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# Obesity Impairs the Action of the Neuroendocrine Ghrelin System

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## Abstract

Ghrelin is a metabolic hormone that promotes energy conservation by regulating appetite and energy expenditure. Although some studies suggest that antagonizing ghrelin function attenuates body weight gain and glucose intolerance on a high calorie diet, there is little information about the metabolic actions of ghrelin in the obese state. In this review, we discuss the novel concept of obesity-induced central ghrelin resistance in neural circuits regulating behavior, and impaired ghrelin secretion from the stomach. Interestingly, weight loss restores ghrelin secretion and function, and we hypothesize that ghrelin resistance is a mechanism designed to protect a higher body weight set-point established during times of food availability, to maximize energy reserves during a time of food scarcity.

## Introduction

Obesity is a multifactorial disease with both nonmodifiable and modifiable risk factors. A key modifiable factor is feeding behavior. As obesity is largely caused by hyperphagia, a better understanding of the mechanisms regulating food intake is crucial to the treatment of this chronic disease. In the current review, we focus our attention on the gut-derived peptide hormone ghrelin, a key modulator of energy metabolism that promotes a shift from negative energy to neutral energy balance, by increasing intake and hepatic glucose production, [1,2]. The role of ghrelin during positive energy balance is less well understood; however, a number of studies suggest that preventing the action ghrelin might attenuate body weight gain and the development of glucose intolerance when fed a high calorie diet [3–6]. In this review, we will examine the impact of diet-induced obesity (DIO) on the physiological function and expression of the neuroendocrine ghrelin axis. We will provide evidence that

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DIO suppresses both the expression of the neuroendocrine ghrelin system and the neural responses to ghrelin feedback, and that postnatal ghrelin helps program hypothalamic feeding circuits, ultimately influencing energy homeostasis and obesity in adulthood. Finally, we define the term central ghrelin resistance and suggest that DIO-induced ghrelin resistance affects homeostatic feeding and reward processing. We hypothesize that ghrelin resistance is a mechanism that protects a higher body weight set-point established during DIO since weight loss reverses ghrelin resistance.

## Ghrelin

Circulating ghrelin is derived mainly from the stomach and duodenum [7]. A unique post-translational addition of a medium-chain fatty acid (normally octanoate) by the enzyme ghrelin O-acyltransferase (GOAT) results in acyl-ghrelin, whereas desacyl-ghrelin, occurs following enzyme-mediated hydrolysis of the acyl moiety [8,9]. Acyl-ghrelin is best characterized for its roles in growth hormone release and food intake, with diverse actions that also influence glucose homeostasis, neuroprotection, stress and anxiety, mood, immunity and inflammation, learning and memory, and olfaction [2]. These effects are mediated through the growth hormone secretagogue receptor 1A (GHSR), a seven-transmembrane G-protein-coupled receptor (GPCR) [10]. Recent studies show that desacyl-ghrelin also regulates aspects of physiology including glucose homeostasis [11] and cerebral blood vessel proliferation [12], although a receptor for desacyl-ghrelin has not yet been identified.

Ghrelin is the only known systemic orexigenic peptide and in lean individuals plasma ghrelin levels fluctuate depending on energy intake. Plasma ghrelin concentration falls postprandially [13–15], suggesting a role as a short-term regulator of energy homeostasis, and with few exceptions is inversely correlated with body weight [13–15]. Calorie restriction and cachexia increase plasma ghrelin concentrations [1,13,16–19], while most obese individuals (not including individuals with Prader–Willi syndrome, who have higher than usual ghrelin) exhibit lower circulating ghrelin and blunted meal-related fluctuations when compared with lean individuals [13,17]. Moreover, attenuated food intake-related decreases in circulating ghrelin occur in ‘emotional eaters’ [13]. Obesity-linked reductions in ghrelin can be reversed by weight loss achieved through caloric restriction [20], whereas weight loss achieved by bariatric surgery has been associated with varying plasma ghrelin results, depending on the procedure and the study [21,22].

## Models to Study Ghrelin Secretion

The fluctuations in plasma ghrelin associated with these different metabolic and disease states are likely influenced by alterations in ghrelin secretion. Several new models and methods are now being used to identify the substrates acting directly on ghrelin cells to modulate ghrelin secretion. These models include genetically engineered mice in which green fluorescent protein reports on the location of ghrelin cells [23–25], primary cultures of dispersed gastric mucosal cells [26], immortalized ghrelinomas cell lines [19,27], transgenic mice expressing Cre recombinase selectively in ghrelin cells [28], and ex vivo stomach explant culture [29,30] systems. Using these models, recent studies have investigated the

direct effects of various peptide hormones, monoaminergic neurotransmitters, macronutrients and their metabolites, GPCRs and potential downstream effector enzymes, and channels on ghrelin secretion.

## Direct Regulation of Ghrelin Secretion by Macronutrients and Their Metabolites

Macronutrient composition in the diet directly influences ghrelin secretion as dietary triglycerides, intralipid infusion, and experimental induction of hyperglycemia reduce ghrelin levels [13,29]. Using cultured, dispersed gastric mucosal cells, ghrelin release was demonstrated to negatively correlate with glucose concentration [25]. As compared with 5 mM glucose exposure, ghrelin release is inhibited by 10 mM glucose (mimicking a hyperglycemic state) and is stimulated by 1 mM glucose (mimicking a hypoglycemic state) [25]. 2-Deoxy-D-glucose prevents the inhibitory effect of high glucose exposure on ghrelin release, suggesting that glucose must enter and be metabolized by ghrelin cells to block ghrelin secretion [25]. mRNAs encoding several channels and enzymes responsible for mediating glucose responsiveness in other cell types, including several facilitative glucose transporters, hexokinases (including glucokinase), and both components of the pancreatic β cell $K_{ATP}$ channel, all are highly expressed within ghrelin cells [25]. These findings suggest that glucose sensing in ghrelin cells regulates the release of ghrelin into the circulation.

Fatty acids and lactate also can reduce ghrelin secretion by directly engaging specialized GPCRs localized to the plasma membranes of ghrelin cells [23,28], including the short-chain fatty acid receptor GPR43 and the long-chain fatty acid receptor GPR120 [28]. Amino acids also likely directly interact with ghrelin cells to affect ghrelin release. For example, exposure of L-glutamine to the ex vivo gastric explant model decreases ghrelin secretion [29]. The calcium-sensing receptor, which has been implicated in sensing both calcium and aromatic amino acids, also is expressed and enriched in ghrelin cells [28]. Its activation by the compound R568 dose-dependently decreases ghrelin release from isolated gastric mucosal cells [28].

## Regulation of Ghrelin Secretion by the Sympathoadrenal System

The sympathoadrenal system also is an important regulator of ghrelin secretion [19,25,28,31]. As such, ghrelin secretion increases when sympathetic nerves are stimulated or when adrenergic agents are infused into the gastric submucosa [32]. $\beta_1$-Adrenergic receptor ($\beta_1$-AR) is the most highly expressed of the adrenergic receptors and of all non-odorant GPCRs within ghrelin cells [19,28]. Norepinephrine, epinephrine, and the β-AR agonist isoproterenol stimulate ghrelin secretion from cultured ghrelin cells [19,25,28,31]. Both reserpine, which depletes adrenergic neurotransmitters from sympathetic neurons, and the selective $\beta_1$-AR antagonist atenolol block the overnight fast-induced increase in plasma ghrelin in mice [19]. Thus, ghrelin secretion during an overnight fast requires $\beta_1$-AR activation.

## Mechanisms Responsible for DIO-Associated Changes to Ghrelin Levels

As mentioned, DIO is associated with lower circulating ghrelin. Furthermore, feeding fails to decrease acyl-ghrelin in people with obesity [33]. To investigate these phenomena, gastric mucosal cells derived from DIO mice were cultured. Unlike cells from lean animals, those from DIO mice do not secrete acyl-ghrelin upon exposure to norepinephrine, nor do they decrease acyl-ghrelin release in response to glucose [21]. This suggests that DIO desensitizes ghrelin cells to these physiological signals of caloric restriction and food intake. DIO also has been associated with a slight increase in ghrelin cell density in the stomachs of both DIO mice [21] and severely obese humans [34], possibly representing a compensatory reaction to the altered sensitivities of cells to norepinephrine and glucose. DIO affects other enteroendocrine cells within the stomach and intestine; for instance, an increase was observed in duodenal somatostatin cell density in DIO mice [21]. As somatostatin is a known inhibitor of ghrelin secretion [28], the increased density of somatostatin cells could potentially provide an increased inhibitory tone to ghrelin cells in the DIO setting.

## The Central Actions of Ghrelin in the Brain Depend on Metabolic State

Following its release into the circulation, ghrelin travels to the central nervous system to enact its effects on food intake and energy homeostasis. These effects are largely mediated by orexigenic neurons in the hypothalamic arcuate nucleus expressing neuropeptide Y and Agouti-related peptide (NPY/AgRP). Ghrelin-induced activation of NPY/AgRP neurons increases NPY and AgRP peptide release from nerve terminals [35], which in turn increase food intake by binding to and activating Y1 and Y5 receptors, and antagonizing melanocortin 4 receptor (MC4R)-containing neurons, respectively, in downstream target areas. Ghrelin induces NPY/AgRP action potential firing [36], and increases NPY and AgRP mRNA levels [37]. The precise mechanisms by which ghrelin mediates its orexigenic effects are reviewed elsewhere [38]. The stimulatory effects of ghrelin on orexigenic NPY/AgRP neurons are complemented by increasing inhibitory GABAergic postsynaptic inputs onto proopiomelanocortin (POMC) cells [36], which prevents the suppressive effect of the anorexigenic neuropeptide $\propto$-melanocyte-stimulating hormone ($\propto$MSH, the cleaved product of POMC) on food intake, allowing even greater orexigenic drive. Ghrelin also acts on several other brain regions, including the midbrain dopaminergic system through which it also increases food intake [39] and engages reward-related behaviors associated with a high-fat diet (HFD) [40,41].

## Negative Energy Balance

Not only is plasma ghrelin increased in response to negative energy balance, the responsiveness of hypothalamic cells to ghrelin, as measured by c-fos induction, is enhanced in fasted rats [42,43] and suppressed by acute signals of positive energy balance such as insulin or leptin, and refeeding [42,43]. Moreover, leptin suppresses ghrelin-induced c-fos activation and feeding behavior [42,44,45], NPY and AgRP gene expression, calcium signaling, and action potential firing rates in identified arcuate NPY neurons [42,44,45]. By contrast, ghrelin and GHSR signaling do not influence the metabolic actions of leptin, despite >90% coexpression of the GHSR and the leptin receptor in the arcuate nucleus [46].

Similarly, insulin inhibits NPY/AgRP gene expression [47], and restricts action potential firing via $K_{ATP}$ channel-induced hyperpolarization in AgRP neurons [48], to suppress food intake. The insulin receptor substrate is localized to NPY/AgRP neurons [49]. Insulin infusion during the fasted state prevents c-fos induction in the arcuate by a ghrelin mimetic [42]. These studies show that leptin and insulin independently modulate the ability of ghrelin to induce food intake through NPY neuronal activation, and suggest a hierarchical regulation of NPY function, whereby leptin and insulin provide tonic inhibition over NPY/AgRP neurons. Fasting is a metabolic state characterized by low insulin and leptin levels, and therefore the fact that negative energy balance potentiates the action of ghrelin in the brain may be due to the removal of the tonic inhibitory actions of insulin and leptin. Also relevant, metabolic state also influences the central actions of ghrelin on liver function and blood glucose regulation, as intracerebroventricular acyl-ghrelin infusion only increases liver gluconeogenic genes and blood glucose in response to a pyruvate challenge, when mice are pair-fed to vehicle controls, and not when allowed to eat *ad libitum* [50]. During severe calorie restriction, ghrelin is required to maintain gluconeogenesis and blood glucose [18,51], and ablation of ghrelin-secreting cells in adulthood causing hypoglycemia only under conditions of calorie restriction [52]. Moreover, fasting increases acyl-ghrelin entry into the arcuate nucleus where it binds to NPY neurons [53]. Collectively, these studies show that the central actions of ghrelin are potentiated by negative energy balance.

## Positive Energy Balance and Ghrelin Resistance

The discovery that ghrelin promotes food intake propelled ghrelin into the limelight as an anti-obesity target. It was widely assumed that the ghrelin system could drive obesity by increasing hyperphagia and weight gain. However, there is overwhelming evidence to show that obesity lowers ghrelin secretion and plasma ghrelin (see above). Also, the transport of ghrelin across the blood–brain barrier (BBB) is impaired in obese mice [54]. Fasting fails to increase ghrelin in DIO mice [55], and ghrelin does not decrease in response to meals in obese humans as it does in lean subjects [33]. Moreover, peripherally administered ghrelin fails to acutely induce food intake in DIO mice [55] with similar results observed in obese Agouti mice [56] and with chronic peripherally administered ghrelin [57].

These studies lead to the hypothesis that the hypothalamic circuitry controlling food intake becomes resistant to ghrelin during obesity [35]. Indeed, a HFD for 12 weeks decreases plasma ghrelin, ghrelin and GOAT mRNA in the stomach, and expression of hypothalamic GHSR mRNA, reflecting a suppression of the neuroendocrine ghrelin axis. Central ghrelin or peripheral ghrelin injections fail to induce food intake, arcuate nucleus c-fos immunoreactivity, NPY and AgRP mRNA expression, and NPY and AgRP peptide secretion in DIO mice. That said, administration of NPY directly into the brain stimulates food intake in both chow-fed and HFD-fed mice, indicating that downstream NPY/AgRP neural targets are unaffected by DIO, and that NPY/AgRP dysfunction is the primary cause of ghrelin resistance (Figure 1, Key Figure). DIO also attenuates fasting-induced hyperphagia by suppressing arcuate neuronal activation and hypothalamic NPY/AgRP mRNA expression [16], showing that DIO affects other physiological cues designed to maintain energy homeostasis. The effects of DIO on ghrelin resistance are not limited to food intake, as ghrelin fails to reduce oxygen consumption in HFD-fed mice relative to chow-fed mice [58].

### Mechanisms of Ghrelin Resistance

Plasma ghrelin and leptin are inversely correlated, consistent with the orexigenic function of ghrelin and the anorexigenic function of leptin, and suggestive of a counter-regulatory relationship. In fact, leptin hyperpolarizes NPY/AgRP neurons via a phosphoinositide 3-kinase (PI3K)–phosphodiesterase (PDE3) pathway [45] and negatively regulates NPY and AgRP mRNA expression [59]. Further, leptin specifically suppresses ghrelin-induced $[Ca^{2+}]_i$ increases in NPY neurons, consequently blocking food intake [45].

Three weeks of HFD-feeding elevated plasma leptin and caused ghrelin resistance in arcuate NPY/AgRP neurons, as demonstrated by lack of ghrelin-induced food intake, reduced NPY action potential firing in response to ghrelin, and reduced activation of c-fos in NPY neurons in response to ghrelin [44]. Interestingly, obese leptin-deficient *ob/ob* mice that are glucose intolerant and obese retain ghrelin sensitivity, although this is abolished upon central leptin administration [44]. Furthermore, *ob/ob* mice fed a HFD for 3 weeks do not become resistant to ghrelin and retain ghrelin sensitivity, and pair-feeding a HFD to match the calorie intake of chow-fed controls did not increase body weight gain or cause central ghrelin resistance. These data suggest that hyperleptinemia may be the cause of ghrelin resistance in DIO mice rather than HFD exposure or obesity *per se*. These findings support previous data that the hypothalamic circuits controlling energy balance are disrupted by obesity and suggest that ghrelin resistance in arcuate NPY/AgRP neurons is a consequence of obesity.

A large body of recent work has focused on DIO-induced hypothalamic inflammation as a key pathogenic process in the development of diabetes and hormonal resistance [60]. Although *ob/ob* mice fed a HFD exhibited increased gliosis in the arcuate nucleus compared with chow-fed *ob/ob* and lean control mice, they remained ghrelin-sensitive, suggesting that hypothalamic gliosis does not cause ghrelin resistance [44]. A recent study by Naznin *et al*. confirmed DIO-induced ghrelin resistance in the hypothalamus and in the nodose ganglion, suggesting that dysregulation of ghrelin signaling in vagal afferent may also be involved in ghrelin resistance [58]. Furthermore, this study observed an increase in inflammation in the hypothalamus and nodose ganglion, and suggested that this may be a cause of ghrelin resistance. Although the presence of inflammation in the hypothalamus and nodose ganglion is associated with ghrelin resistance, it does provide evidence for causation. In fact, normal ghrelin sensitivity in the presence of hypothalamic inflammation in *ob*/*ob* mice on a HFD argues against hypothalamic inflammation as a cause of ghrelin resistance [44].

## Ghrelin Resistance in Non-Hypothalamic Pathways

GHSRs are found in numerous brain regions including the ventral tegmental area (VTA) where they are expressed on dopaminergic neurons. VTA dopamine neurons regulate reward behaviors, and the role of ghrelin in such behaviors has attracted significant attention (reviewed in [41,61,62]. Intriguingly, ghrelin injection directly into the VTA still elicits food intake in DIO mice, similar to lean mice, suggesting that a HFD *per se* does not cause ghrelin resistance directly in the VTA [63]. To determine if DIO affects the ability of ghrelin to influence behavioral tasks associated with an internally rewarding state, conditioned place preference analysis was performed. Indeed, intraperitoneal ghrelin paired with food availability in the conditioning chamber conditioned a place preference in chow-fed but not

DIO mice [63]. Interestingly, intraperitoneal ghrelin in the absence of food during conditioning produced a place aversion in chow-fed and DIO mice, suggesting that separate neural circuits regulate conditioned place preference or aversion [63]. Finger *et al.* investigated whether ghrelin resistance in DIO animals inhibits the reward-associated aspects of ghrelin [64]. In a progressive ratio task, where the subject is required to do an increasing amount of work to gain a reward, the effects of ghrelin receptor ligands are blunted in DIO rats.

As intra-VTA ghrelin increases food intake equally in both chow- and HFD-fed mice, despite ghrelin resistance to reward behaviors, we suggest that ghrelin resistance occurs in neural circuits that regulate VTA dopaminergic pathways, and not the VTA itself. One potential upstream neural target may be hypothalamic AgRP neurons; DIO causes ghrelin resistance in AgRP; AgRP neurons influence dopamine-related behaviors unrelated to feeding, such as cocaine responsiveness [65]; and AgRP neurons increase motivation to obtain food and food-seeking behavior [66].

## Postnatal Overnutrition and Ghrelin Resistance

Intriguingly, ghrelin resistance is not limited to adult mice and is also observed in the small litter mouse model of early overnutrition [67]. Recent data indicate that neonatal overnutrition also has marked effects on circulating ghrelin levels during important periods of growth and development (Box 1). Neonatally overfed mouse pups exhibit elevated ghrelin levels between postnatal days 16 and 22. Surprisingly, normalization of neonatal hypoghrelinemia in neonates raised in small litters does not ameliorate metabolic outcomes, suggesting that neonatally overfed mice are relatively insensitive to neonatal ghrelin and may present ghrelin resistance [67]. Consistent with this hypothesis, the ability of peripheral ghrelin to induce c-fos in the arcuate is markedly attenuated in overnourished pups [67]. However, overnourished pups display a normal response to central ghrelin, raising the possibility that the mechanisms underlying early ghrelin resistance likely include defective transport of the hormone across the BBB to the cerebrospinal fluid or to its sites of action within the brain.

## Physiological Implications of Ghrelin Resistance

We contend that the primary role of ghrelin resistance is to protect a higher body weight set-point established during DIO. The key aspect of this ghrelin resistance hypothesis is that diet-induced weight loss resensitizes the brain to ghrelin by increasing total plasma ghrelin and acyl-ghrelin, relative to DIO mice, and reinstating ghrelin-induced feeding [4]. Indeed, ghrelin knockout mice exposed to a HFD and then placed on a diet restriction weight loss program are less susceptible to rebound weight gain compared with wild-type mice when allowed to eat chow *ad libitum* [4]. The rate of weight loss or restriction of food intake are the likely signals that reinstate ghrelin sensitivity, given that simply switching mice from a HFD to chow does not reinstate ghrelin sensitivity or plasma ghrelin levels [4]. In addition, studies in humans show that plasma ghrelin is regulated by food availability [68]. In healthy weight individuals, ghrelin increases following weight loss [69]. Ghrelin levels also increase following weight loss in obese subjects, even when patients remain overweight [20]. It may

take >12 months for ghrelin to return to normal levels after weight loss [70]. Further, plasma ghrelin is elevated in individuals that frequently intentionally lose weight but cannot maintain weight loss [71]. Thus, we hypothesize that the brain perceives reduced food availability or the rapid loss of body weight during diet-induced weight loss as a form of negative energy balance and responds accordingly by engaging the neuroendocrine ghrelin system to prevent further weight loss. From an evolutionary standpoint, this mechanism enables the defense of a body weight established during periods of relative food abundance.

## Concluding Remarks and Future Perspectives

In this review, we provide multiple lines of evidence that show metabolic state dictates the functional efficacy of the neuroendocrine ghrelin system (Figure 1). First, obesity reduces plasma ghrelin concentrations by actively reducing the rate of secretion from ghrelin-secreting cells in the gut. Second, the central responsiveness to the peripheral actions of ghrelin is reduced through the combined effect of reducing GHSR expression on target neurons, and through altered metabolic endocrine feedback in the DIO state. Third, ghrelin resistance occurs in different neural circuits regulating energy homeostasis, for example, ghrelin resistance in NPY/AgRP and/or nodose ganglion suppresses the hyperphagic effect of ghrelin [35,58], and ghrelin resistance occurs in neural pathways regulating reward processing and motivated behavior [63,64]. Furthermore, ghrelin itself during development programs the adult brain to respond appropriately to metabolic feedback, and impaired ghrelin function in this postnatal period may predispose individuals to obesity in adulthood.

We proposed the hypothesis that ghrelin resistance is a mechanism designed to protect a higher body weight set-point established during times of food availability, to maximize energy reserves during a time of food scarcity (Figure 2). This reflects the physiological role of ghrelin, that is, to defend body weight and glucose homeostasis during times of food shortage. In support of this, ghrelin stimulates many appetitive ingestive behaviors in the mouse, including exploratory sniffing [72], foraging, and food hoarding [73]. Ghrelin also affects complementary parameters of food intake including promoting learning and memory [74], decreasing anxiety [75,76], and enhancing the rewarding properties of food [41,62,77]. Ultimately, ghrelin informs the brain of low energy availability that drives biological adaptations that ensure the ability to locate food, to seek food, and to forage and hunt in dangerous situations.

Many open questions remain that need to be addressed in the future (see Outstanding Questions). One fundamental step forward requires the generation of novel mouse models to allow GHSR population-specific remote control of behavior in a temporal and spatial manner. This will help identify if different GHSR neural circuits control different behavioral outputs, and whether some, but not all, are prone to ghrelin resistance during DIO. Moreover, understanding how (GHSR-sensitive) neural processing in the brain controls ghrelin secretion from the stomach is another fundamental step forward to be addressed in the future. This will allow greater definition of the feedback and/or feed-forward control of ghrelin release into the plasma. Finally, a key question is whether ghrelin resistance is functionally relevant in humans. Current evidence in humans shows that DIO affects aspects of the neuroendocrine ghrelin axis in a similar manner to mice, for example, reduced plasma

ghrelin concentrations. However, whether or not ghrelin resistance in the brain of humans occurs is yet to be determined.

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**Box 1**

### Role of Ghrelin during Development

Although the potent orexigenic efficacy of ghrelin is not yet present in the initial 2–3 postnatal weeks in mice or rats [78,79], ghrelin in early postnatal life does have a lasting developmental effect on the hypothalamic circuits involved in energy homeostasis, and influences body weight in adulthood [80]. Mouse neonates injected with an anti-ghrelin compound between postnatal day 4 (P4) and P22 display increased densities of α-MSH- and AgRP-containing axons innervating the paraventricular nucleus. These structural alterations are accompanied with long-term metabolic defects, including elevated body weight, fat mass, and hyperglycemia [80]. By contrast, treatment of adult mice with the anti-ghrelin compound is relatively ineffective because it does not increase the density of arcuate nucleus projections relative to control mice. Chronic injection of exogenous ghrelin to wild-type mouse neonates between P4 and P12 results in a marked reduction in the density of arcuate nucleus fibers innervating the paraventricular nucleus. These findings suggest that the developmental activity of ghrelin on arcuate projections is restricted to a neonatal critical window. Intriguingly, the density of arcuate nucleus axonal projections is also elevated in ghrelin knockout mouse pups, but it becomes normal in adult knockout animals, indicating that arcuate projections continue to be plastic not just early in development but even during the postweaning period in response to genetically programmed events.

The site of action for the developmental effects of ghrelin likely includes direct action on arcuate neurons. The developing arcuate nucleus contains the highest levels of GHSR expression [80] and direct exposure of isolated explants from the arcuate to ghrelin blunts neurite extension [80]. During neonatal life, ghrelin therefore appears to act as an inhibitory signal, influencing key developmental events in the same hypothalamic pathways that will convey ghrelin signals in mature mice [80]. Thus, while obesity impacts upon the actions of ghrelin in adulthood, the actions of postnatal ghrelin may also impact upon obesity in adulthood.

## Trends

Ghrelin secretion from stomach cells is attenuated during diet-induced obesity (DIO).

During DIO, ghrelin cells in the stomach no longer respond to secretory stimuli, such as norepinephrine and glucose.

When energy is low, ghrelin drives biological adaptations that ensure the ability to seek, locate, and forage food, and enhances the rewarding properties of food.

DIO prevents ghrelin action in the brain, causing a state of central ghrelin resistance; ghrelin can no longer increase food intake due to alterations in homeostatic feeding circuits and reward processing pathways.

Ghrelin resistance is reversed with diet-induced weight loss, and this is a mechanism to defend a higher body weight set-point established during times of food availability, to maximize energy reserves when food becomes scarce.

## Outstanding Questions

How do ghrelin receptor neural circuits regulate differential physiological and behavioral output and how is this affected by DIO? Are the circuits regulating food intake different from circuits regulating anxiety, stress, motivation, or liver function?

How is ghrelin secretion from the stomach regulated by neural processing in the brain? What are the input ghrelin receptor circuits and how do they connect to output circuits via the sympathetic nervous system? Is there a neuroendocrine feedback axis that regulates ghrelin secretion?

Is ghrelin resistance functionally relevant in humans? Can we block the restoration of ghrelin sensitivity to prevent rebound weight gain after diet-induced weight loss? What are the molecular mechanisms that contribute to ghrelin resistance in ghrelin receptor neural circuits?

How does ghrelin, during development, program neural circuits that regulate energy homeostasis in adulthood? What are the indelible molecular mechanisms?

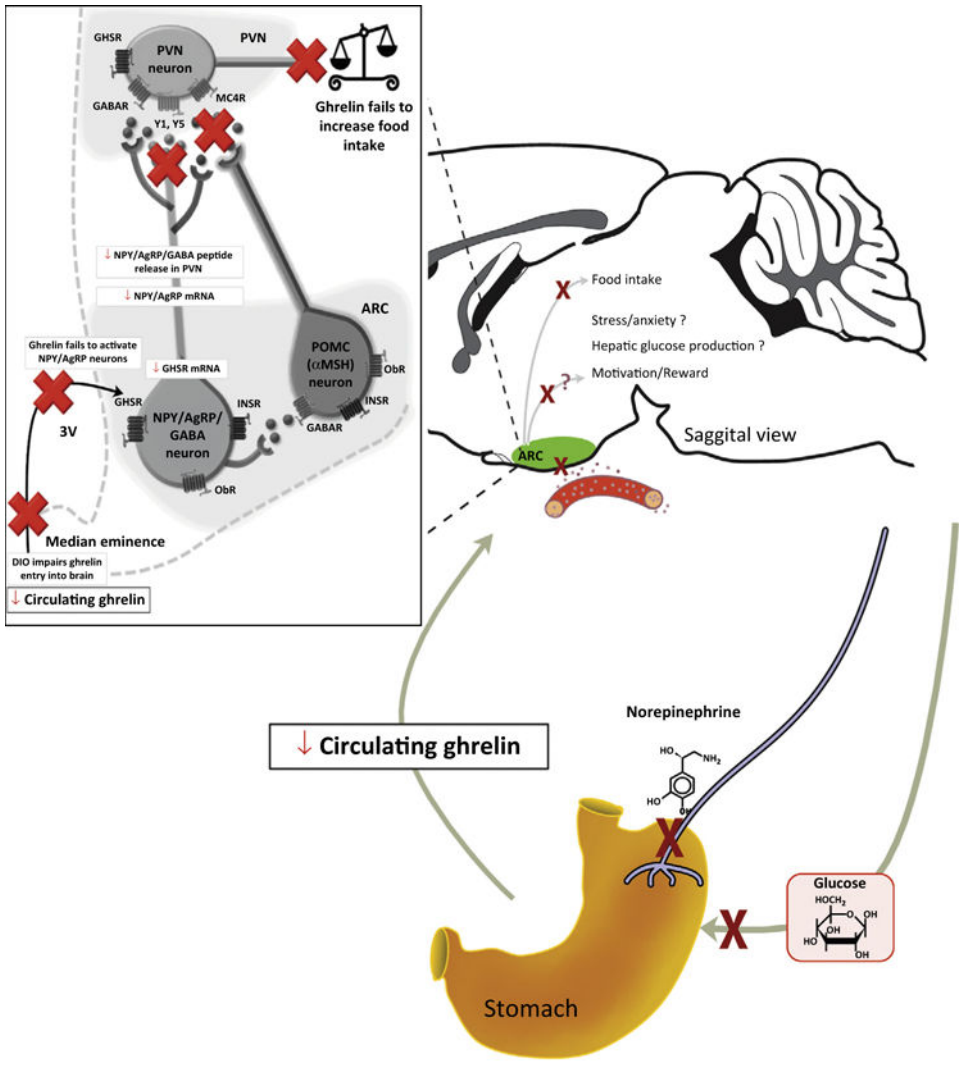


**Figure 1. Diet-Induced Obesity (DIO) Suppresses the Neuroendocrine Ghrelin System**

During DIO, ghrelin-secreting cells in the stomach no longer respond to the stimulatory actions of norepinephrine, nor do they respond to the inhibitory actions of glucose. This leads to lower than normal levels of plasma ghrelin and impaired postprandial changes in plasma ghrelin in DIO mice. In addition to impaired ghrelin secretion, DIO attenuates ghrelin transport across the blood–brain barrier (BBB) and impairs neuropeptide Y and Agouti-related peptide (NPY/AgRP) neural circuits in the hypothalamic arcuate nucleus (ARC) (inset box). With the ARC, ghrelin fails to activate NPY/AgRP neurons, fails to increase NPY and AgRP gene expression or peptide secretion, and ultimately fails to increase food intake in DIO mice. Recent studies also suggest that DIO causes ghrelin resistance in neural circuits regulating motivated behavior, although the origin of these circuits remains unknown (as indicated by the question mark). It is also currently unknown whether DIO causes ghrelin resistance in circuits regulating stress and anxiety or hepatic glucose production.

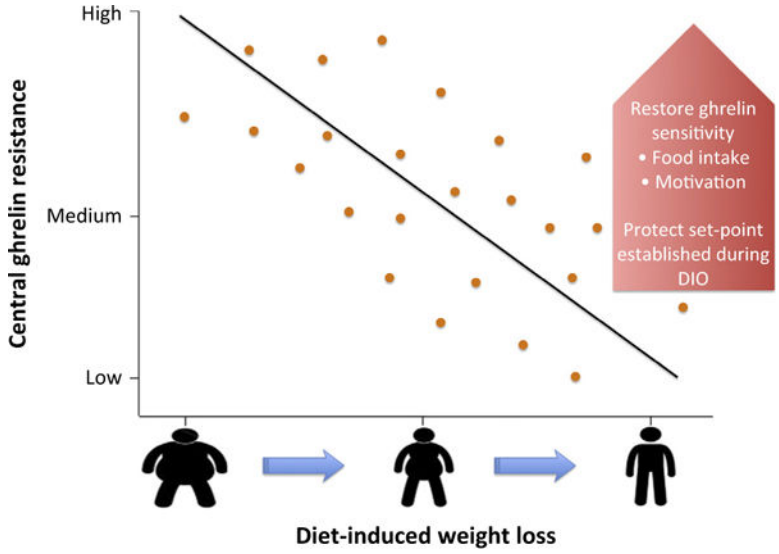


**Figure 2.**

A Theoretical Model that Illustrates the Ghrelin Resistance Hypothesis. Diet-induced weight loss restores ghrelin sensitivity and plasma ghrelin levels are elevated in people following weight loss. We hypothesize that ghrelin resistance is a physiological mechanism that protects a higher body weight set-point established during diet-induced obesity (DIO). The brain perceives reduced food availability or the rapid loss of body weight during diet-induced weight loss as a form of negative energy balance and responds accordingly by engaging the neuroendocrine ghrelin system to increase food intake and motivation to prevent further weight loss. From an evolutionary standpoint, this mechanism enables the defense of a body weight established during periods of relative food abundance.