Review

Advances in understanding the interrelations between leptin resistance and obesity

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HIGHLIGHTS

• Leptin resistance is probably not only a significant symbol for obesity but is also an important risk factor for it.
• The major advances that led to our current understanding of the possible mechanisms underlying leptin resistance
• The role of leptin signals and leptin resistance in the regulation of the pathophysiological processes of obesity
• Some effective treatments of leptin resistance are also discussed.

ABSTRACT

Obesity, which has developed into a global epidemic, is a risk factor in most chronic diseases and some forms of malignancy. The discovery of leptin in 1994 has opened a new field in obesity research. Currently, we know that leptin is the primary signal from energy stores and exerts negative feedback effects on energy intake. However, most individuals with diet-induced obesity (DIO) develop leptin resistance, which is characterized by elevated circulating leptin levels and decreased leptin sensitivity. To date, though various mechanisms have been proposed to explain leptin resistance, the exact mechanisms of leptin resistance in obesity are poorly understood. Consequently, it’s an important issue worth discussing regarding what the exact interrelations between leptin resistance and obesity are. Here, we review the latest advancements in the molecular mechanisms of leptin resistance and the exact interrelations between leptin resistance, obesity, and obesity-related diseases, in order to supply new ideas for the study of obesity.

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Contents

1. Introduction .............................................................. 158
2. Energy homeostasis and obesity ..................................................... 158
3. Leptin and the factors influencing its secretion. .............................................. 158
4. Leptin receptor signals ......................................................... 161
5. Leptin resistance and obesity ...................................................... 161
  5.1. The mechanisms of leptin resistance in obesity ............................................. 161
    5.1.1. Defective transport of leptin into the CNS across the BBB .......................... 161
    5.1.2. Attenuation of ObRb signaling .................................................. 162
    5.1.3. Genetic variations of leptin and LEPR genes ........................................ 162
    5.1.4. Endoplasmic reticulum (ER) stress .............................................. 163
    5.1.5. Others ......................................................... 163

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1. Introduction

Obesity, the global epidemic, has attracted extensive attention worldwide and has led to increasingly serious medical problems [1–3]. Indeed, obesity is currently considered as a significant health threat because nearly 35% of the adult populations in most developed countries are clinically obese (WHO Statistics, 2013). Obesity and being overweight are the fifth leading risk for global deaths and the risk factors in various chronic diseases, including type 2 diabetes mellitus [4], cardiovascular diseases (CVDs) [5], immunity and inflammation [6,7], psychological deficits [8,9] and some malignancy conditions [10], such as colorectal cancer [11] and breast cancer [12]. Thus, the prevalence of obesity, the significance of obesity, and the economic costs of the disease create an urgent need for better therapeutics and understanding of the physiological processes that balance energy intake and energy expenditure. A growing number of researchers have devoted themselves to the study on the molecular mechanisms of obesity and obesity-related diseases [13–18], with the purpose of ascertaining the real root of obesity for reasonable and effective therapies. There have been numerous theories to explain this type of complex physiological network of obesity. However, the exact mechanism of obesity is not clear at present. Two central discoveries in the explanation of this network were those of leptin, which is encoded by the obese gene (OB), and its long receptor, leptin receptor b (ObRb) [12,19]. The hormone leptin, a 16-kDa polypeptide, is mainly synthesized and secreted by the white adipose tissue (WAT). Leptin acting on specific populations of neurons in the brain, including hypothalamic, midbrain, and brainstem neurons, plays a central role in weight control by suppressing food intake and increasing energy expenditure [20–22]. Nevertheless, most individuals with DIO have high levels of circulating leptin [22,23]. More importantly, the injection of additional leptin in obese individuals fails to counteract obesity [20]. Consequently, the concept of leptin resistance emerged to explain the seemingly paradoxical elevated levels of leptin that occur in obesity.

However, a growing number of studies have shown that leptin resistance induced by obesity complicates efforts to distinguish the mechanisms that predispose individuals to weight gain from the mechanisms that result from obesity lately [21]. For this reason, leptin resistance is probably not only a significant symbol for obesity but is also an important risk factor for it. In addition to its association with obesity, leptin is also involved in the regulation of other physiological processes, including reproduction [10,24], bone homeostasis [19] and immune function [12], etc. Therefore, leptin resistance participates in various pathological processes of other diseases, such as CVD [5], osteoporosis [25], systemic inflammation [6,7] and depression [8,9]. In recent years, a growing number of scientists have begun to explore the therapeutic methods for obesity from the point of leptin resistance and also that of leptin resistance-related diseases. Here, we review the major advances that led to our current understanding of the possible mechanisms underlying leptin resistance, the exact interaction between leptin resistance and obesity, and the role of leptin signals and leptin resistance in the regulation of the obese pathophysiological processes. Additionally, some effective treatments of leptin resistance are also discussed.

2. Energy homeostasis and obesity

In recent years, there has been a growing emphasis on the correlation between nutrition and energy homeostasis [26,27], and meanwhile the studies on the effects of adipocytes on calorie homeostasis are in process [28]. Energy homeostasis is the balance between energy intake, energy spent and energy storage [29]. In most adults, although there exists a large variation in daily food intake and energy expenditure, body weight is almost constant [30], which depends on the regulation of the balance between energy intake, in the form of food and drinks, and energy expenditure, in the forms of basal metabolism, physical activity and adaptive thermogenesis [18,28]. The adipocytes act as calorie storage, which makes them well suited to the regulation of energy balance. The adipose tissue also serves as a crucial integrator of energy homeostasis, because a host of regulating hormones for energy balance can be secreted from the adipose tissue [28].

Traditionally, the adipocyte-derived regulating proteins (namely adipocytokines) are categorized as pro-storage of energy including resistin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and retinol-binding protein-4 (RBP-4), and anti-storage of energy such as leptin, adiponectin, visfatin and omentin [231] (Fig. 1). The complicated physiological system for energy homeostasis consists of both afferent signals and efferent effectors [32]. The afferent signals, which are mainly in the form of hormones above, are integrated by the peripheral nerves and the central nervous system in the hypothalamus, brainstem, etc. The integrated signals regulate various types of neuropeptide in return, which modulate energy homeostasis and contribute to the stable state of body weight [33]. Some regions in the hypothalamus, such as the ventromedial hypothalamic nucleus (VMH) and the arcuate nucleus (ARC), have been thought to play central roles in the regulation of energy homeostasis. VMH was clearly associated with increased food intake, morbid obesity and insulin resistance, while damage to more lateral hypothalamic structures was associated with anorexia and adipispa [34]. ARC can produce α-melanocyte stimulating hormone (α-MSH), an anorectic peptide formed from the cleavage of the proopiomelanocortin (POMC) protein [35]. This protein acts on melanocortin receptor types 3 and 4 (MC3/4, respectively) that existed in various hypothalamic nuclei to reduce food intake and energy expenditure in a manner similar to leptin [36]. Most peripheral signals for energy homeostasis, mainly the adipocyte-derived regulating proteins, accept the regulation of mediobasal hypothalamic areas, particularly VMH and ARC, constituting the hypothalamic homeostatic regulating circuits. The lesions of specific sections in the signal pathways will result in the energy metabolism disorders conducing to the development of obesity and related diseases such as diabetes. It is well worth mentioning that leptin has been considered as one of the most important adipocytokines for energy homeostasis.

3. Leptin and the factors influencing its secretion

The word “leptin” originates from the Greek root “leptos”, which means “thin”, and cited by the medical community in 1994 [30]. Leptin, a 16 kDa polypeptide and the product of OB gene, is a cytokine-like circulating hormone produced and secreted predominantly by the...
adipocytes in the WAT, acting through their receptors (LEPRs) on specific populations of neurons in the brain, including the hypothalamic, mid-brain, and brainstem neurons, and whose main function is to counteract obesity [22,37]. Mirroring the body’s fat stores, leptin plays a crucial role in the regulation of numerous neuroendocrine functions, from energy homeostasis to a variety of additional processes such as reproduction [38,39], bone function [19], cardiovascular regulation [5] and immune function [12]. Leptin decreases body weight by both suppressing appetite and promoting energy expenditure by directly targeting the hypothalamic neurons, including the increased expression of the anorexigenic peptide α-MSH, which is derived from POMC cells in the ARC, and the decreased expression of the orexigenic peptide neuropeptide Y (NPY) neurons and agouti-related peptide (AgRP) neurons, which are also located in the ARC [40–42].

Various factors influence leptin secretion and expression (Fig. 2). The most important factors are the distribution of subcutaneous fat

![Diagram](image_url)

**Fig. 1.** Adipocyte-derived regulating proteins with varied effects on energy homeostasis. The pro-storaged proteins for energy including resistin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and retinol-binding protein-4 (RBP-4); The anti-storaged proteins for energy including leptin, adiponectin, visfatin and omentin.

**Fig. 2.** Schematic diagram for biological synthesis, secretion and actions of leptin as well as molecular mechanisms and therapeutic principles of leptin resistance. Notes: +++: high correlation; ++: positive correlation; +: permissive role; −−: inhibition; −: Negative regulation; ●: on study;▲: require further trials; FFAs: free fatty acids; ER: endoplasmic reticulum; ObRb: leptin receptor b.
and the status of its energy stores because leptin is mainly expressed in
the adipose tissue, and circulating leptin concentrations in the fed state
are highly correlated with the degree of adiposity [20,43,44]. Leptin
expression also correlates with insulin level, as evidenced by the fact that
insulin triggers leptin expression directly in isolated adipocytes [45] and
enhances leptin levels when injected into rodents [46], as well as the
discovery that decreased leptin levels accompanied with low insulin re-
sistance states and circulating leptin concentrations increased after in-
sulin treatment [47]. Glucocorticoids directly induce hyperleptinemia
and stimulate leptin synthesis in cultured adipocytes [48], for example,
leptin expression increases in response to the chronic elevation of corti-
sol in humans [49]. In contrast, the secretion of leptin can also enhance
the concentration of circulating glucocorticoids [50]. Therefore, gluco-
corticoids and leptin mutually promote each other. However, other re-
lated studies have demonstrated that there is a negative correlation
between the concentration of circulating leptin and glucocorticoid
levels [51]. Thus, the exact influence of glucocorticoids on leptin
secretion should ultimately be confirmed based on the additional clini-
cal trials in the future.

Other factors influencing the circulating concentration of leptin
were reported in several researches in the field. Glucose and/or its me-
tabolites play permissive roles in the secretion and expression of leptin
[52] and leptin signaling [53], and glucose dose-dependently enhances
leptin signaling and leptin sensitivity, at least in part, by attenuating
the ability of AMPK to inhibit leptin signaling. Circulating free fatty
acids (FFAs) serve as suppressors of leptin secretion, that may be asso-
ciated with the hyposensitivity to food caused by FFAs in circulation
[54]. The administration of thyroid hormone decreases leptin levels in
rodents, but that is not a major determinant of plasma leptin levels
[55]. Meanwhile, there is an interaction effect between leptin, sex hor-
mones and growth hormones, which is likely to result in metabolic dis-
orders involving sex hormones and growth retardation in obese
adolescents [56]. Additionally, infections, endotoxins and some cyto-
kines stimulate leptin synthesis and secretion reported by several

Fig. 3. Schematic overview of ObRb and its role in leptin signaling. The ObRb and other five leptin receptors receptor isoforms all share the same ligand–binding extracellular domain, consisting of two cytokine receptor homology (CRH1 and CRH2) domains separated by an immunoglobulin-like (Ig-like) and followed by two membrane proximal fibronectin III (FN III) domains. The CRH2 domain and Ig-like domain are necessary and sufficient for leptin bind binding and the receptor activation. After the activation of ObRb, Janus kinase 2 (JAK2) combines with the receptor via the recruitment of the box-1 motif (less-well-conserved sequences) adjoining the membrane domain of cell, which leads to the phosphorylation and activation of JAK2. ObRb has three important conserved tyrosine residues in its cytoplasmic domain, indentifying with positions Y985, Y1077 and Y1138, which can be phosphorylated by the acti-
vated JAK2. The process that Y1138 recruits the transcription factor STAT3, together with its phosphorylation by JAK2, dimerisation dimerization and translocation into the arcuate nucleus, regulates the expression and metabolism of POMC, SOCS3, NPY and AgRP. SOCS3 binding to Y985 attenuates ObRb signaling, completing the negative feedback regulatory loop. The re-
cruitment of SHP2 to Y985, which is also a binding site for the negative modulator SOCS3, positively engages the ERK pathway, which improves the effect of leptin on energy homostasis homoestasis. PTP1B mediates the dephosphorylation of JAK2, limiting the extent of leptin action in cultured cells, and also increases the expression of SOCS3 via ER stress. The phosphorylated JAK2 protein also activates and phosphorylates IRS1 and IRS2 (insulin receptorsubstrate substrates 1, 2) after leptin binding to it’s recruitment, which lead to the stim-
ulation of the P13K pathway, and then regulating the balance between food intake and energy consumption by increasing the expression of POMC in the ARC. The phosphorylation of JAK2 suppresses the AMPK expression in hypothalamic acetyl-CoA carboxylase (ACC).
studies [57]. To sum up, there have been so many influencing factors contributing to the level of expression and secretion of leptin that provides a wide field for the further studies on leptin.

4. Leptin receptor signals

Plasma leptin level was found to be highly correlated with the amount of body fat [20] and has a central effect on the regulation of feeding behavior and energy expenditure by activating the hypothalamic leptin receptors (LEPRs) [58–60]. The LEPR, the product of the LEPR gene, is a member of the class I cytokine receptor family, and has at least six splice variants, ObRa–ObRF [61]. Notably, the long receptor ObRb mediates essentially all known physiological effects of leptin in energy homeostasis [62–64] because genetic deficiency of ObRb results in pronounced hyperphagia and morbid obesity in animals [65,66]. Leptin binding to the domain of extracellular of ObRb triggers the domain of activated box-1 and amino acid at 31–36 chain on ObRb, and the recruitment of the tyrosine kinase Janus kinase-2 (JAK2), resulting in the phosphorylation and activation of JAK2 [4,67,68] (Fig. 3). Activated JAK2 phosphorylates three tyrosine residues in the cytoplasmic domain of ObRb, which includes Tyr985, Tyr1077 and Tyr1138 [69]. Of these residues, phosphorylated Tyr1138 (pY1138) recruits signal transducer and activator of transcription 3 (transcription factor,STAT3), which becomes phosphorylated by JAK2 [70,71]. Phosphorylated STAT3 (pSTAT3) then homodimerizes and translocates to the nucleic acid in the hypothalamus, where it increases the expression and neuronal excitability of POMC and inhibits that of AgRP and NPY [40,72,73]. On the one hand, POMC is subsequently processed into α-MSH, which can suppress appetite, promote energy expenditure and decrease body weight. On the other hand, the activation of STAT3 inhibits AgRP expression, which can suppress the activity of NPY and subsequently block NPY inhibition against reproduction and the growth axis, which finally cause the inhibition of appetite and increase energy expenditure [74].

This complicated physiological process is regulated by both positive and negative regulators. The negative regulators act as feedback inhibitors of leptin signaling by binding to pY985 and preventing the activation of the JAK2/STAT3 pathway [75], including suppressor of cytokine signaling protein-3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B). The positive regulator Src-homology 2 domain 1 (SH2B1) markedly enhances JAK2 activity, thereby promoting the activation of the leptin-signaling pathways downstream of JAK2 [76,77]. Additionally, endoplasmic reticulum (ER) stress can also prevent the activation of the JAK2/STAT3 pathway and conduces to the hypersensitivity of leptin and the development of obesity by means of combining with PTP1B to each other.

In addition to the JAK2/STAT3 signaling pathway, the activation of ObRb also results from the activations of extracellular signal-regulated kinase (ERK) and phosphoinositide-3-kinase (PI3K) pathways [78]. The involvement of the ERK pathway is supported by the fact that the inhibition of ERK blocked the effect of leptin on food intake and body weight. SHP2 has been shown to promote signaling through the ERK pathway, though other data indicated that SHP2 might suppress JAK2/STAT3 signaling by decreasing JAK2 phosphorylation [12,79]. Leptin-stimulated activation of hypothalamic phosphatidylinositol-3-kinase (PI3K) is impaired in DIO [80], and the pharmacological inhibition of PI3K activity blocks the anorectic effect of leptin [81,82], while chronic activation of the hypothalamic PI3K pathway by ObRb neuron-targeted deletion of the PIP3 phosphatase PTEN increases leptin sensitivity and decreases adiposity [83,84], suggesting that the PI3K pathway in non-POMC hypothalamic neurons appears to mediate the long-term anorectic effects of leptin. The effect of PI3K promoting leptin actions is mediated by the inhibition of FoxO1, which can antagonize leptin action in vivo [12]. The activation of both pathways has been shown to be required for proper regulation of energy and glucose homeostasis [84].

Corresponding with the abovementioned regulating pathways, including ERK and PI3K, the AMPK and mTOR pathways are also involved in the regulation of leptin signals. Leptin stimulates hypothalamic acetyl-CoA carboxylase (ACC), a key enzyme in fatty acid biosynthesis, via the inhibition of AMPK [85,86]. Dominant negative AMPK expression in the hypothalamus is sufficient to reduce food intake and body weight; in contrast, the fundamental activation of hypothalamic AMPK attenuates leptin’s anorexigenic response [87,88]. It has been reported that glucose and its metabolites play permissive roles in leptin signaling and that glucose enhances leptin sensitivity, at least in part, by attenuating the ability of AMPK to inhibit leptin signaling [54]. Leptin increases the mammalian hypothalamic target of rapamycin (mTOR) activity, and the inhibition of mTOR signaling by rapamycin attenuates leptin’s anorectic effect [89]. Exposure to a high-fat diet decreases mTOR signaling within the hypothalamus, suggesting that reduced hypothalamic mTOR activity contributes to the development of hyperphagia and weight gain during DIO [90].

5. Leptin resistance and obesity

Notably, the reduced abilities of peripheral leptin and central ObRb to collaborate in the suppression of appetite and the promotion of energy expenditure can be taken for the crucial risk factors for the development of obesity. Therefore, elevated peripheral leptin level is likely to control or reverse obesity. However, the hope that leptin might be a "miracle cure" in the treatment of obesity was remote. Rather than being leptin deficient, most obese humans and animals have high levels of circulating leptin [20,91,92], and increased leptin fails to resist the development of obesity [93–95]. Meanwhile, treatment with leptin alone in obese individuals fails to counteract common obesity [94–96]. This transparent leptin ineffectiveness and hypersensitivity is identified as leptin resistance [19,39,97–100]. A large number of studies demonstrated that most individuals with DIO manifest leptin resistance characterized by increased leptin levels in the blood and decreased leptin sensitivity, which is in proportion to the individual's adipose mass and BMI [92,98–103].

5.1. The mechanisms of leptin resistance in obesity

In view of the great influence of leptin resistance on the development of obesity, it's an important subject for treating obesity to elucidate the mechanisms of “leptin resistance”. Currently, several mechanisms have been proposed to explain leptin resistance, including defective leptin transport across the blood–brain barrier (BBB) [104], attenuation of leptin signaling [105,106], the deficiency and variations of LEP and LEPR genes [3,19,21,107], ER stress [67,68,108], inflammation [6], excessive bioavailability of heavy metals such as Zn [109,110], and others (Fig. 2).

5.1.1. Defective transport of leptin into the CNS across the BBB

The results of a large number of studies displayed that obese individuals exhibit high peripheral leptin levels but relatively lower cerebrospinal fluid (CSF) concentrations, suggesting that defective leptin transport into the central nervous system (CNS) across the BBB conduces to leptin resistance [104,111–115]. Indeed, leptin mediates the inhibition of appetite and increased energy expenditure only under the conditions that nomicad leptin is transported across the BBB and ultimately binds to its receptors initiating the signals for energy homeostasis in some hypothalamic populations [104]. By binding to the megalin, a kind of binding protein at the choroid plexus epithelium, leptin triggers its transportation into the CNS in the study of Dietrich et al. [116]. Triglycerides act as the regulators for the process because decreasing triglycerides can strengthen the anorectic effect of leptin by enhancing leptin transport across the BBB [117]. Acute phase C-reactive protein (CRP) is also likely to contribute to leptin resistance by preventing leptin...
from crossing the BBB [118]. The process of leptin transport balance is regulated by two LEPRs, ObRa and ObRe. ObRa mediates leptin transport across the BBB [119,120] and ObRe inhibits leptin transport by counteracting the function of ObRa [121]. Under normal conditions, the actions and number of these two receptors are in balance. Thus, subsequent studies are needed to clarify the equilibrium correlation between ObRa and ObRe under normal and obese states.

5.1.2. Attenuation of ObRb signaling

The attenuation of ObRb signaling is mainly due to two parallel molecular mechanisms including the up-regulation of suppressor of cytokine signaling (SOCS3) in the cytoplasm and that of protein tyrosine phosphatase-1b (PTP1B) in the endoplasmic reticulum. Both of them are all involved in the regulation of ObRb signaling pathway, particularly JAK2/STAT3.

SOCS3 is a critical protein that inhibits the signal transduction process of various cytokines in the body, including leptin [105]. It’s the most crucial procedure for the inhibition of appetite and increased energy expenditure that leptin mediates STAT3 phosphorylation, and then increases POMC expression as well as inhibits the activities of NPY and AgRP. By binding to Tyr985 of ObRb and JAK2, SOCS3 inhibits the leptin-induced phosphorylation of STAT3 signaling through a feedback negative mechanism [121]. Mice with deletions of SOCS3 in the whole brain or POMC are resistant to DIO [122,123]. The incidence of DIO and leptin resistance is decreased in rats with hypothalamic SOCS3 silencing by RNAi, which also confirms the conclusion above [124]. Further studies have indicated that JAK2 and SOCS3 experience leptin-dependent immunoprecipitation [75,105]. This result supports the role of SOCS3 in developing leptin resistance effectively.

PTP1B is a class 1 non-receptor protein tyrosine phosphatase (PTP), which is attached to the cytoplasmic face of the endoplasmic reticulum in the hypothalamic regions where leptin-responsive neurons are located [125]. A high-fat diet is accompanied by elevated PTP1B expression, suggesting that PTP1B may play a crucial role in the etiology of leptin resistance [126]. Both systemic, neuron-specific and POMC neuron-specific deletions of PTP1B improve leptin sensitivity and protect individuals from DIO [127,128]. However, PTP1B in POMC neurons regulates energy expenditure but not food intake, and other neuronal populations are responsible for the impact of PTP1B on both food intake and energy expenditure [30]. PTP1B binds to and dephosphorylates JAK2, thereby inhibiting leptin signaling [125,129]. PTP1B expression is increased by high-fat feeding and inflammation [130] and can be regulated by oxidation, phosphorylation, sumoylation and proteolysis [30]. The exact correlation between the expression and activity of PTP1B and leptin resistance remains unclear; however, this particular correlation is the focus of gene therapy for leptin resistance in the future.

Other mechanisms of leptin resistance focusing on the attenuation of ObRb signaling are explored by some biologists. TC-PTP, a non-receptor, classical PTP in the N1 hypothype, also acts as the critical negative regulator of hypothalamic ObRb signaling because mice that lack TCPPT in neuronal cells have enhanced leptin sensitivity, and are resistant to high-fat-diet-induced weight gain and the development of leptin resistance [46]. The insulin receptor-signaling pathway can interfere with leptin signaling at the level of JAK2 through SHP-2-dependent pathways, which can decrease the level of JAK2 phosphorylation, as the result of hyperinsulinemia contributing to the pathogenesis of leptin resistance [48]. The attenuation of ObRb signaling has been considered as one of the leading factors, and also the primary effect on the central leptin resistance in the hypothalamus. In addition to JAK2/STAT3, other signaling pathways, such as the phosphatidylinositol 3-kinase/Akt (PI3K/Akt), AMPK and mTOR pathways, participate jointly in the regulation of ObRb signal transduction [88,131,132]. Consequently, the studies on the expression level of regulators in ObRb signaling pathways above, and simultaneously explorations on the actions and regulations of other five LEPR signaling pathways can make better sense of leptin resistance in multiple perspectives and also provide the effective academic supports for the treatments on leptin resistance. There are mainly some kinds of regulators in the other signaling pathways of ObRb, including phosphatase and tensin homolog deleted on chromosome 10 (PTEN) [30,39], a type of negative regulator of the PI3K pathway serving as the inhibitor of PI3K phosphatase; ACC [133], a key enzyme in fatty acid biosynthesis and an important intermediary in the attenuation of AMPK for inhibiting food intake; and Akt [12,134,135], the reinforcement for hypothalamic mTOR anorexigenic activity by phosphorylating and inactivating TSC2 for the combination of TSC1/TSC2 complex, etc.

5.1.3. Genetic variations of leptin and LEPR genes

5.1.3.1. Genetic deficiency of LEPR gene. The LEPRs, the product of LEPR gene, mediate the effect of leptin on the regulation of feeding behavior and energy expenditure by accepting the combination of leptin on cytomembrane [136]. A famous study designed by Halaas et al. demonstrated that the phenotype of db/db mice knocked out the ObRb gene was similar to that of leptin-deficient ob/ob mice, including morbidity obesity, hyperlipidemia, decreased insulin sensitivity and infertility [59,62,66], suggesting that the genetic deficiency of LEPR can result in leptin resistance directly. Other related studies also demonstrated that the genetic LEPR deficiency especially lacking of the transmembrane and intracellular domains reduces the binding level of leptin to LEPR, which in turn impairs the LEPR signaling transduction pathway within the CNS, such as JAK2/STAT3, leading to the development of severe obesity and leptin resistance [77,137]. However, an in vivo study in ELKO mice designed by Pan et al. [138] showed that the mutation or deficiency of LEPR expressed in some peripheral regions, such as the endothelium of blood vessels, can provide partial positive resistance to DIO, which is probably the opposite effect compared with neural LEPR. The mechanisms of that have been unclear now, with the possible reason that the endothelial LEPR may merely function as a negative regulator of DIO.

5.1.3.2. Genetic variations of leptin and LEPR genes. Leptin resistance is strongly associated with obesity and simultaneously appears to be a heritable trait characterized by the genetic variations at LEP or LEPR gene between people in different regions [139,140]. The cause of the genetic variation at LEP or LEPR gene is that LEP or LEPR gene contains a number of single nucleotide polymorphisms respectively, which can lead to the impaired LEPR expression, the failure of cellular leptin action, and the development of obesity [37]. Mutations in the polymorphisms above are reported to result in severe obesity in both animal and human models [141,142], and calculating the allelic frequencies of these polymorphisms between different human subjects can effectively show the ethnic variations suffering from obesity and leptin resistance.

5.1.3.2.1. Genetic variation of LEPR gene. Genetic variation at LEPR gene may play a crucial role in the pathophysiology of human obesity, and leptin-resistant state [139]. Numerous studies indicated that the LEPR gene contains a number of single nucleotide polymorphisms, therein, the Lys109Arg, Gln223Arg, and Lys656Asn are the three most common polymorphisms, the association of which with obesity has been evaluated in different populations all over the world [143]. However, the results of previous studies have shown conflicting results. An earlier study reported a lack of relationship between the genetic variation in LEPR gene and obesity in a white British male population [144]. Subsequently, Park et al. [145] identified 35 sequence variants in LEPR gene and some of them associate with BMI in a Korean population, including the LEPR gene Lys109Arg, Gln223Arg and Lys656Asn polymorphisms.

A famous study designed by Masuo et al. [146] explored the correlations between the three polymorphisms above, sympathetic neural activity, blood pressure, and obesity in 129 healthy male individuals. The study showed a significantly higher prevalence of Asn656 allele and Arg223 allele in overweight/obese subjects by measuring the fat mass and plasma leptin levels, and no difference of Lys109Arg frequency between these subjects. However, the study performed by Yiannakouris
et al. [147] reported that only the Arg223 allele variation of LEPR, rather than LEPR Lys109Arg and Lys656Asn polymorphisms, associates with obesity and leptin resistance in a Greek population of young subjects. The conclusions were well verified by the study of Guizar-Mendoza et al. [148] that evaluated the potential role of genetic variation at the LEPR gene in obesity and obesity-related traits in Mexican adolescents. Consequently, it’s pronounced that the specific genetic variation of Gln223Arg in LEPR gene acts as the primary factor for the genetic variation of obesity and leptin-resistant state to a large extent, and which has also been confirmed by a growing number of studies concerned.

The Heritage Family Study showed a significant association between LEPR Gln223Arg and BMI, skin thickness, fat mass, and lean muscle mass in a Quebec family [149]. The Genetic Variation Family Study reported a consistent relationship between LEPR Gln223Arg and BMI in Caucasian men [150]. An association was noted between LEPR Gln223Arg and abdominal fat mass in postmenopausal Dutch women [151]. A study from Brazil with a multiethnic adult group suggested a strong correlation between obesity and LEPR Gln223Arg [152]. Another study by Mattevi et al. [153] in a Brazilian population reported that the association between LEPR Gln223Arg and BMI was stronger in non-smokers than in the general population, which also indicated that smoking serves as one of the risk factors for obesity. Meanwhile, the Gln223Arg variation at LEPR gene is possibly associated with the development of obesity, leptin resistant-states and obesity-related diseases such as hypertension, type 2 diabetes mellitus, and hyperlipidemia in Chinese in some provinces of China [154–157]. Nonetheless, a study of 92 obese and 99 lean Turkish children performed by Komsu-Ornek et al. [158] showed that there is no significant correlation between LEPR gene Gln223Arg and circulating leptin levels, which was also reported by the study of Paracchini et al. [159]. In view of this inconsistency between the results of numerous studies above, authors consider that the primary cause of the inconsistency may be due to the complex pathogenesis of obesity, especially leptin-resistant obesity, which involves a large number of both genetic and environmental factors. However, the genetic variations of LEPR gene exert crucial influences on the susceptibility and morbidity of obesity in terms of the environmental factors.

5.1.3.2.2. Genetic variation of leptin gene. A pedigree-based cross-sectional study by Livshits et al. [160] in an apparently healthy population of European origin strongly suggested that the genetic variations in body mass index, skinfolds, and even body circumferences, as well as of systolic blood pressure are due to genetic variation of circulating leptin levels. They confirmed that this type of genetic variation is also caused by the genetic mutation of leptin gene. LEPR gene polymorphisms are associated with sweet preference and obesity by affecting the molecular mechanisms of leptin activation in the hypothalamic neurons [161]. Numerous studies indicated that the G-2548A polymorphism is the most notable variation in LEPR gene recently. Certainly, LEPR gene G-2548A polymorphism contributes to the developments of obesity and obesity-related diseases to varying degrees in people all over the world, with the different allelic frequencies of G/A in Han nationality children in China [162,163], Aryan in France [164], and the women in Brazil [165]. In most Chinese, the LEPR G−2548 A/G plays a genetic recessive role in the development of extreme obesity, which is similar to that of the women in Brazil, but is opposite to that of Aryan in France. However, Zhang et al. reported that LEPR gene G-2548A polymorphism is not associated with abdominal obesity, but is significantly correlated with abdominal obesity cerebral infarction in Han nationality children in China [166]. Meanwhile, the same result was showed by the study of Xiang et al. [167] in Tujia children in China. The differences between people in various areas may be mainly caused by race, regional or age differences.

As one of the important molecular mechanisms of obesity, the genetic variations in LEPR and/or LEPR genes have been taken seriously in recent years. However, because of the differences of statistical methods, regions, nationalities, ages, etc., there exist multiple results of studies aiming at the association between a kind of genetic variation in specific gene and obesity. Moreover, the pathogenesis of obesity involves not only the genetic factors but also the environmental influences. Further researches should focus on the roles of both gene–gene and gene–environment interaction in the developments of obesity and leptin resistance by means of genomic and proteomic methods.

5.1.4. Endoplasmic reticulum (ER) stress
 Increasing evidence suggests that stress signals can impair endoplasmic reticulum (ER) function, leading to an accumulation of unfolded proteins that result in ER stress [168,169]. The accumulation of unfolded proteins that is toxic to cells markedly inhibits leptin-induced STAT3 phosphorylation in vitro studies performed by Hosoi et al. [170], prevents the activation of the JAK2/STAT3 pathway, and consequently contributes to leptin resistance [171]. Increasing evidence showed that ER stress has been discovered in multiple tissues in leptin-resistant and obese animals, including the hypothalamus, and inhibits leptin signaling. There is a regulatory mechanism of SOCS3 in dysfunction of leptin signaling via activation of IKKβ and ER stress [12]. By binding to the endoplasmic reticulum, PTP1B dephosphorylates JAK2 and activates SOCS3 in the ARC via the activation of IKKβ/NF-κB, and sequentially interrupts central LEPR signaling and leptin action in the hypothalamus leading to leptin resistance. The deactivation of IKKβ significantly conduces to the remission of obesity and leptin resistance [172], indicating that IKKβ acts as one of the most important regulatory factors in leptin resistance associated with the endoplasmic reticulum.

5.1.5. Others
 Activation of inflammatory signaling has also been detected in the hypothalamus of obese rats [43]. WAT is a well known active participant in regulating the physiological processes, and plays a primary role in the development of a triad of hormonal imbalance (leptin resistance, adrenocortical resistance, insulin resistance). Therefore, the dysfunction or intoxication of the WAT might result in leptin resistance and obesity [45]. Moreover, toll-like receptor 4 (TLR4) signaling by fatty acids can also inhibit leptin-induced STAT3 activation [44], which is considered the main mechanism of leptin resistance by inflammatory reactions.

Taneja et al. [110] showed that although the Zn treated rats displayed a higher circulating leptin level, these rats were unable to reduce food intake and gained in body weight compared with the control group, which suggested that excessive bioavailability of Zn induces leptin resistance through the increased uptake of nutrients at the intestinal level, leading to the growth of adipocytes that aggravate leptin synthesis and its release into the blood stream. However, another relevant study indicated that Zn-induced leptin resistance could be attenuated through restoring the ionic balance of Zn, Cu and Mg through the inclusion of an antioxidant-enriched modified poultry egg [50]. Although further clinical studies are required before these eggs are put to use for human consumption, the prospective of the treatments for Zn-induced leptin resistance gives researchers an idea of what to expect.

5.2. The exact interrelations between leptin resistance, obesity and the obesity-related diseases

As discussed above for the de

morbid obesity in case that the positive regulation is damaged or interfered by various internal and external factors such as leptin resistance primarily.

Implicit in the definition of leptin resistance is the idea that a pathophysiological state in which elevated peripheral (adipocyte and circulating) leptin levels coincide with ongoing hyperphagia and morbid obesity, a failure of endogenous hyperleptinemia to reduce appetite or to increase energy expenditure, a decreased responsiveness to the exogenous leptin administration significantly, a failure of leptin to activate key signaling molecules, STAT3 for instance, within the specific area in the hypothalamus from a molecular standpoint, and lastly the processes that promote and/or result from obesity impair leptin action [23, 26, 175]. Individuals that presented reduced leptin sensitivity may predispose to obesity than the normal ones, because these obese strains appear to have a blunted leptin sensitivity even prior to the development of obesity, which also suggests that leptin resistance serves as a predisposing factor for obesity [176, 177]. However, leptin resistance is probably not only an important risk factor but also a significant symbol for obesity. A growing number of biologists have reached extensive consensus on the formulation that leptin resistance is a hallmark of obesity, especially DIO [91], indicating that drugs aimed at ameliorating leptin resistance might prevent the development of DIO. Consequently, understanding the mechanisms that may underlie “leptin resistance” is crucial for both determining the causes of obesity and identifying the potential mechanisms that may be targeted for therapy.

Leptin resistance resulting in the failure of elevated leptin levels to control or reverse obesity enhances the susceptibility to obesity, and obesity can also aggravate leptin resistance, which further sustains obesity, leading to a vicious cycle of escalating metabolic derangement [20, 99, 178, 179]. Studies in the complicated interrelations between obesity and leptin resistance will open a new field for obesity; meanwhile, other potential mechanisms for leptin resistance–related obesity and the studies focusing on obesity-related diseases may provide the basis for a novel treatment of obesity and understanding the role of leptin resistance in these diseases across-the-board.

5.2.2. Leptin resistance, obesity and neoplasia

Obesity increases not only the risk of CVD but also that of various types of neoplasia. The correlation between weight gain and death resulting from cancer was confirmed more than 30 years ago. Since the discovery of ob gene and leptin, a growing number of biologists have devoted themselves to exploring the exact associations between leptin resistance and a variety of neoplasms, hoping to provide the new preventive and therapeutic measures for these tumors.

Leptin resistance may be involved in the development of various types of cancer, such as colorectal cancer and endometrial cancer, which has been testified in recent years. Firstly, a high consistency of elevated circulating leptin level with these types of cancer has been reported in numerous studies [191-195]. It is well known that fat homeostasis is regulated by leptin and that resistance to leptin results in lipotoxicity and cellular lipoapoptosis. The impaired leptin activity leads to the deposition of excess fat in non-adipose tissues where it undergoes oxidative stress and the cells in the target tissues are thus exposed to large amounts of lipid metabolites such as ceramides, nitric oxide and peroxides [196] which can lead to oncogenesis or lipoapoptosis [197]. Secondly, experimental studies have shown that the elevated circulating leptin level has the anti-apoptotic and/or mitogenic effects in certain cancer cell lines, such as the breast, esophagus, colon, and prostate, and that the anti-apoptotic and mitogenic effects can be inhibited by the inhibition of MAPK and PI3K pathways, suggesting that the activation of MAPK and PI3K pathways induces the effects above for the pathological basis of leptin resistance–related neoplasia [199-200]. Lastly, some kinds of neoplasms, including colorectal, breast, endometrial, prostate, and gastric cancers, overexpress LEPR, with a possible explanation that leptin may promote cell proliferation, invasion, and metastasis through JAK2/STAT3 signaling pathway [197, 201-206]. Consequently, though the precise mechanisms of neoplasia above in leptin-resistant obesity have not been confirmed absolutely, it is meaningful for providing the preventive and therapeutic strategies for neoplasms to understand these mechanisms.

5.3. The treatments for leptin resistance-related obesity

5.3.1. Caloric restriction and exercise

It is well-known that caloric restriction plays an important role in the treatment of obesity, which is similar to the role in overcoming leptin resistance [207]. A study by Berriel et al. [208] in DIO mice has shown that three days after abrogating a high-caloric diet, the mice displaying leptin resistance and DIO produced a high sensitivity to leptin again. Some related studies reported that regular and long-term exercise not only decreases the plasma leptin concentration and the expression of OBRb mRNA in the arcuate nucleus in the hypothalamus but also activates STAT3 and AMPK signaling pathways that are suppressed by a high dose of dexamethasone in rats [48, 209]. Zhou [210] demonstrated that free aerobic exercise reduces SOCS3 mRNA expression in the hypothalamus of rats that were fed a high fat diet, and ultimately reduces the inhibition of the JAK2/STAT3 signaling pathway, suggesting that exercise can prevent leptin-resistant obesity. Meanwhile, in vivo study by Tan et al. [211] showed that the effect of an exercise-dietary intervention is superior to that of exercise or diet intervention alone. Therefore, the fundamental and effective therapy for leptin resistance is the combination of caloric restriction and exercise. However, it’s necessary for the majority of severe obesity to explore the gene-targeted drug therapies.

5.3.2. Gene-targeted drug therapy

The primary manifestations of leptin resistance are increased circulating leptin levels and a decreased sensitivity to leptin in the regulation of body weight and energy balance. However, the underlying molecular
mechanisms of leptin resistance are various, which requires the researchers to develop specific therapies targeted at different molecular mechanisms, segments and proteins in LEPR signaling pathway. At present, the gene-targeted drug therapy has become the leading treatment for leptin resistance with the development of gene detection technology and the in-depth study on the molecular mechanisms of leptin resistance.

5.3.2.1. Down-regulation of SOCS3 and/or PTP1B. The central leptin resistance is characterized by diminished hypothalamic LEPRs and impaired leptin signal transduction. The down-regulated expression of LEPR gene-related negative regulating proteins, such as SOCS3 and PTP1B, can increase the signal transduction of ObRb and prevent the development of DIO. Rats with hypothalamic SOCS3 silencing by RNAi are resistant to DIO, leptin resistance and metabolic disorders, suggesting that after the down-regulation of the SOCS3 gene by RNAi, the restoration of JAK2/STAT3 recovers the natural physiological action of leptin and normal body weight [124]. Meanwhile, Liu et al. [212] showed that obese rats with leptin resistance that were injected with SOCS3 small hair RNA (shRNA) lentivirus presented the convalescent actions of leptin. The recombinant adenoviral vector that harbors clock genes (RAV-HCG) in a different study performed by Xie et al. [213] was showed to reduce the obese indexes of DIO mice, increase leptin sensitivity and decrease accumulated SOCS3 in the arcuate nucleus, which suggests not only that RAV-HCG can improve leptin action, but also that the inhibition of clock genes in the central nervous system feeding centers plays an important role in leptin resistance. Consequently, increasing leptin sensitivity through a signal transduction pathway involved SOCS3 is expected to be able to restore the biological function of leptin in regulating the energy balance in obese patients. Leptin resistance is associated with increased activity and expression of PTP1B, which appears to be a promising therapeutic target for the treatment of leptin resistance [214,215]. Inhibition of PTP1B by a selective PTP1B inhibitor, the leptin-induced STAT3 activation was enhanced in cells [216]. Recently, though the inhibiting drugs of PTP1B have not been found, a number of studies have indicated that PTP1B is well accepted as drug target and is now thought as the focus of inhibitor discovery all over the world [217]. Consequently, it’s necessary to understand the structural details of PTP1B molecule relevant to the interactions with inhibitors, and the progress towards compounds with enhanced membrane permeability, affinity, specificity, and potency on intracellular PTP1B [216,218].

5.3.2.2. Activation of POMC directly. The most important leptin-sensitive target regions mediating directly leptin's effects on energy balance are POMC neurons. The central POMC gene therapy, activating POMC, can overcome central leptin resistance in that α-MSH, the product of POMC, can effectively suppress appetite, promote energy expenditure and decrease body weight, which makes POMC neurons the important and useful drug target regions [106,219]. For this reason, the exogenous drugs that activate POMC, such as MT-II reported by Pierroz et al. [220] can effectively bypass central leptin resistance. However, a few researchers demonstrated that direct leptin action on POMC neurons does not reduce food intake, but is sufficient to stabilize the level of glucose in mice lacking LEPRs [221]. Further studies should be concentrated on increasing the activity of POMC in regulating ObRb signals and simultaneously exploring the deep interrelations between POMC in the central and the biological actions of LEPRs.

5.3.2.3. Improvement of leptin transport into the CNS across the BBB. The BBB presents a tremendous challenge for the delivery of drugs to the central nervous system (CNS), especially the hypothalamic regions. A leptin agonist modified by the addition of pluronic block copolymers (P85-leptin) guided by the strategic drug delivery to brain (SDBB) [222] can retain the biological functions of leptin and simultaneously can transport leptin across the BBB independent of some kinds of transporters such as ObRa [223]. The decreasing level of triglycerides may also attenuate leptin resistance generated by the impaired leptin transportation across the BBB in the study by Banks et al. [117]. Though the acute phase CRP is likely to contribute to leptin resistance by preventing leptin from crossing the BBB, a latest study by Hsouchou et al. [118] demonstrated that CRP at a high dose increases the paracellular permeability of the CNS for leptin’s entry. Meanwhile, the improvement of the binding affinity between circulating leptin and its binding proteins like megalin can effectively increase the level of central leptin concentration, which has been confirmed in the study performed by Dietrich et al. [116]. Thus, increasing leptin transport into the CNS across the BBB, which is conducive to LEPR signaling transduction, is a useful strategy to combat the central leptin resistance.

5.3.2.4. Attenuation of ER stress. ER stress has been identified as one of the central mechanisms resulting in leptin resistance, which provides new fields for the treatment of leptin resistance. Hosoi et al. [108] showed previously that fluvoxamine, a selective serotonin reuptake inhibitor used for depression, can effectively attenuate the endoplasmic reticulum stress-induced leptin resistance by reversing the ER-stress-induced impairment of leptin-induced STAT3 phosphorylation. Zheng et al. [224] demonstrated that carbon monoxide (CO), a gaseous molecule, could ameliorate leptin resistance during ER stress induced by thapsigargin or tunicamycin in human cells. Zhou et al. [225] reported that a type of sweet tea (ST) leaf extract can effectively attenuate leptin resistance and produce anti-hyperlipidemic activity by ameliorating the state of oxidative stress of the ER. Meanwhile, Ropelle et al. [226] showed that the recombinant pro-inflammatory cytokine IL-6 and the anti-inflammatory protein IL-10 combined with physical exercise can suppress leptin resistance by inhibiting IKKβ-NFκB and ER stress. In a subsequent study, Jeong et al. [227] reported that AMPK activation by low molecular weight fucoidan (LMWF), a drug for treatments of metabolic disorder widely, could potentially prevent obesity by ameliorating ER stress-related leptin resistance. Consequently, the further explorations on the correlations between leptin resistance and ER stress will provide a novel therapeutic strategy for leptin resistance.

Additionally, other therapies for leptin resistance have been developed in some specific studies. Aubert et al. [228] reported that metformin presents anorectic effects in vivo after its administration was modulated by the hypothalamic ObRb in gene level, which sequentially increase the central sensitivity to leptin. Gong et al. [229] confirmed that electroacupuncture treatment could effectively reduce body weight and noticeably increase the leptin sensitivity of DIO rats through regulating the expression of leptin and ObRb possibly. Moreover, Yan et al. [230] demonstrated that cutgut implantation at acupoint could also appropriately adjust central and peripheral leptin levels and promote hypothalamic OB-R gene expression in leptin-resistant rats. Tam et al. [231] showed that JD5037, a type of peripherally restricted cannabinoid-1 receptor (CB1R) inverse agonist, has anti-obesity effects by reversing leptin resistance, which can effectively decrease appetite and reduce weight mediated by sensitizing DIO mice to endogenous leptin, and sequentially reverse hyperleptinemia by reducing leptin expression and secretion in adipocytes and improving leptin clearance via the kidney. To sum up, increasing treatments, mainly the gene drug therapies, have been developed according to the different leptin-resistant mechanisms and other solutions should also be explored with the discoveries of new targets in LEPR signaling pathways.

6. Discussions

Obesity is a complicated metabolic syndrome that is caused by various factors and molecular mechanisms. Leptin resistance, the central mechanism of obesity, has been a major concern for 20 years. During the two decades at the turn of the century, thousands of researchers have devoted themselves to the studies of leptin resistance and countless research achievements have been published all over the world.
Our hope is not on the quantity of increasing papers in different journals blindly but to bestow the gospels upon obese patients with further research on leptin resistance and obesity. Consequently, the future explorations of leptin resistance should be focused on the following points:

1. The in-depth researches ought to be the regulation of obese gene expression and its individual difference, the exact associations between the leptin gene and/or LEPR gene polymorphisms and the pathogenesis of obesity.

2. The relationships between leptin resistance and the external environment in addition to the genetics, such as diet, gender, age, and work. Meanwhile, the peripheral role of leptin should not be neglected, particularly in the context of enhanced immune responses in autoimmune diseases.

3. The causal and conditional relationships between leptin resistance and obesity.

4. Last but not the least, the exploration of gene-targeted drugs and combined drugs based on leptin signaling pathways should be carried out reasonably for promoting the relief of obesity.

To sum, additional studies in the pathogenesis of leptin resistance, and finding the new therapeutic targets of LEPR signal pathways by sophisticated genetic engineering technology, as well as the exploration of the novel gene-targeted drugs might be the next direction of obesity research and therapy. Meanwhile, the close interrelations between obesity, leptin resistance, and obesity-related diseases, such as CVD and neoplasia, should be elucidated carefully in the future.

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References

[4] Last but not the least, the exploration of gene-targeted drugs and combined drugs based on leptin signaling pathways should be carried out reasonably for promoting the relief of obesity.


H. Pan et al. / Physiology & Behavior 130 (2014) 157–169