Mechanism for the Anti-Diabetic Effect of Leptin

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Summary

Leptin reverses hyperglycemia in T1D though reductions in HPA-mediated lipolysis resulting in reduced hepatic gluconeogenesis through both substrate delivery and allosteric mechanisms.

Leptin treatment reverses hyperglycemia in animal models of poorly-controlled type 1 diabetes (T1D),¹⁻⁶ spurring great interest in the possibility of treating T1D patients with leptin. The antidiabetic effect of leptin has been postulated to occur through suppression of glucagon production and/or suppression of glucagon responsiveness; however, there does not appear to be a direct effect of leptin on the pancreatic α-cell.⁷ Thus the mechanisms responsible for leptin’s anti-diabetic effect remain poorly understood. To this end, we quantified liver-specific rates of hepatic gluconeogenesis and substrate oxidation in conjunction with rates of whole-body acetate, glycerol and fatty acid turnover in three rat models of poorly controlled diabetes including a model of diabetic ketoacidosis (DKA).⁸ We show that higher rates of hepatic gluconeogenesis in all of these models could be attributed to hypoleptinemia-induced activity of the hypothalamic-pituitary-adrenal (HPA) resulting in higher rates of lipolysis, conversion of glycerol to glucose through a substrate push mechanism, and conversion of pyruvate to glucose through greater hepatic acetyl CoA allosteric activation of pyruvate carboxylase flux. Surprisingly these effects could be dissociated from changes in plasma insulin and glucagon concentrations and hepatic gluconeogenic protein expression. All of the altered systemic and hepatic metabolic fluxes were mimicked by infusing rats with Intralipid or corticosterone, and corrected by leptin.

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Author Contributions

R.J.P. and G.I.S. designed the experimental protocols. R.J.P., X-M.Z., D.Z., N.K., J-P.G.C., and G.W.C. performed the studies. All authors contributed to the analysis of data. R.J.P. and G.I.S. wrote the manuscript.

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更换。这些数据证明脂肪分解和底物输送在肝脏中的关键作用，特别是由于低瘦素血症和HPA活性，促进肝糖原生成和高血糖在控制不充分的糖尿病中。

T1D大鼠有严重的空腹高血糖和酮症酸中毒，伴有多达90%的低胰岛素和瘦素水平，以及90%的高胰高血糖素水平（Fig. 1a–d，Table S1），而不影响肝脏和肾脏糖原生成的相对贡献（分别在控制和T1D大鼠中，91±4和91±2%）。正常化血浆瘦素水平通过6小时的动脉内瘦素输注降低了240 mg/dL的血浆葡萄糖浓度和酮症酸中毒，而不会影响胰岛素、胰高血糖素、 adiponectin或FGF-21浓度，或磷酸化glucagon目标cyclic AMP反应元件结合蛋白（CREB）（Fig. 1a–d, Fig. S1a–c）。值得注意的是，血浆胰高血糖素浓度的正常化直到治疗后24小时才发生，这远晚于血浆葡萄糖、皮质醇和ACTH浓度的正常化（Fig. 1c）。瘦素治疗降低了T1D大鼠的肝糖原生成率，通过降低磷酸和甘油的糖原生成（Fig. 1e）。相反，虽然总TCA循环净流量（V_{TCA}）没有变化，脂肪酸氧化在T1D大鼠的肝脏中更高，并通过瘦素治疗恢复（Fig. 1f）。瘦素治疗后甘油对肝糖原生成的贡献降低，与血浆甘油和非酯化脂肪酸（NEFA）浓度的60%降低有关（Fig. 1f–g, Fig. S1e–f）。由于血浆酮酸浓度的降低，表明肝脏的丙酮酸羧化酶（V_{PC}）和丙酮酸脱氢酶（V_{PDH}）净流率的差异。为了确定瘦素治疗后酮症酸中毒改善的原因，我们测量了肝脏的乙酰CoA浓度，这些浓度在糖尿病大鼠中明显降低，并在瘦素治疗后恢复正常（Fig. S1h）。

尽管瘦素可能直接作用于肌肉葡萄糖摄取和/或外周胰岛素敏感性，如一些研究所示1,15,16但不所有研究2,17，我们的数据表明瘦素对快速高血糖的直接作用和与DKA相关的机理可以解释通过瘦素抑制底物输送到肝脏的脂肪分解，导致降低肝脏糖原生成和葡萄糖生成。为了探究脂肪分解的增加及其与瘦素治疗的逆转机制，我们测量了血浆去甲肾上腺素和肾上腺素。与血浆去甲肾上腺素，去甲肾上腺素和肾上腺素相反，血浆胰岛素，胰高血糖素，adiponectin或FGF-21浓度（Fig. 1f–g, Fig. S1e–f）。由于血浆酮酸浓度的降低，表明肝脏的丙酮酸羧化酶（V_{PC}）和丙酮酸脱氢酶（V_{PDH}）净流率的差异。14我们发现，未控制的T1D大鼠中，低血浆酮酸浓度降至正常水平（Fig. S1h）。

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当丙酮酸羧化酶的活性9–12和抑制剂，13观察到的乙酰CoA浓度的升高，表明乙酰CoA浓度在T1D和T1D-leptin治疗组的升高的乙酰CoA净流率，比这些组的动物之间的差异。为了确定原因的改进在酮症酸中毒与瘦素治疗，我们测量肝脏乙酰CoA浓度，以及，一致的与酮症酸中毒的逆转在T1D大鼠，作为由正常化阴离子间隙和降低β-羟基丁酸浓度。14我们发现，未控制的T1D大鼠中，低血浆酮酸浓度降至正常水平（Fig. S1h）。

尽管瘦素可能直接作用于肌肉葡萄糖摄取和/或外周胰岛素敏感性，如一些研究所示1,15,16但不所有研究2,17，我们的数据表明瘦素对快速高血糖的直接作用和与DKA相关的机理可以解释通过瘦素抑制底物输送到肝脏的脂肪分解，导致降低肝脏糖原生成和葡萄糖生成。为了探究脂肪分解的增加及其与瘦素治疗的逆转机制，我们测量了血浆去甲肾上腺素和肾上腺素。与血浆去甲肾上腺素，去甲肾上腺素和肾上腺素相反，血浆胰岛素，胰高血糖素，adiponectin或FGF-21浓度（Fig. 1f–g, Fig. S1e–f）。由于血浆酮酸浓度的降低，表明肝脏的丙酮酸羧化酶（V_{PC}）和丙酮酸脱氢酶（V_{PDH}）净流率的差异。14我们发现，未控制的T1D大鼠中，低血浆酮酸浓度降至正常水平（Fig. S1h）。

在控制不充分的T1D大鼠中，血浆酮酸浓度降至正常水平（Fig. S1h），这与血浆酮酸浓度的降低一致。14我们发现，未控制的T1D大鼠中，低血浆酮酸浓度降至正常水平（Fig. S1h）。

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were not different between groups, and growth hormone, which was lower in T1D rats, we found that plasma corticosterone and ACTH concentrations obtained at 1200 (noon) were markedly greater in the T1D rats (Fig. 1j, Fig. S1i–l). Taken together these data suggest a critical role for the hypothalamic-pituitary-adrenal axis in promoting the higher rates of lipolysis in the T1D group.

To assess whether alterations in plasma insulin concentrations accounted for the hypoleptinemia and/or the elevated gluconeogenesis observed in the T1D model, we also measured these fluxes in rats rendered both hyperglycemic and hyperinsulinemic, due to three-day high fat feeding combined with low dose streptozotocin and nicotinamide treatment (hyperinsulinemic-diabetic rat model), and found that all alterations in hepatic anaplerotic and oxidative fluxes in the T1D rat model were replicated in the hyperinsulinemic-diabetic rats. Hyperinsulinemic-diabetic rats were equally as hyperglycemic as the T1D group despite ~40-fold higher plasma insulin concentrations (Fig. S2a–b), but in contrast to the T1D rats were not ketoacidotic (Table S2). However, hyperinsulinemic-diabetic rats were similarly leptin deficient as compared to the T1D rats (Fig. S2d). Restoring plasma leptin concentrations with a 6 hr intra-arterial leptin infusion normalized plasma glucose, corticosterone, and ACTH concentrations and rates of hepatic gluconeogenesis from pyruvate and glycerol without altering plasma insulin concentrations or $V_{\text{TCA}}$ flux (Fig. S2a–h). Similar to the T1D group, whole-body glycerol, fatty acid, and acetate turnover were reduced, as were hepatic acetyl CoA concentrations (Fig. S2i–l). In contrast, mRNA and protein expression of key gluconeogenic enzymes were not different between the control, hyperinsulinemic-diabetic, and T1D groups (Fig. S3a–g). Taken together, these data imply that excess hepatic substrate flux, independent of insulin, is responsible for the uncontrolled gluconeogenesis of poorly controlled T1D and the improvement in glycemia associated with leptin treatment.

Based on these data, we next examined whether the higher rates of whole-body lipolysis due to leptin deficiency might be playing a role in causing excess gluconeogenesis in the T1D rats. We reasoned that increased glycerol turnover would drive hepatic gluconeogenesis through a substrate-dependent process,$^{3,18}$ whereas an increase in acetate and fatty acid turnover would increase gluconeogenesis through allosteric activation of pyruvate carboxylase by acetyl CoA. In order to determine whether excess substrate availability alone could induce any of the changes to hepatic fluxes measured in poorly controlled T1D rats, we performed a 24-hour infusion of heparin and Intralipid.$^{19}$ Consistent with our hypothesis, this intervention resulted in higher plasma glucose concentrations and gluconeogenesis from both pyruvate and glycerol despite greater plasma insulin concentrations and no changes in plasma glucagon, FGF-21 or adiponectin concentrations (Fig. 2a–b, Fig. S4a–d). As observed in the other diabetic rodent models excess hepatic gluconeogenesis could be attributed to markedly higher rates of whole-body glycerol and fatty acid turnover without any change in total hepatic TCA cycle flux (Fig. 2c–e, Fig. S4e–f). Consistent with the greater $V_{\text{PC}}$ and reduced $V_{\text{PDH}}$ flux, hepatic acetyl concentrations were higher by ~50% in the lipid-infused group (Fig. 2f). To further investigate the role of substrate regulation by leptin, we co-infused leptin-treated T1D rats with Intralipid and heparin and found that raising acetyl CoA, glycerol and fatty acid concentrations by exogenous substrate administration abrogated leptin’s effect to correct hyperglycemia and normalize rates of
hepatic gluconeogenesis from glycerol and pyruvate (Fig. 3a–f, Fig. S5a–f). Taken together, these data demonstrate that excess substrate flux, derived from excess peripheral lipolysis, drives increased rates of hepatic gluconeogenesis in poorly controlled diabetes both through increasing glycerol supply and through allosteric activation of pyruvate carboxylase by increased acetyl CoA. Thus these studies demonstrate the critical role of increased substrate delivery to the liver and allosteric regulation of pyruvate carboxylase activity as the key factors responsible for causing the increased rates of gluconeogenesis in poorly controlled T1D. Furthermore, by demonstrating profound elevations in rates of hepatic gluconeogenesis, independent of changes in hepatic gluconeogenic protein expression, these results also challenge the canonical role for alterations in hepatic gluconeogenic gene and protein expression as being the major contributor to increased hepatic gluconeogenesis in poorly controlled diabetes.

Because hypercorticosteronemia has been observed in leptin-deficient and leptin-resistant rodent models and leptin has been identified as a suppressor of ACTH-dependent cortisol secretion in vitro, we hypothesized that leptin deficiency may be responsible for increased HPA activity leading to higher rates of lipolysis and rates of gluconeogenesis in the T1D rats (Fig. 1e–j). To determine if these elevations in plasma corticosterone concentrations would promote similar increases in rates of lipolysis as observed in the T1D rats we injected high fat fed rats with intra-arterial corticosterone for 24 hr to achieve similar plasma levels of corticosterone (163±19 vs. 187±30 ng/mL, P=0.6) as was observed in the T1D rats. This intervention resulted in more than 100 mg/dl higher plasma glucose concentrations, which was associated with greater hepatic gluconeogenesis from both pyruvate and glycerol, despite three-fold higher plasma insulin concentrations (Fig. 4a–c). In contrast these changes were not associated with any changes in the TCA cycle flux (Fig. 4d). These higher rates of hepatic gluconeogenesis were driven by greater whole-body glycerol, fatty acid and acetate turnover which were associated with higher plasma glycerol, fatty acid, and acetate concentrations as well as hepatic acetyl CoA concentrations similar to what was observed in the T1D rats (Fig. 4e–h, Fig. S6a–c). In contrast, there was no difference in plasma glucagon, FGF-21, or adiponectin concentrations (Fig. 6d–f).

In order to test the mechanism of ACTH-driven increases in corticosterone in driving hyperglycemia in the T1D rat, we treated diabetic rats with mifepristone, a potent glucocorticoid receptor antagonist. Consistent with a major role for increased HPA activity in driving increased lipolysis and hepatic gluconeogenesis, treatment with the glucocorticoid receptor blockade resulted in >200 mg/dl lower fasting plasma glucose concentrations compared to the control T1D group despite identical fasting plasma insulin and glucagon concentrations (Fig. S7a–c). Improvements in glycemia could be attributed to reduced hepatic gluconeogenesis from both pyruvate and glycerol without any changes in total V_{TCA} (Figure S7d–e). Mifepristone-treated T1D rats exhibited 75–85% lower whole-body glycerol, fatty acid and acetate turnover, as well as >40% lower plasma glycerol, fatty acid and acetate concentrations, associated with 60% lower hepatic acetyl CoA concentrations (Fig. S7f–l). Thus the lower fasting plasma glucose concentrations in hyperglycemic T1D rats treated with glucocorticoid receptor antagonism can be attributed to reductions in rates of whole-body lipolysis and in hepatic acetyl CoA content.
In order to determine whether these findings were specific to the acute ketoacidotic state of the streptozotocin-induced T1D rat, we also examined this question in BioBreeding (BB) rats, a genetic rat model of T1D. Similar to the streptozotocin-induced T1D model, the BB rats exhibited severe hyperglycemia associated with hypoleptinemia, as well as hyperglucagonemia and insulinopenia (Fig. S8a–d). Correcting hypoleptinemia normalized plasma glucose concentrations and hepatic gluconeogenesis within 6 hours by correcting lipolysis and normalizing hepatic acetyl CoA concentrations (Fig. S8e–j). Consistent with our findings in other diabetic rat models, hyperglycemia was associated with higher plasma corticosterone and ACTH concentrations, which were corrected in the leptin-infused group (Fig. 8k–l). Also similar to our observations in T1D rats, plasma glucagon concentrations normalized after 24 hours of leptin treatment, which was more than 12 hours following reductions in plasma ACTH, corticosterone and plasma glucose concentrations thus temporally dissociating leptin-induced reductions in plasma glucagon concentrations from leptin-induced reductions in plasma ACTH, corticosterone and glucose concentrations (Fig. S8c).

Taken together, these data identify a key role for hypoleptinemia-induced increases in HPA activity in contributing to the increased rates of hepatic gluconeogenesis, fasting hyperglycemia and ketoacidosis in rodent models of T1D through glucocorticoid-mediated increases in lipolysis (Fig. S9A) and provide the mechanism for correction of hyperglycemia in these animals following systemic or ventromedial administration of leptin (Fig. S9B).2,24,25 Furthermore these results have potential translational significance given previous studies that have documented increases in plasma cortisol concentrations in T1D patients in diabetic ketoacidosis.26–28 Whether this mechanism also contributes to leptin’s ability to reverse insulin resistance and hyperglycemia in patients with severe lipodystrophy, in addition to its ability to decrease hepatic steatosis and intramyocellular lipid content,29 remains to be determined.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Leptin reverses hyperglycemia and excess gluconeogenesis from pyruvate and glycerol in streptozotocin-induced type 1 diabetic (T1D) rats. (a)–(d) Fasting plasma glucose, insulin, glucagon, and leptin concentrations. In panel (d), n=16 for all groups. (e) Hepatic gluconeogenesis from pyruvate (lower bars) and glycerol (upper bars). P-values over the bars represent comparisons of total gluconeogenic flux. Gluconeogenesis from both pyruvate and glycerol was increased (P<0.05 and P<0.0001, respectively) in T1D rats vs. controls, and decreased (P<0.001 and P<0.0001, respectively) in T1D-leptin treated vs. T1D rats. (f)–(h) Whole-body glycerol, fatty acid (palmitate), and acetate turnover. (i) Liver acetyl CoA concentration. (j) 12 p.m. plasma corticosterone concentrations. Data are mean ± S.E. If not otherwise specified, n=6–8 per group. *P<0.05, ***P<0.001, ****P<0.0001 vs. control; ##P<0.01, ###P<0.001, ####P<0.0001 vs. T1D; §§§P<0.001 vs. T1D-leptin 6 hr.
Fig. 2.
24 hr lipid infusion in 3-day high fat fed rats replicates the perturbations to fluxes seen in T1D and hyperinsulinemic-diabetic rats and implicates increased substrate supply in the excess gluconeogenesis of T1D. (a) Plasma glucose. (b) Hepatic gluconeogenesis from pyruvate (lower bars) and glycerol (upper bars). Gluconeogenesis from both pyruvate and glycerol was increased (P<0.001 and P<0.01, respectively) in lipid-infused rats. (c) TCA cycle flux from fatty acid oxidation (lower bars) and through PDH (upper bars). V_{TCA} from fatty acid oxidation was increased and V_{TCA} through PDH was decreased (P<0.05) in lipid-infused rats. (d), (e) Whole-body glycerol and fatty acid (palmitate) turnover. (f) Liver acetyl CoA concentration. In all panels, data are mean ± S.E. of n=6 per group. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.
Fig. 3.
Substrate (Intralipid/heparin) infusion blocks leptin's effect to suppress hepatic gluconeogenesis in T1D rats. (a) Fasting plasma glucose. (b) Hepatic gluconeogenesis from pyruvate (lower bars) and glycerol (upper bars). Gluconeogenesis from both pyruvate and glycerol was increased ($P<0.01$ and $P<0.001$, respectively) in lipid-infused rats. (c) $V_{\text{TCA}}$ from fatty acid oxidation (lower bars) and through pyruvate dehydrogenase (upper bars). $V_{\text{TCA}}$ through PDH was increased ($P<0.05$) in lipid-infused rats. (d), (e) Whole-body glycerol and palmitic acid oxidation. (f) Liver acetyl CoA concentration. In all panels, data are mean ± S.E. of n=6 per group. *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$. 

*Note: The image includes graphs showing the changes in various parameters after substrate infusion and their statistical significance.*
Fig. 4.
Matching plasma corticosterone in high fat fed-corticosterone infused rats to that of T1D animals drives excess lipolysis, gluconeogenesis, and hyperglycemia. (a) Fasting plasma glucose. (b) Hepatic gluconeogenesis from pyruvate (solid bars) and glycerol (dashed bars). Gluconeogenesis from glycerol was increased (P<0.01) in corticosterone-infused rats. (c) Fasting plasma insulin. (d) TCA cycle flux from fatty acid oxidation (solid bars) and through PDH (dashed bars). (e)–(g) Whole-body glycerol, fatty acid (palmitate) and acetate turnover. (h) Liver acetyl CoA concentration. In all panels, data are mean ± S.E.M., n=6 per group. *P<0.05, **P<0.01, ****P<0.0001.