

# One Week's Treatment With the Long-Acting Glucagon-Like Peptide 1 Derivative Liraglutide (NN2211) Markedly Improves 24-h Glycemia and $\alpha$ - and $\beta$ -Cell Function and Reduces Endogenous Glucose Release in Patients with Type 2 Diabetes

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Glucagon-like peptide 1 (GLP-1) is potentially a very attractive agent for treating type 2 diabetes. We explored the effect of short-term (1 week) treatment with a GLP-1 derivative, liraglutide (NN2211), on 24-h dynamics in glycemia and circulating free fatty acids, islet cell hormone profiles, and gastric emptying during meals using acetaminophen. Furthermore, fasting endogenous glucose release and gluconeogenesis (<sup>3</sup>-<sup>3</sup>H-glucose infusion and <sup>2</sup>H<sub>2</sub>O ingestion, respectively) were determined, and aspects of pancreatic islet cell function were elucidated on the subsequent day using homeostasis model assessment and first- and second-phase insulin response during a hyperglycemic clamp (plasma glucose ~16 mmol/l), and, finally, on top of hyperglycemia, an arginine stimulation test was performed. For accomplishing this, 13 patients with type 2 diabetes were examined in a double-blind, placebo-controlled crossover design. Liraglutide (6  $\mu$ g/kg) was administered subcutaneously once daily. Liraglutide significantly reduced the 24-h area under the curve for glucose ( $P = 0.01$ ) and glucagon ( $P = 0.04$ ), whereas the area under the curve for circulating free fatty acids was unaltered. Twenty-four-hour insulin secretion rates as assessed by deconvolution of serum C-peptide concentrations were unchanged, indicating a relative increase. Gastric emptying was not influenced at the dose of liraglutide used. Fasting endogenous glucose release was decreased ( $P = 0.04$ ) as a result of a reduced glycogenolysis ( $P = 0.01$ ), whereas gluconeogenesis was unaltered. First-phase insulin response and the insulin response to an arginine stimulation test with the presence of hyperglycemia were markedly increased ( $P <$

0.001), whereas the proinsulin/insulin ratio fell ( $P = 0.001$ ). The disposition index (peak insulin concentration after intravenous bolus of glucose multiplied by insulin sensitivity as assessed by homeostasis model assessment) almost doubled during liraglutide treatment ( $P < 0.01$ ). Both during hyperglycemia per se and after arginine exposure, the glucagon responses were reduced during liraglutide administration ( $P < 0.01$  and  $P = 0.01$ ). Thus, 1 week's treatment with a single daily dose of the GLP-1 derivative liraglutide, operating through several different mechanisms including an ameliorated pancreatic islet cell function in individuals with type 2 diabetes, improves glycemic control throughout 24 h of daily living, i.e., prandial and nocturnal periods. This study further emphasizes GLP-1 and its derivatives as a promising novel concept for treatment of type 2 diabetes. *Diabetes* 53:1187–1194, 2004

**T**ype 2 diabetes is characterized by insulin resistance and progressive islet cell dysfunction leading to insulin deficiency (1). Increased hepatic glucose release is considered to play a key role in fasting, as well as postprandial hyperglycemia, and increased gluconeogenesis seems to be essential in this scenario (2,3). In addition, elevated glucagon levels (4,5) and a reduced response of glucagon-like peptide 1 (GLP-1) to meals (6) are common features.

The importance of aggressive glucose-lowering therapy to prevent late diabetes complications in type 2 diabetes has been convincingly established (7,8). However, the U.K. Prospective Diabetes Study also demonstrated that the antidiabetic treatment used failed to maintain acceptable glycemic control in the vast majority of the patients, emphasizing the need for more effective antidiabetic agents.

GLP-1 is an incretin hormone secreted from the intestinal mucosa in response to meal ingestion (9). Insulin secretion is stimulated, and glucagon secretion is inhibited; both actions are glucose dependent. Also, GLP-1 has trophic effects on pancreatic  $\beta$ -cells and inhibits their apoptosis (10). Furthermore, it delays gastric emptying (9,11) and may even be a satiety factor. These observations support GLP-1 as a novel candidate for treatment of type 2 diabetes, and the beneficial effects of GLP-1 on

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AUC, area under the curve; DPP-IV, dipeptidylpeptidase IV; EGR, endogenous glucose release; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; GLP-1, glucagon-like peptide 1; GLY, glycogenolysis; GNG, gluconeogenesis; HMT, hexamethylenetetramine; HOMA, homeostasis model assessment; ISR, insulin secretion rate; OHA, oral hypoglycemic agent;  $t_{max}$ , time of occurrence for maximum drug concentration.

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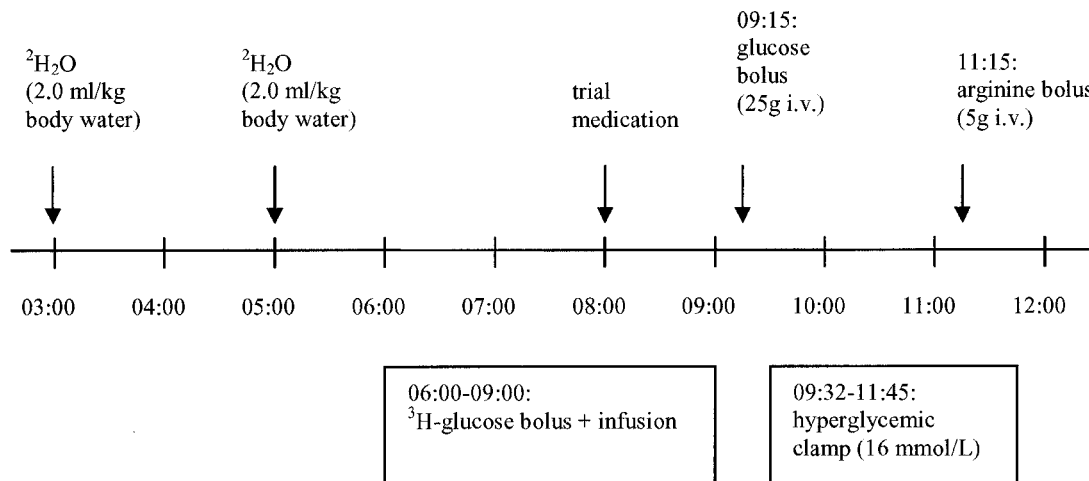


FIG. 1. Flow sheet illustrating the procedures on study day 9. See text for details.

glycemic control in individuals with type 2 diabetes have been demonstrated (12,13). However, native GLP-1 has a very short half-life because of its rapid degradation by the ubiquitous enzyme dipeptidylpeptidase IV (DPP-IV) (14), making GLP-1 per se unsuitable as a therapeutic drug. Derivatives that are resistant to DPP-IV are one way to overcome this failing. Liraglutide is an acylated GLP-1 derivative that binds to albumin. This limits its sensitivity to DPP-IV and delays absorption from its injection site. A prolonged pharmacokinetic profile in humans is attained, the half-life after subcutaneous administration being  $\sim 12$  h (15,16). A single-dose study demonstrated significant glucose lowering in individuals with type 2 diabetes in both the fasting and postprandial states (16), and in a 12-week repeated-dose study, liraglutide lowered blood glucose to the same extent as a sulfonylurea compound (glimepiride) and the prevalence of hypoglycemia was very low (17).

The present study was undertaken to gain further insight into GLP-1 derivatives as novel antidiabetic drugs. We sought to do this by administering liraglutide to individuals with type 2 diabetes for 7 days and assessing the impact on 24-h glucose and hormone levels during daily life conditions, gastric emptying rate, endogenous glucose release (EGR), and various aspects of the pancreatic islet cell function.

## RESEARCH DESIGN AND METHODS

The protocol was approved by the local ethics committee and performed in accordance with the Helsinki Declaration.

**Patients.** Thirteen patients (5 women and 8 men) with type 2 diabetes according to World Health Organization criteria were examined. Their age (mean  $\pm$  SD) was  $56.4 \pm 9.2$  years, BMI was  $31.2 \pm 3.6$  kg/m<sup>2</sup>, last measured HbA<sub>1c</sub> before inclusion was  $7.3 \pm 0.4\%$  (normal range  $<6.4\%$ ), and the duration of diabetes was  $3.0 \pm 2.6$  years (range 5 months to 8 years). At study entry, six patients were treated with diet alone and seven were additionally treated with oral hypoglycemic agents (OHAs; sulfonylurea  $n = 3$ , metformin  $n = 3$ , sulfonylurea and metformin  $n = 1$ ). Concomitant medications were angiotensin-converting enzyme inhibitors ( $n = 4$ ), thiazide diuretics ( $n = 1$ ),  $\beta$ -blockers ( $n = 1$ ), calcium antagonists ( $n = 1$ ), HMG-CoA reductase inhibitors ( $n = 2$ ), and acetyl salicylic acid ( $n = 3$ ). Three patients had retinopathy simplex, and three had nephropathy (microalbuminuria).

**Experimental design.** This crossover trial was double-blinded, placebo-controlled, and randomized. After inclusion, the patients discontinued their OHA for 2 weeks before beginning study medication. Liraglutide (6  $\mu\text{g}/\text{kg}$  body wt) or placebo was injected subcutaneously into the abdomen once daily (at

$\sim 0745$ ) for 9 days using a NovoPen (1.5 with Novofine 30-G, 0.3- to 8-mm needle) as the dispensing device. After 7 days of treatment, the patients arrived at the clinical research unit at 2200. In the next 2 days (days 8 and 9), the following experiments were carried out during continuous treatment.

On day 8 at 0730, a catheter was placed in an antecubital vein for blood sampling. Three standard meals were served at 0800, 1200, and 1800, to be finished within 20 min. Breakfast contained 2,660 kJ (protein 14 E%, carbohydrate 55 E%, and fat 31 E%), lunch contained 2,865 kJ (protein 16 E%, carbohydrate 50 E%, and fat 34 E%), and dinner contained 3,397 kJ (protein 28 E%, carbohydrate 53 E%, and fat 19 E%). With breakfast and dinner, 1 g of acetaminophen dissolved in 150 ml of water was given to assess gastric emptying rate (18). Serum acetaminophen was determined every 15–30 min during the following 4 h, and area under the curve (AUC) for acetaminophen (AUC<sub>acetaminophen</sub>) and the time of occurrence for maximum drug concentration ( $t_{\text{max}}$ ) were calculated. During day 8, blood was collected with changing intervals for determination of glucose, insulin, C-peptide, proinsulin (only fasting and after breakfast), glucagon, free fatty acids (FFAs), and liraglutide.

On day 9, the following procedures were performed (Fig. 1).

**Glucoseogenesis.** At 0300 and 0500, patients drank 2 ml of  $^2\text{H}_2\text{O}/\text{kg}$  body water (99.9% H; Sigma Aldrich; body water was estimated to be 50% of total body weight in women and 60% in men). Water ingested ad libitum thereafter was enriched with 0.4%  $^2\text{H}_2\text{O}$  to maintain isotopic steady state. At 0800, 0830, and 0900, blood was drawn for determination of glucoseogenesis (GNG).

**EGR.** EGR was estimated by use of an isotope dilution technique. At 0600, a bolus of  $^3\text{H}$ -glucose (adjusted priming:  $6 \mu\text{Ci} \times \text{plasma glucose level in mmol/l}$ ) was given followed by a continuous infusion (0.3  $\mu\text{Ci}/\text{min}$ ) until 0900. Blood samples were drawn before the bolus and every 15 min from 0800 to 0900 for determination of glucose  $^3\text{H}$  specific activity. Steady state was achieved during this period in both situations, the average specific activity of glucose being 2,078, 2,180, and 2,160 cpm/mg at time points 0800, 0830, and 0900 during active treatment and 1,927, 1,822, and 1,906 cpm/mg at the same time points during placebo.

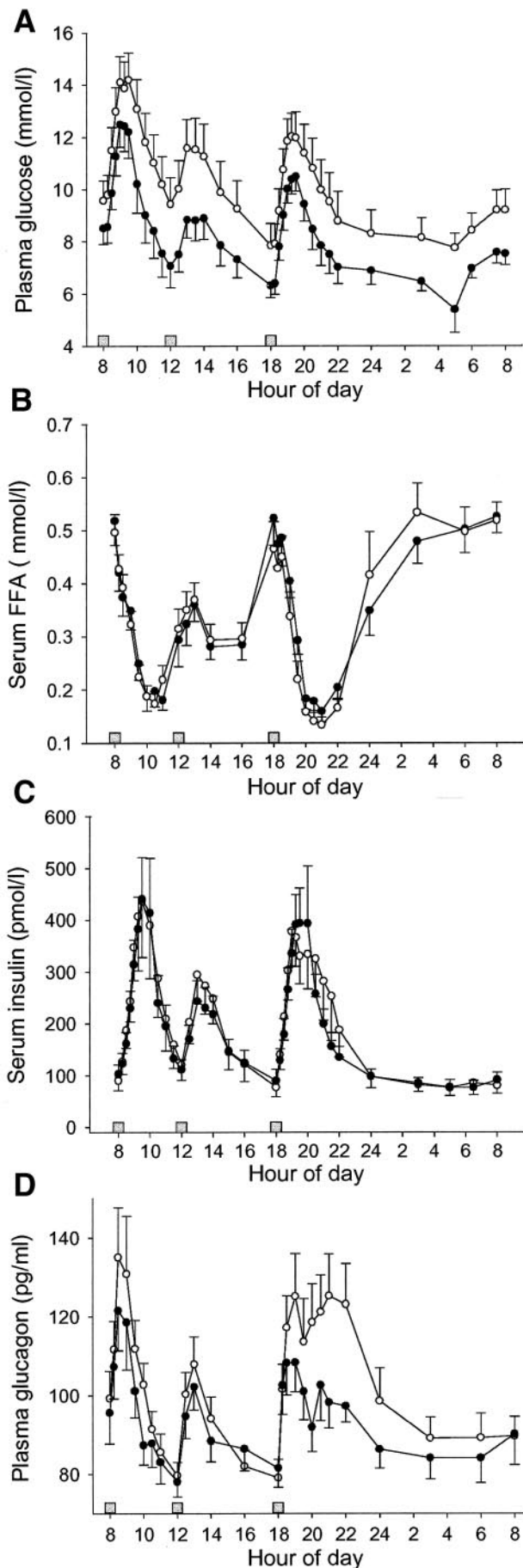
**First-phase insulin secretion.** First-phase insulin secretion was determined using an intravenous glucose bolus. At 0915, 25 g of glucose was administered intravenously over 2 min. Blood was then collected every 2–5 min until 0932 for determining glucose and insulin.

**Hyperglycemic clamp.** From 0932 to 1145, 20% glucose was infused to maintain plasma glucose at 16 mmol/l. After 75 min, when approximate steady state was attained, blood was drawn every 10 min (from 1045 to 1115) for determining plasma glucose and serum insulin, C-peptide, and proinsulin.

**Arginine stimulation test.** At 1115, a bolus of 5 g of arginine was given intravenously over 30 s followed by collection of blood every 5 min until 1145 for determining circulating glucose, insulin, C-peptide, glucagon, and proinsulin.

**Safety.** Adverse events, vital signs, hematology, and biochemistry were monitored throughout the study.

**Assays.** All biochemical analyses were performed in duplicate. Plasma glucose was measured immediately on a glucose analyzer (Beckman Instruments, Palo Alto, CA) using the glucose oxidase technique. All other serum and plasma samples were stored at  $-20^\circ\text{C}$  (C-peptide at  $-80^\circ\text{C}$ ) until analyzed. Serum insulin was determined using a highly specific and sensitive two-site



enzyme-linked immunosorbent assay (ELISA; DAKO Diagnostics, Cambridgeshire, U.K.), and serum C-peptide was measured by a two-site monoclonal-based ELISA with an intra-assay coefficient of variation of 5.1% (DAKO Diagnostics). Serum proinsulin was analyzed by specific immunoassay with no cross-reaction with insulin and C-peptide (DAKO Diagnostics). Serum acetaminophen was determined by high-performance liquid chromatography after extraction. Plasma glucagon was measured using a radioimmunoassay kit (Linco Research). Liraglutide concentrations were analyzed by ELISA using a monoclonal antibody against GLP-1/liraglutide as capture antibody and another monoclonal antibody specific for the NH<sub>2</sub>-terminal part of GLP-1/liraglutide for detection. Before the latter analysis, samples were incubated at 37°C to remove endogenous GLP-1, liraglutide being stable toward this incubation. Determination of 3-<sup>3</sup>H-glucose activity was as previously described (19). Measurement of deuterium enrichment at carbons 2 and 5 glucose was as described by Landau et al. (20). In brief, 15 ml of blood was diluted with 30 ml of demineralized water and deproteinized using 15 ml of 0.3 N ZnSO<sub>4</sub> and 15 ml of 0.3 N Ba(OH)<sub>2</sub>. The samples were then centrifuged at 2,000 rpm for 15 min, and the pellet was diluted in 15 ml of demineralized water to wash out the remaining glucose. Glucose was isolated by high-performance liquid chromatography. For determination of deuterium enrichment on C5, glucose was first converted to xylose, and the carbon 5 with its hydrogens was cleaved by periodate oxidation to formaldehyde, which was condensed with ammonium hydroxide to form hexamethylenetetramine (HMT). The <sup>2</sup>H bound to C2 of glucose was isolated after conversion of glucose to ribitol-5-phosphate and arabitol-5-phosphate and treated to form HMT. Enrichments in the HMTs were measured using a Hewlett-Packard mass spectrometry system with standards of HMTs analyzed along with the unknown samples.

**Calculations and statistics.** Fasting data are presented as the mean values at 0730 and 0800 on days 8 and 9. Furthermore, fasting plasma glucose on day 9 is presented. AUCs of substrates and hormones were calculated by applying the trapezoidal rule and represent the total AUC. EGR was determined using Steele's equations for steady state, as modified by Finegood et al. (21). The fractional contribution of GNG to glucose production equals the ratio between deuterium bound to carbon 5 in glucose and that bound to carbon 2. The rate of GNG in the fasting state was obtained by multiplying the mean EGR from 0800 to 0900 by the mean fractional contribution of GNG during that same hour. The rate of glycogenolysis (GLY) was calculated by subtracting the rate of GNG from total EGR. Insulin secretion rates (ISRs) were estimated by mathematical analysis (deconvolution) of peripheral C-peptide concentrations using a two-compartment model, as described by Polonsky et al. (22) and the standard C-peptide kinetic parameters published by Van Cauter et al. (23). This model allows accurate estimation of ISR also under non-steady-state conditions. Another estimate of  $\beta$ -cell function and an estimate of insulin resistance were calculated by homeostasis model assessment (HOMA-B and R) (24), using glucose and insulin values after a 14-h long fast and 24 h after the last liraglutide injection. As a supplement to the latter, insulin sensitivity was calculated by dividing glucose infusion rate during steady state of the hyperglycemic clamp with mean serum insulin level. Finally, an analog to the disposition index was calculated by multiplying 1/HOMA-R by the peak insulin concentrations during the first-phase insulin secretion test (25).

Statistical analysis was performed using a mixed model with sequence of treatment, visit, and treatment as fixed factors and patient as a random factor. Differences were considered significant at  $P < 0.05$ . All results are given as liraglutide versus placebo.

## RESULTS

### Twenty-four-h profiles of substrates and hormones.

During the 24-h profile, the average concentration of plasma glucose ( $P = 0.01$ ) and postprandial plasma glucose concentrations after all three meals were decreased by ~20% ( $P = 0.01$ ,  $P = 0.02$ , and  $P = 0.02$ , respectively) during liraglutide treatment. Fasting plasma glucose also declined (Fig. 2, Table 1).

Circulating concentrations of fasting insulin, 24-h AUC insulin, postprandial insulin concentrations, and ISR were comparable during treatment with liraglutide and placebo, despite the lower glycemia during active treatment suggesting a relative increase in insulin secretion. The ratio of

**FIG. 2.** Twenty-four-hour profiles of plasma glucose (A), serum FFAs (B), serum insulin (C), and plasma glucagon (D) (means  $\pm$  SEM), day 8.  $\circ$ , placebo;  $\bullet$ , liraglutide;  $\square$ , meals. (Statistical details are listed in Table 1.)

TABLE 1  
Twenty-four-hour profiles and fasting values

	Liraglutide	Placebo	P
<b>Fasting values</b>			
Plasma glucose (mmol/l)	8.06 ± 0.52	9.39 ± 0.76	0.078
Plasma glucose day 9 (mmol/l)	7.56 ± 0.42	9.20 ± 0.78	0.025
FFA (mmol/l)	0.52 ± 0.03	0.51 ± 0.03	0.536
Insulin (pmol/l)	95.6 ± 15.1	87.2 ± 17.9	0.513
ISR (nmol/h)	17.7 ± 2.0	16.1 ± 2.1	0.206
Proinsulin/insulin ratio	0.17 ± 0.04	0.27 ± 0.05	0.009
Glucagon (pg/ml)	92.8 ± 7.2	94.5 ± 5.6	0.586
<b>24-h total AUCs</b>			
Plasma glucose (mmol · l <sup>-1</sup> · h)	187.5 ± 14.0	232.3 ± 21.9	0.014
FFA (mmol · l <sup>-1</sup> · h)	8.54 ± 0.51	8.65 ± 0.68	0.876
Insulin (pmol · l <sup>-1</sup> · h)	3,854 ± 581	4,154 ± 881	0.375
ISR (nmol)	566.1 ± 55.1	561.6 ± 72.6	0.982
Glucagon (pg · ml <sup>-1</sup> · h)	2,179 ± 118	2,371 ± 135	0.037
<b>Postprandial total AUCs</b>			
Plasma glucose 8–12 h (mmol · l <sup>-1</sup> · h)	38.66 ± 3.52	47.51 ± 3.95	0.010
Plasma glucose 12–16 h (mmol · l <sup>-1</sup> · h)	32.52 ± 2.92	41.89 ± 4.54	0.017
Plasma glucose 18–22 h (mmol · l <sup>-1</sup> · h)	33.76 ± 2.50	41.10 ± 3.94	0.016
Insulin 8–12 h (pmol · l <sup>-1</sup> · h)	999 ± 173	1,056 ± 253	0.512
Insulin 12–16 h (pmol · l <sup>-1</sup> · h)	723 ± 107	808 ± 162	0.312
Insulin 18–22 h (pmol · l <sup>-1</sup> · h)	1,017 ± 160	1,117 ± 221	0.392
Proinsulin/insulin ratio 8–12 h	0.12 ± 0.03	0.19 ± 0.04	0.008
Glucagon 8–12 h (pg · ml <sup>-1</sup> · h)	383.0 ± 22.8	413.4 ± 25.6	0.080
Glucagon 12–16 h (pg · ml <sup>-1</sup> · h)	362.6 ± 18.6	374.2 ± 19.5	0.384
Glucagon 18–22 h (pg · ml <sup>-1</sup> · h)	397.3 ± 23.9	470.1 ± 35.2	0.009

Data are means ± SEM. Twenty-four-hour AUCs were calculated from samples obtained from 0800 to 0800. Fasting values were calculated from samples taken at 0730 and 0800 on days 8 and 9.

24-h AUC<sub>insulin</sub>/AUC<sub>glucose</sub> was significantly higher during liraglutide administration ( $P < 0.05$ ), whereas only a trend was observed in the fasting insulin/glucose ratio ( $P < 0.15$ ). The fasting proinsulin/insulin ratio and the proinsulin/insulin ratio after the breakfast meal were markedly reduced during liraglutide administration ( $P < 0.01$ ).

Liraglutide exposure significantly reduced the 24-h AUC of glucagon ( $P = 0.04$ ), primarily as a result of a marked reduction in glucagon concentrations after the protein-rich evening meal ( $P < 0.01$ ). Fasting plasma glucagon concentrations were unaltered. The 24-h AUC insulin/glucagon ratio did not differ in the two situations ( $1.78 \pm 0.28$  pmol/μg vs.  $1.72 \pm 0.32$  pmol/μg;  $P = 0.20$ ). Fasting value and 24-h AUC of FFAs were similar in the two regimens.

**Gastric emptying rate.** AUC<sub>acetaminophen</sub> during the breakfast meal ( $210 \pm 16$  vs.  $210 \pm 12$  μmol · l<sup>-1</sup> · h) and AUC<sub>acetaminophen</sub> during dinner ( $178 \pm 10$  vs.  $179 \pm 9$  μmol · l<sup>-1</sup> · h) were almost identical. Similarly,  $t_{max}$  did not show a significant difference after breakfast ( $40 \pm 8$  min during liraglutide treatment vs.  $32 \pm 4$  min during placebo) or dinner ( $62 \pm 10$  vs.  $50 \pm 7$  min).

**EGR.** The rate of EGR in the fasting state was significantly reduced by liraglutide ( $1.92 \pm 0.06$  vs.  $2.13 \pm 0.09$  mg · kg<sup>-1</sup> · min<sup>-1</sup>;  $P = 0.04$ ). This was due to diminished GLY ( $0.83 \pm 0.04$  vs.  $1.02 \pm 0.04$  mg · kg<sup>-1</sup> · min<sup>-1</sup>;  $P = 0.01$ ). In contrast, GNG did not change (Fig. 3).

**Islet cell function.** β-Cell function in the fasting state, as assessed by HOMA-B analysis, was increased by 30% during liraglutide administration ( $P = 0.01$ ). First-phase insulin response after the intravenous glucose bolus was increased by ~60% ( $P < 0.01$ ). During steady state of the hyperglycemic clamp, there was a 2- to 3.5-fold increase in mean insulin concentration, whereas mean circulating

glucagon concentration was reduced by ~20% ( $P < 0.01$ ). Analogously, the insulin response after arginine infusion was substantially increased during liraglutide treatment ( $P < 0.01$ ), whereas the glucagon response was reduced ( $P = 0.01$ ). The proinsulin/insulin ratio after liraglutide administration was reduced by 40–50% during the hyperglycemic clamp (Fig. 4, Table 2).

**Insulin sensitivity and disposition index.** Insulin sensitivity (as calculated by HOMA-R and glucose infusion rate/mean insulin level) was unaltered during the two treatment regimens. The disposition index increased substantially, almost doubling after liraglutide ( $P < 0.01$ ; Table 2).

**Pharmacokinetic properties.** The half-life of liraglutide in steady state was 17.9 h, and  $t_{max}$  was  $10.1 \pm 3$  h (Fig. 5).

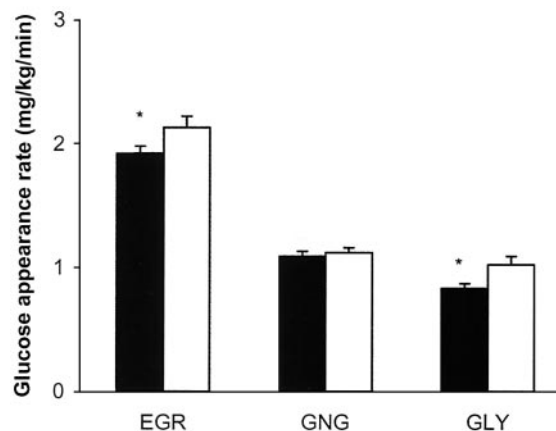


FIG. 3. Fasting EGR, GNG, and GLY, day 9. Data are means ± SEM. □, placebo; ■, liraglutide; \* $P < 0.05$ , liraglutide vs. placebo.

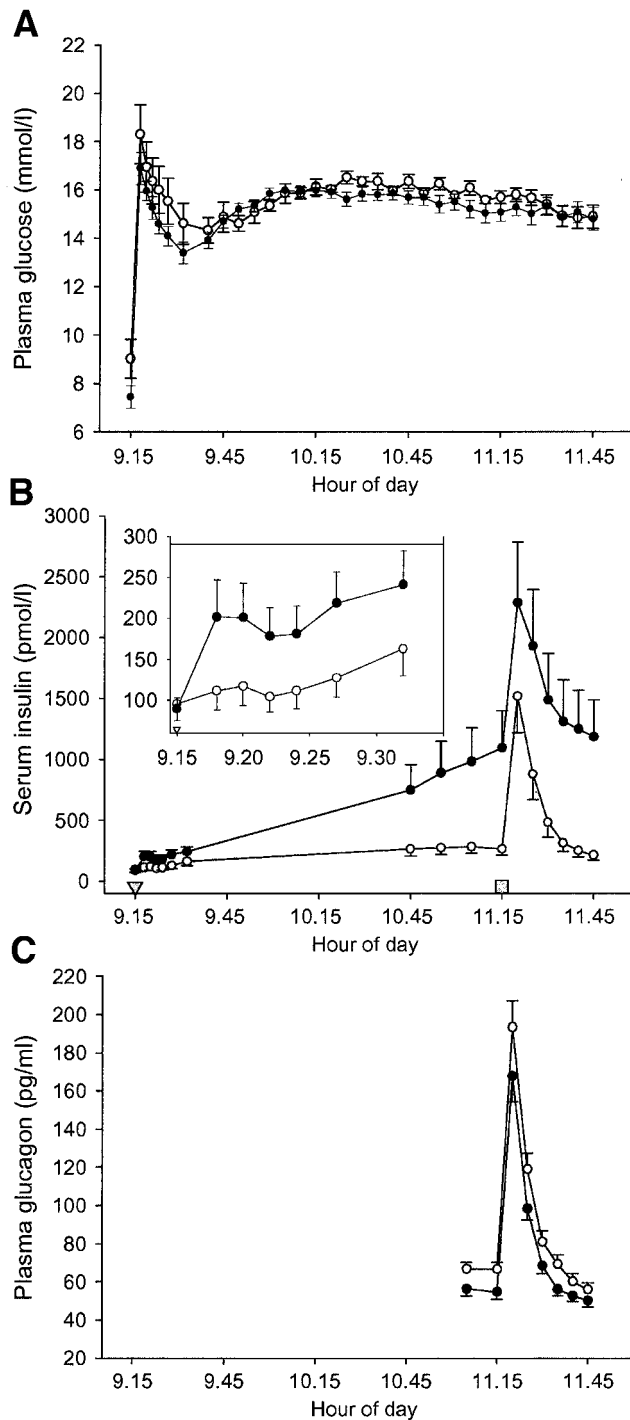


FIG. 4. Plasma glucose (A) and serum insulin (B) concentrations during an intravenous glucose bolus, hyperglycemic clamp, and arginine stimulation test, day 9. ○, placebo; ●, liraglutide; ▽, glucose bolus (25 g); □, arginine bolus (5 g). Plasma glucagon (C) is depicted from 1105. Steady state of hyperglycemic clamp was in the interval 1045–1115. The insert in the middle panel is a blow-up of the first-phase insulin response. Data are means  $\pm$  SEM. (Statistical details are listed in Table 2.)

**Safety.** No hypoglycemic episodes occurred. Three patients experienced gastrointestinal adverse events (nausea and abdominal pain) during the treatment period. In two cases, the symptoms disappeared after the first days of treatment, and in the remaining case, the severity of the gastrointestinal discomfort declined throughout the treat-

ment week. These three were among the patients in whom the highest serum concentrations of liraglutide were found. No other safety concerns were identified.

## DISCUSSION

In the present study, we investigated the effects of 1 week of treatment with the GLP-1 derivative liraglutide (NN2211) on 24-h substrate and hormone profiles under conditions that simulate daily living, on EGR, and on pancreatic islet cell function. A major finding is a markedly reduced circadian plasma glucose level during liraglutide treatment exhibited by fasting, prandial, and nocturnal concentrations. When evaluating the average reduction in plasma glucose ( $\sim 2$  mmol/l), it is important to emphasize that 6 of the 13 patients, even after OHA withdrawal, had a fasting plasma glucose  $< 8.3$  mmol/l, i.e., the level above which action should be taken (26). Moreover, this 1-week study was probably too brief to harvest the additional advantageous effects of decreased glucose toxicity, i.e., no improvement in insulin sensitivity. Finally, the same dose (6  $\mu$ g/kg body wt) of liraglutide was used in all patients. It is likely that some patients would have a further improvement in blood glucose lowering using a higher dose. The current study thus confirms that liraglutide possesses the widely known beneficial effects of native GLP-1 on glycemia in patients with type 2 diabetes (12,13,27) and demonstrates that once-daily dosing of liraglutide is sufficient to ensure 24-h effectiveness (vide infra, pharmacokinetics). It is also important to highlight that no episodes of hypoglycemia occurred despite some of the patients' exhibiting a remarkable glycemic control during liraglutide treatment.

Improved glycemic control during liraglutide treatment is probably orchestrated by changes in insulin and glucagon secretion. Basal and prandial ISRs were unchanged despite the substantial reduction in glycemia, clearly indicating improved  $\beta$ -cell function during daily life conditions. This is in accordance with previous studies exploring native GLP-1 actions (12,13,27) and demonstrates that GLP-1 and/or GLP-1 derivatives augment the ability of the  $\beta$ -cell to respond to prevailing prandial stimuli, e.g., glycemia and glucose dynamics.

A second important observation is that liraglutide significantly reduces 24-h circulating glucagon levels. In particular, the large postprandial glucagon excursion after the protein-rich evening meal was amply decreased. An impaired postprandial inhibition of glucagon is a common feature in type 2 diabetes (5,28), and this contributes notably to the postprandial hyperglycemia (4). Of particular note is that hyperglycemia per se inhibits glucagon secretion, and the decrement in plasma glucagon during liraglutide administration was seen despite a lower glycaemic level.

The lowering effect of native GLP-1 on upper gastrointestinal motility is well established (29). In a single-dose study using liraglutide in patients with type 2 diabetes, reduced gastric emptying rate was also reported (16). In contrast, we did not in the current study observe delayed gastric emptying during liraglutide administration. The data achieved from the acetaminophen assessment technique has been proved to agree with data from using much more sophisticated methods for assessment of gastric

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