Trends in Endocrinology & Metabolism

CellPress REVIEWS

Review

Ghrelin as a Survival Hormone

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Ghrelin administration induces food intake and body weight gain. Based on these actions, the ghrelin system was initially proposed as an antiobesity target. Subsequent studies using genetic mouse models have raised doubts about the role of the endogenous ghrelin system in mediating body weight homeostasis or obesity. However, this is not to say that the endogenous ghrelin system is not important metabolically or otherwise. Here we review an emerging concept in which the endogenous ghrelin system serves an essential function during extreme nutritional and psychological challenges to defend blood glucose, protect body weight, avoid exaggerated depression, and ultimately allow survival.

Trends

The ghrelin system comprises three main components: ghrelin, the ghrelin receptor (the growth hormone secretagogue receptor), and ghrelin-*O*-acyltransferase (GOAT).

Endogenous ghrelin is involved in food anticipatory and food reward behavior but may not play a conspicuous orexigenic role when food availability is plentiful or in diet-induced obese states.

The ghrelin system is essential during certain nutritional and psychological challenges including caloric restriction, cachexia, and psychosocial stress, orchestrating changes in several metabolic processes and behaviors to promote survival.

Activation of the ghrelin system could be a viable pharmacological approach to promote food intake and defend against hypoglycemia, body weight loss, depression/anxiety, and death during extreme nutritional and psychological challenges including severe caloric restriction, cachexia, and psychosocial stress.

Overview

Ghrelin and its cognate receptor **growth hormone (GH) secretagogue receptor (GHSR)** (see Glossary) derive their names from their first recognized functions in mediating GH secretion [1,2]. Shortly after this discovery, the identification of administered ghrelin's action inducing voracious food intake even in satiated rodent models [3–6] and of elevated plasma ghrelin preprandially [7], during fasted states [4,8,9], and following diet-induced weight loss [7] led to the emergence of ghrelin as a candidate peripheral hormone that communicates a negative energy state with neuronal centers to stimulate feeding responses. Thus, the ghrelin system emerged as a potential therapeutic target for intervention in obesity. However, while many subsequent studies have supported those early therapeutic hopes, others have called into question the function of ghrelin as a key member of the hormonal panel that influences feeding responses, leading to the unraveling of this early promise in the minds of many investigators. Although it may be the case that the overall influence of the ghrelin system on food intake and body weight is at most subtle in an environment of plentiful energy stores, such that it makes a poor target for obesity therapy, an alternative emerging notion is that ghrelin acts as an endogenous survival hormone, rising in the bloodstream as a natural adaptive response to nutritional and other stressors that could otherwise lead to significant morbidity and mortality. This review concentrates on the evidence that helps frame the ghrelin system as an essential player in the body's defense against severe **hypoglycemia** and other threats to survival.

Biology of the Ghrelin System

The ghrelin system (Figure 1) comprises three main components: ghrelin, **ghrelin-*O*-acyltransferase (GOAT)**, and GHSR. Ghrelin is a hormone that is produced predominantly by a distinct group of enteroendocrine ghrelin cells localized to the gastric mucosa, where they comprise about one in every 100–300 cells [10]. Significant contributions to the circulating pool of ghrelin are also likely to emanate from ghrelin cells in the duodenum in adults and from pancreatic islets in the fetal period [1,8,11]. Ghrelin is first synthesized as a prohormone that is subsequently processed by **prohormone convertase (PC) 1/3** into its mature form probably in the Golgi, the trans-Golgi network, and/or secretory granules [1,12]. Besides its effects on GH release and mediation of metabolism, ghrelin has been implicated in the regulation of mood, sleep, learning and memory, gastrointestinal motility, gastric acid secretion, bone metabolism, and cardiovascular function among other actions (reviewed previously in [13]).

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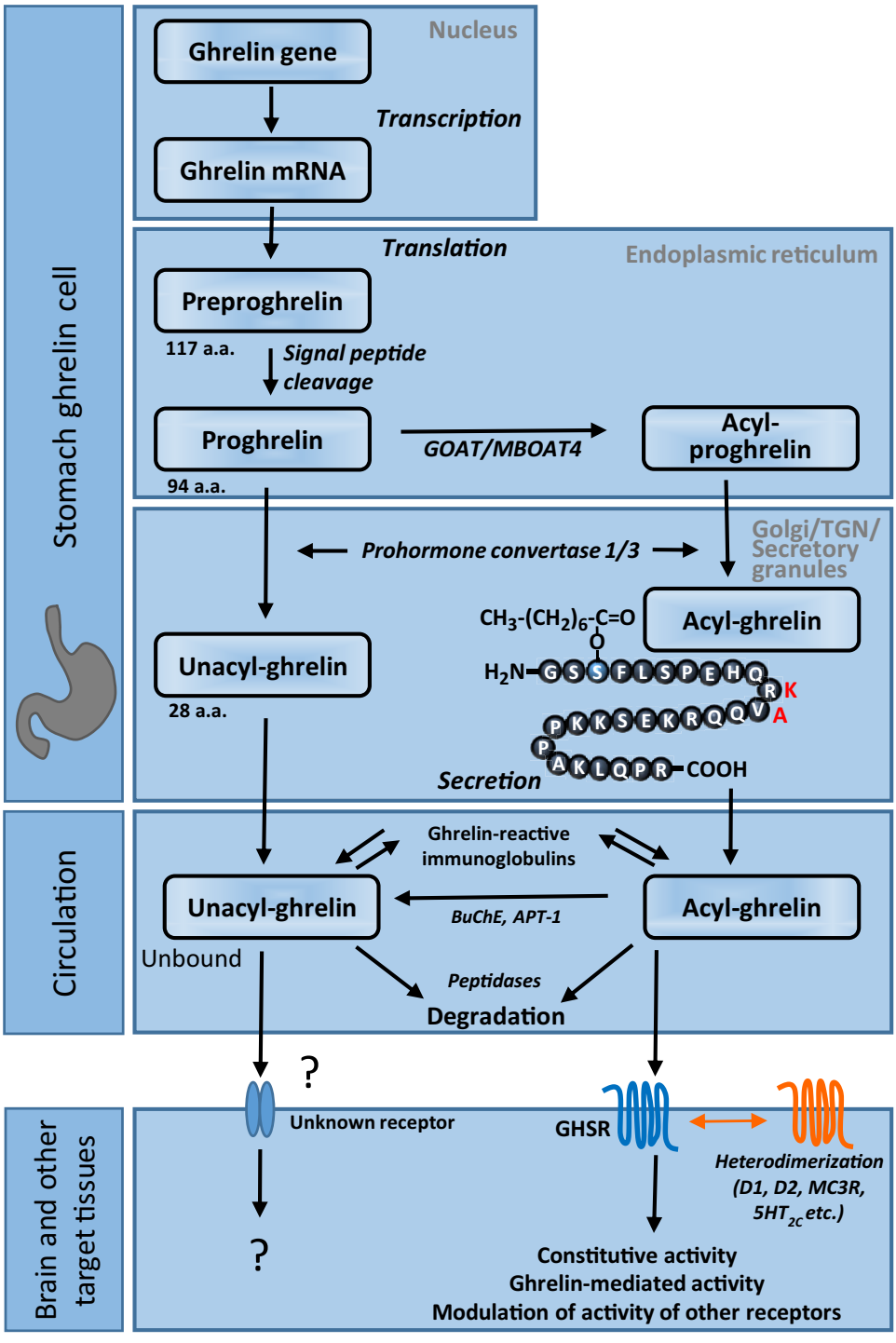

Figure 1. Components of the Ghrelin System. Human ghrelin is predominantly secreted from P/D1-like (X/A-like in rats and mouse) endocrine cells in the gastric mucosa. The ghrelin gene encodes a 117-amino-acid (a.a.) precursor protein – preproghrelin – that yields proghrelin once a signal peptide is cleaved off. A portion of proghrelin is post-translationally acylated (most often octanoylated) at serine-3 of the mature hormone – a unique reaction catalyzed by the enzyme ghrelin-

(Figure legend continued on the bottom of the next page.)

Glossary

Acyl-ghrelin (gene:*Ghrl*): the secreted form of ghrelin in which an acyl group – commonly octanoyl – is attached to the peptide. Acylation is required to engage and activate the GHSR.
Agonist: a factor that binds to and increases the intrinsic activity of a receptor.
Anorexia: reduced desire to eat, either due to primary eating disorders like anorexia nervosa or secondary to chronic disease conditions.
Antagonist: a factor that binds to a receptor, preventing an agonist from activating the receptor.
Autophagy: controlled degradation of cellular components or an organ to meet energy demands during conditions such as starvation or as a method to remove damaged cellular components.
β-blocker: drug that acts to prevent the activity of β-adrenoceptors.
Cachexia: a multiorgan syndrome characterized by severe loss of body weight, fat, and muscle mass often secondary to anorexia or due to another underlying condition such as cancer, heart failure, COPD, or renal failure.
Chronic caloric restriction: food-restriction regimen in which mice are fed 40% of their usual daily food intake (60% caloric restriction) each day for 1 week, emulating a starvation condition (as defined in this review).
Chronic social defeat stress (CSDS): an experimental model of prolonged psychosocial stress featuring aspects of major depression and post-traumatic stress disorder. CSDS subjects male mice to ten daily bouts of social subordination by an older and larger aggressor mouse.
Conditioned place preference (CPP): a preclinical Pavlovian-like behavioral test used to measure the rewarding and/or aversive effects of a test condition.
Diet-induced obesity (DIO): obesity that develops in animals fed a HFD; thought to model the obesity present in most human subjects.
Ghrelin-*O*-acyltransferase (GOAT) (gene: *Mboat4*): also known as membrane-bound *O*-acyltransferase 4 (MBOAT4). GOAT is the only known enzyme that catalyzes the

During the process of protein maturation, a portion of the ghrelin pool (the exact percentage of which remains uncharacterized) undergoes a post-translational acylation – commonly octanoylation – of the serine that ends up at position 3 of the mature hormone. This unique post-translational modification is catalyzed by the enzyme GOAT in the endoplasmic reticulum of ghrelin cells and occurs before the peptide is processed by PC1/3, stored, and secreted [12,14,15]. It also has been claimed that ghrelin can undergo acylation after its secretion, in certain target tissues [16]. Binding of ghrelin to its only known receptor, GHSR, requires this acylation step. The desacyl form of ghrelin (**unacyl-ghrelin**) is also secreted and although most studies agree that it does not bind to GHSR, it nonetheless has been shown to exhibit some, presumably GHSR-independent, biological actions, at least some of which are thought to oppose those of **acyl-ghrelin** (hereafter mostly referred to as 'ghrelin'; reviewed in [17]).

GHSR is a G protein-coupled receptor (GPCR) first isolated from the pituitary gland and later demonstrated to be expressed in several discrete brain regions and peripheral organs including the pancreas, gastrointestinal tract, and heart [1,2,11,18,19]. In the brain, GHSRs are strongly expressed in several sites involved in mediating **homeostatic feeding**, **hedonic feeding** and other reward behaviors, energy homeostasis, and blood glucose [18,20]. Not only are GHSRs activated on binding by acyl-ghrelin – they also possess other fascinating biology the characterization of which remains in its early stages. Namely, compared with most other GPCRs, GHSR has a fairly high ligand-independent, constitutive activity [21] (reviewed in [22]). Thus, even in the absence of ghrelin the capacity for GHSR to engage downstream signaling cascades occurs at nearly 50% of its maximal capacity induced by ghrelin, when assessed in heterologous *in vitro* systems [21]. Supporting an important role for ghrelin-independent GHSR signaling, mutations affecting GHSR constitutive activity have been found in several unrelated Moroccan and Japanese subjects with GH deficiency and short stature [23,24]. Furthermore, GHSRs can heterodimerize with other GPCRs, including dopamine D1 and D2 receptors, serotonin 2C receptors, and melanocortin 3 receptors, such that GHSRs can modulate signaling via these other receptors (reviewed in [22]).

Actions mediated through GHSRs are dependent on the nutritional status of the individual. For instance, fasting was shown to induce an eightfold increase in GHSR mRNA levels [25] and an increase in GHSR sensitivity [26] in the hypothalamus. By contrast, individuals in obese states become resistant to the food intake and reward processing effects of ghrelin (reviewed in [27]).

Of interest as it relates to potential roles in metabolism, plasma ghrelin is negatively correlated in the short term with feeding status and in the long term with body mass index [7,8]. Plasma ghrelin and unacyl-ghrelin levels increase before set meals and fall immediately after refeeding, a phenomenon that can be entrained [4,7,28–30]. Fasting also increases both forms of plasma

acylation of ghrelin. Ghrelin is the only known substrate for GOAT.
Gluconeogenesis: a metabolic pathway that produces glucose from non-carbohydrate sources.
Growth hormone secretagogue receptor (GHSR) (gene: *Ghsr*): also known as the ghrelin receptor. GHSR is the only known receptor for acyl-ghrelin and also serves as the receptor for synthetic growth hormone secretagogues.
Hedonic feeding: eating for pleasure, encompassing behaviors aimed at working hard and efficiently to obtain and consume rewarding foods.
Homeostatic feeding: eating required to sustain life.
Hypoglycemia: low blood sugar; generally, below 70 mg/dl.
Prader–Willi syndrome: a rare genetic disorder due to sporadic loss of or failure to express a set of paternally expressed genes in a 5–6-Mb segment of chromosome 15; involves many different organ systems and is characterized by hypotonia and failure to thrive early in life and hyperphagia and risk of severe obesity in adults.
Prohormone convertase 1/3 (PC1/3): a proteolytic enzyme that catalyzes the cleavage of basic amino acids to convert inactive prohormones into the mature hormone forms.
Unacyl-ghrelin: the form of ghrelin that does not contain an attached acyl group; does not bind to the GHSR, although some GHSR-independent activities have been reported.

O-acyltransferase (GOAT) probably in the endoplasmic reticulum of ghrelin cells. The acylated form (and possibly any remaining unacylated form) of the 94-a.a proghrelin are further processed by prohormone convertase 1/3, probably in the Golgi, trans-Golgi network (TGN), and/or secretory granules, to the mature 28-a.a. ghrelin species. Rat and mouse ghrelin differ from human ghrelin by two amino acids [Lys (K)–Ala (A) instead of Arg (R)–Val (V) at positions 11 and 12]. Acyl-ghrelin is deacylated rather rapidly in the circulation by butyrylcholinesterase (BuChE) or acyl protein thioesterase 1 (APT1) to the unacyl form. Both forms of ghrelin are also degraded by a group of peptidases that remain poorly characterized. While it is in circulation, ghrelin-reactive immunoglobulins may bind ghrelin, which has been shown to delay its degradation. The biological effects of acyl-ghrelin are mediated by binding to growth hormone secretagogue receptors (GHSRs), which are expressed in several discrete brain regions and other target tissues. The GHSR also possesses acyl-ghrelin-independent actions due to its presumed high constitutive activity. GHSRs also alter the activity of other G protein-coupled receptors (GPCRs) through heterodimerization, which in some cases requires ghrelin binding to the GHSRs. Unacyl-ghrelin does not bind the GHSR and the receptor(s) mediating its effects are unknown.

ghrelin in rodents, with the levels increasing several-fold during more **chronic caloric restriction**, although in humans exposed to longer durations of caloric restriction these elevations appear restricted to unacyl-ghrelin as opposed to applying to both ghrelin and unacyl-ghrelin [4,8,30–33]. Plasma ghrelin is generally low with blunted peaks before set meals in most obese individuals compared with lean individuals [7,27,34]. Conversely, levels are prominently elevated in individuals with negative energy balance, including those with **anorexia** nervosa or **cachexia** related to cancer, chronic obstructive pulmonary disease, or congestive heart failure [13,35,36].

True to the hallmark of enteroendocrine cells, ghrelin cells respond directly to nutrients, with glucose, amino acids, and fatty acids negatively influencing its secretion and deficiency of glucose stimulating ghrelin secretion [20,34,37–39]. Also of note, the increase in plasma ghrelin associated with both short-term and more chronic caloric restriction requires sympathetic activation of the highly-expressed β1-adrenergic receptors on ghrelin cells [32]. Other settings associated with high ghrelin include exposure to psychosocial stress [40] and **Prader–Willi syndrome**, although in Prader–Willi syndrome it is unclear how much of this represents unprocessed ghrelin [13,41]. The mechanisms that increase plasma ghrelin in these latter conditions are unclear.

Other emerging components of the ghrelin system that are relatively less well characterized than those mentioned above include enzymes that degrade ghrelin, such as butyrylcholinesterase, which hydrolyzes ghrelin to unacyl-ghrelin [42], ghrelin-reactive immunoglobulins (reviewed in [43]), which may protect ghrelin from degradation, and the truncated transmembrane domain 6- and 7-lacking GHSR1b form of GHSR, which binds and in turn reduces the constitutive activity and cell-surface expression of GHSR [22]. The relative contributions of each of these facets of the ghrelin system to its overall effects on normal physiological processes and disease states deserve further attention.

Does the Endogenous Ghrelin System Affect Body Weight or Not?

Coupled with the early observations of an inverse correlation of plasma ghrelin with energy state were other early observations that administered ghrelin could increase eating, at least in the short term. It is this role of ghrelin as a 'hunger hormone' driving food intake and the development of obesity that has been a major focus of research over the past 15 years and more. Among the early evidence of ghrelin's potent orexigenic action was a human trial primarily investigating GH release in which three of four subjects administered ghrelin reported increased hunger [44]. Subsequent studies in rodents demonstrated potent stimulatory effects on food intake, increased body weight gain, and increased adiposity with either peripheral or central administration of ghrelin [3–6]. The metabolic effects of administered ghrelin are not dependent on its actions as a GH secretagogue as these effects persist in GH-deficient rodents [4,5]. Numerous studies have demonstrated the well-characterized orexigenic hypothalamic arcuate neuropeptide Y (NPY)/Agouti-related peptide (AgRP)-expressing neuron as a prominent direct target of ghrelin action (reviewed in detail in [13,45]). Administration of ghrelin or GHSR **agonists** also lowers energy expenditure and upregulates the gene expression of lipogenic and fat storage-promoting enzymes in white adipose tissue [9,46]. Administered ghrelin also shifts food preference towards fatty diets, shifts fuel preference away from metabolic utilization of fat (presumably allowing fat to be stored instead), and engages many types of hedonic eating behaviors, including motivated lever pressing to obtain food rewards, visual cue-potentiated feeding, **conditioned place preference (CPP)** for food rewards, and locomotor activity in anticipation of a chocolate reward (reviewed in detail in [13]).

Despite these many examples of an increase in food intake and stimulation of processes that would tend to increase energy stores in response to an increase in circulating ghrelin, several studies using genetic models of ghrelin, GHSR, or GOAT deletion suggest that these effects on eating and body weight occur only with the pharmacological administration of ghrelin [33,47–51]. Thus, although ghrelin-system loss-of-function mouse models might have been expected to exhibit marked reductions in food intake, body weight, body fat, and even body length, these manifestations mostly did not materialize – certainly in any extreme sense. That said, although in many cases no differences in these body weight-related indices were observed between ghrelin-system loss-of-function models and wild-type animals, there were some exceptions. While food intake, body weight and/or body composition in *ad lib* chow-fed *ghrl*$^{-/-}$, *ghsr*$^{-/-}$, and *goat*$^{-/-}$ mice were comparable with their respective wild-type littermates [33,47,49,50,52–55], a double *ghrl*$^{-/-}$/*ghsr*$^{-/-}$ exhibited lower body weight when fed standard chow diet although there was no difference in food intake [55]. While fasting-induced rebound food intake was unaltered in *ghrl*$^{-/-}$ and *ghsr*$^{-/-}$ mice [47,49,56,57], pharmacological **antagonism** of GHSR blunted fasting-induced rebound food intake [56] as did chemogenetic inhibition of mediobasal hypothalamic GHSR-expressing neurons [20]. Although *ghrl*$^{-/-}$ mice became obese when they were switched to high-fat diet (HFD) during adulthood [48,49], *ghrl*$^{-/-}$ and *ghsr*$^{-/-}$ mice resisted the full development of **diet-induced obesity (DIO)**, accumulating significantly less fat mass and demonstrating increased energy expenditure when exposed to HFD immediately after weaning [53,58]. Reduced intake of HFD also was observed in the *ghsr*$^{-/-}$ mice but not in the *ghrl*$^{-/-}$ mice [53,58]. Also, the body lengths of the *ghrl*$^{-/-}$ and *ghsr*$^{-/-}$ mice were either normal [48,49,55] or reduced only modestly in *ghsr*$^{-/-}$ and double *ghrl*$^{-/-}$/*ghsr*$^{-/-}$ mice [53,55]. Loss of ghrelin, GOAT, or GHSR did not exaggerate body weight loss on exposure to a 1-week-long caloric restriction protocol in which mice had daily access to only 40% of their usual daily calories [33,51,59–61]. The argument that the failure of *ghrl*$^{-/-}$, *goat*$^{-/-}$, and *ghsr*$^{-/-}$ mice to become anorectic, lean, and runted was linked to compensatory adaptations in other systems was debunked by a study in which ablation of ghrelin cells in adult mice by targeting diphtheria toxin selectively to ghrelin cells nonetheless resulted in body weights and food intake similar to those in mice with intact ghrelin cells [51]. The same study also argued that administered ghrelin could induce feeding only when provided at doses resulting in supraphysiological circulating levels, although another study has suggested that administered ghrelin's orexigenic capacity does occur using doses that result in plasma levels achieved physiologically with fasting, caloric restriction, or stress [19,51].

Overall, the results from many but not all ghrelin-system loss-of-function mouse models suggest that the feeding response and body weight changes mediated by ghrelin are likely to be dispensable when food availability is plentiful. Contrary to the results from many of these loss-of-function mouse models, however, pharmacological intervention to inhibit the ghrelin system by reduction of bioavailable ghrelin or by daily administration of GHSR or GOAT antagonists to HFD-fed mice causes lower body weight and/or reduced food intake [62–65]. Thus, we believe it is still premature to write the definitive biography on the relationship of the endogenous ghrelin system to our overall body weight and feeding control systems. The role of the ghrelin system in the body weight and feeding phenotypes observed under different nutritional environments and situations, in particular, remains enigmatic.

Blood Glucose and Survival

Similar to the discovery of ghrelin's orexigenic potential, a trial in human subjects was among the first to recognize an effect of ghrelin influencing blood glucose. In particular, a bolus intravenous injection of ghrelin induced hyperglycemia accompanied by a fall in plasma insulin and a rise in plasma GH [66], an observation previously noted with GH secretagogues before

the discovery of ghrelin. Corroborating these findings, ghrelin administration to rodents increases blood glucose, lowers insulin levels, and attenuates insulin responses during glucose tolerance testing [67,68]. Similar ghrelin effects on blood glucose and/or insulin release have been demonstrated in isolated rodent islets, isolated pancreata, ghrelin-overexpressing mice, and other human trials [68–70]. Conversely, genetic deletion or pharmacological blockade of ghrelin, GHSR, and GOAT lowers blood glucose. In leptin/ghrelin double-knockout (KO) mice, although the obese hyperphagic phenotype of leptin deficiency persists, hyperglycemia is markedly reduced [71]. A GHSR antagonist restores blood glucose to normal in hyperglycemic HNF1α-deficient mice – a model of maturity-onset diabetes of the young type 3 (MODY3) – presumably via blocking of the glucose-raising actions of what was noted to be increased plasma ghrelin; increased ghrelin is also observed in humans with MODY3 [72,73]. Alone, ghrelin deletion improves glucose tolerance and increases glucose-stimulated insulin secretion from isolated islets [68,71]. GHSR deletion lowers fasting blood glucose, enhances insulin sensitivity, and improves glucose tolerance [53,74]. GHSR deletion also lowers fasting glucagon levels while acute and chronic ghrelin administration and transgenic overproduction of ghrelin both raise glucagon levels [19]. GOAT antagonist and GHSR antagonist markedly improve glucose tolerance [64,65] while exposure of isolated human islets to a GHSR inverse agonist, which decreases constitutive GHSR activity, increases glucose-stimulated insulin secretion [75]. Notably, the effects noted above of ghrelin or GHSR genetic deletion lowering blood glucose are slight in fed conditions but magnified by caloric restriction.

Although the ghrelin system contributes to hyperglycemia and/or hyperphagia in various pathological states linked to diabetes, including leptin deficiency, MODY3, and streptozotocin (STZ) administration [76–78], the blood glucose-raising actions of ghrelin seem more likely to have developed as a defensive strategy to protect against life-threatening hypoglycemia and to prolong survival (Figure 2 and Box 1). Following their initial report on the discovery of GOAT, Drs Brown and Goldstein demonstrated that *goat*$^{-/-}$ mice exhibit a strikingly progressive decline in fasting blood glucose to the point of near-death after being subjected to a caloric restriction protocol (1 week of daily access to 40% of their usual calories) that severely depletes fat stores and mimics a starvation state [33]. Excessive weight loss and an implied resulting poor overall outcome had previously been reported for *ghsr*$^{-/-}$ mice on exposure of individuals weighing 25 g or less to a 1-week restricted access to food (4 h per day) protocol [79].

Findings of marked hypoglycemia similar to that observed in *goat*$^{-/-}$ mice have since been observed on interference with the other main components of the ghrelin system, including in *ghrl*$^{-/-}$ mice, *ghsr*$^{-/-}$ mice, and mice that had undergone ghrelin cell ablation as adults [51,59–61]. Not only do mice with ghrelin cell-selective deletion of β_1-adrenergic receptors fail to appropriately increase ghrelin secretion in response to the chronic 60% caloric restriction protocol – they also experience hypoglycemia and a 36% mortality rate [32]. These findings were unlike another group's experience with *ghrl*$^{-/-}$, *ghsr*$^{-/-}$, and double *ghrl*$^{-/-}$/*ghsr*$^{-/-}$ mice [80], although differences in their protocol [they used older mice that had a higher starting fat mass and were group-housed (which may have prevented equivalent extremes of caloric restriction in all mice)] may have influenced those results.

It is worthwhile noting that in *ad lib*-fed, non-fasted states, blood glucose is not affected in adult mice with deleted ghrelin system components. However, a decrease of blood glucose into the lower range of normal occurs if these mice are fasted overnight or for 24 h, while life-threatening frank hypoglycemia develops with more chronic caloric restriction (such as the 60% caloric restriction protocol). The significance of the ghrelin system in defending against hypoglycemia depends not only on the severity/duration of the caloric restriction but also on the age of the

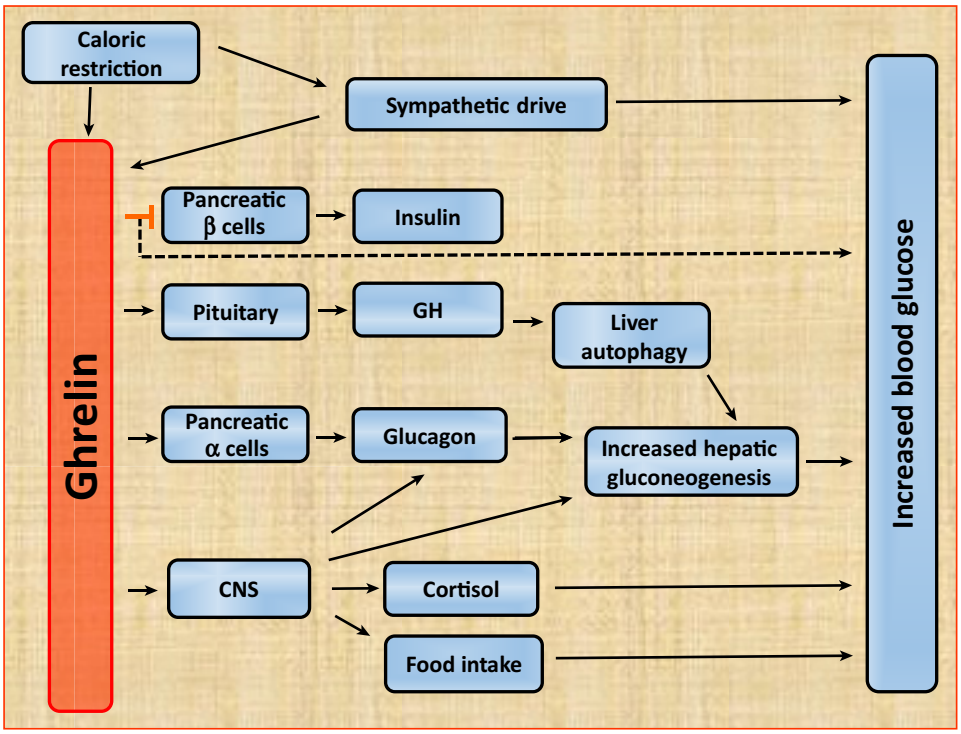

Figure 2. Mechanisms by Which Ghrelin Can Increase or Prevent Falls in Blood Glucose. Caloric restriction induces ghrelin secretion mostly by increasing sympathetic drive onto ghrelin cells and possibly also due to a reduction in the availability of circulating nutrients (e.g., glucose) and in turn a reduction in the nutrients' direct effects on ghrelin cells to decrease ghrelin release. The elevated plasma ghrelin modulates several endocrine and neuronal signals to increase and/or prevent falls in blood glucose. These include: reduction of insulin secretion by direct or indirect actions on pancreatic β cells and reduction of insulin sensitivity; increase in glucagon secretion by direct or indirect actions on pancreatic α cells; actions on the brain to increase cortisol secretion, glucagon secretion, and food intake; and increase in the release of growth hormone (GH), which in the appropriate setting can induce liver autophagy to presumably supply substrates for gluconeogenesis. The increased plasma glucagon and brain actions of ghrelin also stimulate gluconeogenesis by induction of the expression of hepatic gluconeogenic enzymes.

animal and the availability of other counter-regulatory hormones. For instance, hypoglycemia is induced in 24-h-fasted 3-week-old mice but not in 24-h-fasted adult mice on blockade of ghrelin secretion with a **β-blocker**, suggesting that neonates and infants may be more dependent on the blood glucose protective actions of ghrelin than adults [32]. Regarding the availability of other counter-regulatory hormones, whereas genetic deletion of the glucagon receptor prevents the hyperglycemia induced by STZ, the concomitant increase in plasma ghrelin acts in that setting to block the development of hypoglycemia, as administration of a GHSR antagonist further reduces blood glucose levels into the markedly hypoglycemic range in overnight-fasted, STZ-treated glucagon-receptor-KO mice [78].

Psychosocial Stress

Several recent studies have suggested that, besides protective antihypoglycemic actions in settings of caloric restriction, ghrelin also acts in a protective manner to counter anxiety and depression during stressful conditions. Administered ghrelin induces both antidepressant-like and anxiolytic-like behaviors in mice. Similarly, raising plasma physiologically in mice via caloric restriction leads to antidepressant-like and anxiolytic-like behaviors, whereas calorically

Box 1. Ghrelin Regulation of Blood Glucose

There are several mechanisms by which ghrelin can increase or prevent falls in blood glucose (see Figure 2 in main text). Ghrelin can inhibit glucose-stimulated insulin secretion from pancreatic β cells and increase glucagon secretion from pancreatic α cells through direct interactions with those cells [19,61,67,68,70], although two recent studies have suggested that ghrelin instead indirectly influences the activity of those cells via actions on somatostatin-secreting pancreatic D cells [86,87]. Ghrelin also increases glucocorticoid levels [81,88]. A direct effect of ghrelin on GH release appears to be particularly important during the 60% caloric restriction protocol. The usual spike in plasma GH observed after day 6 of this protocol is blunted in *goat*$^{-/-}$ mice, correlating with the exaggerated fall in blood glucose [33,60], and the fall in blood glucose in these *goat*$^{-/-}$ mice is partly corrected by GH infusion during the week of caloric restriction [33,60]. The blood glucose protective effect downstream of GH action in this starvation model involves at least in part increased hepatic **autophagy** and **gluconeogenesis** [59,60,89]. Ghrelin also can influence blood glucose via actions in the brain. For instance, rescue of GHSR expression in hypothalamic arcuate AgRP neurons or in Phox2b-expressing hindbrain neurons in mice lacking GHSRs elsewhere reverses the lowered blood glucose otherwise present in *ghsr*$^{-/-}$ littermates following the 60% caloric restriction protocol and/or an overnight fast [61,90]. Ghrelin's action to promote gluconeogenesis also could involve upregulation of liver gluconeogenic enzymes via GHSRs expressed in the arcuate AgRP neurons [61]. The relative importance of each of these potential mechanisms through which ghrelin acts to counter falls in blood glucose is likely to depend on the severity of caloric restriction. For instance, following an overnight or a 24-h fast, ghrelin's actions to increase glucagon release seem particularly key [19], whereas following the more chronic 60% caloric restriction protocol GH seems to be a key mediator [33].

restricted *ghsr*$^{-/-}$ mice do not exhibit these behaviors, indicating that ghrelin's protective antidepressant-like and anxiolytic-like effects are mediated through GHSRs [40]. Also, *ghsr*$^{-/-}$ mice subjected to **chronic social defeat stress (CSDS)** – an experimental paradigm that models psychosocial stress and major depression – exhibit more pronounced social isolation than do similarly treated wild-type littermates [40,81]. Moreover, CSDS and other forms of psychosocial stress lead to plasma ghrelin elevation in rodents (which in the case of CSDS have been shown to last at least 1 month) and humans [40,82,83]. Stress-induced elevations in ghrelin have also led to a proposal that ghrelin is a mediator of some stress-based eating behaviors – in particular, those that occur in individuals in whom stress leads to comfort-food eating and obesity [81,82]. Wild-type mice exposed to CSDS exhibit hyperphagia and CPP for HFD rewards, whereas CSDS-exposed *ghsr*$^{-/-}$ mice do not [56,81]. A scenario could be imagined in which an activated ghrelin system not only engages antidepressant-like and anxiolytic behaviors that help motivate hungry, prey-susceptible individuals in low-energy states to venture into the world to efficiently locate life-sustaining, calorically dense foods, but also, in the setting of chronic psychosocial stress, might help minimize the development of any related self-destructive behaviors.

Cachexia and Anorexia Nervosa

Ghrelin also is upregulated in a variety of states in which cachexia and/or anorexia are features, as observed in preclinical rodent models bearing tumors, those treated with cisplatin chemotherapy, and those with experimental heart failure and in patients with lung cancer, chronic heart failure, or chronic obstructive pulmonary disease (COPD), probably as a consequence of long-term negative energy balance [13,36,84]. The ghrelin system seems to protect against exaggerated anorexia and cachexia, as pharmacological antagonism of GHSR leads to worsened anorexia and accelerated death in tumor-bearing rats [85]. Exogenous ghrelin administration in experimental cancer models improves food intake and body composition and reduces cachectic symptoms [84,85]. A positive energy balance and improvement in cachexia also has been observed in clinical studies of patients with cancer, COPD, and heart failure [36]. Plasma ghrelin is also elevated in anorexia nervosa (reviewed in [35]). We predict that elevated ghrelin is protective in function in anorexia nervosa, preventing what might otherwise be worsened versions of the anorexia, weight loss, and depressive symptoms that characterize the disorder. Thus, while the importance of ghrelin on feeding under standard

laboratory conditions has been debated, an important role of ghrelin is apparent under extreme conditions of anorexia–cachexia, during which raised ghrelin seems to defend body weight and promote survival.

Concluding Remarks

In conclusion, we believe there is 'a time and a place' for ghrelin (Figure 3, Key Figure). Thus, while in individuals living in stress-free environments and with easy access to food the ghrelin system may be dispensable as it relates to food intake, body weight, and blood glucose control, the literature supports life-preserving, antihypoglycemic actions for the ghrelin system in adults exposed to starvation states. The antihypoglycemic actions of the ghrelin system also become emphasized in young individuals even after shorter, less extreme caloric restriction and in situations in which other hypoglycemia counter-regulatory systems are blocked (e.g., glucagon receptor signaling). We have now come to appreciate the added importance of the ghrelin system in mediating antidepressant-like and anxiolytic-like behaviors during caloric restriction and in defending against exaggerated depressive-like behaviors occurring as a result of chronic

Key Figure

Ghrelin as a Survival Hormone

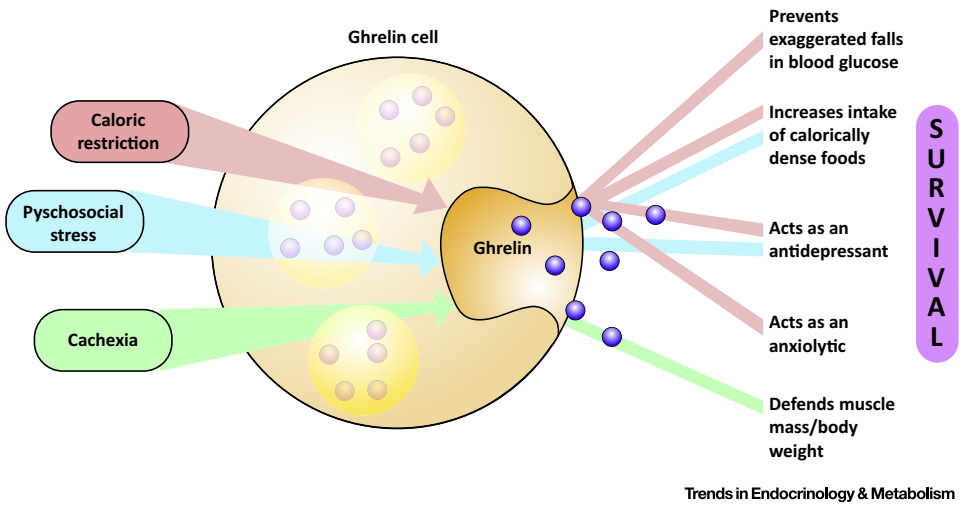

Figure 3. The essential, protective role of ghrelin as a survival hormone becomes apparent during metabolically and psychologically challenging conditions including caloric restriction, psychosocial stress, and cachexia – all of which are associated with increases in plasma ghrelin, presumably from increased ghrelin secretion. The protective roles of ghrelin during these states are summarized as follows. (A) Caloric restriction. During short bouts of caloric restriction, ghrelin limits falls in blood glucose and afterwards mediates rebound hyperphagia. Following slightly longer bouts of caloric restriction, ghrelin mediates food reward behaviors and mediates antidepressant-like and anxiolytic-like behaviors. During more severe/prolonged caloric restriction, ghrelin prevents life-threatening hypoglycemia. Ghrelin also reduces the incidence of hypoglycemia in neonates subjected to short bouts of caloric restriction. (The protective effects of ghrelin during exposure to caloric restriction are emphasized by increasing severity of caloric restriction and by young age.) (B) Psychosocial stress. Following psychosocial stress, ghrelin defends against exaggerated depressive symptoms and increases food reward behaviors including intake of calorically dense food, presumably as a way to more efficiently store energy to thwart any future threats. (C) Cachexia. In conditions characterized by cachexia, ghrelin defends against loss of body weight and muscle mass and extends survival.

Outstanding Questions

Although ghrelin has many potential downstream effectors to influence blood glucose, how does it integrate these many effectors in different situations (e.g., severe caloric restriction in adults vs an overnight fast in neonates)?

How does chronic psychosocial stress increase plasma ghrelin and what components of the ghrelin system are critical in mediating the antidepressant-like and anxiolytic responses to stress?

Is ghrelin secretion enhanced, and thus the ghrelin system's protective actions activated, when sympathetic drive is stimulated by other known stimuli such as cold exposure, aerobic exercise, insulin-induced hypoglycemia, or heart failure?

What are the mechanisms involved in ghrelin resistance?

What is the significance of unacylghrelin and how does it work?

Can ghrelin or ghrelin mimetics work therapeutically in the management of conditions such as cachexia, anorexia nervosa, and stress-associated depression?

psychosocial stress. In addition, even the endogenous ghrelin system's questioned actions in mediating food intake and body weight seem to serve important roles in protecting against exaggerated weight loss and accelerated death in models of cachexia, while also allowing appropriate rebound hyperphagia following an acute fast and mediating several food-reward-related behaviors. Thus, we believe further exploration of ghrelin biology in the settings of starvation states, cancer anorexia/cachexia, cachexia secondary to other illnesses, anorexia nervosa, and youngsters susceptible to hypoglycemia is needed to solidify the therapeutic potential of ghrelin mimetics for treating these and similar conditions such as aging-related sarcopenia (see [36] for a fuller discussion on the therapeutic potential of targeting the ghrelin system; see Outstanding Questions).

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