Targeting inter-organ cross-talk in cardiometabolic diseases

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SUMMARY

Cardiometabolic health is tightly controlled by a complex network of organ communication. Dysfunction of these lines of communication is associated with the development of cardiometabolic diseases, indicating inter-organ cross-talk as a therapeutic target. In this thesis, I explored the therapeutic potential of targeting inter-organ communication in cardiometabolic diseases including obesity, atherosclerotic cardiovascular disease and non-alcoholic steatohepatitis (NASH), based on which I proposed novel therapies to tackle these diseases. **Chapter 1** provides a general introduction on inter-organ cross-talk as a gatekeeper for cardiometabolic health. It explains that various metabolic organ systems can communicate with one another to regulate whole-body metabolic processes by producing signaling molecules, such as peptide/protein hormones, bioactive lipids and functional small molecules.

Gut microbiota-derived metabolites signal to various metabolic tissues and organs in the body, which is part of the cross-talk between the gut and the host and influences cardiometabolic health of the host. For example, gut microbiota metabolize dietary choline into trimethylamine (TMA) that is delivered via the portal vein to the liver where hepatocytes rapidly oxidize TMA by flavin monooxygenases into trimethylamine-N-oxide (TMAO). Studies conducted in *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice showed that TMAO aggravates atherosclerosis by promoting formation of foam cells and activating the inflammatory response. In contrast, the gut microbiota-derived bioactive molecule butyrate has been shown to beneficially modulate the gut microbiota and exert anti-inflammatory and antiatherogenic properties in the same mouse models. Therefore, in **Chapter 2**, we aimed to investigate whether butyrate can alleviate choline-induced atherosclerosis. To this end, we used *APOE*3-Leiden.CETP* mice, a well-established atherosclerosis-prone model with human-like lipoprotein metabolism, and fed these mice with an atherogenic diet alone or supplemented with choline, butyrate or their combination. Interestingly, we observed that choline protected against body fat mass gain, increased the abundance of anti-inflammatory gut microbes, and increased the expression of gut microbial genes involved in TMA and TMAO degradation. Butyrate similarly attenuated fat mass gain and beneficially modulated the gut microbiome, as shown by increased abundance of anti-inflammatory and short chain fatty acid (SCFA)-producing microbes, and inhibited expression of gut microbial genes involved in lipopolysaccharide synthesis. Both choline and butyrate upregulated hepatic expression of flavin monooxygenases, and their combination resulted in highest circulating TMAO levels. Nonetheless, choline, butyrate and their combination did not influence atherosclerosis development, and TMAO levels were not associated with

atherosclerotic lesion size. These data, obtained in a mouse model relevant to human cardiometabolic diseases, may suggest that TMAO lacks atherogenic properties in humans.

Studies in e.g. diet-induced obese (DIO) and *ob*/*ob* mice have linked increased dietary choline consumption also to increased incidence of obesity. However, our study described above and several clinical trials have observed anti-obesity effects of high dietary choline intake. To understand the underlying mechanisms by which choline attenuates obesity in a human-like setting, in **Chapter 3** we aimed to explore the effect of high dietary choline consumption on adiposity by using *APOE*3-Leiden.CETP* mice. We observed that dietary choline reduced body fat by activating brown adipose tissue (BAT), resulting in accelerated triglyceride-rich lipoprotein (TRL) turnover to improve hypercholesterolemia. Besides, choline ameliorated liver steatosis and damage, which was associated with an upregulation of hepatic genes involved in fatty acid oxidation. These data thus provide a mechanistic basis for the observation in human intervention trials that high choline intake is linked with reduced body weight.

Interestingly, recent clinical studies have reported that the narcolepsy drug γ-hydroxybutyric acid (GHB), a SCFA that is structurally similar to butyrate, promotes weight loss via unknown mechanisms. Narcolepsy is a clinical condition of severely disturbed sleep that causes an increase in body weight after disease onset, frequently leading to obesity. Despite being clinically used in the treatment of narcolepsy, GHB is unlikely to be prescribed as anti-obesity drug due to its central effects and its misuse-associated adverse effects (e.g. severe respiratory depression). Nonetheless, elucidating the underlying mechanisms by which GHB reduces body weight may reveal therapeutic handles for the development of effective body weight loss medications. Thus, in **Chapter 4**, we investigated the effect of oral GHB treatment on body weight control in high fat diet (HFD)-induced developing and existing obesity by using C57BL/6J mice. In existing obesity, but not in developing obesity, GHB attenuated HFD-induced fat mass gain, glucose intolerance and insulin resistance. In contrast, GHB alleviated HFD-induced hepatic steatosis and inflammation in both metabolic conditions. This was accompanied by improvement of hepatic mitochondrial dysfunction, as evidenced by upregulated hepatic expression of genes encoding mitochondrial respiratory complex. Accordingly, in developing obesity, GHB alleviated the accumulation of toxic sphingolipids both in the liver and in the circulation. In existing obesity, GHB prevented hepatic loss of retinoids and increased circulating acyl-carnitine, a substrate for combustion by BAT. Consistently, GHB alleviated HFD-induced adipose tissue dysfunction in obese mice, as evidenced by increased uncoupling protein 1 (UCP-1) abundance in BAT and decreased white adipocyte size and white adipose tissue (WAT) inflammation. Moreover, GHB

beneficially influenced the gut microbial composition, as shown by an enrichment of SCFA producers in developing obesity, and anti-inflammatory and succinate-producers in existing obesity. Taken together, GHB promotes metabolic health in developing and existing obesity, which is associated with improved hepatic mitochondrial function and likely involves beneficial modulation of the gut microbial composition. These findings thus uncover previously unknown metabolic effects of GHB related to body weight management, and provide novel insights for new therapeutic handles for treating obesity and its related diseases.

Besides gut-microbiota-associated signaling molecules, various hepatokines produced by the liver can also modulate whole-body metabolic control. A very interesting hepatokine is fibroblast growth factor 21 (FGF21), given that FGF21 analogues are in clinical development to treat obesity and type 2 diabetes. Although their glucose-lowering and insulin sensitizing effects have been largely unraveled, the mechanisms by which they alleviate liver injury have been scarcely addressed. In **Chapter 5**, we unveiled the mechanisms underlying the protective effects of FGF21 on NASH, again using *APOE*3-Leiden.CETP* mice. Liver-specific FGF21 overexpression was achieved in mice using an adeno-associated virus, followed by administration of a high-fat high-cholesterol diet for 23 weeks. We observed that hepatic FGF21 overexpression limited hepatic lipid influx and accumulation through combined endocrine and autocrine signaling, respectively, which prevents Kupffer cell activation and lowers the presence of lipid- and scar-associated macrophages to inhibit fibrogenesis. These findings provide mechanistic insight that further strengthens the therapeutic potential of FGF21 for treatment of NASH and support currently ongoing clinical trials evaluating the impact of long-acting FGF21 on NASH. In **Chapter 6**, we next investigated the importance of FGF21 in other aspects of cardiometabolic health, particularly in lipoprotein metabolism in relation to atherogenesis, by administration of a long-acting recombinant FGF21 to *APOE*3-Leiden.CETP* mice fed an atherogenic diet. We observed that FGF21 treatment reduced plasma total cholesterol levels, explained by a reduction of plasma levels of non-high-density lipoprotein (non-HDL)-cholesterol. Mechanistically, FGF21 promoted BAT activation and WAT browning, thereby enhancing the selective uptake of fatty acids from TRLs into BAT and into beige WAT, consequently accelerating the clearance of the cholesterol-enriched TRL remnants by the liver. In addition, FGF21 reduced body fat, ameliorated glucose tolerance and reduced hepatic steatosis, related to upregulated hepatic expression of genes involved in fatty acid oxidation and increased very-low density lipoprotein (VLDL)-triglyceride secretion. Ultimately, FGF21 largely decreased atherosclerotic lesion area, which was mainly explained by the reduction in non-HDL-cholesterol, as shown by linear regression analysis, decreased lesion severity, and increased atherosclerotic plaque stability index. We have thus provided additional

support for the clinical use of long-acting FGF21 in the treatment of atherosclerotic cardiometabolic diseases.

Finally, in **Chapter 7**, the results of this thesis are placed in the context of the current scientific literature, and novel strategies to combat cardiometabolic disease associated with inter-organ cross-talk disturbance are discussed. In summary, this thesis provides novel insight into targeting gut microbiota- and liver-centered inter-organ cross-talk in the treatment of cardiometabolic diseases. First of all, dietary interventions remain the most effective strategy for modulating the gut microbiota-centered inter-organ communication, and dietary choline and butyrate have beneficial impact on the gut microbiota composition and function and body weight control. In addition, hepatocyte mitochondrial function governs whole-body metabolism through orchestrating local and systemic metabolic substrate metabolism, and hepatocyte mitochondria-targeted therapy holds great promise for combating cardiometabolic diseases. Last but not least, we propose hepatokine FGF21-based pharmacotherapy as a promising strategy for the treatment of fibrotic NASH and atherosclerotic CVD. Thus, the findings described in this thesis emphasize the importance of inter-organ cross-talk for cardiometabolic diseases, and have improved our knowledge on the mechanisms that underlie the risk in the ever-increasing population of individuals who suffer from cardiometabolic diseases.