Additive Glucose-Lowering Effects of Glucagon-Like Peptide-1 and Metformin in Type 2 Diabetes

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OBJECTIVE — The incretin hormone glucagon-like peptide-1 (GLP-1) reduces plasma glucose in type 2 diabetic patients by stimulating insulin secretion and inhibiting glucagon secretion. The biguanide metformin is believed to lower plasma glucose without affecting insulin secretion. We conducted this study to investigate the effect of a combination therapy with GLP-1 and metformin, which could theoretically be additive, in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — In a semiblinded randomized crossover study, seven patients received treatment with metformin (1,500 mg daily orally) alternating with GLP-1 (continuous subcutaneous infusion of 2.4 pmol · kg^{-1} · min^{-1}) alternating with a combination of metformin and GLP-1 for 48 h. Under fixed energy intake, we examined the effects on plasma glucose, insulin, C-peptide, glucagon, and appetite.

RESULTS — Fasting plasma glucose (day 2) decreased from 13.9 ± 1 (no treatment) to 11.2 ± 0.4 (metformin) and 11.5 ± 0.5 (GLP-1) and further decreased to 9.4 ± 0.7 (combination therapy) ($P = 0.0005$, no difference between monotherapy with GLP-1 and metformin). The 24-h mean plasma glucose (day 2) decreased from 11.8 ± 0.5 (metformin) and 11.7 ± 0.8 (GLP-1) to 9.8 ± 0.5 (combination) ($P = 0.02$, no difference between GLP-1 and metformin). Insulin levels were similar between the three regimens, but glucagon levels were significantly reduced with GLP-1 compared with metformin ($P = 0.0003$). Combination therapy had no additional effect on appetite scores.

CONCLUSIONS — Monotherapy with GLP-1 and metformin have equal effects on plasma glucose and additive effects upon combination.

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Type 2 diabetes, which is characterized by insulin resistance, impaired insulin secretion, increased hepatic glucose production, and often obesity, is a progressive disease (1,2). Oral antidiabetic agents, as monotherapy or in combination, initially lower blood glucose effectively, but a deterioration of glycemic control usually develops after some years' duration of disease (1,2). For obese type 2 diabetic patients, the biguanide metformin is recommended as the drug of first choice (3). Metformin effectively reduces plasma glucose by 3–4 mmol/l (4,5). The effect is believed to result from reduction in insulin resistance (6,7) and hepatic glucose production (5), although the latter may predominate (8). Upon progression of type 2 diabetes, metformin is often combined with sulfonylureas, and the combination of metformin and sulfonylureas may reduce fasting plasma glucose by an additional 3–5 mmol/l (4,9). Sulfonylureas stimulate insulin secretion by a glucose-independent closure of the potassium channels in the β-cells (10) and, because of this, may induce severe hypoglycemia (2). However, despite the combination therapy, glycemic control often deteriorates with time (1,2).

The intestinal incretin hormone glucagon-like peptide-1 (GLP-1) stimulates insulin secretion as well as insulin biosynthesis (11,12) and inhibits glucagon secretion (11). Because its effects are glucose-dependent, it does not cause hypoglycemia (11). In addition, GLP-1 reduces meal-induced glucose excursions (13) and reduces appetite (14). Lastly, in animal studies, GLP-1 exerts a trophic effect on pancreatic β-cells (15,16). Although not yet proven, a GLP-1–based therapy could therefore be expected to lead to an improved β-cell function rather than deterioration with time. To date, continuous subcutaneous infusion of GLP-1 is the only way of administering GLP-1 in clinical practice. This is because GLP-1 is inactivated rapidly by the ubiquitous enzyme dipeptidyl peptidase IV, which means that the peptide must be administered continuously (17,18).

Because GLP-1 acts by different mechanisms than metformin, a combination could theoretically have an additive or synergistic effect on plasma glucose. Furthermore, a combination of GLP-1 and metformin might reduce appetite and preserve β-cell function as opposed to a combination of metformin and sulfonylureas. We therefore studied the effect of a a combination of GLP-1 and metformin on plasma glucose and appetite in patients with type 2 diabetes. Because previous studies have evaluated the effects of both GLP-1 (14) and metformin (4) compared with placebo, a placebo study was omitted from the present experiments. Preliminary data have been published in abstract form (19).

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Abbreviations: AUC, area under the curve; GLP-1, glucagon-like peptide-1.

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

RESEARCH DESIGN AND METHODS

Subjects

Five men and two women with type 2 diabetes diagnosed after 40 years of age participated in the study: mean age 53.9 ± 6.5 years (range 49–61); BMI 32.7 ± 1.3 kg/m^2; and HbA_{1c} 9.7 ± 0.5%.

All patients had normal hemoglobin and s-creatinine levels. One patient was treated with diet alone; the other patients were treated with 1,000–2,000 mg metformin daily and/or 2.5–5 mg glipizide daily.

Procedure

The study was designed as a semiblinded crossover study. The patients were randomly allocated to three regimens of treatment. Four patients received treatment according to regimen 1, two patients were treated according to regimen 2, and one patient was treated according to regimen 3, as shown in Fig. 1.

During admittance, the patients were equipped with a portable insulin pump (MiniMed 506; Novo Nordisk Pharmaceuticals, Princeton, NJ) for continuous, subcutaneous infusion of GLP-1 or saline. The infusion rate was 2.4 pmol · kg^{-1} · min^{-1}. The injection site, in the periumbilical region, was changed twice daily, and the syringe was refilled twice daily. GLP-1(7-36)-amide was produced by custom synthesis by Polypeptides AG (Leverkusen, Germany). The correctness of structure and the purity of the peptide (>98%) was ascertained by mass spectrometry and sequence analysis. The peptide was dissolved in sodium phosphate buffer (300 mOsm/l, pH 7.4) to a final concentration of 400 μg/ml. The solution was subjected to sterile filtration and dispensed into glass ampoules and stored frozen under sterile conditions until use. Metformin (Glucophage; Lipha, Glostrup, Denmark) was administered orally as tablets at a dosage of 500 mg three times daily (7:30 A.M., 11:30 A.M., and 5:00 P.M.).

The patients were fed a meal three times daily (8:00 A.M., 12:00 P.M., and 5:30 P.M.). Each patient received the same amount and composition of food on all days. Noncaloric beverages were allowed at all times.

During each admittance, the patients rated sensation of hunger, satiety, fullness, prospective food consumption, nausea, and well-being on 100-mm visual analog scales before and 2 h after each meal. Gastrointestinal side effects such as hiccups, ructus, abdominal distension, flatulence, and abdominal discomfort were rated using a questionnaire 2 h after each meal. Each side effect could be graduated with four options: no symptoms, few symptoms, moderate symptoms, and severe symptoms.

On day 1, plasma glucose was measured before and 90 min after each meal, at 2:00 A.M. and at 7:00 A.M.

On day 2, plasma glucose was measured every 30 min from 8:00 A.M. to 3:00 P.M. and every hour from 3:00 P.M. to 5:00 P.M. In addition, plasma glucose was measured at 5:30 P.M., 7:00 P.M., 2:00 A.M., and 7:00 A.M.

On day 2, blood samples were taken every 30 min from 8:00 A.M. to 3:00 P.M. and every hour from 3:00 P.M. to 5:00 P.M. for determination of plasma concentrations of GLP-1, C-peptide, insulin, and glucagon.

Blood was distributed into sodium fluoride tubes for plasma glucose, into EDTA tubes for GLP-1 and glucagon, and into heparin tubes for insulin and C-peptide determinations.

Trasylol (500 KIU/ml blood; Bayer, Leverkusen, Germany) and valine pyrrolidide (10 μmol/l blood; a gift from Tom Hughes, Novartis, NJ) were added to the EDTA tubes, and Trasylol was added to the heparin tubes. The tubes were immediately chilled in ice and centrifuged at 4°C for 20 min. Plasma for insulin and C-peptide analyses was stored at –80°C. Plasma for glucagon and GLP-1 analyses was stored at –20°C.

Analysis

Plasma glucose concentrations were analyzed using a Beckman Analyzer (Beckman Instrument, Fullerton, CA), except for the nighttime measurements, which were performed on full blood using a Hemocue analyzer (Hemocue, Angelholm, Sweden). Blood glucose concentrations may be converted to plasma glucose concentrations by multiplying by 1.11 (20).

The glucagon assay (21) is directed against the COOH-terminus of the glucagon molecule (antibody code no. 4305) and therefore measures glucagon of mainly pancreatic origin.

GLP-1 is metabolized rapidly while circulating, whereby the truncated biologically inactive metabolite GLP-1(9-36)-amide is formed. Thus, to gauge the total amount of GLP-1 absorbed from the injection site, the sum of intact GLP-1 and its metabolite must be determined. Total GLP-1 was measured by radioimmunoassay using antiserum code no. 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(9-39) (22). Concentrations of intact GLP-1 were measured using an NH_2-terminally directed antibody (code no. 93242) (23).

For glucagon and GLP-1 analysis, plasma was extracted with ethanol (final concentration 70% vol/vol) before analysis. Detection limits and intra-assay coefficients of the assays used were 1 pmol/l and <6% for glucagon, 1 pmol/l and <6% for amidated GLP-1 (antibody code no. 89390), and <5 pmol/l and 7% for intact GLP-1 (antibody code no. 93242).

Insulin and C-peptide concentrations were measured using commercial enzyme-linked immunosorbent assay kits (code nos. K6219 and K6218, respectively; Dako, Copenhagen, Denmark).

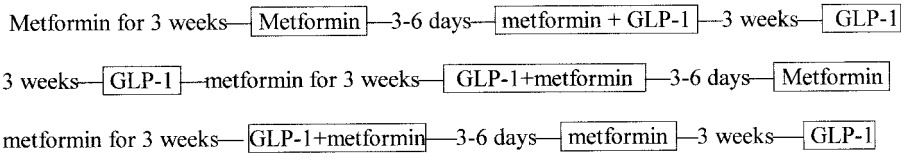

Figure 1—*Study design. Oral antidiabetic agents were discontinued 3 weeks in advance. The patients were admitted to the hospital for 3 × 48 h and received, in randomized order, treatment with GLP-1 for 48 h, alternating with treatment with metformin for 48 h, alternating with combination treatment of GLP-1 and metformin for 48 h. If a patient was randomized to start with metformin or combination of metformin + GLP-1, metformin was started 3 weeks in advance to ensure a steady state. When GLP-1 alone was to be studied, metformin was discontinued 3 weeks in advance to ensure an adequate washout of metformin. A period of 3–6 days was allowed between GLP-1 + metformin and metformin alone to ensure adequate washout of GLP-1. Each box represents treatment during examination days in the hospital.*

The detection limits were 3 pmol/l for insulin assay and 17 pmol/l for C-peptide. Intra-assay and interassay coefficients of variation were 4–10% at 39–1,240 pmol/l for insulin and 3–6% at 380–2,700 pmol/l for C-peptide. The cross-reactivity with intact and split proinsulins in the C-peptide assay was 63–87%. HbA_{1c} was measured at the Steno Diabetes Hospital (Gentofte, Denmark) using an ion-exchange high-performance liquid chromatography method endowing the analysis with an interassay coefficient of variation of 0.15% in the range of 4.7–11.3% (normal range 4.1–6.4).

Statistical analysis and calculations

All results are presented as means ± SEM. For statistical analysis, we used one-way analysis of variance for repeated measurements, Tukey's post hoc test for parametric analysis of normally distributed data, and the Friedman test with Dunn's post hoc test for nonparametric analysis. Statistical significance was set at $P < 0.05$.

Area under the curve (AUC) values were calculated using the trapezoidal rule.

RESULTS

Plasma concentrations of total and intact GLP-1

The mean concentrations of total GLP-1 (intact hormone plus metabolite) increased from 17.7 ± 2.3 pmol/l during monotherapy with metformin (met) to 101.6 ± 14.7 pmol/l during treatment with metformin and GLP-1 (comb) and 108.1 ± 8.2 pmol/l during GLP-1 infusion (GLP-1) ($P = 0.0001$). The mean levels of intact GLP-1 concentrations increased from 20.0 ± 2.1 (met) to 31.8 ± 3.8 (comb) and 35.3 ± 2.7 (GLP-1) pmol/l; $P < 0.0001$, no difference between comb and GLP-1.

Plasma glucose concentrations

On day 1, 24-h mean plasma glucose values, calculated from 9:30 A.M. (allowing the 2.5 h between 7:00 A.M. and 9:30 A.M. for the GLP-1 effect to develop) to 7:00 A.M. the following day, decreased from 11.5 ± 0.9 (GLP-1) and 11.8 ± 0.5 (met) to 9.3 ± 0.4 (comb) mmol/l ($P = 0.006$) (Fig. 2). We found no difference in blood glucose concentrations between monotherapy with GLP-1 and metformin. The corresponding AUC values decreased from 230.9 ± 16.5 (GLP-1) and 236.8 ± 11.1 (met) to 187.6 ± 8.9 (comb) mmol/l × h ($P = 0.006$).

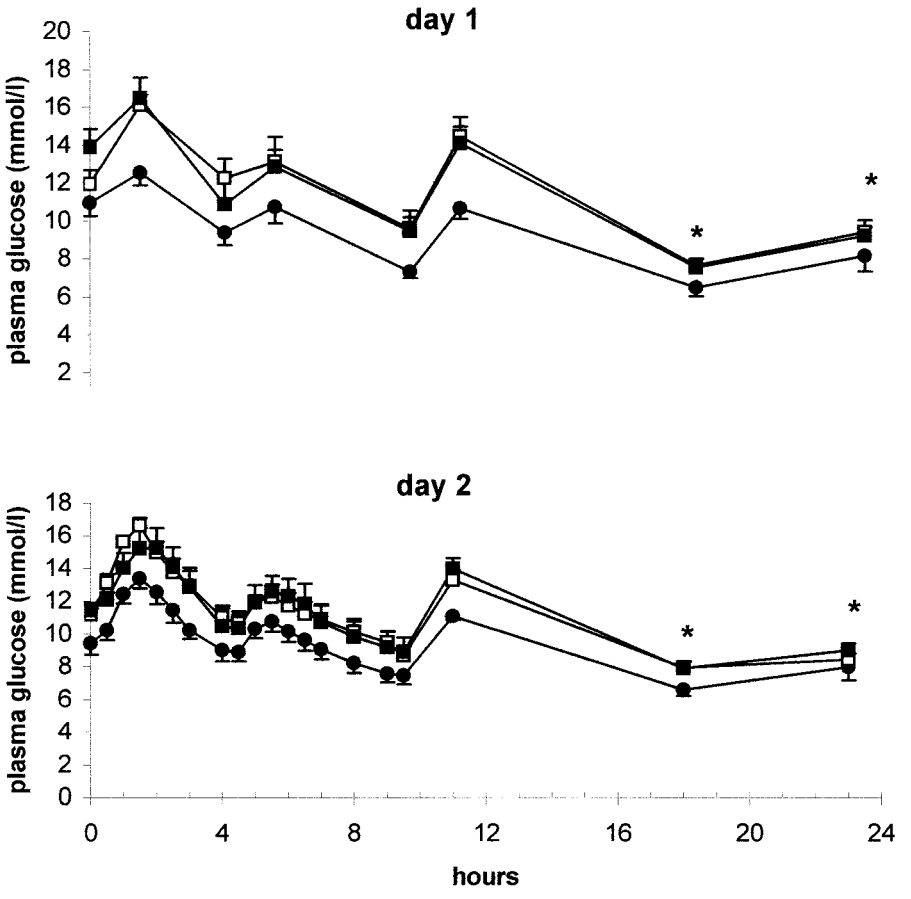

Figure 2—*Plasma glucose concentrations. Values are mean + 1 SEM. Plasma glucose was significantly reduced on combination therapy (●—●) compared with monotherapy with GLP-1 (■—■) and metformin (□—□). There was no difference between monotherapy with metformin and GLP-1. *Blood glucose concentrations as analyzed on a Hemocue analyzer.*

On day 2, fasting plasma glucose decreased from 11.2 ± 0.4 (met) and 11.5 ± 0.5 (GLP-1) to 9.4 ± 0.7 (comb) mmol/l ($P = 0.008$) (Fig. 2). The 24-h mean plasma glucose calculated from 8:00 A.M. to 7:00 A.M. the following day decreased from 11.7 ± 0.8 (GLP-1) and 11.8 ± 0.5 (met) to 9.8 ± 0.5 (comb) mmol/l ($P = 0.02$) (Fig. 2). Corresponding AUC values decreased from 245.3 ± 15.1 (GLP-1) and 244.3 ± 9.0 (met) to 202.9 ± 7.8 (comb) mmol/l × h ($P = 0.004$). No difference was observed between GLP-1 and metformin.

The effect of monotherapy as well as combination therapy compared with the effect of no treatment could be evaluated, in part, by comparison of fasting plasma glucose values from day 1 of GLP-1 treatment (because at that time, the patients had been without treatment for 3 weeks). This value was compared with fasting values from day 2. Fasting plasma glucose decreased from 13.9 ± 1.0 (no treatment) to 11.2 ± 0.4 (met) and 11.5 ± 0.5 (GLP-1) and was further reduced to 9.4 ± 0.7 (comb) mmol/l ($P = 0.0005$ for all treatments compared with no treatment).

Glucagon, C-peptide, and insulin concentrations

Mean plasma glucagon concentration decreased from 13.2 ± 0.8 (met) to 11.7 ± 0.9 (comb) and 10.2 ± 0.8 (GLP-1) pmol/l ($P = 0.0003$ with significance between GLP-1 and metformin, whereas the difference between combination therapy and metformin failed to reach statistical significance) (Fig. 3). Corresponding AUC values decreased from 117.3 ± 8.0 (met) to 104.0 ± 8.1 (comb) and 90.2 ± 7.4 (GLP-1) pmol/l × h ($P = 0.0003$ with significance between metformin and GLP-1 as monotherapy).

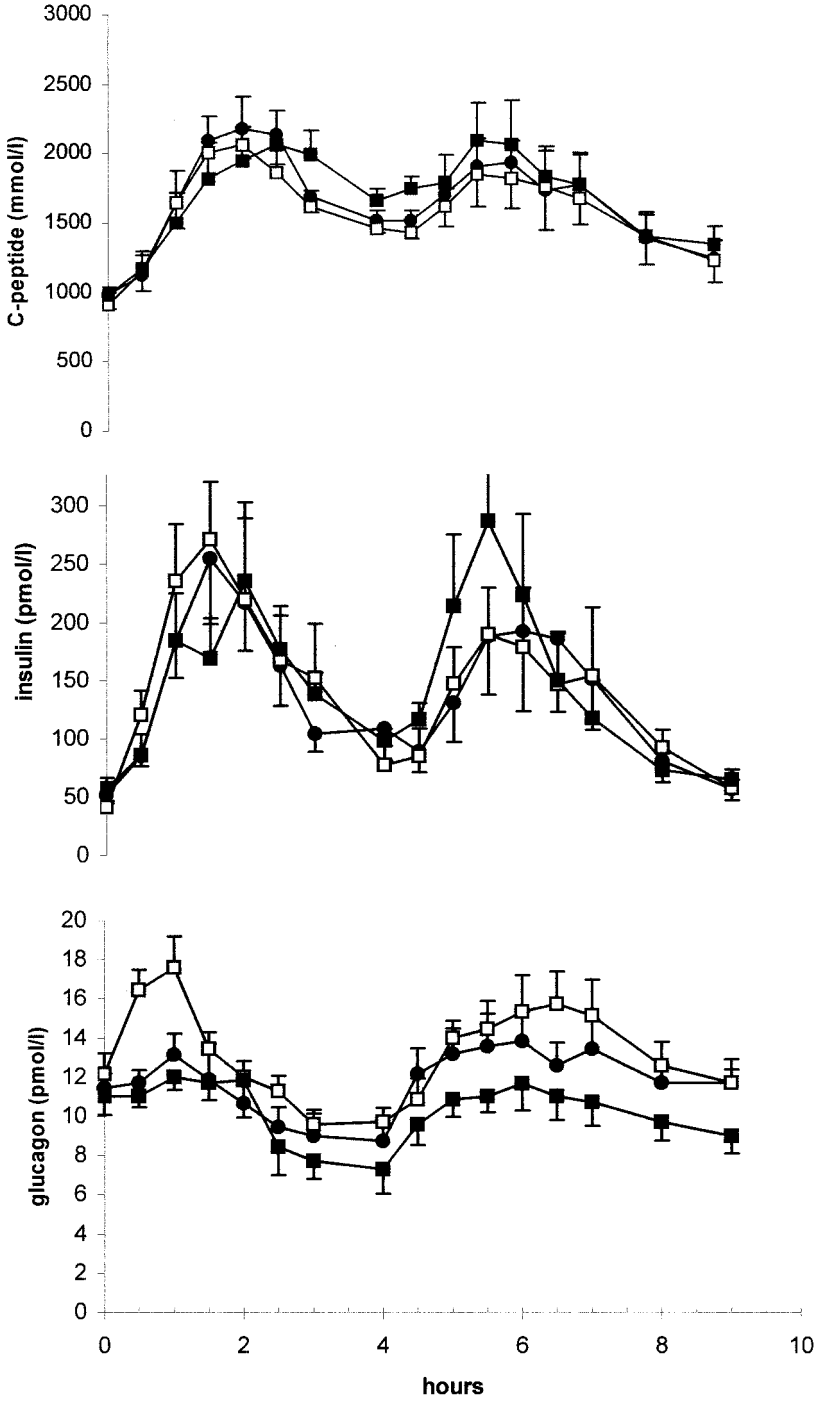

Figure 3—*Nine-hour profiles of the plasma concentrations of C-peptide, insulin, and glucagon concentration. Values are mean + 1 SEM. ●—●, Combination therapy; ■—■, monotherapy with metformin; and □—□, monotherapy with GLP-1. There was no difference between the insulin and C-peptide concentrations at any time of the day on the three regimens. The glucagon concentrations were significantly lower on monotherapy with GLP-1 compared with monotherapy with metformin* ($P = 0.0003$).

We found no significant difference with respect to fasting, 24-h mean-of-the-day, or AUC values for C-peptide and insulin levels between the three regimens (Fig. 3).

Appetite and side effects

For calculation of appetite, we used visual analog scales from day 2 to ensure consistency in appetite.

There was a trend toward reduction in appetite on combination therapy compared with monotherapy (based on an average score, calculated as the sum of reciprocal values of satiety and fullness plus the values for hunger and prospective food consumption). Appetite scores were 143 ± 22 (met), 136 ± 26 (GLP-1), and 122 ± 22 (comb) mm. Corresponding AUC values were 1,607 ± 272 (met), 1,536 ± 340 (GLP-1), and 1,377 ± 277 (comb) mm × h, but none of the changes were significant.

Side effects, recorded as nausea, impaired well-being, hiccups, ructus, abdominal distension, flatulence, and abdominal discomfort, scored very low for all days with no significant difference between the three regimens. Data are not shown.

CONCLUSIONS

— In the present study, a similar degree of glycemic control was obtained by subcutaneous infusion of 2.4 pmol · kg^{-1} · min^{-1} GLP-1 and treatment with 1,500 mg metformin. A further improvement in glycemic control was obtained by combining continuous subcutaneous infusion of GLP-1 and metformin treatment.

Without any treatment, the mean fasting plasma glucose concentration was 13.6 mmol/l, and after GLP-1 and metformin treatment, the corresponding values were 11.5 and 11.2 mmol/l, respectively. This indicates that the treatment with both metformin and GLP-1 lowered plasma glucose by 2–3 mmol/l.

The combination of metformin and GLP-1 reduced plasma glucose by an extra 2 mmol/l, resulting in a mean fasting plasma glucose of 9.4 mmol/l and a 24-h mean-of-the-day of 9.8 mmol/l. Thus, the combination of metformin and GLP-1 seems to elicit additive effects. This would be in agreement with the fact that the two agents lower blood glucose by different mechanisms, namely for metformin, by increasing peripheral insulin sensitivity (6,7) and reducing hepatic glucose production (5) and for GLP-1, by a predominant action on islet secretion of insulin and glucagon (11,12). We used 1,500 mg metformin, a dose with which a decrease in blood glucose level of ~2–4 mmol/l may be expected (1,4,24). For GLP-1, we chose an infusion rate of 2.4 pmol · kg^{-1} · min^{-1}, a dose expected to reduce mean plasma glucose by 2–3 mmol/l (14). The doses of metformin and GLP-1, both of which are submaximal, were chosen to

allow optimal conditions to demonstrate additive effects rather than to demonstrate maximal effects of the two treatments.

The plasma level of intact GLP-1 in the present study increased to 30–35 pmol/l. Continuous intravenous infusion of 1.2 pmol · kg^{-1} · min^{-1} GLP-1 resulted in a plasma concentration of intact GLP-1 of 20–30 pmol/l (18). Thus, the concentrations of intact GLP-1 in the present study correspond to the concentrations of intact GLP-1 after intravenous infusion of 1.2 pmol · kg^{-1} · min^{-1}, supporting the assumption of a bioavailability of 50% after subcutaneous infusions (14). Rachmann et al. (25) used the same infusion rate in a study of the effects of intravenous infusion of GLP-1 in type 2 diabetic patients and obtained a larger reduction in plasma glucose levels. Therefore, it seems that subcutaneous administration is less efficacious than intravenous administration. This could be due to higher concentrations of the antagonistic metabolite GLP-1(9-36)-amide after subcutaneous infusion compared with intravenous infusion. It has been shown that, in high concentrations, its metabolite antagonizes the effect of GLP-1 by competitive inhibition of its receptor activation (26). This important issue clearly requires further investigation.

Metformin was recently described to be able to inhibit GLP-1 degradation (27), causing elevated levels of intact GLP-1. In our study, plasma levels of intact GLP-1 were similar in the presence and absence of metformin. Inhibition of GLP-1 degradation, therefore, cannot explain the additive effect of GLP-1 and metformin therapy.

Surprisingly, we did not find higher insulin and C-peptide levels during GLP-1 treatment compared with metformin alone, taking the identical mean blood glucose levels during the two treatments and the insulinotropic effect of GLP-1 into consideration. Neither the ratio of insulin over glucose nor the ratio of C-peptide over glucose differed between the three groups. The patients were in poor metabolic control with a mean fasting plasma glucose level of 14 mmol/l. As we have previously shown, the insulin response to GLP-1 depends on β-cell function (28); therefore, it is possible that the patients in the present study were incapable of responding to GLP-1 infusion with augmented insulin secretion. Nauck et al. (29) recently demonstrated a preserved insulinotropic effect of GLP-1 in patients with longstanding type 2 diabetes, but these patients were insulin treated and therefore probably less exposed to glucotoxicity than our patients who were all treated with oral agents. Similarly, the identical insulin levels in the metformin and GLP-1 group could be due to a higher degree of glucotoxicity in the GLP-1 group, the members of which were untreated for 3 weeks, whereas the metformin group had been on 3 weeks of therapy.

Still, we observed a glucose-lowering effect of GLP-1 that was similar to that obtained in a previous study using the same infusion rate and the same number of patients (14). However, GLP-1 also inhibits glucagon secretion, and the 24-h mean plasma glucagon level on monotherapy with GLP-1 was significantly reduced compared with monotherapy with metformin. Furthermore, plasma glucagon levels tended to be even lower upon combination of GLP-1 and metformin compared with monotherapy with metformin. The seemingly weaker effect of GLP-1 on the combination day may be due to lower plasma glucose levels, which would tend to increase glucagon secretion. Inhibition of the glucagon level contributes to the glucose-lowering effect of GLP-1; this is supported by the observation that GLP-1 also reduces fasting plasma glucose in type 1 diabetic patients with minimal residual β-cell function (30). In these patients, who could only respond with a marginal insulin secretion to GLP-1, glucagon secretion was significantly inhibited, presumably resulting in decreased hepatic glucose production and, therefore, a decreasing plasma glucose (30).

Another possible advantage of combining GLP-1 and metformin could be an effect on body weight. GLP-1 reduces appetite in diabetic patients (14). The exact mechanism is unknown but could involve inhibition of gastric emptying (13). GLP-1 also inhibits food intake and reduces weight in rats when administered intracerebroventricularly (31). Whether peripherally administered GLP-1 reaches these central appetite-regulating centers is unknown but remains a possibility. In the U.K. Prospective Diabetes Study, metformin improved glycemic control without inducing weight gain; in contrast, treatment with sulfonylurea or insulin (3,4) and metformin may directly reduce weight (5). The mechanism whereby metformin reduces/stabilizes body weight is controversial (6), but reduction in appetite has been proposed (5). We observed a tendency toward reduced appetite during combination treatment compared with monotherapy, but the changes failed to reach statistical significance. Metformin causes side effects such as abdominal discomfort and diarrhea in 20–30% of patients (1). A dose-response study has shown that high doses of GLP-1 cause nausea and vomiting (32), but at therapeutic levels in diabetic patients, no side effects are reported (14). Based on the visual analog scale scores and the questionnaires, our patients seemed to have had very few side effects on either monotherapy, and the combination of GLP-1 and metformin showed no aggravation.

In conclusion, treatment for 48 h with continuous subcutaneous infusion of GLP-1 at a rate of 2.4 pmol · kg^{-1} · min^{-1} seems to equal the effect of 1,500 mg of metformin perorally daily based on fasting and 24-h mean-of-the-day plasma glucose values. Combination of GLP-1 and metformin further reduced plasma glucose by an extra 2 mmol/l with no side effects. Therefore, combination therapy with metformin and GLP-1 seems to have additive effects on plasma glucose in type 2 diabetic patients.

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