GENERAL INTRODUCTION
Cardiometabolic disease represents a cluster of metabolic abnormalities that are risk factors for cardiovascular disease (CVD) including atherosclerosis. The world is facing an epidemic increase in atherosclerosis which is currently one of the major leading causes of deaths worldwide [1]. Cardiometabolic disease is predominantly driven by overweight and obesity and is further characterised by insulin-resistant glucose metabolism, dyslipidemia and hypertension which can ultimately lead to atherosclerosis. The mechanisms responsible for developing cardiometabolic disease are intensively investigated, and it is generally acknowledged that hyperlipidemia and inflammation play a prominent role.

**HYPERLIPIDEMIA AND INFLAMMATION IN CARDIOMETABOLIC DISEASE**

**OBESITY AS A PRECURSOR FOR TYPE 2 DIABETES AND ATHEROSCLEROSIS**

Obesity develops as a result of a long-term positive energy balance where energy intake exceeds energy expenditure. After a meal, dietary triglycerides (TG) and cholesterol are taken up by intestinal cells, which assemble the lipids into TG-rich lipoproteins named chylomicrons, that subsequently travel via the lymph to the blood. From the blood, chylomicrons can provide skeletal muscle and white adipose tissue (WAT) TG-derived fatty acids (FA). The TG-depleted chylomicrons or chylomicron-remnants are cleared by the liver. The majority of the cholesterol in the chylomicrons is ultimately used as a component of cell membranes and as precursor for synthesis of bile acids (BAs), steroid hormones, and vitamin D.

Excess energy intake that is not used for the production of energy in various tissues will be stored primarily in WAT, causing WAT to expand. However, excessive WAT expansion lead to dysfunctional WAT that renders a pro-atherogenic profile (reviewed in [2]). In brief, the balance between TG storage and TG removal from adipocytes determines WAT mass. The removal of TG from WAT is dependent on intracellular lipolysis in which TG are broken down to FA and released from the adipocytes into the circulation. In healthy conditions, circulating free fatty acids (FFA) can be oxidised in other organs such as muscle, liver and brown adipose
tissue. Obesity on the other hand is characterised by hypertrophy of white adipocytes together with increased release of FA into the circulation due to both elevated basal lipolytic rate as well as a reduced regulated lipolysis response to insulin [3,4]. The decreased response of expanded WAT to insulin can be a result of increased WAT inflammation. For example, extensive WAT hypertrophy stresses adipocytes and induces the secretion of inflammatory proteins (adipokines) which subsequently attract pro-inflammatory leukocytes that also release pro-inflammatory cytokines and chemokines into the circulation [5,6]. In the presence of WAT inflammation, the inflammatory cytokines tumour necrosis factor α (TNF-α) and interleukin-6 (IL-6) have been shown to directly inhibit the insulin signalling pathway and thereby induce insulin resistance predominately in WAT itself, liver, and muscle [7–10]. The numbers of pro-inflammatory M1 macrophages and cytotoxic T cells in expanded WAT are increased, whereas the numbers of anti-inflammatory M2 macrophages and regulatory T cells are reduced [11,12]. In addition to macrophages and T cells, increased abundance of B cells and neutrophils and reduced abundance of eosinophils are also hallmarks of WAT inflammation [13–15]. This increased pro-inflammatory state in expanded WAT is also associated with the development of glucose intolerance and type 2 diabetes. After a meal, plasma glucose levels rise and in response insulin is released by the pancreas. Insulin acts on its target tissues including muscle, liver and adipose tissues and induces the uptake of glucose by these tissues which subsequently lowers plasma glucose again [16]. However, inhibition of insulin signalling by e.g. dysfunctional expanded WAT leads to hyperglycemia, signalling the pancreas to produce more insulin. Progressive insulin resistance and compensatory increases in insulin production will ultimately result in failure of the pancreas and the development of type 2 diabetes [9,17,18].

The combination of insulin resistance and the rise in circulating FFA in obesity leads to reduced FFA oxidation and therefore decreased clearance of FFA from the circulation by other organs [19]. The excess FFA that cannot be oxidised by other organs build up in plasma and can serve as a substrate for hepatic TG-rich very-low-density lipoprotein (VLDL) production [20]. Hepatic TG-VLDL are either synthesised de novo, extracted from the circulation as non-esterified FA, or recycled from lipoprotein remnants cleared by hepatic
receptors. As a consequence, plasma levels of TG-VLDL are increased, called hypertriglyceridemia. TG-VLDL particles carry 90% of the plasma TG in the fasted state. TG-VLDL are substrates for the lipolytic enzyme lipoprotein lipase (LPL) mediating TG removal, which finally renders smaller lipoprotein particles with a low density of TG and relatively high in cholesterol, called low-density lipoproteins (LDL). High levels of plasma cholesterol is called hypercholesterolemia. WAT Inflammation, systemic inflammation, hypertriglyceridemia, and hypercholesterolemia form major risk factors for the development of atherosclerosis.

**ATHEROSCLEROSIS**

The development of atherosclerosis is thought to start with some form of damage of the arterial wall, for example due to high shear stress caused by the blood flow, allowing for the retention of LDL to infiltrate and accumulate in the sub-endothelial space of the arterial wall. These trapped lipoproteins are subject to oxidation resulting in oxidised LDL (oxLDL)[21]. The presence of oxLDL activates endothelial cells to express adhesion molecules. Adhesion molecules form the entry point for monocytes to invade the arterial wall where they differentiate towards macrophages. These macrophages scavenge oxLDL which under chronic conditions results in the formation of lipid-laden foam cells and a fatty streak in the arterial wall. This fatty streak is the hallmark of atherosclerosis development [22]. Activated endothelial cells and foam cells also produce pro-inflammatory cytokines and chemokines, leading to proliferation of vascular smooth muscle cells (VMCs) that produce a collagen cap, and attraction of more immune cells towards the lesion. Due to cell death within the lesion, a necrotic core may be formed, containing dead cell debris and cholesterol crystals that are released from foam cells.

The shape and cellular composition of arteries is dynamic and the arterial wall can respond to the presence of lesions by vascular remodelling. Inward vascular remodelling in arteries results in lumen loss, whereas outward vascular remodelling can compensate for lumen loss due to plaque accumulation in the arterial wall [23]. Outward remodelling together with a preserved luminal area often indicates a plaque phenotype that is more vulnerable to rupture [24]. A stable plaque phenotype is defined by a thick collagen cap and a small necrotic core. In
contrast, an unstable plaque is characterised by a low collagen content and thin cap [25], a large necrotic core and a high amount of macrophages within the lesion [21]. An unstable plaque prone to rupture might cause a local thrombotic event and the formation of a thrombus that will stop the blood flow at the site of the lesion, or at some distant site. Occlusion of arteries in the heart can cause an infarction and in the brain it can cause a stroke [21].

Taken together, hyperlipidemia and inflammation play an important role in the development of cardiometabolic disease, including atherosclerosis. Therapeutic interventions that can both decrease hyperlipidemia and inflammation may help to mitigate the underlying pathologies of cardiometabolic disease. Although relatively efficient drugs are available that inhibit the development of atherosclerosis by targeting plasma cholesterol levels and inflammation directly, additional strategies that reduce inflammation and hyperlipidemia are urgently required. Recently, it has become clear that factors deriving from the intestinal tract play a role in the initiation and progression of cardiometabolic disease [26,27].

GUT MICROBIOTA

GASTROINTESTINAL TRACT AND GUT MICROBIOTA COMPOSITION

The gastrointestinal tract comprises the entire path from mouth to stomach, duodenum, jejunum, ileum, cecum, and colon. The major functions of the gastrointestinal tract are to extract and absorb energy and nutrients from food we ingest, and excrete the remaining waste as feces [28]. Although the digestive process starts in the mouth, a substantial part of the enzymatic digestion of food takes place in the small intestine, whereas the remainder of the food components that cannot be digested in the small intestine will reach the cecum and colon of the large intestine. Predominantly complex carbohydrates, but also proteins, which are not digested by host enzymes in the upper gut, are metabolised by gut bacteria in the distal gut [29].

Microorganisms that specifically live within the intestinal tract are collectively known as gut microbiota. This gut microbiota harbours bacteria, viruses, fungi, archaea, and protozoa.
New molecular techniques such as next generation sequencing have made it possible to further identify and study the composition of the human gut microbiota [30–32]. It is estimated that the gut microbiota harbour some $10^{14}$ bacteria and consists of more than 1000 different bacterial species [33,34]. In the jejunum and ileum, the diversity of the microbiota composition is greater than in the duodenum and is composed of mainly anaerobic bacteria. The gut microbiota can be classified using phylogenetic trees, ranging from kingdom, phylum, class, order, family, genus, to species level. The dominant phyla in human gut microbiota are Bacteroidetes and Firmicutes [35], but many other phyla are present, including Actinobacteria, Proteobacteria, and Verrucomicrobia [36]. Most of the species are difficult or impossible to cultivate in vitro, due to required anaerobic conditions and unknown substrate requirements. However, exploitation of new analytical techniques based on genomics, proteomics and metabolomics approaches, makes it possible to obtain more insight into the diversity of the gut microbiota, their metabolic activities, and their functions and effects on the host [37–39].

**GUT MICROBIOTA SYMBIOSIS AND DYSBIOSIS**

Babies acquire intestinal microbiota from the mother and environment during and after birth and are thus affected by mode of delivery and neonatal nutrition. The gut microbiota composition diversifies after the first few years of life, and will eventually converge an adult-like phylogenetic structure [40,41]. Although gut microbiota composition within each individual has been reported to be relatively stable over time [42,43], the intestinal microbiota composition varies greatly between individuals [36,44]. Major differences in gut microbiota composition can be caused by changes in environmental factors such as diet, exercise, medication use, hygiene as well as by specific diseases [39,45–47].

In a symbiotic relationship, interactions between the host and the gut microbiota play a crucial role in the maintenance of normal physiology by extracting remaining energy from food components, shaping the immune system, offering protection against invading pathogens, and by producing essential vitamins [48,49]. Despite strictly controlled interactions between host and gut microbiota, the symbiotic relationship of the host and intestinal microbiota can
become impaired. Dysbiosis is a term for gut microbiota imbalance or maladaptation inside the body and is thought to be associated with disease. Abundant scientific evidence indicates that the increased prevalence of obesity, type 2 diabetes, and atherosclerosis cannot be attributed solely to changes in the human genome, nutritional habits, or reduced physical activity in our daily lives [50], but by the gut microbiota as well [51–55].

**GUT MICROBIOTA DYSBIOSIS, OBESITY AND TYPE 2 DIABETES**

The mechanisms linking gut microbiota and cardiometabolic disease are under intense investigation. The role of gut microbiota in energy homeostasis and obesity development has been pioneered by Bäckhed et al. They found that mice raised in the absence of microorganisms, i.e. germ-free (GF) mice, had about 40% less total body fat than mice with normal gut microbiota, even though GF mice ate 30% more than the conventional mice [56]. When GF mice were conventionalised again with a normal gut microbiota harvested from the cecum of a “normal” mouse, body fat content was increased by 60% and fasting glucose and insulin levels were elevated despite a significantly lower food intake compared to unconventionalised GF mice [56]. The mechanisms behind the weight gain in the conventionalised mice may be ascribed to energy extraction from indigestible food components by the acquired microbiota, an increase in intestinal carbohydrate absorption, and concomitant increase in de novo hepatic lipogenesis which are associated risk factors for the development of obesity and type 2 diabetes.

Besides affecting energy homeostasis, the gut microbiota also influence local and systemic immune responses [57,58]. It has been suggested that high-fat diet (HFD) feeding leads to gut microbiota dysbiosis and inflammation in the small intestine, characterised by activation of specific immune cells and production of pro-inflammatory cytokines [9,59]. These pro-inflammatory factors may contribute to increased intestinal permeability and leakage of lipopolysaccharides (LPS) from the lumen into the circulation. Systemic LPS may lead to activation of circulating immune cells and to infiltration of macrophages in adipose tissue, liver, and muscle. As mentioned previously, increased infiltration of macrophages in e.g. WAT may contribute to the development of systemic inflammation and insulin resistance.
Furthermore, short chain fatty acids (SCFA) produced by the gut microbiota also play a role in the inflammatory phenotype, which will be described in the section ‘Short-chain fatty acids’. Together, this suggests that HFD-induced gut microbiota dysbiosis may also play an indirect role in the development of cardiometabolic disease.

**GUT MICROBIOTA DYSBIOSIS AND ATHEROSCLEROSIS**

Gut microbiota dysbiosis is not only associated with insulin resistance, but recent studies also indicate a prominent role for gut microbiota in the development of atherosclerosis. The gut microbiota may affect atherosclerosis development via different mechanisms, e.g. by affecting the immune system and/or by affecting cholesterol metabolism [60,61].

During bacterial infections, it was found that bacteria can translocate from the intestinal lumen to the site prone to atherosclerotic lesion development, as evidenced by the presence of bacterial DNA in human atherosclerotic plaques [62,63]. Moreover, some infectious microorganisms have been shown to be potentially associated with atherosclerosis. For example, microorganisms such as *A. actinomycetemcomitans*, *C. pneumoniae*, *Helicobacter pylori*, and *P. gingivalis* might contribute to atherosclerosis by increasing atherosclerotic lesion areas in various experimental models [64]. However, although the presence of bacterial DNA in human atherosclerotic plaques is established, it is uncertain whether an infection initiates or promotes atherosclerosis development in humans.

Despite the site of infection (local or systemic), the immune system responds to microbial-derived components, leading to the activation of several inflammatory pathways. For instance, several studies demonstrated that low levels of LPS are detectable in the circulation of healthy humans [65,66], suggesting that LPS can translocate from the intestinal lumen into the bloodstream. It was found that LPS leads to the recruitment of adaptor proteins e.g. MYD88 to the cytoplasmic domain of Toll-like receptors (TLRs). After recruitment of adaptor proteins, downstream signalling cascades can be triggered that lead to the production of pro-inflammatory cytokines and chemokines [67]. It has been suggested that LPS may lead to the induction of a low-grade inflammatory state and can aggravate the progression
of atherosclerosis [61,68].

In addition to affecting inflammation in atherosclerotic plaques, the gut microbiota may also affect atherosclerosis development by influencing the enterohepatic circulation of BAs and therefore cholesterol metabolism. BA metabolism and cholesterol metabolism are interregulated. In the liver, the BAs cholic acid (CA) and xenodeoxycholic acid (CDCA) are synthesised from cholesterol. In rodent liver, most of the CDCA is converted to α-muricholic acid (α-MCA) and β-muricholic acid (β-MCA) [69,70]. After formation, BAs are predominantly conjugated in the liver to either glycine in humans, or to taurine in rodents [71,72], after which primary BAs are secreted in the bile canaliculi and directed to the gall bladder. Upon ingestion of a meal, bile is released into the duodenum to facilitate the digestion and absorption of dietary lipids by pancreatic lipase. Approximately 95% of the conjugated BAs are actively reabsorbed by the apical sodium-dependent bile acid transporter (ASBT) in the distal ileum and returned to the liver via enterohepatic circulation. Although the major component of BA absorption is active, some of the absorption of BAs is passive, less intensive compared to active absorption, and occurs along the entire small intestine and colon. BAs that are unconjugated and more lipophilic such as CDCA and deoxycholic acid (DCA) diffuse more readily through the apical membrane than hydrophilic BAs such as CA. On the other hand, active transport via ASBT is primarily responsible for absorbing conjugated BAs in the ileum which are more hydrophilic [73]. A fraction of the BAs that escape active or passive absorption in the small intestine is subject to bacterial modification in the colon. Gut microbiota that possess active bile salt hydrolase (BSH) activity in the colon first deconjugate BAs, followed by a 7α-dehydroxylation reaction leading to the formation of secondary BAs [74,75] including hyocholic acid (HCA), DCA, and ω-muricholic acid (ω-MCA) [76]. The deconjugated secondary BAs may then be either absorbed passively in the colon or excreted in the feces. Both the absorbed primary BAs and the secondary BAs formed by the gut microbiota are recycled back to the liver via the portal vein and again undergo biotransformation through conjugation to glycine or taurine [77,78]. Thus, gut microbial metabolism of BAs increases BA diversity but also contributes to a more hydrophobic and toxic BA pool. To prevent accumulation of potentially cytotoxic
BAs, BA transport and metabolism are tightly regulated within the liver and intestine. As such, hepatic conversion of cholesterol to BAs balances fecal bile excretion, being the major route for cholesterol catabolism and accounting for almost half of the cholesterol eliminated from the body per day [79]. Therefore, differences in fecal BA excretion affect the enterohepatic circulation of cholesterol and may ultimately affect plasma cholesterol levels [80]. Although it is widely accepted that BAs are important regulators of metabolic homeostasis, the exact relationship between BAs and atherosclerosis development is still elusive.

Finally, the gut microbiota interacts with dietary components and synthesise or convert specific metabolites that, when taken up by the host, directly affect physiological processes and promote or prevent CVD. An example of a CVD-promoting metabolite is trimethylamine N-oxide (TMAO). The group of Hazen et al., found that metabolism of dietary phosphatidylcholine and L-carnitine by intestinal microbiota results in the formation of the metabolite trimethylamine (TMA). TMA enters the circulation and is converted in the liver to TMAO. In patients that are vulnerable to a cardiovascular event, increased TMAO was associated with increased risk of cardiometabolic disease and atherosclerosis [81,82]. The same group recently showed that TMAO directly increases platelet hyperreactivity and thus the risk for a thrombotic event [83]. Synthesised metabolites by the gut microbiota that are linked to the prevention of CVD are e.g. SCFAs. The role of SCFAs in the prevention and onset of CVD is described in the section ‘Short-chain fatty acids’.

Taken together, evidence implicates a role for the gut microbiota in the development of obesity, type 2 diabetes, and atherosclerosis via different pathways. Targeting the gut microbiota may be a promising tool for the prevention and treatment of these diseases including the underlying pathology such as inflammation and hyperlipidemia.
Diet and Gut Microbiota

It has long been known that diet is a major contributor to the risk of developing cardiometabolic disease [84]. Not surprisingly, diet and its components are also a main driver of gut microbiota dysbiosis and underlying pathologies [45,85]. The primary source of energy that sustains the gut microbiota composition is obtained via the fermentation of indigestible carbohydrates [86–88]. Previous studies have shown that a diet rich in indigestible carbohydrates can improve health by increasing bacterial diversity and bacterial richness [89–91]. In contrast, intake of diets rich in fat and sucrose lead to the extinction of several taxa of the gut microbiota [92]. Furthermore, degradation of indigestible carbohydrates by the gut microbiota yields SCFAs, which are acknowledged to have beneficial effects on the intestinal epithelium and gut immune system [93]. Reasonably, this puts indigestible carbohydrates in the spotlight as a tool to modify gut microbiota composition and induce microbial diversity and richness to improve health and prevent disease.

Indigestible Carbohydrates

Indigestible carbohydrates are food ingredients considered as ‘roughage’ material that comprises portions of food that are not broken down by the enzymes of the human digestive tract. They are predominantly plant-derived and abundant in fruits, vegetables, cereals, and legumes.

Prebiotics

Based on the growing evidence for a link between the human diet and the gut microbiota composition in the large intestine [94,95], the term prebiotics was introduced for substances that cause specific advantageous shifts in the gut microbial composition. The term prebiotic, first introduced by Gibson and Roberfroid (1995), was defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health” [96]. This definition was later refined and adjusted with the addition of three criteria: 1) neither hydrolysis
nor absorption in the stomach or small intestine may occur, 2) the ingredient is fermented by intestinal bacteria, and 3) a selective response with regard to beneficial commensal bacteria in the colon is required [97]. Inulin and mannan-oligosaccharides (MOS) are two examples of indigestible carbohydrates with prebiotic activity and/or prebiotic potential that will be discussed below.

**INULIN**

Inulin is a water-soluble polysaccharide present in >45,000 plant species [98] which belongs to a group of indigestible carbohydrates called fructans. Inulin-type fructans are storage carbohydrates of plants containing 1–70 fructose units in their structure linked to a terminal sucrose molecule. Due to the β-configuration of the fructose monomers, these molecules are not absorbed in the small intestine and resistant to hydrolysis by digestive enzymes in the gastrointestinal tract [99]. Instead, inulin-type fructans are fermented by colonic microbiota stimulating the growth and activity of presumably beneficial gut bacteria. Therefore, inulin is an indigestible carbohydrate that meets the three classification criteria for being considered a prebiotic [96].

Inulin is widely studied for its potential to improve intestinal health. Most of the effects of inulin on the gut microbiota composition and function occur via changes in the prevalence and abundance of *Bifidobacteria, Bacteroides*, and *Lactobacilli* [100–102]. In addition, dietary intake of inulin has been associated with a number of health benefits including reduction of gastrointestinal diseases [103], regulation of food intake and appetite [104], but also stimulation of the immune system [105,106], and decreasing hyperlipidemia [107–110]. As immune-related inflammation and increased hyperlipidemia are important risk factors in the development of cardiometabolic disease and its underlying pathology, dietary inulin is an interesting tool for prevention and or treatment of cardiometabolic disease via interactions with the gut microbiota.
MANNAN OLIGOSACCHARIDES

Another important potential, but relatively unknown type of prebiotic, are MOS. MOS can be derived from the outer cell-wall membrane of yeast, plants, or bacteria [111]. The main constituents of the outer cell wall of yeast consists of β(1→3)-D-glucan, β(1→6)-D-glucan, chitin, mannan and proteins [112], which cannot be hydrolysed by host digestive enzymes. MOS obtained from the yeast *Saccharomyces cerevisiae* generally consist of glucomannan complexes [113].

Yeast *Saccharomyces cerevisiae*-derived MOS have been widely used in livestock industry as an alternative to antibiotics and as food supplementation to ameliorate performance by reducing pathogenic contamination [114–116]. However, the mechanism by which MOS exert their effect on the immune system is not fully established. One suggested mode of action by which MOS may improve inflammation is via interaction and modification of the gut microbiota. According to Spring *et al.*, MOS bind to type-1 fimbriae of pathogenic bacteria and prevent their adherence to the intestinal mucosa [117], thereby reducing pathogen-induced inflammation. Although the majority of the studies using MOS were conducted in species such as chickens [118,119], juvenile rainbow trout [120], or turkeys [121], it has been shown that MOS can decrease inflammation both within the gastro-intestinal tract [122] as well as systemically [123,124].

Moreover, in different studies using a variety of experimental animal models, it was shown that dietary supplementation with MOS lowered plasma cholesterol levels [125–127]. Similar to inulin, as inflammation and hyperlipidemia are associated with the onset of cardiometabolic disease, this warrants further research on the effect of MOS in the development of cardiometabolic disease and its underlying pathology.

SHORT-CHAIN FATTY ACIDS

Via interaction with specific gut bacteria, fermentation of indigestible carbohydrates leads to the production of SCFAs. When indigestible carbohydrates reach the colon to be fermented and metabolised by gut microbiota, they form SCFAs. SCFAs can serve as energy substrates,
directly activate G-protein coupled receptors (GPRs), inhibit histone deacetylases (HDAC) [128]. SCFAs consist of 1-6 carbons, of which acetate (C:2), propionate (C:3) and butyrate (C:4) are the most abundant (≥95%) [87,129,130]. Generally, acetate, propionate and butyrate are present in an approximate molar ratio of 60:20:20 in cecum content and feces [87,131]. The production rate and amount of SCFAs depend on the composition and density of the gut microbiota in combination with the type of indigestible carbohydrates available for microbial fermentation [97]. For example, when there is shortage in the supply of indigestible carbohydrates, gut microbes can switch to other sources to support their growth, such as amino acids or dietary fats [95,132]. These less favourable sources of energy lead to reduced fermentative activity of the microbiota and reduced SCFAs as end products [133]. However, supplementation of diets rich in protein or fat with additional indigestible carbohydrates, restores the levels of beneficial gut microbes, and increases SCFAs [134]. SCFAs in turn can be utilised by other bacterial species, or can be readily absorbed by the host. In the cecum and large intestine, 95% of the produced SCFAs are absorbed by the colonocytes, while the remaining 5% is excreted in the feces [135–138].

**TRANSPORT OF SHORT-CHAIN FATTY ACIDS**

Studies that investigated SCFA transport have been performed mostly in colonocytes, which are physiologically exposed to the highest concentrations of SCFAs. SCFAs can be transported across the apical and the basolateral membranes of colonocytes either via passive diffusion or via active transport mediated by a number of different transporters, including MCT1 and SMCT1 for transport across the apical membrane, and MCT4 and MCT5 for the basolateral membrane [139]. Depending on the strength of acidity ($pK_a$) and pH in the gut lumen, either passive diffusion or active transport of SCFAs takes place [139].

Currently, it is still elusive which transporters are exactly responsible for the uptake of SCFAs from the circulation into the tissues. However, OAT2 and OAT7 were identified to transport propionate and butyrate, respectively, across the membranes of hepatocytes [140,141]. Further research is needed to investigate the uptake of SCFA by different organs in order to
better understand the role of SCFAs in various tissues.

**SHORT-CHAIN FATTY ACIDS AS A SUBSTRATE AND THEIR REGULATION OF GLUCOSE AND LIPID METABOLISM**

When SCFAs are taken up, a large part can be used as a substrate for energy. For instance, it is known that humans can use SCFAs for approximately 10% of their daily caloric requirements [142]. Furthermore, around 60-70% of oxidised butyrate is used for the provision of energy in colonocytes [143,144]. Once absorbed, the SCFAs that are produced by gut microbiota in the cecum and colon will end up in the portal vein, the liver, peripheral blood, and in other peripheral tissues [87,145]. For example, while butyrate is hardly absorbed and mainly used as an energy source for colonocytes, acetate and propionate produced after colonic fermentation enter the portal vein of which the majority is taken up by the liver [87,146,147]. In general it is believed that the liver clears a large fraction of propionate from the portal circulation, but absolute values are still unknown. The remainder of the SCFAs will enter the peripheral blood circulation where they will be taken up by other organs and tissues such as adipose tissue, heart, muscle, and kidneys [148].

SCFAs play a role in the regulation of lipid and glucose metabolism [149–153]. For instance, acetate can be used as a substrate for hepatic de novo cholesterol and fatty acid synthesis [147], while propionate inhibits cholesterol synthesis and can be used as a substrate for gluconeogenesis [153]. Variation in the ratio of propionate:acetate can therefore be used to determine either hepatic stimulation or inhibition of lipogenesis which may consequently affect plasma lipid levels [154]. However, the extent to which propionate contributes to energy metabolism in humans is largely unknown due to the lack of data on actual production rates of propionate. SCFAs are able to regulate glucose metabolism by normalising plasma glucose levels and increasing glucose handling via activation of the hepatic AMPK pathway and by increasing the gut hormones peptide YY (PYY) and glucagon-like peptide-1 (GLP-1)[139]. Thus, when SCFAs are taken up and absorbed by various tissues and organs, they play an important role as a substrate in lipid and glucose metabolism.
SHORT-CHAIN FATTY ACIDS AS SIGNALLING MOLECULES

SCFA can serve as signalling molecules either as HDACs inhibitors or via activation of GPRs. Predominantly butyrate and, to a lesser extent, propionate are known to act as HDAC inhibitors, changing the expression of multiple genes with various functions, including proliferation, differentiation, apoptosis, and inhibiting inflammation (reviewed in [128]). Besides its function as HDAC inhibitors, SCFAs predominantly act via activation of the G-protein coupled receptors (GPR) GPR41 and GPR43 [155,156]. They are expressed on various cells residing in the intestine, but also on extra-intestinal cells like adipocytes, pancreatic cells, renal smooth muscle cells, enteric neuronal cells [157,158] and to a lower extend on hepatocytes [159]. Immune cells that can be activated by SCFA are granulocytes, some myeloid cells, macrophages, and dendritic cells [160–163]. SCFAs are well known for their potential to beneficially affect the immune system either directly or via indirect activation their receptors (reviewed in [164,165]). The possible immune-modulatory functions of SCFAs are revealed by a recent study in Gpr43−/− mice [166]. This study showed exacerbated inflammation in various models including colitis, arthritis and asthma. The underlying mechanisms on how SCFAs can modulate the immune system in obesity and atherosclerosis is via the effects of SCFAs on reducing chemotaxis and adhesion of immune cells. By preventing chemotaxis and cell adhesion, SCFAs might prevent infiltration of monocytes in adipose tissue and atherosclerotic lesions and can have a protective effect against systemic inflammation.
As the occurrence of cardiometabolic disease is still increasing, strategies to target the underlying risk factors inflammation and hyperlipidemia are urgently needed.

As evident from chapter 1 of this thesis, the gut microbiota have been strongly associated with the development of cardiometabolic risk factors and disease, including obesity, type 2 diabetes, and atherosclerosis. Since gut microbiota composition and function is highly susceptible to modification via dietary intervention, insight in the role of different dietary components in the modulation of the gut microbiota is crucial. This is a prerequisite for the development of novel strategies to modify risk factors associated with cardiometabolic disease.

Since we exploited the use of high fat and high cholesterol diets in the development of cardiometabolic disease, it was important to have the ability to determine blood lipid composition. Therefore in chapter 2, we describe a method to determine the medium- and long chain fatty acid composition of blood of mice using gas chromatography-mass spectrometry (GC-MS) analysis. Simultaneously, we have exploited the use of indigestible carbohydrates to modulate microbiota activity and composition. The potential role of SCFAs in mediating the beneficial role of plant-derived indigestible carbohydrates necessitates the development of comprehensive, sensitive, and reliable methodologies to quantify the SCFA composition in biological samples such as blood, cecum content and feces. For that reason, in chapter 3 we established a method to determine SCFAs in blood, cecum, and feces samples using GC-MS analysis.

To examine the effects of indigestible carbohydrates on cardiometabolic risk outcome, we performed two mouse studies in which we investigated the effect of the prebiotic inulin on atherosclerosis development. In chapter 4 we investigated the effect of the prebiotic inulin on accelerated atherosclerosis after placement of a non-constrictive perivascular cuff around the femoral artery. In chapter 5 we studied the effect of the prebiotic inulin on cholesterol-driven long-term atherosclerosis development.

Dietary MOS have proven effective at improving growth performance, while also reducing inflammation and hyperlipidemia in livestock. In this thesis, two studies are included
that focus on the effect of dietary supplementation with MOS on diet-induced obesity and atherosclerosis development. In chapter 6 we investigated the effect of MOS on innate immune composition in mesenteric white adipose tissue and liver as well as on diet-induced obesity and glucose intolerance. The effects of MOS on hyperlipidemia and atherosclerosis development were studied in chapter 7.

Finally, in chapter 8, methods to map gut microbiota composition and function, SCFAs as markers for gut microbial function, factors that determine gut microbiota function, the role of the gut microbiota in the development of atherosclerosis, the translatability of mouse models in gut microbiota research, and implications for prebiotics will be discussed.
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