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Central leptin gene therapy ameliorates diabetes type 1 and 2 through two independent hypothalamic relays:

a benefit beyond weight and appetite regulation

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Abstract

Although its role in energy homeostasis is firmly established, the evidence accumulated over a decade linking the adipocyte leptin -hypothalamus axis in the pathogenesis of diabetes mellitus has received little attention in the contemporary thinking. In this context various lines of evidence are collated here to show that (1) under the direction of leptin two independent relays emanating from the hypothalamus restrain insulin secretion from the pancreas and mobilize peripheral organs - liver, skeletal muscle and brown adipose tissue - to upregulate glucose disposal, and (2), leptin insufficiency in the hypothalamus produced by either leptinopenia or restriction of leptin transport across the blood brain barrier due to hyperleptinemia of obesity and aging, initiate antecedent pathophysiological sequelae of diabetes type 1 and 2. Further, we document here the efficacy of leptin replenishment in vivo, especially by supplying it to the hypothalamus with the aid of gene therapy, in preventing the antecedent pathophysiological sequelae-hyperinsulinemia, insulin resistance and hyperglycemia - in various animal models and clinical paradigms of diabetes type 1 and 2 with or without attendant obesity. Overall, the new insights on the long-lasting antidiabetic potential of two independent hypothalamic relays engendered by central leptin gene therapy and the preclinical safety indicators in rodents warrant further validation in subhuman primates and humans.

Keywords

Leptin gene therapy; Hypothalamus; Diabetes mellitus type 1 and 2

1. INTRODUCTION

Insulin produced by pancreatic β -cells facilitates glucose homeostasis by promoting glucose uptake and storage in skeletal muscle, liver and fat cells. Either lack or inefficient use of insulin by these peripheral targets coalesces into diabetes, a chronic disease characterized by hyperglycemia which over time may inflict a spectrum of metabolic and neural diseases and shorten life-span [33,34,53,55,67,72]. Diabetics suffer from either type 1 or type 2 diabetes. Type 1 diabetes is a debilitating autoimmune disease caused by T-cell mediated gradual

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destruction of β -cells, leading to either insufficient or complete lack of insulin production [17,29,33,34,72,82]. Insulin replacement regimens aimed at reproducing the physiological range of blood glucose levels are the treatment of choice for these patients [72,82]. Type 2 diabetes is a progressive chronic disease that can manifest at any age due largely to persistent metabolic imbalance engendered by myriads of internal and external environmental factors, including diet and lifestyle changes [17,29,33,34,72,82]. Increases in episodic basal and post-prandial insulin secretion initiated by these environmental shifts gradually lead to insulin receptor insensitivity, insulin resistance and diminished downstream insulin receptor signaling in target cells. The relentless compensatory insulin hypersecretion to normalize blood glucose levels under these conditions expedites β -cell dysfunction and loss that eventuates into unremitting hyperglycemia [9,33,35,53,55,70,71]. The onset of these pathophysiological sequelae of type 2 diabetes is highly correlated with increasing adiposity and age [29,33-35, 53,55,60,79,80]. Interventional therapies that delay or prevent entirely the inevitable adverse health consequences of type 2 diabetes include insulin administration and injectable and orally effective antidiabetic drugs [33,63,72,82]. However, current therapies for these two etiologically distinct diseases are cumbersome, as they require daily administration or continuous infusion of insulin and antidiabetic drugs, along with constant monitoring by patients and physicians for glycemic control, all of which, in aggregate, substantially escalate medical costs [29,33,53,55,63,72,79,80,82].

Historically, ever since the recognition of insulin as the indispensable signal molecule in maintaining glucose homeostasis, research has been devoted entirely to deciphering the external and internal environmental factors at the systemic, cellular and molecular levels that regulate insulin secretion and endogenous pathways that integrate glucose disposal for tight glycemic control [6,33,55,72,82]. This expanding knowledge has continued to singularly steer research towards improving ways to optimally deliver insulin and identify newer insulin mimetics. The possibility that there may exist additional endogenous signal molecules and alternate peripheral and central pathways, that either on their own or in concert with insulin orchestrate homeostatic cues for tight glycemic control, and may, thus, offer novel therapeutic avenues, has received little attention in contemporary thinking (Fig. 1) [33,34,56,72,82].

Indeed, it has been known for a long time that neural signals from the brain, especially those emanating from the hypothalamus, are quite important in the dynamic operation of the insulin-glucose axis [12,27,37,38,40]. Ablation of either the ventromedial nucleus (VMN) or the paraventricular nucleus of the hypothalamus in rodents accelerated insulin secretion not only prior to the onset of hyperphagia and increased rate of fat accretion but blood insulin levels remained elevated in concert with progression towards morbid obesity [12,27,37,38,40]. These rapid and pronounced permanent shifts in the insulin-glucose axis have long been assumed as a secondary manifestation in response to unregulated ingestive behavior and adiposity. The identification of leptin, primarily produced by white adipose tissue (WAT), as a major hormonal signal in the hypothalamic integration of energy homeostasis [20,35,37,38,55,68, 93], and subsequent unraveling of additional multiple regulatory effects of leptin exerted through the hypothalamus on various physiological systems, re-established a pivotal role of afferent neural relays in the bidirectional communication between the periphery and brain [13,17,21,29,32,35,40,47,55,58-60,78,85].

Consequently, apart from a mandatory regulatory role in energy intake and expenditure [10, 35,38,40,93], independent participation of leptin in the hypothalamic integration of insulin-glucose homeostasis and the new understanding that a breakdown in the cross-talk between WAT and hypothalamus is, indeed, an etiologic factor in the pathogenesis of diabetes is collated in this review. Also, within this context are detailed, (i) hypothalamic relays that influence insulin secretion and glucose disposal under the direction of leptin, (ii) a critical role of antecedent leptin insufficiency in the hypothalamus in causation of pathophysiological

sequelae of diabetes type 1 and 2, and (iii) interventions that reinstate central leptin sufficiency to lessen or eliminate the shortcomings of current therapies.

2. LEPTIN RESTRAINT ON INSULIN SECRETION PRIMARILY THROUGH HYPOTHALAMIC RELAYS

That leptin exerts a dynamic regulatory restraint on insulin secretion is suggested by numerous clinical and animal studies [2,22-24,28,35,66,68-70,74]. Insulin is adipogenic, promotes leptin secretion and fat deposition in the body (Figs. 1,2) [29,33,35,36,47,55]. Since leptin can inhibit insulin efflux from β -cells, a peripheral adipo-insular feedback loop was suggested to tightly regulate leptin and insulin secretion (Fig. 1) [33,47]. On the other hand, subsequent *in vivo* evidence revealed an alternative route that leptin-responsive central, and not peripheral mechanisms, exert a restraint on insulin secretion from β -cells (Fig. 1) [1,2,8,9,20,23-25,31,35,36,46,50,68,70,71,83,90]. The evidence that interruption of leptin-induced neural relays by either lesioning the VMN or surgical transection of descending hypothalamic tracts or deletion of leptin receptors in hypothalamic neurons lets on insulin hypersecretion preceding an increase in fat accretion and morbid obesity [20,27,35,39,46,75], and intraventricular infusion of leptin suppressed blood insulin levels and increased receptor sensitivity prior to any discernable decrease in weight and adiposity endorses the involvement of central leptin receptors [31,41,56,61,76]. The possibility that centrally infused leptin in these paradigms may have leaked into the peripheral circulation in amounts sufficient to restrain insulin secretion directly from β -cells, was ruled out by the observation that when leptin expression was increased locally in the hypothalamus, without leakage to the periphery it exerted a stable and pronounced suppression of blood insulin levels [1,2,9,10,23,25,36,54].

Additionally, it is now amply evident that sufficiency in afferent leptin signaling to the hypothalamus is critical in the regulatory restraint on insulin secretion [35]. Despite existent hyperleptinemia, leptin concentrations in the hypothalamus were consistently found to be significantly lower in obese patients and rodents than those in non-obese counterparts [3-5,15,16,43-45,52,73]. Attenuation of leptin entry across the blood brain barrier (BBB) by an active leptin receptor mediated process by hyperleptinemia was shown to decrease brain leptin levels [43-45]. Further, a gradual transition to leptin insufficiency in the hypothalamus, temporally associated with increases in blood leptin levels, was coincident with the rise in blood insulin levels and fat accrual [3-5,7,10,23,24,70,71]. The evidence that leptin replacement centrally abolished hyperinsulinemia in obese rodents corroborated the formulation that it is leptin insufficiency that normally favors insulin hypersecretion by relieving the restraint on β -cells, a response necessary for enhancing the conversion of excess energy into fat [33,35,40,55,87]. Similarly, hyperinsulinemia was abolished when leptin signaling was reinstated by installation of leptin receptors in discrete hypothalamic sites in leptin receptor mutants [46]. Also, findings from congenital lipodystrophic patients and transgenic mice invoke central leptin insufficiency as a contributor in the genesis of hyperinsulinemia [28,66,69,74,77]. Hyperinsulinemia and diabetes type 2 concomitant with severe leptinopenia in these subjects was completely abrogated by leptin replacement without affecting food intake and weight. Because an intact VMN is necessary for hyperleptinemia to suppress hyperinsulinemia, one can safely conclude that central leptin insufficiency created by leptinopenia in congenital lipodystrophic patients and mice was overcome by increased leptin availability for entry across the BBB to reinstate leptin restraint and suppress hyperinsulinemia by activating descending hypothalamic relays and not by directly inhibiting insulin hypersecretion from β -cells (Figs. 1,2) [35,38,39,50,90].

Thus, taken together with the recent disclosure of distinct neural pathways linking the hypothalamus to the pancreas [14,51], involving the local network of neuropeptide Y and cohorts (Figs. 1,2) [7,40,57], this large body of experimental evidence is consistent with the

view that a dynamic cross-talk between adipocytes and hypothalamus is mandatory in maintaining tonic restraint on insulin efflux from β -cells [9,35].

3. UPREGULATION OF GLUCOSE METABOLISM BY LEPTIN THROUGH HYPOTHALAMIC RELAYS, INDEPENDENT OF INSULIN INVOLVEMENT

Leptin is also a significant player in the regulation of glucose metabolism. Systemic administration of leptin to either leptin-deficient *ob/ob* [19,35,41,61,89] or leptinopenic lipodystrophic, diabetic humans and mice stimulated glucose metabolism and normalized blood glucose concentrations, benefits previously attributed to activation of leptin receptors on hepatocytes, islet cells, adipocytes and skeletal muscle cells [19,29,30,33,47,53,55]. However, these very benefits, i.e. glucose lowering and sustenance of euglycemia, were launched when leptin was provided solely in the hypothalamus of not only WT rodents, but also insulinopenic diabetic Akita mice and streptozotocin-treated rodents with insulinitis [8,9,23,31,35,39,40,48,49,70,71,83,84]. Increased leptin signaling in the hypothalamus in these paradigms modified glucose metabolism, independent of insulin, by a coordinated orchestration of target genes involved in glucose uptake and utilization in liver, skeletal muscle and brown adipose tissue (BAT) (Fig. 1) [8,9,23,31,35,38,40,48,49,56,84,88,91]. Thus, unlike the previous assumption [29,33,47,53,55,68,80], normoglycemia induced by systemic injections of leptin in these paradigms is likely a consequence of augmented glucose disposal imposed by central receptors after its entry across the BBB to the hypothalamus [35,37,43-45]. Furthermore, despite substantially reduced blood insulin concentrations, sustenance of glucose metabolism in leptin over-expressing transgenic Skinny mice [66], in a manner similar to that evoked by hypothalamic leptin receptor activation in lean insulinopenic Akita mice, also invokes participation of central receptors in maintaining elevated rates of glucose disposal [26,84]. Innervations from the hypothalamus conceivably transmit leptin-induced outflow to upregulate glucose metabolism in pancreas, liver, skeletal muscle, WAT and BAT (Fig. 2) [14,51,86].

In sum, apart from food intake regulation, optimal leptin signaling in the hypothalamus is essential for orchestration of glucose homeostasis through independent controls on insulin secretion and glucose metabolism. Leptin insufficiency in the hypothalamus, produced by either leptinopenia or hyperleptinemia, is apparently a common antecedent causal factor in the pathogenesis of diabetes [4,5,15,16,35,43,69,71,74,84,87]. This insight raises an obvious clinically relevant question: can prevention of central leptin insufficiency decelerate or alleviate the pathogenesis of type 1 and type 2 diabetes?

4. AMELIORATION OF DIABETES BY STABLE LEPTIN SUPPLY

Research in gene transfer strategies to develop interventional therapies for various neural diseases has proceeded at a rapid pace [18,35,36,39,40,42,46]. It is now feasible to introduce genes into cells to replace a missing gene in order to correct or augment the target gene function to cure or slow the progression of chronic diseases due to genetic abnormalities, environmental insults and metabolic imbalance. Gene transfer technology thus offers a potentially newer means to harness an optimal supply of bioactive leptin for overcoming leptin insufficiency for extended periods [11,22,36,39,40,64,65,90]. Among the various viral vectors available for gene transfer, recombinant adenovirus (rADV) and recombinant adeno-associated virus (rAAV) encoding leptin have been employed to delineate the role of leptin in integration of energy homeostasis [18,39,40,42]. Although rADV can infect dividing and non-dividing cells with little integration into the host genome, it transduces high level of transgene expression only transiently and rapidly elicits a robust immune reaction to viral protein, an issue of considerable safety concern [18,39,42,65]. On the other hand, replication-deficient rAAV is non-immunogenic and non-pathogenic, it can infect dividing and non-dividing cells with

marked facility, and after integration into the host genome it evokes transgene expression for the lifetime of the cell [18,40,42,64]. Since an ideal anti-diabetic therapy should be safe in establishing relatively rapid and long-lasting glycemic control, without disrupting or only minimally perturbing the homeostatic physiological and neural interactions, the benefits of leptin gene transfer with the aid of either rADV or rAAV on hyperinsulinemia and hyperglycemia, the antecedent pathophysiological sequelae of diabetes associated with or without obesity, are summarized below.

4.1 Diabetes type 2

4.1.1 Insulin hypersecretion—Hyperinsulinemia and insulin resistance, the predisposing conditions for diabetes type 2, manifest either gradually with advancing age or rapidly in response to consumption of energy-enriched diets and sedentary life-style. Increased incidence of diabetes type 2 has been reported to closely correlate with increased weight predominantly due to visceral obesity [24,29,33,35,55,63,80]. Hyperleptinemia produced by a single intravenous or intramuscular injection of either rADV encoding leptin (rADV-lep) or rAAV-lep to either WT rodents or hyperinsulinemic and hyperglycemic obese *ob/ob* mice, markedly decreased blood insulin and glucose levels along with reduced weight and fat depletion [18, 22,50,65], the benefits lasting the duration of experiments, generally for much shorter duration in rADV-lep than in rAAV-lep injected rodents [18,22,50,64,65,92]. However, because of the fact that leptin is pleiotropic, correction of hyperinsulinemia and hyperglycemia by viral vector-induced hyperleptinemia are tempered by health safety concerns. Hyperleptinemia is lipotoxic on adipose and non-adipose tissues, it can adversely impact immune function, bone formation, angiogenesis, the cardiovascular system and renal function [13,21,29,32,36,40,47, 55,58-60,78,82]. Moreover, hyperleptinemia enforces BBB impervious to leptin entry [3-5, 15,16,43-45,73], and the resultant insufficiency in the hypothalamus, in turn, renders subjects vulnerable to a multitude of neural and non-neural diseases [35].

It is possible to circumvent the deleterious consequences of hyperleptinemia by supplying leptin directly into the hypothalamus to engage neural pathways mediating the control on insulin secretion and glucose metabolism [3,56,61,62,76]. Indeed, a single injection of rAAV-lep either intracerebroventricularly (icv) to augment leptin expression in multiple hypothalamic sites that display biologically relevant long form of leptin receptor [8-10,23-25,37-40], or microinjection into a discrete site to elevate leptin transgene expression locally without spread of leptin to either neighboring sites, cerebrospinal fluid or peripheral circulation, was found just as efficacious as peripheral administration of the vector [1,2,9,11,83,84]. After a single icv rAAV-lep administration to prepubertal rodents, various centrally mediated benefits of leptin persisted throughout the lifetime [9,10] and through various phases of the lifecycle, reproduction, lactation and post-lactation after central injection to adult female rats [54]. Specifically, icv rAAV-lep injection suppressed the age-related and energy enriched high fat diet (HFD)-induced risk factors for diabetes type 2, namely, hyperinsulinemia and insulin resistance, accompanied with or without decreases in weight and adiposity [23-25,70,71,83, 84]. This remarkable ability of central leptin transgene expression to repress basal episodic and post-prandial insulin hypersecretion in WT rodents consuming normal rodent chow or HFD was rapid and preceded the changes in weight. A similar diminution of insulin efflux occurred after microinjection of rAAV-lep in discrete hypothalamic sites of rats consuming normal low-calorie rodent chow or HFD [1,2]. To counter a remote possibility that circulating leptin levels, albeit in the low range, participated in lowering insulin levels and enhancing insulin sensitivity, it was shown in subsequent experiments that in the absence of circulating leptin in the *ob/ob* mice, supply of leptin solely in the hypothalamus clamped insulin hypersecretion and extinguished insulin resistance even when these mice were challenged with calorie-enriched HFD diet that normally evokes symptoms of diabetes [9,83,84].

4.1.2 Hyperglycemia—An unanticipated finding of these investigations was that leptin transgene expression in the hypothalamus resulted in euglycemia in all these varied rodent paradigms, despite a robust contemporaneous clamp on insulin secretion from pancreas [1,2,9,10,23-25,41,62,83,84]. Presumably, the heightened insulin sensitivity that accompanied insulinemia after icv rAAV-lep injection was responsible for the sustained euglycemia [9,83,84]. Intriguingly, an additional player assisting in maintaining this divergent outcome, i.e. insulinemia coexistent with euglycemia, was the rise in BAT-mediated thermogenic energy expenditure and up-regulation of glucose metabolism and disposal [9,83,84]. Seemingly, a stable increase in hypothalamic leptin signaling has the potential to optimize glucose metabolism and impose tight glycemic control, with either minimal assistance, as reflected by the prevailing insulinopenia, or independent of insulin participation, as presented in the following section on diabetes type 1.

Nevertheless, these findings clearly illustrate that central leptin gene therapy can correct the predisposing risks of type 2 diabetes by enforcing distinct neurally mediated two-prong action, repression of hyperinsulinemia by a restraint on insulin efflux from β -cells, and up-regulation of glucose metabolism in the body.

4.2 Diabetes type 1

4.2.1 Hyperglycemia—The unanticipated observation that leptin supply in minute amounts directly to central sites can stably confer euglycemia despite a clamp on insulin efflux from pancreas [1,2,9,10,23-25,83,84], channeled investigations in our laboratory towards addressing a highly pertinent question; can enhanced leptin supply similarly impose euglycemia for extended periods in paradigms of type 1 diabetes? In the first paradigm Akita mice that are severely insulinopenic due to a dominant mutation in *Ins 2* gene and suffering from early onset of raging hyperglycemia were employed [60,84]. A single icv injection of rAAV-lep in adult Akita mice abolished hyperglycemia and diabetes mellitus by enhancing the rate of glucose disposal and insulin sensitivity [60,84]. In fact, normoglycemia was imposed within a week after icv rAAV-lep injection and it was sustained for the 7 week duration of the experiment. This euglycemic response was accompanied by increased BAT-mediated thermogenic energy expenditure in a manner similar to that seen in WT and hyperinsulinemic, obese *ob/ob* mice [9,84]. In the second paradigm, the efficacy of rAAV-lep to impel euglycemia was tested in mice with insulinitis produced by an injection of streptozotocin, a research paradigm employed for decades to assess the pathophysiology of type 1 diabetes. STZ-treated rodents suffer from typical symptoms of diabetes mellitus, unremitting hyperglycemia, weight loss despite hyperphagia, and early mortality [48,49]. An icv injection of rAAV-lep shortly after STZ administration, rescued these mice from early mortality, curbed hyperphagia and maintained reduced BW along with sustenance of euglycemia during the entire course of the experiment lasting for one year [48,49]. No behavioral modification or signs of diabetic cardiomyopathy and low grade systemic inflammation were discernible [26,60]. In concordance with these results observed after central rAAV-lep injection, Yu et al. [92] found that elevations in blood levels of leptin several fold above the normal range with the aid of intravenous injection of rADV-lep, first transiently prevented hyperglycemia and then, over time, sustained higher blood glucose concentrations in correlation with decreases in blood leptin, and delayed mortality in STZ-pretreated rats [92]. Similar hyperleptinemia produced by systemic administration of rADV-lep in non-obese diabetic (NOD) mice, a well-established model of diabetes type 1 resulting from an autoimmune disease [72,92], first imposed normoglycemia, but later blood glucose levels rose to a range below that in severely hyperglycemic control NOD mice. Food intake was reduced and BW loss was also minimized in association with hyperleptinemia in these two paradigms of diabetes type 1 for the duration of the experiments. As evidenced by the fact that minute leptin supply solely in the hypothalamus can abrogate hyperglycemia in Akita and STZ-treated mice [48,49,60,84], it is

highly likely that attenuation of hyperglycemia by peripheral hyperleptinemia reported by Yu et al. [92] was orchestrated subsequent to leptin entry across the BBB into the hypothalamus.

5. CONCLUDING REMARKS

A striking inference of these investigations is that leptin in the circulation or available centrally alone, can mimic insulin-like glucose lowering effects in the complete absence of insulin. More importantly, the outcome of the central leptin gene transfer studies illuminates a substantive leptin link in the etiology of diabetes (Fig. 2). Since stable leptin supply blocked the dire life-threatening pathophysiological consequences of diabetes, central leptin gene therapy is potentially a reliable substitute for insulin therapy [10,11,34-36,39,40]. It is amply clear that only a minute amount of leptin within hypothalamic targets can optimally repress hyperinsulinemia for as long as the supply lasts (Fig. 1). Seemingly, post-synaptic response to leptin remains unabated and the leptin-induced downstream intracellular signaling which is apparently distinct from that transduced by insulin [33,81,92], is not compromised by the sustained leptin supply at central targets. As to how, even in the absence of insulin, neural signaling transduced by leptin transgene expression in the hypothalamus alone optimizes the rate of glucose metabolism and improves insulin sensitivity to orchestrate a long-lasting euglycemia (Fig. 2), is not fully understood.

Finally, the incidence of diabetes mellitus in young and adult populations is rising at an alarming pace worldwide [17,29,53,55]. The pandemic has ignited new research endeavors aimed at designing remedies based on newer insights on the genesis of pathophysiological sequelae that eventually inflict type 1 and type 2 diabetes. This drive is also catalyzed by the ever rising cost of treating multiple co-morbidities of diabetes including hypertension, cardiovascular ailments, nervous system damage underlying memory loss and Alzheimer's disease, retinopathy and peripheral neuropathy, kidney diseases and shortened life-span [17, 29,34,39,53,55]. Application of one time central leptin gene therapy to enhance leptin supply locally in the hypothalamus, although unconventional, offers an efficient and durable anti-diabetic treatment paradigm capable of preventing and mitigating both type 1 and 2 diabetes (Fig. 2). Furthermore, it is highly possible that the anti-obesity and anti-aging benefits of central leptin gene therapy demonstrated recently [10,11,35], are causally coupled to the potent anti-diabetic outcome of this therapy. In all, these preclinical indicators of health safety warrant further validation of the long-lasting anti-diabetic potential of central leptin therapy in sub-human primates and humans.

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OBESITY (Age or High Energy Diet-Induced) **Figure 1.**

Peripheral and central sequence of events mediating the obesity-induced type 2 diabetes. For details see text. CNS, central nervous system; OB-Ra, short isoform of leptin receptor; OB-Rb, long isoform of leptin receptor. Adapted with permission from ref. [37].

Figure 2.

Schematic depiction of the role of leptin sufficiency (A) and insufficiency (B) in the hypothalamus in sustaining normoglycemia and diabetes, respectively. A. Leptin sufficiency in the hypothalamus is maintained by an active process of leptin transport across BBB as dictated by circulating levels of leptin produced by WAT. The independent course traversed by leptin-responsive pathways emanating from the hypothalamus involving the NPY network and descending caudally through the brainstem innervate pancreas, liver, skeletal muscle and BAT in the periphery. Information relay along these neural tracts maintains glucose homeostasis. B. Leptin insufficiency in the hypothalamus conferred by either ① restriction of leptin transport engendered by hyperleptinemia due to obesity or that ② manifests due to a deficiency in leptin signaling as in leptin mutants, leptin receptor mutants, lipodystrophy or insulinopenia (type 1 diabetes), cumulatively results ③ in removal of hypothalamic restraint on pancreatic insulin secretion and attenuation of glucose disposal, the antecedent sequelae, hyperinsulinemia and hyperglycemia of diabetes. For details see text. BBB, blood brain barrier; NPY, neuropeptide Y; WAT, white adipose tissue. Adapted with permission from ref. [37].