

Hypothalamic sensing of circulating fatty acids is required for glucose homeostasis

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Increased glucose production is a hallmark of type 2 diabetes and alterations in lipid metabolism have a causative role in its pathophysiology. Here we postulate that physiological increments in plasma fatty acids can be sensed within the hypothalamus and that this sensing is required to balance their direct stimulatory action on hepatic gluconeogenesis. In the presence of physiologically-relevant increases in the levels of plasma fatty acids, negating their central action on hepatic glucose fluxes through (i) inhibition of the hypothalamic esterification of fatty acids, (ii) genetic deletion (Sur1-deficient mice) of hypothalamic K_{ATP} channels or pharmacological blockade (K_{ATP} blocker) of their activation by fatty acids, or (iii) surgical resection of the hepatic branch of the vagus nerve led to a marked increase in liver glucose production. These findings indicate that a physiological elevation in circulating lipids can be sensed within the hypothalamus and that a defect in hypothalamic lipid sensing disrupts glucose homeostasis.

An increased rate of glucose production is the major determinant of fasting hyperglycemia in type 2 diabetes¹. Circulating levels of long-chain fatty acids (LCFAs), which are often elevated in obese and diabetic individuals, have been suggested as an important causative link in the association of obesity with insulin resistance and type 2 diabetes^{2–6}. An elevation in the levels of circulating LCFAs in the presence of hyperinsulinemia markedly diminishes the action of insulin on glucose production^{7–10}. In the presence of postabsorptive insulin concentrations, increasing the levels of plasma LCFAs through lipid infusions stimulates gluconeogenesis, but does not alter glucose production in nondiabetic humans and dogs because of a compensatory decrease in hepatic glycogenolysis^{11,12}. This rapid metabolic adaptation has been referred to as hepatic autoregulation. In type 2 diabetes, hepatic autoregulation seems to be impaired because reciprocal changes in glycogenolysis do not compensate for changes in gluconeogenesis when plasma LCFA concentrations were experimentally manipulated¹³.

The biochemical mechanisms by which changes in circulating LCFA result in reciprocal changes in the rates of hepatic gluconeogenesis and glycogenolysis remain to be elucidated. Circulating LCFAs are metabolized within the central nervous system where they are largely utilized for lipid biosynthesis and to a lesser extent within oxidative pathways¹⁴. In this regard, modulation of lipid metabolism within the hypothalamus can induce dramatic changes not only in energy balance but also in liver glucose metabolism^{15–17}, suggesting that LCFAs may also generate signaling molecules within discrete regions of the hypothalamus. Specifically, we have suggested that the cellular accumulation of LCFA-CoAs within the hypothalamus leads to marked inhibition of glucose

production^{16,17}. The rapid inhibitory effects of the LCFA oleic acid and insulin on liver glucose homeostasis require central activation of ATP-sensitive potassium (K_{ATP}) channels^{16,18}. Furthermore, autonomic neural input to the liver has long been recognized as a potential site for modulation of glucose homeostasis^{19–21}. Here, we postulate that hepatic autoregulation during lipid infusions requires the central sensing of circulating LCFAs, which in turn regulates liver glucose homeostasis (Fig. 1a). Thus, we designed experiments to test whether the central effects of LCFAs normally restrain glucose production during systemic lipid infusions and whether these effects require (i) esterification of LCFA to LCFA-CoAs, (ii) activation of K_{ATP} channels within the mediobasal hypothalamus, or (iii) descending vagal input to the liver.

RESULTS

Activation of K_{ATP} channels by lipids

The effect of intracerebroventricular (ICV) oleic acid on glucose production requires central activation of K_{ATP} channels¹⁶. Here we examined whether selectively blocking the central effects of LCFA through the ICV administration of the K_{ATP} channel blocker glibenclamide is sufficient to alter the effect of a systemic lipid infusion on hepatic glucose fluxes (Fig. 1a,b). We randomized four experimental groups to receive either intravenous saline or lipid infusions and ICV vehicle or glibenclamide (Supplementary Table 1 online). Lipid infusion resulted in increased (about twofold) plasma LCFA levels and hepatic (Fig. 1c,d), and hypothalamic LCFA-CoA levels (from 713 ± 45 to $1,381 \pm 309$ nmol/g protein with saline and lipid treatment, respectively; $P < 0.05$), but not in increased LCFA-CoA levels in skeletal muscle (58

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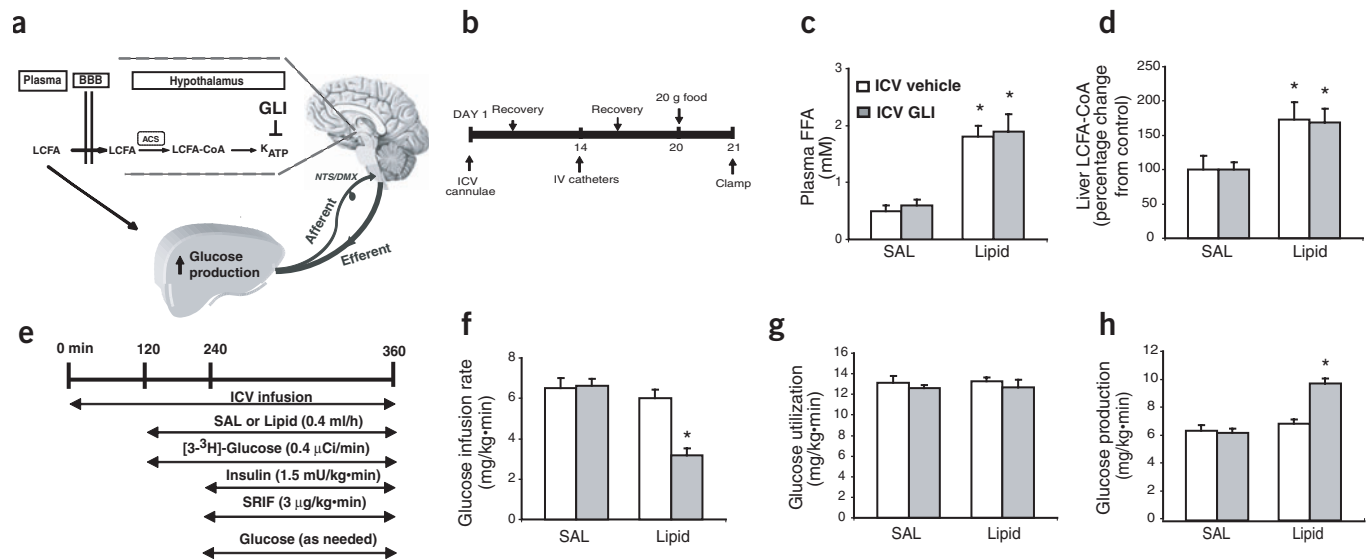


Figure 1 Central activation of K_{ATP} channels is required for hepatic autoregulation during lipid infusion. **(a)** Schematic representation of hypothesis and experimental design. After crossing the blood-brain barrier (BBB), LCFA are esterified by acyl-CoA synthetase (ACS) and can activate K_{ATP} channels. Glibenclamide (GLI) can block this activation. NTS, nucleus of the solitary tract. DMX, dorsal motor nucleus of the vagus. **(b)** Schematic representation of the experimental procedures. **(c)** Plasma free fatty acids (FFA). **(d)** Liver LCFA-CoA levels. **(e)** Schematic representation of the pancreatic clamp. SRIF, somatostatin. **(f)** Glucose infusion rate. **(g)** Glucose utilization. **(h)** Glucose production. * $P < 0.001$ versus saline. SAL, saline.

± 4 and 60 ± 10 nmol/g protein with saline and lipid treatment, respectively). Notably, ICV glibenclamide did not modify these parameters (Fig. 1c,d and Supplementary Table 1 online). We used a combination of ICV infusions and pancreatic-insulin clamp studies (Fig. 1e) in conscious rats. During pancreatic-insulin clamp studies, plasma insulin ($\sim 27 \mu\text{U/ml}$), glucagon and glucose concentrations were kept constant and near basal levels in all groups (Supplementary Table 1 online).

Under these experimental conditions, hyperlipidemia did not alter glucose infusion, glucose uptake or glucose production (Fig. 1f-h) when vehicle was infused ICV. But when ICV glibenclamide

prevented the activation of central K_{ATP} channels by LCFA, lipid infusions resulted in a marked decrease in glucose infusion (Fig. 1f). A significant elevation in glucose production entirely accounted for this decline in glucose infusion (Fig. 1h). Thus, in the presence of similar elevations in plasma LCFA and hepatic LCFA-CoA levels, preventing the activation of central K_{ATP} channels led to a marked ($\sim 50\%$) increase in glucose production.

We next estimated the *in vivo* flux through glucose-6-phosphatase (Fig. 2a,b and Supplementary Table 2 online) and the relative contributions of glucose cycling (Fig. 2c), gluconeogenesis (Fig. 2d) and glyco-

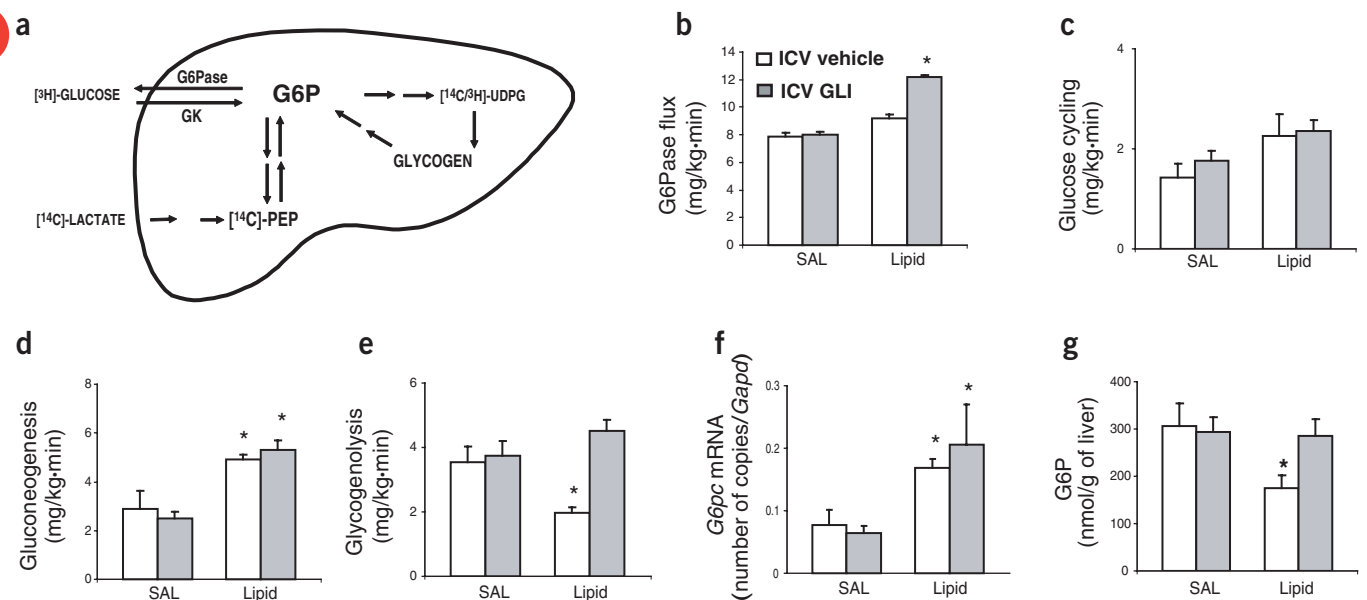


Figure 2 Mechanism by which central antagonism of K_{ATP} channels impairs hepatic auto-regulation in response to lipid infusion. **(a)** Outline of the major pathways and enzymatic steps contributing to glucose production. **(b)** G6Pase flux. **(c)** Glucose cycling. **(d)** Gluconeogenesis. **(e)** Glycogenolysis. **(f)** *G6pc* mRNA. **(g)** Liver glucose-6-phosphate (G6P) content. * $P < 0.01$ versus saline. SAL, saline.

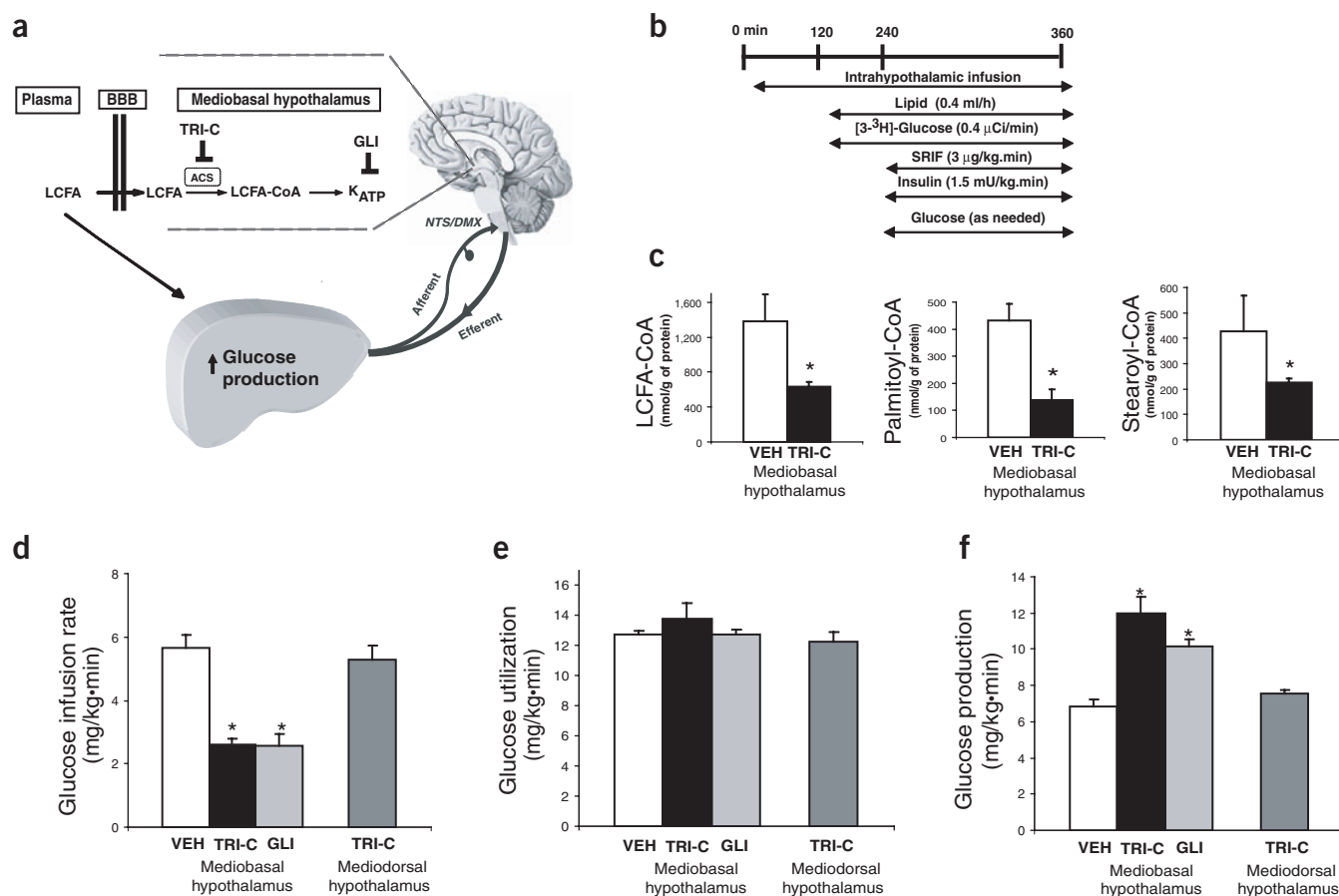


Figure 3 Esterification of LCFA and activation of K_{ATP} within the mediobasal hypothalamus are each required for hepatic auto-regulation during lipid infusions. **(a)** After crossing the blood-brain barrier (BBB) LCFAs are esterified by acyl-CoA synthetase (ACS) and can activate K_{ATP} channels. Triacsin C (TRI-C) is a pharmacological inhibitor of ACS and glibenclamide (GLI) is a K_{ATP} blocker. **(b)** Schematic representation of the pancreatic-insulin clamp procedures. SRIF, somatostatin **(c)** Effect of intrahypothalamic triacsin C on total LCFA-CoAs, palmitoyl-CoA and stearoyl-CoA in the mediobasal hypothalamus. * $P < 0.01$ versus intrahypothalamic vehicle. **(d)** Glucose infusion rate. **(e)** Glucose utilization. **(f)** Glucose production during lipid infusions. * $P < 0.001$ versus intrahypothalamic vehicle. During saline infusions, intrahypothalamic vehicle, triacsin C and glibenclamide during pancreatic-insulin clamp studies did not alter glucose kinetics (data not shown).

genolysis (Fig. 2e) to glucose output. Glucose-6-phosphatase (G6Pase), encoded by *G6pc*, catalyzes the final step in the dephosphorylation of glucose-6-phosphate (G6P) derived from either gluconeogenesis or glycogenolysis to glucose (Fig. 2a). During the pancreatic-insulin clamp studies, lipid infusions alone had no significant effects on G6Pase flux (Fig. 2b). But in the presence of ICV glibenclamide, lipid infusions increased G6Pase flux (Fig. 2b) and modestly increased glucose cycling (Fig. 2c). Lipid infusions significantly and similarly stimulated gluconeogenesis in both ICV vehicle and ICV glibenclamide (Fig. 2d). This increase was compensated for by a similar reduction in glycogenolysis (Fig. 2e) in the presence of ICV vehicle but not during ICV K_{ATP} blockade (Fig. 2e). Finally, lipid infusions induced hepatic *G6pc* expression to a similar extent in both ICV vehicle and ICV K_{ATP} blocker (Fig. 2f). Thus, it is not likely that the marked increase in G6Pase flux observed with ICV K_{ATP} blocker versus ICV vehicle (Fig. 2d) is the result of changes in the hepatic expression of *G6pc*. The hepatic expression of *Pck1*, the gene that encodes phosphoenolpyruvate carboxykinase 1, was comparable in all groups (Supplementary Table 1 online). Of note, despite the marked induction of gluconeogenesis, systemic lipid infusions led to a reduction in hepatic G6P content when ICV vehicle was infused. This decrease

in liver G6P was prevented by the central infusion of glibenclamide (Fig. 2g). We propose that the drop in liver G6P levels with lipid infusions is because of the inhibitory effects of circulating fatty acids on hepatic glycogenolysis (Fig. 2e), which in turn requires the central activation of K_{ATP} channels. These potent metabolic effects of LCFA could be mediated by the activation of K_{ATP} channels anywhere within the central nervous system.

Hypothalamic esterification of LCFA

After their passage across the blood-brain barrier, LCFAs are esterified to LCFA-CoAs within neurons and glial cells. This reaction is catalyzed by the enzyme long-chain acyl-CoA synthetase. Triacsin C (1-hydroxy-3-(E,E,E-2',4',7'-undecatrienylidene) triazene) is a pharmacological inhibitor of this enzyme (Fig. 3a). To investigate the central neuroanatomical localization of the effects of LCFA on liver glucose homeostasis, we next combined systemic lipid infusions with the administration of triacsin C or glibenclamide (Fig. 3a,b) bilaterally within the parenchyma of the medial hypothalamus. Placement of the chronic cannulae was verified by infusion of radioactive tracers followed by sampling of hypothalamic nuclei by micropunches (Supplementary Fig. 1 online).

Lipid infusion resulted in an approximately twofold increase in the concentration of LCFA-CoAs in the mediobasal hypothalamus and this increase was largely accounted for by robust increases in the levels of the saturated LCFA-CoAs palmitoyl-CoA and stearoyl-CoA. This increase was abolished by the concomitant intrahypothalamic infusion of triacsin C (Fig. 3c). Intrahypothalamic infusion of either triacsin C or glibenclamide was sufficient to disrupt hepatic autoregulation with marked decrease in glucose infusion and increase in glucose production (Fig. 3d,f) in response to lipid infusion. Intrahypothalamic triacsin C or glibenclamide infusion alone did not affect glucose kinetics (data not shown). Furthermore, infusion of triacsin C within the paraventricular nuclei of the mediodorsal hypothalamus had no effect on glucose kinetics during lipid infusion (Fig. 3d–f). Thus, the esterification of LCFA to LCFA-CoAs and the activation of K_{ATP} channels within the medial hypothalamus are both required to restrain glucose production in the presence of an increased availability of lipids.

Response to lipids in Sur1-deficient mice

K_{ATP} channels are present in certain hypothalamic neurons. K_{ATP} channels have an octameric structure with a K^+ inward rectifier subunit, K_{IR} 6.1 or 6.2, and a sulfonylurea receptor Sur1 or Sur2 (refs. 22,23). Sur1- K_{IR} 6.2 channels are typically expressed in pancreatic alpha, delta and beta cells as well as in neurons. Sur2A- K_{IR} 6.2 channels are most abundant in cardiac and skeletal muscle, whereas Sur2B- K_{IR} 6.1 channels are found in smooth muscle. Our results in rats indicate that hypothalamic cells expressing K_{ATP} channels are possible targets of LCFA-CoAs. The high sensitivity of these channels to low doses of a sulfonylurea²⁴ is consistent with Sur1 being a component of these LCFA-responsive K_{ATP} channels^{22,25} and with previous observations that Sur1- K_{IR} 6.2 channels are activated by LCFA-CoAs^{26,27}. Sur1 was detectable in the hypothalamus of wild-type but not Sur1-deficient (Sur1KO) mice (Supplementary Fig. 2 online). To investigate whether

the metabolic adaptation to increased plasma lipid levels is selectively impaired in Sur1KO mice, we next performed lipid infusions during pancreatic-insulin clamp studies in conscious Sur1KO and wild-type mice (Fig. 4a and Supplementary Table 3 online). In the presence of modestly elevated plasma insulin levels Sur1KO and wild-type mice showed similar glucose kinetics (data not shown). But in the presence of a physiologically-relevant and similar increase in plasma lipid levels, glucose infusion was markedly lower in Sur1KO compared with wild-type mice (Fig. 4b). This decrease was the result of a pronounced increase in glucose production (Fig. 4c) rather than changes in glucose utilization (Fig. 4d). The increase in glucose production was paralleled by an increased *G6Pase* flux (Fig. 4e) resulting entirely from increased glycogenolysis (Fig. 4f). The rates of glucose cycling (Fig. 4g) and gluconeogenesis (Fig. 4h) were not significantly changed in Sur1KO mice. Taken together with the results of pharmacological loss-of-function studies in rats, these genetic loss-of-function experiments in mice indicate that Sur1-containing K_{ATP} channels are required to restrain glucose production during lipid infusion.

Hepatic branch vagotomy

To investigate the descending pathway that mediates the hypothalamic effect of circulating LCFA on glucose production, we examined whether surgical transection of the hepatic branch of the vagus nerve is sufficient to alter the effect of a systemic lipid infusion on hepatic glucose flux (Fig. 5a). We performed pancreatic-insulin clamp studies in conscious rats who underwent either hepatic branch vagotomy (HVAG) or sham operation (Fig. 5b,e). We initially studied four experimental groups randomized to receive either saline or lipid infusions intravenously in sham and HVAG rats (Supplementary Table 4 online). Lipid infusions resulted in a significant elevation (approximately twofold) in plasma LCFA and liver LCFA-CoAs (Fig. 5c,d) and these effects were not modified by HVAG (Fig. 5c,d).

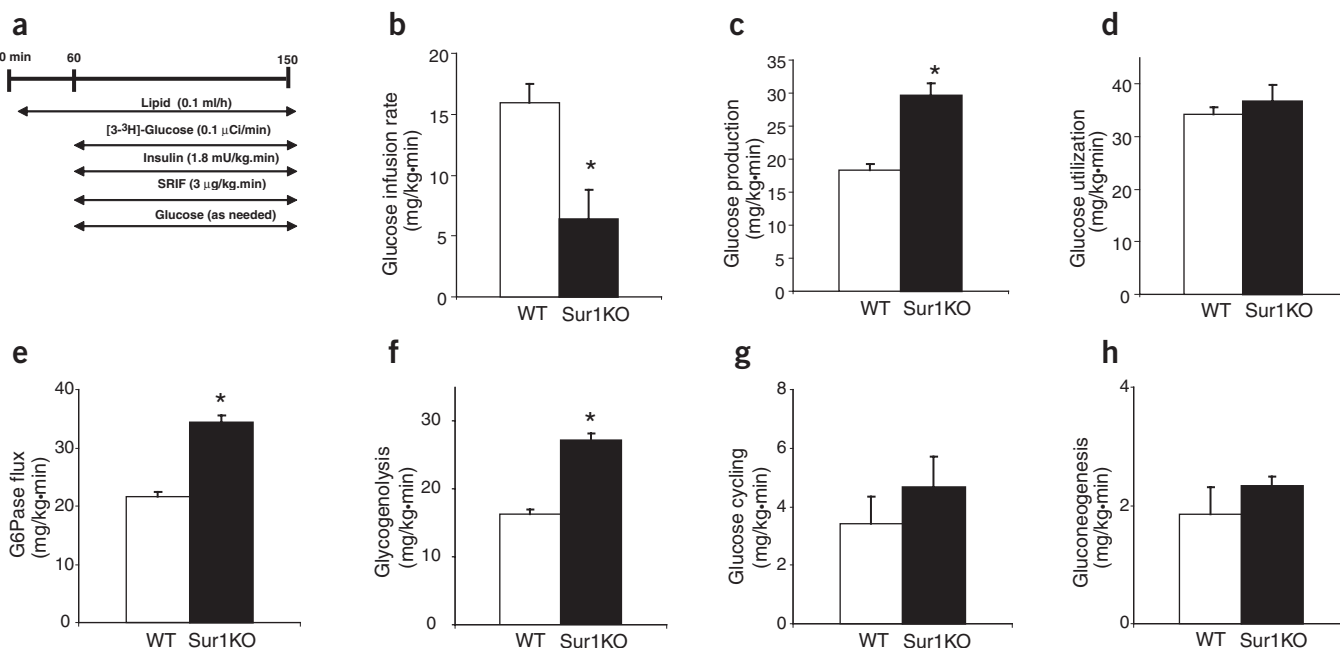


Figure 4 Systemic lipid infusions increased glucose production in Sur1KO but not in wild-type (WT) mice. (a) Schematic representation of the pancreatic-insulin clamp procedures in conscious mice. SRIF, somatostatin. (b) Glucose infusion. (c) Glucose production. (d) Glucose utilization. (e) G6Pase flux. (f) Glycogenolysis. (g) Glucose cycling. (h) Gluconeogenesis. * $P < 0.001$ versus wild-type. In the presence of saline infusions, the *Sur1* genotype had no measurable impact on glucose kinetics during pancreatic-insulin clamp studies (data not shown).

Under these experimental conditions, lipid infusions did not alter the rate of glucose infusion, glucose uptake or glucose production (Fig. 5f–h) in sham rats. In HVAG rats, however, lipid infusions resulted in a marked decrease in glucose infusion (Fig. 5f). Increased glucose production entirely accounted for this decline in glucose infusion (Fig. 5h). Thus, the lack of vagal outflow to the liver resulted in a marked (~50%) increase in glucose production, but only when plasma LCFA and hepatic LCFA-CoA were elevated.

During the pancreatic clamp studies, lipid infusions alone had no significant effects on G6Pase flux in sham-operated rats (Fig. 6a and Supplementary Table 5 online). In HVAG rats, however, lipid infusions resulted in a significant increase in G6Pase flux (Fig. 6a). Glucose cycling did not change significantly in any of the experimental groups (Fig. 6b). Lipid infusions significantly and similarly stimulated gluconeogenesis in both sham-operated and HVAG rats (Fig. 6c). The increase in gluconeogenesis during lipid infusions was compensated by a similar reduction in glycogenolysis (Fig. 6d) in sham-operated rats. But lipid infusions did not decrease glycogenolysis in HVAG rats (Fig. 6d), leading to increased G6Pase flux (Fig. 6a). Conversely, lipid infusions induced hepatic *G6pc* expression to a similar extent in sham-operated and HVAG rats (Fig. 6e) and the hepatic expression of *Pck1* was comparable in all groups (Supplementary Table 4 online). Of note, despite the marked induction of gluconeogenesis, systemic lipid infusions led to a reduction in hepatic G6P content in sham-operated rats. This decrease in liver G6P was prevented by hepatic branch vagotomy (Fig. 6f). Thus, transection of hepatic branch of the vagus nerve was sufficient to disrupt hepatic autoregulation during lipid infusions. This branch is comprised of both efferent and afferent fibers. To address the potential role of afferent vagal mediation of the central effects of LCFA, we performed additional experiments in animals with selective vagal deafferentation (Supplementary Fig. 3 online). These animals have intact descending efferent fibers to the liver but the vagal afferents supplying the hepatic vagal branch are transected at their site of entry in the brainstem. Vagal deafferentation did not alter glucose kinetics in the presence of pancreatic-insulin clamp

and experimental hyperlipidemia (Supplementary Fig. 3 online). Thus, efferent hepatic vagal branch outflow to the liver is required for hepatic autoregulation in response to increased availability of lipid whereas afferent input from the hepatic branch of the vagus nerve to the brainstem is not required.

Response to lipids in rats fed a high-fat diet

The central administration of the LCFA oleic acid did not suppress hepatic glucose fluxes after 3 d of voluntary overeating in rats²⁸. Based on these findings, we postulated that this model of diet-induced insulin resistance²⁹ would also show defective adaptation to an increase in lipid availability. Indeed, a physiological increase in circulating LCFA levels during pancreatic-insulin clamp induced hyperglycemia (Supplementary Fig. 4 online). The increase in circulating glucose levels could not be ascribed to a decrease in glucose clearance (Supplementary Fig. 4 online) but rather resulted from an increase in glucose production (Supplementary Fig. 4 online) in response to lipid infusion.

DISCUSSION

Alterations in lipid homeostasis play a pivotal role in the pathophysiology of the metabolic syndrome and type 2 diabetes^{1–6,30}. Among the pleiotropic actions of LCFAs, the potent stimulatory effect on hepatic gluconeogenesis has long been recognized⁶. This effect can be shown in isolated hepatocytes³¹, perfused liver preparations³² and whole organisms^{33,34} and it is mediated by the stimulation of pyruvate carboxylase through increased levels of acetyl-CoA, the enhanced production of ATP and the increased ratio of NADH to NAD⁺³⁵. Despite these reproducible effects on gluconeogenesis, changes in circulating LCFA levels often do not modify glucose production because of concomitant and opposite changes in the rate of glycogenolysis^{11,12}. The mechanism(s) by which an increase in plasma LCFA levels restrains hepatic glycogenolysis have not been defined. In this regard, recent work indicates that LCFAs can also regulate liver glucose homeostasis through their metabolic

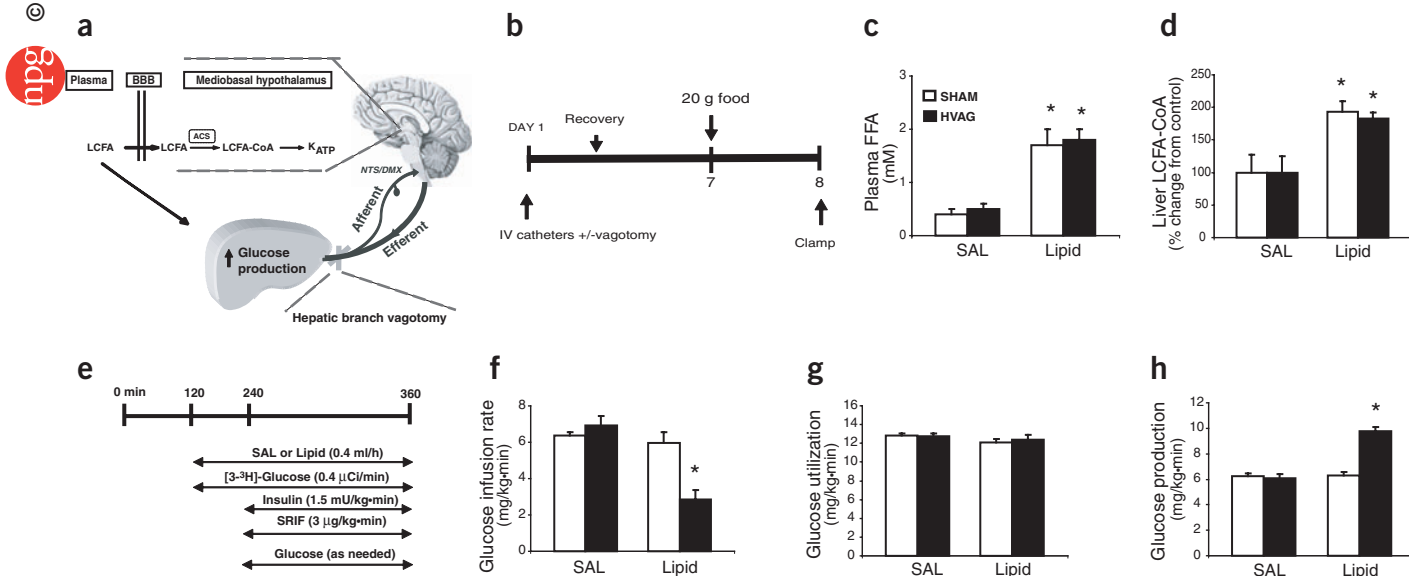


Figure 5 The hepatic branch of the vagus nerve is required for hepatic auto-regulation in response to systemic lipid infusions. **(a)** Schematic representation of hypothesis and experimental design. We negated the central effects of circulating LCFA on liver glucose homeostasis via surgical resection of the hepatic branch of the vagus nerve. **(b)** Schematic representation of the experimental procedures. **(c)** Plasma free fatty acids (FFA) concentrations **(d)** LCFA-CoA levels. **(e)** Schematic representation of the pancreatic clamp. SRIF, somatostatin. **(f)** Glucose infusion rate. **(g)** Glucose utilization. **(h)** Glucose production * $P < 0.001$ versus saline.

signaling within the hypothalamus^{16,17,28,36}. Here we postulate that a prolonged elevation in circulating levels of LCFA modulates hepatic glucose metabolism through distinct biochemical signaling in the liver as well as in the brain (Fig. 1a). The central action of circulating LCFAs is required to counteract LCFA-induced stimulation of gluconeogenesis and to prevent an increase in glucose production, thereby providing an extrahepatic site for hepatic autoregulation (Fig. 1a).

Is the action of LCFAs within the central nervous system required for the effects of systemic hyperlipidemia on liver glycogenolysis? To address this question we combined a physiologically-relevant increase in the levels of circulating lipids with selective loss of function within the central pathway activated by LCFA. Because an elevation in the hypothalamic levels of LCFA-CoAs by either direct ICV administration of the LCFA oleic acid¹⁶ or by inhibition of hypothalamic carnitine palmitoyltransferase-1 activity^{17,36} leads to a marked decrease in glucose production, the esterification of LCFA to LCFA-CoA seems to be a required step in activating the brain-liver circuit that modulates liver glucose homeostasis. Furthermore, increased cellular levels of LCFA-CoA can activate (open) K_{ATP} channels and the ICV infusion of K_{ATP} blockers abolishes the inhibitory effect of ICV oleic acid on glucose production¹⁶. Here we showed that the inhibition of LCFA esterification or of their activation of K_{ATP} channels within the mediobasal hypothalamus is sufficient to disrupt hepatic autoregulation through an increase in hepatic glycogenolysis. Because Sur1-containing K_{ATP} channels do not seem to be expressed in glial cells^{37,38}, it is possible that this pivotal step in hypothalamic sensing of lipid is occurring within neurons. Consistent with this notion, the ability of LCFAs to restrain hepatic glucose production was selectively impaired in Sur1KO mice. Of note, a defect in glucose homeostasis caused by defective counter-regulation to hypoglycemia has also been reported in $K_{IR6.2}$ -null mice^{23,39}. Taken together with the results of gain-of-function experiments¹⁶, these pharmacological and genetic loss-of-function experiments in rats and mice indicate that the central activation of Sur1-containing K_{ATP} channels within the mediobasal hypothalamus is required to restrain hepatic glucose production in response to an increase in the systemic

availability of lipids. Similarly, under near-basal plasma insulin levels, transection of the hepatic branch of the vagus nerve had no significant effects on glucose fluxes, but selectively disrupted hepatic autoregulation in the presence of hyperlipidemia. The requirement of the vagus nerve as the executive branch of the neural circuit activated in response to central sensing of circulating lipids is consistent with the effects of cholinergic agonists and antagonists in the regulation of hepatic glycogen metabolism⁴⁰.

Thus, hypothalamic lipid sensing has a crucial role in regulating glucose production in response to a physiological increase in lipid availability. Notably, a primary impairment in the ability of central lipid to restrain glucose production leads to the breakdown of hepatic autoregulation in response to an elevation in circulating fatty acids. Because the central effects of LCFA on glucose production were markedly impaired after short-term voluntary overeating²⁸, it is reasonable to postulate that central resistance to the metabolic effects of LCFA is also a feature of obesity-associated type 2 diabetes and may impair the normal physiological adaptation to hyperlipidemia.

Because reversing liver insulin resistance is a prominent goal of diabetic therapy, restoring hypothalamic lipid sensing could serve as a new therapeutic approach to this disease. Several metabolic alterations associated with obesity-driven type 2 diabetes have been ascribed to defective adaptation to nutrient excess. Our findings suggest that an important component of this adaptive response requires the sensing of circulating lipids within the mediobasal hypothalamus.

METHODS

Animal preparation. We studied 10-week-old male Sprague Dawley rats. Rats underwent stereotaxic surgery to indwell single catheters in the third cerebral ventricle^{16,18,41} or bilateral catheters into the mediobasal hypothalamus⁴² or mediobasal hypothalamus (targeting paraventricular nuclei; coordinates: 0.5 mm (both sides, left and right) lateral from the midline, 1.9 mm posterior to the bregma and 7.3 mm below the skull surface) 3 weeks before experiments. One week later, we placed indwelling catheters in the internal jugular vein and carotid artery^{16,18,41} (Fig. 1b). We studied 12-week-old male Sur1KO mice and their wild-type littermates in a C57BL/6 genetic background. We catheterized

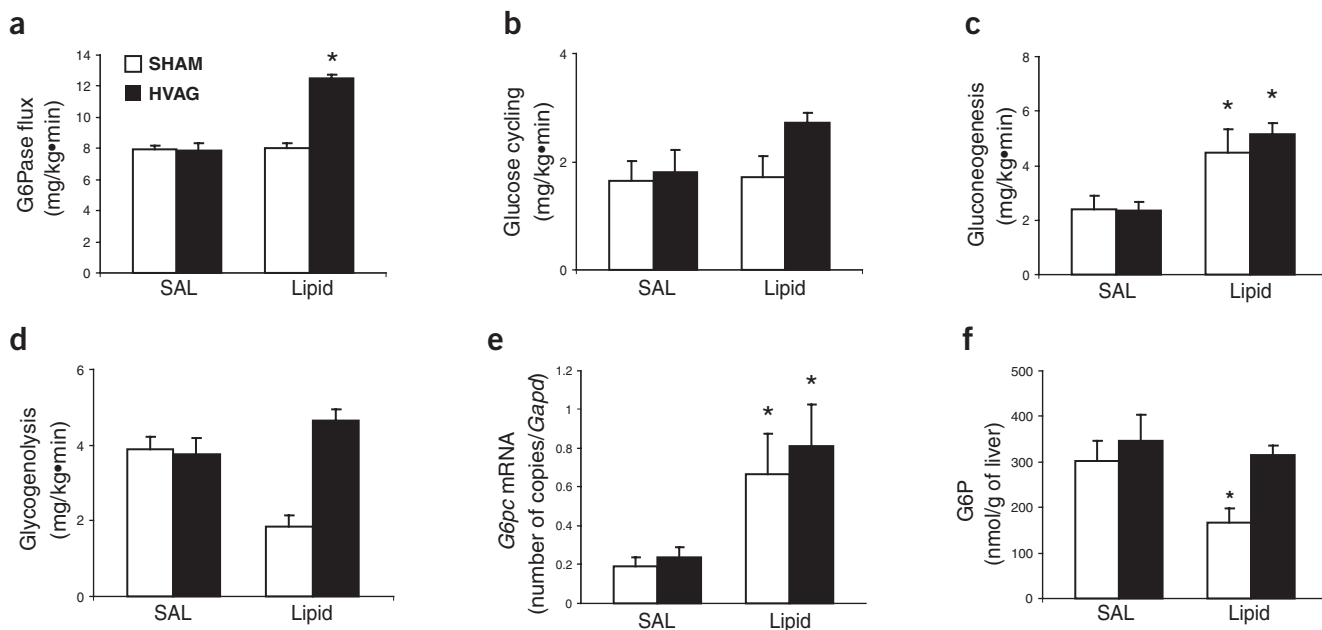


Figure 6 Mechanism by which hepatic branch vagotomy impairs hepatic auto-regulation in response to lipid infusions. (a) G6Pase flux. (b) Glucose cycling. (c) Gluconeogenesis. (d) Glycogenolysis. (e) Liver *G6pc* mRNA. (f) Liver G6P content. * $P < 0.01$ versus saline.

the mice through the right internal jugular vein for infusion⁴³. We collected blood samples from the tail. Recovery from surgery was monitored by measuring daily food intake and weight gain for 4–5 d after surgery. The study protocol was approved by the Institutional Animal Care and Use Committee of the Albert Einstein College of Medicine.

Clamp procedure in rats. We infused intravenous 20% Intralipid (Baxter Healthcare Corporation; mixed with 20 U/ml of heparin, 0.4 ml/h) to elevate plasma free fatty acids concentrations. ICV infusions consisted of glibenclamide⁴⁴ (dissolved in 5% dimethylsulfoxide (DMSO) to 100 μ M; 5 μ l/h; **Fig 1e**). Intrahypothalamic infusions consisted of: triacsin C⁴⁵ (dissolved in 5% DMSO to 40 μ M; bolus, 0.33 μ l; 0.33 μ l/h; **Fig 3b**) and glibenclamide (dissolved in 5% DMSO to 100 μ M; 0.33 μ l/h; **Fig 3b**). The infusion studies lasted 360 min (**Figs. 1e** and **3b**). Briefly, ICV vehicle or glibenclamide (**Fig. 1e**) and intrahypothalamic vehicle or triacsin C or glibenclamide (**Fig. 3b**) were infused throughout the experiments. After 2 h of ICV or intrahypothalamic infusions, intravenous saline or lipids and a primed continuous infusion of [³-³H]-glucose (New England Nuclear; 40 μ Ci bolus; 0.4 μ Ci/min) were initiated at 120 min and maintained throughout the study (**Figs. 1e** and **3b**). Samples for determination of [³H]-glucose-specific activity were obtained at 10-min intervals. A pancreatic clamp was performed in the final 2 h of the study starting at 240 min; continuous infusions of insulin (1.5 mU/kg-min) and somatostatin (3 μ g/kg-min) were administered, and a variable infusion of a 25% glucose solution was started and periodically adjusted to clamp the plasma glucose concentration at ~8 mM. Plasma samples for determination of plasma free fatty acid, insulin, adiponectin, corticosterone and glucagon concentrations were obtained at 30-min intervals during the study. The protocol for individual biochemical analysis can be found in **Supplementary Methods** online. Ten minutes before the end of the studies, [U-¹⁴C]-lactate (New England Nuclear; 20 μ Ci bolus; 1.0 μ Ci/min) was administered to determine the contribution of gluconeogenesis to the pool of hepatic G6P⁴⁶. At the end, rats were anesthetized and tissue samples were freeze-clamped *in situ* with aluminum tongs that had been pre-cooled in liquid nitrogen. All tissue samples were stored at -80 °C for subsequent analysis.

Clamp procedure in mice. We performed lipid infusion studies in Sur1 KO and wild-type mice (**Fig. 4a**). Briefly, mice received a constant infusion of Intralipid + heparin (0.1 ml/h) or saline for 150 min. At 60 min, mice received a constant infusion of [³-³H]-glucose (2 μ Ci bolus, followed by 0.1 μ Ci/min), insulin (1.8 mU/kg-min) and somatostatin (3 μ g/kg-min). A solution of glucose (10%) was infused at variable rates as required to maintain euglycemia. [U-¹⁴C]-lactate (5 μ Ci bolus; 0.4 μ Ci/min) was administered 10 min before the end to determine the contribution of gluconeogenesis to the pool of hepatic G6P. Plasma samples were collected to determine glucose levels and specific activities of [³-³H]-glucose.

Selective hepatic branch vagotomy and vagal deafferentation. We performed lipid infusion studies in rats subjected to hepatic branch vagotomy (**Fig. 5b**) and left vagal rootlet deafferentation (**Supplementary Fig. 3** online). The surgeries were performed as previously described^{47–49}. Please see **Supplementary Methods** online for details.

High-fat diet rats. Male Sprague Dawley rats were fed a high-fat diet for 3 d^{28,29}. These rats developed hyperinsulinemia (basal insulin levels: high-fat diet, 1.6 \pm 0.1 ng/ml versus regular chow, 0.9 \pm 0.2) and hepatic insulin resistance^{28,29}. Rats received intravenous saline or lipids and [³-³H]-glucose for 4 h. A pancreatic clamp was performed in the final 2 h; a continuous infusion of regular insulin (1 mU/kg-min) and somatostatin (3 μ g/kg-min), and a variable infusion of a 25% glucose solution was started and periodically adjusted to clamp the plasma glucose concentration at ~8 mM.

Brain stereotactic micropunches and mediobasal hypothalamic wedge sampling. Brain micropunches of individual hypothalamic nuclei were prepared as previously described¹⁷ and the mediobasal hypothalamus was sampled by dissecting a wedge of tissue including the entire mediolateral and dorsoventral extent of the arcuate nuclei while minimizing ventromedial nucleus tissue.

Hepatic G6P and Pepck and hypothalamic Sur1 gene expression determinations. Please refer to **Supplementary Methods** online for details.

Statistical analysis. Statistical analysis was done by unpaired Student's *t*-test or analysis of variance.

Note: Supplementary information is available on the Nature Medicine website.

ACKNOWLEDGMENTS

We wish to thank B. Liu, J. Liu, C. Baveghems and S. Gaweda for technical assistance. This work was supported by grants from the US National Institutes of Health (to L. Rossetti, DK 45024, DK 48321 and AG 21654; to G. J. Schwartz, DK 47208; to J. Bryan, DK52771; to L. Aguilar-Bryan, DK57671), from the Albert Einstein College of Medicine Diabetes Research & Training Center (DK 20541). S. Obici is the recipient of a Junior Faculty Award from the American Diabetes Association. T.K.T. Lam is supported by a Training Grant from the National Institute of Aging (T32-AG023475).

COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

Received 14 December 2004; accepted 1 February 2005

Published online at <http://www.nature.com/naturemedicine/>

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