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Food for microbes. The interplay between indigestible carbohydrates, gut microbiota, and cardiometabolic disease

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SUMMARY

Cardiometabolic disease such as obesity, type 2 diabetes, and atherosclerosis, are a leading cause of morbidity and mortality in the Western world. Two important risk factors for the development of cardiometabolic disease are hyperlipidemia and inflammation. Recently, evidence strongly indicates a role for the gut microbiota in the development of cardiometabolic disease. Therapeutic approaches are therefore aimed at modifying the gut microbiota composition and function to beneficially affect the development of cardiometabolic disease and its underlying risk factors. A potential candidate to modify gut microbiota composition are indigestible carbohydrates, or prebiotics. In this thesis, we aimed to understand the interplay between various indigestible carbohydrates, gut microbiota composition and function, and the development of obesity, type 2 diabetes, and atherosclerosis.

Chapter 1 serves as a general introduction in which hyperlipidemia and inflammation are introduced as the two main risk factors for cardiometabolic disease. More specifically, the role of the gut microbiota composition, function, and dysfunction (or dysbiosis) will be discussed as modifiable risk factors in the development of cardiometabolic disease. A tool to modify the gut microbiota composition and function and dietary interventions with indigestible carbohydrates are discussed in further detail. Finally, the importance of methods to quantify gut microbiota function is illustrated.

Since we exploit the use of high fat and high cholesterol diets in the development of cardiometabolic disease, it is important to have the ability to determine blood lipid composition. As the various fatty acids play distinct roles in health and disease, methods that can specifically determine the fatty acid profile are needed for fundamental and clinical studies. **Chapter 2** describes a method to determine the medium- and long chain fatty acid composition of blood of mice using gas chromatography-mass spectrometry (GC-MS) analysis. This method quantitatively monitors fatty acid composition using a combination of pentafluorobenzyl bromide (PFBBBr) derivatization, internal standards (IS), and electron-capture negative ionisation (ECNI) in a comprehensive, sensitive, and accurate manner.

In addition, we explore the use of indigestible carbohydrates to modulate microbiota composition and function. Microbial function can be determined by measuring the products

after microbial fermentation of indigestible carbohydrates, short-chain fatty acids (SCFAs).

Chapter 3 describes a method to determine SCFAs in blood, cecum, and feces samples using GC-MS analysis. By applying the combination of PFBBBr derivatization, IS, and ECNI, this method represents a fast, reliable, and reproducible method for the separation and quantification of SCFAs in various mouse-derived samples which can be further exploited for quantification of SCFAs in human studies.

In **chapter 4**, we studied the effect of the indigestible carbohydrate and prebiotic inulin on accelerated atherosclerosis development after placement of a perivascular cuff around the femoral artery of the mice. Previous studies indicated a beneficial role of inulin on inflammation and hyperlipidemia. However, the effect of inulin on atherosclerosis development has not been extensively studied yet. Male APOE*3-Leiden (*E3L*) mice were fed a high-cholesterol diet without or supplemented with inulin for 5 weeks and underwent perivascular cuff surgery in week 3 of the experiment. The combination of this well-established mouse model for human-like lipid metabolism and perivascular cuff placement around the femoral artery, enables us to specifically study the short-term effect of inulin on inflammatory-driven atherosclerosis development. In contrast to our hypothesis, inulin aggravated accelerated atherosclerosis development in these mice, which was accompanied by adverse lesion composition and outward vascular remodelling. Inulin did not affect blood monocyte composition, suggesting that the aggravated atherosclerosis development was driven by the significantly increased plasma cholesterol levels.

In **chapter 5**, we shifted our focus from short-term effects of inulin on atherosclerosis development to long-term atherosclerosis development in a lipid-driven atherosclerotic mouse model. Female APOE*3-Leiden.CETP (*E3L.CETP*) mice were fed a moderate high (0.1%) or high (0.5%) cholesterol diet without or supplemented with inulin for 11 weeks. By combining the use of female *E3L.CETP* mice and different cholesterol-enriched diets, we were able to specifically study the long-term effects of inulin on lipid-driven atherosclerosis development. Inulin combined with a high cholesterol diet clearly showed prebiotic activity, but did not affect plasma cholesterol levels or atherosclerosis development. Surprisingly, inulin combined with

a high (0.5%) cholesterol diet resulted in mild hepatic inflammation. Inulin with a moderate high (0.1%) cholesterol diet did not result in liver inflammation. It was therefore concluded that, although inulin is widely acknowledged as a prebiotic with favourable effects on lipid metabolism and cardiovascular disease, inulin clearly not always exerts beneficial effects.

In **chapter 6**, we switched our attention from a well-known prebiotic to the relatively unknown indigestible carbohydrate MOS, which have great potential to modify gut microbiota composition, inflammation, and hyperlipidemia. MOS have proven effective at improving growth performance, while also reducing inflammation and hyperlipidemia. However, beneficial effects of MOS on inflammation have been shown mainly in the intestines. As obesity is associated with chronic low-grade inflammation that predominantly manifests in extra-intestinal adipose tissue, we aimed to determine the effect of MOS on inflammation in mesenteric white adipose tissue (mWAT) and liver. In addition, we determined the effect of MOS on whole-body glucose tolerance in both lean and high-fat diet (HFD)-induced obese mice. It was found that MOS slightly altered immune cell composition in mWAT and liver of lean mice, but MOS did not ameliorate HFD-induced glucose intolerance or inflammation. Our data therefore indicate extra-intestinal modulatory properties of MOS on immune composition as reported in previous studies. However, the effects are relatively modest.

MOS have proven effective at improving growth performance, while also reducing hyperlipidemia and inflammation in livestock. As atherosclerosis is accelerated both by hyperlipidemia and inflammation, **chapter 7** describes the effect of dietary MOS on atherosclerosis development in hyperlipidemic *E3L.CETP* mice. Mice were fed a high cholesterol diet, with or without MOS for 14 weeks. This study revealed that MOS decreased the onset of atherosclerosis development, via lowering of plasma cholesterol levels. Furthermore, MOS modified the gut microbiota composition and function as was observed by increased cecal butyrate levels and fecal bile acid (BA) excretion. We therefore concluded that MOS presumably decreased atherosclerosis development and lowered plasma cholesterol levels via interactions with the gut microbiota.

In **chapter 8**, the results of this thesis and the value of our research regarding

methods to map gut microbiota composition and function, SCFAs as a marker for gut microbial function, factors that determine gut microbiota function, the role of the gut microbiota in the development of atherosclerosis, the translatability of mouse models in gut microbiota research, and implications for prebiotics are discussed. Taken together, the studies described in this thesis increased our knowledge on the potential of various indigestible carbohydrates in the modulation of the gut microbiota to affect the development of cardiometabolic disease, suggesting a promising strategy to further pursue with some caution.