We hypothesized that the experimental conditions employed (a bolus administration of very high doses of leptin) could explain the absence of an effect of leptin on glycemia in the fasted state. Here we discuss our recent work demonstrating that hypoleptinemia activates the hypothalamic-pituitary-adrenal (HPA) axis, increasing white adipose tissue (WAT) lipolysis and mediating a shift from glucose to fat metabolism to maintain euglycemia in fasted rats.

Throughout history, frequent feast-and-famine cycles have required humans and animals to evolve mechanisms to store energy when food is plentiful, and mobilize energy efficiently when food is scarce, thereby maintaining euglycemia and permitting survival. In the fed state, the body primarily metabolizes glucose, whereas in the fasted state, fat and ketone metabolism prevail1; however the signals that regulate this transition have been the subject of debate for decades. In a classic study published in Nature, Ahima et al. demonstrated hypoleptinemia in fasted mice and performed bolus injections of high doses of leptin to interrogate the physiologic impact of hypoleptinemia. They concluded that leptin mediates the role of the reproductive, thyroid, and adrenal axes to starvation, but did not observe a significant impact of leptin on glycemia or ketosis.2 However, we have recently shown that in diabetic ketosis, hypoleptinemia causes hypothalamic-pituitary-adrenal (HPA) axis activation and resultant increases in white adipose tissue (WAT) lipolysis, hepatic acetyl-CoA content, and hepatic glycogenolysis.3,4 We hypothesized that the experimental conditions employed (a bolus administration of very high doses of leptin, as opposed to a continuous infusion increasing leptin concentrations to the physiologic range in starved rodents) could explain the absence of an effect of leptin on glycemia in the study by Ahima et al. To test this hypothesis, we re-examined the physiologic role of hypoleptinemia in starvation using stable isotope infusions including Positional Isotopomer Nuclear Magnetic Resonance Tracer Analysis5 to examine the impact of hypoleptinemia on lipolysis, hepatic acetyl-CoA content, and rates of glycogenolysis, pyruvate carboxylase flux, and gluconeogenesis from glycerol.6

In our recent study, we examined two key transitions: from the recently fed state (0–8 hr fasted) to moderate-term fasting (16 hr), and from moderate- (16 hr) to long-term starvation (48 hr). In the recently fed to moderate-term fasted transition, reductions in plasma leptin increased plasma corticosterone, thereby increasing WAT lipolysis, hepatic acetyl-CoA content, and hepatic pyruvate carboxylase flux, allowing rats to maintain euglycemia despite exhibiting marked reductions in rates of hepatic glycogenolysis (Fig. 1). We then demonstrated that glycogen depletion, with resultant reductions in plasma glucose and insulin concentrations and adipocyte glucose uptake, were the signal to the fat cell to lower plasma leptin concentrations: treatment of 8 and 16 hr fasted rats with an inhibitor of glycogen phosphorylase and thus of glycogenolysis reduced plasma glucose, insulin, and leptin concentrations and increased plasma corticosterone to levels measured in glycogen depleted, 48 hr fasted rats. These data demonstrate that plasma glucose and insulin concentrations provide a systemic barometer for hepatic glycogen reserves and the resulting reductions in plasma glucose and insulin signal the adipocyte to mobilize energy due to reductions in leptin and increases in HPA axis activity generating increased WAT lipolysis and ketogenesis. The increases in fatty acid and ketone concentrations that then occur due to increased rates of WAT lipolysis and ketogenesis provide fuel for critical organs such as the brain and heart, even as the supply of glucose is declining.

These data beg the question of why prior studies had not observed an effect of leptin on plasma glucose in the fasted or insulinopenic type 1 diabetic (T1D) state.7 However, prior studies have administered large boluses of leptin resulting in plasma leptin concentrations 10–100 times normal. Given the ability of leptin to stimulate catecholamine synthesis,8 we hypothesized that these supraphysiologic leptin concentrations could activate sympathetic nervous system (SNS) activity, obfuscating the effect of leptin to suppress glucocorticoid-driven WAT lipolysis. Consistent with this hypothesis, we found that infusion of leptin to achieve normal physiologic concentrations in 48 hr fasted and T1D rats markedly reduced plasma corticosterone, WAT lipolysis, and hepatic gluconeogenesis, such that the former group required glucose infusion...
to avoid symptomatic hypoglycemia. However, infusion of supraphysiologic lepto
tin to increase plasma leptin concentrations to those observed in a prior study in T1D rats’ stimulated
SNS activity, increasing WAT lipolysis and hepatic gluconeogenesis and reversing the effect of lepto
in to lower plasma glucose concentrations in fasted or T1D rats. These data highlight the critical
importance of interrogating the role of leptin in mediating glycaemia using physiologic leptin infusions rather than treatment with high doses of this agent.

Surprisingly, in the prolonged (48 hr) fasted state, despite increased WAT lipolysis and increased hepatic acetyl-CoA due to reductions in plasma leptin and increases in corticosterone concentrations, rats exhibited 50% reductions in hepatic pyruvate carboxylase flux and hepatic gluconeogenesis. Consistent with prior studies demonstrating reduced alanine turnover in fasted humans, we observed a 65% reduction in whole-body alanine turnover after a 48 hr fast, which resulted in suppression of hepatic mitochondrial anaplerosis (pyruvate carbo
dylase flux) and oxidation (hepatic citrate synthase flux) (Figure). However, infusion of alanine to restore alanine turnover to rates measured in recently fed rats increased both hepatic pyruvate carboxylase flux and hepatic citrate synthase flux rates similar to those measured in fed animals. Thus these data demonstrate that anaplerotic substrate limitation, even in the setting of increases in WAT lipolysis and hepatic acetyl-CoA content, limit hepatic gluconeogenesis during a prolonged fast.

Taken together, our recent study demonstrates that in addition to reductions in plasma insulin concentrations, hypo
gleptinemia is also necessary to maintain euglycemia during starvation in awake rodents. We also describe a novel glucose-fatty acid cycle whereby reductions in hepatic glycogen content lead to reductions in lipolysis, hepatic acetyl-CoA content, and hepatic pyruvate car
boxylase flux in the short-term fasted state. However, in pro
longed starvation, substrate limitation resulting from reduced muscle glucose-alanine cycling impairs both hepatic pyruvate carboxylase flux and hepatic mitochondrial oxidation. These data provide an evolutionary explanation for hepatic insulin resistance with starvation: with prolonged starvation, increased hepatic lipid accumulation due to increased WAT lipolysis minimize hepatic glucose uptake and synthesis of glycogen, thereby shutting any available glucose to critical organs such as the heart and brain. Future studies will be required to dem
onstrate whether this glucose-fatty acid cycle is similarly important to maintain euglycemia in the starved state in humans.

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Figure 1. Proposed mechanism of a glucose-fatty acid cycle permitting the mainte
nance of adequate plasma glucose concentrations in the short-term and prolonged
fasted states. WAT, white adipose tissue; HPA, hypothalamic-pituitary-adrenal axis. Liver, adrenal, and muscle images were obtained from Servier Medical Art.

| Short- to Moderate-Term (8-16 hr Fast) |
|----------------------------------------|
| Pyruvate carboxylase flux | Glucose-alanine cycling |
| Net hepatic glycogenolysis | Plasma glucose | Plasma insulin |
| HPA axis activity |

| Prolonged (>16 hr) Fast |
|------------------------|
| Pyruvate carboxylase flux | Glucose-alanine cycling |
| Net hepatic glycogenolysis | Plasma glucose | Plasma insulin |
| HPA axis activity | WAT lipolysis |

Figure 1: Proposed mechanism of a glucose-fatty acid cycle permitting the maintenance of adequate plasma glucose concentrations in the short-term and prolonged fasted states. WAT, white adipose tissue; HPA, hypothalamic-pituitary-adrenal axis. Liver, adrenal, and muscle images were obtained from Servier Medical Art.