How fat is stored in mammals sparked vigorous scientific debate in the mid-to-late 1800s, yielding conflicting concepts and false starts. Major schools of thought proposed that fat accumulated in unspecialized connective tissue cells in the form of multilocular ‘mulberry’ cells or that ‘wandering’ cells in the plasma accumulated fat and gathered in the connective tissue. Another view posited the current concept that adipocytes occupy a specialized ‘glandular’ tissue, but as late as 1901, this hypothesis was the least appealing in a comprehensive review of the literature. How surprising it would be to these early investigators that adipose tissue is indeed glandular in the true sense, functioning as an endocrine organ that can control systemic functions and that it secretes potent paracrine and autocrine factors that modify its glandular nature. It would be even more startling for these investigators to learn that adipose tissue can be a major heat-generating tissue, critical for many animals to sustain body temperature during extreme cold exposure. Viewed in this context, the multiple roles that adipose tissue has in mammalian physiology are truly remarkable and unexpected.

During the past couple of decades, it was also revealed that adipose tissue has a strong influence on whole-body glucose metabolism and lipid metabolism through its effects on major tissues and organs such as skeletal muscle, liver and brain. Figure 1 (parts 1 and 2) illustrates several well-studied pathways through which this tissue crosstalk has been shown to operate. First, storage of lipids in white adipocytes, where these lipids are mostly derived from hydrolysis and intracellular re-esterification of triglycerides in circulating lipoproteins, serves not only as a reservoir of calories for future use but also as a means to sequester lipids away from peripheral tissues that are vulnerable to lipotoxicity that disrupts insulin’s actions on metabolism. Thus, in obesity, fatty acids derived from dietary intake and white adipocyte lipolysis can increase hepatic glucose production through the generation of the allosteric effector acetyl-CoA and attenuate utilization of glucose in skeletal muscle by inhibiting glucose transport and metabolism. These actions, combined with the dampening of pancreatic islet production of insulin, promote glucose intolerance, leading to type 2 diabetes mellitus (T2DM). By contrast, high levels of fatty acid oxidation, observed in the adipocytes of brown adipose tissue (BAT) and brown-like beige adipocytes in white adipose tissue (WAT) that express UCP1, can also decrease lipid accumulation in peripheral tissues and increase glucose tolerance. Thus, these main functions of white adipocytes to store fat and brown adipocytes...
Reviews

**Key points**

* Adipocytes modulate whole-body metabolism through secretion of endocrine and paracrine factors that modulate local immune cell cytokine secretion, endothelium blood flow and neuronal signalling to the brain.
* Adipocytes, the endothelium and immune cells within adipose tissues secrete factors such as leptin, vascular endothelial growth factor (VEGF) and tumour necrosis factor (TNF) that regulate local sensory nerve fibre functions.
* Adipocyte lipid metabolism communicates with local sensory nerve fibres, sending signals to the central nervous system; conversely, sensory nerve fibres secrete factors such as calcitonin-related gene peptide (CGRP) and substance P that might regulate the metabolism of adipocytes and other adipose-resident cells.
* Increased lipolysis in white adipose tissue in response to sympathetic activation can cause sensory nerve fibres to regulate the metabolic activity of distant brown adipose tissue depots.
* Extensive and dynamic signalling networks among the diverse cell types in adipose tissue integrate and mediate communication through bioactive lipids to local sensory nerve fibres and neurotrophic factors to sympathetic nerve fibres.
* Identifying factors within adipose tissues that regulate the function of sensory and sympathetic nerve fibres might reveal therapeutic strategies for obesity and type 2 diabetes mellitus.

to oxidize fat allow adipose tissue to control systemic insulin sensitivity and susceptibility to metabolic disease. Many reviews have extensively investigated the endocrine regulation of adipocyte lipid metabolism in detail. A growing literature highlights an additional mode of adipose control over systemic metabolism — paracrine signalling from adipocytes to localized nerve fibres. Extensive and dynamic signalling networks among the diverse cell types in adipose tissue facilitate communication through paracrine factors such as monocyte chemoattractant protein 1 (MCP1) and tumour necrosis factor (TNF) that attract or activate macrophages, T and B lymphocytes and other immune cells. In turn, these cells secrete cytokines that can locally disrupt adipocyte lipid sequestration and lipolysis or act systemically to inhibit insulin secretion or insulin action in other tissues.

**Sensory innervation of adipose tissue**

Two modes of communication have been described whereby the metabolic status of adipose tissue is communicated to the brain. The first is exemplified by the WATokine leptin, which is released from adipose tissue into the circulation and acts on the hypothalamus to regulate appetite and systemic metabolism. The discovery of leptin in 1994 definitively established a functional role of adipocytes as endocrine cells. The second mechanism of adipose to brain communication is mediated through the sensory innervation of adipose tissue, which comprises an adipose–nerve tract that can disseminate adipose tissue signals over long distances to the CNS. BOX 2 describes the historical perspective of findings on the sympathetic and sensory innervation of adipose tissue. Using the fluorescent retrograde tracer True Blue, sensory fibres in adipose tissue were clearly observed in rats. Moreover, immunohistochemical analysis of adipose tissue labelled with antibodies against molecular markers selectively expressed in afferent neurons, such as calcitonin gene-related peptide (CGRP), which is produced by an alternative RNA splicing of the Calc gene transcripts and cleavage of inactive precursor protein (BOX 1), proves the existence of adipose sensory innervation in hamsters and rats. Subsequently, a series of elegant studies conducted by Bartness and colleagues showed that Siberian hamster adipose tissue is innervated by sensory nerve fibres that convey signals to the brain. In those studies, the H129 strain of herpes simplex virus (HSV) type 1, an anterograde tract tracer, was used to track routes between adipose tissue and the brain through sensory nerves.

Adipose tissue signals to the CNS through this afferent pathway can in turn trigger peripheral responses through sympathetic outflow, as has been reported for WAT signalling to BAT in Siberian hamsters and mice. This intercommunication between adipose depots might be critical for energy balance and proper control of systemic metabolism. Collectively, these results provide evidence that adipose sensory nerves have an important role in detecting metabolic cues within adipose tissue and in conveying signals to the brain from such cues, contributing to metabolic homeostasis.

**Adipose tissue signals to sensory nerves**

**Leptin.** Progress in identifying the adipose-derived molecular signals that regulate local sensory nerve fibres and ultimately modulate adipose–brain communication has been encouraging. Remarkably, the endocrine factor leptin can function in this capacity as a local paracrine factor that modulates local afferent cells and local sensory and sympathetic nerve fibres within the tissue, and on the potential to exploit these processes to develop strategies for future therapies targeting metabolic disease.
of leptin was also shown to stimulate the sympathetic nervous system (SNS) in skeletal muscle. Altogether, these results suggest that leptin is one adipocyte factor that signals to local afferent nerve fibres, communicating the presence of increased fat stores to the brain. Thus, leptin acts both as a circulating factor to control whole-body metabolism through hypothalamic regulation and as a paracrine factor to directly activate adipose afferent fibres that signal to the CNS. Importantly, leptin release by adipocytes is itself acutely regulated by local SNS activity, creating a highly integrated signalling network.

Fatty acids. Adipose-tissue-derived fatty acids have also been reported to be potent activators of sensory nerve fibres in adipose tissue, which suggests that local afferent fibres sense adipose tissue lipolysis and communicate this metabolic flux to the CNS (Fig. 3). Importantly, in response to local fatty acid release from WAT, the brain increases sympathetic drive to BAT to enhance thermogenesis in Siberian hamsters. Other bioactive lipids such as arachidonic acid, eicosanoids and their derived lipids that are produced by adipocytes as well as other cells resident in adipose tissue, such as immune cells and endothelial cells, have also been shown to act on local sensory nerves. Nonetheless, further investigation is necessary to more clearly determine the physiological relevance of these bioactive lipids in the activation of adipose sensory nerves.

Adipose-derived neurotrophic factors

Another class of agents that is released by cells resident in adipose tissue that might regulate local afferent fibres are neurotrophic factors (Box 1; Fig. 3a). These factors are bioactive molecules that control many aspects of neuronal function in both the peripheral nervous system and CNS. The neurotrophic molecules are essential for neuronal development, regeneration, survival and maintenance. Examples of adipose-derived neurotrophic factors include neuregulin 4 (NRG4), brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF). These proteins are powerful regulators of proliferation, survival, migration and differentiation of neurons and are implicated in several functions of the systemic nervous system. These and other neurotrophic peptides produced by adipose tissue might act on local sensory nerves to expand innervation and/or stimulate electrical activity. Of particular interest, human adipocytes derived from adipocyte progenitors that were differentiated and activated in vitro express neuroendocrine factors and the pro-protein convertase PCSK1 (also known as NEC1), variants of which are strongly associated with obesity. Results demonstrating that rodent sensory neurons express the receptors for NRG4, NGF and BDNF, such as the receptor tyrosine protein kinases ERBB3 or ERBB4, TRKA (also known as high-affinity NGF receptor) and TRKB (also known as BDNF/NT-3 growth factors receptor), respectively, also support this notion. Nonetheless, studies on selective genetic deletion of these receptors in sensory neurons will be necessary to determine whether they actually regulate adipose tissue sensory nerve functions.

![Fig. 1](image_url) **Proposed mechanisms whereby adipose tissue controls systemic insulin sensitivity.** Depicted are pathways in white adipocytes (left semicircle) and beige or brown adipocytes (right semicircle) that have been proposed to affect whole-body insulin sensitivity and systemic metabolism in rodents and humans. At room temperature (22 °C) and above, most of the white adipose tissue (WAT) is composed of white adipocytes (left), whereas at low temperatures (6 °C), brown and beige adipocytes appear (22 °C) and above, most of the white adipose tissue (WAT) is composed of white adipocytes (left). Beige adipocytes display increased mitochondrial density and high capacity for fatty acid oxidation into acetyl-CoA (AcCoA), which fuels heat production via mitochondrial UCP1 within the electron transport chain (part 2). White adipocytes upregulate resident immune cells in obesity, releasing cytokines into the circulation (part 3). Secretion of white adipocyte-derived bioactive molecules (denoted WATokines) and beige or brown adipocyte-derived factors (denoted BATokines) (part 4) might modulate adipose vascularization (part 5) and activate local afferent nerve fibres (part 6). A crosstalk between adipocytes and afferent nerve fibres (replicated by the doubled-headed arrows) might occur, as the afferent nerve can release neurotransmitters to communicate with adipose-resident cells. In addition, these adipokines can be released into the circulation to affect distant tissues. BAT, brown adipose tissue; PKA, protein kinase A.
in vivo. A major caveat of this approach, however, is the lack of tools to ablate receptors selectively in adipose tissue sensory nerve fibres without perturbing sensory nerves in other tissues.

**Vascular endothelial growth factor.** The remarkably close anatomical and functional relationship between vascularization and innervation of adipose tissue has been established for a long time\(^{18-22}\). Morphological analysis revealed that fibres innervating adipose tissue juxtapose the vasculature in rats\(^{18,19}\). Moreover, adipocytes secrete vascular endothelial growth factor (VEGF)\(^{15}\) (Fig. 3a), a peptide known to stimulate angiogenesis and promote vascular sympathetic innervation and increase sensory nerve density\(^{18,19}\). Findings published in the past 10 years have highlighted the role of adipose VEGF in the control of adipose tissue function and in improving systemic energy metabolism and glucose tolerance through the enhancement of adipose vascularization\(^{34,35,37}\).

As sensory neurons express VEGF receptor (VEGFR)\(^{39}\), sensory-neuron-specific genetic deletion of VEGFR would be helpful to decipher whether VEGF acts through a sensory nerve VEGFR signalling pathway to enhance adipose vascularization, sensory innervation and systemic metabolism. In addition, the potential roles of the individual isoforms of VEGF in this regard would be of interest and require further investigation.

**Cytokines.** White adipocytes communicate with immune cells that are resident in the adipose tissue, such as macrophages and lymphocytes, to modulate cytokine production and thus regulate the levels of adipose inflammation, as occurs in humans and rodents with obesity\(^{25,37,40}\). Cytokines secreted from macrophages, such as TNF, IL-1β and IL-6, can act directly on the sensory neuron itself\(^{41-43}\) or indirectly through their pro-inflammatory effects within the adipose tissue microenvironment (Fig. 3a). By contrast, anti-inflammatory cytokines, such as IL-17A and adenosine, which originate from alternatively activated macrophages, regulatory T (T\(_{reg}\)) cells and T helper cells, can also interact directly with sensory nerve terminals or indirectly through modulation of the adipose microenvironment to elicit neuronal responses\(^{44-46}\). As such, the inflammatory status of adipose tissue is likely to be highly influential on afferent signalling to the CNS. Altogether, these observations suggest that many bioactive molecules that are derived from adipose tissue function as paracrine factors to regulate local sensory nerves and that additional such factors are still to be discovered.

**Sensory nerve factors**

**Calcitonin gene-related peptide.** Whereas adipose-resident cells secrete factors that act on local sensory nerve fibres, stimulated sensory nerves might also release factors that modulate adipose tissue function in a way that affects whole-body metabolism (Fig. 3b). This phenomenon, known as the axon reflex, enables afferent fibres to signal directly to the peripheral tissue that they innervate, circumventing central signal integration and response\(^{47}\). An example is CGRP, a member of the calcitonin family of peptides, which is synthesized and released from nerves in the CNS as well as from peripheral nerve fibres\(^{47}\). CGRP expression seems to be concentrated in sensory afferents that innervate the vasculature\(^{47}\), which suggests that the endothelium is a primary target for CGRP released by sensory nerves. Accordingly, CGRP acts as a potent vasodilator when it binds to its cognate calcitonin receptor-like receptor (CALCRL, also known as Cgrp type 1 receptor) in endothelial cells\(^{47}\). The activation of CALCRL signalling has also been reported to promote immune cell adhesion and migration through the endothelium and to modulate tissue inflammation\(^{47}\). In this regard, it is conceivable

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**Box 1 | An explanation of specialist terms**

**β-Adrenergic receptor**
The β-adrenergic receptors belong to a class of G protein-coupled receptors that are activated by catecholamines.

**Afferent nerves**
These nerves consist of sensory nerve fibres that can transmit information from the periphery to the central nervous system.

**Beige adipocytes**
These UCP1-positive, thermogenic adipocytes appear within white adipose tissue (WAT) upon stimulus. These cells are often called inducible brown, beige or b (brown in white) adipocytes.

**Browning of WAT**
This process involves the formation of beige adipocytes and/or the conversion of white adipocytes into beige adipocytes within WAT that occur in response to cold or other stimuli. Through this process, expression of UCP1 protein and the thermogenic capacity of WAT are enhanced.

**Calcitonin gene-related peptide**
Calcitonin gene-related peptide (CGRP) is a 37-amino-acid neuropeptide, produced as a consequence of alternative RNA processing of the calcitonin gene (CALCA) and primarily associated with sensory nerve fibres.

**Crosstalk**
In this Review, we refer to the intercommunication between adipose-resident cells and the local neuronal fibres as crosstalk, which is determined by the interactions between secreted molecules from one cell type with the related receptor or receptors in a distinct cell type.

**Efferent nerves**
These nerves consist of sympathetic nerve fibres that travel away from the central nervous system to innervate adipose tissue and other peripheral tissues.

**Hyperinsulinaemia**
A state of supraphysiological circulating levels of insulin. It is closely associated with glucose intolerance, obesity, hypertension and dyslipidemia.

**Macrophage polarization**
The shift in macrophage phenotypic profile between pro-inflammatory M1 subtypes and anti-inflammatory M2 subtypes depending on external cues.

**Neurogenic inflammation**
Local inflammation generated by signals originating from nerve fibres.

**Neuropathy**
The damage or destruction of nerves.

**Neurotrophic factors**
These are molecules that regulate growth, morphological plasticity and the survival potential of neurons.

**Sympathetic drive**
Sympathetic nerve activity.

**UCP1**
A mitochondrial protein found in beige and brown adipocytes that is involved in thermogenesis. It uncouples mitochondrial respiration from ATP production, converting energy of membrane electrical potential into heat.
Box 2 | Historical perspective of adipose tissue innervation

It has long been known that adipose tissue is innervated, but the extent of adipose innervation and its functional importance has become increasingly apparent in the past few years. Sympathetic nerve fibres within adipose tissue were revealed by the mid-1960s129–131, but evidence of sensory innervation of adipose tissue was not reported until >20 years later132,133. Beginning in the 1990s, a growing number of publications appeared on the role of adipose tissue innervation in adipocyte function. The Bartness laboratory, in particular, generated a deep literature on this topic, and adipose tissue biology experienced a renaissance as well, generating the realization that it is a highly complex endocrine organ with extensive influence over systemic homeostasis. Key discoveries in this field have included the finding that fasting increases adipocyte lipolysis primarily as a result of local release of catecholamines through enhanced adipose sympathetic activity rather than through increased circulating levels of catecholamines132,133. Similarly, it was discovered that the antilipolytic effect of insulin is also in part mediated through inhibition of sympathetic activity at the level of the central nervous system (CNS)122,134. The discovery of leptin in 1994 revealed the capacity of adipose tissue to communicate with the CNS and other distant organs through the circulation, and the discovery of many additional adipokines with various potential activities has continued to reinforce this paradigm133.

The graph, generated from PubMed searches of the terms listed, shows the rapidly expanding discovery of neurotrophic factors emitted by adipose tissue that could directly interact with local nerve fibres and other cell types within the tissue. Such adipocyte–nerve crosstalk has added to our knowledge of the complexity of adipose tissue and its relationship to whole-body metabolism and has created a new perspective on the basis of metabolic disorders such as diabetes mellitus, obesity and cachexia. Targeting these disorders through the neuro–adipose connection offers new strategies for future therapeutic interventions. The number of publications was identified in PubMed and is expressed as the total number of results for the specific keywords “adipose tissue innervation” (blue bars) and “adipose tissue and neurotrophic factors” (red bars).

That CGRP released by adipose tissue sensory nerves has a key role in adipose vascular homeostasis and inflammation and thus systemic metabolism.

Studies published in the past decade have shown that genetic deletion of the Calcα gene causes increased energy expenditure and insulin sensitivity with resistance to diet-induced obesity without changes in food intake90,91. Interestingly, these effects were accompanied by evidence of increased SNS activity, which is consistent with the concept that CGRP dampens activity of sympathetic nerve fibres. Whether these phenotypes noted in mice lacking the Calcα gene are due to changes in adipose tissue vascular function and levels of adipose tissue inflammation has not yet been investigated. However, the concept that CGRP released from sensory nerve fibres within adipose tissue directly modulates adipocyte metabolism is suggested from a study revealing lipolytic responses to CGRP administered systemically92. Further exploration of CGRP as a paracrine factor affecting adipocytes within adipose tissue is warranted.

**Substance P.** Substance P is a neuropeptide released by peripheral sensory nerves that, similar to CGRP, acts as a potent vasodilator and immune cell regulator93–95 (Fig. 3). Substance P exerts its biological activity via the high-affinity neurokinin 1 receptor (NK1R; also known as substance P receptor) in endothelial and immune cells96. Interestingly, substance P stimulates immune cells to express several pro-inflammatory cytokines, such as IL-6 and TNFα, which suggests a role for this neuropeptide as a mediator of adipose tissue inflammation in obesity and metabolic dysfunction. Selective genetic deletion of NK1R receptors expressed in endothelial or immune cells in the adipose tissue will be necessary to definitively determine whether substance P–NK1R signalling modulates adipose tissue functions and whole-body metabolism.

**Sensory dysregulation in obesity**

A hallmark of adipose tissue dysfunction in obese animal models and humans with obesity is a low-grade chronic inflammatory state97–99. Immune cell infiltration, macrophage proliferation and increased pro-inflammatory cytokine production are noted in adipose tissue in rodent and human obesity100–103. As inflammatory mediators trigger neuritis and disrupt sensory neuron activity104,105, the increased production of cytokines in adipose tissue might impair the ability of local sensory nerves to convey information from the adipose tissue to the brain. Indeed, sensory nerve dysfunctions in the initial stages of glucose intolerance in patients with obesity have been reported106. Thus, obesity-associated adipose dysfunction and chronic inflammation that disrupts local afferent signalling to the CNS might have adverse consequences for adipose tissue functions and whole-body metabolism. Studies designed to test this hypothesis will be of high interest.

Sensory nerve fibres in adipose tissue might also contribute to the adipose tissue inflammation in obesity. Hypothetically, the adipose tissue expansion and increased release of leptin and free fatty acids in humans and rodents with obesity could cause hyperactivation of local sensory nerve fibres and consequent release of vasoactive and pro-inflammatory neurotransmitters, such as CGRP and Substance P. This process has been well studied in other peripheral tissues and is known as neurogenic inflammation99,100 (Box 1). This type of inflammation occurs when activated afferent nerves release inflammatory mediators, triggering tissue inflammation99,100. Importantly, neurogenic inflammation also seems to have a role in neuritis and nerve damage, which are pathologies often seen in patients with obesity, type 1 diabetes mellitus and T2DM101,102. Therefore, an exciting hypothesis to test is whether sensory neurogenic inflammation contributes to adipose inflammation and dysfunction in obesity.
Central integration of adipose signals

One key area of focus introduced by the findings that adipose tissue depots are innervated by sensory fibres is determining the destination and central function of these afferent fibres. Analyses conducted by the Bartness group applying anterograde tract tracing with HSV H129 demonstrated that sensory innervation of adipose tissue arises from dorsal root ganglion neurons. This neuronal tracing method also revealed extensive projections of the adipose afferent network as well as surprising results regarding the anatomy of afferent fibres innervating different adipose tissue depots. For instance, subcutaneous inguinal WAT (iWAT), the WAT depot most susceptible to browning, seems to be entirely innervated by spinal afferents that typically associate with somatosensory information. Moreover, no signal arising from the nodose ganglion, which is typically associated with visceral afferents, was detected in iWAT. Thus, this distinct sensory innervation in iWAT probably contributes to its increased ability to promptly respond to changes in physiological conditions such as cold exposure compared with other adipose depots. Importantly, these analyses also revealed that these afferent nerves traversed up the spinal cord, localizing in all areas of the brain — the hindbrain, midbrain and forebrain. Of these areas, the forebrain is highly relevant to systemic metabolism, as it contains the hypothalamus. Essential role of the hypothalamus

The hypothalamus (Fig. 4) is essential for regulation of thermogenesis, energy balance and systemic metabolism, integrating signals from peripheral organs and tissues to generate proper physiological responses. In addition to neuroendocrine signalling to the pituitary, the hypothalamus relies on neuronal circuits and connections to communicate directly with peripheral organs and tissues. One example of such a neuronal circuit is the adipose–hypothalamus–adipose neuronal circuit. As shown in Fig. 4a, the pseudounipolar afferent sensory fibres that innervate WAT depots arise from the dorsal root ganglion proximal to the spinal cord. The dorsal root ganglion, which also projects to the brain via the dorsal horn of the spinal cord, relays sensory information from the periphery to the CNS for integration (Fig. 4a). Then, primarily through this hypothalamic integration, signals can be conveyed as sympathetic
outflow to adipose tissue and peripheral organs via the intermediolateral nucleus of the spinal cord (Fig. 4a).

Subsequent studies utilizing both anterograde HSV H129 tracing and retrograde pseudorabies virus tracing from white and brown adipose depots in Siberian hamsters revealed areas of colocalization within the hypothalamus, suggesting the existence of a reflex circuit that converts adipose sensory cues into sympathetic outflow back into adipose tissue\(^{39,109}\). Corroborating this notion, in an extensive screening of central HSV H129 and pseudorabies virus colocalization from iWAT injections, Bartness and colleagues revealed a high degree of doubly labelled neurons (upwards of 75%), suggesting an extensive sensory-sympathetic iWAT feedback system in Siberian hamsters\(^{47}\). However, an important point to consider related to these findings is the caveats associated with the use of HSV H129. In particular, double labelling of afferent and efferent nerve fibres (Box 1) is possible, making the interpretation of the above results less clear. Moreover, as the HSV H129 tracing experiments were conducted in hamsters, replication from these results in other animal species, such as mice, will be necessary to extend these findings.

An additional remarkable finding was the observation of differential sympathetic outflows to various depots of WAT. Dual pseudorabies virus tracing of mesenteric WAT (mWAT) and iWAT to reveal the sympathetic pathways innervating both tissue depots showed that iWAT had considerably more single-labelled neurons within the brain than mWAT\(^{110}\). However, areas more typically associated with sympathetic regulation of metabolism, such as the paraventricular hypothalamic nucleus, lacked such a striking difference in labelling. This finding suggests that the differences observed in metabolic phenotypes associated with subcutaneous versus visceral adiposity might have a neuronal component\(^{41}\). If the neural origins of sympathetic innervation vary between adipose depots, a hardwired differential sympathetic drive to each tissue might exist, further suggesting specialized, depot-specific roles.

An early study (published in 1998) also showed less pseudorabies virus labelling within the supraciasmatic, arcuate and dorsomedial hypothalamic nuclei following injections into epididymal WAT (eWAT) viral injections than after injections into iWAT\(^{411}\). One should note that when dual-tract tracing is performed, one virus can interfere with the infection of the same neuron by the second, leading to an underestimation of dually labelled neurons. These critical studies indicate that adipose sensory and sympathetic nerves are

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**Fig. 3** Adipose tissue–sensory nerve crosstalk. **a** | Adipose-tissue–resident cells release molecular mediators (represented by yellow circles) that can act on the afferent neuronal pathway and invoke a central response to the tissue microenvironment. The principal component of adipose tissue, the adipocyte, can release various neuroactive and neurotrophic peptides, such as leptin, neuregulin 4 (NRG4), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), free fatty acids (FFAs), arachidonic acid (AA) and eicosanoids, such as eicosapentaenoic acid (EPA) and prostaglandin E\(_2\) (PGE\(_2\)), that act on surrounding cells, including sensory nerve fibres. Adipocytes and the blood vessels within the adipose tissue secrete vascular endothelial growth factor (VEGF), which can promote nerve sprouting and sensory hypersensitization upon interaction with the VEGF receptor (VEGFR) family. Cytokines secreted from macrophages, such as tumour necrosis factor (TNF), IL-1β and IL-6, might act directly on the sensory nerve itself or indirectly through their pro-inflammatory effects within the adipose tissue microenvironment. Anti-inflammatory cytokines, such as IL-17A and adenosine, originating from alternatively activated macrophages and various lymphocytes, including regulatory T (\(T\_{reg}\)) cells, can act in a similar way. **b** | Conversely, signalling from the sensory nerve terminals to cells within adipose tissue has the potential to modulate adipocyte functions. Upon stimulation, the sensory nerve can release calcitonin gene-related peptide (CGRP) and substance P (represented by blue circles) into the innervated tissue that modulates the microenvironment surrounding the sensory nerve.
The DRG also projects to the brain via the dorsal horn of the spinal cord, relaying sensory information from the periphery innervating white adipose tissue (WAT) depots arise from the dorsal root ganglia (DRG) proximal to the spinal cord. Throughout the body, projections into the preoptic area (POA), as well as resident temperature-sensitive neurons, central nervous system, influencing thermogenesis and food intake as well as other critical homeostatic functions, connected and have the potential for communication via hypothalamic relay, as well as via areas of the hindbrain and midbrain. Additionally, diversions in these pathways between WAT depots have emerged as provocative areas to probe when addressing the phenotypic disparity contributed by visceral and subcutaneous adiposity. Although these adipose–hypothalamic–adipose neuronal signalling axes exist as potentially important networks in metabolic communication, traditional regulators of sympathetic drive, such as central leptin and insulin signalling, remain prominent modes of metabolic signalling within the hypothalamus. It is important to emphasize the essential role of central leptin signalling in the regulation of regional sympathetic drive, energy expenditure and metabolic homeostasis. Excellent reviews discussing the contribution of hypothalamic leptin signalling on SNS activation and lipolysis were published in the past few years.

**Hypothalamic insulin signalling**

The functional relevance of adipose–hypothalamic neuronal circuitry for adipose thermogenesis and therefore systemic metabolism was also demonstrated in experiments aimed at investigating the role of hypothalamic insulin signalling in peripheral tissues and systemic metabolism. Insulin was shown to engage with insulin receptors in two neuronal populations: the cocaine-related and amphetamine-related transcript protein–proopiomelanocortin (CART–POMC) and the neuropeptide Y–agouti-related peptide (NPY–AGRP) neurons present within the arcuate nucleus of the hypothalamus. Insulin signalling in these neurons affects neuronal excitability and peptide expression and stimulates the appetite-suppressing population of POMC neurons, which in turn inhibits the appetite-promoting population of AGRP neurons. Importantly, insulin receptor signalling in POMC and AGRP seems to be essential to regulate energy balance and systemic metabolism, as genetic disruption of components in the insulin receptor signalling pathway in those neurons alters systemic metabolism. For instance, insulin receptor deletion in POMC neurons abolishes the ability of insulin to suppress adipose lipolysis, while preserving its ability to suppress hepatic glucose production, and led to the development of hepatic steatosis in mice fed a high-fat diet. Furthermore, deletion of insulin receptors in AGRP neurons impaired insulin’s ability to suppress hepatic glucose production, whereas its action to inhibit lipolysis was preserved.

**Fig. 4 | Central integration of adipose signals and obesity-mediated dysregulation.** a | Afferent sensory nerve fibres innervating white adipose tissue (WAT) depots arise from the dorsal root ganglia (DRG) proximal to the spinal cord. The DRG also projects to the brain via the dorsal horn of the spinal cord, relaying sensory information from the periphery to the central nervous system for integration. b | The hypothalamus is a primary area for metabolic regulation in the central nervous system, influencing thermogenesis and food intake as well as other critical homeostatic functions throughout the body. Projections into the preoptic area (POA), as well as resident temperature-sensitive neurons, relay critical thermoregulatory information to the dorsomedial hypothalamic nucleus (DMH), a core component of the orexigenic system and thermoregulatory function of the hypothalamus. The paraventricular hypothalamic nucleus (PVN), which is proximal to the third ventricle (3V), is involved in food intake, thermoregulation and neuroendocrine functions through projections to the pituitary. The arcuate nucleus (ARC), along with the ventromedial nucleus of the hypothalamus (VMH) and lateral hypothalamus (LH), are also involved in appetitive behaviour and food reward. The suprachiasmatic nucleus (SCN) is a critical area for regulating circadian rhythms. All of these centres have direct or indirect roles in influencing hypothalamic thermogenic regulation. Hypothalamic inflammation has been linked to metabolic dysregulation and obesity-related insulin resistance through excessive gliosis, leading to neuronal damage, particularly noted within the ARC. The hypothalamus sends sympathetic projections to the periphery either directly through the intermediolateral nucleus of the spinal cord (IML), or via relay through the raphe pallidus nucleus (RPa) or the rostral ventrolateral medulla (RVLM) to the IML.
The observations discussed in this section raise an important question regarding the relevance of central integration for metabolic homeostasis. Results supporting an essential role of the CNS and hypothalamus in controlling whole-body metabolism are well established. In one such study, chronic decerebrated rats were shown to be hyperinsulinaemic (BOX 1), hyperadiponectinaemic and hyperleptinaemic compared with their control counterparts. The presence of the forebrain was shown to be critical for maintaining normal circulating levels of insulin, body temperature, energy expenditure and a healthy balance of lean and fat mass. Whether these findings result from a disrupted neuroendocrine axis, disrupted direct sympathetic outflow or a combination of the two should be further investigated. Altogether, these results demonstrate the importance of the central integration of peripheral signals and the hypothalamic regulation of adipose sympathetic drive and systemic metabolism.

Central dysregulation

Obesity causes notable morphological changes within the hypothalamus in a mouse model. In particular, it enhances inflammation and microglial expansion. These observations were extended to individuals with obesity who displayed increased inflammation and gliosis throughout the mediobasal hypothalamus, along with functional impairment. Moreover, results describing mediobasal hypothalamic gliosis linked to obesity and insulin resistance in humans have been published in the past few years. Thus, persistent hypothalamic inflammation positively correlates with metabolic dysfunction in animal models of obesity and humans with obesity. To elucidate whether hypothalamic inflammation is implicated in metabolic dysfunction, studies from the past few years have investigated whether targeting hypothalamic inflammation would improve systemic metabolism. In one study, evidence suggested that the NF-κB pathway is necessary for hypothalamic microgliosis and metabolic dysfunction in obesity in mice. Through either pharmacological depletion of microglia or inhibition of the NF-κB pathway in these cells, hypothalamic inflammation was prevented and a normal systemic energy balance was preserved. Overall, these results strongly support the notion that hypothalamic inflammation in obesity contributes to metabolic dysfunction and energy imbalance. Moreover, persistent hypothalamic inflammation can affect central integration and systemic metabolism, disrupting the adipose afferent reflex by uncoupling the adipose microenvironment from the central relay through sympathetic outflow into the periphery.

Adipose–sympathetic nerve fibre crosstalk

Sympathetic innervation of adipose

Immunohistochemical analysis of selective sympathetic neuron markers, such as the catecholamine biosynthetic enzyme tyrosine hydroxylase, revealed sympathetic nerve fibres in adipose tissue in close anatomical association with the vasculature, similar to what is observed with adipose afferent nerves. Sympathetic innervation of adipose tissue and release of noradrenaline from these nerve fibres are also essential for lipolysis of triacylglycerol in white adipocytes during fasting conditions. By contrast, the secretion of adrenaline by the adrenal glands does not seem to be required for adipocyte lipolysis during fasting, which supports the notion that adipose sympathetic drive is indispensable for mobilization of free fatty acids and their use as fuel in other tissues. The importance of adipose sympathetic drive for the proper control of adipose tissue lipolysis is also illustrated by the experiments from Buettner and colleagues in which central insulin administration suppresses lipolysis in peripheral adipocytes. Thus, insulin acts not only cell autonomously in adipocytes but also in the hypothalamus to attenuate lipolysis. The actions in the hypothalamus occur through insulin-mediated inhibition of sympathetic activity in adipose tissue through unknown mechanisms. Collectively, these observations provide strong evidence that major physiological pathways that mediate both stimulation and inhibition of adipocyte lipolysis are driven by noradrenaline and insulin regulation of sympathetic neurons. Thus, brain to adipocyte communication through sympathetic innervation of adipose tissue seems to be pivotal for homeostatic control of whole-body metabolism.

Sympathetic nerve fibres are readily detected within the adipose parenchyma, and it has been suggested that a cold stimulus triggers sympathetic expansion as well as increased SNS activity in adipose tissue. However, using a whole-tissue clearing method that permits visualization of fluorescently labelled structures in an entire adipose depot, Paul Cohen’s group quantified the degree of arborization of anti-tyrosine hydroxylase antibody-positive structures in iWAT and found (using light sheet microscopy) no change in response to cold exposure. This finding appears to be contradictory to the results previously described here and to western blot analyses that demonstrate an increase in iWAT tyrosine hydroxylase protein levels when mice are housed in the cold. Taken together, the data suggest that tyrosine hydroxylase expression is increased by cold exposure in nerve fibres that are already present in iWAT (that is, cold-induced sympathetic stimulation without structural remodelling). This issue will be of future interest as other laboratories use light sheet microscopy to explore nerve fibres within adipose tissue depots.

The increased sympathetic innervation within WAT and BAT parenchyma is critical for the increased expression of genes encoding protein products involved in thermogenesis in adipose tissue in response to cold exposure. Accordingly, selective sympathetic denervation of WAT and BAT disrupts cold-induced thermogenesis. The importance of adipose sympathetic drive for thermogenesis and systemic metabolism was also consolidated in a report showing that regional sympathectomy impairs adipose thermogenesis and leaves mice susceptible to obesity.
such as IL-6, IL-10 and growth differentiation factor 15 (GDF15). Treg (TNF) and IL-1β tissue. Adipose- resident immune cells, such as macrophages and lymphocytes, secrete cytokines, tumour necrosis factor (TNF), brain- derived neurotrophic factor (BDNF), free fatty acids (FFAs) and endocannabinoids. The vascular sympathetic innervation of adipose tissue. Among these factors are neuronal growth regulator 1 (NEGR1), nerve growth outgrowth. Additionally, white adipocytes synthesize several factors with neurotrophic activity that might enhance adenosine molecules) produce the neurotrophic factor neuregulin 4 (NRG4), which is known to promote neurite outgrowth. Additionally, white adipocytes synthesize several factors with neurotrophic activity that might enhance sympathetic innervation of adipose tissue. Among these factors are neuronal growth regulator 1 (NEGR1), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), free fatty acids (FFAs) and endocannabinoids. The vascular endothelial growth factor (VEGF) secreted by the endothelium and adipocytes elicits sympathetic innervation in adipose tissue. Adipose-resident immune cells, such as macrophages and lymphocytes, secrete cytokines, tumour necrosis factor (TNF) and IL-1β as well as factors demonstrated to affect neurite outgrowth and possibly adipose sympathetic innervation, such as IL-6, IL-10 and growth differentiation factor 15 (GDF15). T_{reg}, regulatory T cell.

**Noradrenaline.** The neurotransmitter noradrenaline and its role in adipose metabolic processes have been well studied for many years and its actions within adipose tissue have been reviewed in the past few years.\(^{148,150}\)

Upon CNS stimulus, sympathetic nerve fibres release noradrenaline within adipose tissue that, upon binding to adrenergic receptors, modulates several metabolic processes, including lipolysis, thermogenesis and adipose tissue remodelling.\(^{148,150}\) (FIG. 5a). Adrenergic receptors activate the classic cAMP signalling pathway that activates protein kinase A (PKA), which activates lipolysis of triacylglycerol within adipocyte lipid droplets in WAT and BAT.\(^{148,150}\) This pathway also induces the browning of WAT through the appearance of beige adipocytes and the BAT thermogenic gene programme, enhancing the expression of mitochondrial and oxidative genes, including the mitochondrial UCP1 (REFS\(^{148,151}\)).

The β-adrenergic receptors (β₁, β₂ and β₃ (BOX 1)) are necessary mediators of noradrenaline’s actions on adipose tissue, as mouse models lacking these three receptor subtypes have dysregulation of energy metabolism and are prone to obesity.\(^{152,153}\) Sustained release of noradrenaline and consequent receptor activation
Neuropeptide Y. Although NPY is largely produced by CNS neurons, this neuropeptide is also synthesized by peripheral sympathetic nerve fibres, acting as a neurotransmitter that affects adipose-resident cells159. FIG. 5a. NPY receptor subtypes 1 and 2 mediate the actions of NPY and belong to the seven-transmembrane (G) protein-coupled receptor family that inhibits adenylyl cyclase and thus the cAMP–PKA pathway, acting antagonistically to adrenergic signalling159. Activation of the NPY receptor suppresses lipolysis in adipocytes160 while also activating the extracellular-signal-regulated kinase (ERK) pathway and adipogenesis161. Similar to noradrenaline, NPY is located in cytosolic granules in the terminals of sympathetic neurons. In mice, its release and actions can be triggered by stressful situations, such as exposure to cold or overfeeding162.

Importantly, signals emanating from NPY receptors promote adipose angiogenesis, along with differentiation of new adipocytes162. In addition, obese mice were found to have elevated circulating levels of NPY and increased expression of its receptors in WAT, which suggests that this neuropeptide has a role in adipose tissue accretion during obesity162. Indeed, this notion was further supported by adipose-targeted knockdown and pharmacological inhibition of the NPY2 receptor, which resulted in reduced obesity and metabolic abnormalities in mice162. Altogether, it seems that NPY produced by sympathetic nervous functions in a system of feedback responses that oppose the actions of noradrenaline. Further studies to identify the signals that trigger the sympathetic production, release and actions of NPY are needed to clarify the exact role of NPY in regulating systemic metabolism and the metabolic syndrome.

ATP. Similar to noradrenaline and NPY, ATP is a co-transmitter released by sympathetic nerve endings163 (FIG. 5a). When secreted from neurons, ATP exerts its effects via the ionotropic purinergic receptor subtypes (P2XRs) and metabotropic purinergic receptor subtypes (P2YRs)164. Evidence suggests that signalling through these purinergic receptors affects several different processes in adipose tissue, including lipid metabolism (enhanced lipogenesis and lipolysis), increased thermogenesis, inflammation and endocrine functions164,165. For example, P2RX5 expression is induced in BAT and WAT upon chronic cold exposure and correlates with UCP1 expression166. Nonetheless, the functional role of these receptor subtypes on adipose and systemic metabolism needs further rigorous investigation, applying both pharmacological and genetic approaches to determine their full functional relevance.

Adipose signals to sympathetic nerves
Neurotrophic factors. It seems probable that adipose-resident cells affected by SNS secretions signal back to local sympathetic nerve fibres within coordinated feedback responses167 (FIG. 5b). Accordingly, several WAT-derived and BAT-derived secreted factors that are regulated by stimulation with noradrenaline have been reported to act on efferent nerves. Among these factors are the neurotrophic factors NRG4 (REFS168–170), neuronal growth regulator 1 (NEGR1)168, NGF169 and BDNF171. Chronic β-adrenergic receptor activation by noradrenaline induces the formation of beige adipocytes in WAT, which also produce some of these neuropeptides172. As these factors act as potent neuronal regulators, it is plausible that they enhance sympathetic innervation in adipose tissue during cold-induced thermogenesis and browning. This positive loop of beige adipocytes promoting their own formation through enhanced sympathetic nerve density might be critical for adipose thermogenesis. In support of this possibility, inhibiting NGF and its receptor TRKA suppresses browning of adipose tissue169. This study suggests that NGF mediates sympathetic plasticity within adipose tissue through enhanced innervation during cold exposure169. Such neuronal plasticity resembles the active remodelling of adipose tissue depots during times of stress. As the TRKA protein is expressed in other tissues, it would be important to selectively inactivate NGF–TRKA signalling in sympathetic nerve endings in adipose tissue to confirm the requirement for this pathway in adipose tissue remodelling during cold exposure.

Fatty acids and bioactive lipid metabolites. Although the effects of fatty acids and bioactive lipids on adipose sensory nerve fibres have been investigated, mechanistic information on how these molecules affect adipose sympathetic nerves is limited (FIG. 5b). Lipolysis of triacylglycerol and de novo biosynthesis of fatty acids in adipocytes may generate fatty acids and lipid metabolites that could act on adipose efferent fibres169. Indeed, in vivo studies have shown that chronic infusion of fatty acids reduces peripheral sympathetic activity in rodents170. However, this effect might be through CNS regulation by fatty acids173, and whether fatty acids also control peripheral sympathetic nerves directly in a physiologically relevant manner remains to be determined.

Endocannabinoids. Studies have demonstrated that adipocytes produce substantial amounts of endocannabinoids172–174 (FIG. 5b). Acting through their receptors (CB1 and CB2), these bioactive lipids stimulate energy intake and inhibit energy expenditure, playing an important role in energy balance175. Importantly, dysregulation of endocannabinoid production in adipose tissue and CB1 activation might contribute to metabolic dysfunction176–179. Thus, in patients with...
obesity, high circulating levels of endocannabinoids are positively correlated with increased visceral fat^185,197^, which suggests that increased levels of endocannabinoids might contribute to metabolic dysregulation during obesity. Furthermore, adipose-specific inactivation of CB1 seems to increase adipose thermogenesis^178^, attenuating obesity-induced metabolic dysfunction in mouse models^179^.

Although some evidence indicates a direct action of endocannabinoids on adipocytes, other studies have suggested an inhibitory effect of endocannabinoids on neural circuits that blunt adipose sympathetic activity and thermogenesis^179,180^, although these effects are probably mediated through central mechanisms, some reports suggest that endocannabinoids might also act directly on peripheral sympathetic nerves^181,182^. In the perfused mesenteric vascular bed of the rat, activation of the CB1 receptor by the endocannabinoid anandamide suppresses the release of noradrenaline and ATP from sympathetic nerve terminals^183^, altogether, these observations suggest that adipose-derived fatty acids and bioactive lipids such as the endocannabinoids might act on local sympathetic fibres, suppressing their activity. Thus, the increased biosynthesis of endocannabinoids in adipose tissue that has been noted in obesity might inhibit adipose sympathetic outflow, contributing to metabolic dysfunction. Endocannabinoids produced in adipose tissue might also signal through afferent nerve fibres, which should be studied in more detail.

**Cytokines and GDF15.** Adipocytes are not the only adipose-tissue-resident cells that secrete neuronal modulators that might affect adipose sympathetic nerve fibres (Fig. 5b). Adipose tissue macrophages (ATMs) produce a number of pro-inflammatory and anti-inflammatory cytokines, such as TNF, IL-1β, IL-6 and IL-10, which are all reported to modulate sympathetic nerve activity^185^. When produced in peripheral tissues, cytokines can enter the circulation and gain access to the brain, enhancing the sympathetic outflow via CNS regulation^183^, however, in chronic inflammatory states (as occurs in adipose tissue during obesity), increased local levels of inflammatory cytokines might restrict sympathetic nerve fibres from regions of inflamed adipose tissue or even damage nerves, depending on the severity of inflammation^181^. This sympathetic neuronal response during a persistent inflammatory state, as occurs in arthritis, has been investigated in depth and previously described^186^. However, evidence supporting the idea that sympathetic nerve repulsion occurs in inflamed adipose tissue remains speculative, and experiments to rigorously test this hypothesis are necessary. Thus, although adipose sympathetic outflow produces an anti-inflammatory response (see subsequent section), the persistent cytokine production and chronic inflammation noted in obesity might disrupt adipose efferent nerve function.

Other reported neuronal modulatory factors potentially produced by adipose-resident cells are growth differentiation factor 15 (GDF15)^180^ and adenosine^185^, although initially identified as a cytokine secreted by macrophages^188^, GDF15 seems to be expressed predominantly in the liver, with lower amounts produced by other tissues^187^. However, under conditions of metabolic stress such as obesity, the adipose expression level of GDF15 is strongly upregulated^188^, which might be owing to increased amounts of ATP in the tissue. Although the effects of GDF15 on energy metabolism are known to be mediated through suppression of food intake mechanisms in the brain^189,190^, peripheral actions of GDF15, including sympathetic neuronal regulation, might also occur. Consistent with this notion, sympathetic neurons express the GDF15 receptor GFRAL^191^, and signalling through this receptor induces a potent neurotropic effect both in vitro and in vivo^190^. Nonetheless, it remains to be tested whether adipose-derived GDF15 has a direct role as a peripheral neuronal modulator.

**Adenosine.** A study published in 2014 indicated that adenosine, through its A1 receptor, induces BAT and WAT thermogenesis^192^. This metabolite is released from adipocytes or from other adipose-resident cells (Fig. 5b). Moreover, adenosine can also be produced from extracellular ATP by Treg lymphocytes and possibly endothelial cells, which express the enzymes ecto-5’-nucleotidases CD33 and CD73, cell surface proteins that are necessary for extracellular adenosine generation from ATP^193^. Importantly, the presence of adenosine receptors was also detected in peripheral efferent fibres in the vasculature, and signalling via these receptors seems to blunt the secretion of neurotransmitters in sympathetic nerve endings^194,195^, which suggests that adenosine within adipose tissue might also be involved in a negative feedback mechanism.

**Sympathetic dysregulation in obesity.** General SNS dysregulation in metabolic disease is well established^194^. As sympathetic outflow into adipose tissue controls such important metabolic processes in adipocytes^196,197,198^, it is not surprising that obesity disrupts catecholamine-mediated adipocyte lipolysis^195,196^, mitochondrial biogenesis^197^ and adipose tissue remodeling^198^, Sympathetic signals emanating from the CNS to adipose tissue might be altered in obesity by the associated hyperinsulinaemia^199^, as insulin receptor signalling in the brain is known to suppress adipose sympathetic outflow in lean mice^200^. Thus, chronic hyperinsulinaemia might cause insufficient sympathetic activity within adipose tissue, attenuating catecholamine-mediated actions. Consistent with this notion, eliminating the hyperinsulinaemia normally observed in mice fed a high-fat diet by genetic deletion of three insulin alleles reprograms WAT to express UCP1 and increase energy expenditure^200,201^. Thus, pathways that are enhanced by catecholamine are promoted upon reduction in circulating levels of insulin. Chronic hyperinsulinaemia in obesity also inhibits β-adrenergic receptor signalling through marked downregulation of β3-adrenergic receptors, as noted in adipocytes from obese mice^202,203^. This decreased adipocyte β3-adrenergic receptor expression and signalling is reversed by treatment of mice with diazoxide to reduce insulin secretion from β-cells^204^.

Although the inhibitory effect of central insulin action on sympathetic outflow into WAT has been
polarization with adipose tissue cell populations. Noradrenaline release from sympathetic terminals leads to adipose macrophage polarization (BOX 1) from a pro-inflammatory (M1) to an anti-inflammatory, alternatively activated (M2) profile. Sympathetic neuron-associated macrophages (SAMs) localize around sympathetic synapses and take up secreted noradrenaline through the solute carrier family 6 member 2 (SLC6A2) noradrenaline transporter. Monoamine oxidase A (MAOA) catalyses the degradation of noradrenaline within the SAMs (part 1). Noradrenaline release from sympathetic nerve endings stimulates the β2-adrenergic receptor (β2-AR) in the vasculature, leading to vascular endothelial growth factor (VEGF) secretion from the endothelium. VEGF stimulates angiogenesis and neurite outgrowth, driving increased irrigation and innervation of the adipose tissue (part 2). ASC β2-AR activation by sympathetic noradrenaline drives beige adipocyte differentiation. These beige adipocytes have an enhanced thermogenic capacity relative to white adipocytes and a brown-like adipokine expression profile, which might include neurotrophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neuregulin 4 (NRG4). These factors can drive increased sympathetic innervation and arborization (part 3). The sympathetic co-transmitter ATP is cleaved by regulatory T (Treg) cells into adenosine via CD73-mediated and CD39-mediated degradation to create the anti-inflammatory purinergic halo of the purinergic P2 receptor family (P2R) to drive beige adipocyte thermogenesis (part 4). PKA, protein kinase A.

previously demonstrated by several studies122,134,200, it is important to emphasize that in other studies insulin signalling in the brain has been claimed to increase sympathetic drive into peripheral tissues, such as kidney and BAT203,204. Thus, insulin signalling in the brain seems to elicit distinct sympathetic effects, depending on the tissue. As central insulin signalling is disrupted during metabolic dysfunction, it would be important to examine whether perturbation of brain insulin signalling affects the increased sympathetic drive into some peripheral tissues.

Similar to adipose sensory innervation, local sympathetic innervation might also be affected by the persistent adipose inflammation observed in obesity123. Interestingly, moderate reduction in hyperinsulinaemia in obese mice decreased adipose inflammation and improved insulin signalling, along with an improvement in systemic metabolism123. However, whether these metabolic enhancements were associated with elevated adipose sympathetic tone and/or activity was not investigated.

**Integrating adipose signalling networks**
The many types of resident cell within adipose tissue communicate with each other through complex signalling networks, as exemplified in FIG. 6a. Sympathetic neurotransmitters control key functions in endothelial cells and multiple types of immune cell in addition to adipocytes, and each of these cell types communicate with adipocytes and each other, adjusting their metabolic pathways and shaping the tissue microenvironment. This adipose tissue complexity also includes
external signals that are integrated within the tissue and then relayed to other tissues from the adipose tissue in the form of secreted factors that act through the circulation or on sensory nerve fibres. Thus, adipose depots act as both sensors and integrators of complex signalling networks, and adipose innervation is critical to this function.

**Neuron–immune cell communication**

A variety of adipose-resident immune cells express adrenoreceptors that can be activated upon the release of noradrenaline by adipose sympathetic nerve endings. ATMs represent one such example of cells that have a pivotal role in adipose inflammation and systemic metabolism. ATMs can be sorted into two major subtypes, denoted M1 and M2, which exert either primarily pro-inflammatory (that is, M1) or anti-inflammatory (that is, M2) functions. Although macrophages probably form a continuum of states between these two extreme types, several studies have shown that, in healthy, functional adipose tissue, the ratio of M1-defined to M2-defined macrophages is low but is shifted towards an M1 profile in unhealthy adipose tissue, as in obesity. Strategies to shift this ratio towards an M2-dominant population seem to improve adipose and whole-body metabolism. Interestingly, catecholamines exert a potent effect on macrophages through β2-adrenergic receptors that promote M1 macrophage differentiation into the M2 state, which indicates that sympathetic signals favour an anti-inflammatory state. This concept is supported by a study showing that sympathetic nerve activity maintains an anti-inflammatory state in adipose tissue by inhibiting TNF expression in macrophages, promoting M2-polarization of ATMs. Thus, the above pathways reveal an anti-inflammatory state in adipose tissue by inhibiting TNF expression in macrophages, promoting M2-polarization of ATMs. This mechanism seems to be distinct from the well-known β3-adrenergic receptor-induced browning of WAT, as it relies on the β1-adrenergic receptor rather than the β3-adrenergic receptor isoform. Interestingly, Adrb1-null mice are intolerant of cold exposure and cannot defend their body temperature, highlighting the importance of β1-adrenergic receptors for adipose thermogenesis and eutermia. Additionally, these perivascular-derived beige adipocytes not only display increased thermogenic activity but also secrete neurotrophic factors to promote adipose sympathetic innervation.

**Neuron–endothelial cell communication**

The close association between adipose nerve fibres and the vasculature indicates interdependency between these two structures that might be essential for their functional role in regulating local and systemic metabolism. Indeed, as shown in FIG. 6b, part 2, noradrenaline secreted by sympathetic nerve endings activates β-adrenergic receptors in the endothelium to induce VEGF production. In turn, endothelium-derived VEGF might promote angiogenesis through stimulation of endothelial cells and increase sympathetic innervation through activation of VEGFR signalling in sympathetic neurons. This physiological response of the endothelium to a sympathetic stimulus might be essential in situations in which increased vascularization and innervation are needed, such as adipose tissue expansion during overfeeding or in cold-induced thermogenesis. Accordingly, inhibition of VEGFR signalling in adipose-resident cells disrupts thermogenesis and browning in WAT induced by cold temperatures. Conversely, overexpression of the VEGF peptide elicits browning in WAT, which is consistent with VEGF having a key role in promoting adipose pathways that benefit systemic metabolism.

**Neuron–adipocyte progenitor communication**

Adipose-resident perivascular cells are a subset of progenitor cells that differentiate into beige adipocytes through activation of their β1-adrenergic receptors. This mechanism seems to be distinct from the well-known β3-adrenergic receptor-induced browning of WAT, as it relies on the β1-adrenergic receptor rather than the β3-adrenergic receptor isoform. Interestingly, Adrb1-null mice are intolerant of cold exposure and cannot defend their body temperature, highlighting the importance of β1-adrenergic receptors for adipose thermogenesis and eutermia. Additionally, these perivascular-derived beige adipocytes not only display increased thermogenic activity but also secrete neurotrophic factors to promote adipose sympathetic innervation.

**Dysregulation of peripheral signals**

Similar to how hypothalamic dysregulation negatively affects systemic metabolism, a failure in peripheral signal integration can occur in obesity and might lead to metabolic dysfunction and T2DM. Dysregulation of a homeostatic mechanism might arise from and result...
in the overproduction or underproduction of potent signalling molecules. The overproduction of NPY in obesity is one such case of signalling molecule dysregulation \(^{162}\). As detailed in a previous section, this neuropeptide favours adipose tissue expansion and has been implicated in obesity-linked metabolic dysfunction. Strategies such as pharmacologic inhibition and genetic deletion aimed at blocking signalling from one of the NPY receptor subtypes (NPY2) were successful in attenuating various metabolic abnormalities during obesity in mice \(^{162}\). Moreover, NPY signalling in vasculature increased adhesion of leukocytes to human endothelial cells \(^{227,228}\), and treatment with a global NPY receptor antagonist effectively improved chronic inflammation in mice \(^{227,229}\). Thus, it is conceivable that persistent activation of adipose-resident cells by increased NPY levels promotes adipose tissue accretion, favours immune cell infiltration and exacerbates adipose inflammation.

Imbalance in adipose tissue due to persistent signals emanating from chronically activated NPY receptors in obesity might contribute to the disruption of the function of adipose tissue, such as local signal integration. However, a study has suggested that NPY derived from macrophages and haematopoietic cells initiates an anti-inflammatory response during the early stages of obesity in mice \(^{230}\). Accordingly, deletion of NPY in these cells promotes inflammation in adipose tissue \(^{230}\). The reason for the discrepancy between this study and the previous results could be the differential effect of NPY at different stages of adipose tissue expansion in obesity, the origin of the NPY (nerve versus immune or other cell types) or the differential signalling by receptor 1 versus receptor 2. Additional studies will be necessary to better understand these complexities of NPY function in adipose tissue.

ATP is a sympathetic co-transmitter; dysregulation of its production and/or its degradation by adipose-resident cells might be implicated in obesity-linked adipose dysfunction and disruption of adipose signalling integration \(^{231}\). An increase in the extracellular levels of ATP is often associated with tissue inflammation and the metabolic abnormalities that occur in adipose tissue during obesity \(^{231,232}\). Consistent with this notion, the adipose tissue from individuals with obesity who are metabolically unhealthy releases increased levels of ATP compared with adipose tissue from lean and metabolically healthy individuals \(^{231}\). Such an increase in the extracellular levels of ATP stimulates purinergic receptor P2RX7 signalling in M1 macrophages to increase pro-inflammatory cytokine production and adipose inflammation \(^{233}\). Therefore, local mechanisms that control the extracellular ATP levels are essential for tissue homeostasis. One such local mechanism that has a role in regulating extracellular concentrations of ATP is the cleavage of ATP into adenosine.

In contrast to the pro-inflammatory role of extracellular ATP, adenosine produced by ATP degradation is known to limit severe inflammation and tissue damage \(^{232}\). The secreted ATP can be metabolized into adenosine by two extracellular ecto-5’-nucleotidases — CD39 and CD73 (REF \(^{225}\)). These two enzymes, which are expressed at the cell surface of T\(_{reg}\) cells \(^{34,35}\) and in the adipose vasculature \(^{214}\) (FIG. 6b, part 4), might have a key role in controlling the extracellular concentrations of ATP and adenosine. Therefore, adipose T\(_{reg}\) cells and endothelial cells can modulate the tissue inflammation state by controlling the conversion of extracellular ATP into adenosine (FIG. 6b, part 4). Disruptions of adipose endothelial function and reduction in T\(_{reg}\) cell content, as noted in obesity, would diminish ATP hydrolysis, increasing the ATP:adenosine ratio in the extracellular compartment. Consequently, high levels of ATP would activate M1 macrophages and favour the chronic inflammatory state typically seen in adipose tissue in obesity.

Hence, the inability of the adipose-resident T\(_{reg}\) cells and endothelial cells to control the extracellular levels of ATP due to disruption of these cells might affect the function of adipose tissue and proper integration of peripheral signals to control systemic metabolism. Consistent with this notion, global genetic deletion of CD39 leads to a decreased ability of endothelial cells to control extracellular ATP levels in the liver, enhanced inflammation and systemic insulin resistance \(^{234}\). It will be important to examine the role of the adipose CD39 and CD73 as modulators of ATP and adenosine signalling in adipose tissue metabolism and possible influences in whole-body metabolism.

In summary, the ability of adipose tissue to integrate central and peripheral signals relies on the accurate regulation of adipose–nerve crosstalk and maintenance of tissue homeostasis. In obesity, adipose expansion triggers tissue inflammation, favouring pro-inflammatory M1 macrophages and diminishing the local sympathetic signalling (FIG. 6b, part 1). The reduction in noradrenaline levels will not only favour a pro-inflammatory state but also blunt VEGF-mediated angiogenesis and adipose innervation (FIG. 6b, part 2). Lower levels of noradrenaline might also reduce differentiation of perivascular-derived beige adipocytes, which affects adipose thermogenesis and potentially innervation (FIG. 6b, part 3). Chronic adipose inflammation might also reduce vascular function and the presence of anti-inflammatory T\(_{reg}\) lymphocytes, resulting in extracellular accumulation of ATP and persistent tissue inflammation (FIG. 6b, part 4). Fine-tuned regulation of these processes might be essential for adipose–peripheral integration and ultimately whole-body metabolic homeostasis. A failure to appropriately regulate such processes might be linked to metabolic disorders in obesity and T2DM.

**Conclusions**

Findings showing dynamic crosstalk between adipose-resident cells and local nerve fibres have opened a fertile area of adipose biology for more detailed study. The revelation that lipolysis of fat stores, which is required for survival in fasting, is stimulated by noradrenaline released from efferent, sympathetic nerve fibres within adipose tissue rather than by circulating catecholamines reinforces the importance of this topic \(^{133}\). Even the dramatic suppression of adipocyte lipolysis during feeding by insulin involves regulation of sympathetic tone in adipose tissue \(^{222,223}\). Similarly, it is plausible that afferent, sensory nerve fibres might also modulate adipose-resident cells, providing an exciting area for further research to identify factors that mediate such putative effects.
Conversely, it is now appreciated that communication moves in the other direction as well: adipocytes and other adipose-resident cell types can signal to nearby sensory nerve fibres that transmit information to the CNS and to sympathetic neurons that control SNS tone. Some factors secreted from adipocytes that act on sensory neurons have been identified, including leptin and fatty acids, but this area of investigation is still at an early stage and offers considerable opportunities for discovery. Importantly, much of the data available and cited in this Review relate to mouse studies, and it is critical for the field to test these findings and ideas in human tissues.

Another fertile area for investigation relates to the key role of the endothelium, adipocyte progenitor cells and immune cells as both targets of neuronal signals within adipose tissue and as generators of signals to local nerve fibres. Noradrenergic and the neurotransmitters CGRP and substance P are vasoactive factors and immune modulators that can regulate the production of vasoactive peptides and cytokines in the endothelium and immune cells within adipose tissue when released by sympathetic and sensory nerve endings, respectively. Importantly, dysregulation of CGRP and substance P might contribute to endothelial dysfunctions and persistent adipose inflammation. This immune reaction resembles the neurogenic inflammation noted in other tissues in type 1 diabetes mellitus and T2DM and, likewise, might lead to adipose neuropathy (Box 1). However, the extent to which adipose sensory nerve dysregulation and afferent nerve-induced neurogenic inflammation contribute to immune cell expansion in adipose tissue in obesity is unknown. Might targeting adipose neurogenic inflammation by blocking CGRP and substance P receptors attenuate chronic adipose inflammation and thus improve systemic metabolism in obesity?

Adipose tissue innervation and the regulation of adipose function by the CNS are probably important targets for disruption by obesity and T2DM. Imbalance in the levels of sympathetic neurotransmitters noradrenergic, NPY and ATP that is associated with metabolic disease is also linked to chronic adipose dysfunction and the failure of adipose tissue to regulate systemic metabolism. Obesity and pro-inflammatory cytokines also reduce the biosynthesis of adipocyte-derived neurotrophic factors, such as NRG4 (REFS 5,27), which has also been shown to be a beneficial circulating agent that controls glucose homeostasis and liver fat metabolism. Similarly, there is high interest in identifying what triggers hypothalamic inflammation and to what extent it causes metabolic dysfunction in obesity. Bioactive lipids, glycosylated proteins, pro-inflammatory cytokines and perhaps hyper-insulinaemia are top candidates among the suspected factors that might elicit hypothalamic inflammation. As this research area is still fairly new, taking advantage of new techniques and developing more refined methods will be critical to optimize progress in understanding communication between adipose-resident cells and local nerve fibres. Rather than relying on genetic deletion of neuronal receptors or ligands in all sensory or sympathetic nerve fibres, more localized perturbations and reporters will be required. Optogenetic5,26 and chemical genetic approaches57 (for example, designer receptors exclusively activated by designer drugs) will be valuable approaches for future studies.

Finally, the discovery that WAT metabolic activity can modulate the function of distant BAT depots through sensory nerve signalling to the CNS to enhance sympathetic outflow represents an exciting future direction for research in this field54,59,69. Coupled with extensive data70 showing that the CNS can regulate metabolic tissues, including liver, skeletal muscle and pancreatic islets, to control systemic glucose homeostasis, these findings infer that adipose tissue might also signal to these other tissues through the brain. Much more work is required to test this idea and to define its full importance, but hopefully this hypothesis will be enticing to many scientists in the field.

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