Control of Appetite in Mouse and Man by the Hormone Ghrelin

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Appetite: an introduction

Appetite refers to the desire to eat food or drink. Appetite can be stimulated in or by many different settings. These include a decrease in the body's energy stores, the experience of food that is pleasurable, environmental cues previously associated with food, and psychosocial stress. All of these aspects of appetite are mediated at least in part by hormones that act in the brain. In addition, there are several brain pathways that help tell us when it is not appropriate to act on our appetite by eating, and in essence help us to suppress the appetite.

Ghrelin: an introduction

Ghrelin is one of the important peripherally-derived hormones mentioned above that acts in the brain to mediate appetite. Ghrelin is a 28-residue peptide synthesized predominantly by specialized endocrine cells of the stomach (1). It was first identified in 1999 as the endogenous ligand of the growth hormone secretagogue receptor (GHSR; ghrelin receptor) and was named for its potent growth hormone-secreting properties (1). Ghrelin is unique among known mammalian peptides in that its bioactive form contains an n-octanoyl group post-translational modification (1). The enzyme that catalyzes this unique n-octanoylation, ghrelin *O*-acyl-transferase, was first identified and described by Drs. Mike Brown, Joe Goldstein and colleagues at UTSW (2). In addition to its role in appetite, which will be discussed below, and its ability to potently stimulate growth hormone secretion, ghrelin also plays a key role in regulating blood glucose levels, gastrointestinal motility and gastric acid secretion, among many other physiological processes and behaviors.

Ghrelin influences several of the mechanisms that regulate appetite and food intake.

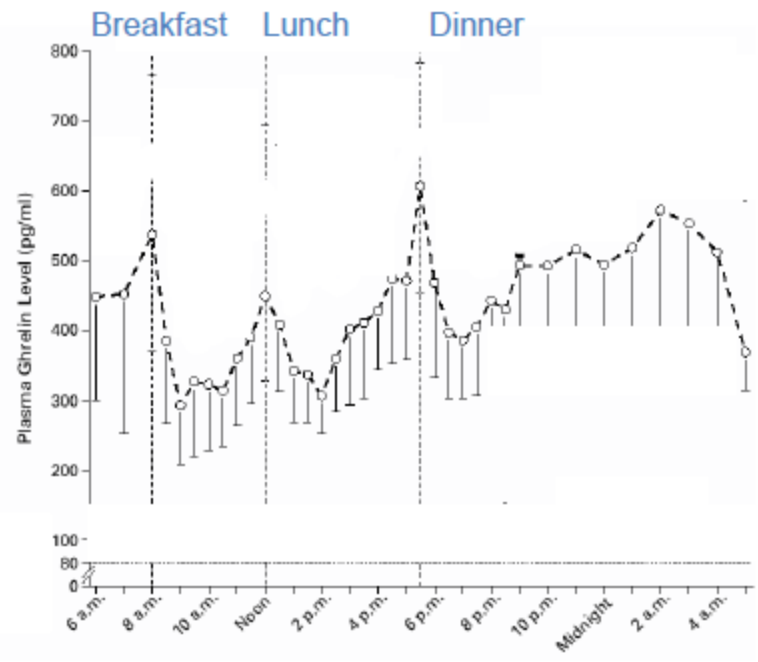
One of ghrelin's main functions relates to its ability to stimulate appetite. It does so by participating in several interrelated mechanisms that influence appetite. These include homeostatic pathways for maintaining body weight, reward pathways responsible for various hedonic (pleasurable) aspects of eating, psychological drives that influence eating, such as stress and depression, and environmental cue-driven appetite behaviors.

Ghrelin's roles in appetite: Stimulation of appetite by a decrease in the body's energy stores.

Body weight homeostatic systems are a group of physiological processes and behaviors that work together to ensure that we always maintain enough energy stores to survive. It is thought that these have developed to their current extent and level of intricacy within warm-blooded mammals because of the absolute requirement to maintain a stable body temperature for survival. This is especially true in cold environments in which maintenance of body temperature involves maintaining a high metabolic rate. Such is achieved by burning fuels, which in turn necessitates that we have sufficient energy stores or easy access to food that will serve as the source of these fuels. Thus, the body has developed an integrated, homeostatic control system in which various peripheral signals of energy availability and gastrointestinal tract activity interact with the central nervous system to modulate food intake and energy expenditure so as to maintain a set body weight.

From a thermodynamic perspective, the regulation of body weight can be described as a linear equation balancing both food intake and energy expenditure (energy balance) to derive the amount of fat stored. Under normal circumstances, a balance in energy intake and energy expenditure results in body weight maintenance. Weight loss would occur if there is a relative increase in energy expenditure and/or a relative decrease in food intake. On the other hand, weight gain would be expected if there is a relative increase in food intake and/or a relative decrease in energy expenditure.

Several pieces of evidence reinforce the notion that ghrelin is important in regulating body weight homeostasis. Administered ghrelin potently stimulates the intake of freely-available food. Furthermore, administered ghrelin reduces energy expenditure (3). The combination of these two effects would be predicted to increase body weight, and indeed, such is observed with chronic ghrelin administration. In particular, ghrelin has a predominant effect of increasing adiposity in normal weight individuals whereas it results in maintenance of body weight or delayed weight loss (with a predominant effect on lean mass) in cachectic individuals. The daily rhythm of plasma ghrelin levels also is suggestive of its role in appetite related to maintaining sufficient fuel stores. As such, ghrelin levels rise before set meals (when energy stores would be at their relative lowest levels in relation to meals) and fall upon food consumption. Ghrelin levels also rise following food deprivation and in response to weight loss resulting from many different situations, including chronic exercise, eating disorders such as anorexia nervosa and bulimia nervosa, and cancer cachexia.

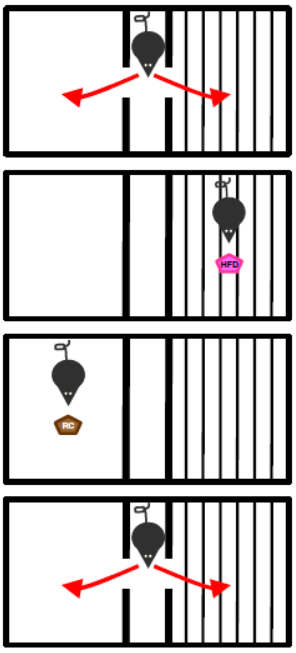


<u>Ghrelin's roles in appetite: Stimulation of appetite by the experience of a food that is pleasurable (reward-based eating)</u>

Reward-based eating refers to a set of behaviors associated with consuming food "rewards." Rewards are things that make us feel better and therefore are LIKED (they give us sensory pleasure and have hedonic impact), WANTED (desired, pursued, and motivate us to work to obtain them), and initiate LEARNING processes which help create cues that predict their availability and help organize efficient behavioral sequences aimed at obtaining them. Reward-based eating is very complex and likely involves interactions with several different intersecting neuronal pathways, including

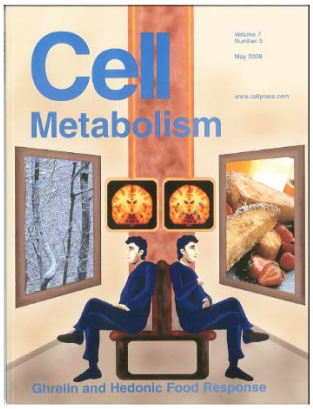
those with neuronal cell bodies in the hypothalamus, midbrain, hippocampus and prefrontal cortex.

There are many different behavioral tests that can be used to investigate reward-based eating using mouse models – and in particular, the role of ghrelin in reward-based eating and the sites where ghrelin is acting. One of these is the operant responding task. This test is thought to focus in on the motivational/wanting aspects of reward. It involves training a mouse to press its nose on a button (or press a lever, in the case of a rat) in order to have a small pellet of delicious food dispensed into the cage. The mice are rewarded with the food pellet only when they perform the nose poking behavior, according to a progressive schedule. In other words, the mouse needs to work harder for each food reward pellet. In our version of the test, the mouse needed to nose poke 5 times to get the first food reward, 10 times to get the second food reward, 20 times to get the third food reward, 30 times to get the 4th food reward, 50 times to get the 5th food reward, and so on. Non-calorically restricted mice will not work as hard as calorically-restricted mice to obtain the food rewards. Administration of ghrelin to sated animals (animals that have had free access to food in their home cages) increases operant-responding for the food rewards. In particular, ghrelin administration increases operant nose-poking or lever-pressing for sucrose, peanut-butter-flavored sucrose, and HFD pellets in rodents (4-6). Conversely, a GHSR (ghrelin receptor) antagonist reduces operant responding for 5% sucrose solution (7). In other words, ghrelin makes the food reward even more rewarding such that the individual works harder to obtain it.



Another behavioral model with which to study reward-based eating using mouse and rat models is the food conditioned place preference (CPP) test. In this test, the animal subject is conditioned to associate one chamber of a CPP apparatus with regular chow and a second, visually and texturally distinct CPP chamber with an equal calorie amount of a more pleasurable, food reward. After several days of conditioning, mice are permitted free access to both chambers in the absence of food, and conditioned place preference for the food reward is then demonstrated by the animal spending more time in the chamber with the environmental cues they have associated with the more rewarding food. Our food CPP studies revealed that pharmacologic administration of ghrelin and endogenous increases in circulating ghrelin induced by caloric restriction both enable acquisition of CPP for fatty/sugary food rewards (4, 8). On the other hand, pharmacologic antagonism of GHSRs or genetic deletion of GHSRs blocks caloric restriction-induced acquisition of CPP for the fatty/sugary food reward.

Ghrelin's actions on food reward are thought to be relevant not only to rodents but also to humans. As such, ghrelin administration to human subjects during functional magnetic resonance imaging has been found to increase the neural response to food pictures in brain regions implicated in hedonic feeding (9). These areas include the



amygdala, insula, orbitofrontal cortex, striatum, the substantia nigra and the ventral tegmental area (VTA), all of which are implicated in reward processing and appetitive behavior. Thus, it is very likely that ghrelin plays an important role in food reward behavior and appetite regulation in humans (9).

Ghrelin's roles in appetite: Cue-potentiated feeding.

The human environment is replete with visual, auditory, and olfactory cues which, via associative learning and Pavlovian conditioning, can become intimately linked to food, resulting in the induction and maintenance of eating (10). Prime examples include logos of commercial enterprises that sell food (11). With continued exposure, these cues can form such a strong association with eating that they may override satiety signals that otherwise would normally lead to eating cessation (10). Recurrent exposure to these cues potentially can lead to an overabundance of food intake resulting in an increased risk for obesity. Of note, the motivational salience of food cues as measured by visual attention is greater in obese individuals than in lean subjects, suggesting that higher sensitivity to cues associated with food may contribute to their lack of control over food intake (12).

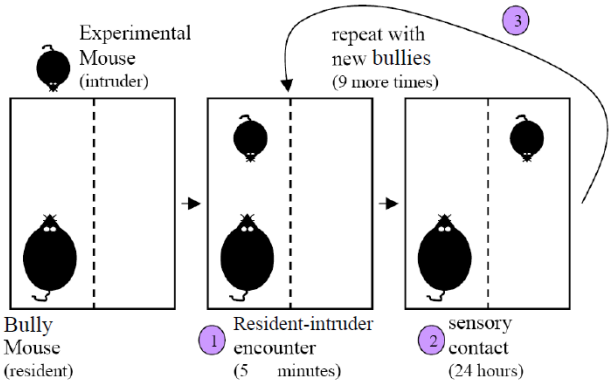
The cue-potentiated feeding paradigm models habitual eating that occurs with strong cue associations linked to food. It was originally designed for study in rats. Several studies have found that food-sated rats increase food consumption after presentation of a conditioned stimulus previously paired with food during a period of caloric restriction (13). These elegant studies were performed with bland pellets similar to regular chow, signifying the strength of a conditioned cue's ability to enhance feeding behavior even without savory taste as a rewarding component.

In a recent study, we postulated that ghrelin has the capacity to enable cue-potentiated feeding. To test this hypothesis, a novel cue-potentiated feeding protocol adapted for use in mice was designed and validated, and then the effects of pharmacologic ghrelin receptor (GHSR) antagonism were assessed. Sated mice indeed demonstrated cue-potentiated intake of grain-based pellets specifically upon presentation of a positive conditioned stimulus (CS+) but not a negative conditioned stimulus (CS-). Treatment with a GHSR antagonist blocked potentiated feeding in sated mice in response to the CS+. Thus, we were able to demonstrate a key role for intact ghrelin signaling pathways in establishing a specific positive cue-food association.

Ghrelin's roles in appetite: Stress-based eating.

Recently, evidence has surfaced linking the ghrelin system to stress-induced eating behaviors (14). Elevations of ghrelin have been observed in several animal and human

stress models (15, 16). For instance, elevations in gastric ghrelin gene expression and plasma ghrelin have been observed in rodents' responses to acute stress, including tail pinch stress and water avoidance stress. Rises in plasma ghrelin levels also are observed in rodents stressed by exposure to a continuously flooded cage or to cold environment. The chronic social defeat stress (CSDS) procedure, which subjects male mice to repeated bouts of social subordination by an older and larger aggressor male, also leads to sustained plasma ghrelin elevations (15, 16). Human beings subjected acutely to psychosocial stress or to the standardized trier social stress test also display increased plasma ghrelin (17).

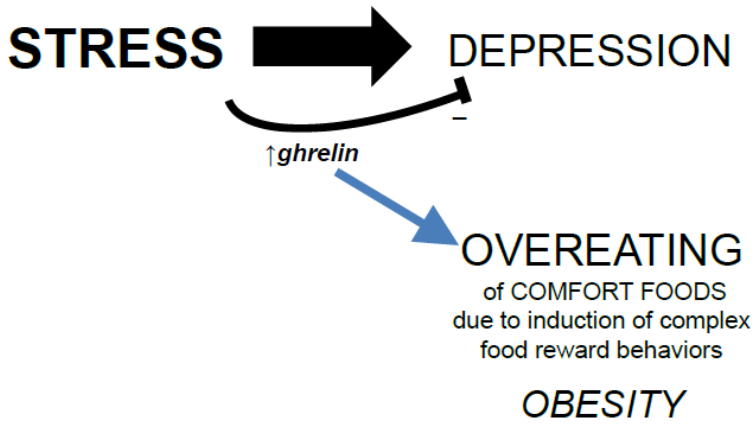


Most humans upon stress report a change in their eating habits – with some eating more and some eating less than prior to the stress (18, 19). Furthermore, most reports show an increase in the intake of highly palatable foods, independent of their general food intake response to the stress (hyperphagia or hypophagia) (18, 19). The complex eating behaviors that are associated with and/or stimulated by stress likely contribute to the increased number of overweight and obese individuals who experience or have experienced stress and depression – e.g. subjects with post-traumatic stress disorder. Interestingly, stress-induced elevations in plasma ghrelin found in "high emotional eaters" -- so-called due to their experienced food cravings and increased consumption of foods high in carbohydrates and fats in response to negative emotions and stress – fail to decline acutely following food consumption (20).

Our group has used the CSDS procedure in mice to specifically investigate the role of ghrelin on stress-induced alterations in eating and food reward behavior. CSDS, which as mentioned above elevates circulating ghrelin, is associated with hyperphagia of freely-available regular chow both during (15) and for at least one month after the defeat period (15, 21, 22). This hyperphagia, which is not observed in mice lacking GHSRs, may contribute to the higher body weight gain observed in CSDS-exposed wild-type mice (15, 21, 22). Not only does CSDS induce a hyperphagic response in wild-type mice, but it also increases CPP for fatty/sugary food rewards (14). Such a stress-induced food reward response seems to be dependent on signaling by ghrelin, as CPP for HFD is not observed in CSDS-exposed GHSR-null mice (14). Furthermore, expression of GHSRs selectively in-tyrosine hydroxylase-containing neurons (which include midbrain dopaminergic VTA neurons) is permissive for the induction of reward-based eating behaviors by the CSDS protocol (14).

Also of note, we have shown that upon CSDS challenge, experimental mice lacking ghrelin receptors (GHSR-null mice) manifest greater social isolation (a marker of depressive-like behavior) than do wild-type littermates (15). In separate studies, we showed that increasing plasma ghrelin levels by calorie restriction or by acute injection

produces anti-depressant-like responses in the forced swim test (15). Anti-depressant effects following ghrelin administration also have been noted in human subjects with major depression (23). Another clinical trial demonstrated lower plasma ghrelin levels in depressed patients (24). Yet another human study found that a particular polymorphism in the ghrelin gene correlated with depression symptomatology (25). Thus, we have suggested that ghrelin may rise in the setting of stress in order to help minimize what would otherwise be worsened depressive-like symptoms. Seemingly as a side-effect, the stress-induced elevations would then result in the comfort-food eating as described above.



<u>Situations that increase ghrelin.</u>
Plasma ghrelin levels are dynamic and fluctuate with metabolic status, rising before a meal and declining after a meal. Furthermore, individuals who lose weight by dieting have been shown to have raised ghrelin levels at all times of the day, suggesting a role for ghrelin in the rebound weight gain often observed in dieters.

Many studies have suggested that an atypical ghrelin response contributes to Roux-en-Y (RYGB) gastric bypass-induced weight loss and improved glycemic control. A majority of human trials have shown low or unchanged ghrelin levels in RYGB subjects, with an exception of a few studies reporting ghrelin increases. This decline in circulating ghrelin may improve the body weights (and also the glycemic control) evident in obese patients who have undergone RYGB.

As mentioned above, elevations of ghrelin also are observed upon stress and in high emotional eaters.

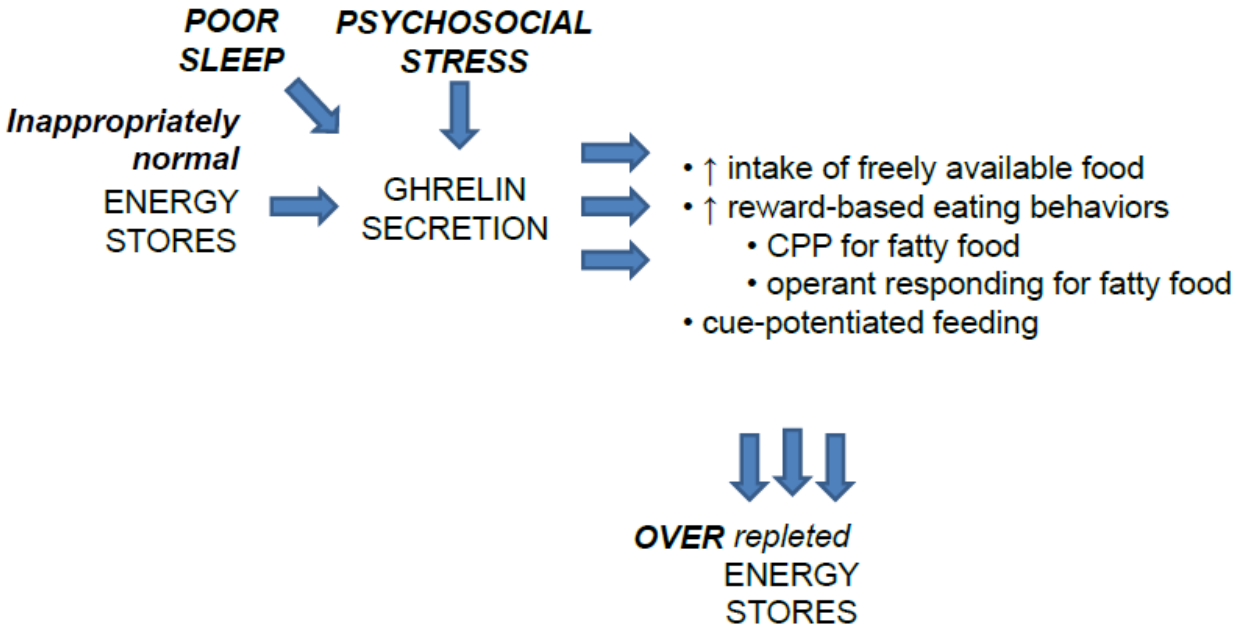
In addition, ghrelin's effects on reward pathways find particular relevance to the obesity and accompanying excessive eating of Prader-Willi Syndrome (PWS). The hyperphagia of PWS is extreme such that PWS individuals often display a significant obsession with food, pica behavior and nearly constant hunger, as well as other disadvantageous feeding behaviors such as food stealing, stealing money to buy food, hoarding, foraging and binge eating. A significant advance in PWS research came with the report of marked elevations of circulating levels of ghrelin in obese adults with PWS. This initial finding has been confirmed in many other studies on adult PWS individuals as well as in obese children and teenagers with PWS. Plasma ghrelin levels in obese PWS individuals have been found to exist at levels 3 to 4.5-fold higher than obese controls. Furthermore, ghrelin cell density is higher in the stomachs of PWS individuals as compared to obese control individuals. It has been postulated that these high ghrelin

levels directly contribute to the voracious appetite, hyperphagia, obesity and extreme food-seeking behaviors that characterize this syndrome.

In addition, in work carried out mainly at the University of Chicago, it has been demonstrated that ghrelin levels are elevated in individuals that have been subjected to sleep deprivation. It has been postulated that this raised circulating ghrelin may contribute to the obesity and other metabolic dysfunctions associated with poor sleep.

Model:

Ghrelin's effects on these many aspects of appetite seem appropriate in situations when energy stores are low and need to be repleted. However, there are several settings when elevations in ghrelin – which may be of benefit in one regard (e.g. minimizing stress-associated depressive behaviors) – lead to elevated appetite and behaviors that result in over-repletion of energy stores.



Conclusions

Studies using mainly rodent models demonstrate an important role for the peptide hormone ghrelin in many different aspects of appetite. These actions are also likely at play in humans, and include effects on eating related to body weight homoeostasis, pleasure, psychological drives, and environmental cues. The challenge ahead will be confirming the relevance of these actions to human disease. A further challenge will be in the design of therapeutic agents that take into account not only ghrelin's actions on eating for survival and eating for pleasure but also its effects on mood.

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