Effects of gut microbiota on obesity and atherosclerosis via modulation of inflammation and lipid metabolism

R. Caesar*, F. Fák* & F. Backhed
Department of Molecular and Clinical Medicine, Sahlgrenska Center for Cardiovascular and Metabolic Research/Wallenberg Laboratory, University of Gothenburg, Gothenburg, Sweden


Recent studies have revealed a close relationship between inflammatory and metabolic pathways, and inflammation is now recognized to have a major role in obesity and metabolic diseases such as insulin resistance and atherosclerosis. The human body is home to a large number of distinct microbial communities, with the densest population in the distal gut (the gut microbiota). Bacteria have long been known to activate inflammatory pathways, and recent data demonstrate that the gut microbiota may affect lipid metabolism and function as an environmental factor that influences the development of obesity and related diseases. Here, we review how the gut microbiota may affect metabolic diseases by activating the innate immune system.

Keywords: atherosclerosis, gut microbiota, obesity, toll-like receptor.

Abbreviations: FFA, free fatty acid; GF, germ-free; LPS, lipopolysaccharide; RCT, reverse cholesterol transport; TLR, toll-like receptor.

Introduction

The gut mucosa is the largest immunologically active organ in the body and protects the host from invading microorganisms, which are collectively known as the gut microbiota. Although these microorganisms may affect inflammation and cause infectious diseases, they are also beneficial and have important functions including nutrient absorption, vitamin production and metabolism of xenobiotic compounds [1].

However, recent evidence indicates a role for the gut microbiota in promoting obesity. For example, germ-free (GF) mice are resistant to diet-induced obesity [2]. The metabolic mechanisms underlying this resistance include decreased absorption of glucose and generation of short-chain fatty acids from the gut lumen, and the associated reduction in hepatic lipogenesis, increase in fatty acid oxidation and decrease in deposition of triglycerides in adipocytes [2–5]. Furthermore, obesity has been shown to be associated with an altered gut microbial composition in mice [6] and humans [7, 8], although this needs to be confirmed in population-based studies. These results support the emerging view that the gut microbiota contributes to metabolic disease by modulating host metabolism [reviewed in [9–15]]. Here, we will discuss how the gut microbiota may affect obesity, and the related metabolic diseases such as insulin resistance and atherosclerosis, by modulating the innate immune system.

The innate immune system affects host metabolism and alters gut microbial ecology

Toll-like receptors (TLRs) are an evolutionarily conserved family of integral membrane pattern-recognition receptors that have a crucial role in the innate immune system, which is the early host defence against invading pathogens [16, 17]. TLR4 mainly recognizes lipopolysaccharide (LPS), whilst other TLRs are activated by microorganism-derived ligands such as flagellin and double- and single-stranded RNA and DNA [18]. Metabolic systems are closely integrated with pathogen-sensing systems [e.g. TLRs], which interfere with insulin signalling [19]. Thus, the gut microbiota may affect host metabolism by modulating inflammatory signalling pathways. Accordingly, mice
deficient in functional TLR signalling have reduced adiposity and improved glucose metabolism [20–23], largely because of modulation of inflammation in peripheral organs. Mice genetically deficient in Tlr5 exhibit hyperphagia and develop hallmark features of metabolic syndrome [23]. It is interesting that these phenotypes correlate with changes in the composition of the gut microbiota and are transmissible as evidenced by the fact that transplantation of the microbiota from Tlr5-deficient to GF mice resulted in obesity and reduced insulin sensitivity [23]. These findings directly demonstrate that modulation of the immune system affects host metabolism by altering the gut microbiota.

The gut microbiota and modulation of metabolic inflammation

Obesity and insulin resistance are associated with increased expression of inflammatory markers in adipose tissue and elevated levels of pro-inflammatory cytokines. It is becoming increasingly clear that this low-grade ‘metabolic’ inflammation constitutes a causative link between obesity and insulin resistance [24]. The human gut lumen serves as reservoir of LPS, which is the major component of the outer membrane of gram-negative bacteria [25]. Several studies have demonstrated that low levels of LPS are detectable in the blood of healthy humans [26–30], suggesting that LPS is continuously absorbed at a low rate from the gut. It is interesting that circulating LPS correlates with insulin levels, and patients with type 2 diabetes have increased amounts of circulating LPS [31].

In a seminal study in mice, Cani et al. [32] demonstrated a direct link between elevated circulating LPS levels and metabolic diseases by chronically infusing low levels of LPS subcutaneously for 4 weeks. The resultant modest twofold increase in circulating LPS matched the level in mice fed with a high-fat diet and caused similar increases in fasting blood glucose and insulinaemia. The increased LPS levels correlated with increased adipose macrophage infiltration and insulin resistance [32]. Ablation of the LPS co-receptor CD14 abrogated LPS- and high-fat diet-induced features of metabolic diseases [32]. Further support for the gut microbiota as a source of pro-inflammatory agents comes from the findings that GF animals have a reduced expression of inflammatory markers [33], decreased abundance of macrophages in adipose tissue (Caesar et al., in preparation) and improved insulin tolerance [2, 3]. As GF mice are not exposed to high levels of LPS, it is likely that the lean phenotype, at least in part, may be a result of reduced stimulation of inflammatory pathways.

Taken together, these findings demonstrate that LPS is sufficient to promote metabolic inflammation and cause insulin resistance.

Mechanisms of intestinal absorption of LPS

Two mechanisms of LPS absorption from the gut to the circulatory system have been proposed: (i) chylomicron-facilitated transport and (ii) extracellular leakage through tight junctions in the epithelial lining (Fig. 1). These mechanisms are not mutually exclusive but may function in parallel, and both are possible links between LPS uptake and metabolic diseases.

Chylomicron-facilitated transport

Chylomicrons are large, triglyceride-rich lipoproteins which are synthesized by enterocytes in the intestinal epithelium and transport dietary lipids to peripheral tissues. LPS is absorbed by enterocytes and transported to the Golgi compartment where chylomicrons are stored before secretion [34, 35]. A recent study showed that LPS secretion from the human epithelial colorectal cell line CaCo-2 is increased when cells are stimulated with fatty acids, which promote chylomicron formation [36]. Conversely, inhibition of chylomicron formation blocked LPS absorption in vivo [36]. Of note, administration of a high-fat meal to healthy human volunteers increases LPS levels during the postprandial period, and a generally high-energy diet results in increased plasma LPS levels [37, 38]. These data support the model of chylomicron-facilitated transport to explain how LPS is transported from the gut lumen into the circulation and how the transport correlates with food intake and composition.

Extracellular leakage through tight junctions in the epithelial lining

The alternative, or possibly complementary, route for LPS movement across the gut epithelium is paracellular transport through tight junctions. Genetically obese mouse strains show increased levels of endotoxaemia [39, 40], which correlate with reduced electrical resistance of the intestinal epithelium and extensive redistribution of the tight junction proteins occludin and ZO-1 [39]. Mice that are obese as a result of a high-fat diet also have increased levels of endotoxaemia, inflammation and gut permeability, which are reduced by manipulating the gut microbiota with antibiotic treatment [40]. In high-fat diet-fed mice, manipulation of the gut microbiota by administration of the prebiotic oligofructose reduces
plasma LPS levels, concentrations of circulating inflammatory cytokines and hepatic inflammation, which correlate with reduced intestinal permeability and improved tight-junction integrity [41]. The increase in gut integrity associated with prebiotic treatment appears to involve glucagon peptide-2 [42].

Together, these data suggest that changes in tight-junction permeability by diet and gut microbiota composition could promote endotoxaemia. However, it is important to note that it has not been directly demonstrated that LPS can indeed be transported through tight junctions.

**Activation of TLRs mediates metabolic inflammation**

Tissue expansion and adipocyte hypertrophy, which are associated with obesity, lead to increased adipocyte cell death and macrophage recruitment to remove cell debris from leaking cells [43]. This results in increased numbers of pro-inflammatory macrophages and increased production of cytokines such as tumour necrosis factor-α and interleukin-6 in adipose tissue of obese individuals [24, 44–46]. Because the gut microbiota may increase macrophage infiltration to adipose tissue both by providing inflammatory stimuli (e.g. LPS) and by promoting energy harvest from the diet [47], which is associated with increased adipocyte hypertrophy [3], it is hard to determine their specific contribution to metabolic inflammation. Furthermore, plasma levels of free fatty acids (FFAs) and LPS co-fluctuate and conditions promoting elevated concentration of LPS, such as obesity and high-fat diet, are also associated with increased concentrations of circulating FFAs.

Mice with defective TLR4 signalling are protected against inflammation and are also partly protected against insulin resistance induced by lipid infusion and high-fat diet [20, 21, 48]. It has been shown that it is the pro-inflammatory CD11c+ macrophage sub-population in adipose tissue that is activated and enriched by fatty acid stimulation of TLR4 and TLR2 [49]. Furthermore, cross-talk between macrophages and adipocytes in adipose tissue involves activation of NF-κB by saturated fatty acids through a TLR4-dependent mechanism [50]. These findings suggest that TLR4 is a molecular link between inflammation, nutrition and metabolism and further support the idea that cellular responses to pathogen infection and nutritional imbalance share signalling pathways.

Using bone marrow chimeras, Saberi et al. [22] demonstrated that TLR4 signalling in macrophages is required for recruitment of macrophages to adipose tissue and development of insulin resistance, but not for diet-induced obesity. Although exactly how TLR4 signalling interferes with insulin signalling remains to be determined, it is known that activation of JNK and IκB kinase, both of which are induced by TLR signalling, mediates insulin resistance by phosphorylation of IRS-1 [51, 52]. Furthermore,
Liver X receptors (LXRs) are nuclear receptors that by which RCT protects against atherosclerosis [61]. Wall macrophages is believed to be a critical first step in peripheral tissues. Efflux of cholesterol from vessel counteracts the accumulation of excess cholesterol. Reverse cholesterol transport (RCT) is a process that TLRs and reverse cholesterol transport biota contributes to atherosclerosis. However, it is not clear whether the ligands that promote atherosclerosis by activating TLR signalling are ever, it is not clear whether the ligands that promote atherosclerosis by activating TLR signalling are.

It should be noted that in addition to LPS, the microbiota is a source of many other pro-inflammatory molecules (e.g. peptidoglycan, lipoproteins and flagellin) that bind to TLRs or other pattern-recognition receptors. It was recently demonstrated that peptidoglycan originating from the gut microbiota has the potential to prime neutrophils in the bone marrow to upregulate their capacity to kill bacteria [55]. This phenotype required functional Nod1 signalling which suggests that microbial components other than LPS may contribute to metabolic diseases.

The role of TLRs and the microbiota in atherosclerosis

Macrophages accumulate in blood vessel walls during hyperlipidaemic conditions, and there they facilitate lipid uptake from the blood stream, eventually forming foam cells. These macrophages have been shown to have a pro-inflammatory profile induced by TLRs. Several TLRs are expressed on macrophages and vascular cells in human atheromas [56], and it is interesting that the TLR4 polymorphisms Asp299Gly and Thr399Ile, which lead to impaired TLR4 signalling, are associated with reduction in the incidence of atherosclerosis [57]. Similarly, ablation of Tlr2 or Tlr4 in mice reduces atherosclerotic plaque formation [58, 59]. Both TLR2 and TLR4 can induce pro-inflammatory gene expression by signalling through the adaptor molecule MyD88 [16]. As expected, Myd88−/− mice are protected from developing atherosclerosis [58, 60]. Cholesterol metabolism and uptake of oxidized LDL are not affected in Myd88−/− mice, but chemokine expression and subsequent macrophage recruitment to the arterial wall are reduced [60]. However, it is not clear whether the ligands that promote atherosclerosis by activating TLR signalling are endogenous or exogenous and whether the gut microbiota contributes to atherosclerosis.

TLRs and reverse cholesterol transport

Reverse cholesterol transport (RCT) is a process that counteracts the accumulation of excess cholesterol in peripheral tissues. Efflux of cholesterol from vessel wall macrophages is believed to be a critical first step by which RCT protects against atherosclerosis [61]. Liver X receptors (LXRs) are nuclear receptors that control cellular and whole-body cholesterol homeostasis and are activated by endogenous oxysterols [62]. Macrophages, liver and small intestine are the main target tissues of LXR agonists of relevance to RCT [62]. LXR agonists increase the expression of ATP-binding cassette transporters A1 and G1 in macrophages, which results in increased cholesterol efflux. Furthermore, bile acid synthesis is elevated by increased hepatic expression of cholesterol 7 α-hydroxylase resulting in increased cholesterol excretion in faeces [61]. Of interest, TLR4 inhibits activation of LXRs, presumably through an MyD88-independent pathway [63] (Fig. 2). Smoak et al. recently demonstrated that TLR2 and TLR4 are essential for RCT as apolipoprotein A-I, but not high-density lipoprotein, utilizes MyD88-dependent signalling [64]. It is noteworthy that RCT induced by endogenous apolipoprotein was also shown to be MyD88 dependent in vivo. Taken together, these findings suggest that TLR signalling modulates cholesterol metabolism and that the gut microbiota may be important for RCT.

Infections and cardiovascular disease

Since the first half of the 19th century, infections have been thought to cause or promote atherosclerosis by augmenting pro-atherosclerotic changes in vascular cells [65]. These changes include increased scavenger receptor expression and activity, enhanced uptake of cholesterol and modified low-density lipoprotein and increased expression of adhesion molecules and inflammatory cytokines leading to atherosclerotic plaque vulnerability [65]. Epidemiological studies have shown a link between periodontitis, Chlamydia pneumoniae and Helicobacter pylori infections and atherosclerosis [66], indicating that infectious agents might be involved in cardiovascular disease initiation or progression. Indeed, C. pneumoniae can induce formation of foam cells in the blood vessel wall, and bacterial DNA can be identified in more than 50% of all plaques [67, 68]. It is possible that C. pneumoniae may infect macrophages and lymphocytes in the lung and through these cells translocate to the vascular wall of the aorta [69]. Because there is a close relationship between infections and cardiovascular disease, attempts have been made to treat atherosclerosis with antibiotics. The results have been inconsistent and no consensus regarding the effect of antibiotics in preventing or reducing atherosclerosis has been reached, and studies using anti-chlamydial antibiotics have not demonstrated any major benefits in patients with arterial disease [70–72].
The normal oral and gut microbiota and atherosclerosis

Impaired dental health is associated with an elevated risk of myocardial infarction [73], and the results of several studies have suggested an oral source for plaque-associated bacteria [74–77]. In support of this finding, the oral pathogen Porphyromonas gingivalis is frequently identified in plaques [75, 77], and direct administration of P. gingivalis to Apoe−/− mice demonstrated that this bacterium may promote development of atherosclerosis [78]. Poor oral hygiene has recently been related to an increased risk of cardiovascular disease [79]. Of interest, it was associated with low-grade inflammation (i.e. elevated C-reactive protein levels), which is associated with cardiovascular events in humans [80]. These data suggest that bacteria from the oral cavity may affect atherosclerosis by promoting low-grade inflammation.

Germ-free mice provide a useful model to test the role of microorganisms as causative agents of atherosclerosis. GF mice on a low-fat chow diet tend to develop atherosclerotic plaques, whereas conventional ApoE−/− mice do not. By contrast, this difference was ameliorated in mice fed with a high-fat diet supplemented with cholesterol [81]. In another study, it was demonstrated that GF ApoE−/− mice had slightly reduced atherosclerosis after 22 weeks of high-fat feeding, which may in part be explained by their resistance to diet-induced obesity [82]. By contrast, preliminary data from our laboratory demonstrated that GF ApoE−/− male mice were protected from atherosclerosis compared with conventional ApoE−/− mice after 12 weeks of high-fat feeding (Fåk et al. in preparation). Thus, bacteria may be important for the initiation but not the progression of atherosclerosis; this may also explain the unsuccessful treatment of atherosclerosis with antibiotics.

Interventions with probiotic bacteria and prebiotics

Obesity is characterized by increased adiposity along with low-grade systemic inflammation and insulin resistance. These traits have all been shown to be modulated by the gut microbiota [9–11, 15], and therefore attempts have been made to alter them by modulating the gut microbiota. Both in rodents and humans, lactobacilli have been shown to reduce blood cholesterol levels [83, 84], possibly as a result of the fact that some bacteria express the bacterial enzyme bile salt hydrolase, which can affect cholesterol re-absorption in the intestine. Perinatal administration of Lactobacillus rhamnosus GG modulated the...
increase in body weight in the early years of life, but had no effect in the later stages of development [85]. Furthermore, administration of the probiotic bacterium \textit{Lactobacillus gasseri} in fermented milk for 12 weeks reduced adiposity and body weight in obese adults, possibly by reducing lipid absorption and inflammatory status [86].

Only a few studies have investigated the effect of bacterial interventions on atherosclerosis development in animal models. Portugal \textit{et al.} investigated whether \textit{Lactobacillus delbrueckii} reduces atherosclerosis by colonizing conventionally raised \textit{ApoE}−/− mice, but observed no significant effects on atherosclerotic lesion size [87], which may be explained by the fact that the bacterium did not alter blood cholesterol levels. By contrast, administration of prebiotics to \textit{ApoE}−/− mice for 16 weeks, which had more dramatic effects on the gut microbiomal composition, reduced atherosclerotic lesion size by 35% [88]. Furthermore, the probiotic bacterium \textit{Enterococcus faecium} improved the lipid profile in rabbits, without affecting atherosclerosis development [89]. Of interest, the probiotic bacterium \textit{Lactobacillus plantarum} 299v affects several biomarkers of cardiovascular disease in smokers (e.g. reduces systolic blood pressure and leptin and fibrinogen levels) [90]. Taken together, these data suggest that different probiotics have specific effects that may improve biomarkers associated with metabolic diseases. The clinical impact and the molecular mechanisms of these probiotics remain to be identified. A potential strategy to modulate host metabolism would be to design a cocktail of probiotics with defined mechanisms of action.

\textbf{Concluding remarks}

Accumulating evidence suggests that bacteria affect host metabolism and may contribute to the development of obesity and diseases such as diabetes and atherosclerosis. One major problem with regard to research in this field is the discrepancy between methods used to determine the gut microbial composition, which may explain different outcomes of studies investigating the role of the gut microbiota in obesity (reviewed in [91]). Standardization and verification of these methods would facilitate future studies, which combined with mechanistically testing in GF mice may reveal the mechanism by which individual bacterial species or defined communities affect metabolic diseases. In the future, it may thus be possible to diagnose and treat these common and fatal diseases by targeting the microbes associated with the human body.

\textbf{Conflict of interest statement}

No conflicts of interest to declare.

\textbf{Acknowledgements}

We thank Rosie Perkins for editing the manuscript. This work was supported by the Human Frontier of Science Program (RGY64/2008), the Swedish Research Council (K2007-65X-20421-01-04), the Swedish Foundation for Strategic Research, the EU-funded ETHERPATHS (FP7-KBBE-222639, http://www.etherpaths.org) and TORNADO projects (FP7-KBBE-222720, http://www.fp7tornado.eu/), Åke Wiberg, and Torsten and Ragnar Söderberg Foundations, and a LUA-ALF grant from Västra Götalandsregionen.

\textbf{References}

Bäckhed F, Crawford PA. Coordinated regulation of the metabolome and lipidome at the host-microbial interface. *Biochim Biophys Acta* 2010; **1801**:240–5.


86 Kadooka Y, Sato M, Imaizumi K et al. Regulation of abdominal adiposity by probiotics (Lactobacillus gasseri SBT2055) in adults with obese tendencies in a randomized controlled trial. Eur J Clin Nutr 2010; 64: 636–43.


89 Cavallini D, Bedani R, Bombespaço L, Vendramini R, Rossi E. Effects of probiotic bacteria, isoflavones and simvastatin on lipid


Correspondence: Fredrik Båckhed, Wallenberg Laboratory, Sahlgrenska University Hospital, S-413 45 Gothenburg, Sweden. (fax: +46-31-82-3762; e-mail: Fredrik.Backhed@wlab.gu.se)