Lead Article

Potential relationship between dietary long-chain saturated fatty acids and hypothalamic dysfunction in obesity

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Diet-induced hypothalamic inflammation, which leads to hypothalamic dysfunction and a loss of regulation of energy balance, is emerging as a potential driver of obesity. Excessive intake of long-chain saturated fatty acids is held to be the causative dietary component in hypothalamic inflammation. This review summarizes current evidence on the role of long-chain saturated fatty acids in promoting hypothalamic inflammation and the related induction of central insulin and leptin insensitivity. Particularly, the present review focuses on the molecular mechanisms linking long-chain saturated fatty acids and hypothalamic inflammation, emphasizing the metabolic fate of fatty acids and the resulting lipotoxicity, which is a key driver of hypothalamic dysfunction. In conclusion, long-chain saturated fatty acids are key nutrients that promote hypothalamic inflammation and dysfunction by fostering the build-up of lipotoxic lipid species, such as ceramide. Furthermore, when long-chain saturated fatty acids are consumed in combination with high levels of refined carbohydrates, the proinflammatory effects are exacerbated via a mechanism that relies on the formation of advanced glycation end products.

INTRODUCTION

The developed world and, increasingly, the developing world are facing an obesity epidemic that imposes major health and economic burdens on society. The obesity epidemic is reaching alarming proportions: in 2014, 39% of adults were overweight, of whom 13% were obese. Even more concerning is that 42 million children under the age of 5 were reported to be overweight or obese in 2013.[1] The health-related problems caused by obesity are immense; indeed, obesity is not an independent pathophysiological condition of excessive adipose tissue accumulation but is a well-recognized risk factor for a plethora of comorbidities, including type 2 diabetes, cardiovascular disease, steatohepatitis, certain types of cancer, and mental health illnesses such as dementia and cognitive impairment.[2–4]

Both environmental and genetic factors contribute to the pathogenesis of obesity. Genetic factors include mutations in the genes that encode leptin, the leptin receptor, the melanocortin-4 receptor (MC4R), and the prohormone convertase enzyme involved in proopiomelanocortin (POMC) processing.[5] Nevertheless, obesity originating from these monogenetic defects is relatively uncommon.[6] Thus, it is more plausible that environmental factors, in combination with a genetic predisposition, are the main players in the obesity epidemic.[7] The major environmental factors contributing to the development of obesity are the ready availability of highly palatable, energy-dense food and a sedentary lifestyle. Of these, a processed, energy-dense diet, particularly one rich in long-chain saturated fatty acids and sugar, also known as the Western diet, is a key contributor to weight gain and the deterioration of metabolic health.[8–12]

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Reversal of obesity through lifestyle modification, ie, caloric restriction and increased energy expenditure, is frequently successful. However, almost all individuals inevitably regain weight within several years.[13] The cause of this is thought to be a compensatory response mediated by the hypothalamus to counteract negative energy balance and, therefore, to protect body weight. In order to achieve this, the hypothalamus enhances appetite and energy efficiency in order to shift the energy balance equation and prevent further weight loss.[14,15] Several studies investigating the effects of lifestyle modification have confirmed the predisposition of humans to regain the weight previously lost via dietary interventions. This is exemplified by the fact that more than 90% of the weight lost through a calorie-restricted diet tends to be regained after a follow-up period of 5 years.[16] More recent evidence shows that individuals who implement lifestyle changes to lose weight, despite being successful in the short-term, fail to maintain long-term weight loss.[17,18] The same paradigm applies in mice. When exposed to cold, mice increase their energy expenditure to maintain body temperature. This is followed by an initial loss in adiposity, which then triggers an increase in food intake to offset the high energy demands needed to avoid further weight loss.[19] Thus, it appears that the systems regulating energy balance respond effectively to negative energy balance.

A great deal of evidence demonstrates there is also an efficient system to prevent weight gain. Indeed, lean human volunteers who were overfed for 6 months in order to increase their body weight by 20% reduced their adherence to the dietary regimen toward the end of the study, despite initial compliance, presumably because of the onset of counterregulatory mechanisms aimed at limiting weight gain.[20] Finally, once the targeted weight gain had been achieved and the participants were free to eat ad libitum, they experienced a profound anorexigenic response, with food intake returning to preintervention levels only when the participants' initial body weight was reestablished.[20] These results have been confirmed in other human and animal studies.[21,22]

Thus, the neuroendocrine system that governs energy homeostasis responds to both positive and negative fluctuations in energy balance to maintain body weight within a tight range. In light of this, it has been speculated that hypothalamic dysfunction, which affects the regulation of energy balance, leads to failure of the energy homeostatic system, which in turn leads to obesity[23,24]

THE HYPOTHALAMUS AS THE MASTER REGULATOR OF ENERGY BALANCE

The first evidence pointing to the hypothalamus as being pivotal in the regulation of energy balance came from studies demonstrating how lesions in different hypothalamic nuclei diversely affect energy homeostasis. In animals, lesions of the ventromedial nucleus of the hypothalamus (VMH) produced obesity,[25] whereas lesions of the lateral hypothalamus (LH) resulted in weight loss characterized by aphagia.[26] The hypothalamus contains highly specialized and diverse neuronal populations that are crucial to the regulation of energy homeostasis. These neurons are structurally and functionally clustered together to form nuclei, encompassing the arcuate nucleus of the hypothalamus (ARC), the VMH, the LH, the paraventricular nucleus of the hypothalamus (PVN), and the dorsomedial nucleus of the hypothalamus (DMH).[27] Of these nuclei, the ARC is known as the master regulator of energy balance, playing a prominent role in controlling feeding behavior. Key to the function of the ARC is its location around the base of the third ventricle, in proximity to the median eminence. The median eminence is where the modified blood-brain barrier allows direct interaction between the hypothalamus and the circulating nutrients and hormones, which convey information related to the peripheral energy status.[23,28,29]

The ARC encompasses 2 major distinct sets of neurons that exert opposite regulatory effects on feeding and energy expenditure. The activity of both groups of neurons is dictated by the ability of these neurons to integrate and respond to peripheral cues that convey information about nutritional status to the hypothalamus. Neurons that are referred to as *orexigenic* promote feeding, inhibit energy expenditure, and express neuropeptide Y (NPY) and agouti-related peptide (AgRP).[30] In contrast, neurons expressing the peptide precursor POMC, together with cocaine and amphetamine-regulated transcript (CART), are termed *anorexigenic* and inhibit appetite while stimulating energy expenditure.[31] Enzymatic cleavage of POMC by the prohormone convertases[32] generates different biological peptides, including α-melanocyte-stimulating hormone (α-MSH), which is crucial to the regulation of energy balance[33] (Figure 1).

POMC/CART and NPY/AgRP neurons, in combination with MC4R-expressing neurons located in the PVN, form the melanocortin system, the role of which in regulating energy balance is demonstrated by the obese phenotype arising from MC4R deletion in both animals and humans.[5,34] POMC/CART and NPY/AgRP neurons represent first-order neurons that receive direct nutritional and hormonal inputs from the periphery, which are then conveyed to the second-order neurons in the PVN. Together, the first- and second-order neurons constitute the melanocortin system. The activation of NPY/AgRP elicits an increase in food intake and a decrease in energy expenditure that is

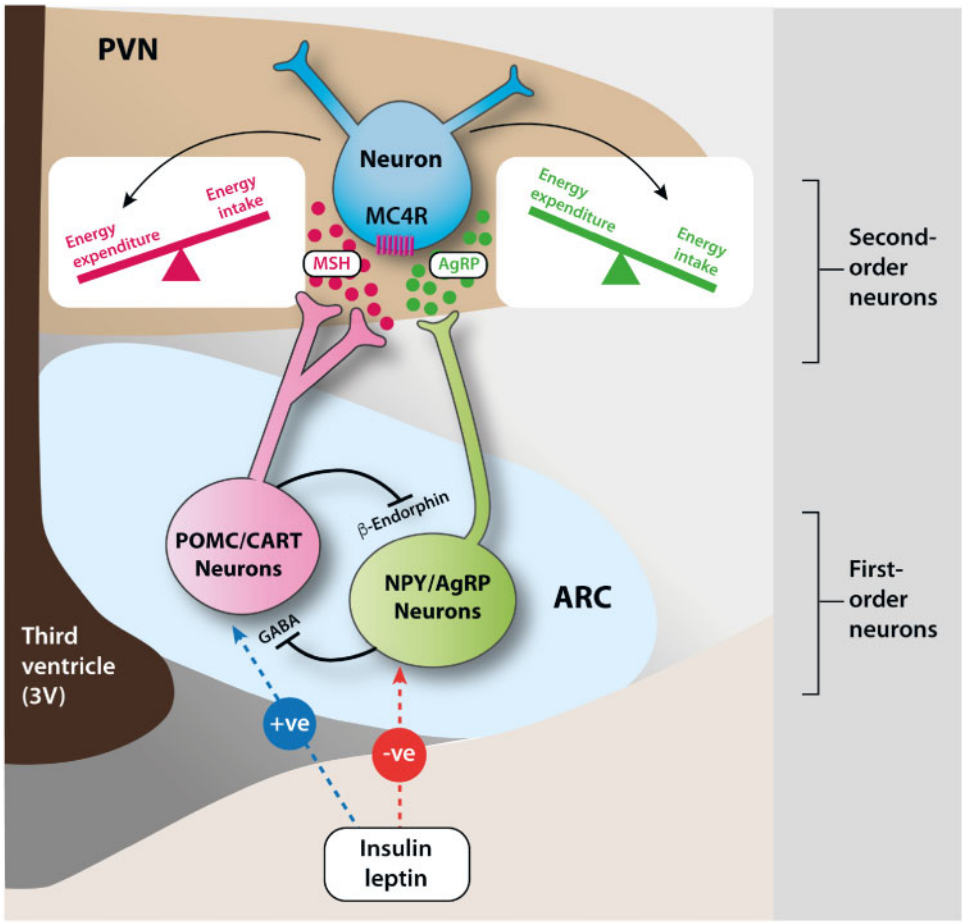

Figure 1 **Synaptic organization of the melanocortin system.** Illustration showing how (POMC/CART) neurons interact with NPY/AgRP-expressing neurons and how these neurons modulate the activity of second-order neurons, such as MC4R-expressing neurons, in the paraventricular nucleus of the hypothalamus. Both α-MSH, synthesized by POMC/CART neurons, and AgRP, the product of NPY/AgRP neurons, compete at the MC4R, with the former acting as an agonist and the latter acting as an antagonist or inverse agonist. The synaptic inputs interconnecting POMC/CART and NPY/AgRP neurons are largely inhibitory and allow a reciprocal fine-tuned regulation. This bidirectional crosstalk between NPY/AgRP and the POMC/CART neurons involves ϒ-aminobutyric acid and β-endorphin. *Abbreviations and symbols*: AgRP, agouti-related peptide; ARC, arcuate nucleus of the hypothalamus; CART, cocaine- and amphetamine-regulated transcript; GABA, gamma-aminobutyric acid; MC4R, melanocortin 4 receptor; MSH, melanocyte-stimulating hormone; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVN, paraventricular nucleus; +ve, positive; –ve, negative.

orchestrated by the downstream activation of the NPY receptors NPY Y1 and/or NPY Y5,[35] while the release of AgRP acts as an inverse agonist[35] or antagonist[36] at the MC4R (Figure 1).

In contrast to the effects of NPY and AgRP, exogenous administration of α-MSH, the product of POMC enzymatic cleavage, suppresses food intake, thereby acting as an MC4R agonist at neurons in the PVN.[37–39] Both α-MSH and AgRP also interact with the melanocortin-3 receptor (MC3R), again acting as an agonist and an inverse agonist, respectively.[40] However, the role of MC3R in energy balance is more controversial, as MC3R is thought to act upstream of MC4R.[41]

Thus, these 2 subsets of neurons in the ARC interact to regulate energy balance. This interaction occurs at 2 different levels: the first level, described above, encompasses the opposing effects of AgRP and α-MSH at the MC4R, while at the second level, the mechanism involves a direct interaction between NPY/AgRP and POMC/CART neurons. Projections from NPY/AgRP neurons directly synapse onto POMC/CART neurons and release NPY and ϒ-aminobutyric acid, both of which inhibit POMC neurons, thereby providing a direct fine tuning of POMC/CART neuronal activity[42] and restraining the activity of POMC/CART neurons while NPY/AgRP neurons are firing. In opposition to this, POMC/CART neurons inhibit NPY/AgRP neurons via both the release of β-endorphin (Figure 1) and the inhibition of AMP-activated protein kinase (AMPK).[43] This reciprocal regulation forms the basis of the homeostatic regulation of energy balance.

The activity of both NPY/AgRP neurons and POMC/CART neurons is modulated by peripheral metabolic cues that convey the nutritional status of an individual, with signals of positive and negative energy balance exerting diametrically opposite effects on this homeostatic system. As expected, fasting stimulates NPY/AgRP neurons, which results in increased food intake and decreased energy expenditure. In contrast, POMC/CART neurons are activated postprandially, eliciting an anorectic response. As described above, the ARC is perfectly anatomically placed, at the border of the blood-brain barrier, to receive peripheral inputs that include insulin and leptin. Both POMC/CART neurons and NPY/AgRP neurons express receptors for the anorexigenic hormones leptin and insulin, which regulate both the gene expression and the firing rate of POMC and AgRP neurons.[44–47]

REGULATION OF ENERGY BALANCE BY LEPTIN AND INSULIN

Leptin

Leptin, a peptide hormone containing 146 amino acids, is produced principally by adipose tissue in proportion to the amount of energy stored as triglyceride.[48,49] It plays a major role in energy balance by signaling long-term energy status to the brain, particularly the hypothalamus, where integration with other homeostatic cues occurs.[50,51] Leptin signals via leptin receptors, which comprise 6 different isoforms identified thus far: LRa, LRb, LRc, LRd, LRe, and LRf.[52,53] These isoforms represent 3 receptor types: soluble, short, and long. The soluble form binds to circulating leptin and is thought to regulate the levels of free circulating leptin,[54] the short form is involved in the transport of leptin across the blood-brain barrier,[55] and the long form of the leptin receptor (LRb), a type I cytokine receptor, is required for leptin signaling.[52,53,56] Leptin binds to LRb, which is expressed in the ARC, DMH, VMH, LH, and the hindbrain.[52,57–61] Activation of LRb by leptin induces the recruitment of Janus kinase 2 (JAK2), which in turn binds to and phosphorylates the intracellular domain of LRb at 3 different tyrosine (Tyr) residues—Tyr_{985}, Tyr_{1077}, and Tyr_{1138}—that are responsible for the biological effects of leptin (Figure 2).[52,62,63] Of these, Tyr_{1138} plays a pivotal role by inducing the recruitment of signal transducer and activator of transcription 3 (STAT3), which, following phosphorylation by JAK2, migrates to the nucleus to regulate gene transcription (Figure 2).[63] The importance of STAT3 in the regulation of energy balance by leptin is further highlighted by the hyperphagia and severe obesity that results from brain-specific knockout of *STAT3* in LRb-expressing neurons.[64,65] Mutation of Tyr_{1138} in LRb also leads to a decrease in energy expenditure and thyroid function as well as hyperphagia, even in the face of hyperleptinemia.[66,67]

Besides activating the JAK/STAT signaling pathway, leptin also activates insulin receptor substrate 2 (IRS2) and phosphoinositide 3-kinase (PI3K) in the mediobasal hypothalamus (Figure 2).[68] The activation of insulin receptor substrate (IRS) requires the protein SH2-B, a JAK2-interacting protein that enhances the activity of JAK2 and induces the recruitment of IRS and its consequent phosphorylation by JAK, ultimately leading to the activation of PI3K.[69,70] Phosphoinositide 3-kinase, a shared mediator of both the insulin and the leptin signaling pathways, exerts its function by catalyzing the conversion of phosphatidylinositol-4,5-bisphosphate (PIP_2) to phosphatidylinositol-3,4,5-trisphosphate (PIP_3). Its activity is inhibited by the tumor suppressor, phosphatase and tensin homolog (Pten), that pushes the reaction in the opposite direction.[71] Leptin directly activates PI3K in POMC neurons but not in AgRP neurons, whereas insulin induces PI3K activity in both neuronal populations.[72] The activation of PI3K by leptin increases the firing of POMC neurons and is required for the acute action of leptin on the neuroelectrical activity of these neurons. Inhibition of PI3K in POMC neurons leads to a decrease in firing rates and an impairment in leptin-induced action potential.[73]

Leptin also inhibits AMPK activity in the PVN and ARC,[74] leading to a decrease in food intake.[74] The mammalian target of rapamycin (mTOR) is a further downstream effector of the PI3K/Akt signaling pathway. It is expressed in both AgRP and POMC neurons and responds to fluctuations in levels of nutrients, particularly amino acids.[75] In contrast to AMPK activity, mTOR activity increases under conditions of energy surplus, and intracerebroventricular administration of leptin increases both mTOR expression and activity.[75] Importantly, deletion of p70 S6 kinase, a downstream target of mTOR, impairs the ability of leptin to reduce food intake in mice.[76] The mTOR/p70 S6 kinase pathway thus mediates the anorectic effect of leptin by promoting the phosphorylation and, therefore, the inhibition of AMPK.[75,77]

The prominent role of leptin in the regulation of energy homeostasis is eloquently demonstrated by the fact that *ob/ob* mice (naturally occurring leptin knockout mice) and leptin-deficient humans are hyperphagic, have decreased energy expenditure, and are extremely obese.[78,79] The same phenotype is displayed by defects of the leptin receptor in mice (*db/db* mice) and humans.[80,81] In summary, leptin exerts its anorexigenic effect by activating POMC/CART-expressing neurons and inhibiting AgRP/NPY-expressing neurons in the

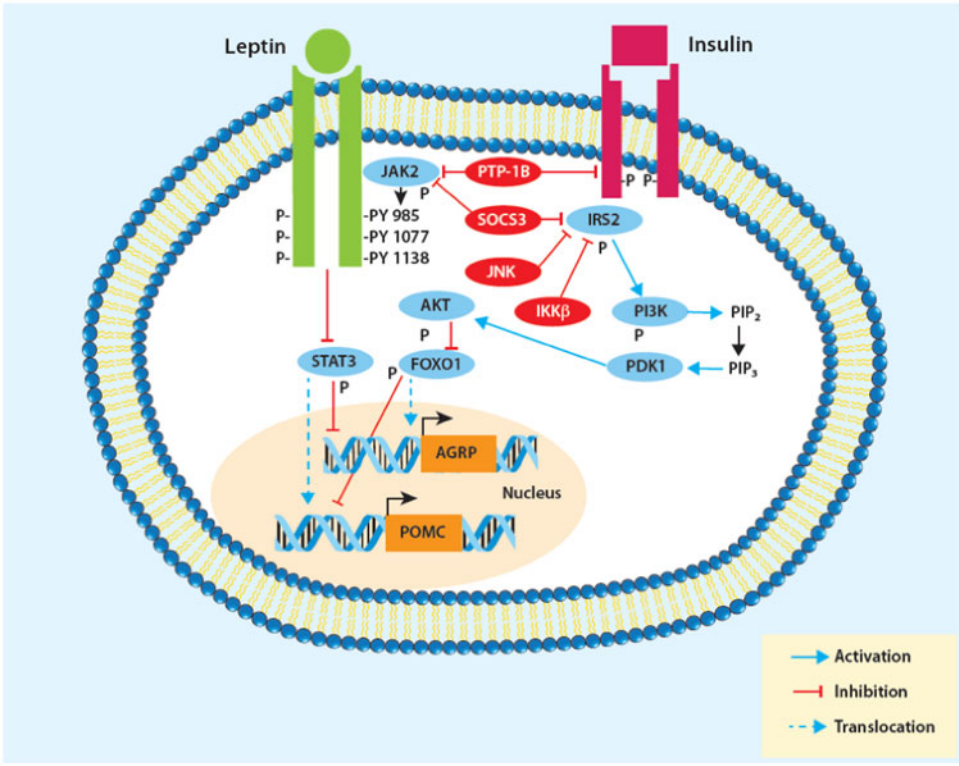

Figure 2 **Hypothalamic leptin and insulin signaling pathways and their inhibition in insulin and leptin resistance.** Leptin binds to the extracellular domain of the LRb, promoting the recruitment and autophosphorylation of JAK2, which in turn phosphorylates the intracellular domain of the LRb at 3 tyrosine residues: Tyr985, Tyr1077, and Tyr1138 (shown as PY 985, PY 1077, and PY 1138). Tyr1138 (PY 1138), in particular, recruits STAT3, which is phosphorylated and activated. Once activated, STAT3 translocates to the nucleus, where it promotes the expression of POMC while inhibiting the expression of AgRP. Leptin also activates IRS2 and the PI3K in the mediobasal hypothalamus. Insulin, upon binding to the insulin receptor, activates the tyrosine kinase activity of the β subunits of the receptor, promoting both autophosphorylation and phosphorylation of IRS2. IRS2 activates PI3K, which converts PIP_2 to PIP_3, which in turn activates PDK1. Finally, PDK1 phosphorylates and activates AKT, which phosphorylates and inhibits FOXO1, preventing the transcriptional events that it mediates. Insulin and leptin signaling are inhibited by a variety of kinases and phosphatases. JNK and IKKβ phosphorylate IRS2 at serine residues, inducing IRS2 inhibition. SOCS3 induces proteosomal degradation of IRS2 and inhibits JAK2, and PTP-1B dephosphorylates both JAK and the insulin receptor. *Abbreviations*: AgRP, agouti-related peptide; AKT, protein kinase B; FOXO1, Forkhead box protein O1; IKKβ, inhibitor of nuclear factor κB kinase β; IRS2, insulin receptor substrate 2; JAK2, Janus kinase 2; JNK, c-Jun N-terminal kinase; LRb, leptin receptor isoform b; P, phosphorylation; PDK1, 3-phosphoinositide-dependent protein kinase 1; PIP_2, phosphatidylinositol 4,5-bisphosphate; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; PI3K, phosphoinositide 3-kinase; POMC, proopiomelanocortin; PTP-1B, protein tyrosine phosphatase 1B; SOCS3, suppressor of cytokine signaling 3; STAT3, signal transducer and activator of transcription 3; Tyr, tyrosine.

ARC. During fasting, circulating leptin levels drop, resulting in an increase in *AgRP* and *NPY* expression and a downregulation of *POMC*, which in turn promotes food intake while decreasing energy expenditure.[82]

In addition to its role in the regulation of feeding behavior, leptin also increases energy expenditure via activity of the sympathetic nervous system. In rodents, the leptin-induced increase in energy expenditure is mediated by an upregulation of uncoupling protein 1 (*UCP-1*) in brown adipose tissue, thereby promoting thermogenesis.[83,84] Besides its role in the regulation of energy balance, leptin plays major roles in the regulation of glucose homeostasis, reproduction, sexual maturation, neuroendocrine and immune function, and bone metabolism.[24,85,86]

Despite leptin being proposed as a panacea for obesity treatment, this initial hope was negated by the fact that hyperleptinemia fails to curtail food intake and promote body weight loss in obese individuals, thus indicating a state of leptin resistance. The defective transport of leptin across the blood-brain barrier and the activation of pathways that disrupt leptin signaling within the hypothalamus, especially in the ARC, have been reported to be pivotal in the onset and progression of leptin resistance.[87–89]

Insulin

Circulating levels of insulin directly reflect and signal whole-body adiposity to the hypothalamus.[90] Insulin modulates feeding behavior by acting via the insulin

receptor in hypothalamic areas that include the ARC, the DMH, and the PVN,[91,92] with intracerebroventricular injection of insulin proving less effective than intrahypothalamic infusion,[93]

The activated insulin receptor is coupled to tyrosine kinase activity, with insulin inducing autophosphorylation of the intracellular domain of the receptor, which in turn recruits and activates the IRS family of adaptor molecules. The IRS2 isoform of the receptor is highly expressed in the ARC,[94] the activation of which is essential for the anorectic effect of insulin, as demonstrated by increased adiposity, hyperphagia, and insulin resistance in mice lacking IRS2.[95,96] Activation of PI3K occurs downstream of IRS2[97] and, in turn, acts on PIP_2 to generate PIP_3, which activates several downstream kinases, including the 3-phosphoinositide–dependent protein kinase 1 (PDK1), Akt, and members of the atypical protein kinase C family. In turn, Akt phosphorylates Forkhead box protein O1 (FOXO1), inducing its relocation from the nucleus to the cytoplasm, which results in downregulation of *AgRP* and upregulation of *POMC* (Figure 2).[98] Phosphoinositide 3-kinase can also modulate neuronal activity by affecting cell excitability via adenosine triphosphate (ATP)-sensitive potassium channels, with activation of PI3K producing hyperpolarization of both AgRP and POMC neurons.[99,100]

Mice lacking insulin develop hyperphagia, but not obesity, in agreement with the known lipogenic role of insulin. On the contrary, intracerebroventricular administration of insulin counteracts hyperphagia and decreases the expression of the orexigenic neuropeptide *NPY*.[47] Inhibition of insulin signaling in the VMH via administration of an anti-insulin antibody produces an increase in feeding behavior,[101] and neuron-specific disruption of the insulin receptor gene results in diet-induced obesity, mild insulin resistance, increased circulating insulin levels, and hypertrygliceridemia.[102]

Insulin signaling in the hypothalamus also plays a crucial role in the regulation of peripheral glucose homeostasis, as demonstrated by the ability of insulin to suppress hepatic glucose production[103] by acting on hypothalamic ATP-sensitive potassium channels to decrease the expression of liver glucose-6 phosphatase and phosphoenolpyruvate carboxykinase, key enzymes in the gluconeogenetic pathway.[104] Deletion of the insulin receptor, specifically in AgRP neurons, impairs the ability of insulin to suppress hepatic glucose production during a euglycemic-hyperinsulinemic clamp, indicating that these neurons play a fundamental role in the regulation of hepatic glucose production by insulin.[99] Knockout of the hypothalamic insulin receptor and re-expression specifically in AgRP or POMC neurons demonstrated that re-expression of the insulin receptor in AgRP neurons, but not in POMC neurons, results in normalization of insulin control over hepatic glucose production.[105]

Beside its role in the control of food intake and glucose homeostasis, insulin also affects energy expenditure. Mice treated with diazoxide, an inhibitor of insulin secretion, show an impaired thermogenic response following carbohydrate ingestion,[106] and insulin injection in the PVN of the hypothalamus results in increased energy expenditure and body temperature.[107]

The mechanisms underlying the regulation of energy balance described thus far emphasize the pivotal roles of both leptin and insulin in the hypothalamic regulation of energy balance and metabolism. Nonetheless, these anorexigenic signals appear to be overridden in obesity, with elevated levels of both hormones failing to effectively inhibit food intake, which implies a state of leptin and insulin resistance. The pathophysiology underpinning leptin and insulin resistance points to the overconsumption of an energy-dense diet rich in long-chain saturated fatty acids and sugars, which, in rodents, results in hypothalamic inflammation and dysfunction characterized by the loss of the hypothalamic response to insulin and leptin (Figure 3).[23,108,109] These studies link nutrient overconsumption, or the "overnutrition" typical of the Western diet, to hypothalamic dysfunction and obesity.[110]

Hypothalamic inflammation

It is widely documented that overnutrition induces a state of low-grade chronic inflammation in the absence of demonstrable local or systemic infection. This has been termed *meta-inflammation*.[2] This atypical low-grade inflammation particularly affects insulin-responsive tissues such as the liver,[111] adipose tissue,[112,113] and muscle[114] and is strongly associated with overnutrition-induced metabolic disorders.

Diet-induced metabolic inflammation, also referred to as meta-inflammation, not only affects peripheral tissues but also occurs in the mediobasal hypothalamus.[108,115,116] The first study that reported high-fat diet-induced hypothalamic inflammation in rats showed a marked upregulation of the proinflammatory cytokines interleukin 1β (*IL-1*β) and tumor necrosis factor α (*TNF-*α), detectable after 16 weeks.[115] This finding was confirmed and extended by studies that reported a more rapid hypothalamic inflammatory response in rodent models of high-fat diet-induced obesity.[108,109,116] In these rats, the hypothalamic inflammatory response appeared after 24 to 72 hours, as indicated by an increased expression of proinflammatory cytokines.[116] Furthermore, acute lipid infusion in mice has been reported to increase hypothalamic TNF-α immunoreactivity within 24 hours.[117] This shows that

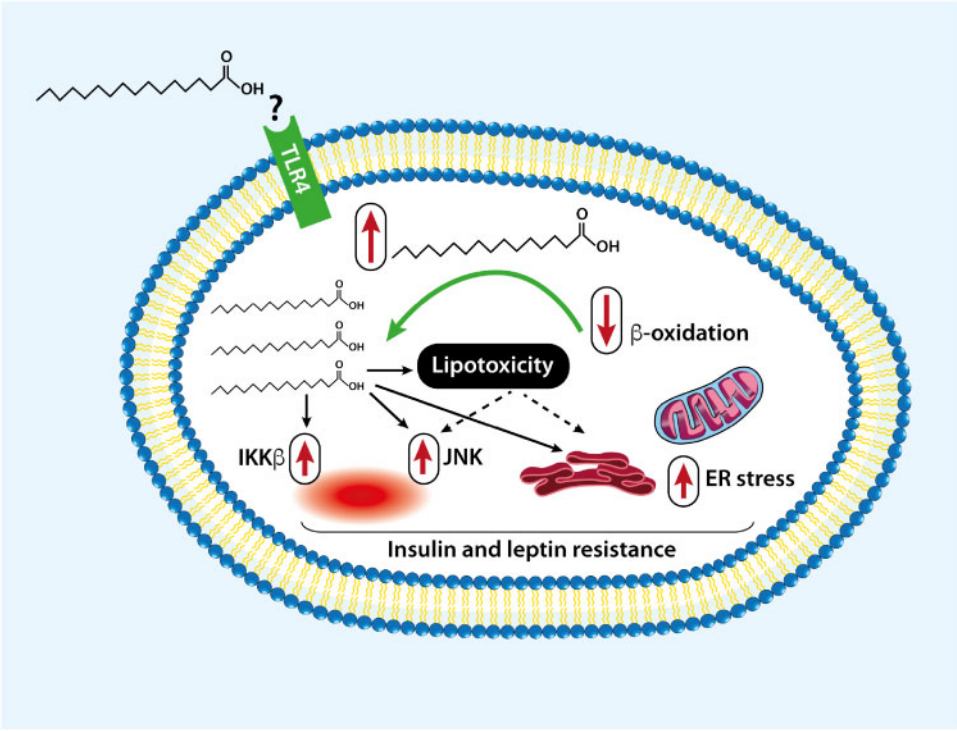

Figure 3 **Activation of intracellular pathways by long-chain saturated fatty acids, which leads to insulin and leptin resistance.** An increase in the supply of long-chain saturated fatty acids to the brain leads to the induction of inflammatory pathways that may involve activation of the TLR4, but this remains controversial. Metabolic overload with long-chain saturated fatty acids, in the face of reduced mitochondrial β-oxidation, promotes activation of IKKβ and JNK as well as ER stress. These pathways are also activated by the build-up of lipotoxic lipid species such as ceramide, which may represent one of the key mediators of fatty acid–induced hypothalamic dysfunction. *Abbreviations*: ER, endoplasmic reticulum; IKKβ, inhibitor of nuclear factor κB kinase β; JNK, c-Jun N-terminal kinase; TLR4, Toll-like receptor 4.

hypothalamic inflammation occurs more rapidly than peripheral inflammation and is not a consequence of the inflammatory response in the periphery. Indeed, in rodents fed a high-fat diet, the accumulation of macrophages in peripheral tissues takes several weeks or months to develop.[113,118] Moreover, hypothalamic inflammation precedes the onset of obesity, implying that it is an early driver of metabolic dysfunction associated with high-fat feeding. While the majority of studies linking hypothalamic inflammation to obesity are based on animal models, hypothalamic inflammation,[119] including gliosis[116] and hypothalamic damage,[120] has also been reported in obese humans. Similar to observations in rodent models, hypothalamic inflammation in humans appears to be driven by dietary fats rather than other dietary components or total energy intake.[119] Additionally, a high-fat diet has been reported to induce hypothalamic inflammation in humans via modulation of the gut microbiome rather than by directly targeting the hypothalamus.[119] However, these later findings should be interpreted with care, especially since dietary intake data in this study relied solely on self-reported nutritional questionnaires.[119]

Two major intracellular signaling mechanisms appear to drive diet-induced hypothalamic inflammation: activation of c-Jun N-terminal kinase (JNK)[115] and the inhibitor of nuclear factor κB kinase (IKKβ)/nuclear factor-κB (NF-κB)[108,109] pathways. The JNK family of stress-activated protein kinases belong to the mitogen-activated protein kinase family and are activated in response to a number of stimuli, including bacterial lipopolysaccharide, oxidative stress, endoplasmic reticulum (ER) stress, growth factors and pro-inflammatory cytokines, particularly TNF-α.[121] JNK exerts its effects on inflammation by stabilizing the messenger RNA (mRNA) of proinflammatory cytokines and other genes involved in the inflammatory process.[121–123] The IKKβ/NF-κB pathway is key to the innate immune response. In the inactive state, the transcription factor NF-κB is sequestered in the cytoplasm by the inhibitor of NF-κB, which is phosphorylated and inhibited by IKKβ, therefore allowing the dimerization and migration of NF-κB to the nucleus, where it elicits the expression of genes related to inflammation.[124]

Genetic and pharmacological approaches that disrupt crucial components of the inflammatory signal transduction pathway in the hypothalamus prevent

both diet-induced inflammation and insulin resistance, implying that meta-inflammation is key in the pathogenesis of insulin resistance and metabolic dysfunction. For example, the expression of a dominant-negative form of IKKβ in the mediobasal hypothalamus decreased high-fat diet-induced weight gain in rodents.[109] Similarly, central pharmacological or genetic inhibition of the IKKβ/NF-κB pathway was associated with increased insulin sensitivity, attenuated high-fat diet-induced glucose intolerance as well as weight gain, and reduced food intake in rats fed a high-fat diet.[108,125] Conversely, induction of hypothalamic inflammation by promoting the expression of a constitutively active form of IKKβ, infusion of tunicamycin in the third ventricle to induce ER stress, or central infusion of low-dose TNF-α recapitulates the metabolic features associated with high-fat feeding.[109,126,127] Furthermore, central pharmacological inhibition of JNK restored insulin signaling and promoted weight loss in rats fed a high-fat diet.[115]

Thus, high-fat diet-induced hypothalamic inflammation is thought to be a causative factor in body weight gain and obesity. A study showing the upregulation of hypothalamic proinflammatory markers 3 days after the start of high-fat feeding, a time frame too short to be reflective of significant weight gain, argues against a primary role of obesity per se in promoting hypothalamic inflammation, suggesting hypothalamic inflammation as a cause, rather than a consequence, of obesity.[116]

Although the association between hypothalamic inflammation and obesity is widely reported, it is imperative to distinguish between diet-induced low-grade chronic inflammation and the inflammatory response typical of the sickness response. While diet-induced chronic low-grade inflammation results in obesity, the robust acute inflammation that occurs as part of the sickness response results in weight loss, despite activating the same pathways.[128] The sickness response produces anorexia characterized by a decrease in body weight and an increase in energy expenditure to sustain elevated core body temperature. This paradox is explained by the different effects exerted by the administration of a low dose vs a high dose of TNF-α into the brain. Central administration of low doses of TNF-α in rats produces antithermogenic effects,[126] while high doses increase the expression of neuropeptides involved in promoting thermogenesis and decrease the expression of the orexigenic peptides *NPY* and melanin-concentrating hormone (*MCH*).[129] Thus, the low-level chronic hypothalamic inflammation elicited by overnutrition results in a different physiological outcome than the acute inflammation caused by the sickness response.

MOLECULAR MECHANISMS LINKING HIGH-FAT DIET, INFLAMMATION, AND INSULIN AND LEPTIN RESISTANCE

Several mechanisms have been proposed to explain how high-fat diet-induced hypothalamic inflammation affects insulin and leptin signal transduction. As described above, JNK activity is upregulated during diet-induced hypothalamic inflammation.[115] This kinase induces serine phosphorylation of IRS, a shared effector of both insulin and leptin signaling pathways, impairing its ability to activate its downstream target, PI3K.[2,130] This, in turn, leads to a decrease in insulin-induced Akt phosphorylation, resulting in functional insulin resistance (Figure 2).[131] Pharmacological inhibition of JNK reverses diet-induced hypothalamic insulin resistance, demonstrating the importance of JNK as a negative modulator of insulin signaling.[115] However, the role of JNK in mediating insulin and leptin resistance is not entirely clear, as central nervous system–specific *JNK* knockout has been shown not to prevent high-fat diet-induced leptin resistance and body weight gain in mice,[132] but neuron-specific *JNK* knockout was found to be protective against diet-induced obesity via increased activity of the hypothalamus-pituitary-thyroid axis.[133]

The IKKβ/NF-κB signaling pathway also contributes to the diet-induced disruption of energy balance by inhibiting insulin and leptin signaling. Overexpression of a constitutively active form of *IKKβ* results in reduced insulin and leptin signaling in the hypothalamus, while neuron-specific *IKKβ* deletion protects against obesity in animals on a high-fat diet.[109] Consistent with this, intracerebroventricular administration of an IKKβ inhibitor is protective against high-fat diet-induced insulin resistance[108] and decreases the expression of suppressor of cytokine signaling 3 (*SOCS3*), a negative regulator of leptin signaling in the ARC, suggesting enhanced leptin sensitivity.[125] In a similar fashion to JNK, IKKβ is also able to induce serine phosphorylation of IRS and can therefore hamper downstream signal transduction (Figure 2).[2,130]

Upregulation of *SOCS3* represents a further mechanism by which inflammation can interfere with both insulin and leptin signaling pathways. *SOCS3* is induced by a high-fat diet as a result of the activation of both the JAK2/STAT3 and IKKβ/NF-κB pathways.[110] Two separate mechanisms are responsible for *SOCS3*-induced leptin and insulin resistance. First, SOCS3 interacts directly with the leptin receptor and leptin-induced STAT3 to block protein-protein interactions, thereby disrupting signal transduction.[134] The second mechanism involves SOCS3-induced ubiquitination and subsequent proteosomal degradation of IRS (Figure 2).[134] Neuron-specific deletion of *SOCS3* improves leptin

sensitivity and exerts a protective effect against diet-induced obesity,[135] while overexpression of *SOCS3* in POMC neurons produces hyperphagia and obesity despite mice consuming a chow diet.[136] Surprisingly, however, upregulation of *SOCS3* in LRb-expressing neurons does not produce the same phenotypical features, indicating that SOCS3 plays specific roles in defined neuronal subtypes.[136]

Both dephosphorylation and phosphorylation of specific amino acid residues within the downstream effectors of both leptin and insulin signaling pathways link inflammation and resistance to these hormones. The protein tyrosine phosphatase 1B (PTP-1B) catalyzes the dephosphorylation and, therefore, the inactivation of the insulin receptor IRS and JAK2 (Figure 2),[137] and feeding mice a high-fat diet leads to an upregulation of PTP-1B in different tissues, including the hypothalamus.[138,139] This effect is mirrored by TNF-α administration, indicating a functional link between inflammation and PTP-1B regulation.[138] In keeping with this, neuronal or POMC-specific deletion of *PTP1B* protects mice against diet-induced obesity by promoting increased energy expenditure and enhanced sensitivity to both leptin and insulin.[140,141] Furthermore, targeting PTP-1B in the hypothalamus of rats by intracerebroventricular administration of antisense oligonucleotides, selectively blunting *PTP1B* expression, improves insulin and leptin signaling and produces a decrease in food intake, body weight, and adiposity following high-fat feeding.[142]

A high-fat diet also triggers ER stress, leading to the accumulation of unfolded proteins and the activation of the unfolded protein response. The unfolded protein response contributes to both high-fat diet-induced hypothalamic inflammation and insensitivity to leptin and insulin.[109,110] Indeed, the induction of ER stress in lean mice produces a leptin-resistant phenotype,[128] and leptin receptor signaling in the hypothalamus can be restored by chemical chaperones that reduce ER stress by enhancing protein folding and reducing the unfolded protein response.[127] Endoplasmic reticulum stress, along with the subsequent unfolded protein response, increases the activity of both JNK and IKKβ/NF-κB in the periphery.[143] However, this is not a unidirectional relationship, as these inflammatory pathways are themselves able to trigger ER stress, thereby generating a vicious cycle. This was confirmed by an increase in ER stress in animals expressing a constitutively active form of IKKβ in the mediobasal hypothalamus. In contrast, neuron-specific *IKKβ* deletion resulted in a decreased unfolded protein response.[109] The unfolded protein response functions to restore ER homeostasis, but when it persists, as in the case of high-fat feeding, it induces apoptosis in hypothalamic neurons, further compromising hypothalamic function.[144]

DIETARY FATTY ACIDS AND HYPOTHALAMIC INFLAMMATION

Long-chain saturated fatty acids, rather than caloric excess per se, are emerging as the major nutritional triggers of hypothalamic inflammation, leading to insulin and leptin resistance and obesity (Figures 3 and 4). Indeed, intracerebroventricular administration of palmitic, stearic, arachidic, and behenic acids in rats has been reported to increase the expression of proinflammatory markers[145] and promote the hypothalamic induction of IKKβ, leading to the consequent decrease in levels of inhibitor of NF-κB.[108] This is accompanied by the activation of JNK and the upregulation of *TNFα*, *IL1β*, and *IL6*.[146] The ability of long-chain saturated fatty acids, and of palmitic acid in particular, to elicit an upregulation in the expression of proinflammatory cytokines as well as ER stress and activation of JNK has been further investigated and confirmed using both hypothalamic neuronal cell lines and primary hypothalamic cultures. Palmitic acid challenge induces the expression of proinflammatory cytokines[147–149] as well as ER stress and activation of JNK in cultured hypothalamic neurons.[150] Interestingly, the deleterious effect of a high-fat diet on the hypothalamus appears to be more marked in male than in female mice.[148,151] This sexually dysmorphic response protects premenopausal female mice from high-fat diet-induced hypothalamic inflammation, as demonstrated by the inability of a high-fat diet to induce the expression of *IL1β*, *IL6*, or *TNFα* solely in female mice.[148] A high-fat diet in animals and palmitic acid in cell cultures have both been reported to significantly decrease the expression of the estrogen receptor α (ERα). This occurs in males, but not in females, suggesting the decrease in ERα is involved in the induction of hypothalamic inflammation in male mice.[148] This was confirmed by the overexpression of ERα and the pretreatment of neuronal cells with 17β-estradiol both being protective against palmitic acid–induced inflammation.[148] The downregulation of ERα was reported to be driven by the downregulation of peroxisome proliferator-activated receptor 1α (PGC-1α), the master regulator of mitochondrial biogenesis and oxidative metabolism. Expression of *PGC-1α* was decreased only in male mice in response to a high-fat diet, implying a protective role of PGC-1α against high-fat diet-induced hypothalamic inflammation in females.[148,151] Moreover, the downregulation of both *PGC-1α* and *ERα* in male mice is associated with an increase in sphingolipids and palmitic acid in the hypothalamus.[148] Thus, given the role of PGC-1α as an important regulator of

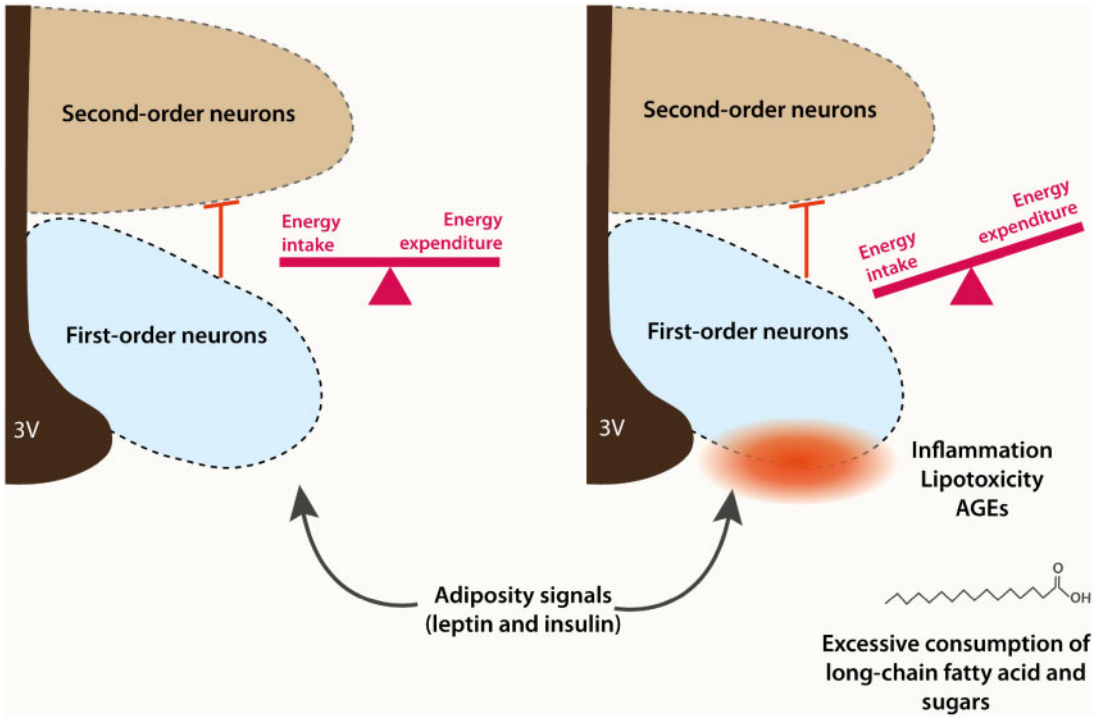

Figure 4 **Effect of long-chain saturated fatty acids on hypothalamic regulation of energy balance.** Energy balance is maintained via hypothalamic sensing and integration of peripheral adiposity signals such as insulin and leptin (left). The excessive consumption of long-chain saturated fatty acids, such as palmitic acid, and sugars promotes lipotoxicity, the build-up of AGEs, and inflammation, which trigger resistance to leptin and insulin, thereby impairing energy balance (right). *Abbreviations*: AGEs, advanced glycation end products; 3V, third ventricle.

energy metabolism,[152] it may be speculated that the positive effect exerted by estrogens and PGC-1α on hypothalamic inflammation may, at least in part, rely on the modulation of hypothalamic fatty acid metabolism.

In support of this, fatty acid metabolism appears to be an important factor in mediating long-chain saturated fatty acid–induced hypothalamic inflammation. Long-chain saturated fatty acids are more obesogenic than isocaloric consumption of other fats, and this fat-type-specific effect may depend on the different rates at which fatty acids are β-oxidized. Indeed, long-chain saturated fatty acids are less prone to β-oxidation than medium-chain fatty acids (carbon chain length $\leq$ 12) and unsaturated fatty acids.[8] This may explain, at least in part, why excessive intake dietary intake of long-chain saturated fatty acids results in metabolic inflammation. In support of this, while enteric gavage of long-chain saturated fatty acids elicits hypothalamic inflammation, the same response is not observed when coconut oil, composed mainly of medium-chain fatty acids (rich in the C12:0 lauric acid), or olive oil, composed primarily of monounsaturated fatty acids (rich in the omega-9 oleic acid), was used.[153] Moreover, the main component of the experimental diet used to induce obesity in rodents is lard, and partial substitution of the fatty acids generally found in a standard high-fat diet with either flax oil (rich in the omega-3 fatty acid linolenic acid) or olive oil (rich in the omega-9 fatty acid oleic acid) reverses the inflammatory response elicited by a lard-based high-fat diet, improves hypothalamic and whole-body insulin sensitivity, decreases food intake, and reduces adiposity.[154] These results were confirmed by intracerebroventricular infusion of either linolenic acid or oleic acid, which resulted in a decrease in the expression of the proinflammatory markers *IL-6* and *TNFα* and an upregulation of the anti-inflammatory cytokine *IL-10*, accompanied by a reduction in both food intake and adiposity.[154] The anti-inflammatory properties of mono- and polyunsaturated fatty acids were further demonstrated using a hypothalamic neuronal cell line in which palmitic acid induced the expression of both *IL6* and *TNFα*, while oleic acid and eicosapentaenoic acid, an omega-9 and a omega-3 fatty acid, respectively, not only were anti-inflammatory but also counteracted palmitic acid–induced upregulation of proinflammatory cytokines.[149] Although long-chain saturated fatty acids have been described as major culprits in promoting hypothalamic dysfunction and consequent obesity, this does not exclude the possibility that other dietary lipids may contribute to hypothalamic inflammation and body weight gain. Of these, linolenic acid appears to be a potential candidate, with studies showing that it elicited greater weight gain compared with saturated fatty acids, independent of hypothalamic

inflammation.[155] Furthermore, a high-fat diet enriched with linoleic acid induced hypothalamic inflammation and body weight gain in rodents.[156] However, a major caveat of these studies is the lack of appropriate controls, ie, a high-fat diet enriched with linoleic acid was compared with a diet high in either saturated fatty acid from coconut oil (rich in the medium-chain fatty acid lauric acid)[155] or polyunsaturated fatty acids from fish oil (rich in omega-3 fatty acids)[156] which have both been reported to be protective against obesity.[154,157] Furthermore, when compared with stearic acid, a long-chain saturated fatty acid, linoleic acid exerted an anti-inflammatory effect in cultured immortalized hypothalamic neurons and enhanced insulin and leptin signaling.[158] High-fat diets rich in oleic acid have also been shown to induce an increase in body weight and adiposity.[159,160] Nonetheless, this remains controversial, with the results of animal and human studies showing a protective effect of oleic acid against body weight gain and increased adiposity. Indeed, while feeding rodents a high-fat diet enriched with lard led to an increase in body weight, this was prevented by a high-fat diet enriched with olive oil (high in oleic acid).[154] Moreover, diets high in monounsaturated fatty acids decreased fat mass and central obesity when compared with diets high in saturated fatty acid[161] or omega-6 polyunsaturated fatty acid.[162] Besides their effects on body weight, monounsaturated fatty acids have been reported to improve insulin sensitivity when compared with dietary saturated fatty acids,[163] further emphasizing the beneficial effect of monounsaturated fatty acids, and oleic acid in particular, on metabolic health.[164]

The notion that the detrimental effects of long-chain saturated fatty acids are, at least in part, dependent on their low rate of β-oxidation has been investigated by modulating hypothalamic fatty acid metabolism. Increasing fatty acid catabolism prevents high-fat diet-induced ER stress and inflammation, thereby ameliorating hypothalamic dysfunction induced by long-chain saturated fatty acids. This was demonstrated by treating a hypothalamic neuronal cell line, mHypoE-44, with the AMPK activator aminoimidazole carboxamide ribonucleotide, which, by promoting fatty acid catabolism, inhibited palmitic acid–induced JNK activation and ER stress and restored insulin signaling.[150] In addition, increasing fatty acid β-oxidation decreases the expression of proinflammatory markers in primary hypothalamic neurons, alleviating the inflammatory response induced by palmitic acid.[147] In contrast, increasing fatty acid catabolism by long-term expression of a constitutively activated form of the enzyme carnitine palmitoyl transferase 1A (CPT1A) in the VMH of rats resulted in hyperphagia, body weight gain, and peripheral insulin resistance, suggesting increasing fatty acid catabolism in the VMH is detrimental to metabolic health.[165] This was further confirmed by inhibition of global hypothalamic CPT1A and the subsequent reduction of fatty acid β-oxidation in rats, by genetic and pharmacological means, which decreased both food intake and hepatic glucose production.[166] These studies shed light on the role of hypothalamic fatty acid catabolism in the regulation of energy balance under normal physiological conditions and in the absence of a high-fat diet, supporting the possibility that increasing hypothalamic fatty acid availability promotes satiety and regulates peripheral glucose homeostasis. However, this does not exclude the possibility that increasing hypothalamic fatty acid catabolism during high-fat feeding may mitigate the detrimental effects of fatty acid overload in the hypothalamus. Apart from genetic approaches, pharmacological agents have also been used experimentally to provide insights into the role of hypothalamic fatty acid metabolism in energy balance. Treatment of rodents with C75, an inhibitor of both fatty acid synthase and carnitine palmitoyl transferase 1 (CPT1),[167] induced a decrease in the expression of *AgRP*, a reduction in food intake, and an increase in energy expenditure,[168–170] further supporting the contention that modulation of hypothalamic fatty acid metabolism has profound downstream effects on energy homeostasis. Nevertheless, this remains a matter of debate. Indeed, intracerebroventricular administration of C89b, a drug that selectively stimulates CPT1 without affecting fatty acid synthase, decreases feeding and body weight in mice,[171] indicating that CPT1 activation, rather than inhibition, may decrease feeding behavior. However, these findings must be interpreted with caution, as the potential off-target effects of this drug are still unknown.

MECHANISMS LINKING LONG-CHAIN SATURATED FATTY ACIDS AND HYPOTHALAMIC DYSFUNCTION

Long-chain saturated fatty acids have been shown to promote inflammation by activating the Toll-like receptor (TLR) 4 and NF-κB pathway (Figure 3).[145,172–175] This appears to be further confirmed in rodent models of high-fat diet-induced obesity, with long-chain saturated fatty acids activating the TLR4 in macrophages and adipose tissue.[176] In vitro studies also support the possibility that long-chain saturated fatty acids can activate TLR2 and TLR4.[172]

The ability of long-chain saturated fatty acids to promote an inflammatory response via the TLR4 also appeared to hold true for hypothalamic inflammation.[145] Although a high-fat diet activates both IKKβ and JNK, the inhibition of TLR4 signaling only affects IKKβ, indicating that the high-fat diet activates JNK via

a mechanism that does not require TLR4 activation.[132] Nonetheless, in agreement with the role of TLR4 as mediator of high-fat diet-induced hypothalamic inflammation, whole-body or brain-specific inhibition of TLR4 counteracts high-fat diet-induced hypothalamic inflammation and body weight gain.[132,145] However, other investigators failed to confirm this, instead showing that ablation of *TLR4* does not result in any differences in body weight, adiposity, circulating levels of free fatty acids, or serum insulin.[177] Indeed, the ability of long-chain saturated fatty acids to directly activate the TLR4 remains a matter of debate. Some reports negate this possibility,[149,178–180] while others suggest that long-chain saturated fatty acids do not bind directly to the TLR4 but instead exert their effects via mediation by an endogenous ligand.[181] Thus, the involvement of the TLR4 in mediating long-chain saturated fatty acid–induced inflammation remains to be fully elucidated.

Lipid overload in tissues not suited for lipid storage is emerging as a key driver of lipid-induced metabolic dysfunction and represents a further mechanism linking long-chain saturated fatty acids with meta-inflammation. When the capacity of adipose tissue to store fat is exceeded, lipid spillover occurs. Excess circulating free fatty acids reach nonadipose tissues, including the liver, pancreatic β-cells, skeletal muscle, heart, and kidneys, where they may be channeled toward non-oxidative pathways, ultimately resulting in the production of toxic lipid species such as ceramide and diacylglycerol,[182,183] a process termed *lipotoxicity*.[184] Lipotoxicity contributes to β-cell failure and the progression to type 2 diabetes,[185] with saturated fatty acids responsible for the accumulation of ceramide in pancreatic β cells[186] as well as in skeletal muscle and liver.[187,188] The hypothalamus is also prone to lipotoxicity (Figure 4). Indeed, a high-fat diet increases levels of hypothalamic ceramide, lysophosphatidylcholine, cholesterol esters, and diacylglycerol. This effect appears to be dependent on the high-fat diet rather than on the obese phenotype per se, as *ob/ob* mice fed a low-fat diet display a healthy hypothalamic lipidomic profile,[189] indicating that increased body weight does not induce the accumulation of lipotoxic compounds within the hypothalamus. However, obese Zucker rats, which lack a functional leptin receptor, have increased ceramide levels in the VMH, indicating that obesity may play a role in ceramide accumulation in the hypothalamus.[190]

Importantly, lipotoxicity, especially long-chain saturated fatty acid–induced ceramide accumulation, produces insulin resistance in peripheral tissues, underlying the negative effects exerted by long-chain saturated fatty acids and their metabolites on metabolic health.[191,192] The ability of ceramide to induce insulin resistance in liver and skeletal muscle suggests that lipotoxicity-induced insulin resistance may also affect the hypothalamus (Figures 3 and 4). A high-fat diet has been shown to induce the hypothalamic build-up of coenzyme A derivatives of stearic acid and palmitic acid (2 long-chain saturated fatty acids), and the accumulation of these derivatives dampens insulin signaling and increases IKKβ activity.[108] Of note, these pathological outcomes are not dependent on total caloric intake but on dietary fat consumption.[108] Levels of oleoyl-coenzyme A, which is monounsaturated and protective against obesity,[193] remain unaffected in the hypothalamus of rats fed a high-fat diet,[108] providing further evidence that long-chain saturated fatty acids are the major contributors to diet-induced hypothalamic dysfunction.

In peripheral tissues, lipotoxicity is manifest through ER stress and inflammation.[194,195] This also occurs in the hypothalamus, where ceramide-induced lipotoxicity leads to ER stress and metabolic dysfunction.[190] Ceramide accumulation within the mediobasal hypothalamus has a detrimental effect on the regulation of energy balance. Central administration of a cell-penetrating ceramide (C6 ceramide) produces an increase in body weight associated with an accumulation of C16 ceramide in the mediobasal hypothalamus.[190] Of note, the increase in body weight is not dependent on increased feeding behavior but relies on decreased energy expenditure, as demonstrated by the decrease in key thermogenic genes in brown adipose tissue.[190] Overexpression of the glucose-regulated protein 78 kDa (*GRP78*), a chaperone that improves protein folding, in the VMH reverses the effect of central C6 ceramide administration in rats. This effect is marked by a decrease in the unfolded protein response, which results in a decrease in adiposity and an increase in body temperature and *UCP1* expression, suggestive of increased thermogenesis.[190] Interestingly, these effects were limited to the VMH, as overexpression of *GRP78* in the ARC does not affect body weight, thermogenesis, or feeding.[190] Inactivation of *GRP78* promotes the unfolded protein response in the VMH, which is associated with an increase in body weight and a decrease in thermogenic markers in the brown adipose tissue,[190] confirming the role of ER stress in mediating the anabolic effects of ceramide. The effect of ceramide-induced ER stress is seen in obese Zucker rats, which have increased ceramide levels in the VMH. Moreover, the overexpression of *GRP78* in the VMH of obese Zucker rats produces a decrease in body weight that, again, is associated with an increase in thermogenesis and a more favorable metabolic profile.[190] Finally, *GRP78* overexpression increases insulin and leptin signaling in the VMH,[190] in agreement with the notion that ER stress negatively affects insulin and leptin sensitivity.[109,127,196,197] Besides the

activation of the unfolded protein response, accumulation of C16 ceramide in the hypothalamus also induces the upregulation of proinflammatory cytokines, confirming the close relationship between lipotoxicity, inflammation, hypothalamic dysfunction, and body weight gain.[190] Palmitic acid was also shown to promote the accumulation of C16 ceramide in a hypothalamic neuronal cell line along with the upregulation of proinflammatory cytokines, confirming the association between lipotoxicity and meta-inflammation. The anti-inflammatory effect of oleic acid and eicosapentaenoic acid was also confirmed and was dependent, albeit in part, on the ability of these fatty acids to counteract palmitic acid–induced ceramide accumulation.[149] Furthermore, palmitic acid–induced impairment of insulin signaling in cultured hypothalamic neurons is restored by inhibition of ceramide synthesis, confirming the role of ceramide in the inhibition of insulin signal transduction. The role of ceramide was also confirmed in vivo in obese Zucker rats. In this rodent model, the inhibition of ceramide synthesis in the hypothalamus improved hypothalamic insulin sensitivity and partially restored glucose tolerance.[198]

Long-chain saturated fatty acids have a detrimental effect on hypothalamic neuroendocrine function, yet lipids are not consumed as an isolated component in the diet. Indeed, dietary lipids, especially in the context of a Western diet, are often consumed together with refined carbohydrates and sugar. It should also be noted that the high-fat diets used to induce obesity in rodents contain sucrose as the primary carbohydrate.[199] The overconsumption of simple carbohydrates, and particularly those contained in soft drinks made with high-fructose corn syrup, has been associated with obesity,[200] the metabolic syndrome, and type 2 diabetes.[201] Simple carbohydrates, especially fructose, are particularly lipogenic and increase de novo lipid synthesis.[202] This does not apply to complex carbohydrates, as evidenced by a reduction in fatty acid synthesis following substitution of dietary starch for sugar.[203] The increase in de novo lipogenesis triggered by fructose is a direct consequence of fructose being rapidly metabolized in the liver, which leads to an increase in lipogenic substrates, such as acetyl coenzyme A, and the upregulation and increased activity of lipogenic enzymes.[204] This is also supported by animal studies showing that fructose, compared with glucose, is converted to fatty acids at a significantly higher rate, thereby causing an increase in circulating triglyceride levels.[205] Although these endogenously produced fatty acids can be transported directly to the hypothalamus via very low-density lipoproteins,[153] whether they can trigger hypothalamic inflammation is still unknown. In any case, an increase in endogenous fatty acid synthesis plays an important role in the development of obesity, especially since dietary carbohydrates, particularly fructose, induce the activity of stearoyl-coenzyme A desaturase 1. Mice lacking this lipogenic enzyme exhibit decreased fatty acid synthesis and increased fatty acid catabolism, both of which confer protection against obesity and insulin resistance.[206–208] Nevertheless, regardless of whether simple carbohydrate-induced de novo lipogenesis promotes hypothalamic dysfunction, sugars, in concert with lipids, have been shown to directly contribute to hypothalamic inflammation.

In the absence of dietary carbohydrates, long-chain saturated fatty acids cause less microglial activation than diets containing high levels of both fat and carbohydrates.[209] The proposed mechanism involves the formation of advanced glycation end products (AGEs) in hypothalamic neurons, which then bind to the receptor for AGEs (RAGE) in microglia, thereby promoting microglial activation and inflammation.[209] Knockout of *RAGE* and the related activated leukocyte cell adhesion molecule (*ALCAM)* receptor results in blunted microglial reactivity and an improved metabolic phenotype, even in animals fed a high-carbohydrate, high-fat diet.[209]

Diet-induced hypothalamic inflammation not only causes obesity but also results in a number of metabolic consequences. These include loss of glycemic control, resulting in part from hypothalamic insulin insensitivity.[24] Increased blood glucose levels further aggravate hypothalamic inflammation by promoting the formation of AGEs, which activate inflammatory and neurotoxic mechanisms that play a crucial role in the pathogenesis of neurodegenerative diseases.[210–212]

CONCLUSION

Hypothalamic inflammation damages the hypothalamic neuroendocrine network governing energy balance and metabolism in rodent models, resulting in body weight gain and impaired glycemic control. However, human studies addressing the cause–effect relationship between hypothalamic inflammation and obesity, as well as the role of long-chain saturated fatty acids in promoting hypothalamic dysfunction, are lacking. Nonetheless, hypothalamic damage, inflammation, and gliosis have been reported in obese humans, supporting the possibility that hypothalamic inflammation may be implicated in the pathogenesis of obesity in humans. Rodents remain useful models to investigate the molecular mechanisms governing diet-induced hypothalamic inflammation and provide invaluable mechanistic insights into the impact of dietary nutrients on hypothalamic dysfunction. Long-chain saturated fatty acids appear to be pivotal in this process, with several mechanisms being

proposed for their metabolically detrimental effect: TLR4 activation, lipotoxicity, and ER stress, all coexisting and reinforcing each other by activating parallel pathways. Remarkably, not all dietary fatty acids exert the same effects on hypothalamic inflammation, with unsaturated fatty acids, particularly omega-3 and omega-9, being protective against hypothalamic inflammation and the subsequent metabolic dysfunction. Finally, when long-chain saturated fatty acids are consumed in combination with high levels of refined carbohydrates, their proinflammatory effects are exacerbated. Thus, it appears that the metabolic fate of long-chain dietary fat is responsible for triggering and sustaining hypothalamic inflammation, an effect that is amplified in the presence of refined carbohydrates. A speculative explanation for this is that on a low-carbohydrate diet, dietary fats become a major source of energy, with fatty acids being more effectively β-oxidized. This enhanced fatty acid catabolism contributes to negating the effects of lipotoxicity and the downstream activation of hypothalamic inflammation, ER stress, and hypothalamic dysfunction.

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