Pharmacokinetics, Pharmacodynamics, Safety, and Tolerability of a Single-Dose of NN2211, a Long-Acting Glucagon-Like Peptide 1 Derivative, in Healthy Male Subjects

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OBJECTIVE — The primary objective of the present study was to investigate the safety, tolerability, and pharmacokinetics of a single dose of NN2211, a long-acting glucagon-like peptide 1 (GLP-1) derivative, in healthy male subjects. The secondary objective was to investigate the pharmacodynamics of NN2211.

RESEARCH DESIGN AND METHODS — In a double-blind, randomized dose, escalation, placebo-controlled study, healthy male subjects were enrolled at eight consecutive dose levels (1.25, 2.5, 5.0, 10.0, 12.5, 15.0, 17.5, and 20.0 μg/kg) with eight subjects per dose level at a 3:1 active:placebo randomization. After subcutaneous dosing with NN2211, 48-h pharmacokinetic, and 24-h glucose, insulin and glucagon profiles were assessed. In addition, three subjects at each dose level were randomly assigned (one placebo/two active) to an intravenous glucose tolerance test (IVGTT) 9 h after the dose (corresponding to the time to maximal plasma concentration of NN2211).

RESULTS — After subcutaneous administration, the half-life of NN2211 was found to be 11–15 h. Overall, although there were no statistically significant differences compared with placebo in the area under the curve (0–9 h for insulin or glucagon), there was a borderline-significant lowering of glucose levels ($P = 0.066$). During the IVGTT, there was a statistically significant increase in insulin secretion ($P = 0.0002$), but there was no significant effect on glucagon levels. Although no significant effect was observed on glucose levels during the IVGTT, there was a dose-dependent increase in the glucose disappearance constant. Whereas no serious adverse events were observed, there was a higher incidence of adverse events after active treatment compared with placebo treatment (notably headache, dizziness, nausea, and vomiting).

CONCLUSIONS — This study provides evidence that NN2211 has a pharmacokinetic profile consistent with once-daily dosing in humans.

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Glucagon-like peptide 1 (GLP-1) is a polypeptide hormone secreted from the L-cells in the gastrointestinal tract (1) that has been shown to possess antidiabetogenic effects in both animal models and patients with type 2 diabetes (2–7). The mechanism of action rests on a suite of effects: notably, a glucose-dependent stimulation of insulin secretion (8), inhibition of glucagon secretion (9), inhibition of gastric emptying (10), and a decrease in appetite (11,12). In addition, a direct stimulation of growth and proliferation of the β-cells has recently been demonstrated in mice (13). Taken together, these effects suggest that GLP-1 is a promising candidate for treating type 2 diabetes. However, the rapid clearance and degradation of GLP-1 by dipeptidyl peptidase IV (DPP-IV) results in a half-life of ~1 h in humans after subcutaneous administration, compromising its therapeutic potential as a new treatment modality (2).

It has previously been demonstrated that derivatization of insulin with fatty acids facilitates binding to serum albumin and provides a protracted action (14,15). This approach has also been reported to result in prolongation of the half-life of GLP-1 after subcutaneous administration in dogs (16). NN2211 is an acylated long-acting GLP-1 derivative, which, in preclinical experiments, has shown prolonged pharmacokinetic properties compared with the native GLP-1 (17) while maintaining its biological action both in vitro (17) and in vivo (18–20).

The primary objective of the present study was to investigate the safety, tolerability, and pharmacokinetics of a single dose of NN2211 in healthy male subjects, and the secondary objective was to investigate the pharmacodynamics of NN2211.

RESEARCH DESIGN AND METHODS

Study protocol

The protocol was approved by the Independent Ethics Committee in Manchester, U.K., and conducted in accordance with the Helsinki Declaration and Good Clinical Practice. Subjects consented to

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Abbreviations: AUC, area under the curve; C_{max}, maximal plasma concentration; DPP-IV, dipeptidyl peptidase IV; ECG, electrocardiogram; GLP-1, glucagon-like peptide 1; IVGTT, intravenous glucose tolerance test; K_g, glucose disappearance constant.

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

participating in the study in writing after a full explanation of the study had been given.

Subjects

Subjects were recruited from a volunteer database kept at Medeval Ltd. A total of 72 healthy male subjects in good general health, as verified by medical history, physical examination, clinical chemistry, hematology, and electrocardiogram (ECG), aged 18–45 years with BMI 20–27 kg/m^2, were included in this study. Mean age was 27 years (range 19–41) and weight 74.5 kg (range 58.0–98.0).

Study design

This was a single-center, randomized, double-blind, placebo-controlled, single-dose, sequential-dose level escalation study. Eight different dose levels of NN2211 were studied: 1.25 (performed twice), 2.5, 5.0, 10.0, 12.5, 15.0, 17.5, and 20.0 μg/kg with eight subjects at each dose level, and a 3:1 active:placebo randomization within each dose level. Subjects were only enrolled at one dose level. In addition, at each dose level, three subjects were randomly assigned (one placebo/two active) to receive a 2-h intravenous glucose tolerance test (IVGTT). Finally, to determine the absolute bioavailability of NN2211, all eight subjects allocated at the 5 μg/kg subcutaneous dose level returned after a 7-day washout to receive an intravenous dose of 5 μg/kg NN2211 or placebo.

Study medication and blinding

NN2211 at a concentration of 5 mg/ml or matching placebo, manufactured in accordance with good manufacturing practice, was provided in cartridges. These cartridges fit into the NovoPen 1.5, which was used for subcutaneous dose administration. The medication was labeled in accordance with the blinded status of the study. For the intravenous administration of NN2211/placebo performed at the 5 μg/kg dose level to determine the absolute bioavailability, a dilution of the trial medication was performed corresponding to 0.1 mg/ml. The intravenous infusion was performed over 1 h (to avoid high peak plasma concentrations), with an infusion rate calculated based on the weight of the individual subject. Sealed codes were kept at the trial site and at Novo Nordisk in the U.K. and Denmark. A copy of the code was provided only to the responsible person performing the analysis of the concentration of NN2211 in blood and was not further disclosed (because this analysis would unblind the study anyway, it was done to reduce the number of placebo samples analyzed). The code was only broken after database lock but could have been broken for individual subjects in case of an emergency.

Experimental procedures

The subjects were admitted to the clinic on the day before dose administration and given a standardized meal at ~7:00 P.M. on the day of admittance and at 6:00 A.M. the following day. NN2211 or placebo was administered between 8:00 and 10:00 A.M. as a single subcutaneous dose in the abdomen. At the first dose level (1.25 μg/kg), the IVGTT was started 5 h postdose; however, because blinded pharmacokinetic data showed that the time to maximal plasma concentration of NN2211 was 9 h in humans, the protocol was amended to start the 2-h IVGTT at 9 h postdose in all subsequent dose levels, including a repeat of the 1.25 μg/kg dose level. A standardized meal was served 11 h after dosing, i.e., after completion of the IVGTT. Blood samples for determination of the concentration of NN2211 were collected at regular intervals from before dosing to 48 h after dosing. Furthermore, a predose to 24-h postdose glucose, insulin, and glucagon profile was obtained in all subjects. In subjects randomized to undergo the IVGTT, additional blood samples for a 2-h glucose, insulin, and glucagon profile were drawn, i.e., between 9 and 11 h after dosing. All subjects were monitored for safety during the study. Adverse events either observed by the investigator or reported spontaneously by the subjects were recorded. In addition, subjects were asked at predose and at 1, 2, 4, 6, 8, 10, 12, 14, 16, 24, and 48 h postdose if they had had any adverse events (including any changes in concomitant illness or new illnesses) since the last evaluation. Clinical laboratory tests (hematology, biochemistry, and urinalysis) were carried out before, during, and after dosing. Blood glucose was measured at regular intervals after dose administration and before discharge using a HemoCue device. Physical examination, ECG (before dosing and 4, 8, 12, 16, 24, and 36 h after dosing), and vital signs, including blood pressure, pulse, and temperature, were recorded. Urine volumes were assessed for the period 0–24 h after dose administration. At the 5-μg/kg dose level, the subjects returned after 1 week for the intravenous administration of NN2211/placebo. At this visit, all procedures were performed as described above, with the exception of the IVGTT, which was not performed.

Laboratory procedures

The concentration of NN2211 was determined by a validated two-site immunoassay using a capturing antibody (GLPB1F1) and a detection antibody (biotin-labeled Mab26.1) as described previously (21). The selectivity of the assay toward NN2211 compared with endogenous GLP-1 was ensured by removing endogenous GLP-1 activity by incubating the samples for 4 h at 37°C before performing the assay. In addition, the interference from a number of peptides was investigated. NN2211 (7-37) isomer interfered positively but <4%, NN2211 (9-37) isomer had negative interference <12%, GLP-1 (15-37) had negative interference <3%, and major pro-glucagon fragment had negative interference <4%. Because all of these interferences were low, and the concentrations of the peptides in the majority of the samples were low compared with the NN2211 concentrations, interference was considered irrelevant. Serum glucose was measured on a Beckman CX5 Delta analyzer using the glucose oxidase technique. Serum insulin was analyzed using the Abbott IMx assay, which shows <0.005% cross-reactivity with human pro-insulin, and glucagon was analyzed with Linco's glucagon radioimmunoassay in accordance with the manufacturer's instructions.

Statistical analysis

Because of the exploratory status of this study (first administration to humans), the sample size of eight subjects per dose level was not based on formal statistical considerations but was considered sufficient to meet the primary objectives of the study. A significance level of 5% was used. Results from the statistical analysis are presented with the mean and 95% CI.

Safety. Urine volume (0–24 h) was analyzed by ANOVA with the following model: the response for each subject was the sum of an overall mean, a fixed dose effect (placebo = zero dose), a random block effect of the dose level groups (in-

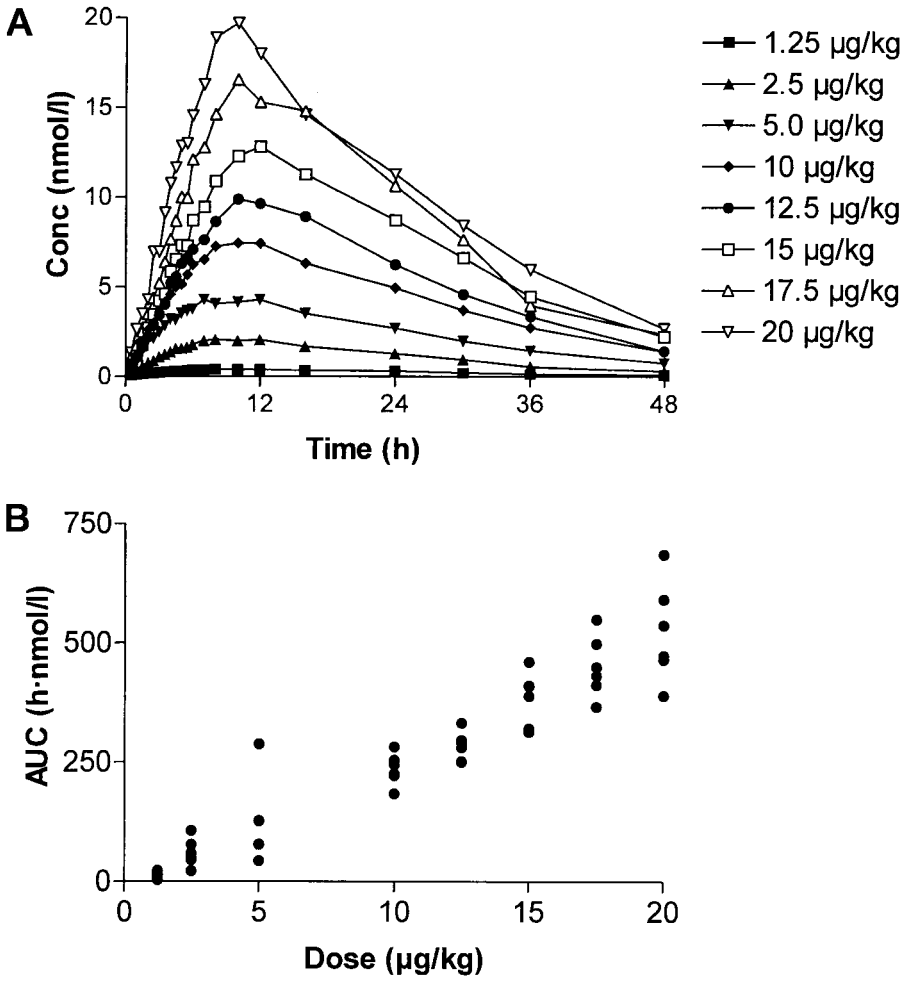

Figure 1—*Mean pharmacokinetic profiles after subcutaneous administration of eight different dose levels of NN2211 to healthy male volunteers* (A) *and a scatter plot showing dose versus AUC of NN2211 after subcutaneous administration* (B).

cluding the two placebo subjects), and a random error. All 72 subjects enrolled were included.

Pharmacokinetics. The pharmacokinetic parameters were assessed by noncompartmental methods from the 48-h NN2211 plasma concentration time profile for the single-dose subcutaneous and intravenous administration. Dose proportionality for subcutaneous dosing was assessed for area under the curve (AUC) to infinity and maximal plasma concentration (C_{max}) by performing linear regression analysis on the log-transformed parameters versus log-transformed dose. An estimate of the slope of the regression line and corresponding 95% CI was calculated. Dose proportionality was assumed if the slope was not statistically significantly different from unity. Of the 54 subjects exposed to active treatment, 3 and 2 subjects were excluded at the 1.25-µg/kg (first cohort only) and 5-µg/kg dose levels, respectively. This exclusion was due to dosing problems (low dosing volume) that caused low exposure when compared with other subjects at the same dose level.

Pharmacodynamics. Only pharmacodynamic data after subcutaneous administration were included in the statistical analysis. The $AUC_{0-9\ h}$ (all subjects) and $AUC_{9-10\ h}$ and $AUC_{9-11\ h}$ (IVGTT-treated subjects only) for glucose, glucagon, and insulin were derived from the respective profiles, applying the trapezoidal rule, and log-transformed. The glucose disappearance constant (K_g) was calculated by linear regression analysis of natural log-transformed glucose data from the IVGTT between 10 and 60 min after glucose administration. For each individual subject, the K_g was defined as the negative slope of the regression line. In one subject receiving placebo, the glucose concentration did not peak until 15 min, and the 10-min data point was therefore omitted for that subject. In another sub-

Table 1—*Pharmacokinetic parameters after NN2211 administration to healthy male subjects*

Dose (µg/kg)	AUC ($n \cdot nmol^{-1} \cdot l^{-1}$)	Absolute bioavailability (%)	C_{max} (nmol/l)	t_{max} (h)	Clearance ($ml \cdot min^{-1} \cdot kg^{-1}$)	Volume of distribution (l/kg)	$t_{1/2}$* (h)
1.25 s.c.†	15 ± 6	—	0.49 ± 0.19	9.6 ± 1.9	—	—	14
2.5 s.c.	60 ± 29	—	2.3 ± 0.70	10 ± 3.4	—	—	11
5.0 i.v.	216 ± 43	—	—	—	0.10 ± 0.02	0.07 ± 0.01	8.1
5.0 s.c.‡	134 ± 108	55 ± 37	4.5 ± 3.4	9.3 ± 3.2	—	—	15
10.0 s.c.	235 ± 33	—	7.9 ± 2.7	12 ± 6.0	—	—	14
12.5 s.c.	283 ± 31	—	10 ± 2.7	11 ± 2.4	—	—	12
15.0 s.c.	384 ± 57	—	13 ± 2.0	11 ± 1.1	—	—	13
17.5 s.c.	451 ± 65	—	17 ± 3.0	11 ± 1.0	—	—	11
20.0 s.c.	523 ± 105	—	20 ± 3.0	10 ± 1.3	—	—	11

Data are mean pharmacokinetic parameters ± SD after intravenous and subcutaneous administration of NN2211 to healthy male subjects (n = 6 unless otherwise indicated). t_{max}, time to maximal plasma concentration; $t_{1/2}$, half-life. *Harmonic mean; † n = 9; ‡ n = 4.

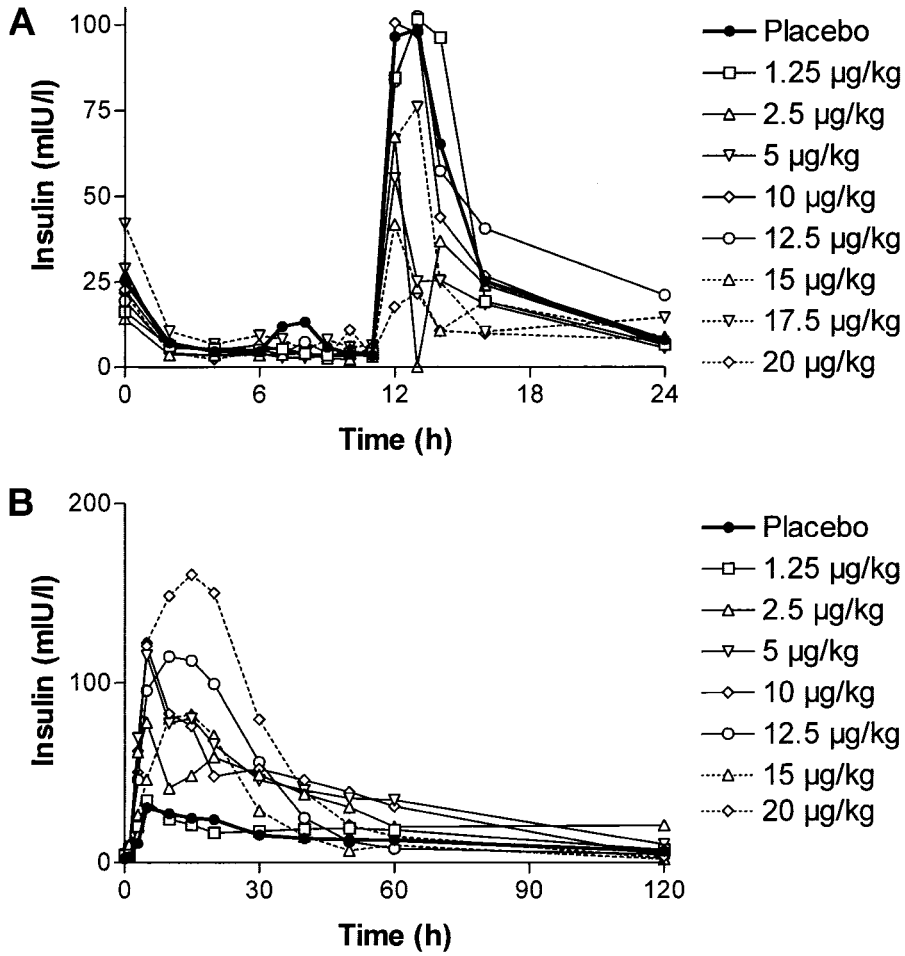

Figure 2—A: *Mean concentration time profiles of insulin after NN2211/placebo administration to healthy male subjects (subjects randomized to the IVGTT have not been included in the figure)* (n = *4 in all dose groups except the placebo, where* n = *8, and at the 5.0 µg/kg dose level, where* n = *3).* B: *IVGTT profiles of insulin after NN2211/placebo administration to healthy male subjects* (n = *2 in all dose groups except the placebo, where* n = *7, and at the 5.0 µg/kg dose level, where* n = *1).*

ject receiving placebo, the glucose concentration had rebounded markedly at the 60-min time point, which was therefore omitted for that subject. Exclusion of these data points represents a conservative approach for the assessment of a possible positive effect of NN2211 on K_g. In the statistical model for end points, the response for each subject was the sum of an overall mean, a fixed-dose effect (placebo = zero dose), a random block effect of the dose level groups (including the one or two placebo subjects), and a random error, and the data were analyzed using ANOVA. All subjects from the first cohort of 1.25 µg/kg were excluded because of the timing of the IVGTT. For the $AUC_{0-9\ h}$ glucose, insulin, and glucagon analysis, two subjects at 5.0 µg/kg were excluded because of low exposure, and one additional subject was excluded because he did not fast (randomized to placebo at the 17.5-µg/kg dose level), giving a total population of 61 subjects. Because no comparison to placebo could be made, all subjects receiving an IVGTT at the 17.5-µg/kg dose level were additionally excluded in the $AUC_{9-11\ h}$ analysis, giving a total population of 20 subjects.

RESULTS — No serious adverse events were reported, and all subjects completed the study. There was a higher number of adverse events reported in subjects on active treatment versus placebo treatment. The higher frequency was noted for headache, dizziness, nausea, and vomiting. Whereas headache and dizziness occurred on the majority of dose levels, nausea and vomiting mainly occurred from 10 and 15 µg/kg, respectively. At the highest dose level, 6 of 6 subjects experienced nausea and/or vomiting compared with 2 subjects experiencing nausea and 1 experiencing vomiting out of 18 subjects in the placebo-treated group. There were no clinically significant changes in vital signs, ECG parameters, physical examination, or safety laboratory parameters (hematology, biochemistry, and urinalysis). There was a tendency toward lower urine volumes at doses ≥12.5 µg/kg, but there was no overall significant difference in urine volumes 0–24 h after dose administration between active and placebo treatment (mean −167; 95% CI [−650 to 316]).

Figure 1*A* shows the mean plasma concentration time profiles of NN2211 after subcutaneous administration, and the derived pharmacokinetic parameters are given in Table 1. NN2211 was slowly absorbed after subcutaneous administration, with maximum plasma concentrations obtained ~9–12 h after dosing and a subsequent mean elimination half-life in the range of 11–15 h. After intravenous administration, NN2211 was shown to have a low volume of distribution (0.07 l/kg) and a mean elimination half-life of 8.1 h. The absolute bioavailability based on the intravenous and subcutaneous crossover administration of 5.0 µg/kg was found to be 55%. Figure 1*B* shows a plot of AUC of NN2211 versus dose, indicating a linear relationship. However, statistical analysis showed linearity only in the dose range of 2.5–20 µg/kg for both C_{max} (mean slope 1.07 [95% CI 0.91–1.24]) and AUC (1.09 [0.94–1.24]) but not in the range of 1.25–20 µg/kg (1.28 [1.17–1.40] and 1.26 [1.15–1.37] for C_{max} and AUC, respectively).

The mean 24-h insulin, glucose, and glucagon time profiles are shown in Figs. 2*A*, 3*A*, and 4*A*, respectively. Furthermore, the derived $AUC_{0-9\ h}$/9 h is shown in Table 2. Overall, in the period before the first meal and the IVGTT corresponding to $AUC_{0-9\ h}$, there was a borderline-significant lowering of glucose levels (P = 0.066; mean 0.97 [95% CI 0.94–1.00]) but no statistically significant difference between active and placebo treatment for insulin (0.97 [0.81–1.16]) or glucagon (0.98 [0.91–1.05]). Furthermore, in connection with the meal (corresponding to 11-h postdose) and at doses >10.0 µg/kg, there was a tendency toward lower insulin and glucose levels after active

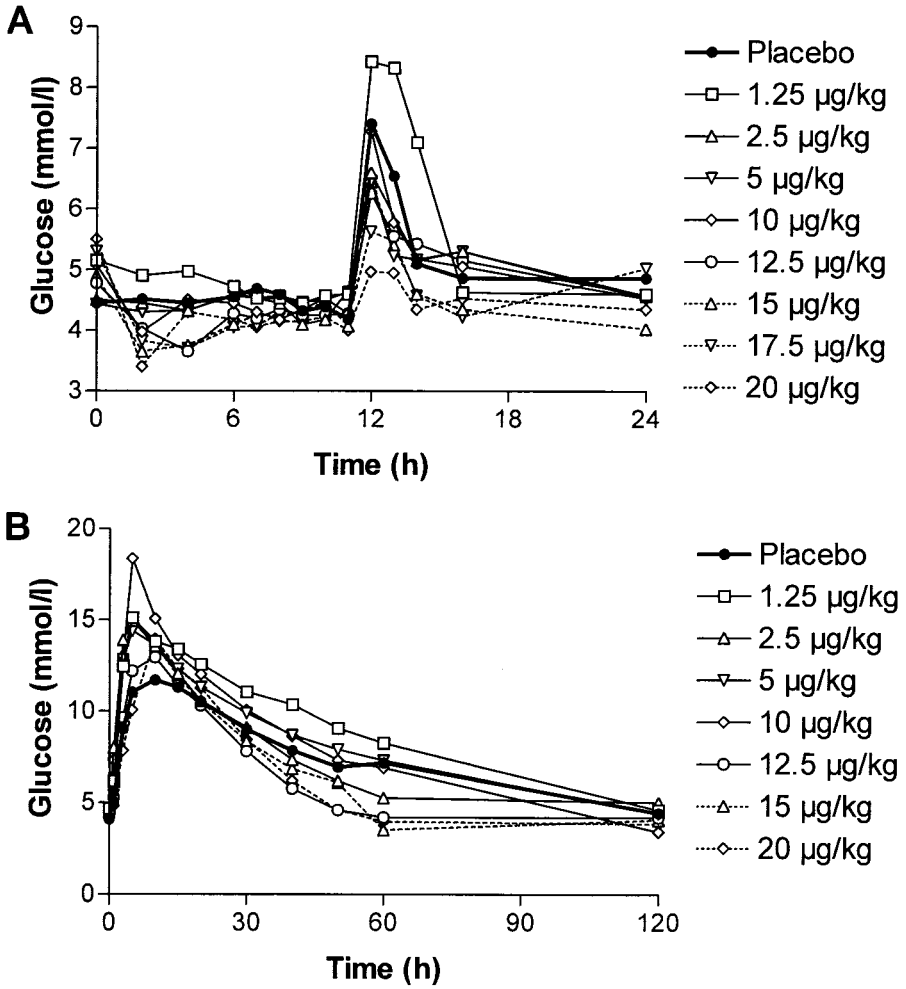

Figure 3—A: *Mean concentration time profiles of glucose after NN2211/placebo administration to healthy male subjects (subjects randomized to the IVGTT have not been included in the figure) (*n = *4 in all dose groups except the placebo, where* n = *8, and at the 5.0 µg/kg dose level, where* n = *3).* B: *IVGTT profiles of glucose after NN2211/placebo administration to healthy male subjects (*n = *2 in all dose groups except the placebo, where* n = *7, and at the 5.0 µg/kg dose level, where* n = *1).*

treatment. Concentration time profiles for insulin, glucose, and glucagon during the IVGTT (9–11 h after dose) are shown in Figs. 2*B*, 3*B*, and 4*B*, respectively. Furthermore, the derived $AUC_{9-10\ h}$/1 h and $AUC_{9-11\ h}$/2 h are shown in Table 2, i.e., corresponding to the first hour of the IVGTT and the complete 2-h IVGTT. Although there was a tendency toward higher glucose levels within the first 5 min after the initiation of the IVGTT in the actively treated subjects, a lowering of glucose occurred from 30 to 120 min in the higher-dose groups. There was a clear dose-dependent increase in insulin secretion during the IVGTT, but there was no effect on glucagon levels. Statistical analysis showed a significant increase in insulin levels after NN2211 administration during the IVGTT ($AUC_{9-11\ h}$) (P = 0.0002; mean 2.29 [95% CI 1.80–2.92]) but no significant effect on glucose (0.97 [0.82–1.14]) or glucagon (1.13 [0.89–1.42]). Statistical analysis of the $AUC_{9-10\ h}$ during the IVGTT showed a similar pattern to that for the complete IVGTT. Thus, there was a statistically significant increase in insulin levels in the NN2211-treated group compared with the placebo group (P = 0.0002; 2.74 [1.75–4.28]), whereas no significant effect on glucose (1.05 [0.89–1.23]) or glucagon (1.06 [0.87–1.29]) was observed. The effect of NN2211 on intravenous glucose tolerance, as assessed by K_g up to 1 h after glucose administration, is shown in Table 2. There was a clear and significant dose-response relationship (P = 0.0078; slope estimate 0.0713 ± 0.0238 [mean ± SE]), such that increasing doses of NN2211 increased the K_g value. However, the statistical analysis did not show a significant overall difference between NN2211 and placebo treatment (P = 0.16; 0.58 [−0.26 to 1.42]), indicating that NN2211 had an effect on K_g at higher, but not at lower, doses.

CONCLUSIONS — The observations in this study of an increase in gastrointestinal side effects and dizziness after NN2211 administration are in accordance with previously published observations after both GLP-1(7-36) amide infusion and NN2211 administration to healthy male subjects and patients with type 2 diabetes (3,9,21,22). The mechanism behind the occurrence of nausea and vomiting is most likely mediated via inhibition of gastric emptying (11,12,23,24).

Native GLP-1 is a polypeptide that undergoes rapid metabolism by DPP-IV and, in addition, is rapidly cleared by renal elimination (1). The half-life after intravenous or subcutaneous administration in humans has been reported to be ~5 min (1) and 1 h (2), respectively. Findings in this study show an elimination half-life of 8.1 h after intravenous administration of NN2211. This increase in the half-life of NN2211 compared with native GLP-1 is most likely mediated via a combination of factors. Notably, NN2211 has been shown to have a lower susceptibility to metabolism by DPP-IV (25) and a high degree of albumin binding of NN2211 (as has been shown for other fatty acid derivatives [14,15]), and after subcutaneous administration, an additional prolongation of the half-life is mediated by a slow absorption of NN2211 from the injection site, as evidenced by the further increase in half-life observed from subcutaneous to intravenous administration. Thus, the pharmacokinetic terminal half-life of NN2211 after the subcutaneous administration observed in this study is in accordance with previous findings in pigs, where the half-life was found to be 14 h (17), and studies with NN2211 in humans (21,22) and strongly supports once-daily administration of NN2211. In addition to improvements in compliance and convenience to the patients by the once-daily formulation, an increase in exposure from 16 to 24 h of native GLP-1 showed further improve-

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