# Additive Effects of Glucagon-Like Peptide 1 and Pioglitazone in Patients With Type 2 Diabetes

METTE ZANDER, MD, PHD[1,2]
ALLAN CHRISTIANSEN, MD[1,2]
STEN MADSBAD, MD, DMSC[1]
JENS JUUL HOLST, MD, DMSC[2]

**OBJECTIVE** — To evaluate the effect of combination therapy with pioglitazone and glucagon-like peptide (GLP)-1 in patients with type 2 diabetes.

**RESEARCH DESIGN AND METHODS** — Eight patients with type 2 diabetes (BMI 32.7 ± 1.3 kg/m$^2$ and fasting plasma glucose 13.5 ± 1.2 mmol/l) underwent four different treatment regimens in random order: saline therapy, monotherapy with continuous subcutaneous infusion of GLP-1 (4.8 pmol · kg$^{-1}$ · min$^{-1}$), monotherapy with pioglitazone (30-mg tablet of Actos), and combination therapy with GLP-1 and pioglitazone. The observation period was 48 h. End points were plasma levels of glucose, insulin, glucagon, free fatty acids (FFAs), and sensation of appetite.

**RESULTS** — Fasting plasma glucose decreased from 13.5 ± 1.2 mmol/l (saline) to 11.7 ± 1.2 (GLP-1) and 11.5 ± 1.2 (pioglitazone) and further decreased to 9.9 ± 1.0 (combination) ($P < 0.001$). Eight-hour mean plasma glucose levels were reduced from 13.7 ± 1.1 mmol/l (saline) to 10.6 ± 1.0 (GLP-1) and 12.0 ± 1.2 (pioglitazone) and were further reduced to 9.5 ± 0.8 (combination) ($P < 0.0001$). Insulin levels increased during monotherapy with GLP-1 compared with monotherapy with pioglitazone ($P < 0.01$). Glucagon levels were reduced in GLP-1 and combination therapy compared with saline and monotherapy with pioglitazone ($P < 0.01$). FFAs during breakfast (area under the curve, 0–3 h) were reduced in combination therapy compared with saline ($P = 0.03$). Sensation of appetite was reduced during monotherapy with GLP-1 and combination therapy ($P < 0.05$).

**CONCLUSIONS** — GLP-1 and pioglitazone show an additive glucose-lowering effect. A combination of the two agents may, therefore, be a valuable therapeutic approach for the treatment of type 2 diabetes.

*Diabetes Care* 27:1910–1914, 2004

Glucagon-like peptide (GLP)-1 is an intestinally produced peptide hormone (1) that stimulates insulin secretion (2), inhibits glucagon secretion (2), reduces plasma glucose levels in patients with type 2 diabetes (2), and has, therefore, been proposed as a new antidiabetic agent. In short-term studies, continuous subcutaneous infusion of GLP-1 reduces plasma glucose levels by 2–3 mmol/l (3), whereas in longer-term studies (6 weeks), fasting plasma glucose levels are reduced by 4–5 mmol/l and $HbA_{1c}$ by 1.3% (4). Furthermore, GLP-1 stimulates proinsulin gene expression and proinsulin biosynthesis (5), and in animal studies GLP-1 receptor agonists stimulate β-cell neogenesis and proliferation (6). Thus, theoretically, long-term treatment with GLP-1 may protect against the deterioration of β-cell function, which inevitably occurs as a part of the natural history of the disease (7). Several studies have shown that GLP-1 inhibits appetite and reduces food intake in humans (8,9); therefore, treatment with GLP-1 may inhibit weight gain or even result in weight loss (4). The peroxisome proliferator–activated receptor-γ agonists, the thiazolidinediones, have been shown to reduce fasting plasma glucose levels by 3–4 mmol/l (10,11) and $HbA_{1c}$ by 1–2% (10) when administered to patients with type 2 diabetes. Thiazolidinediones enhance insulin sensitivity in skeletal muscles (12) and in peripheral adipocytes.

Since type 2 diabetes is a progressive disease, a combination of therapies may be required to bring about acceptable glycemic control (13). Thus, to judge the clinical value of a new agent, it is important to examine the possibility of combining it with other agents.

GLP-1 and the thiazolidinediones have differential mechanisms of action, and combination treatment with both of them might, therefore, result in additive glucose-lowering effects. The purpose of the present study was to assess the effect of GLP-1, administered in combination with the thiazolidinedione, pioglitazone, on glucose, insulin, glucagon, free fatty acids (FFAs), and appetite regulation in patients with type 2 diabetes.

## RESEARCH DESIGN AND METHODS

— Four men and four women with type 2 diabetes diagnosed after the age of 40 years participated. Mean age was 62 years (range 51–71), BMI was 32.7 ± 1.3 kg/m$^2$, and $HbA_{1c}$ was 9.7 ± 0.5%. All patients had normal Hgb and serum creatinine. One patient received combination therapy with metformin and a sulfonylurea. All other patients were treated with either metformin or sulfonylureas.

This study had a crossover design. Each patient received four different treatment regimens in random order: saline therapy, monotherapy with GLP-1, monotherapy with pioglitazone, and combination treatment with GLP-1 and

From the [1]Department of Endocrinology, Hvidovre Hospital, Hvidovre, Denmark; and the [2]Department of Medical Physiology, University of Copenhagen, The Panum Institute, Copenhagen, Denmark.

Address correspondence and reprint requests to Mette Zander, Department of Endocrinology, Hvidovre Hospital, Kettegaards Allé 30, 2650 Hvidovre, Denmark. E-mail: m.zander@dadlnet.dk.

Received for publication 25 March 2004 and accepted in revised form 29 April 2004.

**Abbreviations:** AUC, area under the curve; FFA, free fatty acid; GLP, glucagon-like peptide.

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

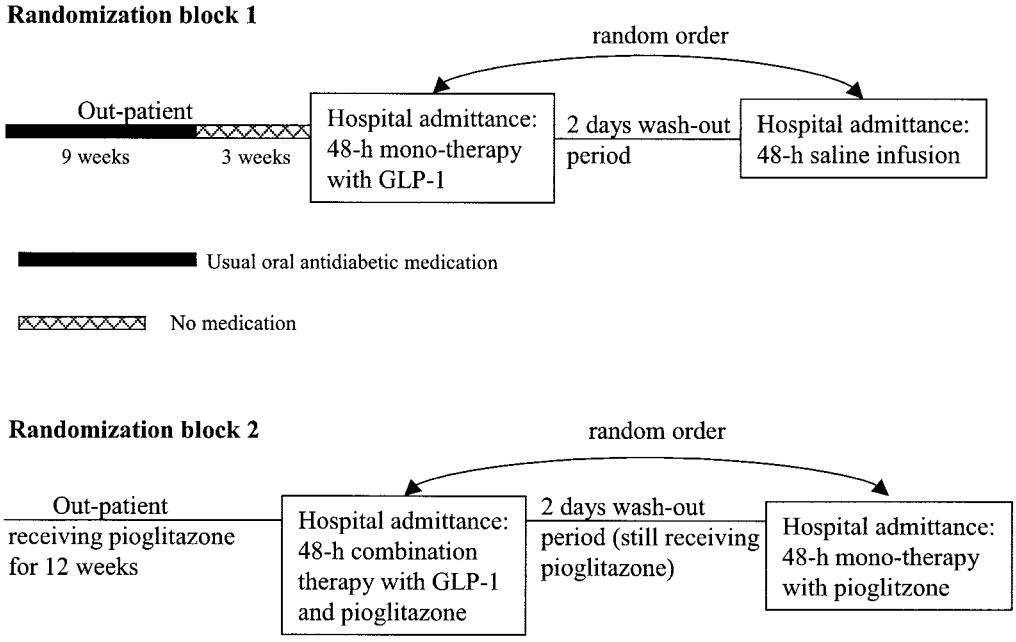


**Figure 1**—*The patients were randomized to start with randomization block 1 or 2. In block 2, the patients were outpatients for 12 weeks, receiving treatment with pioglitazone (30 mg Actos, once daily). Thus the patients received treatment with pioglitazone as outpatients for 12 weeks, during the two 48-h investigation periods and between the two investigations. After the last examination in randomization block 2, pioglitazone was discontinued and the patients initiated their normal oral antidiabetic treatment for 9 weeks. Then the normal oral antidiabetic medication was discontinued, and the patients went without medication for 3 weeks (see block 1). The patients randomized to start with block 1 had their oral antidiabetic medication discontinued for 3 weeks, and during this time they were outpatients. Then they went through the two investigations in block 1. After the last examination, pioglitazone (30 mg Actos, once daily) was initiated for 12 weeks and they went through randomization block 2 as described above. Thus, all patients went through randomization blocks 1 and 2. The observation time in the hospital included four periods of 48 h: 48 h of saline, 48 h of monotherapy with GLP-1, 48 h of monotherapy with pioglitazone, and 48 h of combination therapy with GLP-1 and pioglitazone.*

pioglitazone. The observation period was 48 h; thus each patient was admitted to the hospital for 48 h four times. The order of treatment regimens was blinded to the patients. Due to the extended period required to obtain maximal effect with thiazolidinediones, treatment with pioglitazone was started (30-mg tablet once daily [Actos]) 12 weeks in advance of the time when the effect of pioglitazone was to be examined. Thus, the randomization procedure was built up of two blocks as shown in Fig. 1.

During hospital admittance, the patients were equipped with a portable insulin pump (MiniMed 506) for continuous subcutaneous infusion of GLP-1 or saline. The infusion rate was 4.8 pmol · $kg^{-1}$ · $min^{-1}$. The injection site, in the periumbilical region, was changed twice daily, and the syringe was refilled twice daily. Pioglitazone was given as a 30-mg tablet once daily (Actos). Meals were served three times daily, at 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. 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. Blood samples for glucose, insulin, C-peptide, glucagon, GLP-1, and FFAs were drawn on the second day as a 16-point measurement from 8:00 A.M. to 5:00 P.M. Additionally, blood glucose was measured at 5:30 and 7:00 P.M., at 1:00 A.M., and at 7:00 A.M. on the next morning.

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, Leberkusen, Germany) and valine pyrrolidide (10 μmol/l blood, a gift from Richard D. Carr; Novo Nordisk, Bagsvaerd, Denmark) 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 were stored at −80°C. Plasma for glucagon and GLP-1 analyses were stored at −20°C.

GLP-1 (7-36 amide) was produced by custom synthesis by Polypeptides (Leverkusen, Germany). The correctness of structure and the purity of the peptide (>98%) were ascertained by high-performance liquid chromatography, mass spectrometry, and sequence analysis. The peptide was dissolved in sodium phosphate buffer (300 mosmol/l, pH 7.4) to a final concentration of 400 μg/ml. The solution was subjected to sterile filtration, dispensed into glass ampoules, and stored frozen under sterile conditions until use.

## Analyses

Plasma glucose concentrations were analyzed using a Beckman Analyzer (Beckman Instruments, Fullerton, CA). The coefficient of variation is 2% for intraserial analysis and 4% for interserial analysis. At night, analysis was carried out on full blood using a Hemocue analyzer (Hemocue, Angelholm, Sweden). Blood glucose concentrations were converted to plasma glucose concentrations by multiplication with 1.11 (14).

Glucagon was measured by radioim-

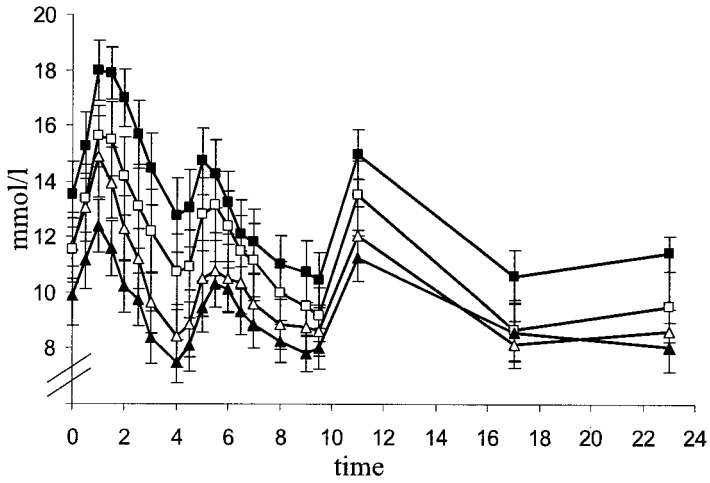


**Figure 2—***Plasma glucose levels. Combination therapy with GLP-1 and pioglitazone showed an additive effect on plasma glucose levels. Time zero = 8:00 A.M. Breakfast was received at time zero, lunch at time 4 (noon), and dinner at time 9.5 (5:30 P.M.).* ■*, saline;* □*, pioglitazone;* △*, GLP-1;* ▲*, combination therapy.*

munoassay using antibody code no. 4,305 (15).

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 (9-36) amide (16). 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 variation for the assays used are 1 pmol/l and <6% for both assays, respectively.

Insulin and C-peptide concentrations were measured using the autoDELFIA immunoassay (Wallac Oy, Turku, Finland). The detection limits of the assays are <5 pmol/l for insulin and <50 pmol/l for C-peptide. Intra- and interassay coefficients of variation are 4–5% at 39–700 pmol/l for insulin and 5–6% at 355–3,700 pmol/l for C-peptide. The cross-reactivity with intact proinsulin was 0.1%, 0.4% with 32-33 split proinsulin, and 66% with Des 64-65 split proinsulin in the insulin assay. In the C-peptide assay, cross-reactivity was 51% with intact proinsulin, 35% with 32-33 split proinsulin, and 92% with Des 64-65 split proinsulin.

$HbA_{1c}$ was measured by an ion-exchange high-performance liquid chromatography (at Steno Diabetes Hospital, Gentofte, Denmark) with an interassay coefficient of variation of 0.15% in the range of 4.7–11.3%.

FFAs were measured by an enzymatic colorimetric method (Wako, Richmond, VA). The intra-assay coefficient of variation was 2%, and the interassay coefficient of variation was 6%.

### Statistical analysis and calculations

All data are normally distributed and analyzed by parametric tests. Data are presented as means ± SE. The differences between the four different treatment regimens were evaluated using one-way ANOVA for repeated measurements. If a significant difference between all of the four groups was found, the following tests were carried out. One-way ANOVA for repeated measurements was carried out for evaluation of the difference between saline and monotherapy with either agent (excluding combination therapy). If a significant difference was obtained, the analysis was followed by the Tukey post hoc test for multiple comparisons for evaluation of the differences between the individual groups. To test for an additive effect, the differences between monotherapy with GLP-1 and pioglitazone and combination therapy (excluding saline) were evaluated by one-way ANOVA for repeated measurements. If a significant difference was obtained, the analysis was followed by Tukey's post hoc test for multiple comparisons for testing significance between the individual groups. Statistical significance was set at $P < 0.05$.

Areas under the curve (AUCs) were calculated using the trapezoidal rule.

**RESULTS** — Plasma levels of GLP-1 increased on days when GLP-1 was infused. Thus, plasma levels of total GLP-1 increased from 20.8 ± 3.2 pmol/l (saline) and 24.1 ± 3.9 (pioglitazone) to 182.5 ± 21.6 (GLP-1) and 194.8 ± 19.5 (combination) ($P < 0.001$).

Fasting plasma glucose decreased from 13.5 ± 1.2 mmol/l (saline) to 11.7 ± 1.2 (GLP-1) and 11.5 ± 1.2 (pioglitazone) and further decreased to 9.9 ± 1.0 (combination) ($P = 0.0004$). By the Tukey post hoc test, fasting plasma glucose levels during pioglitazone therapy were significantly different from that while on saline ($P = 0.033$), and fasting plasma glucose levels were lower on combination therapy compared with monotherapy with either agent ($P = 0.016$).

Twenty-four-hour mean plasma glucose levels were reduced from 13.7 ± 1.1 mmol/l (saline) to 10.6 ± 1.0 (GLP-1) and 12.0 ± 1.2 (pioglitazone) and were further reduced to 9.5 ± 0.8 (combination) ($P < 0.0001$). By the Tukey post hoc test, plasma glucose levels were significantly lower on monotherapy with either agent compared with saline ($P < 0.0001$) and significantly lower on combination therapy compared with monotherapy with either agent ($P < 0.0001$). However, plasma glucose levels were also significantly lower while on monotherapy with GLP-1 compared with monotherapy with pioglitazone ($P < 0.05$). Glucose data are shown in Fig. 2.

Fasting insulin levels were not significantly different between the groups (58 ± 15 [saline], 89 ± 27 [GLP-1], 53 ± 13 [pioglitazone], and 66 ± 13 [combination] pmol/l) ($P = 0.09$).

Mean levels of insulin were 117 ± 27 (saline), 142 ± 35 (GLP-1), 94 ± 17 (pioglitazone), and 99 ± 18 (combination) pmol/l ($P = 0.002$). By the Tukey post hoc test, mean insulin levels were significantly higher on monotherapy with GLP-1 compared with monotherapy with pioglitazone ($P = 0.016$). Insulin levels on combination therapy were not significantly different from insulin levels on monotherapy with either agent.

Fasting C-peptide levels were 983 ± 200 (saline), 1,209 ± 752 (GLP-1), 915 ± 208 (pioglitazone), and 1,117 ± 158 (combination) pmol/l ($P = 0.0008$). By the Tukey post hoc test, fasting C-peptide levels were significantly higher on monotherapy with GLP-1 compared with saline and monotherapy with pioglita-

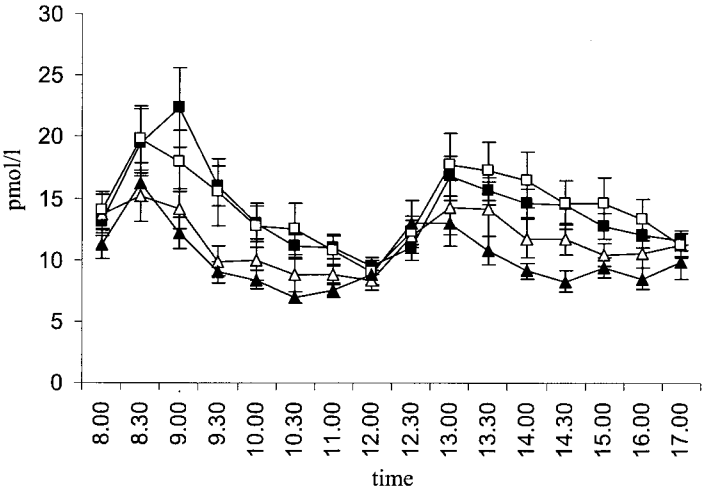


**Figure 3**—*Plasma glucagon levels. ■, saline; □, pioglitazone; △, GLP-1; ▲, combination therapy.*

zone. Fasting C-peptide levels on combination therapy were not significantly different from monotherapy with either agent ($P = 0.08$).

Mean levels of C-peptide were 1,513 ± 269 (saline), 1,805 ± 318 (GLP-1), 1,309 ± 203 (pioglitazone), and 1,484 ± 196 (combination) pmol/l ($P = 0.001$). By the Tukey post hoc test, C-peptide levels on monotherapy with GLP-1 were significantly higher than C-peptide levels on monotherapy with pioglitazone and saline. C-peptide levels on combination therapy were not different from C-peptide levels on monotherapy with GLP-1 and pioglitazone ($P > 0.05$).

Fasting glucagon levels were unaffected by any treatment (13 ± 1 [saline], 14 ± 2 [GLP-1], 14 ± 2 [pioglitazone], and 11 ± 1 [combination] pmol/l) ($P = 0.15$).

Mean levels of glucagon were reduced from 14.1 ± 1.2 pmol/l (saline) and 14.4 ± 1.6 (pioglitazone) to 11.4 ± 1.2 (GLP-1) and 10.1 ± 0.8 (combination) ($P = 0.001$). By the Tukey post hoc test, glucagon levels were significantly lower on monotherapy with GLP-1 compared with saline and monotherapy with pioglitazone ($P = 0.01$). Glucagon levels were lower on combination therapy compared with monotherapy with pioglitazone ($P = 0.013$), but were not different from monotherapy with GLP-1 ($P > 0.05$). Data are shown in Fig. 3.

Neither fasting FFA levels (0.7 ± 0.07 [saline], 0.7 ± 0.08 [GLP-1], 0.6 ± 0.05 [pioglitazone], and 0.5 ± 0.04 [combination] mmol/l; $P = 0.1$) nor mean FFA levels (0.5 ± 0.06 [saline], 0.5 ± 0.06 [GLP-1], 0.4 ± 0.04 [pioglitazone], and 0.4 ± 0.02 [combination] mmol/l; $P = 0.054$) differed between the groups. However, the AUC from breakfast (0 h) and 3 h after was affected by treatment (1.96 ± 0.28 [saline], 1.86 ± 0.22 [GLP-1], 1.67 ± 0.19 [pioglitazone], and 1.4 ± 0.08 [combination] $h \cdot mmol^{-1} \cdot l^{-1}$; $P = 0.03$). By Tukey's post hoc test, no significant differences between saline and monotherapy with either agent were obtained, but FFAs were reduced on combination therapy compared with monotherapy with GLP-1 ($P = 0.04$) and with saline ($P = 0.03$).

Sensation of appetite for the four parameters (hunger, satiety, fullness, and prospective food intake) was significantly reduced on GLP-1 infusions. Data for sensation of hunger and fullness are shown below. Sensation of hunger decreased from 40 ± 4 mm (saline) and 36 ± 4 (pioglitazone) to 28 ± 5 (GLP-1) and 28 ± 6 (combination) ($P = 0.0096$). By Tukey's post hoc test, sensation of hunger was significantly lower on monotherapy with GLP-1 and combination therapy compared with saline ($P = 0.0008$). The decrease in hunger on combination therapy compared with monotherapy with pioglitazone was not significant ($P = 0.08$).

Sensation of fullness increased from 49 ± 3 mm (saline) and 55 ± 3 (pioglitazone) to 60 ± 3 (GLP-1) and 65 ± 5 (combination) ($P = 0.0006$). By the Tukey post hoc test, sensation of fullness increased significantly on monotherapy with GLP-1 compared with saline and monotherapy with pioglitazone ($P = 0.0005$). Sensation of fullness increased significantly on combination therapy compared with monotherapy with pioglitazone ($P = 0.02$).

There were no differences between the groups with respect to registrations of nausea and sensation of well-being.

**CONCLUSIONS** — Several studies (2–4,17) have shown that GLP-1 effectively reduces plasma glucose levels in patients with type 2 diabetes, and therefore it seems suitable as a new agent for treatment of the disease. Due to the progressive nature of the disease, many patients may require combination therapy to reach acceptable glycemic control (13). Thus, to evaluate the potential of GLP-1 as a new agent for the treatment of type 2 diabetes, it is important to investigate the effect of combination therapy with GLP-1 and other antidiabetic agents. We have previously found (18) that combination therapy with GLP-1 and metformin resulted in an additive effect on plasma glucose levels in type 2 diabetic patients. In the present study, we found that monotherapy with both GLP-1 and pioglitazone improved glycemic control in patients with type 2 diabetes, reducing 24-h mean plasma glucose levels by 3.1 and 1.7 mmol/l, respectively, whereas the combination of both agents resulted in significantly lower plasma glucose levels compared with the glucose levels of either of the two monotherapy regimens.

GLP-1 stimulates insulin secretion by binding to GLP-1 receptors on the β-cell. Furthermore, insulin gene expression and de novo insulin synthesis are stimulated (5). Pioglitazone, on the other hand, improves insulin sensitivity (12,19,20). In line with this, insulin and C-peptide levels were higher on GLP-1 therapy compared with pioglitazone. As expected, insulin levels were unaltered on combination therapy because the two therapeutic agents have opposite actions. Glucagon secretion is exaggerated in patients with type 2 diabetes (21), contributing to increased gluconeogenesis and hyperglycemia, and GLP-1 has been shown (2) to reduce glucagon levels in these subjects. In the present study, glucagon levels were lower on monotherapy with GLP-1 compared with saline and monotherapy with pioglitazone, and the effect was maintained on combination therapy. Thus, GLP-1 probably contributes to the additive glu-

cose-lowering effect of combination therapy by lowering levels of glucagon.

GLP-1 has been shown (2,4) to reduce levels of FFAs, most likely due to increased insulin secretion and action, while treatment with thiazolidinediones enhances the insulin sensitivity of the peripheral adipocytes (20), which also results in lower FFA concentrations. High levels of FFAs may cause deteriorating β-cell function (22) and induce insulin resistance (23). Though neither monotherapy with GLP-1 nor pioglitazone reduced FFAs in the present study, FFA concentrations were reduced during combination therapy, indicating a beneficial additive effect of GLP-1 and pioglitazone on FFAs.

GLP-1 reduces appetite and food intake in humans (8,9). In animal studies, GLP-1 is associated with a reduction in body weight (24), and a tendency toward a weight loss has also been shown in humans (4). Because weight gain leads to deterioration of glycemic control and weight loss improves glycemic control, weight loss is desirable in patients with type 2 diabetes. In the present study, appetite scores were reduced during GLP-1 therapy and maintained on combination therapy, and it is likely this will ultimately result in weight loss.

In summary, combination therapy with GLP-1 and pioglitazone had an additive effect on plasma glucose levels in patients with type 2 diabetes. GLP-1 reduced the levels of glucagon, and this effect was maintained during combination therapy, possibly contributing to the additive effect on plasma glucose levels. Furthermore, the levels of FFAs were reduced while on combination therapy. GLP-1 reduced the sensation of appetite, and this effect was maintained on combination therapy, a finding that may have clinical implications after long-term treatment.

**Acknowledgments**— The study was supported by the Danish Diabetes Association and the Danish Medical Research Council.

We thank Susanne Reimer, Hvidovre Hospital, for technical assistance.

**References**

1. Orskov C, Bersani M, Johnsen AH, Hojrup P, Holst JJ: Complete sequences of glucagon-like peptide-1 from human and pig small intestine. *J Biol Chem* 264: 12826–12829, 1989
2. Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W: Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. *Diabetologia* 36:741–744, 1993
3. Toft-Nielsen MB, Madsbad S, Holst JJ: Continuous subcutaneous infusion of glucagon-like peptide 1 lowers plasma glucose and reduces appetite in type 2 diabetic patients. *Diabetes Care* 22:1137–1143, 1999
4. Zander M, Madsbad S, Madsen JL, Holst JJ: Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and beta-cell function in type 2 diabetes: a parallel-group study. *Lancet* 359:824–830, 2002
5. Fehmann HC, Habener JF: Insulinotropic hormone glucagon-like peptide-I(7-37) stimulation of proinsulin gene expression and proinsulin biosynthesis in insulinoma beta TC-1 cells. *Endocrinology* 130: 159–166, 1992
6. Xu G, Stoffers DA, Habener JF, Bonner-Weir S: Exendin-4 stimulates both β-cell replication and neogenesis, resulting in increased β-cell mass and improved glucose tolerance in diabetic rats. *Diabetes* 48:2270–2276, 1999
7. Turner R, Cull C, Holman R: United Kingdom Prospective Diabetes Study 17: a 9-year update of a randomized, controlled trial on the effect of improved metabolic control on complications in non-insulin-dependent diabetes mellitus. *Ann Intern Med* 124:136–145, 1996
8. Flint A, Raben A, Astrup A, Holst JJ: Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 101:515–520, 1998
9. Naslund E, Barkeling B, King N, Gutniak M, Blundell JE, Holst JJ, Rossner S, Hellstrom PM: Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord* 23:304–311, 1999
10. Aronoff S, Rosenblatt S, Braithwaite S, Egan JW, Mathisen AL, Schneider RL, Pioglitazone 001 Study Group: Pioglitazone hydrochloride monotherapy improves glycemic control in the treatment of patients with type 2 diabetes: a 6-month randomized placebo-controlled dose-response study. *Diabetes Care* 23: 1605–1611, 2000
11. Inzucchi SE, Maggs DG, Spollett GR, Page SL, Rife FS, Walton V, Shulman GI: Efficacy and metabolic effects of metformin and troglitazone in type II diabetes mellitus. *N Engl J Med* 338:867–872, 1998
12. Petersen KF, Krssak M, Inzucchi S, Cline GW, Dufour S, Shulman GI: Mechanism of troglitazone action in type 2 diabetes. *Diabetes* 49:827–831, 2000
13. UK Prospective Diabetes Study Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837–853, 1998
14. Fogh-Andersen N, D'Orazio P: Proposal for standardizing direct-reading biosensors for blood glucose. *Clin Chem* 44:655–659, 1998
15. Orskov C, Jeppesen J, Madsbad S, Holst JJ: Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. *J Clin Invest* 87:415–423, 1991
16. Orskov C, Rabenhoj L, Wettergren A, Kofod H, Holst JJ: Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes* 43:535–539, 1994
17. Rachman J, Barrow BA, Levy JC, Turner RC: Near-normalisation of diurnal glucose concentrations by continuous administration of glucagon-like peptide-1 (GLP-1) in subjects with NIDDM. *Diabetologia* 40:205–211, 1997
18. Zander M, Taskiran M, Toft-Nielsen MB, Madsbad S, Holst JJ: Additive glucose-lowering effects of glucagon-like peptide 1 and metformin in type 2 diabetes. *Diabetes Care* 24:720–725, 2001
19. Carey DG, Cowin GJ, Galloway GJ, Jones NP, Richards JC, Biswas N, Doddrell DM: Effect of rosiglitazone on insulin sensitivity and body composition in type 2 diabetic patients [corrected]. *Obes Res* 10: 1008–1015, 2002
20. Mayerson AB, Hundal RS, Dufour S, Lebon V, Befroy D, Cline GW, Enocksson S, Inzucchi SE, Shulman GI, Petersen KF: The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes. *Diabetes* 51:797–802, 2002
21. Reaven GM, Chen YD, Golay A, Swislocki AL, Jaspan JB: Documentation of hyperglucagonemia throughout the day in nonobese and obese patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 64:106–110, 1987
22. Carpentier A, Mittelman SD, Lamarche B, Bergman RN, Giacca A, Lewis GF: Acute enhancement of insulin secretion by FFA in humans is lost with prolonged FFA elevation. *Am J Physiol* 276:E1055–E1066, 1999
23. Boden G: Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* 46:3–10, 1997
24. Larsen PJ, Fledelius C, Knudsen LB, Tang-Christensen M: Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats. *Diabetes* 50:2530–2539, 2001