Leptin: The Satiety Hormone and its Influence on Obesity

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Abstract

The Ob gene, which is known to encode the 16 kDa protein hormone leptin, is one of the main genes that has been linked to the obesity phenotype in humans. Examination of Ob gene expression, as well as leptin's mode of action in the hypothalamus through an intracellular signaling cascade, reveals the complexities of weight regulation (Friedman & Halaas, 1998). Single point mutations in the Ob gene can produce nonfunctional leptin protein due to the disruption of key intramolecular features, resulting in a chronic obesity phenotype (Farooqi et al., 1998; Hager et al., 1998). Current modes of therapy being explored include gene therapy using recombinant adenoviruses, which serve as vectors for leptin cDNA, as well as direct leptin injections (Friedman & Halaas, 1998). While both approaches to correcting leptin deficiency have shortcomings and require further research to improve their effectiveness, they demonstrate potential means of correcting specific metabolic disorders. Such breakthroughs are essential, given the prevalence of obesity in the United States alone and the health threat obesity poses by increasing predisposition to other life threatening conditions (Pi-Sunyer, 2002; Ogden et al., 2008).

Introduction

One of the most prevalent health issues that continues to affect a growing number of Americans in the twenty-first century is obesity. To be considered overweight, an individual must have a BMI of 25-29.9, while an obese individual has a BMI ≥ 30; these designations are determined based on weight ranges that are greater than what is generally considered healthy for a given height (Pi-Sunyer, 2002; Ogden et al., 2008). With the rise of fast food, television, video games, and lifestyles that either promote sedentary tendencies or leave little time for tending to one's personal health, it is no wonder that over the past 50 years the number of overweight and obese individuals has risen to 64% of adults and 25 million children in the U.S. (Ogden et al., 2008). Along with the societal stigmatism of being obese and the limitations it poses for an individual in his or her ability to participate in certain activities, a number of other health conditions can result, such as coronary heart disease, type II diabetes, cancer (including endometrial, breast, and colon), hypertension, dyslipidemia, stroke, liver disease, gallbladder disease, sleep apnea, respiratory problems (asthma), osteoarthritis, and reproductive problems in women (abnormal menses and infertility) (Pi-Sunyer, 2002; Ogden et al., 2008). Therefore, the exploration of different means to combat obesity has been brought to the forefront of medicine over the past decade.

As reported by Pi-Sunyer (2002), obesity not only has an environmental/behavioral component (60-70%) but a genetic component (30-40%) as well. When obesity is the result of environmental/behavioral influences, it is possible to reverse the effects with drastic changes in lifestyle. However, for individuals that are genetically predisposed to weight gain, it is more difficult to control body mass.

According to the most recent update of the Obesity Gene Map, which examined the genetic mutations associated with 176 varying cases of obesity, there are 11 different genes that have been linked to weight gain and fat retention (Rankinen et al., 2006). One of the most well known is the obesity (Ob) gene located on chromosome 7, which codes for the "satiety hormone" leptin (Friedman & Halaas, 1998). Leptin is a 16 kDa protein hormone secreted at high levels by white adipose tissue and at low levels in gastric epithelium and the placenta (Friedman & Halaas, 1998). It appears to aid in the regulation of energy intake and expenditure by curbing appetite and speeding up metabolism (Friedman & Halaas, 1998). When genetic mutations result in abnormal, nonfunctional leptin, individuals have a tendency to gain weight. Therefore, understanding how leptin functions is crucial to understanding one of the common genetic factors that can lead to obesity.

The Discovery of Leptin

Before much research had been completed on the causes of obesity and the discovery of leptin, three main theories existed regarding the way in which the body could potentially regulate body weight: a thermoregulation theory, where maintenance of a basal body temperature through energy expenditure influences weight; a glucostatic theory, where plasma glucose regulates all energy stores; and a lipostatic theory, where a product of fat metabolism circulates in the blood and interacts with certain receptors to either burn or maintain fat stores (Castracane & Henson, 2007). Of the three theories, the lipostatic theory most closely resembles the manner in which leptin functions as a peripheral signal in a feedback loop system. It was first proposed by Coleman (1978) through his studies on obesity and diabetes that the Ob gene encoded a hormone and the Db gene encoded its receptor. It was not until 1994, however, that Friedman's lab at Rockefeller University was able to identify leptin as the product of the Ob gene and linked it back to obesity (Friedman, 1996; Castracane & Henson, 2007). Initially, Friedman et al. (1991) used positional cloning to verify the identity of the Ob gene and found that it encoded a 4.5 kB RNA sequence for the known protein hormone leptin.

Using two strains of mutant mice that expressed a chronically obese phenotype, the ob/ob mouse (production of ineffective leptin protein) and the db/db mouse (production of ineffective leptin receptor), Friedman (1996) and his research team demonstrated that leptin directly influenced weight loss (Castracane & Henson, 2007). Specifically, the ob/ob mice were injected with recombinant mouse leptin, which reduced body weight by 40% after four weeks (Friedman, 1996). This was attributed to an observed reduction in food intake by the mice, as well as an increase in metabolism. Similarly, in wild type mice that were injected with recombinant mouse leptin, there was a sustained 12% weight loss (Friedman, 1996). This demonstrated that leptin served as a means to regulate body fat stores because reintroducing the functional leptin protein to mutant ob/ob mice essentially reversed the chronic obesity phenotype, and in wild type mice leptin also appeared to instigate weight loss.

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The Protein Hormone Leptin

Leptin on the Genomic Level

The Ob gene is exclusively expressed by white adipose tissue, as well as at low levels from gastric epithelium and placental tissue (Freidman & Halaas, 1998). Therefore, leptin is not produced in direct response to food ingestion. Rather, it appears to function in the long-term regulation of body weight (Friedman & Halaas, 1998).

The Ob gene, located on band 31.3 of the long arm of chromosome 7, consists of three exons and two introns that span 18 kb pairs (Gong et al., 1996). Transcriptional regulation of the Ob gene consists of the complex interplay of promoter regions and regulatory elements in response to a number of hormones, proteins, and glucocorticoids. A TATA promoter is located 28 base pairs from the transcriptional start codon (Gong et al., 1996). Additionally, a C/EBP and CCAAT/enhancer binding protein region has been identified, along with a GRE, glucocorticoid response elements, and a CREB, cAMP response element-binding protein (Gong et al., 1996).

Of the proposed molecules that aid in transcriptional regulation, the role of dexamethasone, a glucocorticoid, and insulin have been most widely researched. As confirmed in several studies, transcription of the Ob gene is stimulated by dexamethasone, most likely due to its interaction with GRE regions of the gene (Bradley & Cheatham, 1999; Halleux et al., 1998). However, insulin has no effect on the transcriptional activity of the Ob gene (Bradley & Cheatham, 1999; Halleux et al., 1998). Additional information regarding the synthesis of leptin in its transcriptional and translational pathways is not currently known. However, it was noted by Cohen (1996) that leptin does not undergo any posttranslational modification because the relative mass of the functional protein is roughly equal to its proposed mass, based on the primary structure of the 167 amino acid protein.

Secretion of Leptin and its Role as an Afferent Signal in the CNS

After mature leptin has been produced within adipose cells, some of the protein hormone is released to circulate at basal levels in the plasma and the rest is stored in the endoplasmic reticulum of these cells (Bradley & Cheatham, 1999). Two molecules that stimulate the secretion of stored leptin from adipose cells are dexamethasone and insulin, both of which can elicit up to a twofold increase in leptin plasma levels (Bradley & Cheatham, 1999).

Regardless of when leptin is secreted by adipose cells into the surrounding plasma, it initiates a response pathway by binding to leptin receptors, Ob-R, located primarily in different nuclear regions of the hypothalamus (Friedman & Halaas, 1998; Mercer et al., 1996; Spanswick et al. 1997). A study by Satoh et al.(1997) confirmed that the hypothalamus was an integral part of the leptin signaling pathway by comparing the phenotype of mice with hypothalamic lesions to ob/ob and db/db mice. Significant weight gain was observed in the mice with hypothalamic lesions (Satoh et al., 1997). This conclusion was additionally supported by the results obtained by Halaas et al. (1997), who injected mice both intracerebroventricularly and peripherally with leptin. A decrease in food intake was seen in mice injected intracerebroventricularly but not peripherally, and it took larger doses of peripherally administered leptin to achieve the same level of adipose tissue reduction (Halaas et al., 1997). These results indicate that the hypothalamic region in the brain is the main initiation site for leptin action.

There are five alternatively spliced forms of the Ob-R gene, which result in different leptin receptors: Ob-Ra, Ob-Rb, Ob-Rc, Ob-Rd, and Ob-Re (Friedman & Halaas, 1998). A member of the cytokine family, the leptin receptor protein has both an intracellular and extracellular region that includes two ligand binding domains (Friedman & Halaas, 1998). Of the five isoforms of leptin receptors, the function of only Ob-Ra and Ob-Rb has been examined. Ob-Ra has been implicated in aiding in the transport of leptin across the blood-brain barrier, while Ob-Rb is the hypothalamic receptor leptin that binds to initiate signaling pathways to regulate body weight (Friedman & Halaas, 1998; Mercer et al., 1996; Spanswick et al. 1997).

In order for the Ob-Rb receptor to become activated after leptin is bound, Jak2, an intracellular signaling kinase, must undergo phosphorylation (Friedman & Halaas, 1998). Jak2 then actively phosphorylates STAT at a tyrosine residue, promoting dimerization of STAT monomers. This phosphorlyated STAT dimer is subsequently actively transported into the nucleus, where it binds to inducible promoter regions of specific genes to activate their transcription. In the leptin signaling pathway, neuropeptides, such as melanocyte stimulating hormone (MSH), melanocortin-4 receptor (MC-4), agouti-related transcript (ART), and corticotropin-releasing hormone (CRH), are transcribed (Friedman & Halaas, 1998; Mercer et al., 1996; Spanswick et al. 1997). These neuropeptides act to decrease food intake, increase energy expenditure through

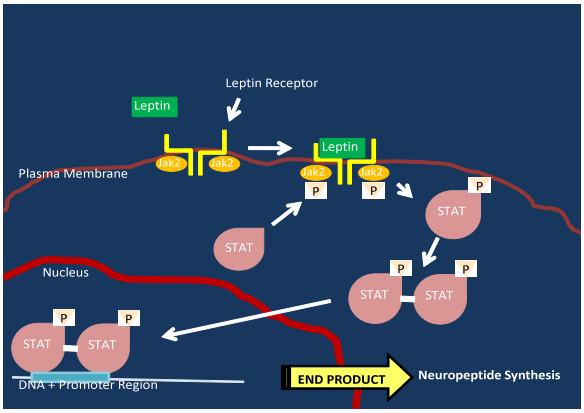

Figure 1 (Blue). Transcriptional signaling pathway of neuropeptides initiated by binding of leptin to Ob-Rb in hypothalamus.

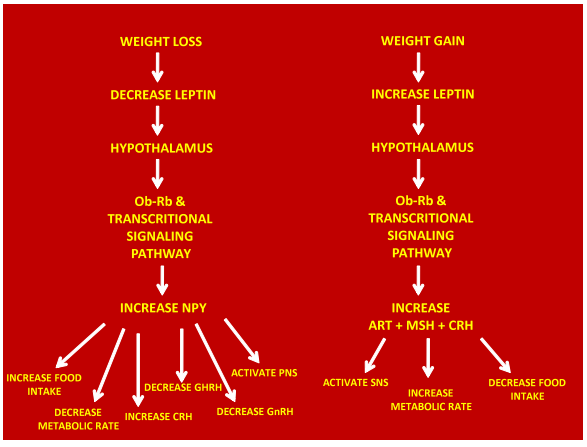

Figure 2 (Red). Flow diagram of biological response to differing leptin levels. (Figures modified from Friedman & Halaas, 1998.)

metabolic processes, and activate sympathetic nervous system function. Therefore, the end result of increased leptin levels ultimately is the reduction of body mass.

Alternatively, in response to little or no leptin present in plasma, transcription of neuropeptide Y (NPY) and its receptor are upregulated through a similar signaling pathway (Friedman & Halaas, 1998). NPY, in turn, increases food intake, decreases energy expenditure by slowing metabolism, activates the parasympathetic nervous system, increases CRH, as well as decreases growth hormone releasing hormone and gonadotropin-releasing hormone (Friedman & Halaas, 1998). Essentially, NPY functions to decrease the amount of energy expended by limiting growth and reproduction, while maintaining "resting state" body functions and increasing energy input. It is currently unclear why NPY is not transcriptionally activated when leptin is circulating at higher levels in the plasma. It has been suggested, however, that higher levels of leptin inhibit NPY or that other neuropeptides produced outweigh the effects of NPY (Friendman & Halaas, 1998).

Mutations in Ob Gene Lead to Low Leptin Plasma Levels and Obesity

Many cases of obesity are often related to the insensitivity of Ob-R receptors to plasma levels of leptin; however, mutations in the Ob gene can result in lower levels of functional leptin and lead to obesity in individuals as well. This observation was demonstrated by Ravussin et al. (1997) in linking chronic obesity in Pima Indians to low levels of leptin. While DNA analysis was not completed in this study to trace lower levels of leptin to a specific mutation in the Ob gene, other studies indicate that this phenotype can be attributed to recessive, autosomally-inherited mutations (Farooqi et al., 1998; Hager et al., 1998).

In one study conducted by Hager et al. (1998), a single point mutation (an adenine to guanine substitution) at position 19 on exon 1 of the Ob gene led to decreased levels of leptin in blood plasma, resulting in obesity. This mutation was seen in two separate Caucasian populations with a high rate of penetrance (Hager et al., 1998). In a separate study completed by Farooqi et al. (1998), a deletion of a guanine nucleotide was detected through genetic analysis in a number of test subjects. This deletion, in turn, disrupted the reading frame of the Ob gene so that 14 aberrant amino acids and a premature stop codon replaced the regular coding sequence in the resulting protein (Farooqi et al., 1998). This mutant leptin protein lacked the C-terminal cysteine necessary for intramolecular disulfide binding. Thus, the mutant leptin protein hormone lacked any biological activity, causing the manifestation of the obesity phenotype.

Treatments for Obesity by Increasing Leptin Levels

Gene Therapy

For individuals that experience a reduction in leptin levels due to mutations in the Ob gene that render the protein nonfunctional, the most direct means of increasing leptin is to alter its expression on the genomic level. The most promising approach in correcting metabolic disorders is through gene therapy using recombinant adenoviruses. Several studies have used these types of vectors to carry cDNA for leptin to induce hyperleptinemia in rats (Chen et al., 1996; Muzzin et al., 1996). In a study conducted by Chen et al. (1996), hyperleptinemia was induced in an experimental group of rats containing no leptin-related mutations. Throughout the experiment and at the end of 28 days, plasma leptin levels, food intake, and weight gain/loss in relation to adipose tissue were examined in the experimental group of rats injected with the recombinant adenovirus. In rats expressing the recombinant leptin protein, leptin plasma levels were approximately four times greater than in control rats, which were normal in Ob gene expression and not injected with recombinant leptin (Chen et al., 1996). Chen et al. (1996) found that with an increase in leptin there was a 30-50% reduction in food intake, and on average there was only a 22 gram weight gain in adenovirus-infected rats in comparison to a 115-132 gram weight gain seen in control rats.

In a related experiment conducted by Muzzin et al. (1996), ob/ob mice were injected with the recombinant adenovirus expressing mouse leptin cDNA to examine whether the chronic obese phenotype could be reversed. Shortly after the administration of the recombinant adenovirus, a drastic reduction of food intake and weight was seen, as well as a normalization of insulin levels and glucose tolerance (Muzzin et al., 1996). After 2-3 weeks, when leptin levels began to decrease in the ob/ob mice injected with the recombinant adenovirus, the chronic obese phenotype resurfaced and mice increased their food intake, resulting in rapid weight gain (Muzzin et al., 1996). This occurrence verified that the functional adenovirus vector was effective in normalizing leptin levels.

The major disadvantage of this approach to therapy is that the duration of expression of adenovirally-expressed genes is limited. This is likely caused by an immune response that destroys the genetic material of these vectors due to specific viral-encoded genes that initiate a host immune response (Chen et al., 1996; Muzzin et al., 1996). Therefore, in the future, specific genes should be turned off to avoid the degradation of the vector. However, this approach to therapy seems promising because of the ability to correct both the obesity phenotype in ob/ob mice and the ability to return to the original obesity phenotype without any adverse effects (Muzzin et al., 1996).

Direct Administration of Leptin Effectively Reverses Obesity Phenotype

Increasing leptin levels through direct injections is another therapeutic method that has been examined in order to correct leptin deficiency as a result of mutations in the Ob gene (Christensen et al., 1999; Friedman & Halaas, 1998; Frühbeck, et al., 1998). Several studies have demonstrated that peripheral administration of leptin shows modest decreases in food intake, resulting in the reduction of adipose tissue mass. In one study conducted by Frühbeck, et al. (1998), ob/ob mice were injected intraperitoneally with doses of leptin. In comparison to ob/ob mice that did not receive the injection, the basal lipolytic activity was significantly increased to over 50% (Frühbeck, et al., 1998).

Using a larger animal model, the rhesus monkey, Christensen et al. (1999) compared the effectiveness of a peripheral versus a central injection of leptin. When leptin was administered peripherally, there was no observed decrease in food intake over the course of the three-day administration period (Christensen et al.,1999). However, when leptin was administered intracerebroventricularly, a sustained reduction of food intake (40-50%) was observed after day two of administration (Christensen et al.,1999). These findings suggest that centrally administered leptin is more effective in producing long-standing effects in the reduction of food intake. Similarly, in a clinical trial, subjects were given daily injections of leptin, and over the course of six months, a group of obese subjects lost 7.1kg in comparison to 1.7kg for a control group that was administered placebos (Friedman & Halaas, 1998). However, not all members of the obese group lost significant amounts of weight, indicating that peripheral leptin

administration is not always completely effective (Friedman & Halaas, 1998).

As indicated by these studies, peripheral leptin administration is not always entirely effective, which stands in contrast to intracerebroventricular injections. However, it would be implausible to administer leptin directly into the brain given the risks it would impose on the individual. Therefore, to improve this approach to therapy, it would be pertinent to consider localized leptin solubility, as well as potential local reactions that could occur at the site of injection that would alter its effectiveness to create an improved "leptin formula" for injections (Friedman & Halaas, 1998).

Conclusion

Given the prevalence of obesity and the numerous negative health effects that are associated with this phenotype, it is pertinent to examine the pathway of leptin in order to determine effective treatment options. Leptin functions through a complex mechanism involving receptors present in specific hypothalamic regions, which means that therapy options must somehow manipulate this pathway in order to be effective. Current treatment options, including both gene therapy and direct leptin injections, have proven to be modestly successful. However, both approaches present serious drawbacks, which would require further research to be completed. For gene therapy, it is necessary to alter the expression of specific viral genes within the adenovirus vectors to ensure that they are not rapidly degraded within the target organism due to an immune response. In order for leptin injections to be effective without being directly administered into brain regions, modifications that do not change the functionality of the protein, but rather the efficiency of its transport mechanism to receptors in the hypothalamus, would be necessary. Both types of therapy, however, show movement in the right direction regarding the battle against genetic predispositions to obesity.

References

Bradley, R.L. & Cheatham, B.. (1999). Regulation of ob Gene Expression and Leptin

Secretion by Insulin and Dexamethasone in Rat Adipocytes. Diabetes, 48, 272-278.

Castracane, V.D. & Henson, M.C. (2007). Leptin. Mineralogical Society Series, 25, 1-9.

Chen, G., Koyama, K., Yuan, X., Lee, Y., Zhou, Y., O'Doherty, R., Newgard, C.B., & Unger, R.H.. (1996). Disappearance of Body Fat in Normal Rats Induced by Adenovirus-Mediated Leptin Gene Therapy. Proceedings of the National Academy of Sciences, 93, 14795–14799.

Christensen, M., Havel, P.J., Jacobs, R.R., Larsen, P.J., & Cameron, J.L.. (1999). Central Administration of Leptin Inhibits Food Intake and Activates the Sympathetic Nervous
System in Rhesus Macaques. Journal of Clinical Endocrinology and Metabolism, 84(2), 711-717.

Cohen, S.L.. (1996). Characterization of Endogenous Leptin. Nature, 382, 589.

Coleman, D.L. (1978). Obese and Diabetes: Two Mutant Genes Causing Diabetes-Obesity Syndromes in Mice. Diabetologia, 14, 141-148.

Farooqi, S., Rau, H., Whitehead, J., & O'Rahilly, S.. (1998). Ob Gene Mutations and Human Obesity. Proceedings of the Nutrition Society, 57, 471-475.

Friedman, J.M. (1996). Leptin and the Control of Body Weight. Proceedings of the Nutrition Society of Australia, 20, 1-2.

Friedman, J.M. & Halaas, J.L.. (1998). Leptin and the regulation of body weight in mammals. Nature, 395, 763-770.

Friedman, J.M., Leibel, R.L., Siegel, D.A., Walsh, J., & Bahary, N.. (1991). Molecular Mapping of the Mouse Ob Gene. Genomics, 11, 1054-1062.

Frühbeck, G., Aguado, M., Gómez-Ambrosi, J., & Martínez, J.A. (1998). Lipolytic Effect of in Vivo Leptin Administration on Adipocytes of Lean and ob/ob Mice, but Not db/db Mice.
Biochemical and Biophysical Research Communications, 250(1), 99-102.

Gong, D., Bi, S., Pratley, R.E., & Weintraub, B.D.. (1996). Genomic Structure and Promoter Analysis of the Human obese Gene. The Journal of Biological Chemistry, 271(8), 3971-3974.

Hager, J., Clement, K., Francke, S., Dina, C., Raison, J., Lahlou, N., Rich, N., Pelloux, V., Basdevant, A., Guy-Grand, B., North, M., & Froguel, P.. (1998). A Polymorphism in the
5' Untranslated Region of the Human ob Gene is Associated with Low Leptin Levels. International Journal of Obesity, 22, 200-205.

Halaas, J.L., et al.. (1995). Physiological Response to Long-Term Peripheral and Central Leptin Infusion in Lean and Obese Mice. Proceedings of the National Academy of Science, 94, 8878-8883.

Halleux, C.M., Servais, I., Reul, B.A., Detry, R., & Brichard, S.M.. (1998). Multihormonal Control of ob Gene Expression and Leptin Secretion from Cultured Human Visceral Adipose Tissue: Increased Responsiveness to Glucocorticoids in Obesity. Journal of Clincal Endocrinology and Metabolism, 83(3), 902-910.

Mercer, J.G., Hoggard, N., Williams, L.M., Lawrence, B.C., Hannah, L.T., & Trayhurn, P. (1996). Localization (Ob-Rb) of Leptin Receptor mRNA and the Long Form Splice Variant in Mouse Hypothalamus and Adjacent Brain Regions by in situ Hybridization. Federation of European Biochemical Societies, 387, 113-116.

Muzzin, P., Eisensmith, R.C., Copeland, K.C., & Woo, S.L.C.. (1996). Correction of Obesity and Diabetes in Genetically Obese Mice by Leptin Gene Therapy. Proceedings of the National Academy of Sciences, 93, 14804–14808.

Ogden C.L., Carroll M.D., & Flegal K.M.. (2008). High Body Mass Index for Age Among US Children and Adolescents, 2003—2006. Journal of American Medical Association, 299(20), 2401—2405.

Pi-Sunyer, X.F. (2002). The Obesity Epidemic: Pathophysiology and Consequences of Obesity. Obesity Research, 10, 97S-104S.

Rankinen, T., Zuberi, A., Chagnon, Y.C., Weisnagel, S.J., Argyropoulos, G., Walts, B., Pe´russe, L., & Bouchard, C.. (2006). The Obesity Gene Map: The 2005 Update. Obesity, 14(4), 529-644.

Ravussin, E., Pratley, R.E., Maffei, M., Wang, H., Friedman, J.M., Bennett, P.H., & Bogardus, C.. (1997). Relatively Low Plasma Leptin Concentrations Precede Weight Gain in Pima
Indians. Nature Medicine, 3, 238-240.

Satoh, N. et al.. (1997). 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, 138, 947-954.

Spanswick, D., Smith, M.A., Groppi, V.E., Logan, S.D. & Ashford, M.L.J.. (1997). Leptin Inhibits Hypothalamic Neurons by Activation of ATP-Sensitive Potassium Channels. Nature, 390, 521-525.