Reviews

Gut Microbiota and Coronary Artery Disease

Tomoya Yamashita, MD, Takuo Emoto, MD, Naoto Sasaki, MD, and Ken-ichi Hirata, MD

SUMMARY

Gut microbiota have been attracting increased attention in many fields of medicine recently. We can perform a comprehensive analysis of gut microbiota using next-generation sequencing techniques together with bioinformatics technology, which expands our knowledge of a large ecosystem consisting of a host and gut microbiota. We summarize some reports about the correlations between gut microbiota and metabolic disorders, particularly atherosclerosis, and discuss future directions for the diagnostic or therapeutic potential of gut microbiota. To take simple examples, we demonstrated that the order Lactobacillales was significantly increased; while the phylum Bacteroidetes was significantly decreased in coronary artery disease (CAD) patients compared with controls or healthy volunteers. The characteristics of gut microbiota in type 2 diabetes and dyslipidemia have been reported. However, these studies have limitations, and the biological significance of gut microbiota and the causal relationships are still controversial. We hope the reports listed in this review article might lead to the development of a novel therapy to prevent CAD via modulating gut microbiota or their metabolites.

Key words: Atherosclerosis, Type 2 diabetes

Coronary artery disease (CAD) is a growing epidemic in developing countries and is the leading cause of death in many industrialized societies. Lowering low-density lipoprotein cholesterol using high potency statins is a cornerstone in the prevention of CAD and cardiovascular (CV) events. However, reducing the residual risk of statin therapy still needs to be addressed. More than 50% residual risk remains even after controlling the proven coronary risk factors. Given this unfulfilled need for effective treatment, there is augmenting interest in targeting a novel pathway, inflammation, that underlies CAD and its risk factors. Further, gut microbiota, which contribute to metabolism and immunity, are current topics as diagnostic and therapeutic targets of CAD.

The role of gut microbiota in common diseases has been explored mainly due to technical advances such as the use of next generation sequencing to profile samples including fecal microbial DNA together with the development of bioinformatics technology. Several clinical and animal studies have demonstrated that the gut microbiota and their imbalance state, dysbiosis, are associated with CAD and its major risk factors including type 2 diabetes (T2D). The contribution of gut microbiota in the development of T2D and CAD is increasingly being recognized and may become a potential therapeutic target. In this review, we discuss the regulation of gut dysbiosis and future direction for potential clinical applications, including the treatment and diagnosis of CAD.

Human gut microbiota; why are they current topics?

Colonization of the gastrointestinal tract begins after birth through maternal physical contact. The gut microbiota becomes more diverse within several weeks, changes and stabilizes to a profile very similar to that of an adult at 2~3 years old, and continues throughout our life. It is estimated that about 100 trillion (10^{14}) bacterial cells, comprised of approximately 1000 bacterial species, are present in the human gastrointestinal tract. The gene content of gut microbiota in a human gut may exceed that of the host by at least 100-fold. This large array of gene products performs a diverse range of biochemical and metabolic activities to complement the host physiology. It has been clarified that host diet, lifestyle, hygiene, host genetics, and bacterial composition of the environment all affect the individual composition of gut microbiota. Recent advances in both sequencing techniques and bioinformatics technology have enabled us to evaluate and understand the gut microbiota and gut microbiome (genes of the microbiota). Most bacterial species in the adult human and mouse gut belong to the phyla Firmicutes and Bacteroidetes, with less abundant bacterial phyla, such as Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia (Figure 1).

From the Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, Kobe, Japan.

This work was supported by Japan Society for the Promotion of Science KAKENHI Grant No. 24591114 (T.Y.), Takeda Scientific Foundation (T.Y.), Mochida Memorial Foundation (T.Y.), Suzuken Memorial Foundation (T.Y. and N.S.), Senshin Medical Research Foundation (T.Y. and N.S.), Yakult Bioscience Research Foundation (T.Y.), Uehara Memorial Foundation (K.H.), Hyogo Science and Technology Association (T.Y.), and The Japanese Circulation Society Translational Research Foundation (K.H.).

Address for Correspondence: Tomoya Yamashita, MD, Division of Cardiovascular Medicine, Department of Internal Medicine, Kobe University Graduate School of Medicine, 7-5-1, Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan. E-mail: tomoya@med.kobe-u.ac.jp

Received for publication August 25, 2016. Revised and accepted September 8, 2016.

Released in advance online on J-STAGE November 4, 2016.

All rights reserved by the International Heart Journal Association.
The 4 major phyla of *Firmicutes*, *Bacteroides*, *Actinobacteria*, and *Proteobacteria* account for more than 99% of all human gut microbiota. Many species of gut bacteria cannot be cultivated in vitro, making the categorization and identification of gut bacteria complicated. Although there is no clear definition of the characteristics of a normal “healthy” gut microbiome in humans, a recent metagenome has allowed 3 major clusters of gut bacteria named “enterotypes” to be distinguished in humans, based on the predominant bacterial genera in fecal specimens; type I is characterized by high levels of *Bacteroides*, type II has high levels of *Prevotella*, and type III has relatively high levels of *Ruminococcus* (Figure 1). Entero-type III can also be characterized by low levels of *Bacteroides* and *Prevotella* rather than a dominant genus. Despite some disagreements regarding enterotype definition, the concept seems to be essentially correct.

The composition of the gut microbiota is remarkably diverse and dynamic over short periods of time because dietary exposures or drug intake significantly affect our microbial community, however, its composition appears to remain remarkably stable over time within individuals and their family members.

Obesity was first reported to be associated with a change of gut microbiota in 2006, an increase of the phylum *Firmicutes* and decrease of the phylum *Bacteroidetes* in obese patients attracted the attention of researchers around the world. Interestingly, Turnbaugh, et al demonstrated that transplanting fecal microbiota from obese mice into germ free (GF) mice resulted in the efficient transmission of the obese phenotype into the recipients, suggesting that gut microbiota could be the cause of some diseases.

**Gut microbiota in immune regulation**

GF mouse experiments indicated the development and differentiation of T cells is directly influenced by the microbiota. The studies on antibiotic-treated or GF animals indicate that the gut microbiota have a crucial role in the differentiation of T helper 17 (Th17) cells. It has been recently demonstrated that specific bacterial species are associated with the differentiation of specific subsets of T cells in the intestine. Both human and mouse *Clostridium* cluster IV and XIVa (Figure 1), the spore-forming component of indigenous intestinal microbiota, have been implicated in the induction of Foxp3+ Tregs in the colon of mice. Furthermore, butyrate, a short chain fatty acid (SCFA) produced by commensal bacteria, promotes Foxp3+ Treg induction. Given these backgrounds, it can be speculated that the propagation or sterilization of some specific bacterial species, which will result in augmented generation of protective Tregs or reduced differentiation of pathogenic T cells, may prevent inflammatory diseases including atherosclerosis. Further studies are needed to prove this hypothesis and may contribute to the development of novel strategies for preventing atherosclerosis through modulation of intestinal immunity.
Gut microbiota and their metabolites: bile acids and short chain fatty acids

Gut microbiota was shown to contribute to the metabolism of bile acids.\(^{23}\) Primary bile acids are synthesized by the oxidation of cholesterol in the liver and are secreted into the intestine to solubilize lipids for absorption. Microbial bile salt hydrolase deconjugates primary bile acids and metabolizes to secondary bile acids. About 95% of bile acids are reabsorbed from the intestine for recycling and 5% are lost in the feces, which is the major route for excretion of cholesterol. Recently, bile acids have been found to play several roles not only in lipid metabolism but also in a wide range of physiologic processes through two main receptors, the farnesoid X receptor (FXR) and the bile acid responsive Takeda G-protein coupled receptor-5 (TGR5).\(^{23,24}\) The precise functions of the bile acids, their receptors, and their relationship with the gut microbiota are still poorly understood and are currently under investigation. The clarification of these functions and mechanisms may lead to the development of novel therapies for not only dyslipidemia but also CAD. Not only bile acid receptor modulators but also interventions directed at gut microbiota are hopeful potential treatments for metabolic disorders including atherosclerosis.

The primary products of bacterial hydrosis are monosaccharides, which are fermented to various SCFAs such as acetate, butyrate, and propionate.\(^{25}\) Acetate and propionate are mostly produced by the phylum Bacteroidetes, while butyrate is produced by the phylum Firmicutes. They have been shown to exert beneficial effects on body weight, glucose homeostasis, and insulin sensitivity.\(^{26}\) The SCFAs are transported by specific monocarboxylate transporters through the mucosal epithelium of the intestine into the blood. SCFAs are ligands for the G-protein coupled receptors GPR43 and GPR41.\(^{27}\) These receptors are widely expressed and play roles in the regulation of energy metabolism, the sympathetic nerve system, and immune cell function. Taken together, these differences in gut bacteria may be related to the amount of SCFAs produced and affect the incidence of several diseases.

Cardiovascular events and gut microbiota derived TMAO

Hazen, et al have reported that the gut microbial-derived metabolites trimethylamine (TMA) and trimethylamine N-oxide (TMAO) are pro-atherogenic in both mice and humans (Figure 2).\(^{28,29}\) First, they used a metabolomic approach to generate unbiased small-molecule metabolomic profiles in plasma that predict CV events.\(^{24}\) Three metabolites of dietary lipid phosphatidylcholine, choline, TMAO, and betaine were identified and these metabolites were associated with atherosclerosis risks in humans and promote atherosclerosis in mice. Plasma TMAO levels were reported to be the strongest positive correlation with CV risk. Oral feeding of choline, rather than parenteral delivery, was necessary to generate the metabolite TMAO, suggesting that a necessary phase in this biochemical pathway was performed within the intestine. Generation of

![Figure 2. Trimethylamine-N-oxide (TMAO) and cardiovascular diseases. Choline (in shrimps and eggs) and carnitine (in meat) are metabolized into trimethylamine (TMA) by gut microbiota. TMA joins the portal vein stream and is metabolized into TMAO by FMOs in the liver. TMAO exacerbates chronic kidney disease (CKD), heart failures, atherosclerosis, and thrombosis. The molecule 3,3-dimethyl-1-butanol (DMB) non-lethally inhibits TMA lyase activity and decreases TMA production.](image-url)
TMAO and its precursor TMA were shown to be dependent on the gut microbiota in both humans and mice. That is, they found that dietary choline is metabolized by intestinal bacteria to TMA, which is subsequently absorbed into the host and metabolized to TMAO in the liver by the hepatic flavin-containing monoxygenase (FMO) family of enzymes (Figure 2). Moreover, dietary supplementation of apolipoprotein E-deficient (ApoE−) mice with choline enhanced atherosclerosis. Deletion of gut microbiota using antibiotics with broad spectrum cancelled the pro-atherosclerotic effect of dietary choline, which was associated with the reduction of plasma TMAO levels in antibiotic-treated mice. They also investigated the relationship between fasting plasma levels of TMAO and the incidence of major adverse CV events (death, myocardial infarction, or stroke) during 3 years of follow-up in 4007 patients undergoing elective diagnostic cardiac catheterization. Increased plasma TMAO levels were associated with an increased risk of a major adverse CV event. Even after adjustment for traditional risk factors, an elevated TMAO level could predict an increased risk of major adverse CV events. Collectively, their findings suggest that pathways that are dependent on the intestinal microbiota may contribute to the pathophysiology of CV disease (CVD) and suggest potential therapeutic targets.

It was also clarified that microbial processing of L-carnitine, which is abundant in red meat and contains a trimethylamine structure similar to that of choline, elevates plasma TMAO concentrations and enhances atherosclerosis in a microbial-dependent manner. It was also recently found that γ-butyrobetaine is the major proatherogenic intermediate in gut microbial metabolism of L-carnitine to TMA and TMAO. The proatherosclerotic mechanisms of TMAO might be increasing macrophage foam cell formation, suppressing reverse cholesterol transport in vivo, and induce the hyperreactivity of platelets. Bacterial taxa belonging to the families Clostridiales and Peptostreptococcaceae were positively associated with TMAO production in humans, suggesting that L-carnitine-metabolizing bacteria may belong to these families. However, the molecular mechanisms through which gut microbial formation of TMAO leads to atherogenic phenotypes are not entirely clear. This study may in part explain why excessive red meat consumption has been associated with increased CVD and mortality risks.

The same group also demonstrated that TMAO is involved in many CVD such as heart failure, chronic kidney disease, and thrombosis. Recently, they demonstrated that the gut microbial enzyme TMA lyase is a key regulator that metabolizes choline to TMA. They found that the molecule 3,3-dimethyl-1-butanol (DMB), which non-lethally inhibits TMA production from cultured gut microbiota, decreased TMAO levels in mice fed a high-choline diet. DMB is a molecule that is detected in some natural safe foods such as balsamic vinegars, red wines, extra virgin olive oils, and grape-seed oils. Therefore, we will be able to take DMB before high choline or L-carnitine diet such as meat or eggs to prevent TMA production as a potential tool for the regulation of atherosclerosis (Figure 2).

Dysbiosis in type 2 diabetes and dyslipidemia

The interaction of obesity and gut microbiota was first observed as an increase of the phylum Firmicutes and a decrease of the phylum Bacteroidetes in obese patients, an observation that attracted the attention of researchers around the world. Some studies have demonstrated the profiles of gut microbiota in metabolic diseases such as T2D, lipid disorders, and atherosclerosis. Two recent papers from the Meta Hit Consortium demonstrated the diagnostic and clinical values of fecal microbiota composition in T2D. In both studies, diabetic subjects were characterized by a reduction of Clostridiales species, including butyrate-producing Roseburia species and Feacalibacterium prausnitzii. Karlsson, et al reported an enrichment of Lactobacillus gasseri and Streptococcus mutans in T2D patients in European women subjects. Qin, et al found that an increased number of Proteobacteria could be a predictor of T2D patients in China. In fecal samples of Japanese T2D patients, the counts of Clostridium cocoides group, Atoptobium cluster, and Prevotella were significantly low, while total Lactobacillus species counts, especially Lactobacillus ruteri and Lactobacillus plantarum, were higher than in those of control subjects, using a sensitive quantitative reverse transcription PCR method. In most of these reports described above, treatment regimens were not matched with control subjects, which implies the profiles of gut microbiota in T2D patients were confounded by the impact of various drugs. Forslund, et al demonstrated that the effect of metformin is one of the confounding factors in the T2D specified gut microbiota profiles reported by the Meta Hit consortium and concluded that it is necessary to disentangle the effects of specific diseases from those of drugs in studies of human microbiota. Pederson, et al identified Prevotella copri and Bacteroides vulgatus as the main species driving the association between the biosynthesis of branched-chain amino acids (BCAAs) and insulin resistance, and that Prevotella copri can induce insulin resistance, aggravate glucose intolerance, and augment circulating levels of BCAAs in mice. They focused on gut microbiota profiles in impaired glucose tolerance before the onset of T2D and taking medication. They successfully adopted transomics methods including metagenomics and metabolomics, and demonstrated a causal relationship of gut microbiota in mice.

Lipid profiles were analyzed using 16S ribosomal RNA sequencing in the LifeLines-DEEP population cohort. After correcting for age and sex, 34 bacterial taxa were associated with body mass index (BMI) and blood lipids. Although some associations were shared and confounded across BMI and lipids, there were also microbial taxa whose proportions were primarily associated with lipids alone, and some of these were newly identified associations with plasma triglyceride and high-density lipoprotein cholesterol levels. For example, the family Pasteurellaceae, the genus Coprococcus, and the species Collinsella stercoris showed strong associations with triglyceride levels.

Shen, et al analyzed the association between the composition of the gut microbiome and lifetime CVD risk profile among subsamples of 55 Bogalusa Heart Study participants with the highest and 57 with the lowest lifetime burdens of traditional CVD risk factors. They reported that while the genera Prevotella and Tyzzerella were enriched, the genera Alloprevotella and Catenibacterium were depleted among those with a high CVD risk profile (Table). Although there are many reports that demonstrated the profiles of gut microbiota in metabolic diseases, especially...
<table>
<thead>
<tr>
<th>Population</th>
<th>Year</th>
<th>Methods</th>
<th>Limitation</th>
<th>Country</th>
<th>Main Findings (↓ = decreased in patients, ↑ = increased in patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2D (Healthy control versus T2D patients)</td>
<td>2012</td>
<td>WGS</td>
<td>Not age-matched Medication</td>
<td>China</td>
<td>Roseburia intestinalis ↓, Faecalibacterium prausnitzii ↓, Akkermansia muciniphila ↑, Bacteroides intestinalis ↑, Enterobacteria coli ↑, Butyrate producing bacteria ↓, Genes involved in cofactors and vitamins ↓</td>
</tr>
<tr>
<td>T2D (Healthy control, IGT patients versus T2D patients)</td>
<td>2013</td>
<td>WGS</td>
<td>Only female Medication</td>
<td>Europe</td>
<td>Faecalibacterium prausnitzii ↓, Roseburia species ↓, Bacteroides intestinalis ↓, Lactobacillus gasseri ↑, Streptococcus mutans ↑, Butyrate producing bacteria ↓, Genes involved in oxidative stress ↑</td>
</tr>
<tr>
<td>T2D (Healthy control versus T2D patients)</td>
<td>2014</td>
<td>quantitative RT-PCR</td>
<td>Not BMI-matched Method (RT-PCR) Medication</td>
<td>Japan</td>
<td>Clostridium cocoides ↓, Atopobium ↓, Prevotella ↓, Lactobacillus (especially reuteri and plantarum) ↑, Live bacteria in blood ↑ (especially Gram positive bacteria)</td>
</tr>
<tr>
<td>IGT (Healthy non-diabetic population)</td>
<td>2016</td>
<td>WGS (+metabolomics)</td>
<td>Not including T2DM patients with impaired insulin secretion</td>
<td>Europe 'Meta Hit'</td>
<td>Prevotella copri ↑, Bacteroides vulgatus ↑ (identified as the main species driving the association between biosynthesis of BCAAs and insulin resistance)</td>
</tr>
<tr>
<td>Dyslipidemia (Healthy population in LifeLines-DEEP Heart Study Cohort)</td>
<td>2015</td>
<td>16S r-RNA sequencing</td>
<td>Lipid levels and BMI are confounding factors</td>
<td>USA</td>
<td>34 taxonomies associated with BMI, TG and HDL: Pasteurellaceae ↓, Coprococcus ↓, Collinsella Stercoris ↑ (associated with triglycerides)</td>
</tr>
<tr>
<td>Cardiovascular disease risk (CVD risk high versus low in Bogalusa Heart Study Cohort)</td>
<td>2016</td>
<td>16S r-RNA sequencing</td>
<td>Small sample size CVD risk profile includes many diseases</td>
<td>USA</td>
<td>Alloprevotella ↓, Catenibacterium ↓, Prevotella ↑, Tyzzerella ↑</td>
</tr>
<tr>
<td>Stroke/TIA (Healthy control and symptomatic patients with stenotic atherosclerotic plaques in the carotid artery)</td>
<td>2012</td>
<td>WGS</td>
<td>Small sample size Medication Setting of controls</td>
<td>Europe</td>
<td>Roseburia ↓, Eubacterium ↓, Bacteroides species ↓, Collinsella ↑, Genes involved in phytoene dehydrogenase ↓, Genes involved in peptidoglycan synthesis ↑, Enterotype III ↑</td>
</tr>
<tr>
<td>Stroke/TIA (Healthy control, asymptomatic atherosclerotic patients versus stroke/TIA patients)</td>
<td>2015</td>
<td>16S r-RNA sequencing</td>
<td>Not age/BMI-matched Medication</td>
<td>China</td>
<td>Bacteroides ↓, Prevotella ↓, Faecalibacterium ↓, Opportunistic pathogens (Proteobacteria, Enterobacter, Megasphaera, Oscillibacter, and Desulfovibrio) ↑, Diversity (Chao index e.t.c.) ↑, TMAO level in the blood ↓</td>
</tr>
<tr>
<td>Coronary artery disease (Healthy control, Control with metabolic diseases versus CAD patients)</td>
<td>2016</td>
<td>T-RFLP</td>
<td>Method (T-RFLP) Medication Small sample size</td>
<td>Japan</td>
<td>Bacteroidetes (Bacteroides + Prevotella) ↓, Lactobacillales ↑, Enterotype III ↑</td>
</tr>
</tbody>
</table>

T2D indicates type 2 diabetes mellitus; IGT, impaired glucose tolerance; WGS, whole genome shot gun sequencing; quantitative RT-PCR, quantitative reverse transcription- polymerase chain reaction; and T-RFLP, terminal-restriction fragment length polymorphism.
T2D, those papers include limitations and inconsistent results. A further prospective large cohort study is necessary to clarify any causal relationship.

Gut microbiota and atherosclerotic cardiovascular events

In the field of atherosclerosis, there are two previous studies that demonstrated the association between cerebral vascular diseases and gut microbiota (Table). Firstly, Karlsson, et al used whole genome sequencing of the gut metagenome and identified several compositional and functional alterations in gut microbiota profiles in patients defined as having stenotic atherosclerotic plaques in the carotid artery leading to cerebrovascular events in 2012. They demonstrated that *Collinsella* was enriched, whereas *Roseburia* and *Eubacterium* were reduced in patients compared with healthy controls. Further characterization of the functional capacity of the metagenomes revealed that the patients were enriched in genes encoding peptidoglycan synthesis and depleted in phytoene dehydrogenase, which were associated with reduced serum levels of *β*-carotene.43 Secondly, Yin, et al demonstrated that patients with symptomatic stroke and transient ischemic attack showed significant dysbiosis of the gut microbiota, and their blood TMAO levels were decreased, while patients with asymptomatic atherosclerosis did not exhibit obvious changes in gut microbiota and blood TMAO levels. They demonstrated that symptomatic patients had more opportunistic pathogens, such as *Enterobacter*, *Megasphaera*, *Oscillibacter*, and *Desalfovibrio*, and fewer commensal or beneficial genera including *Bacteroides*, *Prevotella*, and *Faecalibacterium* and that this dysbiosis was correlated with the severity of the disease (Table).44

On the other hand, we attempted to clarify the specific profile of gut microbiota in CAD patients to investigate the therapeutic potential of gut microbiota.45,46 We compared the profiles of gut microbiota among CAD patients, controls (Ctrl), and healthy volunteers (HV) using terminal restriction fragment length polymorphism (T-RFLP) analysis (Figure 3). T-RFLP analysis is one of the most well-established and reliable 16S ribosomal RNA-based methods, especially when considering its high throughput and reproducibility. T-RFLP using B2I1 could classify gut microbiota into the following 10 groups: *Prevotella*, *Bacteroides*, *Lactobacillales*, *Bifidobacterium*, *Clostridium* cluster IV, *Clostridium* subcluster XIVa, *Clostridium* cluster IX, *Clostridium* cluster XI, *Clostridium* cluster XVIII, and others, combining the operational taxonomic units that belonged to the same group. We found that the order *Lactobacillales* was significantly increased in the CAD group, compared with the Ctrl or HV group. The prevalence of the phylum *Bacteroidetes* (*Bacteroides* + *Prevotella*) was significantly decreased in the CAD group compared with the Ctrl or HV group (Figure 3). The Firmicutes / Bacteroidetes ratio (F/B ratio) was increased in the CAD group compared with the Ctrl group. The order *Lactobacillales* is one of the main components of the human gut microbiota and belongs to the phylum *Firmicutes* (Figure 1). The order *Lactobacillales* is divided into several genera, including *Lactobacillus*, *Streptococcus*, and *Enterococcus*. Because of the study protocol, we could not deny that medication could affect the composition of gut microbiota. High *Lactobacillus* levels were also reported in T2D in several papers including Japanese T2D patients, but not all (Table). However, the reason for the high counts of *Lactobacillus* in both T2D and CAD patients remains unclear and further studies should be conducted to clarify whether the alterations are the causes or the consequences of the diseases. Although there are differences between carotid atherosclerosis and coronary atherosclerosis, we found consistently low levels of the phylum *Bacteroidetes*. *Bacteroides fragilis*, which belongs to the phylum *Bacteroidetes*, affects mucosal T cell homeostasis by promoting regulatory T cell function. Other *Bacteroides* species can also establish mutualistic relationships with the host by being able to flourish in the plant polysaccharide-enriched gut environment and by providing the biological byproducts necessary for the well being of the host.47 For example, *Bacteroides thetaiotaomicron* is a major producer of SCFAs, which are necessary for proper host metabolic and immune functions. Our and previous reports supported the hypothesis that the phylum *Bacteroidetes* might help to prevent coronary atherosclerosis. Therefore, the role of phylum *Bacteroidetes* in CAD should be assessed.

It remains unclear from these studies above whether gut microbiota actively regulates atherosclerosis. It is possible that we just analyze the effects of medication because patients suffering from atherosclerosis take many drugs such as aspirin and statins. A prospective cohort study is needed to identify whether an alteration of gut microbiota precedes the development of atherosclerosis or not. We, for the next step, have to clarify specific bacteria at the species level using next-generation sequencing and determine the biological meanings of dysbiosis in CAD, with a special focus on the phylum *Bacteroidetes*.

How to regulate atherosclerosis by gut microbiota

There are some possible ways to modulate gut microbiota profiles and regulate atherosclerosis; fecal microbiota transplantation (FMT), microbiologic agents using isolated specific strains, probiotics, and dietary habits.

FMT is an established treatment modality for *Clostridium difficile* infection, despite a lack of standardization for the procedure.49 Although there are several FMT trials for *Clostridium difficile* infection or inflammatory bowel diseases, only one small clinical study was performed for metabolic diseases. Vrieze, et al demonstrated the effects of infusing intestinal microbiota from lean donors to male recipients with metabolic syndrome on the microbiota composition and glucose metabolism of the recipients.49 They confirmed that the increased diversity of the gut microbiota by FMT was associated with improved insulin resistance. They concluded that although whether the diversity or changes in specific bacterial species contributed to the insulin resistance is unknown, the results suggested that butyrate produced by certain bacteria prevents the translocation of endotoxic compounds.49 Many problems with respect to FMT remain, such as the method of transferring and the definition of healthy donor samples. It is doubtful whether the administration of functionally effective bacterial strains instead of FMT is useful.

Recently, Li, et al demonstrated that *Akkermansia muciniphila* (A. muciniphila) prevented western diet-induced inflammation and suppressed atherosclerotic lesion formation in ApoE mice, as evidenced by reduced macrophage infiltration and reduced expression of proinflammatory cytokines and
chemokines. This mechanism was explained by an A. muciniphila-mediated reduction in circulating endotoxin level, because A. muciniphila, a mucin-degrading bacterium, can induce intestinal expression of tight junction proteins such as zonula occludens-1 and occludin. They concluded that western-diet induced atherosclerosis was caused partly by a reduction of A. muciniphila in the gut, resulting in a compromised gut barrier and increased endotoxemia, which in turn exacerbate vascular inflammation. However, it is unknown whether A. muciniphila is clinically important for regulating atherosclerosis in humans. Because A. muciniphila was demonstrated to have a protective role against hyperlipidemia and diabetes conditions in mice, clinical trials are needed as a next step. In the future, we would like to select and isolate clinically effective strains for atherosclerosis from another perspective and identify a possible mechanism for suppressing the formation of atherosclerosis.

Oral administration of probiotics such as Lactobacillus and Bifidobacterium species has been demonstrated to suppress atherosclerotic lesion formation in mice. Lactobacillus acidophilus, for example, has been shown to suppress the development of atherosclerosis not only by decreasing serum cholesterol levels but also by inhibiting oxidative stress. In humans, supplementation with two probiotic strains, Lactobacillus curvatus HY7601 and Lactobacillus plantarum KY1032, reduced triglycerides and increased the apolipoprotein A-V and low-density lipoprotein cholesterol particle size. Although there are many studies that examined probiotics, we would like to focus on the immune-suppressive effects of probiotics on atherosclerotic lesion formation, independent of the lipid lowering effects.

As mentioned in the introduction, the diet-gut microbiota...
interaction could be one of the reasons why the number of patients suffering from ischemic cardiac events is increasing in Japan. Factors including age, genetic background, and diet may all influence the composition of gut microbiota. Of these, diet is the most important target for therapeutic intervention. Recent lifestyle changes such as the increased prevalence of a high-fat, high-cholesterol western-diet have altered the genetic composition and metabolic activity of our gut microbiota. We would like to ascertain whether dietary intervention can modulate gut microbiota and can suppress the formation of atherosclerosis. Wu, et al demonstrated enterotypes were strongly associated with long-term diets, particularly protein and animal fat (Bacteroides) versus carbohydrates (Prevotella). They also showed that enterotype identity remained stable during their 10-day study changes in diet pattern. They concluded long-term dietary interventions may allow modulation of an individual’s enterotype to improve health if an enterotype is ultimately shown to be causally related to a certain disease. Many studies have found that fiber influences the composition of gut microbiota. The amount of intake of dietary fiber is decreasing in Japan because of westernization of the everyday-diet. Although some studies have demonstrated dietary fibers are associated with a variety of diseases, the associations between dietary habit, gut microbiota, and atherosclerosis are unknown. We suspected a western-diet, which is high in fat and low in fiber, exacerbates atherosclerosis from the viewpoint of gut microbiota. Dietary habits are suspected to suppress atherosclerotic lesion formation, and we expect to discover new merits and mechanisms of traditional Japanese-style diets.

**Conclusion:** We have summarized the relationship between gut microbiota and metabolic diseases, especially atherosclerosis, in this review paper. The derived molecule, TMAO, has been shown to be involved in atherosclerotic lesion formation. However, other mechanisms are also suspected. Three groups including us revealed a characteristic change of gut microbiota in patients suffering from atherosclerosis, although it is unknown whether medication is one of conflicting factors or whether gut microbiota actively regulates atherosclerosis. In the future, gut microbiota-targeted therapy for the prevention of atherosclerosis should be carried out.

**References**


