

Exenatide Alters Myocardial Glucose Transport and Uptake Depending on Insulin Resistance and Increases Myocardial Blood Flow in Patients with Type 2 Diabetes

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Context: Glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonists provide beneficial cardiovascular effects by protecting against ischemia and reperfusion injury. Type 2 diabetes mellitus patients have reduced glycolysis in the heart.

Objective: We hypothesized that cardioprotection by GLP-1 is achieved through increased glucose availability and utilization and aimed to assess the effect of exenatide, a synthetic GLP-1 receptor agonist, on myocardial glucose uptake (MGU), myocardial glucose transport, and myocardial blood flow (MBF).

Design and Methods: We conducted a randomized, double-blinded, placebo-controlled crossover study in eight male, insulin-naïve, type 2 diabetes mellitus patients without coronary artery disease. Positron emission tomography was used to determine the effect of exenatide on MGU and MBF during a pituitary-pancreatic hyperglycemic clamp with ^{18}F -fluorodeoxyglucose and ^{13}N -ammonia as tracers.

Results: Overall, exenatide did not alter MGU. However, regression analysis revealed that exenatide altered initial clearance of glucose over the membrane of cardiomyocytes and MGU, depending on the level of insulin resistance ($P = 0.017$ and 0.010 , respectively). Exenatide increased MBF from 0.73 ± 0.094 to 0.85 ± 0.091 ml/g \cdot min ($P = 0.0056$). Except for an increase in C-peptide levels, no differences in circulating hormones or metabolites were found.

Conclusions: The action of exenatide as an activator or inhibitor of the glucose transport and glucose uptake in cardiomyocytes is dependent on baseline activity of glucose transport and insulin resistance. Exenatide increases MBF without changing MGU. (*J Clin Endocrinol Metab* 97:E1165–E1169, 2012)

Type 2 diabetes (T2D) will affect 400 million people by 2030 and carries a risk for macrovascular disease. Cardiovascular diseases are the main cause of death in T2D, accounting for 50% of fatalities (1).

Glucagon-like peptide-1 (GLP-1) receptor agonists mimic a range of physiological actions of native GLP-1 on glucose metabolism, including stimulation of insulin secretion, inhibition of glucagon secretion, inhibition of

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Abbreviations: ^{18}F -FDG, ^{18}F -fluorodeoxyglucose; GLP-1, glucagon-like peptide-1; GLP-1r, GLP-1 receptor; GLUT, glucose transporter; HOMA2, homeostasis model of assessment 2; MBF, myocardial blood flow; MGU, myocardial glucose uptake; PET, positron emission tomography; T2D, type 2 diabetes mellitus.

gastric emptying, and reduction of appetite and food intake (2).

GLP-1 exerts its actions through the GLP-1 receptor (GLP-1r), which is present in many tissues including vascular endothelium, cardiomyocytes, endocardium, and smooth muscle cells. Some cardiac effects of GLP-1r agonists may be exerted via other receptors (3).

GLP-1r agonists reduce ischemia-reperfusion injury by reducing infarct size, and long-term studies show that patients treated with exenatide are less likely to experience a cardiovascular disease event. The mechanism remains unclear (4).

We hypothesized that cardioprotection with GLP-1 is afforded by increased glucose availability and utilization and assessed the effects of exenatide on myocardial glucose uptake (MGU), myocardial glucose transport, and myocardial blood flow (MBF) in T2D.

Subjects and Methods

The study was designed in accordance with the Declaration of Helsinki and approved by the ethics committee of Region Midtjylland, Denmark.

Study group

The study group consisted of eight male subjects with a mean age of 58.3 ± 1.7 yr and body mass index of 31.9 ± 1.0 kg/m² with suboptimally controlled T2D (glycosylated hemoglobin, $7.7 \pm 0.2\%$). All were insulin-naïve, nonsmoking Caucasians without coronary artery disease or other significant disease. All received metformin and statins; six received sulfonylurea; three, angiotensin II antagonists; six, angiotensin-converting enzyme inhibitors; four, group 2, and one, group 1 calcium channel blockers.

Study design

We conducted a randomized, double-blinded, placebo-controlled crossover study with 4 wk between positron emission tomography (PET) sessions. Oral antidiabetic drugs were discontinued 48 h before study day, and the remaining medications were discontinued 12 h before clamp start.

Hyperglycemic clamp

A pancreatic-pituitary clamp was performed as earlier described (5). Human insulin infusion rates were 1 mU/kg/min from min 0 to 60 and 0.25 mU/kg/min from 60 to the end. Glucose (200 g/liter) was infused at variable ratios to clamp plasma glucose to 9 mmol/liter.

Patients received iv exenatide (0.066 pmol/kg · min) (6) (Byetta; Amylin Pharmaceuticals, Inc., San Diego, CA; and Eli Lilly, Indianapolis, IN) or placebo.

Assays used for measuring serum insulin, glucagon, C-peptide, fatty acid, GH, cortisol, plasma exenatide, epinephrine, and norepinephrine were described previously (6). Insulin resistance homeostasis model of assessment 2 (HOMA2) was computed as described in Ref. 7, and whole-body glucose uptake M-values as described in Ref. 8.

Positron emission tomography

A PET model EXACT HR47 (Siemens Medical, Knoxville, TN) was used. All PET data were acquired in two-dimensional mode. Scout and transmission scan (⁶⁸Ge rod) preceded metabolic and flow scans.

Myocardial glucose uptake

MGU was quantified by PET using ¹⁸F-fluorodeoxyglucose (¹⁸F-FDG) as the metabolic tracer, 200 MBq ¹⁸F-FDG was injected, and a 23-frame dynamic scan was acquired (6 × 30 sec, 7 × 60 sec, 5 × 120 sec, and 5 × 300 sec). The last frame in the ¹³N-ammonia (¹³N-NH₃) scan was used to obtain a high-contrast image used for region of interest allocation. MGU was quantified by fitting tissue and blood pool (from arterial blood samples) time-activity curves to a three-compartment model for ¹⁸F-FDG. Using this model, we obtained values of unidirectional clearance (K_1^*), efflux coefficient (k_2^*), and phosphorylation rate constant (k_3^*) for ¹⁸F-FDG (rate constants marked with asterisks correspond to ¹⁸F-FDG). MGU was calculated as: $MGU = PG \times K_{glc}/myocardial\ density$. The lumped constant (LC) [the conversion factor between the net uptakes of ¹⁸F-FDG and glucose (9)] was calculated as $LC = \varphi + (\tau - \varphi) \times (k_3^*/(k_2^* + k_3^*))$. The net clearance of glucose was calculated as: $K_{glc} = K^*/LC$. We used an individual spill-in coefficient obtained from the ¹³N-NH₃ scan in the fitting model of the dynamic data. The K_1 values are not adjusted for the spillover coefficient due to the low ¹⁸F-FDG uptake leading to similar concentration of isotopes in left ventricle and the myocardium.

Myocardial blood flow

The MBF was quantified using ¹³N-NH₃ as perfusion tracer (8). For ¹³N-NH₃ imaging, 740 MBq diluted in 10 ml saline was injected. At the time of injection, a dynamic sequence of images (12 × 10 sec, 2 × 30 sec, 1 × 60 sec, and 1 × 900 sec) was collected to obtain time-activity curves from blood pool and myocardium. MBF was quantified by fitting tissue and blood pool (from image-derived left ventricular blood input function) time-activity curves to a three-compartment model for ¹³N-NH₃. Using this model, we obtained values of unidirectional clearance (K_1^*) and the spill-in coefficient V_p . MBF was calculated as $MBF = (K_1\ NH_3 \times 1/(1 - V_p))/myocardial\ density$.

Calculations and statistical analysis

PET data were analyzed using a paired Student's *t* test. Circulating hormones and metabolites were compared using a linear mixed-effects model (repeated measurements ANOVA taking the crossover design into account). A linear regression model was used for regression analysis of the unidirectional clearance and efflux rate constant, and Pearson's *r* test was used to evaluate unidirectional clearance, MGU, and HOMA2 IR. The statistical software used was GraphPad Prism (GraphPad Software, San Diego, CA) and SigmaPlot (Systat Software Inc., Chicago, IL), significance level 5%. Data are presented as mean ± SEM.

Results

Hormones and metabolites

Exenatide resulted in steady-state plasma concentrations of 170 pg/ml. During the PET scan, plasma glucose

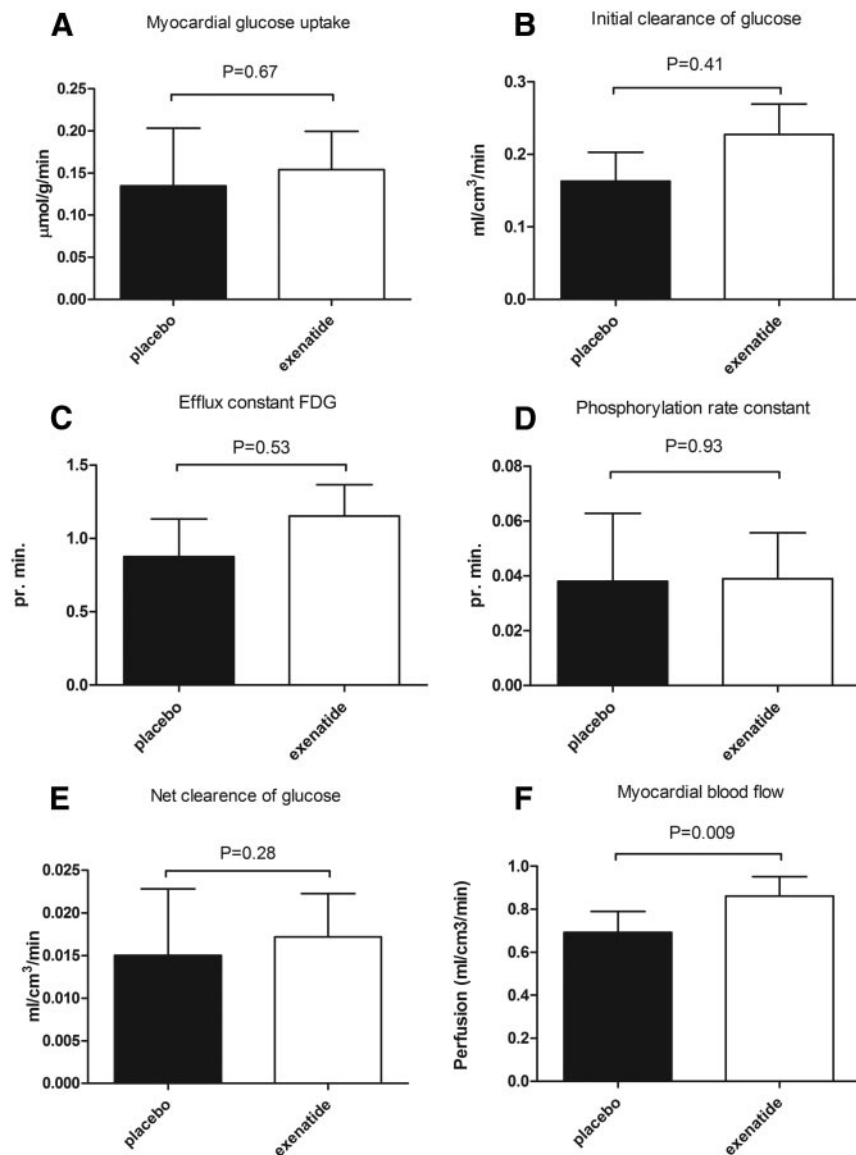


FIG. 1. A, MGU; B, initial clearance of glucose (K_1); C, efflux constant ^{18}F -FDG (k_2^*); D, phosphorylation rate constant for ^{18}F -FDG (k_3^*); E, net clearance of glucose (K_{glc}); and F, MBF during exenatide and placebo infusion. Data are expressed as mean \pm SEM.

concentration was 9.7 ± 0.25 mmol/liter (placebo) and 9.4 ± 0.18 mmol/liter (GLP-1) ($P = 0.88$). Except for an increase in C-peptide ($P = 0.04$) caused by exenatide, no differences in circulating hormones or metabolites were found between sessions. Plasma levels of exenatide differed between sessions ($P < 0.0001$). Insulin levels were comparable to normal fasting state levels during PET scans. Free fatty acid concentrations decreased during the clamp but were not totally suppressed; there were no differences between sessions. Regression analysis showed no correlation between increased MBF and plasma C-peptide or plasma exenatide levels. M-values did not differ between sessions ($P = 0.66$).

Positron emission tomography

Exenatide did not affect MGU (placebo, 0.13 ± 0.07 , and exenatide, 0.15 ± 0.05 $\mu\text{mol/g} \cdot \text{min}$; $P = 0.66$) (Fig.

1A). We found no exenatide-induced changes in the influx of ^{18}F -FDG from blood to cardiomyocytes ($P = 0.42$) (Fig. 1B), in the efflux coefficient from myocytes to blood ($P = 0.53$) (Fig. 1C), or in the phosphorylation coefficient for ^{18}F -FDG ($P = 0.93$) (Fig. 1D). Furthermore, the net clearance of ^{18}F -FDG (Fig. 1E), K_1^*/k_2^* ratios (placebo, 0.30 ± 0.02 ; and exenatide, 0.29 ± 0.01 ; $P = 0.79$), and lumped constant (placebo, 0.51 ± 0.04 ; and exenatide, 0.52 ± 0.05 ; $P = 0.76$) were unchanged with exenatide.

Regression analysis of the unidirectional clearance of glucose (K_1) showed a linear relationship ($P = 0.0017$; $r^2 = 0.83$) between baseline (placebo) unidirectional clearance and alterations in unidirectional clearance after exenatide. Exenatide increased initial clearance of glucose over membranes of cardiomyocytes in patients with low baseline initial clearance and decreased initial clearance in patients with high baseline unidirectional clearance (Fig. 2A). Furthermore, there was a linear relationship between baseline and exenatide-induced alterations in efflux constant (k_2^*) ($P = 0.0016$; $r^2 = 0.83$); a higher response was found with low baseline efflux constants and a decreased response with high baseline efflux constants (Fig. 2B). The alterations of unidirectional clearance correlated positively with HOMA2 IR ($P = 0.017$; $r^2 = 0.64$) (Fig. 2C). We found a positive linear relationship between the exenatide-induced alterations in MGU and IR ($P = 0.010$; $r^2 = 0.69$) (Fig. 2D).

Exenatide significantly increased MBF by 24% from 0.69 ± 0.097 to 0.86 ± 0.09 ml/g \cdot min ($P = 0.0089$) (Fig. 1F).

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Discussion

We demonstrate an exenatide-mediated alteration in glucose transport and MGU in T2D patients dependent on levels of IR. Against our hypothesis GLP-1r activation did not alter acute MGU between sessions in clamped patients with T2D. GLP-1r activation significantly increased MBF with 24%.

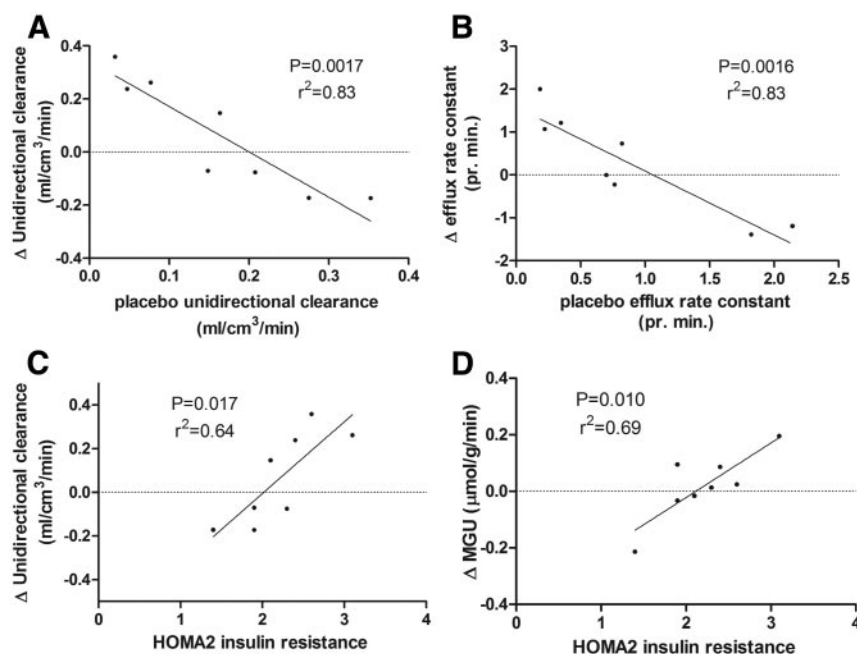


FIG. 2. Relationship between placebo unidirectional clearance and change in unidirectional clearance during exenatide infusion (A), placebo efflux rate constant and change in efflux rate constant (B), HOMA2 IR and change in unidirectional clearance during exenatide infusion (C), and HOMA2 IR and change in myocardial glucose uptake (D).

Myocardial glucose transport and MGU

Glucose mainly enters myocardial cells via glucose transporter (GLUT) 1 and GLUT4. GLP-1 and exenatide enhance GLUT1 translocation and GLUT4 protein levels of the myocardium (10, 11). Hexokinase activity appears not to be directly influenced (12). Our results show that transport across the cell membranes is affected by acute infusion of exenatide. In a three-compartment tracer analysis, K_1 represents the clearance of glucose from blood and extracellular space to myocardial cells, and k_2^* represents efflux of glucose from cardiomyocyte to blood and extracellular space. Therefore, the K_1 and k_2^* values reflect GLUT activity (13) (Fig. 2, A and B). The regression estimates indicate that exenatide raises GLUT translocation in patients with low baseline GLUT activity. Thus, the baseline activity of GLUT, circulating insulin levels, and IR generate a differentiated action of exenatide as inhibitor or activator of glucose transport and uptake (Fig. 2, C and D) in cardiomyocytes. The mechanism of GLP-1r-facilitated GLUT translocation is not clear. The mechanism of the dual effects of exenatide remains unclear but may be caused by receptor-mediated changes or may be a general feature of intracellular IR. The duality may be beneficial because glucose transport and uptake are stabilized by exenatide in patients with low insulin sensitivity.

Despite low circulating free fatty acid levels and hyperglycemia, we observed low rates of MGU (0.13 and 0.15 $\mu\text{mol/g} \cdot \text{min}$, respectively) (Fig. 1A), probably due

to the low insulin infusion mimicking fasting-state insulin levels in T2D. Whether or not myocardial IR is inherent in T2D is unclear. We demonstrated that GLP-1r activation did not change MGU acutely in patients with T2D, contrary to results of animal models [indicating unaltered myocardial IR (8)]. Furthermore, the ^{18}F -FDG efflux constant (k_2^*) exceeded the hexokinase phosphorylation constant (k_3^*) (Fig. 1, C and D). Usually, hexokinase has high affinity for glucose even at low concentrations. T2D diminishes hexokinase synthesis and activity (14). Our findings may reflect that the limiting process in the glycolysis in the myocardial cells is low hexokinase activity in T2D, contrasting normal conditions.

Myocardial blood flow

In a variety of experimental models, GLP-1-related improvements of cardiac function have been demonstrated. It is hypothesized that GLP-1 improves outcome after myocardial ischemia and reperfusion by enhancing blood flow, increasing glucose uptake, and improving metabolism in previously ischemic myocardium (15). GLP-1 acts in the vasculature causing coronary vasodilation (3) and improved postresuscitation coronary microcirculatory function (16). The current study is the first to reveal that GLP-1r activation potentially increases MBF. The ^{13}N - NH_3 scan cannot distinguish between micro- and macrovasculature in the coronary system; however, the literature indicates actions in both domains. The mechanism of increased blood flow induced by GLP-1r activation is not clear. Regression analyses showed no correlation between the C-peptide, exenatide levels, and MBF. The resting-state MBF correlates to heart rate and blood pressure; we determined catecholamines as a surrogate for hemodynamic measures, and no difference was found. Because exenatide does not seem to have any acute hemodynamic effect on heart rate or blood pressure in humans (17, 18) and the rise in MBF was not accompanied by increases in catecholamines, we speculate that there is a direct action through a receptor in the myocardium.

In conclusion, we have demonstrated that acute treatment of T2D patients with exenatide results in activation or inhibition of glucose transport and glucose uptake in cardiomyocytes depending on baseline activity and hence

IR. Furthermore, exenatide increases MBF without changing MGU.

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