The Role of Ghrelin in Body Weight Regulation, Reward Behavior and Mood

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Ghrelin: an introduction

Ghrelin is a 28-residue peptide hormone synthesized predominantly by specialized endocrine cells of the stomach (1) (Figs. 1-2). 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 recently identified at UTSW by Drs. Brown and Goldstein (2). In addition to its role as a growth hormone secretagogue, ghrelin stimulates gastrointestinal motility and gastric acid secretion, affects blood pressure, and regulates blood glucose homeostasis (1, 3-10).

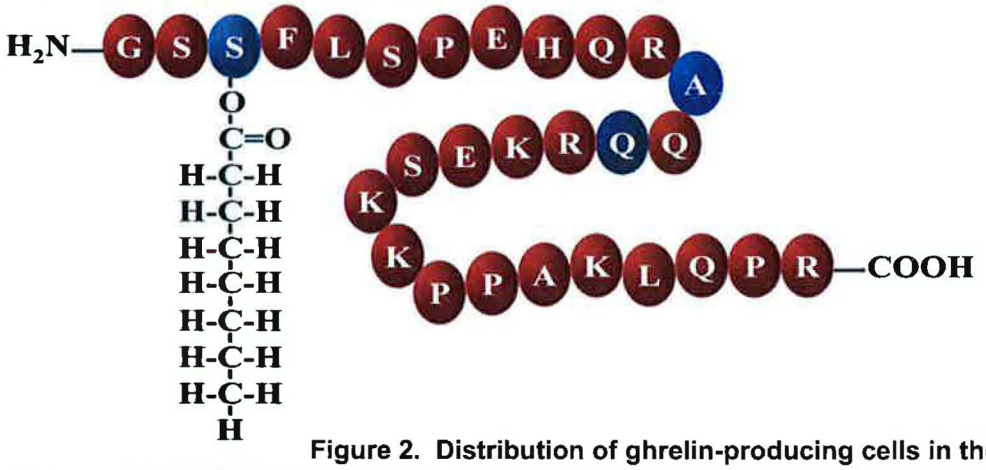
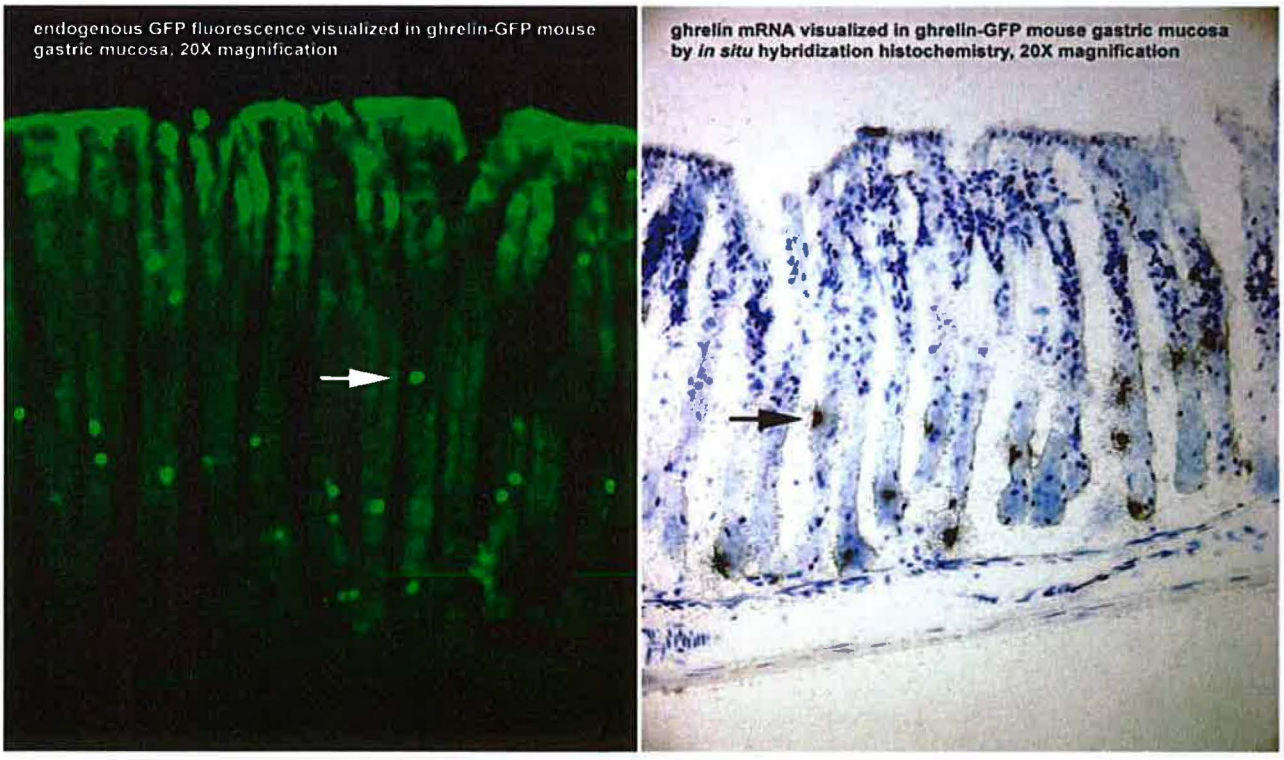


Figure 1. Depiction of amino acid structure and n-octanoyl post-translational modification of mature ghrelin

Figure 2. Distribution of ghrelin-producing cells in the gastric mucosa

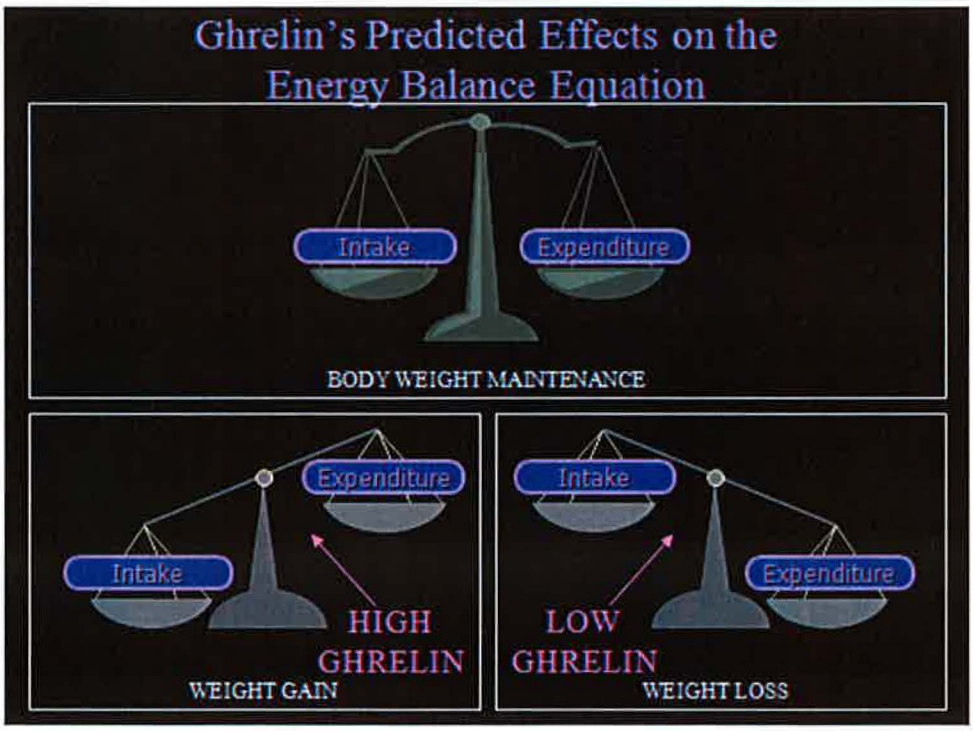


Ghrelin influences several of the mechanisms that regulate appetite and food intake.

One of ghrelin's other functions relates to its ability to stimulate food intake and appetite. It does so by participating in at least three different, interrelated mechanisms that influence appetite. These include roles in homeostatic pathways for maintaining body weight, reward pathways responsible for various hedonic, pleasurable aspects of eating, and psychological drives that influence eating, such as stress and depression.

Ghrelin and body weight homeostasis.

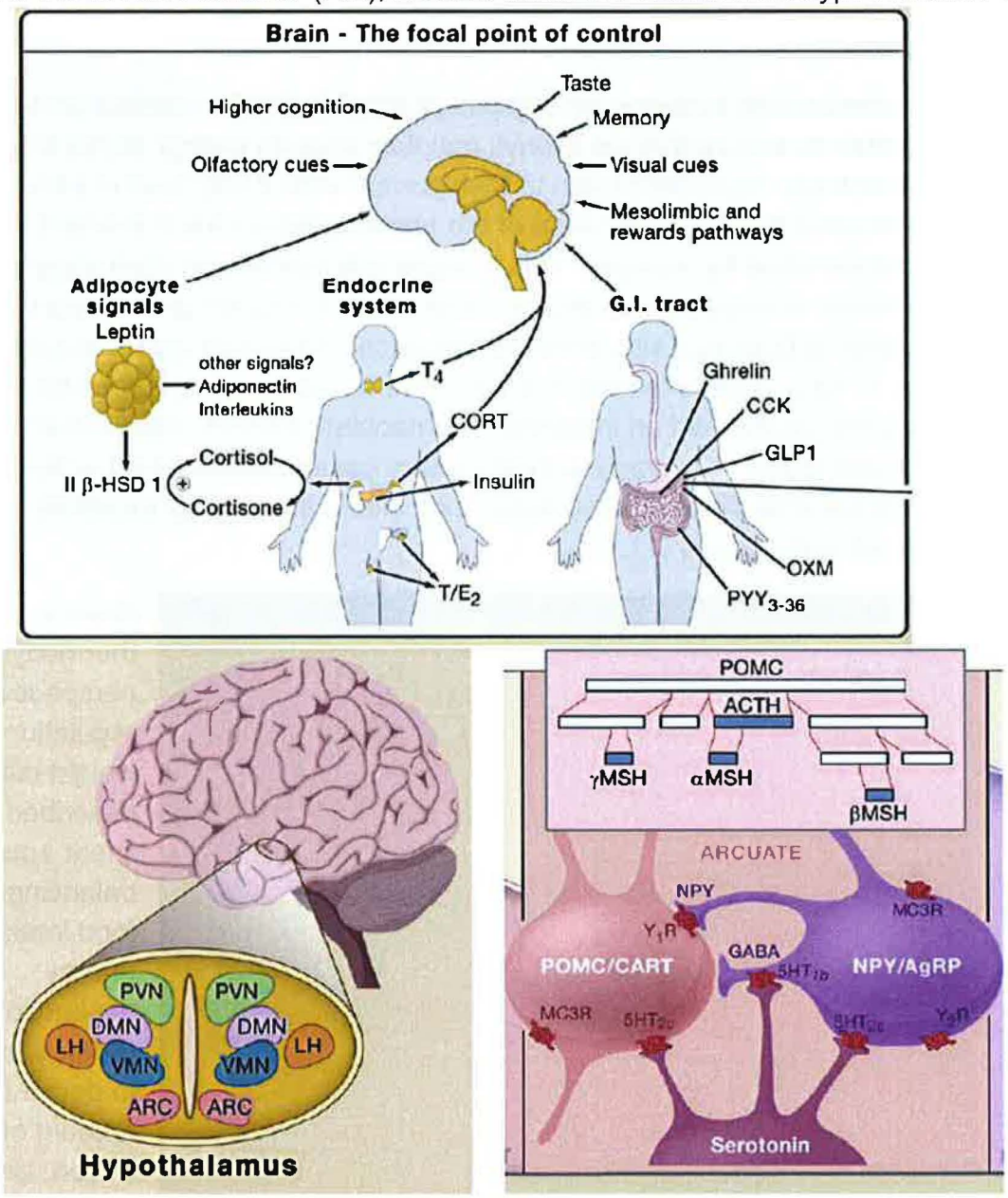
Body weight homeostatic systems are a group of physiological processes and behaviors that work together to ensure that we always maintain enough energy stores to survive. It is thought that these have developed to their current extent and level of intricacy within warm-blooded mammals because of the 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. As such, 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 (11, 12).



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

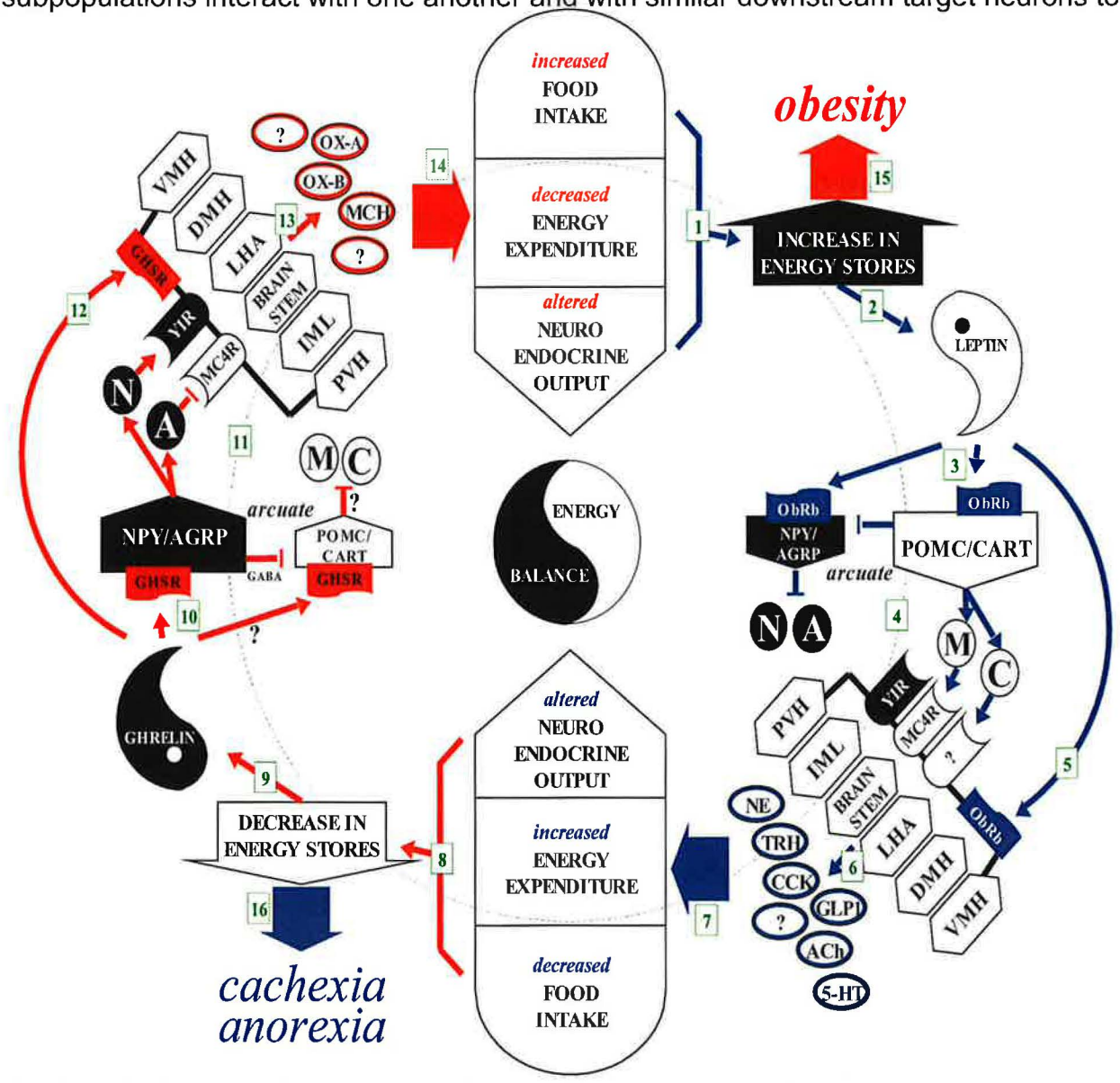
occur if there is a relative increase in energy expenditure and/or a relative decrease in food intake. On the other hand, weight gain would be expected if there is a relative increase in food intake and/or a relative decrease in energy expenditure (13).

Both ghrelin and another hormone, leptin, are examples of hormones made in the periphery that play key roles in body weight homeostasis. One of the key sites where they act is the arcuate nucleus (Arc), located within the basomedial hypothalamus of the brain.



Figures demonstrating the interaction with various peripheral signals of energy availability and gastrointestinal tract activity with different brain systems. This includes the hypothalamus, where ghrelin, leptin and other molecules interact with orexigenic NPY/AgRP neurons and anorexigenic POMC/CART neurons. (14)

Leptin, which is secreted by white adipose tissue, is established as the prototypical hormone released normally in an environment of nutritional plenty. A few years ago, we proposed a model in which in response to a relative increase in energy stores, leptin is released from fat and travels to the Arc (see Figure below) (15). Within the Arc, two distinct ghrelin-responsive cell groups exist. The first is identified by the coexpression of proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) and is often referred to as an anorexigenic population. The second distinct population of neurons is orexigenic and coexpresses the peptides neuropeptideY(NPY) and agouti-related gene product (AgRP). The receptor for leptin (Lepr, ObRb) is expressed on both POMC/CART neurons and NPY/AgRP neurons. These two Arc subpopulations interact with one another and with similar downstream target neurons to



Model for the homeostatic control of body weight by ghrelin and leptin.

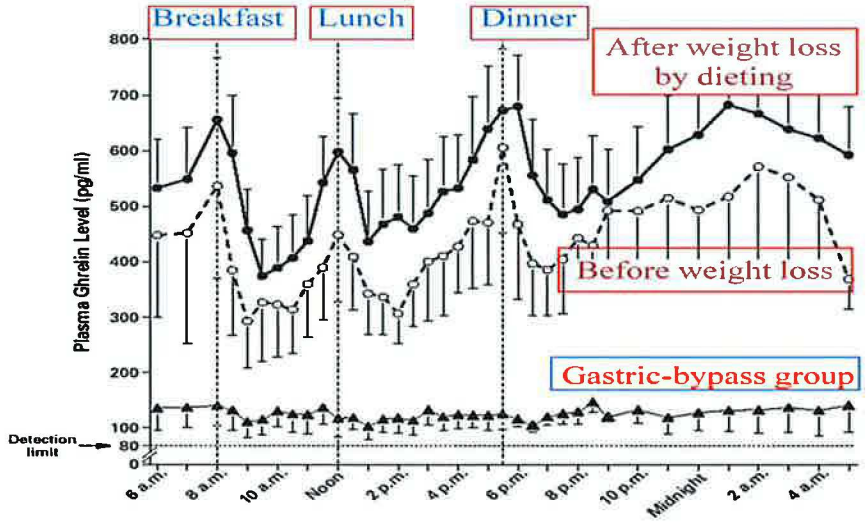
effect changes that ultimately help to regulate body weight. Investigations by a number of investigators have demonstrated that leptin directly binds to its receptors on the POMC/CART neurons to activate them. This results in the release of alpha-melanocyte-stimulating hormone (alpha-MSH), which acts via the melanocortin 4 receptor (MC4R; for which it serves as the endogenous ligand) to engage a coordinated reduction in food intake and increase in energy expenditure. Leptin also directly binds to its receptors on the NPY/AgRP neurons to inhibit them. Once inhibited, the release of the orexigenic NPY neuropeptide is blocked. Also blocked is the release of AgRP, which serves as both a competitive antagonist and an inverse agonist to the melanocortin 4 receptor (MC4R) and thus normally inhibits downstream melanocortin pathways that otherwise decrease food intake and increase energy expenditure. Finally, leptin engagement with NPY/AgRP neurons inhibits the release of the inhibitory neurotransmitter GABA onto neighboring POMC/CART neurons, thereby disinhibiting the anorexigenic POMC/CART neurons.

As a result of leptin action, there is a relative decrease in energy stores. In response, ghrelin is released from the gastrointestinal tract. Ghrelin travels to the Arc, where it activates NPY/AgRP neurons, leading to the release of the orexigenic neuropeptide NPY. Activation of NPY/AgRP neurons by ghrelin also leads to inhibition of the melanocortin pathways via AgRP, which antagonizes MC4Rs, and via GABA, which inhibits POMC/CART neurons. NPY, AgRP, and ghrelin influence the release of various neuropeptides and neurotransmitters from several downstream regulatory regions, which in turn lead to activation of various physiological processes and behaviors that increase energy stores.

Dysregulated stimulation of ghrelin-activated pathways and/or blockade of leptin-activated pathways would be predicted to cause obesity. Similarly, it would be predicted that dysregulated stimulation of leptin-activated pathways and/or blockade of ghrelin-activated pathways cause cachexia. In fact, just such examples of these kinds of dysregulation have been demonstrated in both animal models and humans. For example, ob/ob mice (which are obese, hyperphagic, and have increased adiposity) and db/db mice (which are diabetic with obesity, high insulin, and hyperglycemia) were found to contain mutations in either the leptin gene (ob) or the db gene (leptin receptor). As one might predict from the phenotypes of the ob/ob and db/db mice, interference with the functioning of leptin-engaged circuitry at a number of different places may also lead to obesity and problems with glucose metabolism in humans. Although not common, leptin deficiency does occur in humans and results in hyperphagia, severe obesity, and alterations in immune function and delayed puberty, all of which improve with leptin administration (16, 17). The human equivalent of the db/db mouse occurs much more commonly. In fact, the prevalence of pathogenic leptin receptor mutations

in a cohort of 300 subjects with severe early onset obesity was found to be 3% (18, 19). Even more common is a resistance, or rather impaired responsiveness, to the effects of leptin, which is observed in most obese humans. Human MC4R mutation carriers also exist and have severe obesity, increased lean mass, increased linear growth, hyperphagia, and severe hyperinsulinemia. Mutations in the MC4R appear to be the commonest monogenic cause of obesity thus far described in humans (20).

We now know a lot of information about ghrelin responses and action. Ghrelin levels rise prior to meals (see Figure below), 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 (21-29). Importantly, ghrelin administration potently stimulates feeding and lowers energy expenditure (10, 30, 31). In addition, ghrelin shifts food preference towards diets rich in fat and at the same time shifts fuel preference away from metabolic utilization of fat as an energy source (10, 32). Ghrelin also increases the mRNA expression of many fat storage-promoting enzymes in white adipocytes (33). Collectively, these actions of ghrelin increase body weight (with a predominant effect of increasing adiposity) in normal individuals and result in maintenance of body weight or delayed weight loss (with a predominant effect on lean mass) in cachectic individuals (10, 31, 34, 35).



Interestingly, in most forms of obesity, ghrelin is not presumed to be causative since its levels are usually lower than those in lean individuals (36, 37). Rather, in individuals with the "common" form of diet-induced obesity, ghrelin levels become elevated only after weight loss induced by dieting (Figure above). Notably, this elevation in ghrelin has been hypothesized to contribute to the rebound weight gain commonly observed in dieters (38). Also of note, the marked and prolonged weight loss observed in obese individuals who undergo Roux-en-Y gastric bypass surgery is thought to be due, at least in part, to post-bypass reductions in circulating ghrelin levels (38, 39).

Several studies now support a model that predicts a physiologically important role for naturally-occurring ghrelin in coordinated body weight control. However, such a notion

was challenged in the first published studies using ghrelin- and GHSR-knockout mice, in which no or only modest differences in body weights were noted between mice lacking ghrelin or the ghrelin receptor and wild-type animals (40-42). Counter to these earlier studies, several recent papers do support a required role of intact ghrelin signaling for normal body weight homeostasis and the development of diet-induced obesity. For example, in our own study using GHSR-null mice, we found that ghrelin receptor deficiency was associated with reduced body weight in animals exposed to high-fat diet (Fig. 6). This reduced body weight was due to selective decreases in adiposity, and was associated with both reduced feed efficiency and reduced food intake (43). In parallel, Wortley *et al.* demonstrated that ghrelin deficient (knockout) mice were leaner than wild-type mice after early exposure to high-fat diet. This was due to an effect on adiposity alone and was the result of increased energy expenditure, without any changes in food intake when studied over the short-term (44). Similarly, selective knockdown of GHSR expression in transgenic rats expressing an antisense GHSR transcript (under the control of a tyrosine hydroxylase promoter) also resulted in decreased adiposity and reduced food intake (45). Furthermore, reduction of the bioavailability of naturally occurring ghrelin by use of either a vaccination strategy or a polyethylene glycol-modified L-RNA oligonucleotide capable of specific high-affinity binding to acylated ghrelin resulted in decreased body weight gain, adiposity, food intake and feed efficiency (46, 47). These studies used different methods of inactivating normal ghrelin signaling pathways. However, they all had in common decreased body weight (with a specific effect on fat mass) and increased energy expenditure; the effects on food intake were variable. As such, inhibition of ghrelin action has been touted as a feasible strategy to reduce body weight and food intake (15, 48).

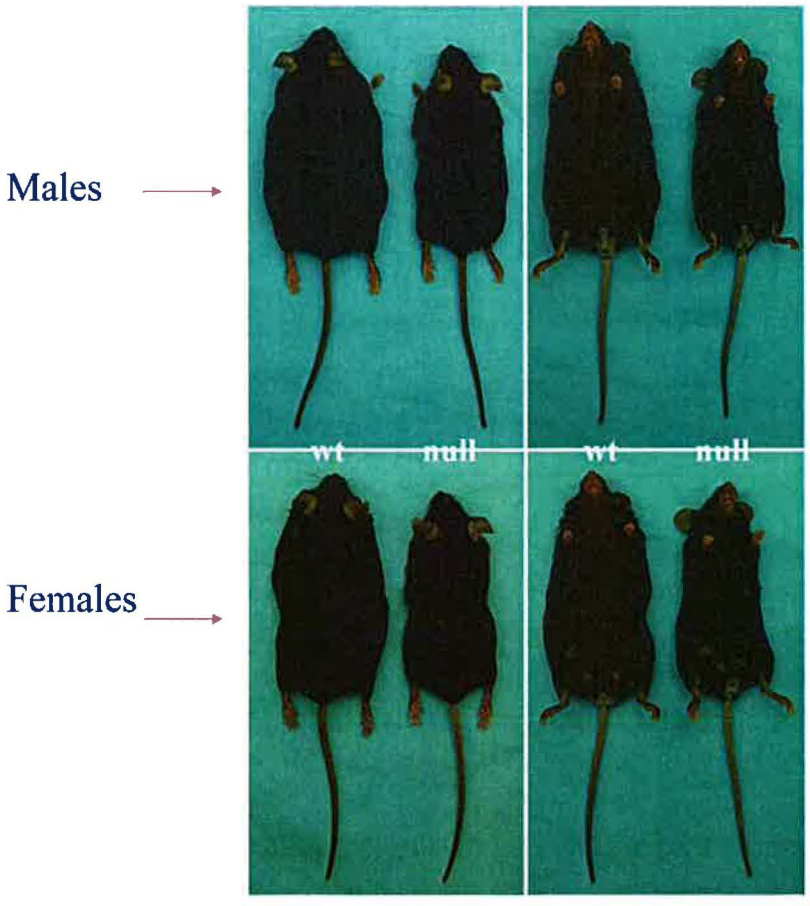


Figure 6. Mice lacking ghrelin receptors gain less body weight when chronically exposed to a high fat diet.

Ghrelin's role in reward behavior

Ghrelin's role in body weight and food intake is not limited to its effects on homeostatic pathways. Several pieces of evidence now suggest that ghrelin, along with the white adipose tissue-derived anorexigenic hormone leptin also affect food intake and body weight by interactions with various brain reward circuits such as the midbrain dopaminergic pathways originating in the ventral tegmental area (VTA).

Receptors for both ghrelin and leptin are highly expressed within both the VTA and the substantia nigra (SN), and we have also carefully documented a high degree of co-expression of GHSR specifically within dopaminergic (tyrosine hydroxylase-immunoreactive) neurons within the VTA and SN (49, 50). This is important because studies mainly from the drug addiction field have identified the nucleus accumbens (NAc) and its dopaminergic inputs from the VTA as playing a critical role in reward (51). Rewards are things that make us feel better and therefore are liked (give us sensory pleasure), wanted (desired, pursued and motivated us to work to obtain them) and initiate learning processes that help create cues that predict their availability and help organize efficient behavioral sequences aimed at obtaining them (51, 52).

Figure 6. Properties of rewarding substances.

Virtually all drugs of abuse increase dopaminergic transmission in the NAc, and this is thought to contribute to the acute rewarding effects of the drugs (53-55). This VTA-NAc pathway is not only one of the most important anatomical substrates for drug reward, but also is important for natural rewards, such as food, sex, and social interactions (53, 55). Studies with leptin have shown that its direct microinjection into the VTA decreases food intake while RNAi-mediated knockdown of leptin receptor within the VTA has the opposite effect (56). Also, leptin administration decreases conditioned place preference for food (57-60), reverses the ability of food deprivation to increase drug reward and relapse (61) and increases intracranial self stimulation thresholds (62). Together, these findings suggest a negative effect of leptin on motivation, and that leptin deficiency increases activation of those centers involved in motivation to obtain food rewards.

More recently, a handful of publications have reported similar types of interactions of ghrelin with these mesolimbic reward circuits. For instance, centrally- and peripherally-administered ghrelin induces dopamine overflow in the NAc, and ghrelin increases action potential frequency in VTA dopamine neurons (63-65). Furthermore, direct microinjection of ghrelin into the VTA increases food intake while direct VTA

microinjection of a GHSR antagonist decreases food intake in response to i.p. ghrelin (63, 66). Also, although only reported as abstracts, ghrelin has been shown to significantly increase appetitive lever pressing for food rewards by rats and mice (67, 68). Ghrelin recently has been shown to stimulate conditioned place preference in mice, thereby mimicking effects previously shown to be induced by drugs of abuse, such as cocaine (69). Importantly, functional magnetic resonance imaging trials in healthy human subjects have demonstrated that ghrelin can increase the neural response to food pictures in regions of the brain, including the amygdala, orbitofrontal cortex, anterior insula, and striatum, implicated in encoding the incentive value of food cues (70). Collectively, these studies seem to indicate that metabolic signals such as ghrelin likely induce food intake, at least in part, by enhancing the hedonic and incentive responses to food-related cues (70).

TABLE 1. Clinical diagnostic criteria for Prader-Willi syndrome

Major criteria (1 point each)
Neonatal and infantile hypotonia
Infantile feeding problems or failure to thrive
Excessive or rapid weight gain between the ages of 1 and 6 yr
Characteristic facial features, including narrow face, almond-shaped eyes, small-appearing mouth with thin upper lip, down-turned corners of the mouth (three or more required)
Hypogonadism (impaired function of the gonads) with underdeveloped genitalia and/or impaired pubertal development
Developmental delay, mental retardation, or learning problems
Hyperphagia, food foraging, or obsession with food
Deletion 15q11-q13 on high-resolution cytogenetic analysis or other abnormality of the Prader-Willi chromosome region
Minor criteria (0.5 points each)
Decreased fetal movement or infantile lethargy
Typical behavioral problems: temper tantrums, violent outbursts; obsessive/compulsive behavior, argumentative, rigid, possessive, stubborn manipulative, stealing, lying (five or more required)
Sleep disturbances or sleep apnea
Short stature for family by the age of 15 yr
Fairer eyes, skin, and hair than expected
Smaller hands and feet than expected for height and age
Narrow hands with straight ulnar border
Esotropia or myopia
Viscous saliva
Speech articulation defects
Skin picking
Supportive criteria (0 points, but help to confirm diagnosis)
High pain threshold
Reduced incidence of vomiting
Temperature control problems
Scoliosis or kyphosis
Early adrenarche
Osteoporosis
Unusual skill with jigsaw puzzles
Normal neuromuscular findings

Ghrelin's effects on reward pathways perhaps find particular relevance to the obesity and accompanying excessive eating of Prader-Willi Syndrome (PWS) (71). 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 (71, 72). A significant advance in PWS research came with the report of marked elevations of circulating levels of ghrelin in obese adults with PWS (73). This initial finding was confirmed in a handful of other studies on adult PWS individuals as well as in obese children and teenagers with PWS (73-77). Plasma ghrelin levels in obese PWS individuals have been found to exist at levels 3 to 4.5-fold higher than obese controls (73-77) (Fig. 4). Furthermore, ghrelin cell density is higher in the stomachs of PWS

individuals as compared to obese control individuals (78). 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 (73, 75).

Psychological Drives influencing food intake and body weight.

There exist many examples in clinical practice of psychiatric illness being associated with alterations of body weight and metabolic function. These include affective disorders, Post traumatic stress disorder, Schizophrenia, and Anorexia nervosa and bulimia nervosa, to name just a few. Despite many recent advances in the understanding of feeding and body weight regulation, relatively little is known about the molecular basis for the link between psychiatric illness and appetite. Some of this metabolic dysregulation is likely influenced by the medications used to treat the psychiatric illness. However, it is also likely the case that certain of the psychiatric diseases cause the metabolic dysregulation with which they are often associated, or vice versa.

Some examples in the literature of this link between psychiatric illness and extremes of body include the following: A cross sectional cross sectional epidemiological study of 9125 adults in the United States, using data from the National Comorbidity Survey Replication (in-person survey of a nationally representative sample of US residents conducted between 2/5/01 – 2/12/03), demonstrated a slightly more than 25% higher odds ratio for mood and anxiety disorders in obese individuals (79). A cross-sectional assessment of a Spanish health management database demonstrated that those individuals being treated for bipolar disorder had a significantly higher prevalence of metabolic syndrome as compared to the reference group (80). Interestingly, in a review of death rates and causes of death after weight loss surgery in Pennsylvania residents between 1995 – 2004, there was an unexpected substantial excess of deaths as a result of suicide and drug overdose (81, 82). A longitudinal study of over 1000 children in a particular town in New Zealand found that major depression in late adolescent girls was associated with a 2.3 fold increased risk of obesity in adulthood, and furthermore that prevalence of obesity in adulthood was positively correlated with the number of episodes of depression during adolescence (83). As a final example, a retrospective study of data obtained from 157 veterans with post-traumatic stress disorder in Richmond, VA demonstrated that these individuals had a combined overweight and obesity prevalence that exceeded those rates in the general population by ~ 20% (84).

A potential role for ghrelin in stress-induced eating.

Thru studies spearheaded by Dr. Michael Lutter in the Department of Psychiatry, we recently have found that rises in ghrelin occur not only in response to states of energy insufficiency but also following chronic stress (85). For example, we have found that ghrelin levels rise in response to chronic social defeat stress (CSDS), a model of depression in laboratory mice (Fig. 8). Our findings are supportive of previous work describing elevations in either gastric ghrelin mRNA or total plasma ghrelin in response to acute stress, including following a tail pinch stress protocol in ddy mice (tail pinch for 10 min every 4 hr for 24 hr) and following a water avoidance stress protocol of 60 min duration in Wistar Kyoto and Sprague-Dawley rats (86, 87).

Next, we examined the potential effects of these stress-induced ghrelin elevations (85). We observed that methods which increase circulating levels of ghrelin, including single subcutaneous injections of ghrelin or 10 days of calorie restriction, resulted in anxiolytic-like and antidepressant-like responses in the elevated plus maze (EPM) and forced swim test (FST), respectively. Conversely, genetic blockade of ghrelin signaling by deletion of ghrelin receptors, as occurs in our GHSR-null mice, negated these anxiolytic-like and antidepressant-like effects when assessed in the EPM and FST.

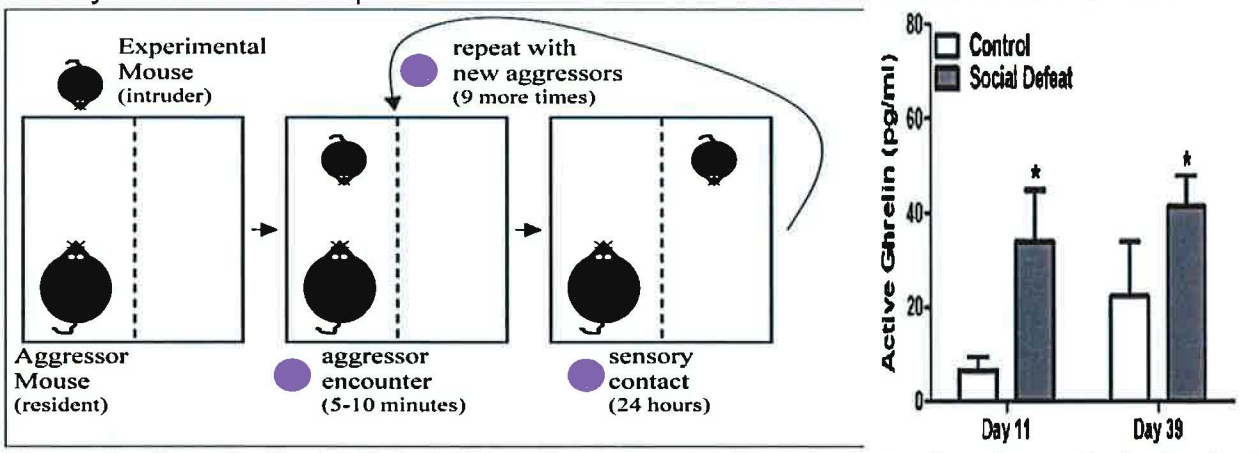


Figure 8. Chronic Social Defeat Stress induces elevations in the active form of ghrelin that persists for at least one month.

Importantly, the majority of wild-type animals that undergo the chronic social defeat stress protocol normally exhibit social isolation behaviors that correlate with increased depressive-like behavior (88, 89). However, we found that deletion of the ghrelin receptor seemed to exacerbate this depressive-like behavior. Furthermore, while those wild-type mice undergoing the social defeat protocol were shown to have hyperphagia, deletion of the ghrelin receptor resulted in blunted food intake following repeated social defeat episodes (85).

Thus, it appears from our research in laboratory mice that methods that increase circulating levels of acylated ghrelin, including subcutaneous injections or caloric

restriction, produce anxiety-lowering and depression-lowering responses. Chronic stress also causes an elevation in ghrelin and furthermore results in increased eating (at least briefly). Animals unable to respond to the elevation in ghrelin (because they lack the receptor for ghrelin) demonstrate even more depressive-like behaviors in response to chronic stress than do wild-type animals, and also do not show hyperphagia. As such, we hypothesize that increases in circulating ghrelin, which occur in response to stress help us cope by generating anxiety-lowering and depression-lowering behavioral adaptations. A side-effect of the stress-induced increased ghrelin is increased food intake/appetite.

The potential significance of these results can perhaps best be illustrated by viewing them in the context of post traumatic stress disorder (see above) or the eating disorders anorexia nervosa and bulimia nervosa. Anorexia Nervosa is an eating disorder characterized by a profound disturbance of body image accompanied by behaviors to maintain body weight below the 85% percentile, including restricted food intake, self-

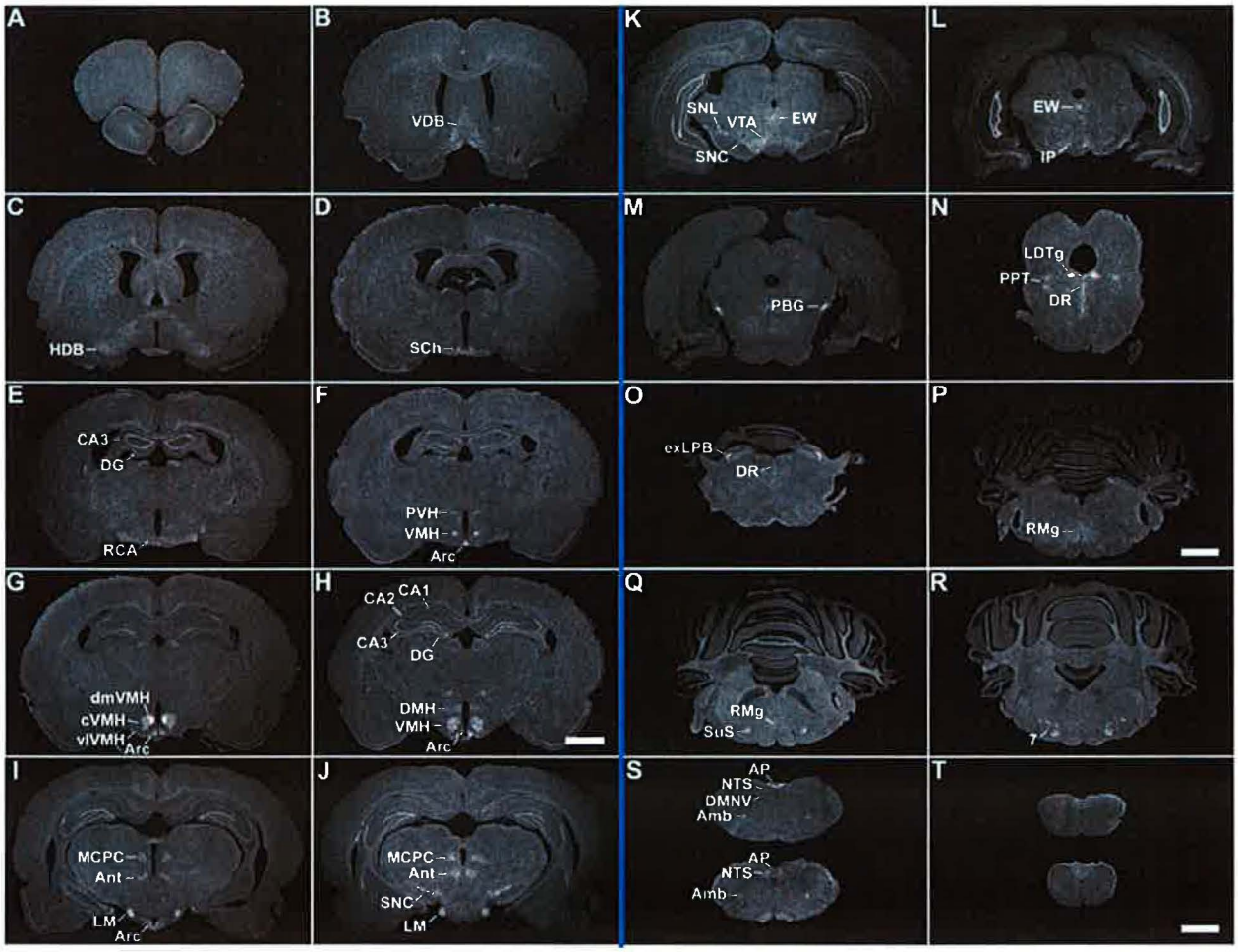


Figure. Distribution of mRNA encoding ghrelin receptor (GHSR) within the rat brain.

induced vomiting, and excessive exercise (90). Bulimia Nervosa is a related eating disorder in which individuals engage in recurrent episodes of excessive calorie intake followed by compensatory purging behaviors such as self-induced vomiting and laxative abuse (90). Both disorders have high rates of co-morbid depressive and anxiety disorders. Major depressive disorder or dysthymic disorder has been reported in up to 50% of anorexia nervosa individuals and similar rates of depression are found in bulimia nervosa (90). In a large clinical sample of 575 patients with anorexia nervosa or bulimia nervosa, almost two-thirds of patients had a lifetime axis one diagnosis of an anxiety disorder (91). It is likely that these co-morbid psychiatric disorders contribute greatly to the progression of both of these illnesses and the frequent relapses that occur during treatment. Importantly, anorexia nervosa and bulimia nervosa are both associated with high levels ghrelin (24, 28, 29). It has been assumed that the primary reason for the elevated ghrelin in anorexia nervosa and bulimia nervosa is as a response to a cachectic state or the habitual binge/purge behaviors (29, 92). However, given our new findings, we hypothesize that the elevated ghrelin levels found in individuals with these eating disorders may rise as a coping strategy for the depression and anxiety that are usually present. Whatever the cause of the ghrelin elevations in anorexia nervosa and bulimia nervosa, our new findings raise the possibility that ghrelin may provide a link between the disordered eating behaviors and some aspects of the psychopathology associated with these conditions.

Conclusions

Ghrelin's actions on body weight involve engagement of various pathways involved in the determination of appetite and food intake, including homeostatic pathways, reward circuitry and psychological drives. This is likely also the case for many other gut hormones and peripheral satiety signals. The challenge ahead will be determining the relevance of these pathways to human disease. A further challenge will be in the design of therapeutic agents that take into account not only ghrelin's actions on homeostatic food intake and food reward seeking behaviors but also its effects on mood and anxiety.

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References

1. **Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K** 1999 Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402:656-660
2. **Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL** 2008 Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. Cell 132:387-396
3. **Ariyasu H, Takaya K, Tagami T, Ogawa Y, Hosoda K, Akamizu T, Suda M, Koh T, Natsui K, Toyooka S, Shirakami G, Usui T, Shimatsu A, Doi K, Hosoda H, Kojima M, Kangawa K, Nakao K** 2001 Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. J Clin Endocrinol Metab 86:4753-4758
4. **Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M** 2000 Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. Endocrinology 141:4255-4261
5. **Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsukura S** 2002 Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. Diabetes 51:124-129
6. **Dezaki K, Hosoda H, Kakei M, Hashiguchi S, Watanabe M, Kangawa K, Yada T** 2004 Endogenous ghrelin in pancreatic islets restricts insulin release by attenuating Ca2+ signaling in beta-cells: implication in the glycemic control in rodents. Diabetes 53:3142-3151
7. **Hosoda H, Kojima M, Matsuo H, Kangawa K** 2000 Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue. Biochem Biophys Res Commun 279:909-913
8. **Lee HM, Wang G, Englander EW, Kojima M, Greeley GH, Jr.** 2002 Ghrelin, a new gastrointestinal endocrine peptide that stimulates insulin secretion: enteric distribution, ontogeny, influence of endocrine, and dietary manipulations. Endocrinology 143:185-190
9. **Prado CL, Pugh-Bernard AE, Elghazi L, Sosa-Pineda B, Sussel L** 2004 Ghrelin cells replace insulin-producing beta cells in two mouse models of pancreas development. Proc Natl Acad Sci U S A 101:2924-2929
10. **Tschop M, Smiley DL, Heiman ML** 2000 Ghrelin induces adiposity in rodents. Nature 407:908-913
11. **Saper CB, Chou TC, Elmquist JK** 2002 The need to feed: homeostatic and hedonic control of eating. Neuron 36:199-211
12. **Palmiter RD** 2007 Is dopamine a physiologically relevant mediator of feeding behavior? Trends Neurosci 30:375-381

13. **Nogueiras R, Tschop MH, Zigman JM** 2008 Central nervous system regulation of energy metabolism: ghrelin versus leptin. Ann N Y Acad Sci 1126:14-19
14. **Coll AP, Yeo GS, Farooqi IS, O'Rahilly S** 2008 SnapShot: the hormonal control of food intake. Cell 135:572 e571-572
15. **Zigman JM, Elmquist JK** 2003 Minireview: From anorexia to obesity--the yin and yang of body weight control. Endocrinology 144:3749-3756
16. **Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, Sewter CP, Digby JE, Mohammed SN, Hurst JA, Cheetham CH, Earley AR, Barnett AH, Prins JB, O'Rahilly S** 1997 Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 387:903-908
17. **Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S** 1999 Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med 341:879-884
18. **Farooqi IS, Bullmore E, Keogh J, Gillard J, O'Rahilly S, Fletcher PC** 2007 Leptin regulates striatal regions and human eating behavior. Science 317:1355
19. **Clement K, Vaisse C, Lahlou N, Cabrol S, Pelloux V, Cassuto D, Gourmelen M, Dina C, Chambaz J, Lacorte JM, Basdevant A, Bougneres P, Lebouc Y, Froguel P, Guy-Grand B** 1998 A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 392:398-401
20. **Farooqi IS, Keogh JM, Yeo GS, Lank EJ, Cheetham T, O'Rahilly S** 2003 Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. N Engl J Med 348:1085-1095
21. **Cummings DE, Foster KE** 2003 Ghrelin-leptin tango in body-weight regulation. Gastroenterology 124:1532-1535
22. **Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS** 2001 A preprandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. Diabetes 50:1714-1719
23. **Nagaya N, Uematsu M, Kojima M, Date Y, Nakazato M, Okumura H, Hosoda H, Shimizu W, Yamagishi M, Oya H, Koh H, Yutani C, Kangawa K** 2001 Elevated circulating level of ghrelin in cachexia associated with chronic heart failure: relationships between ghrelin and anabolic/catabolic factors. Circulation 104:2034-2038
24. **Otto B, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, Heiman ML, Lehnert P, Fichter M, Tschop M** 2001 Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. Eur J Endocrinol 145:669-673
25. **Tolle V, Kadem M, Bluet-Pajot MT, Frere D, Foulon C, Bossu C, Dardennes R, Mounier C, Zizzari P, Lang F, Epelbaum J, Estour B** 2003 Balance in ghrelin and leptin plasma levels in anorexia nervosa patients and constitutionally thin women. J Clin Endocrinol Metab 88:109-116
26. **Wisse BE, Frayo RS, Schwartz MW, Cummings DE** 2001 Reversal of cancer anorexia by blockade of central melanocortin receptors in rats. Endocrinology 142:3292-3301
27. **Kojima S, Nakahara T, Nagai N, Muranaga T, Tanaka M, Yasuhara D, Masuda A, Date Y, Ueno H, Nakazato M, Naruo T** 2005 Altered ghrelin and peptide YY responses to meals in bulimia nervosa. Clin Endocrinol (Oxf) 62:74-78

28. **Misra M, Miller KK, Kuo K, Griffin K, Stewart V, Hunter E, Herzog DB, Klibanski A** 2005 Secretory dynamics of ghrelin in adolescent girls with anorexia nervosa and healthy adolescents. Am J Physiol Endocrinol Metab 289:E347-356
29. **Tanaka M, Naruo T, Muranaga T, Yasuhara D, Shiiya T, Nakazato M, Matsukura S, Nozoe S** 2002 Increased fasting plasma ghrelin levels in patients with bulimia nervosa. Eur J Endocrinol 146:R1-3
30. **Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Ueno N, Makino S, Fujimiya M, Niijima A, Fujino MA, Kasuga M** 2001 Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. Gastroenterology 120:337-345
31. **Wren AM, Small CJ, Abbott CR, Dhillo WS, Seal LJ, Cohen MA, Batterham RL, Taheri S, Stanley SA, Ghatei MA, Bloom SR** 2001 Ghrelin causes hyperphagia and obesity in rats. Diabetes 50:2540-2547
32. **Shimbara T, Mondal MS, Kawagoe T, Toshinai K, Koda S, Yamaguchi H, Date Y, Nakazato M** 2004 Central administration of ghrelin preferentially enhances fat ingestion. Neurosci Lett 369:75-79
33. **Theander-Carrillo C, Wiedmer P, Cettour-Rose P, Nogueiras R, Perez-Tilve D, Pfluger P, Castaneda TR, Muzzin P, Schurmann A, Szanto I, Tschop MH, Rohner-Jeanrenaud F** 2006 Ghrelin action in the brain controls adipocyte metabolism. J Clin Invest 116:1983-1993
34. **Deboer MD, Zhu X, Levasseur PR, Inui A, Hu Z, Han G, Mitch WE, Taylor JE, Halem HA, Dong JZ, Datta R, Culler MD, Marks DL** 2007 Ghrelin Treatment of Chronic Kidney Disease: Improvements in Lean Body Mass and Cytokine Profile. Endocrinology
35. **DeBoer MD, Zhu XX, Levasseur P, Meguid MM, Suzuki S, Inui A, Taylor JE, Halem HA, Dong JZ, Datta R, Culler MD, Marks DL** 2007 Ghrelin treatment causes increased food intake and retention of lean body mass in a rat model of cancer cachexia. Endocrinology 148:3004-3012
36. **Ariyasu H, Takaya K, Hosoda H, Iwakura H, Ebihara K, Mori K, Ogawa Y, Hosoda K, Akamizu T, Kojima M, Kangawa K, Nakao K** 2002 Delayed short-term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. Endocrinology 143:3341-3350
37. **Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML** 2001 Circulating ghrelin levels are decreased in human obesity. Diabetes 50:707-709
38. **Cummings DE, Weigle DS, Frayo RS, Breen PA, Ma MK, Dellinger EP, Purnell JQ** 2002 Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. N Engl J Med 346:1623-1630
39. **Cummings DE, Shannon MH** 2003 Ghrelin and gastric bypass: is there a hormonal contribution to surgical weight loss? J Clin Endocrinol Metab 88:2999-3002
40. **Sun Y, Ahmed S, Smith RG** 2003 Deletion of ghrelin impairs neither growth nor appetite. Mol Cell Biol 23:7973-7981
41. **Sun Y, Wang P, Zheng H, Smith RG** 2004 Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. Proc Natl Acad Sci U S A 101:4679-4684

42. **Wortley KE, Anderson KD, Garcia K, Murray JD, Malinova L, Liu R, Moncrieffe M, Thabet K, Cox HJ, Yancopoulos GD, Wiegand SJ, Sleeman MW** 2004 Genetic deletion of ghrelin does not decrease food intake but influences metabolic fuel preference. Proc Natl Acad Sci U S A 101:8227-8232
43. **Zigman JM, Nakano Y, Coppari R, Balthasar N, Marcus JN, Lee CE, Jones JE, Deysher AE, Waxman AR, White RD, Williams TD, Lachey JL, Seeley RJ, Lowell BB, Elmquist JK** 2005 Mice lacking ghrelin receptors resist the development of diet-induced obesity. J Clin Invest 115:3564-3572
44. **Wortley KE, del Rincon JP, Murray JD, Garcia K, Iida K, Thorner MO, Sleeman MW** 2005 Absence of ghrelin protects against early-onset obesity. J Clin Invest 115:3573-3578
45. **Shuto Y, Shibasaki T, Otagiri A, Kuriyama H, Ohata H, Tamura H, Kamegai J, Sugihara H, Oikawa S, Wakabayashi I** 2002 Hypothalamic growth hormone secretagogue receptor regulates growth hormone secretion, feeding, and adiposity. J Clin Invest 109:1429-1436
46. **Shearman LP, Wang SP, Helmling S, Stribling DS, Mazur P, Ge L, Wang L, Klussmann S, Macintyre DE, Howard AD, Strack AM** 2006 Ghrelin neutralization by a ribonucleic acid-SPM ameliorates obesity in diet-induced obese mice. Endocrinology 147:1517-1526
47. **Zorrilla EP, Iwasaki S, Moss JA, Chang J, Otsuji J, Inoue K, Meijler MM, Janda KD** 2006 Vaccination against weight gain. Proc Natl Acad Sci U S A 103:13226-13231
48. **Zigman JM, Elmquist JK** 2006 In search of an effective obesity treatment: a shot in the dark or a shot in the arm? Proc Natl Acad Sci U S A 103:12961-12962
49. **Zigman JM, Jones JE, Lee CE, Saper CB, Elmquist JK** 2006 Expression of ghrelin receptor mRNA in the rat and the mouse brain. J Comp Neurol 494:528-548
50. **Figlewicz DP, Evans SB, Murphy J, Hoen M, Baskin DG** 2003 Expression of receptors for insulin and leptin in the ventral tegmental area/substantia nigra (VTA/SN) of the rat. Brain Res 964:107-115
51. **Hyman SE, Malenka RC, Nestler EJ** 2006 Neural mechanisms of addiction: the role of reward-related learning and memory. Annual review of neuroscience 29:565-598
52. **Robinson TE, Berridge KC** 1993 The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev 18:247-291
53. **Wise RA** 1998 Drug-activation of brain reward pathways. Drug Alcohol Depend 51:13-22
54. **Di Chiara G, Tanda G, Bassareo V, Pontieri F, Acquas E, Fenu S, Cadoni C, Carboni E** 1999 Drug addiction as a disorder of associative learning. Role of nucleus accumbens shell/extended amygdala dopamine. Ann N Y Acad Sci 877:461-485
55. **Koob GF, Le Moal M** 2001 Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology 24:97-129
56. **Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, Thurmon JJ, Marinelli M, DiLeone RJ** 2006 Leptin receptor signaling in midbrain dopamine neurons regulates feeding. Neuron 51:801-810

57. **Figlewicz DP, Bennett JL, Naleid AM, Davis C, Grimm JW** 2006 Intraventricular insulin and leptin decrease sucrose self-administration in rats. Physiol Behav 89:611-616
58. **Figlewicz DP, Naleid AM, Sipols AJ** 2006 Modulation of food reward by adiposity signals. Physiol Behav
59. **Figlewicz DP, Bennett J, Evans SB, Kaiyala K, Sipols AJ, Benoit SC** 2004 Intraventricular insulin and leptin reverse place preference conditioned with high-fat diet in rats. Behav Neurosci 118:479-487
60. **Figlewicz DP** 2003 Adiposity signals and food reward: expanding the CNS roles of insulin and leptin. Am J Physiol Regul Integr Comp Physiol 284:R882-892
61. **Shalev U, Yap J, Shaham Y** 2001 Leptin attenuates acute food deprivation-induced relapse to heroin seeking. J Neurosci 21:RC129
62. **Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, Maratos-Flier E, Flier JS** 2006 Leptin regulation of the mesoaccumbens dopamine pathway. Neuron 51:811-822
63. **Abizaid A, Liu ZW, Andrews ZB, Shanabrough M, Borok E, Elsworth JD, Roth RH, Sleeman MW, Picciotto MR, Tschop MH, Gao XB, Horvath TL** 2006 Ghrelin modulates the activity and synaptic input organization of midbrain dopamine neurons while promoting appetite. J Clin Invest
64. **Jerlhag E, Egecioglu E, Dickson SL, Douhan A, Svensson L, Engel JA** 2007 Ghrelin administration into tegmental areas stimulates locomotor activity and increases extracellular concentration of dopamine in the nucleus accumbens. Addict Biol 12:6-16
65. **Jerlhag E, Egecioglu E, Dickson SL, Andersson M, Svensson L, Engel JA** 2006 Ghrelin stimulates locomotor activity and accumbal dopamine-overflow via central cholinergic systems in mice: implications for its involvement in brain reward. Addict Biol 11:45-54
66. **Naleid AM, Grace MK, Cummings DE, Levine AS** 2005 Ghrelin induces feeding in the mesolimbic reward pathway between the ventral tegmental area and the nucleus accumbens. Peptides 26:2274-2279
67. **Jewett DC, Lefever TW, Cameron CR, Flashinski DP, Koffarnus MN, Beaudry AR, Masters KM, Grace MK, Levine AS** 2003 Ghrelin increases break point under a progressive ratio 1 reinforcement schedule in rats. Program No. 283.15. 2003 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2003. Online.
68. **Azzara AV, Schuss B, Hong S, Schwartz GJ** 2005 Peripheral ghrelin administration increases food intake, meal size, and progressive ratio responding for food. Society for the Study of Ingestive Behavior: Annual Meeting Sheraton Station Square Hotel, Pittsburgh, PA, USA, 2005
69. **Jerlhag E** 2008 Systemic administration of ghrelin induces conditioned place preference and stimulates accumbal dopamine. Addict Biol 13:358-363
70. **Malik S, McGlone F, Bedrossian D, Dagher A** 2008 Ghrelin modulates brain activity in areas that control appetitive behavior. Cell Metab 7:400-409
71. **Goldstone AP** 2004 Prader-Willi syndrome: advances in genetics, pathophysiology and treatment. Trends in endocrinology and metabolism: TEM 15:12-20

72. **Holm VA, Cassidy SB, Butler MG, Hanchett JM, Greenswag LR, Whitman BY, Greenberg F** 1993 Prader-Willi syndrome: consensus diagnostic criteria. Pediatrics 91:398-402
73. **Cummings DE, Clement K, Purnell JQ, Vaisse C, Foster KE, Frayo RS, Schwartz MW, Basdevant A, Weigle DS** 2002 Elevated plasma ghrelin levels in Prader Willi syndrome. Nature medicine 8:643-644
74. **Goldstone AP, Patterson M, Kalingag N, Ghatei MA, Brynes AE, Bloom SR, Grossman AB, Korbonits M** 2005 Fasting and postprandial hyperghrelinemia in Prader-Willi syndrome is partially explained by hypoinsulinemia, and is not due to peptide YY3-36 deficiency or seen in hypothalamic obesity due to craniopharyngioma. J Clin Endocrinol Metab 90:2681-2690
75. **Haqq AM, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, LaFranchi SH, Purnell JQ** 2003 Serum ghrelin levels are inversely correlated with body mass index, age, and insulin concentrations in normal children and are markedly increased in Prader-Willi syndrome. J Clin Endocrinol Metab 88:174-178
76. **Paik KH, Jin DK, Song SY, Lee JE, Ko SH, Song SM, Kim JS, Oh YJ, Kim SW, Lee SH, Kim SH, Kwon EK, Choe YH** 2004 Correlation between fasting plasma ghrelin levels and age, body mass index (BMI), BMI percentiles, and 24-hour plasma ghrelin profiles in Prader-Willi syndrome. J Clin Endocrinol Metab 89:3885-3889
77. **Tauber M, Conte Auriol F, Moulin P, Molinas C, Delagnes V, Salles JP** 2004 Hyperghrelinemia is a common feature of Prader-Willi syndrome and pituitary stalk interruption: a pathophysiological hypothesis. Hormone research 62:49-54
78. **Choe YH, Song SY, Paik KH, Oh YJ, Chu SH, Yeo SH, Kwon EK, Kim EM, Rha MY, Jin DK** 2005 Increased density of ghrelin-expressing cells in the gastric fundus and body in Prader-Willi syndrome. J Clin Endocrinol Metab 90:5441-5445
79. **Simon GE, Von Korff M, Saunders K, Miglioretti DL, Crane PK, van Belle G, Kessler RC** 2006 Association between obesity and psychiatric disorders in the US adult population. Arch Gen Psychiatry 63:824-830
80. **Sicras A, Rejas J, Navarro R, Serrat J, Blanca M** 2008 Metabolic syndrome in bipolar disorder: a cross-sectional assessment of a Health Management Organization database. Bipolar Disord 10:607-616
81. **Omalu BI, Ives DG, Buhari AM, Lindner JL, Schauer PR, Wecht CH, Kuller LH** 2007 Death rates and causes of death after bariatric surgery for Pennsylvania residents, 1995 to 2004. Arch Surg 142:923-928; discussion 929
82. **Adams TD, Gress RE, Smith SC, Halverson RC, Simper SC, Rosamond WD, Lamonte MJ, Stroup AM, Hunt SC** 2007 Long-term mortality after gastric bypass surgery. N Engl J Med 357:753-761
83. **Richardson LP, Davis R, Poulton R, McCauley E, Moffitt TE, Caspi A, Connell F** 2003 A longitudinal evaluation of adolescent depression and adult obesity. Arch Pediatr Adolesc Med 157:739-745
84. **Vieweg WV, Julius DA, Benesek J, Satterwhite L, Fernandez A, Feuer SJ, Pandurangi AK** 2006 Posttraumatic stress disorder and body mass index in military veterans. Preliminary findings. Prog Neuropsychopharmacol Biol Psychiatry 30:1150-1154

85. **Lutter M, Sakata I, Osborne-Lawrence S, Rovinsky SA, Anderson JG, Jung S, Birnbaum S, Yanagisawa M, Elmquist JK, Nestler EJ, Zigman JM** 2008 The orexigenic hormone ghrelin defends against depressive symptoms of chronic stress. Nat Neurosci 11:752-753
86. **Asakawa A, Inui A, Kaga T, Yuzuriha H, Nagata T, Fujimiya M, Katsuura G, Makino S, Fujino MA, Kasuga M** 2001 A role of ghrelin in neuroendocrine and behavioral responses to stress in mice. Neuroendocrinology 74:143-147
87. **Kristenssson E, Sundqvist M, Astin M, Kjerling M, Mattsson H, Dornonville de la Cour C, Hakanson R, Lindstrom E** 2006 Acute psychological stress raises plasma ghrelin in the rat. Regul Pept 134:114-117
88. **Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, Laplant Q, Graham A, Lutter M, Lagace DC, Ghose S, Reister R, Tannous P, Green TA, Neve RL, Chakravarty S, Kumar A, Eisch AJ, Self DW, Lee FS, Tamminga CA, Cooper DC, Gershenfeld HK, Nestler EJ** 2007 Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131:391-404
89. **Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self DW, Nestler EJ** 2006 Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311:864-868
90. **Kaplan HI, Sadock BJ, Grebb JA** 1994 Kaplan and Sadock's Synopsis of Psychiatry. Seventh Edition ed. Baltimore: Williams & Wilkins
91. **Kaye WH, Bulik CM, Thornton L, Barbarich N, Masters K** 2004 Comorbidity of anxiety disorders with anorexia and bulimia nervosa. Am J Psychiatry 161:2215-2221
92. **Horvath TL, Diano S, Sotonyi P, Heiman M, Tschop M** 2001 Minireview: ghrelin and the regulation of energy balance--a hypothalamic perspective. Endocrinology 142:4163-4169