Appetite Regulatory Peptides

The role of leptin and ghrelin in the regulation of food intake and body weight in humans: a review

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Summary

Leptin and ghrelin are two hormones that have been recognized to have a major influence on energy balance. Leptin is a mediator of long-term regulation of energy balance, suppressing food intake and thereby inducing weight loss. Ghrelin on the other hand is a fast-acting hormone, seemingly playing a role in meal initiation. As a growing number of people suffer from obesity, understanding the mechanisms by which various hormones and neurotransmitters have influence on energy balance has been a subject of intensive research. In obese subjects the circulating level of the anorexigenic hormone leptin is increased, whereas surprisingly, the level of the orexigenic hormone ghrelin is decreased. It is now established that obese patients are leptin-resistant. However, the manner in which both the leptin and ghrelin systems contribute to the development or maintenance of obesity is as yet not clear. The purpose of this review is to provide background information on the leptin and ghrelin hormones, their role in food intake and body weight in humans, and their mechanism of action. Possible abnormalities in the leptin and ghrelin systems that may contribute to the development of obesity will be mentioned. In addition, the potentials of leptin and ghrelin as drug targets will be discussed. Finally, the influence of the diet on leptin and ghrelin secretion and functioning will be described.

Keywords: Ghrelin, humans, leptin, obesity.

Introduction

In most humans, body weight is maintained in a stable condition. Humans can have the same body weight for many years. To have a constant weight, there must be an energy balance; energy intake has to be equal to energy expenditure. However, when the energy balance gets disturbed, this may eventually lead to sustained weight problems like, for example, in obese subjects. A growing number of people, including children, suffer from obesity, particularly in the Western society. In the United States, the prevalence of obesity is very high. In 1999–2002, 65.1% of the adults were overweight, of whom 30.4% were obese (1). In 2002, the prevalence of obesity in Europe ranged from 9% in Italy to 30% in Greece (2). Morbidity and mortality increase gradually with excess of body mass index (BMI) (3). Therefore, many investigators try to identify the mechanisms behind the imbalance between energy intake and energy expenditure.

Body weight is regulated by a complex system, including both peripheral and central factors. Two of the hormones that seem to play an important role in the regulation of food intake and body weight are leptin and ghrelin. Both originate in the periphery and signal through different pathways to the brain, particularly to the hypothalamus (4–6). In the hypothalamus, activation of the leptin or ghrelin receptor initiates different signalling cascades leading to changes in food intake (6,7). As both the leptin and ghrelin systems are disturbed in obesity, it is important to reveal their mechanism of action

for the purpose of developing novel therapeutic interventions.

Leptin is a hormone produced mainly by adipose tissue

In 1994, the human obese (*OB*) gene and its product leptin were identified and characterized by Zhang *et al.* (8). The *OB* gene is located on chromosome 7 (7q31.3) and is composed of three exons and two introns spanning 18 kb (9,10). It encodes a protein consisting of 166 amino acids with a putative signal sequence (11). Only one *OB* mRNA species has been found in abundance in human adipose tissues (11). In addition to adipose tissue, leptin is also produced in small amounts in other human tissues such as the stomach, mammary epithelium, placenta and heart (12–16).

Leptin acts through the leptin receptor (*LEPR* or *OBR*). The *OBR* gene is located on chromosome 1 (1p31), is constituted of 18 exons and 17 introns, and encodes a protein consisting of 1162 amino acids (17,18). One of the splice variants of the *OBR* gene, the one with the longest intracellular domain (*OB-Rb*) and full signalling capabilities, is widely expressed in the human brain (19–21). *OB-Rb* is highly expressed in the hypothalamus and cerebellum (20,22). In addition, the leptin receptor is expressed in other tissues, such as the human vasculature, stomach and placenta (15,23,24).

Importantly, leptin is released into the circulatory system by the adipose tissue as a function of the energy stores (4,25). In 1996, Schwartz *et al.* showed that serum and plasma leptin levels are higher in subjects with a higher BMI and a higher per cent total body fat (26). In addition, it was demonstrated that plasma leptin can cross the blood-brain barrier (BBB), and cerebral spinal fluid (CSF) leptin levels also turned out to be correlated with BMI. After release by the adipose tissue, leptin signals to the brain, giving information about the status of the body energy stores. In rodents and in humans, this results in a decrease in food intake and an increase in energy expenditure to maintain the size of the body fat stores (27–32).

Table 1 gives an overview of several factors that have a regulatory influence on the circulating leptin levels. For example, the expression of leptin by adipose tissue is also influenced by feeding behaviour (25,33–36). Short-term (12 h) or long-term (2 or 8 weeks) overfeeding results in an increase in adipocyte leptin expression and circulating leptin in healthy human subjects (33,36). Furthermore, circulating leptin levels show a diurnal pattern and are influenced by gender, age, exercise and glucose uptake (37–43).

Leptin's role in energy balance is mediated through the hypothalamus

Leptin has been reported to have influence on various biological mechanisms, including reproduction (initiation of human puberty), the immune and inflammatory response, haematopoiesis, angiogenesis, bone formation, and wound healing (44–47). Most interestingly, leptin functions as a feedback mechanism that signals to key regulatory centres in the brain to inhibit food intake and to regulate body weight and energy homeostasis. This has been demonstrated by many studies in rodents (27,28).

Studies in mice and rats have demonstrated that the hypothalamus is the primary centre for regulation of food intake and body weight (48–50). After leptin is released by the adipose tissue into the bloodstream, it crosses the BBB and binds to the hypothalamic leptin receptors, giving information about the status of the body energy stores (6,26,51,52, Fig. 1). By binding to its receptors, leptin influences the activity of various hypothalamic neurones and the expression of various orexigenic and anorexigenic neuropeptides. Orexigenic peptides, which levels are influenced by leptin, include neuropeptide Y (NPY), melanin-concentrating hormone, agouti-related protein (AgRP), galanin, orexin and galanin-like peptide (GALP; 48,52–56). Furthermore, regulation of the effects of ghrelin on hypothalamic neurones (ghrelin blocks leptin's action through the activation of the hypothalamic NPY/Y1 receptor pathway) has been suggested to be one of the important mechanisms by which leptin may control food intake and body weight (6,57,58). However, studies on the effects of leptin on circulating ghrelin levels in humans have produced conflicting results (59–63). It is therefore still possible that leptin is not an upstream regulator of ghrelin.

Anorexigenic peptides, which expressions seem to be modulated by leptin, include pro-opiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript, neurotensin, corticotropin-releasing hormone (CRH) and brain-derived neurotrophic factor (51–53,64,65). The orexigenic and anorexigenic neurones, which are located in the various hypothalamic regions (arcuate nucleus [ARC], lateral hypothalamus, perifornical hypothalamus and paraventricular nucleus), interact with each other (66–68).

Table 1 Regulators of circulating leptin levels

	Effect on circulating leptin
Energy stores (4,25)	↑ With increase in body mass index and per cent total body fat
Food intake (25,33–36)	↑
Gender (38–40)	Higher in females compared with males
Age (40)	↓ With increasing age
Exercise (41,42)	↓
Glucose uptake (43)	↑

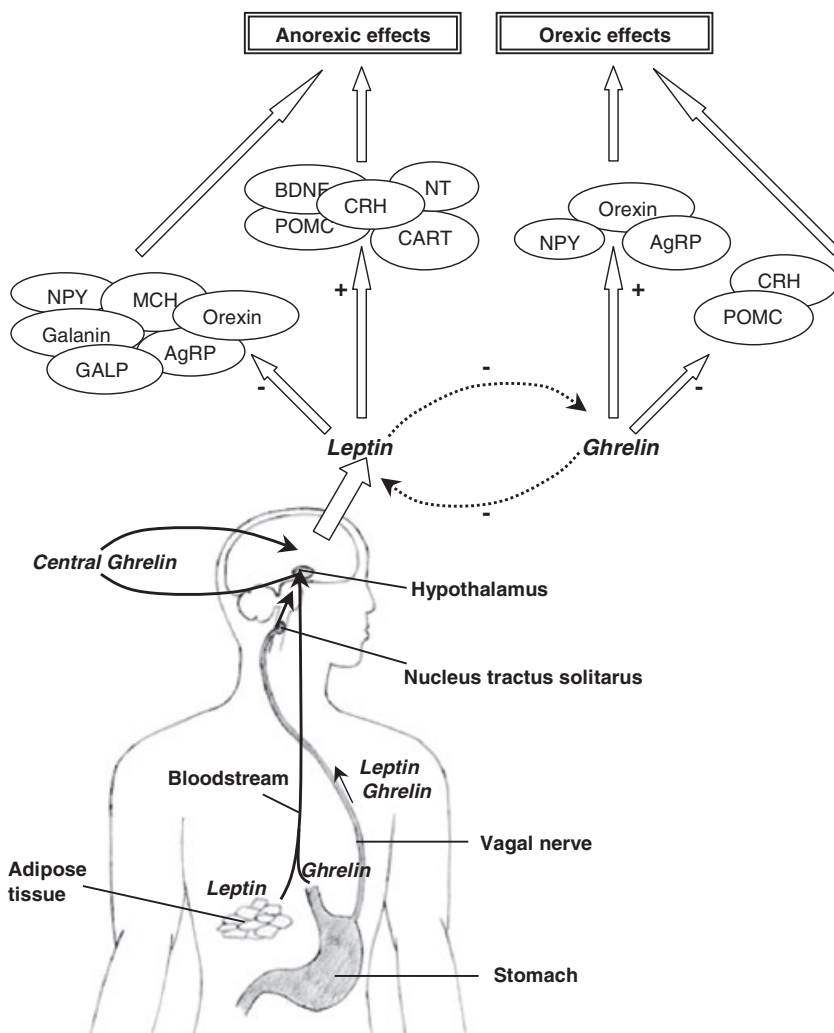The release of leptin by adipose tissue is influenced by various factors.

Figure 1 Pathways by which leptin and ghrelin may have effect on energy balance in humans. This schematic drawing shows the pathways by which leptin and ghrelin may reach the hypothalamus, in order to have an effect on food intake and body weight. Leptin is secreted by adipose tissue and ghrelin is secreted by the stomach. Both hormones may enter the brain through the bloodstream (long arrow with straight line). In addition, ghrelin and gastric leptin may reach the hypothalamus through the vagal nerve and nucleus tractus solitarus (short arrows with straight line). In addition, central ghrelin may affect the energy centre in the hypothalamus (curved arrow). Leptin and ghrelin both stimulate (+) and suppress (–) hypothalamic neurones containing various neuropeptides, resulting in anorexic or orexic effects on energy balance (open arrows). Studies on the effect of leptin on circulating ghrelin levels produced conflicting results; whether ghrelin has influence on circulating leptin levels has not yet been demonstrated (curved arrows with dashed line). AgRP, agouti-related protein; BDNF, brain-derived neurotrophic factor; CART, cocaine- and amphetamine-regulated transcript; CRH, corticotropin-releasing hormone; GALP, galanin-like peptide; MCH, melanin-concentrating hormone; NPY, neuropeptide Y; NT, neurotensin; POMC, pro-opiomelanocortin (6,14,30,32,47,48,51–58,64,65,68,75,84,85,89,103,109-112,116-119).

Compromise in interactions between orexigenic peptides or in their effects on anorexigenic peptides has been suggested to be one of the possible mechanisms of leptin action in the hypothalamus (6).

Leptin induces weight loss by suppression of food intake and by stimulation of metabolic rate

Montague *et al.* provided the first genetic evidence that leptin is an important regulator of energy balance in humans (69). The investigators studied two severely obese children. Congenital leptin deficiency, due to a homozygous frameshift mutation in the *OB* gene, was found to be associated with normal birth weight, followed by a rapid development of severe obesity associated with hyperphagia (over-eating) and impaired satiety. Farooqi *et al.* examined subjects who were heterozygous for the same frameshift mutation (30). Serum leptin concentrations were lower compared with controls and were accompanied by an increased prevalence of obesity. Leptin treatment results in decreased appetite, weight loss, increased physical activity, changes in endocrine function and metabolism, and beneficial effects on ingestive and noningestive behaviour in leptin-deficient patients (30,32). Furthermore, Weigle *et al.* showed that leptin seems to contribute to ongoing weight loss after 12 weeks of dietary fat restriction in healthy humans (70). The effect of leptin on energy expenditure in humans is less clear. Several investigators showed that circulating leptin is not correlated with metabolism in lean or obese subjects (36,39,71,72). On the other hand, Jorgensen *et al.* showed that the serum leptin level is a strong positive determinant of resting metabolic rate (RMR) in healthy men (29). In addition, Jeon *et al.* and Kennedy *et al.* also found a correlation between serum or plasma leptin levels and RMR (31,39).

Until several years ago, leptin had been thought only to play a significant role in long-term regulation of energy

balance. More recent data indicate that leptin also seems to play a role in short-term regulation of food intake and body weight. Leptin is produced not only by adipose tissue, but also in small amount by the stomach (15). Therefore, it has been suggested that leptin might play a role in the control of meal size in cooperation with other satiety peptides (73–75). It has been shown that several intestinal peptides induce gastric leptin release (15,75). In addition, gastric leptin secretion is stimulated by the administration of insulin, which is a hormone released into the bloodstream shortly after food intake (76). Furthermore, high-fat meals and mixed meals lower 24-h circulating leptin levels (77,78). It is, however, possible that gastric leptin serves more as a local stimulus, for example, by playing a role in food digestion and absorption in the intestines (15,74,75). Additional studies are necessary to confirm this hypothesis.

For a long time, many investigators focused their attention on the role of leptin in the pathogenesis of obesity. However, several years ago, many researchers started to realize that leptin might be more importantly involved in adaptation to energy deprivation. Fasting for 36 h (or 3 days) has been shown to result in a significant decrease in plasma leptin concentration (25,34). This decline in plasma leptin was much greater than the change in adipose mass, indicating that this change in adipose mass is not solely responsible for the decrease in circulating leptin concentration. Several studies have demonstrated that leptin is involved in the neuroendocrine response to starvation, including changes in hormone concentrations, and possibly changes in sympathetic nervous system activity and reproductive function (79,80). Disease states like exercise-induced amenorrhoea and anorexia nervosa are also associated with low leptin concentrations and show similar changes in neuroendocrine functioning (81). Importantly, many of the neuroendocrine alterations that occur during fasting are blunted in obese individuals (79,82).

Ghrelin is a hormone secreted by the stomach

The gene coding for human prepro-ghrelin, *GHRL*, is located on chromosome 3 (3p25-26) and is composed of four exons and three introns spanning 5 kb (83,84). Human prepro-ghrelin consists of 117 amino acids, and the mature ghrelin peptide is constituted of 28 amino acids with a fatty acid chain modification (octanoyl group) on the third amino acid (85). Ghrelin peptide was originally isolated from the stomach, but ghrelin protein has also been identified in other peripheral tissues, such as the gastrointestinal tract, pancreas, ovary and adrenal cortex (85–89). In the brain, ghrelin-producing neurones have been identified in the pituitary, in the hypothalamic ARC, and in a group of neurones adjacent to the third ventricle between the dorsal, ventral, paraventricular and arcuate hypothalamic nuclei (68,85,90).

Ghrelin binds to the growth hormone secretagogue receptor (GHS-R). By nucleotide sequence analysis Howard *et al.* identified two types of cDNA encoding for the GHS-R, which were derived from the same gene and were referred to as GHS-R1a and GHS-R1b (91,92). The gene encoding for the human GHS-R1 receptor is located on chromosome 3 (3q26.2) and is constituted of two exons and one intron spanning 4 kb (84,92,93). The GHS-R1a receptor is constituted of 366 amino acids. As to the GHS-R1b variant, it is not clear whether it is transcribed into protein *in vivo*, but theoretically it would code for 289 amino acids (92). The GHS-R1 receptor was originally cloned from the human pituitary and arcuate ventro-medial and infundibular hypothalamus (91). In addition, GHS-R1 receptors have been identified in other human tissues, such as the gastrointestinal tract, ovary and testis (94–96).

The secretion of ghrelin by the stomach depends largely on the nutritional state. Ghrelin levels show preprandial increases and postprandial decreases (59,97,98). In addition, ghrelin levels show a diurnal variation and seem to be influenced by age, gender, BMI, growth hormone (GH), glucose and insulin (Table 2; 59,63,97,99–105). However, several of these correlations could not be confirmed (100,106). Notably, leptin has also been suggested to have influence on circulating ghrelin levels. It has been hypothesized that the satiety-inducing effects of leptin include the suppression of ghrelin secretion (107). Indeed, the effects of leptin on energy homeostasis are opposite (although not complementary) to those of ghrelin; leptin induces weight loss by suppression of food intake, whereas ghrelin functions as an appetite-stimulatory signal. Moreover, leptin has been shown to be an upstream regulator of ghrelin in rodents (57,84,108). However, several studies in humans have produced conflicting results. For example, Tschop *et al.* demonstrated that in obese patients fasting plasma ghrelin levels are negatively correlated with fasting plasma leptin levels (60). However, in another study fasting plasma leptin and ghrelin concentrations were not correlated in obese children and adolescents (61). In addition, intermeal ghrelin levels are displaying a diurnal rhythm

Table 2 Regulators of circulating ghrelin

	Effect on circulating ghrelin
Food intake (59,97,98)	↓
Age (99)	↓ With increasing age
Gender (63,100)	Higher in females compared with males
BMI (97,101,102)	↓ With increasing BMI
GH (103)	↓
Glucose (104)	↓
Insulin (105)	↓

The release of ghrelin by the stomach is influenced by various factors. BMI, body mass index; GH, growth hormone.

that is in phase with that of leptin in healthy humans (59). Furthermore, a recent study showed that leptin administration to healthy volunteers does not regulate ghrelin levels over several hours to a few days (63). These results suggest that leptin does not regulate circulating ghrelin levels. It is therefore possible that the leptin and ghrelin systems function independently of each other in the control of energy homeostasis.

The role of ghrelin in food intake is mediated through the hypothalamus

The effects of ghrelin on energy balance are at least in a large part mediated by the hypothalamus. Korbonits *et al.* proposed three different pathways for the appetite-inducing effects of ghrelin (103). First, after release into the bloodstream by the stomach, ghrelin may cross the BBB and bind to its receptors in the hypothalamus (89,103,109). Second, ghrelin may reach the brain through the vagal nerve and nucleus tractus solitarus (84,103). Third, ghrelin is produced locally in the hypothalamus, where it may directly affect the various hypothalamic nuclei (68,103).

Ghrelin attenuates leptin-induced reduction in food intake and body weight by modulating the expression of various hypothalamic peptides. Ghrelin stimulates the activity of neurones expressing NPY, AgRP and orexin (57,110,111). On the other hand, ghrelin has an inhibitory effect on POMC neurones and CRH-producing neurones (68). Ghrelin does not seem to be a direct regulator of leptin, as fasting produces identical decreases in serum leptin in ghrelin null and wild-type mice (112). The results gathered so far indicate that leptin and ghrelin have different effects on the hypothalamic neurones producing the various orexigenic and anorexigenic peptides, resulting in more or less opposing effects on energy balance (Fig. 1).

Ghrelin presumably functions as an appetite-stimulatory signal

Ghrelin has been shown to regulate the secretion of GH by the pituitary (85). In addition, ghrelin has effect on the gastrointestinal tract, immune cell activation and inflammation (113,114). Interestingly, in 2000, Tschop *et al.* reported that ghrelin seemed to be involved in the regulation of food intake and energy balance in mice and rats (115). Based on the results, it was postulated that ghrelin signals to the hypothalamus when an increase in metabolic efficiency is necessary.

It has been demonstrated that the preprandial increase in ghrelin levels correlates with hunger scores in healthy humans, initiating meals voluntarily in the absence of time- and food-related cues (116). In addition, an intravenous injection or infusion of ghrelin also induces hunger and food intake among healthy and obese humans (117–119). Together, this indicates that ghrelin seems to function as a meal-initiation signal in the system for short-term regulation of energy balance. Based on results of studies with mice, Asakawa *et al.* postulated that this increase in food intake after ghrelin administration is mediated through its stimulatory effect on gastric emptying (120). This might also be the case in humans, as it has been demonstrated that circulating ghrelin levels are correlated with gastric emptying in human subjects (121). Whether ghrelin also has an influence on the regulation of energy expenditure is not clear. It has been reported that rodents show decreased energy expenditure after peripheral administration of ghrelin (115). However, this has not yet been demonstrated in humans.

Besides playing a role in short-term regulation of food intake, ghrelin might also play a role in long-term regulation of energy balance. Peripheral daily administration of ghrelin induces adiposity in rodents by reducing fat utilization (115). In addition, circulating ghrelin concentrations are negatively correlated with BMI in humans, and these levels increase when obese humans lose weight, and decrease when anorexia nervosa patients gain weight. This suggests that ghrelin levels change in response to dieting to maintain body weight (101,102). Also in Prader–Willi syndrome, which is a syndrome resulting from a genetic defect and among other things is characterized by insatiable appetite and obesity, plasma ghrelin concentrations are higher compared with healthy subjects (122). Again, these ghrelin concentrations are negatively correlated with BMI. Furthermore, plasma ghrelin levels decrease after gastrectomy, which most likely contributes to the weight-reducing effect of this procedure (97). However, this might also be due to alterations in other gut peptides involved in regulation of appetite.

Finally, ghrelin does not seem to be crucial for the maintenance of energy homeostasis. Ghrelin knockout mice (*ghrelin*$^{-/-}$) have a normal body size, body composition, bone density, growth rate, gastric emptying, food intake, reproduction, gross behaviour and tissue pathology (112,123). Fasting results in normal decreases in serum insulin and leptin, and ghrelin administration stimulates appetite in *ghrelin*$^{-/-}$ mice. Moreover, *Ghsr*-null mice have a normal appetite, show a normal body size, body composition, body weight and bone density, and show normal serum leptin and insulin responses to fasting (124). However, body weights of mature *Ghsr*-null mice were modestly reduced, which might be related to ghrelin's role in GH release, resulting in subtle changes in body composition. Together this indicates that ghrelin is not critically required for growth, appetite and fat deposition, and is not likely to be a direct regulator of leptin and insulin. It was suggested that other redundant appetite-inducing agents might com-

pensate for loss of ghrelin functioning. Instead, De Smet *et al.* showed that in old mice ghrelin is a mediator of meal initiation triggered by the light/dark cycle, and in young animals ghrelin was suggested to be possibly involved in the selection of energy stores and in the partitioning of metabolizable energy into storage or dissipation as heat (123).

Do abnormalities in leptin and ghrelin or their actions contribute to the development or maintenance of obesity?

Although it would be expected that in obese humans leptin levels are decreased and ghrelin levels are increased, circulating leptin levels turned out to be increased and circulating ghrelin levels showed to be decreased (60,125–127). In addition, obese humans show a disturbed diurnal variation in leptin and ghrelin levels (107). It is still not clear if these abnormalities in the leptin and ghrelin systems are the cause or a consequence of obesity. Although several investigators were able to attribute obesity to polymorphisms in the genes encoding for leptin, ghrelin and their receptors, it seems that defects in these genes are generally not involved in obesity in humans (22,83,126,128–135).

As obese humans show elevated levels of leptin in serum and adipocytes, and show limited effects with leptin treatment, many researchers suggest obese humans to be leptin-resistant (22,26,127,136–138). The development of leptin resistance most likely involves a period of over-eating, resulting in the leptin system getting so disturbed that it leads to sustained defects. Over-eating results in an increase in circulating leptin levels (33,36). This exposure of the hypothalamus to high leptin levels may have damaging effects on the hypothalamus. As a result, the hypothalamus becomes less sensitive to leptin, leading to a sustained increase in leptin levels. It has already been shown that chronic leptin infusion leads to leptin resistance in a rat model (139). In addition, Kolaczynski *et al.* showed that humans develop leptin resistance because of overfeeding (33).

It has been postulated that leptin resistance might be due to defective leptin transport across the BBB. Several studies support this hypothesis (26,127,140). It has been shown that diet-induced obese (DIO) mice develop resistance to peripherally administered leptin, while retaining sensitivity to centrally administered leptin (140). This suggests that these mice have disturbed leptin transport through the BBB. In humans, the ratio between leptin levels in CSF and plasma has been shown to be lower in obese subjects compared with lean individuals (26,127). This suggests that leptin enters the brain by a saturable transport system and that the capacity of leptin transport is lower in obese individuals, thereby providing a mechanism for leptin resis-

tance. However, Levin *et al.* demonstrated that BBB leptin transport was not different between preobese DIO and diet-resistant rats, and impaired leptin transport developed only after DIO rats became obese and/or aged (141). Thus, defects in leptin transport appear to be an acquired defect associated with the development of obesity. In addition, preobese DIO rats had reduced leptin receptor mRNA expression in the ARC, in association with reduced leptin-induced anorexia after peripheral leptin administration. The investigators suggested that a pre-existing reduction in hypothalamic leptin signalling might contribute to the development of diet-induced obesity when dietary fat and calorie intake are increased.

One other possibility is that a defect in leptin receptor expression in the hypothalamus is the cause of altered leptin sensitivity. Hypothalamic leptin receptor mRNA levels are decreased in DIO rats (141). In addition, in obese *db/db* and *ob/ob* mice, *OB-Rb* mRNA levels in the ARC are increased (142). Furthermore, leptin administration reduces *OB-Rb* mRNA levels in the ARC of *ob/ob* mice, and fasting increases *OB-Rb* mRNA levels in the ARC of normal mice. The investigators proposed that hypothalamic *OB-Rb* expression might be sensitive to genetic and physiological interventions that alter circulating leptin levels, and that overexpression of the leptin receptor in the hypothalamus might contribute to increased leptin sensitivity (142). However, it is important to note that in 1996 Considine *et al.* did not find a difference in the amount of leptin receptor mRNA between lean and obese humans (22). Therefore, this concept needs further investigation.

It is also possible that leptin resistance is caused by defects in the downstream mediators of leptin. Based on studies with mice, AgRP and its receptor (Mc4r) have been proposed to be good candidates for human disorders of body weight regulation (143). In addition, changes in gene expression in NPY/AgRP neurones and also POMC neurones have been demonstrated in various animal studies (6). Also, defects in the signalling pathways downstream of the leptin receptor might play a role in reduced leptin response in the hypothalamus. The janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway is one of the major pathways of leptin signal transduction (21,144). El-Haschimi *et al.* demonstrated in studies with DIO mice that peripheral administered leptin was unable to activate hypothalamic Stat3 signalling, and the magnitude of Stat3 activation was substantially reduced after intracerebroventricular leptin (145). Several investigators have reported the negative regulators of leptin signalling (protein tyrosine phosphatase 1B [PTP1B]; SH2-containing phosphatase 2; suppressor of cytokine signalling 3 [SOCS3]) to be potential factors in leptin resistance (146–148). SOCS3 mRNA expression in the hypothalamus is induced by leptin (146). It mediates negative feedback on JAK-STAT activation. Excessive SOCS3 activity might

therefore be involved in leptin resistance. Indeed, in 2004, Howard *et al.* demonstrated that mice with heterozygous *SOCS3* (*SOCS3⁺/⁻*) deficiency display greater leptin sensitivity than wild-type mice; they showed enhanced weight loss and increased hypothalamic leptin receptor signalling after leptin administration (149). In addition, *SOCS3⁺/⁻* mice seemed to be protected against the development of diet-induced obesity. Thus, the level of SOCS3 expression seems to be a determinant of leptin sensitivity and susceptibility for obesity.

Whether an elevated level of circulating leptin causes a reduction in ghrelin levels is still not clear. However, it seems that leptin does not have a direct influence on ghrelin levels. It is possible that decreased plasma ghrelin concentrations represent a physiological adaptation to the positive energy balance associated with obesity (60). This is in line with the observation that circulating ghrelin levels in obese patients increase during weight loss (102). Obese humans do not lose their responsiveness to ghrelin, or have a defect in ghrelin transport at the BBB, as peripheral administration still results in an enhanced appetite in obese subjects (118). It may be that obese patients are oversensitive to ghrelin, for example, because of an overexpression of the GHS-R. It has been shown that a low-dose infusion of ghrelin has no effect in lean people, but does increase *ad libitum* energy intake in obese subjects (150). In addition, a high-dose infusion with ghrelin led to a higher increase in food intake in obese patients compared with lean subjects. However, in mice it has been shown that constitutive overexpression of GHS-R does not affect food intake and adipose tissue response to GHS ligands (151).

Finally, in recent studies conducted by Asakawa *et al.* and Zhang *et al.*, it was demonstrated that desacyl ghrelin and obestatin (which are peptides derived from the same ghrelin gene, that undergo differential post-translational modifications) also play a role in energy balance (120,152). The investigators showed that treatment of rodents with desacyl ghrelin or obestatin induced a negative energy balance by decreasing food intake and delaying gastric emptying, and by decreasing body weight gain. Thus, ghrelin on one hand and desacyl ghrelin and obestatin on the other hand seem to have opposing effects on weight regulation. It might be that dysfunctioning of desacyl ghrelin or obestatin is involved in the pathophysiology of obesity. For example, disturbed post-translational processing of the *GHRL* gene and therefore decreased expression of desacyl ghrelin and obestatin may result in increased food intake and body weight.

The potential of leptin and ghrelin as a drug target for weight regulation

Many studies have been performed to investigate the potential of both leptin and ghrelin as therapeutic targets. Unfor-
tunately, although leptin treatment has been shown to have beneficial effects in patients with leptin deficiency, it shows very limited effects in obese people (136–138). Therefore, several investigators try to find alternatives for the normal leptin hormone and to develop strategies that bypass normal central leptin functioning. In a recent study, Lo *et al.* introduced a superior form of leptin, having enhanced pharmacological properties in comparison with recombinant leptin that has been used in former clinical trials (153). The Fc-leptin immunofusins (consisting of the Fc fragment of an immunoglobulin gamma chain followed by leptin) led to a significant weight loss in non-leptin-deficient mice. In addition, Fc-leptin had an extended circulating half-life. This makes Fc-leptin an interesting compound for the treatment of non-leptin-deficient obese humans. In 2003, Weigle *et al.* showed that leptin contributes to ongoing weight loss after 12 weeks of dietary fat restriction in healthy humans (70). Moreover, in a recent study, Rosenbaum *et al.* showed that daily administration of leptin, in addition to a diet, could prevent adaptations normally occurring during weight loss (154).

Also the potential of the ghrelin system as a therapeutic target for obesity treatment is still under discussion. As it has been demonstrated that circulating ghrelin levels increase when obese humans lose weight, and because obese mice show an increase in sensitivity to ghrelin upon weight loss, blockage of ghrelin could prevent weight regain after weight loss (155). In a recent study with rats, it was demonstrated that anti-ghrelin blocks ghrelin-induced increase in food intake after ghrelin injection (156). In addition, the ghrelin receptor constitutes a potential drug target. The GHS-receptor has been shown to be constitutively active (157). Blocking this constitutive receptor activity was suggested to possibly lower the set point for hunger between meals. It has already been demonstrated that GHS-R antagonists result in a decrease of energy intake in lean and obese mice, and repeated administration gave a decrease of body weight gain in *ob/ob* mice (158). However, as it is possible that the ghrelin system functions differently in humans, similar studies in human subjects are still necessary. Notably, in another study, a novel GHS-R1a antagonist was discovered, which blocks ghrelin-induced GH release in the medial arcuate nucleus, but like ghrelin induces increased body weight gain through the dorsal medial hypothalamus (159). The investigators suggested that the role of ghrelin in weight gain might be mediated by a novel receptor other than GHS-R1a. Therefore, GHS-R1a might not be a potential target to block ghrelin-induced food intake.

One other strategy is to target genes that are involved in leptin or ghrelin functioning, for example, negative regulators of leptin or ghrelin signalling. Howard *et al.* proposed SOCS3, which has been identified as a leptin-induced negative regulator of leptin receptor signalling and potential

mediator of leptin resistance, to be a potential target for therapeutic intervention (149). In addition, PTP1B has been suggested to be a valuable target for the treatment of leptin resistance in human obesity (160). Likewise, the use of agents that stimulate inhibitors of ghrelin signalling may be a potential way to suppress ghrelin's stimulatory effect on food intake and body weight.

Can the diet be modulated to stimulate the secretion or enhance the action of leptin and ghrelin?

Food intake can have significant effects on circulating leptin and ghrelin levels. Overfeeding results in an increase in adipocyte leptin expression and circulating leptin in healthy human subjects (33,36). Fasting (for 20 or 36 h or 3 days) results in a decrease of adipocyte leptin mRNA and serum leptin levels, with a greater decline in leptin levels in lean subjects than in obese subjects (25,34,35). Refeeding is again associated with a rise in serum leptin levels, and leptin levels return to baseline after 24 h (25,34). On the other hand, fasting results in an increase in plasma ghrelin levels, with a nearly twofold increase immediately before each meal (59,97). This preprandial increase in ghrelin levels correlates with hunger scores in humans (116). Feeding results in a decrease in plasma ghrelin levels within 1 to 2 h (59,98).

Not only the size and frequency of meals have an effect on circulating leptin and ghrelin levels, but also the composition of a meal is a determinant of leptin and ghrelin levels in humans (Table 3). For example, low-fat/high-carbohydrate meals result in an increase in circulating leptin concentrations, which is larger, compared with high-fat/low-carbohydrate meals (161). In addition, high-fat meals lower 24-h circulating leptin levels relative to high-carbohydrate meals (78). Hydrolysed guar fibre or protein intake does not seem to have influence on circulating leptin concentrations (162,163).

A low-fat diet seems to have an inhibitory effect on ghrelin levels, as one study reported that a low-fat/high-carbohydrate diet resulted in weight loss, without an increase in plasma ghrelin levels (70). Another study demonstrated that a high-carbohydrate diet caused a larger drop in ghrelin levels than a high-fat diet in healthy women (164). The effect of protein ingestion on ghrelin levels gives conflicting results (163,165,166). Finally, the use of non-caloric Psyllian fibres results in a decrease of plasma ghrelin levels in healthy women (167). Together, these data indicate that for obese subjects it is important to follow a specific diet in order to regulate food intake and body weight.

Conclusion

What becomes clear from this review is that both leptin and ghrelin play major roles in the control system for energy balance in humans. However, leptin is primarily involved in long-term regulation of energy balance; it is released into the circulatory system as a function of energy stores, whereas ghrelin is a fast-acting hormone, of which the circulatory levels show clear meal-related changes. One other difference is that, in contrast to leptin, ghrelin does not seem to be critical for normal appetite and growth. Interestingly, leptin and ghrelin functioning in the system for energy homeostasis involves several overlapping pathways. At present, it is still not clear whether abnormalities in the leptin or ghrelin systems contribute to the development of obesity. Nevertheless, disturbances in both systems seem to play a role in the maintenance of obesity.

Most importantly, obese patients are leptin-resistant, and it is therefore necessary to develop a treatment that overcomes leptin insensitivity or bypasses normal central leptin functioning, for example, by developing novel forms of leptin with stronger physiological properties. The Fc-leptin immunofusins used by Lo *et al*. were shown to have positive effects on body weight in mice (153). Additional studies are warranted to assess the effects of these compounds in humans. Also, ghrelin is still recognized as a potential drug target for weight regulation. When obese patients lose weight, ghrelin levels show an increase, as if to compensate

Table 3 Effects of diet composition on circulating leptin and ghrelin levels

Diet	Effect on circulating leptin	Effect on circulating ghrelin
High-fat	24-h circulating leptin levels ↓ relative to high-carbohydrate meal (78)	↓ (164)
High-carbohydrate		↓ (Larger drop compared with high-fat diet, 164)
Low-fat/high-carbohydrate	↑(Larger compared with high-fat/low-carbohydrate meal, 146)	No increase (70)
High-fat/low-carbohydrate	↑ (146)	
Protein	No effect (148)	Conflicting results (163,165,166)
Hydrolysed guar fibre	No effect (147)	
Non-caloric Psyllian fibres		↓ (167)

The composition of a diet can have increasing or decreasing effect on circulating leptin and ghrelin levels.

for this weight loss (155). Therefore, it seems interesting to try ghrelin antagonists while following a strict diet.

Furthermore, the peptides downstream of leptin and ghrelin constitute possible targets for therapeutic interventions. For example, Makimura *et al.* demonstrated that a reduction of hypothalamic AgRP results in an increase of metabolic rate and a decrease of body weight without affecting food intake in mice. This suggests that agents antagonizing the effect of AgRP may be a useful strategy to treat obesity, without producing unacceptable loss of appetite (168). Interestingly, Belsham *et al.* created a number of hypothalamic neuronal cell lines, which can be used as models to study the regulation of neuropeptides associated with the control of feeding behaviour. Eventually, such studies may provide information that is necessary for the design of anti-obesity agents (169).

As diet and exercise have significant effects on energy homeostasis, the use of solely therapeutic drugs to treat obesity does not seem to be sufficient. Orzano and Scott already showed that the most effective treatment is provided by a combination of diet and exercise (3). Taken together, the best strategy to accomplish long-term changes in body weight seems to be the use of potential anti-obesity agents in combination with a low-fat diet and sufficient exercise.

Conflict of Interest Statement

No conflict of interest was declared.

References

1. Allison A, Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999–2002. *JAMA* 2004; **291**: 2847–2850.
2. Formiguera X, Canton A. Obesity: epidemiology and clinical aspects. *Best Pract Res Clin Gastroenterol* 2004; **18**: 1125–1146.
3. Orzano AJ, Scott JG. Diagnosis and treatment of obesity in adults: an applied evidence-based review. *J Am Board Fam Pract* 2004; **17**: 359–369.
4. Frederich RC, Lollmann B, Hamann A, Napolitano-Rosen A, Kahn BB, Lowell BB, Flier JS. Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. *J Clin Invest* 1995; **96**: 1658–1663.
5. Pralong FP, Gaillard RC. Neuroendocrine effects of leptin. *Pituitary* 2001; **4**: 25–32.
6. Sahu A. Leptin signaling in the hypothalamus: emphasis on energy homeostasis and leptin resistance. *Front Neuroendocrinol* 2004; **24**: 225–253.
7. Schwartz MW. Brain pathways controlling food intake and body weight. *Exp Biol Med* 2001; **226**: 978–981.
8. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; **372**: 425–432.
9. Isse N, Ogawa Y, Tamura N, Masuzaki H, Mori K, Okazaki T, Satoh N, Shigemoto M, Yoshimasa Y, Nishi S, Hosoda K, Inazawa J, Nakao K. Structural organization and chromosomal assignment of the human obese gene. *J Biol Chem* 1995; **270**: 27728–27733.
10. Gong DW, Bi S, Pratley RE, Weintraub BD. Genomic structure and promoter analysis of the human obese gene. *J Biol Chem* 1996; **271**: 3971–3974.
11. Masuzaki H, Ogawa Y, Isse N, Satoh N, Okazaki T, Shigemoto M, Mori K, Tamura N, Hosoda K, Yoshimasa Y, Jingami H, Kawada T, Nakao K. Human obese gene expression. Adipocyte-specific expression and regional differences in the adipose tissue. *Diabetes* 1995; **44**: 855–858.
12. Green ED, Maffei M, Braden VV, Proenca R, DeSilva U, Zhang Y, Chua SC Jr, Leibel RL, Weissenbach J, Friedman JM. The human obese (OB) gene. RNA expression pattern and mapping on the physical, cytogenetic, and genetic maps of chromosome 7. *Genome Res* 1995; **5**: 5–12.
13. Casabiell X, Pineiro V, Tome MA, Peino R, Dieguez C, Casanueva FF. Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. *J Clin Endocrinol Metab* 1997; **82**: 4270–4273.
14. Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, Moizo L, Lehy T, Guerre-Millo M, Le Marchand-Brustel Y, Lewin MJ. The stomach is a source of leptin. *Nature* 1998; **394**: 790–793.
15. Sobhani I, Bado A, Vissuzaine C, Buyse M, Kermorgant S, Laigneau JP, Attoub S, Lehy T, Henin D, Mignon M, Lewin MJ. Leptin secretion and leptin receptor in the human stomach. *Gut* 2000; **47**: 178–183.
16. Hoggard N, Haggarty P, Thomas L, Lea RG. Leptin expression in placental and fetal tissues: does leptin have a functional role? *Biochem Soc Trans* 2001; **29**: 57–63.
17. Chung WK, Power-Kehoe L, Chua M, Leibel RL. Mapping of the OB receptor to 1p in a region of nonconserved gene order from mouse and rat to human. *Genome Res* 1996; **6**: 431–438.
18. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin Chem* 2004; **50**: 1511–1525.
19. Campfield LA, Smith FJ, Burn P. The OB protein (leptin) pathway – a link between adipose tissue mass and central neural networks. *Horm Metab Res* 1996; **28**: 619–632.
20. Burguera B, Couce ME, Long J, Lamsam J, Laakso K, Jensen MD, Parisi JE, Lloyd RV. The long form of the leptin receptor (OB-Rb) is widely expressed in the human brain. *Neuroendocrinology* 2000; **71**: 187–195.
21. Hegyi K, Fulop K, Kovacs K, Toth S, Falus A. Leptin-induced signal transduction pathways. *Cell Biol Int* 2004; **28**: 159–169.
22. Considine RV, Considine EL, Williams CJ, Hyde TM, Caro JF. The hypothalamic leptin receptor in humans: identification of incidental sequence polymorphisms and absence of the db/db mouse and fa/fa rat mutations. *Diabetes* 1996; **45**: 992–994.
23. Henson MC, Swan KF, O'Neil JS. Expression of placental leptin and leptin receptor transcripts in early pregnancy and at term. *Obstet Gynecol* 1998; **92**: 1020–1028.
24. Sierra-Honigmann MR, Nath AK, Murakami C, Garcia-Cardena G, Papapetropoulos A, Sessa WC, Madge LA, Schechner JS, Schwabb MB, Polverini PJ, Flores-Riveros JR. Biological action of leptin as an angiogenic factor. *Science* 1998; **281**: 1583–1585.
25. Weigle DS, Duell PB, Connor WE, Steiner RA, Soules MR, Kuijper JL. Effect of fasting, refeeding, and dietary fat restriction on plasma leptin levels. *J Clin Endocrinol Metab* 1997; **82**: 561–565.

26. Schwartz MW, Peskind E, Raskind M, Boyko EJ, Porte D Jr. Cerebrospinal fluid leptin levels: relationship to plasma levels and to adiposity in humans. *Nat Med* 1996; **2**: 589–593.

27. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995; **269**: 540–543.

28. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995; **269**: 543–546.

29. Jorgensen JO, Vahl N, Dall R, Christiansen JS. Resting metabolic rate in healthy adults: relation to growth hormone status and leptin levels. *Metabolism* 1998; **47**: 1134–1139.

30. Farooqi IS, Keogh JM, Kamath S, Jones S, Gibson WT, Trussell R, Jebb SA, Lip GY, O'Rahilly S. Partial leptin deficiency and human adiposity. *Nature* 2001; **414**: 34–35.

31. Jeon JY, Steadward RD, Wheeler GD, Bell G, McCargar L, Harber V. Intact sympathetic nervous system is required for leptin effects on resting metabolic rate in people with spinal cord injury. *J Clin Endocrinol Metab* 2003; **88**: 402–407.

32. Licinio J, Caglayan S, Ozata M, Yildiz BO, de Miranda PB, O'Kirwan F, Whitby R, Liang L, Cohen P, Bhasin S, Krauss RM, Veldhuis JD, Wagner AJ, DePaoli AM, McCann SM, Wong ML. Phenotypic effects of leptin replacement on morbid obesity, diabetes mellitus, hypogonadism, and behavior in leptin-deficient adults. *Proc Natl Acad Sci USA* 2004; **101**: 4531–4536.

33. Kolaczynski JW, Ohannesian JP, Considine RV, Marco CC, Caro JF. Response of leptin to short-term and prolonged overfeeding in humans. *J Clin Endocrinol Metab* 1996; **81**: 4162–4165.

34. Kolaczynski JW, Considine RV, Ohannesian J, Marco C, Opentanova I, Nyce MR, Myint M, Caro JF. Responses of leptin to short-term fasting and refeeding in humans: a link with ketogenesis but not ketones themselves. *Diabetes* 1996; **45**: 1511–1515.

35. Korbonits M, Trainer PJ, Little JA, Edwards R, Kopelman PG, Besser GM, Svec F, Grossman AB. Leptin levels do not change acutely with food administration in normal or obese subjects, but are negatively correlated with pituitary-adrenal activity. *Clin Endocrinol* 1997; **46**: 751–757.

36. Levine JA, Eberhardt NL, Jensen MD. Leptin responses to overfeeding: relationship with body fat and nonexercise activity thermogenesis. *J Clin Endocrinol Metab* 1999; **84**: 2751–2754.

37. Matkovic V, Ilich JZ, Badenhop NE, Skugor M, Clairmont A, Klisovic D, Landoll JD. Gain in body fat is inversely related to the nocturnal rise in serum leptin level in young females. *J Clin Endocrinol Metab* 1997; **82**: 1368–1372.

38. Saad MF, Damani S, Gingerich RL, Riad-Gabriel MG, Khan A, Boyadjian R, Jinagouda SD, el-Tawil K, Rude RK, Kamdar V. Sexual dimorphism in plasma leptin concentration. *J Clin Endocrinol Metab* 1997; **82**: 579–584.

39. Kennedy A, Gettys TW, Watson P, Wallace P, Ganaway E, Pan Q, Garvey WT. The metabolic significance of leptin in humans: gender-based differences in relationship to adiposity, insulin sensitivity, and energy expenditure. *J Clin Endocrinol Metab* 1997; **82**: 1293–1300.

40. Ostlund RE Jr, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. *J Clin Endocrinol Metab* 1996; **81**: 3909–3913.

41. Hickey MS, Houmard JA, Considine RV, Tyndall GL, Midgette JB, Gavigan KE, Weidner ML, McCammon MR, Israel RG, Caro JF. Gender-dependent effects of exercise training on serum leptin levels in humans. *Am J Physiol* 1997; **272**: E562–E566.

42. Keller P, Keller C, Steensberg A, Robinson LE, Pedersen BK. Leptin gene expression and systemic levels in healthy men: effect of exercise, carbohydrate, interleukin-6, and epinephrine. *J Appl Physiol* 2005; **98**: 1805–1812.

43. Wellhoener P, Fruehwald-Schultes B, Kern W, Dantz D, Kerner W, Born J, Fehm HL, Peters A. Glucose metabolism rather than insulin is a main determinant of leptin secretion in humans. *J Clin Endocrinol Metab* 2000; **85**: 1267–1271.

44. Mantzoros CS, Flier JS, Rogol AD. A longitudinal assessment of hormonal and physical alterations during normal puberty in boys. V. Rising leptin levels may signal the onset of puberty. *J Clin Endocrinol Metab* 1997; **82**: 1066–1070.

45. Lord GM, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; **394**: 897–901.

46. Fantuzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. *J Leukoc Biol* 2000; **68**: 437–446.

47. Takeda S, Elefteriou F, Levasseur R, Liu X, Zhao L, Parker KL, Armstrong D, Ducy P, Karsenty G. Leptin regulates bone formation via the sympathetic nervous system. *Cell* 2002; **111**: 305–317.

48. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. *J Clin Invest* 1996; **98**: 1101–1106.

49. Vaisse C, Halaas JL, Horvath CM, Darnell JE Jr, Stoffel M, Friedman JM. Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nat Genet* 1996; **14**: 95–97.

50. Satoh N, Ogawa Y, Katsuura G, Tsuji T, Masuzaki H, Hiraoka J, Okazaki T, Tamaki M, Hayase M, Yoshimasa Y, Nishi S, Hosoda K, Nakao K. Pathophysiological significance of the obese gene product, leptin, in ventromedial hypothalamus (VMH)-lesioned rats: evidence for loss of its satiety effect in VMH-lesioned rats. *Endocrinology* 1997; **138**: 947–954.

51. Golden PL, Maccagnan TJ, Pardridge WM. Human blood-brain barrier leptin receptor. Binding and endocytosis in isolated human brain microvessels. *J Clin Invest* 1997; **99**: 14–18.

52. Meister B. Control of food intake via leptin receptors in the hypothalamus. *Vitam Horm* 2000; **59**: 265–304.

53. Sahu A. Evidence suggesting that galanin (GAL), melanin-concentrating hormone (MCH), neurotensin (NT), proopiomelanocortin (POMC) and neuropeptide Y (NPY) are targets of leptin signaling in the hypothalamus. *Endocrinology* 1998; **139**: 795–798.

54. Arvaniti K, Huang Q, Richard D. Effects of leptin and corticosterone on the expression of corticotropin-releasing hormone, agouti-related protein, and proopiomelanocortin in the brain of ob/ob mouse. *Neuroendocrinology* 2001; **73**: 227–236.

55. Lopez M, Seoane L, Garcia MC, Lago F, Casanueva FF, Senaris R, Dieguez C. Leptin regulation of prepro-orexin and orexin receptor mRNA levels in the hypothalamus. *Biochem Biophys Res Commun* 2000; **269**: 41–45.

56. Kumano S, Matsumoto H, Takatsu Y, Noguchi J, Kitada C, Ohtaki T. Changes in hypothalamic expression levels of galanin-like peptide in rat and mouse models support that it is a leptin-target peptide. *Endocrinology* 2003; **144**: 2634–2643.

57. Nakazato M, Murakami N, Date Y, Kojima M, Matsuo H, Kangawa K, Matsukura S. A role for ghrelin in the central regulation of feeding. *Nature* 2001; **409**: 194–198.

58. Shintani M, Ogawa Y, Ebihara K, Aizawa-Abe M, Miyanaga F, Takaya K, Hayashi T, Inoue G, Hosoda K, Kojima M, Kangawa

K, Nakao K. Ghrelin, an endogenous growth hormone secretagogue, is a novel orexigenic peptide that antagonizes leptin action through the activation of hypothalamic neuropeptide Y/Y1 receptor pathway. *Diabetes* 2001; **50**: 227–232.

59. Cummings DE, Purnell JQ, Frayo RS, Schmidova K, Wisse BE, Weigle DS. A prandial rise in plasma ghrelin levels suggests a role in meal initiation in humans. *Diabetes* 2001; **50**: 1714–1719.

60. Tschop M, Weyer C, Tataranni PA, Devanarayan V, Ravussin E, Heiman ML. Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001; **50**: 707–709.

61. Ikezaki A, Hosoda H, Ito K, Iwama S, Miura N, Matsuoka H, Kondo C, Kojima M, Kangawa K, Sugihara S. Fasting plasma ghrelin levels are negatively correlated with insulin resistance and PAI-1, but not with leptin, in obese children and adolescents. *Diabetes* 2002; **51**: 3408–3411.

62. Haqq AM, Farooqi IS, O'Rahilly S, Stadler DD, Rosenfeld RG, Pratt KL, LaFranchi SH, Purnell JQ. 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* 2003; **88**: 174–178.

63. Chan JL, Bullen J, Lee JH, Yiannakouris N, Mantzoros CS. Ghrelin levels are not regulated by recombinant leptin administration and/or three days of fasting in healthy subjects. *J Clin Endocrinol Metab* 2004; **89**: 335–343.

64. Kristensen P, Judge ME, Thim L, Ribel U, Christjansen KN, Wulff BS, Clausen JT, Jensen PB, Madsen OD, Vrang N, Larsen PJ, Hastrup S. Hypothalamic CART is a new anorectic peptide regulated by leptin. *Nature* 1998; **393**: 72–76.

65. Bariohay B, Lebrun B, Moyse E, Jean A. Brain-derived neurotrophic factor plays a role as an anorexigenic factor in the dorsal vagal complex. *Endocrinology* 2005; **146**: 5612–5620.

66. Tritos NA, Vicent D, Gillette J, Ludwig DS, Flier ES, Maratos-Flier E. Functional interactions between melanin-concentrating hormone, neuropeptide Y, and anorectic neuropeptides in the rat hypothalamus. *Diabetes* 1998; **47**: 1687–1692.

67. Horvath TL, Diano S, van den Pol AN. Synaptic interaction between hypocretin (orexin) and neuropeptide Y cells in the rodent and primate hypothalamus: a novel circuit implicated in metabolic and endocrine regulations. *J Neurosci* 1999; **19**: 1072–1087.

68. Cowley MA, Smith RG, Diano S, Tschop M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML, Garcia-Segura LM, Nillni EA, Mendez P, Low MJ, Sotonyi P, Friedman JM, Liu H, Pinto S, Colmers WF, Cone RD, Horvath TL. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* 2003; **37**: 649–661.

69. 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. Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 1997; **387**: 903–908.

70. Weigle DS, Cummings DE, Newby PD, Breen PA, Frayo RS, Matthys CC, Callahan HS, Purnell JQ. Roles of leptin and ghrelin in the loss of body weight caused by a low fat, high carbohydrate diet. *J Clin Endocrinol Metab* 2003; **88**: 1577–1586.

71. Roberts SB, Nicholson M, Staten M, Dallal GE, Sawaya AL, Heyman MB, Fuss P, Greenberg AS. Relationship between circulating leptin and energy expenditure in adult men and women aged 18 years to 81 years. *Obes Res* 1997; **5**: 459–463.

72. Aprath-Husmann I, Rohrig K, Gottschling-Zeller H, Skurk T, Scriba D, Birgel M, Hauner H. Effects of leptin on the differentiation and metabolism of human adipocytes. *Int J Obes Relat Metab Disord* 2001; **25**: 1465–1470.

73. Lewin MJ, Bado A. Gastric leptin. *Microsc Res Tech* 2001; **53**: 372–376.

74. Attele AS, Shi ZQ, Yuan CS. Leptin, gut, and food intake. *Biochem Pharmacol* 2002; **63**: 1579–1583.

75. Pico C, Oliver P, Sanchez J, Palou A. Gastric leptin: a putative role in the short-term regulation of food intake. *Br J Nutr* 2003; **90**: 735–741.

76. Sobhani I, Buyse M, Goiot H, Weber N, Laigneau JP, Henin D, Soul JC, Bado A. Vagal stimulation rapidly increases leptin secretion in human stomach. *Gastroenterology* 2002; **122**: 259–263.

77. Dallongeville J, Hecquet B, Lebel P, Edme JL, Le Fur C, Fruchart JC, Auwerx J, Romon M. Short term response of circulating leptin to feeding and fasting in man: influence of circadian cycle. *Int J Obes Relat Metab Disord* 1998; **22**: 728–733.

78. Havel PJ. Role of adipose tissue in body-weight regulation: mechanisms regulating leptin production and energy balance. *Proc Nutr Soc* 2000; **59**: 359–371.

79. Klein S, Horowitz JF, Landt M, Goodrick SJ, Mohamed-Ali V, Coppack SW. Leptin production during early starvation in lean and obese women. *Am J Physiol Endocrinol Metab* 2000; **278**: E280–E284.

80. Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. *J Clin Invest* 2003; **111**: 1409–1421.

81. Chan JL, Mantzoros CS. Role of leptin in energy-deprivation states: normal human physiology and clinical implications for hypothalamic amenorrhoea and anorexia nervosa. *Lancet* 2005; **366**: 74–85.

82. Horowitz JF, Coppack SW, Paramore D, Cryer PE, Zhao G, Klein S. Effect of short-term fasting on lipid kinetics in lean and obese women. *Am J Physiol* 1999; **276**: E278–E284.

83. Ukkola O, Ravussin E, Jacobson P, Snyder EE, Chagnon M, Sjostrom L, Bouchard C. Mutations in the preproghrelin/ghrelin gene associated with obesity in humans. *J Clin Endocrinol Metab* 2001; **86**: 3996–3999.

84. Ueno H, Yamaguchi H, Kangawa K, Nakazato M. Ghrelin: a gastric peptide that regulates food intake and energy homeostasis. *Regul Pept* 2005; **126**: 11–19.

85. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656–660.

86. Date Y, Kojima M, Hosoda H, Sawaguchi A, Mondal MS, Suganuma T, Matsukura S, Kangawa K, Nakazato M. 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* 2000; **141**: 4255–4261.

87. Date Y, Nakazato M, Hashiguchi S, Dezaki K, Mondal MS, Hosoda H, Kojima M, Kangawa K, Arima T, Matsuo H, Yada T, Matsukura S. Ghrelin is present in pancreatic alpha-cells of humans and rats and stimulates insulin secretion. *Diabetes* 2002; **51**: 124–129.

88. Gaytan F, Barreiro ML, Chopin LK, Herington AC, Morales C, Pinilla L, Casanueva FF, Aguilar E, Dieguez C, Tena-Sempere M. Immunolocalization of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in the cyclic human ovary. *J Clin Endocrinol Metab* 2003; **88**: 879–887.

89. Tortorella C, Macchi C, Spinazzi R, Malendowicz LK, Trejter M, Nussdorfer GG. Ghrelin, an endogenous ligand for the growth hormone-secretagogue receptor, is expressed in the human adrenal cortex. *Int J Mol Med* 2003; **12**: 213–217.

90. Korbonits M, Kojima M, Kangawa K, Grossman AB. Presence of ghrelin in normal and adenomatous human pituitary. *Endocrine* 2001; **14**: 101–104.

91. Howard AD, Feighner SD, Cully DF, Arena JP, Liberator PA, Rosenblum CI, Hamelin M, Hreniuk DL, Palyha OC, Anderson J, Paress PS, Diaz C, Chou M, Liu KK, McKee KK, Pong SS, Chaung LY, Elbrecht A, Dashkevicz M, Heavens R, Rigby M, Sirinathsinghji DJ, Dean DC, Melillo DG, Patchett AA, Nargund R, Griffin PR, Demartino JA, Gupta SK, Schaeffer JM, Smith RG, Van der Ploeg LH. A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science* 1996; **27**: 974–977.

92. Petersenn S, Rasch AC, Penshorn M, Beil FU, Schulte HM. Genomic structure and transcriptional regulation of the human growth hormone secretagogue receptor. *Endocrinology* 2001; **142**: 2649–2659.

93. McKee KK, Palyha OC, Feighner SD, Hreniuk DL, Tan CP, Phillips MS, Smith RG, Van der Ploeg LH, Howard AD. Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors. *Mol Endocrinol* 1997; **11**: 415–423.

94. Dass NB, Munonyara M, Bassil AK, Hervieu GJ, Osbourne S, Corcoran S, Morgan M, Sanger GJ. Growth hormone secretagogue receptors in rat and human gastrointestinal tract and the effects of ghrelin. *Neuroscience* 2003; **120**: 443–453.

95. Gaytan F, Barreiro ML, Caminos JE, Chopin LK, Herington AC, Morales C, Pinilla L, Paniagua R, Nistal M, Casanueva FF, Aguilar E, Dieguez C, Tena-Sempere M. Expression of ghrelin and its functional receptor, the type 1a growth hormone secretagogue receptor, in normal human testis and testicular tumors. *J Clin Endocrinol Metab* 2004; **89**: 400–409.

96. Gaytan F, Morales C, Barreiro ML, Jeffery P, Chopin LK, Herington AC, Casanueva FF, Aguilar E, Dieguez C, Tena-Sempere M. Expression of growth hormone secretagogue receptor type 1a, the functional ghrelin receptor, in human ovarian surface epithelium, mullerian duct derivatives and ovarian tumors*. *J Clin Endocrinol Metab* 2005; **90**: 1798–1804.

97. 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. Stomach is a major source of circulating ghrelin, and feeding state determines plasma ghrelin-like immunoreactivity levels in humans. *J Clin Endocrinol Metab* 2001; **86**: 4753–4758.

98. Tschop M, Wawarta R, Riepl RL, Friedrich S, Bidlingmaier M, Landgraf R, Folwaczny C. Post-prandial decrease of circulating human ghrelin levels. *J Endocrinol Invest* 2001; **24**: RC19–RC21.

99. Rigamonti AE, Pincelli AI, Corra B, Viarengo R, Bonomo SM, Galimberti D, Scacchi M, Scarpini E, Cavagnini F, Muller EE. Plasma ghrelin concentrations in elderly subjects: comparison with anorexic and obese patients. *J Endocrinol* 2002; **175**: R1–R5.

100. Barkan AL, Dimaraki EV, Jessup SK, Symons KV, Ermolenko M, Jaffe CA. Ghrelin secretion in humans is sexually dimorphic, suppressed by somatostatin, and not affected by the ambient growth hormone levels. *J Clin Endocrinol Metab* 2003; **88**: 2180–2184.

101. Otto B, Cuntz U, Fruehauf E, Wawarta R, Folwaczny C, Riepl RL, Heiman ML, Lehnert P, Fichter M, Tschop M. Weight gain decreases elevated plasma ghrelin concentrations of patients with anorexia nervosa. *Eur J Endocrinol* 2001; **145**: 669–673.

102. Hansen TK, Dall R, Hosoda H, Kojima M, Kangawa K, Christiansen JS, Jorgensen JO. Weight loss increases circulating levels of ghrelin in human obesity. *Clin Endocrinol* 2002; **56**: 203–206.

103. Korbonits M, Goldstone AP, Gueorguiev M, Grossman AB. Ghrelin-a hormone with multiple functions. *Front Neuroendocrinol* 2004; **25**: 27–68.

104. Nakagawa E, Nagaya N, Okumura H, Enomoto M, Oya H, Ono F, Hosoda H, Kojima M, Kangawa K. Hyperglycaemia suppresses the secretion of ghrelin, a novel growth-hormone-releasing peptide: responses to the intravenous and oral administration of glucose. *Clin Sci* 2002; **103**: 325–328.

105. Anderwald C, Brabant G, Bernroider E, Horn R, Brehm A, Waldhausl W, Roden M. Insulin-dependent modulation of plasma ghrelin and leptin concentrations is less pronounced in type 2 diabetic patients. *Diabetes* 2003; **52**: 1792–1798.

106. Shiiya T, Nakazato M, Mizuta M, Date Y, Mondal MS, Tanaka M, Nozoe S, Hosoda H, Kangawa K, Matsukura S. Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 2002; **87**: 240–244.

107. Yildiz BO, Suchard MA, Wong ML, McCann SM, Licinio J. Alterations in the dynamics of circulating ghrelin, adiponectin, and leptin in human obesity. *Proc Natl Acad Sci USA* 2004; **101**: 10434–10439.

108. Kohno D, Gao HZ, Muroya S, Kikuyama S, Yada T. Ghrelin directly interacts with neuropeptide-Y-containing neurons in the rat arcuate nucleus: Ca2+ signaling via protein kinase A and N-type channel-dependent mechanisms and cross-talk with leptin and orexin. *Diabetes* 2003; **52**: 948–956.

109. Banks WA, Tschop M, Robinson SM, Heiman ML. Extent and direction of ghrelin transport across the blood-brain barrier is determined by its unique primary structure. *J Pharmacol Exp Ther* 2002; **302**: 822–827.

110. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Wakabayashi I. Chronic central infusion of ghrelin increases hypothalamic neuropeptide Y and Agouti-related protein mRNA levels and body weight in rats. *Diabetes* 2001; **50**: 2438–2443.

111. Toshinai K, Date Y, Murakami N, Shimada M, Mondal MS, Shimbara T, Guan JL, Wang QP, Funahashi H, Sakurai T, Shioda S, Matsukura S, Kangawa K, Nakazato M. Ghrelin-induced food intake is mediated via the orexin pathway. *Endocrinology* 2003; **144**: 1506–1512.

112. Sun Y, Ahmed S, Smith RG. Deletion of ghrelin impairs neither growth nor appetite. *Mol Cell Biol* 2003; **23**: 7973–7981.

113. Date Y, Nakazato M, Murakami N, Kojima M, Kangawa K, Matsukura S. Ghrelin acts in the central nervous system to stimulate gastric acid secretion. *Biochem Biophys Res Commun* 2001; **280**: 904–907.

114. Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, Lillard JW Jr, Taub DD. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest* 2004; **114**: 57–66.

115. Tschop M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature* 2000; **407**: 908–913.

116. Cummings DE, Frayo RS, Marmonier C, Aubert R, Chapelot D. Plasma ghrelin levels and hunger scores in humans initiating meals voluntarily without time- and food-related cues. *Am J Physiol Endocrinol Metab* 2004; **287**: E297–E304.

117. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, Dhillo WS, Ghatei MA, Bloom SR. Ghrelin enhances appetite and increases food intake in humans. *J Clin Endocrinol Metab* 2001; **86**: 5992–5995.

118. Tassone F, Broglio F, Destefanis S, Rovere S, Benso A, Gottero C, Prodam F, Rossetto R, Gauna C, van der Lely AJ, Ghigo E, Maccario M. Neuroendocrine and metabolic effects of acute ghrelin administration in human obesity. *J Clin Endocrinol Metab* 2003; **88**: 5478–5483.

119. Schmid DA, Held K, Ising M, Uhr M, Weikel JC, Steiger A. Ghrelin stimulates appetite, imagination of food, GH, ACTH, and cortisol, but does not affect leptin in normal controls. *Neuropsychopharmacology* 2005; **30**: 1187–1192.

120. Asakawa A, Inui A, Fujimiya M, Sakamaki R, Shinfuku N, Ueta Y, Meguid MM, Kasuga M. Stomach regulates energy balance via acylated ghrelin and desacyl ghrelin. *Gut* 2005; **54**: 18–24.

121. St-Pierre DH, Wang L, Tache Y. Ghrelin: a novel player in the gut-brain regulation of growth hormone and energy balance. *News Physiol Sci* 2003; **18**: 242–246.

122. 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. 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* 2004; **89**: 3885–3889.

123. De Smet B, Depoortere I, Moechars D, Swennen Q, Moreaux B, Cryns K, Tack J, Buyse J, Coulie B, Peeters TL. Energy homeostasis and gastric emptying in ghrelin knockout mice. *J Pharmacol Exp Ther* 2006; **316**: 431–439.

124. Sun Y, Wang P, Zheng H, Smith RG. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. *Proc Natl Acad Sci USA* 2004; **101**: 4679–4684.

125. Lonnqvist F, Arner P, Nordfors L, Schalling M. Overexpression of the obese (ob) gene in adipose tissue of human obese subjects. *Nat Med* 1995; **1**: 950–953.

126. Considine RV, Considine EL, Williams CJ, Nyce MR, Magosin SA, Bauer TL, Rosato EL, Colberg J, Caro JF. Evidence against either a premature stop codon or the absence of obese gene mRNA in human obesity. *J Clin Invest* 1995; **95**: 2986–2988.

127. Caro JF, Kolaczynski JW, Nyce MR, Ohannesian JP, Opentanova I, Goldman WH, Lynn RB, Zhang PL, Sinha MK, Considine RV. Decreased cerebrospinal-fluid/serum leptin ratio in obesity: a possible mechanism for leptin resistance. *Lancet* 1996; **348**: 159–161.

128. Li W-D, Reed DR, Lee JH, Xu W, Kilker RL, Sodam BR, Price RA. Sequence variants in the 5-prime flanking region of the leptin gene are associated with obesity in women. *Ann Hum Genet* 1999; **63**: 227–234.

129. Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. *Nat Genet* 1998; **18**: 213–215.

130. 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. A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature* 1998; **392**: 398–401.

131. Baessler A, Hasinoff MJ, Fischer M, Reinhard W, Sonnenberg GE, Olivier M, Erdmann J, Schunkert H, Doering A, Jacob HJ, Comuzzie AG, Kissebah AH, Kwitek AE. Genetic linkage and association of the growth hormone secretagogue receptor (ghrelin receptor) gene in human obesity. *Diabetes* 2005; **54**: 259–267.

132. Carlsson B, Lindell K, Gabrielsson B, Karlsson C, Bjarnason R, Westphal O, Karlsson U, Sjostrom L, Carlsson LMS. Obese (ob) gene defects are rare in human obesity. *Obesity Res* 1997; **5**: 30–35.

133. Matsuoka N, Ogawa Y, Hosoda K, Matsuda J, Masuzaki H, Miyawaki T, Azuma N, Natsui K, Nishimura H, Yoshimasa Y, Nishi S, Thompson DB, Nakao K. Human leptin receptor gene in obese Japanese subjects: evidence against either obesity–causing mutations or association of sequence variants with obesity. *Diabetologia* 1997; **40**: 1204–1210.

134. Bing C, Ambye L, Fenger M, Jorgensen T, Borch-Johnsen K, Madsbad S, Urhammer SA. Large-scale studies of the Leu72Met polymorphism of the ghrelin gene in relation to the metabolic syndrome and associated quantitative traits. *Diabet Med* 2005; **22**: 1157–1160.

135. Wang HJ, Geller F, Dempfle A, Schauble N, Friedel S, Lichtner P, Fontenla-Horro F, Wudy S, Hagemann S, Gortner L, Huse K, Remschmidt H, Bettecken T, Meitinger T, Schafer H, Hebebrand J, Hinney A. Ghrelin receptor gene: identification of several sequence variants in extremely obese children and adolescents, healthy normal-weight and underweight students, and children with short normal stature. *J Clin Endocrinol Metab* 2004; **89**: 157–162.

136. Heymsfield SB, Greenberg AS, Fujioka K, Dixon RM, Kushner R, Hunt T, Lubina JA, Patane J, Self B, Hunt P, McCamish M. Recombinant leptin for weight loss in obese and lean adults: a randomized, controlled, dose-escalation trial. *JAMA* 1999; **282**: 1568–1575.

137. Hukshorn CJ, Saris WH, Westerterp-Plantenga MS, Farid AR, Smith FJ, Campfield LA. Weekly subcutaneous pegylated recombinant native human leptin (PEG-OB) administration in obese men. *J Clin Endocrinol Metab* 2000; **85**: 4003–4009.

138. Westerterp-Plantenga MS, Saris WH, Hukshorn CJ, Campfield LA. Effects of weekly administration of pegylated recombinant human OB protein on appetite profile and energy metabolism in obese men. *Am J Clin Nutr* 2001; **74**: 426–434.

139. Sahu A. Resistance to the satiety action of leptin following chronic central leptin infusion is associated with the development of leptin resistance in neuropeptide Y neurones. *J Neuroendocrinol* 2002; **14**: 796–804.

140. Van Heek M, Compton DS, France CF, Tedesco RP, Fawzi AB, Graziano MP, Sybertz EJ, Strader CD, Davis HR Jr. Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J Clin Invest* 1997; **99**: 385–390.

141. Levin BE, Dunn-Meynell AA, Banks WA. Obesity-prone rats have normal blood-brain barrier transport but defective central leptin signaling before obesity onset. *Am J Physiol Regul Integr Comp Physiol* 2004; **286**: R143–R150.

142. Baskin DG, Seeley RJ, Kuijper JL, Lok S, Weigle DS, Erickson JC, Palmiter RD, Schwartz MW. Increased expression of mRNA for the long form of the leptin receptor in the hypothalamus is associated with leptin hypersensitivity and fasting. *Diabetes* 1998; **47**: 538–543.

143. Wilson BD, Ollmann MM, Barsh GS. The role of agouti-related protein in regulating body weight. *Mol Med Today* 1999; **5**: 250–256.

144. Hubschle T, Thom E, Watson A, Roth J, Klaus S, Meyerhof W. Leptin-induced nuclear translocation of STAT3 immunoreactivity in hypothalamic nuclei involved in body weight regulation. *J Neurosci* 2001; **21**: 2413–2424.

145. El-Haschimi K, Pierroz DD, Hileman SM, Bjorbaek C, Flier JS. Two defects contribute to hypothalamic leptin resistance in mice with diet-induced obesity. *J Clin Invest* 2000; **105**: 1827–1832.

146. Bjorbaek C, Elmquist JK, Frantz JD, Shoelson SE, Flier JS. Identification of SOCS-3 as a potential mediator of central leptin resistance. *Mol Cell* 1998; **1**: 619–625.

147. Carpenter LR, Farruggella TJ, Symes A, Karow ML, Yancopoulos GD, Stahl N. Enhancing leptin response by preventing SH2–containing phosphatase 2 interaction with Ob receptor. *Proc Natl Acad Sci USA* 1998; **95**: 6061–6066.

148. Lam NT, Covey SD, Lewis JT, Oosman S, Webber T, Hsu EC, Cheung AT, Kieffer TJ. Leptin resistance following over-

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expression of protein tyrosine phosphatase 1B in liver. *J Mol Endocrinol* 2006; **36**: 163–174.

149. Howard JK, Cave BJ, Oksanen LJ, Tzameli I, Bjorbaek C, Flier JS. Enhanced leptin sensitivity and attenuation of diet-induced obesity in mice with haploinsufficiency of Socs3. *Nat Med* 2004; **10**: 734–738.

150. Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, Ghatei MA, Small C, Bloom SR. Ghrelin increases food intake in obese as well as lean subjects. *Int J Obes Relat Metab Disord* 2005; **29**: 1130–1136.

151. Lall S, Balthasar N, Carmignac D, Magoulas C, Sesay A, Houston P, Mathers K, Robinson I. Physiological studies of transgenic mice overexpressing growth hormone (GH) secretagogue receptor 1A in GH-releasing hormone neurons. *Endocrinology* 2004; **145**: 1602–1611.

152. Zhang JV, Ren PG, Avsian-Kretchmer O, Luo CW, Rauch R, Klein C, Hsueh AJ. Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. *Science* 2005; **310**: 996–999.

153. Lo KM, Zhang J, Sun Y, Morelli B, Lan Y, Lauder S, Brunkhorst B, Webster G, Hallakou-Bozec S, Doare L, Gillies SD. Engineering a pharmacologically superior form of leptin for the treatment of obesity. *Protein Eng Des Sel* 2005; **18**: 1–10.

154. Rosenbaum M, Goldsmith R, Bloomfield D, Magnano A, Weimer L, Heymsfield S, Gallagher D, Mayer L, Murphy E, Leibel RL. Low-dose leptin reverses skeletal muscle, autonomic, and neuroendocrine adaptations to maintenance of reduced weight. *J Clin Invest* 2005; **115**: 3579–3586.

155. Perreault M, Istrate N, Wang L, Nichols AJ, Tozzo E, Stricker-Krongrad A. Resistance to the orexigenic effect of ghrelin in dietary-induced obesity in mice: reversal upon weight loss. *Int J Obes Relat Metab Disord* 2004; **28**: 879–885.

156. Kobelt P, Helmling S, Stengel A, Wlotzka B, Andresen V, Klapp BF, Wiedenmann B, Klussmann S, Monnikes H. Anti-ghrelin SPIEGELMER NOX-B11 inhibits neurostimulatory and orexigenic effects of peripheral ghrelin in rats. *Gut* 2006; **55**: 788–792.

157. Holst B, Schwartz TW. Constitutive ghrelin receptor activity as a signaling set-point in appetite regulation. *Trends Pharmacol Sci* 2004; **25**: 113–117.

158. Asakawa A, Inui A, Kaga T, Katsuura G, Fujimiya M, Fujino MA, Kasuga M. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* 2003; **52**: 947–952.

159. Halem HA, Taylor JE, Dong JZ, Shen Y, Datta R, Abizaid A, Diano S, Horvath TL, Culler MD. A novel growth hormone secretagogue-1a receptor antagonist that blocks ghrelin-induced growth hormone secretion but induces increased body weight gain. *Neuroendocrinology* 2005; **81**: 339–349.

160. Zabolotny JM, Bence-Hanulec KK, Stricker-Krongrad A, Haj F, Wang Y, Minokoshi Y, Kim YB, Elmquist JK, Tartaglia LA, Kahn BB, Neel BG. PTP1B regulates leptin signal transduction in vivo. *Dev Cell* 2002; **2**: 489–495.

161. Havel PJ, Townsend R, Chaump L, Teff K. High-fat meals reduce 24-h circulating leptin concentrations in women. *Diabetes* 1999; **48**: 334–341.

162. Heini AF, Lara-Castro C, Schneider H, Kirk KA, Considine RV, Weinsier RL. Effect of hydrolyzed guar fiber on fasting and postprandial satiety and satiety hormones: a double-blind, placebo-controlled trial during controlled weight loss. *Int J Obes Relat Metab Disord* 1998; **22**: 906–909.

163. Groschl M, Knerr I, Topf HG, Schmid P, Rascher W, Rauh M. Endocrine responses to the oral ingestion of a physiological dose of essential amino acids in humans. *J Endocrinol* 2003; **179**: 237–244.

164. Monteleone P, Bencivenga R, Longobardi N, Serritella C, Maj M. Differential responses of circulating ghrelin to high-fat or high-carbohydrate meal in healthy women. *J Clin Endocrinol Metab* 2003; **88**: 5510–5514.

165. Erdmann J, Lippl F, Schusdziarra V. Differential effect of protein and fat on plasma ghrelin levels in man. *Regul Pept* 2003; **116**: 101–107.

166. Greenman Y, Golani N, Gilad S, Yaron M, Limor R, Stern N. Ghrelin secretion is modulated in a nutrient- and gender-specific manner. *Clin Endocrinol* 2004; **60**: 382–388.

167. Nedvidkova J, Krykorkova I, Bartak V, Papezova H, Gold PW, Alesci S, Pacak K. Loss of meal-induced decrease in plasma ghrelin levels in patients with anorexia nervosa. *J Clin Endocrinol Metab* 2003; **88**: 1678–1682.

168. Makimura H, Mizuno TM, Mastaitis JW, Agami R, Mobbs CV. Reducing hypothalamic AGRP by RNA interference increases metabolic rate and decreases body weight without influencing food intake. *BMC Neurosci* 2002; **3**: 18.

169. Belsham DD, Cai F, Cui H, Smukler SR, Salapatek AM, Shkreta L. Generation of a phenotypic array of hypothalamic neuronal cell models to study complex neuroendocrine disorders. *Endocrinology* 2004; **145**: 393–400.