review

Sympathetic nervous system control of triglyceride metabolism: novel concepts derived from recent studies

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Abstract Important players in triglyceride (TG) metabolism include the liver (production), white adipose tissue (WAT) (storage), heart and skeletal muscle (combustion to generate ATP), and brown adipose tissue (BAT) (combustion toward heat), the collective action of which determine plasma TG levels. Interestingly, recent evidence points to a prominent role of the hypothalamus in TG metabolism through innervating the liver, WAT, and BAT mainly via sympathetic branches of the autonomic nervous system. Here, we review the recent findings in the area of sympathetic control of TG metabolism. Various neuronal populations, such as neuropeptide Y (NPY)-expressing neurons and melanocortin-expressing neurons, as well as peripherally produced hormones (i.e., GLP-1, leptin, and insulin), modulate sympathetic outflow from the hypothalamus toward target organs and thereby influence peripheral TG metabolism.🔒 We conclude that sympathetic stimulation in general increases lipolysis in WAT, enhances VLDL-TG production by the liver, and increases the activity of BAT with respect to lipolysis of TG, followed by combustion of fatty acids toward heat. Moreover, the increased knowledge about the involvement of the neuroendocrine system in TG metabolism presented in this review offers new therapeutic options to fight hypertriglyceridemia by specifically modulating sympathetic nervous system outflow toward liver, BAT, or WAT.—Geerling, J. J., M. R. Boon, S. Kooijman, E. T. Parlevliet, L. M. Havekes, J. A. Romijn, I. M. Meurs, and P. C. N. Rensen. Sympathetic nervous system control of triglyceride metabolism: novel concepts derived from recent studies. *J. Lipid Res.* 2014. 55: 180–189.

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Dyslipidemia is one of the classical risk factors for cardiovascular diseases (CVD) besides hypertension, type 2 diabetes, and smoking (1). Dyslipidemia is defined as an elevation of plasma LDL-cholesterol (LDL-C), triglycerides (TG), or both, with or without a lowering of HDL-cholesterol (HDL-C) (2). Whereas elevated LDL-C is a well-established major predictor of CVD and has been the primary target for lipid-lowering strategies, evidence suggests that an elevated TG level is an independent risk factor for CVD development as well (3, 4). Plasma TG levels are considered elevated when they exceed 150 mg/dl, which is observed in 31% of the adult US population (5). Although hypertriglyceridemia can be caused by rare monogenic disorders, it is mostly caused by a complex interaction between environmental factors and subtle variations in genes involved in lipoprotein metabolism (5). Current treatments for hypertriglyceridemia are aimed at either increasing TG clearance (e.g., fibrates) (6) or at decreasing lipolysis in WAT (e.g., niacin) (7). In addition, reduction of VLDL-TG production lowers plasma TG levels (e.g., exendin-4) (8).

In recent years, the autonomic nervous system, which consists of a sympathetic and a parasympathetic branch, emerged as an important regulator of metabolic homeostasis. Whereas the role of the sympathetic nervous system (SNS) in the regulation of glucose metabolism has been firmly established (for review, see Ref. 9), considerably fewer studies have focused on its role in TG metabolism. This review provides an update specifically on the role of

the SNS in the regulation of TG metabolism at the level of production (liver), storage (WAT), and combustion (BAT), and it describes novel therapeutic modalities to diminish hypertriglyceridemia by targeting the SNS.

METABOLISM OF TRIGLYCERIDE-RICH LIPOPROTEINS

The transport of lipids in lipoproteins is highly coordinated, and disturbances in lipid transport can cause dyslipidemia. In the blood, cholesterol is primarily transported via LDL and HDL. In contrast, dietary and endogenously derived TGs are transported to various peripheral tissues via large, TG-rich lipoproteins (TRL) (i.e., chylomicrons and VLDL, respectively) (10). After a meal, dietary fat and cholesterol are taken up by the intestine, assembled within chylomicrons, and subsequently released into the circulation. Hepatocytes assemble lipids derived from chylomicrons, as well as endogenous lipids derived from de novo lipogenesis, to form VLDL (11). Once chylomicrons or VLDL arrive in the circulation, lipoprotein lipase (LPL) present on the capillary beds of adipose tissue and muscle hydrolyze TG into glycerol and FFA. The liberated FFA can subsequently be taken up by white adipocytes to be stored as TG or by muscle cells and brown adipocytes to be combusted toward ATP and heat, respectively (12, 13). Cellular uptake of FFA is mediated by various cell surface receptors, including FA transport proteins and CD36 (14). During lipolysis, the TRL become enriched with apoE. The TRL remnant is either rapidly cleared by the liver via binding of apoE to the LDL receptor or LDL-related protein (LRP) (15), or it is further hydrolyzed to generate LDL. Therefore, there is a continuous flux of FA from the intestine and liver (i.e., TG production) toward WAT (storage), muscle and BAT (combustion), followed by clearance of TRL remnants by the liver and subsequent reinitiation of this cycle. In addition, lipolysis of cellular TG in WAT, for example, under conditions of fasting, contributes to a flux of FA from WAT to liver (e.g., for VLDL-TG synthesis) and to muscle and BAT (i.e., combustion).

ANATOMY OF HYPOTHALAMIC SYMPATHETIC OUTFLOW

The various key target organs involved in TG metabolism (i.e., liver, WAT, and BAT) are densely innervated by the sympathetic branch of the autonomic nervous system and are thus, at least in part, controlled by the brain. The major brain region involved in the regulation of general energy balance is the hypothalamus (9, 16). Within the arcuate nucleus (ARC) of the hypothalamus, two neuronal populations, proopiomelanocortin (POMC)-expressing neurons and neuropeptide Y (NPY)/Agouti-related protein (AgRP)-expressing neurons, oppositely regulate energy metabolism (17). Activation of POMC neurons leads to the production of α-melanocyte-stimulating hormone (α-MSH), which in turn stimulates the melanocortin (MC) receptors within the hypothalamic paraventricular nucleus (PVN) to promote a catabolic state of the body (18). In contrast, activation of NPY/AgRP neurons promotes an anabolic state, partly because AgRP acts as an endogenous antagonist for the melanocortin receptors and hereby directly inhibits the actions of POMC-expressing neurons (19).

Within the hypothalamus, separate populations of preautonomic nerve fibers reside that project to either parasympathetic or sympathetic nuclei in the brain stem and spinal cord respectively (20). Sympathetic nerve fibers arise from the intermediolateral (IML) column of the thoracic spinal cord and 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 noradrenalin (i.e., norepinephrin) from their nerve endings (21). Noradrenalin subsequently binds to adrenergic receptors located at the postsynaptic membrane located on the target organ (22). At least nine subtypes of adrenergic receptors, divided into three major classes, have been identified: $\alpha_{1(A/B/D)}$-adrenergic receptors, $\alpha_{2(A/B/C)}$-adrenergic receptors, and $\beta_{(1/2/3)}$-adrenergic receptors (23). All adrenergic 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.

SYMPATHETIC CONTROL OF HEPATIC TG METABOLISM

Sympathetic innervation of the liver

The liver is innervated via both sympathetic and parasympathetic nerve fibers that form two separate but intercommunicating plexuses, which enter the liver at its hilus (24). The sympathetic nerve fibers that innervate the liver arise from two major areas within the hypothalamus: the ventromedial hypothalamus (VMH) and the PVN (for review, see Ref. 25). Both nuclei send sympathetic projections toward the liver via the lower brainstem and the IML column of the spinal cord (**Fig. 1**). Of note, the PVN has many connections with other hypothalamic nuclei involved in energy metabolism (26) and is thereby able to integrate information from other hypothalamic areas with the autonomic control of hepatic energy metabolism (25).

Evidence for a role of the SNS in regulating hepatic TG metabolism

In vivo studies consistently show that sympathetic activation stimulates VLDL-TG production. In rats, hepatic denervation, which decreases local noradrenalin levels in the liver, as well as adrenalectomy, which results in a generalized decrease of circulating plasma (nor)adrenalin levels,
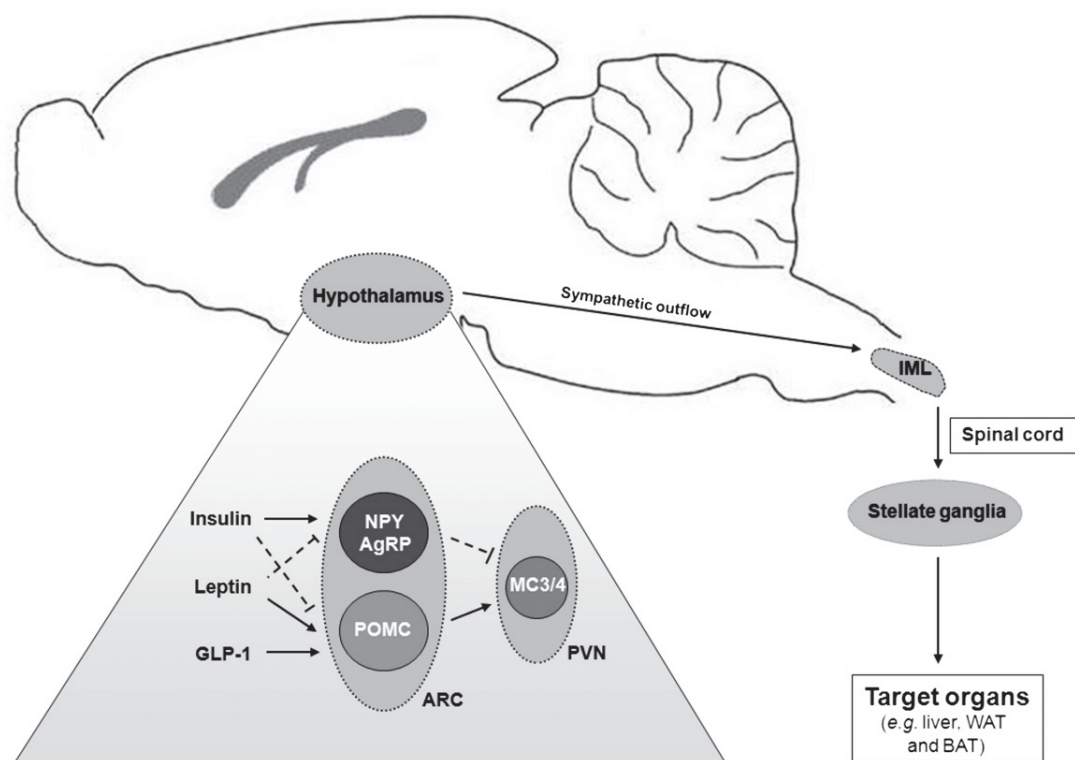
Fig. 1. Sympathetic innervation of key target organs involved in triglyceride metabolism. Within the ARC of the hypothalamus, both NPY/AgRP-expressing neurons and POMC-expressing neurons are involved in TG metabolism. Their actions are partly mediated via neuronal MC3/4 receptor activation in the hypothalamic PVN. Various peripheral hormones (i.e., insulin, leptin, and GLP-1) can impact on the neuronal populations in the ARC and can thereby influence TG metabolism, a process which is at least in part mediated via the sympathetic nervous system. From the hypothalamus, presympathetic nerve fibers are relayed to the IML column of the thoracic spinal cord. Consequently, sympathetic nerve fibers from the IML column project to stellate ganglia that are located just outside the spinal cord and give rise to postsynaptic sympathetic nerve fibers that subsequently innervate the target organs including liver, WAT, and BAT.

reduced incorporation of exogenously administered fatty acids into the plasma VLDL-TG fraction (27), pointing to decreased VLDL-TG secretion. Accordingly, Bruinstroop et al. (28) recently showed that hepatic sympathetic denervation in rats reduced hepatic VLDL-TG secretion. Of interest, this reduction in VLDL-TG secretion was observed in 19 h-fasted rats but not in postprandial, 4 h-fasted rats. These results indicate that the SNS is involved in regulating hepatic VLDL-TG secretion specifically during fasting, a situation in which lipids become the key substrate for energy metabolism (28).

The regulation of hepatic TG metabolism by the hypothalamus is mediated by both hypothalamic NPY- and MC-expressing neurons. Central NPY administration increased hepatic expression of lipogenic genes and VLDL-TG production in rats (29), an effect found to be largely abolished by hepatic sympathetic denervation (28). Likewise, NPY attenuated the suppression of hepatic VLDL-TG production by insulin in mice, as determined under hyperinsulinemic-euglycemic clamp conditions (30).

In contrast to NPY, central administration of MTII, a synthetic MC3/4 receptor agonist, decreased hepatic expression of lipogenic genes in streptozotocin-induced diabetic mice (31) and decreased hepatic TG content in rats (32), suggestive of decreased hepatic VLDL-TG production. In line with this, administration of SHU9119, a MC3/4

receptor antagonist, markedly increased liver TG content in rats, pointing to increased lipogenesis (33). Importantly, the induction of hepatic lipogenesis by blockade of the central MC system appears to require functional endocrine regulation by the hypothalamic-pituitary-adrenal axis (32). Therefore, the exact contribution of the SNS to the MC effect on hepatic VLDL-TG metabolism needs further attention. In addition, MC receptors have been reported to be expressed in rat liver cells (34), suggesting that melanocortins might also directly interfere with TG metabolism in the liver.

Interestingly, various peripherally produced hormones such as glucagon-like peptide-1 (GLP-1) and leptin likely regulate hepatic TG metabolism via the SNS. GLP-1, an incretin hormone secreted upon food intake by intestinal L-cells and the brain (35), mediates its effects via the GLP-1 receptor, which is widely expressed in various tissues, including the hypothalamus (36). Rats with chronically elevated plasma GLP-1 levels showed decreased expression of lipogenic enzymes and reduced hepatic TG content (37). In line with this, continuous subcutaneous infusion of exendin-4, a GLP-1 receptor agonist, decreased hepatic VLDL-TG production in mice (8). Importantly, also central GLP-1 infusion decreased hepatic TG content and expression of lipogenic enzymes in mice, suggesting that GLP-1 can at least in part affect hepatic TG metabolism via a central mechanism (38, 39). In addition, in vitro activation

of GLP-1 receptor signaling in human hepatocytes was shown to directly reduce TG stores (40), thus suggesting that GLP-1 might also directly act on hepatocytes to inhibit lipogenesis. Therefore, the exact role of the SNS in the regulation of hepatic TG metabolism by GLP-1 remains elusive, and experiments combining central and/or peripheral GLP-1 administration with hepatic denervations might prove an effective strategy to resolve this issue.

Leptin, a hormone derived from white adipose tissue (WAT) in case of a positive energy balance, also decreased expression of lipogenic genes and hepatic TG content in mice (41). These effects were dependent on hypothalamic leptin signaling and were, surprisingly, caused by a stimulation of SNS outflow to the liver, as evidenced by an increase in hepatic noradrenalin content (41). In addition, a recent follow-up study showed that leptin administration in 36 h-fasted mice decreased hepatic TG content, lipogenic gene expression, and VLDL-TG production (42). These data, however, should be taken with caution, as 36 h fasting is probably a nonphysiological metabolic condition for mice.

In conclusion, there is clear evidence for a role of the SNS in the regulation of hepatic lipogenesis and VLDL-TG production, in which increased sympathetic outflow to the liver generally leads to an increase in VLDL-TG production, resulting in increased FA availability to be used by peripheral organs (i.e., WAT, BAT, and muscle). This effect is mediated by the NPY system, with increased hypothalamic NPY levels leading to increased sympathetic outflow to the liver and subsequent increased hepatic VLDL-TG output. Furthermore, peripherally produced hormones that are induced upon positive energy balance (e.g., GLP-1 and leptin) reduce hepatic VLDL-TG production.

SYMPATHETIC REGULATION OF TG METABOLISM IN WAT

Sympathetic innervation of WAT

Innervation of WAT by the sympathetic branch of the autonomic nervous system was first evidenced in 1995 by Youngstrom and Bartness by use of anterograde and retrograde fluorescent labeling in Siberian hamsters (43, 44). Of note, they demonstrated that different WAT depots are distinctly innervated by the SNS (44) and that several hypothalamic nuclei involved in regulating peripheral energy metabolism (e.g., the ARC and PVN) are involved in innervation of WAT (45) (Fig. 1).

Evidence for a role of the SNS in regulating TG metabolism in WAT

In general, β-adrenergic SNS stimulation of WAT induces breakdown of TG into glycerol and FFA, a process called lipolysis (46). Stimulation of β_1-, β_2-, or β_3-adrenergic receptors in WAT triggers adenyl cyclase to release cyclic AMP (cAMP) (43). The release of cAMP activates protein kinase A (PKA), which in turn phosphorylates adipose tissue

TG lipase (ATGL), hormone-sensitive lipase (HSL), and perilipins, ultimately resulting in lipolysis of stored TG (43).

Just as in liver, the regulation of TG storage in WAT is mediated by hypothalamic NPY- and MC-expressing neurons. Chronic central NPY infusion in rats promoted lipogenesis and thus lipid storage in WAT, independent of its effects on food intake, which is in line with the anabolic actions of NPY (47). Conversely, in rats, knockdown of the NPY gene in the dorsomedial nucleus of the hypothalamus increased expression of genes related to lipolysis and decreased WAT size, suggesting increased lipolysis (48). Taken together, central NPY signaling may decrease sympathetic outflow toward WAT, resulting in decreased lipolysis and thus enhanced storage of energy. However, direct evidence for a role of the SNS in the NPY-induced changes in lipid metabolism in WAT has not been reported.

In contrast, the catabolic MC neurons appear to oppositely regulate TG storage in WAT by stimulating lipolysis. The melanocortin-4 receptor (MC4R) was found to be expressed in SNS outflow neurons to WAT (49). Furthermore, stimulation of the MC4R, by means of chronic central infusion of the MC3/4R agonist MTII, increased the expression of lipolytic genes in WAT of rats (33), thus suggesting an increase in sympathetic outflow toward WAT and enhanced lipolysis. Conversely, inhibition of the MC4R, via chronic central infusion of the MC3/4R antagonist SHU9119, enhanced the expression of lipogenic genes in WAT (33), suggesting decreased sympathetic outflow to WAT and decreased lipolysis. Importantly, these effects were absent in mice lacking the $\beta_{1,2,3}$-adrenergic receptors, indicating that the central melanocortin system regulates TG lipolysis by regulating β-adrenergic outflow toward WAT.

Just as in the liver, various peripherally produced hormones likely influence TG metabolism in WAT via modulation of sympathetic outflow toward WAT. GLP-1 generally enhances lipolysis in WAT, which plausibly involves both a central and directly peripheral mechanism. Central infusion of GLP-1 in mice decreased TG content in WAT independent of its anorectic effect and decreased expression of lipogenic genes, shifting TG metabolism toward increased lipolysis (38). These effects were indeed mediated by the SNS, as GLP-1 increased sympathetic nerve activity recorded from nerve endings in WAT and the effect was absent in $\beta_{1,2,3}$-adrenoreceptor knockout animals (38). In addition, GLP-1 exerted direct lipolytic actions in isolated rat adipocytes (50).

Within the hypothalamus, insulin and leptin are both inhibitors of NPY/AgRP neurons. However, whereas leptin increased SNS output toward WAT (51, 52), insulin decreased sympathetic outflow, resulting in downregulation of lipolytic genes and upregulation of lipogenic genes in WAT, with net inhibition of lipolysis (53–55). Additionally, we recently showed that circulating insulin stimulates fatty acid retention in WAT in mice, at least in part via the central nervous system (56). Furthermore, insulin directly reduced cellular cAMP levels in WAT, resulting in direct peripheral inhibition of WAT lipolysis (57).

Leptin also regulates TG metabolism in WAT via both central (e.g., via inhibition of NPY/AgRP neurons) and peripheral pathways. First of all, hypothalamic leptin administration in rats decreased lipogenic gene expression (resulting in net lipolysis) in the epididymal WAT depot in rats, an effect abolished after sympathetic denervation of this fat depot (51). Furthermore, leptin administration increased SNS activity in nerves innervating the WAT of rats and this effect was associated with an increase in plasma glycerol and FFA levels (52), indicative of increased WAT lipolysis (51, 52). The differences between insulin and leptin action on WAT lipolysis 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 (58). Therefore, Scherer and Buettner (55) proposed a model in which insulin and leptin, by signaling to different populations of second order neurons, activate (i.e., leptin) or inhibit (i.e., insulin) SNS outflow to WAT, consequently leading to either increased or decreased WAT lipolysis, respectively. Peripheral leptin signaling in WAT also appears to be directly involved in TG metabolism in this tissue, as reduced expression of leptin receptors in WAT following partial knockdown increased adiposity and lowered lipolysis in mice (59).

Thus in general, there is convincing evidence for a role of the SNS in modulating lipid storage in WAT, with increased sympathetic outflow to WAT resulting in increased lipolysis and liberation of FAs. These FAs may subsequently be transported to the liver as substrate for VLDL-TG production, as well as to muscle and BAT to be combusted into ATP and heat, respectively. The hypothalamic NPY/MC system mediates this effect, with activation of hypothalamic NPY neurons leading to decreased WAT lipolysis, whereas stimulation of MC3/4 receptors leads to increased WAT lipolysis. Furthermore, the peripherally produced hormones leptin and GLP-1 both increase WAT lipolysis, while the anabolic hormone insulin decreases lipolysis resulting in increased lipid storage.

SYMPATHETIC REGULATION OF TG METABOLISM IN BAT

Sympathetic innervation of BAT

BAT is densely innervated by the SNS, and SNS-mediated BAT thermogenesis and TG clearance are controlled by an area within the preoptic chiasma/anterior hypothalamic nuclei (PO/AH), located in front of the third ventricle (60, 61). Cooling of this area activates BAT (62, 63), whereas warming suppresses its activity (64). The signal is mediated through the VMH and medulla oblongata and then passes through the spinal cord until it reaches the relevant IML neurons (65). From the consequent stellate ganglia (12), thin unmyelinated nerve fibers directly innervate and activate each brown adipocyte, resulting in a dense innervation of the interscapular BAT depot (12, 61) (Fig. 1). Of note, many other brain regions are involved in the innervation of BAT, such as the brain stem, midbrain (central gray and dorsal raphe nucleus), and various regions in the forebrain (i.e., hypothalamic paraventricular nucleus, dorsomedial hypothalamus, suprachiasmatic nucleus, and arcuate nucleus) (66).

Evidence for a role of the SNS in regulating TG metabolism in BAT

In general, increased SNS outflow toward BAT (e.g., following a cold stimulus) results in increased clearance and combustion of TG into heat. Noradrenalin, released by sympathetic nerve fibers, binds to the β adrenergic receptors on brown adipocytes (12). Of the three subtypes of β-adrenergic receptors, the $β_3$ adrenergic receptor is the most significant in mature brown adipocytes from rodents (12). Binding of noradrenalin to the $β_3$-adrenergic receptor results in activation of its coupled stimulatory G protein, after which adenyl cyclase stimulates the formation of cAMP. cAMP activates PKA, resulting in two important downstream effects. First, PKA stimulates phosphorylation of transcription factors that enhance expression and synthesis of uncoupling protein-1 (UCP-1). Furthermore, PKA phosphorylates and actives intracellular HSL, resulting in increased intracellular lipolysis and, consequently, an increased flux of FA toward the mitochondria to be combusted (12). In addition, FAs may bind to a hydrophobic binding pocket on the UCP-1 protein, resulting in its conformational change. This results in an increased proton flux and uncoupling of ATP synthesis so that heat is produced instead of ATP (12).

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 (67–69). Moreover, animals in which BAT is denervated become rapidly obese and hypertriglyceridemic, underscoring the contribution of BAT to total energy expenditure and TG clearance (70). In addition, Bartelt et al. (71) have shown that housing mice at 4°C for 24 h, a key trigger for sympathetic stimulation of BAT, markedly increased uptake of fatty acids by BAT. This resulted in a rapid normalization of plasma TG levels in hyperlipidemic apoA5 knockout mice (71).

Recently, it became clear that multiple mediators of TG metabolism in liver and WAT also influence thermogenesis in BAT. Since BAT thermogenesis coincides with TG combustion (71), a stimulus that activates BAT is also likely to increase clearance of TRLs toward BAT (71). Unfortunately, most studies that investigated the effect of neuroendocrine factors on BAT thermogenesis have neither focused on lipid metabolism nor performed TG clearance experiments. The effect of these factors on TG clearance therefore remains speculative.

As seen for liver and WAT, the hypothalamic NPY/MC system has also been implied in the regulation of BAT thermogenesis. Knockdown 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 (48). These data suggest that, in the physiological situation, NPY functions to

inhibit BAT activity. Indeed, central infusion of NPY decreased BAT activity and thermogenesis in rats (72) and in mice (M. R. Boon and J. J. Geerling, et al., unpublished data). In addition, NPY signaling in the arcuate nucleus of the hypothalamus decreased sympathetically mediated BAT thermogenesis (73). Though no studies have yet focused on the role of NPY in TG clearance by BAT, the above-mentioned studies suggest that NPY inhibits TG clearance by BAT, which corresponds to its anabolic function.

Similar to NPY-expressing neurons, MC-expressing neurons contribute to the control of SNS-mediated BAT thermogenesis. Since the MC4R colocalizes with SNS outflow neurons to BAT, it might be speculated that the MC4R can affect BAT thermogenesis by modulating the SNS outflow to BAT (74). Indeed, a single injection of MTII, an MC3/4R agonist, into the third ventricle of rats increased BAT thermogenesis and UCP-1 expression (74), which was blocked by surgical sympathetic denervation of BAT (75). On the other hand, three-week ICV infusion of the MC4R antagonist SHU9119 resulted in decreased BAT thermogenesis in rats (76). Thus, activation of the MC4R increases BAT thermogenesis and, as a consequence, likely also increases TG clearance by BAT.

Similar to WAT and liver, peripheral hormones affect BAT thermogenesis and, therefore, likely also affect TG metabolism in BAT. First of all, GLP-1 increases BAT activity, as a recent study in mice showed that central administration of a GLP-1 receptor agonist increased SNS outflow toward BAT, which was accompanied by increased BAT thermogenesis as well as increased expression of LPL (77), pointing to increased TG clearance. Indeed, central administration of the GLP-1 analog exendin-4 specifically increased the uptake of radioactively labeled TG by BAT (E. T. Parlevliet et al., unpublished data). Since GLP-1 may stimulate POMC neurons (78), which results in hypothalamic MC4R activation (79), the effect may have been mediated via this route. Future studies should address the role of the SNS in mediating these effects.

Second, insulin also stimulates BAT thermogenesis. Central injection of insulin into the PO/AH of the hypothalamus induced a dose-dependent increase in BAT thermogenesis and fatty acid oxidation in mice (80). Insulin is known to inhibit NPY/AgRP neurons and to stimulate POMC neurons (80). As these neurons are also involved in mediating BAT activity and thermogenesis (as described above), the effect of insulin on BAT thermogenesis is likely mediated by either increased MC4R activation or decreased NPY activation. On the other hand, 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 Ref. 12). Despite these findings, insulin-resistant mice showed equal if not higher uptake of TRLs by BAT upon cold induction (4°C for 24 h) (71). Furthermore, we recently showed that insulin does not influence TG-derived fatty acid retention in BAT, as assessed under hyperinsulinemic-euglycemic clamp conditions (56). Therefore, insulin is probably not crucially involved in TG clearance by BAT.

Leptin exerts effects on BAT thermogenesis via both central and peripheral pathways. 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 (81–84). This is likely mediated via the MC4R of the MC system, as the above-mentioned effects could be blocked with the MC4R antagonist SHU9119 (85). Leptin may also exert a direct peripheral effect on BAT, as intravenous administration of leptin in rats increased glucose utilization and lipolytic activity in BAT, while ICV infusion of leptin did not elicit these effects (86). Thus, though these studies suggest that leptin actives BAT, no studies have focused yet on the effect of (the absence of) central leptin signaling on TG clearance by BAT.

In conclusion, the SNS is an important mediator of BAT activity and increased SNS activity (e.g., as a result of a cold stimulus) results in increased BAT activation and TG clearance. This is mediated by the hypothalamic NPY/MC system, with activation of hypothalamic NPY neurons leading to decreased BAT activation, whereas stimulation of MC3/4 receptors leads to increased BAT activation. Furthermore, the peripheral hormones leptin and GLP-1 both increase BAT thermogenesis. The FAs that are used as a substrate may in part derive from increased WAT lipolysis as is also induced by these hormones, followed by uptake by BAT. The role of insulin in BAT thermogenesis is probably not substantial.

SUMMARY, CLINICAL IMPLICATIONS, AND FUTURE PERSPECTIVES

The data summarized in this review indicate that SNS is an important regulator of TG metabolism. In general, increased sympathetic output from the SNS toward WAT and liver 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 is transported to the liver to fuel the increased synthesis of VLDL-TG. In addition, concomitant activation of BAT results in combustion of excess FA, to generate heat in case of SNS activation by cold exposure or possibly to prevent the occurrence of FA-induced lipotoxicity. Thus, in general, increased sympathetic output increases substrate availability (see **Fig. 2**). This makes sense physiologically as the SNS is activated by fasting and fight-or-flight responses, both situations in which an organism needs to recruit fuels without being able to eat. In addition, the SNS is not solely involved in innervation of target tissues; it also mediates the effects of various (neuro)peptides and hormones on peripheral TG metabolism (summarized in **Table 1**).

Of note, most insights on the role of the SNS in VLDL-TG metabolism are derived from murine studies. However, accumulating evidence suggests that the SNS is also involved in VLDL-TG metabolism in humans. For instance, in a North European human study cohort, a high level of sympathetic activity was associated with components of the
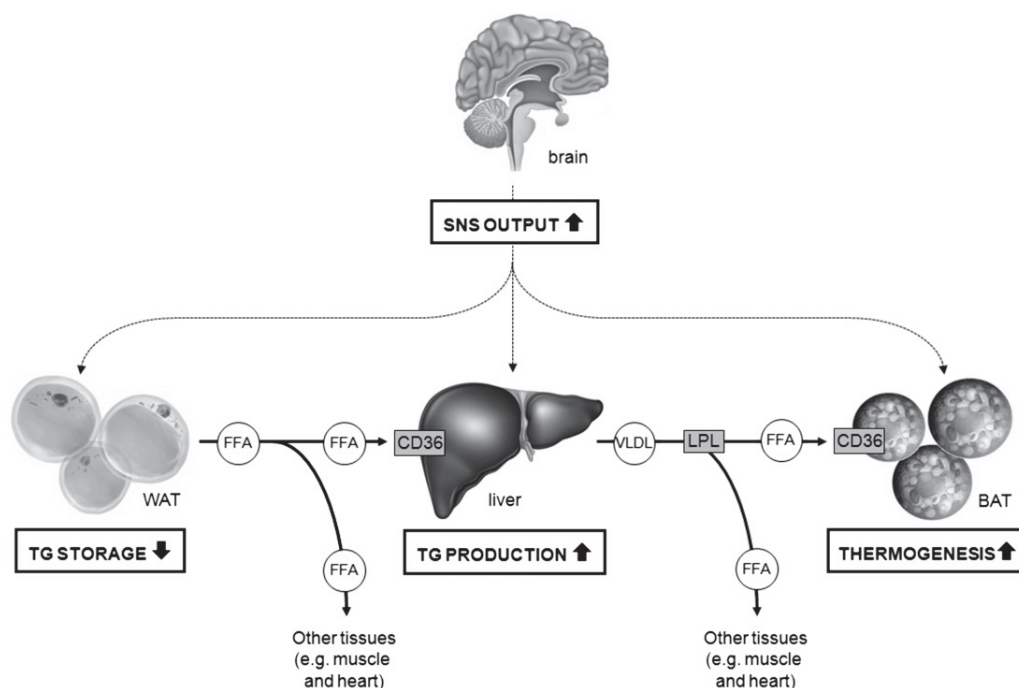
Fig. 2. Sympathetic control of TG metabolism. The SNS is upregulated by fasting and fight-or-flight responses, situations in which an organism needs to recruit fuels without being able to eat, and by cold. The increase in SNS output, in general, increases FA release by stimulating hepatic VLDL-TG production as well as increasing lipolysis in WAT, the latter resulting in release of FFA that are transported to the liver to fuel the increased synthesis of VLDL-TG. In addition, BAT is concomitantly activated to combust FFA, to generate heat in case of cold exposure, or possibly to prevent the occurrence of lipotoxicity.

metabolic syndrome, among which were elevated plasma VLDL-TG levels (87). In line with this, the selective β₁-adrenergic receptor blocker celiprolol decreased plasma TG levels in patients (88). However, treatment with the nonselective β-adrenergic receptor blocker propranolol increased plasma TG levels (88, 89). As it has been shown that β₁-adrenergic receptors are expressed in mature brown adipocytes but are not coupled to any significant extent to intracellular-signaling routes in these cells (64), the decreased plasma TG levels upon celiprolol are presumably caused by a decrease in TG output by both liver and WAT into plasma. In contrast, the β₁/₂-adrenergic receptor blocker does affect BAT adrenergic receptors, as

administration of a low-dose propranolol resulted in a significant reduction of fluorodeoxyglucose (labeled glucose) uptake by BAT in patients undergoing a PET-CT scan, pointing to lowering of BAT activation (90). Therefore, propanolol likely decreases TG combustion in BAT, which can explain the increased TG levels observed in propranolol-treated humans. Since active BAT is present and contributes to energy expenditure in humans (91–94), this might indicate that BAT is crucially involved in TG clearance in humans. We therefore consider BAT as an attractive therapeutic target and propose that future research should be aimed at further delineating the role of this tissue in the regulation of TG metabolism.

TABLE 1.   Effects of various (neuro)peptides and hormones on peripheral TG metabolism

	Liver	WAT	BAT
Hypothalamic mediators			
NPY	↑ TG production (28, 29) ~ TG production (95)	↑ Lipogenesis (47, 48)	↓ Thermogenesis (48, 72, 73)
MC3/4 receptor	↓ TG content (32, 33) ↓ Lipogenesis (31)	↑ Lipolysis (33)	↑ Thermogenesis (74, 76, 96)
Peripherally produced hormones			
GLP-1	↓ TG production (8, 39) ↓ Lipogenesis (37, 38)	↑ Lipolysis (50) ↑ TG content (38) ↓ Lipogenesis (38)	↑ Thermogenesis (77) ↑ LPL expression (77)
Insulin	Not described	↓ Lipolysis (53–55, 57) ↑ FA retention (56) ↑ Lipogenesis (53–55)	↑ Thermogenesis (80)
Leptin	↓ TG content (41, 42)	↑ Lipolysis (51, 52)	↑ Thermogenesis (81–84)

   Effects of hypothalamic mediators and peripheral hormones on the major tissues involved in TG metabolism. For some (but not all) effects, the involvement of the SNS has been reported. For more details, refer to the text.

Current treatments for hypertriglyceridemia are aimed at increasing fatty acid uptake from plasma and subsequent combustion by, for example, the liver (e.g., fibrates). TG lowering can also be reached by decreasing VLDL-TG production by the liver (e.g., exendin-4) (8) or by decreasing WAT lipolysis (e.g., niacin) (7). However, the increasing knowledge about the involvement of the neuroendocrine system in TG metabolism presented in this review offers new therapeutic options. Compounds that specifically modulate SNS outflow toward liver, BAT, or WAT could be attractive therapeutic agents. Therapeutic agents that decrease sympathetic outflow toward liver and WAT (resulting in decreased TG release) while simultaneously increasing outflow toward BAT (resulting in increased TG uptake) could be very effective. Based on this review, GLP-1 receptor agonists might be a promising new therapeutic strategy, as GLP-1 receptor activation decreases hepatic lipogenesis (37, 38) and VLDL-TG production (8), increases lipolysis (38) and decreases lipogenesis in WAT (50), and increases thermogenesis by BAT (77). However, as all these results were found in a preclinical setting using various animal models, dedicated clinical trials are needed to confirm these results in humans.

In conclusion, TG homeostasis is influenced 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 importance to further delineate the involvement of the neuroendocrine system, including the SNS. Dedicated studies combining sympathetic denervation of liver, BAT, and WAT with clearance studies using radiolabeled TG could be useful to conclusively determine the role of the SNS in regulating TG clearance by these organs. In addition, experiments studying SNS outflow to target organs under hyperlipidemic circumstances would increase the general knowledge on the role of the SNS in TG metabolism pathologies, which is essential to optimize future therapeutic strategies.

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