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Author: Geerling, Janine Janetta
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GENERAL INTRODUCTION

Type 2 diabetes mellitus (T2DM) has reached epidemic proportions. T2DM is characterized by, pancreatic β-cell dysfunction, insulin resistance and glucose intolerance [1]. In addition, T2DM is associated with dyslipidemia caused by various disturbances in plasma lipid and lipoprotein metabolism, including increased hepatic apolipoprotein B (apoB) and triglyceride (TG) production [2]. The aim of the present thesis is to gain more insight in the central regulation of peripheral TG metabolism, in the context of diabetic dyslipidemia. This chapter provides an outline of the background of the central regulation of peripheral lipid metabolism.

TRIGLYCERIDE METABOLISM

Lipids including triglycerides (TG) and cholesteryl esters are hydrophobic, and are transported in the blood within the cores of so-called lipoproteins. Lipoprotein metabolism is highly coordinated and disturbances can cause dyslipidemia and ectopic fat deposition. Lipoproteins consist of a hydrophobic core containing TG and cholesteryl esters, covered by a shell containing phospholipid and free cholesterol in which apolipoproteins are embedded [3]. Low-density lipoproteins (LDL) and high-density lipoproteins (HDL) primarily transport cholesterol. The large TG-rich lipoproteins (TRLs), chylomicrons and very low-density lipoproteins (VLDL), transport dietary and liver-derived TG, respectively, to peripheral tissues [4].

After a meal, dietary fat and cholesterol are absorbed in the cells of the small intestine. Within these enterocytes, ApoB is synthesized and lipidated to form TG-rich particles that are then secreted as chylomicrons into the lymphatic system and then enter the blood circulation. In addition, TG-rich lipoproteins are synthesized by the liver. Hepatocytes also synthesize ApoB that is lipidated with endogenous lipids to particles that are secreted as VLDL, a process which is upregulated in times of low food supply (*previously reviewed in* [5]). Once chylomicrons or VLDL enter the blood, their TG are hydrolyzed in capillaries into glycerol and free fatty acids (FA), primarily by lipoprotein lipase (LPL) present on the capillary beds of adipose tissue, heart and skeletal muscle. These FA can be taken up by white adipocytes to be stored as TG, by heart and skeletal muscle to generate ATP, and by brown adipocytes to be combusted as heat, respectively [6].

Uptake of FA by target organs is mediated by various cell surface receptors, including fatty acids transport proteins and CD36 [7]. The rate-limiting step of cellular FA uptake is the local LPL activity [8]. The activity of LPL is influenced by several endogenous peptides and proteins. ApoCI, ApoCIII and Angiopoietin-like proteins (Angptl) 3 and 4 inhibit lipolysis, whereas ApoCII and ApoAV stimulate the lipolytic cascade (*reviewed in* [9]). The importance of these molecules is shown by observations in both humans and mice who lack either ApoCII [10], LPL [11,12] or ApoAV [13,14], all of which resulted in severe hypertriglyceridemia. In contrast, decreased expression of ApoCIII in humans [15] and mice [16], as well as deficiency of Angptl4

in mice [17], is associated with low plasma TG levels. During lipolysis, TRL remnants become enriched with apoE and are rapidly cleared by the liver via binding of apoE to the LDL receptor or the LDL receptor-related protein (LRP) [18]. Hence, there is a continuous flux of FA arising from the intestine and liver and directed towards white adipose tissue (WAT) (storage), muscle and brown adipose tissue (BAT) (combustion), followed by TRL remnant clearance by the liver and subsequent re-initiation of this cycle (see Fig. 1).

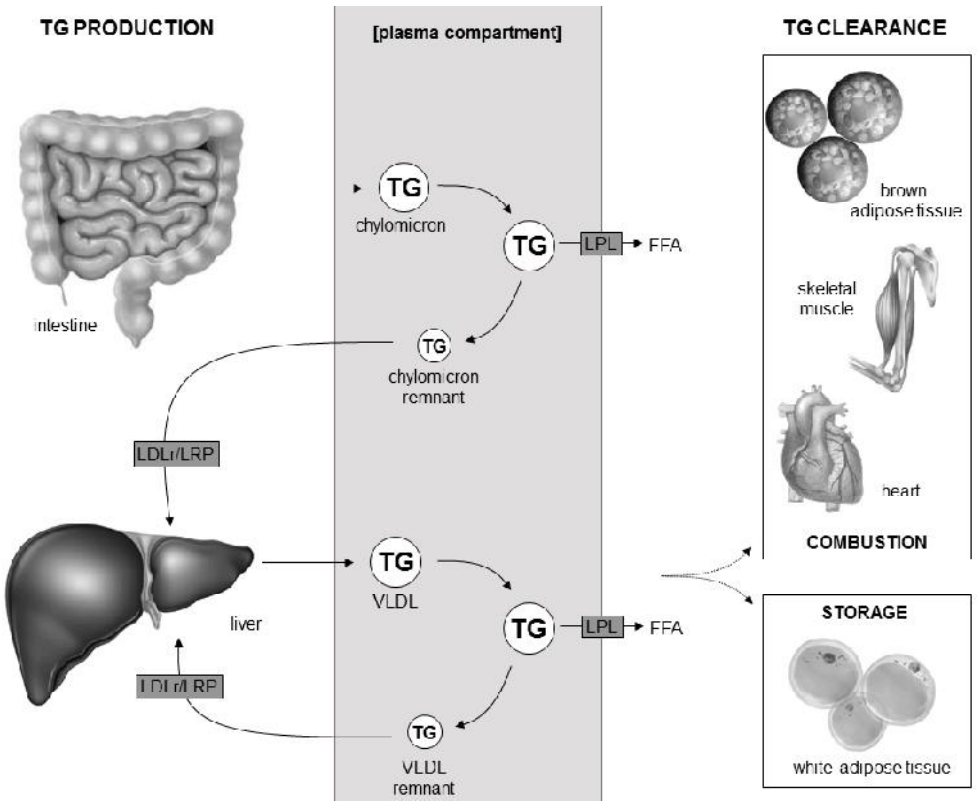

Figure 1. Schematic representation of TG-rich lipoprotein metabolism. See text for details. FFA, free fatty acids; LDLr, low-density lipoprotein receptor; LPL, lipoprotein lipase; LRP, low-density lipoprotein receptor-related protein; TG, triglycerides; VLDL, very low-density lipoprotein.

DEFINITION AND CONSEQUENCES OF DIABETIC DYSLIPIDEMIA

Diabetic dyslipidemia is defined as an elevation of plasma low-density lipoprotein-cholesterol (LDL-C) and/or TG, with or without low high-density lipoprotein-cholesterol (HDL-C) levels [2]. Plasma TG levels are considered to be elevated when they exceed 1.7 mM, which is observed in 31% of the adult US population [19]. Hypertriglyceridemia can be caused by rare monogenic disorders, such as LPL or apoCII deficiency and loss-of-function mutations in the apoAV gene, leading to markedly elevated plasma TG levels that can exceed 10 mM. However, hypertriglyceridemia is mostly caused by complex interactions between environmental factors and subtle variations in genes involved in lipoprotein metabolism [6].

Dyslipidemia, and especially elevated LDL-C, is one of the classical risk factors for cardiovascular disease (CVD) [20]. Therefore, elevated LDL-C has been the primary target for lipid-lowering strategies, with HMG CoA reductase inhibitors (statins) being the most effective and applied class of drugs to lower LDL [19,21]. Importantly, accumulating evidence suggests that an elevated TG level also is an independent risk factor for CVD [21]. LDL and TG-rich lipoprotein remnants can initiate inflammatory processes, resulting in accumulation of monocyte-derived macrophages that scavenge (oxidized) lipoprotein particles and turn into lipid-rich foam cells. This process results in atherosclerosis and ultimately leads to arterial thickening and hardening [22].

Disturbances in TG metabolism not only lead to atherosclerosis, but can also cause liver diseases. Non-alcoholic fatty liver disease (NAFLD) refers to a spectrum of liver pathologies, ranging from mere hepatic TG accumulation ('steatosis') without liver inflammation to non-alcoholic steatohepatitis (NASH), characterized by steatosis combined with infiltration of inflammatory cells including macrophages. Recent data convincingly point to a similar etiology in the development of NASH and atherosclerosis with respect to monocyte recruitment and macrophage activation. Hence, both diseases are considered to represent two aspects of one underlying disease [22].

MOUSE MODELS FOR DYSLIPIDEMIA AND ATHEROSCLEROSIS

The study of dyslipidemia and atherosclerosis in humans is hindered by the complexity of the disease, as well as by technical drawbacks in view of detection modalities [23]. Hence, various animal models have been generated that enable to study the initiation and progression of this disease. As a result of low (V)LDL levels, high HDL plasma levels and a short life span, wild-type mice are fairly resistant to the development of atherosclerosis [23]. Therefore, multiple transgenic mouse models displaying various perturbations of plasma lipoprotein metabolism have been generated. Among these mouse models are ApoE-deficient ($ApoE^{-/-}$) mice, which spontaneously develop atherosclerosis, and mice lacking the LDL receptor ($LDLr^{-/-}$), which develop

atherosclerosis when fed a lipid- and cholesterol-rich diet. A drawback of these mouse models is that they do not readily respond to lipid-lowering interventions, as many of these interventions rely on the increased uptake of lipoprotein remnants via interaction between ApoE and the LDLr [23].

Our lab has generated APOE*3-Leiden (*E3L*) transgenic mice that display strongly increased plasma cholesterol and TG levels when fed a cholesterol-rich diet, attributed to a prominent increase in (V)LDL [24], and develop atherosclerosis with a human-like vascular pathology [23]. In these mice, human APOE*3-Leiden and ApoC1 are expressed, both of which attenuate remnant catabolism. However, as both endogenous ApoE and LDLr are still expressed, they do respond to lipid-lowering interventions. More recently, APOE*3-Leiden mice have been crossbred with mice expressing human cholesteryl ester transfer protein (CETP), a plasma protein that is absent in rodents. CETP shifts the distribution of cholesterol from HDL towards (V) LDL. Therefore, APOE*3-Leiden.CETP mice display lipoprotein metabolism that is even more similar to that of humans [23], and this is a valuable model used in our laboratory to study (interventions in) dyslipidemia and atherosclerosis.

TREATMENT OF DIABETIC DYSLIPIDEMIA

Primary therapies for hypertriglyceridemia are lifestyle modifications, such as body weight control, increased physical activity and cessation of smoking [19]. Pharmacological lowering of plasma TG levels can be achieved by the use of nicotinic acid (niacin) and its derivatives, which lead to a general decrease in plasma (V)LDL levels and a strong increase in HDL, and fibric acid derivatives (fibrates), which lower plasma TG levels with only a modest HDL-raising effect [19].

Interestingly, various therapies used to treat the hyperglycemic aspect of T2DM, show beneficial effects on plasma lipid levels, in addition to their effects on plasma glucose levels. Metformin is the first-line drug for the treatment of T2DM [25]. Although its exact molecular mechanism(s) of action have not been completely resolved, the main effect of metformin is inhibition of the mitochondrial respiratory-chain complex 1 in the liver, hereby acutely decreasing hepatic glucose production [26]. In addition, metformin activates AMP-activated protein kinase (AMPK) in hepatocytes [27], which is likely to occur secondary to its effect on the mitochondria [26]. AMPK is a well-conserved serine/threonine protein kinase and acts as a cellular nutrient and energy sensor and as such is an important regulator of energy homeostasis [28,29]. AMPK is activated by a reduction in hepatocyte energy status. To restore the cellular energy balance, a signaling cascade is initiated, such as glucose transporter type 1 and 4-mediated glucose uptake, CD36-mediated FA uptake, mitochondrial biogenesis and FA oxidation, ultimately resulting in adenosine triphosphate (ATP) production. Concomitantly, AMPK activation inhibits ATP-consuming processes including the synthesis of FA, TG, cholesterol and glucose [29].

Interestingly, although metformin is mostly prescribed for its antihyperglycemic properties, it also improves blood lipid profiles in T2DM patients, as defined by reduced levels of VLDL-C and VLDL-TG levels, and modest increased levels of HDL-C in plasma [30]. The mechanism of action of this lipid-lowering properties of metformin is still unknown, but may include reduced hepatic TG production and/or increased TG clearance.

In addition to metformin, glucagon-like peptide-1 (GLP-1) receptor activation is an interesting therapeutic strategy for the treatment of T2DM. GLP-1, an incretin hormone mainly produced by intestinal L-cells, stimulates insulin secretion [31] and inhibits glucagon secretion [32]] by the pancreas, decreases food intake [33] and slows down gastric emptying [32]. The G-protein-coupled GLP-1 receptor is abundantly expressed in various tissues and mediates the effects of GLP-1 [34]. In plasma, GLP-1 is rapidly degraded by dipeptidylpeptidase-4 (DPP-4). Therefore, current therapeutic strategies encompass inhibitors of DPP-4 as well as synthetic GLP-1 receptor agonists resistant to DPP-4-mediated degradation [31], both aimed at increasing GLP-1 receptor agonistic activity. Like metformin, GLP-1 receptor agonism not only improves glucose metabolism, but also decreases plasma TG levels in T2DM patients [35,36]. As for metformin, the underlying mechanism of its lipid-lowering effect remains to be elucidated.

CENTRAL REGULATION OF ENERGY BALANCE

The brain is strongly involved in maintaining energy balance. The major brain region involved in regulation of energy metabolism is the hypothalamus. The hypothalamus surrounds the third cerebral ventricle and can be subdivided in various hypothalamic nuclei [37,38]. Within the arcuate nucleus (ARC) of the hypothalamus, two neuronal populations, proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript (CART) and neuropeptide Y (NPY)/Agouti-related protein (AgRP) expressing neurons, oppositely regulate energy metabolism [39]. Activation of POMC/CART neurons leads to the production of α-melanocyte-stimulating hormone (α-MSH), which in turn stimulates the melanocortin system to promote an anabolic state of the body, by suppressing energy intake and increasing energy expenditure [40]. In contrast, activation of NPY/AgRP neurons promotes a catabolic state, in part via inhibition of the melanocortin system [41], as AgRP can act as an inverse agonist at melanocortin 3/4 receptors [40].

Various peripherally produced humoral factors can enter the hypothalamus via its median eminence, an area where the blood-brain-barrier is suggested to be incomplete [42]. For example, the pancreatic hormone insulin acts in the brain by activating downstream adenosine triphosphate-dependent potassium channels (K_{ATP} channels) [43]. Furthermore, insulin reduces food intake and promotes energy expenditure by inhibiting hypothalamic NPY/AgRP neurons while concomitantly

stimulating POMC/CART neurons [44]. Additionally, insulin signaling in the ARC inhibits endogenous glucose production by the liver, hereby reducing plasma glucose levels [45]. A similar effect is exerted by leptin, a hormone derived from adipose tissue. Likewise, these hypothalamic pathways are also likely to mediate the anorectic function of GLP-1 [46].]. Interestingly, in contrast to the abovementioned anorectic hormones, the stomach-derived hormone ghrelin is the only gut hormone known to data to exert orexigenic functions via stimulation of hypothalamic NPY/AgRP neurons [42].

AUTONOMIC REGULATION OF TG METABOLISM

In recent years, it has become clear that peripheral TG metabolism is also at least in part controlled by the brain. The key target organs involved in TG metabolism (*i.e.* liver, WAT and BAT) are densely innervated by the autonomic nervous system, which connects these organs with the brain via nerve fibers of the parasympathetic nervous system (PSNS) and/or sympathetic nervous system (SNS). Within the hypothalamus, separate populations of pre-autonomic nerve fibers reside that project to either parasympathetic or sympathetic nuclei in the brain stem and spinal cord respectively: whereas pre-parasympathetic neurons project to the vagal dorsal motor nucleus (DMV) in the brainstem, pre-sympathetic neurons project to the intermediolateral column of the thoracic spinal cord (IML) [47]. Sympathetic nerve fibers arising from the IML project to stellate ganglia located just outside of the spinal cord. In turn, stellate ganglia give rise to postsynaptic sympathetic nerve fibers, which subsequently innervate the target organ. In general, sympathetic neurons transmit their signal by releasing norepinephrine from their nerve endings [48]. Norepinephrine subsequently binds to adrenergic receptors located at the postsynaptic membrane [49]. At least nine subtypes of adrenergic receptors, divided into three major classes, have been identified to date: $\alpha_{1(A/B/D)}$-adrenergic receptors, $\alpha_{2(A/B/C)}$-adrenergic receptors and $\beta_{(1/2/3)}$-adrenergic receptors [50]. All these receptors belong to the G-protein coupled receptor superfamily and couple to G_{α} proteins. Importantly, each class of adrenergic receptors couples to a different G_{α} protein, resulting in different intracellular cascades. α_1-adrenergic receptors couple to the $G_{\alpha q}$ protein to stimulate phospholipase activity. α_2-adrenergic receptors couple to the $G_{\alpha i}$ protein to inhibit adenylyl cyclase activity whereas, in contrast, β-adrenergic receptors stimulate adenylyl cyclase activity by coupling to the $G_{\alpha s}$ protein [50]. Thus, norepinephrine can cause either inhibitory (α_2-adrenergic) or excitatory (α_1-adrenergic and β-adrenergic) sympathetic responses dependent on the function of the target tissue.

Autonomic control of TG metabolism in the liver

The liver is innervated via both SNS and PSNS nerve fibers that form two separate, but intercommunicating plexuses which enter the liver at its hilus [51]. These autonomic nerve fibers arise from three major areas within the hypothalamus: the ventromedial hypothalamus (VMH), the lateral hypothalamic area (LHA) and the PVN (*reviewed in* [52]). While the VMH sends sympathetic projections towards the liver via the lower brainstem and the IML of the spinal cord, the LHA is involved in the parasympathetic innervation of the liver by projecting to the DMV of the brainstem. Interestingly, the PVN sends projections to the liver via both the IML of the spinal cord and the DMV of the brainstem [47] and is thus involved in both parasympathetic and sympathetic innervation of the liver. Additionally, the PVN has many connections with other hypothalamic nuclei involved in energy metabolism [53], and is hereby able to integrate information from other hypothalamic areas with the autonomic control of hepatic energy metabolism [52].

Hepatic TG metabolism is under the control of the SNS. In rats, hepatic sympathetic denervation increases liver fat content, whereas the incorporation of exogenous FA into the plasma VLDL-TG fraction was decreased, pointing to a decrease in hepatic VLDL secretion [54]. In addition, carnitine palmitoyltransferase I and II (CPT-I and CPT-II) activity, as a measure of fatty acid oxidation, were strongly decreased by hepatic sympathetic denervation [54]. Furthermore, hepatic sympathetic denervation impaired the assembly and secretion of VLDL-TG and reduced *Cpt-I* gene expression in overnight fasted rats [55]. Recently, Bruinstroop *et al.*[56] showed that selective sympathetic denervation reduces hepatic VLDL-TG secretion in 17 h-fasted but not in postprandial, 4 h-fasted rats, hereby confirming the importance of the SNS in regulating hepatic VLDL-TG secretion, specifically during fasting, a condition in which lipids become the key substrate for energy metabolism [56].

Hepatic parasympathetic innervation has also been implied in the regulation of hepatic VLDL-TG production, especially in relation to central nutrient sensing. For example, selective central infusion of glucose decrease plasma TG levels via decreased VLDL-TG secretion by the liver [57]. In line with this observation, hepatic activity of stearoyl-CoA desaturase 1 (SCD-1), a key enzyme in FA production/maturation, was also decreased. Hepatic branch vagotomy abolished these effects, indicating a critical role of the parasympathetic nervous system in the regulation of hepatic VLDL-TG production upon central glucose infusion [57].

Autonomic control of TG metabolism in WAT

WAT is innervated by the sympathetic branch of the autonomic nervous system, which was first evidenced in 1995 by Youngstrom and Bartness [58]. By using anterograde and retrograde fluorescent labeling in Siberian hamsters, they showed that different WAT depots are distinctly innervated by the central nervous system [58]. Interestingly, several hypothalamic nuclei involved in regulating peripheral energy metabolism (*e.g.* the ARC

and PVN) are part of this central SNS outflow to WAT [59]. Selective retrograde labeling of the epididymal, inguinal and retroperitoneal WAT depots causes distinct patterns of label distribution at the level of these hypothalamic nuclei [59,60], suggesting that this might coincide with functional differences in WAT innervation [60].

In general, β-adrenergic stimulation induces TG lipolysis in WAT [58]. Stimulation of β_1-, β_2-, or β_3-adrenergic receptors in WAT, by triggering the release of cyclic AMP (cAMP) by adenyl cyclase, activates protein kinase A which in turn phosphorylates hormone-sensitive lipase and perilipins, ultimately resulting in lipolysis of stored TG [61]. Stimulation of α_2-adrenergic receptors, in contrast, decreases TG lipolysis in WAT by decreasing cAMP levels [61].

In contrast to the well-established presence of SNS innervation, the existence of parasympathetic innervation of WAT has caused some debate. In 2002, Kreier *et al.* [62] reported the presence of PSNS innervation of suprarenal fat pads in rats. In addition, they reported that fat pad-specific vagotomy decreased insulin sensitivity and subsequently decreased insulin-mediated glucose and FFA uptake by the denervated fat pad, suggesting that PSNS innervation is not only present but also has a physiological role in WAT [62]. In 2006, however, Giordano *et al.* [63] contradicted this finding by stating that WAT lacks significant PSNS innervation or markers in mice, rats and Siberian hamsters. In fact, the debate on the presence of vagal innervation of WAT is still ongoing [64–68].

Autonomic control of TG metabolism in BAT

The interscapular BAT depot is densely innervated by thin, unmyelinated sympathetic nerve fibers [69,70]. SNS-mediated BAT thermogenesis, and thus TG clearance by BAT, is controlled by an area within the preoptic chiasma/anterior hypothalamic nuclei (PO/AH), located in front of the third ventricle [70,71]. Cooling of this area activates BAT [72,73], whereas warming suppresses its activity [74]. The signal is mediated through the VMH [75]. From the VMH, the thermoregulatory signal is further mediated and finally passes through the spinal cord until it reaches the relevant IML neurons that connect it to the sympathetic chain. From this area, called the stellate ganglia [69], thin unmyelinated fibers directly innervate and activate each brown adipocyte. After release by sympathetic nerve fibers, norepinephrine binds to the brown adipocyte via β_3-adrenergic receptors. This induces activation of its coupled stimulatory G-protein (Gs), after which adenylyl cyclase stimulates the formation of cAMP. cAMP activates protein kinase A, which stimulates thermogenesis by enhancing uncoupling protein-1 (UCP-1) expression and increasing the flux of FA towards the mitochondria via increased intracellular lipolysis [69]. BAT activity is crucially dependent on SNS input, since mice that lack β-adrenergic receptors are unable to increase thermogenesis by BAT upon cold exposure [76–78].

The FA that are an important substrate for BAT thermogenesis, originate from TRLs in the blood and are released upon local LPL-mediated hydrolysis [69]. Uptake and oxidation of lipids by BAT is greatly influenced by sympathetic input. Animals in which

BAT is denervated, become rapidly obese and hypertriglyceridemic, underscoring the contribution of BAT to total energy expenditure and TG clearance [79]. Recently, Bartelt *et al.* [80] have shown that housing mice at 4°C for 4 hours, a key trigger for sympathetic stimulation of BAT, markedly upregulates expression of LPL, CD36 and UCP-1 in BAT, facilitating every step of FA uptake and oxidation. On the other hand, our research group has shown that, when mice are housed at 28°C, a situation in which sympathetic input towards BAT is greatly diminished, lipid uptake and oxidation by BAT are greatly reduced and the size of the intracellular lipid droplets in brown adipocytes is markedly increased [Boon and Rensen, *unpublished observations*].

Treatments known to affect the PSNS, such as vagotomy, alter BAT function, suggesting possible PSNS innervation of BAT. The most convincing and thorough studies attempting to identify PSNS innervation of BAT, however, used immunohistochemical localization of vesicular acetylcholine transporter, an often accepted marker of PSNS innervation. In pericardiac and mediastinal BAT, weak PSNS innervation was found and in interscapular, cervical or perirenal BAT pads no PSNS innervation could be identified at all. Thus, no definitive neuroanatomical data exist for the PSNS innervation of BAT (*reviewed in* [81]).

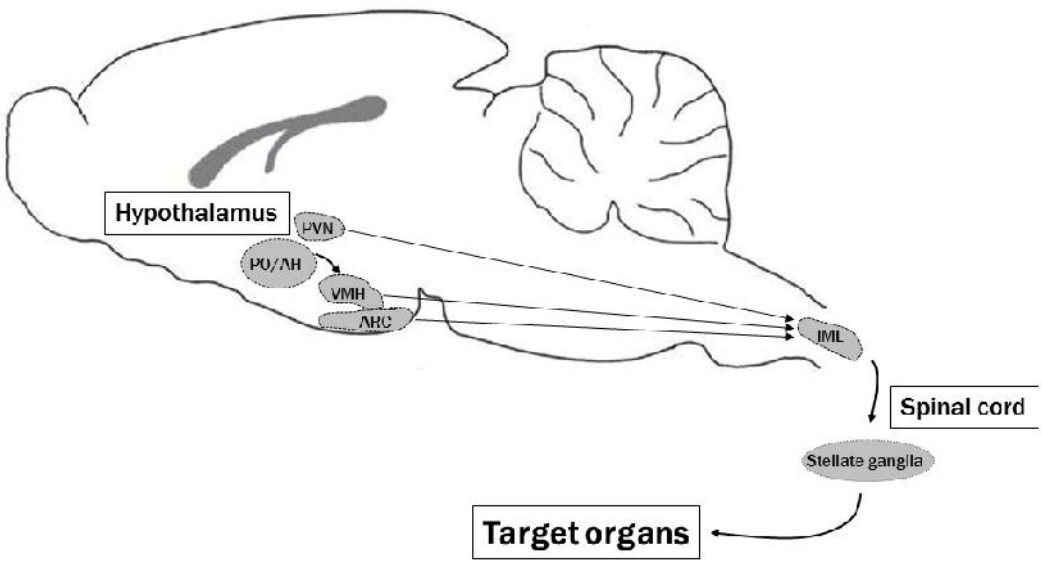

Figure 2. Sympathetic innervation of key target organs involved in triglyceride metabolism. Various key target organs involved in TG metabolism (*i.e.* liver, white adipose tissue and brown adipose tissue) are densely innervated by the sympathetic branch of the autonomic nervous. Pre-sympathetic nerve fibers projecting towards the liver arise in both the paraventricular nucleus (PVN) and ventromedial hypothalamus (VMH) and are relayed to the intermediolateral column (IML) of the thoracic spinal cord. The VMH also sends pre-sympathetic projections towards the white adipose tissue, as does the arcuate nucleus (ARC). Pre-sympathetic nerve fibers projecting towards brown adipose tissue arise from the preoptic chiasma/anterior hypothalamic nuclei (PO/AH), project to the VMH and are subsequently relayed to the IML of the spinal cord. Sympathetic nerve fibers from the IML project to stellate ganglia that are located just outside the spinal cord and give rise to post-synaptic sympathetic nerve fibers that subsequently innervate the target organs.

OUTLINE OF THIS THESIS

The aim of the present thesis is to gain more insight in the central regulation of peripheral TG metabolism, in the context of diabetic dyslipidemia. Circulating insulin suppresses intracellular lipolysis and increases lipogenesis in WAT, ultimately leading to increased WAT mass. As insulin can enter the hypothalamus via the median eminence and subsequently affect hypothalamic neuropeptidergic systems, we set out to investigate whether the brain is involved in the effects of circulating insulin on lipid metabolism in WAT. By blocking central insulin signaling, we determined the extent to which the brain affects insulin-induced retention of both TG-derived FA and albumin-bound FA by WAT, in both chow and high-fat diet-fed animals (**chapter 2**).

Within the hypothalamus, insulin stimulates POMC/CART-expressing neurons, whereas it inhibits neurons expressing NPY/AgRP. Both neuronal subtypes are implied in the hypothalamic regulation of peripheral lipid metabolism. Recently, in rats, central administration of NPY was found to increase hepatic VLDL-TG, which might contribute to the development of atherosclerosis. Since, in contrast to rats, well-suited hyperlipidemic mouse models are available to investigate the effect of hyperlipidemia on atherosclerosis, we set out to investigate whether modulation of central NPY signaling also increases VLDL production in mice (**chapter 3**), ultimately aiming to study whether NPY, by increasing hepatic VLDL production, contributes to the development of atherosclerosis.

T2DM is associated with dyslipidemia caused by various disturbances in plasma lipid and lipoprotein metabolism. Recent studies suggested that GLP-1 receptor activation decreases plasma TG levels in T2DM patients. As the mechanisms underlying this lipid-lowering effect are unknown, we evaluated the effects of GLP-1 receptor agonism on VLDL-TG production and liver TG metabolism in APOE*3-Leiden transgenic mice and wild-type (WT) mice fed a high-fat diet (**chapter 4**). Furthermore, we investigated whether these effects would depend on dietary fat intake and central GLP-1 signaling (**chapter 5**). In addition, we investigated the effects of GLP-1 receptor activation on the development of NASH in addition to atherosclerosis in APOE*3-Leiden.CETP transgenic mice fed a Western-type diet (**chapter 6**).

Although GLP-1 receptor activation receives increasing attention as an additional therapeutic strategy to treat T2DM, biguanides are the most widely used class of antidiabetic drugs and metformin is still considered as a first-line drug for the treatment of T2DM, especially in overweight subjects. In addition to the beneficial effects of metformin on glucose metabolism, metformin also improves plasma lipid profiles, by reducing plasma VLDL-TG and VLDL-cholesterol levels and by modestly increasing HDL-cholesterol levels. As the mechanism(s) by which metformin improves the plasma lipoprotein profile is poorly understood, we first validated that metformin also positively influences lipoprotein metabolism in APOE*3-Leiden.CETP transgenic mice fed a Western-type diet. Subsequently, we investigated the molecular mechanism(s) underlying the metformin-induced changes in plasma lipoprotein metabolism (**chapter 7**).

Mutations in ApoAV, an apolipoprotein described to activate LPL-mediated hydrolysis of VLDL-TG and to increase the uptake of VLDL remnants by the liver, have been associated with hypertriglyceridemia in humans, and ApoAV-deficiency caused hypertriglyceridemia in mice. Interestingly, the *ApoAV*-1131T>C gene variant has also been associated with higher fat intake in humans. Based on this observation, we investigated the role of ApoAV in the regulation of food intake using both ApoAV$^{-/-}$ and WT mice fed a high-fat diet (**chapter 8**). The results of the studies described in this thesis, as well as future perspectives, are discussed in **chapter 9**.

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