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Central leptin insufficiency syndrome: an interactive etiology for obesity, metabolic and neural diseases and for designing new therapeutic interventions

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Abstract

This review critically reappraises recent scientific evidence concerning central leptin insufficiency versus leptin resistance formulations to explain metabolic and neural disorders resulting from subnormal or defective leptin signaling in various sites in the brain. Research at various fronts to unravel the complexities of the neurobiology of leptin is surveyed to provide a comprehensive account of the neural and metabolic effects of environmentally-imposed fluctuations in leptin availability at brain sites and the outcome of newer technology to restore leptin signaling in a site-specific manner. The cumulative new knowledge favors a unified central leptin insufficiency syndrome over the, in vogue, central resistance hypothesis to explain the global adverse impact of deficient leptin signaling in the brain. Furthermore, the leptin insufficiency syndrome delineates a novel role of leptin in the hypothalamus in restraining rhythmic pancreatic insulin secretion while concomitantly enhancing glucose metabolism and non-shivering thermogenic energy expenditure, sequelae that would otherwise promote fat accrual to store excess energy resulting from consumption of energy-enriched diets. A concerted effort should now focus on development of newer technologies for delivery of leptin or leptin mimetics to specifically target neural pathways for remediation of diverse ailments encompassing the central leptin insufficiency syndrome.

Keywords

leptin insufficiency; brain; obesity; metabolic syndrome; cognition; Alzheimer's disease

1. INTRODUCTION

Coleman in 1970's postulated that the absence of a circulating satiety hormone from adipose tissue was responsible for the relentless hyperphagia and morbid obesity in obese *ob/ob* mice [36,37]. Isolation of the *ob* gene from adipose tissue and identification of its protein product, leptin, as a putative "satiety" signal was accomplished in the mid-nineties [143]. On the basis of the long-standing body of clinical and experimental evidence it was generalized that the "satiety" effects of leptin are integrated through the hypothalamus and in the complete absence of leptin, as in *ob/ob* mice and some humans, hyperphagia and the attendant increased rate of bodyweight gain culminate in morbid obesity, and further, leptin normally exerts a tonic restraint on overeating by modulating the interactive appetite regulating network (ARN) in the

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hypothalamus [28,35,52,54,55,59,71,74,77,78,108,114,115,140]. The current worldwide epidemic of obesity attributed to environmental causes and the upsurge in the incidence of a variety of obesity-dependent metabolic afflictions have spurred multidisciplinary research aimed at deciphering the precise nature of the crosstalk between leptin and neurochemical signaling in the hypothalamus [25,52,55,57,64,70,78,79,126,127].

In this context, central leptin resistance has been the cornerstone of the mechanistic insight advocated earlier to explain the environmentally-induced obesity pandemic and related disease cluster of metabolic syndrome [18,27,29,52,54,58,72,74,124]. This hypothesis was construed on the conventional perception that loss of central tonic leptin restraint produced by impedance of leptin transport across the blood-brain barrier (BBB) along with disruptions in leptin-induced intracellular signalings in the ARN, favored overeating and accelerated fat accrual. However, subsequent evidence indicated that unregulated eating was neither universal nor significantly concomitant with dietary obesity in animals and humans [5,20,28,44,54,55,97]. Diminution in blood to brain leptin transfer did not compromise intracellular signaling in the ARN because centrally administered leptin remained effective in suppressing weight in obese rodents [28, 44,59,76,110,135]. Thus, despite the interest generated by this idea, it now appears to be conjectural rather than corroborated by substantial research findings.

An alternate obvious consequence of limiting transport across the BBB is leptin insufficiency at multiple targets in the brain. Indeed, a large body of experimental evidence is consistent with the idea that central leptin insufficiency for extended periods due to dietary and lifestyle changes, orchestrates pathophysiological consequences that include increased rate of fat accrual, decreased energy expenditure and general activity level, hyperinsulinemia, hyperglycemia, neuroendocrine disorders, osteoporosis and impaired learning and memory [7,9,12,13,20,22,31,41,42,45,49,50,67,80,88,95,107,123, Fig. 1]. A further crucial issue here, and one that is usually overlooked is the fact that leptin permeability across the BBB, a first step towards development of leptin insufficiency, is itself subject to modulation by a spectrum of endogenous factors. In addition to adiposity, intrinsic circadian rhythms governing ingestive behavior, daily mealtimes, afferent ultradian rhythms in hormonal feedback signaling critical in hypothalamic integration of the daily feeding pattern, wide ranging blood borne neuroactive metabolic variables and aging itself can affect leptin transport from blood to brain [8–14,29, 73,82–85,98,110–112]. Furthermore, since leptin exerts pleiotropic effects in the brain, BBB-induced suboptimal or defective pattern of leptin signaling at extrahypothalamic targets, either directly or secondarily due to breakdown in hypothalamic integration of energy homeostasis, can potentially contribute to a spectrum of neural disorders.

In light of this new insight into the neurobiology of leptin, this review critically reappraises the body of scientific evidence that argues the merits and demerits of central leptin resistance and leptin insufficiency hypotheses in promoting positive energy balance, the pathophysiological metabolic consequences and CNS disorders. In doing so, an additional goal is to gain a deeper understanding of the dynamic dialogue between leptin and the brain that may be significant for identifying novel loci for designing novel interventional strategies to decelerate the scourge of obesity and related metabolic and neural afflictions.

2. LEPTIN AVAILABILITY IN THE BRAIN

Leptin detected in the two components of the brain, cerebrospinal fluid (CSF) and parenchyma, is derived from the peripheral circulation and synthesis within the brain itself [3,14,27,29,47, 71,85,91,103,112,124,138,139,144]. The major peripheral sources of leptin, adipocytes and stomach, secrete leptin in a pulsatile fashion [6,109,110]. The short isoform of the leptin receptor located in the endothelium of the vasculature and epithelium of the choroid plexus of the circumventricular organs most likely transports leptin across the BBB [19,52,63,85,87,

112]. A variety of endogenous factors that modify the dynamics of leptin entry across BBB are the following:

1. Leptin entry into the brain is temporally correlated with circadian fluctuations in circulating leptin levels. The lowest amount of entry precedes or is coincident with the nadir in circulating leptin concentrations at the onset of the daily dark phase feeding [111,141]. The peak level of leptin entry coincides with rising leptin secretion during the post-prandial period and it subsides progressively thereafter along with the gradual fall in blood leptin levels until the next dark phase feeding [111,141],
2. Blood-to-brain transport of leptin is reduced in association with decreased circulating leptin levels that follow food deprivation or calorie restriction [6,52,72–74,82],
3. Leptin entry into the brain is attenuated in ovariectomized rodents contemporaneously with hypoleptinemia [84,130].
4. Aging affects leptin availability at CNS sites [9,10,12,13]. In outbred aging mice, despite consuming a relatively stable rodent chow diet, the BBB capacity to transport leptin into the brain gradually declined in obesity-prone (OP), moderately heavier hyperleptinemic mice than in obesity-resistant (OR) lean mice [9,10,12,13].
5. At the other end of the spectrum, in severely hyperleptinemic young obese OP rodents and human subjects consuming high energy diets, the supply of leptin to various hypothalamic and extrahypothalamic sites was also reduced [11–13,29,85,91,124].
6. Blood-borne hormones and circulating metabolic variables also independently modulate the BBB transport system to quantitatively affect leptin availability at CNS sites. For example, α -adrenergic agonist epinephrine, insulin and glucose enhance and triglycerides inhibit the transport of leptin across the BBB [8–14,47,83,85,91,98,138].
7. Leptin binding proteins in blood may alter levels of leptin availability for transport. Circulating levels of hepatic C-reactive protein (CRP) that binds leptin independently correlate with plasma leptin levels, and fat depletion suppresses CRP levels [32, 53, and unpublished]. Seemingly, increased leptin binding by elevated CRP levels in hyperleptinemic obese subjects quantitatively restricts leptin availability at the level of short isoform of leptin receptors for transport across BBB. Thus, a feedback loop between adipocytes and hepatic cells operates in the periphery to dampen leptin entry into the brain to modulate varied central responses [32,53].
8. Leptin entry rates at various locations within and outside the hypothalamus in the brain differ significantly under varied physiological challenges [10,47,91]. Hyperleptinemia was reported to be associated with leptin transport to extrahypothalamic sites in the pons, medulla, hippocampus, cerebellum, occipital cortex and midbrain in higher amounts than to the hypothalamic arcuate nucleus (ARC), the site involved in propagation and termination of appetitive drive within the ARN [74,78,140]. The functional implication of these variations in leptin levels in extrahypothalamic sites warrants investigation.

Furthermore, brain is another non-adipocyte site of leptin synthesis, where it is likely to modulate neuronal functions by paracrine/autocrine actions. Wilkinson and coworkers [103, 139] detected leptin mRNA expression in specific areas of rat hypothalamus, cortex and cerebellum. We have consistently detected leptin mRNA expression in the rat and mouse hypothalamus [4,5,17,22,40,41,70,76]. Moreover, fasting decreases leptin mRNA expression in the hypothalamus, as it does in adipose tissue, an observation consistent with the notion that decreased contributions of peripherally and locally derived leptin result in the overall leptin insufficiency at hypothalamic targets in response to fasting-induced depletion of energy stores

[103,139]. Expression of leptin mRNA and leptin protein were also detected in the human hypothalamus [47]. The amount of leptin secreted by the human brain is substantial as leptin outflow was higher in the internal jugular vein when simultaneous arterio-venous blood samples were compared. Interestingly, the spill-over of leptin into the internal jugular vein was greater in obese as compared to lean female subjects [47]. Overall, when one takes into account the pronounced efficacy of enhanced hypothalamic leptin synthesis induced by leptin gene therapy in modulating energy homeostasis [4,5,17,22,40,41], the role of leptin produced by the brain is likely to be significant.

In summary, it is clear that reduced availability of leptin in the brain is a consequence of not only the hyperleptinemia in dietary obese young and aging obese rodents and obese human subjects, but also of the hypoleptinemia in the preprandial interval on a daily basis, and fat depletion in response to fasting and caloric restrictions. This analogous pattern of blood to brain entry in response to two opposite patterns of blood leptin concentrations is a provocative new insight that seriously questions the validity, now in vogue, of the leptin resistance hypothesis in explaining the increased fat accrual and obesity due to environmental causes.

3. ENVIRONMENTAL OBESITY, IS IT DUE TO CENTRAL LEPTIN RESISTANCE OR LEPTIN INSUFFICIENCY?

3.1 A. Leptin resistance

The two tenets, impedance to leptin entry imposed at the BBB and extinction of leptin-induced intracellular signaling downstream of leptin binding to the long form of neuronal receptor LRB in the hypothalamus, constitute the core of the leptin resistance hypothesis promulgated several years ago to explain obesity due to environmental causes [9,12,13,15,18,27,29,52,54,55,58, 59,65,104,105,108,120,121,140]. According to the conventional underpinning, leptin resistance confers chronic overeating that impels storage of excess energy as fat depots which underlies the accelerated rate of weight gain. However, a careful scrutiny of the results of these investigations reveals little support for the contention that rodents forced to consume high fat diets (HFD) to reproduce clinical obesity, display chronic overeating [5,12,20,28,44,59,76, 92,97,110,115]. Nearly one-half the rodents maintained on HFD are OR and do not exhibit increased food intake. The remaining OP subgroup generally reduce their daily intake when it is assessed in terms of the bulk quantity consumed. In fact, increased food consumed in gram quantity is a rare occurrence in rodents maintained on HFD. The high caloric density, and kcal intake derived from modified macronutrients in the diet and not hyperphagia by OP rodents culminates in increased adiposity.

Whether a persistent increase in appetitive drive is an obligatory universal outcome of central leptin resistance in humans consuming energy-enriched diets remains to be ascertained. To invoke consumatory drive, the distinction between the bulk quantity of daily food intake in grams vs. kcal and macronutrients consumed, has yet to be sorted out prospectively in clinical investigations. Thus, it is obvious that, like in rodents, the decreased leptin availability in the brain that follows consumption of energy-enriched diets most likely accelerates fat accrual by mechanisms other than the perceived overeating response in humans [Fig. 2].

The second tenet of the central leptin resistance hypothesis implies marked disruption in leptin-induced intracellular signaling in the hypothalamus. Initial observations that signal transducer and activator of transcription 3 (STAT-3) is augmented in association with leptin-induced attenuation of food intake and breakdown in this association in HFD-consuming rodents led to the hypothesis that impaired intracellular signaling downstream of leptin-Rb activation in ARN targets is a requisite component of leptin resistance in obese rodents [15,52,54,55,58, 105,121]. However, further experimental evidence showed that the restraint on food intake

exerted by leptin persisted independent of STAT-3 signaling, and varying degrees of hyperleptinemia was not correlated with altered STAT-3 signaling [15,105]. Additionally, leptin was recently shown to suppress hypothalamic orexigenic peptidergic signaling in transgenic mice independent of involvement of LRB-STAT-3 signal relay, and that STAT-3 signaling was not required for mediation of leptin-induced tonic restraint on food intake on a daily basis. Thus, the view that suppressed intracellular signaling downstream of leptin-LRB activation in ARN targets is a requisite component of leptin resistance in obese rodents, remains uncertain. A large body of unequivocal evidence demonstrating that leptin, either administered systemically or centrally, continues to inhibit food intake and suppress weight in obese HFD consuming rodents, is inconsistent with the hypothesis [5,28,44,59,115,135].

At another level, because systemic administration of leptin enhanced hypothalamic levels of suppressors of cytokine signaling (SOCS-3), it was additionally proposed that this molecule may mediate the suppression of leptin restraint on appetite [65,104]. Surprisingly, however, SOCS-3-deficient animals continued to display the daily time-related feeding pattern and, although moderately sensitive to exogenous leptin, they maintained a normal response to endogenous circulating leptin because the rate of the age-related increase in fat accrual was undisturbed. Also, partial or complete SOCS-3 deficiency in either the entire brain or solely in the hypothalamus, attenuated, but did not abolish HFD-induced adiposity, and the hypothalamic orexigenic peptidergic signaling, the primary mediators of the inhibitory effects of leptin on food intake and weight, was unaltered in these SOCS-3 deficient animals.

Due to this wide range of ambiguities in the implications that STAT-3 and SOCS-3 intracellular signalings impart central leptin resistance, taken together with several plausible alternate possibilities in these genetically manipulated mice, such as compensatory functional and anatomical reorganizations in the ARN, rearrangements in afferent hormonal feedback signaling involved in weight homeostasis and modifications in leptin transport to the hypothalamus across BBB, the contention that impaired intracellular signaling downstream of leptin entry in the ARN expedites environmentally-induced obesity, remains unsubstantiated.

3.2 B. Leptin insufficiency

A comprehensive assessment of leptin levels in the brain of the non-obese and HFD-induced obese rodents has uncovered an unexpected departure from conventional thinking. As presented earlier, both hypoleptinemia and hyperleptinemia are associated with reduced leptin entry into the brain. It is highly likely that decreased circulating leptin concentrations available for transport across the BBB as seen daily in the preprandial interval and in response to fasting [6,52,72–74,82,111,118,141], are the primary cause of reduced leptin levels in the brain. Though unexpected, in all likelihood, this is a natural defense that initiates and sustains hunger to reinstate energy balance. Indeed, hypoleptinemia promotes the release of orexigenic signals in the ARN for triggering the expression of hunger [74,75,77,78], and as leptin levels rise after commencement of feeding, the release of orexigenic signals and the appetitive drive subside [75,118,141].

If this is the underlying physiological consequence of hypoleptinemia, then what is the mechanism and relevance of a similar attenuation of leptin transport to the CNS in the hyperleptinemic obese rodents and human subjects? In accordance with the current evidence, two mechanisms operate simultaneously to impose reduced leptin entry across BBB in response to hyperleptinemia. The first one is the well-established target receptor unresponsiveness rendered by abnormal increases in rhythmic hormonal signaling [6,73,80]. Hormonal pulsatility is the norm for optimal target-response and excursions from the normal pattern can extinguish target response due to receptor downregulation [73,80]. Therefore, as proposed earlier [110, Fig. 2], we postulate that the HFD-induced increased pulsatile leptin secretion is likely to downregulate leptin receptors in the cerebrovasculature, especially the short

isoform, and thereby markedly attenuate their capacity to transport leptin across the BBB [63,87]. The possibility that various blood borne metabolic variables may also participate exists. Thus, to prevent undereating and starvation, the expected deleterious health consequences of unrestricted leptin passage to the brain under severe hyperleptinemic conditions, leptin receptor down regulation at the blood-brain interphase is a key defense adjustment to strictly curtail leptin supply to the hypothalamus [Fig. 2].

The second line of defense that aids in diminishing CNS leptin levels is the one precedes the point of leptin passage across the BBB. These include increased binding of leptin to CRP and modulation of blood to brain transport by various metabolic variables in the peripheral circulation [8,11,14,32,53,82,83,85,112]. In sum, these two endogenous defensive mechanisms are pressed into high gear to reduce leptin levels in the brain with a common goal of averting undereating and starvation, and initiating those additional hypothalamic mechanisms that promote storage of excess energy as fat, without jeopardizing the daily feeding pattern (see below).

Partial leptin deficiency in humans also is positively correlated with increased fat deposition. Human subjects who inherit one functional copy of *ob* gene and heterozygous *lep^{ob/+}* have markedly reduced circulating leptin levels and display increased mass of adipose tissue as compared to controls [48,108]. Similarly, a minority of Pima Indians with a common form of obesity, not associated with leptin mutations, were hypoleptinemic and exhibited greater fat accrual [119]. Thus, a subthreshold supply of leptin to hypothalamus in these clinical instances is associated with increased adiposity, an inference in line with the bulk of experimental evidence that now endorses central leptin insufficiency in causing dietary obesity.

4. GLOBAL CONSEQUENCES OF CENTRAL LEPTIN INSUFFICIENCY PRODUCED BY ENERGY-RICH DIETS

4.1 OBESITY AND METABOLIC SYNDROME

Compelling evidence links increased visceral adiposity with pathophysiology of the disease cluster of metabolic syndrome. Enhanced rate of fat accumulation is a risk factor for diabetes mellitus, a disease characterized by hyperinsulinemia, insulin resistance, glucose intolerance and hyperglycemia [23,52,57,64,66,68–70,76,80,89,93]. Although the cause and effect relationships between hyperleptinemia, pancreatic insulin secretion and glucose metabolism in the periphery have been explored extensively [68,69,89,93,127], a crucial role of the leptin-hypothalamic axis in monitoring insulin-glucose homeostasis, independently of the fat depots, has only recently been delineated [5,17,20,22,23,42,44,76]. Newer experimental approaches undertaken to assess the selective effects of leptin in the hypothalamus on pancreatic insulin secretion and glucose metabolism in the absence of confounding crosstalk in the periphery, have uncovered multiple new ways whereby peripheral hyperleptinemia and central leptin insufficiency concomitantly participate in the etiology of metabolic syndrome [5,17,20–23, 132,133, Fig. 2].

4.1.1 a. Hyperinsulinemia—Although it is generally held that the fat burden of obesity contributes substantially to cause hyperinsulinemia [57,64,68,69,76,89,93], acute inhibition of insulin secretion by central administration of leptin has also been reported [34,62,89,102, 131]. Suppression of ultradian insulin secretion from the pancreas was consistently observed when leptin expression was selectively enhanced by leptin gene transfer in the hypothalamus or in selected hypothalamic sites such as the medial preoptic area (MPOA), paraventricular hypothalamus (PVN), ventromedial hypothalamus (VMH) or ARC [4,5,17,20,22,41,42,44, 109,110,133]. This stably enhanced leptin signaling in the hypothalamus, without leakage to extrahypothalamic sites or to the periphery, also corrected hyperinsulinemia and averted the

development of insulin resistance in rodents displaying central leptin deficiency due to aging or consumption of HFD [17,20,23,41,42,44]. Seemingly, the combination of restraint on both basal episodic and post-prandial insulin hypersecretion imposed by enhanced leptin signaling in the hypothalamus can sustain circulating insulin concentrations in the normal range [20, 22,23,109,110].

The finding that normally leptin exerts a tonic restraint on pancreatic insulin secretion and HFD-induced central leptin insufficiency in rodents rescinds this restraint to unleash increased insulin secretion, provides a novel etiological insight for the well-documented positive relation in the incidence of obesity and type 2 diabetes. We propose that the positive energy balance generated by consumption of energy-rich diets greatly enhances the rate of storage of excess energy as fat, a process clearly requiring augmented insulin supply [Fig. 2,]. Therefore, leptin-insufficiency conferred by HFD-induced impaired transport of leptin across BBB, meets that challenge by curtailing the tonic leptin restraint on ultradian insulin efflux from pancreas [20,22,23,109,110]. The resultant insulin hypersecretion, in turn, promotes adipogenesis and steers excess energy storage in adipocytes [52,57,68]. An analysis of the ultradian patterns of insulin secretion in OR and OP rodents affirm this proposal [109,110]. We observed that OR rats despite consuming HFD do not display hyperleptinemia as do the OP rats. It turns out that unlike OP rats, OR animals develop neither hyperinsulinemia nor the central leptin insufficiency that allows insulin hypersecretion as seen in OP rats [9,12,109,110].

Central leptin-insufficiency is, thus, another intrinsic defense mechanism to monitor the supply of insulin in accordance with the demands for storage of excess energy as fat in the periphery. The predicted untoward consequences of unabated hyperinsulinemia for extended periods are the antecedent pathophysiological sequelae, insulin receptor downregulation, insulin resistance and impaired intracellular signaling in peripheral targets leading to glucose intolerance, hyperglycemia and diabetes mellitus. The evidence that abrogation of leptin insufficiency by central gene therapy reinstated leptin restraint on ultradian insulin secretion, and simultaneously abolished the risk factors of diabetes, is consistent with our proposal that tonic leptin restraint on the basal ultradian and post-prandial pancreatic insulin hypersecretion is an obligatory hypothalamic regulatory component in insulin-glucose homeostasis.

The clinical reports demonstrating that leptin replacement in non-obese severely leptinopenic, lipodystrophic human and rodent models readily abrogated hyperinsulinemia, insulin resistance and diabetes mellitus also corroborate the role of leptin insufficiency in the etiology of diabetes [46,116,125]. Since leptin supply selectively in the hypothalamus of *ob/ob* mice conferred a stable state of normoinsulinemia [20,132,133], one can reasonably infer that reinstatement of central leptin restraint on insulin secretion underlies the benefits of systemic leptin administration in these lipodystrophic diabetic models.

The results of several tract tracing studies and those obtained after microinjection of leptin transgene in various hypothalamic and extrahypothalamic sites, imply that the leptin-induced signal relay from various hypothalamic sites traverses along the descending effector neural pathways that terminate in the pancreas [4,26,76,90]. Further, it is evident that propagation of insulin inhibiting signals by leptin are mediated by the hypothalamic NPYergic system and the efferent relay bypasses the dorsal vagal complex en route to the pancreas [21,78,79,96].

4.1.2 b. Hyperglycemia and Glucose Metabolism—The precise nature of leptin in the hypothalamic regulation of glucose homeostasis, independent of insulin involvement, was only recently clarified [20,132,133]. Increasing leptin availability selectively in the hypothalamus reduced blood glucose levels and maintained euglycemia in aging rodents as well as in extremely hyperglycemic HFD-consuming rodents and in leptin-deficient *ob/ob* mice for the lifetime [4,5,20,22,23,44,94,132,133]. Remarkably, this perseverance of euglycemia was

evident even in the insulin-deficient streptozotocin treated diabetic mice and genetically-induced severely insulinopenic diabetic Akita mice [132,133,142, and unpublished]. Evidently, a distinct leptin-responsive hypothalamic mechanism orchestrates euglycemia by accelerating glucose metabolism in brown adipose tissue (BAT), liver, skeletal muscles and adipose tissue independent of insulin involvement [34,62,76,99,101,129,131–134,136, Fig. 2].

The mechanisms whereby this newly identified distinct regulatory arm of central leptin action interacts with multiple peripheral players already invoked in glucose homeostasis remain to be delineated. Presumably driven by central leptin insufficiency in obese subjects, two events, a diminution in glucose metabolism that raises blood glucose concentrations, and an easing of the restraint that allows the pancreas to hypersecrete insulin, occur in concert to augment fat accrual. The efferent information generated by leptin from various hypothalamic sites, including the VMH [101], to upregulate glucose metabolism is apparently relayed via distinct descending neural pathways to the liver, BAT, white adipose tissue and skeletal muscles [4, 5,21,43,76,101,129].

4.2 DECREASED ENERGY EXPENDITURE

Normally, by stimulating non-shivering thermogenesis, leptin also assists in the hypothalamic integration of energy balance in rodents [52,54,70,74,76,114]. Distinct efferent pathways relay the leptin-induced messages from the hypothalamic MPOA, PVN and ARC to the BAT to augment energy expenditure [4,5,44]. Consequently, a deficit in leptin signaling from the hypothalamus to BAT, just as found in leptin and leptin receptor mutants, HFD consumption also consistently diminished thermogenic energy expenditure [20,22,23,132,133]. The obvious consequence of reduction in energy expenditure is to conserve energy supply [4,5,22,33,41, 42,67,70,76]. Thus, as with decreased glucose metabolism, diminished energy expenditure in HFD-consuming rodents also boosts the supply of energy fuels for storage as fat [Fig. 2,].

4.3 IMPAIRED BRAIN DEVELOPMENT AND COGNITIVE FUNCTION

Reduced brain weight, myelination and synaptogenesis, the indicators of impaired CNS development and predictors of neurological ailments, have been reported in congenital leptin-deficient humans and rodents [1,16,24,49,50,95,100,108,117,128]. Intriguingly, leptin insufficiency in the brain of obese and diabetic patients, may also adversely impact neural functions [2,38,49,50,58,60]. Diabetes mellitus was associated with cognitive deficits, including psychomotor efficiency, attention, learning and memory, intelligence and executive function. Moreover, even non-diabetic individuals suffering either from hyperinsulinemia and insulin resistance or from poor glucose regulation, displayed impaired learning and memory performance, and atrophy of temporal lobe structures, including hippocampus and amygdala [49,50,56,60,107,113]. Thus, a chronic low-grade decrease in central leptin levels in humans, a consequence of impaired BBB transport [12,27,29,124], may inflict hippocampal damage, volume loss and atrophy, decrease general cognition performance, and memory impairment.

4.4 OSTEOPOROSIS AND BONE REMODELING

That hypoleptinemia, independent of gonadal hormones, is a common denominator in the pathophysiology of bone remodeling, has only recently been appreciated [6,7,67,130]. Severe loss of fat tissue and hypoleptinemia have long been associated with increased incidence of osteoporosis in patients suffering from anorexia nervosa and in long-distance runners [7]. Leptin deficient *ob/ob* mice show stunted bone growth and several other abnormal features of bone density, all of which can be corrected by central leptin repletion [7,67].

5. CAN ONE ALLEVIATE CENTRAL LEPTIN INSUFFICIENCY EXPERIMENTALLY ?

Thus, convergence of evidence concurs with the emerging notion that central leptin insufficiency is a significant contributor to obesity and the disease cluster of metabolic syndrome and certain neural afflictions. Can one alleviate central leptin insufficiency experimentally for extended period to curb obesity and the attending co-morbidities?

Various attempts to increase leptin delivery to the hypothalamus and extrahypothalamic sites with daily injections or continuous infusion systemically and centrally (intracerebroventricular or intrathecal infusion) in pharmacological doses over short periods, have been quite rewarding. Leptin administration to non-obese and HFD-induced obese rodents elicited divergent responses on weight gain and food intake [28,33,39,59,66,114,115,121]. Whereas suppression of weight gain and fat accrual persisted throughout the entire treatment, daily food intake was inhibited only transiently and it gradually normalized [33,121]. Presumably, the short-lived decrease in food intake is, as alluded to earlier, an adaptive endogenous response to prevent undereating and starvation without adversely affecting the daily feeding pattern. Maintenance of severe hyperleptinemia by a systemic injection of adenovirus vector encoding the leptin gene, was highly effective in suppressing weight and adiposity. However, these benefits of hyperleptinemia on weight homeostasis, although mediated through the hypothalamus, were questionable due to the immunogenic nature of adenovirus vector [137]. In a limited clinical trial, systemic administration of leptin was less remarkable in reducing weight in obese subjects, possibly due to extreme heterogeneity in the patient population and inadequate leptin dosing [61,66].

Leptin replacement in various extrahypothalamic sites was also beneficial. Leptin injections reinstated myelination, synaptogenesis, and promoted overall brain growth in *ob/ob* mice [1,16,23,24,60,100,117,128]. Also, the possibility that cognition deficit may be associated with leptin insufficiency has been explored. Leptin-deficient rodents displayed impairment in hippocampal synaptic plasticity and in spatial memory tasks performed in Morris water maze, administration of leptin directly into the hippocampus facilitated hippocampal long-term potentiation (LTP) and improved memory performance in young and aged rats and mice [49]. At the cellular level, leptin was able to convert hippocampal short-term potentiation (STP) into LTP, via enhancing NMDA receptor function [107]. Furthermore, leptin was shown to modulate amyloid plaque formation in vivo and in vitro [50]. Since aggregation of these proteins promote memory loss in Alzheimer's disease, these findings reinforced a role of leptin in learning and memory [2,38]. Consequently, as alluded to earlier, it is highly possible that when leptin transport into the CNS is compromised in obese and aging subjects, and those suffering from metabolic diseases, low levels of leptin in the hippocampus may instill cognitive deterioration [56,60,113]. Leptin insufficiency in the brain either alone, or in concert with hyperglycemia and/or hyperinsulinemia is a significant risk factor for impaired cognitive function in the aging population [56,60,113].

The second approach recently tested for leptin delivery to the brain is by intranasal administration. Circumvention of the BBB with intranasally applied leptin in rodents rapidly raise brain concentrations to the supraphysiological range with higher levels in the hypothalamus than in extrahypothalamic sites [51,86,123]. These high hypothalamic leptin levels, attained independently of circulating concentrations, effectively decreased food intake and weight. The efficacy of intranasal application to deliver leptin directly to various hypothalamic and other brain targets in normal and obese rodents, warrants further investigation.

Finally, central leptin gene therapy, the third modality to deliver leptin is an extremely efficient approach to alleviate leptin insufficiency in anatomically relevant, distinct brain sites [4,17,30,40,42,43,106,122]. A single central injection of non-replicative, non-immunogenic and non-pathogenic recombinant adeno-associated virus (rAAV) encoding the leptin gene, either intracerebroventricularly or selectively into hypothalamic sites, was highly effective in providing a stable lifetime supply of leptin without leakage to extrahypothalamic sites or to the periphery. The efficacy of central leptin gene therapy in various *in vivo* paradigms, such as, non-obese prepubertal and adult rodents, dietary obese, leptin-deficient obese and insulinopenic non-obese rodents has been evaluated [4,5,17,20–23,41,42,44,70,72,76,94,109,110,132,133]. Central leptin gene therapy conferred the following benefits:

1. Suppressed the age-related, ovariectomy-induced and dietary obesities and reduced circulating levels of adipokines, such as leptin, tumor necrosis factor α , adiponectin, triglycerides and free fatty acids, with or without a decrease in food intake.
2. Abolished the gradual age-related rise in circulating insulin in rodents either consuming *ad libitum* either regular rodent chow or HFD, and conferred normoinsulinemia by restraining episodic basal and postprandial insulin hypersecretion.
3. Engendered euglycemia, independent of insulin involvement, as evident in two diabetic rodent models: Akita and streptozotocin-treated mice. Further, increased glucose metabolism in BAT, liver and skeletal muscle, along with enhanced insulin sensitivity at these targets was largely responsible for the observed persistent euglycemic response.
4. Normalized testis weight, abolished the fatty liver response and promoted brain growth in leptin-deficient mice.
5. Estrous cycles, pregnancy and parturition in female rats were unaffected.
6. Normalized, through hypothalamic NPY signal relay, skeletal growth as indicated by femoral length, total bone volume and decreased femoral and vertebral cancellous bone volume in leptin-deficient mice.
7. Ameliorated various life-shortening co-morbidities in leptin deficient *ob/ob* mice, i.e. visceral fat, hyperglycemia, insulin resistance, hyperinsulinemia and decreased insulin-like growth factor-1 levels. These benefits, along with elevated ghrelin levels, more than doubled the lifespan, extending it to the range found in wild type mice.

In aggregate, the excellent safety profile for treating neurological diseases [23,81], coupled with our demonstration of the life-long benefits of overcoming leptin insufficiency [23,81], reinforce the potential of gene transfer technology with rAAV vector in decelerating the incidence of obesity and attendant sequelae of metabolic syndrome and neural disorders of leptin insufficiency [23,70,72,76].

6. CONCLUSION AND THERAPEUTIC IMPLICATIONS

Historically, the term “leptin resistance” was coined to accommodate the presumption that reduced leptin restraint in the hypothalamus imposed by restricted blood to brain transfer of leptin and impaired leptin signal relay within the ARN, elicited varying degrees of unregulated overeating that culminated in overt obesity. The resultant fat burden sustained over extended periods was thought to expedite a spectrum of metabolic afflictions and shorten longevity [23,52,57,64,68,76]. A critical survey of evidence assembled from a variety of physiological, molecular and genetic manipulations in support of the leptin resistance hypothesis is equivocal. In fact, the validity of this concept has been challenged by new knowledge of the dynamic fluctuations in brain leptin levels under diverse physiological and nutritional settings and the

unequivocal demonstration that central leptin replacement, contrary to previous assumptions [27,29,52,54,120,124], can reinstate weight homeostasis in obese rodents consuming energy-enriched diets [5,28,33,44,58,59,115,135]. On the other hand, as proposed earlier [41,42,72,76], the cumulative body of scientific evidence fits better with the alternate concept of hypothalamic leptin insufficiency in causation of obesity and metabolic syndrome. The major tenet of this postulation is that BBB restricts the blood to brain entry of leptin in response to hyperleptinemia consisting of heightened rhythmic leptin drive induced by energy enriched diets and intrinsic daily secretion pattern, and attendant varied blood-borne factors [6,8,11–14,27,29,32,53,73,82–84,109,111,118,141, Fig. 2]. The after effect of central leptin insufficiency is a two part defense mechanism, one, to prevent undereating and starvation, and retain the daily pattern of appetitive drive, and two, to simultaneously set in motion those varied hypothalamic regulatory processes that promote storage of excess ingested energy as fat. These encompass independent leptin-driven hypothalamic neural relays to provide optimal supply of pancreatic insulin and fuels for storage. What sets this new formulation apart from the leptin resistance hypothesis is the corroborative experimental evidence amassed in the last decade demonstrating that alleviation of leptin insufficiency selectively in the hypothalamus and elsewhere in the brain, can curtail the rate of fat accumulation, avert the risks of incurring the disease cluster of metabolic syndrome and an array of other neural co-morbidities.

Collectively, multidisciplinary strategies undertaken to unravel the intricate neurobiology of leptin signaling clearly documents a plethora of pathophysiological symptoms and ailments whose etiology can be traced to leptin insufficiency in the brain [Fig. 1]. This inference makes a major break with the past where traditionally metabolic afflictions singularly focused on persistent central leptin resistance and not suboptimal availability of leptin in the brain that boosts the risks of incurring obesity, diabetes and other related metabolic diseases. Therefore, we propose that central leptin resistance hypothesis should be replaced by the central leptin insufficiency syndrome to embrace a wide spectrum of interdependent peripheral and central afflictions orchestrated by the common underlying chronic suboptimal leptin signaling in the brain. This recognition can aid in designing newer interventional therapies aimed at normalizing, either individually or in unison, the diverse neural central afflictions inflicted by the imbalance in leptin signaling. These include fat accumulation, pancreatic insulin secretion, peripheral glucose metabolism, energy expenditure, bone remodeling and cognitive function [Fig. 1]. A concerted effort in designing new leptin mimetics, carefully assessing the clinical efficacy and safety of those central leptin delivery approaches that have already been evaluated in rodents, and identifying newer ways to improve leptin delivery to specifically targeted neural pathways, will accelerate development of remedial therapies for varied ailments of the central leptin insufficiency syndrome in domestic animals and humans.

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Pathophysiology of Central Leptin Insufficiency Syndrome

- Eating Disorders
- Decreased restraint on episodic pancreatic insulin secretion
- Decreased glucose metabolism in peripheral organs
- Decreased thermogenic energy expenditure
- Increased fat accrual and adipokines secretion
- Neuroendocrine disorders
- Impaired synaptogenesis and brain growth
- Impaired learning and memory

Fig. 1.

A list of various peripheral and central disorders attributed to leptin insufficiency in the brain.

Fig. 2.

A flow chart of sequential metabolic and neural events initiated by consumption of energy-enriched diets leading to leptin insufficiency in the brain and derangements in the hypothalamic regulation of insulin secretion, glucose metabolism and energy expenditure that together promote fat accrual and metabolic disorders including type 2 diabetes. For details see text.