Interplay between dietary fibers and gut microbiota for promoting metabolic health

Mistry, Rima

SUMMARY

Metabolic syndrome is a cluster of several metabolic disorders such as insulin resistance, obesity, type 2 diabetes, dyslipidemia, non-alcoholic fatty liver disease and hypertension, whose prevalence has rapidly increased over the last decades. Unhealthy dietary and lifestyle changes have contributed considerably to this rise. Gastrointestinal microbiota has received increasing interest as a preventive or therapeutic target particularly for its impact on the host metabolism. Dietary fibers are substrates for growth of specific beneficial bacterial species and as a result can shift the microbial population towards a (more) healthy composition. Bacterial fermentation products of dietary fibers are bioactive metabolites such as short-chain fatty acids (SCFA) which can regulate various metabolic processes in the mammalian host. Despite the potential impact of dietary fibers on intestinal microbiota and host metabolism, the underlying mechanism of action has not been elucidated in great depth. In this thesis we aimed to explore the role of different dietary fibers in manipulating gastrointestinal microbiota and subsequently its impact on metabolic health in mouse models. In the first part of the thesis (**chapter 2 and 3**) we focused on in-depth investigation of dietary fibers and its potential impact on various metabolic parameters. In **chapter 4 and 5** we investigated the importance of gastrointestinal microbiota in atheroprotective pathways and whether dietary fiber such β-cyclodextrin could enhance the pathway, using germ-free mice. In **chapter 6** we assessed the long-term metabolic health effects of supplementing galacto-oligosaccharide to Western-type diet.

Inulin, which is a widely used model dietary fiber, has been shown to lower plasma cholesterol in mice. However, some conflicting reports have also emerged on the effects of inulin on cholesterol homeostasis. Several factors contribute to differential effects including chain-lengths of the non-digestible oligosaccharides. In **chapter 2** we investigated the effects of two inulins with different chain-lengths. Wildtype C57BL/6 mice were fed diets supplemented with short- or long-chain inulin (sc and lc; 10%, w/w), after which we determined cholesterol fluxes in the intestine and other metabolic parameters. Most studies have implicated SCFA such as acetate, butyrate

and propionate in lipid metabolism. However, not all SCFA are associated with positive effect on host physiology. Particularly, acetate, for example, is a precursor for cholesterol and fatty acid synthesis and can exert negative effects by elevating cholesterol levels. Our study indicated that sc- and lc-chain inulin feeding substantially increased fecal SCFA levels compared with controls. However, the elevation of SCFA did not result in significant changes in plasma or liver lipid levels. Overall, no adverse effects were measured in mice fed the inulins with respect to intestinal cholesterol absorption, fecal cholesterol excretion or trans-intestinal cholesterol excretion.

Furthermore, we were interested in investigating novel classes of dietary fibers which have shown prebiotic potential in *in vitro* experiments. Isomalto/malto-polysaccharides (IMMP) are one such dietary fibers which have been shown to stimulate growth of *Bifidobacterium* and *Lactobacillus* when incubated with human fecal inoculum. In **chapter 3** we explored in an *in vivo* experiment the potential role of IMMP in modulating cholesterol and bile acid metabolism. Our results indicated that IMMP supplemented (10% w/w) toe diet of C57BL/6 wild-type mice for a period of three weeks increased mass fecal output. However, the overall metabolic response to IMMP feeding remained insignificant with respect to cholesterol metabolism.

Gastrointestinal microbiota is significantly altered in individuals suffering from metabolic diseases. The plasma cholesterol concentration is one of the major surrogate markers for the risk of cardiovascular diseases and preventing the accumulation of cholesterol in the body one of the major therapeutic targets. High-density lipoprotein (HDL) is responsible for transport of cholesterol back to the intestine *via* reverse cholesterol transport pathway (RCT). In **chapter 4** we investigated the role of gastrointestinal microbiota in modulating the atheroprotective pathway of RCT. Our results indicated that depletion of gastrointestinal microbiota can enhance RCT. The significant increase in RCT occurred mainly *via* the fecal disposal of cholesterol in the form of bile acids. In germ-free mice, a significant elevation was seen in alternative bile acid synthesis pathway in the liver. This elevation leads to a higher conversion of macrophage-derived cholesterol into tauro-beta-muricholic acid (T-β-MCA), which is eventually excreted into the feces. In our view, this mechanism explains the major contribution of the bile acid fraction observed in increased RCT in germ-free mice.

Dietary fibers which can modulate cholesterol in the body can also counteract cholesterol accumulation *via* RCT. This could be of particular importance in the light of the relatively modest decline in cardiovascular related events obtained by cholesterol-lowering drugs such as statins. Exploring a potentially additive role of nutritional supplements to existing therapies is therefore desirable. β-cyclodextrin is one such dietary fiber with cholesterol modulating properties. In **chapter 5** we investigated the role of β-cyclodextrin in regulating RCT in mice. Our data demonstrated that

β-cyclodextrin supplementation (10% w/w) in mice significantly enhanced RCT. The outcome is partly due to increased trans-intestinal cholesterol excretion which leads to enhanced cholesterol clearance from the body, independent of the biliary pathway. β-cyclodextrin associates with hydrophobic molecules such as cholesterol and may thereby contribute to decreased cholesterol (re-)absorption and to increased fecal cholesterol excretion. The plausibility of this mechanism is further supported by the lower fecal coprostanol measured in β-cyclodextrin fed mice. Coprostanol is a bacterial product of cholesterol. In addition, our study showed that depletion of intestinal microbiota can augment β-cyclodextrin stimulated RCT. Our finding demonstrated β-cyclodextrin as a dietary fiber with hypercholesterolemic potential which can be used to decrease the risk of developing atherosclerosis. Further studies would be required to explore its benefits as a viable health supplement and or as an additive to standard drug therapies in humans.

Growing consumption of 'Western-type' diets and sedentary lifestyle increase the risk of developing metabolic syndrome. Emerging evidence also associates the risk with a shift in the intestinal microbiota composition induced by Western style diets. Dietary fibers such a galacto-oligosaccharides (GOS) have been shown to stimulate the growth of beneficial gastrointestinal bacteria. In **chapter 6** we investigated long-term metabolic effects of GOS supplementation (7%, w/w) to Western-type diet in mice. Our study showed that GOS supplementation can significantly reduce body weight gain and mitigate development of obesity. Mice fed GOS supplemented diet also showed improvements in dyslipidemia and insulin sensitivity. In addition, we identified delayed intestinal fat absorption and increased intestinal GLP-1 expression as a potential mechanism of action responsible for the lower body weight gain. GLP-1, a satiety signaling hormone have been reported to decrease intestinal chylomicron production *via* a brain gut axis. GLP-1 secretion can be triggered by SCFA and bile acids such as hyodeoxycholic acid which was significantly elevated in GOS fed mice, lending further possibility to the proposed mechanism. Marked increase in beneficial bacterial strains such as *Bifidobacterium* and *Akkermansia* in GOS supplemented group was also observed. Combined our data supports the use of GOS as an attractive dietary supplement in the preventions or treatment of metabolic syndrome related disease risk.

This thesis provides data to further our current understanding of dietary fibers and its gastrointestinal microbiota modulating properties and consequently its role in targeting metabolic syndrome. Given that human physiology is more complex and challenging leading to diverse metabolic response, further efforts in human studies are now required. The ultimate goal would be to identify specific mechanistic pathways utilized by individual dietary fibers which would allow their preventive and/or therapeutic use in a more targeted way.