# The Gut Microbiome and Cardiovascular Disease

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**What is the intestinal microbiome?**

More than 100 trillion bacteria are normally resident in the gastrointestinal tracts of healthy persons; termed the gut microbiome. It is now recognised that the gut microbiome has essential roles not only in digestion and metabolism, but also in the function of the immune system; and that dysbiosis (the alteration of the normal gut microbiome) is associated with multiple diseases including obesity, inflammatory bowel disease, non-alcoholic steatohepatitis, diabetes mellitus and cardiovascular disease. [1] [2] [3] In fact, the microbiome has been described by researchers as "virtually an endocrine organ, arguably the largest". [2] Potential pathways for pathogenesis of disease are both direct and indirect; via small molecule metabolites produced by gut microbiota in digestion, alterations in the intestinal epithelial barrier and via the immune system, although many mechanisms remain unknown. [1] Interest in the gut microbiome has increased markedly over the past fifteen years; both in the role of the microbiome in disease development, and as a potential target for preventative or therapeutic interventions.

**The normal microbiome**

Between individuals, the exact composition (both of species, and the ratios between species) varies, but in healthy subjects *Bacteroides, Firmicutes, Actinobacteria, Proteobacteria*, and *Cerrucomicrobia* are the most common. [2] Variations between individuals occur due to both genetic and environmental factors (such as gut motility, nutrition, and antibiotic exposure). *Bacteroides* and *Firmicutes* comprise >90% of total bacteria, with the highest density of microbes occurring in the colon.

The normal microbiome functions as part of the digestive system, by fermenting carbohydrates and proteins, and metabolising molecules that would otherwise not be broken down by the human gastrointestinal tract. Short chain fatty acids (SCFAs) and other molecules are formed from this process. The SCFAs are then readily absorbed in the distal

gastrointestinal tract, and are used as an energy source in metabolic processes for the host, as well as for other microbes (a phenomenon called “cross-feeding”). Thus, the microbiome has dual roles in digestion and metabolism.

As well as providing an energy source for metabolic processes in humans and microbes, SCFAs reduce the pH of the gastrointestinal tract, helping preserve the epithelial barrier, and act as signalling molecules for the inflammatory response and immune system. Signalling occurs via G-protein coupled receptors GPR41 and GPR43, GPR109 and olfactory factor 78, which are widely expressed in the gastrointestinal tract, as well as in immune cells (neutrophils and monocytes) and adipose tissue. Studies have demonstrated that SCFAs binding to GPR43 reduces the production of inflammatory mediators, and GP43 plays a role in inflammatory bowel diseases. [4] [5] Protection from colonisation by pathogenic organisms by inhibition of growth is also provided by the normal microbiome, offering further immune protection.

Other metabolites produced by the microbiome are biologically active and have roles in inflammation and disease. Trimethylamine-N-oxide (TMAO) is produced by the metabolism of trimethylamine-containing compounds (L-carnitine, choline, phosphatidylcholine), and has important roles in atherosclerosis, heart failure as well as platelet activation and thrombosis, and chronic kidney disease. [6]

The microbiome aids in maintenance of the gut epithelial barrier. Bacteria in the microbiome activate receptors in intestinal epithelial cells – these in turn activate immune responses, and responses to maintain the epithelial barrier. Thus, humans are protected from breaches of the barrier allowing translocation of bacteria.

Finally, small amounts of primary bile acids are present in the colon, and are converted to secondary bile acids by the microbiome. Secondary bile acids may then enter the circulation, where they act as hormones, once again in metabolic and immune pathways.

**Dysbiosis**

Alterations to the microbiome can occur in several ways:

1. Altered diversity of species
2. Altered predominance of species
3. Altered ratios between predominant species
4. Reduced overall number of microbes
5. Alterations in microbial function

Alterations and variations in the microbiome are thought to be caused by genetic differences between hosts, dietary differences, and the use of antibiotics.

Reduction in the diversity of the microbiome is associated with hypertension, and may be associated with disruption of the intestinal epithelial barrier. Dysbiosis can cause excess nitrogenous compound production, which in turn causes disruption of the intestinal epithelial tight junctions. This may allow translocation of intestinal microbes as well as toxins, leading to activation of inflammatory pathways. Inflammation is thought to have an important role in the development of atherosclerosis, and in fact, atherosclerotic plaques have been shown to contain bacterial DNA matching those of the gut microbiome in an individual. [2]

Abundance of certain species appears to be a risk factor for atherosclerosis and inflammation (e.g., *Collinsella, Peptococcaceae, Prevotella*), whilst abundance of other species may be protective against both processes (e.g., *Tenericutes, Christensenellaceae*, *Akkermansia muciniphila*). [2] It is thought that the specific species composition of the microbiome alters both metabolism and metabolic pathways, and inflammation pathways substantially, at that it is through these mechanisms that disease modification and pathogenesis occurs. For example, SCFAs have been demonstrated to alter insulin sensitivity and suppress fat accumulation that is mediated by insulin, as well as stimulating the production of hormones used to signal fullness.

The Firmicutes/Bacteroidetes ratio is a marker for dysbiosis, and appears to be an important risk factor for metabolic and cardiovascular disease, with a lower ratio associated with obesity and with hypertension, and with coronary artery disease. [2] [1] *Bacteroides* species in

particular may be important in regulation of atherosclerosis, with a consistent finding of *Bacteroides* depletion in patients with atherosclerosis and coronary artery disease. [1]

The gut microbiome function may also be altered in disease states, with several studies demonstrating altered function in genes encoding protein synthesis in bacteria – with increased gene expression for the synthesis of lipopolysaccharides, amino acid transport, vitamin metabolism, tryptophan, and TMAO seen in patients with heart failure, and gene expression decreased for the synthesis of amino acids, iron transport, and nucleotide sugar synthesis. [7]

**Cardiovascular disease**

Traditional risk factors for cardiovascular disease (CVD) include hypertension, diabetes mellitus, dyslipidaemia, obesity, sedentary lifestyle, smoking. Recent evidence suggests that dysbiosis is a risk factor for hypertension, obesity and diabetes, and is also an independent risk factor for CVD.

*TMAO and SCFAs*

In 2013, Koeth and colleagues published a paper describing the intestinal microbiome's role in the metabolism of L-carnitine, a trimethylamine containing compound present in red meat. [8] The group described the production of trimethylamine-N-oxide (TMAO) by the metabolism of L-carnitine and identified TMAO as the link between a high red meat diet, and atherosclerosis. Subsequent research demonstrated that TMAO levels were associated with atherosclerosis development only in the presence of gut microflora – when germ free experiments were conducted, TMAO levels did not increase after dietary intake of precursors, and atherosclerosis did not occur. Similarly, when broad spectrum antibiotics were used, and the intestinal microbiome suppressed, TMAO levels were markedly reduced. Elevated levels of TMAO (those subjects in the highest quartile) have now been shown to be associated with a more than double risk of major cardiovascular events, even without the presence of coronary artery disease. [9] TMAO is also associated with increased risk of thrombosis, and enhanced platelet aggregation. Also, TMAO enhances the hypertensive effect of angiotensin II (a potent vasoconstrictor), likely playing a role in the development of

hypertension, and in heart failure (where increased extracellular matrix production is seen, along with an increase in adverse cardiac remodelling). [6] High TMAO levels have been found in patients with stable heart failure compared with normal subjects, and again higher levels have been associated with increased mortality in patients with heart failure, even after risk factor adjustment. [10] Elevated TMAO levels have also been associated with increased coronary artery plaque instability. [11]

It is thought that the combined effects of increased atherosclerosis, hypertension, platelet reactivity, and thrombosis with elevated TMAO levels, contribute to the increased rate of cardiovascular major events.

Signalling via SCFA is associated with reduction in blood pressure, thought to be mediated via GP41 receptors, as mice deficient in GP41 have hypertension.

*Other microbial metabolites*

Indoxyl sulphate is produced by microbial enzymes (tryptophanases), and then the cytochrome P450 system in the liver, by the conversion of dietary tryptophan. Indoxyl sulphate has pro-inflammatory and pro-oxidant effects in cardiac myocytes and fibroblasts, and may affect arterial blood pressure via serotonin signalling pathways. [1]

**Future therapeutics**

*Diet*

A high fibre diet (whilst not exactly novel as a health initiative) increases SCFA-producing microbes, and has been shown to lower blood pressure, reduce cardiac fibrosis, and reduce left ventricular hypertrophy in murine studies. [12] Prebiotics and probiotics may also be helpful in restoring gut microbiome composition: they have been shown to restore the ratio of Bacteroidetes to Firmicutes, replenish depleted species, and in animal studies where specific bacteria have been present in probiotic supplementation, have shown protective effects against diet-induced obesity, hypercholesterolaemia.

*Faecal transplants: can a "skinny healthy poo" transplanted into a patient alter that patient's disease?*

Faecal microbiota transplantation (FMT) is an area of active research. A proven therapy for treatment of *Clostridium difficile* infection, this is currently used in clinical practice. In patients with type II diabetes, faecal transplants from lean donors to obese recipients have been shown to increase insulin sensitivity and increase levels of satiety hormones in the obese recipients. [13] In animal studies, faecal transplants have been associated with weight loss. A team from Brigham and Women's hospital in Boston have recently reported their study, where 22 obese patients received either faecal capsules from a single healthy lean donor (BMI = 17) (11 patients) or placebo (11 patients), for a period of 12 weeks. The team measured glucagon-like peptide 1 and leptin levels (both hormones associated with satiety, and weight gain/loss) in both groups, as well as weight loss, and examined each patient's microbiome at the start, and for 12 weeks following the conclusion of the trial. No differences between the two groups were reported for weight loss, waist circumference, or hormone levels, however the microbiome of the recipients had altered to more resemble that of the transplanted microbiome (with greater diversity and similarities in genetic composition). [14] Further studies are planned examining FMT dose, and the longer-term effects of FMT.

*Drugs*

Antibiotics have a non-selective spectrum of activity, and thus inhibit the normal functional microbiome as well as dysbiosis. Antibiotics are not used currently in management of dysbiosis.

Targeted TMAO inhibitors, and drugs targeting the production of TMAO are currently being investigated. Recent animal studies have demonstrated that TMAO inhibitors and inhibitors of TMAO production have both successfully reduced diet-induced atherosclerosis, without otherwise altering the microbiome. The potential for agents such as these to prevent the development of atherosclerosis and cardiovascular disease is exciting, but as yet unproven and will require further research and long term follow up.

**Summary**

The gut microbiome has clear roles in the pathogenesis of cardiovascular disease, with several pathways, such as TMAO and SCFA production, now identified and characterised. It is now clear that the interplay of human genetics, nutrition, environmental exposures, and microbiome composition and function is important, and it is likely that there are multiple other microbe-mediated pathways for both protection and pathogenesis in cardiovascular and other diseases. Potential future developments include prognostic, diagnostic and therapeutic applications with targeted testing and therapies to known pathways, whilst preserving the normal microbiome.

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