Relations between Metabolic Homeostasis, Diet, and Peripheral Afferent Neuron Biology\textsuperscript{1,2}

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\textbf{ABSTRACT}

It is well established that food intake behavior and energy balance are regulated by crosstalk between peripheral organ systems and the central nervous system (CNS), for instance, through the actions of peripherally derived leptin on hindbrain and hypothalamic loci. Diet- or obesity-associated disturbances in metabolic and hormonal signals to the CNS can perturb metabolic homeostasis bodywide. Although interrelations between metabolic status and diet with CNS biology are well characterized, afferent networks (those sending information to the CNS from the periphery) have received far less attention. It is increasingly appreciated that afferent neurons in adipose tissue, the intestines, liver, and other tissues are important controllers of energy balance and feeding behavior. Disruption in their signaling may have consequences for cardiovascular, pancreatic, adipose, and immune function. This review discusses the diverse ways that afferent neurons participate in metabolic homeostasis and highlights how changes in their function associate with dysmetabolic states, such as obesity and insulin resistance. \textit{Adv. Nutr. 5: 386–393, 2014.}

\section*{Introduction}

Excess calorie intake, inadequate physical activity, and genetic predisposition contribute to metabolic disturbances such as obesity, type 2 diabetes mellitus (T2DM),\textsuperscript{5} and metabolic syndrome (MetS). Although it is well established that these conditions are characterized by an increased state of inflammation and insulin resistance that affect multiple organ systems (i.e., pancreatic, hepatic, and cardiovascular systems) (1,2), regulators of these metabolic phenotypes are still being studied. Of particular interest, the nervous system is a master homeostatic regulator that detects metabolic input to coordinate tissue-specific responses via peripheral hormone and neuronal signaling. Epidemiologic and experimental data are beginning to establish that both the central (CNS) and peripheral (PNS) nervous systems are not immune to the detrimental effects of obesity-associated metabolic dysfunction (3–5). For example, metabolic disturbances in a variety of circulating factors (glucose, TGs, hormones, and cytokines) can influence central insulin and leptin signaling and blood–brain barrier permeability, which in turn can have a negative effect on energy homeostasis and fuel metabolism (6–10). Neuropathy of the peripheral autonomic system is relatively common in diabetes and can impair gastrointestinal, cardiac, genitourinary, and sudomotor functions (5). In obesity, an activated sympathetic nervous system (SNS) was implicated in hypertension, the decline of insulin sensitivity, and renal impairment (11,12).

Despite evidence for diet- or metabolic status–associated disturbances to CNS and autonomic neuronal networks, effects on afferent pathways have been underappreciated. Afferent neuronal networks carry nerve impulses from peripheral tissues toward the CNS and travel along different anatomical pathways, for example, via cranial or spinal nerves. Afferents of the somatosensory system innervate skin, joints, skeletal muscle, and visceral organs, among others, and communicate information regarding touch, temperature, nociception (signaling of tissue injury and noxious insults), and proprioception (one’s place in 3-dimensional space). The cell bodies of spinal somatosensory neurons reside in the dorsal root ganglia (DRGs) and project to the spinal cord in which ascending pathways transmit...
information ultimately to thalamic nuclei and higher centers of the CNS. Vagal afferents innervate visceral organs, such as heart, lungs, liver, and the gastrointestinal tract, and can be activated by mechanical and chemical stimuli. For instance, in the gut, vagal afferents convey information regarding the presence or absence of nutrients and gut-derived hormones. The cell bodies of vagal afferents reside in the nodose ganglia and project centrally to the nucleus tractus solitarius (NTS) (13).

There is increasing evidence that sensory afferents of both these types play an important role in regulating energy balance, metabolic homeostasis, and inflammation and that these functions may be altered under dysmetabolic conditions, such as obesity, MetS, and insulin resistance. Spinal afferent signals coming from adipose tissue participate in the regulation of adiposity (14), and intestinal vagal afferent signaling influences feeding behavior (15,16); therefore, dysfunction in this peripheral communication may contribute to dysregulation of energy balance. Afferent neurons were also implicated in promoting insulin resistance and aging-associated obesity through mechanisms involving the transient receptor potential vanilloid-1 (TRPV1) protein (17,18). Recently, unique PPARγ- and obesity-controlled factors (tumor suppressor candidate 5 and synuclein-γ) were found to display uniquely high coexpression in adipocytes and sensory afferent nerves, possibly indicating a role in metabolism involving both white adipose tissue (WAT) and PNS biology (19–22). Furthermore, although the relation between frank diabetes and peripheral neural dysfunction (i.e., diabetic neuropathy) is well established, there is increasing evidence that sensory nerve fibers are susceptible to earlier consequences of glucose dysmetabolism, such as prediabetes and insulin resistance (23). The focus of this review is to highlight the diverse ways in which peripheral sensory afferents participate in metabolic homeostasis and to describe how metabolic dysfunction is capable of disrupting peripheral sensory neuron biology.

Spinal Afferents Innervate WAT and Control WAT Function

It is increasingly clear that sensory innervation from WAT communicates metabolic information to the CNS and participates in adipose tissue regulation. Neuroanatomical evidence using tract tracing methodology clearly demonstrated spinal afferent projections from fibers emanating from WAT (24,25). Spinal neurons receiving afferent input from WAT project to brain sites that are associated with SNS outflow (brainstem, hypothalamus, paraventricular hypothalamic nucleus, and preoptic area) (14), suggesting the presence of a WAT sensory–SNS feedback loop. Indeed, electrophysiologic activity in sensory neuron fibers innervating WAT is increased in response to heightened SNS drive on lipolysis by 2-deoxy-D-glucose-induced glucose deprivation (25), suggesting that sensory systems report the status of lipid stores to the CNS. Furthermore, WAT sensory–SNS communication may participate in body-weight or adiposity regulation (26). Selective chemical denervation of epididymal WAT sensory neurons resulted in a weight increase of the contralateral nontreated fat pad (27). In other words, the lack of sensory communication coming from WAT to the CNS due to denervation might be interpreted by the CNS as a loss in fat storage from that pad, triggering a compensatory increase of fat storage in an unaffected depot in an attempt to maintain energy balance.

Exactly which adipose-derived signals sensory neurons are responding to is still a matter of investigation but may involve metabolites or adipokines. For instance, leptin has emerged as 1 important mediator of this WAT sensory-induced SNS activation. Expression of the long form of the leptin receptor (Ob-Rb) overlaps with WAT afferent neurons in the DRGs of Siberian hamsters, suggesting that WAT sensory fibers participate in leptin signaling (28). Leptin injection into inguinal WAT increases electrophysiologic activity and activation (measured by c-Fos immunoreactivity) in DRG neurons (28). Furthermore, injection of leptin into WAT of rats not only increases afferent nerve activity (28,29) but results in a concomitant increase in CNS sympathetic outflow to a variety of other tissues, including contralateral WAT (30), brown adipose tissue, adrenal medulla, pancreas, liver (31), and kidney (32). This broader sensory–SNS interaction was termed the adipose afferent reflex (AAR) (29) and was implicated in the pathogenesis of obesity-related hypertension and MetS. Indeed, obese hypertensive rats show an increase in AAR activity, by both an increase in basal WAT afferent activity and a hyperresponsive SNS drive on renal sympathetic nerve activity induced by inguinal WAT sensory activation, resulting in higher mean arterial pressure (33). Whether leptin is involved in the mechanism by which AAR is increased in obesity is unknown, especially in light of the fact that obese Zucker rats display hypertension and increased SNS activity despite having deficient leptin signaling (34,35). Increased SNS activation was also implicated in the decline of insulin sensitivity, renal impairment, and inflammation (11,12,36), raising the possibility that increased AAR activity may also be a factor in these pathophenotypes. However, more research is warranted to determine the exact role, if any, of the SNS in these obesity-associated outcomes.

In addition to its influence on sympathetic activity and adipose biology, sensory signals from WAT can alter food intake and may be important mediators of obesity-associated leptin resistance in the CNS (37). Leptin resistance induced by high-fat feeding was reversed by the introduction of low concentrations of uncoupling protein-1 into intra-abdominal WAT, which reduced food intake, hypothalamic orexigenic neurotransmitters, and body weight compared with control diet–induced obese mice. However, these effects were lost when the manipulated fat pad was chemically denervated with capsaicin, suggesting that WAT sensory neurons played an important role in the leptin-sensitizing effect of uncoupling protein-1. Future research is needed to determine the mechanisms by which adipose–brain
systemic chemical ablation of TRPV1+ neurons improves creatic insulin resistance. Indeed, it is well established that dysfunction through suboptimal insulin release and/or pan-mitomer release at the pancreas, contributing to metabolic Thus, although speculative, it is possible that a dysregulation promote neurogenic inflammation in the pancreas (46). control, and to inhibit insulin action in vivo (44,45). SP can release from tides CGRP and SP (42). CGRP was shown to reduce insulin sensitiveness, potentiating the release of neuropep- mechanism (41), and insulin, in turn, sensitizes TRPV1 on release in a rat pancreatic homeostasis by efferent release of neuro- peptides. For example, capsaicin injection increases insulin release from sensory nerve endings by sending afferent signals to neurons in the spinal cord and higher centers involved in nociception (39). Additionally, spinal TRPV1-expressing (TRPV1+) DRG neurons have efferent capabilities on activa- tion, releasing potent neuropeptides [substance P (SP) and calcitonin gene-related peptide (CGRP)] that can control in- flammation and vasodilation (40). Capsaicin is often used as an experimental tool, both as a TRPV1 agonist and by its ability to desensitize and defunctionalize sensory nerves during continuous application.

Emerging evidence in rodent models suggests that TRPV1 expressed on both sensory neurons and β cells influences pancreatic homeostasis by efferent release of neuro- peptides. For example, capsaicin injection increases insulin release in a rat β-cell line (RIN) via a TRPV1-dependent mechanism (41), and insulin, in turn, sensitizes TRPV1 on sensory nerve endings, potentiating the release of neuropep- tides CGRP and SP (42). CGRP was shown to reduce insulin release from β cells (43), presumably as a negative feedback control, and to inhibit insulin action in vivo (44,45). SP can promote neurogenic inflammation in the pancreas (46). Thus, although speculative, it is possible that a dysregulation in the somatosensory system leads to sustained neurotransmitter release at the pancreas, contributing to metabolic dysfunction through suboptimal insulin release and/or pan- creatic insulin resistance. Indeed, it is well established that systemic chemical ablation of TRPV1+ neurons improves insulin sensitivity and glucose tolerance in obese Zucker rats (47–49). This improved glucose metabolism is accom- panied by a loss of nerve fibers innervating pancreatic islets that coexpress TRPV1 and CGRP (50), yet it has not been established whether the obese phenotype disrupts pancreatic TRPV1 signaling or by what mechanism. TRPV1 and sensory neurons are important to inflammatory immune responses (17,51), so perhaps an obese inflammatory pro- file may contribute to sensory neuron-mediated β-cell dysfunction.

In addition to impaired pancreatic function, TRPV1+ neu- rons were implicated in the development of age-associated obesity. Several examples of systemic capsaicin administration in rat neonates, resulting in ablation of TRPV1+ neurons, demon- strated protection in aging-associated obesity (52,53). Fur- thermore, TRPV1-null mice are protected from diet-induced obesity beginning after 14 wk of high-fat feeding (54). The lean phenotype was attributed to an increase in thermogenesis and not by a change in food intake. Together, this evidence from rodent models suggests that TRPV1 signaling promotes high-fat diet (HFD)-induced weight gain, and inhibition of this signaling is protective against diet-induced obesity. The mechanism for this is unknown but may involve the regulation of CGRP release. CGRP was shown to be increased in the plasma of obese individuals (55) and, as discussed above, can contribute to an insulin resistant phenotype in rats (44,47). Interestingly, CGRP knockout mice are also protected against diet-induced obesity and display improved glucose regulation (56). Furthermore, both TRPV1 and CGRP were re- portedly expressed in non-neuronal cells, such as adipocytes and immune cells (57–59). Therefore, blocking TRPV1 signal- ing by systemic chemical ablation or whole-body knockout models does not address tissue-specific activities of this recep- tor. Future studies are necessary to determine how diet- induced disturbances influences TRPV1 function in these different capacities and to understand whether observations in cells and rodent models apply to the human condition. Additionally, the specific endogenous signaling molecules that regulate TRPV1+ neuron activities and tissue crosstalk remain to be fully elaborated.

**Sensory Neurons and CGRP Control Inflamma- tory Responses**

The PNS is becoming increasingly appreciated for its role in modulating the inflammatory response (60). Spinal and va- gal sensory pathways monitor peripheral tissues regarding the status of injurious challenges and relay this information to the spinal cord or medulla (51,61). Activation of afferent neurons by a variety of inflammatory stimuli (i.e., TNF-α and other cytokines, acids) reflexively initiates autonomic and hormonal (i.e., adrenal) outflow to limit inflammatory mediators to the site of injury (60). For example, cytokine- responsive vagal afferents are thought to mediate a vago- vagal reflex in which acetylcholine release by parasympathetic vagal neurons inhibits cytokine release from immune cells in a variety of tissues (60,61). Additionally, in response to nox- ious stimuli, some spinal somatosensory afferent neurons act in an efferent manner to secrete CGRP, which impinges on inflammation and hemodynamics (51,62). CGRP is a potent vasodilator that controls vascular function by increasing NO release (63). Interestingly, CGRP appears to also have important anti-inflammatory actions (for review, see 51,63). CGRP inhibits the release of type 1 cytokines from immune cells, for example, IL-12 and IFN-γ, and enhances IL-10 pro- duction (64–66). CGRP was also shown to inhibit the produc- tion of TNF-α by inhibiting NF-κB activation in immune cells and to increase endothelial production of PGL2, 1 of the PGs that exerts anti-inflammatory activities (62). In the CNS, CGRP has the ability to reduce LPS-induced activation
of microglia and their release of proinflammatory mediators (67). Additionally, in the gut, spinal afferent release of CGRP acts to dilate mucosal blood vessels, which facilitates bicarbonate secretion to protect against the acidic environment (61). Finally, the local release of CGRP from cardiac and coronary tissues is thought to counteract the effects of ischemic episodes leading to cardio-protective effects (63).

CGRP is primarily released by small-diameter C fibers of the somatosensory system, but under inflammatory conditions such as sepsis, CGRP is more ubiquitously expressed (68,69). Furthermore, CGRP is expressed in rodent and human WAT, in which Linscheid et al. (58,59) showed that both its expression and secretion can be induced by proinflammatory insults. Despite this apparent relation between sensory neurons and inflammation, the potential role of CGRP in obesity-associated inflammation has not yet been established. Furthermore, given evidence that CGRP may contribute to insulin resistance via dysregulated TRPV1 activity (discussed above), future studies are necessary to dissect the role of CGRP in modulating inflammatory phenotypes associated with metabolic dysfunction.

Vagal Afferents Sense Dietary Nutrients and Control Feeding Behavior

Another afferent pathway important to the regulation of energy homeostasis involves vagus nerves emanating from the gut. Vagal impulses are a key neuronal communicator in the gut–brain axis and relay information regarding the quantity and composition of ingested nutrients from the intestine and hepatic portal system to the CNS (70,71). The vagus can be activated by mechanical (gut distension) and chemical stimuli. Gut peptides, such as cholecystokinin (CCK), 5-hydroxytryptamine (serotonin), and peptide YY (3–36) are released by enteroendocrine cells in response to the presence of nutrients in the gut lumen. These peptides bind to specific receptors expressed on vagal neurons (CCK_{\text{g}}, serotonin 5-HT_{3}, and neuropeptide Y_{2} receptors), activating afferent fibers. Vagal afferents can also be activated by lymphatic chylomicrons (72), triggering c-Fos immunoreactivity in the NTS and in the hypothalamus (e.g., paraventricular hypothalamic nucleus, arcuate nucleus, and ventromedial hypothalamus) (73). Activation of vagal afferents results in inhibition of food intake, gastric emptying, and stimulation of pancreatic secretions and may also influence hepatic glucose production (74). Vagal afferents are sensitive to metabolic shifts and can alter the expression levels of gut peptide receptors and peptide transmitters in response to feeding or other hormonal cues (15). Fasting results in a receptor profile that is orexigenic. Conversely, feeding and other hormonal inputs (i.e., CCK) can prompt a shift in expression to reflect an anorexigenic profile. Leptin also plays an important role by enhancing vagal sensitivity to CCK (75). Numerous studies in rodents indicated that a long-term HFD can disturb vagal regulation in a manner that would promote hyperphagia (for review, see 15,16). A HFD reduces intestinal lipid and CCK-induced satiety and is accompanied by a decrease in vagal excitability (76) and reduced neuronal activation in central sites of vagal termination (i.e., NTS) (77,78). In obesity-prone rats, long-term ingestion of a HFD leads to a loss in vagal plasticity such that there is increased expression of genes encoding orexigenic factors [e.g., the endocannabinoid receptor CB_{1} and the ghrelin receptor (growth hormone secretagogue receptor)] in the nodose ganglia compared with obesity-resistant or low-fat–fed animals (79). The mechanism by which HFD causes dysregulation in vagal signaling is still under investigation, but recent evidence suggests that a HFD induces leptin resistance in vagal afferents, which reduces their sensitivity to the satiating effects of CCK (80). Changes in intestinal permeability may also mediate the effects of a HFD on vagal neuron function (81). A HFD alters the expression of tight junction proteins, leading to increased circulating LPS (metabolic endotoxemia), which may contribute to the inflammatory obese phenotype (82,83). Increased LPS coming from the gut lumen may alter vagal activity and contribute to leptin resistance by activating vagal toll-like receptor 4 (81,84). Furthermore, in addition to a HFD, recent evidence demonstrated that T2DM can also impair normal vagal activity, specifically intestinal glucose sensing (85). Interestingly, a recent paper examined the role of PPAR\gamma in vagal nerves and found that a HFD markedly and rapidly reduced PPAR\gamma expression (86), indicating diet-associated alterations of an important metabolic transcription factor in these neurons. The investigators discovered that knockout of PPAR\gamma in murine peripheral afferents led to reduced weight gain, higher energy expenditure, increased prevalence of white adipocytes with brown adipocyte-like phenotype, and enhanced appetitive responsiveness to CCK administration. Because PPAR\gamma expression was found to be most robust in nodose ganglia vs. DRGs (86), altogether these results highlight that associations between metabolic status and peripheral afferent neuron function are context and cell-type specific.

Additionally, efferent activity of the vagus nerve contributes to metabolic phenotype by regulating glucose homeostasis and inhibiting inflammation through cholinergic signaling (87,88). Future studies are necessary to determine whether dietary fat, obesity, or other metabolic challenges influence these efferent functions, either directly or indirectly through altered afferent vagal sensing, which may contribute to the obese inflammatory and insulin-resistant phenotype.

Prediabetic and Dysmetabolic Peripheral Neuropathy

The effects of diabetes on the PNS are well established, and the areas of diabetic somatosensory and autonomic neuropathies have historically been a major focus of research (for review, see 5,89,90). However, diabetes represents 1 of the most harmful and extreme forms of metabolic dysfunction, and in the case of T2DM, the disturbances in metabolism can occur well before diabetes manifests. Prediabetes and insulin resistance represent states in which individuals who exhibit impaired glucose tolerance, hyperinsulinemia, and/
or modestly elevated fasting glucose are at greater risk of developing diabetes and cardiovascular disease (91). This impaired glucose regulation often appears with other hallmarks of MetS, such as obesity, dyslipidemia, and adipose inflammation (92). Prediabetes and MetS are present in a substantial portion of the population, and, for many, the transition to diabetes may take many years or not happen at all (92,93). Somatosensory nerve fibers appear to be particularly susceptible to perturbations of metabolism, because individuals with prediabetes and MetS are at higher risk of various forms of neuropathy (23), with small unmyelinated sensory fibers more likely to be affected than large-diameter fibers (94,95). Interestingly, individuals with idiopathic sensory neuropathy show a higher prevalence of impaired glucose tolerance and prediabetes (96–99).

The pathogenic mechanisms of prediabetic or dysmetabolic neuropathy are still being explored, but MetS risk factors (i.e., chronic hyperglycemia, dyslipidemia, and microvascular dysfunction) were proposed as contributors (99,100). Hyperglycemia is 1 of the main drivers of diabetic peripheral neuropathy, leading to neurotoxicity by increasing oxidative stress (101–103) enhanced by the formation of advanced glycation end products and proinflammatory responses (89). However, emerging evidence indicates that derangements in the sensory nervous system occurs independently of hyperglycemia. Several studies in obese individuals without diabetes, as well as diet-induced obese rodents, showed that impairments in motor and sensory nerve responses are not associated with fasting blood glucose but with hyperinsulinemia and reduced insulin sensitivity (104–107). This suggests a role for insulin resistance and associated metabolic sequelae in the development of sensory nerve dysfunction. Nerve cells do not require insulin for glucose uptake, unlike muscle and adipose tissue that represent the major sites of insulin action. Nevertheless, insulin receptors are present in the PNS (108,109), and insulin signaling stimulates neurite outgrowth and regeneration of sensory neurons (110). Chronic insulin stimulation was shown to induce insulin resistance in mouse DRGs, as evidenced by decreased activation of Akt and its downstream effectors (111,112), and can attenuate the neurotrophic effects of insulin (113). Impaired insulin receptor signaling in peripheral nerves also occurs in mouse models of type 1 diabetes and T2DM that develop diabetic neuropathy (streptozotocin-induced diabetic, db/db and ob/ob) (111,114,115).

Although the evidence for the contribution of insulin resistance to prediabetic neuropathy is compelling, recent evidence in rodents implicated dyslipidemia and not impaired insulin signaling directly as a driver of this condition (116). Sensory neuropathy and oxidative stress in Zucker fatty rats was alleviated with the application of aicipimox, a niacin derivative that selectively reduces serum nonesterified FAs and TGs while maintaining impaired glucose tolerance, suggesting that perturbation in lipid metabolism plays an important role in sensory nerve dysfunction. Indeed, there is evidence that lipid abnormalities (high amounts of LDL and TGs and low amounts of HDL) are more closely associated with MetS-associated neuropathy than glucose variables and are also associated with a greater severity in symptoms (95,117). This suggests that bioactive lipids or other metabolites derived from lipoproteins act on the PNS, raising the possibility that dietary modification and improvements in metabolic health (with attendant changes in lipoprotein profiles and oxidation status) could modify PNS physiology. Future studies identifying the exact mechanisms by which early metabolic disturbances impair sensory neuronal function might provide crucial targets that could be relevant for modulation of disease. Furthermore, in addition to somatosensory neuropathy in the prediabetic/insulin-resistant or MetS-like states, it is possible that these conditions impair normal vagal afferent and efferent functions, as described above. Additional studies are warranted in humans to fully elucidate the magnitude and time course of effects of diet, obesity, insulin resistance, and MetS on afferent peripheral sensory nerve function.

Summary
Sensory afferents are important contributors to metabolic homeostasis and thus need to be considered as part of the integrative network connecting diet and gut, peripheral tissues, and the CNS. WAT somatosensory afferents report lipid status and control adiposity, and increased adipose afferent sensory neuron activity was reported in obese rats. This increased afferent activity may contribute to obesity-related hypertension via the AAR. TRPV1 is a sensory neuron receptor important for pain signaling but may also contribute to insulin resistance and age-associated obesity by dysregulated neurotransmitter release. Vagal afferents stemming from the gut control feeding behavior, and HFDs in ob/ob rats result in an obesogenic receptor expression profile on vagal neurons that may contribute to hyperphagia. Last, sensory neurons are particularly vulnerable to diet-induced metabolic disturbances. Somatosensory neuropathy is prevalent in rodent models of obesity and insulin resistance and can result as a consequence of prediabetes-associated impaired insulin signaling and dyslipidemia. A greater understanding of the afferent sensory neuron contribution to the regulation of metabolism and the peripheral neuron dysfunction that occurs as a consequence of diet-associated and dysmetabolic conditions may identify key pathways that can be targeted for prevention of disease and for optimizing metabolic health.

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