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GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Type 2 diabetes mellitus (T2DM) is not only associated with disturbances in plasma glucose and insulin levels, but also with dyslipidemia. Diabetic dyslipidemia is caused by various dysregulations in plasma lipid and lipoprotein metabolism [1], including increased peripheral triglyceride (TG) hydrolysis in white adipose tissue (WAT), decreased TG burning in brown adipose tissue (BAT) and increased TG production by the liver. The studies described in this thesis aimed to gain more insight into the role of the brain in the regulation of peripheral TG metabolism, in the context of diabetic dyslipidemia. Based on these studies, the current chapter discusses the potential role of the sympathetic nervous system (SNS) in the regulation of TG metabolism at the level of production (liver), storage (WAT) and combustion (BAT) and describes novel therapeutic modalities to treat hypertriglyceridemia by targeting the SNS.

SYMPATHETIC REGULATION OF HEPATIC TG METABOLISM

In general, the hypothalamus appears to influence hepatic TG metabolism via stimulation of the SNS, which is likely mediated by both hypothalamic neuropeptide Y (NPY) and melanocortin (MC) receptor expressing neurons. Some of these effects, however, seem to be subject to species differences. For instance, central NPY administration increases hepatic VLDL-TG production in rats [2], an effect found to be largely abolished by hepatic sympathetic denervation [3] and mediated by Y_1 and, to a lesser extent, Y_2 receptors [4].

In **chapter 3**, however, we did not observe any effect of central administration of NPY, or a Y_1 receptor antagonist for that matter, on hepatic VLDL-TG production in mice, suggesting that NPY-regulated hepatic VLDL-TG production is differentially regulated between these rodent species. This notion is supported by a previous study showing that NPY only affected hepatic VLDL-TG production in mice under hyperinsulinemic-euglycemic clamp conditions, but not under basal conditions [5]. Therefore, the general role of central NPY signaling in the regulation of hepatic lipoprotein metabolism is not unequivocal in various species. As central NPY does affect hepatic glucose production in a similar manner in mice [5] and rats [6], and this effect of NPY in rats is mediated via sympathetic innervation it is likely that central NPY signaling increases sympathetic outflow to the liver in mice as well. The Y_6 receptor, expressed in mice and not in rats, as described in **chapter 3**, might underlie the differences observed between the effects of NPY on hepatic TG and glucose production in both species. Therefore, it would be interesting to investigate whether central Y_6 receptors indeed regulate hepatic TG metabolism in mice and, if so, whether this effect is mediated through counteraction of Y_1 receptor-induced increased sympathetic outflow to the liver. Importantly, it is not known to date whether sympathetic activation of the liver in itself regulates hepatic TG metabolism in a similar manner in mice and rats. Bruinstroop *et al.* [3] showed that sympathetic denervation of the liver decreased VLDL-TG production in rats, specifically

after an overnight fast. It would, therefore, be highly interesting to perform similar studies in mice, to determine whether the basal regulation of hepatic VLDL-TG production by the SNS is comparable in rats and mice.

In addition to NPY, the hypothalamic MC system is involved in the regulation of hepatic TG metabolism, as evidenced by studies showing that central administration of MTII, a synthetic MC receptor 3/4 agonist, decreased the hepatic expression of lipogenic genes in streptozotocin-induced diabetic mice [7]. In line with this observation, administration of SHU9119, a MC3/4 receptor antagonist, markedly increased liver TG content in rats [8] and in mice [Kooijman *et al.*, *unpublished observations*]. Notably, in rats, the induction of hepatic lipogenesis by blockade of central MC receptors requires functional endocrine regulation by the HPA axis [8]. In view of this, it is important to note that, in addition to their central expression, MC receptors have been reported to be expressed in liver cells, at least in rats [9], suggesting that melanocortins might also directly affect the liver. Interestingly, in contrast to the effects of NPY, these studies suggest that MC-regulated hepatic VLDL-TG production is comparable in rats and mice. Nonetheless, the exact contribution of the SNS to the effect of MC on hepatic VLDL-TG metabolism needs further attention. Therefore, studies combining central administration of MC-modulatory agents with sympathetic denervation of the liver are needed to directly address the role of the SNS in the MC-mediated regulation of hepatic TG metabolism.

Interestingly, the SNS also appears to mediate the effect of various peripherally produced hormones on hepatic TG metabolism. For example, the WAT-derived hormone leptin, through hypothalamic signaling, decreased hepatic TG content in rats [10]. This effect was correlated with decreased hepatic lipogenesis and most likely occurred via stimulation of sympathetic outflow to the liver. Additionally, in mice, central leptin administration increased hepatic AMP kinase (AMPK) activity, an effect mediated via α1-adrenergic innervation and correlated with a decrease in hepatic TG content [11]. This increase in hepatic AMPK activity might represent a more general mechanism by which increased sympathetic outflow to the liver regulates hepatic TG metabolism. In the abovementioned study on NPY-mediated hepatic VLDL-TG production in rats, Bruinstroop *et al.* [3] propose that central NPY administration is likely to increase hepatic VLDL-TG production by decreasing hepatic fatty acid (FA) oxidation, indicated by decreased hepatic carnitine palmitoyltransferase-1α (CPT-1α) gene expression levels, which leads to increased substrate availability for TG production. In the liver, AMPK activation, via inhibition of acetyl-CoA carboxylase and subsequent lowering of malonyl-CoA levels, leads to dysinhibition of CPT-1 [12], thus suggesting that the decrease in hepatic CPT-1α expression levels after central NPY administration is possibly mediated via a decrease in hepatic AMPK activity. In addition, administration of the MC3/4 receptor antagonist SHU9119 increased hepatic fatty acid syntahse (FAS) expression levels in rats [8]. As AMPK activation, via inhibition of SREBP1c ultimately leads to decreased FAS expression and FA oxidation

[13], the effects of SHU9119 on hepatic TG metabolism might also be caused by suppression of hepatic AMPK activation. Thus, in general, increased sympathetic outflow to the liver may activate hepatic AMPK and thereby mediate the decrease in hepatic TG content. However, further research is needed to confirm this hypothesis.

In **chapter 4**, we showed that the glucagon-like peptide 1 (GLP-1) receptor is also involved in the regulation of hepatic TG metabolism. In this study, we showed that continuous subcutaneous infusion of both GLP-1 receptor agonists exendin-4 and CNTO3649 in APOE*3-Leiden mice reduced hepatic TG content, inhibited the production of VLDL-TG by the liver and down-regulated the expression of genes involved in hepatic lipogenesis. This is in line with previous results showing that rats deficient for dipeptidyl peptidase-4 (DPP-4), an enzyme causing the rapid degradation of GLP-1 in plasma, showed reduced hepatic TG content associated with decreased hepatic expression of lipogenic genes and increased hepatic FA oxidation [14]. Interestingly, various hypothalamic nuclei contain neurons that are immunoreactive to GLP-1 [15] and GLP-1 receptor mRNA is densely present in both the hypothalamic arcuate nucleus (ARC) and paraventricular nucleus (PVN) [16]. Activation of GLP-1 receptors in these nuclei, similar to activation of hypothalamic leptin receptors, inhibits NPY/agouti-related protein (AgRP) expressing neurons, while proopiomelanocortin (POMC)/cocaine- and amphetamine-regulated transcript expressing neurons are activated, ultimately leading to a decrease in food intake [17]. A previous study showing that central GLP-1 infusion decreases hepatic expression of lipogenic enzymes in mice [18], supports a potential role of central GLP-1 receptors in the regulation of hepatic lipid metabolism. Importantly, this effect occurred secondary to GLP-1's inhibitory effect on food intake, as saline-treated animals pair-fed to GLP-1-treated animals showed a similar decrease in hepatic lipogenic enzyme expression [18]. However, another study by Panjwani *et al.* [19] did show that both central and peripheral administration acutely inhibited hepatic VLDL-TG production. Nonetheless, it was still unclear whether central GLP-1 receptors also mediate the effects of peripherally administered exendin-4 on hepatic VLDL-TG production.

In **chapter 5**, we therefore investigated the role of central GLP-1 receptors in the effects of peripherally administered exendin-4 on hepatic VLDL-TG production by combining subcutaneous administration of exendin-4 with central administration of the GLP-1 receptor antagonist exendin-9. We showed that continuous blockade of central GLP-1 receptors did not impact on the exendin-4-induced reduction in hepatic VLDL-TG production, suggesting that the effects of peripherally administered exendin-4 are not mediated by direct activation of central GLP-1 receptors. Nevertheless, as GLP-1 can also bind and activate vagal sensory afferents in the hepatoportal region and in the liver tissue, which subsequently project to neurons in the nucleus of the solitary tract of the brain stem and finally activate ascending fibers to generate a response in the hypothalamus (*for review* [20]), a potential role of the

brain and thus the SNS cannot be ruled out as yet. Therefore, future studies should aim at combining central and peripheral GLP-1 administration with selective hepatic sympathetic denervation to conclusively determine whether the SNS is involved in the effects of peripherally administered GLP-1 receptor agonists on hepatic TG metabolism.

We continued our research on the beneficial effects of exendin-4 in **chapter 6**, where we demonstrate that exendin-4 treatment significantly protected against the development of both atherosclerosis and non-alcoholic steatohepatitis (NASH) in APOE*3-Leiden.CETP female mice fed a Western-type diet. We showed that exendin-4 reduced oxLDL uptake by peritoneal macrophages in a GLP-1 receptor-dependent manner, and reduced recruitment of monocytes/macrophages both into the liver and into the vessel wall. Intriguingly, this latter effect might be mediated by the central nervous system. In mice, exendin-4 increases neuronal activity of alpha-melanocyte stimulating hormone (α-MSH) expressing neurons in the hypothalamic ARC [21]. Interestingly, central administration of α-MSH was shown to decrease inflammatory markers in mice that received an intraperitoneal injection of lipopolysaccharide [22], suggesting that α-MSH can modulate the immune system. Indeed, central α-MSH administration reduced interleukin 1-β-induced inflammation in the ears of mice, an effect that was abolished by concomitant peripheral administration of a β2-adrenergic receptor antagonist [23]. Furthermore, expression of the α-MSH-specific MC1 receptor was evidenced in human monocytes and expression of this receptor is increased upon activation of these monocytes, illustrating the relationship between monocyte stimulation and MC1 receptor expression [24]. Collectively, these data show that inflammatory responses can be mediated by the SNS, and support the hypothesis that the effect of exendin-4 on macrophage and monocyte recruitment might involve central mechanisms. Therefore, it would be interesting to investigate whether blockade of central α-MSH signaling abolishes the inhibitory effect of peripherally administered exendin-4 on the development of atherosclerosis and NASH.

In summary, there is clear evidence for a role of the SNS in the regulation of hepatic TG metabolism. In general, increased sympathetic outflow to the liver leads to a decrease in hepatic content (see Fig. 1), *e.g.* by increasing hepatic VLDL-TG production [3] or by decreasing hepatic lipogenesis [10]. Future studies should aim at further delineating this role, for instance by assessing hepatic TG metabolism in sympathetically denervated animals.

SYMPATHETIC REGULATION OF TG METABOLISM IN WAT

Like in the liver, the sympathetic regulation of TG metabolism in WAT is mediated by the hypothalamus. Chronic central NPY infusion in rats promoted lipogenesis in WAT, independent of its effects on food intake [25]. Additionally, in rats, knockdown of the NPY gene in the dorsomedial nucleus of the hypothalamus decreased WAT size and increased expression of genes related to lipolysis. It is likely that these effects were mediated by the SNS, as in rats, central NPY administration was found to activate pre-sympathetic neurons in the PVN that project to sympathetic ganglia in the intermediolateral nucleus (IML) of the spinal cord [26]. This suggests that central NPY administration increases general sympathetic outflow to WAT, thereby promoting lipogenesis. However, direct evidence for a role of the SNS in the NPY-induced changes in lipid metabolism in WAT has not been reported yet and thus further studies are warranted. Furthermore, it is yet unknown whether NPY has similar effects on WAT TG metabolism in mice.

In addition to the hypothalamic NPY neurons, MC receptor-expressing neurons appear to be involved in mediating TG storage in WAT. In Siberian hamsters, the MC4 receptor (MC4R) is expressed in sympathetic outflow neurons to WAT, suggesting that the MC4R can affect lipid storage in WAT by modulating sympathetic outflow to this tissue [28]. Indeed, stimulation of the MC4R, by means of chronic central infusion of MTII, increased the expression of lipolytic genes in WAT of rats [28], which is likely mediated by stimulation of sympathetic outflow to this tissue. Conversely, inhibition of the MC4R, via chronic central infusion of SHU9119, induced the expression of lipogenic genes in WAT [28]. As changes in lipogenesis due to central infusion of a MC3/4R agonist or antagonist did not occur in mice lacking the β1,2,3-adrenoreceptors, the stimulation of the central MC system increases WAT TG lipolysis by specifically stimulating β-adrenergic outflow towards this tissue [28].

Like in the liver, the SNS is also involved in the effects of various peripherally produced hormones on lipid metabolism in WAT. For example, leptin regulates TG metabolism in WAT, as central leptin administration increased SNS activity in rats, leading to increased WAT lipolysis [29,30]. Leptin signaling in WAT itself, however, also appears to be involved in lipid homeostasis in this tissue, as reduced expression of leptin receptors in WAT increased adiposity and diminished expression of genes involved in glucose and lipid metabolism in mice [31].

GLP-1 is also implied in the regulation of WAT TG metabolism. First, GLP-1 exerted lipolytic actions in rat adipocytes [32]. Furthermore, central infusion of GLP-1 in mice decreased TG content in WAT independent of its anorectic effect [18]. In accordance, expression of lipogenic genes in WAT was dramatically decreased, again largely independent of the food intake reducing effects of GLP-1. These effects were mediated by increased SNS output, as GLP-1 increased SNS activity recorded from nerve endings in WAT and the effect of central GLP-1 infusion on WAT lipogenic gene expression was absent in β1,2,3-adrenoreceptor knock-out animals [18].

Insulin, derived from the pancreatic β-cells, directly regulates adiposity in WAT by reducing cellular cAMP levels and subsequently decreasing WAT lipolysis [33]. This anabolic hormone, however, also affects WAT lipid metabolism via a central pathway. Within the hypothalamus, insulin and leptin are both inhibitors of NPY/AgRP neurons. However, whereas leptin increases SNS output to WAT [29,30], insulin decreases sympathetic outflow, resulting in downregulation of lipolytic genes and upregulation of lipogenic genes in WAT in both mice and rats [34-36]. In **chapter 2**, we showed that in mice, circulating insulin stimulated FA retention in WAT. This was at least in part mediated via the central nervous system, as blockade of central ATP-dependent potassium channels abolished the insulin-stimulated increase in FA retention in WAT. Thus, insulin stimulates TG storage, whereas leptin favors TG depletion in WAT [29,30]. These differences between insulin and leptin action might be explained by the different signaling cascades evoked by both hormones in AgRP neurons, with leptin inhibiting and insulin stimulating membrane accumulation of the PI3K reporter protein [37]. Therefore, Scherer & Buettner [36] proposed a model in which insulin and leptin, by signaling to different populations of second order neurons, activate (leptin) or inhibit (insulin) sympathetic outflow to WAT, consequently leading to either increased or decreased WAT lipolysis, respectively. We now propose that other factors, such as GLP1, also increase WAT lipolysis by acting in the hypothalamus to increase sympathetic outflow to WAT.

In summary, increased sympathetic outflow generally leads to increased lipolysis and decreased lipogenesis in WAT (see Fig. 1). The regulation of sympathetic outflow from the hypothalamus to WAT likely involves separate neuronal populations, causing either increased or decreased SNS input to WAT. Future studies should aim at defining the exact role of the SNS in mediating the effects of peripherally produced hormones, as well as the hypothalamic NPY/MC system, on TG metabolism in WAT.

SYMPATHETIC REGULATION OF TG METABOLISM IN BAT

Recently, it became clear that multiple factors that regulate TG metabolism in liver and WAT, also influence thermogenesis in BAT. Since thermogenesis in BAT coincides with TG combustion [38], a stimulus that activates BAT is also likely to increase clearance of TG-rich lipoproteins towards BAT. Unfortunately, most studies investigating effects on BAT thermogenesis have not focused on lipid metabolism and have not included TG clearance experiments. The effect of these factors on TG clearance therefore remains speculative.

Like in liver and WAT, the hypothalamic NPY/MC system has been implicated in the regulation of BAT thermogenesis. Knock-down of the NPY gene in the dorsomedial hypothalamus in rats increased the number of brown adipocytes between inguinal WAT and increased thermogenesis in interscapular BAT [39]. These data

suggest that, in the physiological situation, NPY functions to inhibit BAT activity. Indeed, central i.c.v. infusion of NPY decreased BAT activity and thermogenesis, both in mice (Boon and Geerling, *unpublished observations*) and in rats [40]. Furthermore, MC receptor expressing neurons also contribute to the control of SNS-mediated BAT thermogenesis. Just as in WAT, the MC4R co-localizes with sympathetic outflow neurons to BAT, suggesting that the MC4R can affect BAT thermogenesis by modulating sympathetic outflow to BAT [41]. Indeed, a single injection of MTII into the third ventricle of rats increased BAT thermogenesis and uncoupling protein-1 (UCP-1) expression [41], which was blocked by surgical sympathetic denervation of BAT [42]. On the other hand, 3-week i.c.v. infusion of SHU9119 resulted in decreased BAT thermogenesis in rats [43]. Thus, modulation of the MC4R influences BAT thermogenesis and, as a consequence, likely also TG clearance by BAT. Future studies should therefore specifically focus on the role of the hypothalamic NPY/MC system on TG clearance by BAT.

Similar to WAT, leptin exerts effects on BAT thermogenesis via a central pathway. Animals deficient in leptin or its receptor were unable to adapt to acute cold exposure, while activation of central leptin receptors increased SNS output, BAT thermogenesis and UCP-1 expression [44-47]. This was likely mediated via the MC system, as the above-mentioned effects could be blocked with the MC4R antagonist SHU9119 [48]. In addition, intravenous administration of leptin in rats increased glucose utilization and lipolytic activity in BAT [49], suggesting that leptin might also exert direct effects on BAT thermogenesis. In addition to leptin, GLP-1 also seems to affect BAT function, as a recent study in mice showed that central administration of a GLP-1 receptor agonist increased sympathetic outflow towards BAT. In addition to leptin, GLP-1 also seems to affect BAT function, as a recent study in mice showed that central administration of a GLP-1 receptor agonist increased sympathetic outflow towards BAT [50]. Furthermore, it induced BAT thermogenesis with concomitant increased expression of lipoprotein lipase (LPL; [50]), pointing to increased TG clearance. Finally, insulin also affects BAT thermogenesis, as central injection of insulin into the preoptic anterior hypothalamus induced a dose-dependent increase in BAT thermogenesis and FA oxidation in mice [51]. However, insulin may also bind directly to its receptors on brown adipocytes, leading to increased LPL activity and stimulation of TG clearance by BAT (*reviewed in* [52]). Importantly, insulin is likely not crucially involved in TG clearance by BAT, as insulin resistant mice showed equal if not higher uptake of triglyceride-rich lipoproteins (TRLs) by BAT upon cold induction [38]. Furthermore, as described in **chapter 2**, we did not observe a difference in insulin-stimulated FA retention in BAT under hyperinsulinemic-euglycemic clamp conditions. Therefore, the contribution of insulin in TG clearance by BAT is probably not substantial. Importantly, future studies should confirm the role of insulin, as well as of leptin and GLP-1, on TG clearance by BAT and the role of the SNS in mediating these effects.

Metformin, the first-line drug for the treatment of T2DM, 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 showed that metformin improves plasma TG levels in APOE*3-Leiden mice by increasing TG clearance by BAT. This effect was accompanied by an increase in hormone-sensitive lipase and the mitochondrial content, as well as an increase in AMPKα1 activity in this tissue. We therefore proposed that metformin enhances VLDL-TG uptake and intracellular TG lipolysis, followed by an increase in mitochondrial fatty oxidation in BAT and hereby identified BAT as an important player in the TG-lowering effect of metformin. As BAT is crucially dependent on SNS input, it is tempting to speculate on a possible role of the SNS in the increased TG-clearance induced by metformin. Previous kinetic studies in our lab showed that after intravenous injection, only a minute fraction of radiolabeled metformin accumulates in the mouse brain [van den Hoek, *unpublished observations*]. Nonetheless, recent studies showed that metformin was present in the CSF of rats following oral administration [53,54], suggesting that metformin can pass the blood-brain-barrier to impact on central mechanisms. Indeed, phosphorylation of signal transducer and activator of transcription 3 (STAT3) was increased in the hypothalamus of metformin-treated rats, while hypothalamic NPY levels were decreased [54]. As discussed above, NPY decreases BAT activity in both mice [Boon and Geerling, *unpublished observations*] and rats [40], thus suggesting that metformin might increase BAT activity by inhibiting hypothalamic NPY signaling, leading to a dysinhibition of sympathetic outflow to BAT. However, in our study, metformin did not affect cAMP response element-binding protein phosphorylation in BAT (data not shown). As adrenergic receptor activation in BAT is correlated with increased phosphorylation of cAMP response element-binding protein [52], these results suggest that the effect of metformin on TG clearance by BAT was not mediated by the SNS. Nonetheless, studies including sympathetic denervations of BAT are needed to exclusively determine the role of the SNS in the metformin-induced increase in TG clearance by this tissue.

Mutations in apolipoprotein A5 (ApoA5) have been associated with increased plasma TG levels in humans and deficiency for ApoA5 caused hypertriglyceridemia in mice [55]. These effects have been ascribed to ApoA5 being an activator of LPL, thereby stimulating LPL-mediated VLDL-TG hydrolysis and subsequent uptake of VLDL-TG-derived FA by *e.g.* adipose tissue [56-58]. In **chapter 8**, we validated the role of ApoA5 in TG metabolism by showing that the hyperlipidemic phenotype of *ApoA5*$^{-/-}$ mice is correlated with a reduced clearance of TG by various tissues, including BAT. In addition, we described an as of yet unknown function of ApoA5 in the regulation of food intake, based on the observation that *ApoA5*$^{-/-}$ mice become hyperphagic when fed a high-fat diet. We showed that adenovirus-induced hepatic overexpression of ApoA5, as well as intravenous injection of ApoA5-loaded VLDL-like emulsion particles, potently inhibits food intake. As central administration of

ApoA5 also strongly inhibited food intake, we hypothesize that the effects of ApoA5 are at least partly mediated via the brain. As described in **chapter 1**, central regulation of food intake is largely mediated by the hypothalamic NPY/MC system, suggesting that ApoA5 inhibits hypothalamic NPY expressing neurons and/or activates MC3/4 receptor expressing neurons to ultimately decrease food intake. As described above, these neurons are also involved in regulating BAT function, with NPY neurons decreasing and MC3/4 receptor expressing neurons increasing BAT thermogenesis and thus likely TG combustion. This suggests that the decreased TG clearance by BAT observed in *ApoA5*$^{-/-}$ mice might not only be mediated via direct LPL-modulatory actions of ApoA5 in this tissue, but possibly also via decreased sympathetic output from the hypothalamus. Obviously, further research is warranted needed to confirm this hypothesis. Therefore, future studies should aim at measuring norepinephrine content in BAT of *ApoA5*$^{-/-}$ mice and preferably at combining peripheral and/or central ApoA5 administration with sympathetic denervation of BAT, to conclusively determine the role of the SNS in the effects of ApoA5 on TG clearance by BAT.

In summary, the SNS is strongly involved in regulating BAT thermogenesis, with increased sympathetic outflow stimulating BAT thermogenesis. In line with this, TG combustion is also likely to be stimulated upon increased sympathetic outflow to this tissue (see Fig. 1). However, current literature only focus on the role of the SNS in BAT thermogenesis. Therefore, dedicated studies are needed that specifically focus on the role of the SNS in TG metabolism in BAT, for instance by performing TG clearance studies.

CONCLUDING REMARKS AND CLINICAL IMPLICATIONS

The SNS is an important regulator of peripheral TG metabolism. Based on the collective studies described in this chapter, we speculate that increased sympathetic output from the SNS, in general, increases plasma TG levels by stimulating hepatic VLDL-TG production as well as increasing lipolysis in WAT, the latter resulting in release of FA which are subsequently transported to the liver to fuel the increased synthesis of VLDL-TG (see Fig. 1). In addition, BAT is concomitantly activated to combust excess TG, possibly to prevent the occurrence of lipotoxicity as a result of the overall increase in plasma TG. Thus, in general, increased sympathetic output increases substrate availability. This makes physiological sense as the SNS is activated by fasting and fight-or-flight responses, both of which are situations in which an organism needs to recruit fuels without being able to eat. Furthermore, in addition to a direct role of SNS innervation of target tissues, the SNS also has a putative role in mediating the effects of various (neuro)peptides and hormones on peripheral TG metabolism (as summarized in Table 1).

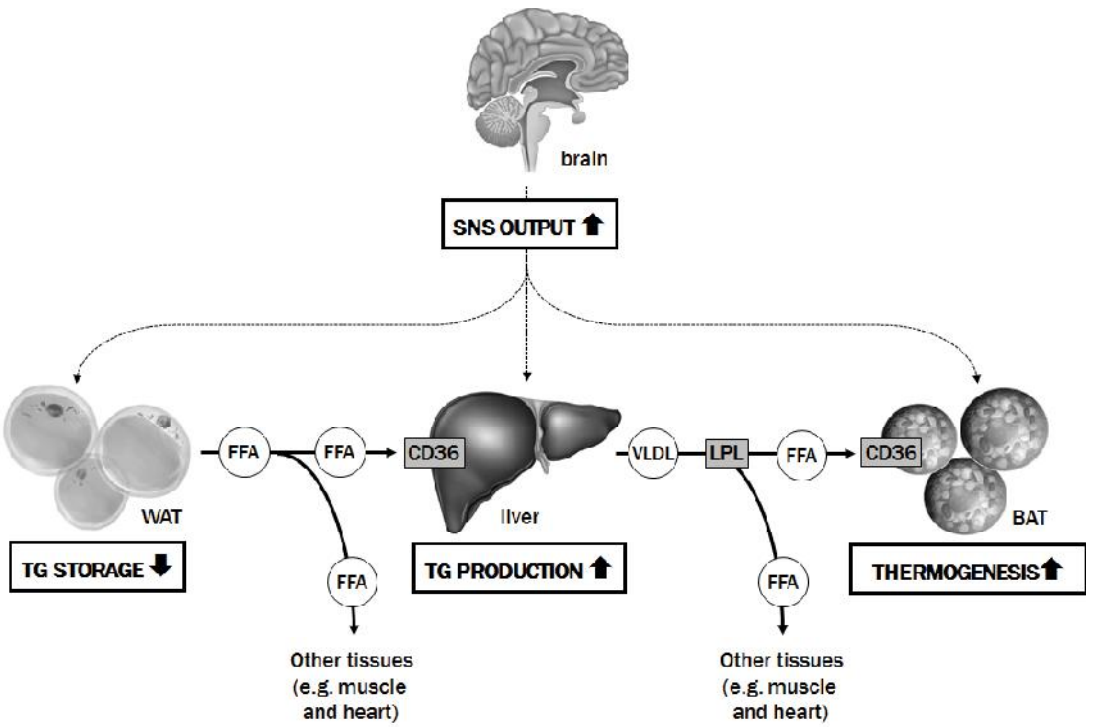

Figure 1. Hypothetical model of sympathetic control of triglyceride metabolism. The sympathetic nervous system (SNS) is upregulated by both fasting and fight-or-flight responses, situations in which an organism needs to recruit fuels without being able to eat. Therefore, the increase in SNS output, in general, increases substrate availability by stimulating hepatic very-low density lipoprotein-triglycerides (VLDL-TG) production, as well as by increasing lipolysis in white adipose tissue (WAT), the latter resulting in release of free fatty acids (FFA) which are transported to the liver to fuel the increased synthesis of VLDL-TG. In addition, brown adipose tissue (BAT) is concomitantly activated to combust excess FFA, derived from VLDL via lipoprotein lipase (LPL) mediated hydrolysis. The incresae in FFA combustion is possibly initiated to prevent the occurrence of lipotoxicity as a result of the increase in plasma TG. Uptake of FFA by target organs is mediated by various cell surface receptors, including fatty acid transport proteins and the cell surface receptor CD36.

Lowering plasma TG levels can be achieved by increasing FA uptake from plasma and subsequent combustion *e.g.* by the liver (*e.g.* fibrates) or BAT (*e.g.* metformin, **chapter 7**), by decreasing VLDL-TG production by the liver (*e.g.* exendin-4, **chapter 4 and 5**) or by decreasing WAT lipolysis (*e.g.* niacin, [59]). However, the increasing knowledge about the involvement of the neuroendocrine system in TG metabolism offers new therapeutic options. Compounds that specifically modulate sympathetic outflow towards target organs involved in TG metabolism (liver, BAT and WAT) could be attractive therapeutics. In this respect, drugs that decrease sympathetic outflow towards liver and WAT, resulting in decreased VLDL-TG and FA release respectively, while simultaneously increase outflow towards BAT, resulting in increased TG uptake, could be very effective. However, it should be noted that decreasing VLDL-TG release

by the liver could lead to hepatic steatosis, whereas decreasing FA release from WAT could lead to obesity. Therefore, further research is needed to determine whether targeting SNS innervation of liver and WAT can be a successful strategy to treat hypertriglyceridemia. Nevertheless, based on this chapter, GLP-1 receptor agonists might be a promising new therapeutic strategy, as individual studies have shown that GLP-1 or GLP-1 receptor agonists decrease hepatic VLDL-TG production (**chapter 4 and 5**) and lipogenesis (**chapter 4** and [14,18]), increase WAT TG storage [18] and increase thermogenesis by BAT [52]. In humans, treatment with exendin-4 was already reported to decrease both plasma TG levels [60] and decrease hepatic steatosis [61]. However, dedicated clinical trials are needed to confirm that GLP-1 receptor agonism concomitantly improves TG metabolism at the level of production (liver) and combustion (BAT).

Recent studies suggest that AMPK activity in the hypothalamus is an important regulator mediating sympathetic outflow towards BAT, and therefore possibly also towards WAT and liver. Various compounds have been shown to lower hypothalamic AMPK activity, leading to increased sympathetic outflow to BAT and induction of thermogenesis and TG combustion by BAT [50,62,63]. Since active BAT is present in humans [64-66], increasing BAT activity might be a new and promising method to fight hypertriglyceridemia. Whether hypothalamic AMPK is also involved in SNS-regulated TG metabolism in WAT and liver, and whether other hypothalamic factors are also involved in this effect, remains to be determined. However, these are promising topics of research, since by influencing peripheral TG metabolism via the brain, multiple organs involved in TG metabolism are targeted at the same time.

In conclusion, peripheral TG homeostasis can be affected by various brain circuits that target multiple key organs involved in TG metabolism. As the exact role of the SNS in the regulation of peripheral TG metabolism remains insufficiently studied, it is of high importance to further delineate the involvement of the neuroendocrine system, including the SNS, in the regulation of TG homeostasis. Dedicated studies combining sympathetic denervation of liver, BAT or WAT with clearance studies using radiolabeled TG are needed to exclusively determine the role of the SNS in regulating TG clearance and uptake by these organs. In addition, experiments studying sympathetic outflow to target organs under hyperlipidemic circumstances will increase the general knowledge on the role of the SNS in TG metabolism pathologies, which is essential to optimize future therapeutic strategies.

Table 1. Neuroendocrine regulation of peripheral TG metabolism. Summary of the neuroendocrine effects on TG metabolism in liver, white adipose tissue (WAT) and brown adipose tissue (BAT), as reviewed in this chapter. NPY, neuropeptide Y; MC3/4R, melanocortin 3/4 receptor; GLP-1, glucagon-like peptide-1.

	Liver	WAT	BAT
NPY	↑ TG production [2-4] ~ TG production [5, **chapter 3**]	↑ lipogenesis [25,39]	↓ thermogenesis [39,40]
MC3/4R	↓ TG content [8] ↓ lipogenesis [8] ↓ TG production [10]	↑ intracellular lipolysis [28]	↑ thermogenesis [41-43]
Leptin	↓ TG content [10]	↑ intracellular lipolysis [29,30,36]	↑ thermogenesis [44-47]
GLP-1	↓ TG production [**chapter 4,5**] ↓ lipogenesis [14,18]	↑ intracellular lipolysis [32] ↑ TG content [18] ↓ lipogenesis [18]	↑ thermogenesis [50] ↑ LPL expression[50]
Insulin	*Not described*	↓ intracellular lipolysis [33,36] ↑ FA retention [**chapter 2**]	↑ thermogenesis [51]

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