Interplay between dietary fibers and gut microbiota for promoting metabolic health

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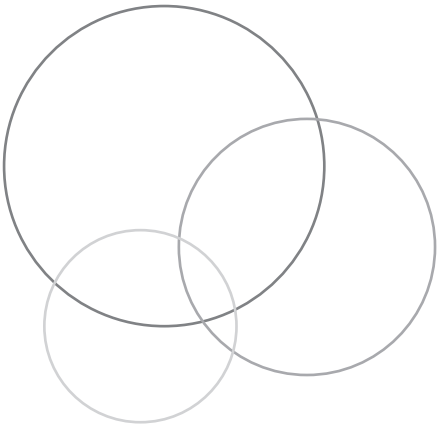

Chapter 1

General Introduction

1. Background

Worldwide, diets and lifestyles have rapidly changed over the last decades with economic development and globalization. Concurrently, the prevalence of metabolic syndrome has also seen a swift increase, indicating one of the negative consequences of an unhealthy diet and lifestyle.[1,2] Metabolic syndrome represents a cluster of several adverse metabolic conditions such as obesity, insulin resistance, type 2 diabetes, dyslipidemia, hypertension and non-alcoholic fatty liver disease. Eventually, such metabolic dysregulations can lead to the development of cardiovascular diseases, kidney failure and liver cirrhosis.

The role of gastrointestinal microbiota on host physiology has received increasing interest for its potential impact on the metabolic syndrome.[3] The human intestinal microbiota is a dynamic component of the human body which expresses approximately 100-fold more genes than the human genome.[4] The intestine is also one of the key organs involved in various processes including regulation of immune function and metabolic pathways of the host. Thus understanding the link between microbiota and manifestation of major epidemic diseases could provide mechanistic insight into its impact and therapeutic potential. Existing evidence supports the role of microbiota in the development of diseases *via* complex chemical interactions between gut microbiota-derived metabolites and the host.[5,6]

Dietary fibers are non-digestible oligosaccharides which are selectively fermented by the gastrointestinal microbiota and form an important element in modulating the composition and biological activities of the intestinal microbiota. Dietary fibers which confer physiological health benefits are also referred to as prebiotics.[7] One known health effect involves enhancing the intestinal barrier function which can prevent pathogen colonization and improve the immune system response.[6,8,9] More recently the impact of intestinal microbiota on the development of metabolic syndrome has become clearer. In this thesis, we have focused on the risk factors that contribute to the development of metabolic diseases such as cholesterol metabolism, insulin sensitivity and obesity. Today there is a growing interest in using dietary fibers in beverages and milk products. Some typical dietary fibers that are widely used to preferentially stimulate the growth of supposed beneficial bacteria and promote health are inulin, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS).[10] There are also other interesting candidates of dietary fiber which have demonstrated cholesterol-lowering potential such as α- and β-cyclodextrin and isomalto-oligosaccharides.[11–14]

Dietary fibers are naturally found in milk, fruits and vegetables such as onion, chicory, rye, barley, wheat and garlic. Their concentration ranges from 0.3% up to 20% of the fresh weight.[15] Dietary fibers are non-digestible because of their chemical

configuration of linked glycosidic bonds, which prevents their digestion by hydrolytic mammalian enzymes.[16] Chemically, dietary fibers are classified based on several factors such as molecular size, the type of monosaccharides, solubility, their degree of polymerization (DP, number of monosaccharides joined by glycosidic linkages) among others. Most dietary fibers are composed of at least three monosaccharide units (Fig.1). FOS for example (inulin, oligofructose) have D-fructose attached by β (2à1) linkages with variable DP. Inulin has a DP ranging from 11-60 whereas oligofructose (OFS) on average has a DP ranging from 3-10. Cyclodextrin has cyclic α (1à4)-linked malto-oligosaccharides with 6, 7 and 8 glucose units.[17]

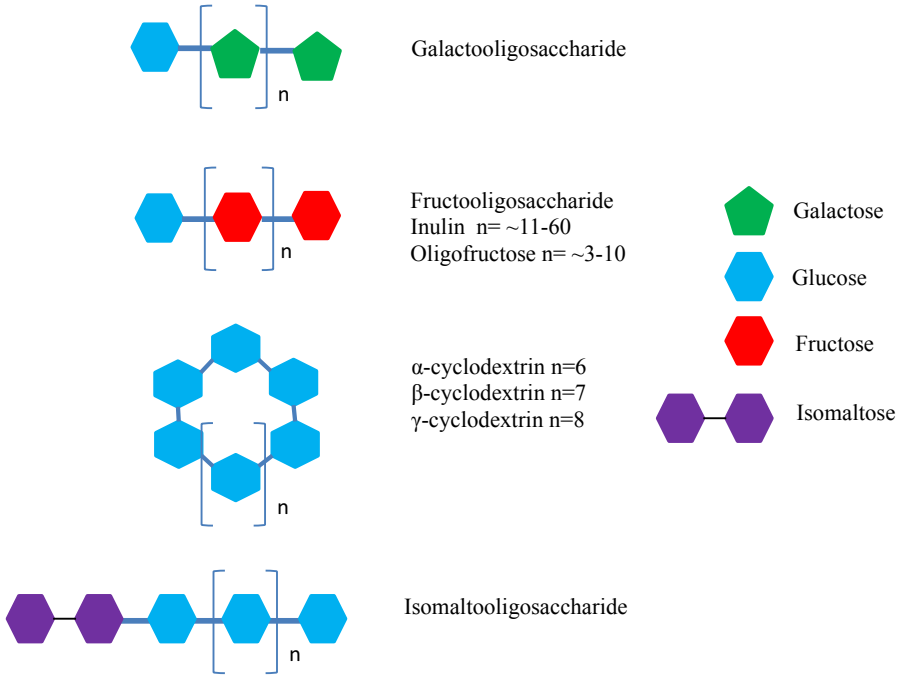

Figure 1: Structural variation of different non-digestible oligosaccharides.

Currently, dietary fibers that are widely used as nutritional products are generated at industrial scale from natural sources by enzymatic processes, only a few fibers are derived from direct extraction or isomerization reactions. Dietary fibers are also low in sweetness which is useful in food products to reduce the overall sweetness and to enhance other flavors. In terms of their physiological properties in humans, dietary fibers are hardly broken down by microorganisms in the mouth and they also resist digestion further on as humans lack enzymes required for hydrolyzing β-links between the monosaccharide units. Thus, most dietary fibers reach the cecum-colon where they can be metabolized by the anaerobic bacteria through fermentation. Specific

dietary fibers act as substrates for specific bacteria and/or result in specific breakdown products, leading to preferential proliferation of bacterial species and thereby altering the microbiota composition. The products released from the fermentation such as CO_2 or H_2 gases are not useful for the host and excreted as such. In contrast, several organic acids such as short-chain fatty acids (SCFA) that are also released by the bacteria have the potential to directly or indirectly regulate other metabolic processes in mammalian hosts.[17]

1.1 Targets of metabolic health

1.1.1 Short-chain fatty acids and their effect on metabolic health

Dietary fibers that undergo microbial fermentation in the colon yield various bioactive metabolites such as the short chain fatty acids (SCFA) acetate, butyrate and propionate.[18] In recent decades many studies have implicated SCFA in glucose and lipid metabolism of the host.[19–26] Microbes may release SCFA as a mean to achieve a redox balance in anaerobic conditions.[27] The amount of SCFA produced mostly depends on the amount and components of the diet. Under normal physiological conditions, SCFA levels were reported to range between 70-140mM in the proximal colon of pigs which decreased to 20-70mM in the distal colon.[22] Since SCFA are volatile organic acids, 95% of produced SCFA are readily absorbed by enterocytes in the cecum and large intestine by passive diffusion. The remaining 5% of the SCFA are excreted in the feces.[22,28] Studies utilizing microbiota from pigs have demonstrated that substantial variation occurs in SCFA production with supplementation of different dietary fibers.[29,30] Under physiological conditions acetate, propionate and butyrate are present in pigs in the ratio of 60:20:20 in colon and feces, which is similar to the colonic ratio of 57:22:21 measured in humans (with an average age of 57) who had died suddenly.[31,32]

As stated, SCFA have been implied in cholesterol homeostasis. Increased cholesterol is one of the major risk factors associated with the development of cardiovascular diseases. Thus, it is important to understand how cholesterol metabolism is regulated by dietary fibers-derived SCFA. Humans with hypercholesterolemia show a moderate increase in fecal propionate and butyrate and a trend towards a decrease in serum cholesterol when treated with inulin.[33] It could be hypothesized that propionate suppresses cholesterol synthesis in the liver as has been demonstrated *in vitro* in rat liver and intestine[34], possibly by lowering the enzymatic activity of hepatic 3-hydroxy-3-methylglutaryl-CoA synthase (HMGCS) and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR).[35] Most studies with dietary fibers, however, did not analyze the effect of the diets on cholesterol synthesis or on the intestinal cholesterol balance which can be

investigated by either measuring the enzymatic activity (HMGCS, HMGCR), stable labeled-isotopes (D_2O, ^{13}C-acetate) and radioisotopes.

Whereas propionate has been implied in decreasing cholesterol synthesis, acetate, which is produced in higher concentrations compared to other SCFA is a major source of acetyl-CoA, a precursor for cholesterol synthesis. Acetate was found to decrease in fecal samples of rats treated for 11 weeks with isomalto-oligosaccharides compared to inulin fed rats but its effect on plasma cholesterol was not measured.[11] Another study in humans has shown that the concentration of acetate increases after consumption of 10g per day of isomalto-oligosaccharides but there is nevertheless decreased plasma cholesterol.[36] Since SCFA have shown to regulate cholesterol metabolism it would be interesting to explore how dietary fibers stimulate the production of specific SCFA and the role of the microbiota in this process. Apart from studies focusing on dietary fibers-derived SCFA, the metabolic effects of SCFA have also been investigated by introducing it directly into the diets. Dietary intervention with SCFA (5% w/w) in mice had a protective effect against high-fat diet induced obesity, improved insulin sensitivity and reduced hepatic steatosis. The protective effect was mediated by adipose and liver expression of peroxisome proliferator-activated receptor-γ (PPARγ).

1.1.2 Bile acids as dynamic signaling molecules

Bile acids are not only detergents required for the intestinal absorption of hydrophobic nutrient molecules including fat, cholesterol, and fat-soluble vitamins but are also important signaling molecules for various metabolic pathways (Fig. 2). In addition, bile acids are a major route for the elimination of cholesterol from the body. Primary bile acids are synthesized in the liver from cholesterol *via* a neutral or acidic pathway involving cascades of enzymatic reactions in hepatocytes. Cholic acid (CA) and chenodeoxycholic acid (CDCA) are two main products of these two pathways, respectively. In rodents, CDCA is further converted into hydrophilic bile acids such as α-muricholic acids (α-MCA) and later into β-muricholic acids (β-MCA). More recently the gene responsible for encoding enzyme known as cytochrome P450 2C70 (Cyp2c70) was indicted in mouse studies to be responsible for the oxidation of CDCA into α-MCA and of ursodeoxycholic acids (UDCA) into β-MCA.[37] The neutral pathway is initiated by cholesterol 7α-hydroxylase (CYP7A1) while the acidic pathway is regulated by sterol 27-hydroxylase (CYP27A1).

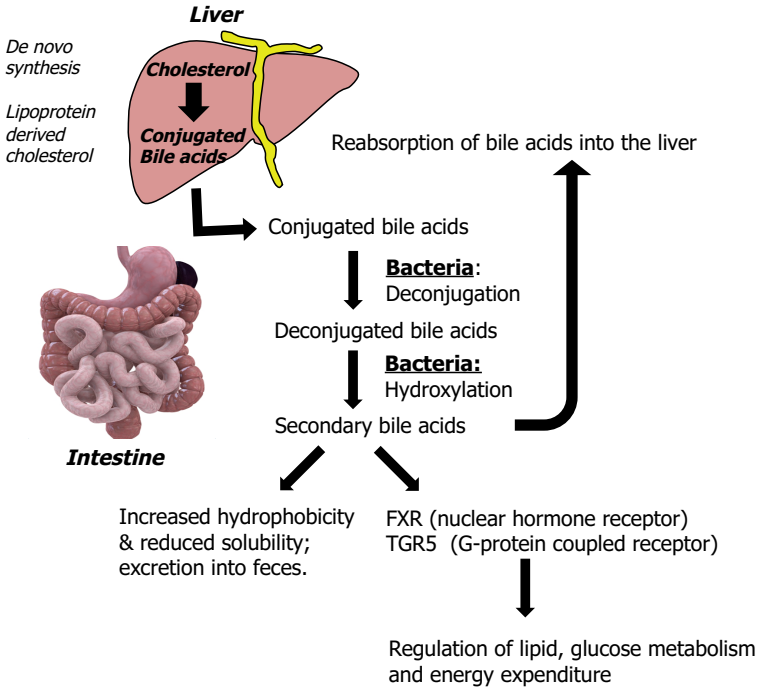

Figure 2: Bile acids: a link between bacterial metabolism and systemic metabolism of the host with major relevance for metabolic regulation

Under physiological conditions, bile contains bile acids as well as cholesterol and phospholipids. Bile is released into the upper small intestine as a response to food intake. Bile acids in the intestine are essential for solubilizing dietary lipids for absorption. Bile acids are secreted into the bile, almost exclusively conjugated with taurine of glycine. The conjugation increases the solubility in the upper intestinal lumen. In humans, bile acids are conjugated with glycine while in rodents they are conjugated with taurine. In the intestine, bacteria play a major role in converting primary bile acids into secondary bile acids by deconjugation and then dehydroxylation.

Most microbial 3metabolism takes place by gram-positive anaerobic bacteria. Dietary fibers such as galacto-oligosaccharides and fructo-oligosaccharides are known to promote the growth of *Bifidobacterium* and *Lactobacillus* in animal and human studies.[38–41] Lactic acid fermenting bacteria such as *Bifidobacterium* and *Lactobacillus* strains are also known to produce bile salt hydrolase (BSH).[42] This enzyme is essential for catalyzing the deconjugation of bile acids and thus could have direct implications on the lipid metabolism of the host. Therefore, modifying intestinal microbiota composition could be an attractive target for regulating bile acid homeostasis, a potential therapy in metabolic diseases. Most bile acids are reabsorbed from the

intestine leaving around 5% which are excreted into the feces per enterohepatic cycle. Vast amounts of bile acids are recirculated *via* enterohepatic circulation and serve as natural ligands for hormone receptors such as farnesoid X receptor (FXR) or G-protein coupled receptors such as TGR5. Activation of these genes has specific regulating effects on energy, fat, glucose and cholesterol metabolism.

1.1.3 Effects of dietary fibers on lipid and glucose metabolism in animal and human studies

Most studies in animal models related to the effects of dietary fibers on lipid metabolism are carried out in rats[43–45], but some have also been conducted in mice[46,47], dogs[48] and hamsters[49]. Mouse models of atherosclerosis namely apolipoprotein E (apoE) and LDL receptor (LDLR) deficient mice have been used to study the effects of inulin and oligofructose. Upon supplementation for 16 weeks of inulin (10%, w/w) to the diet of apoE-deficient atherosclerosis-prone mice cholesterol and triacylglycerol levels decreased by 30% and 50%, respectively. OFS (10%, w/w) supplementation also decreased plasma cholesterol and triacylglycerol levels by about 14% and 48%, respectively. In the same study, when OFS and a long-chain inulin mixture were supplemented together there was a similar effect of lowering plasma cholesterol levels suggesting that it can be effective individually and in combination. In LDLR knockout mice, inulin (Raftiline HP, 10%, w/w) supplementation to the diet for 4-9 weeks reduced plasma cholesterol by 30%, triglycerides levels by 22%, LDL-cholesterol by 25%, VLDL-cholesterol by 37% and IDL-cholesterol by 39% while leaving HDL-cholesterol unaffected. In addition, mice that were receiving inulin showed a slightly lower intima/media (innermost two layers of the arterial wall) thickness ratio compared to control mice.[47] However, the diet of the LDLR knockout mice did not contain cholesterol, and thus mice were only mildly hypercholesterolemic and did not develop advanced atherosclerosis plaques.[50]

The general metabolism of lipoproteins in hamsters is similar to that of humans. For example, hamsters are a species expressing cholesterol esters transfer protein (CETP) and subsequently receptor-mediated uptake of cholesterol originating from HDL *via* the LDLR pathway like in humans.[51–54] In contrast to mice, hamsters produce only apoprotein B100 in the liver and apoprotein 48 in the intestine which resembles the human situation.[55] Furthermore, the lipid and apolipoprotein composition of VLDL produced by hamster liver closely resembles that of human VLDL. The hyperlipidemic hamster model also develops atherosclerotic plaques when induced with a high-fat diet. In hamsters, inulin supplementation had a marked reducing effect on plasma cholesterol (15-29%), which was highest with 16% (w/w) dietary inulin content.[49]

Animals studies have largely shown consistent effects, however, there were also some conflicting reports suggesting that OFS did not significantly decrease plasma cholesterol in rats[43,56,57] or in dogs.[48] In obese Zucker rats, feeding OFS (10%, w/w) for ten weeks had no significant effect on triglyceride or cholesterol level.[57] Another experiment in Wistar rats using a shorter duration (thirty days) of OFS supplementation (10%, w/w) showed no significant decrease in cholesterol, however, triacylglycerol levels decreased by 38%.[56] Similarly, in sucrose-fed insulin-resistant rats, daily consumption of OFS (10%, w/w) for three weeks had no effect on plasma cholesterol, however, it lowered plasma triglycerides levels by about 30%.[43] Substantial evidence associating a decrease in triglycerides and lowered CVD risk has emerged over the years even advocating its use in clinical routine.[58,59]

Few studies are available for other dietary fibers in the context of plasma lipid profile modulation. A six-week study with a galacto-oligosaccharides (GOS) supplemented diet fed to germ-free rats inoculated with human fecal microbiota, showed a significant decrease in plasma cholesterol by 30%. In the same study, GOS and FOS reduced plasma cholesterol by more than 40%. Other dietary fibers such as α-cyclodextrin have shown to reduce total cholesterol in low-density lipoprotein knockout (LDLR KO) mice by 15% when supplemented to a Western diet.[12] In Wistar rats, 2.5% and 5% β-cyclodextrin in the semi-purified diet reduced cholesterol levels by 15% and 20%, respectively[14]. In hamsters a similar effect was shown: when 8% or 12% of β-cyclodextrin was added to the diet for 5-weeks plasma total cholesterol decreased by 28% or 40%, respectively.[13] Although animal models provided initial evidence of cholesterol-lowering effects of dietary fibers there are certain inconsistencies in terms of the strength of the effect.

Human studies have shown contradictory reports with respect to lipid-lowering effects of dietary fibers, especially in healthy subjects. Clinical trials with OFS in healthy subjects found no significant impact on cholesterol.[60,61] More consistent and promising results were obtained in hyperlipidemic and type 2 diabetic patients. OFS administered to male and female non-insulin dependent diabetic subjects in a daily dose of 8g for two weeks recorded a significant decrease in cholesterol levels (6%).[62] The reduction was more pronounced in hypercholesterolemic subjects compared to normocholesterolemic subjects. Another study in hyperlipidemic male and female patients who ingested 8g OFS for a month also showed a decrease in total cholesterol (8%).[63]

Inulin had no effect on plasma cholesterol in sixty-four young women treated with 14g of inulin daily for 4 weeks in a randomized, double-blind study.[64] However, daily consumption of 9g of inulin and OFS mixture for 4 weeks moderately decreased total cholesterol (8%) in twelve healthy male subjects.[65] A more significant decrease in total cholesterol (20%) was observed in a study by Balcazar et al., which involved

hypercholesterolemic and hypertryglyceridemic patients who were administered 7g of inulin daily for 4 weeks. Similarly, a randomized, double-blind study in mild to moderate hypercholesteromia patients ingesting 18g inulin for six weeks showed a slight decrease in total cholesterol (9%).[66] Apart from inulin and OFS which are the two widely studied dietary FOS fibers in humans for their lipid profile improving potential, data for other dietary fibers are scarce. In addition, most studies exclude patients on medication and, therefore, information on whether dietary fibers have an added effect on the lipid profiles of individuals taking lipid modulating drugs such as statins have not been reported.

There are even fewer studies available for other dietary fibers. A study in 200 overweight volunteers showed that when assigned to a 5g mixture of trans-GOS daily for 12 weeks there was a moderate decrease in total cholesterol by 6% and plasma insulin concentrations tended to be lower as well.[67] However, the glucose concentration did not change in these individuals. The participants were included based on an increased cardiovascular risk profile and displayed higher fasting glucose levels, increased blood pressure, dyslipidemia, high TG levels. In another study involving pre-diabetic individuals GOS supplementation for 12 weeks had no impact on insulin sensitivity, body composition or energy metabolism, however, a substantial increase in abundance of *Bifidobacterium* species was observed in feces.[68] Some different oligosaccharides such as alpha-cyclodextrin were studied in 34 healthy adults with no significant change in total cholesterol.[69] Isomalto-oligosaccharides have not been studied for potential cholesterol-lowering effects in animal models, however, one study in humans showed a 10% reduction in total cholesterol levels.[11]

Overall, dietary fibers show more consistent cholesterol-lowering effects in animal than in human studies. It is also clear from human trials that the effect is more pronounced in patients suffering from hyperlipidemia, thus suggesting that the pathway used for cholesterol reduction is specifically enhanced in the subjects suffering from increased cholesterol levels. Since the precise data on the diets are not provided in every study it is difficult to conclude whether in a meta-analysis setting the overall effects in humans are significant or not. Human studies are also more challenging in terms of controlling the daily nutrient intake which may induce variation in the lipid levels. While comparing animal and human studies it is also important to keep in perspective the amount of oligosaccharides ingested compared to the daily food intake. Generally, the food intake varies depending on sex, food availability, age and many other factors. The world health organization (WHO) has suggested that the daily requirement of calories for an average adult ranges from 2500-3400 kcal.[70] Based on this notion, this would correspond to about 2-3% of oligosaccharides (5-14g) ingested in most human studies if we assume a balanced diet involving approximately

400-570g of daily food intake by an average adult. Animal studies used up to 10% or even higher percentages of dietary oligosaccharides. Thus it is to be expected that the effect size of dietary fibers in humans' studies could still be increased by increasing the administered daily dietary fiber amounts. However, it is challenging to implement higher dosages in human studies due to potential gastrointestinal side-effects such as flatulence, intestinal discomfort or diarrhea caused at least in some individuals.[17]

1.2 Intestine

Intestine is one of the key organs in the regulation of metabolism. The presence and absence of (specific) microbial strains in the intestine could have substantial effects on the regulation of metabolism. Moreover, enterocytes are essential for the absorption of dietary monosaccharides, fatty acids and amino acids.[71] These monomers obtained from macronutrients act as substrates for various cellular processes including energy production and consumption. Several transporters at the apical side are involved in the monomer uptake such as Na^+ dependent glucose cotransporter 1 (SGLT1) which transports glucose and galactose, and GLUT5 for the uptake of fructose. At the basolateral membrane of the enterocytes all carbohydrate monomers exit *via* the glucose transporter GLUT2.

Dietary lipids may be taken up by specific transporters such as fatty acid transporter protein (FATP/CD36), or diffuse passively across the enterocytes. The possible role of FATP/CD36 in fatty acid uptake has been challenged recently.[72,73] Triacylglycerol (TAG) which forms more than 90% of dietary lipids consist of three long-chain fatty acids esterified to glycerol. In the duodenum, most TAG are hydrolyzed into free fatty acids (FFA) and monacylglycerols by lipases. The monoacylglycerols and FFA are re-esterified in enterocytes mostly by diglyceride acyltransferase (DGAT) and monoacylglycerol acyltransferase-2 (MGAT). The resynthesized lipids are then incorporated into chylomicrons for transport into the lymph system. Despite the available knowledge of the metabolic function of enterocytes, a comprehensive understanding on the role of nutrients and nutrient-derived metabolites in regulating metabolic properties of intestinal cells is lacking.

As mentioned earlier the intestine is also an important site for microbiota which expresses approximately 100 fold more genes than encoded by the human genome.[74] The intestinal microbiota is not only essential for processing dietary fibers but also responsible for altering bile acid metabolism, thereby having a direct impact on cholesterol turnover in the host. It has previously been demonstrated that specific dietary modulation in the host can alter microbe-host interactions. As a precedence, it has been shown that the production of diet-derived pro-atherosclerotic metabolites such

as trimethylamine-N-oxide increases the development of experimental atherosclerosis and is prospectively associated with a higher risk for incidence of cardiovascular events in humans.[75,76] Microbe-host relationships, especially in the context of the metabolic syndrome, have also been studied by using conventionalized germ-free animals. Germ-free mice on the high-fat diet are resistant to developing insulin resistance and have altered cholesterol metabolism compared to conventional mice.[77,78] However, when germ-free mice are conventionalized it leads to a 60% increase in body fat content and insulin resistance.[79] The difference between gut microbiota in lean and obese individuals was already established a few years back: obese individuals have a greater number of *Firmicutes* (95%) and fewer *Bacteriodetes* (3%).[80–82] Only recently specific strains have been identified such as *Prevotella copri,* which when introduced into conventional animals lead to the inhibition of hepatic glucose production which subsequently increases glucose tolerance.[26] Identifying specific strains and their impact on metabolic parameters have led to the evaluation of new therapeutic probiotics which are live bacteria possessing health-promoting effects.[83–85]

Intestinal microbiota has been demonstrated to regulate the expression of mouse fibroblast growth factor 15 (FGF15) and CYP7A1 *via* FXR activation.[86] FXR negatively regulates bile acid synthesis. Gut microbiota tends to shift the bile acid pool towards a more hydrophobic profile. Hydrophobic bile acids are potent activators of FXR. Presence of gut microbiota suppresses CYP7A1 by reducing tauro-β-muricholic acid. Because muricholic acid is a potent antagonist of FXR, its reduction leads to increased activation of FXR in the enterocytes and upregulation of FGF15 in the liver which suppresses CYP7A1.[86,87]

Intestinal microbiota also contributes to the development of fatty livers in insulin-resistant mice. Dietary fibers and probiotics specifically enhance intestinal barrier function and promote the growth of beneficial intestinal bacteria thus contributing to improved gut permeability.[88,89] Impaired gut permeability causes increased infiltration of bacterial components such as cell wall lipopolysaccharides (LPS). Subsequent binding to pattern recognition receptor in the hepatocytes and adipocytes leads to the production of pro-inflammatory cytokines (IL-6, TNF-α) and insulin resistance. Low-grade inflammation has also been associated with several metabolic disorders, such as obesity and diabetes.[90,91] Dietary fibers such as FOS, when supplemented with a blend of probiotics, have shown to reduce proinflammatory markers in patients with non-alcoholic fatty liver diseases.[92,93] In pigs dietary fiber supplementation reduced colonic expression of pro-inflammatory cytokines. Some dietary fibers have been shown to modulate satiety hormones by increasing gut-derived peptides (glucagon-like peptide 1 and peptide YY) which induce changes in appetite perception thus lowering food intake.[94] Complex factors come into play in the intestine

depending on the type of dietary fibers or probiotics and their respective fermentation properties. However, despite convincing data showing the effects of certain dietary fiber molecules on features of the metabolic syndrome our understanding of the specific underlying molecular mechanisms is not clear.

1.3 Liver

The liver is a central organ for the synthesis and metabolism of cholesterol, triglycerides, glucose and bile acids. The liver is exposed to a variety of different molecules under physiological conditions. It receives blood flow from the portal (intestine) and arterial (systemic) circulation. Although most molecules derived from bacterial activity in the intestine are blocked from actively crossing the barrier, some do escape (due to leakiness of the barrier) and function as signaling molecules. Under conditions of decreased intestinal barrier function, low-grade inflammation can be activated with pro-inflammatory cytokines triggered by intestinal metabolites.[95–97] Liver inflammation is one of the initial hallmarks of potentially developing metabolic disorders and especially non-alcoholic fatty diseases (NAFLD). The pathogenesis of NAFLD is initiated with liver inflammation which further leads to excessive fat storage in the cytoplasm of hepatocytes eventually resulting in non-alcoholic steatohepatitis (NASH).[98,99] The pathogenesis can evolve into liver cirrhosis with a subsequently increased risk for developing hepatocellular carcinoma. Fat accumulation in the liver is a common feature in the pathogenesis of type 2 diabetes and insulin resistance, which makes these conditions of disturbed metabolism one of the prime risk factors for NAFLD/NASH.

Bile acids synthesized from cholesterol in the liver facilitate the absorption of triglycerides, cholesterol and other lipids in the intestine. The synthesis depends on a cascade of at least 14 enzymes in the hepatocytes.[86,100,101] CYP7A1 and CYP8B1 are important enzymes involved in the classical pathway and regulated by activation of the FXR in the liver and ileum (Fig. 3).[100] As mentioned earlier intestinal FXR triggered by bile acids can induce FGF15 (FGF19 in humans) hormone secretion by the distal ileum. FGF15/19 acts through FGFR4 in the liver and inhibit bile acid synthesis by repressing transcription of CYP7A1.

Nutrient signals can also control the expression of CYP7A1. At least in rodents, dietary cholesterol stimulates CYP7A1 expression *via* activation of nuclear receptors such as liver receptor homolog-1 (LRH-1) together with liver X receptor (LXR).[102–104] When cholesterol levels are low, as a negative feedback loop CYP7A1 is downregulated *via* activation of sterol regulatory element binding proteins (SREBP) in order to prevent bile acids accumulation reaching toxic levels.[105,106] In the liver FXR activation

is regulated by another transcription factor, the small heterodimer partner (SHP), which binds to liver receptor homolog-1 (LRH-1) and inhibits the expression of CYP7A1.[100,107–109]

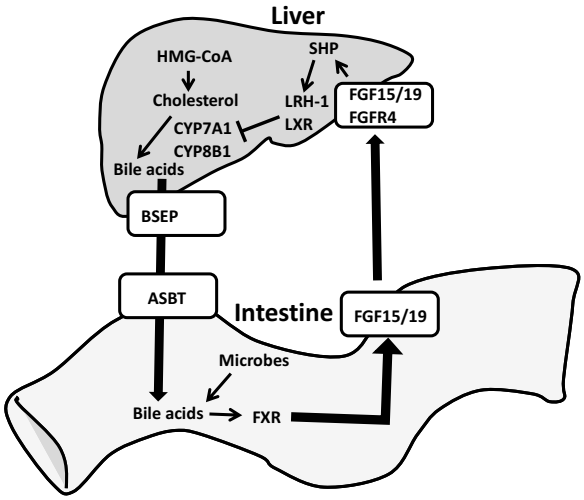

Figure 3: The potential molecular cross-talk between liver and intestine *via* bile acid signaling

After intestinal absorption, lipids are utilized by the peripheral tissues and liver takes up fatty acids in the form of chylomicron remnants. Liver further redistributes lipids over the various tissues of the body. Fatty acids taken up by the liver are esterified into triglycerides. Together with phospholipids, cholesterol and cholesteryl esters are assembled in very-low-density lipoproteins (VLDL) which are then released to be taken up into adipose tissue and muscle. Adipose tissue mainly functions as an energy reservoir especially for storing energy in the form of triglycerides when in times of need. White adipose tissue (WAT) consists of parenchymal cells with large lipid droplet and absence of uncoupling protein 1 (UCP1), consequently, the energy capacity is enhanced by the expansion of fat cells. The increased presence of fat cells thus could be a potential predictor for metabolic disease risk.[110–112] Brown adipose tissue (BAT) on the other hand is abundant in mitochondria with UCP1 which can oxidize fatty acids in their mitochondria and generate heat in the process.[112,113] Therefore, pharmacological and nutritional interventions aimed at activating the thermogenesis process in BAT is an attractive strategy to target metabolic disease especially obesity.

Apart from VLDL lipids being oxidized and/or stored in peripheral tissues, the lipoprotein particles are further metabolized into low-density lipoproteins (LDL). High-density lipoproteins (HDL) are synthesized in the liver (to approximately

70%) and small intestine (contributing approximately 30%). Both VLDL and LDL particles are responsible for delivering fat molecules to peripheral tissues. On the other hand, HDL acquires cholesterol from peripheral tissues and transports it back to the liver and intestine. This process is referred to as reverse cholesterol transport (RCT), which is an atheroprotective pathway.[114,115] Even though the role of enterocytes in the formation of HDL particles is recognized, it is not known whether the gut microbiota has a role in modulating the pathway which is subjected to investigation in this thesis.

2. General aim of the thesis and its applicability

The overall aim of this thesis is to understand the metabolic effects and the underlying mechanisms of different dietary fibers *in vivo*, to allow a targeted application towards treating and preventing the development of the metabolic syndrome. Various dietary fibers or non-digestible oligosaccharides have been studied for their metabolic health effects, particularly on lipid profiles. Dietary fibers can effectively alter gut microbiota and immune function to a varying degree. *In vitro* data suggest promising effects on (surrogate) metabolic markers. Some inconsistent reports, however, have also emerged showing insignificant or even absent effects of dietary fibers or its derived metabolites on metabolic physiology. Similarly, studies in obese and pre-diabetic individuals have shown variable results. However, more consistent results are observed in hypercholesterolemic patients. There are several challenges and limitations in human studies related to dosages and diets. Moreover, specific mechanisms and pathways that are affected by dietary fibers have not been identified. One explanation for this is the sheer diversity of dietary fibers in terms of solubility, monosaccharide composition, glycosidic bond linkages and chain-lengths to mention a few. Thus, the metabolic functionality of fibers depends on specific utilization and fermentation properties in the intestine. Moreover, the dynamic nature of the intestinal microbiota adds another dimension to the existing complexity. Individual bacterial strains can exert differential effects on the metabolic physiology. Therefore, the proliferation of beneficial species over pathogenic species by strategically supplementing favorable substrates in the form of dietary fibers may be crucial for a beneficial metabolic response.

3. Outline

As discussed above, little information is available on whether dietary fibers can lower circulating cholesterol levels by modulating its synthesis, absorption, or excretion.

Inulins are soluble dietary fibers which have previously been studied for their beneficial effects on improvin1g blood glucose and lipid levels although. The health benefits are mostly explained *via* gut microbiota-dependent generation of products such as SCFA. In **chapter 2** we comprehensively examine the effects of two different inulins of different chain lengths on the intestinal cholesterol balance. Wildtype C57BL/6J mice are fed diets supplemented with short- or long-chain inulin 10% (w/w) followed by measuring of metabolically relevant parameters and cholesterol fluxes in the intestine at the end of the dietary intervention period.

Novel classes of dietary fiber with *in vitro* prebiotic potential have recently been identified. Isomaltomalto-polysaccharides (IMMP) are one such class of prebiotics. Earlier *in vitro* studies using human fecal inoculum showed that IMMPs can stimulate growth of *Bifidobacterium* and *Lactobacillus*. IMMP also shows potential modulatory effects on intestinal microbial communities together with accumulation of SCFA. *In vivo* studies pertaining to beneficial effects of IMMP on host metabolism and health are lacking. The data in **chapter 3** are the first *in vivo* study to determine the effects of isomalto/malto-polysaccharides (IMMP) on the microbiota composition and various metabolic biomarkers in a mouse model. We studied the utilization of dietary IMMP by the intestinal microbiota throughout different regions of the intestine. Subsequently, physiologically effects of IMMP on the mice are investigated with a special focus on bile acid and cholesterol metabolism.

Gut microbiota composition is significantly altered in individuals with different metabolic diseases. High-density lipoproteins (HDL) are synthesized in the liver and intestine play an important role in reverse cholesterol transport (RCT). However, whether the pathway can be modulated by intestinal microbiota has never been investigated. In **chapter 4** we employ germ-free mice to investigate the effect of a complete lack of microbiota on RCT. The study demonstrates the therapeutic potential of targeting intestinal microbiota to prevent and treat cardiovascular disease. Abnormal accumulation of cholesterol is known to contribute to the development of atherosclerosis. Despite the vast use of cholesterol-lowering drugs such as statin, the incidence of CVD has only seen a modest decline. β-cyclodextrin has demonstrated potential cholesterol modulating properties in previous studies.[13,14] However, it is not known whether the cholesterol modulating properties of β-cyclodextrin can counteract cholesterol accumulation *via* RCT. In **chapter 5** we investigated whether β-cyclodextrin bears atheroprotective effects on RCT in mice. We further investigate by using germ-free mice to what extent intestinal microbiota are essential in modulating β-cyclodextrin induced RCT.

The growing consumption of the 'Western-type' diet has contributed to

an overwhelming epidemic of the metabolic syndrome. Different varieties of galacto-oligosaccharide (GOS) compounds have been used to improve systemic inflammation and alter gut microbiota in overweight volunteers.[67] However, to our knowledge no study has assessed the effects of dietary GOS on metabolic syndrome and on the underlying mechanistic pathways. In **chapter 6,** we studied the long-term effects of GOS supplementation in Western-type diet fed mice and explored a potential preventive effect on the development of the metabolic syndrome. Finally, in **chapter 7** we discuss the most relevant findings from all studies described in this thesis in relation to the up-to-now current knowledge and the potential implications for future research.

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