Pleotropic Acute and Chronic Effects of Leptin to Reverse Type 1 Diabetes

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Abstract:

Recent studies have demonstrated that leptin can prolong life chronically in rats with poorly-controlled type 1 diabetes (T1D). Multiple explanations have been proposed to explain leptin's chronic antihyperglycemic effect, including suppression of glucagon release and/or signaling, reductions in hyperphagia and ectopic lipid content, and improvements in insulin sensitivity; it is leptin's ability to reduce plasma glucose relies on all of these effects. In addition, leptin reverses hyperglycemia and diabetic ketoacidosis (DKA) acutely, within 6 hours of leptin infusion, by suppressing hypothalamic-pituitary-adrenal (HPA) axis activity in insulinopenic rats. Thus current evidence suggests that leptin's acute, insulin-independent effect to reverse DKA by suppressing HPA axis activity occurs through a different mechanism from its chronic, pleotropic, insulin-dependent effect to reverse hyperglycemia and prolong survival in rodents with T1D. Leptin may therefore represent an attractive therapeutic target to improve glycemic control in humans with poorly-controlled T1D.

Keywords: type 1 diabetes, leptin, corticosterone, diabetic ketoacidosis, hypothalamic-pituitary-adrenal axis

Type 1 diabetes (T1D) occurs when T-cell mediated destruction of the insulin-producing β-cell (1, 2) leads to insulinopenia, hyperglycemia, and ketoacidosis, which can rapidly result in coma and death if left untreated. For reasons that remain unclear, the incidence of T1D is on the rise worldwide (3, 4), and increases in diabetic ketoacidosis (DKA) rates have accompanied the increasing prevalence of T1D (5, 6). For eight decades since its discovery in the early 1920s, insulin was believed to be the only hormone capable of reversing T1D, and consequently it has been prescribed to all T1D patients as a necessary agent to extend life. However, the absolute requirement of treating T1D with insulin was first called into question in the late 1990s. Leptin, a hormone secreted by adipocytes which was first identified by Jeffrey Friedman and colleagues (7) which reduces body weight and normalizes glycemia in leptin-deficient *ob/ob* mice (8-10), resulted in close to normalization of blood glucose concentrations when infused subcutaneously in streptozotocin (STZ)-induced T1D rats (11).

These results have clear clinical implications in humans: although studies have yielded both positive (12-16) and negative results (17-19) regarding an association of leptin with glycemic control in normoglycemic or mildly hyperglycemic T1D patients, patients in diabetic ketoacidosis (DKA) clearly have lower plasma leptin concentrations than healthy controls (20, 21). These results suggest that leptin may be regulated by insulin, an interpretation bolstered by the fact that leptin production by adipocytes rises with increasing insulin concentrations *in vitro* (22), during the acute treatment of DKA in T1D subjects (20) and during acute and chronic hyperinsulinemia in some studies in healthy controls (23-25). However, data are mixed regarding the ability of insulin – such as in the postprandial condition – to acutely regulate leptin secretion in healthy subjects, with the majority of studies finding that leptin gene expression and secretion are not significantly altered with a physiologic, short-term increase in plasma insulin concentrations in non-diabetic subjects (26-28). Taken together, these data indicate that in normal physiology, plasma

leptin concentrations correlate with fat mass, but that this relationship can be altered under pathophysiologic conditions such as DKA.

The striking therapeutic benefit of leptin therapy in rodents with T1D and the potential clinical implications of these findings have spurred great interest in determining the mechanism by which leptin normalizes plasma glucose in T1D rodents. Given the well-established ability of leptin therapy to reduce food intake in *ob/ob* mice (8, 10, 29-31), several groups hypothesized that leptin may exert its antihyperglycemic effect by suppressing food intake in polyphagic T1D rodents, thereby reducing body weight, ectopic lipid, and insulin resistance. In concert with this hypothesis, leptin-treated T1D rodents exhibit marked reductions in plasma and liver triglyceride content (11, 32-34). Not surprisingly, as predicted by the clear association of ectopic lipid deposition with insulin resistance (35-40), lower ectopic lipid concentrations in T1D rats treated with leptin are associated with improvements in both liver and muscle insulin sensitivity (11, 34, 41). However, the ability of leptin to normalize blood glucose in insulin-deficient rodents appears not to be mediated entirely through reductions in food intake: although exogenous leptin treatment or overexpression does suppress hyperphagia in T1D rodents (32, 33, 41-46), pair feeding untreated controls to match their food intake with that of the leptin-treated group was shown by multiple groups to have only minimal impact on blood glucose concentrations (11, 32, 46), suggesting that an alternative mechanism likely mediates part or even all of leptin's antihyperglycemic effect in T1D.

To that end, Roger Unger and colleagues hypothesized that suppression of hyperglucagonemia, which has been demonstrated in animals (32, 44, 47-51) and humans (52-56) with insulinopenic diabetes, may explain leptin's chronic ability to correct glycemia in T1D. Consistent with this hypothesis, these investigators demonstrated that chronic leptin treatment normalizes glucagon concentrations while restoring euglycemia in rodents with T1D (32, 44). Adding further credence to the importance of hyperglucagonemia for the maintenance of T1D, Unger's group found that both glucagon receptor knockout mice and mice treated with a glucagon receptor antibody were protected from hyperglycemia after treatment with STZ to ablate β-cell function.

Importantly, each of the aforementioned leptin treatment studies examined the **chronic** impact of leptin to reverse hyperglycemia in T1D. To our surprise, we recently found that leptin also reverses hyperglycemia **acutely**, with a 6 hour infusion of leptin lowering plasma glucose concentrations by more than 200 mg/dL and reversing ketoacidosis in rats in DKA (57, 58). However, leptin's acute antihyperglycemic mechanism appears different from its chronic mechanism of action. The ability of leptin to normalize plasma glucose concentrations and rates of endogenous glucose production (EGP) preceded its ability to lower plasma glucagon concentrations (57), demonstrating that leptin does not acutely correct DKA by suppressing hyperglucagonemia. However, leptin treatment was associated with suppression of corticosterone-mediated lipolysis, which was both necessary and sufficient to explain leptin's glucose lowering effect by lowering liver acetyl-CoA (57, 58), a critical allosteric activator of the rate-limiting gluconeogenic enzyme pyruvate carboxylase (59-63).

However, in 2015, Morton et al. called these findings into question with data indicating that suppression of hypothalamic-pituitary-adrenal (HPA) axis activity was neither necessary nor sufficient to explain leptin's glucose-lowering effect (64). They showed that although leptin

infusion did lower plasma corticosterone, increasing *in vivo* corticosterone concentrations by exogenous administration of this hormone did not affect glycemia. Even more convincingly, adrenalectomized rats exhibited no difference in plasma glucose relative to intact animals, despite almost negligible plasma corticosterone concentrations, nor did rats treated with a glucocorticoid receptor antagonist. Of note, the plasma insulin concentrations in Morton et al.'s study were ~5-fold higher than those in our experiments, although rats in both studies are markedly hyperglycemic. This discrepancy called into question whether the increase in plasma insulin concentrations from 2 to 10 μU/mL may suppress lipolysis to a sufficient degree to mask any further glucose-lowering effect of leptin (65). Consistent with this hypothesis, infusion of a small amount of insulin to raise plasma insulin concentrations from those measured in our studies to those measured in Morton et al.'s reversed ketoacidosis, lowered plasma glucose and non-esterified fatty acid concentrations, and masked any effect of leptin to lower plasma glucose, EGP, or lipolysis (58). In addition, the majority of studies in Morton's study were performed either in *ad lib* fed rats, or, in the case of the adrenalectomy study, in rats given *ad lib* access to sucrose-containing water. We hypothesized that access to sucrose water in polydipsic diabetic rats may increase plasma glucose concentrations and mask leptin's glucose-lowering effect. Consistent with this hypothesis and with Morton and colleagues' findings, we found that adrenalectomized rats did not exhibit lower plasma glucose concentrations when given sucrose water, but within hours of removing sucrose from their drinking water, plasma glucose concentrations fell to ~120 mg/dL (58). These data indicate that suppression of HPA axis activity (and, consequently, lipolysis) is of minimal importance in mediating glycemia chronically in the fed state, but is of great importance in leptin's effect to lower plasma glucose acutely in the fasted state. It is also important to consider the possibility of dose-dependent effects of leptin on metabolism. In all chronic leptin infusion studies in which plasma leptin concentrations were reported, systemic leptin administration generated supraphysiologic (10-60 fold normal) plasma leptin concentrations (11, 32, 34, 44, 45), whereas our acute leptin replacement studies merely increased plasma leptin to physiologic, non-diabetic concentrations (57, 58). Because supraphysiologic leptin concentrations have been shown to increase energy expenditure in leptin-deficient mice (10, 66), it is conceivable that increased energy expenditure in models in which diabetic rodents are treated chronically with supraphysiologic leptin infusion or leptin overexpression would eventually result in reduced ectopic lipid concentrations. However, this effect would not be seen in a short-term supraphysiologic (64) or physiologic (57, 58) leptin infusion, as days to weeks of increased mitochondrial function are required to generate differences in ectopic lipid accumulation.

Taken together, these studies demonstrate that leptin exerts both insulin-dependent (chronic) (11, 32-34, 42, 44, 58, 64) and insulin-independent (acute) (57, 58) effects to lower plasma glucose concentrations in poorly-controlled diabetes. The acute, insulin-independent effect of leptin appears to be mediated through suppression of HPA axis activity, but requires extreme insulin deficiency and ketoacidosis, a critical state in which rodents cannot survive for more than 24 hours. However, when lipolysis is relatively lower in the moderately insulinopenic state that generates hyperglycemia but not ketoacidosis, the chronic, insulin-sensitizing effects of leptin predominate, and leptin lowers postprandial hyperglycemia likely by a combination of reductions in ectopic lipid due to lower food

intake and/or decreased lipolysis, suppression of hyperglucagonemia, and perhaps also suppression of HPA axis activity.

It is unlikely that leptin will replace insulin as the standard treatment for ketoacidosis; insulin works quickly and successfully in the vast majority of DKA patients to lower plasma glucose rapidly and safely. However leptin would be expected to expected to confer minimal risk of hypoglycemia because leptin replacement does not remove corticosterone or other hyperglycemic hormones, but rather reduces their concentrations to normal levels (57, 58, 64). It may, therefore, be a potential adjunct to insulin in DKA patients, allowing clinicians to reduce insulin doses without slowing the process of lowering plasma glucose concentrations, thereby avoiding potentially dangerous hypoglycemia. Studies in which leptin lowered plasma glucose concentrations as well as insulin in fed rats raise the intriguing possibility that leptin could even be more useful as a chronic therapy to lower ectopic lipid concentrations and both fasting and postprandial glucose concentrations in poorly controlled T1D patients, just as it does in lipodystrophic individuals (67). However, leptin is not expected to have any impact on glucose transporter function. Because of the need for insulin to permit glucose transport into cells, leptin therapy would need to be combined with low doses of insulin in long-term T1D patients who lack all β-cell function.

In summary, recent studies have shown that interventions to raise plasma leptin concentrations acutely in DKA or chronically in poorly-controlled T1D patients may confer a therapeutic benefit in lowering plasma glucose concentrations through an insulin-independent and an insulin-dependent mechanism, respectively. These findings reveal important insights about the pathogenesis of DKA, demonstrating that hypercorticosteronemia and insulinopenia, but not hyperglucagonemia or alterations in other hormones, are necessary for the ketoacidosis characteristic of this critical state. In contrast, chronic insulinopenia without ketoacidosis is associated with a number of physiologic derangements including hypercorticosteronemia, hyperglucagonemia, hyperphagia, increased ectopic lipid content, and insulin resistance, which, together, lead to both fasting and postprandial hyperglycemia. Chronic leptin treatment reverses each of these derangements in rodents and may represent a new therapeutic target, alongside insulin, in patients with poorly controlled T1D.

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