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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.

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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 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.

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).

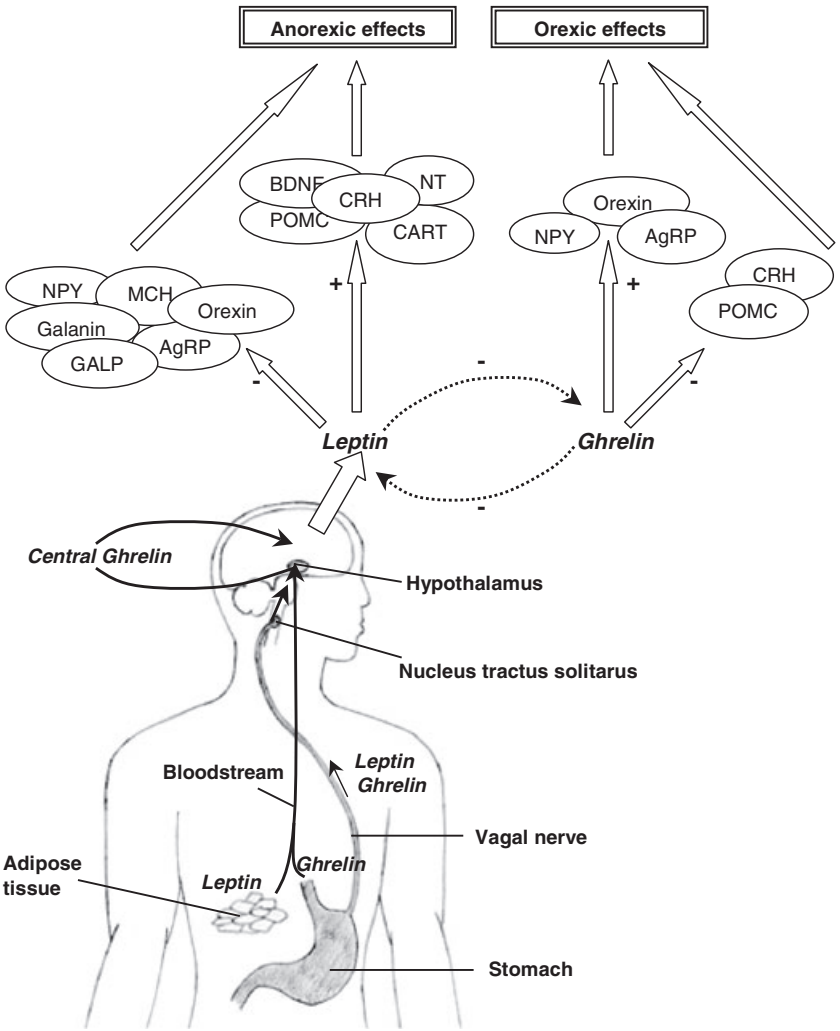

Figure 1 Pathways by which leptin and ghrelin may have effect on energy balance in humans. This schematic drawing shows the pathways by which leptin and ghrelin may reach the hypothalamus, in order to have an effect on food intake and body weight. Leptin is secreted by adipose tissue and ghrelin is secreted 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 resistance. 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. Unfortunately, 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.

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