Central $K_{\text{ATP}}$ channels modulate glucose effectiveness in humans and rodents

Michelle Carey$^{1,2,*}$, Eric Lontchi-Yimagou$^{1,*}$, William Mitchell$^{1,*}$, Sarah Reda$^1$, Kehao Zhang$^1$, Sylvia Kehlenbrink$^3$, Sudha Koppaka$^1$, Sylvan Roger Maginley$^1$, Sandra Aleksic$^1$, Shobhit Bhansali$^1$, Derek M. Huffman$^1$, and Meredith Hawkins$^1$

$^1$Albert Einstein College of Medicine, Bronx, NY
$^2$Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, MD
$^3$Brigham and Women’s Hospital, Harvard Medical School, Boston, MA

*These authors contributed comparably to the manuscript

Address correspondence to:

Dr. Meredith Hawkins
Albert Einstein College of Medicine
1300 Morris Park Avenue, Bronx, NY 10461, USA.
Phone: 718.430.3186; Fax 718.430.8557
E-mail: meredith.hawkins@einstein.yu.edu
ABSTRACT:

Hyperglycemia is a potent regulator of endogenous glucose production (EGP). Loss of this ‘glucose effectiveness’ is a major contributor to elevated plasma glucose concentrations in type 2 diabetes (T2D). ATP-sensitive potassium channels (KATP channels) in the central nervous system (CNS) have been shown to regulate EGP in humans and rodents. We examined the contribution of central KATP channels to glucose effectiveness. Under fixed hormonal conditions (‘pancreatic clamp’ studies), hyperglycemia suppressed EGP by ~50% in both non-diabetic humans and normal Sprague Dawley rats. By contrast, antagonism of KATP channels with glyburide significantly reduced the EGP-lowering effect of hyperglycemia in both humans and rats. Furthermore, the effects of glyburide on EGP and gluconeogenic enzymes in rats were abolished by intracerebroventricular (ICV) administration of the KATP channel agonist diazoxide. These findings indicate that about half of EGP suppression by hyperglycemia is mediated by central KATP channels. These central mechanisms may offer a novel therapeutic target for improving glycemic control in T2D.

Key words: KATP Channels, Endogenous Glucose Production, Glucose Effectiveness, diazoxide, glyburide, gluconeogenic enzymes
INTRODUCTION:

While type 2 diabetes affects over 400 million people globally, many fundamental questions about abnormal glucose metabolism remain, and approximately half of patients remain suboptimally treated (1). Endogenous glucose production (EGP) is an essential process for maintaining plasma glucose concentrations in the fasted state, and is the major source (comprising up to 80%) of elevated glucose levels in type 2 diabetes (T2D) (2). ‘Glucose effectiveness’ is the ability of glucose per se to suppress EGP, independent of its effects on insulin secretion (3). Its relevance to glucose metabolism has been well established by many research groups (3-10). In non-diabetic humans, hyperglycemia inhibits EGP to nearly the same degree irrespective of insulin levels (4). In the presence of identical and constant plasma insulin, glucagon, and growth hormone concentrations, a doubling of plasma glucose levels (to ~10 mmol/l) inhibited EGP by nearly half in non-diabetic subjects (5,6). Of particular note, we and others have demonstrated that glucose effectiveness is impaired in T2D (5,7,11-13). EGP is paradoxically elevated despite sustained hyperglycemia: loss of glucose effectiveness may thus account for about 70 grams of excess glucose production overnight in these individuals (5).

Growing evidence indicates that glucose exerts its effects on EGP by both direct inputs to the liver and via higher signals emanating from the CNS. Within the liver, regulation of EGP by hyperglycemia probably occurs at the level of the glucose-6-phosphate pool, which is regulated by relative flux through glucokinase and glucose-6-phosphatase (5). Additionally, the hypothalamus possesses specific types of glucose-sensing neurons whose activity is either increased or decreased by glucose (14). The balance in activity of these glucose sensing neurons is likely critical for regulating EGP (10). Various nutrients and hormones, including glucose and insulin, have been shown to regulate EGP in rodents by modulating hypothalamic $K_{ATP}$ channels (15-17). In glucose
excitatory (GE) hypothalamic glucose-sensing neurons, increased glucose metabolism raises the ATP/ADP ratio and closes the $K_{\text{ATP}}$ channel. Glucose has been shown to open $K_{\text{ATP}}$ channels in certain hypothalamic glucose inhibited (GI) neurons potentially by raising intracellular pyruvate (18), in association with reductions in EGP (16). This all supports a critical role for central mechanisms in modulating glucose effectiveness.

The human relevance of these findings was first highlighted by parallel studies in non-diabetic rats and humans in which activation of extrapancreatic $K_{\text{ATP}}$ channels reduced EGP under euglycemic conditions (19). Diazoxide was used to activate $K_{\text{ATP}}$ channels under ‘pancreatic clamp’ conditions, in which pancreatic hormone secretion was inhibited by somatostatin (19). Under these fixed hormonal conditions, diazoxide significantly and progressively suppressed EGP in both humans and rats (19). Inhibition of EGP by oral diazoxide in rats was reversed by ICV administration of a $K_{\text{ATP}}$ channel antagonist, suggesting that these effects were centrally mediated (19). Subsequent human studies demonstrated a similar time course and degree of suppression of EGP by intranasal insulin, further supporting the role of the CNS in regulating glucose and energy metabolism in humans (20-23). However, regulation of EGP by central $K_{\text{ATP}}$ channels appears to be impaired in T2D and in diabetic rats (24). This suggests that loss of this regulation contributes to hyperglycemia in diabetes, and that central pathways could be an important target for new diabetes treatments (25).

The purpose of this study was to determine to what extent hyperglycemia suppresses EGP via central $K_{\text{ATP}}$ channels. The $K_{\text{ATP}}$ channel antagonist glyburide blunted the suppression of EGP by hyperglycemia in both humans and rats. Concomitant intracerebroventricular administration of the $K_{\text{ATP}}$ channel activator diazoxide restored glucose effectiveness, confirming that glucose effectiveness is at least in part centrally mediated.
RESEARCH DESIGN AND METHODS:

HUMAN STUDIES:

Study Design:

Nine healthy, non-diabetic adult subjects (4 males, 5 females; age 37.4 ± 15.0 years; BMI, 25.9 ± 4.5 kg/m²) with no family or past medical history of diabetes and not currently on any medications were enrolled in the study. Each participant underwent a pair of identical seven-hour euglycemic-hyperglycemic clamp studies that were performed at least five weeks apart in random order. Following administration of either oral glyburide (10 mg) or identical placebo capsules at t=-180, a pancreatic clamp with replacement of basal glucoregulatory hormones was initiated with infusion of somatostatin (72 ng/kg.min), insulin (1 mU/kg/min), glucagon (0.6 ng/kg.min), and growth hormone (3 ng/kg/min).

Pancreatic clamp studies:

Subjects underwent two paired hyperglycemic pancreatic clamp studies separated by 4-6 weeks. All subjects arrived at the Clinical Research Center (CRC) after an overnight fast, and had one indwelling venous catheter inserted in each arm; an 18-gauge catheter was inserted in an antecubital vein for infusions and a contralateral hand vein was cannulated for blood sampling (26). To obtain arterialized venous blood samples, this hand was maintained 55°C in a thermoregulated sleeve.

Based on pilot studies in which optimal metabolic effects of glyburide were observed at approximately 6 hours after oral administration, our experimental protocols lasted 7 hours and consisted of an initial 3-hour euglycemic pancreatic clamp, followed by a 4-hour hyperglycemic pancreatic clamp study (Figure 1A). At the beginning of the study (t = -180 minutes), subjects received either oral glyburide 10 mg or matched placebo capsules (identical in appearance) in a
randomized, single-blinded fashion. At the same time infusions of basal insulin and somatostatin were initiated with replacement of glucoregulatory hormones (glucagon 0.6 ng/kg/min; growth hormone 3 ng/kg/min) and 20% dextrose (variable infusion if needed to maintain euglycemia) were initiated. Informed by our previous studies (19,26) in which clamped glucagon levels rose slightly above fasting levels, glucagon infusion rates were reduced from 1 ng/kg.min to 0.6 ng/kg.min in the current studies. In so doing we successfully maintained fasting glucagon levels throughout our clamp studies.

The dose of somatostatin was selected to optimally suppress C-peptide levels in the presence of both hyperglycemia and glyburide. Following a careful review of the literature, we selected somatostatin infusion rates of 72 ng/kg.min (27), i.e. a 44% increase above our prior studies. This was successful in preventing significant rises in endogenous insulin levels, although there were small rises in C-peptide with combined glyburide and hyperglycemia (see Results). Of the 10 subjects who completed the paired clamp studies, one was excluded due to unsuppressed C-peptide levels during hyperglycemia.

To measure glucose fluxes, [6,6-\(^2\)H\(^2\)] glucose (primed infusion of 200 mg/m\(^2\)/min for 3 minutes, then 4 mg/ m\(^2\)/min thereafter) was infused throughout the entirety of the clamp studies. Glucose kinetics and the estimated volume of distribution of glucose were used to calculate the bolus and continuous infusion rates for [6,6-\(^2\)H\(^2\)] glucose using the mathematical models of Finegood and Steele (28,29).

Once basal insulin infusion rates were established and ‘steady state’ euglycemic conditions were achieved, glucose metabolism was quantified for the euglycemic phase of the clamp. After 3 hours of euglycemia, the hyperglycemic portion of the clamp was initiated (at t = 0 minutes) and was continued for 4 hours. Plasma glucose levels were rapidly raised to approximately 180 mg/dL via
variable infusion of labeled 20% dextrose. Once steady state hyperglycemic conditions were achieved, glucose kinetics were quantified for the hyperglycemic phase of the clamp.

Throughout the entire 420 minutes of the study, plasma glucose concentrations were measured at 5 to 10 minute intervals. From time -180 to -30 minutes, optimal insulin infusion rates were established by making frequent adjustments (~ every 25 minutes) to maintain euglycemia with the intent of preventing the need for exogenous glucose infusion. In a few subjects in whom glucose levels fell below target (90 mg/dl) during the final hour of euglycemia, we infused small amounts of glucose to avoid precipitous drops in glucose that could stimulate counter-regulatory hormone release and thereby impact EGP, consistent with our previous studies (24). Most of the average glucose infusion rate was accounted for by one subject who required glucose infusion in both studies to avoid drops in glucose; excluding this subject did not impact EGP results.

Despite the occasional need for small amounts of exogenous glucose infusion, basal insulin levels were still achieved: with an anticipated portal/systemic insulin ratio of approximately 2.4:1 (30) and fasting systemic levels of insulin ~ 11 μU/ml in our subjects, we would expect corresponding fasting portal insulin levels of ~ 25 μU/ml. These portal insulin levels would have been attained with insulin infusion during our clamp studies, since systemic insulin levels in our studies were ~19-23 μU/ml. Since peripheral vein insulin infusion in depancreatized dogs resulted in portal insulin levels that approximated (within 20%) systemic insulin levels (31), it is likely that the liver was exposed to insulin levels similar to the subjects’ habitual fasting levels. The individual’s optimal insulin infusion rate was then kept constant between t = -30 and 0 minutes.

At t = 0 minutes, we initiated a bolus of 20% dextrose designed to raise plasma glucose concentrations to 180 mg/dl within 7.5 minutes. Specifically, a volume-based bolus of 0.7 x weight was administered, with half over the first 2.5 minutes and the subsequent half over the next 5
minutes. Optimal 20% dextrose infusion rates to maintain plasma glucose levels at approximately 180 mg/dL were established in a similar fashion between \( t = 0-180 \) minutes, with the goal of keeping 20% dextrose infusion rates constant between \( t = 180-240 \) minutes. From \( t = -180 \) to \( t = 240 \) minutes, blood samples were obtained for determinations of plasma glucose, insulin, glucagon, C-peptide, cortisol, growth hormone, free fatty acids, glycerol, lactate, and \([6,6-^{2}H_{2}]\text{glucose}\).

All infusions except 20% dextrose were stopped at \( t = 240 \) minutes. Post-clamp meals were provided to each participant with strict glucose monitoring following study completion, and 20% dextrose infusion rates were decreased as appropriate to maintain euglycemia. Subjects who received glyburide were admitted overnight to the CRC for hourly blood-glucose monitoring, and continued to receive 20% dextrose infusions as needed to maintain euglycemia.

**Glucose turnover:**

Glucose turnover data represents the mean values during the steady state portion of the study, as previously described. Rates of glucose appearance (Ra) and disappearance (Rd) and other indices of glucose turnover were estimated using Steele equations (6), using the assumption that Ra=Rd for steady state, and using the following calculation: 

\[
R_d = \frac{\text{Basal } [6,6-^{2}H_{2}]\text{glucose infusion rate} + D20/[6,6-^{2}H_{2}]\text{glucose infusion rate}}{\text{APE fraction/ wt (kg)}}.
\]

Endogenous glucose production (EGP) was determined by subtracting the rates of glucose infusion from the tracer-derived Ra. GC/electron impact-mass spectrometry analysis were performed in the CTSA Analytic Core Laboratory for the \([6,6-^{2}H_{2}]\text{glucose determinations}\) (32). Plasma samples for Gas Chromatography-Mass Spectrometry (GC-MS) were derivatized after protein precipitation to the aldehyde penacetate with hydroxykamine hydrochloride-acetic anhydride.
Plasma hormone and substrate determinations:

Plasma glucose, insulin, C-peptide, FFA, and glucagon levels were measured as previously described (19), and radioimmunoassay was used to measure plasma cortisol levels (Immunobiological Laboratories America) (33).

RODENT STUDIES:

Study Design:

Nineteen male Sprague Dawley rats (Charles River Laboratories; age: 12-14 weeks, weight: 386.1 ± 7.2 g) received cannulae for ICV, followed by subsequent clamp studies. One week following ICV cannulae placement, animals underwent a second surgery for placement of arterial and venous catheters (19). Following recovery, animals were divided into three experimental groups. Groups 1 and 2 received either oral (gavage) saline (n = 7) or oral glyburide (n = 7), respectively. Group 3 received oral (gavage) glyburide with ICV infusion of a K$_{ATP}$ channel agonist, diazoxide (n = 5).

Pancreatic clamp studies:

All rats underwent hyperglycemic pancreatic clamp studies of 4 hours’ duration. The rats were anesthetized and received either saline or glyburide (2.5 mg/kg) ~20 minutes prior to the 4 hours clamp studies (Figure 2A). At t = -20 minutes, ICV infusions of diazoxide (group 3: 0.08 μl/min; n = 5) or comparable volumes of artificial cerebral spinal fluid (aCSF; groups 1 and 2) were initiated. A primed continuous intravenous infusion of [3-$^3$H]-glucose was initiated at t = 0, and maintained for 4 hours (240 min) to assess glucose kinetics. A hyperglycemic clamp with basal peripheral insulin levels was performed during the final two hours (t = 120–240 minutes) (33). Prior to the infusion study (t = -20 minutes), rats were anesthetized with isofluorane and received normal saline or glyburide (2.5 mg/kg) by oral gavage. For the remainder of the studies, the rats
were conscious and unrestrained. An ICV infusion of diazoxide (9.69 µg/µl, 0.006 µl/min) or saline was started at t = -20 minutes. At t = 0 minutes (~20 hours after saline or glyburide gavage), a primed continuous IV infusion of [3- 3 H]-glucose (400 µl/min, 40 µl/min thereafter, Perkin Elmer) was begun and maintained throughout the study to assess glucose kinetics in vivo. To assess [3- 3 H]-glucose specific activity, plasma sampling was performed during the time period when steady state was anticipated. A euglycemic clamp with target glucose of 95-120 mg/dl was performed at t = 0-120, and a hyperglycemic clamp with a target glucose of 250-270 mg/dl was performed at t = 120-240. During the final two hours of the infusion study (t = 120-240 minutes), a peripheral basal insulin (1 mU/kg/min) pancreatic-hyperglycemic clamp was performed as previously described (12). At the onset of the hyperglycemia clamp studies (t = 120 min), plasma insulin levels were comparable among groups (S=0.75±0.10, G=0.91±0.08, GLB+DZX = 0.72±0.08, ng/ml; p > 0.05 for all groups). The data for insulin, C-peptide, and glucose are presented as an average of values obtained from multiple time points during the infusion studies. Following the study, rats were anesthetized with ketamine (150 mg/kg). Liver tissue samples were obtained by freeze clamping.

**Real-time Reverse Transcriptase Polymerase Chain Reaction (rt-PCR):**

To examine expression of Pepck and G6pase in rat liver, real-time rt-PCR was performed using a Roche LightCycler and SYBR Green I (Qiagen), as previously described (19). Expression of ‘housekeeping genes’ B2m, Rpl-19, Gapdh, β-actin and 18s were also examined. Five ‘housekeeping genes’ were used to normalized the gene expression data. The primer sequences are provided in the Supplementary Table 1. Relative gene expression was calculated as the ratio of target gene divided by the geometric mean of the expression of housekeeping genes (19,34).
**Study approval:**

All procedures for humans and rodents were approved by the Albert Einstein College of Medicine Institutional Review Board and the Einstein Institutional Animal Care and Use Committee, respectively.

**STATISTICAL ANALYSIS:**

We performed paired Student’s two-tailed $t$-tests to compare glyburide and placebo studies in the human subjects, and repeated measures Analysis of Variance (ANOVA) to compare multiple time points within the same study type. Rodent studies were analyzed by one-way ANOVA to compare main effects, and post hoc tests with Bonferroni or Dunnett $t$ (2-sided)$^b$ adjustment were performed when appropriate. $P$-values of $< 0.05$ were considered significant. Data are presented as mean ± SEM.

**Data and Resource Availability:** All data generated or analyzed during this study are included in the published article (and its online supplementary files).

**RESULTS:**

*Extrapancreatic $K_{ATP}$ channels play a substantial role in the regulation of EGP in humans.*

Given the importance of glucose effectiveness in regulating EGP, we examined whether central $K_{ATP}$ channels contribute to hyperglycemia’s suppressive effects. Paired studies were conducted in which non-diabetic subjects ($n = 9$) received either glyburide (10 mg) or placebo on separate occasions. ‘Pancreatic clamp’ methodology was employed to attain basal levels of insulin and glucoregulatory hormones; this was particularly important in preventing the stimulatory effects of glyburide and hyperglycemia on insulin secretion (Figure 1A). EGP was measured during a 3-hour period of euglycemia (90 mg/dl), and again during a subsequent 4-hour period of hyperglycemia (180 mg/dl). Blood glucose levels and the percent enrichment of plasma glucose
with deuterated [6,6-2H2] glucose (atom percent enrichment; APE) were stable throughout both euglycemia and hyperglycemia, confirming steady state conditions (Figure 1B and C).

Hyperglycemia suppressed EGP by 59% by the final hour of the placebo studies, as compared to baseline euglycemia (EGP: euglycemia = 2.38 ± 0.23 vs hyperglycemia = 0.98 ± 0.17 mg/kg.min, \( p < 0.001 \); Figures 1D and 1E). This is consistent with many previous observations \((3,5,8,9,12,35)\) that hyperglycemia potently inhibits EGP in the face of basal insulin levels, and highlights the importance of glucose effectiveness.

Blocking \(\text{K}_{\text{ATP}}\) channels with glyburide blunted the ability of hyperglycemia to suppress EGP: under these conditions, hyperglycemia suppressed EGP by only 32% (EGP: glyburide= 1.56 ± 0.29 vs placebo= 0.98 ± 0.17 mg/kg.min, \( p = 0.047 \); Figures 1D and 1E). While substantial glucose infusion rates (GIR) were required to maintain hyperglycemia, rates of GIR were lower with glyburide, consistent with the higher EGP in those studies (table 1). Similar insulin, free fatty acids (FFA), cortisol, and glucagon concentrations were maintained throughout all clamp studies, and somatostatin effectively suppressed C-peptide levels (table 1).

**Extrapancreatic \(\text{K}_{\text{ATP}}\) channels do not impact glucose uptake in humans.** As expected based on previous findings \((3,5)\), there was a rapid doubling in rates of glucose disappearance (Rd) with hyperglycemia. However, Rd did not differ between glyburide and placebo (Rd during the final hour of the clamp: glyburide = 4.31 ± 0.57 mg/kg/min vs placebo = 4.05 ± 0.39 mg/kg/min, \( p = 0.26 \); Figure 1F). These results are consistent with our observations that central activation of \(\text{K}_{\text{ATP}}\) channels does not affect peripheral Rd \((19)\).

**Glyburide’s effects on EGP are blocked by ICV diazoxide in rats.** Complementary rodent studies were performed to confirm that the effects of glyburide on EGP are central in origin. We first
determined the response of EGP to oral glyburide, then infused ICV diazoxide to antagonize glyburide’s effects on central $K_{\text{ATP}}$ channels. Normal male Sprague Dawley rats ($n = 19$) were assigned to the following groups: 1) oral glyburide, 2) oral saline, 3) and oral glyburide with concurrent ICV diazoxide, after establishing optimal dose and time course. Similar to the human studies, EGP was comparable among groups during the euglycemic phase of the rodent studies ($p = 0.25$ by ANOVA) and was suppressed by about 48% under hyperglycemic conditions (EGP: euglycemia $= 12.58 \pm 1.69 \text{ vs }$ hyperglycemia $= 6.60 \pm 0.47 \text{ mg/kg.min, } p=0.031$). Likewise, plasma glucose, insulin, and C-peptide concentrations during clamped conditions did not differ among groups, and insulin infusion rates were also comparable (Table 2). The addition of glyburide blunted the suppressive effects of hyperglycemia on EGP from 48% to 25% (EGP during hyperglycemia: glyburide $= 9.21 \pm 0.75 \text{ vs saline }= 6.60 \pm 0.47 \text{ mg/kg.min, } p = 0.046$; Figure 2D). Thus, inhibiting $K_{\text{ATP}}$ channels diminished hyperglycemia’s suppressive effect on EGP by about half. Simultaneous activation of central $K_{\text{ATP}}$ channels with ICV diazoxide abolished the effects of glyburide on EGP (EGP: diazoxide plus glyburide $= 5.78 \pm 0.57 \text{ vs saline }= 6.60 \pm 0.47 \text{ mg/kg.min, } p = 0.12$). EGP during hyperglycemia was significantly different between the glyburide and the glyburide + diazoxide group (EGP: glyburide $= 9.21 \pm 0.75 \text{ vs glyburide + diazoxide } = 5.78 \pm 0.57 \text{ mg/kg.min, } p = 0.034$; Figure 2D).

**Expression of key hepatic gluconeogenic enzymes in rats are modulated by central $K_{\text{ATP}}$ channels.** Following hyperglycemic clamps, hepatic phosphoenolpyruvate carboxykinase ($\text{Pepck}$) expression was significantly higher after glyburide administration (relative gene expression: saline $= 2.13 \pm 0.13 \text{ vs glyburide }= 4.59 \pm 0.40, p = 0.001$; Figure 2F), consistent with glyburide’s effect on EGP. This effect of glyburide was reversed by concomitant ICV administration of diazoxide (relative $\text{Pepck}$ expression: diazoxide plus glyburide $= 1.97 \pm 0.25, p = 0.88 \text{ vs saline}$). Similarly, there was a significant increase in glucose-6-phosphatase (G6pase)
gene expression with glyburide (relative gene expression: glyburide = 0.47 ± 0.09 vs saline = 0.10 ± 0.04, p = 0.004), which was also abolished by ICV diazoxide (relative gene expression: diazoxide plus glyburide = 0.06 ± 0.02, p = 0.85 vs saline; Figure 2G). This suggests that central K\(_{\text{ATP}}\) channel activation increases hepatic p-STAT3, a known mechanism for regulating gluconeogenic enzyme transcription and translation (19,36,37).

**DISCUSSION:**

These parallel studies in humans and rodents are the first to demonstrate that central K\(_{\text{ATP}}\) channels contribute to glucose effectiveness, a critical component of the regulation of EGP. Consistent with extensive reports in the literature (3,5,8,9,12,35), hyperglycemia suppressed EGP by approximatively half in both non-diabetic humans and normal Sprague Dawley rats under carefully maintained, fixed hormonal conditions. We have shown that either a fourfold increase in insulin levels or a doubling of plasma glucose levels results in a similar, approximately 50% suppression of EGP (7). Collectively, these observations support the importance of glucose *per se* in the regulation of EGP. Indeed, the relevance of ‘glucose effectiveness’ to the regulation of glucose metabolism has been well established by many research groups (3-10).

Here we show that antagonism of K\(_{\text{ATP}}\) channels with glyburide significantly reduced the EGP-lowering effect of hyperglycemia in both humans and rats. The effects of glyburide on EGP and gluconeogenic enzymes in rats were abolished by intracerebroventricular (ICV) administration of the KATP channel agonist diazoxide, suggesting that about half of EGP suppression by hyperglycemia is mediated by central K\(_{\text{ATP}}\) channels. These findings are consistent with the previous observations that nutrient and hormonal signals regulate EGP by modulating central K\(_{\text{ATP}}\) channels (16,19,38,39). This regulation appears to be impaired in T2D (24) and in insulin resistant humans (52), potentially consistent with the observation that obese diabetic rats have reduced
expression of Kir 6.2 subunit in the medial basal hypothalamus (40). Therefore, future studies examining the role of KATP channels in modulating glucose effectiveness in human and rodent models of insulin resistance and type 2 diabetes are clearly warranted.

Indeed, CNS regulation of metabolism is likely to be highly complex, comprising many types of highly specialized neurons and inter-connected central and peripheral regulatory pathways. As noted above, glucose has been shown to open $K_{ATP}$ channels in hypothalamic glucose inhibited (GI) neurons (41). The resulting decrease in activity of these GI neurons would be expected to reduce EGP, consistent with our current observations, and suggest that GI neurons contribute to ‘glucose effectiveness’. Of note, activation of SF-1 neurons (which define the VMH) under basal conditions increased whole-body glucose utilization and glucose uptake into skeletal muscle, while simultaneously increasing glucose production, thus maintaining glucose levels. In contrast, activation of the same SF-1 neurons suppressed gluconeogenic gene expression and glycogen phosphorylase a activity in the liver under hyperinsulinemic conditions. Thus, there are clearly cells in the VMH associated with both glucose-lowering and glucose-raising mechanisms which are differentially activated under different metabolic conditions.

Notably, diazoxide inhibits binding of gliburide to the sulfonylurea receptor (42), such that the two substances may compete for binding to the $K_{ATP}$ channel-sulfonylurea receptor (SUR1) complex in the brain. This provides reassurance that even if diazoxide were more immediately available in the CNS, it would still block binding of gliburide. Therapeutic doses of gliburide, though rapidly extruded from the CNS, reach concentrations in brain parenchyma that are substantially higher than the EC50 of SUR1, with abundant amounts of gliburide detected in CSF four hours after intraperitoneal delivery of gliburide, ie. a comparable time course to this study (43). Furthermore, biological effects of sulfonylureas often last much longer than their
plasma half-life, given the duration of receptor interaction and the formation of active metabolites (44). This is all consistent with our findings that EGP did not change over time in the group that received glyburide + diazoxide. Finally, ICV diazoxide blocked glyburide’s effects on EGP, providing further evidence for regulation of EGP by central K\textsubscript{ATP}-dependent mechanisms.

The effect of glyburide on EGP became significant after about 200 minutes of hyperglycemia in the human clamp studies. This delay likely reflects the time required for orally administered agents to interact centrally and for the subsequent effects of central signals to affect transcription and translation of hepatic gluconeogenic enzymes. This time course is consistent with previous findings demonstrating that central regulation of EGP requires several hours to manifest in humans, in contrast with the more acute changes in EGP that are mediated by insulin and glucose signaling within the liver (19,20). Indeed, the importance of subacute regulation of glucose metabolism is highlighted by several transgenic models, in which chronic disruption of central signals causes abnormal glucose homeostasis (39,45-47). This is further evidenced by the association of various neurologic disorders with abnormal glucose metabolism (24,48-51).

In conclusion, these novel studies in humans and rodents highlight the key role of central signals in the regulation of glucose effectiveness. Given the urgent need for additional treatment options in patients with T2D, central pathways may offer promising therapeutic targets.

**Author Contributions:** MC, ELY and WM conducted experiments, acquired and analyzed data, and wrote the manuscript. KZ and DMH conducted experiments, acquired and analyzed data, and provided reagents. SR, SB and SA analyzed data and contributed to writing the manuscript. SK, SK, and SRM all conducted experiments, acquired and analyzed data. MH designed research studies, analyzed data and wrote the manuscript.
Acknowledgements: The authors thank Robin Sgueglia, Daniel Stein, Oana Sandu, Nora Tomuta, Stephen Marsh, Akankasha Goyal, Rebekah Gospin, Kenny Ye, Pooja Raghavan, Harsha G Jayatillake, Kevin Jordan, Morgan Drucker, Riana Jumamil, Ankur Srivastava, Marc Ganz, Juan Lin and Tatyana Harris for their assistance. This work was supported by the National Institutes of Health (DK048321, DK069861, DK079974), by the Einstein-Montefiore NIH CTSA Grant UL1TR001073 from the National Center for Research Resources (NCRR), the Einstein-Mt. Sinai Diabetes Research Center (5P30DK020541-41) and the Nathan Shock Center (P30AG038072). Its contents are solely the responsibility of the authors and do not necessarily represent the official views or policies of the FDA, NCRR or NIH.

Dr. Meredith Hawkins is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of Interest Statement:
The authors have declared that no conflict of interest exists.
REFERENCES:

1. Pharmacologic Approaches to Glycemic Treatment: Standards of Medical Care in Diabetes-2018. Diabetes Care. 41(Suppl 1): p. S73-s85 (2018).
2. M. Riddle, G. Umpierrez, A. DiGenio, et al., Contributions of basal and postprandial hyperglycemia over a wide range of A1C levels before and after treatment intensification in type 2 diabetes. Diabetes Care. 34(12): p. 2508-14 (2011).
3. M. Ader, T.C. Ni, and R.N. Bergman, Glucose effectiveness assessed under dynamic and steady state conditions. Comparability of uptake versus production components. J Clin Invest. 99(6): p. 1187-99 (1997).
4. M.M. Wolfe and G.M. Reel, Inhibition of gastrin release by gastric inhibitory peptide mediated by somatostatin. Am J Physiol. 250(3 Pt 1): p. G331-5 (1986).
5. M. Hawkins, I. Gabriely, R. Wozniak, et al., Glycemic control determines hepatic and peripheral glucose effectiveness in type 2 diabetic subjects. Diabetes. 51(7): p. 2179-89 (2002).
6. J. Tonelli, P. Kishore, D.E. Lee, et al., The regulation of glucose effectiveness: how glucose modulates its own production. Curr Opin Clin Nutr Metab Care. 8(4): p. 450-6 (2005).
7. M. Mevorach, A. Giacca, Y. Aharon, et al., Regulation of endogenous glucose production by glucose per se is impaired in type 2 diabetes mellitus. J Clin Invest. 102(4): p. 744-53 (1998).
8. A.A. Alzaid, S.F. Dinneen, D.J. Turk, et al., Assessment of insulin action and glucose effectiveness in diabetic and nondiabetic humans. J Clin Invest. 94(6): p. 2341-8 (1994).
9. A. Basu, C. Dalla Man, R. Basu, et al., Effects of type 2 diabetes on insulin secretion, insulin action, glucose effectiveness, and postprandial glucose metabolism. Diabetes Care. 32(5): p. 866-72 (2009).
10. E. Ferrannini, R.A. DeFronzo, and R.S. Sherwin, Transient hepatic response to glucagon in man: role of insulin and hyperglycemia. Am J Physiol. 242(2): p. E73-81 (1982).
11. R. Basu, A. Basu, M. Nielsen, et al., Effect of overnight restoration of euglycemia on glucose effectiveness in type 2 diabetic subjects. J Clin Endocrinol Metab. 84(7): p. 2314-9 (1999).
12. G.J. Morton, M.E. Matsen, D.P. Bracy, et al., FGF19 action in the brain induces insulin-independent glucose lowering. J Clin Invest. 123(11): p. 4799-808 (2013).
13. S. Nagasaka, K. Tokuyama, I. Kusaka, et al., Endogenous glucose production and glucose effectiveness in type 2 diabetic subjects derived from stable-labeled minimal model approach. Diabetes. 48(5): p. 1054-60 (1999).
14. V.H. Routh, L. Hao, A.M. Santiago, et al., Hypothalamic glucose sensing: making ends meet. Front Syst Neurosci. 8: p. 236 (2014).
15. M. Carey, S. Kehlenbrink, and M. Hawkins, Evidence for central regulation of glucose metabolism. J Biol Chem. 288(49): p. 34981-8 (2013).
16. T.K. Lam, R. Gutierrez-Juarez, A. Pocai, et al., Regulation of blood glucose by hypothalamic pyruvate metabolism. Science. 309(5736): p. 943-7 (2005).
17. A. Pocai, T.K. Lam, R. Gutierrez-Juarez, et al., Hypothalamic K(ATP) channels control hepatic glucose production. Nature. 434(7036): p. 1026-31 (2005).
18. J.A. Gonzalez, F. Reimann, and D. Burdakov, Dissociation between sensing and metabolism of glucose in sugar sensing neurones. J Physiol. 587(1): p. 41-8 (2009).
19. P. Kishore, L. Boucau, K. Zhang, et al., Activation of K(ATP) channels suppresses glucose production in humans. J Clin Invest. 121(12): p. 4916-20 (2011).
20. S. Dash, C. Xiao, C. Morgantini, et al., Intranasal insulin suppresses endogenous glucose production in humans compared with placebo in the presence of similar venous insulin concentrations. Diabetes. 64(3): p. 766-74 (2015).
21. C. Benedict, S. Brede, H.B. Schioth, et al., Intranasal insulin enhances postprandial thermogenesis and lowers postprandial serum insulin levels in healthy men. Diabetes. 60(1): p. 114-8 (2011).
22. M. Heni, R. Wagner, S. Kullmann, et al., *Hypothalamic and Striatal Insulin Action Suppresses Endogenous Glucose Production and May Stimulate Glucose Uptake During Hyperinsulinemia in Lean but Not in Overweight Men*. Diabetes. **66**(7): p. 1797-1806 (2017).

23. S. Gancheva, C. Koliaki, A. Bierwagen, et al., *Effects of intranasal insulin on hepatic fat accumulation and energy metabolism in humans*. Diabetes. **64**(6): p. 1966-75 (2015).

24. Y.B. Esterson, M. Carey, L. Boucai, et al., *Central Regulation of Glucose Production May Be Impaired in Type 2 Diabetes*. Diabetes. **65**(9): p. 2569-79 (2016).

25. M.A. Bentsen, Z. Mirzadeh, and M.W. Schwartz, *Revisiting How the Brain Senses Glucose-And Why*. Cell Metab. **29**(1): p. 11-17 (2019).

26. M. Hawkins, J. Tonelli, P. Kishore, et al., *Contribution of elevated free fatty acid levels to the lack of glucose effectiveness in type 2 diabetes*. Diabetes. **52**(11): p. 2748-58 (2003).

27. P. Shah, A. Vella, A. Basu, et al., *Elevated free fatty acids impair glucose metabolism in women: decreased stimulation of muscle glucose uptake and suppression of splanchnic glucose production during combined hyperinsulinemia and hyperglycemia*. Diabetes. **52**(1): p. 38-42 (2003).

28. D.T. Finegood, R.N. Bergman, and M. Vranic, *Estimation of endogenous glucose production during hyperinsulinemic-euglycemic glucose clamps. Comparison of unlabeled and labeled exogenous glucose infusates*. Diabetes. **36**(8): p. 914-24 (1987).

29. R. Steele, *Influences of glucose loading and of injected insulin on hepatic glucose output*. Ann N Y Acad Sci. **82**: p. 420-30 (1959).

30. E. Sikuler, J. Polio, R.J. Groszmann, et al., *Glucagon and insulin metabolism in a portal-hypertensive rat model*. Am J Physiol. **253**(2 Pt 1): p. G110-5 (1987).

31. T. Ishida, Z. Chap, J. Chou, et al., *Effects of portal and peripheral venous insulin infusion on glucose production and utilization in depancreatized, conscious dogs*. Diabetes. **33**(10): p. 984-90 (1984).

32. R. Hovorka, H. Jayatillake, E. Rogatsky, et al., *Calculating glucose fluxes during meal tolerance test: a new computational approach*. Am J Physiol Endocrinol Metab. **293**(2): p. E610-9 (2007).

33. S. Obici, Z. Feng, A. Arduini, et al., *Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production*. Nat Med. **9**(6): p. 756-61 (2003).

34. S.A. Bustin, V. Benes, J.A. Garson, et al., *The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments*. Clin Chem. **55**(4): p. 611-22 (2009).

35. S. Dube, I. Errazuriz-Cruzat, A. Basu, et al., *The forgotten role of glucose effectiveness in the regulation of glucose tolerance*. Curr Diab Rep. **15**(6): p. 605 (2015).

36. A. Pocai, S. Obici, G.J. Schwartz, et al., *A brain-liver circuit regulates glucose homeostasis*. Cell Metab. **1**(1): p. 53-61 (2005).

37. H. Inoue, W. Ogawa, M. Ozaki, et al., *Role of STAT-3 in regulation of hepatic gluconeogenic genes and carbohydrate metabolism in vivo*. Nat Med. **10**(2): p. 168-74 (2004).

38. H. Inoue, *Central insulin-mediated regulation of hepatic glucose production [Review]*. Endocr J. **63**(1): p. 1-7 (2016).

39. A.C. Konner, R. Janoschek, L. Plum, et al., *Insulin action in AgRP-expressing neurons is required for suppression of hepatic glucose production*. Cell Metab. **5**(6): p. 438-49 (2007).

40. A. Gyte, L.E. Pritchard, H.B. Jones, et al., *Reduced expression of the KATP channel subunit, Kir6.2, is associated with decreased expression of neuropeptide Y and agouti-related protein in the hypothalami of Zucker diabetic fatty rats*. J Neuroendocrinol. **19**(12): p. 941-51 (2007).

41. D. Burdakov, L.T. Jensen, H. Alexopoulos, et al., *Tandem-pore K+ channels mediate inhibition of orexin neurons by glucose*. Neuron. **50**(5): p. 711-22 (2006).

42. I. Niki and S.J. Ashcroft, *Characterization and solubilization of the sulphonylurea receptor in rat brain*. Neuropharmacology. **32**(10): p. 951-7 (1993).
43. C. Lahmann, H.B. Kramer, and F.M. Ashcroft, Systemic Administration of Glibenclamide Fails to Achieve Therapeutic Levels in the Brain and Cerebrospinal Fluid of Rodents. PLoS One. 10(7): p. e0134476 (2015).
44. D. Sola, L. Rossi, G.P. Schianca, et al., Sulfonylureas and their use in clinical practice. Arch Med Sci. 11(4): p. 840-8 (2015).
45. L. Koch, F.T. Wunderlich, J. Seibler, et al., Central insulin action regulates peripheral glucose and fat metabolism in mice. J Clin Invest. 118(6): p. 2132-47 (2008).
46. S.A. Paranjape, O. Chan, W. Zhu, et al., Chronic reduction of insulin receptors in the ventromedial hypothalamus produces glucose intolerance and islet dysfunction in the absence of weight gain. Am J Physiol Endocrinol Metab. 301(5): p. E978-83 (2011).
47. L.E. Parton, C.P. Ye, R. Coppari, et al., Glucose sensing by POMC neurons regulates glucose homeostasis and is impaired in obesity. Nature. 449(7159): p. 228-32 (2007).
48. J.C. Bruning, D. Gautam, D.J. Burks, et al., Role of brain insulin receptor in control of body weight and reproduction. Science. 289(5487): p. 2122-5 (2000).
49. A. Campayo, P. de Jonge, J.F. Roy, et al., Depressive disorder and incident diabetes mellitus: the effect of characteristics of depression. Am J Psychiatry. 167(5): p. 580-8 (2010).
50. S.M. de la Monte, Brain insulin resistance and deficiency as therapeutic targets in Alzheimer's disease. Curr Alzheimer Res. 9(1): p. 35-66 (2012).
51. R.S. McIntyre, J.K. Soczynska, J.Z. Konarski, et al., Should Depressive Syndromes Be Reclassified as "Metabolic Syndrome Type II"? Ann Clin Psychiatry. 19(4): p. 257-64 (2007).
52. C. Xiao, Dash S, Stahel P, Lewis GF. Effects of intranasal insulin on endogenous glucose production in insulin-resistant men. Diabetes Obes Metab. 2018 Jul;20(7):1751-1754.
Table 1. Human plasma hormone concentrations, insulin infusion rate (IIR), plasma glucose concentration, and glucose infusion rate (GIR).

| Variable                  | Group | 0            | 120 to 180 | 180 to 240 |
|---------------------------|-------|--------------|------------|------------|
| Insulin (μU/ml)           | PLC   | 22.76 ± 2.44 | 22.82 ± 2.38 | 22.51 ± 2.74 |
|                           | GLB   | 23.06 ± 4.59 | 19.13 ± 2.51 | 20.03 ± 3.11 |
| C-peptide (ng/ml)         | PLC   | 0.20 ± 0.02  | 0.26 ± 0.04  | 0.26 ± 0.05  |
|                           | GLB   | 0.25 ± 0.04  | 0.30 ± 0.06  | 0.44 ± 0.09  |
| FFA (mmol/l)              | PLC   | 0.09 ± 0.02  | 0.08 ± 0.01  | 0.08 ± 0.01  |
|                           | GLB   | 0.12 ± 0.06  | 0.10 ± 0.05  | 0.08 ± 0.01  |
| Cortisol (μg/dl)          | PLC   | 13.70 ± 2.54 | 14.65 ± 2.69 | 13.70 ± 2.01 |
|                           | GLB   | 13.39 ± 2.00 | 11.71 ± 1.25 | 10.75 ± 1.78 |
| Glucagon (pg/ml)          | PLC   | 68.04 ± 11.85| 65.22 ± 7.37 | 64.48 ± 7.98 |
|                           | GLB   | 60.82 ± 5.95 | 58.90 ± 5.29 | 65.31 ± 4.42 |
| Lactate (mg/dl)           | PLC   | 8.16 ± 1.69  | 7.89 ± 1.41  | 7.30 ± 1.04  |
|                           | GLB   | 7.74 ± 0.81  | 7.56 ± 0.84  | 7.32 ± 0.94  |
| IIR (mU/kg/min)           | PLC   | 0.18 ± 0.01  | 0.18 ± 0.01  | 0.18 ± 0.01  |
|                           | GLB   | 0.17 ± 0.01  | 0.17 ± 0.01  | 0.17 ± 0.01  |
| Glucose (mg/dl)           | PLC   | 91.3 ± 2.71  | 182 ± 0.50   | 181 ± 0.55   |
|                           | GLB   | 97.3 ± 5.72  | 182 ± 0.55   | 183 ± 1.32   |
| GIR (mg/kg/min)           | PLC   | 0.20 ± 0.17  | 2.91 ± 0.15  | 3.03 ± 0.50  |
|                           | GLB   | 0.56 ± 0.23  | 2.62 ± 0.13 *| 2.76 ± 0.42 *|

PLC: Placebo, GLB: Glyburide, FFA: Free fatty acid, IIR: insulin infusion rate, GIR: glucose infusion rate. Repeated measures ANOVA was used to compare hormone and substrate concentrations among time intervals. Paired t-tests were used to compare values between study...
conditions (separately for euglycemia vs. hyperglycemia) for glucose and GIR. * Significantly different between placebo and glyburide for that time period (p < 0.05, using t-test).

Table 2. Rodent plasma hormone and glucose concentrations.

| Variable           | Group     | 0 to 120      | 180 to 240     |
|--------------------|-----------|---------------|----------------|
| Insulin (μU/ml)    | NS        | 0.75 ± 0.10   | 1.47 ± 0.30    |
|                    | GLB       | 0.91 ± 0.08   | 1.46 ± 0.16    |
|                    | GLB+DZX   | 0.72 ± 0.08   | 1.53 ± 0.18    |
| C-peptide (ng/ml)  | NS        | 0.14 ± 0.03   | 0.57 ± 0.11    |
|                    | GLB       | 0.16 ± 0.02   | 0.53 ± 0.05    |
|                    | GLB+DZX   | 0.17 ± 0.02   | 0.71 ± 0.13    |
| Glucose (mg/dl)    | NS        | 118.8 ± 8.60  | 263.57 ± 3.81  |
|                    | GLB       | 100.35 ± 5.91 | 273.16 ± 7.43  |
|                    | GLB+DZX   | 95.60 ± 5.58  | 253.61 ± 2.40  |

NS: Normal saline, GLB: Glyburide, GLB+DZX: Glyburide + Diazoxide.

One-way ANOVA was used to compare values among the three study conditions.
Figure 1. Effect of $K_{ATP}$ channel inhibition on the ability of glucose effectiveness to regulate EGP and glucose disposal in humans. (A) Schematic of human hyperglycemic pancreatic clamp protocol. (B) Blood glucose levels during the clamps. (C) Atom percent excess (APE) during the steady state period. (D) Time course of EGP during euglycemic baseline (-30 to 0 min) and hyperglycemic clamp (0–240 min). (E) Mean EGP during the final hour of hyperglycemic clamp studies (Hyperglycemia) and during euglycemic baseline (Euglycemia). (F) Rate of glucose disappearance during the final hour of hyperglycemic clamp. *$p<0.05$; Repeated measures ANOVA (Figure 1D), 2-tailed t test (Figure 1E, F). Data represent the mean ± SEM. PLC: Placebo, GLB: Glyburide; $n=9$. 

Diabetes
Figure 2. Effect of $K_{\text{ATP}}$ channel inhibition on the ability of glucose effectiveness to regulate EGP and glucose disposal in rodents. (A) Schematic of rodent hyperglycemic pancreatic clamp protocol. (B) Schematic of glyburide and diazoxide’s opposing actions at different sites of the same $K_{\text{ATP}}$ channel. (C) Time course of EGP during euglycemic baseline (60 to 120 min) and hyperglycemic clamp (180–240 min) (D) Mean EGP during the hyperglycemic clamp studies and during euglycemic baseline. (E) Rate of glucose disappearance during the final hour of hyperglycemic clamp. (F) Levels of Pepck and (G) G6Pase expression in normal saline, glyburide, and glyburide + ICV diazoxide rodents. *p<0.05; ANOVA with Bonferroni adjustment. Data represent the mean ± SEM. NS: Normal saline (n=7), GLB: Glyburide (n=7), GLB+DZX: Glyburide+Diazoxide (n=5).
SUPPLEMENTAL MATERIAL:

Human subject recruitment: All procedures were approved by Einstein’s Institutional Review Board. The purpose, nature, risks, benefits and procedures of the study were explained to all potential subjects and their voluntary, informed, written consent was obtained. All subjects completed a clinical screening evaluation consisting of medical history, physical examination, and laboratory evaluation including hematologic, lipid, and chemistry parameters (including fasting glucose level), and baseline electrocardiogram.

Rats: A total of fifty-four studies were performed on 12-14 week-old male Sprague Dawley rats (Charles River Breeding Laboratories). Pilot studies were performed on thirty-five of these rats, to assess appropriate time course, glucose levels, and optimal glyburide and diazoxide doses. Nineteen rats with an average weight of 386.1 ± 7.2 g were studied under the following conditions:

1. Oral (gavage) normal saline control (NS, n=7).
2. Oral (gavage) Glyburide (GLB, n=7).
3. Oral (gavage) Glyburide with intracerebroventricular (ICV) infusion of the K\textsubscript{ATP} channel activator blocker Diazoxide (GLB+DZX, n=5).

Twenty minutes prior to a four-hour pancreatic clamp study, rats matched for age and weight received oral normal saline (NS) or 2.5 mg/kg of Glyburide (GLB) by gavage in parallel with the human studies (Figure A). To determine whether the effects of oral glyburide on EGP are mediated through central mechanisms, a third group of rats received the same dose of oral glyburide with an ICV infusion of the K\textsubscript{ATP} channel activator Diazoxide (GLB+DZX, 9.6 mg/dl) two hours prior to the pancreatic clamp study (Figure 2B). As previously described, rats were prepared for the in vivo experiments with implantation of an ICV cannula under anesthesia occurred two weeks prior to the study and implantation of carotid and internal jugular catheters occurred one week prior (1,2).
Intracerebroventricular (ICV) Cannulation:

Three weeks prior to the clamp studies, all ICV cannulae were implanted into the third cerebral ventricle by stereotaxic surgery, performed in Einstein’s Chronobiosis and Aging/Metabolism of Aging (CEAC) animal care facility following the well-designed and histologically verified methodology developed by Rossetti’s group (3, 4, 5, 6).

Specifically, each rat was fixed in a KOPF stereotaxic apparatus (DAVID KOPF INSTRUMENT, Tujunga, CA) with ear bars and a nose piece set at +5.0 mm. A 22-gauge stainless steel guide cannula (C313GSPCXC, Plastics One, Roanoke, VA) was chronically implanted into the third ventricle using the following coordinates from bregma: anterior-posterior; +0.2 mm, dorsal-ventral; −9.0 mm, medial-lateral; 0.0 directly on the midsagittal suture. A mating dummy cannula (C313DC/SPC, Plastics One, Roanoke, VA) was inserted to prevent clogging of the guide cannula. The implant is secured to the skull with dental cement, and the skin is closed over the implant using wound clips. All surgeries were performed under anesthesia by intraperitoneal ketamine (Ketaset, 87 mg/kg) and xylazine (Rompun, 11 mg/kg). Recovery was monitored until body weight was within 3% of the pre-operative weight (5–6 days). Intravenous infusion of diazoxide or saline was performed as described (2). Following the studies, the established methodology included verification of implantation histologically for each animal, as described (2).

Supplemental Table 1

Forward and reverse primer sequences for the gluconeogenic enzymes phosphoenolpyruvate carboxykinase (Pepck), glucose-6-phosphatase (G6pase), and six housekeeping genes (B2m, Rpl19, Gapdh, βactin, 18s).

| Genes   | Forward Sequence | Reverse Sequence |
|---------|------------------|------------------|
|         |                  |                  |
| Rat Pepck | GGAAAGACAAAAAACGGCAAG | ACGTAGCCAATGGGAGTGAG |
|----------------------|-----------------------|-----------------------|
| Rat G6pase | TGCTGCATCTCTTTTGACTCG | TTGTGTGTTGTCGCCAGGGAG |
| Rat B2m | CTGCTACGTCGTCTCAGTCCAC | TGCAAGCATATACATCGGTCTC |
| Rat Rpl19 | GACCTGGATGCGAAGGATGA | CCATGAGAATCCGCTTGTIT |
| Rat Gapdh | AAACCCATCACCATTTCCA | GTGGTTCAACCCCACACAAA |
| Rat βactin | GCTACAGCTTCACCACCAACA | AGGAAGGAAGGCTGGAAGAG |
| Rat 18s | AGGGTTCGATTTCCGGAGAGG | CAACCTTAATATACGCTATTGG |

REFERENCES

1. Pocai, A., Obici, S., Schwartz, G.J., and Rossetti, L. 2005. A brain-liver circuit regulates glucose homeostasis. Cell Metabolism 1:53-61.

2. Preeti Kishore, Laura Boucai, Kehao Zhang, Weijie Li, Sudha Koppaka, Sylvia Kehlenbrink, Anna Schiwek, Yonah B. Esterson, Deeksha Mehta, Samar Bursheh, Ya Su, Roger Gutierrez-Juarez, Radhika Muzumdar, Gary J. Schwartz, Meredith Hawkins. Activation of K(ATP).

3. Rossetti L, Smith D, Shulman DI, Papachristou D, DeFronzo RA: Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. J Clin Invest 79:1510–1515, 1987.

4. Silvana Obici, Zhaohui Feng, Kimyata Morgan, Daniel Stein, George Karkanias, Luciano Rossetti Central Administration of Oleic Acid Inhibits Glucose Production and Food Intake. Diabetes 51(2):271-5, 2002.

5. Muzumdar R, Ma X, Yang X, Atzmon G, Bernstein J, et al. (2003) Physiologic effect of leptin on insulin secretion is mediated mainly through central mechanisms. Faseb J 17: 1130–1132.
6. Liu L, Karkanias GB, Morales JC, Hawkins M, Barzilai N, et al. (1998) Intracerebroventricular leptin regulates hepatic but not peripheral glucose fluxes. J Biol Chem 273: 31160–31167.