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Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease

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Nyström, Thomas, Mark K. Gutniak, Qimin Zhang, Fan Zhang, Jens Juul Holst, Bo Åhrén, and Åke Sjöholm. Effects of glucagon-like peptide-1 on endothelial function in type 2 diabetes patients with stable coronary artery disease. *Am J Physiol Endocrinol Metab* 287: E1209–E1215, 2004. First published September 7, 2004; doi:10.1152/ajpendo.00237.2004.—GLP-1 stimulates insulin secretion, suppresses glucagon secretion, delays gastric emptying, and inhibits small bowel motility, all actions contributing to the anti-diabetogenic peptide effect. Endothelial dysfunction is strongly associated with insulin resistance and type 2 diabetes mellitus and may cause the angiopathy typifying this debilitating disease. Therefore, interventions affecting both endothelial dysfunction and insulin resistance may prove useful in improving survival in type 2 diabetes patients. We investigated GLP-1's effect on endothelial function and insulin sensitivity (S_I) in two groups: 1) 12 type 2 diabetes patients with stable coronary artery disease and 2) 10 healthy subjects with normal endothelial function and S_I . Subjects underwent infusion of recombinant GLP-1 or saline in a random crossover study. Endothelial function was measured by postischemic FMD of brachial artery, using ultrasonography. S_I [$\text{in } (10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})/(\mu\text{U/ml})$] was measured by hyperinsulinemic isoglycemic clamp technique. In type 2 diabetic subjects, GLP-1 infusion significantly increased relative changes in brachial artery diameter from baseline FMD(%) (3.1 ± 0.6 vs. $6.6 \pm 1.0\%$, $P < 0.05$), with no significant effects on S_I (4.5 ± 0.8 vs. 5.2 ± 0.9 , $P = \text{NS}$). In healthy subjects, GLP-1 infusion affected neither FMD(%) (11.9 ± 0.9 vs. $10.3 \pm 1.0\%$, $P = \text{NS}$) nor S_I (14.8 ± 1.8 vs. 11.6 ± 2.0 , $P = \text{NS}$). We conclude that GLP-1 improves endothelial dysfunction but not insulin resistance in type 2 diabetic patients with coronary heart disease. This beneficial vascular effect of GLP-1 adds yet another salutary property of the peptide useful in diabetes treatment.

nitric oxide; insulin resistance

A LOT OF INTEREST has been engendered in glucagon-like peptide-1 (GLP-1) as an emerging new drug in the treatment of type 2 diabetes (19, 26). Exogenous administration of GLP-1 has been shown to lower blood glucose in both type 1 and type 2 diabetic patients (11, 18, 23). Furthermore, GLP-1 may improve insulin resistance and glucose utilization in patients with type 2 diabetes (13). In addition, part of the antihyperglycemic effect of

GLP-1 may be attributable to decreases in gastric and small bowel motility, effects dependent on nitric oxide (NO) (31).

High-affinity receptors for GLP-1 are also present in extrapancreatic and intestinal tissues, i.e., nervous system, heart, kidney, and vascular smooth muscle (6, 22, 27). Apart from glycemic actions, cardiovascular effects by GLP-1 have been described. GLP-1 improves severe left ventricular heart failure in humans suffering from a myocardial infarction (24). Additionally, GLP-1 relaxes precontracted pulmonary artery rings in rats, an effect described as NO dependent (17, 27). Recently, Yu et al. (34) demonstrated that GLP-1 infusion improves endothelial function in a salt sensitivity rat model (34). Endothelial function in response to reactive hyperemia can reliably be measured with ultrasonography, i.e., flow-mediated vasodilation (FMD), and is considered to be NO mediated (10). Furthermore, endothelial dysfunction of FMD in the brachial artery highly correlates with endothelial dysfunction in the coronary circulation (2). Endothelial dysfunction is strongly associated with insulin resistance and type 2 diabetes mellitus and may be causing the angiopathy typifying this debilitating disease (7). Intervention affecting both endothelial dysfunction and insulin resistance may prove useful in patients with type 2 diabetes.

Therefore, the aim of this study was to evaluate the effect of GLP-1 on endothelial function in type 2 diabetic patients with coronary artery disease (CAD) and whether such an effect correlates to insulin sensitivity. Because there are differences in endothelial function between insulin-resistant subjects and healthy subjects, we also studied healthy subjects. Finally, we also investigated whether GLP-1 receptors are expressed on human coronary endothelial cells, an issue that remains elusive.

MATERIALS AND METHODS

Subjects

Twelve middle-aged, moderately obese Caucasian male patients with type 2 diabetes of short duration with an established CAD and 10 young, healthy, unmatched Caucasian male subjects participated in the study. Baseline characteristics of the study groups are shown in Table 1. The study was approved by the Karolinska Institutet ethics committee, and written informed consent from the study subjects was obtained.

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Table 1. Baseline clinical and biochemical characteristics of the study groups

	Type 2 Diabetes Subjects (n = 12)	Healthy Subjects (n = 10)
<i>Clinical characteristics</i>		
Age, yr	61±3	29±4
Duration of diabetes, yr	5±1	—
BMI, kg/m ²	28±1	24±0.6
Smoker, yes/no	1/11	0/10
Systolic blood pressure, mmHg	126±4	110±2
Diastolic blood pressure, mmHg	78±2	71±2
Urinary protein, mg/l	71±53	<4
<i>Patients treated with</i>		
Insulin, yes/no	10/2	
Insulin dose, units/day	52±4	
Sulfonylurea, yes/no	3/9	
Metformin, yes/no	4/8	
ACE inhibitor/ARB, yes/no	11/1	
Beta-blocker, yes/no	12/0	
Statin, yes/no	8/4	
Calcium channel blocker, yes/no	3/9	
Acetyl salicylic acid, yes/no	12/0	
<i>Biochemical characteristics</i>		
Serum hemoglobin, g/l	139±3	146±4
Serum Hb A _{1c} , %	6.9±0.4	4.5±0.1
Serum creatinine, μmol/l	78±3	84±2
Serum total cholesterol, mmol/l	4.3±0.2	3.8±0.3
Serum HDL-cholesterol, mmol/l	0.9±0.1	1.3±0.2
Serum LDL-cholesterol, mmol/l	2.6±0.1	2.0±0.2
Serum triglycerides, mmol/l	1.7±0.2	1.1±0.2

Data are means ± SE. ARB, angiotensin receptor blocker; BMI, body mass index; ACE, ANG I-converting enzyme.

Type 2 diabetes subjects. In the diabetes group, three patients showed signs of microalbuminuria, but no one had any overt microvascular complications such as nephropathy, neuropathy, or retinopathy. However, they had all suffered a myocardial infarction. All but one were nonsmokers, and five were ex-smokers. All but one were non-snuff users. Lipid control was satisfactory, whereas glycemic control was moderately impaired. Eight subjects were on antihypertensive treatment. Everyone was receiving secondary preventive drugs against CAD. Metoprolol was the β-blocker used in all patients. Drugs were withheld the evening before the experiments. Glycemic control was achieved by diet alone (in one patient) or diet plus oral hypoglycemic agents (metformin or sulfonylurea or both), or insulin alone or insulin plus oral hypoglycemic agents (Table 1). In all cases, 3–6 mo passed between the myocardial infarction and the commencement of the study procedure.

Healthy subjects. In the group of healthy subjects, questionnaires revealed no family history of diabetes or heart disease. Furthermore, all were free from pharmacological treatment or any diseases. All were nonsmokers and non-snuff users.

Study Protocol

This was a pilot study with a randomized crossover design. A scheme of the test protocol is given in Fig. 1. After a 12-h overnight fast, subjects underwent intravenous infusion of human recombinant GLP-1 (2 pmol·kg⁻¹·min⁻¹; Restoragen, Lincoln, NE) or saline (0.9% NaCl; Baxter, Deerfield, IL) with an insulin pump (Medtronic, Minneapolis, MN) in a single-blind (blinded for patient) random crossover study, with a washout period of 1 wk. Subjects were not allowed to eat or drink anything but water, and they refrained from their medicines on the morning of the test day; otherwise, subjects were taking their medicines as usual between the two procedures.

GLP-1 was infused 15 min after priming for the hyperinsulinemic clamp and throughout the clamp, thus in total for 105 min. To assess as correctly as possible GLP-1 effects on sensitivity to insulin-mediated glucose disposal under the conditions normally prevailing in each patient, we chose to clamp patients at their actual fasting glucose levels (isoglycemic) rather than at statistically “normal” glucose levels (euglycemic). Thus the steady-state glucose level for diabetics is higher than that for controls. Another advantage of isoglycemic clamp is that one avoids artifacts caused by acute lowering of glucose levels.

Brachial Artery Flow and Diameter

The diameter of the target artery was measured from two-dimensional ultrasound images with a 7.0-MHz linear array transducer and a standard 128XP/10 system (Accuson, Mountain View, CA) according to the forearm cuff occlusion technique (9, 10). After a 30-min resting time, the subject's left arm was immobilized, and the transducer was fixed in the same position throughout the study, with the assistance of a mechanical arm. The position of the transducer was marked to retrieve the same position of the artery in the second study. The brachial artery was scanned longitudinally, and the transmit (focus) zone was set to optimize images of the lumen arterial wall interface. The B-mode images were magnified by a resolution box and obtained with gating from the R wave of the electrocardiogram as triggering mode. The condition of reactive hyperemia was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 300 mmHg for 4.5 min, followed by release [endothelial-dependent vasodilation (FMD)]. Measurements were made at baseline (after 30 min of supine rest) and at 45 and 60 s after cuff release. Then, after 10 min of rest, 0.4 mg of glyceryl trinitrate spray was applied, and new images were obtained 4 min later [endothelial-independent vasodilation (nitroglycerin-mediated dilation; NTG)]. The relative changes in vessel diameter are expressed as percentages after reactive hyperemia [FMD(%)] and after nitroglycerine administration [NTG(%)], from baseline scan. Brachial artery diameter, the means of two images, was measured with an automated, computerized, analyzing system, according to the sonomorphological definition of Wendelhag et al. (32). In brief, this analyzing program is a PC/Windows-based software with digitized ultrasound image. The starting point of the measurement area is set by the operator, and a 10-mm box is automatically drawn. The different echo interfaces are automatically outlined. If obvious errors are detected, it is possible to modify the measurement by marking a correct echo in the ultrasound image. In this case, only one or two manually marked points are needed to guide the automatic system to the correct interface. This automated system has proven superior to manual measurement systems, with a dramatically improved reproducibility in both inter- and intraobserver coefficients of variation (32).

Arterial flow velocity rates were obtained with a pulsed Doppler signal at 70° angles to the vessel in the center of the artery. Ultrasound images were recorded 15 s after cuff release with the freeze mode. The volume flow was calculated by multiplying the velocity time integral of the Doppler flow signal for the mean of three pulse waves by the heart rate and vessel cross-sectional area. Calculations of blood flow

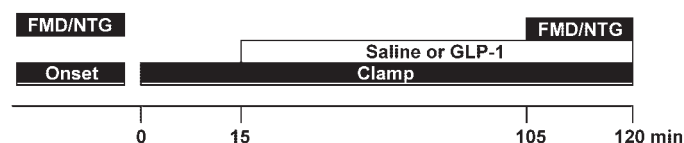


Fig. 1. Experimental design. Before any infusions (Onset), subjects underwent flow-mediated vasodilation (FMD) and nitroglycerine vasodilation (NTG) measurements. Hyperinsulinemic isoglycemic clamps (Clamp) for a total of 120 min were then undertaken in a crossover study with saline or glucagon-like peptide-1 (GLP-1; 2 pmol·kg⁻¹·min⁻¹) infusions. At 105 min, subjects were reexamined with FMD and NTG. See MATERIALS AND METHODS for details.

and changes in arterial vasodilation were done unaware of the subjects or the procedure.

The mean (SE; range) coefficients of repeatability and variance were determined in all subjects (pooled data) between the first and second visit at basal condition, according to guidelines for ultrasound assessment (10). The mean (SE; range) for FMD(%) was 6.0 (1.3; 3.3–8.7) at the first visit and 7.0 (1.1; 4.6–9.3) at the second visit. The mean (SE; range) for NTG(%) was 18.4 (1.3; 15.8–20.1) at the first visit and 15.6 (1.3; 12.9–18.2) at the second visit. Correlation (r) between the first and second visit was $r = 0.8$ for FMD(%) and $r = 0.6$ for NTG(%). Repeatability coefficients (RCs) were calculated with the formula $RC = 2 \cdot \sqrt{(\sum D_i^2/n)}$, where D_i is the absolute difference between measurements at first and second visit, and n is the number of measurements (5). RC was 7.1 for FMD(%) and 11.9 for NTG(%). Correlation of variation between the first and second visit was 9.8% and 14.7% for FMD(%) and NTG(%), respectively.

Hyperinsulinemic Isoglycemic Glucose Clamp

Hyperinsulinemic clamps were performed according to DeFronzo et al. (12). In brief, a superficial dorsal hand vein was cannulated in retrograde fashion with a 21-gauge butterfly needle and kept patent by a slow infusion of saline solution. The hand was kept warm by an electric device for intermittent sampling of arterialized venous blood. After that, one intravenous catheter was inserted into the left antecubital vein for substrate (insulin/glucose) and drug infusion (GLP-1/saline). During the 120 min of the test, insulin ($40 \text{ mU} \cdot \text{m}^{-2} \cdot \text{min}^{-1}$ Human Actrapid; Novo Nordisk, Bagsværd, Denmark) was infused along with 20% dextrose (Fresenius Kabi, Copenhagen, Denmark). The rate of dextrose infusion was adjusted to achieve a blood glucose level comparable with the fasting glucose levels of subjects, on the basis of arterialized samples withdrawn every 5 min from the dorsal hand vein catheter (heated-air box at 55°C ; Dept. of Physiology and Pharmacology, Univ. of Nottingham, Nottingham, UK). The glucose clamp-derived index of insulin sensitivity [S_I ; in $(10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})/(\mu\text{U/ml})$] was calculated from glucose infusion rate (GIR), corrected for body weight, during the final 30 min as follows: $S_I = \text{GIR}_{\text{SS}}/\text{G}_{\text{SS}} \times \Delta I_{\text{SS}}$, where GIR_{SS} is the steady-state GIR (mg/min), G_{SS} is the steady-state blood glucose concentration (mg/dl), and ΔI_{SS} is the difference between basal and steady-state plasma insulin concentrations ($\mu\text{U/ml}$). This calculation is assumed to correct for differences in prevailing glucose and insulin concentrations.

Cell Culture

Human coronary aortic endothelial cells (HCAEC) were purchased from Clonetics (San Diego, CA) and cultured in endothelial growth medium according to the manufacturer's instructions. A rat insulinoma-derived cell line, BRIN-BD11 (BRIN), known to express the GLP-1 receptor (14) served as a positive control. Cells were maintained in sterile tissue culture flasks at 37°C in an atmosphere of 5% CO_2 -95% air. HCAEC were obtained from nondiabetic Caucasian males without any symptoms of coronary heart disease. Cells stain positive for acetylated LDL and von Willebrand (factor VIII) antigen and stain negative for smooth muscle α -actin. These cells also express various adhesion molecules.

Western Blotting

HCAEC and BRIN cells were harvested by gentle trypsinization. Cells were washed three times in phosphate-buffered saline and solubilized in SDS-PAGE sample buffer. Twice as much protein from HCAEC than from BRIN cells was subjected to SDS-PAGE under reducing conditions. Proteins in the gel were subsequently electrotransferred onto nitrocellulose membranes. The membrane was blocked overnight in Tris-buffered saline-0.05% Tween 20 (TBS-T) with 5% nonfat dry milk, followed by an overnight incubation with $5 \mu\text{g/ml}$ of rabbit anti-NH₂-terminal GLP-1 receptor antibody (a gen-

erous gift of Dr. Bernard Thorens) in TBS-T-1% BSA at 4°C . The membrane was further incubated with horseradish peroxidase-labeled goat anti-rabbit IgG in TBS-T-1% BSA for 1 h at room temperature. The immunostained proteins were visualized by enhanced chemiluminescence (21).

Analytical Methods

Blood glucose levels were determined by the glucose oxidase method with a glucose analyzer (2300 STAT PLUS; Yellow Springs Instruments, Yellow Springs, OH). Arterialized plasma samples were anticoagulated with EDTA, centrifuged for ~ 15 min, separated, and stored at -20°C until assayed. Plasma samples for each subject were run in the same assay to eliminate interassay variations. Insulin and C-peptide were measured with the use of enzyme-linked immunosorbent assays (Pharmacia, Uppsala, and Mercodia, Uppsala, Sweden, respectively).

Total GLP-1 was determined with a radioimmunoassay using antiserum-89390, which is highly specific for the COOH terminus of GLP-1 and therefore measures the sum of GLP-1-(7–36) amide and its metabolite GLP-1-(9–36). For GLP-1 and glucagon analysis, plasma was extracted with ethanol (final concentration 70% by volume). The glucagon assay uses an antiserum directed against the COOH terminus of the glucagon molecule (antibody code no. 4305).

Statistical Analysis

Results are shown as means \pm SE. Comparisons between treatments and time were made by analysis of variance (ANOVA) for repeated measures. Furthermore, by use of a sequence factor (regarding the order in which subjects were examined) in the ANOVA model, any carryover effects were corrected for. Significant differences by ANOVA were followed by the post hoc Sheffé's test. Normality tests revealed that all parameters, except triglycerides, showed bell-shaped Gaussian curves. $P < 0.05$ was deemed statistically significant.

RESULTS

Healthy Subjects

Hyperinsulinemic clamp data. All data are given in Fig. 2. Basal levels of plasma insulin [32 ± 6 vs. $35 \pm 4 \text{ pmol/l}$, $P = \text{not significant (NS)}$], C-peptide (325 ± 19 vs. $359 \pm 18 \text{ pmol/l}$, $P = \text{NS}$), glucagon (7.7 ± 1.5 vs. $8.0 \pm 1.4 \text{ pmol/l}$, $P = \text{NS}$), GLP-1 (5.0 ± 1.4 vs. $5.0 \pm 1.3 \text{ pmol/l}$, $P = \text{NS}$), and blood glucose (4.6 ± 0.1 vs. $4.7 \pm 0.1 \text{ mmol/l}$, $P = \text{NS}$) were similar between GLP-1 and saline infusions. During GLP-1 infusion, plasma levels of insulin, C-peptide, and GLP-1 significantly increased, whereas plasma glucagon levels did not change (Fig. 2). At steady-state clamp, during the last 30 min, GLP-1 infusion affected neither GIR (11.8 ± 1.0 vs. $13.3 \pm 0.7 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, $P = \text{NS}$) nor S_I [14.8 ± 1.8 vs. $11.6 \pm 2.0 (10^{-4} \text{ dl} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})/(\mu\text{U/ml})$, $P = \text{NS}$].

FMD and NTG responses. All data are given in Table 2. Baseline brachial artery diameters for FMD and NTG were similar between GLP-1 and saline infusions, both at onset and at steady-state clamp. At steady-state clamp, brachial artery diameter tended to increase, albeit not significantly, compared with onset. FMD(%) as well as NTG(%) were above 10 and 15%, respectively. GLP-1 infusion did not affect FMD or NTG responses.

Brachial artery flow data. All data are given in Table 2. The postischemic stimulus measured as brachial artery blood flow did not differ between the procedures, nor did GLP-1 infusion affect brachial artery blood flow.

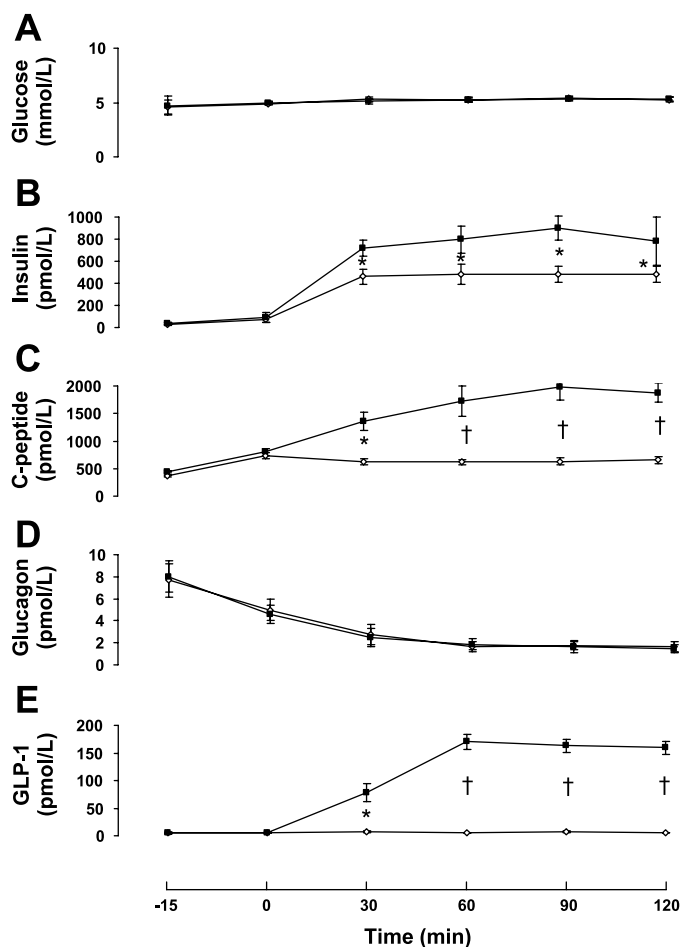


Fig. 2. Healthy subjects: hyperinsulinemic isoglycemic clamp data. Changes in blood glucose, plasma insulin, C-peptide, glucagon, and GLP-1 levels during hyperinsulinemic isoglycemic clamp when saline (\diamond) or GLP-1 (\blacksquare) was infused in healthy subjects. Results are means \pm SE. * $P < 0.05$ and † $P < 0.01$, saline vs. GLP-1 at given time point (ANOVA). See RESULTS for further details.

Heart rate and blood pressure data. Neither systolic nor diastolic blood pressure changed during GLP-1 infusion. GLP-1 infusion also did not affect heart rate (Table 2).

Type 2 Diabetes Subjects

Hyperinsulinemic clamp data. All data are given in Fig. 3. Basal levels of plasma insulin (182 ± 84 vs. 187 ± 72 pmol/L, $P = \text{NS}$), C-peptide (597 ± 93 vs. 533 ± 96 pmol/L, $P = \text{NS}$), glucagon (12.6 ± 1.3 vs. 11.3 ± 1.1 pmol/L, $P = \text{NS}$), GLP-1 (9.6 ± 2.1 vs. 8.4 ± 1.2 pmol/L, $P = \text{NS}$), and blood glucose (6.5 ± 0.7 vs. 6.4 ± 0.8 mmol/L, $P = \text{NS}$) were similar between GLP-1 and saline infusions. During GLP-1 infusion, plasma levels of C-peptide and GLP-1 significantly increased, whereas plasma insulin levels did not change (Fig. 3). Plasma glucagon levels decreased during GLP-1 infusion (Fig. 3). At steady-state clamp, GLP-1 infusion affected neither GIR (3.7 ± 0.3 vs. 4.2 ± 0.4 mg·kg⁻¹·min⁻¹, $P = \text{NS}$) nor S_I (4.5 ± 0.8 vs. 5.2 ± 0.9 (10⁻⁴ dl·kg⁻¹·min⁻¹)/(μU/ml), $P = \text{NS}$).

FMD and NTG responses. All data are given in Table 3. Baseline brachial artery diameters for FMD and NTG were similar between GLP-1 and saline infusions, both at onset and at steady-state clamp. At steady-state clamp, brachial artery

diameter tended to increase, albeit not significantly, compared with onset. There was a poor increase in FMD(%) seen in this group. However, NTG(%) seemed not to be diminished. At steady-state clamp, GLP-1 infusion significantly increased FMD(%) (3.1 ± 0.6 vs. $6.6 \pm 1.0\%$, $P < 0.05$). Patients with higher blood glucose levels did not react better in FMD(%) than patients with lower blood glucose during isoglycemic clamp (blood glucose range 4.4–9.1 mmol/L).

Brachial artery flow data. All data are given in Table 3. The postischemic stimulus measured as brachial artery blood flow did not differ between the procedures, nor did GLP-1 infusion affect brachial artery blood flow.

Heart rate and blood pressure data. Neither systolic nor diastolic blood pressure changed during GLP-1 infusion. GLP-1 infusion also did not affect heart rate (Table 3).

Side Effects of GLP-1

Two of 12 subjects in the type 2 diabetes group and 3 of 10 subjects in the healthy group experienced a transient mild sensation of nausea during infusion of GLP-1 compared with saline. It is known from several clinical trials that nausea frequently occurs at pharmacological doses of GLP-1.

Human Coronary Artery Endothelial Cells Express the GLP-1 Receptor

Because there is ambiguity as to the exact location of the GLP-1 receptor in human blood vessels, we determined whether it is expressed in pure human coronary artery endothelial cells. Western blotting of the cell lysates prepared from HCAEC revealed a single band with a molecular mass of 67 kDa, corresponding to the GLP-1 receptor seen in BRIN cells. It indicates the expression of the GLP-1 receptor in the endothelial cells with less abundance than in insulin-secreting cells (Fig. 4).

Table 2. Brachial artery, heart rate, and blood pressure data in healthy subjects

Variable	Saline		GLP-1	
	Onset	Clamp	Onset	Clamp
Baseline diameter FMD, mm	3.8 \pm 0.1	4.0 \pm 0.2	3.8 \pm 0.2	4.0 \pm 0.2
Maximal diameter FMD, mm	4.2 \pm 0.2	4.4 \pm 0.2	4.2 \pm 0.2	4.4 \pm 0.2
Δ FMD(%)	11.4 \pm 1.5	11.9 \pm 0.6	10.1 \pm 0.7	10.3 \pm 1.0
Baseline diameter NTG, mm	3.8 \pm 0.2	4.0 \pm 0.2	3.9 \pm 0.2	4.0 \pm 0.2
Maximal diameter NTG, mm	4.6 \pm 0.2	4.6 \pm 0.2	4.5 \pm 0.2	4.7 \pm 0.2
Δ NTG(%)	19.6 \pm 1.2	16.5 \pm 1.3	16.9 \pm 1.5	15.2 \pm 1.4
Baseline flow FMD, ml/min	31 \pm 2	35 \pm 2	37 \pm 3	36 \pm 4
Maximal flow FMD, ml/min	156 \pm 18	164 \pm 15	167 \pm 14	166 \pm 23
Baseline flow NTG, ml/min	35 \pm 4	40 \pm 4	36 \pm 3	40 \pm 3
Maximal flow NTG, ml/min	45 \pm 5	57 \pm 8	53 \pm 5	56 \pm 6
Heart rate, beats/min	52 \pm 2	55 \pm 3	52 \pm 2	56 \pm 3
sBP, mmHg	112 \pm 2	111 \pm 2	107 \pm 1	108 \pm 1
dBP, mmHg	71 \pm 3	69 \pm 2	72 \pm 1	69 \pm 1

Data are means \pm SE. Brachial artery, heart rate, and blood pressure data in healthy subjects at onset and at steady-state hyperinsulinemic clamp during glucagon-like peptide-1-(7–36) amide (GLP-1) or saline infusion. Onset, resting state without any procedure or infusions; Clamp, 90- to 120-min interval in hyperinsulinemic clamp; FMD, flow-mediated dilation (endothelial-dependent vasodilation); NTG, nitroglycerin-mediated dilation (endothelial-independent vasodilation); Δ FMD(%), relative increases in FMD; Δ NTG(%), relative increases in NTG; sBP, systolic blood pressure; dBP, diastolic blood pressure

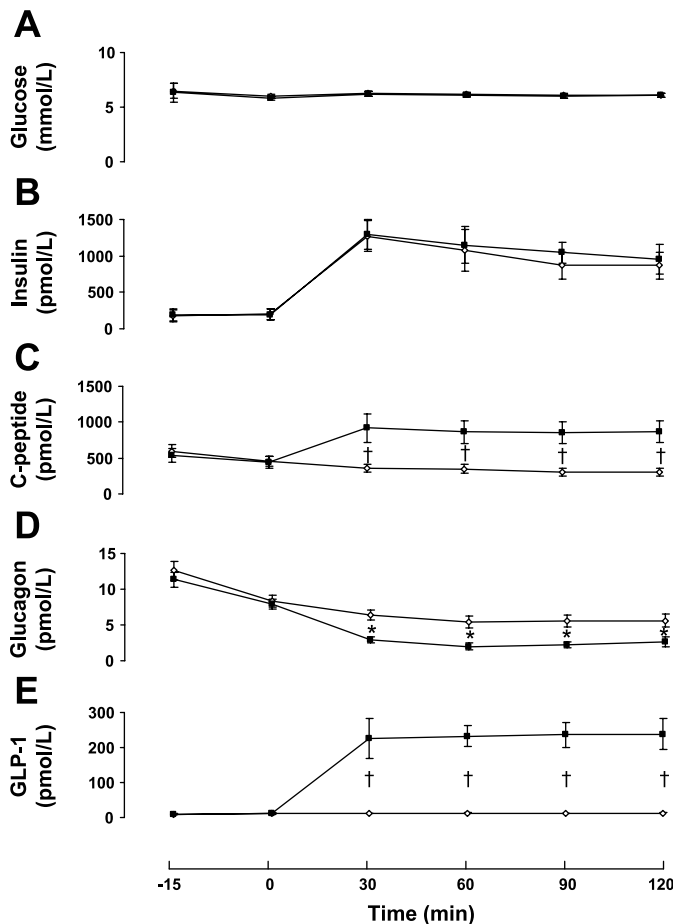


Fig. 3. Type 2 diabetes subjects: hyperinsulinemic isoglycemic clamp data. Changes in blood glucose, plasma insulin, C-peptide, glucagon, and GLP-1 levels during hyperinsulinemic isoglycemic clamp when saline (\diamond) or GLP-1 (\blacksquare) was infused in type 2 diabetes subjects. Results are means \pm SE. * $P < 0.05$ and $\dagger P < 0.01$, saline vs. GLP-1 at given time point (ANOVA). See RESULTS for further details.

DISCUSSION

We report in this study the novel finding that GLP-1 improves endothelial dysfunction in type 2 diabetes patients with established coronary artery disease without affecting whole body glucose uptake. It is well known that type 2 diabetes subjects are characterized by impaired endothelial-dependent relaxation, which may be a consequence of a disturbed synthesis or increased destruction of NO or a combination thereof (8). In agreement with this, the currently studied type 2 diabetes subjects showed a poor FMD response indicative of severe endothelial dysfunction. Infusion of GLP-1 significantly improved this FMD response in type 2 diabetes subjects without any effects in healthy subjects. It has been reported that GLP-1 directly relaxes pulmonary artery vessel rings in a rat organ bath model (27). This relaxation was abolished after removal of the vascular endothelium, indicating the requirement of an intact endothelium in GLP-1 vasorelaxation (16, 27). The exact location of the GLP-1 receptor in human blood vessels has not been unequivocally shown, but our present data demonstrate that the receptor is expressed in pure human coronary artery endothelial cells. However, it remains to be studied whether GLP-1 functions as an endogenous vasodila-

Table 3. Brachial artery, heart rate, and blood pressure data in type 2 diabetes subjects

Variable	Saline		GLP-1	
	Onset	Clamp	Onset	Clamp
Baseline diameter FMD, mm	4.2 \pm 0.1	4.4 \pm 0.2	4.3 \pm 0.1	4.4 \pm 0.2
Maximal diameter FMD, mm	4.3 \pm 0.1	4.6 \pm 0.1	4.3 \pm 0.1	4.7 \pm 0.2
Δ FMD(%)	2.0 \pm 0.7	3.1 \pm 0.6	2.2 \pm 0.5	6.6 \pm 1.0*
Baseline diameter NTG, mm	4.3 \pm 0.1	4.5 \pm 0.2	4.3 \pm 0.1	4.5 \pm 0.2
Maximal diameter NTG, mm	5.0 \pm 0.1	5.1 \pm 0.1	5.0 \pm 0.1	5.2 \pm 0.1
Δ NTG(%)	17.5 \pm 2.0	14.5 \pm 2.0	16.7 \pm 1.5	16.5 \pm 2.4
Baseline flow FMD, ml/min	56 \pm 4	52 \pm 4	60 \pm 10	54 \pm 5
Maximal flow FMD, ml/min	166 \pm 9	161 \pm 11	162 \pm 12	167 \pm 18
Baseline flow NTG, ml/min	58 \pm 4	53 \pm 4	57 \pm 4	58 \pm 4
Maximal flow NTG, ml/min	64 \pm 4	62 \pm 3	67 \pm 4	69 \pm 4
Heart rate, beats/min	61 \pm 3	60 \pm 3	60 \pm 3	62 \pm 3
sBP, mmHg	127 \pm 5	125 \pm 4	124 \pm 4	127 \pm 3
dbp, mmHg	78 \pm 2	77 \pm 2	78 \pm 2	78 \pm 2

Data are means \pm SE. Brachial artery, heart rate, and blood pressure data in type 2 diabetes subjects at onset and at steady-state hyperinsulinemic clamp during GLP-1 or saline infusion. See Table 2 legend for additional information. * $P < 0.05$ compared with saline (onset and clamp) and with GLP-1 (onset), ANOVA.

tor. GLP-1 vasorelaxation was also demonstrated by Golpon et al. (17), who suggested that this effect was NO dependent. Interestingly, chronically administered GLP-1 improves vasodilator response in precontracted aortic rings in a salt-sensitive rodent model (34). In the present study, GLP-1 may have increased endothelial NO synthase activation, thereby improving endothelial function. However, the possibility that the amelioration of the impaired FMD by GLP-1 noted in our type 2 diabetes subjects is an effect of GLP-1 per se cannot be excluded at this point. The effect of GLP-1 on small bowel motility (31) as well as the vasorelaxant influence on the pulmonary artery (17) is NO dependent.

It has become increasingly clear that insulin also has vasoactive properties that are NO dependent (28) and diminished in insulin-resistant states (29). However, in the present study, plasma insulin levels during the hyperinsulinemic clamps were identical between GLP-1 and saline infusions in type 2 diabetes subjects. Therefore, the salutary effect on endothelial function by GLP-1 seems not to be mediated by insulin. In contrast, there was a robust increase in plasma insulin in healthy subjects. There may be several reasons for this discrepancy; i.e., both insulin resistance and type 2 diabetes are well-known factors in β -cell dysfunction, leading to diminished insulin and C-peptide secretions. This may explain the higher insulin and C-peptide concentration levels seen in healthy subjects during



Fig. 4. Human coronary artery endothelial cells (HCAEC) express the GLP-1 receptor. Western blot analysis shows GLP-1 receptor (GLP-1R) expression on HCAEC and BRIN-BD11 (BRIN) cells. GLP-1 receptors were detected after separation by gel electrophoresis and Western blotting with an antibody against the NH₂-terminal region of the GLP-1 receptor.

GLP-1 infusion. Nonetheless, the difference in plasma insulin levels between saline and GLP-1 infusions in healthy subjects makes it difficult to determine whether GLP-1 also may affect a normal, functional endothelium.

Endothelial dysfunction is associated with insulin resistance and type 2 diabetes mellitus and may be causing the angiopathy typifying this debilitating disease. Improvement of insulin resistance may also improve endothelial function. Recently, it was shown that long-term treatment with GLP-1 enhances both insulin sensitivity and β -cell function in type 2 diabetic patients along with secondary metabolic improvements, e.g., reduced glucotoxicity and lowering of free fatty acids (35). However, these authors could not rule out a direct effect of GLP-1 on the improved insulin sensitivity (35). In our type 2 diabetes subjects, there was a relative increase of 16% in insulin sensitivity, albeit not significant. This could in part be an issue of a beta-error, because the correlation of variation in the clamp method is reported to be 15% compared with that in the ultrasound method (10%) in the current study. However, in short-term studies, GLP-1 does not seem to affect insulin sensitivity (1, 25), lending support to our present findings. Therefore, it seems unlikely that the improvement in endothelial function is secondary to the small increase in insulin sensitivity index seen in type 2 diabetes patients.

A robust increase in C-peptide levels was seen after GLP-1 administration in both groups. There are reports showing an increase in blood flow in patients with type 1 diabetes after C-peptide infusion, supporting the view that C-peptide elicits NO-dependent vascular effects (15, 20). However, in these studies, C-peptide levels were as much as four times higher compared with our present study. Furthermore, it remains to be proven that C-peptide increases blood flow in type 2 diabetes subjects with cardiovascular disease. Thus, although these differences make it difficult to compare these findings with the present results, the possibility remains that the increased blood flow by GLP-1 observed in our study could partly be mediated by the increase in C-peptide levels.

No changes were seen in blood pressure or heart rate during GLP-1 infusion. Recent rat studies have demonstrated an increase in systolic and diastolic blood pressure during GLP-1 treatment, an effect believed to be mediated by a central increase in sympathetic outflow (3, 4, 33). Concerns have therefore been raised as to whether GLP-1 may be suitable for type 2 diabetic patients, in whom hypertension is common and adversely influences their survival (33). In contrast, Yu et al. (34) demonstrated antihypertensive effects by chronic GLP-1 treatment in a rat model. In a human study, continuous GLP-1 infusion tended to lower both systolic and diastolic blood pressure (30). The reason for the apparent discrepancies is not clear, although differences in species, dose, and treatment duration may be involved. Our findings nonetheless do not support previous concerns about blood pressure raising actions of GLP-1.

In conclusion, we show here for the first time that GLP-1 ameliorates endothelial dysfunction in type 2 diabetic patients with an established coronary artery disease. This beneficial vascular effect of GLP-1 adds yet another salutary property of the peptide, increasing its clinical utility in type 2 diabetic patients, in whom endothelial dysfunction is a salient feature that adversely affects their survival.

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