Published in final edited form as:

Postdoc J. 2017 January ; 5(1): 3–11.

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

Rachel J. Perry, PhD

Department of Internal Medicine, Yale University, New Haven, CT 06510

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, pleiotropic, 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 $\mu\text{U/mL}$ 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 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.

Acknowledgments

The author thanks Dr. Gerald I. Shulman for helpful discussions. This work was supported by NIH T32 DK10019 and NIH MICROMouse grant 16AU3737 (to RJP).

References

1. Bottazzo GF, Dean BM, McNally JM, MacKay EH, Swift PG, Gamble DR. In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulinitis. *The New England journal of medicine*. 1985; 313(6):353–60. PubMed PMID: 3159965. DOI: 10.1056/NEJM198508083130604 [PubMed: 3159965]
2. Bach JF. Insulin-dependent diabetes mellitus as an autoimmune disease. *Endocr Rev*. 1994; 15(4): 516–42. PubMed PMID: 7988484. DOI: 10.1210/edrv-15-4-516 [PubMed: 7988484]
3. Gale EA. The rise of childhood type 1 diabetes in the 20th century. *Diabetes*. 2002; 51(12):3353–61. PubMed PMID: 12453886. [PubMed: 12453886]
4. Egro FM. Why is type 1 diabetes increasing? *J Mol Endocrinol*. 2013; 51(1):R1–13. PubMed PMID: 23733895. DOI: 10.1530/JME-13-0067 [PubMed: 23733895]
5. Venkatesh B, Pilcher D, Prins J, Bellomo R, Morgan TJ, Bailey M. Incidence and outcome of adults with diabetic ketoacidosis admitted to ICUs in Australia and New Zealand. *Crit Care*. 2015; 19:451. PubMed PMID: 26715333; PMCID: PMC4699354. doi: 10.1186/s13054-015-1171-7 [PubMed: 26715333]
6. Rewers A, Dong F, Slover RH, Klingensmith GJ, Rewers M. Incidence of diabetic ketoacidosis at diagnosis of type 1 diabetes in Colorado youth, 1998-2012. *JAMA*. 2015; 313(15):1570–2. PubMed PMID: 25898057. DOI: 10.1001/jama.2015.1414 [PubMed: 25898057]
7. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994; 372(6505):425–32. PubMed PMID: 7984236. DOI: 10.1038/372425a0 [PubMed: 7984236]
8. Halaas JL, Boozer C, Blair-West J, Fidathusein N, Denton DA, Friedman JM. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. *Proceedings of the National Academy of Sciences of the United States of America*. 1997; 94(16):8878–83. PubMed PMID: 9238071; PMCID: PMC23177. [PubMed: 9238071]
9. Schwartz MW, Baskin DG, Bukowski TR, Kuijper JL, Foster D, Lasser G, Prunkard DE, Porte D Jr, Woods SC, Seeley RJ, Weigle DS. Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. *Diabetes*. 1996; 45(4):531–5. PubMed PMID: 8603777. [PubMed: 8603777]
10. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science*. 1995; 269(5223):540–3. PubMed PMID: 7624776. [PubMed: 7624776]
11. Chinookoswong N, Wang JL, Shi ZQ. Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat. *Diabetes*. 1999; 48(7):1487–92. Epub 1999/07/02. PubMed PMID: 10389859. [PubMed: 10389859]
12. Hecht, Baldauff N., Tfayli, H., Dong, W., Arena, VC., Gurtunca, N., Pietropaolo, M., Becker, DJ., Libman, IM. Relationship of adiponectin and leptin with autoimmunity in children with new-onset type 1 diabetes: a pilot study. *Pediatr Diabetes*. 2016; 17(4):249–56. PubMed PMID: 25754190. DOI: 10.1111/pedi.12267 [PubMed: 25754190]
13. Safai N, Eising S, Hougaard DM, Mortensen HB, Skogstrand K, Pociot F, Johannesen J, Svensson J. Levels of adiponectin and leptin at onset of type 1 diabetes have changed over time in children and adolescents. *Acta diabetologica*. 2015; 52(1):167–74. PubMed PMID: 25059225. DOI: 10.1007/s00592-014-0630-y [PubMed: 25059225]
14. Wedrychowicz A, Stec M, Sztelfko K, Starzyk JB. Associations between bone, fat tissue and metabolic control in children and adolescents with type 1 diabetes mellitus. *Exp Clin Endocrinol Diabetes*. 2014; 122(8):491–5. PubMed PMID: 24918536. DOI: 10.1055/s-0034-1375666 [PubMed: 24918536]

15. Pham MN, Kolb H, Mandrup-Poulsen T, Battelino T, Ludvigsson J, Pozzilli P, Roden M, Schloot NC, European CPT. Serum adipokines as biomarkers of beta-cell function in patients with type 1 diabetes: positive association with leptin and resistin and negative association with adiponectin. *Diabetes Metab Res Rev*. 2013; 29(2):166–70. PubMed PMID: 23197433. DOI: 10.1002/dmrr.2378 [PubMed: 23197433]
16. Thrailkill KM, Jo CH, Cockrell GE, Moreau CS, Lumpkin CK Jr, Fowlkes JL. Determinants of undercarboxylated and carboxylated osteocalcin concentrations in type 1 diabetes. *Osteoporos Int*. 2012; 23(6):1799–806. PubMed PMID: 22068385; PMCID: PMC3471372. DOI: 10.1007/s00198-011-1807-7 [PubMed: 22068385]
17. Majewska KA, Majewski D, Skowronska B, Stankiewicz W, Fichna P. Serum leptin and adiponectin levels in children with type 1 diabetes mellitus - Relation to body fat mass and disease course. *Adv Med Sci*. 2016; 61(1):117–22. PubMed PMID: 26647091. DOI: 10.1016/j.advms.2015.10.002 [PubMed: 26647091]
18. Neumann T, Lodes S, Kastner B, Franke S, Kiehnopf M, Lehmann T, Muller UA, Wolf G, Samann A. Osteocalcin, adipokines and their associations with glucose metabolism in type 1 diabetes. *Bone*. 2016; 82:50–5. PubMed PMID: 25888930. DOI: 10.1016/j.bone.2015.04.017 [PubMed: 25888930]
19. Geyikli I, Keskin M, Kor Y, Akan M. Increased resistin serum concentrations in patients with type 1 diabetes mellitus. *J Clin Res Pediatr Endocrinol*. 2013; 5(3):189–93. PubMed PMID: 24072088; PMCID: PMC3814534. DOI: 10.4274/Jcrpe.1072 [PubMed: 24072088]
20. Hathout EH, Sharkey J, Racine M, Ahn D, Mace JW, Saad MF. Changes in plasma leptin during the treatment of diabetic ketoacidosis. *The Journal of clinical endocrinology and metabolism*. 1999; 84(12):4545–8. Epub 1999/12/22. PubMed PMID: 10599716. DOI: 10.1210/jcem.84.12.6184 [PubMed: 10599716]
21. Kitabchi AE, Umpierrez GE. Changes in serum leptin in lean and obese subjects with acute hyperglycemic crises. *The Journal of clinical endocrinology and metabolism*. 2003; 88(6):2593–6. Epub 2003/06/06. PubMed PMID: 12788860. DOI: 10.1210/jc.2002-021975 [PubMed: 12788860]
22. Cammisotto PG, Gelinas Y, Deshaies Y, Bukowiecki LJ. Regulation of leptin secretion from white adipocytes by insulin, glycolytic substrates, and amino acids. *American journal of physiology Endocrinology and metabolism*. 2005; 289(1):E166–71. PubMed PMID: 15741239. DOI: 10.1152/ajpendo.00602.2004 [PubMed: 15741239]
23. Kolaczynski JW, Nyce MR, Considine RV, Boden G, Nolan JJ, Henry R, Mudaliar SR, Olefsky J, Caro JF. Acute and chronic effects of insulin on leptin production in humans: Studies in vivo and in vitro. *Diabetes*. 1996; 45(5):699–701. PubMed PMID: 8621027. [PubMed: 8621027]
24. Boden G, Chen X, Kolaczynski JW, Polansky M. Effects of prolonged hyperinsulinemia on serum leptin in normal human subjects. *The Journal of clinical investigation*. 1997; 100(5):1107–13. PubMed PMID: 9276727; PMCID: PMC508285. DOI: 10.1172/JCI119621 [PubMed: 9276727]
25. Saad MF, Khan A, Sharma A, Michael R, Riad-Gabriel MG, Boyadjian R, Jinagouda SD, Steil GM, Kamdar V. Physiological insulinemia acutely modulates plasma leptin. *Diabetes*. 1998; 47(4):544–9. PubMed PMID: 9568685. [PubMed: 9568685]
26. Dagogo-Jack S, Fanelli C, Paramore D, Brothers J, Landt M. Plasma leptin and insulin relationships in obese and nonobese humans. *Diabetes*. 1996; 45(5):695–8. PubMed PMID: 8621026. [PubMed: 8621026]
27. Clapham JC, Smith SA, Moore GB, Hughes MG, Azam H, Scott A, Jung RT. Plasma leptin concentrations and OB gene expression in subcutaneous adipose tissue are not regulated acutely by physiological hyperinsulinaemia in lean and obese humans. *International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity*. 1997; 21(3):179–83. PubMed PMID: 9080255.
28. Vidal H, Auboeuf D, De Vos P, Staels B, Riou JP, Auwerx J, Laville M. The expression of ob gene is not acutely regulated by insulin and fasting in human abdominal subcutaneous adipose tissue. *The Journal of clinical investigation*. 1996; 98(2):251–5. PubMed PMID: 8755631; PMCID: PMC507424. DOI: 10.1172/JCI118786 [PubMed: 8755631]
29. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science*. 1995; 269(5223):546–9. PubMed PMID: 7624778. [PubMed: 7624778]

30. Stephens TW, Basinski M, Bristow PK, Bue-Valleskey JM, Burgett SG, Craft L, Hale J, Hoffmann J, Hsiung HM, Kriauciunas A, et al. The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature*. 1995; 377(6549):530–2. PubMed PMID: 7566151. DOI: 10.1038/377530a0 [PubMed: 7566151]
31. Levin N, Nelson C, Gurney A, Vandlen R, de Sauvage F. Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proceedings of the National Academy of Sciences of the United States of America*. 1996; 93(4):1726–30. PubMed PMID: 8643697; PMCID: PMC40010. [PubMed: 8643697]
32. Wang MY, Chen L, Clark GO, Lee Y, Stevens RD, Ilkayeva OR, Wenner BR, Bain JR, Charron MJ, Newgard CB, Unger RH. Leptin therapy in insulin-deficient type I diabetes. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(11):4813–9. Epub 2010/03/03. PubMed PMID: 20194735; PMCID: 2841945. DOI: 10.1073/pnas.0909422107 [PubMed: 20194735]
33. Fujikawa T, Chuang JC, Sakata I, Ramadori G, Coppari R. Leptin therapy improves insulin-deficient type 1 diabetes by CNS-dependent mechanisms in mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107(40):17391–6. Epub 2010/09/22. PubMed PMID: 20855609; PMCID: 2951430. DOI: 10.1073/pnas.1008025107 [PubMed: 20855609]
34. Denroche HC, Levi J, Wideman RD, Sequeira RM, Huynh FK, Covey SD, Kieffer TJ. Leptin therapy reverses hyperglycemia in mice with streptozotocin-induced diabetes, independent of hepatic leptin signaling. *Diabetes*. 2011; 60(5):1414–23. PubMed PMID: 21464443; PMCID: PMC3292314. DOI: 10.2337/db10-0958 [PubMed: 21464443]
35. Shulman GI. Ectopic fat in insulin resistance, dyslipidemia, and cardiometabolic disease. *The New England journal of medicine*. 2014; 371(12):1131–41. Epub 2014/09/18. PubMed PMID: 25229917. DOI: 10.1056/NEJMr1011035 [PubMed: 25229917]
36. Samuel VT, Shulman GI. The pathogenesis of insulin resistance: integrating signaling pathways and substrate flux. *The Journal of clinical investigation*. 2016; 126(1):12–22. PubMed PMID: 26727229. PMCID: PMC4701542. DOI: 10.1172/JCI77812 [PubMed: 26727229]
37. Perry RJ, Samuel VT, Petersen KF, Shulman GI. The role of hepatic lipids in hepatic insulin resistance and type 2 diabetes. *Nature*. 2014; 510(7503):84–91. PubMed PMID: 24899308. [PubMed: 24899308]
38. Samuel VT, Shulman GI. Mechanisms for insulin resistance: common threads and missing links. *Cell*. 2012; 148(5):852–71. Epub 2012/03/06. PubMed PMID: 22385956; PMCID: 3294420. [PubMed: 22385956]
39. Asrih M, Jornayvaz FR. Metabolic syndrome and nonalcoholic fatty liver disease: Is insulin resistance the link? *Mol Cell Endocrinol*. 2015; 418(Pt 1):55–65. PubMed PMID: 25724480. DOI: 10.1016/j.mce.2015.02.018 [PubMed: 25724480]
40. Boren J, Taskinen MR, Olofsson SO, Levin M. Ectopic lipid storage and insulin resistance: a harmful relationship. *J Intern Med*. 2013; 274(1):25–40. PubMed PMID: 23551521. DOI: 10.1111/joim.12071 [PubMed: 23551521]
41. Fujikawa T, Berglund ED, Patel VR, Ramadori G, Vianna CR, Vong L, Thorel F, Chera S, Herrera PL, Lowell BB, Elmquist JK, Baldi P, Coppari R. Leptin engages a hypothalamic neurocircuitry to permit survival in the absence of insulin. *Cell metabolism*. 2013; 18(3):431–44. Epub 2013/09/10. PubMed PMID: 24011077; PMCID: 3890693. DOI: 10.1016/j.cmet.2013.08.004 [PubMed: 24011077]
42. Xu Y, Chang JT, Myers MG Jr, Xu Y, Tong Q. Euglycemia Restoration by Central Leptin in Type 1 Diabetes Requires STAT3 Signaling but Not Fast-Acting Neurotransmitter Release. *Diabetes*. 2016; PubMed PMID: 26822087; PMCID: PMC4806656. doi: 10.2337/db15-1160
43. Lin CY, Higginbotham DA, Judd RL, White BD. Central leptin increases insulin sensitivity in streptozotocin-induced diabetic rats. *American journal of physiology Endocrinology and metabolism*. 2002; 282(5):E1084–91. Epub 2002/04/06. PubMed PMID: 11934674. DOI: 10.1152/ajpendo.00489.2001 [PubMed: 11934674]
44. Yu X, Park BH, Wang MY, Wang ZV, Unger RH. Making insulin-deficient type 1 diabetic rodents thrive without insulin. *Proceedings of the National Academy of Sciences of the United States of*

- America. 2008; 105(37):14070–5. Epub 2008/09/10. PubMed PMID: 18779578; PMCID: 2544580. DOI: 10.1073/pnas.0806993105 [PubMed: 18779578]
45. Miyanaga F, Ogawa Y, Ebihara K, Hidaka S, Tanaka T, Hayashi S, Masuzaki H, Nakao K. Leptin as an adjunct of insulin therapy in insulin-deficient diabetes. *Diabetologia*. 2003; 46(10):1329–37. PubMed PMID: 12928770. DOI: 10.1007/s00125-003-1193-6 [PubMed: 12928770]
 46. Hidaka S, Yoshimatsu H, Kondou S, Tsuruta Y, Oka K, Noguchi H, Okamoto K, Sakino H, Teshima Y, Okeda T, Sakata T. Chronic central leptin infusion restores hyperglycemia independent of food intake and insulin level in streptozotocin-induced diabetic rats. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2002; 16(6):509–18. PubMed PMID: 11919153. [PubMed: 11919153]
 47. Patel YC, Ruggere D, Malaisse-Lagae F, Orci L. Alterations in somatostatin and other islet cell functions in the spontaneously diabetic BB Wistar rat: biochemical and morphological characterization. *Metabolism: clinical and experimental*. 1983; 32(7 Suppl 1):18–25. PubMed PMID: 6135136. [PubMed: 6135136]
 48. Dobbs R, Sakurai H, Sasaki H, Faloona G, Valverde I, Baetens D, Orci L, Unger R. Glucagon: role in the hyperglycemia of diabetes mellitus. *Science*. 1975; 187(4176):544–7. PubMed PMID: 1089999. [PubMed: 1089999]
 49. Vranic M, Pek S, Kawamori R. Increased "glucagon immunoreactivity" in plasma of totally depancreatized dogs. *Diabetes*. 1974; 23(11):905–12. PubMed PMID: 4430418. [PubMed: 4430418]
 50. Mashiter K, Harding PE, Chou M, Mashiter GD, Stout J, Diamond D, Field JB. Persistent pancreatic glucagon but not insulin response to arginine in pancreatectomized dogs. *Endocrinology*. 1975; 96(3):678–93. PubMed PMID: 1116479. DOI: 10.1210/endo-96-3-678 [PubMed: 1116479]
 51. McGarry J, Wright PH, Foster DW. Hormonal control of ketogenesis. Rapid activation of hepatic ketogenic capacity in fed rats by anti-insulin serum and glucagon. *The Journal of clinical investigation*. 1975; 55(6):1202–9. PubMed PMID: 1133169; PMCID: PMC301874. DOI: 10.1172/JCI108038 [PubMed: 1133169]
 52. Sperling MA, Aleck K, Voina S. Suppressibility of glucagon secretion by glucose in juvenile diabetes. *The Journal of pediatrics*. 1977; 90(4):543–7. PubMed PMID: 839365. [PubMed: 839365]
 53. Muller WA, Faloona GR, Unger RH. Hyperglucagonemia in diabetic ketoacidosis. Its prevalence and significance. *The American journal of medicine*. 1973; 54(1):52–7. PubMed PMID: 4629972. [PubMed: 4629972]
 54. Gerich JE, Lorenzi M, Bier DM, Tsalikian E, Schneider V, Karam JH, Forsham PH. Effects of physiologic levels of glucagon and growth hormone on human carbohydrate and lipid metabolism. Studies involving administration of exogenous hormone during suppression of endogenous hormone secretion with somatostatin. *The Journal of clinical investigation*. 1976; 57(4):875–84. PubMed PMID: 820717; PMCID: PMC436731. DOI: 10.1172/JCI108364 [PubMed: 820717]
 55. Alberti KG. Role of glucagon and other hormones in development of diabetic ketoacidosis. *Lancet*. 1975; 1(7920):1307–11. PubMed PMID: 49515. [PubMed: 49515]
 56. Hare KJ, Vilsboll T, Holst JJ, Knop FK. Inappropriate glucagon response after oral compared with isoglycemic intravenous glucose administration in patients with type 1 diabetes. *American journal of physiology Endocrinology and metabolism*. 2010; 298(4):E832–7. PubMed PMID: 20103744. DOI: 10.1152/ajpendo.00700.2009 [PubMed: 20103744]
 57. Perry RJ, Zhang XM, Zhang D, Kumashiro N, Camporez JP, Cline GW, Rothman DL, Shulman GI. Leptin reverses diabetes by suppression of the hypothalamic-pituitary-adrenal axis. *Nature medicine*. 2014 Epub 2014/06/16. PubMed PMID: 24929951.
 58. Perry RJ, P L, Abulizi Abudukadier, Kennedy L, Cline GW, Shulman GI. Mechanism for leptin's acute insulin-independent effect to reverse diabetic ketoacidosis. *The Journal of clinical investigation*. In press.
 59. Perry RJ, Camporez JP, Kursawe R, Titchenell PM, Zhang D, Perry CJ, Jurczak MJ, Abudukadier A, Han MS, Zhang XM, Ruan HB, Yang X, Caprio S, Kaech SM, Sul HS, Birnbaum MJ, Davis RJ, Cline GW, Petersen KF, Shulman GI. Hepatic acetyl CoA links adipose tissue inflammation to

- hepatic insulin resistance and type 2 diabetes. *Cell*. 2015; 160(4):745–58. Epub 2015/02/11. PubMed PMID: 25662011. DOI: 10.1016/j.cell.2015.01.012 [PubMed: 25662011]
60. Scrutton MC, Keech DB, Utter MF. Pyruvate Carboxylase. Iv. Partial Reactions and the Locus of Activation by Acetyl Coenzyme A. *The Journal of biological chemistry*. 1965; 240:574–81. Epub 1965/02/01. PubMed PMID: 14275106. [PubMed: 14275106]
61. Utter MF, Keech DB. Formation of oxaloacetate from pyruvate and carbon dioxide. *The Journal of biological chemistry*. 1960; 235:PC17–8. Epub 1960/05/01. PubMed PMID: 13840551. [PubMed: 13840551]
62. Utter MF, Keech DB. Pyruvate Carboxylase. I. Nature of the Reaction. *The Journal of biological chemistry*. 1963; 238:2603–8. Epub 1963/08/01. PubMed PMID: 14063279. [PubMed: 14063279]
63. Utter MF, Keech DB, Scrutton MC. A possible role for acetyl CoA in the control of gluconeogenesis. *Advances in enzyme regulation*. 1964; 2:49–68. Epub 1964/01/01. PubMed PMID: 5863094. [PubMed: 5863094]
64. Morton GJ, Meek TH, Matsen ME, Schwartz MW. Evidence against hypothalamic-pituitary-adrenal axis suppression in the antidiabetic action of leptin. *The Journal of clinical investigation*. 2015; 2015 PubMed PMID: 26529250; PMCID: PMC4665796. doi: 10.1172/JCI82723
65. Perry RJ, Lee S, Ma L, Zhang D, Schlessinger J, Shulman GI. FGF1 and FGF19 reverse diabetes by suppression of the hypothalamic-pituitary-adrenal axis. *Nature communications*. 2015; 6:6980. PubMed PMID: 25916467; PMCID: PMC4413509. doi: 10.1038/ncomms7980
66. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science*. 1995; 269(5223):543–6. PubMed PMID: 7624777. [PubMed: 7624777]
67. Petersen KF, Oral EA, Dufour S, Befroy D, Ariyan C, Yu C, Cline GW, DePaoli AM, Taylor SI, Gorden P, Shulman GI. Leptin reverses insulin resistance and hepatic steatosis in patients with severe lipodystrophy. *The Journal of clinical investigation*. 2002; 109(10):1345–50. Epub 2002/05/22. PubMed PMID: 12021250; PMCID: 150981. DOI: 10.1172/JCI15001 [PubMed: 12021250]