20 Leptin Therapy as a Substitute for Insulin Replacement in Experimental Models of Diabetes: Clinical Implications in Humans

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Introduction

The minute to minute participation of the central nervous system (CNS) in imposing glucose homeostasis for lifetime was firmly established in the early twentieth century when ablation of selected sites in the hypothalamus or interruption of afferent signals to the periphery reliably affected ingestive behavior, adiposity, and metabolic balance, accompanied by accelerated secretion and excretion of glucose [1–5]. On the one hand, this revelation led to an upsurge in research focused on a deeper understanding of the ways the brain regulates appetite and adiposity accretion in response to ever-changing daily energy intake and disposal during lifetime, and on the other, it engendered an appreciation of the notion that hormones are indispensable for relaying regulatory signals between the CNS and peripheral organs for sustenance of metabolic homeostasis [2–6]. A major breakthrough emanating from these endeavors was the identification, isolation, chemical characterization, and soon, thereafter, availability of pancreatic insulin for treatment of diabetic patients in the 1920s [3, 4, 7]. Following the recognition of insulin as the indispensable signal in sustaining glucose homeostasis and as a miracle molecule of modern medicine, multidisciplinary research has been devoted to the better understanding of (1) the external and internal environmental factors at systemic, cellular, and molecular levels critical in regulation of insulin secretion, (2) delineation of pathways in the body that participate in integration of glucose disposal for glycemic control and (3) fine-tuning the insulin delivery technology to optimally simulate the normal pattern of glucose fluctuations on a 24-h basis in diabetics. Looking back on this history, one is stuck by the revelation that until the 1970s, there was a paucity of research aimed both at identifying the existence of additional signal molecules, as efficient as insulin, that regulate glucose homeostasis and at mapping metabolic and neural pathways that orchestrate and mediate the homeostatic cues for tight glycemic control. Fortunately, history is replete with instances when serendipitous observations herald a revolution in our understanding of the etiology of diseases that lead to the discovery of newer therapies. In this context, one can assign the serendipitous discovery of a few morbidly obese, diabetic mutants in a rodent colony and the diligent pursuit by elegant experimentation in vivo aimed to decipher the underlying pathogenesis of these metabolic aberrations, that culminated decades later, first in the discovery and isolation of leptin hormone from adipocytes [6, 8–12], and subsequently establishing the adipocyte leptin-hypothalamus axis as obligatory in maintenance

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of glucose homeostasis [5, 11–14]. The outcome as summarized here is an ongoing mini revolution in the newer and deeper understanding of the etiology of diabetes mellitus type 1 (T1DM) and type 2 (T2DM), coupled with the findings from ingenious experimental paradigms that have compellingly endorsed the therapeutic potential of leptin.

New Conceptual Revelations

Pancreatic Insulin-Adipocyte Leptin-Hypothalamus Axis

Pancreatic insulin: Normally insulin is secreted in a pulsatile manner characterized by stable pulse frequency and wide amplitude fluctuations throughout the day. The strength of pulse discharge is quantitatively regulated by blood glucose concentrations, the higher range prevailing postprandially and return to basal range during the inter-meal interval. Hyperinsulinemia is invariably a consequence of increased episodes of insulin discharge in response to increments in quantity and quality of macronutrient intake [15–18]. Furthermore, substantial experimental evidence supports the emerging notion that sustenance of increased insulin pulse amplitude results in loss of target effectiveness engendered initially by down regulation of target receptor response followed sequentially by development of insulin insensitivity, resistance and extinction of intracellular signaling, breakdown in glucose homeostasis, thereby, progressively precipitating T2DM, fat accumulation, and obesity [7, 17, 18]. This new understanding of the chain of events in the pathophysiology of T2DM and obesity radically contrasts the widely prevailing conventional wisdom that it is the sustenance of increased fat depots over considerable periods that precipitates the development of insulin resistance leading to T2DM, instead of the antecedent increased energy intake-dependent hyperinsulinemia, insulin resistance, hyperglycemia, and T2DM [7, 19, 20] being the initial etiologic sequences that promote increased accretion of body fat and obesity.

Adipocyte Leptin

A critical reappraisal of recent experimental evidence has also affirmed that insulin is adipogenic and stimulates the secretion of a host of adipokines, including leptin [12, 13, 16, 21–24]. Quantitatively, rhythmic fluctuations in blood leptin levels are driven by pulsatile variations in insulin which, in turn, are dependent upon the strength of blood glucose signals generated in response to energy intake [25–28]. Further, just as observed in the case of hyperinsulinemia, hyperleptinemia readily engenders leptin receptor down regulation, leptin insensitivity and/or resistance at peripheral targets [17, 25, 29].

Hypothalamus and Leptin Insufficiency

As expected, soon after the identification of leptin as an anorexigenic peripheral signal, it became evident that the hypothalamus was the singular site of leptin action in inhibiting appetite, an essential component of brain control of body weight homeostasis [5, 6, 17, 26, 30–33] mediated by activation of leptin receptors located on the interconnected network of orexigenic and anorexigenic neurotransmitter producing neurons (Fig. 20.1) [5, 6, 17, 26, 30–33]. A major breakthrough that subsequently uncovered the etiology of hyperglycemia, diabetes, and augmented rate of fat accretion was the obligatory participation of the blood–brain barrier (BBB) in the control of leptin transport into the CNS [34–42]. It was observed that a sustained rise in circulating leptin levels readily down regulated leptin receptors on endothelial cells involved in the transport of leptin, leading thereby to decreased availability of leptin in various CNS sites, including the hypothalamus. Indeed , the resultant leptin insufficiency for extended periods in the hypothalamus was shown to be intimately associated with breakdown in glucose homeostasis as reflected by hyperglycemia, hyperinsulinemia, and rise in circulating adipokines titers, the major symptoms of T2DM

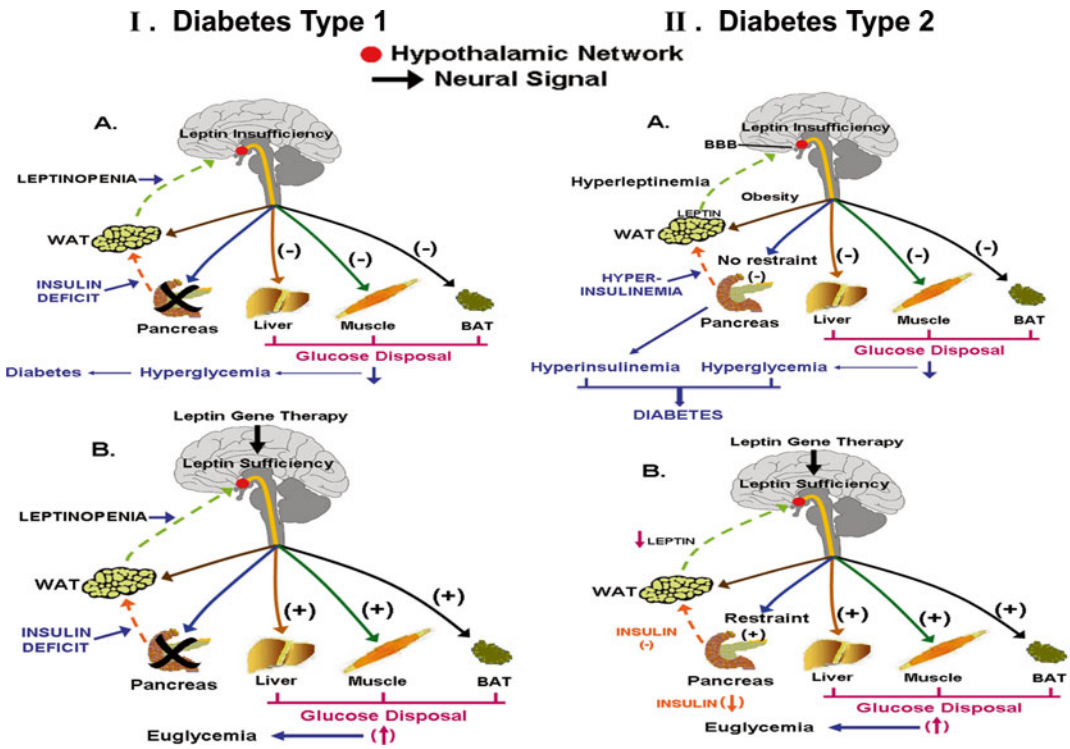

Fig. 20.1 Schematically depicts the role of hypothalamic leptin insufficiency and sufficiency reinstated with leptin gene therapy on glucose homeostasis. (I) Diabetes type 1 (*left*) (**a**) absence of insulin due to a loss of pancreatic b-cells secondarily results in leptinopenia, which, in turn, produces leptin insufficiency in the hypothalamus responsible for neurally reducing (−) glucose disposal in liver, skeletal muscle, and brown adipose tissue (BAT) in the periphery, and facilitating the development of chronic hyperglycemia (for details see text). (**b**) Leptin sufficiency in the hypothalamus transduced by leptin gene therapy optimally reinstates euglycemia by restoring enhanced rate (+) of glucose metabolism in peripheral sites, even in the absence of insulin (for details see text). (II) Diabetes type 2 (*right*) (**a**) through descending relays, leptin insufficiency in the hypothalamus produced by imperviousness of BBB transduced by environmentally acquired hyperleptinemia due to obesity, curtails (−) the central restraint on pancreatic insulin secretion and glucose disposal in peripheral targets leading to the development of chronic hyperinsulinemia and hyperglycemia, respectively. These two metabolic abnormalities in concert, sustain diabetes type 2. (**b**) Leptin sufficiency in the hypothalamus transduced by leptin gene therapy reinstates euglycemia by neurally suppressing hyperleptinemia, restoring the regulatory restrain on insulin secretion and upregulation of glucose metabolism in peripheral sites (+) (for details see text, reproduced from [43])

[19, 31, 41, 43], and increased rate of fat deposition . Remarkably, a similar hypothalamic insufficiency manifests in T1DM primarily due to the prevalence of extreme leptinopenia attributed to lack of insulin-induced leptin secretion from adipocytes [5, 12, 19]. That optimal leptin transport to the hypothalamus across BBB is mandatory was amply endorsed when leptin replenishment locally readily abrogated these metabolic disorders and reinstated glucose homeostasis in T1DM models [5, 42–44].

Leptin Targets in the Hypothalamus

A complex interconnected network of leptin receptor producing neurons spanning almost the entire basal hypothalamus was diligently mapped during the last decade (Fig. 20.1) [6, 17, 19, 45–48]. Rostrally from the medial preoptic area (MPOA), this circuit projects caudally and ventrally to the arcuate nucleus (ARC) and caudally and laterally to the paraventicular nucleus (PVN), ventromedial hypothalamus (VMH), and lateral

hypothalamus (LH). The neurons within this circuitry coexpress diverse neuropeptides and amino acid neurotransmitters that communicate with each other and relay regulatory messages to multiple peripheral targets for energy balance under the direction of the ever-changing leptin milieu in the hypothalamus. More recently, the existence and dynamic operation of a discrete anatomical substrate within this circuitry concerned singularly with leptin-glucose homeostasis, has been detailed extensively [5, 6, 12–14, 17, 32, 48] and is presented in condensed form in the following sections.

Descending Neural Pathways

Extensive tracing studies aimed at delineating the paths of the leptin-induced descending relays from various nuclei in the hypothalamus revealed three distinct pathways namely, the appetite regulating network (ARN) to the gastrointestinal tract to control energy intake, the energy expending network (EEN) to brown adipose tissue to stimulate nonthermogenic energy expenditure, and the fat accrual network (FAN) to the pancreas to control insulin efflux and adipogenesis, and to skeletal muscles, liver, and white adipose tissue (WAT) to upregulate glucose disposal (Fig. 20.1) [48–55]. It is now well established that a coordinated operation of these afferent signals to the periphery is necessary for maintaining glucose homeostasis and a breakdown in any of the afferent relays rapidly precipitates hyperglycemia, diabetes, and fat accumulation [5, 12, 18, 19, 56].

Temporal Relationship Between Centrally Mediated Adipocyte-Leptin and Pancreatic-Insulin Feedback Underlying Glucose Homeostasis

Close evaluation of the temporal relationships among circulating concentrations of insulin and leptin and CNS leptin levels showed that either the complete absence or diminution of insulin secretion as seen in T1DM or that inflicted experimentally, is always concomitant with markedly low levels of leptin in the circulation as well as in the CNS [13, 15, 19, 21, 23, 34, 35, 38, 39, 57–59]. That this leptinopenia is the major etiologic cause of disruption in glucose homeostasis is reenforced by extensive experimental evidence showing that replenishment of leptin alone, either by systemic or by local central routes, reinstated glucose homeostasis, even in the complete absence of insulin in the circulation and unchanged fat depots [40, 43, 44, 57, 59–62]. On the other hand, insulin hypersecretion in response to increased energy intake is followed by augmented leptin secretion, but attenuated transport across BBB and disruption of glucose homeostasis in young and adult wild (WT) rodents [38, 41, 42, 60]. Under these conditions, whereas leptin administration by systemic routes was found to be completely ineffective, direct delivery into the CNS rapidly decreased insulin secretion and corrected glucose homeostasis along with a gradual decrease in body fat during the entire course of treatment [5, 12, 13, 26, 27, 29, 49, 55–57, 60]. Consequently, operation of the feedback loop between pancreatic insulin and adipocytic leptin was found to be essential in the regulation of glucose homeostasis via the hypothalamus [19, 32, 40, 43, 46, 56, 60]. This feedback loop is now known to normally operate as follows: rising blood glucose levels in response to food intake stimulate insulin secretion from pancreas, which in turn stimulates leptin secretion from adipocytes and transportation of leptin into the hypothalamus. Glucose homeostasis is then reinstated by activation by leptin of FAN and EEN afferent signaling in order to restrain insulin secretion and activate glucose disposal, as elaborated in the preceding sections.

Leptin, a New Therapy for Diabetes

The highlights of the new insight enumerated above are that optimal leptin signaling to the hypothalamus is mandatory for sustaining glucose homeostasis which is regulated by a tight centrally mediated feedback relationship between insulin and leptin. The follow-up discovery that leptin insufficiency in the hypothalamus is the common etiologic factor in causation of T1DM

and T2DM [19], has triggered research towards comprehensively evaluating leptin as a new therapy to replace insulin for both types of diabetes in a variety of animal and clinical paradigms [5]. An account of these endeavors in the last decade is summarized in the following sections.

Type 1 Diabetes

Leptin mutant ob/ob mice are morbidly obese due to incessant hyperphagia in association with hyperglycemia and hyperinsulinemia [8–10]. Leptin replacement by systemic routes resulted in diminished energy intake, loss of fat depots, a slight diminution in insulin secretion accompanied by euglycemia, all of these metabolic changes were initially attributed solely to the disappearance of adiposity [3, 6, 7, 11, 22, 33]. In another leptinopenic rodent model exhibiting lipodystrophia but with severe hyperglycemia and hyperinsulinemia, daily leptin replacement systemically also corrected these pathologies [5, 60]. Most interestingly , these metabolic benefits were reproduced by central infusion of leptin alone with a slightly disparate temporal sequence, an antecedent decrease in circulating insulin levels and euglycemia followed by decrements in appetite and weight in various T1DM paradigms [5, 14, 18, 21, 23, 26, 28, 29, 31, 32, 41, 46, 56, 59]. These revelations clearly reinforced the notion that leptin action alone in the hypothalamus, while restraining insulin secretion from the pancreas, concurrently augmented glucose metabolism in order to impose a stable state of euglycemia (Fig. 20.1). To reaffirm the singularity of central site of leptin action in promoting stable euglycemia over extended intervals, a series of follow up investigations employed central leptin gene therapy to replenish leptin selectively within the hypothalmic sites for local actions, without diffusion either to adjoining brain sites or into the general circulation for actions on the peripheral targets (Fig. 20.1, Table 20.1).

This novel paradigm of selective and stable availability of biologically active leptin in minute amounts not only corroborated the earlier findings of metabolic benefits, but further showed that even in WT young and aging rodents leptin action selectively in the hypothalamus suppressed blood glucose and insulin levels (Table 20.1) [18, 19, 23, 26, 29, 32, 43, 57, 58]. Most surprisingly, similar central leptin gene therapy imposed euglycemia during the extended course of the experiment, lasting lifetime, in the T1DM rodent model, the insulin-deficient diabetic Akita mice and the leptin-deficient hyperinsulinemic diabetic ob/ob mice [44, 57–61]. Notably, whereas stable euglycemia manifested in both rodent models, it manifested independent of any appreciable change in energy consumption and body weight in Akita mice [58]. Recently, long-term normoglycemia was also established by hyperleptinemia induced in the progeny of these insulin deficient Akita mice crossbred with leptin-expressing transgenic (lepTG) mice [63], another experimental modality affirming that lack of leptin in insulin-deficient Akita mice underlies the diabetic state.

The robust ability of leptin delivered by systemic or central routes to ameliorate hyperglycemia and early mortality in the absence of insulin in Akita mice was replicated in WT adult mice pretreated with streptozotocin (STZ) to induce insulitis following destruction of pancreatic beta cells [59]. In another paradigm, skin transplants from transgenic mice overexpressing leptin, also induced glycemia when grafted into the leptin-deficient ob/ob mice, thereby reaffirming the ability of systemic leptin in insulin-deficient T1DM subjects [64]. As far as institution of stable glycemic control, leptin therapy was found to be superior when compared with insulin therapy in the commonly employed T1DM model, the nonobese diabetic (NOD) mouse (Table 20.1) [44].

Genetic defects in leptin signaling in target cells due to receptor gene mutation as in db/db mice, fa/fa Zucker rats and f/f Koletsky rats result in obesity, accompanied by hyperinsulinemia and hyperglycemia [19, 65]. Selective instillation of leptin receptor (OB-Rb) in discrete hypothalmic site(s) with the aid of leptin receptor gene therapy also markedly decreased body weight along with circulating insulin and normalization of glucose concentrations [65]. Consequently, all the adverse consequences on glucose homeostasis inflicted

Table 20.1 Effects of leptin on diabetes in animal paradigms

	Paradigm	Pathophysiology	Route of administration	Benefits
T1DM	ob/ob	Hyperglycemia, hyperinsulinemia, leptinonemia	Systemic, central	Euglycemia
	Akita	Hyperglycemia, insulinemia, leptinopenia	Systemic, central	Euglycemia
	NOD	Hyperglycemia, insulinopenia	Systemic	Euglycemia
	WT + STZ	Hyperglycemia, insulinemia	Central	Euglycemia
	Congenital lipodystrophy	Hyperglycemia, hyperinsulinemia	Systemic	Euglycemia
T2DM	WT aging, obese	Hyperglycemia, hyperinsulinemia, hyperleptinemia	Central	Euglycemia
	WT HFD, obese	Hyperglycemia, hyperinsulinemia, hyperleptinemia	Central	Euglycemia
	UCD-T2DM	Hyperinsulinemia, hyperinsulinemia, hyperleptinemia	Systemic	Euglycemia

by the loss of intracellular signal relay resulting from mutations in leptin receptor gene can be mitigated by reinstatement of leptin receptor, just in those hypothalmic sites previously found to be essential for glucose homeostasis [5, 32, 46].

Seemingly, in diabetics displaying either coexistent hyperinsulinemia and leptinopenia or insulinemia and leptinopenia, leptin therapy is highly effective in restoring normoglycemia. Further, it is now well established that insulin stimulates leptin secretion from adipocytes and it is this lack of insulin stimulus due to loss of pancreatic beta cells, the hallmark of T1DM, produces a state of leptinopenia leading sequentially to leptin deficiency in the hypothalamus and absence of neural afferents involved in sustenance of glucose homeostasis [5, 32, 46, 57–59, 61]. That leptinopenia is the singular etiologic factor of T1DM is further corroborated by the finding that leptinopenia due to experimentally induced lipodystrophia also results in breakdown in glucose homeostasis and it is readily corrected by leptin replacement [20, 57, 60, 66]. Consequently, optimal supply of leptin to hypothalmic targets either by systemic routes or directly into the hypothalamus is the treatment of choice for T1DM (Table 20.1) [67–73] and it can replace the currently in-vogue insulin monotherapy which is riddled with a host of shortcomings due to difficulties in simulating the normal minute-to-minute blood glucose fluctuations, ensuing frequent episodes of hypoglycemia and several related multiple metabolic inflictions emanating over the extended periods of energy imbalance [5, 12, 19, 32].

Type 2 Diabetes

The pathophysiology of T2DM characterized by high titers of circulating insulin and glucose, concomitant with insulin resistance and decrease in the rate of glucose disposal is commonly observed in association with obesity manifesting either gradually in an aged-related fashion or rapidly in response to consumption of calorie-rich high fat diet (HFD) [6, 17, 27, 46]. Furthermore, according to the conventional notion, the onset and sustenance of T2DM is engendered initially by a steady rise in the rate of visceral fat accumulation which impels the development of insulin resistance and decrements in glucose disposal to culminate in hyperglycemia [5, 7, 20, 24]. However, as described earlier, the age-related as well as HFD-induced obesity are also associated with the BBB-induced diminished leptin transport to the CNS in response to hyperleptinemia [19] and it is the ensuing insufficient leptin signaling within the hypothalmic targets that evokes diminished afferent relays to the periphery and breakdown in glucose homeostasis [5, 19]. That central leptin insufficiency in obese rodents may be the primary underlying cause of T2DM was amply affirmed by the findings that reinstatement of

optimal leptin supply selectively in the hypothalmic targets ameliorated the age-related and HFD-induced T2DM, as reflected by the observation of stable glycemia, normalization of insulin sensitivity, and rate of glucose disposal (Fig. 20.1, Table 20.1) [2, 17, 18, 23, 26–29, 61].

In accord with these various lines of evidence gathered from WT animals, it was recently shown that UC Davis-type 2 diabetes mellitus (UCD-T2DM) rats that exhibit adult onset of obesity, diabetes, insulin resistance, and hyperglycemia when administered leptin subcutaneously for extended periods normalized blood glucose, by imparting increased insulin sensitivity in conjunction with diminution in insulin secretion in these rats [74]. Overall, it is clear that leptin insufficiency in the CNS engendered by imperviousness of the BBB due to increased adiposity-dependent hyperleptinemia underlies the development of T2DM (Fig. 20.1, Table 20.1) [5, 17].

Mode of Action of Leptin in the Hypothalamus in Regulation of Glucose Homeostasis

Detailed mapping of neuronal targets in the hypothalamus disclosed an interconnected network of two primary populations of neurons in the ARC, one that coexpresses neuropeptide Y, agouti-related peptide, and gamma-aminobutyric acid (GABA) and the other that coexpress proopiomelanocortin and GABA. These populations project into various sites within the hypothalamus to regulate leptin-induced afferent relays along the ARN, EEN, and FAN pathways associated with energy homeostasis (Fig. 20.1) [6, 13, 17, 46]. Evidently, inhibition of appetite by leptin is mediated by ARN that operates independently of its control on glucose homeostasis [18, 46]. Activation by leptin of EEN from the MPOA to brown adipose tissue controls nonthermogenic energy expenditure, while activation of FAN from ARC, VMH, and LH restrains pulsatile insulin secretion and insulin sensitivity, on the one hand, and stimulates glucose metabolism from skeletal muscles, liver, and WAT, on the other (Fig. 20.1) [18, 19, 32, 42, 60–62, 67–83]. Thus, diminution in leptin signaling in the hypothalamus, as it manifests in response to either leptinopenia or impaired entry of leptin across BBB, results in the breakdown in these hypothalamic regulatory relays on energy intake and glucose homeostasis, all of which can be rapidly reinstated with resupply of leptin [17–19, 30, 31, 42, 43, 46, 56, 57].

A Perspective: Future Clinical Testing

Early results of clinical studies to evaluate the benefits of leptin therapy delivered systemically on appetite and obesity were disappointing [7, 12, 84]. These failures are attributable now to multiple shortcomings, such as dose and frequency of administration in relation to preexisting levels of leptin, combined with a lack of appreciation of the pivotal role of BBB in transporting leptin to targets in the hypothalamus [12, 14, 19, 22, 38, 39, 46]. Soon after, however, it was reported that both in patients harboring congenital leptin-deficiency and a small population of obese patients displaying low circulating concentrations of leptin due to unknown causes, leptin replacement effectively imposed normoglycemia concomitant with reduced food intake and body weight [20, 22, 24, 33, 83]. Indeed, a small population of congenitally leptin-deficient pediatric and adult patients on leptin therapy has displayed improved glycemia for years without any discernible untoward metabolic disruptions. In response to these reports, leptin replacement therapy has also been tested worldwide in patients suffering from impaired glucose homeostasis in association with either leptinopenia due to congenital lipodystrophy or hypoleptinemia of acquired lipoatrophy. Leptin administration readily reinstated diverse centrally mediated metabolic responses, notably diabetes, for the entire course of treatments lasting several months to years [20, 22, 24, 33, 59, 66, 69, 70, 73, 75, 79–83]. Consequently, these clinical successes have boosted the discovery emanating from diverse experimental paradigms in rodents that the adipocytic leptin is an obligatory signal in maintenance of glucose homeostasis (Table 20.1).

Another significant outcome of these clinical investigations is that systemic administration of leptin only 2–3 times a day engendered stable glycemia. Because hypoleptinemia normally exists in newly diagnosed untreated T1DM patients and the fact that leptin under conditions of hypoleptinemia can restore euglycemia, leptin replacement therapy alone, or in adjunct with insulin therapy, is being clinically investigated.

In contrast, leptin administration systemically in obese, hyperleptinemic subjects suffering from T2DM has generally failed to exert any appreciable impact on blood glucose concentrations [7, 12, 84]. This outcome is not surprising in view of the clear evidence expounded earlier that BBB is rendered impervious by hyperleptinemia in humans and animals, alike. Therefore, one can easily surmise that raising blood leptin levels further in hyperleptinemic human subjects is unlikely to provide optimal leptin signals to the hypothalamus required to reinstate glucose homeostasis. On the other hand, the possibility that leptin delivery directly to the hypothalamus, as demonstrated by irrevocable findings from experiments in rodents (Table 20.1, Fig. 20.1), would ameliorate T2DM remains to be tested clinically.

Collectively on balance, to ameliorate both types of diabetes and to reduce or alleviate the accompanying well-documented adverse metabolic consequences, leptin therapy has immense potential to replace or be employed as a conjunct to insulin monotherapy [5, 12, 18, 32, 33]. As the incidence of diabetes has attained epidemic status worldwide [85], the societal burden of ever soaring treatment costs, in concert with the new expanding knowledge of the diabetes-associated multiple metabolic and neurological disorders, including cognition impairment [5, 12, 19], this discovery is highly significant . Consequently, there is a dire urgency to improve and identify new long-acting leptin mimetics that can bypass BBB enroute to CNS, and rigorously evaluate in humans the efficacy of leptin and /or mimetics delivery directly to CNS sites via well-tested gene therapy and intranasal routes [5, 18, 19, 86–88].

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