Targeting adipose tissue to improve cardiometabolic health

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Summary

The broad availability of high-caloric food in modern society has led to a high prevalence of obesity and obesity-associated diseases, including type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD) and cardiovascular diseases (CVDs). CVDs, with atherosclerosis as a major underlying pathology, are the current leading cause of death worldwide, and available cholesterol-lowering medication is only capable of preventing one third of atherosclerotic cardiovascular events. The residual risk is at least partly explained by high circulating triglycerides (TGs) within TG-rich lipoprotein (TRL) remnants. Although lifestyle changes are a suitable treatment option to lower TGs, long-term adherence hereto is often challenging and not always feasible. Novel strategies that improve lipid metabolism beyond the effects of classical cholesterol-lowering medication are therefore warranted to further decrease atherosclerotic CVD risk.

Chapter 1 serves as a general introduction, in which I first described the mechanisms that drive atherosclerosis development including the role of lipids and inflammation. Available therapeutic strategies to attenuate risk for atherosclerosis were also discussed, as well as how improving the lipid buffering capacity of white adipose tissue (WAT) and stimulating the thermogenic activity of WAT and brown adipose tissue (BAT) might contribute to an anti-atherogenic lipid profile. In addition, I discussed the use of animal models to study novel strategies to improve lipid metabolism and cardiometabolic health by targeting WAT and BAT.

In chapter 2, I investigated the possibility of targeting the endocannabinoid system (ECS) to improve lipid metabolism and atherosclerosis, as previous research suggested that signaling via the cannabinoid type 1 receptor (CB1R) might suppress adrenergic stimulation of thermogenic activity in BAT and WAT. Treatment of female APOE*3-Leiden.CETP mice that were fed a cholesterol-rich diet, a well-established mouse model for human-like lipid metabolism and atherosclerosis development, with the CB1R inverse agonist rimonabant promoted browning of WAT, and stimulated the uptake of TG-derived fatty acids (FAs) by WAT. The increase in lipolytic activity in adipose tissue was coupled to accelerated hepatic uptake of TRL remnants. In combination with a decrease in hepatic very-low-density lipoprotein (VLDL)-TG production, this resulted in improved dyslipidemia and less atherosclerosis development. While rimonabant itself will most likely not be re-introduced in the clinic as related to previously observed side effects, this study provides proof-of-concept that inhibiting the ECS is a valuable strategy to lower atherosclerosis development. Peripherally restricted CB1R blockage, or inhibition of endocannabinoid synthesis enzymes may prove to be safe alternatives in the future.

Glucagon-like peptide-1 receptor (GLP1R) agonism is a viable strategy to activate BAT, and we hypothesized that its efficacy could be increased by inducing a timely flux of FAs towards the BAT. Since glucose-dependent insulinotropic polypeptide receptor (GIPR) agonism increases the uptake of lipids by WAT in the postprandial state while stimulating the release of FAs from WAT by increasing intracellular lipolysis in the fasted state, in chapter 3 we investigated the effects of combined GIPR and GLP1R agonism on atherosclerosis development in female APOE*3-Leiden.CETP mice fed a cholesterol-rich diet. Combined GIPR/GLP1R agonism decreased atherosclerosis severity, while treatment with the individual agonists showed only non-significant improvements. Mechanistically, the combined treatment strongly reduced plasma TG levels by increasing TG-derived FA uptake by BAT and WAT, coupled to an increased uptake of VLDL core remnants by the liver, and by decreasing hepatic VLDL-TG production. Strikingly, both agonists also lowered markers of systemic low-grade inflamma-

tion, which may be the result of a direct effect on immune and endothelial cells. Because the APOE*3-Leiden.CETP mouse is a well-established model for human lipoprotein metabolism and atherosclerosis development, these data suggest that combined GIPR/GLP1R agonism may be a promising strategy to lower cardiometabolic risk in humans as well.

Given the actions of GIPR and GLP1R agonism on both lipid metabolism and inflammation, in chapter 4 we hypothesized that combined GIPR/GLP1R agonism could also be an effective strategy to counteract NAFLD development. To study this, male APOE*3-Leiden.CETP mice were fed a diet rich in cholesterol and fat, and treated with a GIPR agonist, a GLP1R agonist, or both agonists combined. Combined GIPR/GLP1R agonism additively lowered hepatic steatosis as evidenced by a reduced hepatic lipid content and hepatocellular hypertrophy, and decreased macrovascular and microvascular steatosis. Mechanistically, combined GIPR/GLP1R agonism lowered food intake and increased fecal energy excretion, which likely reflects reduced intestinal FA absorption caused by reduced fecal bile acid excretion. In line with the results of the experiments described in chapter 3, combined GIPR/GLP1R agonism also increased TG-derived FA uptake by BAT. The combined treatment furthermore reduced hepatic inflammation, as evidenced by a lowered frequency of monocyte-derived (pre-)Kupffer cells in the liver, which coincided with a decreased hepatic expression of genes encoding chemoattractants. Overall, these data suggest that combined GIPR/GLP1R agonism is a promising strategy to attenuate NAFLD development in addition to attenuating atherosclerosis development.

We anticipated that insight into the mechanisms underlying the naturally occurring day-night rhythm in BAT could provide insight into how to optimally target BAT. Therefore, in chapter 5, BAT samples of wild type C57BL/6J mice were collected at 3-hour intervals throughout a 24-hour period to perform RNA-sequencing. The oscillating genes that were in synchrony with metabolic BAT activity were enriched in genes involved in (lipid) catabolic processes. This coincided with peak expression of lipoprotein lipase (*Lpl*), encoding the enzyme responsible for the liberation of FAs from TGs, and was predicted to be driven by peroxisome proliferator-activated receptor gamma (PPARγ) activity. Using chromatin immunoprecipitation (ChIP)-sequencing in the same BAT samples, we correspondingly observed enrichment in (lipid) catabolic processes only among the subset of these genes that showed oscillated PPARγ binding. We also identified oscillated binding of PPARγ to *Lpl*, peaking just prior to the peak in *Lpl* gene expression. Interestingly, of the known LPL modulators, expression of the inhibitor angiopoietin-like 4 (*Angptl4*) showed a strong diurnal oscillation, in a phase opposite to *Lpl*. In subsequent mechanistic experiments using *Angptl4* knockout and overexpression mouse models, we showed that ANGPTL4 modulation flattens oscillation of LPL activity and TG-derived FA uptake by BAT within the range of its physiological day-night rhythm. Taken together, these data suggest involvement of PPARγ and a critical role of ANGPTL4 in mediating the day-night rhythm in TG-derived FA uptake by BAT, and imply that targeting PPARγ or inhibiting ANGPTL4 at the onset of the resting phase may be clinically most relevant for stimulating TG-derived FA uptake by BAT.

Whilst we showed that activating thermogenic activity in WAT and BAT coincides with an anti-atherogenic lipid profile in APOE*3-Leiden.CETP mice (see also chapters 2-4), others showed that BAT activation by cold or the β3-adrenergic receptor agonist mirabegron in ApoE and LDLR knockout mice aggravates, rather than attenuates, atherosclerosis development. We suspected, however, that the latter results were artifacts of the mouse models used, and do not represent the human situation. In chapter 6 we therefore performed a similar experiment with mirabegron in female APOE*3-Leiden.CETP mice. Mirabegron stimulated the uptake

of TG-derived FAs from TRLs by BAT and WAT, which was coupled to an increased hepatic uptake of TRL remnants. Mirabegron also increased the hepatic VLDL production, likely as a result of a higher flux of FAs from the WAT towards the liver. The combination of these two effects of mirabegron led to a transient increase in plasma TGs, but a strong reduction in plasma TGs thereafter. Importantly, mirabegron tended to lower plasma cholesterol levels and attenuate atherosclerosis development. We suggest that the observed pro-atherogenic effects of mirabegron in the ApoE and LDLR knockout mice are the result of complete abolishment of the ApoE-LDLR clearance pathway that is essential for the hepatic uptake of cholesterol-enriched TRL remnants after delipidation by thermogenic adipocytes. Overall, our data emphasize the critical importance of the choice of mouse model when studying BAT-targeted interventions in relation to lipoprotein metabolism and atherosclerosis development.

In order to improve the experimental design of studies in mice beyond the choice of mouse model, in chapter 7, I described the development of a novel software tool that I have named RandoMice. RandoMice is a user-friendly software tool that allows researchers at the start of an (animal) experiment to easily randomly divide mice, or other experimental units, into experimental groups of predefined sizes. Because from an ethical perspective the sample size in animal experiments should be kept at a minimum, RandoMice can also take randomization one step further by taking baseline variables and covariates into account when dividing the experimental units into groups. This allows users to identify random group divisions that are well-balanced for the provided baseline characteristics, as was also done in chapter 3, 4 and 6, thereby reducing the risk of bias.

Finally, in chapter 8 the results of the experiments described in this thesis were appraised within the context of current scientific literature. Limitations and translational challenges of these findings were discussed, and future prospective was addressed. Taken together, the results described in this thesis increased our insight into the mechanisms underlying the beneficial effects of ECS modulation and combined GIPR/GLP1R agonism on lipid metabolism and affirmed the therapeutic potential of these strategies to attenuate atherosclerotic CVD and/or NAFLD risk. The results also provided insight into the mechanisms underlying the day-night rhythm in BAT, and furthermore emphasize the critical importance of the choice of mouse model when studying BAT-targeted interventions, as well as the necessity of utilizing randomization in animal studies.