Interplay between dietary fibers and gut microbiota for promoting metabolic health

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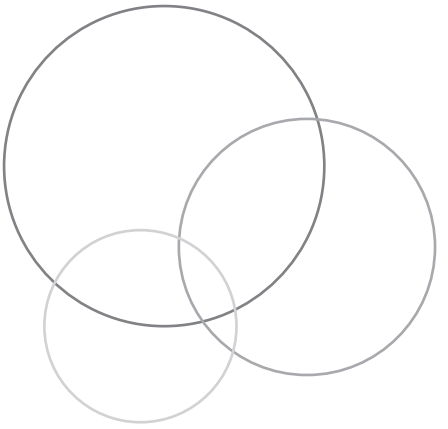

Chapter 7

General discussion

General Discussion

In the last few decades, the numbers of individuals suffering from or being at risk of developing metabolic syndrome have increased drastically.[1] Several factors have contributed to this phenomenon. Nutrition and lifestyle, together with individual genetic factors, influence various metabolic parameters such as cholesterol, triglycerides, fatty acids, glucose and insulin secretion. Consumption of Western-type diets has become increasingly popular. In addition, highly processed foods that are widely consumed lack complex carbohydrates. Prolonged exposure to such diets eventually leads to lipid and glucose metabolic dysregulation and ultimately to the development of components of the metabolic syndrome, particularly cardiovascular disease, obesity, type 2 diabetes and non-alcoholic fatty liver disease. Currently, the main strategy for targeting metabolic dysregulation involves prevention, lifestyle and drug interventions. An alternative concept such as manipulating gut microbiota for promoting health, which was proposed several decades ago, has on the other hand also gained increasing interest. Gut microbiota is a dynamic system which has been implicated in the development of the metabolic syndrome. Several therapeutic tools have been developed to target gut microbiota varying from fecal transplantation, the introduction of specific microbial strains and bioactive substrates.[2,3]

Dietary fibers, also referred to as prebiotics, which remains undigested throughout the upper section of the gastrointestinal tract, can manipulate and stimulate the growth of health-promoting bacterial strains. Although the definition of dietary fibers has been updated several times[4], the common agreement in the definitions has remained the ability to selectively stimulate the growth of specific bacterial groups and the production of bioactive metabolites. Some of the widely used classes of prebiotics include fruto-oligosaccharides (FOS) and galacto-oligosaccharides (GOS). The diversity between and within these two classes of prebiotics leads to different effects on bacteria. Two main genera of bacteria considered to be health-promoting genera are *Bifidobacterium* and *Lactobacillus*. In contrast, an abundance of other genera such as the *Bacteriodes* and *Clostridia* are considered to be largely not favorable for the health of the host. Both the genera *Bifidobacterium* and *Lactobacillus* have been found to proliferate in the gut when supplied with specific dietary fibers. FOS induce a broad effect on the microbiota in terms of shifting the relative abundance of specific genera. For example, in ob/ob mice supplementation of oligofructose in high-fat diet resulted in an increase in up to 102 taxa which could potentially impact host metabolism.[5] On the other hand, GOS has a largely bifidogenic effect on microbial populations.[6]

Carbohydrates and especially dietary fibers are also the largest source of fuel for the intestinal bacteria, even though substantial amounts of other nutrients such as amino

acids are available. This is because intestinal bacteria mostly undergo saccharolytic fermentation which largely occurs in the proximal region of the colon and the main byproducts released are short-chain fatty acids (SCFA). The other type of fermentation which is known as proteolytic fermentation occurs mostly in the distal region of the colon and produces nitrogenous metabolites (amines and ammonia) which are considered less favorable. Specific clusters within the bacterial genome are responsible for expression of saccharolytic enzymes which metabolize different dietary fibers.[7] For example in *Bacteriodes*, the gene cluster responsible for utilizing fructan is found to be conserved in varying degrees in different species. Through genetic and structural analyses, the hybrid two-component (HTC) family of signaling sensors was identified in bacteria. The HTC system involves several multi-protein regulators in bacteria which enables sensing and response to environmental stimuli. The system is especially important in processes such as symbiosis, the formation of biofilms, cells division and antimicrobial activity.[8–11] Mutation or deletion in HTC *in vitro* leads to loss of capacity to utilize fructan substrates, thus preventing the growth of these bacteria.[12] Various dietary fibers have been used in the past for studying its effect on shifting intestinal microbial composition and subsequently impact metabolic pathways. The aim of the experiments described in this thesis was to understand through *in vivo* studies the functions of different dietary fibers in manipulating intestinal microbiota and to investigate its metabolic effects. The ultimate goal is to strategically supplement dietary fibers in order to stimulate the growth of favorable bacterial composition in the host's intestine and thereby improve its metabolic health.

The fermentation of a given fiber can be complex and will depend on solubility, monosaccharide composition, glycosidic bond linkages and chain lengths. C**hapter 2** describes two different chain lengths of inulin and their effect on (chole-)sterol metabolism including intestinal cholesterol handling, a topic that had not been investigated earlier. Wildtype C57BL/6 mice fed short-chain (sc) or long-chain (lc) inulin for two weeks showed a 2.5-fold increase in fecal SCFA compared to controls. Several studies have previously shown that a number of metabolic pathways in the liver could potentially be regulated by SCFA.[13–15] For example, it is known that gut-derived SCFA can serve as precursors for the synthesis of cholesterol and fatty acids in the liver.[16] However, we observed that the increase in SCFA had no significant impact on plasma and liver lipids. Similarly, bile acids which can be modified by microbial activity through deconjugation and hydroxylation showed no major changes in composition in fecal and bile samples. These data suggest that sc- and lc-inulin feeding in wild-type mice did not induce modifications of bile acids *via* microbiota modulation. The subtle changes in plasma would suggest potential changes in absorption of certain bile acid species. These changes, however, we believe had no substantial impact on the

metabolic physiology. Combined, our investigation indicated that two different chain lengths of inulin with a considerable effect on SCFA had no impact on cholesterol absorption, trans-intestinal cholesterol excretion or mass fecal cholesterol excretion. In **chapter 3** we investigated the effects of IMMP on metabolic parameters in an *in vivo* study. The study was designed to measure microbiota composition in different sections of the gastrointestinal tract as well as fermentation as judged by the levels of SCFA, the main fermentation products. C57BL/6 wildtype mice fed IMMP for a period of three weeks had an increased fecal bulk compared to the control mice. A higher fecal bulk output is associated with several benefits ranging from stimulating defecation, distribution of intracolonic pressure and diluting toxins.[17–20] However, no major impact on bile acids or (chole-)sterol balance was found. The overall metabolic response to IMMP feeding, especially on lipid biomarkers such as cholesterol, triglycerides, free fatty acids and bile acids, remained comparable between control and IMMP. The insignificant effect on fermentation and microbial communities in different regions of the intestine in our view may explain the moderate metabolic response to IMMP supplementation. In the context of cholesterol and fatty acid metabolism, the proximal part of the intestine becomes more relevant. A weaker effect in the proximal part of the intestine as observed could potentially also explain the overall weaker metabolic response to IMMP. Previous studies have demonstrated the role of SCFA in regulating pathways associated with lipid and glucose metabolism.[13–15] Therefore, we determined the production of specific SCFA at different time intervals. After three weeks of IMMP supplementation, a significant increase in propionic acid and lactic acid was detected in IMMP-fed animals. While propionic acid has been shown to attenuate lipid biosynthesis in the liver, lactic acid is an intermediate substrate which together with acetic acid synthesizes butyric acid.[21,22] Moreover, lactic acid being a natural ligand for GPR81, exhibits a signaling function leading to inhibition of lipolysis.[23] However, it is important to point out that luminal SCFA shift may not reflect intestinal SCFA uptake by the host or its potential metabolic effect.[24]

Our data seem to underline the role of the host diversity in response to different dietary fibers. Some dietary fibers (IMMP, long-chain and short-chain inulins) induced subtle shifts in fecal bile acid composition with negligible effect on cholesterol metabolism, whereas others (galacto-oligosachharide and β-cyclodextrin) induced significant shifts in fecal bile acid composition and cholesterol metabolism. Such differential responses in hosts are not uncommon in metabolic (dys)regulation. For example, it is known that hypercholesterolemia could either develop due to increased synthesis of cholesterol or due to increased absorption of dietary cholesterol. As a consequence, this might explain why certain drugs such as statins (which supposedly inhibit synthesis) are more effective in some and ezetimibe (which inhibits intestinal

absorption) is more effective in others. Thus, depending on whether the host is a synthesizer or an absorber a differential response to drug treatment can be expected. Similarly, hosts with different intestinal microbial compositions may trigger a specific response to certain types of dietary fibers more than the others. Further studies in disease models and humans would be needed to investigate the, perhaps rather personalized, utilization of dietary fibers.

The intestinal microbiota is a crucial part of the human gut and can exert a direct impact on the (patho)physiology of the metabolic syndrome. Previous reports have indicated that specific diet-microbe-host interactions lead to generation of metabolites such as TMAO in conventional mice. TMAO was shown to directly increase atherosclerosis formation.[25,26] The hallmark of atherosclerotic plaques are macrophage foam cells. Increased levels of apoB-containing lipoproteins in the circulation represent a major cardiovascular risk factor. The reverse cholesterol transport (RCT) pathway on the other hand counteracts the formation of atherosclerosis by stimulating high-density lipoprotein (HDL)-mediated removal of cholesterol from foam cells within the vessel wall.[27] In **chapter 4** we demonstrated the role of the microbiota on the atheroprotective pathway of RCT. We studied the impact of (absence of) microbiota on RCT by comparing conventional and germ-free mice. We found that a complete absence of microbiota has no influence on mass fecal sterol excretion either in the form of neutral sterols or bile acids. To assess *in vivo* RCT, mice were injected with macrophage foam cells loaded with acetylated LDL and ^{3}H-cholesterol. Increased ^{3}H-cholesterol tracer recovery measured in plasma at different times points demonstrated that cholesterol efflux, the first step of RCT, was significantly enhanced in germ-free mice. In feces, tracer recovery was 2-fold higher in germ-free mice. Interestingly, most cholesterol tracer was recovered from the bile acid fraction of the fecal samples despite unchanged fecal bile acid mass excretion suggesting that the overall synthesis was comparable in conventional and germ-free mice.

Liver mRNA expression of Cyp7a1 and of Cyp8b1 which are responsible for the synthesis of bile acids *via* the neutral pathway, were lower in germ-free animals while Cyp27a1 which mediates the alternative acidic pathway was unaltered. Correspondingly, β-MCA, a main product of the alternative pathway was found in higher quantity in the feces of the germ-free compared to the conventional animals. These measurements together with the increased biliary bile acid secretion under germ-free conditions indicated an increasing bile acid cycling. More experimentation would be required to understand the role of intestinal microbiota in regulating pathways associated with cholesterol and bile acid metabolism including conventionalization of germ-free with specific bacterial strains to enhance the effect. Bile acid sequestrants which are used as drugs for lowering cholesterol as it prevents reabsorption of cholesterol and

increases fecal cholesterol excretion, can also be used to further stimulate the effects.[28] The present work was important in demonstrating the impact of gut microbiota on the RCT pathway which has relevance to prevention and treatment of atherosclerosis and CVD.

The dependency of gut microbiota on dietary fibers is poorly understood especially in the context of their beneficial effects via generation of SCFA. What is known is that dietary fibers require gut microbiota for fermentation and generation of bioactive metabolites. However, whether metabolic pathways are partially or entirely dependent on the diet-microbe interaction is not clearly understood. Moreover, the role of dietary fibers in altering the RCT pathway has not been investigated earlier. We hypothesized that ßCD, a non-digestible dietary fiber with cholesterol lowering potential could enhance atheroprotective pathway of RCT. Given that intestinal microbiota mediates fermentation of dietary fibers, the presence or absence of microbiota can modulate the effect of ßCD on RCT. In **chapter 5** we compared germ-free and conventional mice in order to determine the dependency on intestinal microbiota for the proposed lipid modulating effects of ßCD. Our study demonstrated that ßCD supplementation to the diet reduced plasma cholesterol and increased fecal neutral sterol excretion both in germ-free and conventional mice. This observation is partly due to a stimulating effect of the TICE (trans-intestinal cholesterol excretion) pathway. Stimulation of the TICE pathway can increase fecal neutral sterol excretion and thereby enhance cholesterol clearance independent of biliary pathway.[29] We observed in our study that ßCD containing diet stimulated the TICE pathway significantly (about seven-fold) compared to the control diet. However, we also suspect that ßCD partly contributes to the process of clearing cholesterol from the body by forming inclusion complexes with hydrophobic molecules, such as cholesterol.[30] The decreased levels of coprostanol in feces of ßCD-fed conventional mice further substantiates the plausibility of such a hypothesis by indicating the lack of cholesterol availability to the microbiota, which is essential for generation of coprostanol by intestinal bacteria.

Importantly, the RCT study revealed a novel potential anti-atherogenic effect of ßCD supplementation in mice, namely to increase RCT, an observation that was more pronounced in germ-free mice. We measured a higher contribution of bile acids in the recovery of RCT-relevant cholesterol in the feces of the germ-free mice compared to the conventional mice. Both RCT-derived neutral sterol as well as the bile acid excretion contributed to the increase in total recovery of RCT-relevant cholesterol in feces of germ-free mice. The intestinal microbiota plays an important role in converting primary into secondary bile acids. Some secondary bile acids such as DCA, CDCA and CA are known FXR agonists in the liver.[31–33] Thus, increasing the concentration of these bile acids can activate FXR activation in the liver and consequently lower bile

synthesis. In contrast, MCA is an antagonist of FXR and therefore can enhance bile acid synthesis in the liver.[34] This could partially explain the observation made with respect to increased recovery of RCT-relevant cholesterol in fecal bile acids fraction.

Studies in the past have shown cholesterol lowering, anti-atherogenic effects of other kinds of ßCD such hydroxypropryl-ßCD and methyl-ßCD.[35,36] Some studies have used a subcutaneous route for administration while others have investigated *ex-vivo* incubation of human atherosclerotic plaque tissue with ßCD.[32,37] These studies have shown that the atheroprotective effect of hydroxypropryl-ßCD was mediated *via* a LXR-dependent mechanism which resulted in enhanced RCT and expression of anti-inflammatory genes in atherosclerotic plaques. In our study we have demonstrated that the dietary route can effectively increase fecal neutral sterol excretion as well as RCT. Based on our finding future studies are warranted to explore the potential benefits in human trials. Dietary fibers in humans are beneficial supplements in food comprising of high compliance with nutritional recommendations and represent potential additives to standard drug therapies such as statins. Challenges in both animal and human studies would likely revolve around consistency in experimental design especially in terms of the diet, duration and dosage. In such studies intake of cholesterol lowering drugs such as statins and ezetimibe would also provide novel insights as to whether dietary fiber could give an additive therapeutic effect. It remains to be assessed whether individuals with dyslipidemia would experience larger or added benefits. Since ßCD has no known side effects for up to a very high intake of 20% in rats and hamsters, it may offer a potentially viable health supplement to the standard drug therapy used by patients at risk of atherosclerosis.[38,39]

Dietary fibers lower the risk of developing metabolic syndrome

Increased consumption of "Western" diets low on complex dietary fibers has contributed to rising risk of developing metabolic syndrome.[1] In **chapter 6** we demonstrated in wildtype mice fed "Western" diet for a long duration that supplementation with galacto-oligosaccharides (GOS) has the potential to reduce body weight gain and mitigate development of obesity. In addition, GOS supplementation to these mice significantly improved dyslipidemia and insulin sensitivity compared to control group. Previous reports on body weight showed that increased fiber intake is associated with decreased body weight gain which we believe is contributed by lower amounts of adipose tissue.[40–43] Actual body weight loss due to consumption of fiber can be attributed to several factors. Firstly, satiety inducing hormones such as glucogon-like peptide (GLP-1) and peptide YY (PYY) have been shown to increase in animals

administered dietary fibers.[44,45] However, some studies have indicated no effects on satiety.[46–48] In our study, we found that both groups did not differ in food intake in these ad libitum fed mice indicating that GOS did not increase satiety. In terms of lipid profile, GOS was effective in lowering plasma cholesterol and triglycerides levels. Lipoprotein profiles demonstrated that LDL cholesterol tended to be lower in GOS-fed mice compared to the control group. A significant increase was seen in fecal neutral sterol excretion, contributed particularly by higher coprostanol levels, in GOS-fed mice. Therefore, in the human situation where statins are used to promote LDL clearance, supplementation of GOS could prove to be useful in complementing the mainstay of anti-atherosclerotic therapy and thus contribute to normalizing proatherogenic lipoprotein profiles.

A lower body weight gain can be induced *via* SCFA action on energy metabolism. For example, butyrate when administered to obese mice causes loss of body weight which is majorly due to increased energy expenditure and fat oxidation by activating brown adipose tissue (BAT).[49,50] Similar outcomes have been reported with the administration of acetate and propionate in mice fed high-fat diet.[51] In our study, however, unaltered energy expenditure and respiratory exchange ratio in both groups indicated that energy metabolism was comparable despite a somewhat higher *Ucp1* mRNA expression measured in BAT of GOS-fed animals. Interestingly, epididymal and perirenal fat depots were substantially lower in GOS-fed mice compared to the control group. We identified delayed intestinal fat absorption rate and subsequently reduced adipose tissue accumulation with GOS supplementation, which may contribute to its protective effect against high-fat diet induced obesity.

Combined, the experiments in this thesis provide evidence to support dietary fibers and their beneficial effect on preventing or treating metabolic syndrome. Further efforts are needed to understand the mechanism of action of prebiotics either individually or in combinations of pre- *and* probiotics which could help us utilize dietary fibers with a more targeted approach. The use of disease models to gain molecular insights could be particularly useful in identifying specific mechanistic pathways and further substantiating the beneficial effects of dietary fibers. Ultimately the goal is to translate the beneficial effects of dietary fibers into human. Human research is particularly important for studying metabolic effects of dietary fibers given that human physiology encompasses several challenges including differences in intestinal microbiota, host genome and differential metabolic and immune responses. Currently, most efforts of research regarding dietary fibers are geared towards extracting potential health benefits in order to encourage their incorporation into ongoing therapies, promote a healthy lifestyle as well as to reduce the risk of developing metabolic syndrome across the world.

References

1. WHO | Diet, nutrition and the prevention of chronic diseases Report of the joint WHO/FAO expert consultation. *WHO* (2016).
2. Holmes, E. *et al.* Therapeutic modulation of microbiota-host metabolic interactions. *Sci. Transl. Med.* **4,** 137rv6 (2012).
3. Roberfroid, M. *et al.* Prebiotic effects: metabolic and health benefits. *Br. J. Nutr.* **104,** S1–S63 (2010).
4. Gibson, G. R. & Roberfroid, M. B. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J. Nutr.* **125,** 1401–12 (1995).
5. Everard, A. *et al.* Responses of Gut Microbiota and Glucose and Lipid Metabolism to Prebiotics in Genetic Obese and Diet-Induced Leptin-Resistant Mice. *Diabetes* **60,** 2775–2786 (2011).
6. Davis, L. M. G., Martínez, I., Walter, J., Goin, C. & Hutkins, R. W. Barcoded pyrosequencing reveals that consumption of galactooligosaccharides results in a highly specific bifidogenic response in humans. *PLoS One* **6,** e25200 (2011).
7. Fuller, P. *Gut Flora, Nutrition, Immunity and Health.* (John Wiley & Sons, 2008).
8. Domian, I. J., Quon, K. C. & Shapiro, L. Cell type-specific phosphorylation and proteolysis of a transcriptional regulator controls the G1-to-S transition in a bacterial cell cycle. *Cell* **90,** 415–24 (1997).
9. Lynch, J. B. & Sonnenburg, J. L. Prioritization of a plant polysaccharide over a mucus carbohydrate is enforced by a Bacteroides hybrid two-component system. *Mol. Microbiol.* **85,** 478–91 (2012).
10. BÃ©langer, L., Dimmick, K. A., Fleming, J. S. & Charles, T. C. Null mutations in *Sinorhizobium meliloti exoS* and *chvI* demonstrate the importance of this two-component regulatory system for symbiosis. *Mol. Microbiol.* **74,** 1223–1237 (2009).
11. Petrova, O. E. & Sauer, K. The Novel Two-Component Regulatory System BfiSR Regulates Biofilm Development by Controlling the Small RNA rsmZ through CafA. *J. Bacteriol.* **192,** 5275–5288 (2010).
12. Sonnenburg, E. D. *et al.* Specificity of Polysaccharide Use in Intestinal Bacteroides Species Determines Diet-Induced Microbiota Alterations. *Cell* **141,** 1241–1252 (2010).
13. Canfora, E. E., Jocken, J. W. & Blaak, E. E. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat. Rev. Endocrinol.* **11,** 577–591 (2015).
14. De Vadder, F. *et al.* Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. *Cell Metab.* **24,** 151–157 (2016).
15. De Vadder, F. *et al.* Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. *Cell* **156,** 84–96 (2014).
16. den Besten, G. *et al.* Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am. J. Physiol. Gastrointest. Liver Physiol.* **305,** G900–G910 (2013).
17. Nicholson, J. K. *et al.* Host-gut microbiota metabolic interactions. *Science (80-.).* **336,** 1262–1267 (2012).
18. Krishnan, S., Alden, N. & Lee, K. Pathways and functions of gut microbiota metabolism impacting host physiology. *Curr. Opin. Biotechnol.* **36,** 137–45 (2015).
19. Gu, F. *et al.* In vitro fermentation behavior of isomalto/malto-polysaccharides using human fecal inoculum indicates prebiotic potential. *Mol. Nutr. Food Res.* **62,** 1800232 (2018).
20. Davidson, M. H. & McDonald, A. Fiber: Forms and functions. *Nutr. Res.* **18,** 617–624 (1998).
21. Monro, J. A. Faecal bulking index: A physiological basis for dietary management of bulk in the distal colon. *Asia Pac. J. Clin. Nutr.* **9,** 74–81 (2000).
22. Macfarlane, G. & Cummings, J. The colonic flora, fermenta- tion and large bowel digestive function. *large Intest. Physiol. patho- Physiol. Dis.* 51–92 (1991).
23. Schneeman, B. O. Dietary fiber and gastrointestinal function. *Nutr. Res.* **18,** 625–632 (1998).

24. Demigné, C. *et al.* Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. *Br. J. Nutr.* **74,** 209–219 (1995).
25. Nishina, P. M. & Freedland, R. A. Effects of propionate on lipid biosynthesis in isolated rat hepatocytes. *J. Nutr.* **120,** 668–673 (1990).
26. Portugal, L. R. *et al.* Effect of Lactobacillus delbrueckii on cholesterol metabolism in germ-free mice and on atherogenesis in apolipoprotein E knock-out mice. *Brazilian J. Med. Biol. Res.* **39,** 629–635 (2006).
27. den Besten, G. *et al.* The short-chain fatty acid uptake fluxes by mice on a guar gum supplemented diet associate with amelioration of major biomarkers of the metabolic syndrome. *PLoS One* **9,** e107392 (2014).
28. Wang, Z. *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472,** 57–63 (2011).
29. Schiattarella, G. G. *et al.* Gut microbe-generated metabolite trimethylamine-N-oxide as cardiovascular risk biomarker: a systematic review and dose-response meta-analysis. *Eur. Heart J.* **38,** 2948–2956 (2017).
30. Annema, W. & Tietge, U. J. Regulation of reverse cholesterol transport - a comprehensive appraisal of available animal studies. *Nutr. Metab. (Lond).* **9,** 25 (2012).
31. Rosenbaum, D. P., Petersen, J. S., Ducharme, S., Markham, P. & Goldberg, D. I. Absorption, distribution and excretion of GT31-104, a novel bile acid sequestrant, in rats and dogs after acute and subchronic administration. *J. Pharm. Sci.* **86,** 591–5 (1997).
32. Tietge, U. J. F. & Groen, A. K. Role the TICE?: Advancing the Concept of Transintestinal Cholesterol Excretion. *Arterioscler. Thromb. Vasc. Biol.* **33,** 1452–1453 (2013).
33. Trautwein, E. A., Forgbert, K., Rieckhoff, D. & Erbersdobler, H. F. Impact of β-cyclodextrin and resistant starch on bile acid metabolism and fecal steroid excretion in regard to their hypolipidemic action in hamsters. *Biochim. Biophys. Acta - Mol. Cell Biol. Lipids* **1437,** 1–12 (1999).
34. Makishima, M. *et al.* Identification of a nuclear receptor for bile acids. *Science* **284,** 1362–5 (1999).
35. Howard, W. R., Pospisil, J. A., Njolito, E. & Noonan, D. J. Catabolites of cholesterol synthesis pathways and forskolin as activators of the farnesoid X-activated nuclear receptor. *Toxicol. Appl. Pharmacol.* **163,** 195–202 (2000).
36. Parks, D. J. *et al.* Bile acids: natural ligands for an orphan nuclear receptor. *Science* **284,** 1365–8 (1999).
37. Hu, X., Bonde, Y., Eggertsen, G. & Rudling, M. Muricholic bile acids are potent regulators of bile acid synthesis via a positive feedback mechanism. *J. Intern. Med.* **275,** 27–38 (2014).
38. Atger, V. M. *et al.* Cyclodextrins as catalysts for the removal of cholesterol from macrophage foam cells. *J. Clin. Invest.* **99,** 773–80 (1997).
39. Liu, S. M. *et al.* Cyclodextrins differentially mobilize free and esterified cholesterol from primary human foam cell macrophages. *J. Lipid Res.* **44,** 1156–66 (2003).
40. Zimmer, S. *et al.* Cyclodextrin promotes atherosclerosis regression via macrophage reprogramming. *Sci. Transl. Med.* **8,** 333ra50 (2016).
41. Férézou, J. *et al.* Hypocholesterolemic action of beta-cyclodextrin and its effects on cholesterol metabolism in pigs fed a cholesterol-enriched diet. *J. Lipid Res.* **38,** 86–100 (1997).
42. Riottot, M. *et al.* Hypolipidemic effects of beta-cyclodextrin in the hamster and in the genetically hypercholesterolemic Rico rat. *Lipids* **28,** 181–8 (1993).
43. Tucker, L. A. & Thomas, K. S. Increasing Total Fiber Intake Reduces Risk of Weight and Fat Gains in Women. *J. Nutr.* **139,** 576–581 (2009).
44. Brockman, D. A., Chen, X. & Gallaher, D. D. Hydroxypropyl methylcellulose, a viscous soluble fiber, reduces insulin resistance and decreases fatty liver in Zucker Diabetic Fatty rats. *Nutr. Metab. (Lond).* **9,** 100 (2012).

45. Blom, W. A. M. *et al.* Effects of gastric emptying on the postprandial ghrelin response. *Am. J. Physiol. Metab.* **290,** E389–E395 (2006).
46. van Nieuwenhoven, M. A., Kovacs, E. M., Brummer, R. J., Westerterp-Plantenga, M. S. & Brouns, F. The effect of different dosages of guar gum on gastric emptying and small intestinal transit of a consumed semisolid meal. *J. Am. Coll. Nutr.* **20,** 87–91 (2001).
47. Brubaker, P. L. The Glucagon-Like Peptides: Pleiotropic Regulators of Nutrient Homeostasis. *Ann. N. Y. Acad. Sci.* **1070,** 10–26 (2006).
48. Plaisancié, P., Dumoulin, V., Chayvialle, J. A. & Cuber, J. C. Luminal peptide YY-releasing factors in the isolated vascularly perfused rat colon. *J. Endocrinol.* **151,** 421–9 (1996).
49. van der Beek, C. M. *et al.* The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men. *Metabolism* **87,** 25–35 (2018).
50. Belobrajdic, D. P., King, R. A., Christophersen, C. T. & Bird, A. R. Dietary resistant starch dose-dependently reduces adiposity in obesity-prone and obesity-resistant male rats. *Nutr. Metab. (Lond).* **9,** 93 (2012).
51. Keenan, M. J. *et al.* Effects of Resistant Starch, A Non-digestible Fermentable Fiber, on Reducing Body Fat*. *Obesity* **14,** 1523–1534 (2006).
52. Gao, Z. *et al.* Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* **58,** 1509–17 (2009).
53. Li, Z. *et al.* Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. *Gut* **67,** 1269–1279 (2018).
54. den Besten, G. *et al.* Short-Chain Fatty Acids Protect Against High-Fat Diet–Induced Obesity via a PPARγ-Dependent Switch From Lipogenesis to Fat Oxidation. *Diabetes* **64,** 2398–2408 (2015).