866-017-1090-7 PMID: 28818050
56. Rajkumar H, Mahmood N, Kumar M, Varikuti SR, Challa HR, Myakala SP. Effect of probiotic (VSL#3) and omega-3 on lipid profile, insulin sensitivity, inflammatory markers, and gut colonization in overweight adults: a randomized, controlled trial. Mediators Inflamm. 2014;2014:348959. DOI: 10.1155/2014/348959 PMID: 24795503
57. Yang YJ, Sheu BS. Probiotics-containing yogurts suppress Helicobacter pylori load and modify immune response and intestinal microbiota in the Helicobacter pylori-infected children. Helicobacter. 2012;17(4):297-304. DOI: 10.1111/j.1523-5378.2012.00941.x PMID: 22759330