LEPTIN RESISTANCE: A PREDIPOSING FACTOR FOR DIET-INDUCED OBESITY

Running title: Leptin resistance

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ABSTRACT

Obesity is a resilient and complex chronic disease. One potential causative factor in the obesity syndrome is leptin resistance. Leptin behaves as a potent anorexic and energy-enhancing hormone in most young or lean animals, but its effects are diminished or lacking in the obese state associated with a normal genetic background. Emerging evidence suggests that leptin resistance predisposes the animal to exacerbated diet-induced obesity (DIO). Elevation of central leptin in young, lean rats induces a leptin resistance that precludes obesity on a chow diet but accelerates high-fat (HF)-induced obesity. Similarly, chronic dietary fructose consumption evokes a leptin resistance that causes obesity only upon HF exposure. Inherent central leptin insensitivity also contributes to dietary weight gain in certain obesity-prone rats. Conversely, aged, leptin resistant animals are obese with continuous chow feeding and demonstrate aggravated obesity when challenged with a HF diet. Additionally, a submaximal central blockade with a leptin antagonist leads to obesity on both chow and HF diets, as is the case in rodents with leptin receptor deficiency of genetic origin. Despite the differences in the incidence of obesity on a chow diet, all these forms of leptin resistance predispose rodents to aggravated HF-mediated obesity. Moreover, once leptin resistance takes hold, it aggravates DIO, and the leptin resistance and obesity compound one another, promoting a vicious cycle of escalating weight gain.

Key words: leptin resistance, age, STAT3, leptin antagonist, fructose
INTRODUCTION

Leptin, a product of the obesity gene (or ob gene) [68], is a key regulator of feeding and energy expenditure [14]. This adipocyte-derived hormone was once heralded to be an anti-obesity agent. Whilst effective in certain individuals bearing congenital leptin deficiencies [17] or lipodystrophies [45, 46], as a monotherapy, leptin has been disappointing in humans and rodents with common obesity, that is, obesity associated with elevated serum leptin under a normal genetic background [23, 30, 65]. Leptin production increases proportionally with adiposity, and leptin levels are high in rodent and human models of diet-induced or adult-onset obesity. Yet, the increased leptin fails to curtail the progression of obesity [22, 23, 30, 34, 65]. This apparent leptin ineffectiveness is identified as leptin resistance.

The notion of leptin resistance conjures different interpretations, and its complex nature gives way to several definitions. In the most general terms, leptin resistance is described as the failure of elevated circulating leptin to reduce common obesity. This resistance may be due to an inability of leptin reaching target sites within the brain (resistance to peripherally administered leptin) [4] and/or impaired cellular responses within selected neurons in defined brain regions (central leptin resistance) [15, 42, 43]. In rodents, leptin resistance is often noted as the reduced sensitivity with respect to the anorectic response to exogenous leptin introduced either peripherally or centrally.

Extensive research efforts have been made to examine the cause(s), characteristics and metabolic consequences of the leptin resistance. This review will summarize some recent data and evidence arguing that leptin monotherapy, in-and-of-itself, is destined to be a failed strategy to treat obesity and that leptin resistance is a predisposing factor for diet-induced obesity (DIO).

Animal models of leptin resistance

There are several models of naturally occurring, programmed leptin resistance, including that displayed in seasonal animals [27, 28, 63] or during preganancy/lactation [2, 21] where increased food consumption and weight gain are necessary to meet the particular biological demands. Additionally, several genetic models of obesity associated with defective or absent leptin receptors reflect a tachyphylaxis to leptin responses [17]. In reality, in most models of leptin resistance such as DIO, the resistance is relative, characterized by significant reductions in either leptin sensitivity or efficacy, but some leptin receptor activity remains. The underlying mechanisms are not clearly delineated. One form of leptin resistance is associated with a defective transport of serum leptin across the blood brain barrier (BBB) [4] and thus, the ratio of cerebral spinal fluid leptin to serum leptin level is diminished [10]. This resistance to peripherally administered leptin is characteristic of high-fat (HF) induced obesity [47, 64], occurs relatively soon after initiating HF feeding and at a time
when rodents remain responsive to centrally administered leptin [47]. The mechanism may involve the interference of elevated triglycerides with leptin BBB transport [3], presumably, resulting in insufficient leptin levels within the brain. The second form of leptin resistance is characterized by blunted responses to centrally administered leptin (central leptin resistance), and often referred to as cellular leptin resistance [41, 43]. It is associated with impaired leptin signaling events within specific brain regions. Molecular mechanisms contributing to this cellular leptin resistance and impaired signaling may include increased activity of phosphotyrosine phosphatase 1B (PTP1B), elevated levels of suppressor of cytokine signaling 3 (SOCS3). The latter mediates its inhibitory action through binding to leptin-stimulated phosphorylation of Tyrosine-985 on the long form functional leptin receptor [43]. On the other hand, manipulations that enhance leptin signaling confer a lean or dietary-resistant phenotype [7, 8, 25, 40], suggesting that diminished leptin receptor signaling may indeed be responsible for cellular leptin resistance. The reader is referred to recent reviews for an in depth analysis of leptin signaling events associated with leptin resistance [42, 43].

**Leptin resistance in diet-induced obese animals**

Leptin resistance is a hallmark of diet-induced obesity. Rodents usually become obese when fed a HF diet, and the temporal development of diet-induced leptin resistance is dependent on the species and strain of the rodent [22, 35, 64]. Obese rodents have elevated leptin levels but blunted leptin sensitivity. They initially develop a resistance to peripheral leptin administration, but continued HF feeding will eventually induce central leptin resistance [13, 35, 64]. The leptin resistant rats display reduced leptin transport across BBB [4], decreased signal transducer and activator of transcription protein 3 (STAT3) phosphorylation and P-STAT3 transcription binding capacity [13], and marred melanocortin release in certain areas in the hypothalamus [15]. This leptin resistance is presumably reversible if the HF diet is withdrawn [65].

The degree of DIO is not uniform. For example, most Sprague Dawley (SD) after weaning spontaneously divide into two groups when exposed to HF, those resistant to the diet-induced weight gain (referred to as Diet Resistant) and those particularly susceptible to the weight gain (referred to as Dietary Prone) [30]. The susceptibility to DIO may be due to inherent differences in leptin sensitivity [31, 32]. When SD rats selectively bred to develop or resist dietary obesity were subject to an acute leptin challenge, the Dietary Prone rats demonstrated diminished hypothalamic leptin-induced STAT3 phosphorylation compared with the Dietary Resistant rats prior to the development of obesity [31]. Although no cause and effect was established, this evidence supports the notion that leptin resistance promotes obesity. Despite advancing knowledge, the exact cause for the diet-induced leptin resistance is still unclear and many questions remain: is it due to the
energy-rich diet, the elevated leptin, increases in compensatory mechanisms and/or a gradual neural network rewiring following chronic HF feeding? Because the HF food consumption has more to do with the reward properties of the palatable food rather than the caloric value, the endocannabinoid system and/or mesolimbic dopamine circuits may also have a role the HF feeding behavior and the resultant obesity [6, 12, 24, 37].

**Leptin resistance in aging animals**

Besides DIO, another common form of obesity is age-related or adult-onset obesity. Humans demonstrate a steady increase in body weight and adiposity through early senescence followed by a decline in latter life [60]. In our aging rat model, the Fisher 344 x Brown Norway (F344xBN) rat, body weight and adiposity follow a steady increase into early senescence (from 3 to 24 months) and then a decline from 24 to 30 months [34]. For the purpose of this review, we will consider only the portion of the adult life span in which body weight and adiposity are increasing, and refer to this as age-related obesity. At 24 mo, aged F344xBN rats have 3-4 times higher serum leptin relative to that at 3 mo [34]. Despite this hyperleptinemia, obesity persists, and the adiposity level at 24 mo amounts to ~ 400% of that at 3-mo. These aged-obese rats exhibited little or no anorectic or weight loss responses to peripherally infused leptin [26] as opposed to a dose dependent food suppression and weight reduction in young rats in response to similarly infused leptin, demonstrating a leptin resistance associated with age-related obesity. Similar leptin resistance is also indicated in aged Wistar rats [18], and caloric restriction was shown to be effective in these animals to partially restore leptin responsiveness [19].

The age-related leptin resistance consists of both a peripheral and central component [69]. Young rats respond more robustly to either peripheral or central leptin administration than the aged [26], but the responses to central leptin infusion in the aged are greater compared to the minimal responses observed following peripheral infusion [26, 51, 52].

**Leptin-induced leptin resistance in young lean animals**

There is clear evidence that prolonged leptin treatment in young, lean rodents can induce leptin resistance. Male Long-Evans rats receiving a 21-day chronic subcutaneous leptin infusion lost the anorectic response to leptin half way into the treatment. A subsequent peripheral high-dose leptin challenge failed to alter 24hr food intake in the leptin-treated rats [36]. Significantly diminished hypothalamic leptin receptor message and protein levels appeared to provide one explanation for this leptin resistance [36]. In another case in which leptin was infused chronically into the lateral
cerebroventricle in adult male Sprague Dawley rats for 28 days, the animals had an initial decrease in food intake, but then developed resistance to the satiety action of leptin after three weeks [49]. In a study from Friedman laboratory, a supra-physiological dose of leptin was used first to deplete fat mass and reduce body weight. Upon an abrupt withdrawal of the leptin supplement, a sudden peripheral leptin deficiency was created that generated diminished immune and reproductive functions. When half of the mice were allowed to eat, subsequently, *ad libitum*, they displayed hyperphagia and regained the lost weight. The other half of the mice though, with their food intake restricted to the normal pre-experimental consumption, continued to display low levels of leptin and blunted responses to an exogenous leptin challenge both in terms of prevention of the regain of lost weight and restoration of deficient immune and reproductive function [39]. It was concluded that high-dosage leptin treatment induced a state of acquired leptin resistance. Using transgenic mice overexpressing human leptin within adipose tissue, Qiu et al. found that the mice had lower body weight and less adiposity at young age, but regained the lost adiposity and body weight at older age [48]. Such data again demonstrate that leptin insensitivity develops over time.

We have established our own rat model of central leptin elevation-induced leptin resistance. This was achieved using rAAV-based gene transfer system to deliver the gene coding for rat leptin (rAAV-leptin) directly into the brain and intended to gain insights into the relationship between elevated central leptin and leptin resistance. Leptin overexpression in the hypothalamus of the lean F344xBN rats continued up to over 300 days without evidence of abatement and resulted in a 75% elevation in cerebrospinal fluid (CSF) leptin levels [57]. The animals initially responded to rAAV-leptin with reduced food intake, elevated oxygen consumption, and a substantial decrease in body weight, and the body fat reduced to near zero by day 10 [57]. However, food intake slowly returned to control level over time and the enhancement in oxygen consumption was also lost [58]. Despite lower body mass in the rAAV-leptin-treated versus control rats at day 300, the rAAV-leptin rats maintained their regular food intake whereas control rats displayed the expected food suppression in response to a 7-day leptin icv infusion, confirming a leptin-induced leptin resistant state [58]. The fact that leptin overexpression in the CNS could evoke hypothalamic leptin resistance in rats devoid of obesity argues that elevated central leptin is one independent factor causal to acquired leptin resistance.

In humans, elevated serum leptin predicts the subsequent development of metabolic syndrome at 5 and 10 years [20], and CSF levels of leptin are augmented with obesity in humans, even though the ratio of CSF to peripheral leptin is diminished [10]. However, the physiological significance of increased central leptin remains unclear. Conceivably, the CSF leptin levels in common obesity may be abnormally high due to peripheral hyperleptinemia and could contribute to
diet-induced central leptin resistance or these levels are, in fact, insufficient owing to the defect in leptin transport across the BBB associated with DIO.

The multiple examples of leptin treatment described, whether transgenic, high pharmacological leptin administration, or leptin central gene therapy, may not have a naturally occurring physiological and/or pathological counterpart; nevertheless, they provide valuable research tools to study the role of leptin or hyperleptinemia per se in homeostasis regulation. Moreover, the results from these various studies demonstrate a common phenomenon of leptin-induced leptin resistance and underscore the destined failure of using leptin monotherapy to combat obesity.

**Dietary Fructose and Leptin Resistance**

We discovered a unique model of leptin resistance associated with chronic high fructose feeding in which neither obesity nor leptin treatment was involved [62]. Epidemiological studies suggest a relationship between the consumption of fructose-enriched products and increased rates of obesity [9]. We hypothesized that leptin resistance may provide the link between dietary fructose and obesity. We fed a group of Sprague-Dawley rats a fructose-free control or a high fructose diet (60% fructose) for six months and tested for leptin sensitivity both before and after the diet. Initially, all rats displayed an anorectic response to intraperitoneal leptin injection, but after six months, only the control rats responded [62]. At this point, both groups had similar body weights, total body adiposity, or serum leptin, insulin or glucose except for elevated serum triglycerides with high fructose consumption. The fructose-fed rats nonetheless showed blunted food response and decreased hypothalamic STAT3 phosphorylation compared with controls [62], demonstrating a chronic fructose consumption-induced leptin resistance. Similar findings indicating a fructose-induced leptin resistance were reported in an abstract from the Harris group [11]. Elevated Serum triglycerides due to high fructose consumption may leptin transport across the blood-brain barrier [3], which implicates an potential insufficiency in leptin reaching target sites within the brain resultant from the fructose-induced leptin resistance.

**Leptin resistance predisposes rodents to diet-induced obesity**

It is clear in animals with normal endogenous levels of circulating leptin that leptin therapy, be it by administration or overexpression, initially induces a weight and adipose loss, but eventually those animals regain the lost weight and adiposity and achieve body weight parity with control counterparts. Despite the presence of acquired leptin resistance, body weight of the leptin-induced leptin resistant animals never exceeds that of control animals. The amount of chow diet consumed
in these animals without leptin supplement likely represents homeostatic caloric requirement for natural growth (with little reward incentive). The growth-related body weight appears to be rigorously defended by animals despite the transient period of negative energy balance due to exogenous leptin treatment. These observations are consistent with the so-called “Adiposity (Weight) Set-Point” theory [29, 33] in which animals fiercely protect a predetermined level of adiposity (or body weight) presumably to ensure critical immune and reproductive functions for survival. By and large, the acquired leptin-induced leptin resistance appears to be “quiescent” in chow-fed animals as long as additional metabolic challenges are not superimposed.

However, upon exposure to a HF diet, a different picture emerges: leptin resistant animals display exacerbated weight and adiposity gain compared with corresponding HF-fed, non-leptin resistant controls. In fact, all leptin-induced, aged-related, and fructose-fed leptin resistant animals faired worse in face of the HF exposure.

In our leptin-induced leptin resistant model, leptin resistance was produced following 94 days of central rAAV-leptin gene therapy in the young-lean rats [54]. When switched to a HF diet the rAAV-leptin-treated, HF-fed rats consumed a 36% greater amount of calories, grew considerably heavier (Fig. 1A), and accumulated 26% more visceral fat relative to HF-fed leptin sensitive controls. Hence, the acquired leptin resistance predisposes these rats to DIO. A similar outcome was discovered with the transgenic mice overexpressing human leptin in adipose tissue. The leaner transgenic mice gained considerably more body weight and adiposity on a HF diet compared to the wide type control mice [44].

A similar scenario occurred in aged leptin resistant rats. For instance, the 30-month-old F344xBN rats consumed more calories and exhibited a larger weight gain relative to young-lean rats on a HF diet (Fig 1B) [26], implicating a heightened susceptibility to dietary obesity associated with the age-related leptin resistance.

In the unique case of chronic fructose consumption, rats developed leptin resistance despite the normalcy of all other metabolic markers except for the increased serum triglycerides [62]. There was no evidence of obesity until a subsequent two-week long HF challenge was applied. The rats pre-exposed to fructose consumed more calories, gained more body weight, and had increased adiposity compared with correspondingly fed rats pre-exposed to the fructose-free diet (Fig 1C). This leptin resistance resembles that of leptin-induced leptin resistance, in that, weight gain was avoided unless a HF diet was provided.

The presence of a continuous high-energy diet complicates the DIO leptin resistance model. We describe here two aspects of leptin responsiveness: prior to HF feeding and after consumption of a
HF diet. With respect to the former, is the case of dietary prone rats that exhibit rapid weight gain with HF feeding. These rats have diminished response to leptin prior to HF feeding, and presumably, this leptin resistance predisposes the rats to dietary obesity [31]. Conversely, those rats that are diet resistant have corresponding greater responses to leptin prior to HF feeding, suggesting that leptin sensitivity protect against DIO. These data support the idea that leptin resistance predisposes to DIO.

Feeding a HF or combination of high fat, sugar, and salt (Western diet) during pregnancy was reported to subject offspring to develop later obesity [5, 38]. Although leptin resistance was not examined in these studies, consumption of the HF diet during gestation, lactation or post-weaning resulted in hyperleptinemia, indicative of a presumed leptin resistant state. These facts seem to imply that maternal/parental leptin resistance may be an important contributing factor predisposing the offspring to obesity.

While the issue about whether the leptin resistance associated with DIO is secondary or causal to obesity is unsettled, a new question arises – does this leptin resistance sustain and/or worsen obesity? Little information is available to address this concern at the present time. One of our recent studies may give some clues. We subjected diet-induced obese rats (5-mo HF fed) to central rAAV-leptin gene therapy; they responded with a transient, mild anorexia and weight reduction, reflecting a partial but not complete leptin resistant state [61]. The lost body weight was regained in 14 days. However, as the leptin therapy continued, the trajectory of HF-mediated increase in weight gain became two-fold greater in the rAAV-leptin-treated compared with HF-control animals (Fig 1D). Thus, central leptin therapy in rats with existing dietary obesity evidently accelerated diet-induced weight gain. We interpret these findings to indicate that the partially leptin resistant DIO rats developed a full leptin-induced leptin resistance rather quickly, which triggered the heightened weight gain in response to the continued HF feeding. A further contention would be that the leptin-induced resistance worsens dietary obesity.

The physiological relevance of leptin resistance evoked by pharmacological leptin therapy or central leptin overexpression remains to be tested, but animals possessing this kind of acquired leptin resistance as well as those with diet-induced or age-related leptin resistance, all lack the ability to properly regulate energy balance when challenged with a HF diet. Collectively, leptin resistance is a predisposing factor for diet-induced obesity. On the contrary, increasing leptin sensitivity (thus dampening leptin resistance) through genetic disruption of inhibitory factors of leptin signaling events reduces body weight and protects against DIO [7, 25, 40]. These findings seem to lay some ground for claiming a physiological significance of leptin resistance in obesity, especially in the context of HF feeding.
Insights from a leptin antagonist

Leptin resistance is associated with reduced leptin receptor activity

Whether central leptin resistance is partially due to reduced leptin receptors is controversial, but mounting evidence demonstrates diminished leptin receptor-mediated signaling including both STAT3 and phosphatidylinositol-3-OH kinase (PI3 kinase) pathways is associated with leptin resistance [50, 53]. In addition, leptin up-regulation of the downstream anorectic neuropeptide pro-opiomelanocortin (POMC) and the release of its cleaved product α-melanocyte-stimulating hormone (α-MSH) is impaired in the hypothalamus with leptin resistance [15, 57]. Alternatively, disruption of the inhibitory components of the leptin signaling pathway, including PTP1B [7, 66] or SOCS3 [25, 40] enhances leptin sensitivity and/or confirms resistance to dietary obesity. Thus, diminished leptin receptor activity appears to be integral to leptin resistance. Leptin receptor activity, for the purpose of this discussion, refers to both the stimulation of the immediate leptin-receptor signaling events and all subsequent signaling and downstream physiological events that are dependent on the initial ligand-receptor interaction. The obesity phenotype resultant from either lack of leptin or leptin receptors due to genetic mutations highlights the importance of leptin receptor pathway in long-term body weight maintenance. Conceivably, even a minor impairment in the leptin receptor pathway could result in obesity over time. The recent availability of a leptin receptor antagonist has afforded us a special tool to simulate various degrees of leptin blockade and examine the long-term physiological consequences of this form of leptin resistance. We predict minimal metabolic effects from leptin antagonist treatment in leptin-resistant versus leptin-responsive rats if leptin resistance is the result of down-regulated leptin receptors, diminished signaling, or receptors functionally uncoupled from downstream physiological responses. In this case, we consider the extent of the metabolic responses to the leptin antagonist to be a measure of functional leptin receptor activity.

Leptin receptor blockade.

The in vivo action of the leptin antagonist was first characterized in our young, lean F344xBN rats maintained on a chow diet [67]. Simultaneous central administration of leptin and increasing doses of the leptin antagonist revealed a dose-dependent inhibition of leptin-induced hypothalamic STAT3 phosphorylation. A 7-day infusion of the leptin antagonist alone produced an increase in food intake and weight gain [67]. When administered in the presence of exogenous leptin during the infusion, the leptin antagonist blocked the leptin-mediated anorexic effects and body weight reduction as well as the increase in brown adipose tissue (BAT) UCP1 protein and hypothalamic STAT3 phosphorylation. Although long-term antagonist infusion is impractical, if this feat was
accomplished, we would anticipate a gradual build-up of fat mass and body weight and eventual obesity. Another group also found the same leptin antagonist protein useful to investigate the effects of early postnatal leptin disruption on long-term leptin sensitivity and metabolic phenotype [1].

The leptin antagonist was next employed to gain insights into the relationship between leptin resistance, leptin receptor activity and metabolic outcomes. Central leptin resistance was induced in lean rats by chronic leptin overexpression mediated by rAAV-leptin vector delivery [55]. At day 153, rAAV-leptin and rAAV-Control rats were given centrally either vehicle or a rat leptin antagonist for 14 days [55]. Food intake, body weight, adiposity and BAT UCP1 expression increased with the antagonist infusion in controls but elevated only marginally in rAAV-leptin rats [55]. The lack of significant physiological responses to the antagonist treatment is indicative of diminished leptin receptor activity accompanying the central leptin resistance and resonates with the notion that defective leptin receptor function underlies leptin resistance.

The leptin antagonist also revealed the role of leptin receptor activity in hedonic high-fat consumption [67]. When rats were provided with a HF diet (60% kcal as fat) in the presence of the antagonist (25 μg/d, lateral ventricle infusion) or vehicle for 7 days and compared with vehicle-infused chow-fed control rats, the daily caloric intake of both HF groups peaked on day 2. However, the HF-induced caloric hyperphagia was normalized to a level isocaloric with regular chow intake by day 7 in the vehicle-infused HF group, whereas the hyperphagia remained in the HF antagonist-infused group (Fig 2A). Moreover, the HF-mediated weight gain was exaggerated by the leptin antagonist treatment (Fig 2B). These results indicate an important inhibitory function of endogenous leptin or leptin receptor activity in the counter-regulation of hedonic feeding (reward aspect of eating behavior).

**Sub-maximal chronic leptin receptor blockade.**

Generally, the development of leptin resistance upon HF feeding, with age, or prolonged leptin therapy is gradual, involving a process complicated by interactions between the diet, aging and various leptin-dependent and independent pathways controlling food consumption and energy expenditure. We introduced a chronic sub-maximal leptin receptor blockade via central gene delivery of a mutated rat leptin gene coding the leptin antagonist protein [59], attempting to directly address the role of daily central leptin receptor activity in the control of energy homeostasis in naturally growing, young-lean animals in a chronic manner. Young-lean, chow-raised F344xBN rats received rAAV-Control or rAAV-leptin antagonist for 190 days. During this time, the rats were maintained on a chow diet for 80 days, then switched to a 60% HF diet until day 140, after which
they were returned to the chow diet until the end of the experiment [59]. The normal growth-related and diet-induced body weight gain was exacerbated in the antagonist group. Feed efficiency expressed as the body weight gain per unit of food consumed, was increased during both the chow and HF feeding periods respectively. Because full leptin receptor blockade achieved via central infusion of the leptin antagonist protein in previous study abolished the normalization of HF-induced hyperphagia (Fig 2A) [67] as opposed to only a small delay in the normalization process upon HF exposure with antagonist overexpression, we consider that the rAAV-leptin antagonist produced just a sub-maximal central receptor blockade. In addition to its impact on energy intake, the antagonist also affected physical activity. The rAAV-leptin antagonist rats displayed substantially less wheel running (WR) activity when provided free access to running wheels for 6 days during both the chow and HF feeding periods. On the contrary, WR activity was increased by more than 2-fold in response to leptin overexpressing in the hypothalamus [59]. At death, adiposity and serum leptin levels were greater in the antagonist group. In aggregates, sub-maximal central leptin receptor blockade leads to greater adiposity, accelerates dietary weight gain, and diminishes WR activity. These observations validate the critical role of fully responsive leptin receptors in long-term regulation of energy homeostasis and hedonic feeding.

**Perspective and Significance:**

This review examined several models of leptin resistance and the role of leptin resistance in the susceptibility to dietary obesity. Only some of the leptin-resistance models (leptin antagonist blockade and aged-obese rats) exhibit heightened weight and adiposity gain on a chow diet, whilst all models discussed demonstrate obesity in the presence of a high-fat diet. Thus, the leptin resistance appears to be reinforcing “reward eating” beyond caloric energy requirements. Over-consumption of palatable food could be mediated by activation of reward circuitry involving opioids and dopamine or an impairment in a pathway (or pathways) mediating satiation of the palatable diet [16].

Whereas the hypothalamus, in particular, the arcuate nucleus, has been identified as important in regulating the caloric requirements, other regions in the hypothalamus such as lateral hypothalamus and extrahypothalamus areas including the amygdale, prefrontal cortex, nuclear accumbens and ventral tegmental area (VTA) are implicated in the reward properties of food [12, 43]. Leptin receptors are identified on dopamine containing neurons within the VTA and found to suppress dopaminergic neuron firing rate [24]. They act through the JAK-STAT signaling pathway and decrease food consumption upon leptin action. The fact that a chronic reduction in leptin receptor activity in the VTA by siRNA knockdown enhances sensitivity to
highly palatable food underscores an important role of leptin receptor function in the regulation of reward feeding behavior [24]. Our own data also support a counter-regulatory mechanism by which leptin modulates HF feeding: leptin receptor blockade prolonged the caloric hyperphagia induced by a HF diet [67]. Although, the inputs from multiple brain regions are integrated to determine food ingestion, hedonic feeding driven by the VTA has been suggested to be able to overcome the caloric requirements of homeostatic regulatory properties of the hypothalamus [12].

Two of the leptin resistance models described herein, leptin-induced leptin resistance [54] and fructose-induced leptin resistance [62] accelerated the onset and exacerbated the extent of HF-induced obesity. However, in neither case, did leptin resistance result in obesity on chow diet. For example, rats with central overexpression of leptin initially respond to leptin with a reduction in body weight, then as leptin resistance develops, the lost body weight is regained until it reaches parity with control animal [54, 56, 58]. Despite this leptin resistance, the body weight of the leptin-induced leptin resistant animals never exceeded that of control animals unless a HF diet was provided. A similar outcome is manifested in fructose-induced leptin resistant rats, i.e., obesity only develops with HF feeding [62]. Conversely, aged, leptin resistant animals with a deficit in leptin signaling and blunted leptin responses display a steady gain in adiposity on standard chow diet and demonstrate aggravated obesity when challenged with a HF diet [26, 34, 51]. Likewise, treatment with a leptin antagonist at a submaximal blockade level leads to obesity on chow diet and increased susceptibility to HF-induced weight gain [59]. This resonates with genetic models of leptin receptor deficiency that also result in obesity on both a chow and HF diet. It appears that acquired leptin resistance including leptin-induced and fructose-induced promote obesity only in association with over-consumption of palatable diet, whereas the other forms of leptin resistance evoke obesity regardless of diets. This suggests that the former forms of acquired leptin resistance are fundamentally different than partial blockade or full disruption of leptin receptor activity, and possibly involve preferential dysregulation of hedonic eating rather than long-term homeostatic regulatory mechanisms.

In a presumed scenario when leptin receptor activity within the hypothalamic homeostatic brain circuitry falls below a threshold level, obesity may occur irrespective of diet compositions, whereas specific defects within the brain reward circuitry may increase the susceptibility to HF-induced obesity. By such a speculation, if leptin-induced leptin resistance differentially impairs the leptin receptors involved in the reward properties of food ingestion as opposed to those involved the homeostatic regulation of the caloric requirement for animals, then the consequences of the former aspect of the resistance would only be revealed with the
introduction of a HF diet. The HF-induced hyperphagia is usually transient, indicating that diminished energy expenditure and/or increased food efficiency are other likely mechanisms underlying long-term leptin-resistance-mediated obesity. These ideas remain to be tested.

The diet-induced obesity model is complex, and the relationship between leptin resistance and the development of this type of obesity is unclear. Data from dietary prone animals point to a potential causal role of preexisting deficient leptin receptor signaling (i.e., some degree of leptin resistance) in predisposing the animals to HF-induced obesity [31]. Additionally, chronic leptin treatment in DIO rats exacerbates HF-induced obesity (Fig. 1D, [61]), an observation implicating a preferential disruption in counter-regulation of motivational eating rather than homeostatic feeding. Lacking definitive evidence, our current discussion of the potential impact of leptin resistance on dietary obesity remains speculative and likely too simplistic, and further investigations in this regard are warranted. The quest ahead is to elucidate the exact factors initiating leptin resistance, the role of this resistance in hedonic feeding and adaptation to positive energy balance in relationship to obesity development, and more importantly, if restoration of leptin responses in selective brain regions reverses diet-induced obesity.

In today's society with an overabundance of readily available high caloric food, leptin resistance is likely to be prevalent. As leptin resistance takes hold, each subsequent exposure to high-density food may escalate dietary weight gain, causing a vicious spiral of increasing obesity. The predictable devastating metabolic consequences associated with ever perpetuating obesity underpin the importance in unraveling the mystery of leptin resistance for both the prevention and intervention of obesity.

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Figure Legends

Fig. 1.

A. Body weight gain following a high fat diet in rats pre-treated for 94 days with control vector (open circles) or rAAV-leptin (closed circles) compared with chow fed rats pre-treated with control vector (closed squares). Values represent the mean ± SE of 8 rats per group. In some cases standard error bars are less than the size of the data point. \( P < 0.001 \) for difference in body weight gain between all pairs by one-way ANOVA. Modified from [70].

B. Body weight gain following HF feeding in 3-month old (open circles) and 30-month old (closed circles) rats. Data represents mean ± SE of 21 3-month old and 5 30-month old. Body weight gain in the 30-month-old rats was greater than the 3-month-old rats beginning at day 3 (\( P < 0.01 \), one-way ANOVA). Adapted from [26].

C. Body weight gain in rats pretreated with a fructose free (circles) or high fructose (squares) diet for 6 months, and then either maintained on the fructose-free (open circles) or high-fructose (open squares) diets or switched to a 60% high fat diet (respective closed symbols). Values represent the mean ± SE of 5-6 animals per group. \( P < 0.01 \) for difference between high fructose/ high fat and control/ high fat weight gain beginning at day 1. Adapted from [62].

D. Body weight gain following administration of control vector (circles) or rAAV-leptin (squares) in DIO rats. The rAAV-leptin or control vectors were administered in rats raised on a high-fat diet for 5 months and continued on the high-fat diet throughout the experiment. Values represent the mean ± SE of 6 rats per group. \( P < 0.05 \) for difference in slopes beginning at day 20 after vector administration. Adapted from [61].

Fig. 2. Daily caloric intake (A) and body weight gain (B) in HF-fed rats following a 7-day infusion of antagonist (25 \( \mu \)g/d) or vehicle and in chow-fed rats following vehicle infusion. The antagonist or vehicle infusion and HF feeding started at day 0. Values represent the mean ± SE of 9 Antagonist/HF, 8 Control/HF and 8 Control/Chow rats. Food intake data are expressed as caloric intake per day, based on 3.10 kcal/g of chow and 5.24 kcal/g of HF diet. Caloric intake and body weight significantly differed between Antagonist/HF and Control/HF beginning at day 1 (\( P < 0.01 \)). Adapted from [67].