Exenatide Regulates Cerebral Glucose Metabolism in Brain Areas Associated with Glucose Homeostasis and Reward System

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Abstract

GLP-1 receptors have been found in the brain, but it currently is unknown whether GLP-1R agonists (GLP-1RA) influence brain glucose metabolism. The study aim was to evaluate the effects of a single injection of the GLP-1RA, exenatide, on cerebral and peripheral glucose metabolism in response to a glucose load.

In 15 male subjects with HbA1c=5.7±0.1%, fasting glucose 114±3mg/dl and 2h-glucose 177±11mg/dl, exenatide (5 µg) or placebo were injected in double blind, randomized fashion subcutaneously 30 min before an oral glucose tolerance test (OGTT). Cerebral glucose metabolic rate (CMR$_{\text{glu}}$) was measured by PET following injection of $^{18}$F-FDG before OGTT and rate of glucose absorption (RaO) and disposal were assessed using stable isotope tracers.

Exenatide reduced RaO$_{0-60\text{min}}$ (4.6±1.4 vs. 13.1±1.7 µmol/min-kg) and decreased the rise in mean glucose$_{0-60\text{min}}$ (107±6 vs. 138±8 mg/dl) and insulin$_{0-60\text{min}}$ (17.3±3.1 vs. 24.7±3.8 mU/l). Exenatide increased CMR$_{\text{glu}}$ in areas of the brain related to glucose homeostasis, appetite and food reward, despite lower plasma insulin concentrations, but reduced glucose uptake in the hypothalamus. Decreased RaO$_{0-60\text{min}}$ after exenatide was inversely correlated to CMR$_{\text{glu}}$.

In conclusion, these results demonstrate, for the first time in man, a major effect of a GLP-1RA on regulation of brain glucose metabolism in the absorptive state.

Trial registration. ClinicalTrials.Gov. NCT01588418

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**Introduction**

Increasing experimental and clinical evidence suggest that brain glucose metabolism is altered in patients with type 2 diabetes mellitus (T2DM) and contributes to the disturbance in whole body glucose homeostasis (1; 2).

The brain receives multiple inputs including: (i) hormones such as leptin, ghrelin, peptide YY (PYY), glucagon like peptide-1 (GLP-1), insulin; (ii) nutrient-related signals that regulate fat accumulation in adipose tissue and hepatic and peripheral glucose metabolism; (iii) gastrointestinal tract (1; 2).

GLP-1 receptors have been found in several brain areas (3). Intestinal cells secrete GLP-1 in response to glucose and both deficiency of and resistance to GLP-1 have been described in T2DM patients (4; 5). The main actions of GLP-1 are to stimulate insulin and inhibit glucagon secretion, although other pleiotropic effects of GLP-1 have been described (2; 5). Secreted GLP-1 can gain access to central nervous system (CNS), bind to GLP-1 receptors, and modulate neuronal activity in different areas of human brain (3; 6). Following administration of radiolabelled GLP-1, non-saturable uptake has been demonstrated in CNS (6). We have shown that native GLP-1, secreted in response to meal, potentiates insulin secretion and glucose sensing (7) and inhibits hepatic glucose production without altering peripheral glucose disposal (8).

Although GLP-1 and some GLP-1 receptor agonists (GLP-1RA) can cross the blood brain barrier (9; 10), their effects on CNS are poorly understood. Exenatide, a GLP-1RA, readily crosses the blood brain barrier, even more efficiently than native GLP-1 (10). Both liraglutide and lixisenatide accumulate in mouse brain increase functional hypothalamic connectivity in brain areas involved in glucose homeostasis (11). Most previously published studies were performed under fasting conditions or
during intravenous glucose and/or insulin administration and not following meal or glucose ingestion, which represents the normal physiologic route. In the present study, we examined whether subcutaneous exenatide injection stimulates cerebral, as well as peripheral, glucose metabolism in prediabetic/newly discovered diabetic patients using PET and stable isotope tracers to measure glucose fluxes.

**Methods**

**Subjects**

Fifteen male subjects with HbA1c 5.7-6.4%, fasting plasma glucose (FPG) 100-125 mg/dl and 2h plasma glucose (2h-PG) during OGTT>140 mg/dl were included in this randomized, double-blind, placebo-controlled trial (ClinicalTrial.gov, clinical trial reg. no. NCT01588418). Protocol was approved by IRB of University of Texas Health Science Center at San Antonio and informed written consent was obtained from all subjects prior to participation.

The primary aim was to examine the acute effect of exenatide on brain glucose metabolism after glucose load. The relation between brain glucose metabolism and postprandial peripheral and hepatic glucose metabolism was secondary aim.

**Study Protocol**

The study design, represented in Figure 1, is a randomized, double-blinded, placebo-controlled crossover study. After screening, subjects underwent two Positron Emission Tomography (PET) scans during a double tracer 75g-OGTT as previously described (7). Prior to double tracer OGTT subjects received subcutaneous injection of exenatide (EX) or placebo (PLC) in random order. The two OGTTs were completed within 26 days.
A primed-constant infusion of [6,6-2H₂]glucose was started at T = -120 min and continued throughout the study to measure total rate of glucose appearance (RaT), rate of glucose disposal (Rd) and glucose clearance (Cglu) (**Figure 1**). At T = -30 minutes EX (5µg) or PLC (Amylin Pharmaceuticals, San Diego, CA) was injected and subject was positioned in PET scanner. At T=0 subjects received intravenous injection of 185 ± 37 MBq (5 mCi) of [{¹⁸}F]-2-fluoro-2-deoxy-D-glucose (¹⁸F-FDG) over 15s while laying supine and PET scanning was started. Simultaneously with FDG, subjects drank 75 g glucose containing 1.5 g of U-¹³C₆-glucose in 5 minutes (**Figure 1**) to measure endogenous glucose production (EGP). Arterialized venous samples were drawn for determination of plasma ¹⁸F-FDG radioactivity and glucose concentrations at -10, 0, 5, 10, 15, 30, 45, 60, 65, 70, 75, 80, 85, 90, 105, 120 minutes. Plasma insulin and tracer enrichments were measured every 15 minutes as previously described (7). Urine was collected at end of OGTT, and urine volume, FDG radioactivity and glucose concentrations were measured.

**Positron Emission Tomography**

At T=0 min a dynamic scan of the heart was performed (34 frames, 12 x 120, 6 x 180, 5 x 300, 4 x 600, 6 x 1800, 1 x 600 s). At T=60 min a 2D dynamic brain scan was performed (6 frames of 300 seconds each), followed by a 10 min static 3D scan using a 63-slice Siemens/CTI HR+ scanner. Regions of interest (ROI) were drawn in the left ventricular heart chamber for radioactivity measurement in arterial blood and in cortical and subcortical grey-matter regions of brain. In addition, 2 main ROIs were defined to pool brain regions/nuclei involved in specific and similar brain functions: (i) glucose homeostasis regulation areas comprised of the nucleus of solitary tract, brainstem, insula, putamen, caudate, amygdala, limbic system,
hypothalamus, and (ii) food reward system comprised of orbitofrontal lobe, thalamus, anterior and posterior cingulate, putamen, caudate, amygdala, limbic system.

**Imaging and data analysis**

Cerebral glucose metabolic rate (CMR\textsubscript{glu}) was calculated from the slope $K_i$ of the linear phase of Gjedde-Patlak plot. To account for FDG/substrate competition, we used a model based on the integral of plasma specific activity, $SA(t)$, rather than of plasma FDG concentration.

$$\frac{C_t(t)}{C_p(t)} = K_i \cdot \frac{\int_0^t SA(z)dz}{C_p(t)} + \text{const.}$$

where $C_t$ and $C_p$ are tissue and plasma radioactivity levels at time ($t$), $SA(t)=C_p(t)/G(t)$ and $K_i$ represents the uptake rate ($\mu\text{mol/ml min}$) (see online appendix for details). CMR\textsubscript{glu} is calculated as $K_i$ divided by appropriate lumped constant (LC=0.81).

$Ra_T$, $R_d$, and $C_l$glu were calculated from 6,6-$^2$H-glucose kinetics and $Ra_O$ from U-$^{13}$C-glucose kinetics using nonsteady state modeling, as previously described (7).

Data are expressed as mean±SE. Comparison between study variables was performed using parametric or nonparametric tests (e.g., paired t-test or Wilcoxon test) for variables normally or non-normally distributed, respectively. Non-normally distributed variables were log transformed (natural logarithm) before correlation analysis.

**Results**

*Subject Characteristics.* We studied 15 male subjects, age=57±2 years, BMI=29.4±0.9 kg/m\textsuperscript{2}, with FPG=114±3 mg/dl and 2h-PG=177±11 mg/dl. The group
included 10 IFG/IGT, 2 IGT and 3 newly discovered T2DM during screening OGTT. At screening HbA1c was 5.5±0.1 in IGT and 5.7±0.2% in T2DM (p=0.45), and fasting plasma insulin (FPI) was 10.4±1.4 mU/l.

Effect of Exenatide on Peripheral Glucose Metabolism.

Exenatide did not affect either the FPG or FPI. At T=0, i.e., 30 min after EX or PLC, FPG=105±8 and 105±3 mg/dl, respectively, and FPI=8.2±1.4 and 7.0±1.3 mU/l, respectively.

During 2 hour OGTT, plasma glucose concentration remained close to fasting level following EX, while it rose progressively to ~200 mg/dl following PLC. In both EX and PLC, plasma insulin levels increased, reaching a plateau between 60-120 min, with an ~2-fold greater rise in PLC versus EX. The lower rise in plasma glucose following EX was explained by a significant delay/reduction in postprandial glucose absorption (RaO\textsubscript{0-120} = 5.5±1.4 vs 15.4±1.2 µmol/min•kg). Glucose clearance was similar in EX and PLC studies (Cglu\textsubscript{0-120} 1.8±0.1 vs 1.7±0.1 ml/min•kg).

Effect of Exenatide on Brain Glucose Metabolism

Cerebral glucose metabolic rate (CMR\textsubscript{glu}) during OGTT, quantitated by \textsuperscript{18}F-FDG-PET, was significantly increased in EX versus PLC in total gray matter, total cortex and collectively in the brain areas involved in glucose homeostasis regulation (frontal, occipital, temporal, parietal lobes, limbic system, insula, putamen) and food reward system (orbitofrontal lobe, thalamus, anterior and posterior cingulate) (Figure 2). In contrast, CMR\textsubscript{glu} decreased significantly in hypothalamus following EX (Figure 2).
Correlations Between Brain and Peripheral Glucose Metabolism

Glucose RaO and RaT correlated strongly and negatively with brain areas involved in glucose homeostasis regulation ($r=-0.80$ and $r=-0.76$, $p<0.0005$; respectively) and food reward system ($r=-0.75$ and $r=-0.75$, $p<0.0008$; respectively) after EX, but not after PLC (Figure 3, Table 1). This negative correlation was most pronounced in the insula, thalamus, limbic system, orbitofrontal, occipital, parietal and temporal lobe, anterior and posterior cingulate and nucleus of solitary tract (all $r>0.60$, $p<0.01$, Table 1); no significant correlations were observed with other areas or after PLC and no correlation was observed between CMR$_{glu}$ and EGP, glucose Rd, or clearance (Cglu).

Discussion

The present results demonstrate for the first time that, following glucose ingestion, exenatide has profound effects on brain glucose metabolism in male subjects with mild postprandial hyperglycemia. Compared to PLC, EX stimulated CMR$_{glu}$ in brain areas involved in regulation of glucose homeostasis and associated with reward system/satiety/food intake, including orbitofrontal lobe, thalamus, insula, posterior cingulate, putamen and limbic system after a glucose load.

In contrast to previous studies that infused GLP-1 during pancreatic euglycemic (12) and hyperglycemic clamps (13), our study evaluated the effect of GLP-1 under physiologic conditions by combining $^{18}$F-FDG with stable isotope tracer infusion during OGTT and quantitated CMR$_{glu}$, rate of oral glucose absorption and total glucose appearance. We found that RaO and RaT were inversely correlated with CMR$_{glu}$ but only after EX and not after PLC. These findings could explain the appetite-suppressant effect of GLP-1RAs.
A recent paper in mice generated with either neuronal/visceral nerve-specific deletion of GLP-1R showed that CNS, but not peripheral nervous system GLP1-Rs are necessary for weight loss and anorexogenic effect of liraglutide, while the glucose lowering-effects were independent of CNS activation (14). The results are in agreement with ours since we found a correlation between CMR_{glu} and delayed oral glucose appearance but no correlation with suppression of EGP or peripheral glucose clearance.

Our study is the first in humans to show that EX injection, prior to an oral glucose load, increases glucose uptake in cerebral tissues involved in regulating peripheral glucose metabolism and food intake, despite reduced insulin concentrations compared to placebo. The protocol reflects the effect of normal route of exenatide administration (sc) on the normal route of glucose administration (oral). Previous studies used intravenous infusion of glucose, GLP-1 and somatostatin, and found a trend to decrease in CMR_{glu} during normoglycemia (12) and an increase in CMR_{glu} during hyperglycemia (13). However, in the Gejl study (13) insulin concentrations were three times those observed during placebo, and thus a separate effect of insulin could not be excluded. It is likely that brain glucose uptake might result potentiated after a long acting GLP-1RA, given the higher insulin response after a meal.

Of note, we observed a significant decrease in CMR_{glu} in the hypothalamus. Similarly, a previous study demonstrated that GLP-1 infusion under euglycemic conditions reduced CMR_{glu} in hypothalamus and brainstem (3). We previously demonstrated, using fMRI, that glucose ingestion was associated with a rapid inhibition of MRI signal in several hypothalamic nuclei before any changes in plasma glucose concentration occurred (15). Therefore, we hypothesize that exenatide, in absence of change in plasma glucose concentration, suppresses CMR_{glu} in
hypothalamic neurons through GLP-1 receptor activation and glucokinase inhibition, as previously suggested (3; 16). It has been shown that hypothalamic neurons act as glucose sensors of extracellular glucose concentration and modulate their firing activity accordingly (16; 17). Further, reduced glucokinase activity in arcuate nucleus of mouse hypothalamus is associated with reduced food intake (18). This could represent an important mechanism via which GLP-1RAs modulate appetite. However, the study could distinguish between direct and indirect brain-mediated GLP-1 effects, but provides evidences of what happens in .

Our study has several potential limitations. First, during glucose ingestion is difficult to selectively clamp insulin and/or glucose concentrations and thus evaluate the separate contributions. Insulin crosses the blood brain barrier via saturable transport system (19). In normal glucose tolerant subjects, some studies have failed to show any effect of insulin on brain glucose metabolism (20), while other studies have demonstrated that insulin increases CMR$_{glu}$ (21). However, in our study, the rise in both plasma insulin and glucose concentrations following EX was reduced compared to placebo making this an unlikely explanation for the increase in CMR$_{glu}$. Instead, the observed EX effect may have been even underestimated, and group differences may have been further amplified if insulin and glucose levels had been as elevated in EX as they were in placebo studies. Second, 3 of the 15 subjects were found to have diabetes (mean±SD 2h-PG = 230±4 mg/dl) during the OGTT. However, the effect of EX on brain glucose metabolism was similar whether or not these 3 subjects were included. Third, our study included only males. Fourth, brain imaging was initiated at T=60 minutes, so we could examine only mean brain glucose uptake and not the time course of the effect of EX.
In conclusion, our results demonstrate that, following glucose ingestion, exenatide exerts a profound effect on glucose metabolism in multiple brain areas involved in regulation of glucose homeostasis and the reward system. Further studies are warranted to investigate whether similar results will be observed in T2DM and obese nondiabetic subjects following both acute and chronic GLP-1RA administration.

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AUTHOR CONTRIBUTIONS:
A.G. 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. A.G. study design; G.D., M.M.C., C.T. performed the studies; G.D., P.I., J.L., P.F., A.G., D.C. data analysis; G.D., P.I., P.F., N.M., D.T., C.T., R.A.D., A.G., contributed to discussion; G.D., P.I., R.A.D., A.G. wrote the manuscript. All authors reviewed the manuscript prior to submission. RAD’s salary is, in part, provided by the South Texas Veterans Administration-Audie Murphy Division.
DISCLOSURE


E.C. Speaking engagement for Takeda and Janssen.

C.T. Speaker's Bureau: Astra Zeneca.

R.A.D. Advisory Board: Amylin, Takeda, Bristol Myers Squibb, Astra Zeneca, Novo Nordisk, Janssen, Lexicon, Boehringer-Ingelheim; Research Support: Amylin, Bristol Myers Squibb, Boehringer-Ingelheim, Takeda; Speaker's Bureau: Novo-Nordisk, Bristol Myers Squibb, Astra Zeneca, Janssen.


No other potential conflicts of interest relevant to this article were reported. Amylin-BMS-AZ played no role in the study design, data collection/analysis, or manuscript preparation/review.
References


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FIGURE LEGEND

**Figure 1.** Protocol of the study: each subject underwent two studies, one with Exenatide and one with Placebo (in double blind order). In each session, tracer infusion and oral glucose tolerance test (75 gr OGTT) were performed.

**Figure 2.** Top panel, Image of FDG brain glucose uptake during placebo (A, C, D) and Exenatide (B, E, F) in one study subject. Lower panel (G), mean cerebral glucose uptake after oral glucose load (OGTT) was increased after exenatide (black bars) compared to placebo (white bars) in total grey matter, cortex, areas involved in glucose homeostasis and food reward but not in hypothalamus where glucose uptake was reduced.

**Figure 3.** Top Panels: Correlation between oral glucose Ra (panels A) or systemic glucose Ra (Panels B) and cerebral glucose uptake in brain regions associated with glucose homeostasis (left panels) or food reward (right panels). (Data were log transformed before analysis). Bottom panels (C): mean insulin and glucose concentrations during the first hour of OGTT; mean glucose fluxes, i.e., oral rate of appearance (RaO), endogenous glucose production (EGP) and peripheral glucose disposal (Rd) during the first hour of OGTT.
Table 1. Correlations between Cerebral Glucose Metabolic Rate and Oral (RaO) or Total (RaT) Rate of Glucose Appearance in different brain areas

<table>
<thead>
<tr>
<th>CMRglu µmol/(ml min)</th>
<th>Placebo RaO</th>
<th>Exenatide RaO</th>
<th>Placebo RaT</th>
<th>Exenatide RaT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p value</td>
<td>r</td>
<td>p value</td>
</tr>
<tr>
<td>Total Gray Matter</td>
<td>-0.22 0.46</td>
<td>-0.77 0.0005</td>
<td>-0.33 0.25</td>
<td>-0.78 0.0003</td>
</tr>
<tr>
<td>Cortex</td>
<td>-0.19 0.53</td>
<td>-0.44 0.10</td>
<td>-0.37 0.19</td>
<td>-0.54 0.035</td>
</tr>
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<td>Glucose Homeostasis</td>
<td>-0.23 0.46</td>
<td>-0.80 0.0001</td>
<td>-0.28 0.36</td>
<td>-0.76 0.0005</td>
</tr>
<tr>
<td>Reward System</td>
<td>-0.18 0.56</td>
<td>-0.75 0.0008</td>
<td>-0.22 0.48</td>
<td>-0.75 0.0007</td>
</tr>
<tr>
<td>Solitary Nucleus</td>
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<td>-0.67 0.0075</td>
<td>-0.19 0.54</td>
<td>-0.65 0.010</td>
</tr>
<tr>
<td>Brainstem</td>
<td>-0.09 0.76</td>
<td>-0.31 0.27</td>
<td>-0.25 0.40</td>
<td>-0.44 0.10</td>
</tr>
<tr>
<td>Insula</td>
<td>-0.40 0.18</td>
<td>-0.66 0.006</td>
<td>-0.31 0.32</td>
<td>-0.65 0.008</td>
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<tr>
<td>Putamen</td>
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<td>-0.43 0.11</td>
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<tr>
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<td>-0.30 0.28</td>
<td>-0.51 0.06</td>
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<td>Amygdala</td>
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<td>-0.32 0.25</td>
<td>-0.07 0.81</td>
<td>-0.23 0.41</td>
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<tr>
<td>Limbic System</td>
<td>-0.12 0.71</td>
<td>-0.78 0.0003</td>
<td>-0.08 0.80</td>
<td>-0.70 0.002</td>
</tr>
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<td>Hypothalamus</td>
<td>-0.38 0.19</td>
<td>-0.19 0.50</td>
<td>-0.48 0.08</td>
<td>-0.36 0.19</td>
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<tr>
<td>Thalamus</td>
<td>-0.19 0.52</td>
<td>-0.70 0.002</td>
<td>-0.07 0.80</td>
<td>-0.65 0.007</td>
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<tr>
<td>Anterior Cingulate</td>
<td>-0.21 0.51</td>
<td>-0.69 0.003</td>
<td>-0.36 0.24</td>
<td>-0.65 0.007</td>
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<td>-0.75 0.0007</td>
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<td>-0.25 0.40</td>
<td>-0.78 0.0003</td>
</tr>
<tr>
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<tr>
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<td>-0.61 0.015</td>
<td>-0.27 0.36</td>
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<td>Temporal Lobe</td>
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<td>-0.80 0.0002</td>
<td>-0.22 0.45</td>
<td>-0.76 0.0005</td>
</tr>
</tbody>
</table>

Data were log-transformed before analysis
Heart scan (input function)

Oral load
(75g of glucose plus 1.5g of U^{13}C-glucose)

[^{18}F]FDG 5 mCi IV injection

-120  -30   0   30   60   90  120min

5mcg Exenatide or Placebo SC injection

[6,6-^{2}H_{2}] glucose primed-continuous IV infusion

Blood drawn for plasma radioactivity counts

Brain scan

Oral load

Diabetes
Cerebral Glucose Uptake

**Placebo**  **Exenatide**

- **Total Grey Matter**
- **Total Cortex**
- **Glucose Homeostasis**
- **Food Reward**
- **Hypothalamus**

*Statistically significant difference.
Diabetes

A

Ln of CMRglu in Glucose Homeostasis Regulation (nmol/ml min)

Ln of CMRglu in Food Reward System (nmol/ml min)

B

Ln of Tot Ra (umol/min/kg)

Ln of CMRglu in Glucose Homeostasis Regulation (nmol/ml min)

Ln of CMRglu in Food Reward System (nmol/ml min)

C

Insulin

Glucose

EGP or Ra

Rd

Placebo  Exenatide
Online appendix

Subjects inclusion/exclusion criteria

Inclusion criteria were: (i) age = 18-65 years; (ii) BMI=25-40 kg/m²; (iii) FPG = 100-125 mg/dl or HbA1c = 5.7-6.4%; (iv) 2-hour glucose concentration above 140mg/dl after 75g glucose load, i.e., having impaired glucose tolerance or newly diagnosed type 2 diabetes. Exclusion criteria included: (i) previous treatment with medications known to affect glucose metabolism (ii) blood pressure > 140/90 mmHg; (iii) serum creatinine > 1.6 mg/dl; (iv) hematocrit < 35%, (v) evidence of major organ system disease as determined by medical history, physical exam, and routine screening blood chemistries.

Positron Emission Tomography

PET Scanner: Scanning was performed using a Siemens/CTI HR+ scanner (Siemens, Inc, Knoxville, TN) which is a 63-slice, high-sensitivity, high-resolution (4.1 mm in 3D mode) whole body scanner capable of 2D and 3D data acquisition. The sensitivity of the scanner at the center of field of view is 5.24 cps/kBq in 2D and 36.57 cps/kBq in 3D mode.

PET Imaging: The position of the subject was optimized in the scanner in order to scan the heart and a 20-min transmission scan was obtained after exposure of a retractable ⁶⁸Ge ring source to correct all subsequent emission data for tissue attenuation of γ-photons. Then, all subjects received an intravenous injection of 185 ± 37 MBq (5 mCi) of ¹⁸F-FDG, while lying supine on the scanner bed at T=0. At the same time of the injection of ¹⁸F-FDG subjects consumed a flavored drink containing 75 grams of glucose while lying in the supine position. At T=0 minutes a 60 minute dynamic scan was obtained to image the heart (34 frames, 12 x 120, 6 x 180, 5 x 300, 4 x 600, 6 x 1800, 1 x 600 s). At T=60 minutes the PET scanner was focused on the head. A transmission scan of the brain to serve as attenuation correction map and anatomic reference was performed and followed by 2D dynamic imaging (6 frames of 300 seconds each), and then by a 10-minute static 3D scan. Arterialized blood
samples were collected throughout the scan to measure whole blood and plasma $^{18}$F-FDG radioactivity over time.

**Image Processing:** Initially, each subject’s brain PET images were spatially normalized to support the use of standard ROI. Thus, brain images were registered within the Talairach brain space to properly label the anatomical brain areas. This was a two-step process in which low-resolution PET images were aligned with high-resolution anatomical MR images of reference and MR images were aligned with the high-resolution brain template. Alignment transforms for the PET images (PET-to-MRI) were concatenated with those of the MR images (MRI-to-Talairach) to register the PET images in Talairach space. Linear affine transforms were used for alignment (1). Standard regions of interest defined in Talairach space were then used to analyze regional $^{18}$F-FDG levels within the registered PET images. The primary focus of the study was on cortical and subcortical grey-matter regions of the brain. Brain ROIs were drawn in the nucleus of solitary tract, brainstem, insula, putamen, caudate, amygdala, limbic system, hypothalamus, orbitofrontal lobe, thalamus, and the anterior and posterior cingulate cortices. In addition, 2 main ROIs were defined to pool brain regions/nuclei involved in specific and similar brain functions: (i) glucose homeostasis regulation areas comprised of the nucleus of solitary tract, brainstem, insula, putamen, caudate, amygdala, limbic system and hypothalamus, and (ii) food reward system comprised of orbitofrontal lobe, thalamus, anterior and posterior cingulate, putamen, caudate, amygdala and limbic system.

Regional analysis was performed using the ROI analysis tool implemented in the Java-3D based program Mango developed by one of the authors (1) (http://ric.uthscsa.edu/mango/). Each ROI was further optimized in terms of boundary accuracy using morphological operations. For each brain region evaluated, the ROI was drawn with the same volume in EX and PLC scans for each participant to allow comparison between treatments and to evaluate the effect of EX on $^{18}$F-FDG levels.
Radioactivity concentrations, measured in arterialized plasma samples over time, were used as the input function for calculations of brain glucose uptake, as detailed below. These were integrated by image-derived blood activity data in the first few minutes after injection. Regions of interest (ROI) were drawn in the left ventricular chamber of the heart for the measurement of radioactivity in arterial blood; special attention was paid to avoiding contamination from surrounding myocardial tissue. Image-derived blood values over time were converted into plasma concentrations using the hematocrit, according to Phelps et al. (2). Later points of the curve were corrected for spillover by *in vitro* measurement of arterialized plasma radioactivity.

**Calculation of brain glucose uptake rate using a modified Gjedde-Patlak analysis**

The Gjedde-Patlak plot is a model-independent approach that enables one to calculate the net transfer rate $K_i$ (influx constant), independent of the number of compartments (3-5), Figure A1. For brain studies the net transfer rate $K_i$ (influx constant) is often calculated using a few time frames from the later times assuming that equilibrium is achieved and the relationship is linear (6).

![Figure A1. Two-compartment model by Patlak et al (4) where Cp represents plasma $^{18}$F-FDG radioactivity, Ct is the tissue radioactivity, and $K_i$ (min$^{-1}$) is the fractional uptake rate.](image)

The Gjedde-Patlak plot has the integral of the blood $^{18}$F-FDG concentration from time 0 to t min (kBq × min/ml) normalized by blood $^{18}$F-FDG concentration on the X axis vs. brain tracer concentration (for example from 60-90 min (kBq/ml) normalized by blood $^{18}$F-FDG concentration at time t on the Y axis:}
\[
\frac{C_t(t)}{C_p(t)} = K_i \cdot \int_0^t \frac{C_p(z)}{C_p(t)} \, dz + \text{const.}
\] 

equation A1

where \( C_t \) and \( C_p \) are tissue and plasma radioactivity levels at each sampling time point \( t \).

The slope of the linear phase represents the fractional uptake rate \((K_i, \text{min}^{-1})\), i.e., \( K_i \) represents the amount of accumulated tracer in relation to the amount of tracer that has been available in plasma.

The Gjedde-Patlak analysis was developed to measure tissue glucose uptake of FDG under conditions of constant plasma glucose concentrations, either during fasting or during a hyperinsulinemic euglycemic or hyperglycemic clamp. The brain glucose uptake rates (CMR\(_{\text{glu}}\) expressed in \( \mu \text{mol/min per ml of tissue} \)) are obtained by multiplying times the plasma glucose concentration (\( G(t) \)) divided by appropriate lumped constant (LC=0.81) (7):

\[
\text{CMR}_{\text{glu}} = K_i \cdot \frac{\text{mean}G(t)}{LC}
\]

equation A2

However, this does not take into account possible changes in blood glucose concentration during the period before starting brain image acquisition (i.e., from 0-60min).

In this set of experiment, we infused the same dose of FDG under two different conditions (ie during exenatide or placebo injection 30 min before administration of \( ^{18}\text{F-FDG} \)) that generated different changes in plasma glucose and insulin concentrations but similar systemic rates of glucose disposal (See Figure A2).
Figure A2. Panel A: plasma $^{18}$F-FDG Concentration Following Acute IV Administration. Plasma glucose (Panel B) and insulin (Panel C) concentrations and systemic glucose disposal (Panel D) during the first 60 min after FDG bolus and oral glucose ingestion are displayed.

When glucose concentrations are changing several approaches have been used (8). The previously proposed methods multiply Ki times the average glucose concentration. Since changes in plasma glucose concentration were time dependent and different in placebo vs. EX studies, this approach did not account for real tracer(tracee) competition. The great majority of $^{18}$F-FDG clearance occurred during the first 5-10 min after $^{18}$F-FDG injection, when plasma insulin and glucose concentrations were not different from baseline and were superimposable in both studies. Higher plasma glucose concentrations in the placebo versus Exenatide group were observed only after 30 minutes of FDG injection. This allows one to hypothesize that substrate competition was similar in the two studies during the first 5-10 min, when most of $^{18}$F-FDG disappeared from the plasma, while after 30 minutes a higher glucose concentration in the placebo group could lead to reduced tissue FDG uptake. This is justified by the fact that during both studies systemic Rd was similar and not substantially different from baseline (figure 3), due to small changes in the plasma insulin concentration.
In order to account for substrate competition and differences in glucose concentrations we used a modified Gjedde-Patlak plot based on the integral of plasma specific activity (SA(t)=Cp(t)/G(t)) (equation A3) rather than a plot based on the plasma FDG concentration (equation A1)

\[
\frac{Ct(t)}{Cp(t)} = K_i \cdot \frac{\int_0^t SA(z)dz}{Cp(t)} + \text{const.} \quad \text{equation A3}
\]

where \( K_i \) represents the tissue uptake rate (\( \mu \text{mol/min per ml of tissue} \)). CMR\text{glu} was then calculated as \( K_i \) divided by appropriate lumped constant (LC=0.81, equation A4):

\[
\text{CMR}_{\text{glu}} = \frac{K_i}{LC} \quad \text{equation A4}
\]

Please note that equation A3 is similar to the formula previously proposed and validated by Dunn et al (5).

We tested and verified that the modified Gjedde-Patlak plot was linear (see in figure A3 an example plot from one subject). The slope of the plot, i.e. the new \( K_i \), is the brain tissue glucose uptake rate (expressed in \( \mu \text{mol/min per ml of tissue} \))

![Figure A3. Linear relationship between tracer concentration in tissue and integral of plasma specific activity normalized by tracer plasma glucose concentration in one study subject.](image-url)
References