The Role of Ghrelin and Leptin in Obesity: Is Exogenous Administration of These Hormones a Possible Drug Therapy?

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The Role of Ghrelin and Leptin in Obesity: Is Exogenous Administration of these Hormones a Possible Drug Therapy?

PERI ECKSTEIN

ABSTRACT

Ghrelin and leptin 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, playing a role in meal initiation. As a growing number of people suffer from obesity, understanding the mechanisms by which various hormones and neurotransmitters influence energy balance has been a subject of intense research. This paper provides background on 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. The role of gut hormones on hunger and satiety as well as the effect of sleep deprivation on these hormones will be briefly described. Finally, the potentials of leptin and ghrelin as drug targets will be described (Klok, et al., 2006).

INTRODUCTION

The incidence of obesity has increased dramatically worldwide. The prevalence of obesity is a major health problem because excessive body weight is a risk factor for development of chronic diseases such as cardiovascular disease and type II diabetes mellitus (Park, 2010). Overweight is a result of a deregulation of calorie intake and energy expenditure. An individual’s genetics and the environment create a system that controls appetite and energy expenditure. Throughout the past few decades the type and cost of food has changed. The food industry encourages people to eat fast foods which are relatively inexpensive but high in calories. Snacks and beverages high in sugar have also been added to the daily diet. Physiologically, the body secretes hormones which are responsible for controlling appetite and satiety. Genetic defects in this control system manifest itself in obesity (Skelton & Rudolph, 2007). The overwhelming percentage of overweight people created the need to develop new treatments. In pursuit of this goal, researchers have dissected the mechanism through which satiety and hunger manifests (Jayasena & Bloom, 2008). Further research has led to the discovery of gut hormones and the role of adipose tissue as participants in controlling the body’s physiologic and pathologic processes (De Luis, et al., 2008).

Body weight is regulated by a complex system. Two hormones that play an important role in the regulation of food intake and body weight are leptin and ghrelin. Both hormones are part of the peripheral nervous system and signal the brain, specifically the arcuate nucleus (ARC) of the hypothalamus, through different pathways. In the hypothalamus, activation of leptin and ghrelin

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receptors set off different signals leading to changes in food intake (Klok, et al., 2006). One of the pathways consists of neurons that express neuropeptide Y (NPY) and agouti gene related protein (AgRP), stimulators of food intake. Other arcuate neurons express proopiomelanocortin (POMC), cocaine and amphetamine-regulated transcript (CART), which suppress food intake (Nogueiras, et al., 2004).

DISCUSSION

Leptin and its Functions

The human obese (OB) gene and its product leptin were discovered in 1994. The OB gene is on chromosome 7 (Klok, et al., 2006). It encodes leptin, a protein consisting of 167 amino acids which is secreted primarily from adipocytes. Leptin is also secreted by skeletal muscles, the placenta, and the stomach (Feldman, Freidman, & Brandt, 2010). Leptin acts through the leptin receptor (OBR). The OBR gene is located on the first chromosome and encodes a protein consisting of 1162 amino acids. OB-Rb, a part of the OBR gene, is expressed in the hypothalamus and the cerebellum. Leptin functions through a feedback mechanism that signals regulatory centers in the brain to inhibit food intake and to regulate body weight and energy homeostasis (Klok, et al., 2006). Studies on rodents have shown that the hypothalamus is the primary center for body weight and food intake regulation. After leptin is released by adipose tissue, it crosses the blood brain barrier and binds to the hypothalamic leptin receptors, conveying information regarding the status of body energy stores. Leptin inhibits the expression of orexigenic, or appetite stimulating neuropeptides, and stimulates anorexigenic, or appetite inhibiting neuropeptides in the arcuate nucleus of the hypothalamus (Neary, et al., 2003).

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Figure 1.

When leptin is secreted, it activates anorexigenic peptides proopiomelanocortin and cocaine and amphetamine-regulated transcript neurons and inhibits orexigenic peptides neuropeptide Y and agouti-related protein neurons which results in inhibition of eating and an increase in energy expenditure (Nogueiras, et al., 2004).

Leptin is involved in different signaling pathways in the hypothalamus. Leptin is a member of the cytokine family of signaling molecules. There are different forms of leptin receptors throughout the body. The “long form” leptin receptor is a type of cytokine-receptor, which is located in the hypothalamic nuclei. Leptin binds to and activates Janus Kinase (JAK-2), tyrosine kinases involved in intracellular cytokine signaling, beginning a positive feedback mechanism. Activation of JAK-2 leads to phosphorylation of members of the signal transduction and transcription family of proteins (STAT). As a result, STAT proteins activate transcription of leptin target genes (Figure 2). The “long form” of leptin receptor is required for normal energy homeostasis. Studies have shown that mutations of this gene result in obesity in rodents (Kronenberg, et al., 2008).
Leptin also activates other pathways in the hypothalamus. Leptin activates the insulin receptor substrate phosphatidylinositol-3 OH kinase (IRS PI 3-Kinase) pathway in the hypothalamus which is also required for leptin regulation of food intake. Leptin also increases hypothalamic mammalian target of rapamycin (mTOR) activity, an enzyme which regulates growth. This pathway is required for leptin-induced anorexia, or lack of appetite. Signaling through these pathways in the hypothalamus is necessary for leptin to function to reduce food intake and body weight.

In addition, the “long form” of leptin receptor is expressed in the hippocampus, hindbrain, and mesolimbic areas. The mesolimbic areas are involved in reward and include the ventral tegmental area (VTA) and the substantia nigra. This leads to the possibility that leptin may reduce food intake through direct action on brain motivation/reward circuitry, in addition to the action of leptin on areas of the brain involved with energy homeostasis (Morton, et al., 2009).

A study was performed to examine the mechanism by which leptin signaling in the VTA reduces food intake (Morton, et al., 2009). Two questions arose: Firstly, does leptin activate the Janus Kinase - signal transduction and transcription family of proteins, insulin receptor substrate phosphatidylinositol-3 OH kinase, and hypothalamic mammalian target of rapamycin signal transduction pathways in the ventral tegmental area and substantia nigra as it does in the hypothalamus? Secondly, is activation of these pathways required for VTA leptin-induced anorexia? The data collected suggested that JAK-2 signaling in the ventral tegmental area is activated by leptin. JAK-2 is required for anorexia induced by VTA leptin action, but leptin signaling of IRS-PI 3-kinase and mTOR do not participate in ventral tegmental area leptin induced anorexia.
The experiment was performed on adult male Wistar rats held in a temperature controlled room and provided with ad libitum access to water and food. A stainless steel cannula was placed in the third cerebral ventricle in the animals to examine the signal transduction pathways activated by intracerebroventricular administration of leptin. In experiments with intra ventral tegmental area injections, a bilateral stainless steel cannula was implanted to the ventral tegmental area. The rats had 7 days to recover after the surgery, and daily intake of food and body weight was recorded. An hour before the injection of recombinant mouse leptin into the VTA, the food was removed. A dosage of 0.05, 0.25, or 0.50 micrograms was administered. To determine the role of JAK-STAT signaling in VTA leptin action, the leptin injection was counterbalanced with either a pretreatment of JAK-2 inhibitor or, as a control, its vehicle (a substance of no therapeutic value used to convey an active medicine for administration).

It had been demonstrated previously that this inhibitor blocks the central effect of leptin to reduce food intake and body weight. The food was then replaced. Similar injections were administered to determine the role of insulin receptor substrate phosphatidylinositol-3 OH kinase and hypothalamic mammalian target of rapamycin on leptin action in the ventral tegmental area.

The results showed that bilateral intra VTA leptin administration in rats reduced food intake by 25%. The reduction of food intake was accompanied with a decrease in body weight in the animals that received leptin as opposed to the control group. In the case of the JAK-STAT pathway, it was found that central administration of leptin activated the JAK-STAT pathway in the ventral tegmental area and the substantia nigra. To determine if leptin acts directly in the VTA to activate the pathway, leptin was delivered directly into the VTA. It was noted that intra-VTA administration of leptin induced tyrosine phosphorylation of STAT3, a mediator of leptin receptor signaling in this part of the brain. However, intra-VTA pretreatment of the JAK-2 inhibitor, when administered alone blocked leptin in the VTA from reducing food intake and decreasing body weight.

The experiment continued to determine whether leptin in the VTA signals through the IRS-PI 3-Kinase pathway. The question to address was, does intracerebroventricular leptin or direct administration of leptin in the ventral tegmental area increase phospho protein kinase B, a marker of PI 3-Kinase activation? The result was that leptin administration failed to induce phospho protein kinase B in the VTA. An additional group of rats was used to determine if the mTOR pathway is affected by leptin action in the VTA. Neither intracerebroventricular nor direct intra-ventral tegmental area administration of leptin activated the mTOR signaling pathway in the VTA. Although the hypothalamus plays a major role in leptin signaling and activation, this experiment supports the growing body of evidence that leptin receptors are expressed in extrahypothalamic sites as well, particularly the VTA (Morton, et al., 2009).

**Leptin and Obesity**

Researchers suggest that obesity in humans is due to leptin resistance. Leptin resistance is thought to involve a period of overeating. Overeating results in an increase in leptin levels, which may damage the hypothalamus. As a result, the hypothalamus becomes less sensitive to leptin, leading to a sustained increase in leptin levels (Klok, et al., 2006).

**The Role of Ghrelin**

Ghrelin plays a central role in weight regulation. In 1999, attempts to identify the endogenous ligand for the growth hormone secretagogue receptor (GHS-R) led to the discovery of ghrelin (Higgins, et al., 2007). The gene that codes for human prepro-ghrelin is located on chromosome 3.
Prepro-ghrelin consists of 117 amino acids, and the mature ghrelin constitutes 28 amino acids, with a fatty acid chain on the third amino acid. Ghrelin peptide was first isolated in the stomach and is most abundant in the gastric fundus, but is also found in the pancreas and adrenal cortex. Ghrelin is produced by endocrine cells known as P/D1 cells that are categorized as opened or closed. The open cells are exposed to the lumen of the stomach and are in contact with gastric contents. The closed type is in close proximity to the capillary network of the lamina propria. Both types of cells secrete the hormone into the bloodstream (Feldman, et al., 2010). In the brain, ghrelin producing neurons are found in the pituitary, hypothalamic arcuate nucleus, and between the dorsal, ventral, paraventricular, and arcuate hypothalamic nucleus (Klok, et al., 2006).

Ghrelin stimulates growth hormone secretion, increases food intake, and produces weight gain. Ghrelin is a member of the motilin family of peptides, stimulates gastric contraction and enhances stomach emptying (Feldman, et al., 2010). Ghrelin levels show preprandial (prior to meals) increase and postprandial (after meals) decreases. Ghrelin levels are also low in obesity, while high during fasting and in anorexia nervosa. The postprandial reduction of ghrelin levels is smaller in obese subjects than in subjects of normal weight. This explains how ghrelin leads to excessive eating. First, the diminished reduction of postprandial ghrelin levels increases the length of time that subjects feel hungry. Second, because of relatively higher ghrelin levels, the speed of gastric emptying may not be reduced, which causes a lack of feeling satisfied. Without feelings of satiety, obese individuals eat more than they need and gain weight. Furthermore, ghrelin levels are influenced by age, gender, BMI, growth hormone, glucose, and insulin. Leptin also affects ghrelin levels. Ghrelin plays a central role in neurohormonal regulation of food intake and energy homeostasis.

The expression of orexigenic peptides in the hypothalamus regulates eating behavior. Obesity may develop from deregulation of these pathways. Intravenous and central administration of ghrelin stimulates hunger and food intake. Ghrelin levels are decreased in obese subjects, which are thought to be the body’s response to reduce hunger and food intake in those with excessive energy balance.

In the following study, the diets of healthy lean men were supplemented with a moderate amount of fat-rich food, to establish the effect of overfeeding on plasma ghrelin levels. Six men between the ages 21-34 with a BMI of 21.4-24.3 were included in the study. Over a period of three weeks, the participating students visited the lab for 8 experimental visits, 4 visits for postprandial fat loading tests and 4 visits for the measurement of gastric emptying. The day before postprandial testing, subjects were given a low fat dinner, and refrained from exercise. The day of the testing, an oral fat tolerance test consisting of 125 ml of dairy cream was administered and blood samples were taken at 30 minute intervals for 2 hours and then at 4 and 6 hours postprandially. The same preparation was done the day before gastric emptying measurement: a low fat dinner, and no exercise the night before. In the morning of the testing, subjects consumed 500 ml of water. A nasogastric tube was positioned. A 300 ml intralipid was injected into the stomach over a period of 2 minutes. The contents of the stomach were mixed by aspiration and 20-30 ml was reinjected 10 times using a catheter tip syringe. This cycle continued for 60 minutes. Following the initial tests, subjects were provided with a high fat dietary supplement to be eaten daily for 3 weeks. Subjects were tested on days 7, 14, and 21 for postprandial testing, and on days 8, 15, and 22 for gastric emptying measurements. Blood samples were collected to analyze plasma fatty acids, leptin, ghrelin, and pancreatic polypeptide. During the study, the mean weight gain was 2.1 kg. Results showed that the inhibitory response of ghrelin to oral fat was enhanced after overfeeding. Before
the study, maximal suppression of ghrelin occurred 90 minutes postprandially. After only one week into the study, the maximal suppression occurred at 120 minutes. Leptin concentration was increased after the 3 week dietary supplementation. Triglyceride concentration was also changed by dietary supplementation. It has been suggested that ghrelin levels respond to changes in body weight and not to changes in energy supplied to the GI tract. However, these results imply that changes in ghrelin concentration begin before significant increases in body weight, since a weight gain of 3% was associated with an 18% decrease in ghrelin concentration in the study.

The Mechanism of Action of Ghrelin

Ghrelin is considered a natural growth hormone secretagogue (GHS), or a substance that stimulates the secretion of growth hormone. Generally, the administration of growth hormone secretagogues, which are synthetic peptide and nonpeptide compounds, stimulates the release of growth hormone. Administration of growth hormone secretagogues synthetic peptide also stimulates feeding behavior. Growth hormone secretagogues receptor (GHS-R) is expressed in the pituitary gland, hypothalamus, and several other areas. In the hypothalamus, GHS-R mRNA is expressed in neuropeptide Y, agouti-related protein, proopiomelanocortin, and growth hormone releasing hormone (GHRH) containing neurons.

Similarly, research shows that ghrelin administration increases food intake in rodents. An experiment was done on male Sprague–Dawley rats, weighing 250-280 grams. They were given food and water ad libitum. A stainless steel cannula was implanted into the right lateral ventricle. Only the rats whose cerebrospinal fluid flowed into the cannula were used. The rats were injected

**Figure 3.** These two diagrams show the result of the above experiment, indicating that the primary hypothalamic target of ghrelin are NPY and AGRP, proving that ghrelin is an orexigenic peptide (Kamegai, et al., 2001).
with the peptide 14 days after the cannula was placed. Rat ghrelin or saline was injected every 12 hours over a 72 hour period. Food intake and body weights were measured daily. Around 4 hours after the last ghrelin injection, the rats were killed. The blood was taken to test glucose, insulin, leptin, and growth hormone levels. The brains were preserved, and then coronal sections were cut and mounted onto slides. The results revealed an increase in food intake; however, they did not affect plasma insulin, glucose, leptin, and GH concentrations. Figure 3 shows that neuropeptide Y mRNA levels were increased, as well as arcuate nucleus agouti-related protein mRNA levels in ghrelin treated rats. However the levels of proopiomelanocortin and growth hormone releasing hormone mRNA in the arcuate nucleus did not differ between the ghrelin treated and saline treated rats. These results show that the primary hypothalamic targets of ghrelin are neuropeptide Y and agouti-related protein neurons and that ghrelin is an orexigenic peptide in the brain and stomach. The fact that ghrelin administration did not alter GHRH levels or the release of growth hormone indicates that the orexigenic action of ghrelin is not likely to be due to GH secretion (Kamegai, et al., 2001).

In contradiction to this experiment, experiments on Zucker rats led to the conclusion that ghrelin may be GH dependent (Nogueiras, et al., 2004). The purpose of this study was to investigate a number of properties affecting ghrelin.

1. Leptin may alter the expression of Growth hormone secretagogues receptor, thereby inhibiting the effect of ghrelin.
2. The effect of leptin administration on the expression of Growth hormone secretagogues receptor in the arcuate nucleus and ventromedial nuclei.
3. The influence of ghrelin administration on Growth hormone secretagogues receptor expression.
4. The influence of ghrelin on the expression of leptin receptors Ob-Rb.
5. The role of growth hormone in the effect of ghrelin.

The rats used were male between the ages of 8 and 12 weeks. For short term ghrelin and leptin effects, intracerebroventricular cannulas were implanted in the lateral ventricle of the rats. One group of rats was fed ad libitum, and the other group was deprived of food for 48 hours. The rats were then injected with a single injection of ghrelin or its vehicle. In a second experiment, rats were injected with 0.5, 2.0, or 5.0 microgram of rat ghrelin or its vehicle. In both cases, the rats were killed 2 hours after the injection. In another experiment, rats were fed ad libitum or fasted for 48 hours. A single injection of recombinant human leptin or its vehicle was given, and the animals were killed 2 hours after. For long term ghrelin effects, a cannula was placed in the lateral ventricle. Rats received ghrelin or its vehicle for 24 hours, 48, hours, or 7 days. For leptin testing, either human recombinant leptin or its vehicle was infused for a period of 7 days into the lateral ventricle. The last 48 hours of the infusion, the rats were either fed ad libitum or fasted.

The results showed the following. The GHS-R expression was higher in the arcuate nucleus of the obese Zucker rats than the lean rats, but the expression in the ventromedial nuclei did not differ significantly. Plasma ghrelin levels were unchanged in the fatty rats in comparison to the lean ones. The GHS-R mRNA of the arcuate nucleus in fasted rats was higher than in ad libitum fed rats. No change was seen in its expression in the ventromedial nuclei.

The effect of leptin on GHS-R differed for short and long term infusions. A single injection of leptin did not affect the level of GHS-R expression in the arcuate nucleus or ventromedial nuclei in the fed animals. However, the long term infusion of leptin decreased food intake and body weight. Additionally, long term infusion of leptin caused a decrease in GHS-R levels in the ARC
but not in the ventromedial nuclei in both ad libitum and fasted animals. The effect of leptin on GHS-R was dose dependent. No change was seen at 1 microgram/day, but at 5 and 15 microgram/day, GHS-R expression was lower.

Ghrelin also had a significant effect on GHS-R expression in the ARC. After short-term treatment with ghrelin, GHS-R mRNA levels increased in the arcuate nucleus. The administration of ghrelin caused an increase in food intake. The increase in GHS-R gene expression was also seen in the fasting animals. No change was seen in the ventromedial nuclei in any of the treatments. The effect of ghrelin seemed to be dose-dependent: gene expression of GHS-R was higher after 5 micrograms compared with 2 micrograms. In long term infusion of ghrelin, GHS-R mRNA was only significantly increased after 1 week and not after 24 hours or 48 hours.

Interestingly, short term treatment of ghrelin did not increase GHS-R mRNA levels in the arcuate nucleus of growth hormone deficient rats. It was concluded that ghrelin and leptin are both involved in the regulation of GHS-R in the arcuate nucleus, but not in the ventromedial nuclei. Intracerebroventricular ghrelin increases GHS-R expression while intracerebroventricular leptin decreases GHS-R mRNA. During fasting (high levels of ghrelin and low levels of leptin) and in obese rats insensitive to leptin, GHS-R mRNA expression is increased in the arcuate nucleus. GHS-R mRNA seems to be regulated by ghrelin through a GH dependent mechanism since ghrelin fails to stimulate GHS-R expression in the absence of GH (Nogueiras, et al., 2004).

Ghrelin is released from the GI tract and provides input to the brain through three mechanisms.

1. Directly, via the blood stream, by entering the anterior pituitary gland and other areas of the brain not protected by the blood brain barrier.
2. Directly, by crossing the blood brain barrier, via a saturated transport system.
3. Indirectly, via the vagus nerve. Ghrelin levels rise before a meal due to the reduced inhibitory controls of the vagus nerve on ghrelin.

Ghrelin acts in the arcuate nucleus, a part of the brain important in the regulation of feeding and appetite. Growth hormone secretagogues receptors are found in the arcuate nucleus on neurons that release neuropeptide Y and agouti-related protein, stimulators of weight gain, where ghrelin acts to increase the output from these neurons. GHS can be found on presynaptic nerve endings, influencing the release of neurotransmitters.

Ghrelin promotes appetite in two ways. It can depolarize the orexigenic neuropeptide Y and agouti-related protein neurons, or it can increase the inhibition exerted by the NPY and AgRP neurons over the anorexigenic proopiomelanocortin and cocaine and amphetamine-regulated transcript neurons. Both of these enhance appetite (Higgins, et al., 2007). Ghrelin stimulates the activity of neurons expressing neuropeptide Y, agouti related protein, and orexin. On the other hand, ghrelin inhibits proopiomelanocortin neurons and corticotrophin releasing hormone producing neurons (Figure 4) (Klok, et al., 2006).

**Gherelin and Obesity**

Ghrelin functions as a meal-initiation signal in the system for short term energy balance regulation. This is demonstrated by two things. Firstly, the preprandial increase in ghrelin levels initiates meals voluntarily even in the absence of food. Secondly, an intravenous injection of ghrelin induces hunger and food intake in healthy and obese humans (Klok, et al. 2006). In addition to the involvement of ghrelin in short term regulation of energy balance, it also plays a role in long term energy balance regulation. Ghrelin concentration is negatively correlated with BMI. Ghrelin
levels change in response to dieting to maintain body weight. This is seen in obese and anorexic subjects. When obese people lose weight, these levels increase, and they decrease when anorexic patients gain weight (Klok, et al. 2006).

**Gut Hormones and their Effect on appetite**

Although ghrelin is the only gut hormone that stimulates food intake, there are other hormones in the GI tract that have an effect on hunger and satiety (Jayasena & Bloom, 2008). In 1982, Peptide YY (PYY) was isolated from colonic extracts. Peptide YY is a 36 amino acid peptide with tyrosine residue at both the C and the N terminals. PYY is secreted from the endocrine L cells of the small and large bowel. Two forms of peptide YY exist, PYY 1-36 and PYY 3-36. The maximum concentration is found in the rectum, while lower concentrations are found in the small intestine, terminal ileum, and colon. In contrast to ghrelin, PYY levels are suppressed in a fasting state, but increase after a meal. PYY levels in the body are lowest in the morning, and steadily increase throughout the day, reaching a peak after the evening meal (Neary, et al., 2003). Peptide YY is released into the blood following a meal, in proportion to the calorie intake. PYY acts on the hypothalamus as a satiety signal. It has multiple effects that slow the passage of nutrients through the gut. PYY decreases food intake by delaying gastric emptying, and inhibiting gallbladder contraction, pancreatic exocrine secretions, and gastric acid secretion (Jayasena & Bloom, 2008.)
Peptide YY reduces the appetite stimulating hormone ghrelin, and diminishes the preprandial rise in ghrelin. Low levels of peptide YY in obese subjects point to PYY deficiency contributing to obesity (Popovic & Duntas, 2005).

Another gut hormone involved in the increase or decrease of food intake is cholecystokinin (CCK). CCK is a peptide hormone expressed in the small intestine and is released after a meal. Cholecystokinin stimulates pancreatic and gallbladder exocrine secretions, inhibits gastric emptying, and increases intestinal mobility. Cholecystokinin also acts as a neurotransmitter. Injections of cholecystokinin decrease the amount eaten but increase meal frequency without any change in body weight. However, because the ability of cholecystokinin to reduce food intake does not last long, it is not the best target as a drug to treat obesity (Jayasena & Bloom, 2008).

Ghrelin and Leptin as Drug Therapy

Because of the increasing cases of obesity throughout the world, scientists found a need to find an effective drug therapy to aid in weight loss. This has led to extensive research on recombinant hormones, as well as lipase inhibitors as possible paths to increase or decrease ghrelin and leptin as needed.

Current Drug Therapy For Obesity

Pharmacotherapy options to treat obesity are very limited. There are two weight loss medications that are approved in the United States and Europe, Orlistat® and Sibutramine®. Very recently, Rimonabant® was approved in some European counties. The FDA recommends pharmacotherapy only after dieting and exercise have failed and the BMI is greater than 30kg/m². Orlistat® (Figure 5) was approved by the FDA in 1999.

Orlistat® is a gastrointestinal lipase inhibitor. It decreases fat absorption by binding to pancreatic lipase, which blocks hydrolyses of triglycerides into fatty acids and monoglycerides, and increases fat excretion in fecal material by 30%. In clinical trials, weight loss was 3% greater in subjects taking Orlistat® compared to those taking the placebo. Orlistat® has gastrointestinal side effects such as oily fecal splotting, fecal urgency, abdominal pain, and fecal incontinence. In addition, Orlistat causes a loss of fat soluble vitamins.

Sibutramine® (Figure 6) was approved by the FDA in 1997. It inhibits serotonin and norepinephrine reuptake. Sibutramine® was originally created as an antidepressant and reduces food intake by reducing appetite. It has been demonstrated, that long term Sibutramine® along with a reduced calorie diet resulted in weight loss over a period of 1-2 years. In clinical trials, weight loss was 5% greater in subjects taking Sibutramine® than those taking the placebo. Maximum weight loss was achieved after six months, and was dose related. 10 mg/day resulted with an average of 7.4 kg compared to those taking the placebo.
with 3.6 kg for the placebo, and 15 mg/day resulted in 10.3 kg weight loss compared with 1.3 kg in the placebo group. Because Sibutramine® can increase blood pressure and heart rate, patients taking it are monitored carefully.

Although these anti obesity drugs do promote weight loss of 3-5% of initial body weight, most patients are still overweight or obese after treatment. In addition, they regain the weight lost after drug withdrawal. Therefore different strategies are being researched to achieve weight loss, such as stimulating anorexigenic signals and blocking orexigenic signals. Possible antiobesity therapy currently being investigated includes leptin receptor superagonists, neuropeptide Y receptor antagonists, peptide YY analogues, ghrelin receptor antagonists, and growth hormone receptor agonists (Isidro & Cordido, 2009).

A study was done comparing leptin and ghrelin levels in obese subjects who took Orlistat® and those only on a dietary regimen of 55% carbohydrates, 25% each of fat and protein over a span of 12 weeks. Twenty-one obese patients, 6 males and 15 females, and ten control subjects were involved in the experiment. The obese patients were divided into two groups: one group consisting of 11 people who took Orlistat® 3 times daily, while the second group of 10 received dietary treatment only. At the end of the 12 week period, there were decreases in BMI, weight, waist perimeter, glucose, insulin, and cholesterol in the Orlistat® group, while there was only a decrease in BMI, weight, and insulin levels in the dietary treatment group. Ghrelin levels were increased in both groups. Leptin levels were reduced in the Orlistat® experimental group, but did not decrease significantly in the dietary treatment group. The results of this study have shown that patients who change their lifestyle and eating habits have similar success rates as those who are treated with Orlistat®. The elevated leptin levels and reduced ghrelin in obesity are reversed by weight loss regardless of the type of treatment, Orlistat® vs. dietary treatment (Ozkan, et al., 2009). However, the above experiment does not have a proper control and experimental group. The group that was given Orlistat® was also on dietary treatment, making it difficult to discern whether the weight loss and change in BMI, glucose and insulin levels were due to changes in food habits or to the treatment of Orlistat®.

**Leptin As Pharmacotherapy**

Leptin directs the onset and termination of appetite and restraint between meals at two sites. At the first site, leptin opposes the orexigenic action of ghrelin directly and indirectly by restraining the output of excitatory neuropeptide Y signaling in the arcuate nucleus- paraventricular nucleus axis. Secondly, leptin suppresses ghrelin output from gastric glands to restrain appetite between meals. Deficiency in this complex system of leptin restraint leads to weight gain in humans and rodents. Several experiments were performed to try to prevent an insufficiency of leptin with the goal that it will assist those with obesity.

One experiment was done with gene transfer technology with viral vectors. The non-replicative, non-immunogenic, and non-pathogenic vector, recombinant adeno-associated viral vector encoding the leptin gene was engineered for testing in rodents. It was found that a single intravenous injection increased circulating leptin and normalized body weight in obese leptin mutant mice. Similar effects were produced when it was injected centrally into either intracerebroventricularly or into hypothalamic sites on leptin mutant rats and mice (Kalra, 2007).

Another experiment was done to determine whether leptin administration affects body weight. As mentioned earlier, leptin signals regulatory centers in the brain to inhibit food intake and regulate body weight. Overeating can cause a lack of sensitivity to leptin receptors and signal-
ing resulting in obesity. This insensitivity is thought to be overcome by administration of exog-

A previous study had shown that a minimum of 3 injections daily of recombinant leptin
was required to have an effect; however, this dose was intolerable. Therefore, this study (Zelissen,
et al., 2005) was done to determine whether a lower dosage of leptin would be tolerated better. The
recombinant leptin would be injected at night to mimic the normal diurnal rhythms. Diurnal varia-
tion in endogenous leptin peaks between 12:00 and 2:00 AM, and is less pronounced in obese sub-
jects. It was hypothesized that diurnal variation could be improved by recombinant leptin admin-
istered once daily in the evening, which might result in greater weight loss. For 3 weeks, patients
with a BMI between 27.5 and <37.5 kg/m2 were given a 2100 calorie diet. All participants were
given 2-ml injections of a placebo. Those that lost more than 5 kg over this period were excluded
from the rest of the experiment. Patients were divided into three groups of 12 week treatment regi-
mens.
• Group 1: administered once daily in the morning; 70 patients with recombinant leptin and 25
with placebo.
• Group 2: administered once daily at night; 70 patients with recombinant leptin and 24
with placebo.
• Group 3: administered twice daily; 71 patient with recombinant leptin and 24 with placebo.

The results did not show overall significant changes between the placebo groups and the
leptin groups. Although only slight, the difference in weight change was greater in the twice daily
recombinant leptin and placebo than in the once daily doses. The weight loss of subjects with low
serum leptin levels was not much different from those with high serum leptin levels. Similarly,
feelings of hunger were compared between the groups, and no change was found. The same was
true for calorie intake from before the study and the end of the study. Other studies have shown
that when pegylated leptin was administered in addition to a restricted diet, greater weight loss was
observed (Zelissen, et al., 2005). As in Ozkan, et al.’s study (2009), more research has to be done
to conclude whether physical activity and diet restriction combined with leptin administration is a
valuable treatment for obesity.

Just as in the previous study done by Zelissen, et al., (2005) in which the use of recombi-
nant leptin was used to determine its affect on weight loss, the following study (Hukshorn, et al.,
2002) was done using pegylated recombinant leptin (PEG-OB) to examine if its administration
will decrease food intake and promote weight loss. Twenty-eight obese subjects, 16 women and
12 men, partook in the study. Subjects were between the ages of 18-65 years and women had to be
either sterile or postmenopausal. Anyone with a medical condition requiring pharmaceutical treat-
ment was excluded. After a 4 week lead in diet, only those who lost 1.75 kg or more were allowed
to continue treatment. Treatment consisted of 60 mg pegylated recombinant leptin or a matching
placebo, which was administered once a week over 8 weeks. In addition to the injections, subjects
were on a hypoenergetic diet designed to reduce energy intake by 3200kJ/day. Body weight and
height were measured weekly, as well as the BMI. Blood and urine were collected throughout the
study. The results after 8 weeks were similar in the pegylated recombinant leptin group and the pla-
cebo. Both groups lost about the same amount of weight, and no real difference in change of BMI
between the two groups was recorded. There was also no significant difference in the decrease
of cholesterol, triglycerides, glucose and insulin levels between the treatment groups. However,
pegylated recombinant leptin levels and total leptin levels showed an increase a week after the last
dose. These results show that exposure to 60 mg of PEG-OB weekly for 8 weeks did not influence
weight loss in obese subjects, even though pegylated recombinant leptin levels were elevated after the study. Although this dosage of PEG-OB was ineffective, it may be possible that when PEG-OB is administered during a severe energy restriction or total leptin deficiency, it will cause weight loss. In addition, the small number of subjects studied, as well as the short duration of the study, may also explain the lack of effectiveness of pegylated recombinant leptin administration (Hukshorn, et al., 2002). It is interesting that the pegylated recombinant leptin did not produce a weight loss, while gene transfer in a viral vector did contribute to an increase in leptin levels and weight loss.

The Effect of Sleep on Ghrelin and Leptin

Average sleep duration in the United States has declined over the past several decades. At the same time, obesity rates have increased. Studies indicate that sleep may regulate hunger and satiety through hormones including ghrelin and leptin. Previous studies have indicated that sleep deprivation leads to an increase in ghrelin and a decrease in leptin, which affects appetite and hunger. It is hypothesized that sleep brings about these changes by changes in the hypothalamic control of autonomic nervous system activity which influence ghrelin and leptin secretion.

A study was done (Littman, et al., 2007) on moderate to intense physically active post menopausal women to establish the relationship between exercise, sleep, BMI, ghrelin, leptin, and weight change over a 12 month period. Before the experiment, the scientists hypothesized what the outcome would be.

1. Improvement in sleep might lead to greater weight loss by influencing satiety and hunger hormones.
2. Exercise induced decreases in leptin and increases in ghrelin would be greater in those whose sleep worsened as compared to those whose sleep remained the same or improved.

Participants in the study were aged 50-75 years who exercised less than 60min/week of moderate or vigorous activity. Participant’s BMI had to be greater than 25.0 kg/m2. The exercise group had 87 subjects, while the control group contained 86. Blood was taken after a 12 hour overnight fast at the baseline, 3 months, and 12 months. Participants in the experimental group performed a minimum of 45 minutes of moderate to intense aerobic exercise 5 days a week for 12 months. Those in the control group attended a 45 minute session of stretching once a week for a year. Subjects in both groups maintained their usual diet. The results revealed that the average BMI in women who slept more than 9 hours per night was higher than that of women who slept 8 or fewer hours per night. Ghrelin levels increased during the 12 month trial among both groups. This increase was mainly due to weight loss. The difference in ghrelin levels was greater in those who experienced improved sleep as opposed to those whose sleep remained the same. Leptin decreased more among the exercise group than the control group. Leptin increased with worsening of sleep quality in the experimental group. The difference in leptin levels in both groups was less among those with improved sleep quality. The observed trends were in the opposite direction of the proposed hypothesis. The weight loss difference between the groups was greater for those who slept less. Ghrelin levels increased and leptin levels decreased among those with improved sleep compared with those with decreased sleep quality. In addition, exercise induced weight loss was greater in those who slept < 6 hours per night in direct opposition to the hypothesis that improved sleep would increase weight loss (Littman, et al., 2007).

On the other hand, recent studies have shown that short sleep duration not only increases BMI, but also reduces circulating leptin levels which suppress appetite and increase ghrelin levels.
that promote hunger. Experiments on subjects who slept 4 hours in comparison to 10 hours for two consecutive nights revealed that they experienced decreased leptin levels and increased ghrelin levels, overall increasing feelings of hunger and appetite. To further determine the influence of short term sleep curtailment on leptin and ghrelin secretion, fasting morning levels of these hormones were taken from healthy men after nights of different amounts of sleep.

The study was done (Schmid, et al., 2008) on nine men whose ages ranged from 20-40 years. The BMI of these subjects fell between 20.7 and 25 kg/m². Subjects had normal sleep cycles and were not following any particular diet. Average sleep was 7-8 hours with bedtime between 10:00pm-1:00am and waking time 6:00-8:00am. Subjects were examined after three conditions, spaced 2 weeks apart. 1) a night of total sleep deprivation. 2) a sleep restriction to 4.5 hours during the first part of the night. 3) a night with 7 hours of sleep. Participants in the study did not drink caffeine for 10 hours before and alcohol for 24 hours before the experiment. In addition, subjects were told to avoid intense physical activity. Blood samples were taken, and then subjects rated their feelings of hunger on a scale from 0 (not at all) to 9 (severely).

Results showed (Figure 7) that subjects had stronger feelings of hunger after total sleep deprivation than after 7 hours or 4.5 hours of sleep. The difference between 7 and 4.5 hours in hunger ratings was not significant. Ghrelin levels were $22 \pm 10\%$ higher after total sleep deprivation than after 7 hours of sleep. After 4.5 hours of sleep, ghrelin levels were increased $11 \pm 5\%$. Interestingly, leptin levels were completely unaffected by sleep restriction or deprivation. A single night of sleep deprivation increased feelings of hunger and plasma levels of the hunger-promoting hormone ghrelin (Schmid, et al., 2008). There are still factors that have to be taken into account and further researched. What would be the effect of sleep deprivation and restriction on women? In addition, this study was done only over a short period of time and after one night of each category of sleep restriction. The question still remains, what effect will long term sleep deprivation have on ghrelin levels as well as leptin levels?

In the study done by Littman, et al. (2007), leptin levels did decrease in those that experienced less sleep, while in the study done by Schmid, et al., (2008), leptin levels were unaffected by sleep change. It is possible that the physical activity done by subjects in the former experiment was the driving factor behind the change in leptin levels, and not actual change in sleep. It is unclear, since sleep and exercise were combined into one experiment. Furthermore, the changes in ghrelin levels show an opposite trend in the two studies. In Littman, et al., (2007), ghrelin levels increased with improved sleep, while in Schmid, et al., (2008), ghrelin levels increased when subjects experienced less sleep.

Obstructive sleep apnea syndrome, (OSAS) is a common disease associated with obese subjects. The actual effect of obstructive sleep on ghrelin and leptin levels is further complicated...
by the following study whose results seem to differ from the research done by Littman, et al. (2007) and Schmid, et al. (2008). The subjects of the study were 65 obese men with obstructive sleep apnea syndrome. Subjects underwent polysomnography overnight to measure the degree of obstructive sleep. All subjects had fasting blood samples taken between 7:00-8:00 AM. Cholesterol, triglyceride, leptin, and ghrelin levels were tested. Thirty of the 65 obese subjects had moderate to severe obstructive sleep apnea syndrome. Twenty two subjects did not have OSAS and became the control group. The results showed that leptin levels were higher in patients with moderate-severe obstructive sleep apnea syndrome compared to the control group. There was no difference in ghrelin levels between the two groups; however, ghrelin levels were correlated with BMI, while leptin levels were not (Ciftci, et al., 2005). This study is another factor pointing to the relationship of sleep, obesity, and corresponding ghrelin and leptin levels.

CONCLUSION

Leptin and ghrelin both play major roles in energy balance in humans. It is unclear whether abnormalities in the leptin or ghrelin systems contribute to the development of obesity. However, disturbances in these systems do maintain obesity. Obese patients are leptin resistant, and it is therefore necessary to develop a treatment that overcomes it or bypasses normal leptin functioning. Ghrelin also is seen as a potential drug target for weight regulation, as obese patients are ghrelin-sensitive. Diet and exercise have significant effects on energy homeostasis. The use of therapeutic drugs alone is not sufficient to treat obesity. As seen from a number of experiments, the most effective treatment is provided by a combination of diet and exercise. The best strategy to accomplish long term changes in body weight is the use of potential anti-obesity agents in combination with a low fat diet and sufficient exercise.

REFERENCES


