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Central Regulation of Glucose Production May Be Impaired in Type 2 Diabetes



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The challenges of achieving optimal glycemic control in type 2 diabetes highlight the need for new therapies. Inappropriately elevated endogenous glucose production (EGP) is the main source of hyperglycemia in type 2 diabetes. Because activation of central ATP-sensitive potassium (K_{ATP}) channels suppresses EGP in nondiabetic rodents and humans, this study examined whether type 2 diabetic humans and rodents retain central regulation of EGP. The K_{ATP} channel activator diazoxide was administered in a randomized, placebo-controlled crossover design to eight type 2 diabetic subjects and seven age- and BMI-matched healthy control subjects. Comprehensive measures of glucose turnover and insulin sensitivity were performed during euglycemic pancreatic clamp studies following diazoxide and placebo administration. Complementary rodent clamp studies were performed in Zucker Diabetic Fatty rats. In type 2 diabetic subjects, extrapancreatic K_{ATP} channel activation with diazoxide under fixed hormonal conditions failed to suppress EGP, whereas matched control subjects demonstrated a 27% reduction in EGP ($P = 0.002$) with diazoxide. Diazoxide also failed to suppress EGP in diabetic rats. These results suggest that suppression of EGP by central K_{ATP} channel activation may be lost in type 2 diabetes. Restoration of central regulation of glucose metabolism could be a promising therapeutic target to reduce hyperglycemia in type 2 diabetes.

Substantial evidence indicates that optimal glycemic control is associated with better clinical outcomes in type 2 diabetes (1,2). However, despite a sizeable therapeutic armamentarium that targets pathways in liver, muscle, and

pancreas, 50% of patients with type 2 diabetes are unable to achieve adequate glycemic control (3). Therefore, new approaches to improve glucose homeostasis are urgently needed. Increased endogenous glucose production (EGP) is the major source of both fasting and postabsorptive hyperglycemia in type 2 diabetes (4,5). Although EGP is suppressed by both insulin and glucose in nondiabetic humans, this effect is considerably impaired in individuals with type 2 diabetes (4). Therefore, this inappropriately elevated EGP is an important target for intervention in type 2 diabetes.

Importantly, evidence for regulation of glucose homeostasis by the central nervous system (CNS) has been accumulating in both rodents and humans. Several rodent studies have demonstrated that the CNS is involved in the regulation of glucose metabolism through its detection of nutrients and hormones, subsequent signaling through hypothalamic ATP-sensitive potassium (K_{ATP}) channels, and transduction of those signals to the liver via vagal efferent fibers (6–14). We recently reported that oral administration of diazoxide, a K_{ATP} channel activator, significantly reduces EGP in nondiabetic humans under fixed hormonal conditions (15). Additionally, we presented supporting evidence in rats that diazoxide's suppressive effects on EGP are abolished with central administration of the K_{ATP} channel blocker glibenclamide, suggesting that these effects are centrally mediated (15). Furthermore, intranasal administration of insulin at doses previously shown to increase cerebrospinal fluid (CSF) insulin concentrations approximately twofold suppressed glucose production to a similar extent and over a similar time course, likely through activation of central K_{ATP} channels (16). Collectively, these studies in rodents and humans suggest a role

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for hypothalamic K_{ATP} channels in regulation of glucose metabolism.

Of note, a number of studies in obese or diabetic rodents have indicated that central sensing mechanisms are ineffective at maintaining glucose homeostasis in these models (17–20). In fact, it has been hypothesized that dysregulated CNS circuits may contribute to impaired glucose homeostasis in type 2 diabetes (21). Therefore, it is essential to establish whether central regulation of glucose homeostasis remains intact in humans with type 2 diabetes. If so, hypothalamic K_{ATP} channels represent a potential therapeutic target to counteract excessive EGP and improve hyperglycemia in type 2 diabetes. Conversely, if central regulation of EGP is lost in type 2 diabetes, future therapies could be directed at restoring these pathways.

Given the evidence supporting regulation of EGP by a brain–liver pathway in humans without diabetes (15), the current randomized, placebo-controlled crossover study was designed to determine whether activation of central K_{ATP} channels would suppress EGP in individuals with type 2 diabetes. Importantly, we conducted parallel studies in a group of specifically recruited age- and BMI-matched control subjects without diabetes. To exclude any effects of diazoxide on insulin secretion, these studies were performed under euglycemic pancreatic clamp conditions. Complementary studies in Zucker Diabetic Fatty (ZDF) rats were also performed to assess diazoxide's ability to cross the blood brain barrier and to suppress EGP in this animal model of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Human Studies

Eight subjects with moderately to poorly controlled type 2 diabetes were studied (Table 1). Eligible subjects were

diagnosed with type 2 diabetes within the past 10 years and otherwise in good health. Seven healthy age- and BMI-matched control subjects without diabetes were also studied (Table 1). The purpose, nature, risks, and benefits of the study were explained to all subjects in the Clinical Research Center (CRC) prior to their enrollment in the study, and their voluntary, informed, written consent was obtained. All subjects had an initial screening visit to allow for a clinical evaluation that included history, physical examination, hematologic, lipid, and chemistry screening (including fasting glucose levels), baseline electrocardiogram, and consent procedures. A 2-h oral glucose tolerance test was performed to ensure normal glucose tolerance in control subjects without diabetes. Each subject received the experimental agents in random order, and the agents were identical in appearance.

Euglycemic Pancreatic Clamp Procedures

All experiments consisted of basal insulin and somatostatin (250 μ g/h) infusions with replacement of glucoregulatory hormones (glucagon 0.6 ng/kg/min; growth hormone 3 ng/kg/min) starting at $t = -120$ min. From $t = -120$ to $t = 0$ min, insulin infusion rates were adjusted every 20–25 min to determine optimal insulin infusion rates to maintain euglycemia. Finer calibration of insulin infusion rates were performed from $t = 0$ to $t = 120$ min to establish individualized basal insulin infusion rates by 120 min (15). In 8 of the 30 studies ($n = 1$ nondiabetic placebo, $n = 2$ nondiabetic diazoxide, $n = 2$ diabetic placebo, and $n = 3$ diabetic diazoxide), minor changes to the insulin infusion rates were made from $t = 120$ to $t = 170$ min to prevent hypoglycemia. There were no significant changes in either insulin infusion rates or plasma insulin concentrations between 0 and 240 min, emphasizing the

Table 1—Subject characteristics (N = 15)

	Subjects with diabetes ($n = 8$), mean (SE)	Subjects without diabetes ($n = 7$), mean (SE)	<i>P</i> value
Continuous variables			
Age, years	49.75 (1.39)	47.14 (2.57)	0.37
BMI, kg/m ²	31.04 (0.54)	30.28 (0.98)	0.49
Weight, kg	95.64 (3.40)	97.20 (3.47)	0.75
HbA _{1c} , %	9.15 (0.44)	5.26 (0.14)	<0.0001
HbA _{1c} , mmol/mol	76.62 (4.81)	34.14 (1.58)	<0.0001
Fasting plasma glucose, mg/dL	199.57 (17.74)	95.6 (7.10)	<0.001
Fasting insulin, μ U/mL	42.61 (12.30)	12.54 (2.33)	0.04
Fasting C-peptide, ng/mL	0.31 (0.10)	1.41 (0.54)	0.04
Baseline systolic blood pressure, mmHg	138.9 (6.47)	139.0 (7.90)	0.99
Baseline diastolic blood pressure, mmHg	83.63 (4.77)	81.71 (3.74)	0.76
Baseline heart rate, bpm	66.63 (3.71)	73.00 (3.51)	0.24
Categorical variables, N (%)			
Race			
Black	3 (37.5)	3 (42.9)	
Hispanic	4 (50.0)	1 (14.3)	
White	0 (0)	2 (28.6)	
Other	1 (12.5)	1 (14.3)	
Sex			
Male	8 (100.0)	7 (100.0)	
Female	0 (0)	0 (0)	

fact that any changes were minor. This careful approach to attaining individualized, basal insulin infusion rates, avoiding the need for virtually any exogenous glucose infusion, permits highly sensitive measures of glucose production without overinsulinization (15,22). Plasma glucose concentrations were measured at 5-min intervals during the 240 min of the study and maintained at normal fasting concentrations (~ 90 mg/dL), using low infusion rates of dextrose 20% if needed. All infusions were stopped at $t = 240$ min, and subjects received a standard meal with subsequent plasma glucose monitoring for 60 min after the completion of the study before being discharged from the CRC. Data for glucose turnover represent the mean values during the final 60 min of the studies ($t = 180$ – 240 min).

Each subject underwent two paired euglycemic pancreatic clamp studies separated by 4–6 weeks. After an overnight fast, subjects without diabetes were admitted to the CRC on the morning of the study. Subjects with type 2 diabetes were admitted to the CRC the night prior to the clamp study for gradual lowering of plasma glucose levels with intravenous insulin infusions (22,23) and were also fasted overnight prior to the study. Because an algorithm was used for insulin infusion rates, subjects received progressively lower rates of insulin infusion as their glucose levels dropped. Sulfonylurea agents and metformin were discontinued for 72 h prior to all admissions, and thiazolidinediones were held for 8 days prior to admission. Long- or intermediate-acting preparations of insulin were discontinued prior to admission such that subjects received no long-acting insulin for 24 h prior to the study and no intermediate-acting insulin for at least 12 h. An 18-gauge catheter was inserted in an antecubital vein for infusions, and a contralateral hand vein was cannulated in a retrograde fashion for arterialized venous blood sampling. To obtain arterialized venous blood, the hand was kept in a warming pad maintained at 55°C . During initial pilot studies (24), it was determined that optimal metabolic effects of diazoxide were observed ~ 6 to 7 h following drug administration.

At $t = -180$ min, the subjects were administered either oral diazoxide 4–6 mg/kg or placebo in a double-blinded fashion (Fig. 1). Vital signs were recorded at $t = -180$ min and hourly thereafter. Primed continuous infusions of 6–6 glucose (D2G) tracer were initiated at $t = -120$ min (200 mg/ m^2 bolus, then 2 mg/ min/m^2) to measure glucose fluxes under pancreatic clamp conditions (15). From $t = 0$ to $t = 240$ min, blood samples were obtained for determinations of plasma glucose, insulin, glucagon, C-peptide, cortisol, free fatty acids, glycerol, lactate, and 6–6 glucose determinations. Each subject returned to the CRC for a second study (either placebo or diazoxide) after 4–6 weeks had elapsed.

Plasma Hormone and Substrate Determinations

Plasma glucose was measured at the bedside with a Beckman glucose analyzer (Beckman Coulter, Fullerton, CA) by use of the glucose oxidase method. Measurements of plasma insulin, C-peptide, and glucagon were undertaken in order

to evaluate the inhibitory effects of somatostatin on insulin secretion and the consistency of hormone replacement. Plasma insulin, C-peptide, glucagon, and cortisol concentrations were measured by radioimmunoassay in the Diabetes Research Center Hormone Assay Core (25). Plasma lactate, free fatty acids, and glycerol were measured using spectrophotometric techniques (26–28). 6–6 Glucose concentrations were measured by gas chromatography mass spectrometry, as previously described (29,30). The rates of EGP were compared during euglycemic clamp studies following diazoxide administration versus following placebo in each subject. Rates of glucose appearance (R_a) and disappearance (R_d) and other indices of glucose turnover were estimated by using Steele equations (31), the assumption that $R_a = R_d$ for steady state, and the following equation: $R_d = (\text{Basal } [6,6\text{-}^2\text{H}_2] \text{ glucose infusion rate} + D_2O/[6,6\text{-}^2\text{H}_2]) \text{ glucose infusion rate/atom percent excess fraction/weight (kg)}$, with data averaged over 60-min segments of each experiment. EGP was determined by subtracting the rates of glucose infusion from the tracer-derived R_a .

Rat Studies

Eleven-week-old male ZDF rats ($n = 12$) (Charles River Laboratories; Wilmington, MA), with an average weight of 354.5 ± 10.6 g, were studied under the following conditions: 1) oral (gavage) saline control ($n = 6$); and 2) oral (gavage) diazoxide ($n = 6$) (Fig. 5A). The night before infusion studies were performed, each animal received Neutral Protamine Hagedorn insulin (3–5 units/kg) to slowly correct hyperglycemia prior to the study. Each infusion study lasted 240 min. At 120 min prior to infusion studies, rats were anesthetized with isoflurane, and either saline or diazoxide (100 mg/kg) was administered by oral gavage. For the remainder of the studies, rats were conscious and unrestrained. Insulin infusion (3–6 mU/kg/min) was then initiated to slowly lower blood glucose to ~ 140 – 150 mg/dL prior to initiation of the study. At $t = 0$ min, a primed continuous i.v. infusion of $[3\text{-}^3\text{H}]\text{-glucose}$ was begun and maintained for 4 h to assess glucose kinetics (40 μCi bolus followed by 0.4 $\mu\text{Ci}/\text{min}$ infusion; PerkinElmer). Blood samples were obtained at 10-min intervals during the final hour of the clamp to assess $[3\text{-}^3\text{H}]\text{-glucose}$ -specific activity. A peripheral basal insulin pancreatic-euglycemic clamp was performed for the final 2 h of the infusion study ($t = 120$ – 240 min), as previously described, using continuous i.v. somatostatin infusion (15). This specific protocol was followed, without glucagon infusion, in order to replicate previous studies examining central regulation of glucose production (12,15).

Rats were prepared for the in vivo experiments with implantation of carotid and internal jugular catheters 1 week prior to the study. Following the study, rats were anesthetized with ketamine (150 mg/kg). CSF samples were obtained by ventricular puncture and liver tissue samples were obtained by freeze clamping. CSF was analyzed for diazoxide content by NMS Laboratories (Willow Grove,

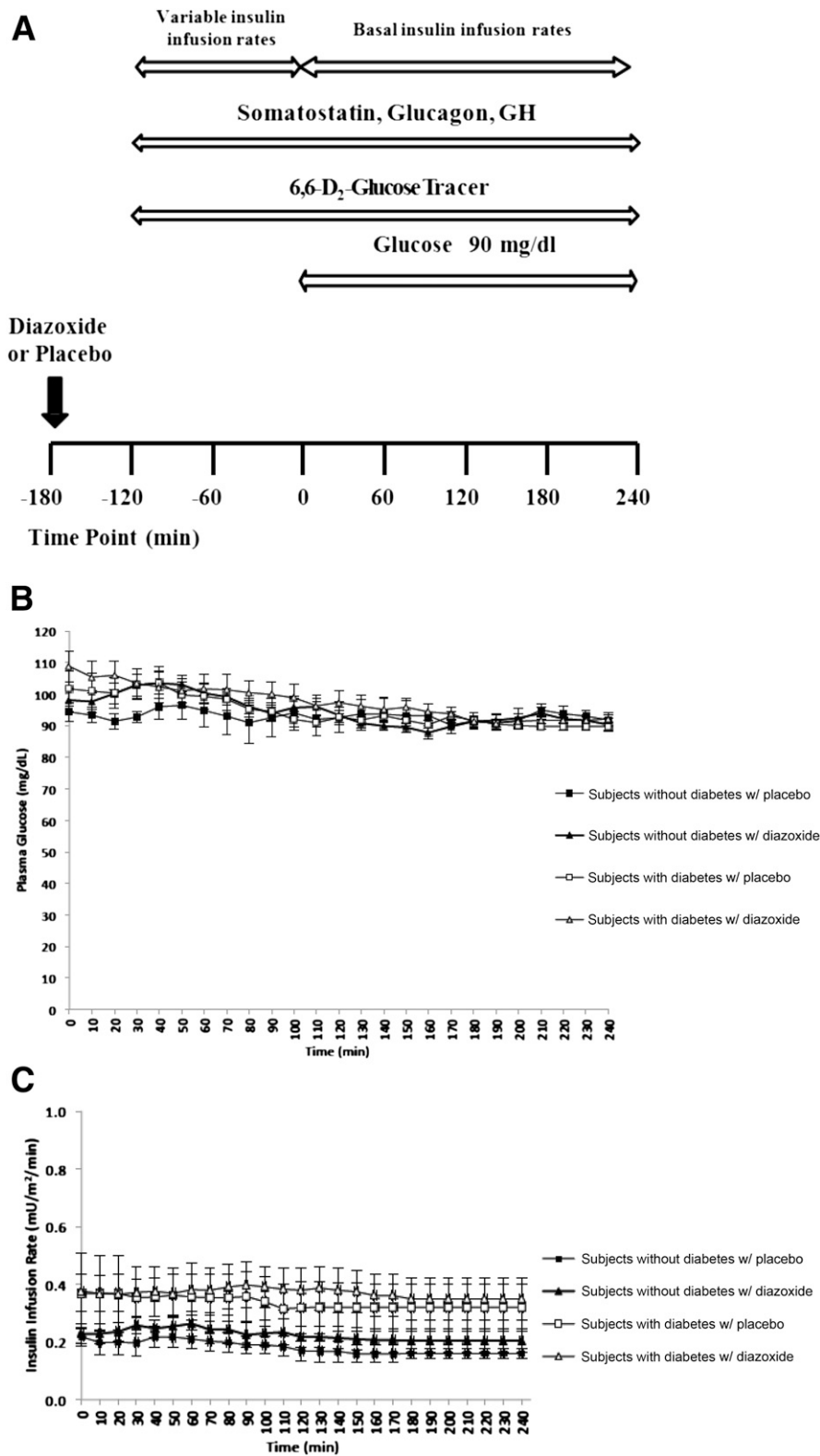


Figure 1—A: Euglycemic pancreatic clamp protocol scheme. B: Plasma glucose levels for each study group throughout the clamp studies. C: Average insulin infusion rates for each study group throughout the clamp studies. GH, growth hormone.

PA) using liquid chromatography-tandem mass spectrometry. Real-time RT-PCR was performed to examine gene expression and protein levels of phosphoenolpyruvate

carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) in rat liver, using a Roche LightCycler and SYBR Green I (Qiagen). Relative gene expression was calculated

as the ratio of target gene divided by the geometric mean of housekeeping genes.

Statistical Analysis

Comparison of EGP, rate of glucose disappearance, glucose infusion rate, and hormone and substrate levels during diazoxide versus placebo studies within each group were performed using paired Student *t* tests. Unpaired Student *t* tests were used to compare the percent change in EGP (diazoxide vs. placebo studies) and subject characteristics between the two groups. Repeated-measures ANOVA was used to assess the stability of average percent enrichment of 6-6 glucose in the plasma during the last 2 h of the clamp studies. Student *t* tests and ANOVA were performed under the assumptions of equality of variances and normality. Equality of variances was met when the SDs of the variables differed by less than an order of magnitude. Normality of data could not be fully assessed given that the classical tests of normality are not robust to smaller sample sizes. Therefore, the significance or non-significance of each parametric test result was confirmed with the alternative nonparametric test (Wilcoxon matched-pairs signed-rank sum test for comparison of medians of paired data, Mann-Whitney tests for comparison of medians of two independent groups, and Kruskal-Wallis test for comparison of medians of three or more independent groups). The α value was set at 0.05 for all tests. Data are reported as mean \pm SE unless otherwise noted.

Study Approval

All procedures were approved by the Institutional Review Board of Albert Einstein College of Medicine.

RESULTS

Human Subject Characteristics

Eight subjects with diabetes and seven control subjects without diabetes were frequency matched for age and BMI. Additional subject characteristics are presented in Table 1. Because a number of rodent studies suggest that central regulation of metabolic homeostasis is impaired with both obesity and aging (18–20), we specifically recruited an age- and BMI-matched control group without diabetes for these studies. All subjects with type 2 diabetes who completed both placebo and diazoxide clamps were males. We included only male control subjects to maintain consistency between groups. Of note, analysis of our previously published data reveals that the decrease in EGP seen with diazoxide administration relative to placebo administration in healthy subjects was not significantly different in females versus males ($P = 0.22$) (15). Furthermore, because study recruitment aimed to be representative of the ethnic composition of the Bronx, NY, both groups were racially/ethnically heterogeneous, although there were more Hispanic subjects in the group with diabetes versus the group without diabetes (4 vs. 1, respectively). Although the effects of diazoxide were comparable among all subjects in both groups, this study was not sufficiently powered for a subgroup analysis to discern ethnic differences.

Human Clamp Conditions

Over the interval of measurement, average percent enrichment of 6-6 glucose in the plasma during diazoxide studies remained stable in both subjects with diabetes ($P = 0.42$) and control subjects without diabetes ($P = 0.96$). Similarly, average percent enrichment during placebo studies remained stable in both subjects with diabetes ($P = 0.99$) and control subjects without diabetes ($P = 0.89$). Plasma hormone levels were measured to confirm that the clamp conditions prevented pancreatic hormone secretion. There were no statistically significant differences in plasma levels of insulin, glucagon, cortisol, free fatty acids, C-peptide, glycerol, or lactate in response to diazoxide versus placebo in subjects with diabetes (Table 2) or in control subjects without diabetes (Table 3). Although this likely reflects a lack of difference, we cannot rule out type II error in light of the relatively small sample sizes common to all resource-intensive physiologic studies. Of note, baseline C-peptide levels were suppressed following overnight insulin infusion in the subjects with diabetes. Average insulin infusion rates were similar during the final hour of the clamp under both experimental conditions in subjects with diabetes (0.35 ± 0.08 mU/m²/min with diazoxide vs. 0.32 ± 0.07 mU/m²/min with placebo; $P = 0.52$) and in control subjects without diabetes (0.21 ± 0.04 mU/m²/min with diazoxide vs. 0.16 ± 0.02 mU/m²/min with placebo; $P = 0.09$). Although diazoxide has the potential to lower blood pressure at high doses, there were no differences in mean systolic blood pressure ($P = 0.36$ in subjects with diabetes; $P = 0.69$ in control subjects), mean diastolic blood pressure ($P = 0.17$ in subjects with diabetes; $P = 0.17$ in control subjects), or mean heart rate ($P = 0.39$ in subjects with diabetes; $P = 0.44$ in control subjects) with diazoxide versus placebo versus baseline.

Glucose Fluxes

We previously reported that oral administration of diazoxide caused a 29% decrease in EGP in healthy human subjects (15). In the current study, we determined that oral administration of diazoxide to subjects with diabetes under fixed hormonal conditions did not affect EGP (1.55 ± 0.08 mg kg⁻¹ min⁻¹ with diazoxide

Table 2—Plasma hormone and substrate levels in subjects with diabetes from $t = 180$ – 240 min during the clamp studies ($N = 8$)

	Diazoxide, mean (SE)	Placebo, mean (SE)	<i>P</i> value
Insulin, μ U/mL	35.0 (5.6)	41.1 (8.4)	0.26
Glucagon, pg/mL	61.2 (5.0)	73.6 (6.4)	0.10
Lactate, mmol/L	0.8 (0.1)	0.8 (0.1)	0.73
Glycerol, μ mol/L	32.2 (4.5)	28.8 (4.8)	0.63
Free fatty acid, μ mol/L	385.8 (64.7)	329.7 (70.1)	0.58
Cortisol, μ g/dL	11.1 (1.9)	7.2 (1.1)	0.12
C-peptide, nmol/L	0.07 (0.01)	0.05 (0.01)	0.07

Table 3—Plasma hormone and substrate levels in control subjects without diabetes from $t = 180$ – 240 min during the clamp studies ($N = 7$)

	Diazoxide, mean (SE)	Placebo, mean (SE)	<i>P</i> value
Insulin, $\mu\text{U/mL}$	21.0 (3.3)	17.4 (2.5)	0.14
Glucagon, pg/mL	76.8 (8.6)	77.3 (6.3)	0.90
Lactate, mmol/L	0.5 (0.06)	0.5 (0.04)	0.74
Glycerol, $\mu\text{mol/L}$	19.8 (3.6)	18.6 (5.0)	0.82
Free fatty acid, $\mu\text{mol/L}$	134.2 (29.7)	163.2 (41.4)	0.26
Cortisol, $\mu\text{g/dL}$	9.3 (0.7)	10.1 (1.5)	0.60
C-peptide, nmol/L	0.08 (0.02)	0.08 (0.01)	0.96

vs. $1.59 \pm 0.08 \text{ mg kg}^{-1} \text{ min}^{-1}$ with placebo; $P = 0.74$) (Figs. 2A and 3A). This is in contrast to the 27.4% decrease in EGP after diazoxide administration in age- and BMI-matched control subjects without diabetes ($1.14 \pm 0.07 \text{ mg kg}^{-1} \text{ min}^{-1}$ with diazoxide vs. $1.61 \pm 0.14 \text{ mg kg}^{-1} \text{ min}^{-1}$ with placebo; $P = 0.002$) (Figs. 2A and 3B). Relative to basal rates of EGP, there was a $0.4 \pm 8.4\%$ increase in EGP in the placebo studies and a $26.4 \pm 3.0\%$ decrease in the diazoxide studies between time -120 and 240 min in the subjects without diabetes. The diazoxide-induced suppression of EGP in the control subjects without diabetes thus differed significantly from the complete lack of response in the subjects with diabetes ($P = 0.01$) (Fig. 2B). Of note, our intent in designing the current studies was to avoid overinsulinization of the liver, and in fact, it should be noted that rates of EGP remained unsuppressed throughout the placebo studies relative to basal EGP at time -120 min, prior to the onset of the clamp. Furthermore, among subjects in whom insulin infusion rates were unchanged after 120 min, there was a 32% suppression of EGP by diazoxide in the group without diabetes and no change in the group with diabetes. However, it would be interesting to know whether diazoxide exerts an effect on EGP in the presence of hepatic hyperinsulinemia in humans.

Diazoxide did not alter the rate of glucose disappearance in subjects with diabetes ($1.91 \pm 0.14 \text{ mg kg}^{-1} \text{ min}^{-1}$ with diazoxide vs. $1.96 \pm 0.17 \text{ mg kg}^{-1} \text{ min}^{-1}$ with placebo; $P = 0.77$) or in control subjects without diabetes ($2.24 \pm 0.38 \text{ mg kg}^{-1} \text{ min}^{-1}$ with diazoxide vs. $2.29 \pm 0.29 \text{ mg kg}^{-1} \text{ min}^{-1}$ with placebo; $P = 0.81$) (Fig. 4A). Of note, because these studies were conducted under basal insulin conditions, glucose infusion rates required to maintain euglycemia were minimal in all groups (subjects with diabetes: $0.43 \pm 0.17 \text{ mg kg}^{-1} \text{ min}^{-1}$ with diazoxide vs. $0.41 \pm 0.08 \text{ mg kg}^{-1} \text{ min}^{-1}$ with placebo, $P = 0.94$; control subjects without diabetes: $1.02 \pm 0.36 \text{ mg kg}^{-1} \text{ min}^{-1}$ with diazoxide vs. $0.65 \pm 0.20 \text{ mg kg}^{-1} \text{ min}^{-1}$ with placebo, $P = 0.24$) (Fig. 4B).

Rat Studies

Complementary rodent studies were performed in $n = 12$ ZDF rats (average weight, 354.5 ± 10.6 g). Due to limited sample volume from each rodent, pooled CSF collected at the end of the clamp studies was analyzed and demonstrated

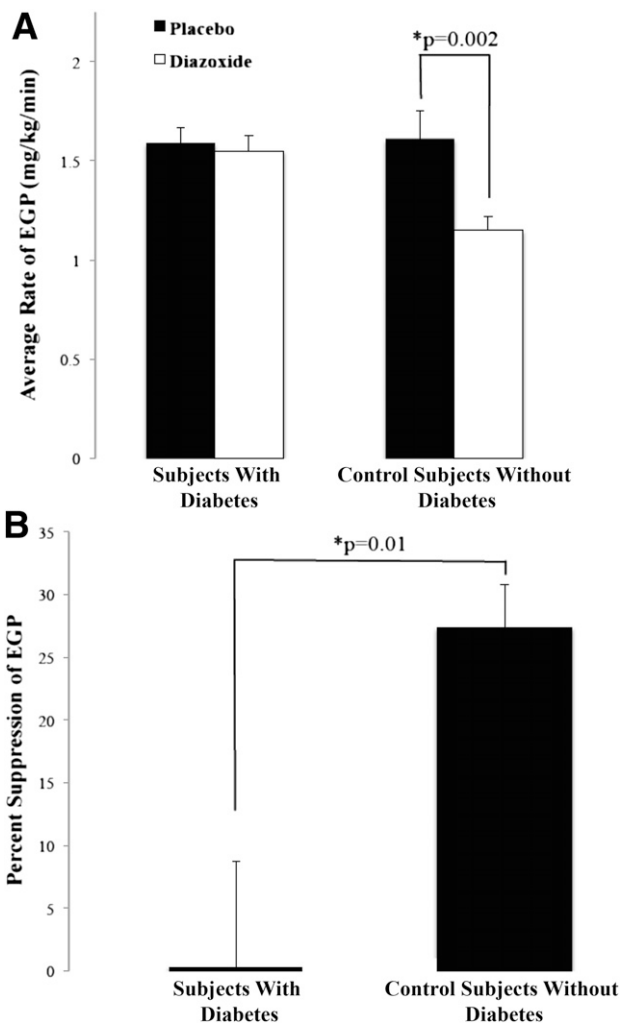


Figure 2—A: The average rate of EGP during the final hour of clamp studies was significantly suppressed with diazoxide administration relative to placebo administration in control subjects without diabetes ($*P = 0.002$) but not in subjects with diabetes. **B:** The percent suppression of EGP by diazoxide was significantly impaired in subjects with diabetes relative to control subjects without diabetes ($*P = 0.01$).

measurable levels of diazoxide 6 h after administration ($1 \mu\text{g/mL}$, with a reporting limit of $0.5 \mu\text{g/mL}$) by liquid chromatography-tandem mass spectrometry, comparable with CSF diazoxide levels reported in our studies in Sprague Dawley rats (15). Tail stick blood glucose levels the night before the clamp studies were elevated in both groups of ZDF rats prior to treatment with Neutral Protamine Hagedorn insulin (blood glucose, $409.5 \pm 50.3 \text{ mg/dL}$ in the diazoxide group and $474.2 \pm 44.3 \text{ mg/dL}$ in the saline group; $P = \text{not significant [NS]}$). During the steady-state phase of the clamp studies, average plasma glucose levels were similar between the diazoxide and saline groups ($143.1 \pm 1.3 \text{ mg/dL}$ with diazoxide vs. $145.9 \pm 1.0 \text{ mg/dL}$ with saline; $P = \text{NS}$). Insulin levels were also similar for the two groups during the steady-state phase of the clamp ($143.0 \pm 21.7 \mu\text{U/mL}$ with diazoxide vs. $130.41 \pm 35.87 \mu\text{U/mL}$ with saline; $P = \text{NS}$). Consistent with our

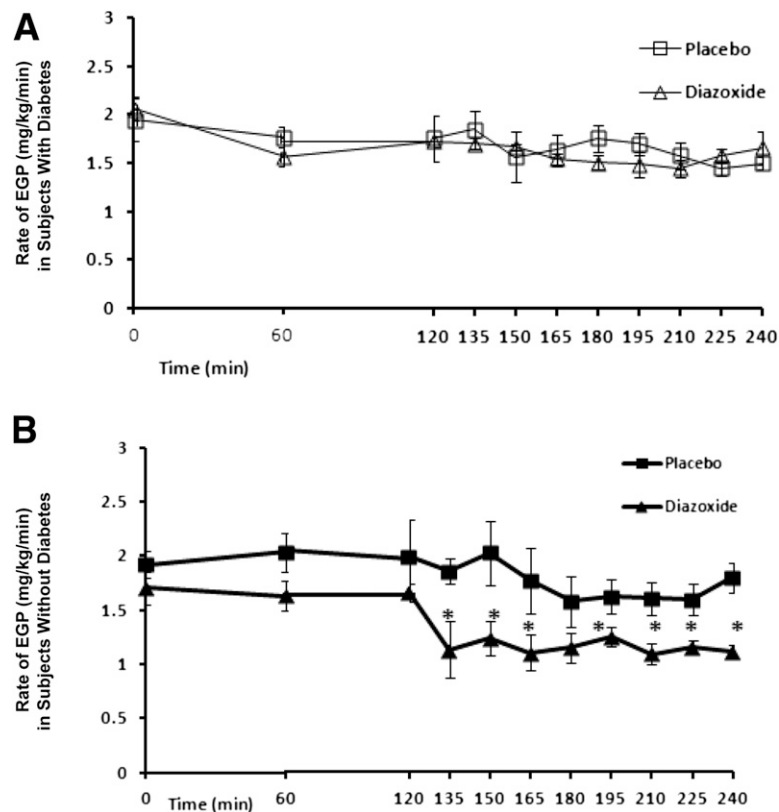


Figure 3—Time course of EGP during the clamp studies in subjects with diabetes (A) and in control subjects without diabetes (B) (* $P < 0.05$).

findings in human subjects with diabetes, there was no significant difference in average rates of EGP following administration of diazoxide compared with saline gavage (3.5 ± 0.9 mg kg⁻¹ min⁻¹ with diazoxide vs. 2.8 ± 0.8 mg kg⁻¹ min⁻¹ with saline; $P = 0.52$) (Fig. 5B).

Intriguingly, although these rates of EGP are lower than previously published clamp results in ZDF rats (32), clamp studies performed in the absence of glucagon infusion were associated with EGP rates that were similar to those observed in the current studies (M. Shiota, personal communication). The reason for performing these clamp studies without glucagon infusion was to reproduce study conditions previously used to examine the impact of diazoxide on central regulation of EGP (12,15).

Gene expression of hepatic gluconeogenic enzymes PEPCK and G6Pase also showed no significant differences following diazoxide versus saline administration (relative PEPCK gene expression: 1.12 ± 0.12 with diazoxide vs. 1.04 ± 0.15 with saline, $P = 0.68$; relative G6Pase gene expression: 0.10 ± 0.01 with diazoxide vs. 0.09 ± 0.02 with saline, $P = 0.72$) (Fig. 5C).

DISCUSSION

EGP is a critical component of the homeostatic mechanisms that maintain blood glucose at appropriate levels, and its dysregulation in type 2 diabetes contributes importantly to hyperglycemia. Given that activation of

extrapancreatic K_{ATP} channels is able to suppress EGP in both animal models and healthy humans (12,15), the current study examined the ability of the K_{ATP} channel activator diazoxide to regulate EGP in ZDF rats and humans with moderately to poorly controlled diabetes under fixed hormonal conditions. Our results indicate that central regulation of EGP is impaired in both rats and humans with type 2 diabetes.

Given the potential that age and obesity might impact central regulation of glucose metabolism (33), we specifically recruited a group of age- and BMI-matched subjects without diabetes as a comparison group for this study. Of note, the EGP response to diazoxide in these overweight, middle-aged subjects was consistent with our previous observations in younger, leaner subjects (15). An additional methodologic point pertains to diazoxide's ability to activate K_{ATP} channels in the plasma membrane of pancreatic β -cells, thereby inhibiting insulin secretion (34,35). Therefore, the current study used somatostatin, known to suppress insulin secretion via G-protein-coupled somatostatin receptors and inhibit intracellular calcium ion translocation (36). The absence of any differences in plasma hormone levels confirms the adequacy of the pancreatic clamp technique to allow us to isolate diazoxide's extrapancreatic effects.

Furthermore, because hyperglycemia would be expected to suppress EGP in the subjects with diabetes, it

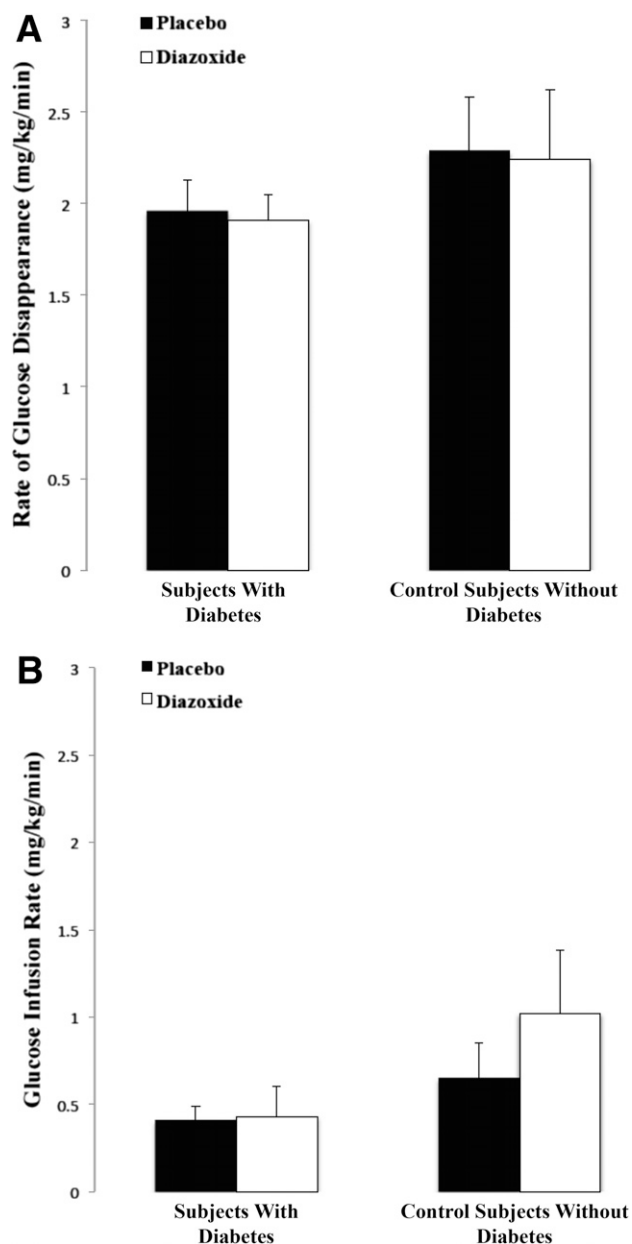


Figure 4—A: The average rate of glucose disappearance during the final hour of clamp studies did not significantly differ between placebo and diazoxide studies in either subjects with diabetes or control subjects without diabetes. **B:** The average glucose infusion rate during the final hour of clamp studies did not significantly differ between placebo and diazoxide studies in either subjects with diabetes or control subjects without diabetes.

was important to correct hyperglycemia prior to the onset of the clamp studies. Indeed, the overnight insulin infusions in the group with diabetes were designed to attain comparable basal rates of EGP and plasma glucose levels in the two groups. Additionally, insulin requirements progressively fell with correction of glucose toxicity overnight, such that insulin infusion rates averaged 0.24 ± 0.07 mU/kg/min by the final hour prior to the clamp studies (Supplementary Fig. 1), and rates of EGP were similarly

unsuppressed at the onset of the studies in both groups. Although we cannot exclude the possibility that some suppression of basal EGP by overnight insulin might have attenuated the effect of diazoxide on EGP in the diabetic rats and humans with diabetes, because hepatic hyperinsulinemia may mask CNS effects on the liver (37–39), it is important to note that insulin infusion rates were no greater than basal for >6 h prior to the study interval when EGP was calculated. Future studies using an SGLT2 inhibitor to lower glucose levels prior to the clamp could further address this question.

Furthermore, activation and deactivation of K_{ATP} channels are very rapid phenomena (40), making residual activating effects of insulin on K_{ATP} channels unlikely.

Of note, plasma insulin infusion rates were approximately doubled in the subjects with diabetes versus the subjects without diabetes in the placebo studies. This reflected mild insulin resistance despite correction of glucose toxicity in the subjects with diabetes. The comparable rates of EGP in both subject groups demonstrated that we were successful in selecting appropriate insulin infusion rates to study both groups under basal EGP conditions. Furthermore, attaining individualized, basal insulin infusion rates during the clamp studies avoided the need for virtually any exogenous glucose infusion and permitted highly sensitive measures of glucose production without overinsulinization (15,23).

The results of these studies in humans and rats are consistent with prior literature in rodents, suggesting that metabolic disturbances including those present in obesity and type 2 diabetes disrupt central regulation of glucose homeostasis. Inhibiting insulin action in the arcuate nucleus by a number of experimental approaches including insulin antibodies and inhibition of phosphatidylinositol 3-kinase results in a diminished ability of insulin to suppress EGP (41,42). Furthermore, insulin is unable to activate central K_{ATP} channels in obese rats (20), consistent with our findings in ZDF rats. Indeed, high-fat feeding of even short duration activates hypothalamic S6 kinase, a putative mediator of insulin resistance, which in turn impairs the ability of circulating insulin to suppress EGP (14). Additionally, hypothalamic signaling via the insulin receptor substrate–phosphatidylinositol 3-kinase pathway, an important mediator of insulin action, is impaired in rats with streptozotocin-induced diabetes (17). Of note, it is likely that poor metabolic control contributes to the lack of EGP response in our subjects with diabetes, because subjects with diabetes in comparably poor control showed a lack of suppression of EGP by hyperglycemia (likely mediated at least in part by central K_{ATP} channels), whereas subjects with diabetes in good control showed normal suppression of EGP (43). This is also consistent with the observation that centrally administered diazoxide was able to modulate EGP in high-fat-fed yet normoglycemic rats (41). Furthermore, although intranasal insulin (presumably via central mechanisms) increased hepatic energy metabolism and reduced lipid storage in healthy

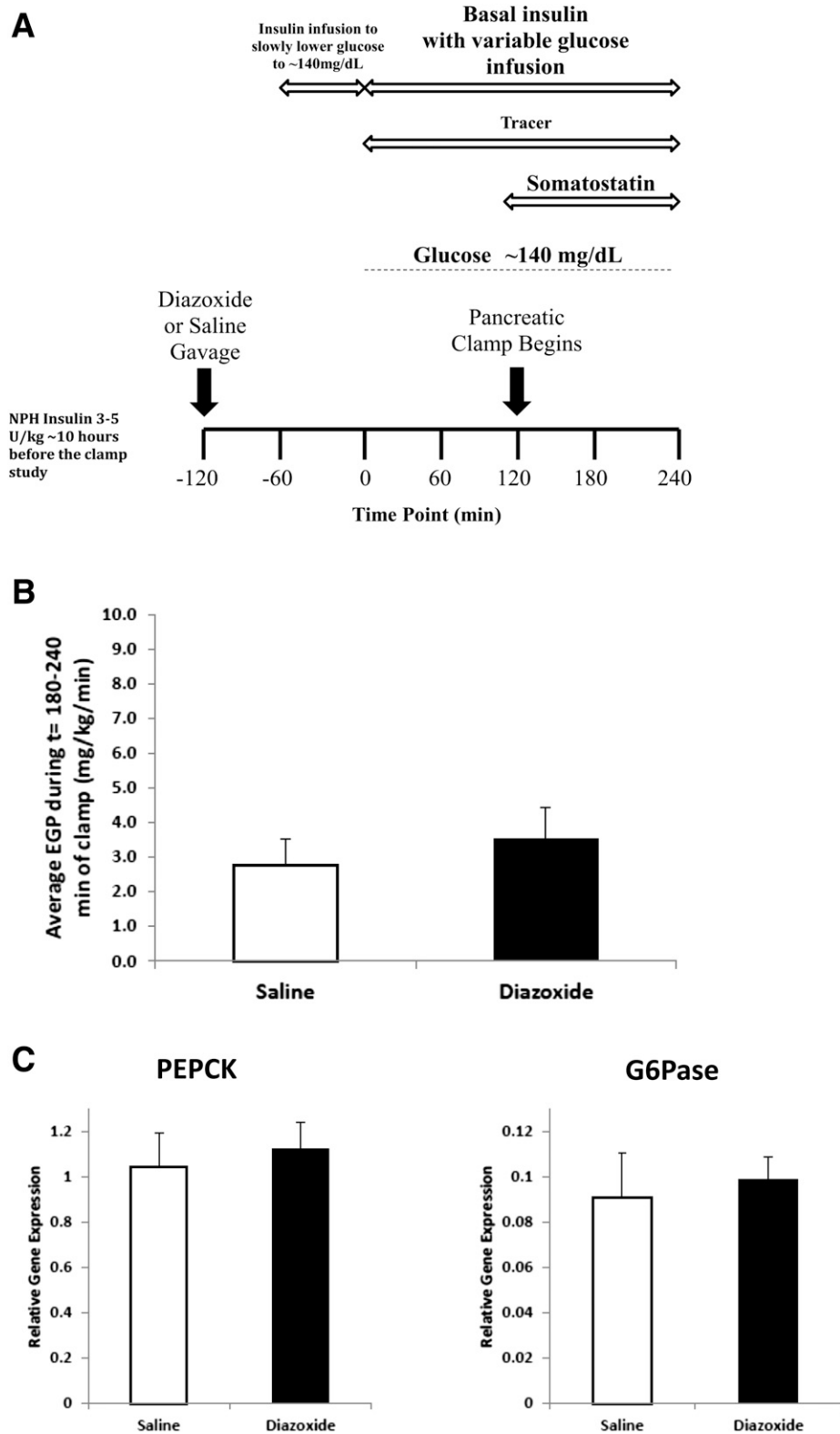


Figure 5—A: ZDF rat euglycemic pancreatic clamp protocol scheme. B: The average rate of EGP during the final hour ($t = 180$ – 240 min) of the study. EGP was not significantly suppressed in ZDF rats after diazoxide administration relative to saline control. C: Hepatic PEPCK and G6Pase gene expression levels in diazoxide-treated and saline-treated ZDF rats.

humans, this effect was absent in patients with type 2 diabetes (44).

Collectively, these findings highlight the need for interventions to restore central signaling mechanisms. Various molecular targets have been proposed that could restore the brain's sensitivity to nutrients in type 2 diabetes. These targets include regulators of insulin and leptin action (such as protein tyrosine phosphatase 1B, c-Jun-N terminal kinase, and SRC homology 2B) as well as peripheral modulators of glucose metabolism (such as glucagon-like peptide-1) (42). A critical challenge lies in developing medications that specifically target the brain without acting peripherally. One potential model is the established treatment for Parkinson disease, L-dopa, which is delivered with a peripheral decarboxylase inhibitor to prevent it from being metabolized prior to passing through the blood-brain barrier (45). In fact, even short-term improvement in an individual's metabolic state may restore the integrity of the brain-liver pathway in type 2 diabetes (43). It will also be important to identify the critical stage(s) at which therapies targeting restoration of the brain-liver pathway would be most beneficial. Although delineating whether central or hepatic mechanisms are responsible for the lack of response in diabetes was not the goal of the current studies, we hope to perform future studies specifically designed to delineate the central and/or peripheral site(s) at which the response is altered.

Although it has long been accepted that type 2 diabetes is associated with malfunctions of the β -cell, muscle, and liver, growing evidence suggests that the brain also plays a key role in the pathogenesis of type 2 diabetes. The results of the current study are also relevant to recent work demonstrating that neurologic disorders such as Alzheimer disease, major depressive disorder, Parkinson disease, Huntington disease, and vascular dementia are all associated with metabolic derangements. Specifically, these diseases feature impaired systemic glucose metabolism and insulin resistance, as well as poor cerebral glucose utilization, cerebral insulin resistance, cerebral insulin deficiency, and abnormal expression of genes that are typically regulated by insulin (46–49). Indeed, a great deal of evidence supports a role for the brain in metabolic disease and, conversely, for metabolic derangements in the pathophysiology of neuropsychiatric disorders. This work also highlights a potential concern with the use of sulfonylurea agents in the long-term treatment of type 2 diabetes, especially in patients with waning β -cell reserve. Inhibition of central K_{ATP} channels by these agents could increase EGP and hence contribute to deterioration of glycemic control in these individuals (50).

Thus, we present the first study in humans to show that regulation of EGP through activation of extrapancreatic K_{ATP} channels is impaired in type 2 diabetes, providing further insight into the CNS basis for the pathogenesis of metabolic disorders. Complementary rodent studies in a rat model of type 2 diabetes support our findings in humans. Given that unrestrained EGP is the chief source of

hyperglycemia in type 2 diabetes, restoring the brain's sensitivity to nutrient signals would be a promising therapeutic target.

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