*Anna Gajos

The intestinal microbiota in the mechanism of obesity and atherosclerosis

Udział jelitowej flory bakteryjnej w mechanizmie otyłości i miażdżycy

Department of Clinical Physiology, Medical Center of Postgraduate Education, Warszawa
Head of Department: prof. Andrzej Beręsewicz, MD, PhD

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Address/adres:
*Anna Gajos
Department of Clinical Physiology
Medical Center of Postgraduate Education
ul. Marymoncka 99/103, 01-813 Warszawa
tel. +48 (22) 569-38-40
anna_gajos@wp.pl

Summary

Gut flora consists of a combination of microorganism species that live in the digestive tracts of animals and is the largest reservoir of human flora. At birth, the human intestines are rapidly colonized by gut microbes. Owing to their vast number, species heterogeneity, and capacity to ferment nutrients and secret bioactive compounds, gut microbiota may affect the host's physiology and metabolism negatively or positively. Thus, a number of studies describe characteristic differences between the composition and/or the activity of the gut microbiota of lean individuals and those with obesity. Recent studies in animals and humans have shown a mechanistic link between intestinal microbial metabolism of dietary phosphatidylcholine and L-carnitine and atherosclerosis and coronary artery disease through the production of a proatherosclerotic metabolite, trimethylamine-N-oxide. Furthermore, flavonoids have been recently demonstrated to exert their antiatherogenic activity via their gut microbiota metabolite protocatechuic acid. The following review discuss the microbiota-obesity and the microbiota-atherosclerosis relationships and proposed mechanisms by which the gut microbiota are thought to influence weight gain and to support the development of atherosclerotic lesions.

Streszczenie

Organizm człowieka jest środowiskiem życia ogromnej ilości mikroorganizmów. Wraz z porodem przewód pokarmowy noworodka jest kolonizowany przez bakterie. Jelitowa flora bakteryjna (JFB) jest bardzo liczna, zróżnicowana gatunkowo i stopień tego zróżnicowania zależy od czynników środowiskowych, w tym od diety. JFB rozkłada różne składniki diety i, w zależności od jej składu gatunkowego, produkty tego rozkładu mogą mieć korzystne lub niekorzystne działania zdrowotne. W tym kontekście wykazano charakterystyczne różnice w składzie gatunkowym JFB między osobami szczupłymi i otyłymi. Niedawne badania u zwierząt i ludzi wykazały, że JFB uczestniczy w rozkładzie pokarmowej fosfatydylocholiny i L-karnityny oraz że produkt tego rozkładu – tlenek-N-trójetyloaminy (TMAO) – ma działanie promiażdżycowe, a TMAO w surowicy jest czynnikiem ryzyka choroby niedokrwiennej serca. Okazało się ponadto, że flawonoidy działają przeciwmiażdżycowo poprzez produkt ich bakteryjnego rozkładu – kwas protokatechowy. W prezentowanym artykule opisano związek między składem gatunkowym JFB a rozwojem otyłości i miażdżycy oraz mechanizmy, w jakich JFB wpływa na wagę ciała i powstawanie zmian miażdżycowych.

INTRODUCTION

Since 2008 there has been an international research project called Human Microbiome Project analyzing the biological roles of commensalistic bacteria settled in different areas of human body. This and other research suggest involvement of intestinal bacterial flora (IBF) in the mechanisms of different diseases, including such civilization diseases like obesity (1-8) and arteriosclerosis (9, 10). In this context it was proved that the bacterial inhabitants of the human gastrointestinal tract break down various alimentary composition and that the products of this process may have beneficial or unbeneficial biological effects. There are three kinds of substances important to obesity and arteriosclerosis development:

1. Short chain fatty acids, which are produced in the disintegration of complex polysaccharides and take part in the development of obesity.
2. **Trimethylamine (TMA)**, which is a product of lecithin and L-carnitine bacterial metabolism and which, after oxygenation by the hepatic enzyme into TMAO, **has a pro-arteriosclerotic effect.**
3. **Protocatechuic acid (PCA)**, which is a product of plants flavonoids bacterial metabolism and which has an **anti-arteriosclerotic effect.**

The current hypothesis is that there are individual differences in the IBF composition and that some quantitative and qualitative proportion of IBF have beneficial and others unbeneficial effects. It is widely known that lean

and obese people have different kinds of IBF. In this article I present IBF content and biology as well as its role in development of obesity and arteriosclerosis.

THE COMPOSITION AND BIOLOGY OF INTESTINAL BACTERIAL FLORA

Alimentary tract of a newborn mammal is sterile. With birth the process of alimentary tract colonization by bacteria begins. In humans the more or less final composition of IBF is settled when the child is introduced on the same diet as adult family members (11). The amount of intestinal bacteria in an adult human is estimated to 10^{13}-10^{14} microorganisms (~1 kg of bacteria), which means that we have ~10 times more bacteria that our own cells in the intestines only (12). Human IBF (but also mice's) contain mainly anaerobic bacteria belonging to five phyla: *Firmicutes* (64%), *Bacteroidetes* (23%), *Proteobacteria*, *Actinobacteria* i *Verrucomicrobia*, consisting of totally 1000-1500 bacterial species (13). In human intestine there are also viruses, protozoons, archeons and fungi (14).

There are individual differences in IBF composition. These may be quantitative differences (in the percentage of different phyla/species in the whole amount of bacteria) and qualitative (in the amount of IBF species). The differences in IBF species composition are determined by: geographical area of origin, environmental hygiene conditions and genetics (in uniovular twins the differences in IBF composition are minimal) (1). The species composition of the human intestinal microbiota is also determined by: (a) prematurity – in premature infants there were mainly anaerobes (*Klebsiella*, *Enterobacter*) and *Bifidobacterium*, *Enterobacteriaceae* and *Lactobacillus* bacteria occur much later than in children born in term (15); (b) the kind of delivery – natural vs by cesarean section (16); (c) the kind of feeding – in children fed with breast milk there are mainly *Bifidobacterium* bacteria and in children fed with modified milk IBF is more differentiated (16, 17); (d) feeding habits in adult period, e.g. "carnivores" vs vegetarians/vegans (9, 18, 19); (e) undergoing antibiotic therapy (20); (f) past bariatric operation (21); (g) pregnancy (22-24) and (h) ageing (25).

IBF has numerous beneficial biological effects, including intestinal peristalsis stimulation, influences the development of intestinal villi and rebuilding of epithelium and has also positive effect on maturation and activity of alimentary tract immunological system (26). Lately there have been numerous reports on the role of IBF in development of different pathologies.

BACTERIAL ORIGINS OF OBESITY

There are the following arguments for the engagement of IBF in obesity development:

1. Germ-free mice (deprived of intestinal microflora) are thinner than mice with IBF even if they are fed in the same way. It partly is a result of the fact that germ-free individuals do not have intestinal villi developed, which may impair intestinal absorption. Another reason is that IBF takes part in digestion of complex polysaccharides (like fiber), which are not digested by the host's alimentary tract. The products of bacterial fermentation of these substances are short-chain fatty acids (acetic acid, lactic acid, propionic acid, butyric acid), which when absorbed are the additional source of energy. In humans, IBF "delivers" about 80 to 200 kcal per day, which is 4-10% of the daily energy demand (2). The same fatty acids are, additionally, agonists of specific G protein-coupled receptors – GPR41 and GPR43. These receptors are also known as the free fatty acids receptors – FFAR3 and FFAR2. Their activation causes increased intestinal absorption and adjusting fat tissue metabolism into increased fat accumulation (27).
2. Infection of control mice group with IBF from the obese mice (mice ob/ob deprived of the leptin gene) caused faster weight gaining of control mice, apart from the fact that both groups were fed in the same way (2). At the same time it was proved that in IBF of ob/ob mice the percentage of *Firmicutes* bacteria was lowered and *Bacteroidetes* bacteria was increased in comparison with control mice (28).
3. In humans the composition of IBF also correlates with obesity. It was proved that in obese people, in comparison with control group, IBF is richer in *Firmicutes* bacteria and poorer in *Bacteroidetes* bacteria and that this composition normalizes within a year of weight-loss diet application (18). In MetaHIT research in Denmark including 169 obese patients and 123 lean patients there was a group with a large amount of genes in their IBF (which means a lot of species in the IBF) and a group with little amount of genes (which means little species in IBF). It occurred that less species differentiation of IBF is a risk factor of obesity occurrence, insulin resistance, dyslipidemia and pro-inflammatory phenotype (29).

INVOLVEMENT OF BACTERIAL PRODUCTS OF PHOSPHATIDYLCHOLINE AND L-CARNITINE DISINTEGRATION IN ARTERIOSCLEROSIS MECHANISM

Phosphatidylcholine (lecithin) is present in diet containing eggs, milk, liver, red meat, poultry, shell fish and fish. The main source of L-carnitine is red meat. Phosphatidylcholine and L-carnitine molecules contain choline and choline-like trimethylamine (TMA) respectively. Final product of phosphatidylcholine and L-carnitine digestion by IBF is TMA, which is next oxygenated by a hepatic enzyme FMO3 (*flavin monooxygenase 3*) to pro-arteriosclerotic oxygenated TMAO (*trimethylamine N-oxide*) (30-32) (fig. 1). Arguments for the TMAO involvement in the arteriosclerosis and/or cardio-vascular disease development are:

1. Metabolomic research in the group of 1876 patients attending routine cardiologic appointment showed that in their serum, among thousands of other micromolecular substances there are three derivatives of phosphatidylcholine – choline, TMAO and betaine. At the same time it was proved that increased concentration of these substances was a risk factor

of coronary artery disease and other forms of cardio-vascular disease occurrence (10).

2. In mice (10) and in healthy volunteers (33) fed with phosphatidylcholine rich diet (phosphatidylcholine stress test) a transient increase in TMAO level was observed. This effect was prevented by antibiotic therapy, sometime after antibiotic exclusion the phosphatidylcholine stress test again resulted in the increase of TMAO in the serum, which proves the role of IBF in TMAO production. Also other research proved that antibiotic therapy is effective in lowering TMAO level in blood (33-35).
3. In mice fed with choline, TMAO or betaine rich diet there was an increased expression of scavenger receptors (CD36 and SRA) which take part in pro-arteriosclerotic accumulation of oxidized LDL by macrophages and accelerate the development of atherosclerosis. All these effects were prevented by antibiotic therapy.
4. The ability to produce TMAO correlates with cardio-vascular risk. Patients (n = 4007) referred to coronarography had a phosphatidylcholine stress test performed (2 cooked eggs) and had the following increase in TMAO concentration in the serum measuredwhich could be prevented by the earlier antibiotic therapy. It was observed that the level of this growth appeared to be a risk factor of cardio-vascular events (death, heart attack, brain stroke) in a three-year observation (33).
5. In the study involving 2595 patients who reported to routine cardiologic appointment it was proved that the level of L-carnitine allowed predicting the increased risk of cardio-vascular disease occurrence and in people with an increased TMAO also the risk of cardio-vascular events (death, heart attack, brain stroke) (9).
6. IBF takes part in L-carnitine metabolism into TMAO. Similarly to phosphatidylcholine also diet including red meat resulted in the increase in the level of TMAO (and L-carnitine) in human and mice blood as well as accelerated the development of atherosclerosis in mice. These effects were prevented by antibiotic therapy and then reactivated after discontinuing antibiotic therapy (9).
7. Feeding habits, and thus the species composition of IBF, decide about the intensity of L-carnitine metabolism to TMAO. Vegetarians and vegans, unlike "carnivores" had significantly lower control concentration of TMAO in blood and did not show increase in TMAO concentration in L-carnitine stress test. At the same time it occurred that carnivores and vegetarians/vegans had significantly different IBF composition (9). This last result was confirmed also by another research team (36). In addition, it was proved that diet enriched in L-carnitine changed the species IBF profile in mice, which resulted in increased production of TMA and TMAO as well as accelerated the development of atherosclerosis These effects were not present in germ-free mice (9).

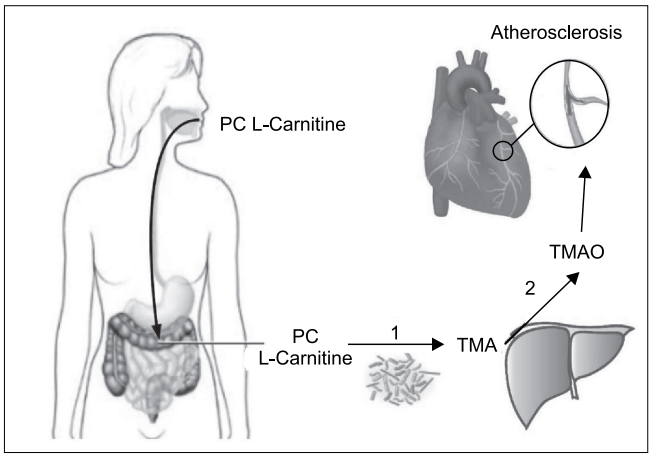

Fig. 1. Pathways Linking Dietary Phosphatidylcholine, Intestinal Microbiota, and Incident Cardio-vascular events.
Intestinal microbial metabolism of phosphatidylcholine (lecithin), the major dietary source of total choline, producing trimethylamine (1). TMA is rapidly further oxidized to trimethylamine-N-oxide (2) by hepatic flavin-containing monooxygenases (FMOs). TMAO enhances the accumulation of cholesterol in macro-phages, the expression of CD36 and SRA, the accumulation of foam cells, thereby promoting atherosclerosis.

PRODUCTS OF BACTERIAL FLAVONOID FERMENTATION VS ARTERIOSCLEROSIS

Flavonoids are a part of plants polyphenols and are quite differentiated. They are widespread in the plant kingdom where play an important role protect against ionizing radiation and to provide coloration (plant pigments) function as antioxidants and natural substances protecting plants against pathogens. They are a daily component of our diet and their daily intake is ~1 g (37). Flavonoids, particularly anthocyanins, have a confirmed anti-arteriosclerotic and preventive effect in the cardio-vascular disease (37-44). These substances are very weakly absorbed in the alimentary tract and, in case of anthocyanins, it was showed that only 0.1% of their amount present in the diet is absorbed (43). At present it is known that not the anthocyanins themselves have the anti-arteriosclerotic effect but their well absorbed metabolite protocatechuic acid (PCA) (43). Anthocyanins at first are bio-transformed into Cyanidin-3-O-beta-glucoside, which is next metabolized by bacteria present in the large intestine to above mentioned PCA (fig. 2).

Recent research on ApoE$^{-/-}$ mice (the strain susceptible to arteriosclerosis development) showed that (40):

1. Cyanidin-3-O-beta-glucoside transformation into PCA does involve IBF (the transformation is prevented by antibiotic therapy and it is not present in germ-free animals).
2. Clinically observed PCA concentrations (but not Cyanidin-3-O-beta-glucoside) increase reversetransport of cholesterol from macrophages by stimulation of reversetransport transporters ABCA1 and ABCG1 expression. The mechanism is that PCA suppresses expression of micro-RNA-10b, which is a repressor of ABCA1 and ABCG1 expression.
3. Diet enriched in Cyanidin-3-O-beta-glucoside suppresses development of arteriosclerosis and eradication of IBF with antibiotics reduces this effect.

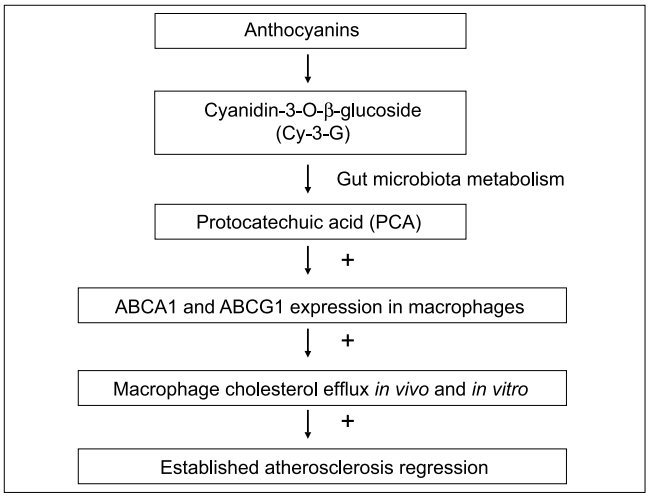

Fig. 2. Model depicting the relationship between a diet rich in anthocyanins and metabolism of gut microbiota in the promotion of macrophage RCT and atherosclerosis regression in the ApoE-deficient mice (ApoE-/-).

These research implication is that IBF, dependent of the species composition, may either be the source of anti-arteriosclerotic flavonoid biodegradation products or it may deprive the organism of their beneficial activity.

CONCLUSIONS

IBF takes part in biodegradation of diet elements and by easily absorbed products of this degradation influences the host's organism. Whether this influence is beneficial or not depends on individual species differentiation of IBF. In this article I present IBF involvement in the mechanism of obesity and arteriosclerosis as an example of this problem. Discussed data create new directions of treatment of these diseases, which are set to health-oriented modification of IBF species composition. At present, the tested trials of such modifications include application of so called prebiotics and/or probiotics. However, clinical benefits of such therapy were not yet confirmed.

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