“A LEAP 2 conclusions? Targeting the ghrelin system to treat obesity and diabetes”

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ABSTRACT

Background: The hormone ghrelin stimulates food intake, promotes adiposity, increases body weight, and elevates blood glucose. Consequently, alterations in plasma ghrelin levels and the functioning of other components of the broader ghrelin system have been proposed as potential contributors to obesity and diabetes. Furthermore, targeting the ghrelin system has been proposed as a novel therapeutic strategy for obesity and diabetes.

Scope of review: The current review focuses on the potential for targeting ghrelin and other proteins comprising the ghrelin system as a treatment for obesity and diabetes. The main components of the ghrelin system are introduced. Data supporting a role for the endogenous ghrelin system in the development of obesity and diabetes along with data that seemingly refute such a role are outlined. An argument for further research into the development of ghrelin system-targeted therapeutic agents is delineated. Also, an evidence-based discussion of potential factors and contexts that might influence the efficacy of this class of therapeutics is provided.

Major conclusions: It would not be a “leap to” conclusions to suggest that agents which target the ghrelin system — including those that lower acyl-ghrelin levels, raise LEAP2 levels, block GHSR activity, and/or raise desacyl-ghrelin signaling — could represent efficacious novel treatments for obesity and diabetes.

1. INTRODUCTION TO THE GHRELIN SYSTEM

This review focuses on the potential for targeting ghrelin and other proteins comprising the ghrelin system as a new treatment for obesity and diabetes. The ghrelin system encompasses several key components, including ghrelin, growth hormone secretagogue receptor (GHSR; ghrelin receptor), ghrelin-acyltransferase (GOAT), liver-enriched antimicrobial peptide 2 (LEAP2), and melanocortin receptor accessory protein-2 (MRAP2) (Figure 1). Ghrelin is a hormone which, in adults, is produced mainly by a distinct population of neurons and GHSR-expressing peripheral cells that regulate food intake, body weight, and blood glucose [1,3–5]. Ghrelin also potentiates growth hormone (GH) release, stimulates gastrointestinal motility, induces gastric acid release, and has anti-depressant-like properties, among other effects [3]. In both humans and rodents, plasma ghrelin increases upon fasting and declines in obese states [3,6–11]. Plasma ghrelin levels are also dynamically influenced by feeding status, with levels rising pre-prandially and falling after the consumption of a meal [11,12]. While ghrelin is found in circulation as both acyl-ghrelin and desacyl-ghrelin, only acyl-ghrelin, which receives its unique post-translational acylation via interaction with the enzyme GOAT, binds GHSRs with high affinity [13,14]. Some studies have shown that desacyl-ghrelin also possesses biological activity, which presumably is GHSR-independent, including the ability to reduce food intake and/or block the orexigenic effect of acyl-ghrelin [15–22]. GHSRs not only mediate the metabolic actions of acyl-ghrelin (which will be referred to as “ghrelin” for the remainder of the review), but also impact the activity of other G-protein coupled receptors with which it forms heterodimers, including melanocortin 3 receptor (MC3R), dopamine 1 receptor (D1R), and dopamine 2 receptor (D2R) [23–26].

The hormone LEAP2 is the newest component to be identified as a key member of the ghrelin system [27]. LEAP2, which is highly expressed by the liver and jejunum, serves as a second endogenous ligand for GHSR [27]. Upon binding to GHSR, LEAP2 both blocks ghrelin action and reduces ghrelin-independent GHSR activity, or rather, constitutive

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Abbreviations: GH, Growth hormone; GHSR, Growth hormone secretagogue receptor; GOAT, Ghrelin O-acyltransferase; KO, Knockout; LEAP2, Liver-enriched antimicrobial peptide 2; MRAP2, Melanocortin receptor accessory protein-2; PWS, Prader–Willi Syndrome; ZDF, Zucker diabetic fatty

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GHSR activity [27–29]. As is the case for ghrelin, plasma levels of LEAP2 are highly regulated by body weight and feeding status, albeit in the opposite direction from ghrelin in adult humans and mice in most scenarios studied to date [28]. GHSR activity is also modulated intracellularly by MRAP2. In particular, MRAP2 potentiates ghrelin-dependent GHSR signaling via Gqx-mediated inositol phosphate-3 accumulation, inhibits constitutive GHSR signaling via Gq, and decreases ghrelin-stimulated β-arrestin recruitment [30,31]. Although MRAP2 regulates other G-protein-coupled receptors than GHSR, we characterize it as a key component of the ghrelin system given its many effects on GHSR signaling and since its expression is required for ghrelin-induced food intake [30,31]. Other components of the ghrelin system include enzymes such as butyrylcholinesterase (BChE) and acyl-protein thioesterase 1 (APT1), which hydrolyze ghrelin to desacyl-ghrelin, ghrelin-reactive immunoglobulins, 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 [32–35].

2. DATA SUPPORTING A ROLE FOR THE ENDOGENOUS GHRELIN SYSTEM IN THE DEVELOPMENT OF OBESITY

Soon after the identification of ghrelin as an endogenous ligand for GHSR and a potent growth hormone secretagogue, it was shown that ghrelin administration increases food intake and body weight gain [3,4,7,36]. The administration of ghrelin and/or GHSR agonists also lowers energy expenditure and upregulates gene expression of fat storage-promoting enzymes in white adipose tissue [3,8,37]. Based on the early characterization of those functions, limiting ghrelin action was predicted to reduce food intake, body weight gain, and adiposity [3,4,7,8,12,38,39]. Indeed, neutralization of bioavailable ghrelin and administration of GHSR antagonists or GOAT antagonists to mice fed a high-fat diet lowers body weight and/or food intake [40–43]. Administration of a GOAT antagonist to mice reduces weight gain in response to a diet enriched in medium-chain triglycerides [42]. Pharmacological antagonism of GHSR and chemogenetic inhibition of mediobasal hypothalamic GHSR-expressing neurons blunt fasting-induced rebound food intake in mice [44,45]. The administration of LEAP2 or LEAP2-derived peptide analogs acutely reduces baseline food intake and/or ghrelin-induced food intake in mice [27,29]. Some genetic models targeting the ghrelin system also support a role for an intact ghrelin system for normal eating behaviors and/or body weight responses to chronic high-fat diet exposure [44,46–48]. For example, when male ghrelin-knockout (KO) mice were placed on a high-fat diet early in life, they accumulated 30% less body weight over 20 weeks relative to wild-type controls [46]. Similarly, when female mice lacking GHSR (GHSR-null mice) were placed on a high-fat diet soon after weaning, they showed a robust phenotype relative to their wild-type littermates, such that after 19 weeks, they weighed 13% less and had nearly 50% less fat mass [47]. Male GHSR-null mice weighed 11% less and exhibited a 17% lower fat mass than wild-type littermates following high-fat diet exposure [47]. Female GHSR-null mice also accumulated less body weight than their wild-type littermates when fed regular chow, albeit not to the degree of those fed high-fat diet [47]. Another study demonstrated not only that male ghrelin-KO mice exhibited attenuated body weight gain when placed on a high-fat diet for 12 weeks, but also that following a 13-day caloric restriction period, they exhibited less rebound body weight gain upon resumption of ad libitum food access [48]. Simultaneous genetic deletion of both ghrelin and GHSR lowered body weight in animals fed regular chow [49]. Additionally, selective neuronal deletion of GHSRs was shown to almost completely prevent the development of diet-induced obesity, via upregulating energy expenditure [50].

The effects of ghrelin on food intake are particularly apparent when examining hedonic eating behaviors in preclinical models [44,51–55]. As examples, ghrelin administration increases the rewarding value of palatable foods, such that rodents are motivated to work harder to obtain palatable food rewards [44,55]. In contrast, calorically-restricted GHSR-null mice, similarly restricted wild-type mice given a GHSR antagonist, and chronic psychosocial stress-exposed GHSR-null mice all lack conditioned place preference for high-fat diet food rewards [44,52,56]. Furthermore, administered ghrelin induces cue-potentiated feeding behavior, whereas this feeding behavior is disrupted in mice with blocked GHSR signaling [53,54].
3. DATA SUPPORTING A ROLE FOR THE ENDOGENOUS GHRELIN SYSTEM IN THE DEVELOPMENT OF DIABETES

The GH secretagogue MK-677 was first identified as having the capacity to elevate fasting blood glucose in healthy human volunteers prior to the identification of GHSR and ghrelin [57]. In 2001, it was reported for the first time that acute administration of ghrelin increases blood glucose in healthy human volunteers [58]. A series of other studies support these findings, consistently reporting that ghrelin, as delivered by bolus or continuous infusion, increases blood glucose in both lean and obese human participants [22,59–62]. In line with data in humans, both central and peripheral ghrelin administration increase blood glucose in rodents [58,63–71].

Ghrelin has many known glucoregulatory actions that likely contribute to its ability to increase blood glucose. For instance, administration of ghrelin reduces insulin sensitivity, restricts insulin secretion, increases circulating cortisol, and stimulates glucagon, somatostatin, and growth hormone secretion [65,68,72–76]. These effects of ghrelin occur, at least in part, via direct interactions with GHSR-expressing neurons in the hypothalamic arcuate nucleus (ARC), the caudal brainstem, and GHSR-expressing delta cells within pancreatic islets [75–78]. Perhaps counterintuitively, ghrelin also increases circulating concentrations of glucagon-like peptide 1 (GLP-1), although presumably this serves to attenuate the overall effect that ghrelin has on blood glucose [72,78,80].

In contrast, chronic pharmacological blockade of GHSRs and genetic ablation of other ghrelin system components that normally engage GHSR signaling improve glucose tolerance and/or insulin sensitivity in diet-induced obese mice [46,49,81–84]. An intact ghrelin system is also required to prevent the development of life-threatening hypoglycemia in a mouse starvation model. More specifically, ghrelin-KO mice exhibit a progressive decline in fasting blood glucose to the point of near-death following a week-long calorie restriction regimen that provides 40% of usual daily calories and depletes body fat to <2% [85]. Hypoglycemia under this regimen also occurs in mice with ablated ghrelin cells, GOAT-KO mice, GHSR-null mice, mice with ghrelin cell-selective deletion of β1-adrenergic receptors (which exhibit impaired ghrelin secretion), mice carrying a GHSR mutation (A203E) that ablates its constitutive activity, mice overexpressing LEAP2, and mice with hepatocyte-selective GHSR receptor deletion [9,27,77,83,86–88]. Under the severe calorie restriction regimen, ghrelin release is stimulated in wild-type mice, which in turn induces GH release, followed by activation of hepatocyte GHSR receptors, stimulation of autophagy in the liver, and then enhanced gluconeogenesis [88,89]. Additionally, both GHSR-KO and ghrelin-KO mice require a higher glucose infusion rate during hyperinsulinemic-hypoglycemic clamps to maintain hypoglycemia [90–92]. Conversely, GHSR agonist administration reduces the glucose infusion rate required by ghrelin-KO mice during the hypoglycemic clamps, potentially via its actions to increase plasma corticosterone and plasma GH [91].

The literature also contains numerous reports linking the ghrelin system to hyperglycemia in models of diabetes [73]. For instance, ghrelin deletion attenuates hyperglycemia in leptin-deficient (ob/ob) mice, which are normally hyperphagic, obese, and diabetic [93] despite the obese phenotype of leptin deficiency remaining unaltered in the ob/ob mice with a ghrelin-KO background [93]. Furthermore, the administration of a GHSR inverse agonist improves glucose tolerance in Zucker diabetic fatty rats (ZDF; fa/fa; leptin-receptor deficient) [84]. In contrast to ob/ob mice, leptin receptor-deficient (db/db) mice, and ZDF rats, all of which exhibit relatively low plasma ghrelin levels [94], humans with maturity-onset diabetes of the young type 3 (MODY-3), and a MODY-3 mouse model [hepatocyte nuclear factor 1-alpha (HNF1α)-deficiency] exhibit elevated plasma ghrelin [95,96]. Notably, GHSR antagonist administration normalizes blood glucose in the otherwise hyperglycemic mice modeling MODY-3 [99]. Ghrelin is also higher in humans with the MODY-2 (glucokinase–MODY) form of diabetes mellitus [96].

The streptozotocin (STZ) model of type 1 diabetes, in which STZ administration to rats and mice chemically ablates pancreatic β-cells, resulting in hyperglycemia as well as hyperphagia, is also associated with alterations in plasma ghrelin. Several studies demonstrate elevated plasma ghrelin in STZ-treated animals [92,97–103]. Of particular interest, genetic ablation of ghrelin and pharmacological inhibition of GHSR cause significant reductions in STZ-associated hyperphagia [10,101–104]. Genetic ablation of GHSR or genetic ablation of ghrelin also lowers fasting blood glucose in STZ-treated mice [10,92]. Furthermore, without ghrelin, STZ-treated mice are unable to mount the usual counterregulatory response to insulin-induced hypoglycemia [92].

4. DATA THAT DO NOT NECESSARILY SUPPORT A ROLE FOR THE ENDOGENOUS GHRELIN SYSTEM IN THE DEVELOPMENT OF OBESITY AND DIABETES

Although there are many examples of administered ghrelin and endogenously high ghrelin increasing food intake, food reward behaviors, adiposity, body weight, and blood glucose as well as numerous examples of pharmacologic and genetic manipulations that decrease ghrelin levels or ghrelin/GHSR signaling attenuating the development of obesity and lowering blood glucose, the literature does not universally support a role for the endogenous ghrelin system in the development of obesity and diabetes. Indeed, some studies have shown little to no effect of genetic or pharmacologic interference with ghrelin signaling on body weight and food intake [105,106]. For example, initial experiments of ghrelin-KO and GHSR-KO mice reported an insignificant effect of ghrelin deletion on body weight, cumulative food intake, and compensatory hyperphagia following a fast and only a modest reduction in body weight for GHSR-KO mice fed regular chow relative to wild-type controls [107,108]. In a different ghrelin-KO model, mice were resistant to diet-induced obesity only when introduced to high-fat diet soon after weaning, but not when challenged with high-fat diet exposure later in life [46,109]. The initial reports of GOAT-KO mice demonstrated either insignificant or only modest effects on body weight [83,110]. Additionally, ablation of ghrelin cells from adult mice had no effects on either food intake or body weight in mice fed regular chow or high-fat diet [86]. Moreover, while genetic deletion of ghrelin from ob/ob mice resulted in marked improvement in the hyperglycemia characteristic of leptin-deficiency, genetic deletion of GHSR from ob/ob mice paradoxically had the opposite effect—namely, it worsened the hyperglycemia of the ob/ob mice [111]. Typically, plasma ghrelin levels are lower in individuals with obesity than in lean individuals. With some exceptions, plasma ghrelin is consistently reported as being inversely correlated with body weight. This correlation has been found in both adult humans with obesity or metabolic syndrome and in mouse models of diet-induced obesity [28,112–119]. In diet-induced obese mice, fasting fails to increase plasma ghrelin [120], whereas in humans with obesity, plasma ghrelin levels are not suppressed after consumption of a meal [121]. Furthermore, plasma levels of the GHSR antagonist and inverse agonist LEAP2 are higher in diet-induced obese mice and adult humans with obesity as compared to lean controls [28]. In particular, plasma LEAP2 is positively correlated with body mass index, percentage body fat,
plasma glucose, homeostatic model assessment of insulin resistance (HOMA-IR), serum triglycerides, visceral adipose tissue volume, visceral adipose tissue volume to subcutaneous adipose tissue volume ratio, and intrahepatocellular lipid content in humans and with fat mass and body weight in mice [28]. These findings translate to an increase in the mean plasma LEAP2/ghrelin molar ratio in subjects with obesity when compared with lean subjects [28]. If ghrelin were a major driver of obesity and the glucose intolerance associated with obesity, one might predict that plasma ghrelin would be increased and plasma LEAP2 decreased relative to levels in lean control subjects as opposed to the reverse.

Not only is plasma ghrelin lower and LEAP2 higher in obese states, but ghrelin transport across the blood—brain barrier is also impaired in obese mice [115,122,123]. These findings lead to the hypothesis that the hypothalamic circuitry controlling food intake becomes ghrelin-resistant during obesity [35]. As examples of this specific ghrelin resistance, ghrelin fails to acutely induce food intake in diet-induced obese mice and in obese Agouti mice, whether acutely or chronically administered [120,124,125]. Administered ghrelin also fails to reduce energy expenditure in diet-induced obese mice unlike its clear effect to reduce energy expenditure in mice fed regular chow [126]. Ghrelin-induced GH release is also attenuated in human subjects with obesity [127]. Among the pathways proposed to mediate the ghrelin resistance of obesity is the above-described coordinated rise in plasma LEAP2 and fall in plasma ghrelin observed in humans and mice with obesity [28].

Similar to the lower levels of plasma ghrelin observed in obesity, lower levels of plasma ghrelin have been reported in patients with type 2 diabetes mellitus. Often, the plasma ghrelin is negatively correlated with circulating insulin levels and obesity in these participants, suggesting that the higher insulin levels observed in early stages of type 2 diabetes and in obesity may drive reductions in plasma ghrelin [128,129]. As mentioned in the discussion above for obesity, we might have predicted plasma ghrelin to be high in type 2 diabetes if it were driving the hyperglycemia. Further, despite the STZ model of type 1 diabetes mellitus being associated with elevated plasma ghrelin, consistent changes to plasma ghrelin in humans with type 1 diabetes mellitus have not been observed [96,130—135].

As another example, Prader–Willi Syndrome (PWS) [136—140], which is characterized by failure-to-thrive, GH deficiency, and hypotonia in early-childhood and unrelenting hunger, hard-to-control food-seeking behaviors, hyperphagia, and obesity in late childhood and adulthood, is associated with high plasma ghrelin—approximately 3- to 4.5-fold higher than in matched control subjects with non-syndromic obesity [116,141—150]. As mentioned in the discussion above for obesity, we might have predicted plasma ghrelin to be high in type 2 diabetes if it were driving the hyperglycemia. Further, despite the STZ model of type 1 diabetes mellitus being associated with elevated plasma ghrelin, consistent changes to plasma ghrelin in humans with type 1 diabetes mellitus have not been observed [96,130—135].

Thus, for years, PWS has been touted as a prime example of a condition in which high ghrelin may be contributing to hyperphagia and obesity [151]. It has also been proposed that the occurrence of high ghrelin in youngsters with PWS may prime their brain for the eventual occurrence of obesity [143], as is supported by work suggesting a key role for proper expression of ghrelin for normal development of hypothalamic feeding circuits [152]. Yet, impaired prohormone processing, including that of proghrelin, has been reported in PWS, as has detection of both mature ghrelin and unprocessed proghrelin by the standard ghrelin assay kits, suggesting that perhaps the levels of biologically-active, mature ghrelin might actually not be elevated in PWS [153]. Indeed, ghrelin deletion and GHSR deletion have only minimal effects on the metabolic profile of Snord116del mice modeling PWS [154]. Instead, daily administration of a GHSR agonist to Snord116del neonates markedly improves their survival, suggesting that boosting ghrelin/GHSR signaling might be beneficial in the early stages of PWS [154].

Furthermore, others have proposed that a high plasma desacyl-ghrelin to acyl-ghrelin ratio contributes to the failure-to-thrive phenotype in infants with PWS [147,155]. Whether or not increased endogenous plasma desacyl-ghrelin contributes to the failure-to-thrive phenotype in youngsters with PWS, a cyclized desacyl-ghrelin analog (AZP-531) has been shown in a two week-long, proof-of-concept multicenter, randomized, double-blind, placebo-controlled trial of 47 adult patients with PWS to significantly improve food-related behaviors and reduce appetite [156]. A short-term course of this desacyl-ghrelin analog also reduced hemoglobin A1c and body weight when tested in a small cohort of patients with type 2 diabetes [157].

5. IS THERE A ROLE FOR THERAPEUTIC TARGETING OF THE GHRELIN SYSTEM TO TREAT OBESITY AND DIABETES?

As evidenced in the above discussion, the literature does not draw a straight line from altered ghrelin system functioning to obesity or diabetes. Yet, would it be a leap to conclusions to suggest that therapeutics which lower acyl-ghrelin, raise LEAP2, block GHSR activity, and/or raise desacyl-ghrelin signaling, might be beneficial in obesity or diabetes? Examples of agents with these properties are numerous in the literature and include the following classes depicted in Figure 1: anti-ghrelin L-RNA aptamers (anti-ghrelin vaccine), GHSR antagonists, GHSR inverse agonists, GOAT inhibitors, cyclized desacyl-ghrelin analogs, LEAP2, and LEAP2-derived peptide analogs [29,41,157—159]. Our simple answer is no. Instead, it is our strong opinion that ample preclinical and clinical data exist supporting further research and development in this area. Moreover, as outlined in Figure 2, we believe there are several factors which may influence the efficacy of such agents. These factors are described in more detail below.

As mentioned, ghrelin levels are low in obesity, and at least some of ghrelin’s metabolic actions are blunted in obesity due to a state of ghrelin resistance. Similarly, plasma LEAP2 depends on both the long-term, underlying metabolic state (e.g., body mass and adiposity) as well as more short-term, meal-dependent changes in nutrient availability [28]. These state- and meal-dependent LEAP2 changes are mostly opposite of those of ghrelin. Notably, the concentration of plasma LEAP2 in the ad libitum—fed state is more than 20-fold higher than that of plasma ghrelin in both mice and humans [28]. Also, the potency of LEAP2 as a GHSR antagonist is very close to the potency of ghrelin as a GHSR agonist when assessed in vitro [27,29,160]. Therefore, we have hypothesized that in the fed state, LEAP2 serves as the dominant ligand of GHSR, prominently antagonizing ghrelin actions [28]. In contrast, in the fasted state, the fall in the plasma LEAP2/ghrelin molar ratio seems to favor a relatively higher degree of ghrelin–GHSR binding. This potentially explains why genetic deletion of endogenous ghrelin does not have pronounced metabolic effects in ad libitum—fed conditions, but does upon energy restriction [65]. The same reasoning can be used to predict a dominant role for LEAP2 in the setting of obesity. As indicated in Figure 3, we have proposed that in obese states, LEAP2 rises and ghrelin falls, shifting the plasma LEAP2/ghrelin molar ratio higher so as to limit ghrelin’s capacity to exacerbate obesity and glucose intolerance by raising food intake, body weight, and blood glucose. Furthermore, our model predicts that therapeutic interventions that raise plasma LEAP2 or further reduce plasma ghrelin, in an effort to raise the plasma LEAP2/ghrelin molar ratio even higher, would serve to limit the development of obesity and glucose intolerance in obeseogenic environments.
Why might raising LEAP2 be helpful in the setting of obesity? Importantly, plasma LEAP2 is positively correlated with body mass index, and postprandial increases in plasma LEAP2 have been observed only in subjects with body mass indices greater than 35–40 kg/m² [28]. This suggests that less severe degrees of obesity (e.g., Class I obesity) may not be associated with what is presumed to be compensatory elevations of LEAP2 which would otherwise limit food intake and body weight gains. As such, individuals with less severe obesity might benefit from therapies that would raise plasma LEAP2 (for instance, to curb appetite and reduce food reward). So too might individuals who have achieved weight loss through lifestyle interventions, but who run the risk of rebound weight gain, as weight loss induces decreases in plasma LEAP2 and reciprocal increases in plasma ghrelin [28].

Why might lowering plasma ghrelin even further in obesity be helpful? Several studies have reported an effect of insulin administration to markedly lower plasma ghrelin [91,92,161–163]. However, despite this drop in plasma ghrelin, for instance to curb appetite and reduce food reward, So too might individuals who have achieved weight loss through lifestyle interventions, but who run the risk of rebound weight gain, as weight loss induces decreases in plasma LEAP2 and reciprocal increases in plasma ghrelin [28].

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Why might lowering plasma ghrelin even further in obesity be helpful? Several studies have reported an effect of insulin administration to markedly lower plasma ghrelin [91,92,161–163]. However, despite this drop in plasma ghrelin, for instance to curb appetite and reduce food reward, So too might individuals who have achieved weight loss through lifestyle interventions, but who run the risk of rebound weight gain, as weight loss induces decreases in plasma LEAP2 and reciprocal increases in plasma ghrelin [28].
Figure 3: Model illustrating the coordinated response of ghrelin and LEAP2 to obesity and the proposed metabolic effects of a therapeutic intervention that would further raise the plasma LEAP2/ghrelin molar ratio. Top: Obesity is associated with an increase in plasma LEAP2 and a decrease in plasma ghrelin. The resulting increased plasma LEAP2/ghrelin molar ratio serves to limit food intake, body weight, and blood glucose, or rather, processes that would otherwise exacerbate obesity and glucose intolerance. Bottom: Therapeutic interventions that increase plasma LEAP2 and/or decrease plasma ghrelin, such as many of those illustrated in Figure 1, would further increase the plasma LEAP2/ghrelin molar ratio. In turn, food intake, body weight, and blood glucose would be further limited, as would the development of obesity and glucose intolerance in obesogenic environments.

orexigenic effects of administered ghrelin. Ghrelin’s effects are reduced upon estrogen delivery to male and ovariectomized female rats. Also, ovariectomized GHSR-null mice resist the gains in food intake and body weight normally induced by ovariectomy. Age might also be an important factor. For instance, similar to humans, fasted young mice are susceptible to hypoglycemia upon beta blocker administration, potentially as a result of reducing ghrelin secretion, whereas adult mice are not [9]. Aged mice also appear to be particularly susceptible to ghrelin or GHSR deletion [185,186]. Thus, different age brackets should be investigated to determine if one or the other is more responsive to targeting the ghrelin system. In nearly all studies, ghrelin administration drives hedonic eating, whereas GHSR and GOAT deletion or GHSR antagonism diminish it [187–189]. Accordingly, those individuals whose obesity is driven by food reward behaviors might benefit more from targeting the ghrelin system. It is also certain that specific genetic mutations might enhance performance of ghrelin system-targeting therapies. As described above, some monogenic forms of diabetes, such as MODY-3 and MODY-2, are associated with elevated plasma ghrelin, and a GHSR antagonist reversed hyperglycemia in a preclinical MODY-3 mouse model. Also, ghrelin deletion improved hyperglycemia in leptin-deficient mice. Furthermore, the cyclized desacyl-ghrelin analog shows promise in reducing appetite in patients with PWS.

6. THEORETICAL DOWNSIDES TO MODULATING THE GHRELIN SYSTEM AS A MEANS TO TREAT OBESITY AND DIABETES

There are potential drawbacks to pharmacologically reducing GHSR signaling as a means to treat obesity and diabetes. One drawback relates to actions of ghrelin and GHSR to stimulate adult hippocampal neurogenesis, while genetic GHSR deletion reduces adult hippocampal neurogenesis in the settings of chronic social defeat stress (a model of chronic psychosocial stress) and caloric restriction [190–192]. Such reductions in adult hippocampal neurogenesis have been linked to exaggerated depressive-like behavior and deficiencies in learning and memory and may be particularly relevant in neurodegenerative disorders, such as Alzheimer’s disease and Parkinson’s disease [190–192]. Consequently, individuals with diabetes or obesity suffering from depression or a neurodegenerative disorder may not be optimal candidates for therapeutic interventions that would decrease ghrelin/GHSR signaling. Also, ghrelin possesses gastrokinetic activity, leading to the investigation of GHSR agonists as a potential treatment for diabetic gastroparesis [193]. As a result, diabetic individuals complicated by gastroparesis may not be the best candidates for treatment with a GHSR antagonist.

Another theoretical drawback to blocking ghrelin/GHSR signaling relates to the proposed function of ghrelin as a survival hormone [4]. In particular, we and others have hypothesized that the endogenous ghrelin system serves an essential function during extreme nutritional and psychological challenges to defend blood glucose, protect body weight, and ultimately allow survival [4]. Highlighting these protective actions of ghrelin, ghrelin-KO mice and related genetic models that decrease ghrelin/GHSR signaling experience marked hypoglycemia and increased mortality upon exposure to a prolonged caloric restriction regimen that depletes body fat to <2%, as mentioned above [9,27,77,85–89]. Ghrelin prevents hypoglycemia from occurring in fasted, beta blocker-treated 3-week-old mice, modeling the hypoglycemia occasionally experienced by beta blocker-treated human infants and toddlers when they have not been eating [9]. During hyperinsulinemic-hypoglycemic clamp procedures, ghrelin-KO and GHSR-KO mice require greater glucose infusion rates to prevent blood glucose levels from falling beneath the target range [90–92]. GHSR-null mice exhibit reduced exercise tolerance when run on treadmills [183]. Also, pharmacological antagonism of GHSR and GHSR-deletion worsen anorexia/cachexia and accelerate death in tumor-bearing rodents [194,195]. Given these findings, activating the ghrelin system could be a viable pharmacological approach to promote food intake and defend against hypoglycemia, body weight loss, poor exercise endurance, and death during extreme nutritional challenges including severe caloric restriction and cachexia [4,183]. In contrast, it likely would be appropriate to avoid treatment with agents that reduce ghrelin/GHSR signaling in the presence of anorexia/cachexia syndromes, such as those associated with cancer, heart failure, chronic kidney disease, or severe chronic obstructive pulmonary disease [4]. It also might be prudent to avoid the use of drugs that reduce ghrelin/GHSR signaling in diabetic patients susceptible to insulin-induced hypoglycemia.

7. CONCLUSIONS

We believe that ample data support further research and development of classes of drugs that act to lower plasma ghrelin, raise plasma LEAP2, block GHSR activity, and/or raise desacyl-ghrelin signaling as a means of treating obesity and diabetes. While the literature suggests
certain scenarios in which these compounds may have negative consequences, the literature also highlights scenarios and contexts in which these compounds may be particularly beneficial as novel treatments for obesity and diabetes.

**AUTHOR CONTRIBUTIONS**

Deepali Gupta: writing — review and editing. Sean Ogden: visualization, writing — review and editing. Kripa Shankar: writing — review and editing. Salii Varshney: writing — review and editing. Jeffrey Zigman: conceptualization, visualization, writing — original draft preparation, writing — review and editing, funding acquisition.

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**CONFLICT OF INTEREST**

None declared.

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