Gastrointestinal transit time, gut microbiota and metabolic health

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PROEFSCHRIFT

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CHAPTER 1

General Introduction

The obesity problem

Over the last five decades, the global prevalence of obesity has risen to pandemic proportions. In 2016, worldwide more than 39% of the adult population was overweight (BMI > 25 kg/m^2) while more than 13% had obesity (> 30 kg/m^2) [1]. Obesity is a major risk factor for type 2 diabetes mellitus (T2DM), cardiovascular diseases, fatty liver disease, depression and certain types of cancers. The increasing obesity prevalence is a major socio-economic burden for public health. It is evident that obesity is a complex, relapsing, and chronic disease process that requires intervention [2]. Additionally, interventions should not only address the individual but also the obesogenic environment [3].

Current first-line obesity treatment mainly focuses on the counseling of lifestyle adaptions such as caloric restriction and physical activity. These lifestyle adaptions can effectively reverse progression to T2DM. However, adherence to lifestyle changes is challenging and more than 20% of the individuals do not respond effectively to lifestyle intervention. Despite rapid initial weight loss, more invasive treatments such as bariatric surgery result often in limited long-term weight maintenance. Hence, there is an urgent need to improve and advance current treatment strategies to tackle obesity and obesity-related disorders.

Obesity, glucose homeostasis and insulin resistance

A disbalance between energy intake and energy expenditure, when in favor of energy intake, may lead to obesity and its related comorbidities. Next to genetic predisposition and other environmental factors, a sedentary lifestyle and increased intake of energy-dense food causes excess energy to be stored as fat in the adipose tissue and consequently leading to body weight gain and adiposity. Functional disturbances in adipose tissue metabolism have been implicated in the development of insulin resistance and T2DM. The adipose tissue stores meal-derived lipids and provides free fatty acids to other organs during energy-demanding conditions. During a chronic positive energy balance, failure to differentiate new adipocytes upon increased demand for fat storage results in enlargement of existing adipocytes which are more insulin resistant and are associated with impairments in buffering capacity [4]. Consequently, excess lipids spill over into the circulation leading to hyperlipidemia [5]. Together with impairments in the capacity to oxidize lipids upon increased lipid supply (i.e., metabolic inflexibility), this may lead to ectopic fat accumulation in tissues such as skeletal muscle and the liver. Increased

lipid storage, in particular the accumulation of bioactive lipid metabolites (e.g., long-chain fatty acids-Coenzyme A, diacylglyceride, ceramides, and acylcarnitine) may disrupt insulin-mediated glucose uptake in skeletal muscle and liver and hence, may contribute to hyperglycemia [6].

In the long term, an expanded adipose tissue mass and a reduced adipose tissue functionality are associated with the development of insulin resistance, T2DM and cardiovascular diseases [7]. Obesity-associated insulin resistance is characterized by decreased insulin sensitivity of important organs involved in energy regulation such as adipose tissue, skeletal muscle and liver. The onset of insulin resistance is initially compensated by an increased insulin secretion and can develop into T2DM, when insulin secretion deteriorates and a prediabetic state and subsequently T2DM may develop. The decline in insulin secretion may be due to glucolipotoxicity and/or genetic predisposition [8, 9]. The prediabetic state is described by an impaired fasting blood glucose (fasting glucose > 6.1 - 7.0 mmol/l) or increased blood glucose after an oral glucose challenge (impaired glucose tolerance > 7.8 – 11 mmol/l) according to the World Health Organization [10]. Chronic postprandial hyperglycemia together with hyperlipidemia and hyperinsulinemia are frequently observed in obesity and linked to the onset of T2DM and cardiovascular disease. Already in the healthy state, frequent high excursions of postprandial glucose and glycemic variability may be deleterious to metabolic homeostasis and are suggested to increase overall risk of obesity and onset of insulin resistance [11].

One of the main tasks of the gastrointestinal (GI) tract is the digestion and absorption of the macronutrients, i.e., protein, carbohydrate and lipids as well as vitamins and minerals as described in **Chapter 2**. Moreover, the GI tract is a major endocrine organ that secretes hormones involved in central appetite regulation, GI motility and pancreatic endocrine functions; hence, it is strongly connected to the regulation of whole-body energy and substrate metabolism beyond digestion. Importantly, the GI tract and in particular the large intestine, harbors millions of microorganisms termed *gut microbiota*, which complement the human metabolism as will be detailed later. The following sections discuss the role of GI functioning, the gut microbiota and its metabolites in the context of metabolic health and will briefly introduce prebiotic fibers as well as the site-specific colonic fermentation as novel nutritional approaches to prevent or treat metabolic disorders.

Gut functioning and metabolic health

Gut functioning

With a surface area of 300-400 m^2, the GI tract is the largest organ system in the human body. The GI tract and its related organs control the passage of food to ensure effective nutrient breakdown and absorption as well as reabsorption of endogenous factors until food wastes are excreted. Food intake and subsequent digestion and absorption induce numerous physiological processes involved in the regulation of nutrient handling, and regulatory signaling towards the brain. The entry as well as the transit time of food traversing through the stomach, small intestine and colon induces site-dependent metabolic responses as reviewed in **Chapter 2**.

Upon food intake, digestion begins in the mouth with chewing and breakdown of starch and fats by the action of amylase and lingual lipase, respectively. Within seconds, food passes through the esophagus to the stomach, where it is mixed with several digestive enzymes, which mainly facilitate protein breakdown. In a controlled manner, food is passed in small portions from the gastric antrum into the small intestine. This process termed *gastric emptying* takes up to two hours depending on meal size and composition. The gastric emptying rate contributes to approx. 35 % of postprandial glucose variability as it determines the rate of nutrient, i.e., saccharide appearance into the duodenum [12, 13]. The rate of gastric emptying is tightly controlled by hormonal and neural mechanisms to ensure a constant downstream flux of 1-4 kcal/min for solid meals [14].

The small intestine is divided into three regions, the duodenum, jejunum and distal ileum. The liver and pancreas excrete bile acids and pancreatic juices into the duodenum to further break down macronutrients to amino acids, small peptides and fatty acids, while oligo- and disaccharides and small peptides are further broken down to monosaccharides and amino acids by brush border enzymes on the intestinal epithelium. Subsequently, amino acids and small peptides as well as monosaccharides are absorbed into the portal circulation via diffusion or membrane transporters, while fatty acids are packed in chylomicrons which enter the systemic circulation via the lymph. Arrival of nutrients in the duodenum induces gut hormone secretion from enteroendocrine cells upon receptor-dependent nutrient stimulation. These hormones inhibit gastric emptying, induce satiety and stimulate insulin and bile acid secretion (i.e., cholecystokinin (CKK), glucagon-like peptide-1 and 2 (GLP-1, GLP-2), gastric inhibitory polypeptide (GIP) and

peptide YY (PYY)) [15]. Independent of meal size, this negative feedback regulation ensures a constant nutrient flow to the duodenum in order to control efficient duodenal nutrient absorption to prevent nutrient spill over into the colon. In particular, GLP-1 amplify insulin-mediated glucose uptake to prevent hyperglycemia, while both GLP-1 and PYY induce central satiety mechanism [12]. Small intestinal transit time of a solid meal is estimated to take up to 6 hours depending on gastric emptying rate as well as meal composition and size [16]. The small intestine ends into the cecum, the most proximal part of the colon, which is divided into the caecum, ascending, transverse, descending and sigmoid colon, rectum and the anus. The primary task of the colon is reabsorption of water, electrolytes and vitamins from the food residues. Food residues that reach the colon are mainly indigestible food compounds such as dietary fibers, but also residues of slowly digestible and indigestible proteins. These residues are fermented and metabolized by the gut microbiota yielding a variety of metabolites influencing human metabolism, as will be detailed later. The colonic transit of food residues can take up to 70 hours until feces excretion [17].

The passage of food through the intestine, i.e., the GI transit time plays a crucial role in postprandial nutrient metabolism and may affect gut microbiota composition. Thus, modulation of the GI transit time may impact glucose homeostasis and metabolic health. A more detailed description of methodologies to measure GI transit is given in **Chapter 2** of this doctoral thesis. In the following section, the role of the gut microbiota and its metabolites in human metabolism will be discussed in further detail.

The gut microbiota, diet and metabolic health

Gut microbiota composition and its covariates

The GI tract harbors more than $>10^4$ microorganisms including bacteria, archaea, eukaryotes and viruses. This thesis will focus on the intestinal bacteria and archaea, which are the most studied microorganisms of the gut microbiota. Shortly after birth and depending on the mode of delivery, the GI tract is populated by a microbiota similar to vaginal and skin microbiota, yet with the introduction of solid foods, the infant gut microbiota gradually resembles the adult microbiota [18, 19]. This maturation is influenced by use of medication i.e., antibiotics, as well as contact with intestinal pathogens in early life [20]. In adulthood, the gut microbiota composition is more stable

but unique to a given individual due to environmental and genetic influences [21, 22]. This leads to a great interindividual variability as exemplified by Qin *et al*. [23] observing that in 90% of individuals, the abundances of the most common 57 species varied greatly between 12- to 2,187-fold. This dichotomy between composition plasticity and robustness makes it challenging to define a 'healthy' gut microbiota composition. However, the existence of a core microbiota has been broadly accepted, which includes the most abundant phyla *Firmicutes* with around 200 gram-positive genera (incl. *Clostridium*, *Eubacterium*, *Faecalibacterium*, *Lactobacillus*, *Roseburia* etc.) and *Bacteriodetes* with around 20 gram-negative genera (incl. *Bacteriodes*, *Prevotella* etc.) and the less abundant *Actinobacteria* (i.e., *Bifidobacteria*), *Proteobacteria* (i.e., *Desulvibrio*, *Escherichia*) and *Verrucomicrobia* (i.e., *Akkermansia muciniphila*) [24]. The small intestine is colonized by lower density of bacteria (10^3-10^8 cells/g feces) compared to the colon (10^{11} cells/g feces) [25, 26], which may be partly due to gradients in luminal pH caused by pancreatic and gallbladder secretions.

Even relatively stable in adult life, the gut microbiota composition fluctuates and can be strongly determined by several extrinsic factors including mode of delivery, host genome, immune response, diet, medication (including antibiotics), infections, circadian rhythm, and the microbial external environment [27]. Dietary changes such as switching from animal to plant-based protein, increasing dietary fiber intake or changing the fat to carbohydrate ratio can impact the gut microbiota within days [28-30]. Yet, one has to critically evaluate the level of evidence for microbiota modulating factors especially since not all of them may affect gut microbiota variation to the same extent and persistence. Large-scale human population studies can clarify the role of host-related and extrinsic factors that may drive microbiota variation. In fact, a large-scale metagenome-wide association study included data on lifestyle, diet, medication, blood and bowel parameters of almost 4000 participants of the Flemish Gut flora Project and the Dutch LifeLine-DEEP study. This study revealed that 98 covariates could explain a cumulative 7,7% of microbiota community variation, which, in the light of the in-depth phenotyping, was strikingly low. These data clearly indicate that not all microbiota covariates are identified yet. Stool consistency as a proxy of GI transit was the top covariate explaining 4.9% of community variation, followed by medication, age and sex [31]. These associations have been confirmed in a Chinese population-wide study (Guandong Microbiome Project) including 7000 participants [32]. The influence of the GI transit on the gut microbiota composition has been reported earlier in two small scale human cross-sectional studies using stool consistency ratings (Bristol stool scores) or the more quantitative radio-opaque marker

method [33, 34], as described in **Chapter 2**. *Vice versa*, the gut microbiota can produce many metabolites, which directly stimulate GI motility such as methane [35], short-chain fatty acids (SCFA) [36-38], bile acids [39], bacterial lipopolysaccharides [40], or indirectly via host factors such as serotonin [41], GLP-1 or PYY. In humans, little is known about the complicated, reciprocal relationship between GI transit, gut microbiota and microbial metabolite production. Based on the aforementioned studies, Falony *et al*. [42] introduced the concept of GI transit-dependent maturation of microbial ecosystem hypothesizing that with longer transit, alongside water reabsorption and saccharolytic substrate depletion, adaption occurs favoring species more equipped to deal with this ecological selection pressure. Yet, it remains to be investigated how GI transit and its reciprocal relationship with the gut microbiota may consequently affect human metabolism.

Gut microbiota metabolites and host metabolism

The gut microbiota itself harbors a large bacterial genome (i.e., the microbiome), which outnumbers the human genome by approximately 100-fold [22]. Naturally, the microbiome encodes genes not represented by the human genome and thereby complements the human metabolism in processes such as maturation of immunity, defense against pathogenic overgrowth, endocrine and neurologic signaling, vitamin synthesis, conjugation of bile salts, and diet-related metabolism [43]. Commonly, taxonomical data in human studies include abundance of bacteria on genus or even species level and estimates of microbial diversity. Besides somewhat complex and not always conclusive compositional changes, microbiota diversity (i.e., microbial richness and evenness) has been broadly accepted as biomarker for a healthy, stable and resilient gut microbiome [44-47]. Furthermore, there is great functional redundancy within the gut microbiota already on genus level, which makes it challenging to link functionality to composition [48]. Yet, still some bacteria have been more consistently associated with beneficial effects on host health (i.e., *Akkermansia municiphila*, *Bifidobacterium spp.*, *Lactobacillus spp.*, *Feacalibacterium prausnitzii*) [49-51]. Nevertheless, in order to obtain consistent evidence for the relationship between gut microbiota and the host, not only taxonomical data but also microbiota functionality measured by metagenomics, metatranscriptomics, metaproteomics and metabolomics needs to be taken into account [52]. In the following, the major bacterial metabolites are described with a focus on their effect on metabolic health.

Short-chain fatty acids and metabolic health. Indigestible carbohydrates are the main source of energy for the gut microbiota. Fermentation of these carbohydrates leads to production of the SCFA acetate, propionate, butyrate and to smaller amounts of valerate, caproate as well as gases (CH_4, CO_2, H_2), lactate and succinate. Acetate is produced by many enteric bacteria including *Akkermansia muciniphila*, *Bacteroides spp*., *Bifidobacterium spp*., *Prevotella spp.*, *Ruminococcus spp*., whereas butyrate is mainly produced by *Anaerostipes spp*., *Coprococcus catus*, *Eubacterium rectale*, *Eubacterium hallii, Faecalibacterium prausnitzii*, *Roseburia spp.*, however some species such as *Faecalibacterium prausnitzii* may also switch to acetate production under certain conditions. Lastly, propionate is mainly produced by *Bacteroides spp*., *Phascolarctobacterium succinatutens*, *Dialister spp*. and *Veillonella spp*. [53-55]. Substrate availability is an obvious rate limiting factor for SCFA production, but also GI transit time, gut microbiota composition and microbial cross-feeding have been reported to influence colonic SCFA concentrations [56]. Acetate is the most abundant SCFA, followed by propionate and butyrate, which have been measured in a ratio of 3:1:1 in the colon of sudden death victims [57]. Here, SCFA concentrations were highest in the proximal colon (123 ± 12 mmol/kg luminal content range) and slightly lower in the distal colon (100 ± 30 mmol/kg). Venous acetate concentrations range from 5 to 400 µmol/l, and lower concentrations ≤13 µmol/l and ≤12 µmol/l for circulating propionate and butyrate, respectively as observed in [58, 59] and **Chapter 5** of this thesis. Around 5-10% of total SCFA are excreted into feces [57, 60].

A plethora of rodent studies [61-70] as well as acute [71-75] and a few longer-term human studies [76-78] demonstrated beneficial effects of SCFA on body weight regulation, food intake and glucose homeostasis and, thus, SCFA are currently the main microbial-derived targets in the context of interventional and nutritional approaches to prevent metabolic disorders. In the colon, most butyrate is used as energy source for colonocytes, whereas most of the microbially produced acetate and propionate are absorbed into the circulation [56]. Colonic SCFA interact with the colonic epithelium via G-coupled protein receptors 41 and 43 (GPR41/43) [79, 80]. SCFA binding to GPR41/43 on enteroendocrine L-cells stimulates secretion of GLP-1 and PYY, which reduce postprandial glycemia by amplifying glucose-dependent insulin secretion, inhibition of glucagon production and induction of satiety as reported in mice models [62, 63]. Additionally, based on a rat colon model, receptor-independent mechanisms have been proposed suggesting that enteroendocrine cell use luminal as well as visceral SCFA as energy source which in turn induces hormone secretion [81]. In mice, SCFA supplementation increased

intestinal gluconeogenesis which was accompanied by decreases hepatic gluconeogenesis. The latter may be responsible for the induction of central satiety mechanisms, increased energy expenditure and improved glucose tolerance in these mice [82].

As shown in humans, SCFA concentrations in the portal vein draining the proximal colon are lower than SCFA in inferior mesenteric veins draining the descending colon, sigmoid and rectum [83]. Once in the liver, acetate and butyrate can act as substrates for lipogenesis, while propionate is mostly used for gluconeogenesis [84, 85]. Moreover, animal studies suggested that in the liver, butyrate and acetate may increase hepatic AMP-activated protein kinase (AMPK) phosphorylation by increasing hepatic AMP to ATP ratio and consequently increase peroxisome proliferator-activated receptor (PPAR) α involved in hepatic fatty acid and glucose metabolism [70, 86, 87]. SCFA in the peripheral circulation may interact with various regulatory pathways in skeletal muscle and adipose tissue. In murine adipocyte and human adipocytes cell studies, predominantly acetate has been shown to decrease intracellular lipolysis by GPR43-dependent attenuation of hormone sensitive lipase activation as well as to increase triglyceride extraction (lipid buffering capacity) through increasing lipoprotein lipase expression [88-90]. These SCFA-mediated effects may reduce lipid overflow and attenuate ectopic fat accumulation, thus beneficially affect insulin sensitivity. In rodent models, short-term administration of SCFA were reported to increase skeletal muscle glucose transporter type 4 (GLUT4) expression and glycogen storage, while decreasing glycolysis potentially via AMPK- and PPARδ-related mechanisms [91, 92]. Indirectly, the SCFA-induced increase of GLP-1 and PYY may also increase glucose-dependent uptake in skeletal muscle as observed with GLP-1 infusion in rats [93].

Short-term human intervention studies with acute rectal or intravenous infusion of SCFA mixtures in concentrations reflecting a high dietary fiber intake reported decreases in glucagon and free fatty acid concentrations, increases in PYY and whole-body fat oxidation mostly without effects on glucose and insulin [71-76]. In overweight adults, 24-week supplementation of 10 g/day inulin-propionate ester as compared to inulin only increased postprandial PYY and GLP-1 concentrations and reduced energy intake, which was accompanied by weight loss and a decrease in hepatocellular lipid content [94]. Interestingly, oral intake of 4 g/day of sodium butyrate in capsule form for 4 weeks improved peripheral and hepatic insulin sensitivity in lean but not in obese males with metabolic syndrome indicating that there may be an impaired SCFA metabolism in metabolically compromised conditions [78]. Yet, it is debatable if encapsulated orally

ingested butyrate is comparable to or even mimics metabolic effects with colonic SCFA production.

Furthermore, controversy remains whether the additional extra energy derived from fermentation-derived SCFA may contribute to obesity which may be supported by the observation that fecal SCFA are higher in obese compared to lean participants [95, 96]. However, fecal SCFA are the net result of microbial SCFA production as well as absorption may thus not be a good indicator for SCFA production [97-100]. In mice, *in vivo* SCFA rate of appearance in the circulation measured via isotopically labelled SCFA, but not cecal SCFA absorption correlated with improved markers of metabolic health after a dietary fiber intervention, indicating that SCFA metabolism and flux but not concentrations *per se* are linked to metabolic health [101]. In a recent study in the Dutch LifeLines cohort, a mendelian randomization approach was used to investigate causal relationships between gut microbiota, host genetics and fecal SCFA [102]. The authors showed that the microbial pathway involved in 4-aminobutanoate (GABA) degradation, which results in butyrate and propionate production, was related to an improved insulin secretion after an oral glucose-tolerance test. However, fecal butyrate levels did not correlate with the expression of the GABA degradation pathway, which supports the notion that fecal SCFA should not be regarded as a good biomarker of colonic SCFA production. Thus, it remains to be further investigated how SCFA are linked to human energy metabolism as well as the causality of this relationship.

Protein fermentation and metabolic health. In the distal intestines, proteolysis of residual dietary proteins is partly facilitated by the gut microbiota and the resulting amino acids are preferably incorporated into bacterial proteins. Especially diets low in dietary fiber may exacerbate depletion of saccharolytic substrates in the colon causing a metabolic switch to energetically less efficient microbial protein fermentation yielding proteolytic end-products such as indole, phenol, *p*-cresol and *p*-cresyl sulfate derived from branched chain amino acids (BCAA), hydrogen sulfide, short and branched-chain-fatty acids (isobutyrate, isovalerate and 2-methyl-butyrate) and to lesser extent SCFA (including valerate, caproate) [103]. Known protein fermenters are from the families *Bacteriodes*, *Eubacterium*, *Clostridium*, *Desulfvibrio*, *Peptostreptococcus* and *Peptococcus* [104]. In general, excessive protein fermentation in the colon has been associated with detrimental effects on gut health due to the increased production of potential toxic byproducts [103]. Animal and *in vitro* studies report a compromised colonic epithelium and barrier function with increased ammonia, phenols, and hydrogen sulfide concentrations [105, 106].

Colonic protein fermentation has been less studied in the context of chronic metabolic diseases. There is conflicting evidence for an either protective [107] or harmful [108] role of hydrogen sulfide in the development of T2DM. Phenolic compounds and BCAA were increased in plasma of insulin-resistant and T2DM patients, which was linked to an increased potential of microbial BCAA synthesis [109]. Furthermore, *p*-cresyl sulfate administration for four weeks induced increased ectopic fat accumulation in the liver and skeletal muscle and peripheral insulin resistance in mice [110]. On the other hand, short-term consumption of low carbohydrate/high protein diets may support body weight loss, yet it is still unclear to what extent a high protein diet may affect the gut microbiome. High protein diets have been consistently reported to reduce fecal butyrate, yet genotoxicity, cytotoxicity or inflammation is not changed upon short-term high protein diets [111]. To conclude, there is still little knowledge on the health effects of protein-derived microbial metabolites, mostly due to the lack of well-controlled long-term human intervention or association studies.

Dietary fat, bile acid metabolism and metabolic health. Upon dietary fat intake, bile acids are secreted from the gall bladder into the small intestine to activate pancreatic lipase and form bile salt micelles to facilitate dietary lipid solubilization and absorption. Thus, most of the dietary fat is absorbed in the small intestine. However, it is estimated that around 7% of free fatty acids from dietary triacyclglycerols are secreted in stool [112]. Despite the fact that dietary fat may only have minor direct effects on the gut microbiota, rodent studies show that diets high in saturated fat are accompanied by alterations in gut microbiota composition such as reduced diversity [113, 114], decreased *Bacteroidetes* and increased *Firmicutes* and *Proteobacteria* [115, 116]. In humans, the gut microbiota might be involved in inflammatory responses to n-6 polyunsaturated fatty acids (PUFA)) intake. In healthy adults, 6 months of high-fat diet (total fat 40 %E, of which were 7 %E saturated fatty acids, 9 %E monounsaturated fatty acids and 24 %E n-6 polyunsaturated fatty acids (PUFA)) decreased *Faecalibacterium* compared to an isocaloric low-fat diet, which also correlated with reduced fecal butyrate [117]. Furthermore, the high-fat diet was associated with increased fecal content of indole, palmitic acid, stearic acid, arachidonic acid and indoleacetic acid, whereby fecal arachidonic acid was positively correlated with pro-inflammatory plasma metabolites.

One of the suggested mechanisms how the gut microbiota contribute to the adverse metabolic effects of high fat diets may be through a stimulation of the translocation of bacterial lipopolysaccharide (LPS, also termed *endotoxin*) via

chylomicrons into the circulation causing low-grade systemic inflammation [118]. In rodents, the entry of LPS into the circulation stimulates toll like receptor 4 (TLR4) ubiquitous to the innate immune system, which consequently contributes to systemic low-grade inflammation, i.e., metabolic endotoxemia, one of the hallmarks of the metabolic syndrome [119]. Furthermore, acute [120] and long-term [121] intervention studies with high fat diets report increased circulating LPS-binding protein (LBP) in healthy participants, and increased plasma LPS concentrations have been associated with increased risk of incident T2DM [122]. These studies provide some evidence for a role of LPS in the context of human metabolic disorders. In humans, a mechanistic link between the gut microbiota and metabolic endotoxemia still needs to be demonstrated.

Additionally, dietary fat intake increases secretion of primary bile acids, which are antimicrobial and thus may attenuate the growth of species less resistant to bile [123]. Furthermore, alterations of the primary bile acid pool may also affect secondary bile acid conversion by the gut microbiota [124]. Bile resistant genera support the reabsorption of bile acids into the host circulation. *Bacteroides*, *Clostridium*, *Lactobacillus* and *Bifidobacterium* are able to deconjugate bile acids, while genera such as *Eubacterium* and *Clostridium* dehydroxylate bile acids to secondary bile acids, which improves their permeability and thus increases reabsorption into the circulation [125]. Besides lipid digestion, primary bile acids are ligands for the farnesoid X receptor (FXR) and secondary bile acids are ligands for G protein–coupled bile acid receptor 1 (TGR5) expressed on hepatic and intestinal tissue [126]. In rodents, intestinal FXR inhibition is protective against high fat diet induced obesity [127], while TGR5 activation may improve glucose homeostasis by stimulating GLP-1 secretion [128]. Thus, dietary fat intake, bile acids and microbial bile acid conversion are linked to energy and glucose metabolism in rodents [124]. However, human studies are scarce and less straight forward. One-week administration of the antibiotic vancomycin substantially changed the fecal microbiota composition accompanied by decreased fecal secondary bile acids and decreased peripheral insulin sensitivity in participants with metabolic syndrome [129]. In obese, prediabetic men, one-week administration of vancomycin similarly altered the gut microbiota composition with concomitant decreases in secondary bile acids, yet without changes in insulin sensitivity, GLP-1 concentrations or glucose homeostasis [130]. In a study with naïve T2DM patients, therapeutic acarbose treatment (which increases carbohydrate availability and thus bacterial fermentation) reduced body weight and increased insulin sensitivity compared to classic antidiabetic treatment. These beneficial effects were accompanied by increased conversion to secondary bile acids, which was

most pronounced in participants with initial low secondary bile acid concentrations [131]. To conclude, there is not much known about the fate of dietary fat in the colon as well as its impact on gut microbiota composition. Data mostly based on rodent data describes an obesity-like microbiota profile with diets high in saturated fat. Whether the underlying mechanisms involve direct or indirect effects induced by changes of the primary and secondary bile acid pool warrants further investigation.

The gut microbiota in obesity and T2DM

The gut microbiota has been linked to metabolic disorders, namely obesity, metabolic syndrome and T2DM [132-134]. Early mice experiments reported that the obese differed from the lean gut microbiota composition. Furthermore, transplantation of the obese gut microbiota of mice and humans induced the same obese phenotype in germ-free mice [18, 135-137]. Similarly, antibiotic-induced depletion of gut microbiota protects against diet-induced obesity and metabolic impairments in mice models [138, 139]. Thus, rodent data support a causal link between gut microbiota and obesity and metabolic disorders. Yet, findings of human studies comparing obese and lean gut microbiota are less straightforward [140]. Early human studies described an increased *Firmicutes* to *Bacteroidetes* ratio in obese compared to lean humans [137]; however, others did not confirm this finding [137, 141]. BMI has a small but significant explanatory power for gut microbiota composition in large-scale population studies [31, 32]. However, in cross-sectional studies, the obese microbiota is associated with a reduced microbial diversity in some but not all studies [142-145] and a low abundance of certain taxa, i.e., *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* [146, 147]. The latter two taxa are reported to beneficially affect metabolic health, possibly via increased acetate and butyrate production [148, 149]. Furthermore, the obese microbiota has been associated with enrichment of genes involved in energy extraction from indigestible food compounds [150]. It has been estimated that SCFA may contribute to 10% of total energy intake [151], however whether this energy surplus from fermentation plays a significant role in human obesity development remains to be investigated. Similarly, in T2DM, fecal microbiota profiles are associated with a reduced abundance of *Bifidobacteria* [152, 153], *Akkermansia muciniphila* [31, 154] and butyrate – producing bacteria such as *Roseburia* and *Faecalibacterium prausnitzii* [155], and increased potential for BCAA production [147, 156-158].

Studies in humans using antibiotics [129, 130] or fecal transplantation [159, 160] reported conflicting findings. One-week supplementation of the antibiotic vancomycin reduced microbiota diversity, fecal SCFA, plasma secondary bile acid concentrations, yet without major changes in insulin sensitivity in obese, prediabetic men [129, 130]. Fecal transplantation of lean donor feces resulted in moderate improvement of insulin sensitivity at six weeks [159] but not at 18 weeks after transplantation in obese, prediabetic men [160]. In this study, the initial gut microbiota composition could predict improved insulin sensitivity after lean donor fecal transplantation [160], indicating that microbiota-based personalized approaches may be more successful to target metabolic health outcomes. In fact, in a pioneer study, an algorithm integrating blood metabolites, habitual diet, anthropometrics, physical activity as well as gut microbiota composition was developed to predict variation in postprandial glycemic responses after meals [161]. The authors showed that a diet based on the algorithm prediction lead to reduced postprandial glucose responses in a subsequent one-week intervention trial. However, whether microbiome-based approaches can be translated to the longer term and ameliorates or prevents metabolic diseases still needs to be investigated.

Together, recent data suggest that the gut microbiota is linked to metabolic disorders. Nevertheless, causal evidence is mostly derived from rodent studies. Therefore, well-controlled human studies incorporating more personalized intervention approaches are urgently needed to prove the directionality and causality between gut microbiota and metabolic disorders.

Prebiotics as nutritional strategy to modify gut microbiota

Prebiotics and metabolic health

Dietary fibers are the indigestible food compounds in the human diet such as oligosaccharides, lignin and polysaccharides. Based on physico-chemical properties, dietary fibers can be classified as insoluble, soluble, fermentable or viscous [162]. These properties strongly determine the effect they exert on GI transit time and GI responses after food intake, which has been reviewed in **Chapter 2**. Foods rich in insoluble fibers such as grains and cereals have been consistently associated with a reduced risk of developing T2DM and prevention of weight gain [163-165]; however, the underlying mechanisms are poorly understood. Soluble and fermentable fibers are only moderately associated with

disease risk reduction in observational studies, probably due to the limited resolution of dietary fiber types in food frequency questionnaires [166].

Prebiotic fibers are soluble, fermentable and defined as food compounds that selectively alter gut microbiota to confer host benefits [167]. Well-studied prebiotics include fructans (inulin, fructo-oligosaccharides (FOS)), galacto-oligosaccharides (GOS) and other less known oligosaccharides (i.e., xylo-oligosaccharides, mannan-oligosaccharides). Intake of inulin, FOS and GOS results in an increased abundance of bacterial species such as *Bifidobacteria* and *Lactobacillus,* which have been proposed to be associated with beneficial health effects [168-171]. These genera have been reported to strengthen the gut barrier, improve host mucosal immunity, increase SCFA production and protect against opportunistic gut pathogens [171]. Recent advances in metagenomics extended previous findings reporting that species such as *Faecalibacterium prausnitzii* and *Anaerostipes spp.* are increased upon prebiotic supplementation as well [172, 173]. Fructans (inulin, FOS) and GOS have been extensively studied in diet- or genetically-induced obese rodent models. Supplementation with GOS and FOS in these rodent models improved glucose homeostasis, reduced serum lipids and weight gain [174-176]. A recent meta-analysis concluded that FOS intervention decreases hyperglycemia in obese rodent models [177]. Proposed mechanisms are mainly based on an increase of colonic SCFA production, stimulation of enteroendocrine cell proliferation and increased gut hormone secretion but also modulation of the bile acid metabolism and a reduction of systemic inflammation [178-180]. Human intervention trials are less conclusive and either report improved postprandial glucose and insulin concentrations in healthy and obese volunteers [172, 181, 182] or no changes [183, 184]. A recent systematic review summarized randomized controlled trials with supplementation of inulin-type fructans and GOS (7.4g to 30g/d during a period of 20 days up to 6 months) in healthy, obese and T2DM individuals. This meta-analysis showed that fasting insulin (mean difference -4.01 U/L; $P < 0.0001$) and fasting glucose (mean difference -0.42 mmol/L; $P = 0.09$) concentrations were only reduced in T2DM individuals but not in overweight/obese, normoglycemic participants [185]. Different prebiotic types, fermentation efficiency and site of fermentation, as well as heterogeneity in study populations make it difficult to draw consistent conclusions. Thus, human studies looking at specific subgroups and different fiber types and/or combination of fibers could provide more efficient strategies of prebiotic intake with the aim to improve body weight control, insulin sensitivity and metabolic health.

Arabinoxylan-Oligosaccharides - a potential prebiotic?

Novel prebiotic fibers are currently under investigation to more efficiently target the gut microbiota composition and functionality in the context of metabolic health. Arabinoxylans are compounds in the cell wall of many grains and wheat bran and their hydrolysis products Arabinoxylan-Oligosaccharides (AXOS) have recently been described to fulfill the prebiotic criteria: AXOS is an indigestible, fermentable fiber , which stimulates the growth of specific bacteria namely *Bifidobacteria spp., Roseburia and Prevotella* in animals and humans [186].

AXOS supplementation to the high-fat diet of mice protected against weight gain, increased PYY and GLP-1 concentrations, improved gut barrier function and reduced inflammatory IL-6 mRNA expression in adipose tissue [187]. Moreover, AXOS supplementation in mice fed a western style diet increased *Butyricicoccus* and *Bifidobacterium* abundances, which correlated with reduced body weight gain and fat mass [188]. In healthy humans, acute supplementation of AXOS decreased postprandial glucose concentrations, accompanied by increased colonic fermentation measured as breath H_2 and by plasma SCFA concentrations [189]. In addition, AXOS may affect GI transit as AXOS intake increased stool consistency and frequency in healthy adults [190-192]. So far, only one human study investigated AXOS-induced gut microbiota modulation determined by 16S rRNA sequencing. In a 4-week crossover design with supplementation of 10.4 g AXOS/day, participants with more than two symptoms of metabolic syndrome had increased abundance of fecal *Bifidobacterium*, *Faecalibacterium*, *Ruminococcus*, *Dorea*, and *Eubacterium*, but no effects on fasting glucose, insulin and plasma lipids were demonstrated [193]. To conclude, AXOS is a potential novel prebiotic with promising beneficial effects on both metabolic and GI health in the short term, however the long-term effect on gut microbiota modulation, GI functioning and metabolic health remains to be investigated.

Targeting distal fermentation as novel approach to improve metabolic health

Along the distal colon, carbohydrate availability declines and protein fermentation increases, while luminal content consistency acidifies and solidifies due to colonic water absorption and increased mucus viscosity [42, 194]. These longitudinal gradients form the proximal towards the distal colon impact the gut microbiota composition and functionality as observed by variation in microbiota composition along the colon: the proximal colon is enriched with lactobacilli and proteobacteria, whereas in the more distal colon, Firmicutes (*Lachnospiraceae*, *Ruminococcaceae*), Bacteroidetes such as *Bacteroidaceae*, *Prevotellaceae*, and *Rikenellaceae*) are more abundant [195, 196]. Mostly dependent on substrate availability, microbial SCFA fermentation seems to differ along the colon as well. Colonic SCFA concentrations are highest in the proximal colon, while paradoxically, plasma SCFA concentrations are highest in the inferior mesenteric veins draining from the distal rather than proximal intestine [57, 83]. This indicates potential differences in epithelial uptake and utilization of SCFA in the proximal vs. distal colon and implies that, depending on the colonic site, SCFA uptake and flux into the circulation may vary.

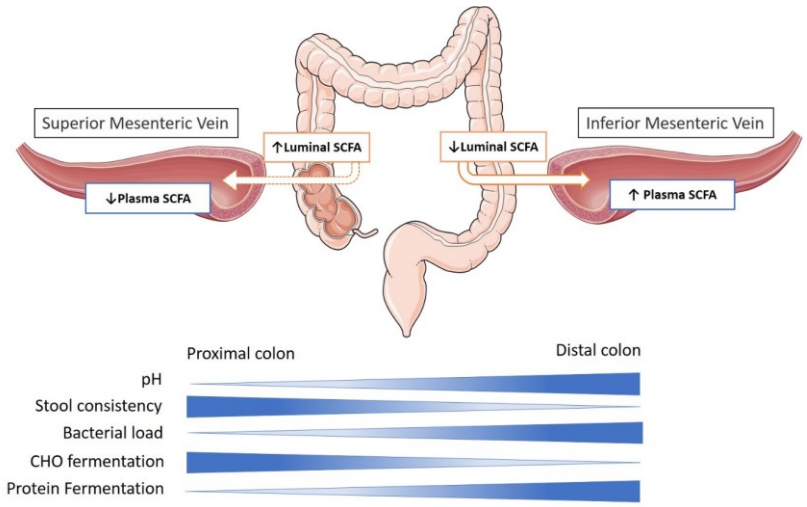

Figure 1. Luminal and plasma SCFA concentrations as well as colonic gradients along the GI tract. Gradients of pH, stool consistency, bacterial load, CHO and protein fermentation along the longitudinal axis in the colon. Luminal SCFA concentrations are highest in the proximal colon and lower in the distal colon, mostly due to decreased indigestible carbohydrate availability. Plasma SCFA concentrations in superior mesenteric vein draining to the liver are lower compared to the distal inferior mesenteric vein indicating differential proximal epithelial uptake and utilization of SCFA by the host (indicated by the dashed line) compared to the distal colon. Yet, data are based on few small human studies [57, 83].

This may have important implications for the effect of SCFA on the human metabolism. In fact, acute infusion of the SCFA acetate in the distal but not in the proximal colon showed metabolic effects, including an increased fasting fat oxidation, increased PYY and decreased lipolysis in overweight men [71, 72]. One of the explanations for these findings could be that distally administered acetate reaches the peripheral circulation in greater concentrations when absorbed via the inferior mesenteric veins of the anus and rectum. Thereby, it partly circumvents hepatic clearance resulting in higher systemic acetate concentrations and thus, might exert more pronounced effects on peripheral tissues like adipose tissue and skeletal muscle. Indeed, with distal infusion acetate concentrations were higher than with proximal infusion and there was a positive association between circulating acetate and increases in fat oxidation and energy expenditure [71]. In pigs, a combination of readily fermentable resistant starch and insoluble wheat bran fiber was used to investigate if the wheat bran-induced accelerated colonic transit would increase delivery of resistant starch into the distal colon. In fact, the combination, but not wheat bran or resistant starch alone led to 133% increase of fecal butyrate and reduced ammonia in the distal colon [197]. Canfora *et al.* suggested to target distal SCFA production by intake of a combination of slow-fermentable, long-chain fibers and fast-fermentable fiber in order to obtain more pronounced effects on metabolic health [104]. In the proximal colon, the gut microbiota is then saturated by the fast-fermentable fiber so that the long-chain, slow-fermentable fiber would reach the distal colon in significant concentrations. Hence, distal delivery of dietary fibers may be a potential novel strategy to increase circulating SCFA (and in particular acetate) in order to prevent metabolic disorders.

Outline of this thesis

This doctoral thesis focuses on the complex relationship between diet, GI functioning and the gut microbiota composition in the context of metabolic health. **Chapter 2** provides an overview of the potential underlying mechanisms of the interaction between GI transit, glucose homeostasis and metabolic health, and how dietary fibers may exert beneficial metabolic effects via modulation of GI functioning. Physiological and methodological aspects of (the measurement of) GI transit (time) are discussed in relation to glucose homeostasis. Short- and long-term human interventions with soluble, insoluble, fermentable or viscous dietary fiber are discussed with respect to their effect on GI transit, microbiota modulation and glucose homeostasis.

Given the available human evidence that short-term AXOS intake may induce beneficial effects on colonic fermentation, stool parameters as well as glycemia, we aimed to investigate the effect of long-term AXOS intervention on GI transit, GI health and metabolic health in normoglycemic participants with slow GI transit. In this randomized, placebo-controlled parallel trial (**Chapter 3**), we thoroughly characterized parameters of gut functioning, gut microbiota composition and activity as well as the metabolic profile of the participants.

The GI transit may be a determinant of the gut microbiota composition, yet human studies looking at associations between quantitative measures of GI transit, gut microbiota composition and microbial fermentation are lacking. In **Chapter 4**, we investigated the associations of colonic transit measures, gut microbiota diversity and composition while taking into account diet composition and host demographics.

Human studies on the role of SCFA in host metabolism are hampered by the fact that most often only fecal SCFA are measured, reflecting only 5% of the microbially produced SCFA. In **Chapter 5,** we studied the association between fecal and circulating SCFA and relevant metabolic markers including plasma metabolites, hormones, substrate oxidation and insulin sensitivity in a large cohort of healthy to prediabetic participants.

It has been suggested that acetate produced in the distal colon is more likely to escape hepatic clearance and reach the circulation in higher concentration where it may have beneficial effects on peripheral tissues involved in energy homeostasis. In **Chapter 6**, we aimed to target distal acetate production with acute supplementation of a slow-fermentable, long-chain fibers and fast-fermentable fiber lean and overweight/obese men. Lastly, in **Chapter 7** the main findings of this thesis are discussed and integrated into a broader perspective with regards to potential implications and future research.

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CHAPTER 2

Gastrointestinal Transit Time, Glucose Homeostasis and Metabolic Health: Modulation by Dietary Fibers

Running title: Gut transit, dietary fiber and metabolic health

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Abstract

Gastrointestinal transit time may be an important determinant of glucose homeostasis and metabolic health through effects on nutrient absorption and microbial composition, among other mechanisms. Modulation of gastrointestinal transit may be one of the mechanisms underlying the beneficial health effects of dietary fibers. These effects include improved glucose homeostasis and a reduced risk of developing metabolic diseases such as obesity and type 2 diabetes mellitus. In this review, we first discuss the regulation of gastric emptying rate, small intestinal transit and colonic transit as well as their relation to glucose homeostasis and metabolic health. Subsequently, we briefly address the reported health effects of different dietary fibers and discuss to what extent the fiber-induced health benefits may be mediated through modulation of gastrointestinal transit.

Introduction

Food intake activates several gastrointestinal (GI) processes. The transit of food through the stomach, small intestine and colon is crucial for digestion and absorption of nutrients. Upper intestinal transit includes gastric emptying (GE) and small intestinal motility and plays a major role in satiety and appetite regulation, glycemic control and gut hormone signaling [1,2]. Furthermore, the rate of lower intestinal namely the colonic transit has a major impact on the gut microbiota [3], which may be involved in many physiological functions in energy and substrate metabolism, metabolic cross-organ signaling and insulin sensitivity [4]. Thus, altered GI transit may play a role in the etiology of metabolic diseases such as obesity and type 2 diabetes mellitus (T2DM) [5]. Food-based approaches are under investigation among which indigestible dietary fibers are long acknowledged to be beneficial in the prevention of chronic metabolic diseases. Indeed, observational studies showed that intake of dietary fibers from various plant sources decrease the prevalence of obesity and T2DM [6,7]. Nevertheless, data from human intervention studies are less consistent and may depend, among other factors, on type and amount of dietary fibers, overall diet composition and the metabolic phenotype of the individual [8]. The underlying mechanisms of the beneficial effects of dietary fibers are not completely understood, but the GI transit might play a role in these mechanisms. Indeed, dietary fibers may affect the GI transit of nutrients via various mechanisms depending on their physical-chemical properties (i.e., viscosity, fermentability and water solubility) [9]. This review discusses how GI transit may relate to glucose homeostasis and metabolic health and to what extent the positive metabolic effect of dietary fiber intake may be mediated by modulation of GI transit. First, we provide an overview on methodologies to assess GI transit. Then, we discuss the (patho)physiological consequences of an altered GI transit on glucose homeostasis, gut hormonal secretion and gut microbiota composition. Secondly, we review available human evidence of insoluble, soluble viscous and non-viscous fiber on glucose homeostasis. Finally, we discuss to what extent these effects may be mediated GI transit.

Gastrointestinal Transit: Methodology and (Patho)physiology

Gastric Emptying and Its Regulation

After ingestion, food passes through the esophagus and reaches within seconds the stomach. Initially, food intake induces gastric accommodation in the proximal part of the stomach thereby increasing its capacity to store ingested foods. Contractile activity patterns mix gastric liquids and push solid food particles towards the distal antrum and pylorus. The antrum repetitively contracts and grinds food particles against the closed pylorus facilitating mechanical and chemical break down of solid foods. Consequently, the pylorus opens to gradually release chyme with a size of 1–2 mm at a rate of 4 kcal/min into the duodenum; a process termed gastric emptying (GE) [10]. GE rate is often reported as initial lag phase (T_{lag}, i.e., the phase between ingestion and start of emptying) and GE half-time ($T_{1/2}$, i.e., the time at which half of the meal is emptied). GE rate adapts to energy density, volume and digestibility of the meal and differs greatly between solid and liquid foods. Macronutrients generally slow down GE, an effect that is mainly dependent on the caloric content of the macronutrient rather than type of macronutrient [2,11]. Non-caloric liquids are rapidly released from the stomach into the duodenum in an exponential manner. Solid food empties in a biphasic manner described by T_{lag} which takes few minutes up to an hour followed by a linear emptying phase [10]. The gold standard to measure GE rate is imaging with radionuclide scintigraphy. It visualizes the retention of a stable isotope labeled meal in the stomach [12]. Due to the exposure to ionizing radiation, other validated non-invasive methods have been developed using paracetamol or non-radioactive 13C stable isotopes. After ingestion of a 13C-isotope or paracetamol labeled meal, rapid duodenal absorption enables detection of 13CO2 in the expired breath or paracetamol in the blood, respectively [13].

The regulatory mechanisms coordinating GE rate are complex and involve the central nervous system (CNS), enteric motor neurons and the gastric smooth muscle cells. Normal GE rate is stimulated by contractile activity of the stomach and small intestine, which is coordinated by the CNS, the vagus nerve and neurohumoral peptides [14]. During the fasting and postprandial state, numerous neurohumoral gut peptides are secreted from enteroendocrine cells in the gastric and intestinal mucosa (i.e., ghrelin, cholecystokinin (CKK), glucagon-like peptide-1 and 2 (GLP-1, GLP-2) and peptide YY (PYY)). Food intake inhibits ghrelin secretion and stimulates secretion of CCK, PYY and GLP-1, which in turn modulates gastric and intestinal motility by activating receptors on sensory,

vagal and intrinsic afferent neurons. Thus, GLP-1, PYY and CCK delay GE rate and induce satiety via central signaling [15–18]. This negative feedback loop controls food transit through the upper gut to optimize digestion and nutrient absorption.

Gastric Emptying and Blood Glucose Homeostasis

GE rate determines the appearance rate of glucose in the duodenum and its subsequent absorption and systemic appearance [19]. In healthy individuals [20,21], as well as in individuals with obesity [22] and T2DM [23], GE rate explained around 30% of the variance in peak circulating glucose concentrations after an oral glucose load. Notably, a higher initial GE rate is directly related to the initial rise in postprandial glucose concentrations [2]. Over time, elevated postprandial blood glucose may play a role in the development of insulin resistance and T2DM [24]. In the overweight or obese state, GE rate has been reported to be faster, similar or delayed compared to lean individuals [25,26]. However, a recent, large-scale cross-sectional analysis reported faster GE rates measured via scintigraphy and reduced fasting plasma PYY concentrations in overweight and obese compared to lean adults [27]. Therefore, it is intriguing to speculate that accelerated GE in obesity may lead to rapid postprandial blood glucose peak, thus imposing a constant challenge to postprandial glucose homeostasis. However, data on altered GE rates in overweight and obesity is still inconclusive, which is likely due to different methodologies, test meal composition and study population heterogeneity.

To conclude, controlled release of nutrients from the stomach affects blood glucose appearance and in turn is regulated by nutrient-induced release of neurohumoral gut peptides. There are indications that GE rate is altered in overweight or obese individuals which might contribute to postprandial hyperglycemia. Thus, modulation of GE rate might be a potential target to modulate postprandial glycemia, thereby reversing or preventing cardiometabolic risk.

Small Intestinal Transit and Its Regulation

Food transit through the small intestine depends on GE rate and involves simultaneous propulsion, mixing and segmentation of chyme along all intestinal segments. Regional small contractions mix the luminal content with digestive secretions and increase contact of chyme with the mucosal surface to optimize absorption of nutrients. When the stomach is empty, organized repetitive contractions, termed the migrating motor complex

(MMC), spread from the stomach along the small intestine. These contractions facilitate efficient nutrient absorption and evidentially cleansing of undigested food particles to prevent small intestinal bacterial overgrowth [28]. Small intestinal flow patterns are measured with intestinal manometry or impedance catheters that capture intestinal pressure waves and the transit of a food bolus. In the clinic and research setting, less invasive, indirect breath tests are usually used to measure small intestinal transit time. Breath tests measure oro-cecal transit, which describes the time from food ingestion to arrival of chyme in the caecum. After ingestion of an indigestible, fermentable carbohydrate (i.e., lactulose or inulin), exhaled breath hydrogen is measured in intervals. Once entered into the cecum, colonic bacteria rapidly ferment the indigestible carbohydrates leading to increased hydrogen excretion [29,30]. However, oro-cecal transit measured via hydrogen exhalation showed a high inter-individual variability indicating that it might only detect significant differences in case of extreme delayed/accelerated transit [31]. Recently, the wireless SmartPill has been introduced which measures intestinal transit time based on luminal pH differences along intestinal segments [32].

Small Intestinal Transit and Glucose Homeostasis

Small intestinal motility patterns likely influence the extent of glucose absorption, but little is known about the underlying mechanisms. Luminal carbohydrate breakdown, small intestinal flow patterns and mesenteric blood flow may play a role in intestinal glucose absorption. The major part of glucose is absorbed in the proximal intestine via luminal sodium-glucose co-transporter (SGLT1) and glucose transporter 2 (GLUT2) at the baso-lateral membrane [33]. Drug-induced attenuation of small intestinal luminal flow markedly reduced the early postprandial plasma glucose peak in healthy humans [34]. Other studies using nutrient infusion into different sites of the small intestine indicated that increasing the intestinal area that is exposed to nutrients may have beneficial effects on postprandial glycemia, satiety, hunger and hormonal responses [35].

Only a few studies have assessed small intestinal transit time in overweight and obese adults. In obese women and lean participants, intestinal contents were sampled via a tube inserted in the proximal 70 cm of the intestine. After intake of a high-fat liquid test meal, the energy content of the luminal sample was lower in obese women compared to lean participants, indicating a more effective rate of intestinal nutrient absorption [36]. Upon prostaglandin-induced stimulation, intestinal smooth muscle strips from obese showed an increased contractility measured with isometric transducers compared to

intestinal muscle strips from lean controls [37]. In contrast, the oro-cecal transit of 100 mL water measured via lactulose breath test was slower in obese compared to normal weight controls [38]. Another study reported no differences in oro-cecal intestinal transit in obese compared to lean participants despite higher postprandial glucose concentrations after a liquid test meal in individuals with obesity [22]. In addition, no association was found between body mass index (BMI) and oro-cecal transit assessed by scintigraphy in a cross-sectional analysis including lean and obese individuals [39].

To summarize, evidence suggests a role of small intestinal transit in postprandial glucose absorption. However, data on altered small intestinal transit in obesity is yet inconclusive. Among other factors, studies lack standardized methodology such as harmonized test meal compositions. Further, well-designed human studies are warranted to study putative differences in small intestinal transit patterns in overweight/obese vs. normal weight individuals including their contribution to glycemic control.

Colonic Transit and Its Regulation

The main function of the colon is to absorb water and electrolytes, a process much slower than small intestinal nutrient absorption, and storage of food waste [40]. The enteric nervous system largely controls colonic motor function. Colonic motor activity occurs in high-amplitude propagated contractions compartmentalizing colonic segments which eventually results in stool formation [41]. Gut hormones involved in colonic motility are somatostatin, neurotensins, motilin and corticotrophin-releasing factor (CRF) [42]. There is no consensus on the golden standard for measuring colonic transit. In clinic and research settings, scintigraphy, wireless SmartPills and radio-opaque marker methods are commonly used. The latter involves ingestion of a defined number of radio-opaque markers in 24 h intervals. After 4 to 7 days of ingestion, an abdominal x-ray is taken, and colonic transit time is calculated based on the amount of markers visible on the x-ray [43]. Stool consistency assessed via the Bristol Stool Chart (BSC) is often used as a proxy for colonic transit. Individuals classify stool consistency from very firm to loose stool. Low BSC scores, i.e., firm stool, indicates slow colonic transit and high BSC scores, i.e., soft stool, indicate normal to fast transit. BSC scores correlate well with outcomes of scintigraphy imaging or the radio-opaque marker method and to a lesser extent with defecation frequency [44].

Colonic Transit and Metabolic Health

The role of colonic transit in metabolic health is not well studied. A meta-analysis of 21 cross-sectional studies concluded that GI symptoms such as diarrhea, but not constipation, were associated with increased BMI [45]. Small scales studies assessing colonic transit are less conclusive. Studies reported slower [46,47] as well as faster colonic transit [39] in obese compared to lean individuals. In T2DM, constipation is one of the most common reported GI symptoms [48] and underlying processes are likely to be related to the higher occurrence of autonomic and enteric neuropathy in T2DM [49]. Furthermore, colonic transit may play an indirect role in metabolic health via its reciprocal relationship with the gut microbiota, which is discussed in following section.

Gut Microbiota and Colonic Transit

The colon is the ecological habitat of trillions of bacterial cells termed the gut microbiota. Species belonging to the phyla *Firmicutes* with around 200 g-positive genera (incl. *Clostridium*, *Eubacterium*, *Faecalibacterium*, *Lactobacillus*, *Roseburia* etc.) and *Bacteriodetes* with around 20 g-negative genera (incl. *Bacteriodes*, *Prevotella* etc.) are the most abundant (>80%). Less abundant species belong to the phyla *Actinobacteria* (i.e., *Bifidobacteria*), *Proteobacteria* (i.e., *Desulvibrio*, *Escherichia*) and *Verrucomicrobia* (i.e., *Akkermansia muciniphila*) [50]. The symbiotic relationship between the microbiota and the human host plays a role in many physiological functions within the energy metabolism, metabolic cross-organ signaling, gut barrier integrity and (mucosal) immune system [51]. Although a healthy gut microbiota composition remains yet to be defined, evidence from animal and human studies suggests that alterations of gut microbiota contribute to the etiology of obesity and T2DM [52,53]. Microbial metabolites such as short chain fatty acids (SCFA) interact with metabolic signaling and inflammatory pathways on intestinal and systemic level [4,54]. Hence, they physiologically link the gut microbiota composition and activity with the host metabolic phenotype and consequently to metabolic disorders. In T2DM, fecal microbiota profiles have been reported to be associated with a reduced abundance of *Bifidobacteria* [55,56], *Akkermansia muciniphila* [57] and butyrate-producing bacteria such as *Roseburia* and *Faecalibacterium prausnitzii* [58–62]. *F. prausnitzii* may be an indicator for gut health [63] and might be involved in low-grade inflammation in obesity and T2DM [64]. The obese microbiota is characterized by a low abundance of certain taxa i.e., *Akkermansia* [62,65] and a reduced overall microbial

diversity [66]. With respect to chronic metabolic diseases, modulation of gut microbiota composition and activity might consequently be a potential target for prevention or treatment opportunities.

Colonic transit time is an important host factor shaping the microbiota ecosystem since it regulates water and nutrient availability as well as the rate of luminal washout [67,68]. First observations on the relation between microbiota composition and GI transit stem from germ-free (GF) rodent models, which showed a delayed GE rate and delayed whole gut transit compared to conventionally raised animals [69]. Re-colonization with *Lactobacillus* and *Bifidobacterium* could normalize GI transit in GF rodents [70–72]. The gut microbiota might affect gut motility via different mechanisms. Microbially-derived metabolites and molecules such as SCFA, lipopolysaccharide (LPS), secondary bile acids and methane may affect colonic motility via neural and humoral (i.e., GLP-1, PYY, motilin, serotonin) pathways [73,74]. Vice versa, colonic transit may impact gut microbiota composition and its functionality. Early human studies with loperamide-induced delayed or sienna-induced accelerated colonic transit showed that fecal microbial mass either increased with accelerated, or decreased with delayed colonic transit [75,76]. With more sophisticated 16S rRNA sequencing techniques available, several studies have revisited the interaction between colonic transit and the gut microbiota. In a population analysis with 1335 participants, stool consistency (measured by BSC score) was the top covariate explaining most of the microbiota community variation observed [3]. In healthy women, softer stool was associated with increased abundance of *Bacteroides* while firmer stool was associated with increased abundance of methane-producing archaea and increased species richness [77]. A prospective cohort study including 1126 participants reported no association between stool consistency and species richness but a similar positive correlation between abundance of methanogens (*Methanobrevibacter*) and *Clostridiaceae* with BSC scores. Interestingly, *F. prausnitzii* was strongly associated with softer stool consistency [78]. A recent cross-sectional study with 85 overweight to obese participants with increased risk for metabolic syndrome showed that slower colonic transit was associated with increased *Methanobrevibacter* and reduced *F. prausnitzii* abundance as well as an increased production of microbial protein catabolism end-products (i.e., p-cresol, indole) [79]. The authors conclude that with a delayed colonic transit, indigestible carbohydrates are more and more depleted driving bacteria to switch to protein fermentation. However, the authors did not investigate colonic transit and gut microbiota in relation to the metabolic phenotype of the participants.

In summary, a slow colonic transit is associated with a methanogenic profile and increased bacterial protein catabolism. Slow transit may be accompanied by carbohydrate deprivation, and a subsequent microbial energy metabolism shift towards protein fermentation. This may result in an increase of metabolites potentially detrimental for metabolic health (i.e., branched-chain fatty acids (BCFA), ammonia and aromatic derivatives of amino acids) [80]. On the other hand, faster transit was associated with higher *F. prausnitzii* abundance, which is associated with gut health and reduced low-grade inflammation. Thus, modulating colonic transit might be a potential target to counteract microbial dysbiosis related to chronic metabolic diseases.

Dietary Fibers

Dietary fibers are a heterogeneous group of food compounds. Generally, dietary fibers are indigestible carbohydrates (i.e., cellulose, hemicelluose, β-glucans, pectins, gums fructans and resistant starch) and lignins intact and intrinsic in fruits, vegetables, legumes and cereals. Chemically, dietary fibers include non-starch polysaccharides such as cellulose, hemicellulose, β-glucans, polyfructoses (i.e., inulin), natural gums and heteropolymers (i.e., pectin) as well as natural or synthetically produced oligosaccharides (i.e., fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS)) [81]. These compounds vastly differ in their structural, physical and chemical properties, namely water solubility, viscosity, binding and bulking ability and fermentability [82].

Effect of Insoluble Dietary Fibers on Glycemia

Insoluble fibers include cellulose, hemi-celluloses and lignin, which are major components of plant cell walls. Insoluble fibers are the main constituent of dietary fiber fractions in cereals, grains, vegetables and fruits. However, it is important to note that most fiber–rich foods contain soluble, insoluble, (non-) fermentable fibers in varying ratios [83]. Foods rich in insoluble fibers such as whole grains and cereals are consistently associated with a reduced risk of developing T2DM in observational studies [6,84–87]. A recent meta-analysis with more than 15,000 participants concluded that an increase in cereal fiber intake of 10 g/day decreased the risk of developing T2DM by 25% independent of BMI [7]. In line, a meta-analysis of 17 prospective cohort studies reported that an increase in cereal fiber intake of 2 g/day reduced the risk to develop T2DM by 6% (RR 0.94, 95% CI 0.93–0.96) [88]. Only a few intervention studies investigated the impact of foods

rich in insoluble fiber on GI transit and glucometabolic effects. In an acute crossover study, 14 healthy women consumed either bread enriched with 10.4 g wheat fibers or 10.6 g oat fibers or a white bread as a control [89]. Intake of both high fiber breads increased early postprandial insulin peak and reduced postprandial glucose concentration, but stool consistency did not change. In another crossover study, 50 overweight or obese participants fulfilling one or more metabolic syndrome criteria consumed a whole grain diet (179 ± 50 g/day) or a control diet with refined grain for 8 weeks. Circulating low-grade inflammatory markers and body weight (from 85.4 kg ± 13.4 kg to 85.2 kg ± 13.4 kg, $p < 0.001$) were reduced after the whole grain diet, which correlated with a reduced total energy intake. The whole grain intervention had no effect on fasting blood glucose, insulin, lipid or GLP-1 concentrations and no major changes in fecal gut microbiota or whole gut transit measured by radio-opaque markers were observed [90].

Underlying Mechanism: A Role of GI Transit? To date, it is not fully understood which mechanisms are responsible for the beneficial effects of insoluble fiber intake on health. With regards to GI transit, it is generally accepted that laxation is one of the major health benefits of insoluble and cereal fiber intake. Insoluble fibers increase fecal bulking and stool water content due to their high water-binding capacity which in turn mechanically stimulates mucus secretion and peristalsis [91–93]. A weighted regression analysis of 65 interventions studies in healthy participants showed that an increase of cereal and wheat fiber of 1 g/day increased stool wet by 3.9 g/day. Interestingly, only in individuals with a GI transit time above 48 h, the increased intake of 1 g/day cereal and wheat fiber led to a decrease in colonic transit of 0.78 h/day [91]. Fiber-induced fecal bulking affects the rheological structure of the food matrix which might impact the bioavailability and bio-accessibility of macronutrients within the food matrix [94]. Thus, macronutrients might be less accessible for digestion and absorption and consequently reduce energy intake, which may contribute to the observed beneficial effects on metabolic health. Besides, the replacement of available carbohydrates by indigestible carbohydrates per se might reduce energy content of the diet. Further, microbial modulation might also occur due to fiber-induced changes of colonic habitat such as increased water availability and trapping of nutrients within the stool matrix. Microbial derived SCFA have been proposed as one of the potential beneficial mechanisms explaining the improved insulin sensitivity with diets high in insoluble fiber [95]. However,

the degree of fermentability of insoluble fibers and the possible effect on metabolic health is controversial [96–98].

It should be kept in mind, that most sources of insoluble fiber are consumed as whole grains or partly processed cereal grains, which may differ in functionality (grain source and refining procedure). Furthermore, they also contain varying amounts of soluble fibers and bioactive phytochemicals, such as polyphenols, that might also contribute to the observed health benefits [99]. It is evident that further human studies investigating the relation between insoluble fiber intake, GI transit and metabolic health are warranted.

Effects of Soluble, Viscous Fibers and Postprandial Glycemia

Soluble, viscous fibers include polysaccharides such as plant-derived pectins, β-glucans, psyllium/ispaghula husks, natural gums, galactomannans and alginates. Once dissolved in water, viscous fibers form gels and/or thicken, a physico-chemical characteristic that may impact intestinal motility and absorption rates of glucose, triglycerides and cholesterol [100]. Randomized, controlled studies reported that viscous and/or gel-forming fibers may improve glycemic and insulinemic responses [101]. In fact, the European Food Safety Agency (EFSA) authorized the health claim that consumption of 4 g of β-glucans (derived from barley and oats) per 30 g of available carbohydrates is sufficient to reduce postprandial glucose concentration in the range of clinical significance [102]. The viscous fiber psyllium has been reported to lower postprandial glucose concentrations and improve insulin sensitivity in healthy, obese and T2DM individuals. This positive effect on postprandial glycemia was reported in intervention studies ranging from 6 weeks to 6 months with doses of 10–14 g psyllium per day [103]. A meta-analysis of 4 studies (duration 2 to 24 weeks) in T2DM individuals showed that psyllium intake decreased fasting glucose concentration by 37 mg/dL and reduced HbA1c (−10.6 mmol/mol) compared to placebo [104]. Similar, 6-week supplementation of 10 g/d natural partly hydrolyzed guar gum reduced fasting glucose and insulin concentration in healthy men [105] and HbA1c concentration in T2DM patients [106]. However, other studies with hydrolyzed guar gum supplementation in T2DM patients reported no effect on glycemia [107,108]. Acute reduction of subjective appetite rating and acute energy intake was reported after intake of pectins (mean dose 14.2 g/day and 4.8 g/day, respectively) and β-glucans (mean dose 6.2 g/day and 5.8 g/day, respectively) compared to the control food [109].

Underlying Mechanisms: Importance of Viscosity. It has been suggested that viscosity is crucial to exert beneficial fiber-specific effects on glucose homeostasis and appetite regulation. High food viscosity induces gastric distension i.e., feeling of fullness [110], delays GE and/or physically prevents absorption of nutrients in the small intestine [111,112]. Pharmacologically or fiber-induced delayed GE rate was associated with reduced feelings of hunger and increased satiety [113]. Hence, this suggests that viscous fibers might improve glycemic control by delaying GE rate. Table 1 gives a summary of available human intervention studies on viscous fiber intake on GE rate and postprandial glycemia. Early scintigraphy studies showed that both healthy volunteers and T2DM individuals had a delayed GE after the consumption of 20 g apple pectins/day for 4 weeks compared to an isocaloric low fiber diet, which was accompanied by reduced postprandial glucose concentrations only in T2DM individuals [114,115]. In healthy volunteers, acute intake of liquid drink supplemented with 2.5 g pectin delayed GE measured by 13C-acetate breath test, but no changes were observed in postprandial glucose concentrations [116]. Acute intake of 5 g sodium-alginate with a liquid meal reduced postprandial glucose and insulin concentrations in T2DM patients, which was correlated with a delayed GE assessed by scintigraphy [117]. In another acute study, intake of high viscous β-glucans attenuated the early postprandial glucose response and delayed GE rate in healthy volunteers compared to the less viscous ß-glucan [118]. In contrast, acute supplementation of 1.7 g psyllium to a solid meal neither reduced GE, nor affected postprandial glucose and insulin concentrations compared to a control meal in healthy volunteers [119]. Acute intake of β-glucans with low or high viscosity in an oat bran beverage resulted in higher postprandial GLP-1 and PYY concentrations and faster GE rate with the low-viscosity compared to high viscosity beverage in healthy volunteers [120]. In contrast, acute meal supplementation of 23 g psyllium led to reduced postprandial glucose and GLP-1 concentrations in healthy volunteers as compared to an isocaloric low fiber meal. The authors conclude that a psyllium-dependent increase in luminal viscosity physically impairs the efficient stimulation of L-cells and concomitant GLP-1 and PYY release [121].

Post-gastric events might also contribute to the observed effects of viscous fiber on postprandial glycemic control, GE and satiety. There is limited evidence that viscosity is maintained throughout the small intestine, since the digesta is diluted with gastric secretions during passage from stomach to duodenum. Besides, the degree of viscosity depends on structural and chemical composition of the fiber type and can differ across pH gradients in the GI tract [122]. Viscous, gel-like digesta can, to some extent, bind and

thereby limit diffusion of nutrients within the gel matrix. This reduces the contact of nutrients with digestive enzymes and absorption from the intestinal epithelium [100,112]. In rat intestinal cells, incubation with β-glucans suppressed glucose uptake, which was accompanied by reduced expression of SGLT1 and GLUT2 [123]. Also, postprandial intestinal contractility is prolonged (indicating a delayed small intestine transit) after 5 g guar gum supplementation to solid or liquid meals in healthy individuals [124]. On the other hand, several acute studies reported no differences in small intestinal transit time after ingestion of guar gum or psyllium as measured by indirect hydrogen breath tests [119,125,126].

Hence, there is a robust link between acute viscous fiber intake and delayed GE, however this is not consistently associated with an improved postprandial glucose response. Differences in methodology for measuring GE rate, interactions with other meal compounds, and degree of viscosity might play a role in the variability of the interaction between viscous fiber, GE and glycemia. Evidence from animal and in vitro studies suggests that viscous fiber might interfere with intestinal glucose uptake and absorption. This might partly explain the observed beneficial effects on glycemia in humans. However, well-controlled human studies are warranted to further elucidate the underlying mechanism of viscous fiber on GE rate, postprandial glycemia and intestinal glucose absorption.

Effects of Soluble, Non-Viscous Fibers on Glycemia

Soluble, non-viscous fibers are fructans (inulin, FOS, GOS, xylo- and arabinoxylan oligosaccharides (A(XOS)), resistant starches and analogous polysaccharides such as polydextrose that are fermentable by the gut microbiota in the colon [127]. Some soluble fibers such as inulin, FOS, GOS and XOS are classified as indigestible prebiotics defined as ‘a substrate that is selectively utilized by host microorganisms conferring a health benefit’ [128]. Intake of inulin, FOS and GOS results in an increased abundance of bacterial species associated with beneficial health effects such as Bifidobacteria and Lactobacillus [129–132]. These genera have been associated with various beneficial effects including strengthening of gut barrier function, improving host mucosal immunity, increased SCFA production and protection against opportunistic gut pathogens [132].

Table 1. Summary of human intervention studies with viscous dietary fiber on GE rate and postprandial glucose metabolism

Participants	Design	Intervention	Method	GE Rate	Metabolic Outcomes	Ref.
13 healthy adults (6 men, 7 women)	2-wk isocaloric low-fiber diet followed by 4-wk low fiber diet + supplement	20 g/day apple pectin baked in muffins or 20 g/day cellulose supplement as control fiber	Scintigraphy Solid meal (545 kcal, 74% CHO, 23% Protein, 1% fat)	↑ $T_{1/2}$	↔ glucose	[115]
12 non-insulin dependent T2DM patients (7 men, 5 women)	2-wk isocaloric low-fiber diet followed by 4-wk low fiber diet + supplement	20 g/ day apple pectin baked in muffins	Scintigraphy Solid meal (690 kcal, 43% CHO, 43% Protein, 23% fat)	↑ $T_{1/2}$	↓ iAUC glucose	[114]
7 male T2DM patients (BMI 20–30 kg/m^2)	Acute crossover study	5 g sodium-alginate, control drink without supplement	Scintigraphy Semi-solid meal (340 kcal, 48% CHO, 13% protein, 39% fat)	↑ $T_{1/2}$	↓ postprandial peak insulin ↓ postprandial peak glucose	[117]
10 healthy men	Acute crossover study	2 g agar or 4 g pectin, control drink without supplement	^{13}C-acetate breath test Semi-solid meal (400 kcal, 32% CHO, 8% protein, 39% fat)	↑ $T_{1/2}$ ↑ T_{lag}	↔ AUC glucose	[116]
10 healthy adults (4 men, 6 woman)	8 Acute crossover study	Pasta meal supplemented with 1.7 g psyllium and with or without added sunflower oil	Paracetamol absorption High-fat solid meal (510 kcal, 45% CHO, 1% protein, 52% fat) Low-fat solid meal (240 kcal, 96% CHO, 3% protein)	↔ AUC paracetamol	↔ glucose ↔ insulin ↔ GLP-1	[119]
15 healthy adults (3 men, 12 women)	Acute crossover study	High molecular weight 12.8 g, β-glucan (25% purity), low molecular weight 3.6 g β-glucan (75% purity), control without supplement	^{13}C-acetate breath test Liquid meal (189–192 kcal, 60–67% CHO, 7–10% protein, 27–29% fat)	↑ $T_{1/2}$ ↑ T_{lag}	↓ $iAUC_{0–60min}$	[118]

CHO carbohydrate; $T_{1/2}$ gastric emptying half time; T_{lag} initial gastric emptying rate; AUC area under the curve; iAUC incremental area under the curve; % Percentage of total energy intake

Fructans (inulin, FOS) and GOS have been extensively studied in diet-induced obese or ob/ob rodent models. The supplementation with GOS and FOS in these rodent models improved glucose homeostasis, reduced serum lipids and a reduced weight gain during a high-fat diet [82,133,134]. With respect to glycaemia, human intervention trials are less conclusive and either report improved postprandial glucose and insulin concentrations in healthy and obese volunteers [135–137] or no changes [138,139]. A recent systematic review summarized 20 randomized controlled trials with 607 healthy, obese and T2DM patients with inulin-type fructans ITF (FOS, GOS, inulin and mixes) supplementation ranging from 7.4 g to 30 g/d during a period of 20 days up to 6 months. Overall analysis showed that fasting insulin and glucose concentrations were only reduced in T2DM individuals [140].

Underlining Mechanisms: A Role for Microbial Functionality? The potential underlying mechanism of prebiotics may involve the modulation of microbial metabolite production (SCFA, secondary bile acids) and reduction of bacterial constituents (LPS). SCFA and bile acids are tightly involved in energy homeostasis, insulin signaling, fat accumulation and inflammatory signaling, as review elsewhere [4,141–144]. Besides, these microbial metabolites might also indirectly affect GI transit as described in Section 2.7. Animal and in vitro studies reported that many bacterial metabolites i.e., SCFAs, hydrogen, LPS and secondary bile acids interact with enteric nerves and smooth muscles function possibly stimulating GI transit time [73]. The few existing human studies indicate that inulin might potentially improve colonic transit and potential delay GE rate. Inulin intake promotes bowel movements and softer stool consistency indicating a faster colonic transit, however not in the same magnitude as insoluble dietary fiber [145]. In a crossover studies with healthy men, daily inulin-enriched (11% inulin) pasta intake over 5 weeks decreased GE rate and decreased fasting glucose concentrations [146].

Yet, there is no data on other prebiotics available, making it difficult to draw valid conclusions. To sum up, well-controlled human intervention studies are warranted to substantiate the effect of prebiotic fibers on improvement of postprandial glycemia and the potential involvement of GI transit.

Conclusions

To summarize and conclude, there are site-specific effects of GI transit on postprandial glucose homeostasis and metabolic health (Figure 1). GE and small intestinal transit are mainly involved in central appetite signaling, initial glucose appearance in the circulation and gut peptide secretion. The underlying mechanisms of insoluble fibers in improving glucose homeostasis and reducing T2DM risk may (partly) be related to effects on GI transit. Modulation of GI transit, which in turn may also affect the microbiota composition, might be an underlying mechanism and should be considered in future human intervention studies. Viscous fibers can delay postprandial glycemia mediated by changes in gastric emptying, yet the role of gut peptide secretion in this process is not fully understood. Further studies are warranted to understand the underlying mechanisms linking gut motility, dietary fiber and glucose homeostasis.

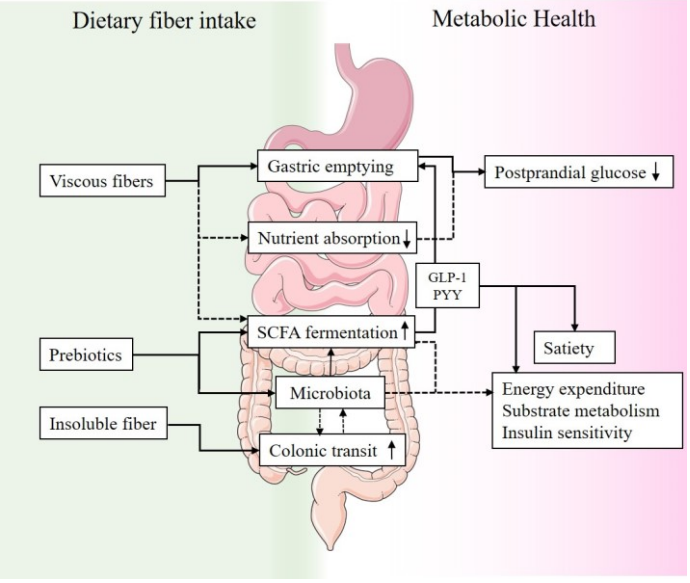

Figure 1. The complex relationship between dietary fiber intake, the gastrointestinal tract and host metabolism. Viscous fibers increase gastric emptying, may inhibit nutrient absorption and contribute to SCFA fermentation that may lead to reduced postprandial glucose appearance and an increased release of incretin and satiety-stimulating hormones (GLP-1, PYY), which might influence energy intake and peripheral tissue metabolism. Prebiotics modulate microbiota composition and SCFA production thereby affecting energy homeostasis and insulin sensitivity. Insoluble fibers are most effective in increasing colonic transit time thereby possibly affecting microbiota composition, and vice versa microbial metabolites may stimulate colonic motility. Solid lines indicate well studied effects of dietary fiber, dashed line indicate more controversial findings. Abbreviations: SCFA short-chain fatty acids; GLP-1 glucagon-like peptide 1; PYY peptide YY.

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CHAPTER 3

Effect of wheat bran derived prebiotic supplementation on gastrointestinal transit, gut microbiota and metabolic health: a randomized controlled trial in healthy adults

Running title: Prebiotics, gut transit and metabolism

Mattea Müller, Gerben D.A. Hermes, Emanuel E. Canfora, Jens J. Holst, Erwin G. Zoetendal, Hauke Smidt, Freddy Troost, Frank G. Schaap, Steven Olde Damink, Johan W.E. Jocken, Kaatje Lenaerts, Ad A.M. Masclee, and Ellen E. Blaak

In Review

CHAPTER 4

Long distal colonic transit is linked to gut microbiota diversity and microbial fermentation in humans

Running title: Colonic transit and gut microbiota

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In Review

CHAPTER 5

Circulating but not fecal short-chain fatty acids are related to insulin sensitivity, lipolysis and GLP-1 concentrations in humans

Running title: Circulating and fecal SCFA in metabolic health

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Abstract

Microbial-derived short-chain fatty acids (SCFA) acetate, propionate and butyrate may provide a link between gut microbiota and whole-body insulin sensitivity (IS). In this cross-sectional study (160 participants, 64% male, BMI: 19.2-41.0 kg/$m^{2,}$ normal or impaired glucose metabolism), associations between SCFA (fecal and fasting circulating) and circulating metabolites, substrate oxidation and IS were investigated. In a subgroup (n=93), IS was determined using a hyperinsulinemic-euglycemic clamp. Data were analyzed using multiple linear regression analysis adjusted for sex, age and BMI. Fasting circulating acetate, propionate and butyrate concentrations were positively associated with fasting GLP-1 levels. Additionally, circulating SCFA were negatively related to whole-body lipolysis (glycerol), triglycerides and free fatty acids levels (standardized (std) β adjusted (adj) -0.190, P = 0.023; std β adj -0.202, P = 0.010; std β adj -0.306, P = 0.001, respectively). Circulating acetate and propionate were, respectively, negatively and positively correlated with IS (M-value: std β adj -0.294, P < 0.001; std β adj 0.161, P = 0.033, respectively). We show that circulating rather than fecal SCFA associated with GLP-1 concentrations, whole-body lipolysis and peripheral IS in humans. Therefore, circulating SCFA are more directly linked to metabolic heath, as compared to fecal SCFA, which indicates the need to measure circulating SCFA in human prebiotic/probiotic intervention studies as a biomarker/mediator of effects on host metabolism.

Introduction

In obesity and type 2 diabetes mellitus (T2DM), alterations in the gut microbiota composition and functionality may contribute to disease etiology. The gut microbiota ferments indigestible carbohydrates (e.g. dietary fibers) and major end-products thereof are the short- chain fatty acids (SCFA) acetate, propionate and butyrate [1]. Acetate, propionate and butyrate are present in the colon in a ratio of approximately 3:1:1, respectively [2, 3]. Most butyrate is utilized by colonocytes as energy source [4]. Via the portal vein, SCFA reach the liver where acetate and propionate are metabolized and partly oxidized or used as substrate in gluconeogenesis and lipogenesis [5]. Consequently, a small proportion of microbial-derived SCFA enters the peripheral circulation whereby acetate reaches the highest concentrations compared to propionate and butyrate [6, 7]. SCFA are ligands to G-protein coupled receptors (GPR) 41 and 43 expressed on intestinal, adipose, skeletal muscle, liver and pancreatic tissues [8-10], indicating their important role in the crosstalk between the gut and peripheral tissues. Several rodent studies showed that oral, intravenous and colonic infusion of SCFA as well as microbial-derived SCFA beneficially affect the functioning and metabolism of the aforementioned tissues and consequently improve insulin sensitivity, substrate metabolism and body weight regulation [11]. In humans, distal colonic acetate infusions increased fasting fat oxidation, energy expenditure, and PYY secretion whilst whole body lipolysis was decreased [12, 13]. Other acute studies show decreased circulating free fatty acid (FFA) concentration after rectal SCFA infusion in healthy participants [14, 15]. In addition, 24 weeks of 10 g/day inulin propionate ester protected against body weight gain as compared to inulin only in overweight individuals [16]. Potential mechanisms may include a SCFA-induced inhibition of energy intake possibly mediated via the stimulation of glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) secretion, increased intestinal gluconeogenesis, increased skeletal muscle fat oxidation and improved lipid buffering capacity of adipose tissue [11].

However, increased microbially-derived acetate formation has been associated with increased body weight gain and insulin resistance in diet-induced obese rats [17]. Additionally, increased fecal SCFA have been reported in overweight and obese compared to lean participants [2, 3, 8, 18, 19], yet it is difficult to interpret the latter data since fecal SCFA reflect the net result of colonic production and absorption [20, 21]. Even though fecal SCFA are commonly used as an indicator of microbial fermentation, fecal SCFA may not accurately reflect *in vivo* colonic fermentation since approximately 95% of colonic SCFA are absorbed and only the remaining 5% are excreted in feces [22-25].

|Circulating and fecal SCFA in metabolic health

To obtain more information on the validity of fecal SCFA as biomarker for metabolic health effects, the associations between fecal and circulating SCFA concentrations and parameters of metabolic health were studied in a relatively large cohort of 160 participants with a wide range of body mass indices (BMI) and glucometabolic status. Using multiple regression analysis models, we analyzed the relationship between fecal and fasting circulating SCFA with fasting glucose, insulin, circulating lipids (free fatty acids (FFA), triacylglycerols (TAG), glycerol), insulin resistance index (homeostasis model assessment of insulin resistance (HOMA-IR)), gut hormone concentrations (PYY, GLP-1), fasting substrate utilization and inflammation markers including lipopolysaccharide-binding protein (LBP), tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and interleukin 8 (IL-8). We further investigated the relationship between fecal and fasting circulating SCFA profiles and peripheral insulin sensitivity index (M-value) as measured via the gold standard hyperinsulinemia-euglycemic clamp technique.

Results

In the total group, the mean age was 50 ± 15 years and 66.2 % of participants were male with a mean BMI of 29.8 ± 4.4 kg/m^2, a mean fasting glucose of 5.6 ± 0.6 mmol/L and a mean HOMA-IR of 3.7 ± 1.5 (Table 1). In the subgroup, peripheral insulin sensitivity (M-value) was measured in 93 overweight to obese, prediabetic men (n=72) and women (n=21) with mean age of 59.0 ± 7.1 years and a mean BMI of 31.8 ± 3.1 kg/m^2, respectively.

Table 1. Characteristics of participants

Characteristic	**Mean ± SD**	**Range**
Male/Female, *n*	106/54	
Age, *y*	50 ± 15	20 – 69
Body Weight, *kg*	90.8 ± 15.7	52.8 – 125.9
BMI, *kg/m^2*	29.8 ± 4.4	19.2 – 41.0
Waist: Hip ratio	0.93 ± 0.13	0.64 – 1.22
HOMA-IR	3.7 ± 1.5	1.0 – 9.0
Fasting plasma insulin, *mU/L*	14.9 ± 9.4	2.4 – 82.7
Fasting plasma glucose, *mmol/L*	5.6 ± 0.6	3.1 – 7.5
Fasting plasma free fatty acids, *µmol/L*	672 ± 243	140.3 – 1620
Fasting plasma triacylglycerol, *µmol/L*	1178 ± 613	311.0 – 3944
Fasting plasma free glycerol, *µmol/L*	102.1 ± 46	27.1 – 372.1
Fasting plasma acetate, *µmol/L*	65.2 ± 64.7	2.8 – 429.4
Fasting plasma propionate, *µmol/L*	2.0 ± 1.6	0.06 – 12.0
Fasting plasma butyrate, *µmol/L*	1.7 ± 1.8	0.07 – 6.7
Fecal acetate, *µmol/g*	44.2 ± 16.1	6.80 – 102.8
Fecal propionate, *µmol/g*	13.0 ± 7.9	2.53 – 71.2
Fecal butyrate, *µmol/g*	10.9 ± 5.9	0.00 – 33.8
Subgroup hyperinsulinaemic-euglycaemic clamp		
Male/Female, *n*	72/21	
BMI, *kg/m^2*	31.8 ± 3.1	26.1- 41
Age, *y*	59.0 ± 7.1	72 – 69
Fasting glucose, *mmol/L*	6.0 ± 0.5	5.0 – 7.5
2h glucose, *mmol/L*	7.2 ± 1.8	3.3 – 11.2
M-value, *mg*kg^{-1}*min^{-1}*	3.6 ± 1.5	1.7 – 8.3

BMI, body mass index; HOMA-IR, Homeostasis model assessment of insulin resistance; M-value, mean glucose infusion rate at steady state during insulinemic- euglycemic clamps

Associations between fecal and circulating SCFA concentrations

Fecal acetate and butyrate were not associated to their respective circulating concentrations, while fecal propionate was positively associated with circulating propionate (standardized (std) std β = 0.262, P = 0.002).

Circulating, but not fecal, SCFA are associated with BMI

Fecal acetate was positively associated with BMI (std β = 0.245, P = 0.004), however, after adjustment for age and sex, the association was not significant anymore (std β adj = 0.092, P = 0.214). Fecal propionate and butyrate were not significantly associated with BMI with or without adjustment for age and sex (fecal butyrate std β = 0.162, P = 0.055, fecal propionate std β = 0.023, P = 0.746). In contrast, circulating butyrate and propionate were significantly inversely related to BMI, also after adjustment for age and sex (circulating butyrate std β = -0.599, P < 0.001, circulating propionate std β = -0.290, P < 0.001, Supplementary Figure 1 K, L). Circulating acetate was negatively associated with BMI (std β = -0.285, P < 0.001), yet not after adjustment for age and sex (std β = -0.115, P = 0.108).

Fecal SCFA were not related to metabolic parameters

None of fecal SCFA were significantly associated with fasting GLP-1, PYY, FFA, TAG, glycerol, glucose, insulin concentrations, HOMA-IR, inflammatory markers or fasting substrate oxidation either with or without adjustment for age, sex and BMI. In the subgroup analysis fecal SCFA were not associated with peripheral insulin sensitivity (Table 2).

Table 2. Simple and multiple linear regression coefficients between fecal SCFA and metabolic parameters in fasting state

	Fecal acetate				**Fecal propionate**				**Fecal butyrate**			
	std ß	P	std β adj	P	std ß	P	std β adj	P	std ß	P	std β adj	P
Metabolites												
FFA, *µmol/L*	0.052 ±0.090	.569	-0.048 ±0.082	.558	-0.098 ±0.089	.276	-0.092 ±0.080	.249	0.001 ±0.090	.991	-0.030 ±0.081	.707
TAG, *µmol/L*	0.058 ±0.090	.519	-0.057 ±0.084	.498	0.129 ±0.089	.151	0.037 ±0.081	.647	0.094 ±0.090	.296	-0.007 ±0.082	.931
Glycerol, *µmol/L*	-0.047 ±0.090	.603	-0.152 ±0.086	.079	-0.019 ±0.090	.836	-0.022 ±0.084	.797	-0.185 ±0.145	.205	-0.160 ±0.084	.059
Hormones												
Insulin, *mU/L*	0.275 ±0.082	.001	0.122 ±0.071	.088	0.185 ±0.084	.029	0.114 ±0.069	.101	0.207 ±0.084	.015	0.102 ±0.070	.146
GLP-1, *pmol/L*	-0.081 ±0.092	.379	0.114 ±0.074	.125	0.017 ±0.092	.852	0.140 ±0.071	.053	-0.037 ±0.092	.692	0.116 ±0.072	.113
PYY, *pmol/L*	-0.194 ±0.106	.070	-0.166 ±0.112	.140	-0.093 ±0.107	.386	-0.087 ±0.110	.428	-0.156 ±0.106	.146	-0.142 ±0.110	.199
Insulin sensitivity												
M-value, *mg/kg/min*	-0.071 ±0.106	.502	-0.035 ±0.075	.640	-0.029 ±0.105	.783	-0.050 ±0.073	.490	-0.100 ±0.105	.345	-0.111 ±0.073	.130
HOMA-IR	0.267 ±0.084	.002	0.080 ±0.068	.244	0.193 ±0.085	.025	0.091 ±0.066	.169	0.214 ±0.085	.013	0.076 ±0.067	.254
Glucose, *mmol/L*	0.174 ±0.084	.040	-0.029 ±0.067	.661	0.112 ±0.084	.187	0.006 ±0.065	.925	0.086 ±0.085	.310	-0.066 ±0.066	.315
Inflammatory markers												
LBP, *pg/ml*	0.152 ±0.091	.095	0.033 ±0.086	.699	0.024 ±0.091	.789	-0.010 ±0.084	.909	0.155 ±0.090	.088	0.092 ±0.084	.274
IL-6, *pg/ml*	0.164 ±0.085	.055	-0.010 ±0.071	.892	0.012 ±0.085	.887	-0.088 ±0.069	.204	0.039 ±0.085	.651	-0.094 ±0.069	.178
IL-8, *pg/ml*	0.157 ±0.085	.067	0.106 ±0.088	.231	0.164 ±0.084	.053	0.118 ±0.085	.170	0.115 ±0.085	.179	0.061 ±0.086	.479
TNF-α, *pg/ml*	0.196 ±0.084	.021	0.070 ±0.078	.170	0.063 ±0.085	.459	-0.046 ±0.076	.548	0.156 ±0.084	.067	0.039 ±0.076	.612
Substrate Oxidation												
Fat, *E%*	0.036 ±0.092	.691	0.001 ±0.095	.996	-0.002 ±0.091	.982	0.004 ±0.093	.970	0.013 ±0.092	.885	0.004 ±0.094	.969
CHO, *E%*	-0.092 ±0.094	.329	-0.084 ±0.098	.397	-0.034 ±0.094	.716	-0.042 ±0.096	.666	-0.110 ±0.094	.243	-0.116 ±0.096	.233

β, standardized β coefficient + standard error of coefficient of fecal acetate, propionate and butyrate as dependent variable in a simple regression and multiple regression analysis adjusted for age, sex and BMI. M-value (n=93), PYY (n=107). Adj, adjusted; std, standardized; E%, percentage of energy expenditure; FFA, free fatty acids; TAG, triglycerides; GLP-1, glucagon like peptide 1; PYY, peptide YY; HOMA-IR, homeostatic model assessment of insulin resistance; LBP, lipopolysaccharide binding protein; IL-6, interleukin 6; IL-8, interleukin 8; TNF**α**, tumor necrosis factor alpha

Table 3. Simple multiple linear regression coefficients between circulating SCFA and metabolic parameters in fasting state

	Circulating acetate				Circulating propionate				Circulating butyrate			
	std ß	P	Std β adj	P	std ß	P	std β adj	P	std ß	P	std β adj	P
Metabolites												
FFA, *µmol/L*	-0.032 ±0.084	.701	0.003 ±0.080	.974	-0.302 ±0.079	.000	-0.127 ±0.078	.103	-0.381 ±0.077	.001	-0.306 ±0.090	.001
TAG, *µmol/L*	-0.249 ±0.081	.003	-0.077 ±0.081	.340	-0.225 ±0.081	.006	-0.202 ±0.078	.010	-0.356 ±0.078	.001	-0.137 ±0.094	.150
Glycero, *µmol/L*	-0.204 ±0.082	.014	-0.190 ±0.083	.023	-0.025 ±0.083	.768	0.155 ±0.081	.059	-0.286 ±0.080	.001	-0.173 ±0.098	.079
Hormones												
Insulin, *mU/L*	-0.187 ±0.079	.019	0.003 ±0.070	.968	-0.234 ±0.078	.003	-0.066 ±0.068	.336	-0.406 ±0.073	.001	-0.040 ±0.082	.625
GLP-1, *pmol/L*	0.402 ±0.078	.001	0.187 ±0.070	.009	0.318 ±0.080	.001	0.218 ±0.068	.002	0.574 ±0.069	.001	0.274 ±0.081	.001
PYY, *pmol/L*	0.125 ±0.097	.201	0.107 ±0.107	.317	0.080 ±0.097	.413	0.018 ±0.105	.868	0.182 ±0.096	.060	0.142 ±0.126	.263
Insulin sensitivity												
M-value, *mg/kg/min*	-0.224 ±0.102	.031	-0.294 ±0.071	.001	0.327 ±0.099	.001	0.161 ±0.074	.033	0.164 ±0.103	.113	-0.066 ±0.091	.469
HOMA-IR	-0.247 ±0.079	.002	-0.009 ±0.067	.892	-0.160 ±0.080	.047	0.007 ±0.065	.914	-0.461 ±0.072	.001	-0.054 ±0.078	.494
Glucose, *mmol/L*	-0.228 ±0.078	.004	0.005 ±0.066	.939	-0.177 ±0.078	.025	-0.044 ±0.064	.499	-0.506 ±0.068	.001	-0.200 ±0.075	.001
Inflammatory markers												
LBP, *pg/ml*	-0.075 ±0.088	.399	0.038 ±0.087	.667	-0.262 ±0.085	.003	-0.117 ±0.085	.168	-0.330 ±0.083	.001	-0.116 ±0.102	.254
IL-6, *pg/ml*	-0.298 ±0.077	.001	-0.098 ±0.070	.163	-0.177 ±0.079	.027	-0.027 ±0.053	.690	-0.406 ±0.073	.000	-0.021 ±0.082	.803
IL-8, *pg/ml*	-0.079 ±0.080	.324	0.024 ±0.086	.778	0.126 ±0.079	.113	0.164 ±0.084	.051	-0.023 ±0.080	.775	0.185 ±0.100	.067
TNF-α, *pg/ml*	-0.296 ±0.076	.001	-0.105 ±0.076	.168	-0.095 ±0.079	.232	-0.048 ±0.075	.523	-0.330 ±0.075	.001	-0.047 ±0.089	.598
Substrate Oxidation												
Fat, *E%*	-0.156 ±0.084	.065	-0.021 ±0.086	.812	-0.117 ±0.084	.167	-0.052 ±0.085	.543	-0.317 ±0.080	.001	-0.138 ±0.101	.172
CHO,*E%*	0.074 ±0.087	.396	0.159 ±0.094	.094	-0.092 ±0.087	.291	-0.167 ±0.092	.072	0.002 ±0.087	.986	0.047 ±0.111	.672

β, standardized β coefficient + standard error of coefficient of fasting circulating acetate, propionate and butyrate as dependent variable in a simple regression and multiple regression analysis adjusted for age, sex and BMI. M-value (n=93), PYY (n=107). Adj, adjusted, std standardized; E%, percentage of energy expenditure; FFA, free fatty acids; TAG, triglycerides; GLP-1, glucagon like peptide 1; PYY, peptide YY; HOMA-IR, homeostatic model assessment of insulin resistance; LBP, lipopolysaccharide binding protein; IL-6, interleukin 6; IL-8, interleukin 8; TNF**α**, tumor necrosis factor alpha

Fasting, circulating SCFA were related to fasting GLP-1, lipid metabolites and insulin sensitivity

All three circulating SCFA were positively associated with fasting GLP-1 concentrations. Additionally, circulating acetate, propionate and butyrate negatively associated with fasting glycerol, TAG and FFA, respectively. Also, circulating butyrate was negatively associated with fasting glucose. These relationships remained after adjustment for age, sex and BMI (Table 3, Supplementary Figure 1). Circulating SCFA were not associated with fasting PYY, LBP, IL-6, IL-8 and TNF-α. Circulating SCFA were not related to fat and carbohydrate oxidation, expressed as percentage of energy expenditure. In the subgroup analysis of overweight/obese, prediabetic individuals, circulating acetate was negatively associated with peripheral insulin sensitivity (M-value). Contrary, circulating propionate was positively related to peripheral insulin sensitivity (Table 3, Supplementary Figure 1). These relationships remained significant after adjustment for age, sex and BMI.

Discussion

We investigated the relationship between fecal and fasting circulating SCFA with fasting plasma metabolites, gut hormones, substrate metabolism and inflammatory markers in a cohort with a wide range of BMI and glucometabolic status. This study shows that only circulating but not fecal SCFA concentrations were related to fasting plasma glucose, FFA, TAG and glycerol, GLP-1 and insulin sensitivity, also after adjustment for age, sex and BMI. Contrary to previous human studies, fecal SCFA were not related to BMI, whereas circulating butyrate and propionate were inversely associated with BMI. Circulating plasma propionate seems to be the most reflective of its respective fecal concentrations, whilst fecal acetate and butyrate were not related to their respective circulating concentrations. In line, previous literature reports that SCFA flux into the circulation and uptake in peripheral tissues rather than microbial SCFA production *per se* is of importance for metabolic health [26-28]. Our data emphasize the need to measure circulating SCFA in human prebiotic/probiotic intervention studies as a biomarker/mediator of effects on host metabolism.

To our knowledge, this is the first study providing evidence that fasting circulating SCFA are positively associated with fasting plasma GLP-1 in humans. High colonic SCFA production is linked to increased GLP-1 and PYY secretion through binding of SCFA to GPR41/43 on the enteroendocrine L-cell [29]. Further, a one-year dietary fiber intervention (wheat bran, 24g/d) increased circulating SCFA concentrations accompanied by increased levels of GLP-1 concentrations in hyperinsulinemic participants [30]. Yet, there is little known about the contribution of circulating SCFA to GLP-1 secretion during the fasted state. Circulating SCFA may stimulate GLP-1 secretion from the visceral, basolateral side of L-cells as observed in isolated rat colons [31]. Besides L-cells, pancreatic α-cell have been suggested to contribute to GLP-1 concentrations in the fasted state [32, 33], but whether SCFA can act as stimuli warrants further investigation. Although the mechanisms remain to be elucidated, the present data indicate that circulating SCFA are a more accurate proxy for *in vivo* gut-derived SCFA production as compared to fecal SCFA. We did not find an association between circulating and fecal SCFA with fasting PYY. This contrasts with human and *in vitro* studies reporting a stimulatory effect of SCFA on PYY secretion [12, 34, 35], however to what extent SCFA and/or dietary fibers contribute to fasting PYY secretion remains to be investigated. Although the mechanisms still remain to be elucidated, the present data indicate that despite being the net result of production,

uptake and tissue utilization, circulating SCFA are more directly linked to metabolic health as compared to fecal SCFA.

In our study population, only circulating, but not fecal SCFA were associated with fasting plasma metabolites. Circulating acetate was negatively associated with fasting free glycerol, an indicator of whole-body lipolysis. This is consistent with *in vitro* and human *in vivo* studies reporting that acetate has an anti-lipolytic effect [13, 36-38]. This may be beneficial for metabolic health in the long term, since partial inhibition of adipose tissue lipolysis may reduce systemic lipid spill-over thereby attenuating ectopic lipid accumulation [39]. Further, circulating propionate was negatively associated with fasting TAG, which might be explained by the activating effect of propionate on lipoprotein lipase (LPL) in adipose tissue leading to increased TAG extraction as shown *in vitro* [40]. Circulating butyrate was negatively associated with fasting FFA concentrations. *In vitro* data about the lipolytic effect of butyrate are contradictive showing pro- and antilipolytic effects of butyrate in white adipose tissue models [38, 41]. Thus, circulating SCFA may be negatively related to glycerol or FFA and/or TAG suggesting that increased circulating SCFA may reduce systemic lipid overflow with a potential beneficial effect on ectopic lipid accumulation and insulin sensitivity.

Nevertheless, with respect to markers of insulin sensitivity, neither fasting circulating nor fecal SCFA were related to fasting insulin or HOMA-IR in the total study population. Yet, fasting circulating butyrate, but not acetate and propionate, was negatively associated with fasting glucose. This is consistent with rodent studies showing that butyrate administration has glucose lowering effects and improves insulin sensitivity in the postprandial state [42, 43]. In obesity, insulin resistance and T2DM, the abundance of butyrate-producing bacteria is reduced, which may explain to some extent the inverse association between circulating butyrate and fasting glucose in our study [44-46].

In the subgroup analysis including prediabetic individuals with obesity, circulating acetate was negatively associated with peripheral insulin sensitivity. This is in contrast with previous rodent studies reporting a beneficial role of acetate on insulin sensitivity [34] and with two small-scale human cross-sectional studies including obese women or morbidly obese individuals reported either none or a positive association of circulating acetate and insulin sensitivity measured via hyperinsulinemic-euglycemic clamp, respectively [47, 48]. Additionally, when acetate is administered colonically, overweight participants showed increases in fasting fat oxidation, energy expenditure, and PYY secretion [12, 13], reflective of positive effects on metabolic health. Interestingly, a kinetic study showed that

intravenously infused acetate remains longer in the circulation in individuals with T2DM suggesting a disturbed acetate tissue uptake and metabolism in the context of metabolic disorders [34]. Further, exogenous and endogenous acetate production but not colonic acetate absorption differed between hyperinsulinemic and normoinsulinemic individuals after rectal infusion of sodium-acetate [37, 49, 50]. Thus, our findings may reflect an altered endogenous acetate metabolism rather than an altered microbially derived acetate production in metabolically compromised individuals. In contrast to fasting circulating acetate, fasting circulating propionate was positively associated with clamp-derived insulin sensitivity. Propionate has been reported to stimulate glucose uptake in 3T3-L1 adipocytes and C2C12 skeletal muscle cells *in vitro* and improve insulin sensitivity (HOMA-IR) in mice fed a high fat diet [51, 52]. Possible mechanisms include increase in peripheral glucose uptake via increased GPR41 stimulation, suppression of hepatic *de novo* lipogenesis and increase formation of beneficial odd chain fatty acids in the liver [53]. The main limitation of our study is the cross-sectional design, which limits causal suppositions. Further, we cannot account for endogenous SCFA production, splanchnic and liver extraction or tissue utilization in this study [54, 55]. Secondly, measures of GLP-1 and SCFA in the postprandial state would have been valuable. However, the study's major strength is the availability of fecal and fasting circulating SCFA in combination with metabolic markers in a relatively large cohort with a broad range of BMI and metabolic health status. This enabled us to investigate the relationship between fecal and fasting circulating SCFA concentrations with markers of lipid and energy metabolism as well as insulin sensitivity measured by the gold standard hyperinsulinemic-euglycemic clamp. For the first time, we confirmed that fasting circulating but not fecal SCFA were related to whole body-lipolysis, fasting GLP-1 and insulin sensitivity in the fasted state. Furthermore, our study calls for urgently needed mechanistic studies in humans concerning the relationship between SCFA, GLP-1 secretion and lipid metabolism.

In conclusion, our data show that circulating but not fecal SCFA are linked to circulating GLP-1 concentrations, whole-body lipolysis and peripheral insulin sensitivity in humans. Of note, this highlights that circulating SCFA are more directly linked to metabolic heath parameters. Therefore, our data indicate the need to measure circulating SCFA as a biomarker/mediator of effects on host metabolism in future human prebiotic/probiotic intervention studies. This may provide interesting leads for future research, which should aim to modulate the SCFA availability in the systemic circulation and its impact on peripheral tissue function.

Methods

Study participants

This cross-sectional analysis included 160 Caucasian men and women aged 20 – 70 years with a BMI between 19.2 and 41.0 kg/m^2 from the general population in the vicinity of Maastricht, The Netherlands during August 2013 and December 2016. Individuals had normoglycemia, impaired fasting glucose (IFG, $\geq$ 5.6 mmol/L) and/or impaired glucose tolerance (IGT, 2 hour plasma glucose of 7.8 – 11 mmol/L after 75g oral glucose challenge) according to the diagnostic criteria of the American Diabetes Association, 2010 [56]. Eligibility of the participants was assessed via a general health questionnaire, medical history and anthropometry during an initial screening visit. Exclusion criteria were as follows: use of antibiotics, prebiotics, or probiotics 3 months before the study, diagnosis of T2DM, gastrointestinal or cardiovascular diseases, abdominal surgery, participants with life expectancy shorter than 5 years and participants following a hypocaloric diet. Participants did not use β-blockers, lipid- or glucose-lowering drugs, anti-oxidants, or chronic corticosteroids. All protocols were reviewed and approved by the local Medical Ethical Committee (MUMC+) and conducted in accordance with the Declaration of Helsinki (revised version, October 2008, Seoul, South Korea). Written informed consent was obtained from all participants.

Study Design

This cross-sectional analysis included metabolic parameters as well as fecal and fasting circulating SCFA concentrations from the baseline investigation day of previously performed intervention studies [12, 13, 57-59]. In the present study, we collated and analyzed study data at baseline and thus prior to the respective interventions. In all studies, sample collection was performed after an overnight fast, and measurements were conducted according to the same standard operating procedures**.** Two days prior to the baseline investigation day, participants were asked to refrain from intense physical activity and alcohol consumption, and to collect a fecal sample. In the evening before the investigation day, the participants consumed a standardized low-fiber meal.

Used data sets

The data set included baseline data from the following intervention human in vivo studies. These include an intervention study in prediabetic, overweight-obese individuals on the effect of antibiotics on insulin sensitivity (Clinical trial No. NCT02241421) [58], an intervention study in prediabetic, overweight-obese individuals on the effect of dietary fiber (galacto-oligosaccharides) on insulin sensitivity (Clinical trial No. NCT02271776) [57] an intervention study in normoglycemic, normal to overweight individuals on the effect of dietary fibers on gastrointestinal transit (Clinical trial No. NCT02491125) [59] and lastly two acute studies investigating the effect of different mixtures of SCFA in normoglycemic, overweight to obese individuals on human substrate and energy metabolism (Clinical trial No. NCT01826162 and No. NCT01983046) [12, 13].

Baseline investigation day

After an overnight fast (>10 h), participants came to the laboratory by car or public transport. Anthropometry was measured including height, weight and waist to hip ratio. After inserting a cannula into the antecubital vein, blood samples were taken to measure plasma metabolites, hormones and inflammatory markers in the fasted state. After the blood sampling, participants were in a resting, half-supine position and fasting substrate oxidation was measured for 30 min using an open circuit ventilated hood system (Omnical, MUMC+, Maastricht, the Netherlands). Fat and carbohydrate oxidation were calculated according to the equations of Weir and Frayn [60, 61], assuming that protein oxidation accounted for 15% of total energy expenditure.

Hyperinsulinaemic-euglycaemic clamp

Peripheral insulin sensitivity was determined in a subgroup of participants via hyperinsulinaemic-euglycemic clamps as previously described [57, 58]. In short, a cannula was inserted into an antecubital vein for infusion of glucose and insulin. To measure blood glucose, a second cannula was inserted into a superficial dorsal hand vein, which was arterialized by placing the hand into a hotbox (~50 °C). A priming dose of insulin infusion (Actrapid, Novo Nordisk, Gentofte, Denmark) was administered during the first ten min (t0 – t10 min) and insulin infusion was thereafter continued at 40 mU/m^2/min for 2 h (t10 – t120 min). By variable infusion of a 20% glucose solution, plasma concentrations were

maintained at 5.0 mmol/L. Peripheral insulin sensitivity (M-value, mg*(kg*min)-1) was calculated from the mean glucose infusion rate during the steady-state of the clamp (last 30min, stable blood glucose concentration at 5.0 mmol/L) [62]. A high M-value represents high insulin sensitivity (i.e., more glucose needs to be infused to maintain euglycemia during insulin infusion).

Analysis of fecal and circulating SCFA

Fecal samples were collected at home and stored in the subjects' freezer at -20 °C maximum of two days before the baseline investigation day, transported on dry ice, and stored on arrival at the university at -80 °C. Faecal acetate, propionate, and butyrate were measured by gas chromatography-mass spectrometry (Dr. Stein and Colleague Medical Laboratory, Mönchengladbach, Germany) as previously described [63]. Plasma sample preparation for circulating SCFA analysis were performed as reported previously [64]. In short, deproteinization was performed by mixing 1-part plasma (v/v) with 2 parts methanol acidified with 1.5 mmol/l hydrochloric acid. Subsequently, samples were vortex-mixed vigorously and immediately centrifuged at 50000xg in a model Biofuge Stratos (Hereaus, Dijkstra Vereenigde, Lelystad, the Netherlands) for 15 min. at 4°C. 100 µl aliquots of the clear plasma supernatant were transferred into glass micro-insert vials and stored in the Combi-Pal until analysis. Samples were calibrated against external standards. The reversed phase separation was performed on a X-select ODS 2.5 µm column (150mm x 2.1mm I.D., Waters, Breda, the Netherlands), mounted in a Mistral Spark column oven (Separations, H.I. Ambacht, the Netherlands), set to 45°C. Samples were completely separated from other components into the individual SCFA in a 25 min. gradient cycle between an aqueous 1 mmol/l solution of sulfuric acid and ethanol. Post-column, the solvent pH was enhanced to about 9, by mixing with 150 mmol/l ammonia in ethanol to maximize negative ionization. Samples were processed using a Combi-PAL sample processor (Interscience, Breda, the Netherlands) with Peltier chilled sample storage compartments set to 10°C. The system was equipped with a 50 µl sample loop. Separated SCFA were detected using a model LTQ XL linear ion trap mass spectrometer (Thermo Fisher Scientific, Breda, the Netherlands), equipped with an ion-max electrospray probe. The MS was operated in MS-MS full scan negative mode.

Blood collection and biochemical analysis

Blood was collected in pre-chilled EDTA tubes (0.2 mol/L EDTA; Sigma, Dorset, UK) for SCFA, insulin, glucose, FFA, TAG, free glycerol, LBP, GLP-1, TNF-α, IL-6 and IL-8 analyses during fasting conditions. For GLP-1 and PYY analysis, 20 µl of dipeptidyl peptidase-IV inhibitor (Milipore Merck, Billerica, MA, USA) was added to EDTA and Aprotinin (Becton Dickinson, Eysins, Switzerland) tubes, respectively. Samples were centrifuged at 3500 g, 4 °C for 10 minutes; plasma was aliquoted and directly snap-frozen in liquid nitrogen and stored at -80 °C until analysis. Plasma glucose concentrations were determined using commercially available reagent kit (Glucose Hexokinase CP, Horiba ABX Pentra, Montpellier, France) involving a two-step enzymatic reaction with hexokinase followed by Glucose-6-phosphate-dehydrogenase resulting in D-gluconate-6-phosphate. The colorimetric reaction was measured using an automated spectrophotometer (ABX Pentra 400 autoanalyzer, Horiba ABX Pentra). Plasma FFA concentrations were measured using a commercially available kit (NEFA-HR(2) assay, Wako, Sopachem BV, Ochten, the Netherlands) with a two-step enzymatic reaction involving acylation of Coenzyme(Co) A followed by acyl-CoA oxidase resulting in the production of hydrogen peroxide as substrate that in the presence of peroxidase yields a blue purple pigment, measured with a colorimetric reaction measured using an automated spectrophotometer (ABX Pentra 400 autonalyzer, Horiba ABX Pentra). Plasma TAG were determined using a commercially available kit (Triglycerides CP, Horiba ABX Pentra) based on enzymatic reactions involving lipoprotein lipase, glycerolkinase and glycerol-3-phosphate oxidase resulting in the production of hydrogen peroxide as substrate of a colorimetric reaction measured using the automated spectrophotometer (ABX Pentra 400 autonalyzer, Horiba ABX Pentra). Plasma glycerol was measured after precipitation with an enzymatic assay (Enzytec TM Glycerol, Roche Biopharm, Basel, Switzerland) involving phosphorylation of glycerol to L-glycerol-3-phosphate by glycerokinase and the colorimetric reaction is measured using an automated spectrophotometer (Cobas Fara, Roche Diagnostics, Basel, Switzerland). Plasma insulin was determined with a commercially available radioimmunoassay (RIA) kit (HI-14K Human Insulin specific RIA, Millipore Merck) according to the manufacture's protocol. Plasma IL 6, IL-8 and TNF-α were determined with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Human Proinflammatory II 4-Plex Ultra-Sensitive kit, Meso Scale Diagnostics, MD, USA). Plasma samples were assayed for total GLP-1 immunoreactivity using an antiserum that reacts equally with intact GLP-1 and the primary (N-terminally truncated) metabolite as previously described [65]. PYY

concentrations were determined using a commercially available radio-immunoassay (RIA) kits (Human PYY (3-36) Specific RIA, Millipore Merck). Plasma LBP was measured as previously described [66]. In short, plates (Greiner Mocrolon 600 high binding; Sigma Aldrich, St. Louis, MO) were coated with polyclonal anti-human LBP antibodies. Diluted plasma samples (1:5000) and a standard dilution series with recombinant LBP were added to the plate. Detection occurred with a biotinylated polyclonal rabbit anti-human LBP IgG, followed by peroxidase-conjugated streptavidin and substrate. The detection limit for the LBP assay was 200 pg/ml.

Statistical analysis

Normality of data was assessed with the Gaussian distribution and Kolmogorov-Smirnov procedure, and ln or Z-score transformation was used if assumption of normality was not met. HOMA-IR was calculated as previously described [67]. In case of missing data, the participant was excluded from the analysis. Multicollinearity was checked using variance inflation factor index < 10. First, we used simple linear regression to investigate the associations between fecal and circulating concentrations of acetate, propionate and butyrate (as dependent variables) and metabolic parameters (as independent variables) i.e., insulin sensitivity (M-value), insulin resistance (HOMA-IR), circulating glucose, insulin, circulating lipids (TAG, FFA and glycerol), circulating inflammatory markers (IL-6, IL-8, TNF-α and LBP) and fasting substrate oxidation. Subsequently, we used multiple linear regression to test whether the associations between fecal and circulating SCFA and the aforementioned metabolic parameters were independent of the covariates sex, age and BMI. All data were analyzed using SPSS 22.0 (IBM, Armok, U.S.) with significance set at $P < 0.05$.

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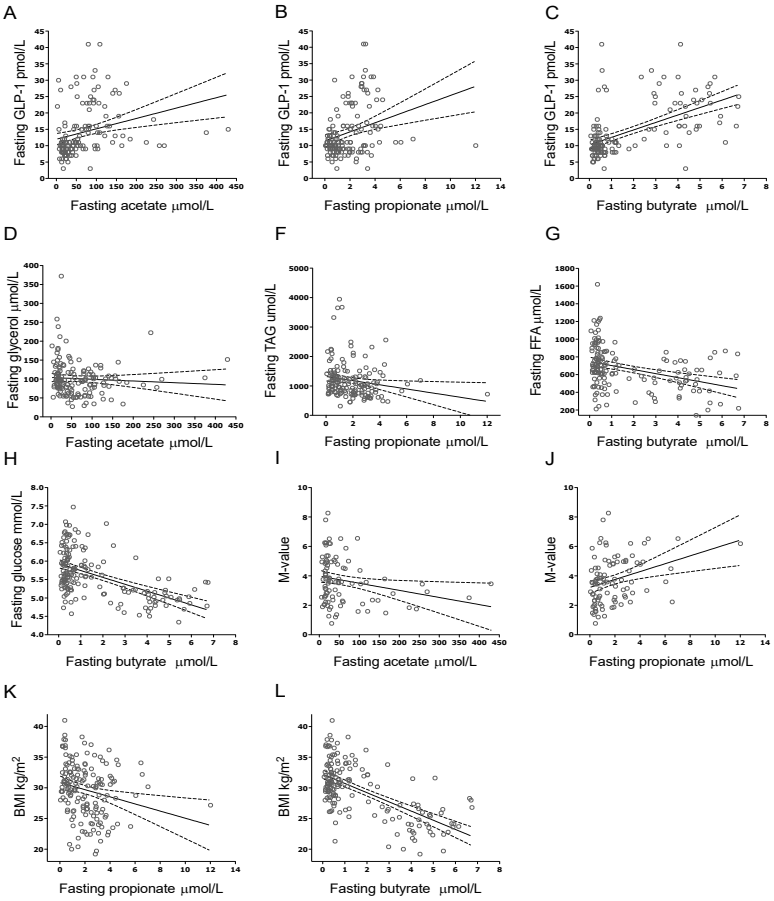
Supplementary



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Supplementary Figure 1 Associations between metabolic parameters, BMI and insulin sensitivity and fasting circulating SCFA. Linear regression scatter plots of fasting circulating SCFA with fasting GLP-1 (**A-C**), fasting acetate with fasting glycerol (**D**), fasting propionate with fasting TAG (**F**), fasting butyrate with FFA (**G**) and fasting glucose (**H**), fasting acetate with M-value (**I**), fasting propionate with M-value (**J**) and BMI (**K**) and fasting butyrate with BMI (**L**). GLP-1 glucagon like peptide 1, TAG triacylglycerol, FFA free fatty acids, BMI body mass index

CHAPTER 6

A novel fiber mixture promotes microbial fermentation in the distal colon, increases energy expenditure and postprandial insulin sensitivity in lean, but not prediabetic, overweight men

Running title: Fiber mixture in lean and prediabetic men

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In Preparation

CHAPTER 7

General Discussion

The obesity pandemic has become a worldwide public health issue contributing to increasing incidences of chronic metabolic diseases such as type 2 diabetes (T2DM) and cardiovascular disease [1]. A sedentary lifestyle and intake of energy-rich diet lead to a positive energy balance and excessive fat accumulation in adipose tissue and reduced adipose tissue functionality which may lead to insulin resistance and an impaired metabolic health. Together, the steep increase of obesity prevalence combined with the moderate long-term success of current prevention and treatment strategies demand novel approaches in tackling the obesity problem.

The gastrointestinal (GI) tract as the initial site of nutrient digestion and absorption plays an important role in energy and substrate metabolism and metabolic health. One important function of the GI tract is to facilitate the transit of foods, a process which strongly impacts nutrient absorption [2], nutrient-induced gut derived signals [3] and the ecological habitat of the gut microbiota [4]. Besides, the gut microbiota itself has been linked to metabolic diseases in human association studies [5-7]. Evidence mainly derived from rodent studies point towards a role for microbial-derived metabolites, mainly the fermentation-derived short-chain fatty acids (SCFA) acetate, propionate and butyrate as a link between microbiota and metabolic health [8, 9]. By specifically targeting compositional and metabolic changes within the gut microbiota, prebiotic dietary fiber supplementation has the potential to influence GI functioning, to selectively increase beneficial microbes and SCFA production thereby improving energy and substrate metabolism and metabolic health [10, 11]. The present thesis aimed to investigate the effect of dietary fibers on GI transit time, the gut microbiota composition and its metabolites in relation to human metabolic health and glucose homeostasis and insulin sensitivity.

Complex interactions between dietary fibers, GI transit and the gut microbiota in human metabolism

The modulation of GI functioning by dietary fibers might have a key role in their effects on metabolic health and glucose homeostasis. **Chapter 2** summarizes human studies investigating the interaction between different types of dietary fibers i.e., soluble, insoluble, fermentable or viscous fibers and site-specific GI transit times i.e., gastric emptying, small intestinal and colonic transit. Insoluble dietary fibers are known to accelerate GI transit due to increased fecal stool bulking and have been associated with reduced T2DM risk in epidemiological studies [12]. Further, there is consistent evidence

from human intervention studies showing that the intake of viscous fibers delays gastric emptying, which may potentially reduce postprandial glycemia. In the last decades, soluble, prebiotic fibers have gained increasing attention due to their ability to specifically stimulate growth of specific microbes and stimulation of colonic SCFA production [10]. Yet, until now, only a few, small scale human studies investigated the effect of prebiotics, and mainly inulin, on GI transit in relation to metabolic responses with inconsistent findings [13-16]. Lastly, the most consistent evidence for alterations of GI transit times in overweight/obese insulin resistant or prediabetic individuals points towards a faster gastric emptying in the obese phenotype, while evidence for alterations in small intestinal and colonic transit is scarce or inconsistent. As pointed out in **Chapter 2**, there is an urgent need for well-controlled intervention studies focusing on the complex interaction between prebiotic fibers, gut functioning, gut microbiota, and the implications on metabolic health to further unravel the potential of prebiotics in the prevention of metabolic disorders.

Insights from a prebiotic intervention with Arabinoxylan-Oligosaccharides

Intervention effects on GI transit and gut microbiota

In healthy participants, preliminary short-term interventions with the prebiotic fiber Arabinoxylan-Oligosaccharide (AXOS) derived from wheat bran have demonstrated beneficial effects on GI functioning i.e., stool consistency and frequency [17-19], on colonic fermentation as well as on metabolic parameters i.e., decreased glycemia [20, 21]. Therefore, in **Chapter 3**, we investigated the effect of long-term AXOS intake on GI transit parameters, gut microbiota composition and on metabolic health parameters in normoglycemic, lean to overweight (20 -30 kg/m^2) adults with a slow GI transit. We demonstrated that 12-week intake of 15 g/day of AXOS greatly increased *Bifidobacterium spp.* abundance, and to a lesser extent *Akkermansia*, *Prevotellaceae* NK3831 group and *Lactobacillus* abundances. These findings are in line with *in vitro* data showing that AXOS is selectively used by *Bifidobacteria spp.,* and to a lesser extent by *Lactobacilllus spp*. in a simulator of the human intestinal microbial ecosystem [22]. We observed that AXOS intake led to softer stool consistency (i.e., Bristol stool score, BSS), but did not change gastric emptying rate, small intestinal, colonic transit times or stool frequency. The mechanisms by which AXOS may improve stool consistency could be an increased quantitative abundance of *Bifidobacteria* (i.e., by increasing fecal bacterial biomass), since

probiotic supplementation with *Bifidobacterium spp*. has been shown to accelerate GI transit in healthy and constipated humans [23-25] However, since we observed no changes in GI transit with AXOS , other mechanisms like an increased water binding capacity and/or stimulation of host mucus production may have been more important [26]. In general, there are limited numbers of randomized trials studying the effect of prebiotics on quantitatively measured GI transit time. Thus, systematic reviews and meta-analysis have mostly focused on surrogate markers such as stool frequency, wet weight and consistency. A pooled analysis of 29 studies showed an increased stool frequency (mean increase of 0.36 ± 0.06 defecations per day) and softer stool consistency (mean difference - 0.23, 95% CI 0.05, 0.40 Bristol stool score) after intake of mean 12 g/day short-chain fructans for 7 to 27 days in healthy participants [27]. With similar effect size, a systematic review of five randomized trials in patients with functional constipation reported a mean difference of 1.0 defecations per week (95% CI: 0.04-1.99), and a mean difference in stool consistency (Bristol Stool Score) of −0.59, (95% CI: −1.16 to −0.02) with fructans and galacto-oligosaccharides (GOS) supplementation (prebiotic dose 10-15 g/day, study duration 7-56 days) [28]. Therefore, the difference in stool consistency (difference - 0.43, 95% CI: - 0.93 to -0.02) after AXOS intervention observed in **Chapter 3** has a similar effect range as other prebiotic interventions.

With softer stool consistency, AXOS intake also decreased microbial alpha diversity, which should be interpreted in the light of recent population studies showing that increased microbiota alpha diversity associates with harder stool consistency [29-31]. These studies led to the expansion of current understanding of microbial diversity as a biomarker for a healthy microbiota and metabolic profile [32]. Essentially, microbial alpha diversity is suggested to reflect the ecological diversification along the colon due to substrate depletion and microbial competition [4]. Thus, AXOS supplementation may have affected alpha diversity partly by increasing *Bifidobacterium* (i.e., bacterial biomass) and/or by softening stool consistency. By using a machine learning approach, we discovered that the overall gut microbiota composition could differentiate responders with faster GI transit from non-responders with unchanged or slower GI transit after AXOS intervention. Responders were characterized by increased *Senegalimassilia* ASV (family *Coriobacteriaceae*, phylum Actinobacteria), and an unclassified genus from the family *Lachnospiraceae* (phylum *Firmicutes*), while the other predictive species were common members of the core gut microbiota. Most importantly, *Bifidobacterium spp.* was not amongst significant predictors substantiating that the prebiotically-induced increase of *Bifidobacteria* abundance does not affect GI transit time in healthy adults with slow GI

transit. Other gut functioning parameters i.e., site-specific gut permeability and plasma lipopolysaccharide-binding protein (LBP) were not changed upon AXOS intervention. Additionally, fecal and systemic inflammation markers as well as GI symptom and quality of life ratings were in a healthy range before the start of the study, thus making further improvement in these parameters unlikely.

Intervention effects on postprandial energy metabolism and metabolic health

In addition to gut functioning, we demonstrated that 12-week AXOS supplementation to the habitual diet did not induce major changes in fasting and postprandial glucose, insulin, fecal and plasma SCFA or circulating lipid, substrate oxidation and energy expenditure, and markers of adipose tissue function (adipocyte morphology, lipolytic gene expression) in **Chapter 3**. However, we observed that the early postprandial glucagon-like peptide (GLP) 1 response (AUC 0 - 90 min) was reduced after AXOS intervention, while fasting and postprandial peptide YY (PYY) and ratings of hunger and satiety were unchanged. Early postprandial GLP-1 stimulation is induced by the stimulation of the glucose-stimulated SGLT-1 receptor on L-cells [2], whereas SCFA-induced GLP-1 stimulation may play a role in the late postprandial phase or fasting state as speculated in **Chapter 6**. Furthermore, the action of AXOS on GLP-1 may differ depending on time of consumption. The intake of 18.4 g AXOS in bread the evening before has no effect on postprandial GLP-1 after a breakfast the following morning [33] similar to what was observed in **Chapter 3.** In contrast, acute consumption of 15 g AXOS in cereal increased postprandial GLP-1 concentration [34]. Further, AXOS may be already fermented by small intestinal bacteria, as plasma concentration of arabinose and xylan increase after ingestion of wheat-bran rich pasta in healthy participants compared to control wheat bread [35]. Further, arabinose itself has been shown to partly inhibit sucrase and maltase activity in *in vitro* and in rodent models [36-38] . Arabinose may have potentially attenuated the digestion of carbohydrates affecting glucose-dependent GLP-1 stimulation providing a possible explanation for the reduced early postprandial GLP1 response after AXOS intervention. Nonetheless, we did not observe any changes in early postprandial insulin or glucose concentrations. Thus, it remains to be elucidated to what extent AXOS is already fermented in the small intestine and whether arabinose may affect the activity of glucose-degrading brush border enzymes in the long-term.

GI transit is differentially linked to microbiota diversity and composition

In large population studies, variation of the gut microbiota composition seems to be clearly linked to stool consistency [29, 39]. To date, there is little knowledge on the interaction between quantitative measures of GI transit, microbiota composition and microbial metabolism. Therefore, in **Chapter 4,** we investigated the associations between the self-reported Bristol stool score (BSS), colonic and segmental colonic transit measured via radio-opaque marker method as well as markers of microbial fermentation in feces and plasma of healthy participants with a slow GI transit. We extend previous findings by showing that site-specific transit along the colon may be of importance in the diversification of the gut microbiota. More specific, transit through the left distal colon was correlated with increased microbiota alpha diversity, whereas total colonic transit and stool consistency were not related to alpha diversity. We speculate that a longer distal colonic transit time may have resulted in a prolonged period of low carbohydrate conditions in the descending colon, which, together with increased microbial competition, may have driven microbiota diversification specifically in the descending colon.

Furthermore, we also studied the relation between GI transit and the similarity of gut microbiota composition between participants in **Chapter 4**. Depending on the metric used for gut microbiota composition, we observed that some well-known covariates such as sex, age and stool consistency, and also dietary fat and protein intake, plasma LBP and fecal calprotectin, but surprisingly not colonic transit, were explanatory variables of variation of the gut microbiota composition. When using Bray Curtis dissimilarity as microbiota diversity metric, only stool consistency, but not demographics, diet or colonic transit, was an explanatory variable for the observed microbiota variation. This link between stool consistency as assessed by BSS and gut microbiota composition may be explained by processes like colonic water absorption and peristaltic mixing [29, 30]. Indeed, besides being a proxy for colonic transit, stool consistency correlates with fecal moisture [40] and potentially reflects colonic peristalsis movement. Increased water absorption along the colon concentrates the luminal content leading amongst other factors to increased fecal SCFA accumulation, lower pH and slowing down nutrient mobility along the colon, all of which are factors affecting growth conditions of the microbiota [41]. Of note, stool consistency was not related to colonic transit time, determined by radio-opaque markers in the study population of the AXOS study (**Chapter 3** and **Chapter 4).** Several human cross-sectional studies ($n < 70$) using radio-opaque markers have reported a negative correlation [42, 43] or no correlation [44]. Thus, BSS

may not be a good surrogate marker for quantitatively determined colonic transit time and may rather be a more accurate reflection of colonic water absorption and peristalsis potentially explaining the consistent associations between BSS and gut microbiota variation determined by Bray Curtis dissimilarity.

Further, total dietary intake of fat and protein was significantly related to gut microbiota composition. Dietary fat is rarely metabolized by the gut microbiota but may affect the gut microbiota via other mechanisms for example by increasing antimicrobial bile acid secretion [45, 46]. High dietary fat intake was strongly associated with decreased relative abundance of *Bifidobacterium spp.* in **Chapter 4** which is consistent with data of animal high-fat diet models [47-50] and humans [51-53]. Dietary proteins can be used as energy source by species capable to ferment proteins or peptides and in **Chapter 4**, protein intake was positively correlated to relative abundances of gut health related *Bifidobacterium* and *Blautia,* a genus known to ferment a variety of substrates including proteins. Notably, both dietary fat and protein may enter the colon only in small amounts, thus other factors such as quality and complexity of the proteins and fats could have potentially contributed to the relation between dietary fat and protein intake and microbiota composition [54]. Furthermore, age was positively correlated with the relative abundance of *Methanobrevibacter* consistent with previous human observations [55, 56]. *Methanobrevibacter* was inversely related with *Blautia* and *Bifidobacterium* which may partly be due to their competition for H_2 as energy substrate [53]. To conclude, we demonstrate that distal colonic transit time was related to microbial alpha-diversity, fecal SCFA and plasma acetate concentrations, while microbiota composition was related to age, sex and dietary intake and only to a minor extent to stool consistency.

Short-chain fatty acids, GI transit and metabolic health

Distal GI transit is a determinant of fecal SCFA and plasma acetate

Colonic SCFA production depends on dietary nutrient intake, the fermentative capacity of the microbiota as well as cross-feeding mechanisms amongst the microbes, while the fecal SCFA concentrations may also vary with colonic transit time [57-59]. In fact, in **Chapter 4**, the fecal SCFA profile was strongly associated with a longer sigmoid transit time suggesting that fecal SCFA may reflect SCFA production and/or absorption in the very distal colon thus not necessarily reflect production and/or absorption of other more

proximal colonic sites. Further, longer sigmoid transit may exacerbate low-nutrient conditions and attenuate microbial energy metabolism which is partly reflected by lower SCFA concentrations in feces. Additionally, longer distal colonic transit time was associated with lower fasting plasma acetate, but not with propionate and butyrate concentrations. In the distal colon, SCFA absorption is highest [60], hence the longer transit particular through the distal colon may influence acetate absorption into the circulation.

Importance of plasma SCFA over fecal SCFA in energy metabolism and metabolic health

Based on studies reporting higher fermentative capacity, higher energy extraction from dietary fibers and increased fecal SCFA concentration in obesity, it has been suggested in several studies that the obese state is characterized by increased SCFA production [61-65]. Yet, this is in contrast with rodent [66-75] and human short-term [76-80] and long-term [81, 82] interventions showing a beneficial effect of SCFA on energy metabolism, insulin sensitivity and prevention of weight gain. Indeed, there are some major limitations in using fecal SCFA as a biomarker for colonic production, since only approximately 5-10% of colonic SCFA are excreted, reflecting probably only the net result of the most distal colonic production and absorption as observed in **Chapter 4** and by others [83-86]. In **Chapter 5**, we compared fecal and fasting plasma SCFA in a large cohort of healthy, normoglycemic lean to obese, prediabetic participants (n=160) and their associations with a variety of fasting metabolites, hormones and substrate oxidation. Interestingly, only fasting plasma SCFA but not fecal SCFA concentrations were related with metabolic parameters and BMI. More specifically, fasting acetate, butyrate and propionate were positively associated with fasting GLP-1 but not with PYY concentrations in the total study population. Since rodent studies mostly report GLP-1 stimulation by SCFA from the luminal site, it is surprising that plasma but not fecal SCFA are associated with fasting GLP-1 concentrations. However, this underlines that plasma SCFA may be more reflective of *in vivo* colonic SCFA production than fecal SCFA. Besides luminal SCFA, circulating SCFA may stimulate GLP-1 secretion from the visceral, basolateral side of L-cells as observed in isolated rat colons [87]. In general, little is known about GLP-1 secretion in the fasted state. However, when considering that healthy colonic transit takes at minimum 12 h [88], colonic SCFA fermentation can still occur after overnight fasting and thus, may affect fasting GLP-1 secretion via stimulation of colonic L-cells, either via the luminal or basolateral site. In addition, fasting SCFA were negatively associated with markers of

whole-body lipolysis and lipid metabolism in the total study population in **Chapter 5**. Notably, acetate was negatively associated with fasting free glycerol, circulating butyrate was negatively associated with fasting free fatty acid concentrations, while propionate associated negatively with fasting triglycerides (TAG). These findings are consistent with *in vitro* and human *in vivo* studies reporting an anti-lipolytic effect of acetate [76, 77, 89-91] and improved lipid buffering capacity [92-94] upon SCFA supplementation. Propionate may activate lipoprotein lipase (LPL) in adipose tissue leading to increased TAG extraction as shown *in vitro* [95]. *In vitro* evidence for lipolytic effect of butyrate showing pro- as well as and antilipolytic effects in white adipose tissue models [91, 96]. Together, increased circulating SCFA may reduce systemic lipid overflow with a potential beneficial effect on ectopic lipid accumulation and insulin sensitivity.

Additionally, we investigated the associations between plasma SCFA and peripheral insulin sensitivity measured by the gold standard hyperinsulinemic-euglycemic clamp in an obese, prediabetic subgroup in **Chapter 5**. Fasting acetate was negatively, and propionate positively associated with peripheral insulin sensitivity in this overweight/obese, prediabetic subgroup. While propionate has been reported to stimulate adipose tissue and skeletal muscle glucose uptake *in vitro* and improve insulin sensitivity in mice fed a high fat diet [97, 98], the association of increased fasting acetate with a worsening of insulin sensitivity seems contrary to previous rodent studies [66-75] , human intervention studies [76-82] and small-scale human association studies [99, 100] on acetate and insulin sensitivity and metabolic health. Yet, evidence from human studies comparing acetate metabolism in metabolically disturbed to normoglycemic lean individuals shows that acetate production and uptake may be altered in the metabolically disturbed phenotype [82, 90, 101, 102]. Intravenously infused acetate remains longer in the circulation in individuals with T2DM [82], while exogenous and endogenous acetate production but not colonic acetate absorption differs between hyperinsulinemic and normoinsulinemic individuals after rectal infusion of sodium acetate [90, 101, 102]. Hence, based on the associations reported in **Chapter 5**, we hypothesize that with progression towards a diabetic state, acetate metabolism may be disturbed in terms of acetate uptake by peripheral tissues and/or altered exogenous and endogenous production.

To conclude from **Chapter 4**, fecal SCFA concentrations decrease with longer sigmoid colonic transit and are not related to proximal colonic transit, indicating that fecal

SCFA concentrations may be most reflective of colonic SCFA production in the very distal colon. Even though plasma SCFA concentrations are subject to colonic, splanchnic and hepatic extraction and are influenced by exogenous SCFA production [103], they may be more informative about colonic SCFA production than fecal SCFA concentrations based on the findings of **Chapter 5**. Thus, based on this data, plasma SCFA seem to be a more accurate proxy of colonic SCFA production and may need to enter systemic circulation to interact with peripheral organs in the regulation of energy and substrate metabolism.

Distal SCFA fermentation – site does matter

The observations from **Chapter 4** and **Chapter 5** indicate that colonic SCFA production may be site-specific, and that acetate metabolism may differ in the overweight/obese prediabetic phenotype compared to healthy, lean phenotype. In addition to these findings, the colonic site of SCFA production may be a potential target to improve effectiveness of dietary fiber interventions in terms of increasing circulating SCFA concentrations. SCFA produced in the distal colon may partly circumvent hepatic extraction via their absorption into the inferior mesenteric veins, and thus may reach the peripheral tissues in higher concentrations compared to proximal produced SCFA [104]. Further, the density of PYY and GLP-1 producing L-cells is higher in the distal compared to the proximal colon [105], thus distal SCFA may additionally stimulate PYY and GLP-1 secretion inducing beneficial effects on glucose homeostasis and food intake regulation.

Indeed, rectal infusion studies showed that distal, but not proximal, infusion of acetate elicits beneficial effects on whole body lipolysis, PYY concentrations and energy expenditure in healthy overweight volunteers [76, 77]. Acetate as the most abundant SCFA seems to be a key metabolite in the cross-talk between gut microbiota and host metabolism [106]. Thus, targeting distal acetate fermentation by indigestible carbohydrates may increase circulating acetate concentrations exacerbating its beneficial metabolic effects on peripheral tissues and thus improve metabolic health as observed in several rodent studies [107-116]. We hypothesized that with the ingestion of a mixture of a more 'rapidly fermentable' and a 'slowly fermentable' dietary fiber, the rapidly fermentable resistant starch would satiate the gut microbiota in the proximal colon so that slowly fermentable long-chain inulin would reach the distal colon in higher concentrations, thus leading to increased distal acetate production. Therefore, we combined *in vitro* and *in vivo* approaches in **Chapter 6**. Firstly, different fiber mixtures were evaluated with regards to their acetate and total SCFA production in an *in vitro* model of the human colon

(TIM-2 system) [117]. Subsequently, by means of an acute double-blind, placebo-controlled, randomized, crossover study, we investigated whether one day supplementation of the fiber mixture would affect plasma and fecal SCFA, glucose as well as energy homeostasis measured on the following morning in lean, normoglycemic men and overweight/obese prediabetic men.

The combination of a long-chain inulin (INU) combined with a rapidly fermentable resistant starch (RS) yielded high acetate, butyrate and total SCFA concentrations compared to INU alone in the distal colon mimicked by the TIM-2 model using microbiota of lean, but not of overweight/obese prediabetic, individuals. In the human study, acute *in vivo* one-day supplementation of INU+RS did not increase plasma acetate in lean men but increased fasting plasma butyrate, hydrogen breath excretion, energy expenditure and carbohydrate oxidation, and improved postprandial insulin sensitivity. In contrast to the lean group, one-day supplementation INU+RS did increase circulating acetate in overweight/obese, prediabetic men, yet without changes in hydrogen breath, glucose homeostasis, substrate oxidation and energy expenditure. The discrepancy between *in vivo* and *in vitro* findings might be due to differences of the experimental setup i.e., between fermentation of INU+RS fermentation on SCFA *in vitro* versus SCFA production the morning after INU+RS intake *in vivo*.

We hypothesize that in INU+RS in the distal colon could have initially been fermented to acetate, however due to microbial cross-feeding mechanism [103], was further converted to butyrate which may partly explain the increased fasting butyrate concentrations after INU+RS in lean men. Increased fasting butyrate may have further promoted beneficial metabolic effects since butyrate supplementations improved insulin sensitivity in high fat diet mice models as well as lean normoglycemic human individuals [109, 110, 118]. Other studies adding RS to prebiotic of dietary fiber supplementation report similar increased in plasma SCFA, breath hydrogen and improved glucose homeostasis the morning after supplementation intake in healthy participants [33, 119]. In contrast to the lean group, one-day supplementation of INU+RS increased fasting and postprandial acetate in overweight/obese, prediabetic men, yet without changes in hydrogen breath, glucose homeostasis, and substrate oxidation and energy expenditure. The reason why metabolic effects were only observed in the lean but not in the overweight/obese, prediabetic phenotype despite the increase of plasma acetate may be explained by differences in composition and/or activity of the microbiome and/or SCFA metabolism/handling in peripheral tissues. As pointed out in **Chapter 5,** the obese,

prediabetic state can be characterized by alterations in SCFA metabolism. These can occur at the level of the gut such as an altered microbiota composition and microbial SCFA production efficacy in the colon. Further, the microbiota of overweight/obese prediabetic individuals may harbour less butyrate producing species in the distal colon [120, 121], therefore less conversion from acetate to butyrate due to cross-feeding may have occurred in prediabetic individuals. Additionally, it may be possible that INU+RS fermentation occurred already earlier in the proximal colon during the night which may partly explain why breath hydrogen after INU+RS supplementation the morning after in the prediabetic individuals. Furthermore, SCFA metabolism may also be altered regarding their peripheral tissue uptake and utilization (e.g. in the skeletal muscle and adipose tissue). Together, several human studies and **Chapter 6** of this thesis showed that the metabolic response after oral SCFA administration, intravenous acetate or vinegar interventions is more effective in improving glucose homeostasis and insulin sensitivity in lean compared to metabolically compromised participants [82, 122-125].

Implications and future perspective

This thesis focused on the complex relationship between dietary fibers, GI transit and the gut microbiota composition in the context of metabolic health. The following gives an overview of the main outcomes of this doctoral thesis as well as its implications for future research:

1. We show that long-term wheat bran derived AXOS intake induces a bifidogenic effect and softens stool consistency in healthy adults with a prolonged GI transit time. However, 12-week AXOS intake does not improve prolonged GI transit time. Further, in this normoglycemic study population, long term AXOS intake had no effect on SCFA production, glycemic, insulinemic responses, lipid metabolism or energy metabolism, but decreased early GLP-1 response. In the future, AXOS interventions should closely monitor its breakdown products to arabinose and xylan to further shed light on the putative effects of AXOS on small intestinal glucose metabolism.

2. Given that the overall gut microbiota composition may classify responders with increased GI transit upon AXOS intake, a prediction independent of the increased *Bifidobacterium spp.* abundance, our data substantiate current findings that there is an interaction between GI transit and microbiota composition, while the directionality is still not well understood. Therefore, human studies aiming to study the underlying mechanism could investigate alterations in gut microbiota composition and activity after pharmacological (e.g., with loperamide) alteration of the colonic transit in healthy participants, and /or substantiate data on site specific GI transit alteration in metabolically compromised individuals. These studies would shed further light on the direction of the interaction between GI transit, microbiota diversity and composition in the context of metabolic health. Dietary fiber interventions targeting improvements GI transit or in functional constipation should in fact take microbiota based personalized approaches into account.

3. In the present thesis, distal colonic transit time strongly relates to gut microbiota diversity, fecal SCFA and plasma acetate, while microbiota composition is more related to host demographics and dietary intake. Our findings suggest that future studies looking at fecal SCFA concentrations should ideally take colonic transit time into account. Furthermore, stool consistency (commonly regarded as proxy for GI transit), but not colonic transit, was related to microbiota composition. Thus, indicate that stool consistency may reflect other microbiota-shaping parameters of the colon such as colonic water absorption and peristaltic mixtures. Based on these data, stool consistency as an easy non-invasive proxy of colonic ecology should be included as covariates in future microbe-host studies, yet the underlying processes connecting stool consistency, colonic transit and gut microbiota should be further investigated.

4. Plasma, but not fecal, SCFA associate with fasting GLP-1, whole body lipolysis and insulin sensitivity in a cross-sectional analysis of healthy as well as prediabetic individuals. We conclude that plasma SCFA seem to be a more accurate proxy of colonic SCFA production as compared to fecal SCFA, and/or plasma SCFA may need to enter systemic circulation to interact with

peripheral organs in the regulation of energy and substrate metabolism. In line with point 3, we conclude that future studies looking at prebiotic, probiotic or SCFA supplementations should prefer circulating SCFA over fecal SCFA measurements, or even more accurate, use isotope labelled tracer studies to disentangle exogenous and endogenous SCFA production.

5. In a novel approach to target the distal colonic SCFA production, we show that one-day supplementation of a mixture of resistant starch and long-chain inulin increased colonic bacterial fermentation and improved substrate metabolism and postprandial insulin sensitivity demonstrated in lean, but not in overweight/obese, prediabetic individuals. If these findings translate into longer term metabolic effects, the fiber mixture could be a nutritional tool to prevent body weight gain and insulin resistance. Further, these findings extend previous findings indicating that participants with disturbed glucose metabolism are not responsive to changes in acetate production upon acute intake of the fiber mixture. Studies comparing SCFA metabolism in normoglycemic and metabolically compromised phenotypes could use isotope labelled tracer studies combined with in *in vivo* tissue biopsies of skeletal muscle and adipose tissue to shed light on the fate of SCFA in human energy metabolism. Finally, these findings emphasize that more subgroup based, or personalized approaches are needed to more efficiently target the gut microbiota with nutritional interventions to improve metabolic health.

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Addendum

Abbreviations

AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
ASV	Amplicon sequence variant
AXOS	Adipose tissue
AUC	Area under the curve
AXOS	Arabinoxylan-oligosaccharides
CLA	Conjugated linoleic acid
BCAA	Branched-chain amino acids
BCFA	Branched-chain fatty acids
BMI	Body mass index
CID	Clinical investigation day
CCK	Cholecystokinin
eCB	Endocannabinoid system
FFA	Free fatty acids
FOS	Fructo-oligosaccharide
FXR	Farnesoid X receptor
GE	Gastric emptying
GI	Gastrointestinal
GABA	4-aminobutanoate
GLP-1	Glucagon-like peptide-1
GPR	G-protein coupled receptor
GOS	Galacto-oligosaccharide
HOMA-IR	Homeostasis model assessment of insulin resistance
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
LBP	Lipopolysaccharide-binding protein
LPS	Lipopolysaccharide
LPL	Lipoprotein lipase
OCTT	Oro-cecal transit time
PUFA	Polyunsaturated fatty acids
PPAR	Peroxisome proliferator-activated receptor
PYY	Peptide YY
OGTT	Oral glucose tolerance test
rRNA	Ribosomal ribonucleic acids
ROC	Receiver operating characteristic
SCFA	Short-chain fatty acids
T2DM	Type 2 diabetes mellitus
TAG	Triacylglycerol
TGR5	G protein–coupled bile acid receptor 1
TNF-α	Tumor necrosis factor-α
IL	Interleukin
WGTT	Whole-gut transit time

Summary

Alterations of the gut microbiota and microbial metabolites may play an important role in the etiology of metabolic diseases such as obesity and type 2 diabetes mellitus. Key microbial metabolites are the short-chain-fatty acids (SCFA) acetate, butyrate, and propionate derived from microbial fermentation of dietary fibers. SCFA are suggested to be involved in body weight regulation and insulin sensitivity as shown in rodent studies and short-term human interventions. The passage of food through the intestine, i.e., the GI transit time plays a crucial role in postprandial nutrient metabolism and may affect gut microbiota composition. Thus, modulation of the GI transit time may impact glucose homeostasis and metabolic health. Recently, *in vitro* and population-wide studies suggested a bidirectional relationship between the gastrointestinal (GI) transit time and the gut microbiota composition. Using different methodological approaches, this thesis aims to provide insights into the interaction between dietary fibers, the gut microbiota and SCFA production in relation to GI transit time and metabolic health in humans.

First, **Chapter 2** provides an overview of the potential underlying mechanisms of the interaction between GI transit, glucose homeostasis, and metabolic health, and how dietary fibers may exert beneficial metabolic effects via modulation of GI functioning. Short- and long-term human interventions with soluble, insoluble, fermentable or viscous dietary fiber are discussed with respect to their effect on GI transit, microbiota modulation, and glucose homeostasis. Different types of dietary fibers i.e., soluble, insoluble, fermentable or viscous fibers influence GI transit time in a site-specific manner. Insoluble fibers accelerate colonic transit due to increased fecal stool bulking and have been associated with reduced T2DM risk in epidemiological studies. Viscous fibers can delay postprandial glycemia mediated by changes in gastric emptying, yet the role of gut peptide secretion in this process is not fully understood. From this literature review, it becomes evident that there is a lack of high-quality and well-controlled human intervention studies investigating the effect of prebiotic fibers on GI transit in the context of metabolic health.

Next, we studied the prebiotic wheat-bran derived dietary fiber arabinoxylan-oligosaccharides (AXOS), which induced beneficial effects on colonic fermentation, stool parameters as well as glycemia as shown in short-term human studies. In a randomized, placebo-controlled 12-week intervention study, we investigated the effect of 15 g/day AXOS intake on whole gut transit time (measured via radio-opaque markers) and

metabolic health in normoglycemic participants with slow GI transit (**Chapter 3**). Secondary outcomes were changes in gut microbiota composition, gastric emptying, oro-cecal transit, gut permeability, fecal and plasma SCFA, energy and substrate metabolism, local inflammatory markers and glycemic and insulinemic responses. The 12-week of AXOS intervention strongly increased fecal *Bifidobacterium* abundance, reduced microbiota diversity and softened stool consistency without changes in GI transit, SCFA fermentation and metabolic health parameters. Contrary to probiotic studies, prebiotic stimulation of Bifidobacterium was not effective in improving GI transit or markers of metabolic health in this study population. However, the overall gut microbiota composition accurately classified responders with faster GI transit indicating that changes in GI transit were associated with gut microbiota composition independent of *Bifidobacterium* increases.

To further elucidate this interaction between GI transit and gut microbiota composition, we investigated to what extent quantitative measures of (segmental) colonic transit time were related to gut microbiota composition, microbial metabolites and gut-related parameters, dietary intake and host demographics in a human cross-sectional study (**Chapter 4**). Linear regression models and redundancy based-analysis demonstrated that distal colonic transit time was related to microbial alpha-diversity, fecal SCFA and plasma acetate concentrations, while microbiota composition was related to age, sex, and dietary intake and only to a minor extent to stool consistency. The study's findings suggest that the distal colonic transit may affect not only microbiota diversity but also microbial SCFA production. Thus, when targeting the microbial fermentation to improve the host health, the colonic site of fermentation should be considered in future interventional studies.

Contrary to the accumulating evidence of the beneficial effects of SCFA on energy metabolism, human cross-sectional studies have also shown increased fecal SCFA concentrations in overweight and obese compared to lean participants. Yet, it is well known that fecal SCFA reflect only approximately 5% of the microbially produced SCFA and are the net result of production and absorption. In **Chapter 5**, we studied the association between fecal and fasting circulating SCFA with fasting glucose, insulin, circulating lipids (free fatty acids, triacylglycerols, glycerol), insulin resistance index (homeostasis model assessment of insulin resistance (HOMA-IR)), gut hormone concentrations in 160 healthy to prediabetic participants. In a subgroup of prediabetic participants, peripheral insulin sensitivity was measured by the hyperinsulinemic-euglycemic clamp technique. Using adjusted multiple linear regression models, we showed that fasting circulating acetate, propionate and butyrate concentrations were positively associated with fasting glucagon-

like peptide (GLP) 1 concentrations. Further, circulating SCFA were negatively related to whole-body lipolysis (glycerol), triglycerides and free fatty acids concentrations. In the subgroup analysis of the prediabetic participants, circulating acetate was negatively and propionate positively associated with peripheral insulin sensitivity. Contrary to previous studies on acetate, we observed higher circulating acetate concentrations in prediabetic participants, which may reflect an altered endogenous acetate metabolism in these metabolically compromised individuals. Strikingly, none of the fecal SCFA were associated with any measured metabolic parameter. We concluded from these data that circulating SCFA are more directly linked to metabolic heath indicating, which indicates the need to measure circulating SCFA in human prebiotic/probiotic intervention studies as a biomarker/mediator of effects on host metabolism.

As shown in this thesis, the site of colonic fermentation may be of importance when studying the effect of dietary fiber fermentation on metabolic health. We previously demonstrated that acetate infusions in the distal, but not proximal, colon improved fat oxidation and metabolic health in men. In a proof-of-concept study (**Chapter 6**), we hypothesized that combining acetogenic fibers, especially if 'slowly fermentable', with a more 'rapidly fermentable' fiber will enhance microbial acetate production in the distal colon, thereby improving human energy and substrate metabolism. We first identified an optimal fiber mixture that yielded high distal colonic acetate in an *in vitro* model of the human colon (TIM-2). Long-chain inulin (INU) was selected as slow fermentable fiber combined with a more rapidly fermentable resistant starch (RS), which together yielded a high distal colonic acetate production in the TIM-2 model. Subsequently, in a randomized crossover study, lean and prediabetic men with overweight/obesity received equicaloric supplements (3 times a day) of either 12g long-chain inulin + 7.5g resistant starch (INU+RS), 12g long-chain inulin alone plus 5.4g maltodextrin (INU) or 11.4g maltodextrin only (placebo, PLA), a day prior to a clinical investigation day. One-day supplementation of RS combined with INU versus INU and PLA. promoted microbial fermentation, increased fasting and postprandial energy expenditure and carbohydrate oxidation and increased postprandial insulin sensitivity in lean individuals the following day. Contrary to our hypothesis, circulating acetate was not increased after INU and RS supplementation in lean men.

In overweight/obese prediabetic men, INU+RS versus INU and PLA increased circulating acetate concentrations but did not result in improvements in the energy and substrate metabolism which substantiates again the notion that acetate metabolism may be altered in the metabolically disturbed phenotype. Further research has to elucidate

whether in the prediabetic phenotype a longer-term supplementation period is required to demonstrate beneficial effects on metabolic health.

To conclude, the main findings of this thesis provide further evidence for the connection between the (distal) GI transit, gut microbiota composition and microbial fermentation in humans. Further, this thesis highlights the importance of measuring circulating SCFA in the context of metabolic health outcomes and provides evidence of altered SCFA metabolism in the metabolically disturbed phenotype. Personalized approaches are warranted to more efficiently target the gut microbiota composition and metabolism with nutritional interventions to improve gastrointestinal and metabolic health.

Samenvatting

Veranderingen in de darmmicrobiota en microbiële metabolieten kunnen een rol spelen in de etiologie van stofwisselingsziekten zoals obesitas en type 2 diabetes. De belangrijkste microbiële metabolieten zijn de korte-keten vetzuren (SCFA) acetaat, butyraat en propionaat afkomstig van microbiële fermentatie van voedingsvezels. Proefdierstudies en korte termijn humane interventie studies tonen aan dat SCFA betrokken zijn bij de regulering van het lichaamsgewicht en insulinegevoeligheid. De passage van voedsel door de darm, d.w.z. de transittijd speelt een cruciale rol in het postprandiale nutriëntenmetabolisme en kan de ook de samenstelling van de darmmicrobiota beïnvloeden. Zo kan modulatie van de GI transittijd van invloed zijn op de glucosehomeostase en de metabole gezondheid. Het blijkt uit recente *in vitro* en populatie brede studies dat er een bidirectioneel verband is tussen de gastro-intestinale (GI) transittijd en de samenstelling van de darmmicrobiota. Met behulp van verschillende methodologische benaderingen wil dit proefschrift inzicht geven in de interactie tussen voedingsvezels, de darmmicrobiota en SCFA-productie in relatie tot de GI-transittijd en de metabole gezondheid.

Hoofdstuk 2 geeft een overzicht van de potentiële onderliggende mechanismen van de interactie tussen GI-transittijd, glucose homeostase en metabole gezondheid, en hoe voedingsvezels gunstige metabole effecten kunnen hebben via de modulatie van GI functies. Korte en lange termijn humane interventie studies met oplosbare, onoplosbare, fermenteerbare, prebiotische of viskeuze voedingsvezels worden besproken met betrekking tot hun effect op de GI-transittijd, microbiota modulatie en glucose homeostase. Verschillende soorten voedingsvezels, d.w.z. oplosbare, onoplosbare, fermenteerbare of viskeuze vezels, beïnvloeden de GI transittijd op een locatie-specifieke manier. Onoplosbare vezels versnellen de transittijd door de colon als gevolg van een verhoogde ontlasting van de ontlasting en zijn in epidemiologisch onderzoek in verband gebracht met een verminderd T2DM-risico. Viskeuze vezels kunnen de postprandiale bloedsuikerspiegel, gemedieerd door veranderingen in de maaglediging, verbeteren, maar de rol van de secretie van specifiek darm hormonen in dit proces wordt niet volledig begrepen. Verder blijkt uit dit literatuuroverzicht blijkt dat er een gebrek is aan kwalitatief hoogstaande en goed gecontroleerde humane interventiestudies die het effect van prebiotische vezels op de GI-transit in de context van metabole gezondheid onderzoeken.

Vervolgens bestudeerden we de prebiotische van tarwezemeel afgeleide voedingsvezel arabinoxylan-oligosacchariden (AXOS), die gunstige effecten kan hebben op

de fermentatie en ontlasting in de colon en op de controle van de bloedglucosespiegel zoals aangetoond in korte-termijn humane studies. In een gerandomiseerde, placebo-gecontroleerde 12 weken durende interventiestudie onderzochten we het effect van 15 g/dag AXOS inname op de transittijd van de gehele darm (gemeten via radio-opake markers) en de metabole gezondheid bij normoglycemische deelnemers met een trage GI transit (hoofdstuk 3). Secundaire uitkomsten waren veranderingen in de samenstelling van de darmmicrobiota, maaglediging, oro-cecale passagetijd, darmdoorlaatbaarheid, fecale en plasma SCFA, energie- en substraatmetabolisme, lokale inflammatoire markers en glykemische en insulinemische responses. De 12 weken durende AXOS-interventie verhoogde de aanwezigheid van fecale Bifidobacteriën, verminderde de microbiota diversiteit en resulteerde in een zachtere ontlasting zonder veranderingen in de GI-transittijd, SCFA-fermentatie en metabole gezondheidsparameters. In tegenstelling tot probiotische studies was de prebiotische stimulatie van *Bifidobacterium* niet effectief om in deze studiepopulatie de GI-transittijd of markers van metabole gezondheid te verbeteren. Echter, de samenstelling van de darmmicrobiota kon deelnemers met snellere GI transittijd na AXOS inname nauwkeurig classificeren, wat aangeeft dat veranderingen in de GI-transittijd geassocieerd zijn met de samenstelling van de darmmicrobiota onafhankelijk van aanwezigheid van *Bifidobacterium*.

Om deze interactie tussen de GI transittijd en de samenstelling van de microbiota verder te onderzoeken, hebben we de associaties van kwantitatieve maten van de (segmentale) transittijd door het colon en de diversiteit en samenstelling van de darmmicrobiota van deze deelnemers bepaald door middel van een cross-sectionele studie (**Hoofdstuk 4**). Lineaire regressiemodellen toonden aan dat de transit door het distale colon gerelateerd was aan de microbiële alfa-diversiteit, fecale SCFA en plasma-acetaatconcentratie. Daarentegen was de samenstelling van de microbiota gerelateerd aan leeftijd, geslacht, en de voedingsinname en slechts in geringe mate aan de consistentie van de ontlasting. Dit suggereert dat de distale colon transit niet alleen van invloed kan zijn op de diversiteit van de microbiota, maar ook op de microbiële stofwisseling. Dit suggereert dat voor het verbeteren van de metabole en damgezondheid rekening moet worden gehouden met de plaats in het colon waar de microbiële fermentatie plaatsvindt.

In tegenstelling tot het toenemende bewijs van de gunstige effecten van SCFA op het energiemetabolisme, hebben cross-sectionele studies aangetoond dat fecale SCFA-concentraties bij overgewicht en obesitas hoger zijn in vergelijking met slanke deelnemers. Niettemin, fecale SCFA weerspiegelen slechts 5% van de microbieel geproduceerde SCFA en zijn het nettoresultaat van productie en absorptie. In **Hoofdstuk 5** bestudeerden we

het verband tussen fecale en circulerende SCFA met gevaste concentraties van glucose, insuline, circulerende lipiden (vrije vetzuren, triacylglycerol, glycerol), insulineresistentie-index (homeostasemodel bepaling van de insulineresistentie (HOMA-IR)) en darmhormoonconcentraties in 160 gezonde tot prediabetische deelnemers. In een subgroep van prediabetische deelnemers met overgewicht/obesitas werd de perifere insuline gevoeligheid gemeten met behulp van de hyperinsulinemische-euglycemische clamp techniek. Met behulp van multivariate lineaire regressiemodellen toonden we aan dat circulerende acetaat-, propionaat- en butyraatconcentraties positief geassocieerd waren met glucagon-like peptide (GLP) 1 concentraties. Verder waren circulerende SCFA negatief gerelateerd met de lipolyse (glycerol), triglyceriden en vrije vetzuurconcentraties. In de subgroep analyse van de prediabetische deelnemers was circulerend acetaat negatief en propionaat positief geassocieerd met perifere insuline gevoeligheid. In tegenstelling tot eerdere studies over acetaat, zagen we hogere circulerende acetaatconcentraties bij prediabetische deelnemers, die een veranderde endogene acetaatstofwisseling bij deze metabool verstoorde personen kunnen weerspiegelen. Opvallend genoeg werd geen van de fecale SCFA geassocieerd met metabole parameters. We concluderen uit deze resultaten dat de circulerende SCFA direct gerelateerd zijn aan parameters van de metabole gezondheid, wat aangeeft dat we in interventiestudies, die de effecten van pro- of prebiotica bekijken, circulerende SCFA moeten meten als een reflectie van de effecten op de stofwisseling.

Zoals aangetoond in dit proefschrift is de plaats waar vezels gefermenteerd worden van belang bij het bestuderen van het effect van voedingsvezelfermentatie op de metabole gezondheid. In vorige studies hebben we aangetoond dat met name distale toediening van acetaat in het colon uitgesproken effecten heeft op de metabole gezondheid, terwijl proximale toediening in het colon geen effecten had op de stofwisseling. In een proof-of-concept studie (**hoofdstuk 6**), was onze hypothese dat het combineren van acetogene vezels, vooral de combinatie van 'langzaam fermenteerbare' met 'sneller fermenteerbare' vezels de microbiële acetaatproductie in het distale colon zal verhogen, wat meer uitgesproken effecten op de metabole gezondheid zal hebben.

We hebben eerst in een *in vitro* model van het humane colon (TIM-2) het optimale vezelmengsel geïdentificeerd wat leidt tot de hoogste acetaat productie in het distale deel van de colon. Lange keten inuline (INU) werd geselecteerd als langzaam fermenteerbare vezel in combinatie met een snel fermenteerbaar, resistent zetmeel (RS). Vervolgens hebben we een gerandomiseerde, placebo-gecontroleerde studie uitgevoerd met acute supplementatie van het vezelmengsel bij normoglycemische mannen met

normaalgewicht en bij mannen met prediabetes en overgewicht/obesitas. Het was een eendaagse interventie met inname (3 keer per dag) van ofwel 12g lange-keten inuline + 7.5g resistent zetmeel (INU+RS), 12g lange-keten inuline + 5.4g maltodextrine (INU) of 11.4g maltodextrine alleen (placebo, PLA) een dag voorafgaand aan een klinische onderzoeksdag. Eendaagse suppletie van RS in combinatie met INU versus INU en PLA bevorderde de microbiële fermentatie, verhoogde nuchter en postprandiaal energieverbruik en koolhydraatoxidatie en verhoogde postprandiale insuline gevoeligheid bij slanke mannen de volgende dag. In tegenstelling tot onze hypothese, werd het circulerende acetaat na INU en RS supplementatie bij slanke mannen niet verhoogd

Bij mannen met prediabetes en overgewicht/obesitas verhoogde de inname van INU+RS versus INU en PLA de circulerende acetaatconcentraties, maar dit resulteerde niet in verbeteringen in het energie- en substraatmetabolisme. Deze bevindingen bevestigen het idee dat het acetaatmetabolisme verstoord kan zijn bij mensen met een hoog risico om diabetes te ontwikkelen. Samen suggereren deze resultaten dat dit vezelmengsel een strategie zou kunnen zijn om gewichtstoename en insulineresistentie te voorkomen, maar verdere lange termijn suppletiestudies zijn gerechtvaardigd.

De belangrijkste bevindingen van dit proefschrift leveren bewijs voor het verband tussen de passagesnelheid in de (distale) dikke darm, de samenstelling van de darmmicrobiota en de microbiële fermentatie bij de mens. Verder benadrukt dit proefschrift het belang van het meten van circulerende SCFA in de context van metabole gezondheid en levert het bewijs dat een verstoorde metabole gezondheid gepaard kan gaan met een veranderd acetaatmetabolisme Gepersonaliseerde interventies zijn noodzakelijk voor een meer doelgerichte verandering van de microbiële samenstelling en functionaliteit om op deze manier een optimale gezondheidswinst te bewerkstelligen

Zusammenfassung

Veränderungen der Darmmikrobiota und deren Stoffwechselprodukte spielen möglicherweise eine wichtige Rolle in der Ätiologie von Stoffwechselerkrankungen wie Adipositas und Diabetes Mellitus. Wichtige mikrobielle Metabolite Stoffwechselmetabolite sind in diesem Zusammenhang die kurzkettigen Fettsäuren (SCFA) Acetat, Butyrat und Propionat, welche bei der Fermentation von Ballaststoffen im Darm produziert werden. Sowohl Tierstudien als auch akute Humane Interventionsstudien , die sich auf den akuten Effekt von SCFA fokussieren, zeigen, dass SCFA eine wichtige Rolle in Stoffwechselprozessen innerhalb der Regulierung des Körpergewichts und der Insulinsensitivität beteiligt sind. spielen. Der Transit von Nahrung durch den Darm, d.h. die gastrointestinale (GI) Transitzeit spielt eine entscheidende Rolle im postprandialen Nährstoffstoffwechsel und kann die Zusammensetzung der Darmmikrobiota beeinflussen. Auf diese Weise könnten Veränderungen der GI-Transitzeit die Glukosehomöostase und die metabolische Gesundheit beeinflussen. Aktuelle *in vitro* und bevölkerungsweiten Studien geben Hinweise auf einen bidirektionalen Zusammenhang zwischen der GI Transitzeit und der Zusammensetzung der Darmmikrobiota. Diese Interaktion zwischen der Einnahme von Ballaststoffen, der Darmmikrobiota und SCFA-Produktion in Bezug auf die GI-Transitzeit und den Stoffwechsel wurde in der vorliegenden Doktorarbeit mittels verschiedener Methodiken untersucht.

Im **Kapitel 2** der vorliegenden Doktorarbeit werden die potenziellen Mechanismen beschrieben, welche der Interaktion zwischen GI-Transit, Glukosehomöostase und Glukosehomöostase und weiteren wichtigen Stoffwechselfaktorenmetabolischer Gesundheit zugrunde liegen und erläutert weiterhin, wie Ballaststoffe durch die Modulierung von GI-Funktionen den Stoffwechsel positiv beeinflussen. Akut- und Langzeitstudien mit löslichen, unlöslichen, fermentierbaren, präbiotischen oder viskosen Ballaststoffen wurden hinsichtlich ihrer Auswirkungen auf den GI-Transit, die Zusammensetzung der Darmmikrobiota und die Glukosehomöostase diskutiert. Verschiedene Arten von Ballaststoffen, d.h. lösliche, unlösliche, fermentierbare oder viskose Ballaststoffe beeinflussen die GI-Transitzeit ortspezifisch. Unlösliche Ballaststoffe beschleunigen den Transit durch das Kolon aufgrund von erhöhtem Stuhlvolumen und wurden gleichzeitig in epidemiologischen Studien mit einem reduzierten T2DM-Risiko in Verbindung gebracht. Viskose Ballaststoffe können die postprandiale Glykämie verzögern indem sie Entleerung des Mageninhaltes in den Dünndarm verlangsamen. Weiterhing geht aus dieser Literaturrecherche hervor, dass es

ein Mangel gibt an qualitativ hochwertigen und gut kontrollierten Studien gibt, die die Wirkung von unlöslichen und als auch präbiotischen Ballaststoffen sowohl auf den GI-Transit als auch auf den Stoffwechsel untersuchen.

In **Kapitel 3** wurden die Effekte von einer 12-wöchigen Intervention mit dem von Weizenkleie abstammender Ballaststoff namens Arabinoxylan-Oligosaccharid (AXOS) untersucht. Akute Humanstudien haben gezeigt, dass die Einnahme von AXOS positive Auswirkungen auf die Fermentation im Darm, die Stuhlkonsistenz und als auch die postprandiale Glukoseregulierung haben kann. In einer randomisierten, Placebo-kontrollierten 12-wöchigen Interventionsstudie wurde die Wirkung von 15 g/Tag AXOS auf GI-Transit (gemessen am Transit durch den gesamten Gastrointestinaltrakt) und verschiede Stoffwechselfaktoren die metabolische Gesundheit bei normoglykämischen Teilnehmern mit langsamen GI-Transit untersucht. Sowohl die Darmbarrierefunktion, die Zusammensetzung der Darmmikrobiota und Fermentation als auch das Stoffwechselprofil der Teilnehmer wurde charakterisiert. Die 12-wöchige AXOS-Intervention erhöhte das Vorkommen von Bifidobakterien im Stuhl, reduzierte die mikrobielle Diversität und führte zu einer weicheren Stuhlkonsistenz, jedoch ohne den GI-Transit, die SCFA Produktion oder das Stoffwechselprofil zu beeinflussen. Im Gegensatz zu probiotischen Studien war der AXOS induzierte Anstieg von Bifidobakterien nicht ausreichend um den GI-Transit zu verbessern, d.h. zu beschleunigen. Jedoch konnten wir auf Basis der generellen Zusammensetzung der Darmmikrobiota vorhersagen, welche Teilnehmer nach der AXOS Einnahme einen beschleunigten, also verbesserten, GI-transit hatten. Dies deutet daraufhin, dass, unabhängig von der Zunahme von Bifidobakterien, Änderungen im GI-Transit mit der Zusammensetzung der Darmmikrobiota assoziiert sind. Um diese Interaktion weiter zu untersuchen, analysierten wir mögliche Zusammenhänge zwischen GI-Transitparametern, hierbei mit besonderem Fokus auf die Transitzeit durch die unterschiedlichen Segmente im Kolon, der mikrobiellen Diversität und Zusammensetzung unter Berücksichtigung von Ernährungsprofil, Alter, und Geschlecht der Studienteilnehmer in **Kapitel 4**. Lineare Regressionsmodelle und redundanzbasierte Analysen zeigten, dass vor allem eine hohe Transitzeit durch das distale Kolon mit höherer mikrobieller Diversität, fäkalen SCFA und niedrigeren zirkulierende Acetatkonzentrationen zusammenhing. Die mikrobielle Zusammensetzung hingegen hing am stärksten mit dem Alter, Geschlecht und Ernährungsprofil der Teilnehmer und in geringem Maße mit der Stuhlkonsistenz zusammen. Dies deutet darauf hin, dass der Transit durch das distale Kolon nicht nur die Diversität der Mikrobiota, sondern auch den mikrobiellen SCFA-Stoffwechsel beeinflussen kann. Dies sollte bei zukünftigen interventionellen Studien

berücksichtig werden, welche darauf abzielen, durch erhöhte mikrobielle Fermentation und Diversität die Gesundheit des Darmes und des Stoffwechsels positiv zu beeinflussen.

Im Gegensatz zu den sich häufenden Beweisen über den positiven Effekt von SCFA auf den Energiestoffwechsel, zeigen manche humane Querschnittsstudien wiederum erhöhte SCFA-Konzentrationen im Stuhl von Teilnehmern mit Übergewicht und Adipositas im Vergleich zu normalgewichtigen Teilnehmern. Diese Studien werden jedoch durch die Tatsache beeinflusst, dass fäkale SCFA nur circa 5% der mikrobiell produzierten SCFA im Kolon reflektieren und das Nettoergebnis von Produktion und Absorption sind. In **Kapitel 5** haben wir den Zusammenhang zwischen fäkalen und zirkulierenden SCFA und Glukose, Insulin, verschiedener Lipide (freie Fettsäuren, Triacylglycerol, Glycerol) als auch dem Inuslinresistenz-Index (Homeostasis Modell Assessment Test) und darmspezifischer Hormone bei 160 Teilnehmern mit Normalgewicht, als auch Übergewicht und Adipositas mit Prädiabetes untersucht. In einer Subgruppe von Teilnehmern mit Prädiabetes wurde außerdem die periphere Insulinsensitivität mit der Hyperinsulinämischer-euglykämischer Glukose-Clamp-Test gemessen. Mittels multivariaten, linearen Regressionsmodellen zeigten wir, dass die Konzentrationen von zirkulierendem Acetat, Propionat und Butyrat positiv mit den Konzentrationen von Glucagon-like Peptiden (GLP-1) assoziiert waren. Weiterhin assoziierten zirkulierende SCFA negativ mit Markern von Lipolyse (Glycerin), Triglyceriden und freien Fettsäurekonzentrationen. In der Subgruppenanalyse der prädiabetischen Teilnehmer waren zirkulierendes Acetat negativ und Propionat positiv mit der peripheren Insulinsensitivität assoziiert. Im Gegensatz zu früheren Studien beobachteten wir höhere zirkulierende Acetatkonzentrationen bei Teilnehmern mit Prädiabetes, welches möglicherweise auf einen gestörten endogenen Acetatstoffwechsel bei diesen Teilnehmern hindeuten könnte. Auffallend war, dass keiner der fäkalen SCFA mit den gemessenen Stoffwechselparametern assoziiert war. Aus diesen Daten schlossen wir, dass im Kontext eines funktionierenden Stoffwechsels die im systemischen Blutkreislauf gemessene SCFA aussagekräftiger sind als fäkale SCFA Konzentrationen. Dies sollte in zukünftigen Interventionsstudien mit Ballaststoffen berücksichtigt werden.

Wie in dieser Arbeit gezeigt wurde, hatte der anatomische Ort der Fermentation im Kolon einen Einfluss auf die Fermentation von Ballaststoffen Wir haben in früheren Studien bereits gezeigt, dass Acetatinfusionen in das distalen, aber nicht ins proximale Kolon die Fettverbrennung und die metabolische Gesundheit bei Männern mit Übergewicht/Adipositas verbessern kann. In einer Proof-of-Concept-Studie (**Kapitel 6**) stellten wir die Hypothese auf, dass die Kombination von einem acetogenen Ballaststoff, insbesondere wenn sie "langsam fermentierbar" sind, mit einem "schnell

fermentierbaren" Ballaststoff die mikrobielle Acetatproduktion im distalen Kolon erhöhen könnte, wodurch sich auch der Energie- und Substratstoffwechsel verbessern könnte. Wir identifizierten zunächst eine optimale Ballaststoffmischung, die in einem *in-vitro*-Modell des menschlichen Dickdarms (TIM-2) zu erhöhter Acetatproduktion im distalen Kolon führte. Hierbei ergab sich, dass langkettiges Inulin (INU) als langsam fermentierbare Ballaststoff in Kombination mit der schnell fermentierbaren resistenten Stärke (RS) im TIM-2-Modell zu einer hohen distalen Acetatproduktion führte. Anschließend führten wir eine randomisierte, Placebo-kontrollierte Studie mit akuter Ballaststoffmix-Supplementierung bei normoglykämischen Männern mit Normalgewicht und bei Männern mit Prädiabetes und Übergewicht/Adipositas durch. Die Intervention bestand aus einer dreimal täglichen Einnahme von 12 g langkettigem Inulin + 7,5 g resistenter Stärke (INU+RS), oder 12 g langkettigem Inulin + 5,4 g Maltodextrin (INU) oder nur 11,4 g Maltodextrin (Placebo, PLA). Die eintägige Supplementierung von RS in Kombination mit INU versus INU und PLA alleine förderte die mikrobielle Fermentation, erhöhte den nüchternen und postprandiale Energieverbrauch und Kohlenhydratoxidation und erhöhte postprandiale Insulinempfindlichkeit bei normalgewichtigen Männern bei der Messung am Morgen nach der Einnahme. Entgegen unserer Hypothese wurde zirkulierendes Acetat nach INU- und RS-Supplementierung bei mageren Männern nicht erhöht. Im Gegensatz dazu erhöhte die Einnahme von INU+RS versus INU und PLA die Acetatkonzentrationen von Teilnehmern mit Übergewicht und Prädiabetes, führten aber nicht zu einer Verbesserung der untersuchten Stoffwechselparameter. Dies deutet daraufhin, dass der Acetatstoffwechsel im metabolisch kompromittierten Phänotyp verändert scheint. Zusammengenommen deuten diese Ergebnisse darauf hin, dass diese Ballaststoffkombination ein Ernährungsstrategie sein könnte, um den Glukosehaushalt zu verbessern, jedoch sind weitere langfristige Studien mit der Ballaststoffkombination notwendig um diese Ergebnisse zu untermauern.

Zusammenfassend liefern die Ergebnisse dieser Doktorarbeit weitere Beweise für den bidirektionalen Zusammenhang zwischen dem (distalen) GI-Transit, der Darmmikrobiota und der mikrobiellen Fermentation beim Menschen. Darüber hinaus unterstreicht diese Arbeit, dass, im Kontext von Stoffwechselkrankheiten, die Messung von zirkulierender SCFA im Plasma der Messung von fäkalen SCFA vorzuziehen ist. Es wird deutlich, dass in zukünftige Ernährungsinterventionen, welche über die Darmmikrobtioa und mikrobielle Fermentation die Gesundheit des Magen-Darm-Traktes und des Stoffwechsels verbessern sollen, mit personalisierten Ansätzen möglicherweise erfolgreicher sein könnten.

Valorization

The present thesis discusses the potential interaction between the gastrointestinal tract, the gut microbiota and the effect of dietary fiber intake in the context of metabolic health. Further, the future implications and the relevance of the present results will be put into a societal and economical context.

The most recent world health organization (WHO) report indicates that obesity has tripled in the last forty decades with more than 1.9 billion adults being overweight and more than 650 million being obese in 2016. Obesity develops as a result of positive energy balance caused by increased food intake accompanied by declining physical activity, most commonly manifesting in gradient weight gain over years. Further, obesity is a strong risk factor in the onset of other metabolic diseases such as type II diabetes mellitus (T2DM), and is also associated with development of certain cancers, depression and cardiovascular diseases. Thus, despite being a preventable disease, the obesity pandemic and its comorbidities pose a massive challenge to healthcare systems worldwide. Moreover, due to rising childhood obesity, the challenges of the obesity pandemic will only foreseeable accelerate in the future. Current obesity treatment mainly focuses on the counseling of lifestyle adaptions such as caloric restriction and physical activity. These lifestyle adaptions can effectively lead to weight loss and reverse progression to T2DM, but adherence to lifestyle changes is challenging. The lack of implementing a healthy life style in the long term inevitably leads to treatment failure. Hence, there is an urgent need to advance current treatment strategies to tackle the obesity pandemic and obesity-related disorders.

Most recently, much attention has been drawn to the role of the gastrointestinal (GI) tract and especially the gut microbiota in the etiology of obesity. Thus, industry, as well as academia have put much effort into the development and study of dietary components targeting the gut microbiota and specific “beneficial” microbial species in order to improve parameters of human metabolism. However, there are still huge gaps in our understanding of the complex interactions between dietary components, the physiological functions of the GI tract, the gut microbiota and its metabolites in the context of metabolic health. The studies conducted in this thesis have been partly funded by the Top Institute for Food and Nutrition, (TIFN), a public-private partnership on pre-competitive research in food and nutrition combining resources and expertise from both food industry and academia. By using industrial and academic resources, this thesis aimed to elucidate some of these interactions by means of different methodological approaches.

|Valorization

Our findings indicate that long-term supplementation with a dietary fiber (Arabinoxylan-Oligossacharide, AXOS) that clearly stimulates Bifidobacteria does not translate into beneficial metabolic changes in a healthy, normoglycemic population. Yet, AXOS- stimulated growth of Bifidobacteria may help to improve stool consistency and thus may have potential applications in patients suffering from functional GI disorders. Further, the supplementation of AXOS led to a decreased microbial diversity, which is associated with several GI diseases. Yet, these findings need to be interpreted in a microbiological/ecological context, a perspective that seems to lack in many prebiotic interventional studies. Further, our results show that microbial diversity may also depend on the colonic transit time, thereby adding an important covariate which should be considered in future interventional studies targeting the gut microbiota. Further, the results from this thesis may suggest more careful interpretation of microbial diversity in the context of prebiotic intervention studies. Further large-scale cross-sectional studies including segmental colonic transit are needed to validate our findings. Nevertheless, the results from this thesis elucidate the complex interaction between GI transit, the gut microbiota and dietary fibers in the context of metabolic health and further research, especially in humans is required to identify underlying mechanisms and drivers.

Many human and animal studies emphasize the beneficial potential for microbially produced short-chain fatty acids on the human metabolism. Yet, in order to efficiently target SCFA production, it is important to understand their metabolism and covariates thereof. This thesis gave important insights into the latter, emphasizing that the gastrointestinal transit time is linked to fecal SCFA concentrations, a parameter often used in prebiotic intervention studies to indicate fermentation. Further, we emphasize that rather than fecal SCFA, the measurement of plasma SCFA may be more meaningful in the context of metabolic processes, since only plasma SCFA concentrations associated with several important metabolic markers. These results provide interesting considerations for future studies targeting lifestyle interventions thereby increasing their effectiveness to prevent chronic metabolic diseases.

In this thesis, we report results from a proof-of concept studie funded by a partnership between the Netherlands Organization of Scientific Research and the Carbohydrate Competence Center, a private- public organization aiming to “generate, develop and share high-value knowledge in the field of carbohydrates, to promote innovation worldwide and contribute to healthy and sustainable societies”. The study reported in this thesis gave import insights into the effectiveness of dietary fiber combinations with the approach of directly translating *in vitro* findings into an acute *in*

vivo human interventional study. We show that a dietary fiber mixture induces beneficial metabolic effects on energy expenditure and postprandial insulin tolerance after one day intake in lean men. These results should encourage further research, especially investigating long-term beneficial effect of dietary fiber mixtures specifically targeting increased distal acetate production as a nutritional strategy to improve or metabolic health. Also, these results may provide direct lead for food industry to develop products with specific (combinations of) dietary fiber(s) which are most effective in counteracting or preventing obesity and type 2 diabetes mellitus.

The studies described in this thesis have been or will be published in scientific journals in the field of nutrition and microbiology and thus will be partly available for the public and scientific community. Industrial partners as well as academic partners have contributed to this project by funding and knowledge exchange during meetings and presentations. Thus, given the interesting implications of this thesis, the development of novel food products such as dietary fiber mixtures may provide an interesting strategy within the existing collaborations between academia and industrial partners and may provide leads for industrial product development. Ultimately, and from the public health perspective, novel recommendations regarding specific fibers and mixtures may be implemented in dietary guidelines to more efficiently prevent development of T2DM and to combat obesity.

Dankwoord

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|Dankwoord

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Mattea

Curriculum Vitae

Mattea Müller was born on 7th November 1988 in Preetz, Germany. After completing secondary school at the Toni-Jensen Gesamtschule in Kiel, Germany in 2007, she began an apprenticeship as medical-technical lab assistant in Lübeck, Germany. In 2008, she started a Bachelor of Science in Cell Biology at the University of Osnabrück, Germany, where she graduated in 2011. After internships at the Karolinska Institute in Stockholm, Sweden and the German Institute for Human Nutrition in Potsdam, Germany, she obtained her Master of Science degree in Molecular Life Science at the Humboldt University in Berlin, Germany in 2014. In January 2015, she began as a PhD candidate at the Department of Human Biology of Maastricht University Medical Centre+ in Maastricht, the Netherlands. Her research, performed under the supervision of Prof. Dr. Ellen Blaak and Dr. Emanuel Canfora, focused on the effect of dietary fibers on the gut microbiota, gastrointestinal functioning and metabolic health. These results were funded by the Top Institute for Food and Nutrition and were presented on several national and international conferences.

List of publications

Müller M, Canfora EE, Blaak EE. Gastrointestinal Transit Time, Glucose Homeostasis and Metabolic Health: Modulation by Dietary Fibers. Nutrients. 2018;10(3):275.

Müller M*, M. A. González Hernández*, G.H. Goossens, D. Reijnders, J. J. Holst, J.W.E. Jocken, H.van Eijk, E.E. Canfora, and Ellen E. Blaak. Circulating not fecal short-chain fatty acids are related to insulin sensitivity and GLP-1 concentrations in humans. *Scientific Reports* **volume 9**, Article number: 12515 (2019) *Shared first authors

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Presentations

2019 Circulating not fecal short-chain fatty acids are related to insulin sensitivity and GLP-1 concentrations in humans. 27th International Symposium on Diabetes and Nutrition, Netherlands

2018 Arabinoxylan-Oligosaccharide intake increases Bifidobacterium, softens stool consistency without changes in gut transit and metabolic health in healthy adults. Annual Dutch Diabetes Meeting, Netherlands

2018 The effect of long term Arabinoxylan-oligosaccharide supplementation on gastrointestinal functioning and metabolic parameters: A randomized controlled trial. Netherlands Association for the Study of Obesity, Netherlands

2018 The effect of long term Arabinoxylan-oligosaccharide supplementation on gut functioning and metabolic parameters: A randomized controlled trial. 36th International symposium on Diabetes and Nutrition, Croatia, *Poster Prize*

2018 The effect of 12-week Arabinoxylan-oligosaccharide intake on gastrointestinal functioning and host metabolism. 25th European Congress on Obesity, Austria

2017 The effect of 12-week Arabinoxylan-oligosaccharide intake on gastrointestinal functioning and host metabolism. Diabetes Symposium Maastricht - Lille – Düsseldorf