Regulation of Body Weight by Leptin, with Special Reference to Hypoxia-induced Regulation

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Abstract

Since first cloned and reported by Zhang et al in 1994 (Nature 372:425), the obese gene and its product—leptin has been studied profoundly. Our knowledge in body weight regulation and the role played by leptin has increased substantially. Leptin serves as an adiposity signal to inform the brain of the adipose tissue mass in a negative feedback loop regulating food intake and energy expenditure. Many articles have reported weight loss at high altitude, but the explanation has been limited to loss of appetite. New ideas were highlighted after studies by Grosfeld et al and Ambrosini et al on the obese gene under hypoxia condition. Cells with hypoxia treatment upregulated obese gene transcription and suggested that enhancement of leptin secretion in vivo under hypoxia environment may be one of the potential therapeutic methods for obesity treatment.

Key words: obese gene; leptin, leptin receptor, hypoxic environment

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Introduction

In recent years, obesity and regulation of body weight are rapidly becoming the center of attention for public health experts in the industrialized world as well as in developing countries (1). In the Western countries, especial in America, about half the adult population is considered overweight as defined by body mass index [BMI, ratio of body weight (kg) divided by height squared (m²)] in excess of 25. Of these individuals, half are clinically obese (with a BMI in excess of 30). A more disturbing trend is the increased early onset of obesity in children. This alarming trend has prompted the World Health Organization to declare obesity a worldwide epidemic (2).

Obesity is a disorder characterized by increased mass of adipose tissue that results from a systemic imbalance between food intake and energy expenditure (3). And is a public health challenge because it is associated with many complications, including type 2 diabetes, hypertension, coronary heart disease, and increased mortality rate (4). Now, obesity, which has taken the place of diseases due to infection and cacotrophia, is becoming the main risk for human health.

Several genes that function in the control of body weight have been cloned. Of these, the adipose tissue-derived hormone leptin has emerged as a central player (5). Recently, research findings showed the obese (ob) gene contains hypoxia response element (HRE) site that can be bound by HIF-1 in 5´-untranslated region (6, 7). A possible new method for lowering weight has been found on the gene level, this has opened up broad possibilities for losing weight under hypoxia or living in highlands. Here, we introduce the structure and physiological function of ob gene, its regulation under hypoxia and the relationship between obesity and hypoxia.

Structure of Obese Gene

In 1994, the obese (ob) gene was first identified in genetic obesity mouse adipose tissue through a positional cloning approach by Zhang et al (5). The length of mouse ob gene is 2.9 kilobases (kb), with 501 base-pare (bp) opening frame and 3.7 kb untranslated region. Latter, Isse et al (8) identified the human ob gene spanned ~2.9 kb and contained three exons separated by two introns. The first intron, ~10.6 kb in size, occurred in the 5´-untranslated region, 29...
bp upstream of the ATG start codon. The second intron of 2.3 kb in size was located at glutamine +49. By rapid amplification of 5’-cDNA ends, the transcription initiation sites were mapped 54~57 bp upstream of the ATG start codon. The 172 bp 5’-flanking region of the human ob gene contained a TATA box-like sequence and several cis-acting regulatory elements (three copies of GC boxes, an AP-2-binding site, and a CCAAT/enhancer binding protein-binding site). By the fluorescence in situ hybridization technique, the ob gene was assigned to human chromosome 7q31.3. Then, Gong et al (9) found there are seven SP-1, three C/EBP, two CREB/cAMP response element-binding proteins, and a GRE/glucocorticoid response element site in the 5’-untranslated region. This study should establish the genetic basis for ob gene research in humans, thereby leading to the better understanding of the molecular mechanisms underlying the ob gene. Recently, scientists identified the ob gene contain the hypoxia response element (HRE) site in the 5’-flanking region, the core sequence is 5’-gcacgt-3’ and is located between -121~-116 bp. The studies showed that it was upregulated under the hypoxic condition and bound with HIF-1 (6, 7); further, a study indicated that it is a hypoxia inducible gene (10). Sequence analyzing showed the rat ob gene has 96% homology with that of mice and the nucleotide and protein was 84% and 82% homologous to those of humans, respectively (11).

**Leptin receptor**

The leptin receptor (LR), is a member of the class I cytokine receptor family. The extracellular (EC) domain is composed of two so-called cytokine receptor homology (CRH) domains, a membrane distal CRH1 and a membrane proximal CRH2. Both domains are separated by an immunoglobulin-like (Ig) domain and are followed by two fibronectin type III (FNIII) domains proximal to the membrane. The CRH2 domain is necessary for leptin binding (17). Despite the lack of any binding affinity for the ligand, the two FNIII and the Ig domains are needed for receptor activation (17, 18). Because of alternative splicing and ectodomain shedding, the LR can exist as six isoforms: a LR long form (LRlo or LRb), four short forms (LRa, LRc, LRd, and LRf), and a soluble isoform (LRe).

Leptin signals utilizing the Jak-STAT pathway by leptin receptor were analyzed using the electrophoretic mobility shift assay (EMSA) (19). Signal transduction by this family of receptors generally depends on ligand-induced phosphorylation of the soluble receptor tyrosine kinase JAK2. The kinase in turn phosphorylates tyrosine residues on the receptor and/or phosphorylation of such proteins initiates signal transduction (20). Data suggest that SHP-2 is a component of the leptin signal transduction pathway and may indicate that it attenuates leptin signaling by decreasing the level of phosphorylation of JAK2.

LRb, which contains the full cytosolic domain and is the only isoform capable of signaling, is highly expressed in the hypothalamus as assayed by in situ hybridization and RNase protection analysis, suggesting that this brain region is an important site of leptin action (21, 22). Ventromedial hypothalamus lesioned rats exhibit serum leptin concentrations (12). Structure and function analyses showed that the structure that consists of amino acid peptide from 106-140 is the most important for its function. With similar molecular weight of 16024 to the deduced leptin amino acid from DNA sequences; the relatively weight of the leptin amino acid separated from human serum is 16026±9. Thus, leptin is not processed after translation. Subcutaneous fat tissues of the whole body, omentum fat, peritoneum fat and mesenteriy fat can express leptin; the expression level in subcutaneous fatty tissue is the highest among them. The differentiation of the fatty cell is closely related to the expression of ob gene. 3T3-L1 fatty cell completely differentiated can express ob mRNA on a high level, this dependent differentiation may be bound to the CCAAT/Enhancer Binding Protein α (C/EBP α) transcriptional factor. Leptin was originally identified as a satiety factor secreted by adipose tissue (5). Studies demonstrated that the ob gene is also produced in human placenta (13), stomach (14), muscle tissue (15), and cerebral adenohypophysis and hypothalamus (16). The mRNA expression of the ob gene is regulated by hormones, cold environment and hypoxia (6, 7).

**Physiological Function and Mechanism of Weight Regulation**

**Leptin**

Leptin, a 167 amino acid peptide hormone that is the product of the ob gene, a 146 amino acid peptide without the 21 amino acids of the N-terminor signal peptide, is the mature leptin. The molecular weight of leptin is 16 kDa with high hydrophilia, and as a single monomer in plasma

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Figure 1. Body weight regulation by leptin.
Figure 2. Schematic representation of the effects of leptin limiting further weight gain in response to a condition of chronic positive energy balance. This concept is mainly supported by data obtained in rodents. Solid arrows, main mechanisms; dashed arrows, less important mechanisms.

Mechanism of weight regulation

A chronic minor imbalance between energy intake and energy expenditure may lead to obesity. Both lean and obese subjects eventually reach energy balance and their body weight regulation implies that the adipose tissue mass is “sensed”, leading to appropriate responses of energy intake and energy expenditure. Leptin provides a system signaling the amount of adipose energy stores to the brain, which does not only suppress food intake but also increases energy expenditure. After leptin binding to LRb in the hypothalamus, leptin stimulates a specific signaling cascade that results in the inhibition of several orexigenic neuropeptides, while stimulating several anorexigenic peptides. The orexigenic neuropeptides that are downregulated by leptin are neuropeptide Y (NPY), melanin-concentrating hormone (MCH), orexins, and agouti-related peptide (AGRP). The anorexigenic neuropeptides that are upregulated by leptin are alpha-melanocyte-stimulating hormone (alpha-MSH), which acts on melanocortin-4 receptor (MC4R), cocaine and amphetamine-regulated transcript (CART) and corticotropin-releasing-hormone (CRH) (27) (Fig. 1).

The demonstration that leptin plays a role in mouse body weight regulation stems from the observation that its chronic injection into ob/ob mice causes the animals to lose weight and maintain their weight loss (28-30). Leptin appears to have a dual action; it decreases the animal food intake and increases its energy expenditure, causing the animal to oxidize more fat (Fig. 2). It has been reported that the metabolic effects of leptin (stimulation of metabolic rate with normalization of body temperature) in treated ob/ob mice precede its effects on appetite and body weight (30). When leptin was injected at the doses of 5 mg/kg/day intraperitoneally for 33 days, ob/ob mice exhibited a decrease in body weight within 4 days, and lost 40% of their body weight after 33 days (29). Food intake of treated ob/ob mice was less than that of control mice after 2 days and stabilized at 40% the intake of control mice at all points after 4 days. In another experiment, untreated ob/ob mice were pair-fed with ob/ob mice receiving leptin injections. The latter lost more weight than the former, indicating that leptin not only decreases food intake but also increases energy expenditure (29). Collins et al (31) pointed out that leptin bound with leptin-receptor increases the basic tone of sympathetic nervous system and then periphery noradrenalin release is increased, and noradrenalin activates membrane β-3 receptor of the fatty cells. Energy expenditure increasing after the expression of UCP was upregulated.

In the past, the physiological function of leptin was considered to be mainly passed by binding leptin-receptor in hypothalamus. In recent years, studies have shown that leptin-receptor is also present in many peripheral organs including adipose tissue, so leptin can accelerate lipophagia by...
binding leptin-receptor in peripheral tissues. Studies of Frühbeck et al (32) showed exogenous leptin dependent lipoprotein in ob/ob mice adipose cell with mutated leptin gene. Sarmiento et al (33) proved that injecting external leptin 20 mg/kg/day in normal mice can quickly increase synthesis of lipoprotein protease and decrease fatty acid synthetase in brown and white steatolysis, resulting in immediate lipoprotein.

**Regulation of Obese Gene under Hypoxia**

**HIF-1**

HIF-1 is a transcription factor that binds specifically in hypoxia to a 5′-RCGTG-3′ hypoxia-responsive element (HRE) in the promoter of various hypoxia-inducible genes (34), which include erythropoietin, vascular endothelial growth factor, glucose transporters, and glycylcotic enzymes, as well as genes involved in iron metabolism and cell survival (34, 35). HIF-1 is a heterodimer composed of a 120-kDa HIF-1α subunit and a 91-94-kDa HIF-1β/ARNT subunit, both of which are members of the basic helix-loop-helix (bHLH)-PAS family (34, 36). The HIF-1β subunit is constitutively expressed. By contrast, HIF-1α is maintained at a low level in normoxic cells through proteasomal degradation of the protein.

The von Hippel-Lindau tumor suppressor protein is a component of the complex that targets HIF-1α for poly-ubiquitination and degradation (37). Two recent observations indicate that von Hippel-Lindau protein binds to HIF-1α when a proline residue at codon 564 is hydroxylated (38, 39). Hydroxylation of HIF-1α is controlled by a Fe²⁺-dependent hydroxylase activity that is inhibited by decreased oxygen. This mechanism accounts for HIF-1α stabilization in hypoxic cells, allowing nuclear translocation and dimerization with HIF-1β. Stabilization of HIF-1α is also induced by chelating or substituting Fe²⁺ with desferrioxamine and cobalt chloride (CoCl₂), respectively. This provides a molecular mechanism accounting for the ability of these agents to mimic the effect of hypoxia in experimental cell systems.

In addition to HIF-1α, a structurally and functionally related protein designated HIF-2α, which is the product of the EPAS1 gene, can also heterodimerize with HIF-1β. HIF-1α: HIF-1β and HIF-2α:HIF-1β heterodimers appear to have overlapping but distinct target gene specificities. Unlike HIF-1α, HIF-2α is not expressed in all cell types, and when expressed it can be inactive as a result of cytoplasmic sequestration. A third protein, designated HIF-3α, has also been identified. Its role has not been well defined, although a splice variant, designated IPAS, has been shown to bind to HIF-1α and inhibit its activity (40).

**HRE in ob gene**

Special DNA sequences in the enhancer or promoter of hypoxia inducible genes can be upregulated by binding with HIF-1 under hypoxia condition are called hypoxia/O2-response/regulation elements (O2-RE/HRE). 5′-RCGTG-3′ is a core sequence located in the 5′ or 3′ flanking region which can be bound with HIF-1α/β complex under hypoxia condition, and then start transcription.

Ambrosini et al (6) found HRE site in the 5′ flanking region of human ob gene by deletion analysis method under hypoxia condition when they studied the relationship between ob gene and hypoxia. They showed that the leptin gene is transcriptionally activated in response to hypoxia through a mechanism that involves binding of the heterodimer HIF1α/β to a functional HRE site located within the proximal promoter region.

Ambrosini et al (6) analyzed the sequence first and revealed the presence of a 5′-RCGTG-3′ HIF-1 binding consensus sequence HRE within the 0.146-kb promoter fragment. Then they constructed 7 fragments of leptin promoter sequence upstream of the luciferase reporter-gene: p(1872)luc, p(1718)luc, p(979)luc, p(213)luc, p(171)luc, p(146)luc, p(116)luc and p(-63+28)luc as a control. Reporter constructs containing 7 lengths of the leptin gene promoter region were transfected into BeWo cells. For each construct, the fold increase in luciferase activity elicited by either HIF-1α overexpression, CoCl₂ treatment, or a combination of the two was determined over non-stimulated cells. A similar pattern of reporter gene expression was observed for several constructs containing up to 0.146 kb of the leptin gene 5′-flanking region. Both HIF-1α and CoCl₂ individurally stimulated luciferase activity by 5 to 7-fold. The effect of the two stimuli in combination was always greater than that elicited by HIF-1α or CoCl₂ alone. However, when combined, these effects were never fully additive. In contrast to all other deleted constructs, the p(116)luc reporter vector was unresponsive to HIF-1α or CoCl₂. This analysis revealed that the first 146 bp of the leptin promoter harbor a sequence responsive to CoCl₂ and exogenous HIF-1α, which is missing or disrupted in the p(116)luc construct.

This putative HRE, located between -120 and -116 on the noncoding strand, is disrupted in the p(116)luc construct. To assess its functional importance, the sequence was mutated in the p(146)luc reporter vector. Constructs containing two distinct mutated fragments, p(146)lucmut and p(146)lucmut, were transfected in BeWo cells, and their capacity to respond to CoCl₂ treatment and HIF-1α over expression was tested. The luciferase activity produced by both mutated leptin promoter fragments was not increased by these stimuli. This certified that this HRE consensus sequence is required for hypoxia-mediated induction of leptin promoter activity (6, 7).

**Obesity and Hypoxia**

Now, many scientists are studying how to treat human obesity with leptin, further progress has also been obtained by animal experiments. Injecting the exogenous leptin in normal and diet-induced mice, the effect was between the injection to the sensitive ob/ob mice and the non-reactive
that remains unresolved is whether changes in nutrient intake and loss of body weight (44). An intriguing question free mass typically observed with reductions in total energy strength and function but may not attenuate the loss of fat-resistance exercise to a weight loss intervention will increase the long-term maintenance of weight loss. The addition of 300 min (3.3-5.0 h) of exercise per week, as recent scientific evidence indicates that this level of exercise facilitates the long-term maintenance of weight loss. The addition of resistance exercise to a weight loss intervention will increase strength and function but may not attenuate the loss of fat-free mass typically observed with reductions in total energy intake and loss of body weight (44). An intriguing question that remains unresolved is whether changes in nutrient intake or body composition secondarily affect spontaneous physical activity. If this were the case, physical activity would represent a major adaptive mechanism for body-weight control (45).

Many studies have been carried out under hypoxic condition. However, none have reported the treatment of obesity under hypoxic condition. Some studies report that loss of appetite and weight are frequently observed at high altitudes. Proper acclimatization to altitude and high caloric intake minimizes, but can not completely prevent significant weight loss under the influence of hypobaric hypoxia (46, 47). Other studies have shown that the expression of glycolysis enzyme mRNA can be upregulated when exposed to low O2 environment; this is one kind of reaction when the cell is stimulated by environment changes. Enhancing the glycolysis pathway is an important way to produce energy and increase the expenditure under hypoxic condition, and finally regulate body weight.

Furthermore, Ob gene as a hypoxia inducible gene can be upregulated by hypoxia with a high leptin concentration in serum; for obese people to stay at high altitudes for weeks in their spare time or vacation and have regular physical activity less than 3000 m may be a good method to expend adipose to increase energy, and lose body weight. It is regulated by itself and is not harmful. So appropriate hypoxia is good for humans and also can treat obesity in a natural way. With further researches, good ways to treat obesity will likely be discovered.

References

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