

Influence of Glucagon-Like Peptide 1 on Fasting Glycemia in Type 2 Diabetic Patients Treated With Insulin After Sulfonylurea Secondary Failure

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OBJECTIVE — Glucagon-like peptide 1 (GLP-1) has glucose-dependent insulinotropic and glucagonostatic actions in type 2 diabetic patients on diet and on oral agents. It is not known, however, whether after secondary sulfonylurea failure, GLP-1 is still effective.

RESEARCH DESIGN AND METHODS — Therefore, 10 type 2 diabetic patients (6 women, 4 men; age 65 ± 10 years, BMI 30.4 ± 5.1 kg/m², HbA_{1c} $8.2 \pm 1.5\%$, 6 ± 3 [2–13] years after starting insulin treatment) were examined in the fasting state after discontinuing NPH insulin on the evening before the two study days. GLP-1 (1.2 pmol · kg⁻¹ · min⁻¹) or placebo (NaCl with 1% human serum albumin) were infused over 6 h. Plasma glucose (glucose oxidase) insulin (IMx), and C-peptide (enzyme-linked immunosorbent assay) were measured. Statistical analysis was performed using repeated measures analysis of variance.

RESULTS — Fasting plasma glucose was 9.4 ± 0.5 mmol/l and was reduced by GLP-1 to 5.3 ± 0.3 (3.9–7.3) mmol/l (placebo: 8.2 ± 0.7 mmol/l; $P < 0.0001$). GLP-1 transiently increased insulin (from 115 ± 31 to 222 ± 64 pmol/l at 150 min; $P < 0.0001$) and C-peptide (from 1.00 ± 0.12 to 1.90 ± 0.23 nmol/l at 120 min; $P < 0.0001$) with no effect of placebo. Glucagon and free fatty acids were lowered transiently. After normalization of plasma glucose, insulin and C-peptide concentrations became lower again during the ongoing administration of exogenous GLP-1, and no hypoglycemia occurred.

CONCLUSIONS — It is concluded that exogenous GLP-1 effectively lowers plasma glucose concentrations in advanced type 2 diabetes long after sulfonylurea secondary failure. These findings may broaden the applicability of GLP-1-derived drugs as a new treatment to nearly all type 2 diabetic patients.

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Glucagon-like peptide 1 (GLP-1) (7-36 amide) is an insulinotropic (incretin) (1–3) and glucagonostatic (4–6) hormone produced in and secreted from L-cells located mainly in the ileum, colon, and rectum (7,8). Together with gastric inhibitory

polypeptide (GIP, also called glucose-dependent insulinotropic polypeptide), it contributes to the postprandial insulin response (2,3). In pharmacological concentrations, which were approximately two- to fourfold higher than typical postprandial values,

exogenous GLP-1 stimulated insulin, lowered glucagon, and normalized glucose concentrations in hyperglycemic type 2 diabetic patients (6,9). In addition, in healthy as well as in type 2 diabetic subjects, gastric emptying is decelerated (10,11), leading to a slower absorption of nutrients after meals and a reduction in postprandial glucose responses. The full glucose-lowering potential of GLP-1 can only be achieved by intravenous infusion (6,9) or subcutaneous injections repeated at short intervals (12,13) because of a rapid enzymatic degradation (14) and elimination (2,6,15). The development of additives meant to slow absorption from subcutaneous depots and of peptide and, possibly, nonpeptide agonists that are better suitable as antidiabetic drugs is time-consuming and expensive. Therefore, the question arises of whether a beneficial use of GLP-1 is restricted to certain stages of type 2 diabetes or if all type 2 diabetic patients could profit from GLP-1-like agents. In that respect, GLP-1 stimulated insulin and reduced glucagon secretion in mild (diet-treated) diabetic patients (6), but also in patients who were in poor metabolic control on sulfonylurea treatment (9). Thus, these patients were at the point of sulfonylurea secondary failure (16). In light of recent concepts based on the long-term observation of large cohorts of type 2 diabetic patients (U.K. Prospective Diabetes Study [17]), it has become clear that type 2 diabetes is a slowly progressive disease and that glucose control seems to worsen with time independent of treatment. Therefore, patients long beyond the start of insulin treatment should be the ones least expected to respond to an insulinotropic agent like GLP-1.

It makes sense to assume a differential responsiveness toward sulfonylureas and GLP-1 because the former bind to a specific receptor that is coupled to a potassium channel, which is closed, leading to a depolarization of β -cells and a subsequent increase in intracellular calcium concentrations (opening of a voltage-dependent calcium channel) (18), whereas the latter mainly act by augmenting cyclic AMP synthesis (1,19).

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Abbreviations: ANOVA, analysis of variance; ELISA, enzyme-linked immunosorbent assay; GLP-1, glucagon-like peptide 1; HOMA, homeostasis model assessment.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—Patient characteristics at the time of the present study

Patient number	Sex (M/F)	Age (years)	BMI (kg/m ²)	Plasma glucose (mmol/l)		HbA _{1c} (%)	Duration of insulin treatment (years)	Insulin dose (U/day)	β-Cell secretion (%)	Insulin resistance*
				Fasting	Random postprandial					
1	M	61	35.1	6.9	10.2	6.5	6	72	106	4.8
2	F	65	36.2	9.8	11.7	9.8	10	48	38	6.5
3	M	64	27.0	8.1	12.4	8.1	4	88	148	32.6
4	M	43	25.5	9.8	12.3	9.8	13	102	45	6.3
5	M	61	28.6	5.6	11.2	5.6	2	48	46	4.8
6	F	69	35.6	7.9	6.5	7.9	5	80	36	4.0
7	F	74	36.6	8.0	11.1	8.0	5	56	59	5
8	F	76	22.9	7.3	8.2	7.3	4	80	54	4.3
9	F	57	29.6	9.4	18.7	9.4	5	70	17	5.4
10	F	78	26.8	9.7	17.3	9.7	5	124	176	13.7
Mean ± SD	4/6	65 ± 10	30.4 ± 5.1	8.0† ± 1.8	11.7† ± 5.9	8.2† ± 1.5	6 ± 4	77 ± 24	72 ± 53	8.7 ± 8.8

β-Cell secretion and insulin resistance were calculated according to HOMA analysis (25). *Insulin resistance units are relative to healthy subjects; †significantly different from the value at secondary sulfonylurea failure ($P < 0.05$, t test).

It was the aim of the present study to examine the effect of exogenous GLP-1 on insulin and glucagon secretion and on glycemia in the postabsorptive state in type 2 diabetic patients who had been successfully treated with sulfonylurea drugs in the past but had been treated with insulin for a minimum of 2 years (20).

RESEARCH DESIGN AND METHODS

Study protocol

The study protocol was approved by the ethics committee of the medical faculty of Ruhr-University, Bochum, before the study. Written informed consent was obtained from all participants.

Subjects

A total of 10 type 2 diabetic patients were studied. Their patient characteristics are shown in Table 1. They were selected from our hospital patients based on well-documented use of sulfonylurea drugs in the past and insulin treatment since secondary failure (indicated by insufficient metabolic control with sulfonylurea drugs, in one case in combination with metformin). The patients had been treated by insulin for a minimum period of 2 years. Overall known diabetes duration was 12 ± 6 years. Hence, a patient group with prevalent diabetic complications was studied. There was evidence for coronary disease in 9 of 10 patients, including 3 with previous myocardial infarction. One patient had peripheral vascular disease, and two patients had previous cerebrovascular

events. Retinopathy was present in four patients, and neuropathy in eight. Microalbuminuria was present in eight patients; one was normoalbuminuric, and one had macroalbuminuria. Plasma creatinine was 1.1 ± 0.2 mg/dl. Metabolic control at the time of the study was acceptable on insulin treatment on average (HbA_{1c} $8.2 \pm 1.5\%$) and was significantly improved over that documented around the time of sulfonylurea secondary failure (based on fasting and postprandial plasma glucose as well as on HbA_{1c}; Table 1).

Study design

All participants were studied in the morning (after an overnight fast) in random order on two occasions: either GLP-1 was administered intravenously at an infusion rate of $1.2 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 360 min, or placebo was infused for the same duration and at the same infusion rate (5 ml/h).

Peptides

Synthetic GLP-1 was purchased from Saxon Biochemicals (Hannover, Germany) and had a net peptide content of 88%. The peptide was dissolved in 0.9% NaCl:1% human serum albumin, filtered through $0.2 \mu\text{m}$ nitrocellulose filters (Millipore, Bedford, MA), and stored frozen at -30°C as previously described. High-performance liquid chromatography profiles (provided by the manufacturer) showed that the preparation was $>99\%$ pure (single peak coeluting with appropriate standards). Samples were analyzed for bacterial growth (standard culture techniques) and for pyro-

gens (Limulus amoebocyte lysate endo-LAL; Chromogenix AB, Mölndal, Sweden). No bacterial contamination was detected. Endotoxin concentrations in the GLP-1 stock solutions always were <0.03 U/ml.

Experimental procedures

The tests were performed in the morning after an overnight fast. On the evenings before the experiments, only the regular insulin component (at the usual dose) was injected before dinner, and NPH insulin was omitted, to achieve some degree of hyperglycemia on the morning of the experiments (Fig. 1). Other medications (e.g., antihypertensive treatment) were continued. There was 1 day allowed between the two experiments with a normal eating rhythm and regular medication (including insulin at the usual dose). Two forearm veins were punctured with a teflon cannula (Moskito 123, 18 gauge; Vygon, Aachen, Germany) and were kept patent using 0.9% NaCl (for blood sampling and GLP-1 administration, respectively).

After drawing basal blood specimens, at 0 min, an intravenous infusion of GLP-1(7-36) amide was started and continued for 360 min using 0.9% NaCl with 1% human serum albumin (Human-Albumin 20% salt-poor; Behringwerke, Marburg, Germany) as vehicle. Physiological saline with 1% human serum albumin was used as placebo. Blood was drawn before (-30 and 0 min) and every 30 min after the start of GLP-1/placebo infusions over a period of 360 min. Plasma glucose was determined immediately.

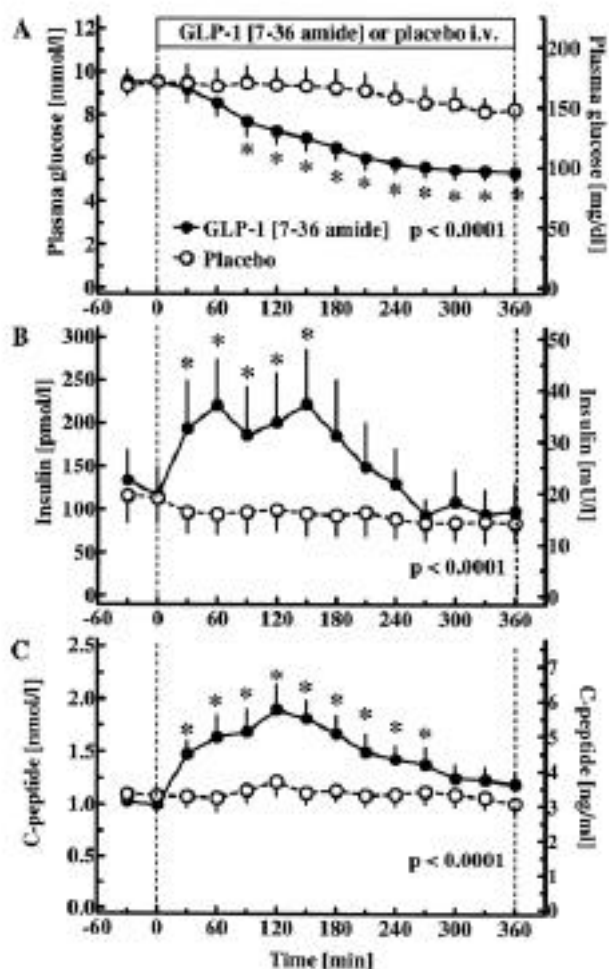


Figure 1—Plasma concentrations of glucose (A), insulin (B), and C-peptide (C) during the intravenous (i.v.) infusion of GLP-1 in 10 insulin-treated type 2 diabetic patients. Data are means \pm SEM. Values represent the interaction of treatment (GLP-1 versus placebo) and time as calculated by repeated-measures ANOVA. *Significantly different ($p < 0.05$ by Student's *t* test) at individual time points.

Blood specimens

Blood was drawn into tubes containing EDTA and aprotinin (Trasylol, 20,000 kallikrein inhibitor units [KIU]/ml, 200 μ l per 10 ml blood; Bayer, Leverkusen, Germany). A sample (\sim 100 μ l) was stored in NaF (Microvette CB 300; Sarstedt, Nümbrecht, Germany) for the measurement of glucose. After centrifugation, plasma for hormone analyses was kept frozen at -30°C .

Laboratory determinations

Glucose was measured using a glucose oxidase method with a Glucose Analyser 2 (Beckman Instruments, Munich, Germany). Plasma immunoreactive insulin and C-peptide were determined using commercial immunoassay kits. Insulin was measured using an insulin microparticle

enzyme immunoassay (MEIA) (IMx Insulin; Abbott Laboratories, Wiesbaden, Germany). Assay sensitivity was 6 pmol/l, and the working range without sample dilution was up to 1,800 pmol/l. Intra-assay coefficients of variation were 2.5–4.0% for samples containing 49.5–725 pmol/l. Interassay coefficients of variation were 3.6–4.5%. This insulin assay has <math><0.005\%</math> cross-reactivity with human proinsulin and none with C-peptide. Recovery of insulin added to plasma samples was 94–107%. Human insulin was used as standard. Low-titer anti-insulin antibodies (as might be present in patients treated with human insulin) do not interfere with this insulin immunoassay.

C-peptide was measured using C-peptide antibody-coated microtiter wells (C-peptide enzyme-linked immunosorbent

assay [ELISA]) from Dako Diagnostics (Cambridgeshire, U.K.). The detection limit was 0.017 nmol/l, and the working range was ≤ 5.0

Plasma proinsulin was assayed using an ELISA commercially available from DRG Instruments (Marburg, Germany). Assay sensitivity is 1 pmol/l. This assay also detects des (65,66) split proinsulin (cross-reactivity 55–64%), but has no cross-reactivity with des (31,32) split proinsulin (<math><1.5\%</math>) or insulin (0%). The detection limit was <math><3</math> pmol/l, and intra-assay coefficients of variation were 2.9–7.4% for samples containing 7–60 pmol/l. Interassay coefficients of variation were 5.5–6.8% for samples containing 7–65 pmol/l. Recovery of added proinsulin was between 93 and 101%. Human proinsulin was used as standard.

GLP-1 was determined in ethanol-extracted plasma as previously described (21), using antiserum 89,390 (final dilution 1:150,000) for the measurement of GLP-1 and synthetic GLP-1(7-36) amide for tracer preparation and as standard. Recovery of GLP-1 standards after alcohol extraction was $75 \pm 8\%$. The experimental detection limit (2 SDs over samples not containing GLP-1) was <math><5</math> pmol/l. Antiserum 89,390 binds to the amidated COOH-terminus of GLP-1 (22). Intra-assay coefficients of variation were <math><8\%</math>.

Pancreatic glucagon was assayed in ethanol-extracted plasma using antibody 4305 as previously described (23).

Plasma-free fatty acids (nonesterified fatty acids) were assayed using reagents from Wako Chemicals (Neuss, Germany) on a Hitachi 709 autoanalyzer. Triglycerides and total cholesterol were measured using standard clinical chemistry.

Each patient's set of plasma samples was assayed at the same time to avoid errors due to interassay variation.

Indirect calorimetry

In the basal state (-25 to 0 min), after 3 h of infusion GLP-1 (155–180 min) or placebo, and at the end of the GLP-1 infusion period (335–360 min), each patient's head was placed under a translucent gas-tight hood. Oxygen consumption and carbon dioxide production was measured using a Deltatrak MBM 100 metabolic monitor (Datex; Hoyer-Engström, Bremen, Germany). Respiratory quotients, substrate oxidation rates, and energy expenditure were calculated according to standard equations (24).

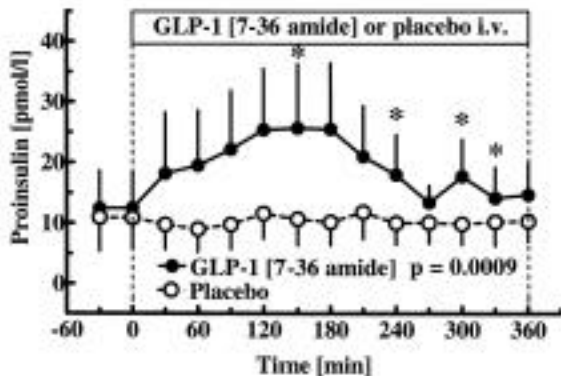


Figure 2—Plasma concentrations of proinsulin during the intravenous (i.v.) infusion of GLP-1 in insulin-treated type 2 diabetic patients (●, GLP-1; ○, placebo). Data are means \pm SEM. values represent the interaction of treatment (GLP-1 versus placebo) and time as calculated by repeated-measures ANOVA. *Significantly different ($P < 0.05$ by Student's t test) at individual time points.

Statistical analysis

Results are reported as means \pm SEM. β -Cell secretory capacity and insulin resistance were calculated according to homeostasis model assessment (HOMA) as described by Matthews et al. (25), based on mean fasting plasma glucose and insulin concentrations. β -Cell secretion of 100% and an insulin sensitivity of 1 are based on reference values obtained from normal subjects aged 40–68 years.

All statistical calculations were carried out using repeated-measures analysis of variance (ANOVA) using NCSS Version 5.01 (Jerry Hintze, Kaysville, Utah). If a significant effect of GLP-1 or an interaction of treatment and time was documented ($P < 0.05$), values at single time points were compared by Student's t test (paired analyses). A two-sided P value < 0.05 was taken to indicate significant difference.

RESULTS

Patient characteristics

The pertinent patient characteristics are presented in Table 1. At the time point of secondary sulfonylurea failure, the patients had had a BMI of 28.8 ± 5.1 kg/m², a fasting and (random) postprandial plasma glucose of 11.8 ± 2.6 and 15.2 ± 3.6 mmol/l, respectively, and an HbA_{1c} level of $9.8 \pm 1.7\%$. The duration of sulfonylurea treatment was 6 ± 4 (range 0.5–21) years, and the glibenclamide dose was 7.0 ± 3.7 mg/day. One patient, in addition, had received metformin (850 mg/day) at that time.

Plasma glucose

Basal plasma glucose concentrations (Fig. 1A) were 9.6 ± 0.4 mmol/l (before infusing

GLP-1) and 9.4 ± 0.5 mmol/l (before the administration of placebo). GLP-1 reduced plasma glucose concentrations to 5.3 ± 0.3 mmol/l. These values were reached after ~ 4 h and were more or less maintained during the last 2 h of the study. The lowest plasma glucose measured during the ongoing infusion of GLP-1 was 3.8 mmol/l. Plasma glucose concentrations in the hypoglycemic range did not occur. With placebo, only a minor reduction in plasma glucose to 8.2 ± 0.7 mmol/l occurred ($P < 0.0001$ by repeated measures ANOVA).

Plasma insulin, C-peptide, and proinsulin

Basal plasma insulin concentrations (Fig. 1B) were 126 ± 34 pmol/l (before infusing GLP-1) and 115 ± 31 pmol/l (before the administration of placebo). GLP-1 elevated insulin concentrations to 222 ± 64 pmol/l (peak after 150 min), which was 1.8-fold over basal concentrations. Plasma C-peptide concentrations (Fig. 1C) followed a similar time course, as did plasma proinsulin values (Fig. 2). The proportion of proinsulin relative to insulin and C-peptide did not change significantly from baseline values (data not shown).

During the ongoing administration of GLP-1, insulin concentrations returned to basal values again when plasma glucose concentrations (Fig. 1A) approached the normal fasting range. A similar time course was observed for C-peptide (Fig. 1C) and proinsulin (Fig. 2) during the latter half of the experiments.

GLP-1 concentrations

The intravenous administration of GLP-1

raised plasma levels to 215 ± 31 pmol/l (Fig. 3A), which was significantly higher than with placebo (GLP-1 concentrations ~ 5 pmol/l).

Glucagon concentrations

Basal plasma glucagon concentrations (Fig. 3B) were 13 ± 2 pmol/l (before infusing GLP-1) and 15 ± 2 pmol/l (before the administration of placebo). GLP-1 transiently lowered glucagon concentrations to 9 ± 1 pmol/l (after ~ 120 min). With placebo, glucagon concentrations tended to fall during the entire duration of the experiment. With repeated-measures ANOVA, no significant effect of GLP-1 was documented. However, paired t tests revealed significant differences between experiments with GLP-1 and placebo at 30 min ($P = 0.03$), 60 min ($P = 0.004$), 120 min ($P = 0.007$), 300 min ($P = 0.03$), and 360 min ($P = 0.04$).

Free fatty acids, triglycerides, and cholesterol

During the period of stimulated insulin (Fig. 1B) and suppressed glucagon (Fig. 3B) secretion, the concentration of nonesterified fatty acids was significantly reduced with GLP-1 from basal 1.00 ± 0.15 mmol/l to 0.66 ± 0.14 mmol/l at 180 min ($P = 0.0012$; data not shown). However, free fatty acids returned to basal values when plasma glucose concentrations approached the normal fasting concentration range (Fig. 1A). Triglyceride and cholesterol concentrations did not change significantly during the study period.

Indirect calorimetry

As shown in Fig. 4, none of the parameters measured by indirect calorimetry changed significantly when the basal state was compared with 3 and 6 h of exogenous GLP-1 administration or if experiments with GLP-1 and placebo were compared.

CONCLUSIONS— The present study extends previous findings that GLP-1 is able to normalize fasting plasma glucose in type 2 diabetic patients on diet treatment (mild type 2 diabetes) (26) and at the time point of sulfonylurea secondary failure (9,26). In other studies, more mixed groups of type 2 diabetic patients have been studied and the uniform result was that GLP-1 was able to truly normalize glycemia in any hyperglycemic type 2 diabetic patient independent of age, sex, weight, previous oral antidiabetic treatment, and other patient characteristics of

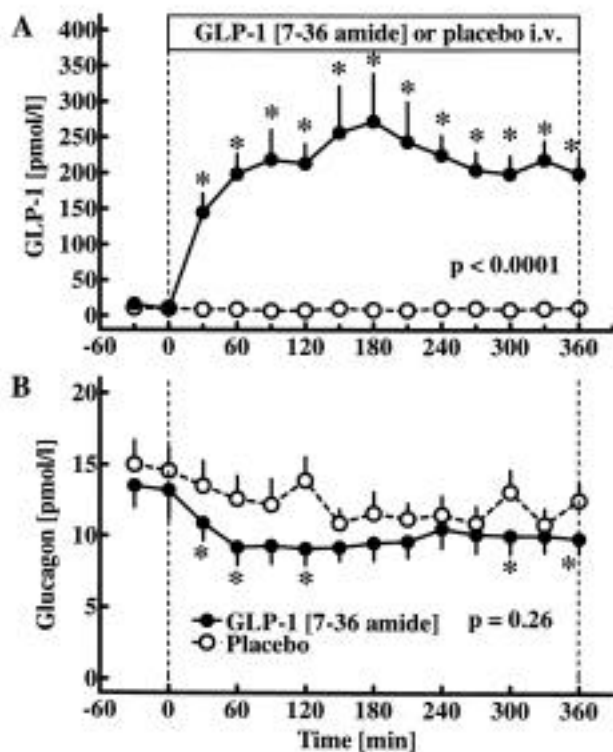


Figure 3—Plasma concentrations of GLP-1 (A) and glucagon (B) during the intravenous (i.v.) infusion of GLP-1 in 10 insulin-treated type 2 diabetic patients. ●, GLP-1; ○, placebo. Data are means \pm SEM. P values represent the interaction of treatment (GLP-1 versus placebo) and time as calculated by repeated-measures ANOVA. *Significantly different ($P < 0.05$ by Student's *t* test) at individual time points.

potential influence. According to the present results, a 4-h intravenous infusion of GLP-1 is sufficient to normalize plasma glucose even in long-term insulin-treated type 2 diabetic patients made hyperglycemic by the withdrawal of long-acting insulin the evening before the experiments (Fig. 1A). It is obvious from the insulin and C-peptide responses (Fig. 1B and C) that the stimulation of insulin secretion also plays an important role for the glucose-lowering effect in late stages of type 2 diabetes. β -Cell secretory responses (as estimated from insulin and C-peptide concentrations in peripheral plasma) display the same pattern that has been known from previous studies: there is an initial rise (due to the insulinotropic action of GLP-1 [9,13,27]), but with normalization of glycemia, there is a reduction in insulin and C-peptide values (9,26) (Fig. 1). This is most likely explained by the well-known glucose-dependence of GLP-1 effects on insulin secretion (28,29). In line with this interpretation, in the present study no hypoglycemic reaction toward GLP-1 infusion was observed, even though the period of GLP-1 administration was longer than in our previous studies in less-

advanced stages of type 2 diabetes (9). This indicates that a similar glucose threshold for GLP-1-induced insulin secretion is still active in patients with true sulfonylurea secondary failure. The good response to GLP-1 in patients no longer clinically responding to sulfonylureas may be taken as additional evidence for the differences in the mechanism of action between sulfonylureas (18) and GLP-1 (19).

The present study does not make a direct comparison between the GLP-1 effect in normal versus type 2 diabetic patients or between different stages of type 2 diabetes. Comparison with previous studies suggests that in metabolically healthy subjects and in type 2 diabetic patients who are still responsive to sulfonylurea treatment, insulin secretion is stimulated to a greater degree than in the present study (6,9).

The reduction in plasma glucagon concentrations (Fig. 3B) was not as prominent as in previous projects studying other patient groups (6,9,26). It appears possible that the longer duration of the experiments (6 instead of 4 h) makes the overall differences appear smaller and that the statistical

analysis by repeated-measures ANOVA, therefore, does not detect a significant difference. Given the clear results obtained by paired *t* tests, it appears justified to conclude that glucagon was suppressed also in the present experiments.

Previous examinations of GLP-1 effects, however, in metabolically healthy subjects, described a marked insulinotropic effect at a hyperglycemic glucose concentration (~ 8 mmol/l), which was accompanied by an increase in energy expenditure, respiratory quotient, and carbohydrate oxidation (30), and a decrease in fat oxidation. Similar changes were not observed in the present study (Fig. 4), despite a major influence of GLP-1 on glucose (Fig. 1A) and free fatty acid concentrations (i.e., extracellular substrate availability) and on insulin (Fig. 1B), as well as glucagon (Fig. 2B), concentrations. In comparison with the study by Shalev et al. (30), however, the increase in insulin was smaller (222 ± 64 vs. $\sim 1,500$ pmol/l). Thus, in the present study, the increases in insulin concentrations and the changes in glucose versus free fatty acid concentrations were too small to elicit, e.g., a preferential oxidation of carbohydrates. Furthermore, the rates of (intracellular) substrate oxidation may not reflect extracellular substrate availability in type 2 diabetic patients, in whom impaired glucose transport or oxidation is a typical characteristic (31).

A priori, one would not expect a prominent insulinotropic effect (Fig. 1B and C) in a group of type 2 diabetic patients to be characterized by a rather low number of β -cells, prominent islet amyloidosis, and impaired insulin responses. These are the peculiarities assumed to be associated with sulfonylurea failure in type 2 diabetes (16). At least with GLP-1 as a stimulus, there is no obvious reduction in its insulinotropic effect in the presently studied group of patients. These patients, on the other hand, were clinically characterized as having passed the stage of sulfonylurea secondary failure (Table 1). This purely clinical classification may appear a little imprecise, but no clearer criteria are available at present. This is evident from the wide range of β -cell secretion estimated by HOMA analysis. No additional pharmacological tests were performed to test β -cell secretory capacity in comparison with the response to exogenous GLP-1. Such additional studies might shed more light on the pathophysiological basis of sulfonylurea secondary failure.

One may object that insulin treatment will put the endocrine pancreas at rest and

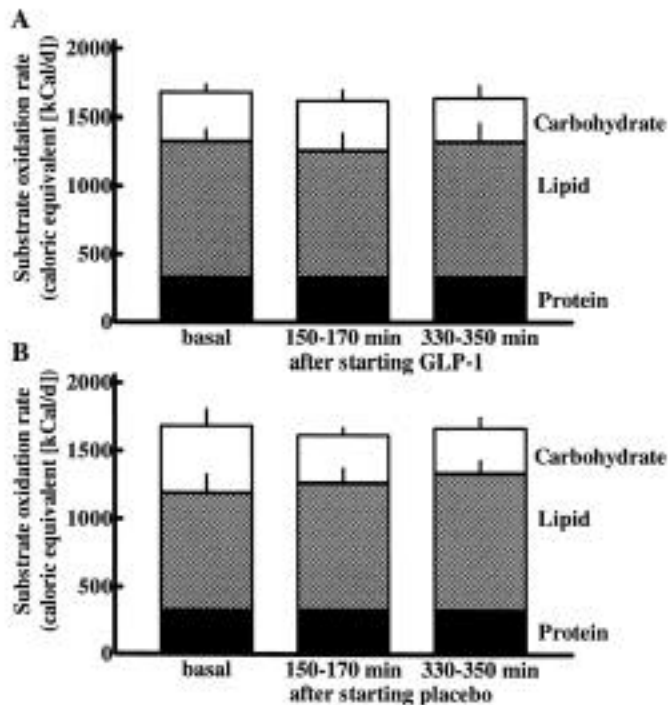


Figure 4—Substrate oxidation rates as determined by indirect calorimetry in units of energy expenditure during the intravenous infusion of GLP-1 (A) or placebo (B) in 10 insulin-treated type 2 diabetic patients. Data are means \pm SEM. There was no significant change with time or between experiments with GLP-1 or placebo, respectively, by repeated-measures ANOVA.

will—if successful as judged by glucose concentrations and HbA_{1c} levels (Table 1)—lead to a partial recovery of β -cell secretion due to reduced glucose toxicity (32,33). In that case, a well-maintained insulinotropic response to GLP-1 may not be viewed as a surprise. Following this line of reasoning, the previously studied group of type 2 diabetic patients at the stage of sulfonylurea secondary failure (9), in retrospect, may have been in a more extreme metabolic situation.

It appears from the HOMA analysis that there was reduced β -cell secretion in the present group of type 2 diabetic patients (72 \pm 17% of normal subjects). On the other hand, insulin resistance was much more prominent (8.7-fold higher relative to healthy subjects) (Table 1). The latter finding is supported by the degree of obesity present (Table 1) and by the daily insulin dose of 77 \pm 24 U used. Therefore, the present results may primarily characterize the action of GLP-1 in type 2 diabetic patients requiring insulin because of prominent insulin resistance (rather than severe β -cell failure). Future studies should preferentially include patients with less prominent insulin resistance and inadequate insulin secretory responses to glucagon,

arginine, or other pharmacological secretagogues. Theoretically, such prerequisites might be anticipated to preclude clinically meaningful effects of GLP-1, although lack of effectiveness of GLP-1 (regarding its ability to lower or even normalize plasma glucose) has not yet been observed in any single type 2 diabetic patient. The present study was designed to test GLP-1 effects in a group of patients considered a priori to be the ones least likely to respond sufficiently. Along that line, the results should encourage further efforts to develop GLP-1 as a new treatment for type 2 diabetes (34) because the likelihood of treatment failures appears to be very low, from the mild initial stages to patients who have progressed far and who presently need insulin. Clinical studies, however, will have to wait until a GLP-1 preparation will be available that has a longer duration of action on subcutaneous injection (35).

GLP-1 plasma levels were higher in the present study than previously reported in type 2 diabetic patients receiving a similar infusion regimen (6,9,11,26). The most likely reason for this is the more progressed stage of the patients' nephropathy. Microalbuminuria was present in a large proportion of patients (7 of 10), and plasma creatinine

concentrations exceeded normal values in four. GLP-1 is eliminated via the kidneys (36), and in uremic patients, elevated GLP-1 concentrations have been described (37). The impaired elimination of proglucagon (1-61) [glicentin (1-61)], the circulating concentration of which builds up in uremia (38), may also help to explain the apparent nonsuppression of glucagon concentrations (Fig. 3) because this molecule cross-reacts in our glucagon assay without displaying a similar biological activity. Other unknown factors related to the patients' clinical characteristics, however, may contribute to the apparently slower elimination of GLP-1 in the present study.

The proportion of proinsulin relative to intact insulin or C-peptide (Fig. 2) is in line with the previously reported hyperproinsulinemia of type 2 diabetic patients (39,40).

In conclusion, GLP-1 normalizes fasting plasma glucose concentrations in type 2 diabetic patients with sulfonylurea secondary failure who are usually controlled by conventional insulin treatment. In this patient group, as in previous studies, insulin secretion was stimulated and glucagon was suppressed transiently, and prolonged administration of GLP-1 over 6 h did not lead to hypoglycemia. These results should broaden the applicability of GLP-1-derived drugs to later stages of type 2 diabetes. In such patients, GLP-1 may become an alternative to insulin treatment.

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