Review

MOLECULAR METABOLISM

Role of hypothalamic *de novo* ceramides synthesis in obesity and associated metabolic disorders

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ABSTRACT

Background: Sphingolipid-mediated signalling pathways are described as important players in the normal functioning of neurons and non-neuronal cells in the central nervous system (CNS).

Scope of review: This review aims to show role of *de novo* ceramide synthesis in the CNS in controling key physiological processes, including food intake, energy expenditure, and thermogenesis. The corollary is a condition that leads to a dysfunction in ceramide metabolism in these central regions that can have major consequences on the physiological regulation of energy balance.

Major conclusions: Excessive hypothalamic *de novo* ceramide synthesis has been shown to result in the establishment of central insulin resistance, endoplasmic reticulum stress, and inflammation. Additionally, excessive hypothalamic *de novo* ceramide synthesis has also been associated with changes in the activity of the autonomic nervous system. Such dysregulation of hypothalamic *de novo* ceramide synthesis forms the key starting point for the initiation of pathophysiological conditions such as obesity — which may or may not be associated with type 2 diabetes.

Keywords Hypothalalumus; Ceramides; Food Intake; Obesity; Type 2 diabetes

1. INTRODUCTION

Nervous control of the energy balance through dynamic adjustment of energy fluxes according to the needs of the body, is a key regulatory mechanism that allows an organism to maintain optimal functioning of many metabolic processes such as glycemia and body weight [1]. Specialized regions of the central nervous system (CNS) are constantly receiving vagal nerve afferent signals or circulating information from hormones and nutrients reflecting the body's energy status and calorie needs [1]. Once these signals have been integrated, the CNS can, in turn, transmit information to peripheral tissues such as liver, skeletal muscles, and adipose tissue— to regulate anabolic pathways such as lipogenesis and glycogenesis, and catabolic pathways including lipolysis and glycogenolysis, and also to islets of Langerhans [2]. In the prandial and resting state, the anabolic pathways predominate; whereas, the reverse is true for the catabolic pathways during periods of fasting or physical activity [3]. Thus, energy balance is finely tuned throughout life and allows an organism to maintain stable body weight and glucose homeostasis. In addition to the regulation of metabolic pathways, the nervous system also regulates food behaviour [3]. Energy imbalance arising from excessive storage of calories, mainly as triglycerides (TG) in adipose tissue, will lead to body weight gain and the development of obesity and its comorbidities such as type 2 diabetes (T2D), hypertension, and cardiovascular diseases.

The central areas involved in the nervous control of energy balance and food intake include the hypothalamus [4], which regulates metabolic homeostasis, while other areas including the striatum, nucleus accumbens, and ventral tegmental area are involved in eating and hedonic "liking" for food [5]. These regions possess neuronal populations specialized in sensing nutritional information and are sensitive to changes in blood glucose, free fatty acids (FA) concentration, or circulating triglycerides [6]. Additionally, they also express leptin or insulin receptors and maybe therefore susceptible to the development of leptin and insulin resistance during dyslipidemia [7].

Lipids are among the nutrients which reach these central regions and play an informative role in controlling energy balance by modulating the activity of neurons, astrocytes, and microglia [6]. A growing body of evidence suggests that a dysfunction of central lipid sensing may be at least partly responsible for the deregulation of the energy balance and the development of obesity and associated insulin resistance. Recent evidence suggests that *de novo* synthesis of ceramides, a class of complex sphingolipids, are key players in the nervous control of energy balance.

This review will summarize the present state-of-the-art on the role(s) played by *de novo* ceramide synthesis on the nervous control of the

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energy balance, and how the dysregulation of *de novo* ceramide synthesis can lead to the development of obesity and its repercussions on metabolic diseases such as T2D.

2. HYPOTHALAMIC *DE NOVO* CERAMIDE SYNTHESIS AND CONTROL OF ENERGY BALANCE

Circulating FA are not used by neurons as a source of energy, and hence, they cross the blood—brain barrier weakly; it was long believed that they do not play an important role in the nervous control of energy balance compared to glucose sensing by neurons [8]. However, there is a growing body of evidence which shows that circulating FA is sensed by neurons and astrocytes and can control energy balance by regulating insulin secretion, hepatic glucose production, or food intake through changes in sympathovagal balance [9—11].

Interestingly, recent data suggest that *de novo* synthesis of ceramides in the hypothalamus may also play a role in FA sensing. A possible link between FA and *de novo* ceramide synthesis could be the lipoprotein lipase (LPL) and the hydrolysis of TG-enriched particles. LPL is present in the hypothalamus, hippocampus, or nucleus accumbens and functions to control energy balance by the local delivery of FA to target cells [12]. The partial deletion of LPL in the ventromedial hypothalamus (VMH) of mice leads to obesity, without hyperphagia, but is associated with a reduction in energy expenditure and thermogenesis [13]. The deletion of LPL in VMH was also associated with dysregulated *de novo* ceramide synthesis [13]. LPL is also expressed in astrocytes and its specific deletion in these cells also induces an increase in food intake, the development of obesity, and ceramide accumulation in the hypothalamus [14]. Taken together, these data suggest that selective metabolism of lipoproteins in the neurons and astrocytes located in the hypothalamus could regulate energy homeostasis with an antiobesogenic role of LPL related to the regulation of *de novo* ceramide synthesis.

The oxidation of FA in mitochondria also plays a central role in the regulation of energy homeostasis, with the mitochondrial carnitine palmitoyl-transferase 1a and 1c isoforms (CPT-1a, CPT-1c). Increased malonyl-CoA, a fatty acid metabolism intermediate, inhibits CPT-1a, which prevents the transfer of long-chain acyl-CoA from the cytoplasm to mitochondria [15]. The accumulated long-chain acyl-CoA in the cytoplasm function as a satiety signal by down regulating the expression of the orexigenic neuropeptides, Agouti-related protein (AgRP) and neuropeptide Y (NPY), in the arcuate (ARC) nucleus of the hypothalamus [16—18] (Figure 1). Opposite regulation by leptin and ghrelin of hypothalamic AMPK, which inhibits malonyl-CoA synthesis, is central for their effect on energy homeostasis [19] (Figure 1).

Recent studies highlighted a role for CPT1c as a link between FA sensing and ceramide synthesis in regulating energy balance (Figure 1). Gao et al. suggested that the endoplasmic reticulum (ER)-localised CPT-1c could be a downstream target of malonyl Co-A during anorectic signalling, whereby malonyl-CoA inhibits CPT-1c to down-regulate *de novo* ceramide biosynthesis [20]. Further molecular studies have shown that CPT-1c exhibits very low acyl—transferase activity, approximately 20—300 times less than CPT-1a, and uses palmitoyl-CoA as the preferred substrate [21]. Importantly, palmitoyl-CoA is a substrate of the serine palmitoyl-transferase (SPT); the rate-limiting enzyme of *de novo* ceramide synthesis (Figure 2) [22]. Additionally, Gao et al. also demonstrated that overexpression of CPT-1c in the ARC resulted in increased ceramide levels, whereas deletion of CPT-1c had the opposite effect. Interestingly, *de novo* ceramide synthesis in the ARC was necessary for the anorectic actions of leptin (Figure 1) [20]. The authors further demonstrated that CPT-1c and high levels of malonly-coA levels are necessary to mediate the anorectic action of leptin in mice [23]. These studies indicate that the anorectic action of leptin impacts ceramide metabolism through malonyl-CoA and CPT-1c (Figure 1). Other studies show that the orexigenic effect of ghrelin is also because of hypothalamic activation of AMPK, which reduces the concentration of malonyl-CoA. This will decrease the inhibition of CPT-1c and favour *de novo* ceramide synthesis by ghrelin (Figure 1) [24]. CPT-1c activity, as explained above, promotes high *de novo* ceramide synthesis, which leads to the expression of the AgRP and NPY genes, and consequently, an increase in food intake (Figure 1). It is interesting to note that the inhibition of ceramide synthesis by myriocin — a selective inhibitor of SPT (Figure 2) — cancels the opposite action of leptin and ghrelin through the normalization of the levels of orexigenic neuropeptides, emphasising the direct role of hypothalamic *de novo* ceramides biosynthesis in controlling dietary intake [24]. More recently, CPT1c expressed in the VMH has been shown to be necessary for the activation of brown adipose tissue (BAT) thermogenesis driven by leptin in mice under short high-fat diet (HFD) exposure [25]. This study underscores the importance of CPT1c located in VMH in activating BAT thermogenesis to counteract diet-induced obesity. Considering that CPT1c is a regulator of *de novo* ceramide biosynthesis, it will be important to determine whether alteration of ceramide levels in VMH plays a role in the adaptation of thermogenesis to HFD. Together, these studies evidenced a key role of CPT1c/*de novo* ceramide synthesis in the regulation of energy homeostasis. The *de novo* synthesis of ceramides, which occurs in the ER, involved several enzymatic steps (Figure 2) [22]. This synthesis starts from the condensation of palmitoyl-coA with serine catalysed by serine palmitoyltransferase (SPT) to produce 3-ketosphinganine [26]. SPT is a heterodimer comprising SPT1 and SPT2, or SPT3 isoforms, in which, SPT1 bears the catalytic activity [27]. This complex is associated with regulatory components such as small subunit-SPT and ORMDL3 proteins [28,29]. Biochemical and recent structural analysis showed that the catalytic site is localized in the cytoplasmic side of ER where the substrates, serine, and palmitoyl-CoA, are present [27,30]. Interestingly, the topology of the catalytic site of the SPT complex does not fit with the requirement for palmitoyl-CoA to be transported by CPT1c in the lumen of the ER. As such, the precise role of palmitoyl-CoA uptake in the lumen of the ER by CPT1c on the regulation of the *de novo* ceramide synthesis has yet to be determined.

The first intermediate in *de novo* sphingolipid synthesis, 3-ketosphinganine, is rapidly reduced to dihydrosphingosine (DH-Sph) by 3-ketosphinganine reductase, and the resulting DH-Sph acts as a substrate for ceramide synthases (CerS) (Figure 2), leading to the production of dihydroceramides [31,32]. Dihydroceramides are further transformed into ceramides by dihydroceramide desaturase (DES1) [33]. Recent studies established that dihydroceramide could be biologically active [34]. To date, studies have demonstrated a role for hypothalamic *de novo* ceramide biosynthesis in the regulation of food intake. However, the specific roles that dihydroceramide and ceramide play in the regulation of food intake have yet to be determined. Moreover, CerS are responsible for the synthesis of several ceramide species which have selective functions [35,36]. We showed that specific ceramide produced by CerS4 was responsible for palmitate-induced pancreatic β cell death [37]. More recent studies showed that the expression of CerS isoforms in peripheral tissues plays selective roles in the development of metabolic diseases [35,36]. Whether specific CerS expressed in the hypothalamus play a role in the regulation of energy homeostasis has yet to be demonstrated.

Finally, the roles of LPL and CPT1c in the regulation of *de novo* ceramide synthesis to modulate AgRP expression in the hypothalamus

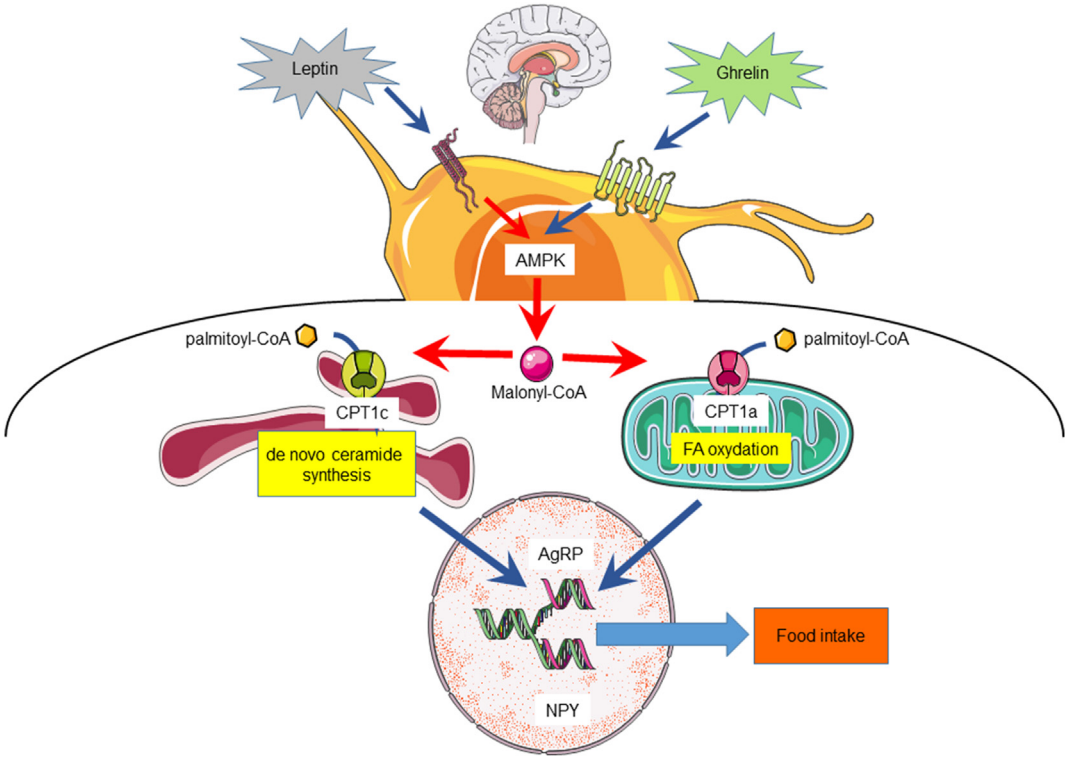

Figure 1: Role of CPT1 isoforms in the regulation of food intake by fatty acids. When the AMPK pathway is activated by grhelin, the resulting decrease of malonyl-CoA levels relieves its inhibitory action on CPT1a, and therefore, favours FA oxidation in mitochondria. Decrease of malonyl-CoA also favours the entry of FA through CPT1c in ER and their metabolism into ceramide. Both the increase of FA mitochondrial oxidation and *de novo* ceramide synthesis stimulate the expression of orexigenic neuropeptides AgRP and NPY, which increased food intake. In contrast, the inhibition of AMPK by leptin results in the increase of malonyl-CoA levels, and inhibition of FA oxidation and ceramide synthesis, and therefore, decrease food intake. Red arrow: inhibition; blue arrow: stimulation. AMPK: AMP-dependent protein kinase; AgRP: agouti-related protein; CPT1: carnitine palmitoyl-transferase; ER: endoplasmic reticulum; FA: fatty acid; NPY: neuropeptide Y.

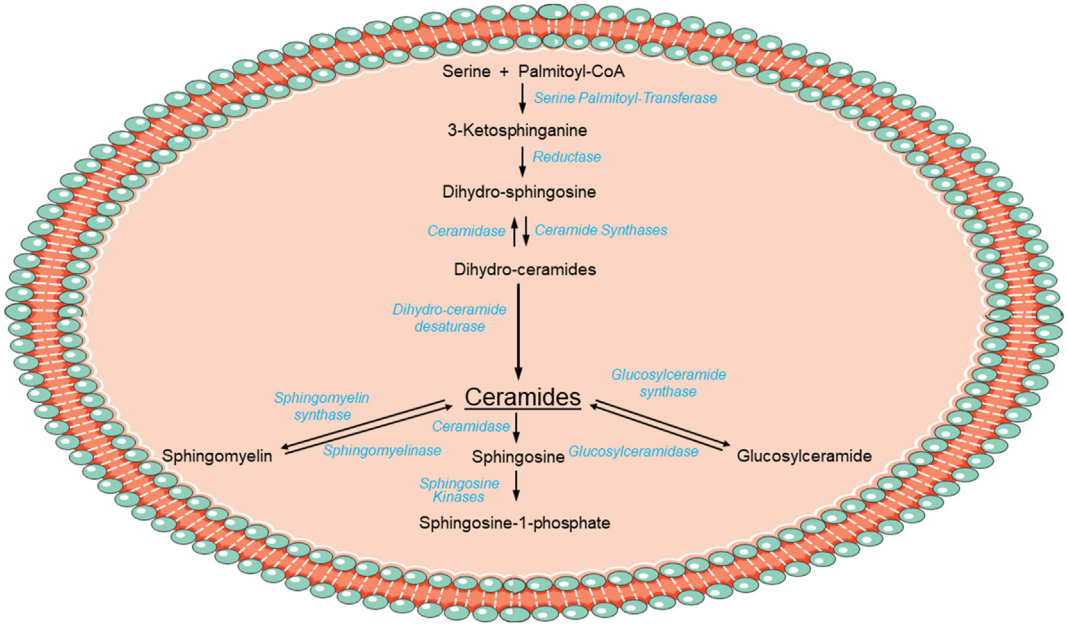

Figure 2: De novo ceramide synthesis and metabolism. De novo ceramide synthesis starts with the condensation of palmitate and serine to form 3-keto-sphinganine. This reaction is catalyzed by the serine palmitoyltransferase. Then, 3-keto-dihydrosphingosine is reduced to dihydrosphingosine, which will be acylated by ceramide synthases to produce dihydroceramide. Finally, dihydroceramides will be desaturated to give ceramides. Ceramides can be further metabolized into sphingomyelin or glucosylceramide. Ceramides can be deacylated by ceramide synthase to generate sphingosine. Sphingosine will be phosphorylated by two sphingosine kinases to form sphingosine-1-phosphate.

appear to be diametrically opposed. CPT1c was slightly decreased in 6-months old neuron-specific LPL-deficient mice [38] suggesting that this phenomenon is an adaptive response that is secondary to the obesity phenotype. Therefore, it will be interesting to determine whether the inhibition of food intake by LPL could be related to the inhibition of a CPT1c-dependent *de novo* ceramide biosynthesis.

3. DOES *DE NOVO* CERAMIDE BIOSYNTHESIS PLAY A ROLE IN HYPOTHALAMIC LIPOTOXICITY-INDUCED OBESITY?

Having established the key role that *de novo* ceramide synthesis plays in central lipid sensing for energy homeostasis, the question arises as to whether an excessive accumulation of ceramide may be responsible for hypothalamic lipotoxicity as described in the peripheral organs [22], and if so, will it contribute to the development of obesity and deregulation of glucose homeostasis to favour T2D.

It is well established in insulin-sensitive tissues such as the liver, heart, and skeletal muscle that the ectopic accumulation of reactive lipid species, such as DAG and ceramides, is a risk factor for the development of insulin resistance that can lead to T2D and cardiovascular complications [39]. In pancreatic β cells, the accumulation of ceramides can also lead to apoptosis [40]. This lipotoxicity includes the appearance of inflammation and ER stress [22,41,42]. In particular, studies have shown that ER stress and activation of the unfolded protein response play a key role in the promotion of insulin resistance in peripheral tissues [43]. In the hypothalamus, ER stress also induces insulin resistance and leptin resistance, leading to weight gain [42,44]. In addition, chronic excess lipids have been shown to impair the functioning of FA-sensitive neurons, and this deregulation may contribute to the development of hepatic insulin resistance through changes in the activity of the autonomic nervous system [9,45]. In peripheral tissues, lipotoxicity is mediated partly by an increase in *de novo* ceramide synthesis [22]. In mammals, it has been shown that enzymes of the *de novo* ceramide synthesis pathway are expressed in different CNS areas, including the hypothalamus and hippocampus [46,47]. Additionally, recent studies showed that the accumulation of ceramides under lipotoxic conditions could play a role in the deregulation of the energy balance in the hypothalamus [46,48–50]. The first evidence was provided by Contreras et al. [46] where they found that the intrahypothalamic content of ceramides increased in genetically obese animal models such as the Zucker rat [46]. In addition to this local endogenous production of ceramides, they also showed that exogenous ceramides can induce hypothalamic lipotoxicity, ER stress, and a decrease in sympathetic tone, leading to a decrease in thermogenesis and weight gain without variation in food intake in rats (Figure 3) [46]. In this study, genetic overexpression of chaperone GRP78 in rat VMH attenuated the deleterious action of ceramides by reducing hypothalamic ER stress and increasing thermogenesis in obese animals, leading to weight loss [46]. This study identified a signalling network involving central ceramides, hypothalamic lipotoxicity/ER stress, and thermogenesis as a pathophysiological mechanism of obesity (Figure 3). Furthermore, alleviation of ER stress by overexpression of GRP78 did not impact ceramide levels in obese Zucker rats, which remain elevated relative to their lean littermates [51]. Therefore, this evidence indicated that ER stress regulation of thermogenesis is downstream of *de novo* ceramide biosynthesis [51]. However, it is important to note that ER stress could also lead to increased ceramide synthesis. It has been shown in rodents that ER stress is concomitant with hepatic insulin resistance and is capable of activating the SREBP-1c cleavage [43], and inducing the entire hepatic lipogenic program that leads to steatosis and increased ceramide content [52]. A similar mechanism could work perfectly well in the brain. Accumulation of ceramide in VMH could also play a role in hypothalamic ER stress and the dysregulation of thermogenesis induced by short-term HFD. However, deletion of CPT-1c — a regulator of *de novo* ceramide synthesis in VMH — prevented leptin-induced BAT thermogenesis during short-term HFD [25]. In the absence of CPT1c, leptin was unable to restrain *de novo* ceramide synthesis. As the flux through SPT occurs largely by kinetic effects caused by changes in palmitoyl-CoA availability [53] which is increased by HFD, this will drastically raise ceramide levels, and therefore, inhibition of BAT thermogenesis through changes in sympathetic nervous system tone. In addition to the induction of ER stress by the accumulation of hypothalamic ceramide, *de novo* synthesis of ceramide also induced insulin resistance in the hypothalamic cell line GT1-7 by the activation of the PKCζ (Figure 3) [49]. In muscles, activated PKCζ interacted and phosphorylated the pleckstrin homology (PH) domain of Akt on a Thr34/Ser34 residue; thus inducing its sequestration in caveolin-enriched microdomains (CEM) at the plasma membrane, which in turn prevented the stimulation of the kinase by insulin [36,54]. The genetic *in vivo* overexpression of chaperone GRP78 increased the levels of phosphorylated PI3K and pAKT, the downstream targets of the insulin receptor in the VMH of obese Zucker rats [46]. In contrast, the levels of SOCS3, a potent inhibitory regulator of insulin signalling were reduced in these rats, lending credence to the idea that hypothalamic ceramide/ER stress induces central insulin resistance [46]. Another potential mechanism linking ceramide metabolism to hypothalamic lipotoxicity is the regulation of neuroinflammation which plays a key role in central insulin resistance [55]. L-cycloserine, a potent inhibitor of SPT activity, counteracted palmitate-induced intracellular ceramide accumulation leading to the downregulation of IL-6 and TNFα in the mHypoE-N42 hypothalamic cell line (Figure 3) [56]. However, the role of hypothalamic ceramide in neuroinflammation *in vivo* remains to be clarified.

Recent studies highlight that *de novo* synthesis of ceramides in VMH could be modulated by different hormones to regulate body weight gain and obesity. Lopez and colleagues showed that the central action of thyroid hormones (T3) on hepatic lipogenesis is mediated by a reduction in hypothalamic ceramide-induced ER stress, which promoted BAT thermogenesis [57]. Importantly, The AMP-activated protein kinase expressed in steroidogenic factor 1 neurons in VMH appeared to be crucial for the effect of T3 (Figure 4). Estrogen is another hormone that can regulate hypothalamic ceramide metabolism [58]. Ovarian insufficiency, characterized by a depletion in estrogens, increased the expression of SPT1 and SPT2 isoforms and ceramide levels in the hypothalamus. In contrast, central E2 decreased ceramide levels and ER stress in VMH, leading to increased BAT thermogenesis, weight loss, and metabolic improvement [58] (Figure 4). These data suggest that dysregulation of hypothalamic ceramide metabolism may play a role in energy homeostasis changes observed during menopause in women. More recently, hypothalamic ceramide metabolism has been involved in precocious puberty induced by obesity [59]. In this study, Heras et al. evidenced that early-onset obesity, as observed in childhood, increased expression of SPT1 in the paraventricular nucleus of the hypothalamus, thereby advancing the maturation of the ovarian noradrenergic system [59]. Taken together, these recent data showed that hypothalamic ceramide metabolism could be regulated by peripheral signals and can open new avenues for future treatments of obesity. This approach is supported by evidence that the antiobesity effect of telmisartan — an angiotensin II receptor type 1 antagonist — is

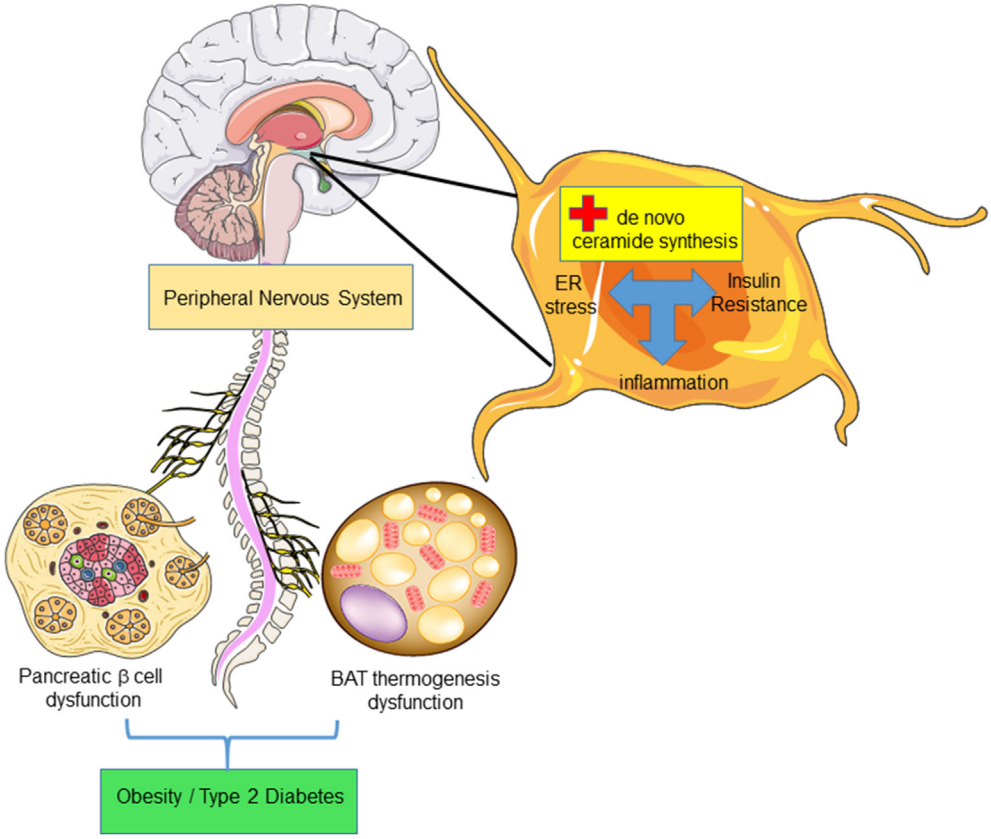

Figure 3: Regulation of obesity/type 2 diabetes by *de novo* ceramide synthesis in the hypothalamus. When ceramide levels are increased by *de novo* ceramide synthesis in the hypothalamus, the resulting ER stress, central insulin resistance, and inflammation will impair the function of the peripheral nervous system. Hypothalamic ceramides reduce BAT sympathetic nerve activity which decreases thermogenesis, and therefore, favours body weight gain. Hypothalamic ceramides also decrease parasympathetic activity, which results in pancreatic β cell dysfunction, and therefore, favours hyperglycemia. Both deregulation of body weight gain and glycemia by hypothalamic ceramide would be the premise to develop obesity and type 2 diabetes. ER: endoplasmic reticulum; BAT: brown adipose tissue.

mediated by preventing hypothalamic ceramide accumulation (Figure 4) [60].

Having established a role of the axis CPT1c/*de novo* ceramide synthesis in the regulation of neuropeptides in the ARC, it will be important to establish the effect of excessive ceramide levels in this area on food intake during obesity. Recent studies showed that palmitate induces inflammation and ER stress in the mHypoA, expressing anorexigenic neuropeptide proopiomelanocortin (POMC) neuron cell line independently from *de novo* ceramide synthesis [61]. These data suggest that ceramide would regulate inflammation in another neuronal population that could be the NPY/AgRP neurons, in which, palmitate had induced inflammation [62]. In NPY/AgRP hypothalamic neurons, autophagy-regulated lipid metabolism has been shown to modulate neuropeptide levels which have immediate effects on food intake [63]. Interestingly, Milanski and colleagues recently showed that palmitate-mediated induction of autophagy in the NPY-expressing neuronal cell model mHypoE-46 is dependent on *de novo* ceramide biosynthesis [64]. These results suggest that increased *de novo* ceramide biosynthesis in different neuronal populations is involved in the regulation of energy homeostasis. However, specific targeting of *de novo* ceramide synthesis in these neuronal populations *in vivo* will be required to conclusively determine the role of *de novo* ceramide synthesis in energy homeostasis regulation.

As mentioned above, it has been shown in peripheral organs that the length and saturation of the ceramide chain are crucial to mediate their effect on glucose homeostasis [36,54]. In the hypothalamus, Lopez and colleagues demonstrated an accumulation of C18-ceramide in obese Zucker rats, whereas exogenous ceramide infusion in the hypothalamus increases C16-ceramide [46]. Using the hypothalamic GT1-7 cell line treated with deutered palmitate, the palmitate induced an increase in ceramide levels by serving as a substrate for both SPT and CerS [49]—resulting in two pools of ceramide, with 50% coming from the use of palmitate by SPT and the other 50% from the use of palmitate by CerS. In the latter pool, it appears that palmitate could be elongated before its use by CerS. Importantly, the relative contributions of the two ceramide pools to insulin resistance have yet to be determined. It is envisioned that *in vivo* approaches targeting CerS isoforms in the hypothalamus will enable us to gain a better understanding of the role of specific ceramide species in the regulation of energy homeostasis.

4. DOES *DE NOVO* CERAMIDE BIOSYNTHESIS PLAY A ROLE IN HYPOTHALAMIC LIPOTOXICITY-INDUCED DEREGULATION OF GLUCOSE HOMEOSTASIS?

Deregulation of energy homeostasis, which favours obesity, is known to promote the development of T2D by affecting glucose homeostasis. Peripheral ceramide is involved in inducing insulin resistance and pancreatic β cell death and dysfunction [22,35,36]. As the hypothalamus is an area of the brain which includes the ARC and VMH, which is

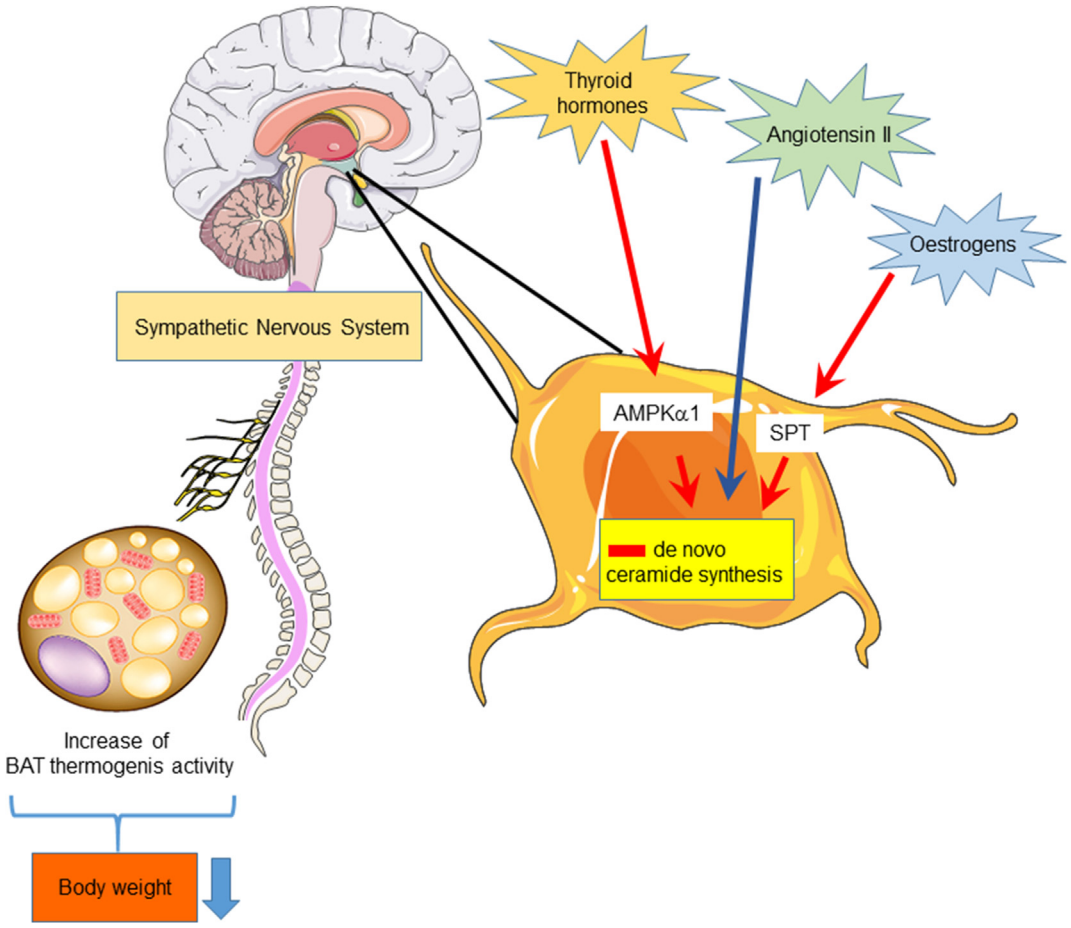

Figure 4: Pathophysiological regulation of hypothalamic *de novo* ceramide synthesis: impact on body weight gain. Thyroid hormones decrease *de novo* ceramide synthesis in the hypothalamus indirectly through the inhibition of AMPK1a. Oestrogen is also a potent inhibitor of *de novo* ceramide synthesis in the hypothalamus by negatively regulating the expression rate-limiting enzyme, SPT. In contrast, angiotensin II increases ceramide levels in the hypothalamus by an unknown mechanism. Regulation of hypothalamic ceramide levels contributes to the effect of these hormones on body weight gain. In the case of thyroid hormones and oestrogens, relieving the inhibition of sympathetic nerve activity and BAT thermogenesis by hypothalamic ceramide will decrease body weight gain and obesity. *De novo* ceramide synthesis in the hypothalamus appears to be a central regulator of body weight gain in response to altered production of thyroid hormones (hyperthyroidism) and oestrogens (menopause). AMPK: AMP-dependent protein kinase; BAT: brown adipose tissue; SPT: serine palmitoyltransferase.

involved in the regulation of glucose homeostasis [65], the question arises as to whether hypothalamic ceramide could alter glucose homeostasis. Lopez's group showed that genetic overexpression of chaperone GRP78 in rat VMH attenuated the deleterious action of ceramides that leads to the improvement of glucose homeostasis [46]. Indeed, impaired insulin sensitivity in obese Zucker rats was reversed by the GRP78 adenovirus in the VMH [46]. However, overexpression of GRP78 was insufficient to restore glucose tolerance in them. In contrast to overexpression of GRP78 in VMH, intracerebral infusion of myriocin, the potent inhibitor of SPT, improved tolerance of obese Zucker rats to glucose [49]. In this obese rat model, glucose intolerance is associated with insulin resistance, but also dysfunction of pancreatic insulin secretion (Figure 3) [49]. Decreasing the hypothalamic content of ceramides was sufficient to improve glucose-stimulated insulin secretion *in vivo,* and consequently, carbohydrate homeostasis [49]. Glucose regulates insulin secretion partly through its action on the parasympathetic nervous system [65,66]. In obese rats, the basal parasympathetic tone was elevated at the basal state, but not simulated by glucose; this reflects an impairment of hypothalamic glucose sensing. *De novo* ceramide biosynthesis was responsible for this defect since myriocin treatment restored glucose activation of parasympathetic tone in obese rats [49]. The parasympathetic tone is also known to regulate pancreatic β cell mass proliferation [67]. Lesions of the VMH increased β cell proliferation through parasympathetic hyperactivity [67]. Importantly, inhibition of *de novo* ceramide synthesis in the hypothalamus was associated with a potent increase in β cell area with the presence of a high number of small new islets [49]. To date, the identity of the neurons regulating insulin secretion is largely unknown; although it has been demonstrated that glucose can excite the POMC neurons in the ARC of the hypothalamus, resulting in the regulation of parasympathetic activity [68]. Whether *de novo* ceramide biosynthesis is a regulator of POMC neuron activity remains to be elucidated. Glucokinase, which is highly expressed in the ARC, plays a key role in glucose sensing [69]. Lowering glucose sensing in the ARC has been shown to induce glucose intolerance by inhibiting insulin secretion [69]. Moreover, when POMC neurons are unable to sense glucose under high-fat diet feeding, glucose tolerance is impaired [70]. There is no evidence that ceramide could modulate glucose sensing or glucokinase activity/expression in POMC neurons. However, as myriocin can restore glucose stimulation of

parasympathetic tone in obese Zucker rats, the ability of ceramide to modulate glucokinase should be tested. A recent study showed that the loss of mitochondrial flexibility in POMC neurons altered glucose sensing, resulting in defective insulin secretion [71]. Importantly, ceramide metabolism has been linked to the regulation of mitochondria dynamics [50]. It is therefore possible that an increase in hypothalamic *de novo* ceramide synthesis could inhibit glucose sensing in POMC neurons by affecting mitochondrial function, which will consequently block insulin secretion regulated by the parasympathetic tone.

Acetylcholine (Ach) release is one of the parasympathetic mechanisms found to stimulate insulin secretion. The cholinergic pathway is formed by preganglionic fibres that originate in the dorsal motor nucleus of the vagus nerve (DMNX) which travels to the pancreas [72]. This brain area is directly innervated by POMC neurons. *De novo* ceramide biosynthesis in the hypothalamus could indirectly alter Ach metabolism and release, resulting in defective glucose-induced insulin secretion. Interestingly, Ach metabolism is altered in the brain of obese Zucker rats [73]. Long-term defects in Ach signalling could have profound effects on the secretory properties of pancreatic islets. Importantly, central myriocin treatment restored the capacity for glucose-induced insulin release from isolated obese Zucker islets [49], and the insulin content of obese Zucker rats was also increased following myriocin treatment. These observations strongly suggest that hypothalamic ceramide controls the expression of intrinsic pancreatic β cell proteins that are crucial for insulin secretion; probably through the indirect regulation of parasympathetic tone.

A recent study showed that hypothalamic Glut4 expressing neurons could regulate insulin secretion [74]. Accordingly, knockout (KO) mice for hypothalamic Glut4 are glucose intolerant because of impaired insulin secretion. At the periphery, insulin resistance induced by *de novo* ceramide synthesis is associated with a defect in the translocation of Glut4 to the plasma membrane and a decrease in glucose uptake [75]. Accumulation of hypothalamic ceramide induces central insulin resistance [46,49]. Whether hypothalamic insulin resistance induced by ceramide is also associated with an alteration of Glut4 activity, which will alter glucose sensing, should be a subject of future studies.

VMH lesion and stimulation studies suggested that this hypothalamic area has a direct neural influence on pancreatic function [76]. In particular, the VMH is important for hypoglycemia detection and the counterregulatory response [77]. Even though chaperone GRP78 injection in VMH does not improve glucose tolerance in obese Zucker rats, this does not preclude a role for *de novo* ceramide biosynthesis in the regulation of glucose-induced insulin secretion through other specific signalling pathways in the VMH. Steroidogenic factor 1 (SF1) neurons of the VMH are known to control insulin sensitivity and glucose tolerance [78]. Whether *de novo* ceramide synthesis could alter SF1 neuron activity and glucose homeostasis will need to be examined.

Altogether, it appears that excessive *de novo* ceramide synthesis in specific nuclei of the hypothalamus, namely ARC and VMH, could alter glucose homeostasis——by targeting insulin secretion and sensitivity during the development of obesity.

5. CERAMIDE METABOLISM IN HYPOTHALAMUS AND THE REGULATION OF ENERGY HOMEOSTASIS

De novo ceramides produced in the ER are not end products as they are transported to the Golgi apparatus where they are further metabolized into sphingomyelin or glycosphingolipids [79] (Figure 2). Gangliosides produced by glycosylation of ceramide by glucosylceramide synthase (GCS) are acidic glycosphingolipids that are highly expressed by neurons [80]. They contribute to the formation of membrane microdomains, called lipid rafts, that are enriched in cholesterol and sphingolipids; lipid rafts have been shown to regulate intracellular signal transduction, *e.g.*, insulin receptor signalling [81]. At the periphery, glycosphingolipids have been shown to regulate insulin signalling in different tissues. However, the ganglioside sialosyllactosylceramide (GM3) suppresses activation of the insulin receptor signalling pathway that results in reduced glucose uptake in adipocytes [82]. Moreover, KO mice for the GM3 synthase or pharmacological inhibition of glucosylceramide synthase (GCS) are protected from high-fat diet—induced insulin resistance [83,84]. Based on these studies, it appears likely that in addition to ceramides, their glycosphingolipid metabolites also play important roles in the development of peripheral insulin resistance. Gangliosides are found in all mammalian tissues, but are most abundant in the brain [85]. Interestingly, KO mice for GCS in hypothalamic neurons fail to respond to peripheral energy signals such as leptin that result in obesity in these mice [86]. In contrast, complementation of GCS by adenoviral approaches in the ARC of KO mice was sufficient to improve the regulation of energy homeostasis [86]. GCS is expressed in both POMC and AgRP/NPY neurons and its deletion in both cell types renders them unresponsive to leptin in obese mice [86]. Gangliosides likely form complexes with leptin receptors to facilitate signal transduction to regulate energy homeostasis [86]. It has been shown that the deletion of GCS in hypothalamus neurons in KO mice resulted in the accumulation of *de novo* ceramides under HFD, which is sufficient to counteract leptin's action. These data support the notion that both ceramides and gangliosides are regulators of antagonistic behaviour on leptin signalling in the hypothalamus.

In contrast, it was observed that MBH insulin receptor levels were elevated and signalling activated in GCS KO mice. This suggests that gangliosides could function as negative regulators of insulin in the hypothalamus, as at the periphery [87]. Inhibition of hypothalamic insulin signalling was associated with defective fasting-induced lipolysis, suggesting that hypothalamic gangliosides could regulate glucose homeostasis. However, the evidence for the improvement of peripheral insulin resistance or deregulation of pancreatic β cell function in GCS KO mice is lacking. Further studies will be required to differentiate the respective roles of excessive *de novo* ceramide production and its conversion into gangliosides in the regulation of energy and glucose homeostasis by the hypothalamus.

Among the other ceramide metabolites, sphingomyelin has been shown to function also as a signalling molecule [26]. Similar to gangliosides, sphingomyelin is also enriched in lipid rafts. In peripheral tissues, conversion of ceramide into sphingomyelin is rather protective against insulin resistance. This is supported by evidence that shows the inhibition of the ceramide transporter CERT in muscles and is associated with a higher attenuation of insulin signalling by palmitate [75]. Moreover, it has been shown that when sphingomyelin synthesis in islets of Langerhans is affected, this is associated with a defect in insulin secretion, likely because of mitochondrial dysfunction [88]. To date, there is no direct evidence for a specific regulatory role of hypothalamic sphingomyelin in the regulation of energy and glucose homeostasis. In the hypothalamus, obesity has been shown to decrease the interaction of AKT with lipid rafts enriched in cholesterol and sphingolipids [89]. This effect was correlated with the recruitment of TANK-binding kinase 1 (TBK1), a negative insulin receptor signalling [89]. The exact mechanism of TBK1 recruitment to lipid rafts and whether sphingomyelin is required is presently unknown.

It has been shown that changing the flux of ceramide metabolism towards the synthesis of less harmful lipids, such as sphingosine 1-

phosphate (S1P), is a way to prevent the deleterious effects of saturated fatty acids. At the periphery, forcing S1P synthesis in pancreatic β cells protected them from apoptosis induced by palmitate [90]. Moreover, KO mice for sphingosine kinase 1 (SphK1) predisposed them to T2D, induced by obesity [91]. Whether ceramide metabolism into S1P plays a role in the hypothalamus remains to be determined. However, a recent study showed that intracerebroventricular injection of S1P reduced food intake and promoted the elevation of body temperature [92]. S1P mediates its cellular effect through the activation of specific G-protein coupled receptors [92]. In the hypothalamus, S1P1 receptor disruption also increased food intake [92]. Interestingly, intracerebroventricular infusion of leptin increased S1PR1 levels in the hypothalamus of rats, lending credence that S1P signalling in the hypothalamus could contribute to the anorectic effect of leptin. If this is the case, leptin signalling in the hypothalamus is expected to inhibit CPT1c-dependent *de novo* ceramide biosynthesis and stimulate S1P1 signalling to decrease food intake. This regulation would be altered in obese mice [92]. The S1P1 receptor is highly enriched in hypothalamic POMC neurons of rats [92], suggesting that leptin will regulate both POMC and AgRP/NPY neurons by targeting different sphingolipid pathways/metabolism. In concert, leptin will decrease *de novo* ceramide biosynthesis AgRP/NPY neurons and stimulate the S1P1 receptor in POMC neurons to decrease food intake. Targeting the balance between ceramide and S1P that could be used as a novel therapeutic approach is an interesting hypothesis [22]. Interestingly, a recent study has shown that the accumulation of cholesterol and ceramide observed in the neurologic disease Niemann-Pick type C is reduced by a sphingosine kinase 1 (SphK1) activator [93].

6. CONCLUSIONS AND PERSPECTIVES

In the last decade, several studies evidenced that *de novo* ceramide synthesis in specific areas of the hypothalamus plays a central role in the physiological regulation of energy homeostasis through orexigenic neuropeptides AgRP/NPY. Most importantly, excessive accumulation of ceramide from *de novo* synthesis in the hypothalamus under lipotoxic conditions may play a key role in the deregulation of energy balance and lead to food intake disorders, obesity, and the associated perturbation of glucose homeostasis.

A recent study combining lipidomic analysis and metabolic phenotyping in mouse models of obesity, and more importantly, in human prospective cohorts (DESIR) evidenced that plasma ceramides are potential diabetes susceptibility biomarker candidates [94]. Quantitative analysis revealed that specific long-chain fatty acid-containing dihydroceramides, the precursor of ceramide (Figure 2), were significantly elevated in the plasma of individuals who are likely to develop diabetes [94]. The rise in the levels of dihydroceramides in the plasma is probably a reflection of an increase in the flux of *de novo* ceramide synthesis. Studies suggest that early onset of deregulation of hypothalamic homeostasis could be the premise for the later development of obesity and metabolic disorder associated in mice [95]. Whether an early upregulation of hypothalamic *de novo* ceramide synthesis could play a central role in the later development of obesity and T2D will require further experimentation.

Additionally, a deep molecular analysis of the role of ceramide metabolism, including the role of gangliosides and sphingomyelin, will help to understand the precise role of these sphingolipids in metabolic disease at the level of the brain. As many pharmacological targets exist for ceramide reduction in preclinical studies, and some medications which inhibit ceramide production are presently approved for human use [96], novel therapies targeting ceramide accumulation in the brain may represent the future of obesity management and better prevention of T2D. In particular, acid sphingomyelinase inhibitors hold promise for new therapies for Alzheimer's disease and depression, while acid ceramidase inhibitors are potential agents for cancer therapies as reviewed by Kornhuber et al. [96]. The inhibitor of GCS, Genz123346, could be also of interest as it could target the peripheral and hypothalamus to prevent insulin resistance [87,97].

In conclusion, *de novo* ceramide synthesis in the hypothalamus appears to be a physiological and pathophysiological signal in the regulation of energy and glucose homeostasis. Future studies will be required to determine the nature of neural cells which produce and/or are the target of ceramide. It also remains to be determined whether the regulatory effects of ceramides are specific to particular species of ceramides. Therefore, a better knowledge of ceramide metabolism and action in the hypothalamus may lead to an earlier and more successful diagnosis and therapeutic options for patients suffering from obesity and associated metabolic disorders.

AUTHOR CONTRIBUTIONS

CM and HLS: conceived the idea, wrote the article, and approved the final version.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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