Improved Glycemic Control With No Weight Increase in Patients With Type 2 Diabetes After Once-Daily Treatment With the Long-Acting Glucagon-Like Peptide 1 Analog Liraglutide (NN2211)

A 12-week, double-blind, randomized, controlled trial

OBJECTIVE — Liraglutide is a long-acting glucagon-like peptide 1 analog designed for once daily injection. This study assessed the efficacy and safety of liraglutide after 12 weeks of treatment in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — A double-blind, randomized, parallel-group, placebo-controlled trial with an open-label comparator arm was conducted among 193 outpatients with type 2 diabetes. The mean age was 56.6 years and the mean HbA1c was 7.6% across the treatment groups. Patients were randomly assigned to one of five fixed-dosage groups of liraglutide (0.045, 0.225, 0.45, 0.60, or 0.75 mg), placebo, or open-label sulfonylurea. Patients were from the 1Hvidovre Hospital, University of Copenhagen, Denmark; the 2University Hospital of Aarhus, Aarhus, Denmark; 3Novo Nordisk A/S, Bagsvaerd, Denmark; and the 4Oxford Centre for Diabetes, Endocrinology & Metabolism, University of Oxford, Oxford, U.K.

RESULTS — A total of 190 patients were included in the intention-to-treat (ITT) analysis. HbA1c decreased in all but the lowest liraglutide dosage group. In the 0.75-mg liraglutide group, HbA1c decreased by 0.75 percentage points (P < 0.0001) compared with placebo. Improvement in glycemic control was evident after 1 week. Body weight decreased by 1.2 kg in the 0.45-mg liraglutide group (P = 0.0184) compared with placebo. The proinsulin-to-insulin ratio decreased in the 0.75-mg liraglutide group (P = 0.0244) compared with placebo. Patients treated with glimepiride had a 1.2 kg weight loss after treatment with GLP-1 (11). These mechanisms make this hormone an attractive candidate for the treatment of type 2 diabetes. However, native GLP-1 has a very short half-life (1 min) (12), being rapidly metabolized by the enzyme dipeptidyl peptidase IV (13). It has been shown that GLP-1 must be present continuously in the blood stream to exert its actions (14).

CONCLUSIONS — A once-daily dose of liraglutide provides efficacious glycemic control and is not associated with weight gain. Adverse events with the drug are mild and transient, and the risk of hypoglycemia is negligible.

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Abbreviations: GLP-1, glucagon-like peptide 1; HOMA, homeostasis model assessment; ITT, intention to treat; OHA, oral hypoglycemic agent.

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Improved glycemic control with liraglutide (NN2211)

RESEARCH DESIGN AND METHODS—This trial was a 12-week, multicenter, double-blind, randomized, parallel-group, dosage-response trial in six treatment arms. In addition, an open-label sulfonylurea (glimepiride) arm was included as a reference group. Patients were instructed to maintain their diet, exercise program, and daily routines during the course of the trial. After a 4-week wash-out period during which current oral hypoglycemic agent (OHA) treatment was discontinued, patients were equally randomized to double-blind treatment of one of six arms (one of five dosages of liraglutide or placebo) or to the open-label reference group (glimepiride). The trial was conducted between December 2000 and October 2001 in Scandinavia and the U.K. The trial was conducted in accordance with the Helsinki Declaration (19), and the protocol was approved by each institution’s independent ethics committee. The trial was explained to all patients, and their written informed consent was obtained before any trial-related procedures were initiated.

Eligible patients were men and women age ≥30 years who had a type 2 diabetes diagnosis (according to American Diabetes Association criteria) (20), had BMI ≤40 kg/m², were being treated with diet or an OHA, and had an HbA₁c ≥9.5% (OHA) or 7.5–10.0% (diet). For patient safety, an upper limit for HbA₁c was defined as a 4-week wash-out period and a placebo arm was included in the trial. Patients were excluded if any of the following were present: liver or renal disease, heart failure (New York Heart Association class III and IV) (21), unstable angina pectoris, myocardial infarction within the previous 12 months, concomitant treatment with thiazolidinediones or other investigational drugs, or other significant conditions likely to affect a patient’s diabetes and/or ability to complete the trial. Women who were pregnant, breast-feeding, or not using an adequate method of contraception were also excluded. At randomization, fasting blood glucose had to be 6–13 mmol/l. Patients were withdrawn from the trial if fasting plasma glucose was >15 mmol/l.

In total, 311 patients were screened and 193 patients were randomized to a treatment group (Table 1). The reasons for failing screening were not meeting any inclusion criteria or meeting any exclusion criteria (n = 86), not meeting randomization criteria (n = 7), withdrawn consent (n = 6), and other (n = 19). The majority of patients (n = 158) were being treated with OHA(s) and the remaining 35 patients were being treated with diet only. Most patients were on monotherapy with either metformin (65 patients) or sulfonylureas (55 patients); 22 patients received a combination of metformin and sulfonylureas, 14 patients received repaglinide, and 2 patients received acarbose treatment. In all, 190 patients were exposed to the experimental protocol; 3 patients withdrew consent before receiving randomized treatment.

Patients were recruited from the participating investigators’ outpatient clinics or by local advertisements. Each patient was seen on seven occasions: screening; baseline; after 1, 4, 8, and 12 weeks of treatment; and follow-up. Current treatment with oral antidiabetic medication was discontinued at screening. Patients were supplied with a blood glucose meter (One Touch Profile Glucometer) and instructed in its use. Fasting blood glucose was measured every morning. HbA₁c, fasting serum glucose, insulin, C-peptide, and glucagon were measured every 4 weeks. Fasting serum glucose was also measured after the first week of treatment. Proinsulin was assessed in a subset of patients (n = 74, equally distributed among the groups). Fasting samples were obtained before administration of the trial drug. Safety parameters (adverse events, hypoglycemic episodes, weight, standard hematology and biochemistry profile, vital signs, and electrocardiogram) were assessed at each visit. Liraglutide antibodies were measured before and after treatment.

The trial was double blind for the five dosage levels of liraglutide and placebo, and open label for glimepiride. The blinding was kept until database release.

Liraglutide and placebo (Novo Nordisk A/S, Bagsvaerd, Denmark) were administered as a once-daily injection (subcutaneously) in the morning before breakfast. The five dosages of liraglutide administered were 0.045, 0.225, 0.45, 0.60, and 0.75 mg. These dosages were chosen based on observations from previous trials (16–18). In one of those trials, a single dose of 10 µg/kg (corresponding to ~0.80 mg) showed significant effects on glycemia, but 2 of 11 patients reported nausea (18). Further, once-daily dosing for 7 days showed a 40% increase in Cₘₚ₉ in the steady state (17). Therefore, to avoid causing unacceptable side effects, the highest dosage included in this trial was 0.75 mg, corresponding to 7.5 µg/kg (equivalent to 10.5 µg/kg in steady state) for a person weighing 100 kg. The lowest dosage of 0.045 mg was considered too low to have any significant effect on glycemic control. Glimepiride (Amaryl; Aventis Pharma, Frankfurt, Germany) was supplied as 1- and 2-mg tablets for oral use, with the dosage adjusted according to glycemic control during the first 4 weeks, with the aim to achieve a fasting plasma glucose level <7 mmol/l. The mean glimepiride dosage during the trial was 2.7 mg. Compliance was assessed by drug accountability and plasma concentration measurements.

Analytic methods
HbA₁c was analyzed with a Unimate HbA₁c assay (Roche Diagnostics; normal range 4.5–5.7%). Liraglutide antibodies were determined by a radioimmunoassay developed by the Department of Immunoochemistry at Novo Nordisk A/S. The serum concentrations of insulin, C-peptide, and proinsulin were analyzed by enzyme-linked immunosorbent assay methods. Plasma glucagon was analyzed by MDS Pharma Services (Wangen, Switzerland) using a radioimmunoassay (Linco Research, St. Charles, MO). Standard laboratory analyses were performed by a central laboratory (Novo Medical Medi-Lab, Clinical Trials Lab, Copenhagen, Denmark, if not otherwise stated).

Statistical analysis
Pretrial calculation showed that a two-sided test of the highest dosage versus the placebo group required 30 patients per group to detect a difference in mean HbA₁c of at least 1% unit with 5% significance and 95% power.

The primary end point HbA₁c, as well as secondary end points (fasting serum glucose, fasting C-peptide, glucagon, insulin, homeostasis model assessment
[HOMA], proinsulin-to-insulin ratio, and weight] were analyzed in a mixed-effects model with treatment, visit, and center as fixed effects and patient as the random effect. The interaction term, baseline HbA1c by visit, was included in the model as a covariate. Adjusted end point levels at 12-week follow-up were calculated for each treatment group by means of this model.

The HOMA(S) (β-cell function) was determined as β-cell function (%) = 20 × insulin/glucose − 3.5. HOMA(R) (insulin resistance) was determined as resistance = insulin/(22.5e−[ln(glucose)](22)).

All analyses were performed for the intention-to-treat (ITT) population (i.e., all patients who received at least one dose of a trial drug). Statistical analyses were performed using SAS software (version 8; SAS Institute, Cary, NC).

**RESULTS**

**Enrollment**

The 193 patients randomized in this trial were evenly distributed across treatment groups (Table 1). Baseline clinical characteristics of the 190 patients exposed to the experimental protocol are given in Table 1. No apparent differences were seen among the treatment groups with regard to baseline characteristics.

**Effect on glycemic control**

After 12 weeks of treatment, HbA1c was decreased in all groups except the lowest liraglutide dosage group (Fig. 1A). Treatment with the two highest dosages reduced HbA1c significantly more than placebo (Table 2). At these dosage levels, the effect of liraglutide was comparable with that of glimepiride with respect to effect on HbA1c. The effect of liraglutide increased with duration of treatment. The largest decreases in HbA1c levels were observed at the end of the 12-week treat-
ment period for the highest dosages (Fig. 1B), and it seemed that HbA1c levels were still decreasing at the end of the treatment period at these dosages. We observed that 59% of patients completing the trial in the two highest liraglutide dosage groups achieved HbA1c \(\leq 7\%\) after 12 weeks.

As was seen with HbA1c, fasting serum glucose levels decreased in most treatment groups during the trial, with the decreases being statistically significant for the 0.225-, 0.60-, and 0.75-mg liraglutide dosage groups compared with placebo (Table 2). In accordance with the observations for HbA1c, the effect of the highest dosages of liraglutide was comparable with that of glimepiride with respect to fasting serum glucose (Fig. 1C). In the liraglutide groups, maximal effect on fasting serum glucose was evident after the first week of treatment (Fig. 1D).

**Effect on body weight**

Treatment with liraglutide did not increase body weight (Fig. 2). Furthermore, for the 0.45-mg liraglutide dosage group (Table 2), a statistically significant decrease, compared with placebo, was observed.

**Effect on islet cell function**

HOMA (22) was used to assess \(\beta\)-cell function and insulin resistance after 12 weeks of liraglutide treatment. Mean \(\beta\)-cell function (derived from fasting insulin and glucose) was significantly higher in the 0.75-mg liraglutide group after 12 weeks than in the placebo group (Table 2). The effect obtained with the highest liraglutide dosage was similar to that observed with glimepiride. For insulin resistance, no differences were seen among the three treatments (liraglutide, glimepiride, and placebo; data not shown). Further, the proinsulin-to-insulin ratio decrease was statistically significantly after treatment with 0.75 mg liraglutide compared with placebo (Table 2). No change in the proinsulin-to-insulin ratio was demonstrated after treatment with glimepiride. For insulin resistance, no differences were seen among the three treatments (liraglutide, glimepiride, and placebo; data not shown).

**Safety evaluation**

Of the 135 patients exposed to liraglutide, 1 (in the 0.60-mg group) experienced minor hypoglycemia (defined in the study protocol as blood glucose <2.8 mmol/l) and 7 reported symptoms of hypoglycemia only. These incidences seemed lower than in the glimepiride group (n = 26), where four patients experienced minor hypoglycemia and five patients reported symptoms of hypoglycemia.

The number of patients with adverse events (based on spontaneous adverse event reporting) was comparable across the liraglutide groups and the placebo group (60% [81 of 135]) vs. 55% [16 of 29] of patients, respectively) and was lower in the open-label reference group (35% [9 of 26] of patients). For gastrointestinal events, the incidence seemed to increase with increasing doses of liraglutide; nausea was reported by 1–2 patients in each of the lowest dose groups and by 5 of 28 patients in the highest dose group (in total, reported by 10 of 135 patients exposed to liraglutide) compared...
with 1 of 29 patients in the placebo group. Other gastrointestinal events included diarrhea (5 of 135 liraglutide-treated patients), vomiting (3 of 135 patients), and constipation (3 of 135 patients). None of these events were reported in the placebo or glimepiride groups, with the exception of one patient experiencing vomiting in the glimepiride group. Approximately two-thirds of the gastrointestinal events reported during treatment with liraglutide were resolved within 1–3 days. None of the patients withdrew due to gastrointestinal events (Table 1). Overall, the most frequent adverse events were headache and nausea, which were also the events most often assessed as related to the trial product. Generally, adverse events were mild or moderate and completely resolved. Only 1 of the 135 patients exposed to liraglutide experienced two mild injection site reactions, described as an “urticarial reaction at injection place.” No antibody formation against liraglutide could be detected in this trial.

No safety issues with regard to vital signs, electrocardiogram, or laboratory analysis were raised by this trial.

**CONCLUSIONS** — This trial was the first to demonstrate a sustained improvement in glycemic control after long-term treatment with the GLP-1 analog liraglutide, administered once daily. After 12 weeks of treatment with 0.60 or 0.75 mg liraglutide, both HbA1c and fasting serum glucose levels were significantly lower than with placebo. Glycemic control was maintained throughout the treatment period and was as effective at the highest liraglutide dosages as that provided by the open-label reference therapy glimepiride.

### Table 2—Repeated measures analysis after 12 weeks, comparison with placebo in ITT population

<table>
<thead>
<tr>
<th></th>
<th>Liraglutide (mg)</th>
<th>Glimepiride</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.045</td>
<td>0.225</td>
</tr>
<tr>
<td>n</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>0.25 (0.1905)</td>
<td>-0.34 (0.0877)</td>
</tr>
<tr>
<td>Fasting serum glucose (mmol/L)</td>
<td>0.74 (0.1499)</td>
<td>-1.37 (0.0090)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>-0.03 (0.9602)</td>
<td>-0.74 (0.1544)</td>
</tr>
<tr>
<td>Proinsulin-to-insulin ratio</td>
<td>-0.04 (0.6468)</td>
<td>-0.12 (0.1314)</td>
</tr>
<tr>
<td>β-Cell function (%)</td>
<td>-6.07 (0.3546)</td>
<td>7.55 (0.2514)</td>
</tr>
<tr>
<td>Fasting insulin (mmol/L)</td>
<td>-2.44 (0.8425)</td>
<td>-4.15 (0.7381)</td>
</tr>
<tr>
<td>Fasting C-peptide (mmol/L)</td>
<td>-0.09 (0.4033)</td>
<td>-0.01 (0.9449)</td>
</tr>
<tr>
<td>Fasting glucagon (mmol/L)</td>
<td>12.74 (0.3619)</td>
<td>-15.84 (0.2710)</td>
</tr>
</tbody>
</table>

*P* value given in parentheses.
at an average dosage of 2.7 mg (a near-maximal dosage) (23), with trends toward less hypoglycemia and weight loss. At the highest dosage levels, there was a trend toward improved HbA1c with duration of treatment. This suggests that further improvements in HbA1c levels may be seen with extended (>12 weeks) treatment. More than half the patients in the two highest dosage liraglutide groups had HbA1c ≤ 7% after 12 weeks of treatment. Furthermore, treatment with liraglutide caused no weight increase, and, at the highest dosage, the difference with glimepiride was statistically significant. This observation was in accordance with findings that GLP-1 suppresses energy intake in humans (8).

The full dosage-response curve for liraglutide remains to be elucidated, and future trials will investigate higher dosages of drug, but the current trial confirmed the proposed once-daily dosing regimen for liraglutide.

The proinsulin-to-insulin ratio decreased during 12 weeks’ treatment with liraglutide. This ratio is known to be elevated in type 2 diabetes and is considered an index of insulin secretory dysfunction (24,25). It is believed that the hyperproinsulinemia is caused by an increased demand placed on the β-cell by hyperglycemia and insulin resistance, which in turn leads to secretion of an increased amount of incompletely processed granules containing proinsulin. This is also known as the “overworked β-cell” hypothesis (26). An alternative hypothesis is that the increased proinsulin concentration is related to an intrinsic β-cell defect in type 2 diabetes (24). In both cases, improvement of the proinsulin-to-insulin ratio, and thus of β-cell function, would be a desirable effect of a potential new treatment for type 2 diabetes. β-Cell function, as determined by HOMA analysis, was significantly improved at the highest liraglutide dosage compared with placebo. There was no effect on the α-cells in the fasting state, in so far as the glucagon concentrations were unaltered.

The safety profile of liraglutide was favorable, with the expected very low risk of hypoglycemic episodes as anticipated with the glucose-dependent mode of action of native GLP-1 (27). Adverse events were isolated, with the most frequent being headache and nausea. Usually the events were transient and of mild or moderate severity and resolved without intervention. Although both nausea and vomiting were reported, neither of these was of a severity that caused the patient to withdraw. Most gastrointestinal events were transient (i.e., they resolved within 1–3 days). Side effects within the gastrointestinal system are known to occur after GLP-1 treatment and seem to be dosage related (14,28,29). These effects probably result from the inhibition of gastric emptying by GLP-1 (9,30–32). No formation of antibodies toward liraglutide was detected after 12 weeks of treatment. The sensitivity of the assay has been found to be 30 ng/ml (data on file) using a monoclonal antibody. The liraglutide antibody assay is essentially similar to a previously published assay used to assess insulin and insulin aspart antibodies with a similar and acceptable sensitivity (33).

Another approach for investigating the potential of GLP-1 includes administering native GLP-1 by means of continuous infusion (11,14). Continuous intravenous infusion of GLP-1 for 1 week resulted in an instant decrease in blood glucose levels in patients in whom sulfonylurea therapy failed to be effective (14). A 6-week trial with GLP-1 administered as continuous subcutaneous infusion via an insulin pump demonstrated significant effects on fasting and 8-h plasma glucose and HbA1c, as well as an inhibition of gastric emptying and body weight loss (11). The latter trial was conducted in patients with poorly controlled HbA1c levels, indicating that the GLP-1 treatment concept may be applied to a wide range of glycaemia in type 2 diabetes. Also, the peptide exenatide (synthetic exendin-4) has demonstrated effects similar to those of GLP-1 (34). In this 28-day study, adding exenatide to the existing treatment two or three times daily showed a significant effect in controlling patients’ HbA1c levels, which were previously not satisfactorily controlled with diet and/or OHA.

In conclusion, the long-acting GLP-1 analog liraglutide shows considerable promise as a once-daily therapy in type 2 diabetes for lowering blood glucose without weight gain or substantial risk of hypoglycemia.

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