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Title: Central nervous system control of triglyceride metabolism

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In our Western society, type 2 diabetes mellitus (T2DM) has reached epidemic proportions. T2DM is hallmarked by insulin resistance, pancreatic β -cell dysfunction and glucose intolerance, and is associated with various disturbances in plasma lipid and lipoprotein metabolism. These disturbances include elevation of plasma (V)LDL and/or triglyceride (TG) levels, which are often accompanied by reduction of HDL levels, collectively termed diabetic dyslipidemia. Plasma TG levels are determined by the balance between intestinal and hepatic TG production and TG uptake from the plasma by peripheral organs that subsequently either store or combust the TG-derived fatty acids. The brain is known to innervate the key organs involved in these processes via the sympathetic and/or parasympathetic nervous system, and is therefore a likely key mediator in TG metabolism. In **chapter 1**, we provide an overview on the role of the sympathetic nervous system in the regulation of TG metabolism at the level of production (liver), storage (white adipose tissue) and combustion (brown adipose tissue).

The pancreatic hormone insulin has recently been demonstrated to modulate TG metabolism by, in addition to inhibiting hepatic VLDL-TG production, increasing lipogenesis at the level of the white adipose tissue (WAT), thereby ultimately increasing WAT mass. As insulin can enter the brain and has been shown to influence hypothalamic neuropeptidergic systems, we hypothesized that the brain is involved in the effects of circulating insulin on TG metabolism in WAT. Therefore, in **chapter 2**, we assessed tissue-specific fatty acid retention under hyperinsulinemic-euglycemic clamp conditions in insulin-sensitive wild-type mice, in combination with a concomitant intracerebroventricular infusion of the K_{ATP} -channel blocker tolbutamide, hereby blocking central insulin signaling in these mice. We showed that circulating insulin specifically increases the retention of both TG-derived and albumin-bound fatty acids in WAT, and that this effect was largely abolished after central infusion of tolbutamide. We therefore concluded that in insulin-sensitive mice, circulating insulin stimulates WAT-specific retention of fatty acids at least in part through activation of K_{ATP} -channels in the brain.

Hypothalamic regulation of peripheral energy metabolism is mediated by two major neuronal subtypes: proopiomelanocortin/cocaine- and amphetamine-regulated transcript-expressing neurons and neuropeptide Y (NPY)/agouti-related protein-expressing neurons. Previous studies suggested that NPY regulates hepatic lipid metabolism, as central NPY administration increases hepatic VLDL-TG production in rats, which might increase plasma levels of atherogenic lipoproteins and thereby contribute to the development of atherosclerosis. In **chapter 3**, we set out to investigate whether NPY also increases hepatic VLDL-TG production in mice, ultimately aiming to study the role of central NPY signaling in the development of atherosclerosis using hyperlipidemic mouse models. We injected NPY in the lateral or third ventricle of mice and subsequently assessed hepatic VLDL production. In contrast to previous studies in rats, acute central administration of NPY did not affect hepatic VLDL-TG production in mice. We did, however, observe the well-known increase in food

intake upon NPY injection. This apparent species difference in the effects of NPY, specifically on hepatic VLDL-TG production, is of great significance for future animal studies on the central regulation of hepatic VLDL production.

Agonism of the receptor of the incretin hormone glucagon-like peptide-1 (GLP-1) is known to improve glucose intolerance. Recent studies, however, suggest that it also beneficially impacts on lipid metabolism by decreasing plasma TG levels in humans and mice. In **chapter 4**, we evaluated the effect of GLP-1 receptor agonism on VLDL-TG production and liver TG metabolism in high-fat diet-fed APOE*3-Leiden transgenic mice, a well-established mouse model to study human-like lipoprotein metabolism. After 4 weeks of continuous subcutaneous administration of the GLP-1 peptide analogues CNTO3649 or exendin-4 (EX4) as compared to vehicle, we determined hepatic VLDL production, lipid content, and expression profiles of selected genes involved in lipid metabolism. Both CNTO3649 and EX4 improved glycemic parameters and reduced hepatic VLDL-TG and VLDL-apoB production, indicative of decreased production of VLDL particles rather than a decrease in apoB lipidation. Furthermore, hepatic content of TG, cholesterol and phospholipids was markedly reduced by both compounds, an effect accompanied by a down-regulation of genes involved in hepatic lipogenesis and apoB synthesis. These data suggested that GLP-1 receptor agonism, in addition to improving glycemic control, might also ameliorate dyslipidemia and reduce hepatic steatosis in T2DM patients.

In **chapter 5**, we investigated whether the EX4-induced reduction in hepatic VLDL-TG production depends on dietary fat content or central GLP-1 receptor signaling. To study the effects of dietary fat content, wild-type mice were fed either a high-fat diet or a low-fat diet and treated with a continuous subcutaneous infusion of EX4 or vehicle for four weeks. EX4 decreased hepatic VLDL-TG production in both high-fat and low-fat diet-fed mice. To study the role of central GLP-1 receptor signaling, wild-type mice were fed a high-fat diet and treated with a continuous subcutaneous infusion of EX4 or vehicle, while receiving a concomitant continuous central infusion with the GLP-1 receptor antagonist exendin-9 or vehicle. Co-administration of exendin-9 had no effect on the EX4-induced reduction in hepatic VLDL-TG production. We therefore concluded that EX4 reduces hepatic VLDL-TG production independently of dietary fat content and central GLP-1 receptor signaling.

Hepatic steatosis, together with hepatic infiltration of inflammatory cells including macrophages, is a hallmark for non-alcoholic steatohepatitis (NASH). Interestingly, the development of NASH shares common inflammatory mechanisms with the development of atherosclerosis, *e.g.* the infiltration of macrophages in the liver and vessel wall, respectively. EX4 reduces hepatic steatosis (**chapter 4**) and was recently shown to inhibit development of atherosclerosis as well, but the effect of EX4 on NASH was understudied to date. In **chapter 6**, we therefore investigated the effects of EX4 on NASH in addition to atherosclerosis. APOE*3Leiden.CETP transgenic mice were fed a cholesterol-containing Western type diet and treated with EX4 or vehicle for four weeks. Although EX4 only slightly affected plasma lipid and lipoprotein levels, it potently decreased atherosclerosis development. Furthermore, EX4 reduced

hepatic cholesterol and macrophage content and macrophage recruitment to the liver, indeed showing that EX4 attenuated diet-induced NASH. We therefore concluded that, in addition to atherosclerosis, EX4 protects against NASH development and that EX4 might be a valuable strategy to treat patients suffering from both NASH and cardiovascular disease.

Like GLP-1 receptor agonists, metformin, the first-line drug for the treatment of T2DM, also beneficially affects diabetic dyslipidemia by reducing plasma VLDL-cholesterol and VLDL-TG levels in a manner that was incompletely understood to date. In **chapter 7**, we therefore aimed at unraveling the underlying molecular mechanisms behind the lipid-lowering effect of metformin in APOE*3-Leiden.CETP mice fed a Western-type diet. We showed that metformin potently decreased plasma VLDL-cholesterol and VLDL-TG levels. Metformin did not affect hepatic VLDL-TG production or VLDL particle composition, but selectively enhanced the clearance of glycerol tri³H]oleate-labeled VLDL-like emulsion particles by brown adipose tissue (BAT). This effect was accompanied by an increase in mitochondrial content, lipoprotein hormone-sensitive lipase (HSL) protein expression and higher AMPK- α 1 activity in this tissue. These data suggested that metformin lowers plasma VLDL-TG levels by enhancing VLDL-TG uptake by BAT, accompanied by an increase in intracellular TG lipolysis and subsequent mitochondrial fatty acid oxidation in BAT. We hereby identified BAT as a novel key player in the TG-lowering action of metformin and therefore proposed that targeting this tissue might be of strong therapeutic interest in the treatment of (diabetic) dyslipidemia.

Mutations in apolipoprotein AV (APOA5) have been associated with increased plasma TG levels in humans, and deficiency for *Apoa5* caused hypertriglyceridemia in mice. These effects have been ascribed to APOA5 being an activator of LPL, thereby stimulating LPL-mediated VLDL-TG hydrolysis and subsequent uptake VLDL-TG-derived FA by *e.g.* adipose tissue. For this reason, we hypothesized that *Apoa5*-deficient mice would be resistant to diet-induced obesity. Therefore, in **chapter 8**, we set out to investigate the effect of APOA5 in the development of high-fat diet-induced obesity, by feeding both wild-type and *Apoa5*^{-/-} mice a chow or high-fat diet. Surprisingly, we found that, compared to wild-type animals, *Apoa5*^{-/-} mice become more obese when fed a high-fat diet, as explained by hyperphagia. The hyperphagic effect was prevented by adenovirus-mediated hepatic overexpression of APOA5. Furthermore, both intravenous and intracerebroventricular injection of APOA5 acutely inhibited food intake in wild-type mice. We thus showed that APOA5 plays a role in the central regulation of food intake and hereby described a novel function for this apolipoprotein in addition to its role in the regulation of LPL-mediated TG clearance.

In **chapter 9**, we evaluated the results of this thesis in light of the sympathetic innervation of the liver, WAT and BAT (as described in **chapter 1**) and discussed how the sympathetic nervous system might mediate the various results reported in the previous chapters. Furthermore, we describe novel therapeutic modalities that might diminish hypertriglyceridemia by targeting the sympathetic nervous system.

Collectively, the studies described in this thesis offer more insight into the central regulation of peripheral TG metabolism in the context of diabetic dyslipidemia. We expanded the knowledge on the involvement of two hormones, insulin and GLP-1, in the production and clearance of TG and described a novel function of APOA5 in the central regulation of food intake. Furthermore, we report a crucial discrepancy in the central regulation of hepatic TG production between mice and rats, which might be of great significance for future animal studies within in this specific area of research. Finally, we unraveled the mechanisms underlying the lipid-lowering properties of two commonly used anti-diabetic drugs, exendin-4 and metformin, and hereby presented new therapeutic options for these drugs.

