CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-341

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

Office of Clinical Pharmacology

Memo to File

NDA	22-341
Submission Date	05/23/08
Drug	Victoza (Liraglutide) 6 mg/mL
Memo Date	September 29, 2009
Reviewer	Manoj Khurana, Ph.D.
Team Leader	Sally Choe, Ph.D.
Sponsor	Novo Nordisk
Subject	Clinical Site Deficiencies cited by The Division of Scientific Investigations (DSI) on the Pivotal Bioequivalence Trial.

Background Information:

The Division of Scientific Investigations (DSI) audited and issued the Form 483s to the analytical and clinical sites of the following pivotal bioequivalence trial, IMP NN2211-1692: "*A Randomized, Double-Blind, Single-Center, Two-Period, Cross-Over Trial in Healthy Subjects Investigating the Bioequivalence Between the Phase 3a Formulating of Liraglutide (Formulation 4) and the Planned Phase 3b Formulation (Final Formulation 4)*". The response to analytical site deficiencies of the trial was found acceptable by both Office of Clinical Pharmacology (see QBR Memo Dated 07/10/09) and DSI (See review dated 08/31/09 in DAARTS) reviewers.

DSI Findings:

DSI mentioned in their review (dated 02/25/2009) that following the inspection of the clinical site, Lund University Hospital, Lund, Sweden (January 26 - 29, 2009), a 2-item Form 483 was issued. DSI reported their evaluation of the significant findings, reported in form 483 and the clinical site's response letter (February 18, 2009) to the deficiencies, as follows:

"1) Failure of the clinical site to maintain the blinding code to identify that the study formulations administered to the individual subjects followed the randomization code. The site was unable to provide assurance that the individual test articles administered to subjects or retained for reserve samples contained a specific formulation.

2) Source data were not signed and dated by the individual collecting the data. For example; collection time points for the pharmacokinetic blood samples were not attributable to either of the two study staff present at the time of collection, corrections to the raw data in 3 of 4 occurrences were performed approximately 3 months after the date of collection and cannot be verified, and those corrections were not performed by the staff present during the collection."

The Sponsor's Response:
1) The firm's response letter stated that the sealed codes were handled according to the ICH GCP guideline, section 8.4.6. However, in DSI reviewer's opinion this was not in accordance with Agency's Final Rule and mentioned that the "*Guidance for Industry: Handling and Retention of BA and BE Testing Samples*" clearly addresses this issue. DSI also noted that during the inspection, the sponsor emailed a document, which meant to represent the blinding code, but this document was generated on 05/22/07, which is after the conduct of the study. There was no assurance that this code was identical to that provided to the site at the time of randomization for the trial. Therefore, this document does not assure the identity of the drug products administered to subjects or the reserve samples.
2) The firm's response letter stated they agreed that the design of the Case Report Forms (CRFs) used to capture the source data was not optimal, as it did not allow the staff collecting the samples to date and sign the collection time. However, they had created a schedule for blood collection time points and that the staff had been instructed to follow that schedule. They stated that the investigator and research coordinator, who had not performed the blood collection task, had evaluated and corrected the source data after receiving a Date Correction Form (DCF) from the sponsor monitor. No corrective actions were purposed by the firm for the significant observation.

Reviewer's Comments and Conclusions:
Based on the review of the responses provided by the sponsor and DSI's review, the following were noted:

- The site's handling of codes was in accordance to the ICH guidance document E6 item 8.4.6 that states that randomization codes should be retained by the Sponsor after the study ends. However, the sponsor failed in appropriately directing the site to ensure compliance with the FDA guidance and later did not provide adequate responses to the DSI during inspection.
- Even though the randomization codes were not handled as per the FDA guidance, this reviewer considers that site did demonstrate adherence to the ICH guidance document E6: Good Clinical Practice item 8.4.6, and considers it to be rather a procedural failure at the sponsor's part. The trial results are acceptable based on this fact and the totality of study results.

Recommendation:
The Office of Clinical Pharmacology/Division of Clinical Pharmacology-II (OCP/DCP-II) has reviewed the results of the Division of Scientific Investigations (DSI) audit conducted on a pivotal bioequivalence trial for NDA 22-341 (liraglutide) and found the trial results to be acceptable.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22341	ORIG-1	NOVO NORDISK INC	VICTOZA (LIRAGLUTIDE)

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/s/

MANOJ KHURANA
11/04/2009

SALLY Y CHOE
11/05/2009

Office of Clinical Pharmacology

Memo to File

NDA	22-341
Submission Date	05/23/08
Brand Name	Victoza (Liraglutide) 6 mg/mL
Memo Date	July 10, 2009
Clinical Pharmacology Reviewer	Manoj Khurana, Ph.D.
Clinical Pharmacology Team Leader	Sally Choe, Ph.D.
Sponsor	Novo Nordisk
Subject	Sponsor's response to FDA letter (dated 04/17/09) requesting information on a summary of the pivotal bioequivalence and other clinical pharmacology studies including a by-study summary of the percentage of duplicate values in the analytical standards.

Background Information:
In a letter dated March 5, 2009, the Agency requested Novo Nordisk (hereafter referred as sponsor) for responses to deficiencies cited during the January 2009 analytical site inspections conducted at [redacted]. The sponsor provided their response in March 27, 2009 with revised clinical pharmacology assessments, which were incorporated in the final QBR. However, in another letter dated 04/17/09 Agency asked the sponsor for further clarification in this regard: b(4)

"In Agency's opinion, your approach of (1) accepting one of duplicate determinations when the mean of the duplicates are outside +/-30% and %CV >30% and excluding single determination that deviates most from the nominal value, and (2) exclusion of one of the duplicates for both calibrators and QCs due to technical errors (reported after generation of results), is not completely objective and may introduce bias. Please provide us your justification for using these criteria."

Additionally, to evaluate the impact of this approach on the overall clinical pharmacology program the following request was made:
"We also request you to provide us a summary for the pivotal BE and other clinical pharmacology studies, covered in the responses submitted on March 27th and March 30th, 2009, and which should include the following:

(a) A by-study summary of the percentage of duplicate values in the analytical standards (Calibration and QC) as well as test samples that fall into above mentioned criteria (i.e. where single values were reported instead of means) and the percent of analytical runs affected.

(b) Listing of subject IDs for each study for which these criteria were used."

Current Submission:
The sponsor provided their response in May 08, 2009 submission. The sponsor mentioned that for the review of raw data, they prospectively decided to consistently adhere to the assay run acceptance criteria set by ——— at the time of sample analysis. In addition, they supplemented the acceptance criteria with CV requirements for duplicate determinations, in order to be able to reject single determinations objectively. All analytical runs not meeting the criteria were rejected, unless a reason for the data evaluation was documented in the raw data. Thus, the approach of accepting one of duplicate calibrator determinations when the mean accuracy of the duplicates are outside +/-30% and CV >30%, and excluding the single determination that deviated most from the nominal value was adopted. b(4)

Further, the sponsor elaborated that "*The rationale for keeping calibrator points as single determinations, instead of excluding the calibrator level if the mean did not fulfill the criteria, relates to the fact that the calibration curve has been split and validated in two parts in the liraglutide bioanalytical assay performed at ——— . Thus, exclusion of central calibrator levels could affect the whole calibration curve. The rationale for keeping the single determination that is closest to the nominal value is that this value most likely will result in the best fit for the calibration curve.*" b(4

Sponsor also provided re-analysis results for the pivotal BE study analysis after excluding all runs accepted based on single determinations of calibrators or QCs (20% runs; 18% samples). The result of the updated analysis is presented in summary in Table 1.

Table 1–1 Comparison between Formulations (Final Formulation 4 / Formulation 4) – Primary Endpoints – Trial 1692

		Original Analysis Final Formulation 4 / Formulation 4	Updated Analysis Final Formulation 4 / Formulation 4
AUC(0-t)			
	Estimate	0.99	0.92
	Lower 90% limit	0.92	0.83
	Upper 90% limit	1.06	1.02
Cmax			
	Estimate	1.02	0.97
	Lower 90% limit	0.91	0.88
	Upper 90% limit	1.14	1.06

Based on the review of the responses provided by the sponsor, the following were noted: b(4)

- *In this reviewer's opinion, overall, the use of uniform criteria for data review is a better approach over the inconsistent approach followed by the ——— and the revised analysis did not impact the interpretation of clinical pharmacology studies. (See the Clinical Pharmacology NDA review in DAARTS dated 04/24/09).*
- *In this reviewer's opinion, the justification in support of retaining single values is not clear as to how the retention of central calibrator is related to the splitting of standard curve during validation, when the context is the calibration standards used during the analysis of study samples. If the objective of keeping a value*

close to nominal value is to get the best fit for the calibration curve, then this objective is not bias free. Though one can argue that this will not impact the back calculation of unknown concentrations in test samples, one should not deviate from the objective of calibration standards and QCs. The objective of calibration standards and QCs is to capture the analytical performance of assay on any given day using the known concentrations.

- *This reviewer agrees with the conclusion that the results of the pivotal BE study were not affected by the further exclusion of data based on single determinations in the calibration/QC standards. For other clinical pharmacology studies 6-17% of samples were affected by this issue and do not impact the interpretation of results.*

Recommendation:

Overall, from Clinical Pharmacology perspective, the sponsor still did not provide adequate justification regarding the bias-free nature of the approach they followed in retaining single determinations. However, we agree that the impact of this approach is minimal and does not affect the interpretation of clinical pharmacology data. No further action is recommended with respect to the data integrity issues.

Nevertheless, in future submissions, the sponsor is strongly advised to proactively undertake the responsibility of providing the assurance of analytical data integrity. Since the analytical data from one vendor supported the pivotal clinical pharmacology program in this submission, the sponsor should have been considerate of the associated impact of the vendor's performance on quality of the data submitted for regulatory approval. This recommendation should be sent to the sponsor as appropriate.

Linked Applications	Submission Type/Number	Sponsor Name	Drug Name / Subject
NDA 22341	ORIG 1		VICTOZA (LIRAGLUTIDE)
NDA 22341	ORIG 1		VICTOZA (LIRAGLUTIDE)

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/s/

MANOJ KHURANA
08/13/2009

SALLY Y CHOE
08/13/2009

CLINICAL PHARMACOLOGY REVIEW

NDA: 22-341	Submission Date(s): 05/30/2008
Brand Name	Victoza™
Generic Name	Liraglutide
Clinical Pharmacology Reviewer	Manoj Khurana, Ph.D.
Clinical Pharmacology Co-Reviewer	Ritesh Jain, Ph.D.
Clinical Pharmacology Team Leader (Acting)	Wei Qiu, Ph.D.
Primary Pharmacometrics Reviewer	Manoj Khurana, Ph.D.
Secondary Pharmacometrics Reviewer	Rajanikanth Madabushi, Ph.D.
Pharmacometrics Team Leader	Christoffer Tornoe, Ph.D.
OCP Division	Clinical Pharmacology -2
OND division	Metabolic and Endocrine Products
Sponsor	Novo-Nordisk
Submission Type; Code	NDA 505(b)(1); Standard
Formulation; Strength(s)	Solution for SC Injection
Proposed Indication	An adjunct therapy to diet and exercise to improve glycemic control in subjects with type 2 diabetes.

List of Figures and Tables

1 Executive Summary

1.1 Recommendation

The Office of Clinical Pharmacology/Division of Clinical Pharmacology 2 (OCP/DCP-2) has reviewed the clinical pharmacology data submitted in support of NDA 22-341 for liraglutide and found it acceptable, pending an acceptable resolution of the deficiencies found in the Division of Scientific Investigation with regards to the bio-analytical method.

Required Office Level OCP briefing was held on 25th March, 2009. Attendees included Dr. Chandrahas Sahajwalla, Dr. Isam Zineh, Dr. Kellie S Reynolds, Dr. Nam Atiqur Rahman, Dr. Hae Young Ahn, Dr. Sally Y Choe, , Dr. Christoffer Tornoe, Dr. Rajanikanth Madabushi, Dr. Partha Roy from Office of Clinical Pharmacology and Dr. Mary H Parks, Dr. Hylton Joffe from Office of New Drugs.

1.2 Phase IV Commitments

None

1.3 Summary of Important Clinical Pharmacology Findings

Novo Nordisk is seeking an approval of Victoza™ (Liraglutide) for the indication of improving glycemic control in patients with type 2 diabetes mellitus (T2DM). Liraglutide is intended as an adjunct to diet and exercise to achieve glycaemic control in T2DM patients. Liraglutide is developed for once-daily administration as:

- Monotherapy
- Combination therapy with one or more oral antidiabetic drugs (metformin, sulphonylureas or a thiazolidinedione) when previous therapy does not achieve adequate glycaemic control.

Liraglutide is a human Glucagon-Like Peptide-1 (GLP-1) analog with 97% homology to human GLP-1 that binds to and activates the GLP-1 receptor. The GLP-1 receptor is the target for native GLP-1, which is an endogenous incretin hormone that potentiates the glucose-dependent insulin secretion from the pancreatic beta cells. Unlike GLP-1, liraglutide has a pharmacokinetic and pharmacodynamic profile in human suitable for once daily administration. Following subcutaneous administration, the protracted action profile is based on three mechanisms: self-association, which results in slow absorption, and binding to albumin and enzymatic stability towards the DPP-IV enzyme both resulting in a long plasma half-life.

The liraglutide formulation is a clear, colorless solution (6 mg/mL) for subcutaneous injection, provided in a multi-dose, disposable pre-filled pen. The proposed dosing regimen is that liraglutide is administered once daily at any time, independent of meals, and can be injected subcutaneously in the abdomen, in the thigh or in the upper arm. The injection site and timing can be changed without dose adjustment. For all patients liraglutide should be initiated with a dose of 0.6 mg for at least one week, after which the dose should be increased to 1.2 mg. Based on clinical response and after at least one week the dose can be increased to 1.8 mg to achieve maximum efficacy. No dose adjustment is recommended by the sponsor either based on age, race, body weight and body mass index, or for elderly subjects, subjects with renal impairment, and subjects with hepatic impairment.

Overall, the liraglutide clinical development program comprised 38 completed trials that were conducted world-wide, with the majority being conducted in Europe. The therapeutic confirmatory trial program investigated the benefits of liraglutide as a:

- monotherapy (Trial 1573)
- combination with metformin (Trial 1572)
- combination with an SU (glimepiride) (Trial 1436)
- combination with a TZD (rosiglitazone) and metformin (Trial 1574)
- combination with an SU (glimepiride) and metformin (Trial 1697)

The five long-term therapeutic confirmatory trials were all randomized, double-blind, double-dummy (including liraglutide and/or OAD placebo) trials, providing long-term efficacy and safety data.

The clinical pharmacology program performed to evaluate the pharmacokinetic and pharmacodynamic properties of liraglutide included 26 clinical pharmacology trials. These comprised 19 trials in healthy subjects (including bioequivalence trials, trials in elderly subjects, subjects with renal or hepatic impairment and Japanese subjects) and 7 trials in subjects with type 2 diabetes (including one trial in Japanese subjects). The program was supported by evidence from 5 phase 2 trials, a population pharmacokinetic analysis from the therapeutic confirmatory Trial 1573 and from 10 *in vitro* studies performed with human biomaterials, i.e. cells, recombinant enzymes, plasma or plasma proteins.

The 14 week Phase 2 monotherapy trial evaluated effect of 0.65 mg, 1.25 mg, and 1.9 mg once daily subcutaneous administration on lowering of fasting plasma glucose and glycosylated hemoglobin (HbA1c), the primary surrogate efficacy endpoint for anti-diabetic treatment. The Phase 2 exposure-response data demonstrated that the two doses, 1.25 mg and 1.9 mg, achieved maximal reduction in HbA1c from baseline, and 0.65 mg appeared to be close to the ED50. However, to improve the tolerability profile with regards to gastro-intestinal adverse events, sponsor evaluated the three doses (0.6 mg, 1.2 mg and 1.8 mg) in the Phase 3 monotherapy trial using titration with the 0.6 mg as the lowest starting dose. Overall, treatment with liraglutide (as monotherapy as well as in combination treatment) resulted in a substantial and clinically relevant lowering of HbA1c. Treatment with liraglutide consistently reduced HbA1c more than placebo and to at least the same extent as comparator treatment. In most cases liraglutide treatment was also superior to the glucose lowering effect of the comparator treatments. The estimated mean decrease from baseline in HbA1c after treatment with liraglutide ranged from 0.60% points (liraglutide 0.6 mg, Trial 1436) to 1.48% points (liraglutide 1.2 and 1.8 mg, Trial 1574). Please refer to the clinical and pharmacometric reviews for more detail on liraglutide safety and efficacy.

Liraglutide is metabolized by DPP-IV (dipeptidyl peptidase-IV) and NEPs (neutral endopeptidases) that are present ubiquitously in the body, and hence the elimination is not organ specific. Primary component in the systemic circulation was unchanged peptide; liraglutide (89-100%). In plasma two other components were detected that were slightly more lipophilic and represented $\leq$9% and $\leq$5% (respectively) of the total exposure (2-24 h). No unchanged liraglutide was detected in urine or feces. The three metabolites in urine were detected and accounted for around 3% of the administered radioactivity. The three metabolites detected in feces comprised 3-5% of the administered radioactivity. The structure of these metabolites or peptide fragments is not characterized.

For the clinical pharmacology assessments, liraglutide was quantitated in plasma and other biomatrices using ELISA assay. The liraglutide assay was validated for analyzing liraglutide in

plasma and serum samples in terms of recovery, linearity, accuracy, precision and sensitivity. The storage stability was demonstrated for a maximum period of two years with no decrease in the measured liraglutide concentrations. Antibodies against GLP-1 were shown to interfere with the ELISA assay. However, sponsor mentioned that only very few subjects in the phase 2 and 3 trials had GLP-1/liraglutide binding antibodies, and the antibody levels in these subjects were low, suggesting that bias due to interference from antibodies is limited.

PK in healthy subjects and T2DM patients: The mean liraglutide apparent clearance was 0.7 L/hr and apparent volume of distribution was 12.5 L after a single subcutaneous dose of 0.7 mg. The maximum concentrations were achieved at 12 hr median t_{max} and liraglutide eliminated with a half-life of 13 hr, thus suggesting that liraglutide follows a flip-flop pharmacokinetics after subcutaneous administration. The dose-proportionality assessment revealed that the liraglutide exposure increased in proportion to the increase in dose up to 20 μg/kg (equivalent to 1.8 mg dose based on 90 kg median weight in Phase 3 trial). There was slight accumulation (R_A of 1.4-1.5) after multiple once daily subcutaneous administrations. On average the absolute bioavailability of liraglutide is around 55% following subcutaneous administration.

Pharmacodynamics: The pharmacodynamic effects of liraglutide on glucodynamics were also demonstrated. The liraglutide administration resulted in increased insulin secretion in response to glucose. There was a significant reduction in post-prandial glucose over 24 hour period, slight increase in post-prandial insulin and significant post-prandial glucagon suppression. There was also a substantial increase in the first phase insulin secretion as assessed during a hyperglycemic clamp.

QT/QTc: No significant QT prolongation effect of liraglutide (Once daily s.c. doses of 1.8 mg, titrated in weekly steps of 0.6 mg) was detected in the thorough QT study. The largest upper bounds of the 2-sided 90% CI for the mean difference between liraglutide (1.8 mg and 1.2 mg) and placebo were below 10 ms (2.7 ms and 0.9 ms), the threshold for regulatory concern as described in ICH E14 guidance.

Body weight, Age, BMI, Gender and Race: Liraglutide AUC_{0-t} was declared equivalent in young and elderly subjects after a single 1 mg dose. There appeared to be a difference between male and female subjects based on the time-concentration profiles and the corresponding derived parameters. However, when adjusting for body weight there were no statistically significant differences between male and female subjects in this study. The effect of various covariates e.g. Weight, Age, BMI, Gender and Race was assessed in the population pharmacokinetic analysis. Weight was found to be a significant predictor of CL/F of liraglutide. There was no effect of age or BMI on liraglutide clearance. Based on the weight adjusted clearance, the females were found to have 34% lower weight adjusted clearance than the males. However, after accounting for Weight and Gender effects, the Race effect could not be ascertained as claimed by the sponsor. Although both weight and gender were found to affect the clearance, the effect appears to be only statistically important. Considering that steady state exposures (Cavg), resulting from 1.2 and 1.8 mg doses, were in the maximal response region of the exposure-relationship for primary efficacy variable (HbA1c), these differences are not clinically meaningful to warrant a dose-adjustment.

Renal and Hepatic Impairment: No dose adjustment is proposed for renal and hepatic impairment subjects. Overall on average, the $AUC_{0-\infty}$ of liraglutide was around 19 - 35% lower in the renally impaired subjects than the normal subjects. Total apparent clearance (CL/F) varied slightly across the renal groups; however, no trend with respect to renal function was seen. However, severe hepatic impairment has an impact on the liraglutide pharmacokinetics in terms of around two-fold increase in clearance and 42% lower mean $AUC_{0-\infty}$ of liraglutide. The exposure-

response relationship seen in the efficacy studies suggest that the proposed doses are adequate in the type 2 diabetic subjects who also have severe hepatic impairment.

Relative Bioavailablity from Different Injection Sites: The relative bioavailability of liraglutide after subcutaneous administration was estimated as 78% in thigh versus abdomen, 87% in upper arm versus abdomen and 110% in upper arm versus thigh based on primary parameter $AUC_{0-\infty}$ as well as C_{max} and AUC_{0-t}. There was no effect on tmax. Even if thigh showed consistently lower exposures of liraglutide, the magnitude does not appear to be clinically meaningful. Based on these findings liraglutide can be administered interchangeably at these injection sites.

Drug-drug Interactions: Several drug interactions were evaluated by the sponsor with an objective to establish the effect of liraglutide on gastro-intestinal motility and how does it impact the pharmacokinetic profile of other drugs, especially those sensitive to these physiological changes. Paracetamol (BCS Class I), Atorvastatin (Class II), Griseofulvin (Class II), Lisinopril (Class III), and Digoxin (Class IV) showed the changes expected for these BCS class representatives based on reduction in gastric emptying rate due to liraglutide. The results from digoxin DDI study also showed that liraglutide does not prolong the intestinal transit time. Drug interaction study with single dose administration of ethinylestradiol and levonorgestrel combination, showed no effect of co-administration with liraglutide on ethinylestradiol total exposure ($AUC_{0-\infty}$). However, the levonorgestrel $AUC_{0-\infty}$ was 18% higher during liraglutide treatment. C_{max} was 12% and 13% lower for ethinylestradiol and levonorgestrel, respectively, during liraglutide treatment.

Co-administered Drug	Time of Administration Relative To Liraglutide	Cmax	AUC
Atorvastatin (40 mg)	After 5 hr	**↓38 %**	↔
Digoxin (1 mg)	After 7 hr	**↓31 %**	**↓16 %**
Lisinopril (20 mg)	After 5 minutes	**↓27 %**	**↓15 %**
Griseofulvin (500 mg)	Co-administered	**↑37 %**	↔
Ethinylestradiol (0.03 mg)	After 7 hr	↓12%	↔
Levonorgestrel (0.15 mg)	After 7 hr	↓13%	18%↑

↔ No change

To-be-marketed vs. Phase 3 formulation: The pivotal BE study demonstrated that the Phase 3 formulation (Liraglutide formulation 4) and to be marketed formulation (liraglutide final formulation 4) were bioequivalent with respect to the primary endpoints AUC_{0-t} and C_{max}. The bioequivalence were also demonstrated for intermediate changes in the formulations in separate studies.

Bioanalytical Issues: The bioanalytical site for the pivotal bioequivalence study (Study NN2211-1692) was audited by the Division of Scientific Investigation (DSI). There were serious deficiencies identified during the audit, and based on the conclusions of DSI review, the reliability of analytical data and hence the study results became uncertain as the laboratory need to re-assess the analytical data using an unbiased acceptance and rejection criteria for all analytical runs. The DSI also cited serious deficiencies during the inspection of clinical site in

Lund University Hospital, Lund, Sweden. Two separate information requests were issued to the sponsor for resolution of these issue for the BE evaluation. Division also communicated its concern to the sponsor regarding the other clinical pharmacology studies for which, the same laboratory has performed liraglutide assay. Please see DSI memos dated 02/18/2009 and 03/09/09 in DFS for further details.
Sponsor submitted their response to the information request letters (see 03/27/2009 and 03/30/2009 in EDR), and provided reanalysis of pivotal BE results and other clinical pharmacology study results that were used to make labeling claims and for which [redacted] conducted the bioanalysis. b(4)

Prior to the review of the raw data, Sponsor decided to consistently adhere to the assay run acceptance criteria set by [redacted] at the time of sample analysis. All assay runs not meeting the pre-set uniform criteria were rejected.

Thus, the sponsor re-assessed the raw data based on a standard uniformly applied run acceptance criterion for the calibration and QCs, and reanalyzed the reduced data sets for major clinical pharmacology studies except the population PK analysis where, only 6% of samples were affected by the bioanalytical inconsistencies, and we agree that it will not impact the study results. From a Clinical Pharmacology perspective, the sponsor's response to information request has not completely addressed the issues raised due to the deficiencies related to the bioanalytical method. The revised standard criteria used by the sponsor for re-evaluation of the analytical data was not objective and bias free when selecting single observations instead of mean values of the duplicate values for the ELISA runs. The impact of this is unknown and additional information has been requested from sponsor. Therefore, based on the review of original and revised information, overall the clinical pharmacology assessments conducted under this NDA are considered appropriate and acceptable provided there is an acceptable resolution of the deficiencies in the bioanalytical methods as explained above.

2 Question-Based Review (QBR)

2.1 General Attributes

Liraglutide is a human Glucagon-Like Peptide-1 (GLP-1) analog with 97% homology to human GLP-1 that binds to and activates the GLP-1 receptor. The GLP-1 receptor is the target for native GLP-1, an endogenous incretin hormone that potentiates glucose-dependent insulin secretion from the pancreatic beta cells.

The incretin based approaches for the management of type 2 diabetes are based on the current understanding that this function is impaired in type 2 diabetes. Treatment with GLP-1 can help to compensate for this defect as GLP-1 has been shown to reduce hyperglycaemia in subjects with type 2 diabetes. Studies with native GLP-1 have shown that the primary mechanisms of action are to:

- stimulate insulin secretion and decrease glucagon secretion in a physiological and glucose dependent manner
- delay gastric emptying
- reduce appetite

These properties make GLP-1 a suitable candidate for the treatment of type 2 diabetes. However, due to the very short half-life of native GLP-1 ($t_{1/2}$ <1.5 minutes after i.v. administration) and short duration of action, the native hormone is not a useful therapeutic agent. The short half-life is due to rapid degradation by dipeptidyl peptidase-IV (DPP-IV).

Following subcutaneous administration, the prolonged action profile of liraglutide is based on three mechanisms: self-association, which results in slow absorption, and binding to albumin and enzymatic stability towards the DPP-IV enzyme both resulting in a long plasma half-life in contrast to the short half-life of the endogenous GLP-1.

Physiologically, GLP-1 is processed from the pre-proglucagon gene in the L-cells of the intestine as well as in the brain. The amino acid sequence of GLP-1 is preserved in mammals and only one receptor, the GLP-1 receptor, has been identified. The GLP-1 receptor is a so-called G-protein coupled receptor belonging to the B family. There is close homology between the GLP-1 receptor in different mammalian species, with rat and human GLP-1 receptor having as high as 90% homology and monkey and human 99%. The cellular action of GLP-1 is mediated through the Gs protein and the adenylate cyclase (Figure 1) leading to cAMP accumulation, and in pancreatic beta-cells to a subsequent activation of PKA and increase in intracellular cytosolic Ca2+ and PI3-kinase leading to exocytosis of insulin-containing granules and activation of mitogenic pathways.

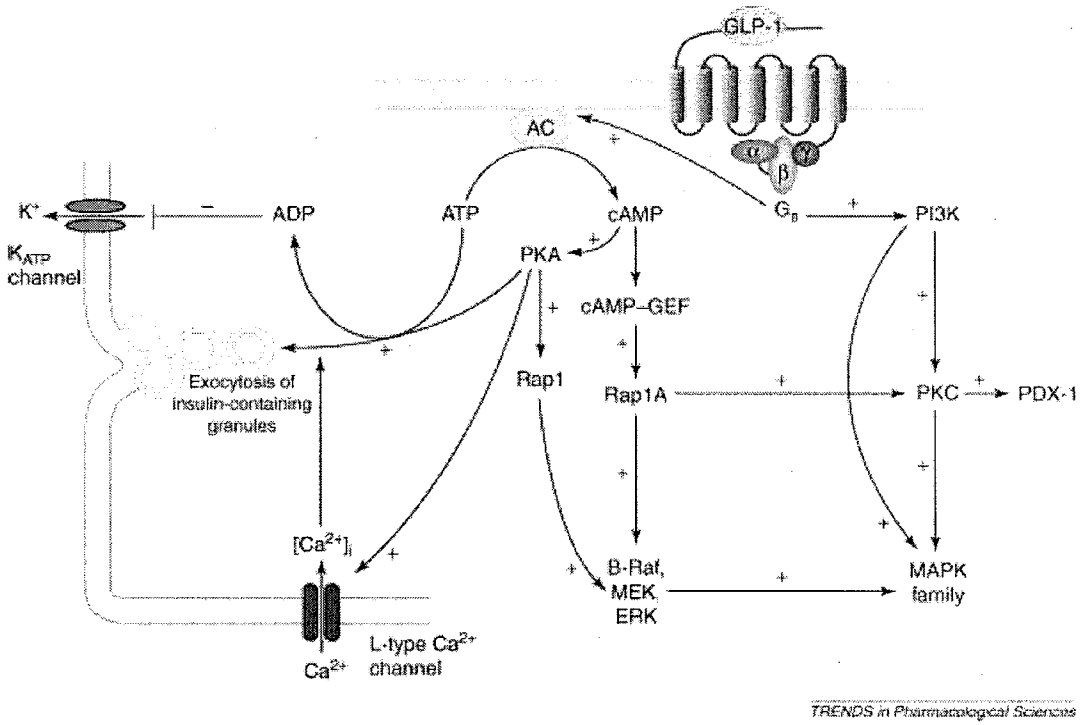


Figure 1 GLP-1 receptor activated signaling pathways in pancreatic beta-cells.

Liraglutide's mechanism of action, being a GLP-1 analog, is also proposed to be multifocal and mediated via a specific interaction with GLP-1 receptors, leading to an increase in cAMP, stimulation of insulin secretion, improvement in beta-cell function in a glucose dependent manner, lowering of inappropriately high glucagon secretion, also in a glucose dependent manner. Furthermore, the mechanism of blood glucose lowering is also believed to involve a minor delay in gastric emptying and effects like reduced hunger and lowered energy intake.

Proposed indications for liraglutide are as an adjunct to diet and exercise to achieve glycaemic control in patients with type 2 diabetes mellitus. Liraglutide is developed for once-daily administration as:

- Monotherapy
- Combination therapy with one or more oral antidiabetic drugs (metformin, sulphonylureas or a thiazolidinedione) when previous therapy does not achieve adequate glycaemic control.

Liraglutide is proposed to be administered once daily at any time, independent of meals, and can be injected subcutaneously in the abdomen, in the thigh or in the upper arm. The injection site and timing can be changed without dose adjustment. For all patients liraglutide should be initiated with a dose of 0.6 mg for at least one week, after which the dose should be increased to 1.2 mg. Based on clinical response and after at least one week the dose can be increased to 1.8 mg to achieve maximum efficacy.

2.1.1 What are the highlights of the Liraglutide drug product as they relate to clinical pharmacology review?

Liraglutide is an Arg^{34}-GLP-1 analogue substituted on the ε-amino group of the lysine in position 26 with a Glu-spaced palmitic acid. The structural formula is $Arg^{34}Lys^{26}$-(N-ε-γ-Glu (N-α-hexadecanoyl)))-GLP-1[7-37]. The molecular formula of liraglutide is $C_{172}H_{265}N_{43}O_{51}$. The theoretical molecular mass of liraglutide is 3751.20 atomic mass units. The analogue is produced as the polypeptide precursor by r-DNA technology with *Saccharomyces cerevisiae* strain YES2085 as the production strain. Substitution with the side chain is performed during down-stream processing. Liraglutide has the chemical structure illustrated in Figure 2 below:

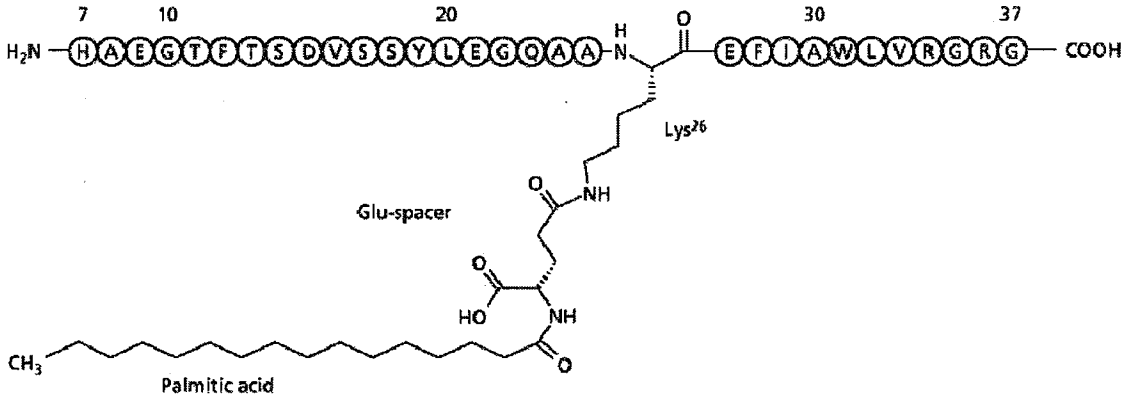


Figure 2 Chemical Structure of liraglutide.

The proposed drug product is a clear colorless solution for injection containing 6.0 mg/ml of the active ingredient liraglutide. Liraglutide 6.0 mg/ml will be marketed as a pre-filled pen in the following presentations:

b(4)

2.1.2 What is the composition of to-be-marketed formulation of Liraglutide?

Liraglutide 6.0 mg/ml, 3 ml cartridge is a clear colorless solution containing liraglutide in a 3 ml cartridge. The pH of the product is 8.15. The composition of liraglutide 6.0 mg/ml, 3 ml cartridge is listed in Table 1 below.

Table 1 Quantitative Composition of To-Be-Marketed Liraglutide 6.0 mg/mL, 3 mL Cartridge

Name of ingredients	Quantity per mL	Function	Reference to standards
Active substance			
Liraglutide	6.0 mg	Active drug substance	Novo Nordisk A/S
Excipients			
Disodium phosphate, dihydrate	1.42 mg	[redacted]	Ph. Eur., USP
Phenol	5.5 mg[1]	[redacted]	Ph. Eur., USP, JP
Propylene glycol	14.0 mg	[redacted]	Ph. Eur., USP, JP
[redacted]			Ph. Eur., USP, JP
			Ph. Eur., USP, JP
Water for Injections	To make [redacted]	Solvent	Ph. Eur., USP, JP

1. [redacted]

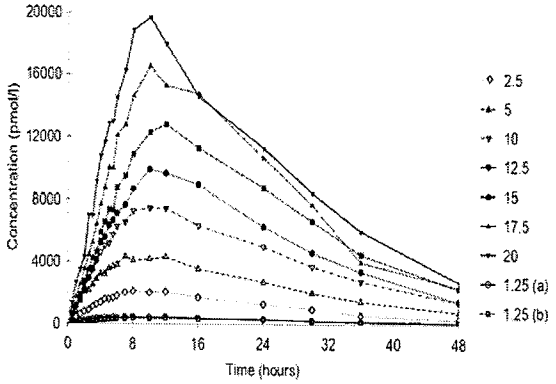
b(4)

2.2 General Clinical Pharmacology

2.2.1 What are the PK characteristics of liraglutide after subcutaneous administration and how do they relate to the dose?

Single Dose:
Study NN2211-1149, a randomized, double-blind, placebo-controlled, dose escalation trial of single doses of liraglutide (NNC 90-1170) evaluated the tolerability, pharmacokinetics, pharmacodynamics and absolute bioavailability in healthy male subjects. Upon s.c. administration, the liraglutide was slowly absorbed into the systemic circulation and maximum concentrations were achieved between 9-12 hr. Across these dose groups, the elimination half-life ranged from 11 to 15 hr. The mean plasma concentration-time profiles of liraglutide are illustrated in the Figure 3 below.

(a) Concentration-time plot (Linear Scale)



(b) Concentration-time plot (Semi-log Scale)

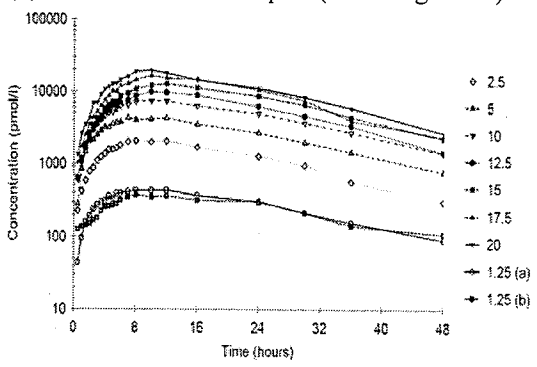


Figure 3 Mean plasma concentration time profile of liraglutide after single rising s.c. doses (1.25 to 20 μg/kg)

The mean and SD of pharmacokinetic parameters for liraglutide are presented in Table 2 below.

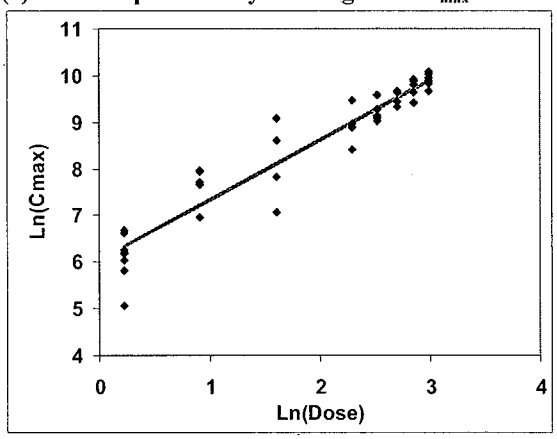
Table 2 Pharmacokinetic parameters of liraglutide after single rising subcutaneous doses from 1.25 to 20 μg/kg.

Dose		AUC0-∞	AUC0-t	F	Cmax	tmax	$t_{1/2}$
μg/kg		pmol*h/L	pmol*h/L	%	pg/mL	h	h
1.25a	Mean	14501	12503	-	451	9.2	14
	SD	3205	2361	-	73	1.8	-
1.25b	Mean	16058	10095	-	390	10	13
	SD	8702	9091	-	329	2.2	-
2.5	Mean	60155	54169	-	2303	10	11
	SD	29012	22949	-	696	3.4	-
5	Mean	134076	117244	55	4478	9.3	15
	SD	108073	93978	37	3412	3.2	-
10	Mean	234834	206160	-	7911	12	14
	SD	33386	33545	-	2750	6	-
12.5	Mean	283376	258268	-	10102	11	12
	SD	30570	32138	-	2250	2.4	-
15	Mean	383629	339763	-	13136	11	13
	SD	56975	45773	-	1973	1.1	-
17.5	Mean	450776	411611	-	17253	11	11
	SD	64623	58426	-	3044	1	-
20	Mean	523224	477591	-	20209	10	11
	SD	104999	80834	-	2981	1.3	-

[b]First dose level was repeated for safety assessment.

The dose-proportionality assessment revealed that the liraglutide exposure increased in proportion to the increase in dose up to 20 μg/kg.

(a) Dose Proportionality in Liraglutide C_{max}



(b) Dose Proportionality in Liraglutide AUC_{0-inf}

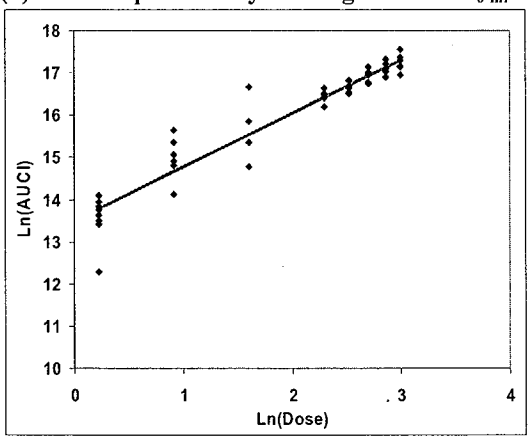


Figure 4 Dose Proportional Increase in Liraglutide C_{max} and AUC_{0-inf}

Pharmacokinetic profile from the to-be-marketed (Final formulation 4) and Phase 3 formulation (Formulation 4) was also similar to those observed in the early clinical evaluations. The

pharmacokinetic parameters of liraglutide after single s.c. administration of 0.7 mg dose are summarized in Table 3, and the concentration-time profile is shown in Fig. 5 below:

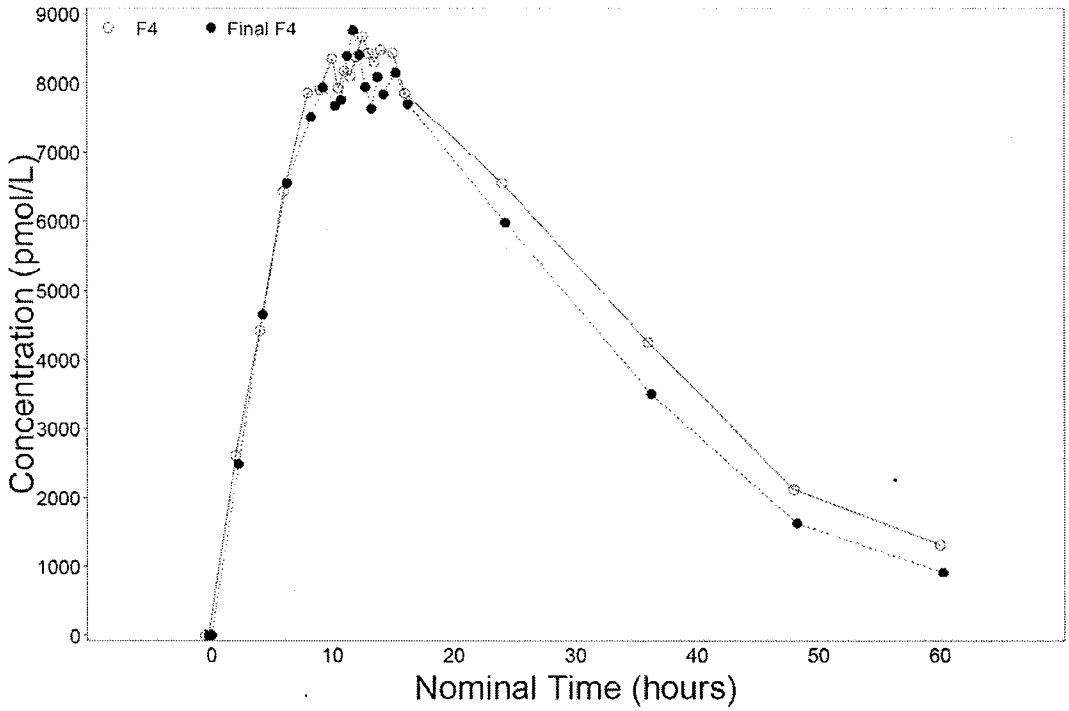


Figure 5 Plasma concentration-time profile of liraglutide after oral administration.

Table 3 Pharmacokinetic parameters of liraglutide after single 0.7 mg s.c. dose in pivotal BE study using Formulation 4 (Phase 3) and Final formulation 4 (to-be-marketed)

Cmax:

	Original Analysis		Updated Analysis	
	Final F4	F4	Final F4	F4
N	21	21	13	12
Mean	10365	9771	9333	9682
SD	4320	1671	1677	1351
Median	9378	9960	9158	9910
GeoMean	9799	9630	9201	9593
Min				
Max				

AUC:

b(4)

	Original Analysis		Updated Analysis	
	Final F4	F4	Final F4	F4
N	21	21	12	11
Mean	271304	271945	256057	265692
SD	59415	44960	54223	50463
Median	243829	280024	240358	249830
GeoMean	265463	269474	251192	261851
Min			180120	221780
Max				

The mean liraglutide apparent clearance was 0.7 L/hr and apparent volume of distribution was 12.5 L. The maximum concentrations were achieved at 12 hr median t_{max} and liraglutide eliminated with a mean half-life of 13 hr, which was longer than the 8 hr half-life observed after i.v. dose, thus suggesting that liraglutide follows a flip-flop pharmacokinetics after s.c. administration.

Multiple Once Daily Doses:
Mean plasma concentrations increased with an increase in dose both following single (Day 1) and multiple (Day 11) once daily administration of liraglutide. The results suggested a slight accumulation of liraglutide following multiple dose once daily s.c. administration, as indicated by the accumulation index that ranged betwen1.4 – 1.5 based on ratio of AUC_{0-24}.on Day 11 and Day 1.

Table 4 Mean liraglutide pharmacokinetic parameters after single dose (on Day 1) and multiple once daily s.c. dose

Dose		Day 1		Day 11[a]	
(µg/kg)	Parameter	Mean	SD	Mean	SD
7.5[#]	AUC (h·nmol/l)	149	22	216	54
	AUC_{0-24} (h·nmol/l)	86	32	138	32
	$AUC_{0-24,day11}/AUC_{day1}$	-	-	0.9	0.1
	R_{ac}	-	-	1.4	0.3
	C_{max} (nmol/l)	6	3	8	2
	t_{max} (h)	14	7	10	0
	Cl/f (ml/min/kg)	0.20	0.03	-	-
	V_z/f (l/kg)	0.22	0.06	-	-
	λ_z (h^{-1})	0.0561	0.0067	0.0558	0.0008
	$t_{½}$* (h)	12.4	-	12.4	-
10[#]	AUC (h·nmol/l)	246	43	351	60
	AUC_{0-24} (h·nmol/l)	138	26	225	33
	$AUC_{0-24,day11}/AUC_{day1}$	-	-	0.9	0.07
	R_{ac}	-	-	1.5	0.14
	C_{max} (nmol/l)	8	1	11	2
	t_{max} (h)	12	0	11	1
	Cl/f (ml/min/kg)	0.16	0.03	-	-
	V_z/f (l/kg)	0.17	0.03	-	-
	λ_z (h^{-1})	0.0541	0.0056	0.0540	0.0055
	$t_{½}$* (h)	12.8	-	12.8	-
12.5	AUC (h·nmol/l)	254	75	361	122
	AUC_{0-24} (h·nmol/l)	152	46	247	80
	$AUC_{0-24,day11}/AUC_{day1}$	-	-	1.0	0.2
	R_{ac}	-	-	1.4	0.2
	C_{max} (nmol/l)	9	3	14	4
	t_{max} (h)	11	1	10	2
	Cl/f (ml/min/kg)	0.19	0.06	-	-
	V_z/f (l/kg)	0.19	0.05	-	-
	λ_z (h^{-1})	0.0598	0.0040	0.0536	0.0029
	$t_{½}$* (h)	11.6	-	12.9	-

N equals 4 unless otherwise indicated. *) Harmonic mean. #) Day 11, mean of three.

2.2.1 What are the pharmacodynamic characteristics of liraglutide after subcutaneous administration and how do they relate to the dose?

GLP-1 is an insulinotropic hormone that is released from the L cells in the intestine. It stimulates insulin secretion and at the same time decreases glucagon secretion. Both actions are glucose-dependent, appearing at elevated glucose levels. Being a GLP-1 analog, liraglutide's insulinotropic effects were evaluated by assessing beta-cell function and post-prandial glucose in two PD studies.

Beta Cell Function:

The effects of single subcutaneous administration of 7.5 µg/kg liraglutide (NNC 90-1170) on beta cell insulin secretion (in response to increasing glucose concentrations) were examined in the type 2 diabetes patient population, and compared to the beta cell insulin secretion patterns of healthy individuals (Study NN2211-2063).
The primary objective of the efficacy analysis was to compare the effects of liraglutide and placebo on the beta-cell responsiveness to graded glucose infusion, as assessed by the primary endpoint: AUC of insulin secretion rate (ISR) over the 5-12 mmol/L glucose interval (corresponding to times of 40 to 220 minutes). The ISR was derived from the C-peptide concentration profile. $AUC_{(40-220)}$ is the area under ISR curve in the interval from 40 to 220 minutes, and it was calculated using the trapezoidal method (ISR as vertical axis and time as horizontal axis).
The secondary objective of the efficacy analysis was to compare liraglutide and placebo with respect to the following secondary endpoints:

- Slope of the mean ISR vs. mean glucose dose response relationship. The mean ISR and mean glucose was derived for each of the glucose infusion intervals. Regression model of mean ISR on mean glucose was used to estimate the slope.
- AUC of glucagon concentration over the 40-220 minutes time interval, which was calculated in a similar way to that of $AUC_{(40-220)}$ of ISR.
- Insulin clearance: Mean ISR divided by mean insulin concentration.

The results showed that:

- The average response to liraglutide treatment showed a restoration of Cpeptide levels to those approximating normal individuals. Liraglutide significantly increased the $AUC_{(40-220)}$ for the insulin secretion rate (over the 90-216 mg/dL glucose interval, times from 40 to 220 minutes) as compared with placebo, suggesting that liraglutide improves beta-cell responsiveness to increasing blood glucose concentrations in subjects with type 2 diabetes. $AUC_{(40-220)}$ ISR values for liraglutide were not significantly different from those obtained for healthy volunteers over the same glucose interval (**Fig. 7 and Table 5**), further suggesting that liraglutide restores beta-cell function.
- The slope of the mean ISR vs. mean glucose level for liraglutide was significantly greater than that for placebo, and similar to that seen in healthy volunteers. Insulin clearance and the $AUC_{(40-220)}$ for glucagon were not significantly different between placebo treatment, liraglutide treatment, and healthy individuals.
- The mean AUC for liraglutide plasma concentration (from time 0 to 17 hours) was 610 nmol.hr/L for type 2 diabetes subjects. The mean C_{max} for liraglutide was 5.9 nmol/L. The mean T_{max} for liraglutide was 13.1 hours.

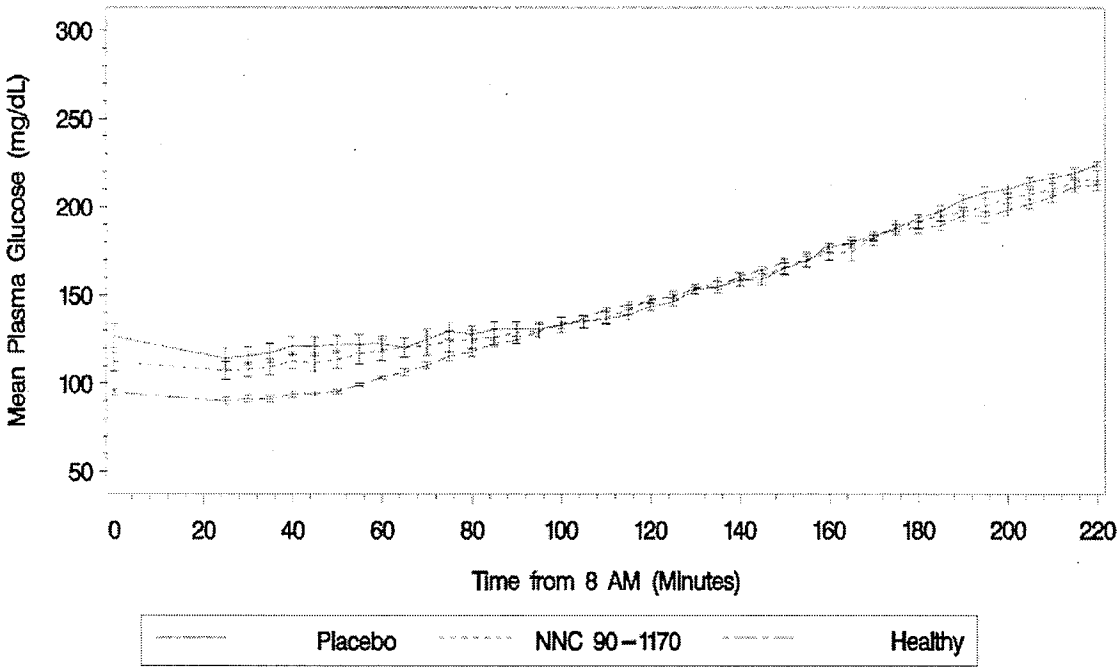


Figure 6 Mean plasma glucose profile for placebo treated, liraglutide treated and healthy subjects (control).

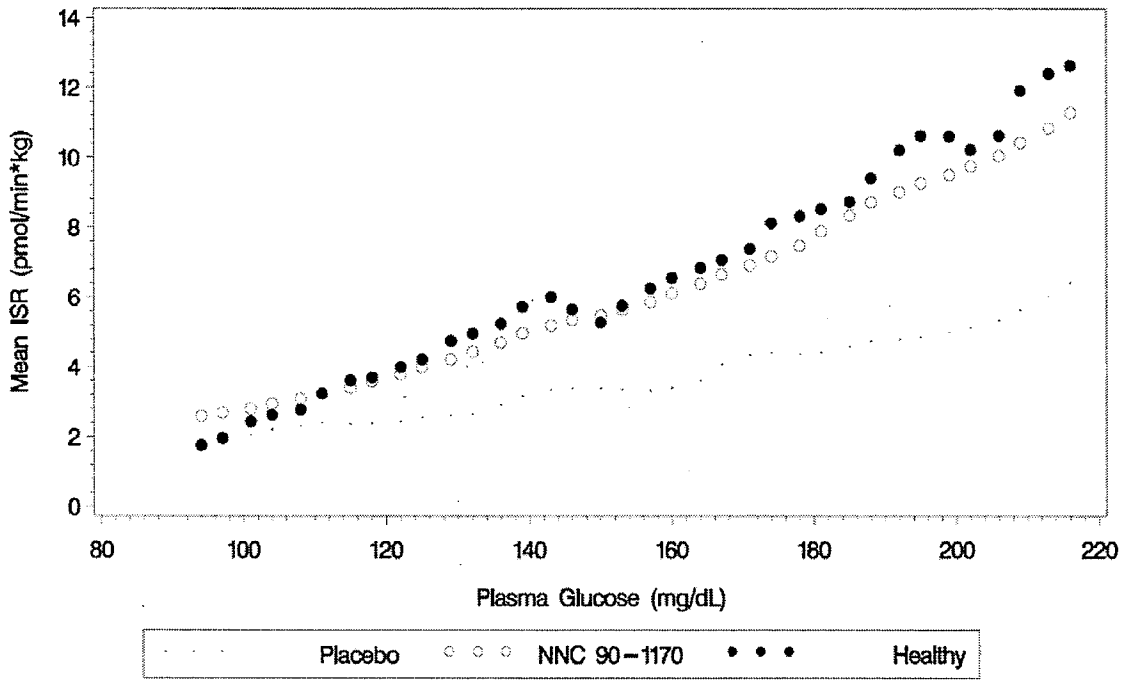


Figure 7 Mean Insulin secretion rate versus plasma glucose profile for placebo treated, liraglutide (NNC 90-1170) treated and healthy subjects (control).

Table 5 AUC(40-220) of insulin secretion rate (ISR) values in T2DM by treatment, versus healthy subjects

	Placebo (N = 10) (pmol/min*kg)	NNC 90-1170 (N = 10) (pmol/min*kg)	Healthy (N = 10) (pmol/min*kg)
Mean (SD)	667.6 (336.39)	1129.7 (473.94)	1206.9 (314.23)
Median	592.0	1077.2	1183.3
Min. – Max.			
p-value vs. NNC 90-1170	< 0.001		0.487

b(4)

Post-prandial glucose, First-phase Insulin Secretion:

The effect of liraglutide on 24-hour glucose and hormonal profiles, gastric emptying, and fasting gluconeogenesis in type 2 diabetic subjects was evaluated in trial NN2211-1332. The trial was a single-centre, randomized, double-blind trial in subjects with type 2 diabetes. Liraglutide (6 µg/kg; corresponding to 0.55 mg dose using the mean body weight of 91.4 kg in the trial) and placebo were injected subcutaneously for 9 to 10 days in a cross-over design. Previous treatment with oral hypoglycaemic agents (OHAs) was discontinued 2-3 weeks before each treatment period.

Primary endpoint was 24-hour glucose profiles after three fixed meals and the secondary endpoints included (i) 24-hour insulin secretion profiles after three fixed meals, (ii) first phase and maximal secretory capacity after a hyperglycaemic clamp and arginine bolus, (iii) endogenous glucose release (EGR), glycogenolysis (GLY), and gluconeogenesis (GNG) where EGR was expressed in mg/kg/min using a labeled glucose method and GNG was expressed in mg/kg/min using a labeled water method, (iv) 24-hour glucagon and free fatty acids (FFA) profiles after three fixed meals, (v) gastric emptying rate-4-hour paracetamol profiles after two fixed meals, (vi) pharmacokinetic profile of liraglutide in steady state-30-hour profile (AUC, Cmax, tmax, t½), (vii) 4-hour leptin profile after a fixed meal (dinner), and (viii) 4-hour pro-insulin profile after a fixed meal (breakfast).

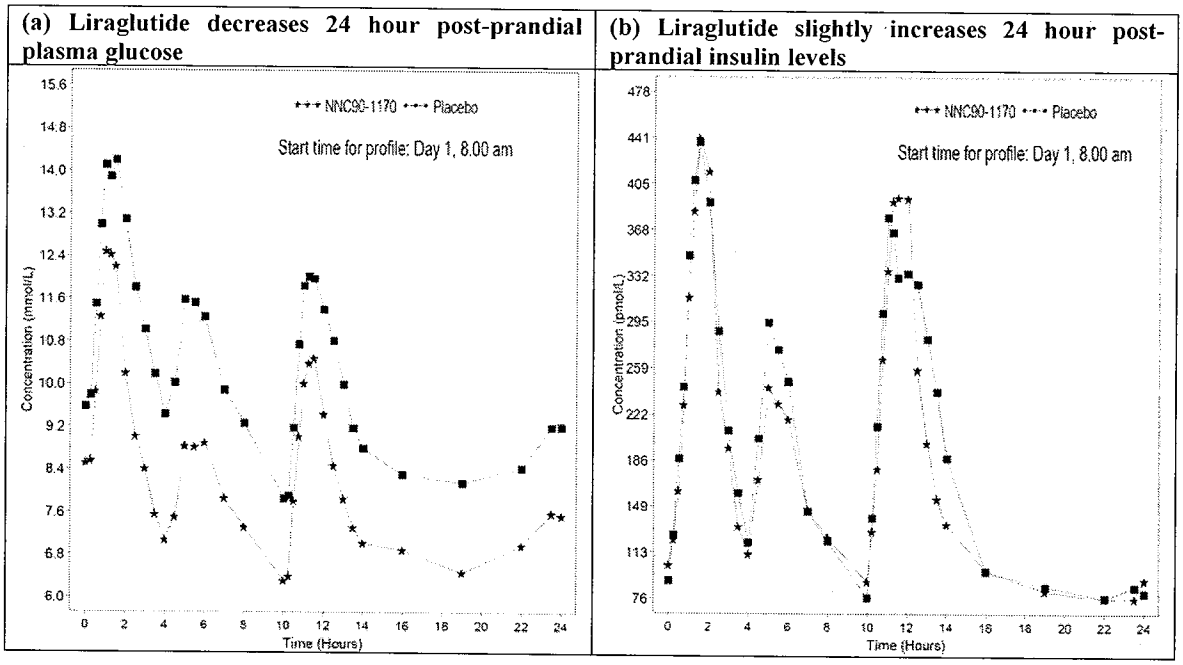


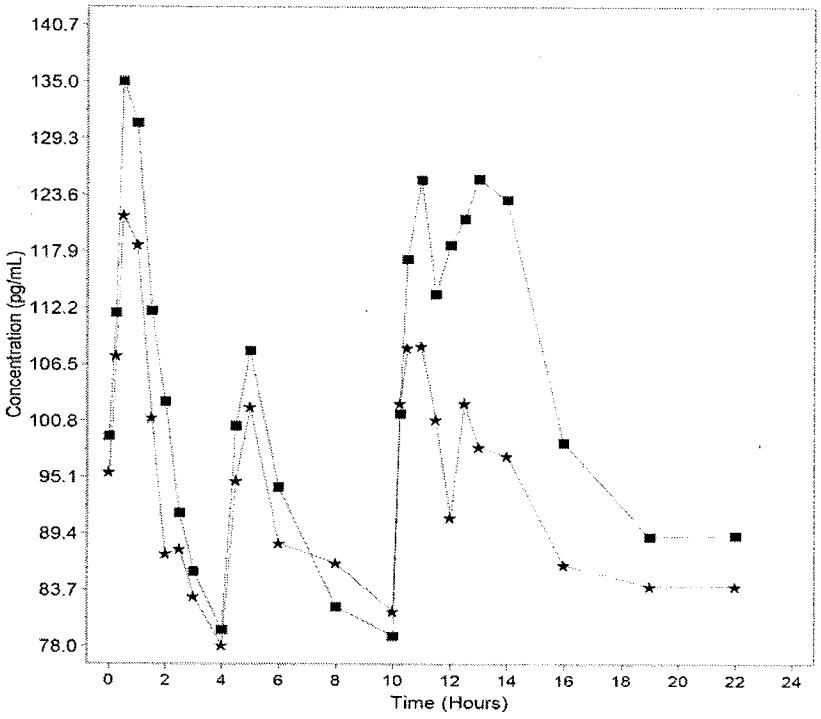
Figure 8 Mean 24 hour Profiles for Plasma Glucose (a) and insulin (b).

Table 6 Statistical Analysis of 24-hour plasma glucose

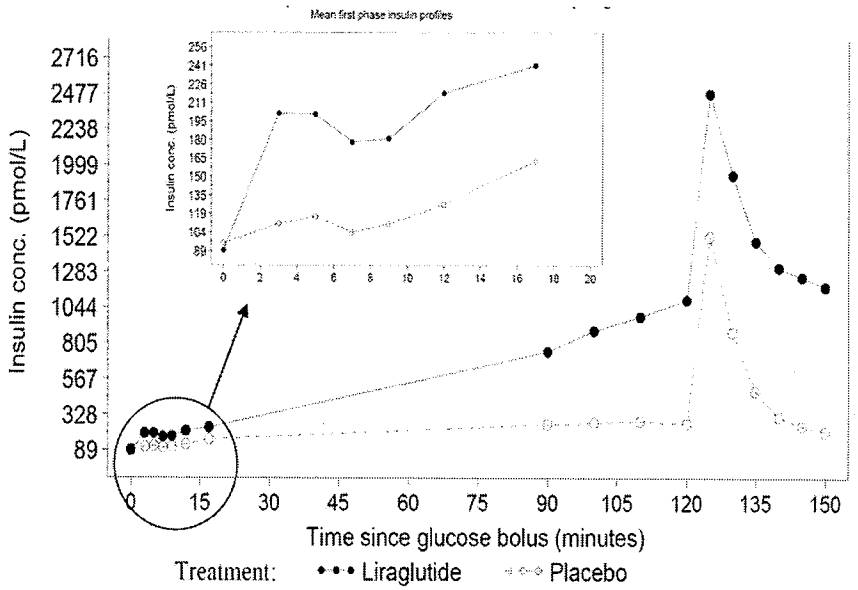
	Liraglutide	PBO	Liraglutide-PBO
24-hour glucose concentration, AUC - mmol/L*h			
N	13	13	26
Mean (S.E.M.)	187.46 (14.02)	232.30 (21.94)	-44.55 (15.27)
Min - Max			
95% C.I.			[-78.15 ; -10.95]*
p-value			0.0140

b(4)

(a) Liraglutide suppresses 24 hour post-prandial plasma glucagon



b) Liraglutide significantly increases first-phase insulin during hyperglycemic clamp



Glucose bolus (first phase): 0-17 min; Hyperglycaemic clamp (second phase): 90-120 min; Arginine stimulation test (maximum insulin secretion): 120-150 min

Figure 9 (a) Mean 24 hour Profiles for Plasma Glucagon on Day 1 and (b) Mean First-phase Insulin assessed during the hyperglycemic clamp on Day 2.

Table 7 Statistical Analysis of 24-hour plasma glucagon

	Liraglutide	PBO	Liraglutide
Fasting Glucagon concentration, AUC - pg/mL*h			
N	13	13	26
Mean (S.E.M.)	92.84 (7.24)	94.47 (5.57)	-1.87 (3.32)
Min - Max			
95% C.I.			[-9.18 ; 5.45]
p-value			0.5858
24-hour Glucagon concentration, AUC - pg/mL*h			
N	13	13	26
Mean (S.E.M.)	2179.1 (118.0)	2371.2 (135.0)	-197.3 (82.98)
Min - Max			
95% C.I.			[-379.9 ; -14.65]*
p-value			0.0367

b(4)

Table 8 Statistical Analysis of First-Phase Insulin during hyperglycemic clamp

	Liraglutide	PBO	Liraglutide-PBO
Insulin concentration, AUC - pmol/L*h			
N	13	13	26
Mean (S.E.M.)	55.45 (9.93)	34.26 (6.40)	20.97 (6.43)
Min - Max			
95% C.I.			[6.81 ; 35.13]*
p-value			0.0076
Insulin concentration, Incremental AUC - pmol/L*h			
N	13	13	26
Mean (S.E.M.)	30.15 (9.05)	7.32 (2.85)	22.83 (6.87)
Min - Max			
95% C.I.			[7.70 ; 37.95]*
p-value			0.0068
Insulin concentration, Max - pmol/L			
N	13	13	26
Mean (S.E.M.)	262.62 (47.51)	166.46 (32.43)	95.43 (28.93)
Min - Max			
95% C.I.			[31.76 ; 159.10]*
p-value			0.0071
Glucose concentration, Max - mmol/L			
N	13	13	26
Mean (S.E.M.)	17.18 (0.57)	18.32 (1.22)	-1.13 (1.03)
Min - Max			
95% C.I.			[-3.40 ; 1.15]
p-value			0.2986

b(4)

PBO = Placebo. The statistics are obtained from a mixed model with subjects as a random factor and visit and treatment as fixed factors. An asterisk indicates statistical significance.

Efficacy results showed that:

(1) Primary and secondary end point based on 24 hour profiles:

- For the primary objective, 24-hour glucose profiles, liraglutide treatment provided 24-hour glycaemic control, as glucose AUC(0-24) was statistically significantly lower compared with placebo (**Figure 8 and Table 6**).
- The overall glucagon level (glucagon AUC(0-24)) was significantly inhibited after treatment with liraglutide (**Figure 9a and Table 7**).
- Fasting value of pro-insulin was statistically significantly lower after liraglutide treatment than after placebo.

- No statistically significant difference could be demonstrated for free fatty acids, insulin secretion rate (ISR), fasting glucose (p=0.0782), and AUC(0-24) or fasting values of insulin and C-peptide.

(2) Hyperglycaemic clamp:

- Treatment with liraglutide increased insulin levels throughout the entire hyperglycaemic clamp. In addition, first phase insulin response improved after liraglutide treatment (**Figure 9b and Table 8**).
- During the steady state part of the clamp, treatment with liraglutide increased mean levels of insulin, C-peptide, and pro-insulin, while glucagon and pro-insulin/insulin ratio were decreased.
- Maximum insulin and C-peptide concentrations after arginine infusion were significantly increased after liraglutide treatment, compared with placebo. No statistically significant difference was seen for maximum concentration of pro-insulin, while the maximum glucagon concentration was statistically significantly lower after liraglutide treatment.

(3) Other endpoints:

- β-cell function (insulin secretion, $ISEC_{HOMA}$) was statistically significantly increased after treatment with liraglutide, whereas no statistically significant differences were seen between treatments for insulin resistance ($IRES_{HOMA}$) or insulin sensitivity (ISEN).
- No statistically significant differences were found between the two treatments with regard to gastric emptying rate.
- Treatment with liraglutide resulted in a statistically significant lowering of endogenous glucose release, which could be contributed to a decrease in glycogenolysis, as no effect was seen on gluconeogenesis. No statistically significant effect was seen for the indirect calorimetry parameters.

Hypoglycemic counter-regulation

Glucagon inhibition is part of GLP-1's mode of action, and hence disturbance of the hypoglycaemic counter-regulation under the influence of GLP-1 could be expected. Since liraglutide is believed to have a mode of action similar to GLP-1, the effect of liraglutide on hypoglycaemic counter-regulation was evaluated in this stepwise hypoglycaemic clamp study in subjects with type 2 diabetes. The primary endpoint was glucagon secretion measured as mean glucagon for each of the four 40-minute clamps at 78, 66, 54 and 42 mg/dL (4.3, 3.7, 3.0 and 2.3 mmol/L) glucose concentrations performed over the 240-minute period.

At baseline, plasma glucagon level was approximately 77 pg/mL and increased steadily with progressive hypoglycaemia by approximately 1.5-fold over basal glucagon concentration for both treatment groups (**Fig. 10**).

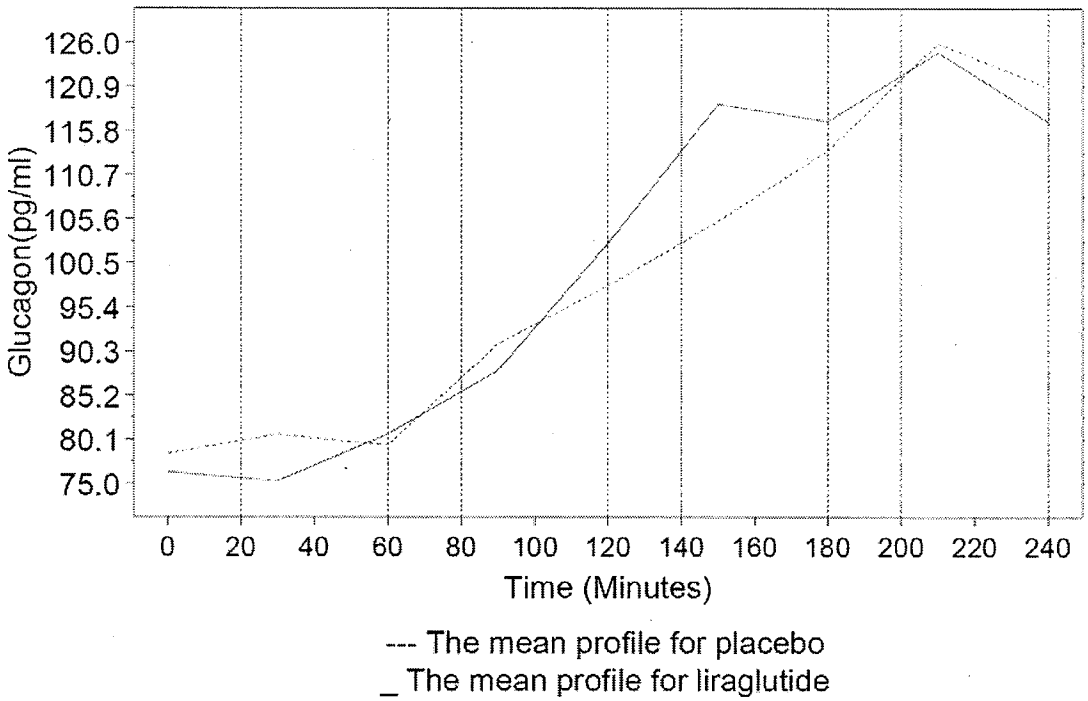


Figure 10 Liraglutide does not impair the glucagon response to hypoglycemic counter-regulation

The results demonstrated that after a single s.c. dose of 7.5 µg/kg, liraglutide did not affect the glucagon response to hypoglycaemia and did not impair the overall hypoglycaemic counter-regulation response. In accordance with the glucose-dependent stimulation of insulin secretion, ISR was borderline significantly increased at the two highest glucose levels (78 and 66 mg/dL), but not at the two lower glucose levels (54 and 44 mg/dL). It induced minor statistically significant differences for adrenaline and growth hormone (suppressed release relative to placebo).

2.2.2 Is major route of elimination in humans identified?

In vitro and in vivo metabolism and excretion studies demonstrated that liraglutide is fully metabolized in the body by sequential cleavage of the peptide with no excretion of liraglutide and only very limited excretion of closely related metabolites in the feces or urine in all animal species and humans. Minor components of the peptide or the palmitic acid part of liraglutide were observed in feces and urine. The extensive metabolism of ^{14}C-liraglutide after s.c. administration in rats resulted in the excretion of ~ 70% $^{14}CO_2$ in the expired air. In plasma from all species including man, small amounts of metabolites more lipophilic than liraglutide have been observed. This is in agreement with the metabolites identified *in vitro* in hepatocytes and following incubation with dipeptidyl peptidase IV (DPP-IV) or neutral endopeptidase (NEP), both known to be important in the metabolism of native GLP-1. Liraglutide was metabolized by DPP-IV and NEP in similar positions in the peptide as observed for native GLP-1. The metabolite profile of [^{3}H]-liraglutide was studied *in vitro* in rat, mouse, monkey and human hepatocytes (NN 205145).

Several metabolites were detected in increasing amounts over time and the overall pattern was similar across species. Metabolism and excretion of [^{3}H]-liraglutide have also been studied *in vivo* in rats (NN 205265 and NN 206387), mice (NN 205264) and monkeys (NN 205399). Circulating metabolites have been found in plasma for all three species. Most metabolites (up to 10) were found in rat plasma whereas only 2 metabolites were found in mice and up to 5 in cynomolgus monkey plasma. All metabolites were minor compared to the total exposure level and more lipophilic than liraglutide suggesting truncation of the peptide backbone with an intact fatty acid side chain in these animal species.

The sponsor conducted one mass balance study in which metabolic fate of [^{3}H]-Liraglutide (**Fig. 11**) was evaluated in vivo following single s.c. administration in humans. This trial investigated the profile of liraglutide metabolites in plasma, urine and feces in healthy subjects. Due to the chemical structure of liraglutide, being a large peptide with a fatty acid side chain, a degradation of liraglutide into peptides, amino acids, fatty acid fragments, water and products from recycling pathways was expected. Hence, a full mass balance profile (i.e. a radioactivity recovery of ≥ 95%) was not possible to obtain. A single dose of 0.75 mg liraglutide (containing 12.0 MBq) was given as s.c. injection in the abdomen. Plasma was collected for 4 elimination half-lives (approximately 60 h) of liraglutide, and urine and feces were collected until excreted levels of tritium ([^{3}H]) reached the end criteria level of 1000 dpm/g in pooled 24-hour samples, or until a maximum of 14 days post dose.

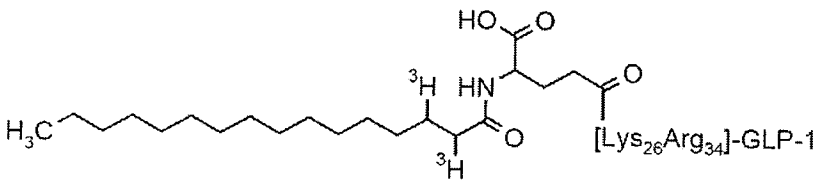


Figure 11 Location of tritium label in [^{3}H]-liraglutide

The samples from plasma, urine and feces were analyzed by means of HPLC and radio-chromatography. Results from these chromatograms were used for semi-quantification of components as concentration equivalents (plasma) and percentage of the administered radioactivity (urine and feces). The structure of these metabolites (peptide fragments) is more or less unknown.

Pharmacokinetic properties based on labeled drug

Time to maximum liraglutide plasma concentration (t_{max}) was 11.7 h and t½ was estimated to 15.4 h. Liraglutide was primarily distributed in plasma compared to blood (ratio approximately 0.6).

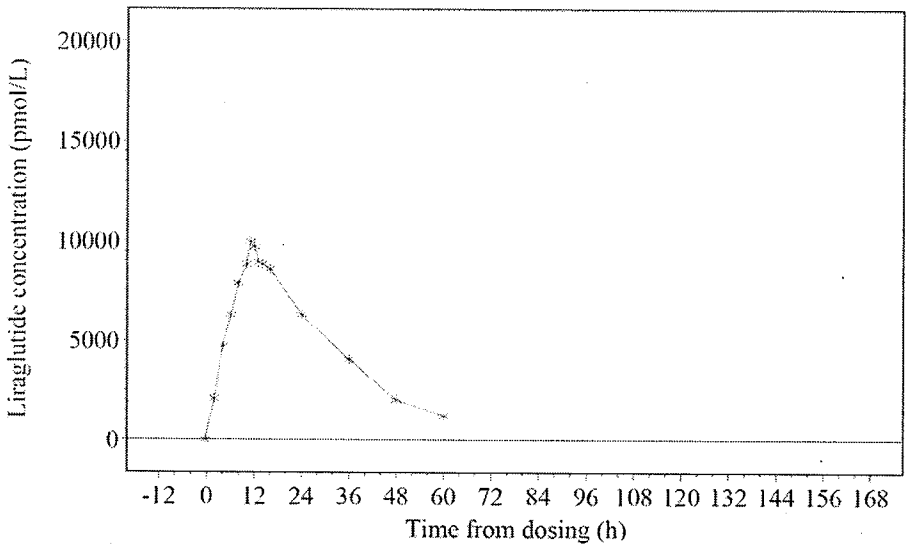


Figure 12 Mean plasma concentrations of [^{3}H]-liraglutide following a single SC dose of 0.75 mg.

Excretion of Radioactivity in Urine and Feces

In feces three metabolites F1, F2 and F3 were detected. All of these were more lipophilic than the parent compound, and two of them were detected in the majority of the subjects. No quantification of the individual components was possible. Until Day 14, 26.3% of the total radioactivity was excreted in urine and feces. Excretion of non-volatile radioactivity (liraglutide-related radioactivity) by urine and feces was 11.5% of the total radioactivity (6.4% and 5.1%, respectively). Excretion of volatile radioactivity (e.g. tritiated water) was 14.8% of the total radioactivity (13.7% in urine and 1.1% in feces).

Metabolite Profile of Liraglutide in Plasma, Urine and Feces

- In plasma three components were detected. The major component was unchanged liraglutide (89-100%) while the two metabolites P1 and P2 were slightly more lipophilic and represented ≤9% and ≤5% (respectively) of the total exposure (2-24 h).
- No unchanged liraglutide was detected in urine or feces.

In urine three metabolites U1, U2 and U3 (only in one subject) were detected. All of these had much lower retention times than the parent compound. The major component U1 was excreted as 3% of the administered radioactivity.

2.2.3 Does this drug prolong the QT or QTc Interval?

A formal consult to review the thorough QT study was submitted to the IRT. Based on the IRT review the following observations were made:

No significant QT prolongation effect of liraglutide (1.8 mg) was detected in this TQT study. The largest upper bounds of the 2-sided 90% CI for the mean difference between liraglutide (1.8 mg and 1.2 mg) and placebo were below 10 ms (2.7 ms and 0.9 ms), the threshold for regulatory concern as described in ICH E14 guidance. The largest lower bound of the two-sided 90% CI for the ΔΔQTcI for moxifloxacin was greater than 5 ms, and peaked at hour 2. In this randomized,

double blinded, two-period crossover, placebo-controlled trial study, 52 healthy subjects received liraglutide 1.2 mg, liraglutide 1.8 mg, placebo, and a single oral dose of moxifloxacin 400 mg (positive control). Overall summary of findings is presented in **Table 9**.

Table 9 Point Estimates and the 90% CIs Corresponding to the Largest Upper Bounds for Liraglutide (1.8 mg and 1.2 mg) and the Largest Lower Bound for Moxifloxacin

Treatment	Time (hour)	ΔΔQTcI (ms)	90% CI (ms)
Liraglutide 1.8 mg	8	0.3	(-2.1, 2.7)
Liraglutide 1.2 mg	3	-1.7	(-4.3, 0.9)
Moxifloxacin 400 mg*	2	12.4	(8.9, 15.9)

*Multiple endpoint adjustment is not applied. The largest lower bound after Bonferroni adjustment for 4 timepoints is 7.6 ms.

The relationship between ΔΔQTcI and liraglutide concentrations is visualized in **Figure 13** with no evident exposure-response relationship.

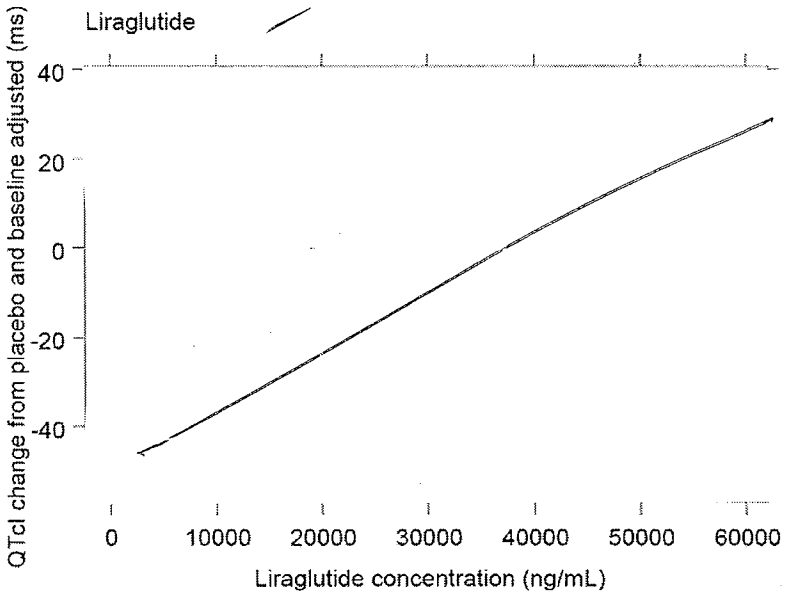
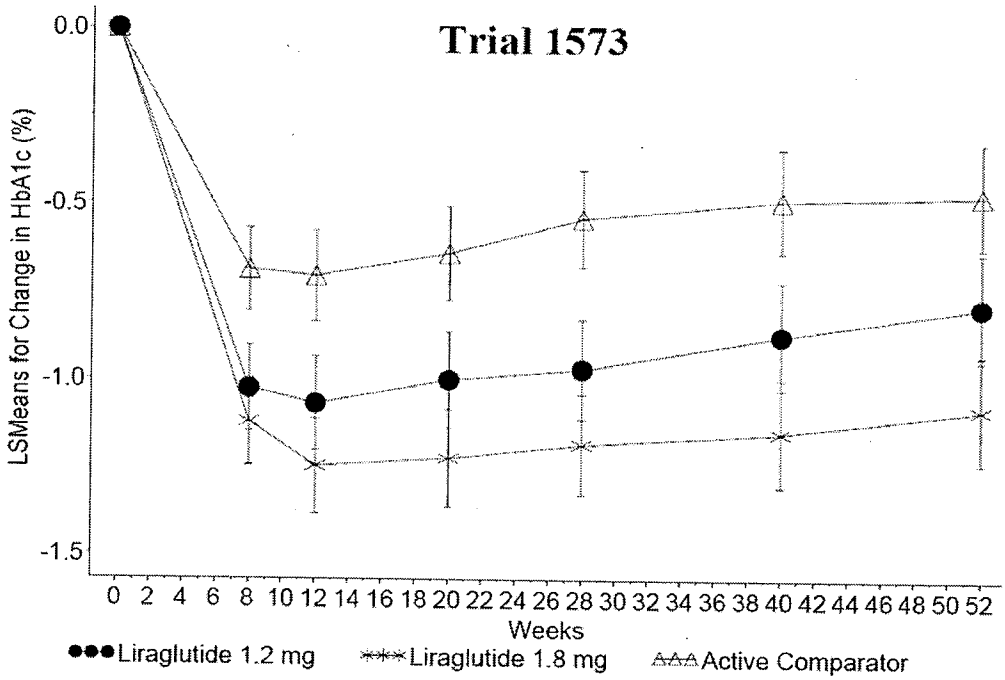


Figure 13 ΔΔ QTcI vs. Liraglutide Concentration

2.2.4 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) with regards to efficacy?

The change in HbA1c versus time profile from Phase 3 study showed that the maximal mean reduction in HbA1c from baseline is achieved by week 12 (**Fig. 14a**), thus allowing the comparison of week 12 data among Phase 3 and Phase 2 monotherapy trial, the latter were of 12 to 14-weeks duration. Graphically, the response with 0.6 mg was in reasonable proximity to half-the maximal response (**Fig. 14b**). Graphical analysis of pooled dose-response data from Phase 2 and Phase 3 studies showed that the liraglutide treatment is associated with a dose dependent reduction in HbA1c from baseline (see **Fig. 14b**). The maximal effect is achieved at 1.2 mg dose with a numerical advantage of 1.8 mg over 1.2 mg with regards to maximal HbA1c reduction.

(a)



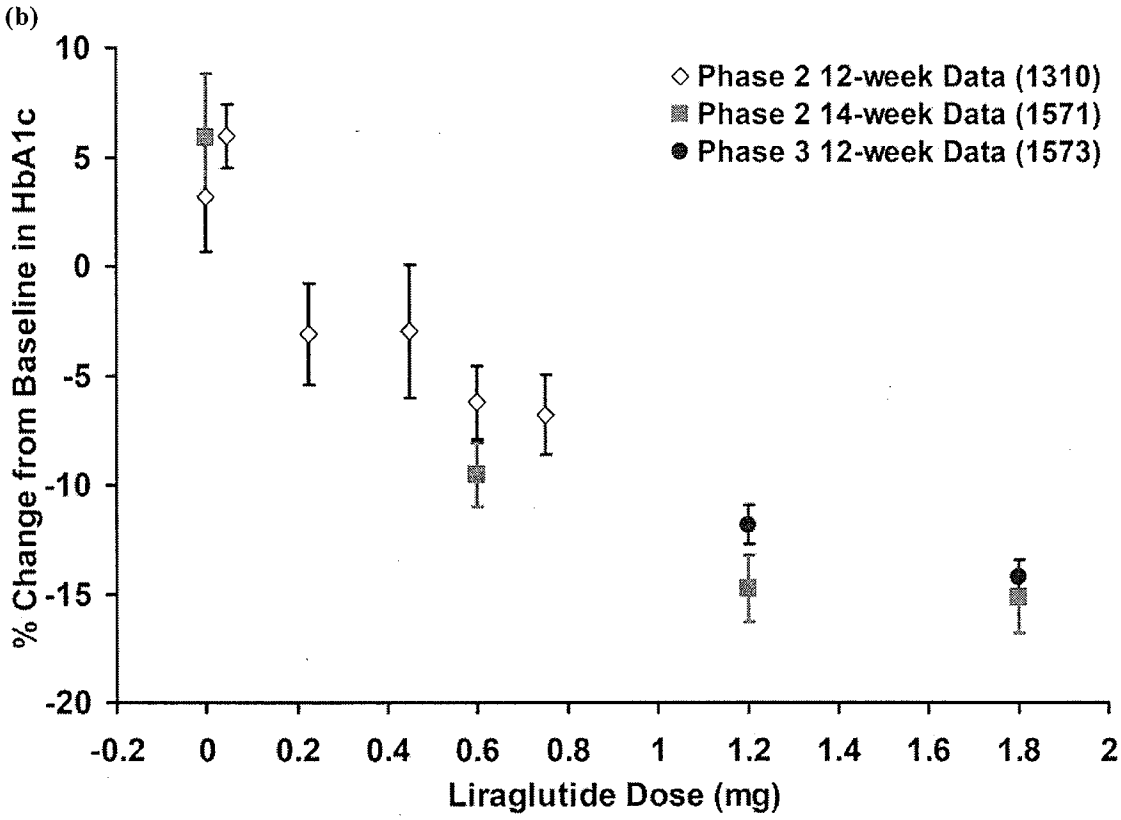


Figure 14 (a) Time course of change from baseline in HbA1c from the 52-week Phase 3 confirmatory trial (1573) and, (b) Dose dependent increase in effectiveness of liraglutide based on Mean(±SE) %change from baseline in HbA1c from 12-week Phase 2 trial (1310), 14-week Phase 2 trial (1571), and 12-week data from the 52-week Phase 3 confirmatory trial (1573).

The % change from baseline in FPG and HbA1c decreased with increasing liraglutide concentration (**Fig. 15**). The early 12 week Phase 2 evaluation by sponsor revealed that the ED_{50} was around 0.65 mg. An exploratory PKPD analysis of the 14 week phase 2 data revealed that the liraglutide concentrations resulting from the doses 0.65 mg and above exceeded the expected EC_{50} value of ~4 nmol/L estimated from the exploratory analysis of liraglutide-FPG relationship (*see PM review under Appendix 4.3 for details*). Since there was a considerable overlap in the exposures for 1.2 mg and 1.8 mg doses, the two doses could not be differentiated using a dose-response analysis. In patients with body weight 160 kg the expected mean Cavg is 9 nmoL/L and 13 nmol/L using 1.2 mg and 1.8 mg dose, respectively. However, the liraglutide concentration-response (%change from baseline HbA1c) suggests that maximum effect is achieved at or above 7 nmol/L liraglutide concentration (which is the lowest limit of 2nd quartile) (**Fig. 15a**). This was consistent for the Phase 3 data where the concentrations resulting ranged from 5 nmol/L to 45 nmol/L (**Fig. 15b**). Hence, it can be inferred that the proposed doses provide adequate liraglutide exposures over the body weight range of 40-160 kg, and does not warrant for any weight based dose adjustment in this population. The sponsor's proposed fixed dose titration is acceptable from clinical pharmacology perspective.

(a) *Mean(SE) %change in HbA1c from baseline versus mean liraglutide of each quartile range (Phase 2)*	(b) *Mean(SE) %change in HbA1c from baseline versus mean liraglutide of each quartile range*

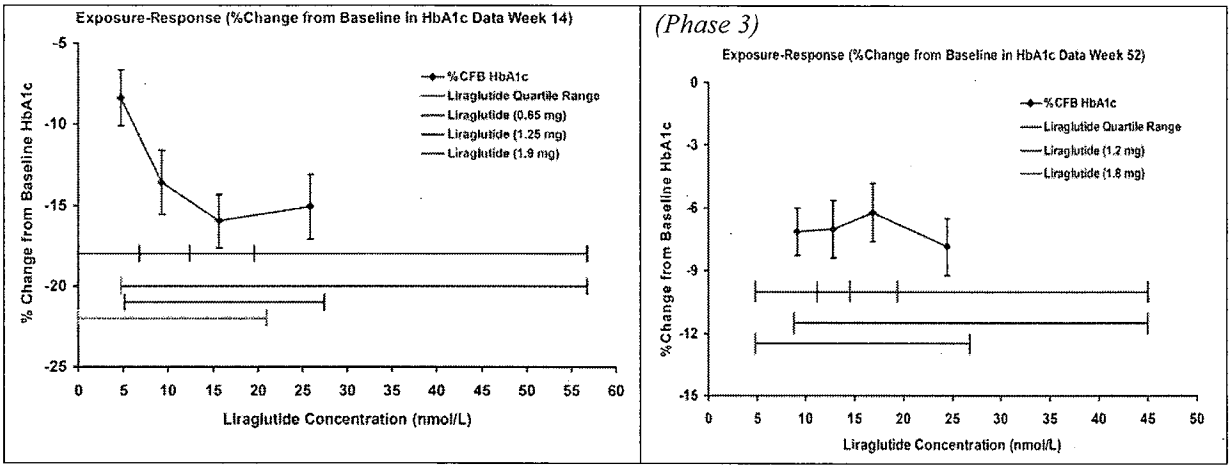
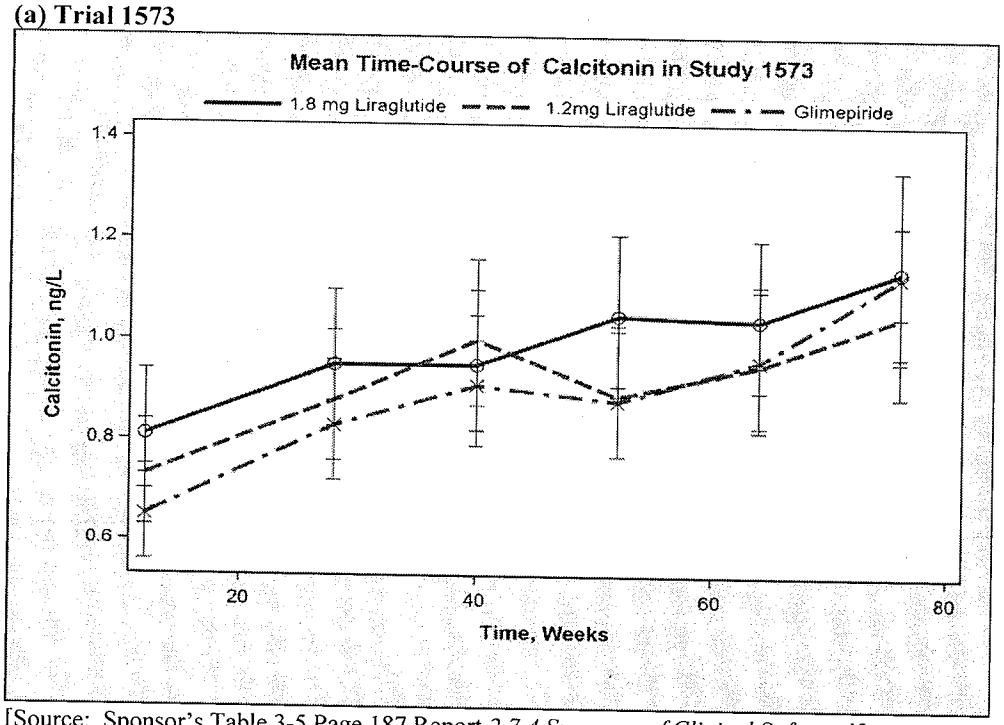


Figure 15 Exposure-response relationship of liraglutide in (a) Phase 2 exploratory and (b) Phase 3 confirmatory trial.

2.2.4 What are the characteristics of the exposure-calcitonin relationships (dose-response, concentration-response) with regards to thyroid safety?

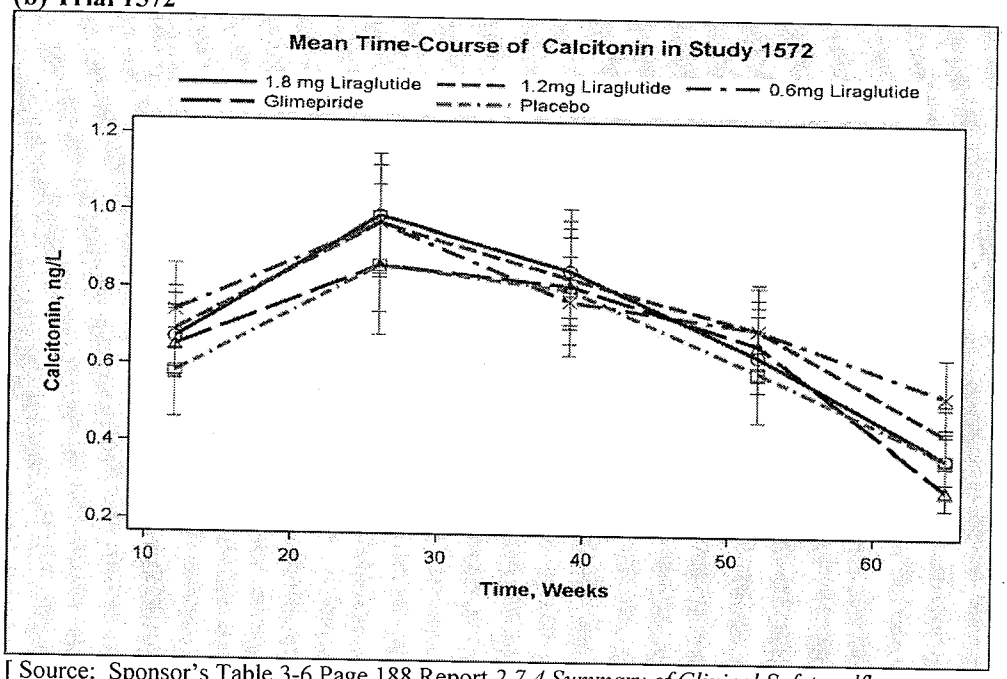
Liraglutide caused Thyroid c-cell tumors in mice and rats in long-term toxicity studies at or above equivalent human exposure. This finding was associated with dose dependent increase in incidence of tumor and an increase in serum Calcitonin levels, which is a hormone secreted from c-cells. The serum Calcitonin was also measured in the efficacy trials as part of thyroid safety investigation following long-term liraglutide administration. The mean Calcitonin versus time profile from Phase 3 monotherapy trial showed that there was a gradual increase in Calcitonin for liraglutide and active comparator. However, among the liraglutide treatment arms, dose-response was not consistent at all the time points. Although in general mean Calcitonin levels appeared to be higher for 1.8 mg dose in comparison to 1.2 mg dose, there was considerable overlap in 95% CI at all time points (**Fig. 16a**). Further, the add-on to metformin trial (Study 1572) also did not reveal a consistent increase in calcitonin levels and dose levels of 0.6, 1.2 and 1.8 mg were indistinguishable with regards to the serum Calcitonin levels at all the time points (**Fig. 16b**) (see PM review for additional details).

(a) Trial 1573



[Source: Sponsor's Table 3-5 Page 187 Report *2.7.4 Summary of Clinical Safety.pdf*]

(b) Trial 1572



[Source: Sponsor's Table 3-6 Page 188 Report *2.7.4 Summary of Clinical Safety.pdf*]

Note: The LS mean estimates presented in these figures are from a repeated measurements analysis for normal censored data with time, treatment, gender and treatment by time interaction as fixed effects and subject as random effect. Error bars represent 95% confidence intervals around the LS mean.

Figure 16 Time course of Calcitonin from (a) 52-week Phase 3 confirmatory Trial 1573 and (b) Add-on to metformin Phase 3 Trial 1572.

Further, the graphical evaluation of exposure-response data revealed that change from baseline in Calcitonin at week 26 was not related to steady-state liraglutide exposure (see **Fig. 17**).

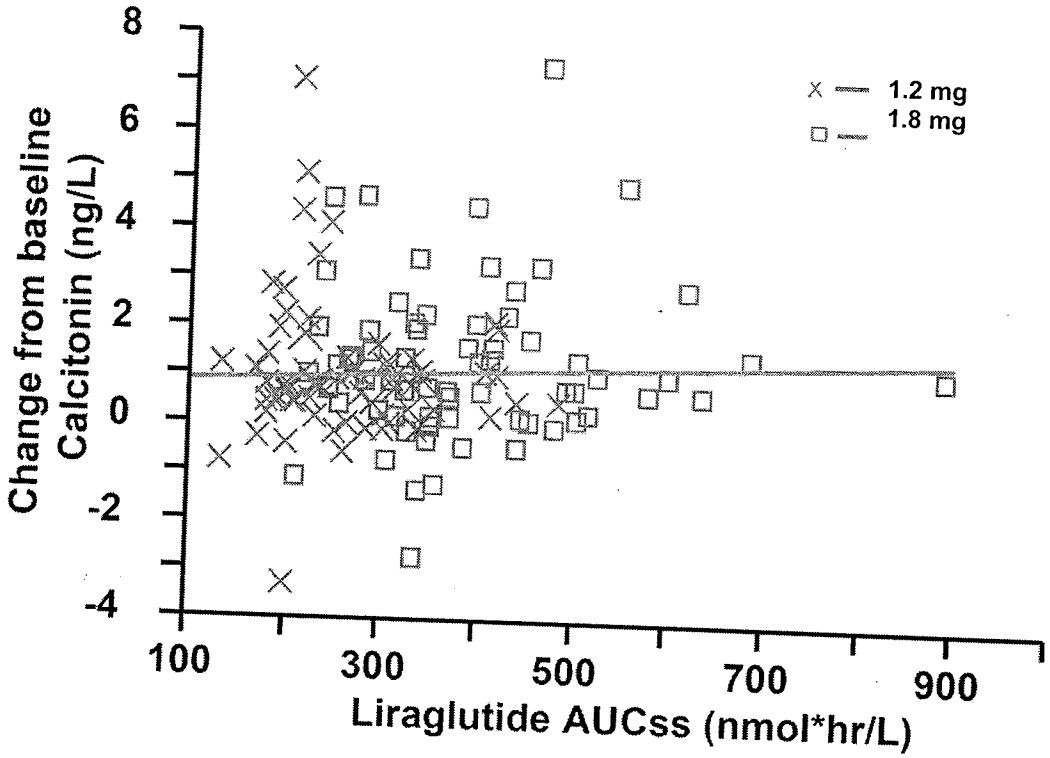


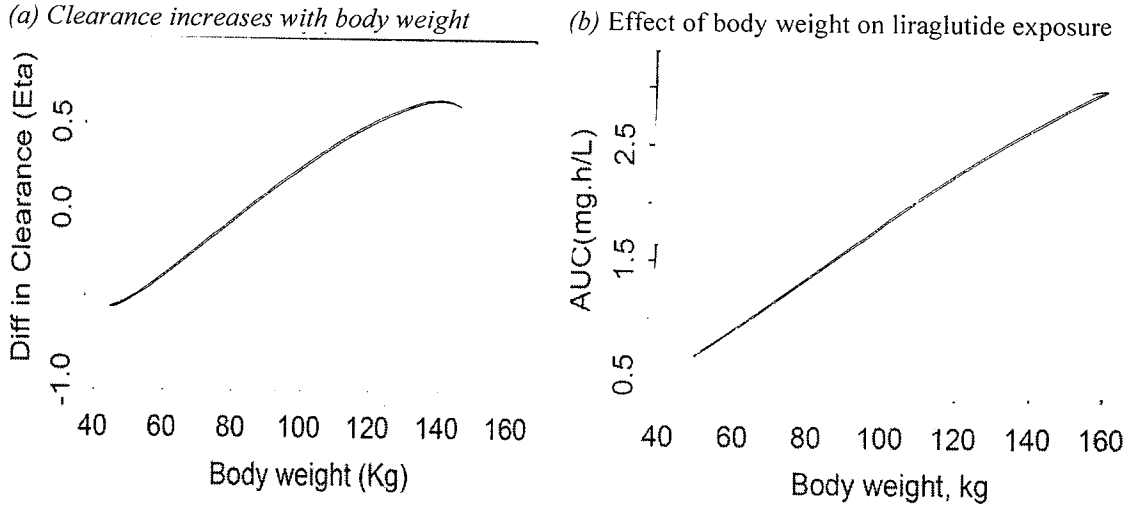
Figure 17 Flat liraglutide exposure-change from baseline in Calcitonin relationship at week 26 from the 52-week Phase 3 confirmatory trial (1573).

2.3 Intrinsic Factors

2.3.1 What intrinsic factors (e.g., weight, gender, race, age, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

The effect of various covariates e.g. Weight, Age, BMI, Gender and Race was assessed in the population pharmacokinetic analysis. The details are mentioned in the Pharmacometric review under **Appendix 4.3**. Highlights of the results are described below:

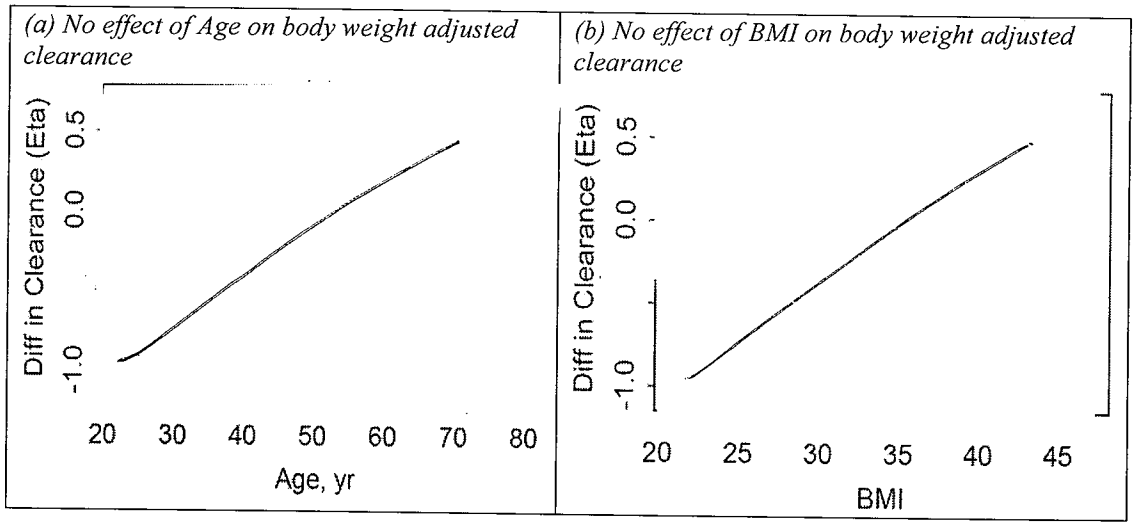
Body Weight: Weight was found to be a significant predictor of CL/F of liraglutide. This relationship was established from the population PK analysis of Phase 3 trial 1573 (**Fig. 18a, 18b**).



b(4)

Figure 18 Effect of body weight on liraglutide clearance pharmacokinetics.

Age and BMI: There was no effect of age or BMI on liraglutide clearance as illustrated in **Figure 19** below.



b(4)

Figure 19 No effect of Age and BMI on liraglutide clearance.

Gender and Race: Based on the weight adjusted clearance, the females were found to have 30% lower weight adjusted clearance than the males. However, after accounting for Weight and Gender effects the Race effect could not be ascertained as claimed by the sponsor, as illustrated in **Figure 20** below.

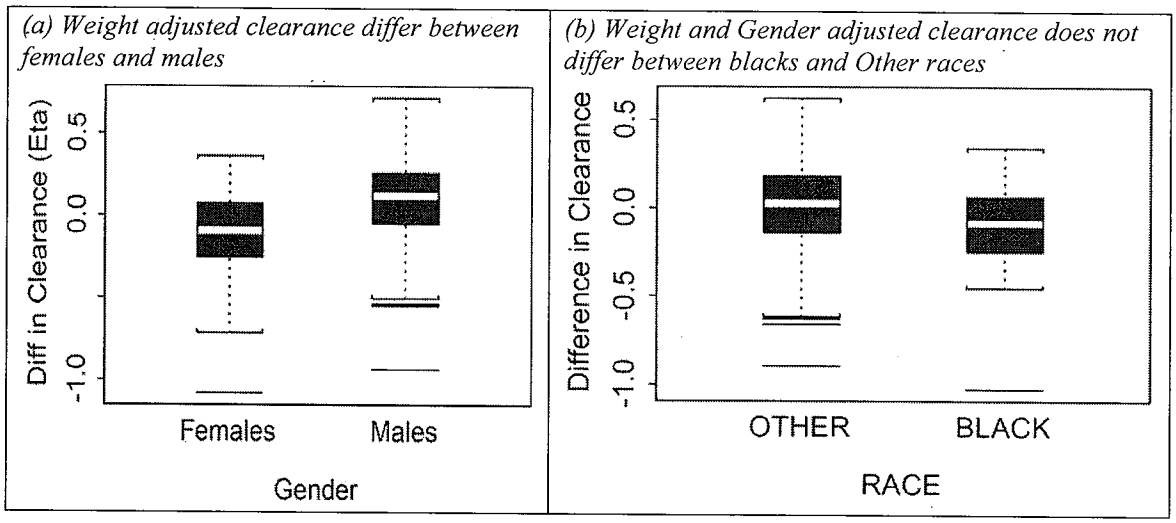


Figure 20 Effect of gender and race on fenofibric acid pharmacokinetics.

Although both weight and gender were found to affect the clearance, the effect is only statistically important. Considering that exposures (Cavg), resulting from 1.2 and 1.8 mg doses, were in the maximal response region of the exposure-relationship for primary efficacy variable (HbA1c), these differences do not appear to be clinically meaningful to warrant a dose-adjustment.

2.3.2 Does the renal function affect Liraglutide pharmacokinetics?

In the renal impairment study, a single-centre, single-dose, parallel group, open-label trial investigating pharmacokinetic profiles of liraglutide in five groups of subjects with normal, mild, moderate, severe and end-stage renal impairment. Liraglutide was administered as a single dose of 0.75 mg. The dose was injected subcutaneously, into the abdomen of the trial subjects. The classification of renal impairment was based on creatinine clearance (glomerular filtration rate) estimated with the Cockcroft & Gault formula. It was aimed to have six subjects in each of the five trial groups in order to cover the complete range of creatinine clearance (**Table 10**).

Table 10 The classifications of renal impairment groups

Description	Estimated Creatinine Clearance (mL/min)
Normal renal function	>80 mL/min
Mild renal impairment	>50-≤80 mL/min
Moderate renal impairment	>30-≤50 mL/min
Severe renal impairment	≤30 mL/min
End-stage renal disease	Requiring dialysis

The results of statistical comparison of AUC are summarized in the following table and presented in **Figure 21.**

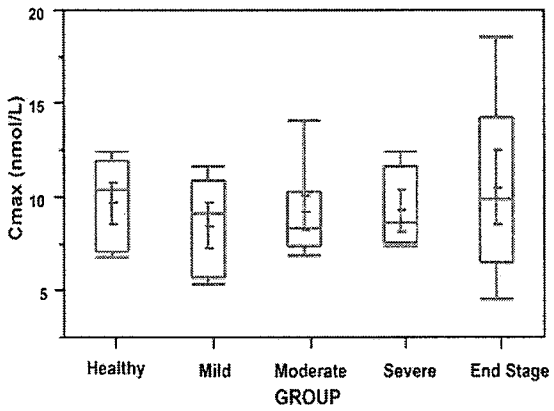
Table 11 Pharmacokinetic parameters of liraglutide in healthy and renally impaired subjects

	Mild/Normal	Moderate/Normal	Severe/Normal	End stage/Normal
Original Analysis				
AUC				
Estimate	0.67	0.86	0.73	0.74
Lower 90% limit	0.54	0.70	0.57	0.56
Upper 90% limit	0.85	1.07	0.94	0.97
Updated Analysis				
AUC				
Estimate	0.65	0.81	0.71	0.70
Lower 90% limit	0.51	0.65	0.55	0.53
Upper 90% limit	0.83	1.01	0.92	0.91
fu, all three concentrations				
Estimate	0.81	1.37	0.60	0.87
Lower 90%	0.49	0.85	0.35	0.48
Upper 90%	1.34	2.21	1.02	1.55

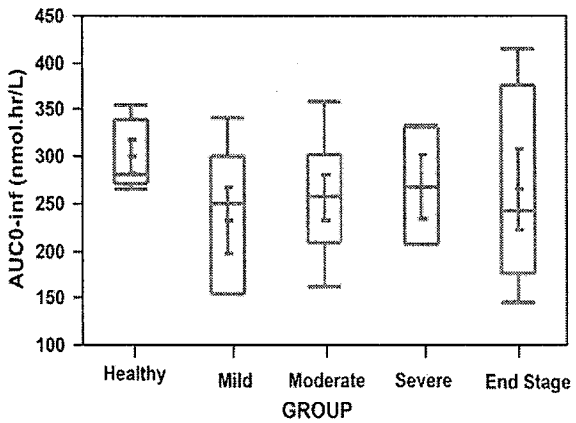
fu = fraction of unbound liraglutide

Based on the updated analysis, overall the average $AUC_{0-\infty}$ of liraglutide was around 19 - 35% lower in the renally impaired subjects than the normal subjects. This was in agreement to the original analysis. Total apparent clearance (CL/F) varied slightly across the renal groups; however, no trend with respect to renal function was seen. V/F was highest in subjects with mild renal impairment and in subjects with normal renal function. The apparent volume of distribution was similar or lower for the subjects with moderate, severe and end-stage renal disease. These differences couldn't be explained based on the unbound concentrations, which were similar across different groups (**Figure 21d**).

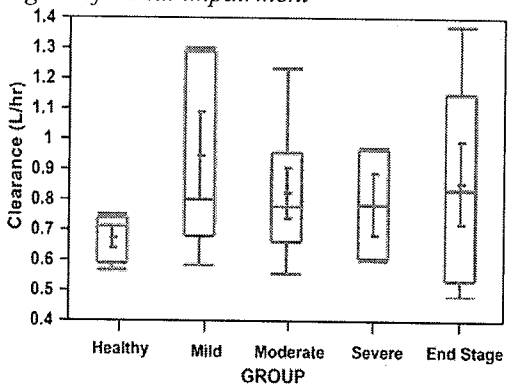
(a) No trend apparent in Liraglutide Cmax with degree of renal impairment



(b) No trend apparent in Liraglutide AUC(0-∞) with degree of renal impairment



(c) No trend apparent in Liraglutide CL/F with degree of renal impairment



(d) Fraction unbound across various treatments

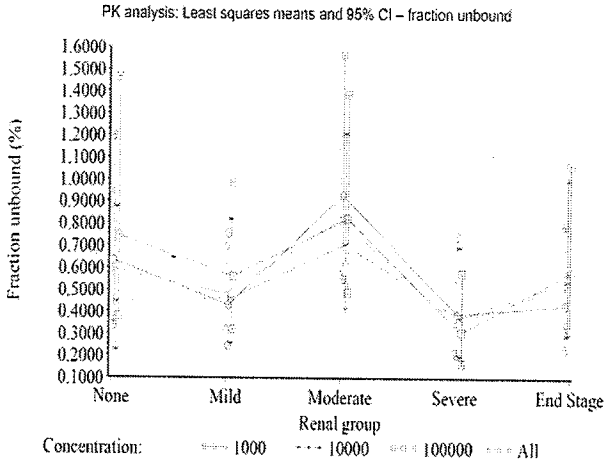


Figure 21 Liraglutide pharmacokinetic profile in healthy and renal impairment subjects

Based on these findings:

- It cannot be concluded that reduced renal function has an impact on the liraglutide pharmacokinetics.
- Subjects with type 2 diabetes who also suffer from renal impairment, including subjects with end-stage renal disease, should use standard treatment regimens for liraglutide and be dosed according to their glycaemic control.

2.3.3 Does the hepatic function affect Liraglutide pharmacokinetics?

The effect of hepatic impairment was assessed in a single-centre, single-dose, parallel group, open-label trial investigating pharmacokinetic profiles of liraglutide in four groups of subjects with normal, mild, moderate, and severe hepatic impairment. The classification of hepatic impairment was based on the Child-pugh scores (**Table 12**) and was in accordance with that defined by the Guidance document. Six subjects were evaluated in each group as planned.

Table 12 The classifications of hepatic impairment groups

	Points Scored for Observed Findings		
	1	**2**	**3**
Encephalopathy grade*	none	1 or 2	3 or 4#
Ascites	absent	slight	moderate
Serum bilirubin (μmol/L)	<34.2	34.2 – 51.3	>51.3
Serum albumin (g/L)	>35	28 – 35	<28
Prolonged Prothrombin time	<4	4 – 6	>6

*Grade 0: normal consciousness, personality, neurological examination, electro encephalogram.
*Grade 1: restless, sleep disturbed, irritable/agitated, tremor, impaired handwriting, 5 cps waves.
*Grade 2: lethargic, time-disoriented, inappropriate, asterixis, ataxia, slow triphasic waves.
*Grade 3: somnolent, stuporous, place-disoriented, hyperactive reflexes, rigidity, slower waves.
*Grade 4: unrousable coma, no personality/behaviour, decerebrate, slow 2-3 cps delta activity.
#Patients with encephalopathy grade 3 and 4 are excluded.
Conversion from points to groups of different degrees of hepatic impairment:
Child-Pugh Grade A, mild hepatic impairment (5-6 points)
Child-Pugh Grade B, moderate hepatic impairment (7-9 points)
Child-Pugh Grade C, severe hepatic impairment (10-15 points)

The impact of hepatic impairment on relevant pharmacokinetic parameters of liraglutide is depicted below in **Figure 22**.

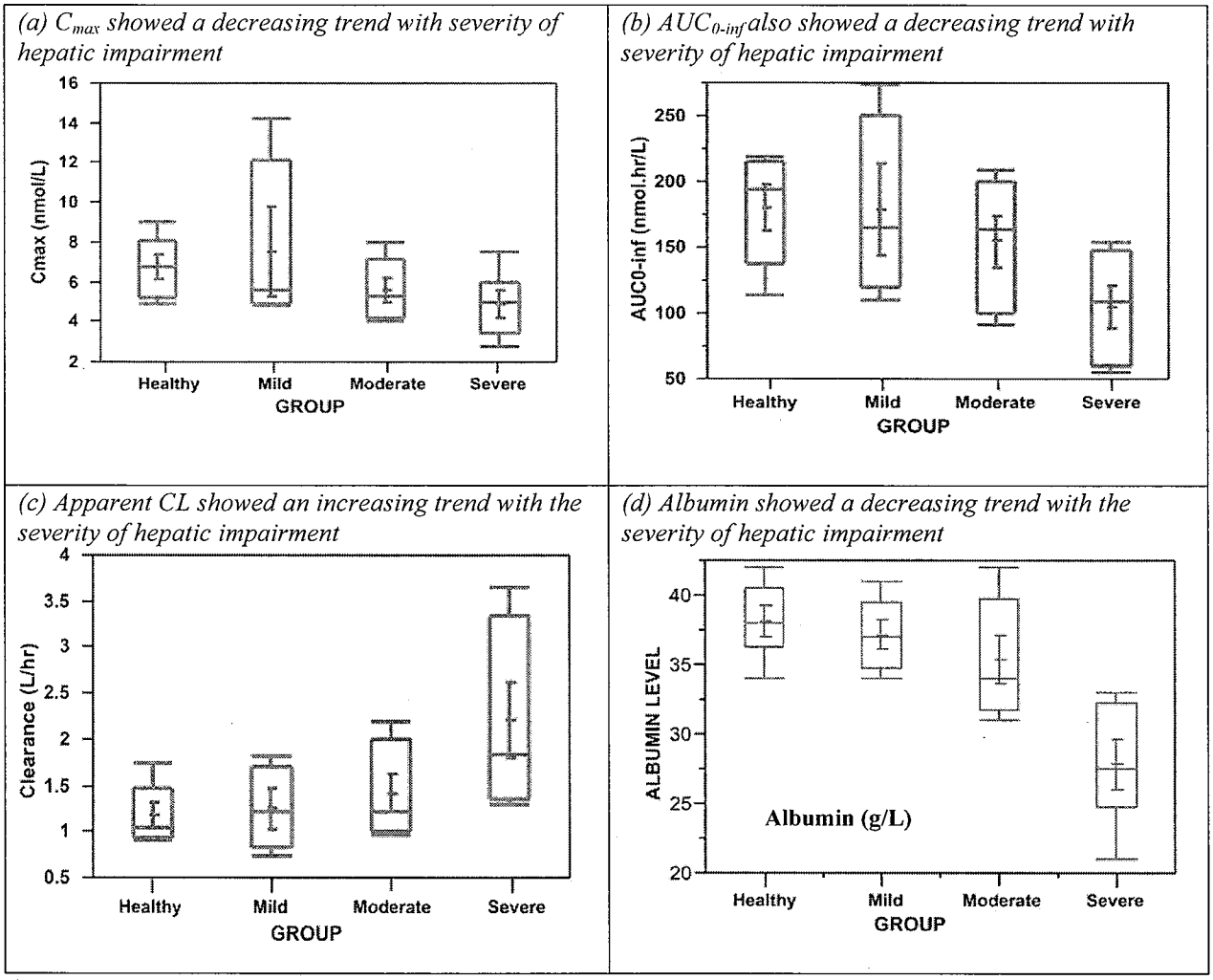


Figure 22 Liraglutide pharmacokinetic profile in healthy and hepatic impairment subjects after single 0.75 mg dose

Results of the statistical analysis of pharmacokinetic parameters from the original and updated analysis are summarized in the following table.

Table 13 Statistical comparison of $AUC_{0\text{-}inf}$ of liraglutide in healthy and hepatic impairment subjects

	Hepatic group		
	Mild/Normal	Moderate/Normal	Severe/Normal
Original Analysis			
AUC			
Estimate	0.77	0.87	0.56
Lower 90% limit	0.53	0.60	0.39
Upper 90% limit	1.11	1.25	0.81
Updated Analysis			
AUC			
Estimate	0.89	0.86	0.58
Lower 90% limit	0.57	0.60	0.40
Upper 90% limit	1.40	1.25	0.85

Results showed that:

- Equivalence with respect to AUC(0-∞) was not demonstrated between the groups of severely hepatically impaired subjects and healthy subjects (estimated ratio of 0.58 with a 90% confidence interval of [0.40, 0.85]), with severely hepatically impaired subjects having a lower exposure to liraglutide.
- Equivalence with respect to AUC(0-∞) was not demonstrated between any of the other groups of hepatically impaired subjects and healthy subjects either (estimated ratios and 90% confidence intervals of 0.89 [0.57, 1.40] and 0.86 [0.60, 1.25] for mild/normal and moderate/normal, respectively).
- Albumin was found to have a statistically significant effect on $AUC_{0\text{-}\infty}$ and does appear to explain the observed difference in clearance in severe hepatic impairment. Since the drug is highly protein bound, ~1.5 fold lower albumin content does go well with similar fold difference in clearance. However, no clear association between the unbound fraction of liraglutide and hepatic group was seen. Moreover, the group of subjects with severe hepatic impairment did not have a higher unbound fraction compared to the group of subjects with normal hepatic function.

Based on these findings:

- Severe hepatic impairment has an impact on the liraglutide pharmacokinetics in terms of two-fold increase in clearance. However, the exposure-response relationship seen in the efficacy studies suggests that the proposed doses are adequate in the hepatic impairment subjects.
- The altered protein binding is known to result into only a transient change in free drug concentration that is buffered by a compensatory change in clearance or a change in bioavailability. The restores the free drug concentration to its pre-alteration state, although the total drug concentration may be reduced. The findings with the liraglutide can be explained on this basis.
- Subjects with type 2 diabetes who also suffer from hepatic impairment can use standard treatment regimens for liraglutide and be dosed according to their glycaemic control.

2.4 Extrinsic Factors

2.4.1 What is the effect of different injection sites on the bioavailability of Liraglutide?

The relative bioavailability of liraglutide after s.c. administration in the thigh, upper arm and abdomen was assessed in a randomized, open-label, single-centre, three period, cross-over trial (Study 1745) where single doses of liraglutide were administered s.c. in the evening to 21 healthy male and female subjects on three different occasions, each separated by a 1-3 weeks wash-out period counting from time of dosing. The site of administration differed between the dosing days, either in the abdomen, thigh or upper arm. The dose was given as a fixed dose (0.60 mg) in a fixed volume (100 µl). The results are summarized in **Table 14** below. The pharmacokinetic profile of liraglutide and exposure by injection site data is presented in **Figure 23** and **Error! Reference source not found.**, respectively.

Table 14 Effect of injection site on liraglutide pharmacokinetics after single 0.6 mg SC administration in thigh, abdomen, and upper arm using formulation 4

	Thigh / Abdomen	Upper arm / Abdomen	Upper arm / Thigh
Original Analysis			
AUC(0-inf)			
Estimate	0.81	0.90	1.11
Lower 90% limit	0.76	0.83	1.03
Upper 90% limit	0.86	0.96	1.19
Updated Analysis			
AUC(0-inf)			
Estimate	0.78	0.87	1.10
Lower 90% limit	0.72	0.78	1.01
Upper 90% limit	0.86	0.96	1.21

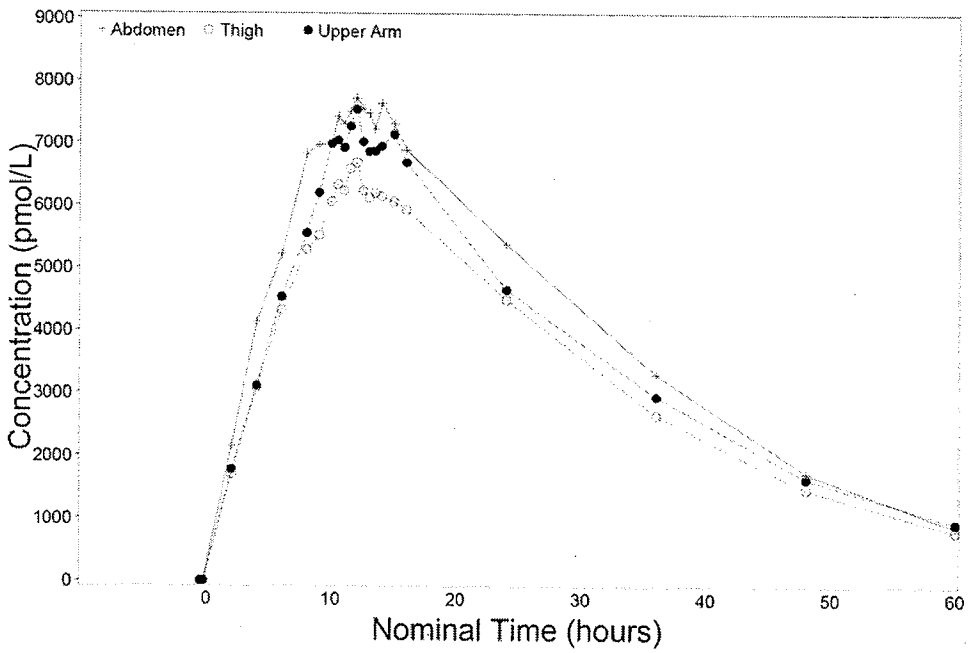


Figure 23 Mean liraglutide plasma concentrations from different injection sites

The relative bioavailability of liraglutide after s.c. administration was estimated as 81% in thigh versus abdomen, 90% in upper arm versus abdomen and 111% in upper arm versus thigh based on primary parameter $AUC_{0-\infty}$ as well as C_{max} and AUC_{0-t}. There was no effect on t_{max}. Even if thigh showed consistently lower exposures of liraglutide, the magnitude is not clinically meaningful. Based on these findings liraglutide can be administered interchangeably at these injection sites.

2.4.2 Drug-Drug Interactions

2.4.2.1 What is the CYP inhibition potential of Liraglutide?

The potential inhibitory effect of liraglutide (NNC 90-1170) on the important human drug metabolising cytochrome P450s was examined *in vitro* using human liver microsomes. The model substrate activities determined and the isoforms which these assessed were as follows: ethoxyresorufin-*O*-deethylase (CYP1A2), coumarin 7-hydroxylase (CYP2A6), paclitaxel 6α-hydroxylase (CYP2C8), tolbutamide 4- hydroxylase (CYP2C9), *S*-mephenytoin 4-hydroxylase (CYP2C19), bufuralol 1- hydroxylase (CYP2D6), *p*-nitrophenol hydroxylase (CYP2E1) and testosterone 6β-hydroxylase (CYP3A4). The summary of estimated IC50 values for liraglutide for human drug metabolizing cytochrome P450s is presented in table 15 below. This assessment was based on the fact that observed maximal inhibition of all the investigated CYP isoforms was 15.5 % at concentrations ranging from 0.1-100 μM liraglutide and with no dose dependency. Calculation of IC50 values is therefore was not applicable and it was concluded that a possible IC50 value could be >> 100μM for the investigated CYP isoforms.

Table 15 In vitro CYP enzyme inhibition potential of liraglutide

CYP	CYP substrate	Concentration (μM)	IC_{50} (μM)
1A2	7-Ethoxyresorufin	0.5	>100
2A6	Coumarin	5	>100
2C8	Paclitaxel	8	>100
2C9	Tolbutamide	150	>100
2C19	*S*-Mephenytoin	100	>100
2D6	Bufuralol	25	>100
2E1	*p*-Nitrophenol	100	>100
3A4	Testosterone	250	>100

Sponsor concluded that liraglutide at concentrations up to 100 μM did not inhibit or only very slight inhibited all the human cytochrome P450s studied (CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2D6, CYP2C19, CYP2E1 and CYP3A4). Thus, liraglutide is not expected to cause any drug-drug interactions related to inhibition of cytochrome P450s and we agree to sponsor's conclusions.

2.4.2.2 What is the effect of Liraglutide co-administration on the pharmacokinetics of other drugs?

Several drug interactions were evaluated by the sponsor with an objective to establish the effect of liraglutide on gastro-intestinal motility and how does it impact the pharmacokinetic profile of other drugs, especially those sensitive to these physiological changes.

Atorvastatin, Lisinopril, Griseofulvin, and Digoxin:
The summary of liraglutide drug interaction study designs are presented in **Table 16** below.

Table 16 Summary of Pharmacokinetic Drug Interaction Study Design

Study Design	Co-administered Drug	Dose	Dosing Duration	N
Part A: Randomized, double-blinded, placebo-controlled, two-way cross-over trial	Liraglutide/ Placebo	0.6 , 1.2 and 1.8 mg	Weekly increasing doses	40/42
	Atorvastatin	40 mg	Single at steady state of placebo/liraglutide 1.8 mg	40/42
	Lisinopril	20 mg	Single at steady state of placebo/liraglutide 1.8 mg	40/42
Part B: Randomized, double-blinded, placebo-controlled, two-way cross-over trial	Liraglutide/ Placebo	0.6 , 1.2 and 1.8 mg	Weekly increasing doses	27/28
	Griseofulvin	500 mg	Single at steady state of placebo/liraglutide 1.8 mg	27/28
	Digoxin	1 mg	Single at steady state of placebo/liraglutide 1.8 mg	27/28

The mean concentration profiles for atorvastatin, lisinopril, griseofulvin, and digoxin when co-administered at liraglutide steady state conditions compared to during placebo treatment are presented in figure below.

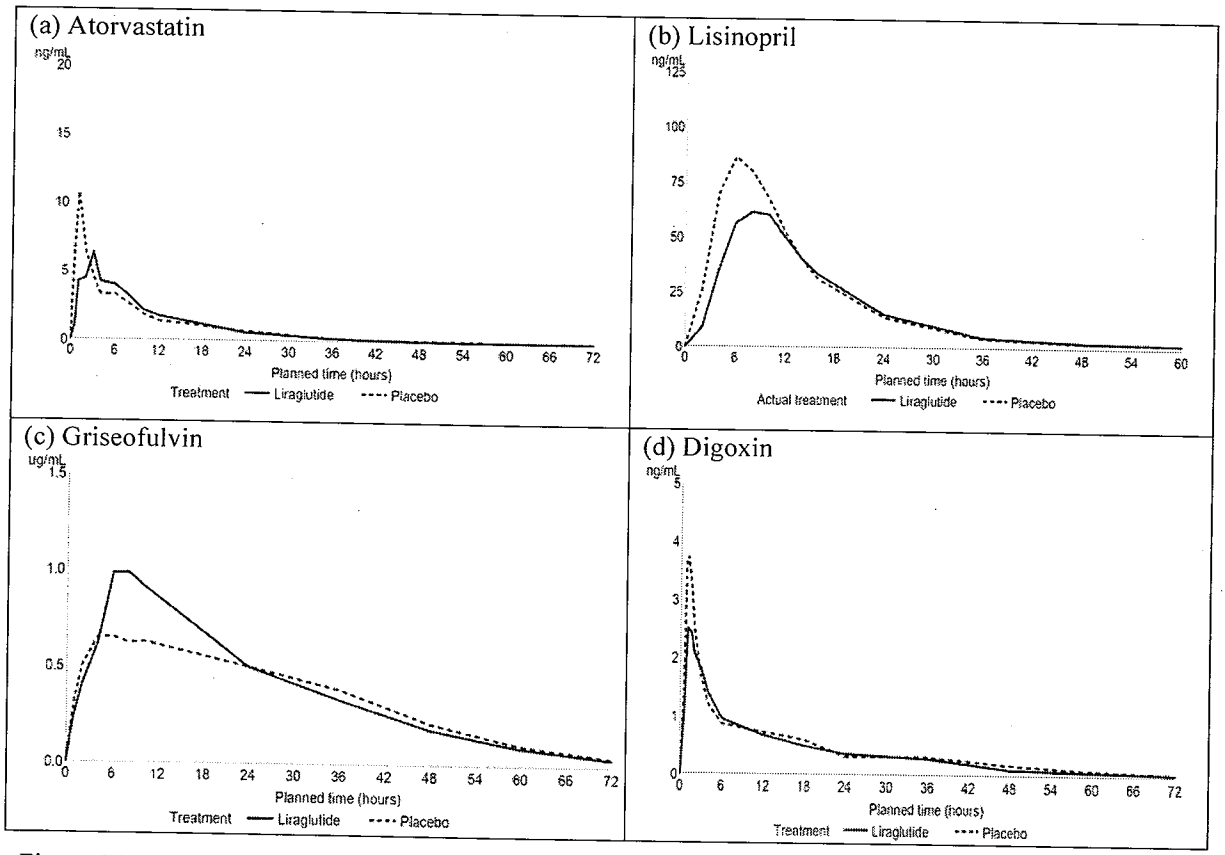


Figure 24 Mean concentration-time profiles for the co-administered drugs

The statistical analysis of pharmacokinetic parameters is summarized in the **Table 16** below.

Table 17 Effects of Liraglutide on Pharmacokinetic Parameters of Atorvastatin, Lisinopril, Griseofulvin, and Digoxin

		liraglutide/placebo
atorvastatin		
$AUC_{0-\infty}$	N	42
	Estimate	0.946
	Lower 90% limit	0.886
	Upper 90% limit	1.010
lisonopril		
$AUC_{0-\infty}$	N	40
	Estimate	0.849
	Lower 90% limit	0.747
	Upper 90% limit	0.966

		liraglutide/placebo
atorvastatin		
Cmax (ng/mL)	N	42
	Estimate	0.619
	Lower 90% limit	0.533
	Upper 90% limit	0.720
lisonopril		--
Cmax (ng/mL)	N	40
	Estimate	0.730
	Lower 90% limit	0.630
	Upper 90% limit	0.846

		liraglutide/placebo
griseofulvin		
AUC	N	22
	Estimate	1.096
	Lower 90% limit	1.013
	Upper 90% limit	1.185
digoxin		
AUC(0-72h)	N	27
	Estimate	0.843
	Lower 90% limit	0.722
	Upper 90% limit	0.984

		liraglutide/placebo
griseofulvin		
Cmax	N	27
	Estimate	1.369
	Lower 90% limit	1.243
	Upper 90% limit	1.507
digoxin		
Cmax	N	27
	Estimate	0.691
	Lower 90% limit	0.602
	Upper 90% limit	0.794

- Single concomitant administration of atorvastatin (40 mg) at steady-state of liraglutide (1.8 mg) resulted in equivalent $AUC_{0-\infty}$ as the 90% CI for the estimated ratio of $AUC_{0-\infty}$ (liraglutide/placebo treatment) was within the pre-specified limits for equivalence, i.e. within 0.80 to 1.25. For atorvastatin, equivalence was not demonstrated for C_{max} or $t_{½}$. Cmax was 38% lower, $t_{½}$ was 17% shorter and t_{max} appeared approximately 1.25 h later at liraglutide steady state conditions compared to during placebo treatment.

- Single concomitant administration of griseofulvin (500 mg) at steady-state of liraglutide (1.8 mg) resulted in equivalent $AUC_{0-\infty}$ as the 90% CI for the estimated ratio of $AUC_{0-\infty}$ (liraglutide/placebo treatment) was within the pre-specified limits for equivalence, i.e. within 0.80 to 1.25. However, equivalence was not demonstrated for C_{max}, which was 37% higher at liraglutide steady state conditions compared to during placebo treatment.

- For single administration of lisinopril (20 mg), equivalence was not demonstrated for $AUC_{0-\infty}$, when the drug was given at liraglutide steady state conditions compared to during placebo treatment. The $AUC_{0-\infty}$ for lisinopril was 15% smaller at liraglutide treatment than during placebo [estimated ratio 0.85 (90% CI (0.75; 0.97])]. The equivalence was also not demonstrated for C_{max} or Vz/F. C_{max} was 27% lower and V/F was 16% larger. The t_{max} appeared approximately 2 h later at liraglutide steady state conditions compared to during placebo treatment. Equivalence was demonstrated for t½.

- For single administration of digoxin (1 mg), equivalence was not demonstrated for AUC_{0-72h}, respectively, when the drug was given at liraglutide steady state conditions compared to during placebo treatment. The AUC_{0-72h} for digoxin was 16% smaller at liraglutide treatment compared to during placebo [estimated ratio 0.84 (90% CI (0.72; 0.98)]. For

digoxin, equivalence was also not demonstrated for $AUC_{0-\infty}$ or C_{max}. The $AUC_{0-\infty}$ was 21% lower and C_{max} was 31% lower when digoxin was given at liraglutide steady state conditions compared to during placebo treatment. The t_{max} for digoxin appeared approximately 1.1 h later at liraglutide steady state conditions compared to during placebo treatment.

- There were only minor changes in gastric pH over a 24-hour period

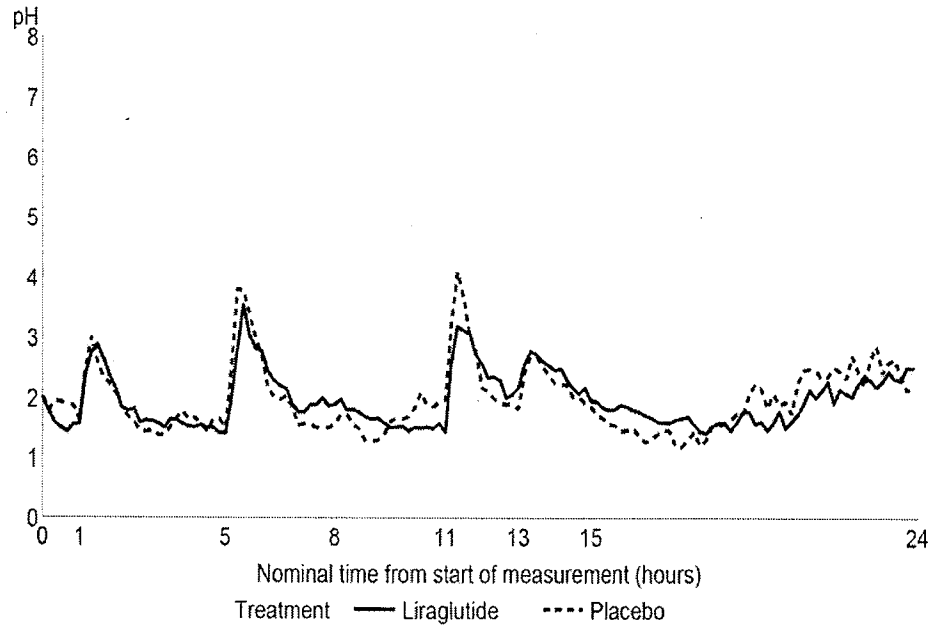


Figure 25 Effects of Liraglutide on the gastric pH

Paracetamol

Study design for the DDI evaluation for paracetamol is summarized in the **Table 18** below:

Table 18 Summary of Paracetamol Pharmacokinetic Drug Interaction Study Design

Study Design	Co-administered Drug	Dose	Dosing Duration	N
Randomized, placebo controlled, double-blind, two-period cross-over trial	Liraglutide/ Placebo	1.8 mg	Liraglutide: Weekly increments of 0.6 mg (0.6, 1.2 and 1.8 mg/day)	17/18
	Paracetamol	1 g	Single	

The mean concentration profiles for paracetamol when co-administered at liraglutide steady state conditions compared to during placebo treatment are presented in **Figure 26** below.

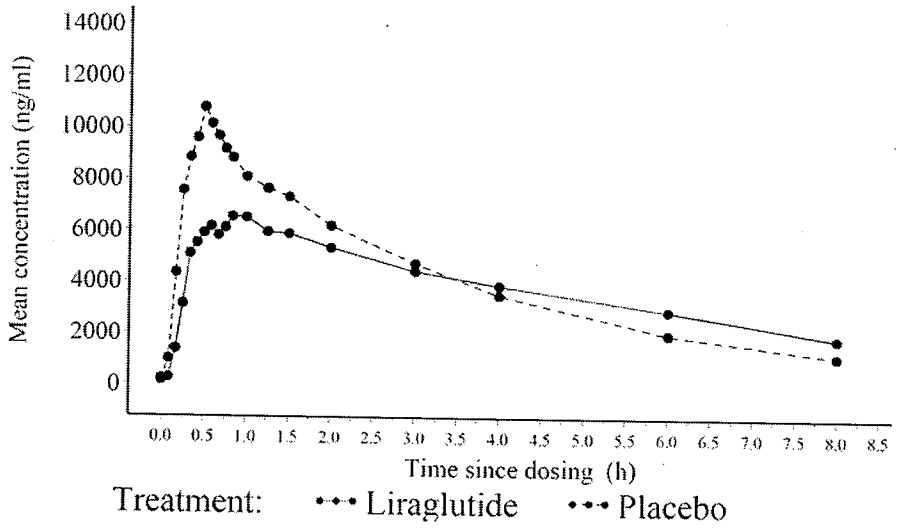


Figure 26 Effects of Liraglutide on the Pharmacokinetics of Paracetamol

Table 19 Summary of Primary and Secondary Pharmacokinetic Parameters of Paracetamol

Primary		Secondary	
	Liraglutide 1.8 mg/Placebo 1.8 mg		Liraglutide 1.8 mg/Placebo 1.8 mg
AUC(0-inf)		Cmax	
Estimate	1.04	Estimate	0.69
Lower 90% limit	0.97	Lower 90% limit	0.56
Upper 90% limit	1.10	Upper 90% limit	0.85
AUC(0-480)		tmax (min)	
Estimate	0.95	Estimate	15.00
Lower 90% limit	0.89	Lower 90% limit	0.00
Upper 90% limit	1.01	Upper 90% limit	92.50

Administration of paracetamol at tmax of liraglutide at steady state on the 1.8 mg dose level showed equivalence on the primary endpoints $AUC_{0-\infty}$ and $AUC_{0-480min}$, reflecting the overall exposure of paracetamol. The analysis of C_{max} of paracetamol did not demonstrate equivalence. Cmax was about 31% lower after liraglutide treatment compared with placebo indicating a delayed rate of initial absorption thus representing a delay in gastric emptying. T_{max} of paracetamol occurred later after liraglutide treatment compared to placebo, however, the estimated median difference between tmax corresponding to the two treatments was limited to 15 min.

Interpretation of DDI evaluation for five representative BCS class drugs is summarized in the **Table 20** below:

Table 20 Expected and Observed effects of liraglutide co-administration on four representative BCS class drugs

Drug (BCS CLASS)	Solubility/ Permeability	Expected Effect of Reduction in GI Motility on PK	Observed Effect of GI Motility Change on PK
Paracetamol (Class I)	High solubility and permeability	↓ Cmax, unchanged AUC and tmax	31%↓ Cmax, unchanged AUC and tmax
Atorvastatin (Class II)	Low solubility and High permeability	↑ Cmax, delayed tmax, increased or unchanged AUC [↑ residence time in the stomach: higher degree of dissolved drug available for absorption immediately after entrance into the small intestine]	38%↓ Cmax, unchanged AUC, tmax ↑ of 1.25 h [Dissolution occurs at neutral pH in intestine]
Griseofulvin (Class II)	Low solubility and High permeability	↑ Cmax, delayed tmax, increased or unchanged AUC [↑residence time in the stomach: higher degree of dissolved drug available for absorption immediately after entrance into the small intestine]	37% ↑ in Cmax, unchanged AUC and tmax
Lisinopril (Class III)	High solubility and Low permeability	unchanged AUC, ↓ in Cmax and delayed tmax [intestinal permeability is rate limiting]	27%↓ Cmax, delayed tmax (2 h), 15%↓ AUC [no intestinal transit time prolongation]
Digoxin (Class IV)	Low solubility and permeability	whether solubility or permeability is the rate limiting step in the overall absorption	↓ Cmax (31%) and delayed tmax (1.125 h), [intestinal permeability is rate limiting], 16%↓ AUC [no intestinal transit time prolongation]

Oral Contraceptive (levonorgestrel and ethinylestradiol)

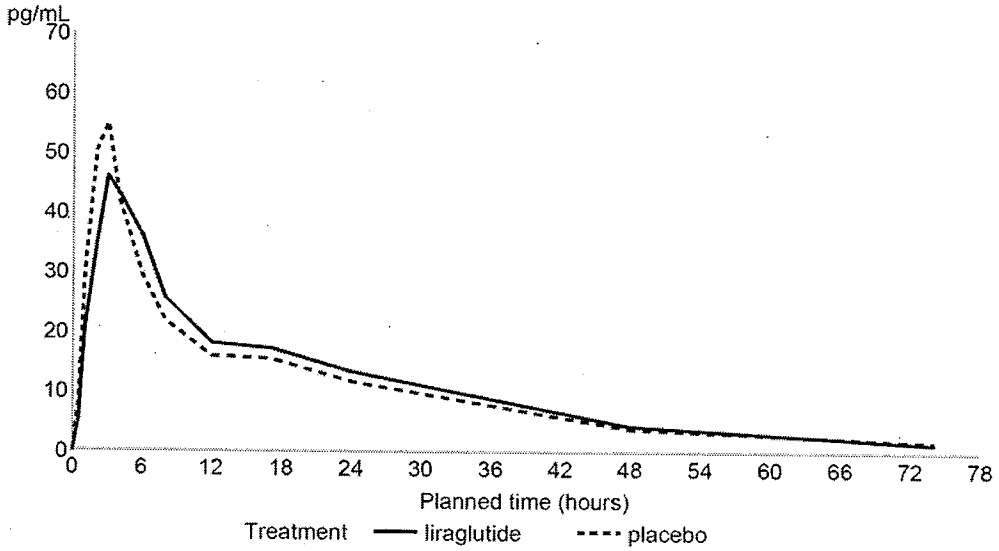
Study design for the DDI evaluation for oral contraceptive is summarized in the **Table 21** below:

Table 21 Summary of Oral Contraceptive Pharmacokinetic Drug Interaction Study Design

Study Design	Co-administered Drug	Dose	Dosing Duration	N
Randomized, placebo controlled, double-blind, two-period cross-over trial	Liraglutide/ Placebo	1.8 mg	Weekly increments of 0.6 mg (0.6, 1.2 and 1.8 mg/day)	
	Neovletta®; ethinylestradiol and levonorgestrel	Ethinylestradiol: 0.03 mg; Levonorgestrel: 0.15 mg	Single	14/21 21/21

The effect of liraglutide on pharmacokinetics of ethinylestradiol and levonorgestrel is presented in **Figures 27a and 27b**, respectively. The effect of liraglutide on the absorption of an orally administered contraceptive drug (Neovletta®) was investigated at highest steady state dose of liraglutide (1.8 mg). The oral contraceptive administered after 7 hours of liraglutide administration resulted in mean plasma ethinylestradiol and plasma levonorgestrel concentration time profiles that were characterized by a reduced C_{max} and t_{max}.

(A) Ethinylestradiol



(B) Levonorgestrel

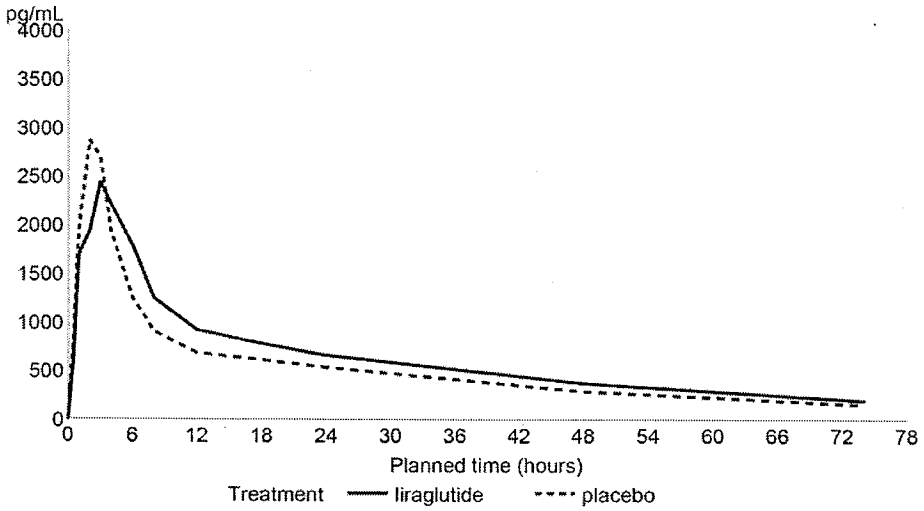


Figure 27 Mean Plasma ethinylestradiol (A) and levonorgestrel (B) following single dose administration of oral contraceptive (Neovletta; 0.03mg ethinylestradiol, 0.15 mg levonorgestrel)

The effect of liraglutide on the primary PK parameter ($AUC_{0-\infty}$) of ethinylestradiol and levonorgestrel is summarized in Table 21. $AUC_{0-\infty}$ was not calculated if the extrapolated part was more than 20% of the total AUC. This was observed with 3 ethinylestradiol profiles and 18 levonorgestrel profiles.

Table 22 Statistical Comparison between Treatments (Liraglutide/Placebo) for Ethinylestradiol and Levonorgestrel

		liraglutide/placebo
ethinylestradiol		
$AUC_{0-\infty}$	N	21
	Estimate	1.057
	Lower 90% limit	0.988
	Upper 90% limit	1.131
levonorgestrel		
$AUC_{0-\infty}$	N	14
	Estimate	1.182
	Lower 90% limit	1.040
	Upper 90% limit	1.343

After statistical analysis for ethinylestradiol, equivalence was demonstrated with respect to $AUC_{0-\infty}$ as the 90% CI for the estimated ratio of $AUC_{0-\infty}$ (liraglutide/placebo treatment) was within the pre-specified limits for equivalence, i.e. within 0.80 to 1.25.

However, for levonorgestrel, equivalence was not demonstrated with respect to $AUC_{0-\infty}$ as the 90% CI for the estimated ratio of $AUC_{0-\infty}$ (liraglutide/placebo treatment) was outside the pre-specified limits for equivalence. The estimated ratio of $AUC_{0-\infty}$ (liraglutide/placebo treatment)

was 1.18 and the 90% CI was 1.04 to 1.34; i.e. the levonorgestrel $AUC_{0-\infty}$ was 18% higher during liraglutide treatment.

2.5 General Biopharmaceutics

2.5.1 What is absolute bioavailability and disposition of Liraglutide?

On average the absolute bioavailability of liraglutide is around 55% following s.c. dose. This was assessed from the comparison of AUCs from an IV dose of 5 µg/kg, given as 1 hour infusion to 5 subjects. These subjects were also given a s.c. dose of 5 µg/kg at a different occasion for AUC comparison.

Table 23 Pharmacokinetic parameters of liraglutide after intravenous administration

Dose µg/kg		$AUC_{0-\infty}$ pmol*h/L	AUC_{0-t} pmol*h/L	$t_{½}$* h
5	Mean	215674	211251	8.1
	SD	42519	40520	-

*Harmonic mean

Table 24 Absolute bioavailability (%) of liraglutide after subcutaneous administration

Dose µg/kg	Subject	AUC pmol*h/L	AUC_{0-t} pmol*h/L	F %
5	Mean	134076	117244	55
	SD	108073	93978	37

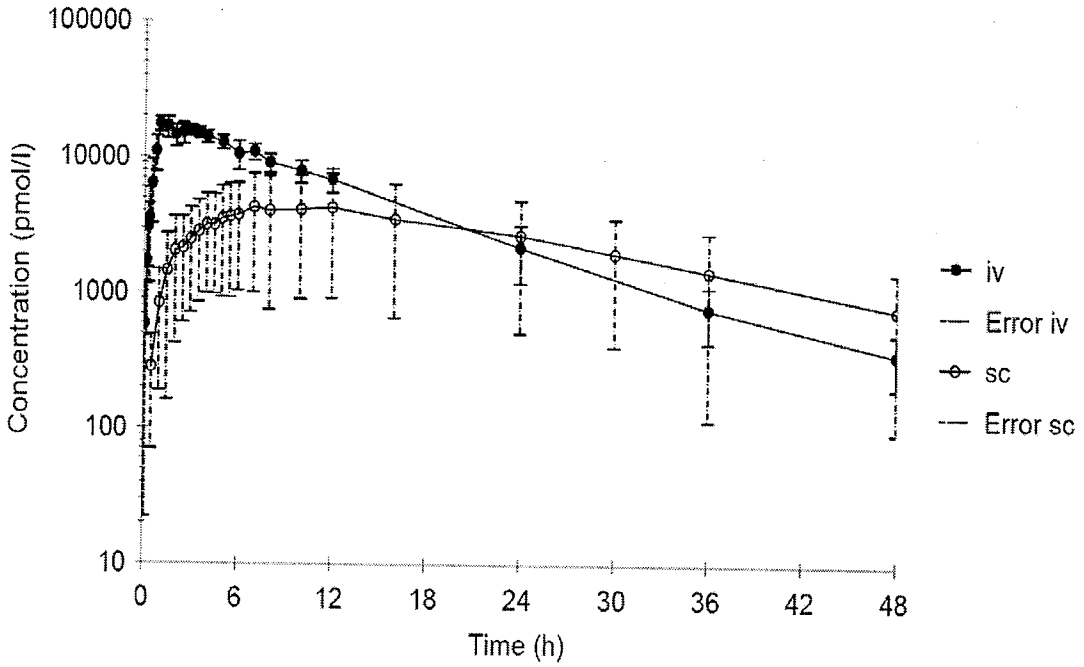


Figure 28 Mean plasma liraglutide profiles after 5 µg/kg liraglutide given as 1 hour IV infusion and as single SC administration

2.5.2 Is bioequivalence established between the to-be-marketed formulation and the Phase 3 trial formulation and how does it relate to the overall product development?

Throughout the development of liraglutide, changes were made within chemistry, manufacturing and control to give a more robust drug substance manufacturing process suitable for commercial production. In addition, the drug product formulation and manufacturing process were gradually modified to [redacted] b(4)

[redacted] of the liraglutide formulation 2, used in the trials NN2211- 1326, 1551 (phase 1) and 1310, 2072, 1499, 1571 (phase 2), a new formulation of liraglutide at pH 7.7 (formulation 3) was produced. The bioequivalence of liraglutide formulations 2 and 3 was demonstrated in trial NN2211-1331. The liraglutide formulation 3 was produced at three different pH (pH 7.7, 7.9 and 8.15), and their bioequivalence was demonstrated in trial NN2211-1636. The liraglutide formulation 3 (pH 7.7) was used in trials NN2211-1328, 1329, and 1334 (phase 1 and 2). [redacted] liraglutide formulation 4 (pH 8.15). Formulation 4 was found to be bioequivalent with formulation 3 in trial NN2211-1693. Formulation 4 has been used in phase 3a trials in the EU and US.

In liraglutide final formulation 4, the drug substance manufacturing process was optimized and the drug product manufacturing process has been up scaled from [redacted]. Final formulation 4 is the formulation planned to be used in phase 3b trials and the formulation planned to be marketed. The bioequivalence between the to-be-marketed and the Phase 3 trial formulations was established in the definitive bioequivalence study (Study NN2211-1692). This was a randomized, double-blind, single-centre, two-period, cross-over trial designed to test for b(4)

bioequivalence between the phase 3a formulation of liraglutide (formulation 4) and the phase 3b formulation (final formulation 4).

Two single s.c. doses of liraglutide were administered to healthy male and female subjects in the evening on two different occasions, separated by a 14 (±2) day wash-out period. The liraglutide formulations, each in a dose of 0.72 mg, were given in the abdomen as a fixed volume of 120 µL (12 clicks) from a FlexPen®. All subjects were administered liraglutide at approximately 9-10 pm. The rationale for evening administration was to utilize the pharmacokinetic profile of the drug with Cmax at 10-13 h after administration. Serial blood samples for estimation of the liraglutide plasma concentrations were drawn at Visit 2 and 3; before dosing at -30 and -15 minutes, and at 2, 4, 6, 8, 9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 15, 16, 24, 36, 48 and 60 h after dosing.

The summary of various bioequivalence assessments carried out for various stages of liraglutide formulation development is presented in **Table 25.**

Table 25 Results of bioequivalence analysis from milestone BE evaluations including the pivotal study (Study NN2211-1692) from original submission

Trial ID N (M/F)	Formulation (Dose)	Estimate [90% CI]			
		Relative AUC0-∞	Relative AUC0-t	Relative Cmax	Difference Tmax (h)
1331 22 (13/9) **1636** 24 (13/11)	Formulation 3 vs. 2 (1 mg) Formulation 3 (0.75 mg)	0.98 [0.92; 1.04]	0.98 [0.92; 1.04]	0.96 [0.89; 1.04]	-0.25 [-1.00; 0.25]
	pH 7.9 vs. pH 7.7	0.94 [0.90; 0.98]	0.93 [0.89; 0.98]	0.93 [0.88; 1.00]	-0.75 [-2.00; 0.25]
	pH 8.5 vs. pH 7.7	1.02 [0.98; 1.07]	1.02 [0.97;1.07]	1.02 [0.96; 1.09]	-0.50 [-2.00; 1.00]
1693 22 (19/3)	Formulation 4 vs. Formulation 3 (0.71 mg, s.c.)	1.06 [1.00; 1.12]	1.06 [1.00; 1.13]	1.04 [0.95; 1.13]	0.00 [-1.50; 1.00]
1692 21 (19/2) **(Original)**	Formulation 4 vs. Final Formulation 4 produced with the final drug substance manufacturing process (0.72 mg, s.c.)	0.99 [0.92; 1.07]	0.99 [0.92; 1.06]	1.02 [0.91; 1.14]	-0.50 [-1.50; 0.75]

Based on the statistical analyses

- Liraglutide formulation 4 and liraglutide final formulation 4 were bioequivalent with respect to the primary endpoints AUC_{0-t} and C_{max}.
- The bioequivalence were also demonstrated for intermediate changes in the formulations.

While this review was being complied, the bioanalytical site for the pivotal bioequivalence study (Study NN2211-1692) was audited by the Division of Scientific Investigation (DSI). There were serious deficiencies identified during the audit, and based on the conclusions of DSI review, the reliability of analytical data and hence the study results became uncertain as the laboratory need to re-assess the analytical data using an unbiased acceptance and rejection criteria for all analytical runs. The DSI also cited serious deficiencies during the inspection of clinical site in Lund University Hospital, Lund, Sweden. Two separate information requests were issued to the sponsor for resolution of these issue for the BE evaluation. Division has also communicated its

concern to the sponsor regarding the other clinical pharmacology studies for which, the same laboratory has performed liraglutide assay. Please see DSI memos dated 02/18/2009 and 03/09/09 in DFS for further details.

Sponsor submitted their response to the information request letters (see 03/27/2009 and 03/30/2009 in EDR), and provided reanalysis of pivotal BE results and other clinical pharmacology study results that were used to make labeling claims.

Prior to the review of the raw data, Sponsor decided to consistently adhere to the assay run acceptance criteria set by ——————————— at the time of sample analysis. Accordingly, the following objective acceptance criteria were used for evaluation of each analytical run: b(4)

Acceptance criteria – Calibration curve:

- The mean (n=2) of the back-calculated calibrators shall be within ±20% of the nominal values, except for calibrators at concentrations <129 pmol/L, where the mean (n=2) of the back-calculated calibrators shall be within ±30% of the nominal values
- A maximum of 3 calibration levels may be rejected
- No single calibrator determination can be excluded unless the mean of the double determination is outside ±30% (<129 pmol/L) or ±20% (≥129 pmol/L), and the CV% of the double determination is >30%. The single determination excluded must be the determination deviating most from the nominal value
- Exclusion of single determinations for calibrators were allowed where technical errors were annotated the raw data

Acceptance criteria – QC samples:

- Maximally one mean QC sample from each QC level may have an inaccuracy greater than ±20% from the target value, and in total, no more than two out of six mean determinations of QC-samples (2 x low, 2 x medium and 2 x high) may have an inaccuracy greater than ±20% from the target values
- Exclusion of single determinations for QC samples were allowed where technical errors were annotated the raw data

All assay runs not meeting the above criteria were rejected.

The results of the original (full data) and the revised BE analysis (based on the reduced data-set) are summarized in Table 26 below.

Table 26 Summary of bioequivalence analysis for pivotal BE study 1692

		Original Analysis Final Formulation 4 / Formulation 4	Updated Analysis Final Formulation 4 / Formulation 4
AUC(0-t)			
	Estimate	0.99	1.01
	Lower 90% limit	0.92	0.91
	Upper 90% limit	1.06	1.12
Cmax			
	Estimate	1.02	0.97
	Lower 90% limit	0.91	0.88
	Upper 90% limit	1.14	1.07

Based on the statistical analyses of reduced data set it can be concluded that

- Liraglutide formulation 4 and liraglutide final formulation 4 were bioequivalent with respect to the primary endpoints AUC_{0-t} and C_{max}.

2.6 Analytical

2.6.1 Is the analytical method for Liraglutide appropriately validated?

The liraglutide assay was validated for analyzing liraglutide in plasma and serum samples in terms of recovery, linearity, accuracy, precision and sensitivity. The storage stability was demonstrated for a maximum period of two years with no decrease in the measured liraglutide concentrations. The presence of GLP-1/liraglutide antibodies decreased the response whereas the presence of haemolysis in the samples increased the analyzed liraglutide concentrations. Liraglutide in plasma was analyzed using a specific enzyme-linked immuno-sorbent assay (ELISA) that measured both protein bound and unbound liraglutide. The ELISA was a sandwich immunoassay with two monoclonal antibodies directed against different epitopes on liraglutide. The capture antibody, coated on the microtitre plate, was directed against the N-terminal part of the amino acid chain. The detection antibody, labeled with biotin, was directed against the C-terminal part. Cross-reactivity with native glucagon-like peptide-1 (GLP-1) was eliminated by degradation of native GLP-1 by pre-incubation of the plasma sample for 4 hours at 37°C. Liraglutide was shown to remain intact at these conditions.

Table 27 Summary of analytical method used for the CPB studies

Parameter	Results	Study ID
Range	——— pmol/L	NN990090
Recovery	Within the limits of ———	NN990090
Dilution linearity	Dilution ——— within the limits of ———	NN990090
Intermediary precision	——— %	NN990090
Sensitivity	LLOQ = 18 pmol/L Limit of detection = 3 pmol/L	NN990090
Stability	Frozen samples for 2 years ———	NN990092 NN990091
Interference	Presence of antibodies against GLP-1/liraglutide Occurrence of ———, in samples	NN990091

LLOQ: Lower limit of quantification

b(4)

The ELISA assay was developed and validated by Novo Nordisk A/S. All analyses were performed at Novo Nordisk A/S or ——— . A cross-validation was performed to transfer the assay from Novo Nordisk A/S to ———

b(4)

Antibodies against GLP-1 were shown to interfere with the ELISA assay. However, only very few subjects in the phase 2 and 3 trials had GLP-1/liraglutide binding antibodies, and the antibody levels in these subjects were low, suggesting that bias due to interference from antibodies is limited. Another source of potential interference with the assay was ———in the samples which has been shown to increase the measured concentrations. ——— samples were, however, excluded from the pharmacokinetic assessments.

b(4)

27 Page(s) Withheld

_____ Trade Secret / Confidential (b4)

✓ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

*Withheld Track Number: Clin Pharm/Bio-*_____

4.2 Individual Study Reviews

4.2.1 Initial PK and Tolerability Study in Healthy Subjects

Single Dose (NN2211-1149):

Title of Trial	A randomised, double-blind, placebo-controlled, dose escalation trial of single doses of NNC 90-1170 to assess tolerability, pharmacokinetics, pharmacodynamics and absolute bioavailability in healthy male subjects
Trial ID	NN2211-1149
Development Phase	I
Generic Name	NNC 90-1170
Indication	Type-2 Diabetes
Investigator	Paul Rolan, MD, FRACP, FFPM
Trial Site	Medeval Ltd Skelton House Manchester Science Park Lloyd Street North Manchester M15 6SH
Trial Initiated	25 March 1999
Trial Completed	20 December 1999
Sponsor	Health Care Development Novo Nordisk A/S Novo Allé 2880 Bagsværd, Denmark
International Trial Manager	Grethe Jakobson, MSc, Novo Nordisk A/S
International Clinical Trial Manager	Boldil Elbrønd, MD, Novo Nordisk A/S
Local Trial Manager	Michaela Viel, Novo Nordisk
Statistician	Soren Larsen, MSc, Novo Nordisk A/S
Report Writer	[redacted] Medeval
Report Date	10 August 2000

b(4)

Title of Study: A randomized, double-blind, placebo-controlled, dose escalation trial of single doses of NNC 90-1170 to assess tolerability, pharmacokinetics, pharmacodynamics and absolute bioavailability in healthy male subjects

Objective:

Primary Objectives:

- To assess the safety and tolerability (maximum tolerated dose) after ascending 8 s.c. single doses from 1.25 µg/kg up to 20 µg/kg of NNC 90-1170 and after a single i.v. dose of 5µg/kg.

- To assess the pharmacokinetic profile at all dose levels following s.c. administration and following 5 µg/kg i.v. administration. The absolute bioavailability of 5 µg/kg s.c. dose was estimated using the i.v. data.

Secondary Objectives:

- To assess the pharmacodynamics of NNC 90-1170 using an intravenous glucose tolerance test (IVGTT).

- To evaluate the effect of NNC 90-1170 on diuresis and serum leptin concentrations at all dose levels.

Methodology:

This was a single-centre, randomized within dose group, double-blind, placebo-controlled, parallel groups dose-escalation trial of 8 subjects at each dose level (6 active, 2 placebo). NNC 90-1170 and placebo were administered as single s.c. doses of 1.25 µg/kg up to 20 µg/kg with subjects receiving the dose level 5 µg/kg s.c. in addition received, after a wash-out of at least 7 days, a single i.v. dose of 5 µg/kg, or corresponding placebo. In addition, three subjects at each dose level (2 active and 1 placebo) received an IVGTT for 2 hours, starting approximately 9 hours post-dosing.

Number of Subjects (Planned and Analyzed):

Sixty-four (64) healthy male subjects were planned for inclusion. Seventy-two (72) were randomized following the repeat of the 1.25 µg/kg dose level. All 72 subjects completed the trial. Healthy male volunteers of any ethnic origin between 18 and 45 years of age, inclusive were included in the study.

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

NNC 90-1170, 5 mg/ml provided in 1.5 ml PenFill® cartridges for subcutaneous injection (Batch No. 317901) was used.

Pharmacokinetic Assessment:

Following assessments were made: Forty-eight (48) hour NNC 90-1170 profiles and 24-hour glucose, insulin, glucagon, leptin and diuresis profiles in all subjects and 2-hour profiles for glucose and insulin in subjects receiving IVGTT between 9 and 11 hours post-dosing.

Safety:

Blood glucose measurements, adverse events, clinical laboratory tests (haematology, biochemistry, urinalysis), physical examination, vital signs including temperature, blood pressure, pulse and ECG.

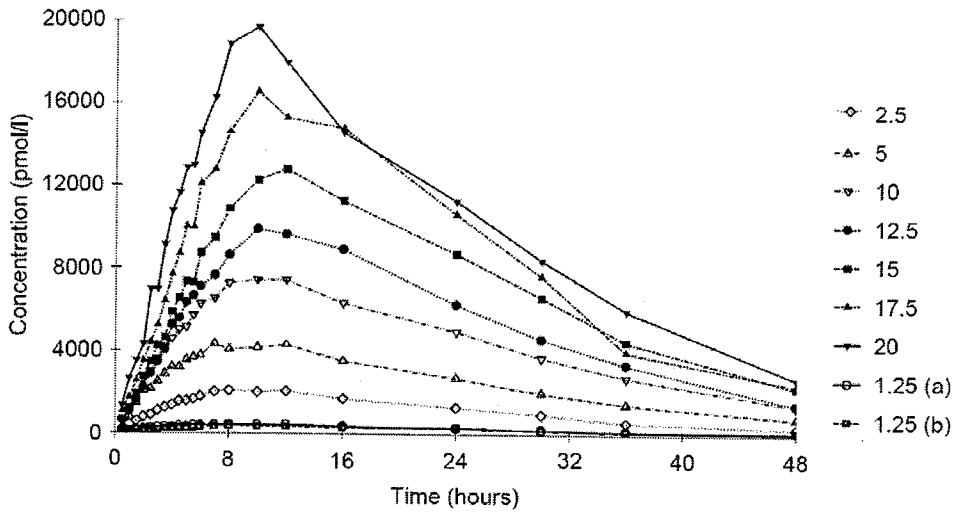
Statistical Methods:

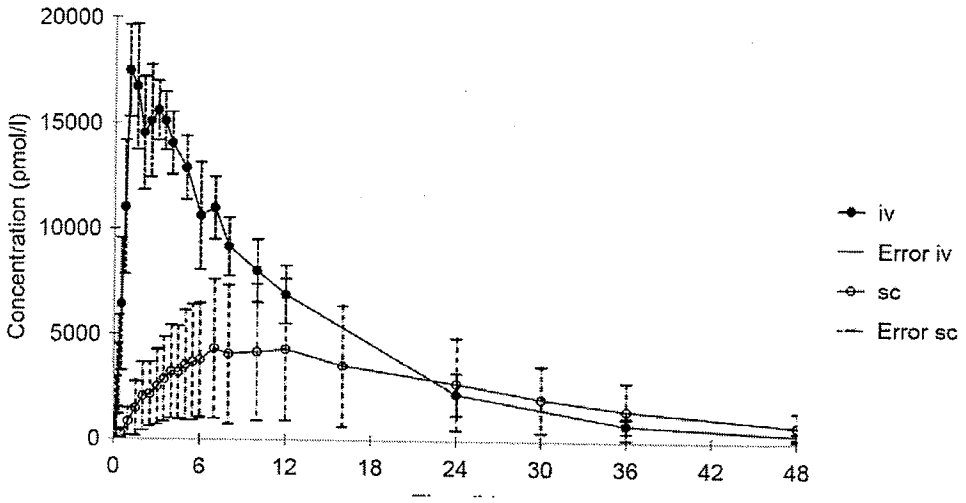
NNC 90-1170 Pharmacokinetics: Non-compartmental analysis was performed and the primary endpoint $AUC_{0-\infty}$ and the secondary endpoints C_{max}, t_{max}, λz and Vz/F (Vz following i.v.) were derived from the 48-hour plasma profiles. Except for t_{max}, all parameters following s.c. administration were logarithmically transformed prior to analysis and then subjected to an analysis of variance (ANOVA). Following back-transformation, estimated least squares means for each dose level with corresponding 95% confidence intervals (CI) were calculated. t_{max} was subjected to non-parametric analysis using Wilcoxon test. Dose proportionality was assessed for $AUC_{0-\infty}$ and C_{max} by performing regression analysis on log transformed parameter and log-transformed dose. An estimate of the slope of the regression line and corresponding 95% CI were calculated and dose proportionality was assumed if the slope was not statistically significantly different from unity.

Pharmacokinetic:

NNC 90-1170 Pharmacokinetics

Illustrated below are mean plasma concentration time profiles following 8 s.c. doses of NNC 90-1170 as well as following a 1-hour i.v. infusion and s.c. administration of 5 µg/kg.





The absolute bioavailability for the 5 µg/kg s.c. dose was 55%. The statistical analysis showed a dose-proportional increase in C_{max} and $AUC_{0-\infty}$ for doses between 2.5 and 20 µg/kg, but not when data from the 1.25 µg/kg dose level are included.

Results of dose proportionality assessment for C_{max} and $AUC_{0-\infty}$ using data from all dose levels

Parameter	Slope*	Lower 95% CI	Upper 95%CI	p-value
C_{max}	1.283	1.168	1.399	0.00002
$AUC_{0-\infty}$	1.263	1.153	1.374	0.00003

*Slope is different from unity based on 95% CI and p-value.

Results of dose proportionality assessment for C_{max} and $AUC_{0-\infty}$ excluding individual C_{max} and $AUC_{0-\infty}$ from the 1.25 µg/kg dose level

Parameter	Slope*	Lower 95% CI	Upper 95%CI	p-value
C_{max}	1.074	0.912	1.237	0.256
$AUC_{0-\infty}$	1.091	0.943	1.239	0.181

*Slope is not significantly different from unity based on 95% CI and p-value.

Safety Results:

A higher proportion of the subjects had AEs following the various doses of NNC 90-1170 (50-100%) than placebo (39-50%). Most of the AEs at all dose levels were related to the central and peripheral nervous system (dizziness and headache) following NNC 90-1170 (17-67%) and placebo (28%). All 6 subjects receiving 20 µg/kg NNC 90-1170 experienced gastrointestinal system disorders. The vast majority of the adverse events were mild with probable or possible

relation to NNC 90-1170. There were no SAEs and no AE related withdrawals. There were three adverse events of severe intensity which were headache, nausea and vomiting, one was following 20 µg/kg NNC 90-1170 and two following placebo.

Sponsor's Conclusions:

- The absorption of NNC 90-1170 following s.c. administration was slow reaching a maximum approximately 9-12 hours post-dosing.

- The increase in C_{max} and $AUC_{0-\infty}$ of NNC 90-1170 was dose-proportional in the investigated dose range of 2.5-20 µg/kg s.c., but not when including data from the 1.25 µg/kg dose level.

- The absolute bioavailability of s.c. NNC 90-1170 was calculated to be 55% at 5 µg/kg.

- For glucagon, the overall and within dose analysis of active versus placebo for all subjects and for subjects receiving IVGTT only, showed no statistically significant trend towards a difference.
- For glucose, the overall analysis of the active versus placebo in all subjects showed a trend towards lower average glucose levels following active treatment (p = 0.0538 for AUC9-11, Glucose/2 and p=0.0658 for AUC0-9, Glucose/9). However, there was no trend towards a difference in subjects receiving IVGTT.
- For insulin, the overall analysis of active versus placebo in all subjects showed no trend towards a difference. However, in subjects receiving IVGTT there was a clear trend (overall and with dose levels) towards higher insulin levels following active treatment.
- Serum leptin levels were not significantly affected by NNC 90-1170, compared to placebo, at any of the dose levels.
- In general, diuresis was not significantly affected by NNC 90-1170, compared to placebo, at any of the dose levels.
- There were no SAEs and no adverse event related discontinuations or withdrawals.
- There were no safety concerns for single doses up to the 17.5 µg/kg dose level.
- AEs were more frequent and intense at the 20 µg/kg dose level and this dose level was poorly tolerated.
- Hence, a single s.c. dose of 17.5 µg/kg was the maximum tolerated dose of NNC 90-1170 in healthy male volunteers.

Reviewer's Comment:

Overall, the study conduct and assessments were appropriate and the concentration data was supported by the analytical method. However, sponsor reported in another PK study in Japanese subjects that there was 10% degradation of the active ingredient in the formulation used for the study. Hence, the CL/F and V/F values were not reliable from this study, though it did not affect the dose-proportionality assessment. There were no other major protocol violations affecting the study outcome. The sponsor's conclusions regarding the other PK parameters (Cmax, AUC, $t_{1/2}$) are reasonable from a clinical pharmacology perspective.

Single and Multiple Dose (NN2211-1189):

TITLE OF TRIAL A Randomised, Double-Blind, Placebo-Controlled, Dose Escalation, Parallel-Group, Single and Multiple Dose Trial of NNC 90-1170 in Healthy Volunteers and Patients with Type 2 Diabetes to Assess Tolerability, Pharmacokinetics and Pharmacodynamics
INVESTIGATOR Paul Rolan, MD, FRACP, FFPM
TRIAL SITE Medeval Ltd, Skelton House, Manchester Science Park, Lloyd Street North, Manchester M15 6SH, UK
PUBLICATIONS None

TRIAL PERIOD	**DEVELOPMENT PHASE**
4 August 1999 till 23 December 1999	Phase I

OBJECTIVES

Healthy Volunteers and Type 2 Diabetic Patients:

Primary Objectives

- To assess the safety and tolerability after single and multiple s.c. doses of NNC 90-1170 in healthy volunteers and in patients with Type 2 diabetes

Secondary Objectives

- To assess the multiple dose pharmacokinetics of NNC 90-1170
- To evaluate the effect of NNC 90-1170 on diuresis and leptin plasma concentrations in all subjects at all dose levels
- To assess the effect of NNC 90-1170 on calorie intake and evaluate changes in body mass index and weight
- To assess antibody production against NNC 90-1170; and IgE levels following NNC 90-1170

Type 2 Diabetic Patients:

- To assess the pharmacodynamics of NNC 90-1170 in Type 2 diabetic patients only, by measuring plasma glucose, glucagon and insulin profiles on Days 1,5, 8 and 11, and performing an IVGTT at expected C_{max} of NNC 90-1170 on Day 0 (baseline), Days 5 and 11.

Healthy Volunteers:

- To assess the effect of NNC 90-1170 on insulin and glucose levels in healthy volunteers.

METHODOLOGY

This was a single-centre, randomised within dose group, double-blind, placebo-controlled, parallel-groups dose-escalation trial, with initial single, and subsequent multiple s.c. doses of NNC 90-1170 to subjects at five levels (1.25, 5, 7.5, 10 and 12.5 µg/kg). Twelve subjects were to be enrolled at each dose level, divided into 6 healthy volunteers and 6 Type 2 diabetic patients, randomised within the group at an active:placebo ratio of 2:1. Each subject was allocated to one dose level only. At each dose level NNC 90-1170/placebo were administered as single and multiple s.c. doses. Following single dose administration on Day 1, initiation of once daily multiple dosing started on Day 5 and continued for 7 consecutive days. In Type 2 diabetic patients only, a baseline intravenous glucose tolerance test (IVGTT) was performed on Day 0 at each dose level, when no drug was administered, and subsequent IVGTTs were performed 9 hours following dosing on Days 5 and 11.

NUMBER OF SUBJECTS PLANNED AND ANALYSED

Healthy Volunteers:

Thirty (30) subjects were enrolled in the study, 6 at each dose level (4 active and 2 placebo). Three subjects on active treatment were withdrawn due to adverse events following the start of repeated once daily administration (Days 5-11). Only data from subjects dosed and exposed to trial product on all study Days 1 and 5-11 were included in the pharmacokinetic and pharmacodynamic statistical analysis. All subjects were included in the safety analysis

Type 2 Diabetic Patients:

Four subjects were enrolled in the study, 3 at the 1.25 µg/kg dose level (2 active and 1 placebo) and 1 at the 5 µg/kg dose level (placebo). Three rather than 6 patients were included at the 1.25 µg/kg dose level due to recruitment difficulties. One subject on active and another on placebo at the 1.25 µg/kg dose level were withdrawn due to adverse events and hyperglycaemia, respectively. Data from all patients were included in the safety analysis and from the remaining 1 patient on the 1.25 µg/kg and another on the 5 µg/kg dose levels in the pharmacokinetic analysis. Data was not subjected to any statistical analysis.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

Healthy male and female volunteers of any ethnic origin aged 18-45 years, inclusive, and male and female subjects diagnosed with Type 2 diabetes of any ethnic origin aged 40 - 70 years, inclusive.

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

NNC 90-1170, 5 mg/ml provided in 1.5 ml PenFill® cartridges for subcutaneous injection of 1.25, 5, 7.5, 10 and 12.5 µg/kg dose levels. Batch No. 317901.

DURATION OF TREATMENT

Subjects received single (Day 1) and repeated once daily s.c. administration (Days 5-11) of either NNC 90-1170 or matching placebo and were followed-up between 1-2 weeks after dosing for check-up. In addition, Type 2 diabetic patients received IVGTT over 2 hours on Day 0, when no drug was administered, and starting approximately 9 hours post-dosing on Days 5 and 11.

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

NNC 90-1170 injection medium, provided in 1.5 ml PenFill® cartridges for subcutaneous injection. Batch No. 317903.

CRITERIA FOR EVALUATION – EFFICACY

Healthy Volunteers

- Eighty-four (84) hour NNC 90-1170 profiles following dosing on Days 1 and 11.
- 24-hour glucose and insulin profiles following dosing on Days 1 and 11.
- 16-hour leptin profiles following dosing on Days 1, 4 and 10.
- Daily caloric intake on all study days and body weight on Days 0 and 14.

Type 2 Diabetic Patients

- Eighty-four (84) hour NNC 90-1170 profiles following dosing on Days 1 and 11.
- 16-hour glucose, glucagon and insulin profiles following dosing on Days 1, 5, 8 and 11.
- 16-hour leptin profiles following dosing on Days 1, 4 and 10.
- 2-hour glucose, glucagon and insulin profiles on Day 0 (baseline) and Days 5 and 11 following receiving IVGTT between 9 and 11 hours post-dosing.
- Daily caloric intake on all study days and body weight on Days 0 and 14.

CRITERIA FOR EVALUATION - SAFETY

Blood glucose measurements, adverse events, clinical laboratory tests (haematology, biochemistry, urinalysis), physical examination, vital signs including temperature, blood pressure, pulse, ECG, 24-hour diuresis (Days 1, 4 and 11) and antibody levels against NNC 90-1170 in all pharmacokinetic samples.

STATISTICAL METHODS

- **NNC 90-1170 Pharmacokinetics**

Non-compartmental analysis was performed and the primary endpoints AUC_{0-24} and $AUC_{0-\infty}$, the secondary endpoints C_{max}, t_{max} and λ_z, and the accumulation ratio ($R_{ac}=AUC_{0-\infty,Day11}/AUC_{0-\infty}$) were derived following dosing on Days 1 and 11.

For healthy volunteers only, AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, λ_z and R_{ac} were logarithmically transformed prior to analysis and then subjected to ANOVA. Following back-transformation, estimated ls-means for each dose level with corresponding 95% CI were calculated. T_{max} was subjected to non-parametric analysis and median values were obtained. Dose proportionality was assessed for AUC_{0-24}, $AUC_{0-\infty}$ and C_{max}, excluding data from the 1.25 µg/kg dose level, and an estimate of the slope of the regression line and corresponding 95% CI were calculated. Dose proportionality was seen if the slope was not statistically significantly different from unity. Linearity in the pharmacokinetics was assessed statistically for the ratio of $AUC_{0-24\ Da\ 11}/AUC_{0-\infty\ Da\ 1}$, using two-sided t-test, where the null

hypothesis was that the ratio should not be statistically significantly different from unity.

- **Pharmacodynamics**

Healthy Volunteers

24-hour profiles of glucose and insulin on Days 1 and 11 were subjected to non-compartmental analysis. The AUC_{0-24} values on Days 1 and 11 were included in the statistical analysis following log-transformation. A comparison between a) the actively treated healthy volunteers and the corresponding placebo treated ones for Days 1 and 11 separately and combined and b) Day 1 against Day 11 was performed. An ANOVA was carried out and estimated ls-means ratios for each dose level with corresponding 95% CI was calculated.

Healthy Volunteers and Type 2 Diabetic Patients

For leptin, the AUC_{0-16} values on Days 1, 4 and 10 were included in the statistical , following log-transformation. A comparison between the actively treated healthy volunteers and the corresponding placebo treated ones was made. The Day 4 profile served as the baseline, the profiles on Days 1 and 10 represented respectively the single and multiple dose effect. An ANOVA was carried out and estimated ls-means ratios for each dose level with corresponding 95% CI were calculated.

Type 2 Diabetic Patients

16-hour profiles of glucagon, glucose and insulin on Days 1, 5, 8, and 11 and profiles between 9 and 11 hours on Days 0, 5 and 11, immediately following glucose challenge, were subjected to non-compartmental analysis. AUC_{0-16} and AUC_{9-11} were computed and average glucose, insulin and glucagon levels on Days 1, 5, 8, and 11 were calculated as $AUC_{0-16}/16$ and on Days 0, 5 and 11 as $AUC_{9-11,glucose}/2$.

- **Diuresis**

The 24 h diuresis on Days 1, 4 and 11 from healthy volunteers were subjected to an ANOVA and estimated differences in ls-means for each dose level with corresponding 95% CI were calculated.

- **Caloric Intake and Body Weight**

The daily caloric intake was calculated for all study days. Body weight was compared between active and placebo, as an overall and within each dose level.

Due to the small number of patients exposed to NNC 90-1170 data from patients were not subjected to any of the above statistical analysis.

EFFICACY RESULTS

- **NNC 90-1170 Pharmacokinetics**

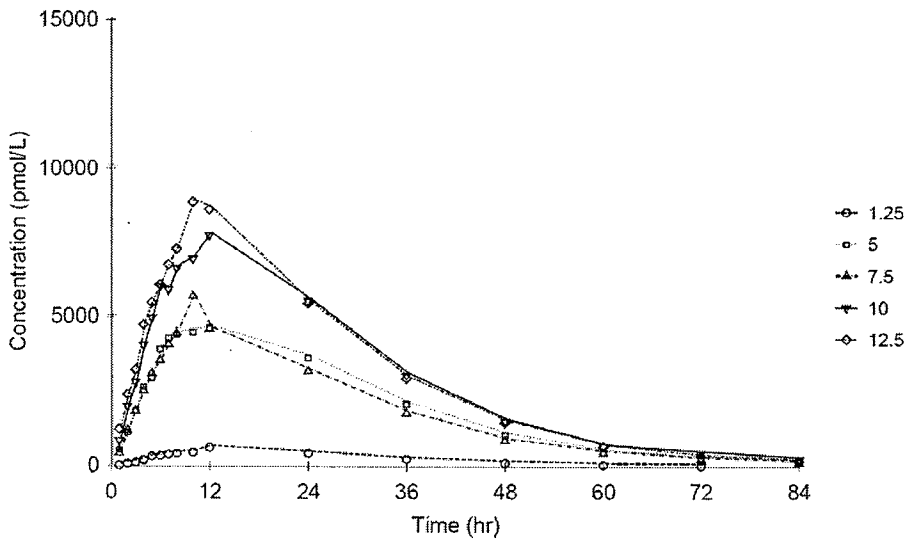
Illustrated below are mean plasma concentration time profiles following single and repeated once daily s.c. administration of NNC 90-1170 to healthy volunteers. Mean ± SD pharmacokinetic parameters (only AUC, AUC_{0-24}, C_{max}, t_{max} and $t_{½}$) following the 7.5, 10 and 12.5 µg/kg doses are summarised below:

Day	Parameter	7.5 µg/kg	10 µg/kg	12.5 µg/kg
1	AUC (h·nmol/l)	149 ± 22	246 ± 43	254 ± 75
	AUC_{0-24} (h·nmol/l)	86 ± 32	138 ± 26	152 ± 46
	C_{max} (nmol/l)	6 ± 3	8 ± 1	9 ± 3
	t_{max} (h)	14 ± 7	12 ± 0	11 ± 1
	$t_{½}$* (h)	12.4	12.8	11.6
11	AUC (h·nmol/l)	216 ± 54	351 ± 60	361 ± 122
	AUC_{0-24} (h·nmol/l)	138 ± 32	225 ± 33	247 ± 80
	C_{max} (nmol/l)	8 ± 2	11 ± 2	14 ± 4
	t_{max} (h)	10 ± 0	11 ± 1	10 ± 2
	$t_{½}$* (h)	12.4	12.8	12.9
	*harmonic mean.			

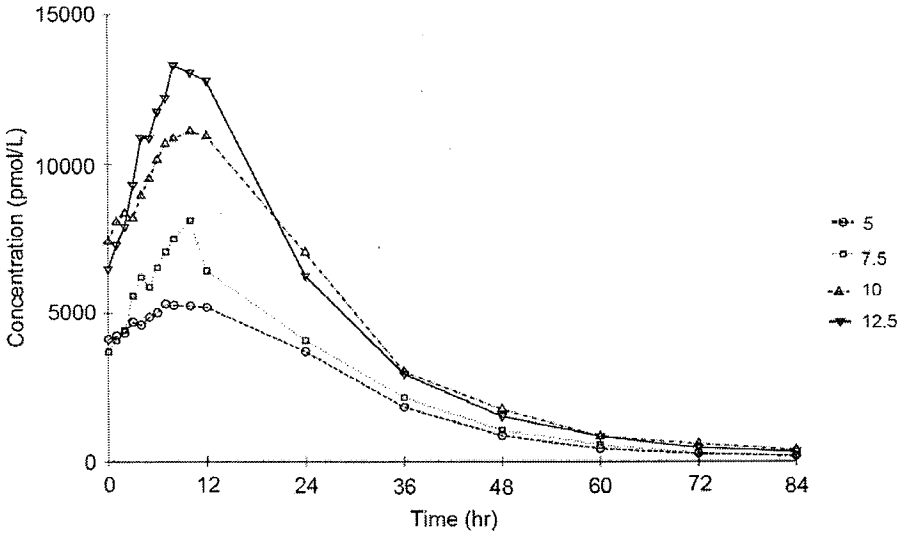
The statistical analysis showed a dose-proportional increase in C_{max} , AUC_{0-24} and $AUC_{0-\infty}$ following single and repeated once daily s.c. administration of doses between 5-12.5 µg/kg to healthy subjects. There was small, but statistically significant, accumulation (R_{ac}=1.4-1.5) of NNC 90-1170 following repeated once daily administration, except for the 5 µg/kg dose level. The ratio of steady-state $AUC_{0-24,Day11}/AUC_{0-\infty,Day1}$ was not statistically significant from unity (p=0.096), indicating linear pharmacokinetics of NNC 90-1170 following repeated s.c. administration.

Data were available from just two Type 2 diabetic patients, but they seemed to be in accordance with what was observed for the healthy subjects.

Day 1



Day 11



- **Pharmacodynamic Endpoints**

Healthy Subjects

- There was a slight, but inconclusive, increase in insulin AUC_{0-24} and subsequently a decrease in glucose AUC_{0-24} with an increase in exposure to NNC 90-1170 (i.e., an increase in AUC_{0-24} for NNC 90-1170) following both single and multiple s.c. administration.
- Statistically, there was no significant trend towards a difference between active treatment and placebo in glucose AUC_{0-24}, except lower AUC_{0-24} for the 10 µg/kg dose level. Within dose level comparison showed no trends towards a difference except a higher insulin AUC_{0-24} following active treatment for the 12.5 µg/kg dose level on Day 1 only.
- Statistically, there was no overall significant trend towards a difference between active treatment and placebo in insulin AUC_{0-24}, except lower AUC_{0-24} for the 12.5 µg/kg dose level and contradictory higher AUC_{0-24} for the 5 µg/kg dose level. Within dose level comparison, there was no significant trend towards a difference between active treatment and placebo in AUC_{0-24} following single (Day 1) and multiple (Day 11) s.c. dosing, except on Day 1 for the 12.5 µg/kg dose level where AUC_{0-24} was lower following active treatment and on Day 11 for the 5 µg/kg dose level where AUC_{0-24} was higher following active treatment.
- For leptin, there was no overall trend towards an effect except statistically significantly lower AUC_{0-16} following active treatment for the 12.5 µg/kg dose level. There was no statistically significant trend towards a difference between active treatment and placebo except on Day 10 for the 12.5 µg/kg dose level where AUC_{0-16} for active treatment was significantly lower than placebo.
- There was no overall statistically significant difference between active and placebo in body weight. However, there was an overall trend towards an increase in body weight following active treatment for the 10 µg/kg dose level only (p=0.002), with a statistically significant trend towards higher body weight following active treatment on Days 0 and 14.
- The above finding in healthy subjects should be viewed with great caution due to the small number of subjects receiving either active treatment or placebo at each dose level.

Type 2 diabetic patients

- No conclusion could be drawn regarding insulin, glucose, glucagon and leptin due to limited data obtained in this population.

SAFETY RESULTS

• Adverse Events

A higher proportion of the subjects had AEs following NNC 90-1170 (91%) than placebo (75%). Most AEs were central and peripheral nervous system disorders (dizziness and headache) following both active treatment and placebo and decreased appetite and somnolence, mainly following active treatment. Thirteen of the 22 subjects on active treatment at all dose levels experienced gastrointestinal system disorders, mainly decreased appetite and nausea, as compared to 5 of the 12 placebo subjects. The vast majority of all adverse events were of mild intensity with probable or possible relation to NNC 90-1170, particularly nausea. There were two severe adverse events, one case of hyperglycaemia in a Type 2 diabetic patient following 1.25 µg/kg NNC 90-1170 and another of pharyngitis in a healthy subject following 7.5 µg/kg NNC 90-1170.

Four subjects were withdrawn due to AEs, all occurring during the multiple dose phase of the study (Days 5-11): one healthy subject with mild-to-moderate dizziness and one Type 2 diabetic patient with severe hyperglycaemia and polydipsia at the 1.25 µg/kg dose level, one healthy subject with mild nausea, dizziness, headache, appetite decrease and dry mouth at the 7.5 µg/kg dose level and another with moderate nausea and diarrhoea at the 10 µg/kg dose level. All subjects withdrawn were receiving active treatment. None of the adverse events were serious and the randomisation was not broken due to these AEs related withdrawals. One Type 2 diabetic patient receiving placebo at the 1.25 µg/kg dose level was withdrawn because of hyperglycaemia. Subjects either recovered or were with mild intensity of AEs when they were discharged from the clinical unit. There were no deaths in the study.

• Clinical Laboratory Tests

There was no indication of an impact of NN 90-1170 on haematology, biochemistry and urinalysis.

• Vital Signs, Physical Examination and ECG

There were no changes in vital signs, including BP, pulse, temperature and respiratory rate. There were no changes of clinical significance in physical examination findings. As judged by the investigator there were no changes of clinical significance in ECG evaluation.

• Diuresis

Overall, there was a general trend towards lower diuresis following active treatment.

• Antibodies against NNC 90-1170

All samples from healthy volunteers and Type 2 diabetic patients were tested negative for antibodies to NNC 90-1170 following single or repeated s.c. administration of NNC90-117 at all dose levels.

CONCLUSIONS

- Neither the safety, tolerability, pharmacokinetics nor pharmacodynamics of NNC 90-1170 could be assessed in Type 2 diabetic patients due to the small number of patients enrolled in the study.
- There were 4 AE-related withdrawals due to trial product, all occurring during the multiple dose phase of the study (Days 5-11). One healthy subject due to mild-to-moderate dizziness and one Type 2 diabetic patient due to severe hyperglycaemia and polydipsia, both at the 1.25 µg/kg dose level. One healthy subject experienced mild central and peripheral nerves system disorders at the 7.5 µg/kg dose level and another moderate nausea and diarrhoea at the 10 µg/kg dose level.
- Based upon adverse AEs, clinical laboratory results, vital signs, physical examination, ECG, diuresis and antibodies against trial product, the safety of NNC 90-1170 following single and repeated once daily s.c. administration was acceptable in healthy subjects.

- The tolerability of NNC 90-1170 in healthy subjects was difficult to assess due to variation in adverse events between the examined dose levels.
- A dose proportional exposure was demonstrated for AUC and C_{max} in healthy subjects, indicating that linear kinetics applies in the dose range studied, with a slightly significant accumulation of NNC 90-1170 following once daily s.c. repeated administration. Only limited data were available from Type 2 diabetic patients, but they seemed to be in accordance with what was observed for healthy subjects.
- Overall, there was no statistically significant difference between active treatment and placebo for body weight in healthy subjects.
- The statistical analysis showed no conclusive trend towards an effect of NNC 90-1170 on insulin, glucose and leptin levels in healthy volunteers. In Type 2 diabetic patients there were a small number of subjects at each dose level and thus no conclusion could be made on any of the pharmacodynamic effects of NNC 90-1170 in this population.

Reviewer's Comment: The study assessments were exploratory and can only be used to determine trends as it was conducted in small number of subjects/dose group. The PK data on two Type 2 DM subjects evaluated in the study is preliminary and inconclusive.

4.2.2 Mass-Balance Study (NN2211-1699)

Title: A Single-Centre, Open Label Trial Investigating the Metabolites in Plasma, Urine and Feces after a Single Subcutaneous Dose of [^{3}H]-Liraglutide to Healthy Subjects

Investigator and Study Center(s):
Jan J. Van Lier, MD, Pharmaceutical Research Associates Group B.V., Zuidlaren, Netherlands

Trial Sites
Pharmaceutical Research Associates Group B.V.,
Zuidlaren, The Netherlands

Study Sponsor:
Novo Nordisk A/S, Denmark

Bioanalytical Analysis:

b(4)

STUDY PERIOD: 15 November 2006 (Trial Initiated) to 17 December 2006 (Trial Completed)

Objective:
The primary objective of this study was to characterize the metabolic profile of liraglutide in plasma, urine and faeces after a single s.c. injection of [^{3}H]-liraglutide, and to estimate the total tritium and tritium-labeled compound and metabolites excreted in urine and faeces.

The secondary objective of the study was to

- To determine the pharmacokinetic profile of liraglutide in plasma.
- To determine the distribution of [^{3}H]-liraglutide in whole blood versus plasma.
- To assess the safety of liraglutide after a single s.c. injection of [^{3}H]-liraglutide.

Rationale for the Trial:
The rationale of this trial was to investigate the profile of liraglutide metabolites in plasma, urine and feces after s.c. administration in healthy subjects.

Study Design:
The trial was a single centre, open label trial investigating the liraglutide metabolite profile in plasma, urine and feces in healthy subjects given [^{3}H]-liraglutide. A single dose of 0.75 mg liraglutide (containing 12.0 MBq) was given as a s.c. injection in the abdomen. Plasma was collected for 4 elimination half-lives (approximately 60 h) of liraglutide, and urine and feces were collected until excreted levels of tritium ([^{3}H]) reached the end criteria level of 1000 dpm/g in pooled 24-hour samples, or until a maximum of 14 days post dose.

The subjects attended a minimum of 3 visits, a screening visit, a dosing visit and a follow-up visit. For subjects not fulfilling the end criteria of 1000 dpm/g at the last day of the in-house

dosing visit, one more visit were to take place before the follow-up visit. The total trial duration for the individual subject was a maximum of 5 weeks.

At Visit 1 (screening), the eligibility of the subjects was assessed. Visit 2 (dosing), occurred within 3 weeks from Visit 1. At Visit 2, administration of [^{3}H] liraglutide was performed on Day 1, and subjects stayed in-house at the clinic for 10 days post dose for collection of plasma, urine and faeces samples. Quick counts were measured in urine and feces from Day 5 onwards. If the excreted levels of radioactivity had not reached the end criteria before discharge at Day 10 post dose, the subjects were to continue to sample urine and feces at home until the end criteria were met or until Day 14 post dose, whichever came first. If applicable, the subjects were asked to attend the clinic for Visit 3 (extra visit) at Day 12 post dose to bring in samples for quick counts. At Visit 4 on Day 14 post dose, follow-up was carried out for all subjects.

Blood samples were collected for analysis of total liraglutide concentration in plasma (radiolabelled and unlabelled compound). Blood samples were drawn at 15 time points during Visit 2 (baseline (within -30 to -15 min pre-dosing) and at 2, 4, 6, 8, 10, 11, 12, 13, 14, 16, 24, 36, 48 and 60 h post dose). Urine was sampled at baseline, 0-4, 4-8, 8-12, 12-24 h post dose and then at 24 h intervals until End of Trial (Day 10 on Visit 2, or until reaching end excretion criteria level at Day 12 or until Day 14 post dose). Feces were sampled at baseline and at 24 h intervals until End of Trial (Day 10 on Visit 2, or until reaching end excretion criteria level at Day 12 or at Day 14 post dose).

Study Population:
A total of 7 subjects were exposed to trial product and all subjects completed the trial. All subjects were male, between 47 and 60 years of age and with BMI between 22.7 and 27.0 kg/m^2. Table 1 below shows the demographics of the enrolled patients.

Investigational Product and Dose Selection:
The maximum radiation dose of [^{3}H]-liraglutide to be given in this trial was calculated to be 325 µCi which, given the specific radioactivity of the radiolabelled compound, corresponded to approximately 0.025 mg liraglutide or approximately 3% of the active pharmaceutical ingredient. Hence, to obtain a total dose of 0.75 mg liraglutide in this trial, a mixture of [^{3}H]-liraglutide and unlabelled compound was required.

Labeled and Unlabeled Liraglutide
The following trial products were supplied by Novo Nordisk A/S, Denmark:

- [^{3}H]-Liraglutide (47 µg/mL, 828 µCi/mL) at pH 8.15 in liraglutide 3 mL Penfill® injection medium.
- Unlabelled liraglutide (6.0 mg/mL) at pH 8.15 in pre-filled 3 mL Penfill® cartridges for s.c. injection.

Table 2: Batch number of product used in this trial

Trial Product	Concentration	Batch Number	Expiry Date
[^{3}H]-Liraglutide	Radiochemical: 828 µCi/mL Chemical: 47 µg/mL	29WW	14 December 2006
Liraglutide	6.0 mg/mL	RQ50575	29 March 2007

The final formulation of the trial product was manufactured at the trial site by mixing labeled and unlabeled liraglutide injection solutions followed by sterile ——— of the mixture. The final formulation was injected s.c. by a standard syringe and needle no later than 4 h after sterile ——— . b(4)

Table 1: Baseline Demographics of Study Population.

Sex (N (%))	
Males	7 (100%)
Ethnic Origin (N (%))	
White	5 (71.4%)
Black or African American	1 (14.3%)
Asian	1 (14.3%)
Age (Y)	
N	7
Mean	56.6
SD	4.5
Median	57.0
Min	47.0
Max	60.0
Height (m)	
N	7
Mean	1.73
SD	0.07
Median	1.74
Min	1.64
Max	1.86
Weight (kg)	
N	7
Mean	74.5
SD	9.4
Median	71.1
Min	64.0
Max	88.7
BMI (kg/m2)	
N	7
Mean	24.7
SD	1.7
Median	24.5
Min	22.7
Max	27.0

Bioanalysis:

Quantitative assessment of liraglutide was done using enzyme immunoassays (ELISA). The lower limit of quantification of the assay was 18 pmol/L. The calibration curves were analyzed at liraglutide concentration range of 20 pmol/L to 5521 pmol/L. The precision of the assay, as determined from analysis of quality control samples ranged between ——— . The mean accuracy (% Bias) ranged between ——— Between-batch precision (%CV) results of the calibration standards of liraglutide was less than or equal to 6.4 % and accuracy (%Bias) ranged from ——— Analysis ^{3}H-radioactivity levels in human plasma, whole blood, urine and feces samples as wet samples and as reconstituted freeze dried samples was conducted by means b(4)

of validated liquid scintillation counting methods. Metabolite profiling analyses of plasma, urine and feces samples were analyzed by means of HPLC and radiochromatography.

Results:

Metabolite Profile of Liraglutide in Plasma, Urine and Feces

The samples from plasma, urine and feces were analyzed by means of HPLC and radiochromatography.

Plasma

In plasma, 3 peak components were detected in the radiochromatography analyses. Liraglutide (mean Rt 37.7 min) was the major component at all time points from all subjects and in addition two metabolites P1 and P2 (mean Rt 39.7 and 43.2 min, respectively) were detected. The retention times relative to liraglutide (RtR) were 1.05 for P1 and 1.15 for P2. The exposure levels (2-24 h) for P1 and P2 were estimated from relative peak areas in pooled plasma to be ≤9% and ≤5% of the total exposure (respectively).

Urine and Feces

No unchanged liraglutide was detected in urine or feces. In urine, three peak components (U1, U2 and U3) were detected in the radiochromatography analyses. U1 was the major component in the urine and was excreted in average as 3% of the administered radioactivity. U1 (Rt 3.4 min) was detected in all subjects, U2 (Rt 4.2 min) was detected in 5 of 7 subjects and U3 (Rt 12.9 min) was only detected in 1 subject.

In feces, three peak components (F1, F2 and F3) were detected in the radiochromatography analyses. The peaks were close to the detection limit, thus no quantitative data were generated. The total excreted levels were 3-5% of the administered radioactivity.

Excretion of Radioactivity in Urine and Feces

Mean cumulative excretion is presented graphically for urine in Figure 1, for feces in Figure 2 and for urine and feces in Figure 3.

Figure 1: Mean cumulative excretion of radioactivity in Urine

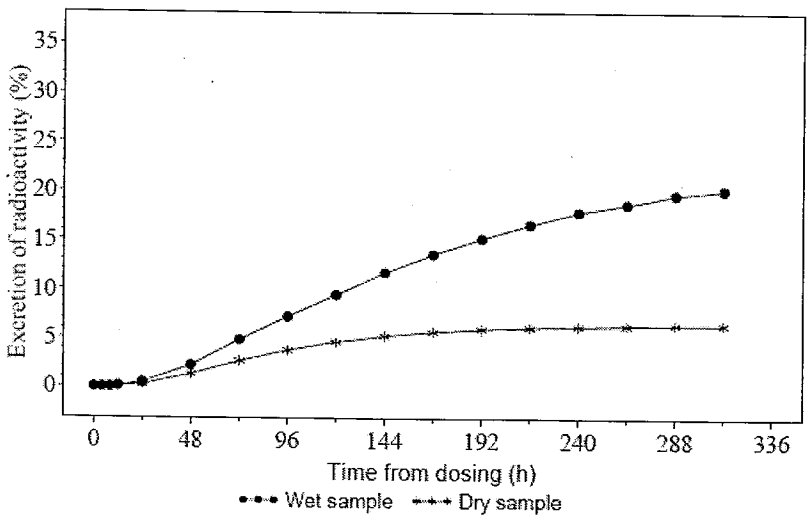


Figure 2: Mean cumulative excretion of radioactivity in Feces

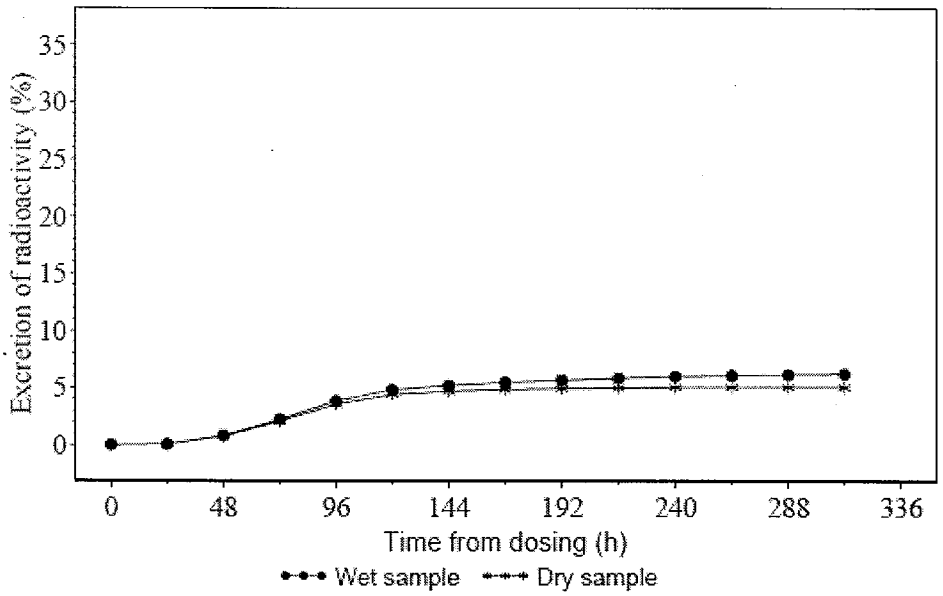
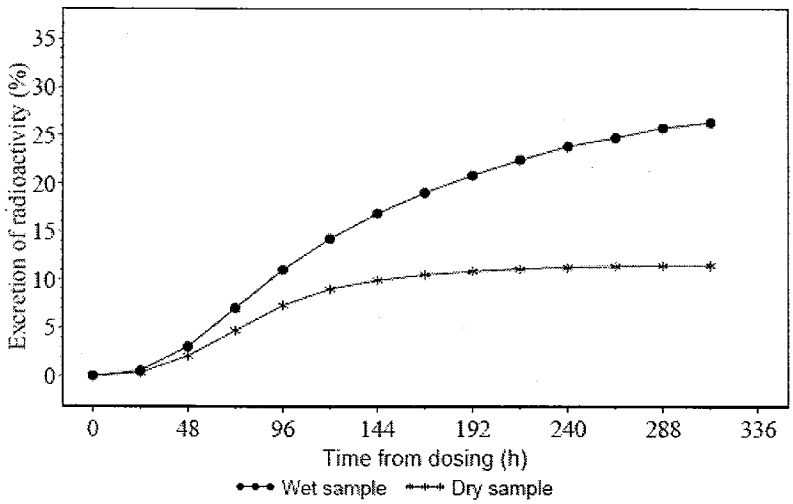


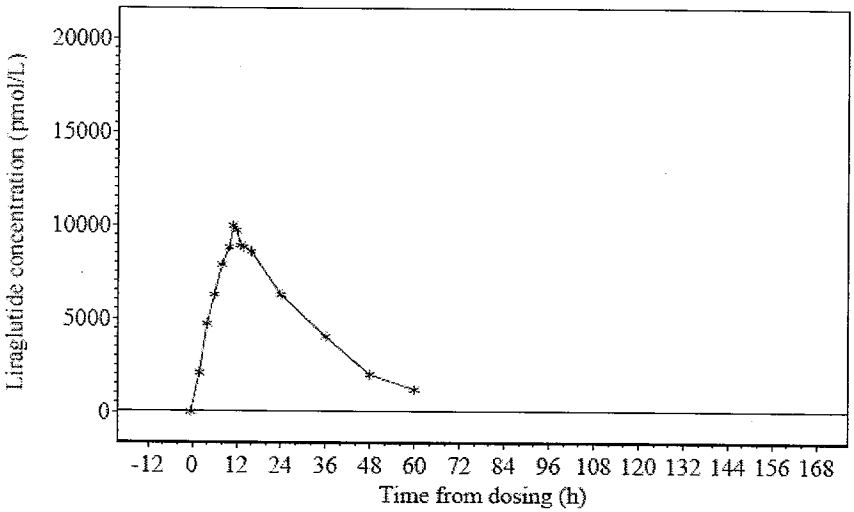
Figure 3: Mean cumulative excretion of radioactivity in Urine and Feces



In excreta continuously collected until Day 14, 26.3% of the total radioactivity was excreted in urine and feces, 20.1% in urine and 6.2% in feces (as based on the wet samples). A total of 11.5% of the dosed radioactivity was excreted as non-volatile liraglutide-related metabolites, 6.4% in urine and 5.1% in feces and 14.8% was excreted as volatile components (13.7% in urine and 1.1% in feces).

Pharmacokinetics Results:
The mean plasma concentration time profile of liraglutide is shown in Figure-4.

Figure 4: Mean Plasma profile of liraglutide by formulation-linear scale



The summary of pharmacokinetic parameters of liraglutide is presented below in Table-3

Table 3: Summary of Pharmacokinetic Parameter of Liraglutide.

AUC(pmol*h/L)		t1/2(h)	
N	7.0	N	7.0
Mean	311122.4	Mean	15.6
SD	61767.8	SD	2.1
Geometric Mean	306036.7	Harmonic Mean	15.4
CV	19.9	CV	13.5
Median	320162.8	Median	15.4
Min		Min	
Max		Max	
AUC(0-t)(pmol*h/L)		Cl/F(L/h)	
N	7.0	N	7.000
Mean	282964.2	Mean	0.682
SD	62811.2	SD	0.128
Geometric Mean	277314.2	Geometric Mean	0.671
CV	22.2	CV	18.775
Median	292569.4	Median	0.636
Min		Min	
Max		Max	
Cmax(pmol/L)		Vz/F(L)	
N	7.0	N	7.000
Mean	10585.6	Mean	15.568
SD	3202.2	SD	4.242
Geometric Mean	10233.8	Geometric Mean	15.027
CV	30.3	CV	27.251
Median	10077.0	Median	15.310
Min		Min	
Max		Max	
tmax(h)			
N	7.0		
Mean	11.7		
SD	1.0		
Geometric Mean	11.7		
CV	8.1		
Median	11.0		
Min			
Max			

b(4)

Distribution of Liraglutide in Blood and Plasma:

The ratio between total radioactivity in blood versus plasma is displayed in Figure 5 (wet samples) and Figure 6 (dry samples). The mean ratio between the total radioactivity in blood versus plasma was around 0.6 thus indicating that liraglutide was primarily distributed in plasma.

Figure 5: Mean Total Radioactivity Ratios in Blood versus Plasma (Wet Sample).

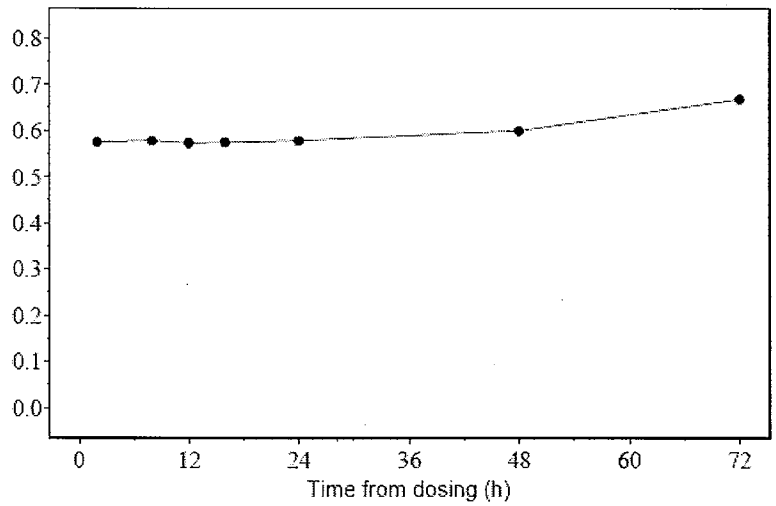
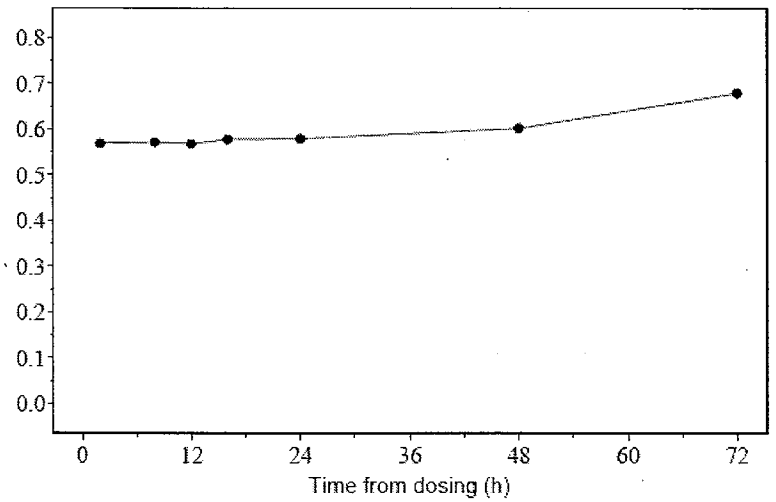


Figure 6: Mean Total Radioactivity Ratios in Blood versus Plasma (Dry Sample).



Metabolite Profile of Liraglutide in Plasma, Urine and Feces

- In plasma three components were detected. The major component was unchanged Liraglutide (89-100%) while the two metabolites P1 and P2 were slightly more lipophilic and represented ≤9% and ≤5% (respectively) of the total exposure (2-24 h).
- No unchanged liraglutide was detected in urine or feces.

- In urine three metabolites U1, U2 and U3 were detected. All of these had much lower retention times than the parent compound. The major component U1 was excreted as 3% of the administered radioactivity. U3 was only detected in one subject.
- In feces three metabolites F1, F2 and F3 were detected. All of these had higher retention times than the parent compound, and two of them were detected in the majority of the subjects. No quantification of the individual components was possible, but it was estimated that these components in total comprised 3-5% of the administered radioactivity.

Excretion of Radioactivity in Urine and Feces

- In excreta continuously collected until Day 14, 26.3% of the total radioactivity was excreted in urine and feces.
- Excretion of non-volatile radioactivity (liraglutide-related radioactivity) by urine and feces was 11.5% of the total radioactivity (6.4% and 5.1%, respectively).
- Excretion of volatile radioactivity (e.g. tritiated water) was 14.8% of the total radioactivity (13.7% in urine and 1.1% in feces).

Pharmacokinetic Properties

- Time to maximum liraglutide plasma concentration (tmax) was 11.7 h and t½ was estimated to 15.4 h.

Distribution of Liraglutide in Blood versus Plasma

- Liraglutide was primarily distributed in plasma compared to blood (ratio approximately 0.6).

Safety Conclusions

- A total of 7 treatment emergent adverse events were reported in 5 subjects, the most commonly reported event was dizziness.
- All adverse events except one (dizziness, moderate severity) were evaluated as mild.
- One treatment emergent adverse event (nausea) was evaluated as possibly related to trial product. All other events were evaluated as unlikely to be related.
- No serious adverse events were reported and no adverse events led to withdrawal.
- There were no clinically relevant findings in other safety parameters including clinical laboratory tests and vital signs.

Overall Conclusions

- The radioactivity excretion profiles indicate that the metabolic fate and clearance of Liraglutide is similar to that of large peptides
- All detected liraglutide metabolites were minor and obtained in very low amounts and therefore no structural identification was carried out.
- The pharmacokinetic profile of radiolabelled liraglutide in plasma was comparable with the profiles seen in previous trials using unlabelled liraglutide.
- The blood to plasma distribution of radioactivity demonstrated that liraglutide was primarily distributed in the plasma compartment.
- No safety concerns were raised during the trial.

Reviewer's Comment:

Overall the study design and results obtained seems reasonable. Adequacy of the analytical method results could not be commented as analytical report on the HPLC and radio-

chromatography was not provided with the study report to review. This reviewer agrees that, due to the chemical structure of liraglutide, being a large peptide with a fatty acid side chain, a degradation of liraglutide into peptides, amino acids, fatty acid fragments, water and products from recycling pathways was a reasonable expectation, and hence, a full mass balance profile (i.e. a radioactivity recovery of $\geq$ 95%) could not be obtained. Also study revealed the existence of some minor metabolites of liraglutide whose identities are unknown.

APPEARS THIS WAY
ON ORIGINAL

4.2.3 Single-Dose PD Study (NN2211-2063)

TITLE OF TRIAL NNC 90-1170 Mechanism of Action: A Double-blind, Randomized, Single-center, Placebo Controlled, Crossover Study to Examine Beta-cell Responsiveness to Graded Glucose Infusion in Subjects with Type 2 Diabetes	
INVESTIGATOR Jeffrey B. Halter	
TRIAL SITE University of Michigan Turner Geriatric Clinic Department of Internal Medicine 1500 E. Medical Center Drive Ann Arbor MI 48109-0920	
PUBLICATIONS None	
TRIAL PERIOD 27 February 2001 – 30 October 2001	**DEVELOPMENT PHASE** Phase I
OBJECTIVES **Primary Objective** • To assess the effect of NNC 90-1170, compared to placebo, on beta-cell responsiveness to increasing blood glucose concentrations in subjects with type 2 diabetes. **Secondary Objective** • To compare beta-cell responsiveness to increasing blood glucose concentrations in NNC 90-1170-treated subjects with type 2 diabetes with beta-cell responsiveness to the same conditions in a control group of healthy volunteers of similar age and BMI.	
METHODOLOGY The trial was a double-blind, randomized, single-center, placebo-controlled, two period crossover trial to evaluate beta-cell responsiveness to graded glucose infusion in subjects with type 2 diabetes. A single-dose of NNC 90-1170 (7.5 µg/kg) or placebo was administered by subcutaneous injection in a random order to subjects with type 2 diabetes. There was a three-to six-week interval between dosing periods. A control group of healthy volunteers of similar age and BMI was included. This control group did not receive any trial medication, and only received the graded glucose infusion during Period 1. Subjects with type 2 diabetes on prior oral anti-diabetic agent (OAD) monotherapy had prior treatment discontinued before dosing in each of the study periods: one week prior for subjects on insulin secretogogues and one day prior for subjects on all other OADs. The medication was restarted two days after all of the procedures were completed in each study period. In addition, regular insulin was given to all subjects to maintain the plasma glucose at 90 mg/dL (5 mmol/L), if needed, prior to the initiation of the graded glucose infusion in each period. The study consisted of an initial screening visit for healthy subjects or subjects with type 2 diabetes. At a time 1-4 weeks later, Visit 2 occurred and all subjects received a graded glucose infusion procedure (with a test dose of placebo or NNC90-1170 for type 2 diabetes subjects, no test substance for healthy subjects). For subjects with type 2 diabetes, visit 3 occurred 3-6 weeks later, when another graded glucose infusion procedure was performed accompanied by a test dose of placebo or NNC90-1170. Finally, a followup Visit 4 occurred 2-7 days after Visit 3 (type 2 diabetes) or after Visit 2 (healthy subjects).	

NUMBER OF SUBJECTS PLANNED AND ANALYZED

Planned: up to 15 subjects with type 2 diabetes, up to 15 healthy subjects.
Evaluable subjects required: 10 with type 2 diabetes, 10 healthy subjects.
Enrolled and analyzed: 10 subjects with type 2 diabetes, 10 healthy subjects.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

The subjects with type 2 diabetes can be either newly diagnosed with at least two months on diet or on OAD monotherapy for at least three months. Subjects with type 2 diabetes will have a screening $HbA_{1C} < 12\%$ with a fasting plasma glucose (FPG) ≤ 216 mg/dL the day prior to Visits 2 and 3 (for subjects with a screening $HbA_{1c} > 9$ and $< 12\%$). All subjects will be aged ≥ 18 and ≤ 75 years and will have a BMI ≥ 24 to ≤ 35 kg/m^2.

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

NNC 90-1170 injection 5mg/mL 1.5 mL Penfill® cartridge; Batch number: 317010

Subjects with type 2 diabetes were to receive a single-dose of NNC 90-1170 (7.5 µg/kg) or placebo, in random order, administered by subcutaneous injection.

DURATION OF TREATMENT

Subjects with type 2 diabetes received a single dose of test drug or placebo. There was a 3-to 6-week interval between dosing periods. The control group of healthy volunteers did not receive any trial medication, and only received the graded glucose infusion during Period 1. The duration of the trial for subjects with type 2 diabetes from screening to the last follow-up safety visit was from 5 weeks to 11 weeks. The duration of the trial for the healthy volunteers from screening to the last follow-up safety visit was from 2 to 6 weeks.

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

NNC 90-1170 injection vehicle 1.5 mL Penfill® cartridge; Batch number: 317050

CRITERIA FOR EVALUATION - EFFICACY

The efficacy assessments were insulin, C-peptide, and glucagon (beta-cell function).
Primary endpoints:

- AUC of Insulin Secretion Rate (ISR) over the 90-216 mg/dL glucose interval (40-220 minutes); ISR was derived from the C-peptide concentration profile.

Secondary endpoints:

- Slope of the mean ISR versus mean glucose dose response relationship.
- AUC of glucagon concentration over the 40-220 minutes time interval.
- Insulin Clearance: Mean ISR divided by mean serum insulin concentration.

PHARMACOKINETIC EVALUATION

- NNC 90-1170 plasma concentrations

CRITERIA FOR EVALUATION - SAFETY

The safety assessments include:

- Adverse events
- Safety laboratories (biochemistry and hematology)
- Physical examination
- Vital signs
- ECG
- Hypoglycemic episodes

OTHER EVALUATIONS

- Proinsulin, leptin, epinephrine, norepinehrine, triglycerides, free fatty acids, cortisol, and growth hormone.

STATISTICAL METHODS

AUC of ISR and glucagon concentration over the 40-220 minutes time interval was analyzed using analysis of variance (ANOVA) for the crossover design based on log-transformed data. Insulin clearance was analyzed using ANOVA for the crossover design based on log-transformed data. The slope of the ISR vs. glucose was tabulated and graphically presented for NNC 90-1170 and placebo subjects. All these parameters were also summarized for healthy subjects as a reference.

Frequencies of hypoglycemic episodes and subjects with hypoglycemia were tabulated.

The frequency of shifts from baseline to end of study in physical examination, vital signs, and ECG were summarized. Descriptive statistics at baseline to end of study using observed and change from baseline data were calculated for vital signs.

Descriptive statistics such as mean, median, standard deviation, and range at baseline to end of study using observed and change from baseline data were summarized overall for laboratory data. Scatter plot of end of study vs. baseline data were displayed.

Treatment-emergent adverse events (TEAEs) were defined as adverse events occurring during the time from the first dose of study treatment up to times 7 days after study treatment was terminated.

DEMOGRAPHY OF TRIAL POPULATION

The demographic and baseline characteristics of the trial population are tabulated by treatment group in Table 1.

Table 1. Demographic and Baseline Characteristics of Subjects

	Type 2 Diabetes Subjects (N = 10)	Healthy Subjects (N = 10)
	Mean ± SD	Mean ± SD
Age, years	62.9 ± 7.6	61.3 ± 7.3
Gender, M/F	6/4	5/5
Weight, kg	88.1 ± 18.9	75.2 ± 13.6
BMI, kg/m^2	30.1 ± 4.2	26.0 ± 2.8
HbA_{1C}, %	6.5 ± 0.8	5.1 ± 0.4
Duration of diabetes, years	5.4 ± 5.7	---

Cross-reference: End-of-Text Table 2

All enrolled subjects were of Caucasian ethnic background.

EFFICACY RESULTS

- The average response to NNC 90-1170 treatment showed a restoration of C-peptide levels to those approximating normal individuals. NNC 90-1170 significantly increased the $AUC_{(40-220)}$ for the insulin secretion rate (over the 90-216 mg/dL glucose interval, times from 40 to 220 minutes) as compared with placebo, suggesting that NNC 90-1170 improves beta-cell responsiveness to increasing blood glucose concentrations in subjects with type 2 diabetes (Table 2). $AUC_{(40-220)}$ ISR values for NNC 90-1170 were not significantly different from those obtained for healthy volunteers over the same glucose interval, further suggesting that NNC 90-1170 restores beta-cell function.
- The slope of the mean ISR vs. mean glucose level for NNC 90-1170 was significantly greater than that for placebo, and similar to that seen in healthy volunteers (Table 2).

- Insulin clearance and the $AUC_{(40-220)}$ for glucagon were not significantly different between placebo treatment, NNC 90-1170 treatment, and healthy individuals (Table 2).
- The mean AUC for NNC 90-1170 plasma concentration (from time 0 to 17 hours) was 6.1×10^4 pmol/L for type 2 diabetes subjects. The mean Cmax for NNC 90-1170 was 5.9×10^3 pmol/L. The mean Tmax for NNC 90-1170 was 13.1 hours.

Table 2. Efficacy Endpoints

	Treatment Phase		
	Placebo	**NNC 90-1170**	**Healthy**
No. of Subjects Treated	10	10	10*
Primary Efficacy Endpoints			
AUC(40-220) of ISR	(pmol/min*kg)	(pmol/min*kg)	(pmol/min*kg)
Mean (SD)	667.6 (336.39)	1129.7 (473.94)	1206.9 (314.23)
Median	592.0	1077.2	1183.3
Min. – Max.	278.2 – 1212.2	686.5 – 2034.5	693.0 – 1847.0
p-value vs. NNC 90-1170	< 0.001		0.487
Secondary Efficacy Endpoints Slope of Mean ISR vs. Glucose	(pmol*dL/ min*mg*kg)	(pmol*dL/ min*mg*kg)	(pmol*dL/ min*mg*kg)
Mean (SD)	0.033 (0.024)	0.070 (0.049)	0.083 (0.033)
Median	0.023	0.055	0.076
Min. – Max.	0.008 – 0.079	0.019 – 0.177	0.048 – 0.165
p-value vs. NNC 90-1170	0.014		0.472
AUC(40-220) of Glucagon	(pg*min/mL)	(pg*min/mL)	(pg*min/mL)
Mean (SD)	15154 (5111)	14462 (4426)	11828 (2431)
Median	13265	13526	12090
Min. – Max.	9902 – 24333	8577 – 22221	8136 – 15020
p-value vs. NNC 90-1170	0.325		0.156
Insulin Clearance	(L/min*kg)	(L/min*kg)	(L/min*kg)
Mean (SD)	0.055 (0.017)	0.057 (0.019)	0.054 (0.013)
Median	0.052	0.058	0.052
Min. – Max.	0.029 – 0.096	0.031 – 0.093	0.030 – 0.080
p-value vs. NNC 90-1170	0.349		0.669

* Data for 9 healthy volunteer subjects were analysed for AUC of glucagon.

SAFETY RESULTS

- There were no serious adverse events during this clinical trial.
- Three patients (3/10) treated with NNC 90-1170 experienced treatment-emergent adverse events, all of which were considered mild: headache, anemia, and diarrhea. Two placebo-treated patients (2/10) experienced TEAE: mild diarrhea and a procedural site reaction. No healthy volunteers experienced any adverse events.
- There were no hypoglycemic events reported.

Sponsors Conclusion:

- Liraglutide effectively restored beta-cell responsiveness to increasing blood glucose concentrations in subjects with type 2 diabetes. Insulin secretion rates with liraglutide were significantly increased over those seen with placebo, and reached levels similar to those seen in healthy subjects. Responsiveness to increasing glucose was further evidenced by the slope of ISR vs. glucose, where liraglutide treatment yielded a slope similar to that achieved in healthy volunteers, but significantly greater than that seen with placebo treatment.

- Liraglutide was well tolerated in patients with type 2 diabetes. There were no hypoglycemic events or serious adverse events. Treatment emergent AEs were all mild and showed no noteworthy patterns.

Reviewer's Comment:

Overall, the study conduct and assessments were appropriate with regards to assessing the pharmacodynamics of liraglutide. There were no major protocol deviations affecting the study outcome. The sponsor's conclusions are also reasonable from a clinical pharmacology perspective.

APPEARS THIS WAY
ON ORIGINAL

4.2.4 Multiple-Dose PD Study (NN2211-1332)

Title of Trial	Effect of Liraglutide on 24-hour glucose and hormonal profiles, gastric emptying, and fasting gluconeogenesis in type 2 diabetic subjects. A double-blind, placebo-controlled, randomised, cross-over trial
Trial ID	NN2211-1332
Development Phase	Phase 2
Generic Name	Liraglutide (NNC 90-1170)[i]
Indication	Diabetes mellitus
Investigator	Prof. Ole Schmitz, MD
Trial Site	Department of Medicine C, University Hospital of Aarhus, Denmark
Trial Initiated	9 May 2001
Trial Completed	11 February 2002
Sponsor	Clinical Drug Development, Novo Nordisk A/S Novo Allé, 2880 Bagsværd, Denmark
International Medical Officer	Milan Zdravkovic, M.D., Ph.D. Novo Nordisk A/S
International Clinical Project Manager	Bodil Elbrønd, M.D. Novo Nordisk A/S
International Trial Manager	Grethe Jakobsen, M.Sc. Pharm. Novo Nordisk A/S
Local Trial Manager	Dorte Skydsgaard, Novo Nordisk Scandinavia A/B
Statisticians	Tu Duyen Le Thi, M.Sc., Novo Nordisk A/S Jonas Ranstam, Ph.D., Novo Nordisk A/S
Medical Writer	Trine Kruse, M.Sc., Novo Nordisk A/S
Report Date	17 November 2003

TITLE OF TRIAL Effect of liraglutide on 24-hour glucose and hormonal profiles, gastric emptying, and fasting gluconeogenesis in type 2 diabetic subjects. A double-blind, placebo-controlled, randomised, cross-over trial
INVESTIGATOR Dr. Ole Schmitz
TRIAL SITE Department of Medicine C, University Hospital of Aarhus, Denmark
PUBLICATIONS KB Degn, CB Juhl, J Sturis, G Jakobsen, V Chandramouli, B Landau, O Schmitz: One week's treatment with NN2211, a long-acting GLP-1 derivative, markedly ameliorates 24-h glycemia and β-cell function and reduces fasting endogenous glucose production in type 2 diabetic patients. Diabetes 2003; 52(suppl 1): A116. KB Degn, CB Juhl, J Sturis, G Jakobsen, V Chandramouli, J Rungby, BR Landau, OE Schmitz: One week's treatment with NN2211, a long-acting GLP-1 derivative, significantly improves first phase insulin response and other markers of β-cell function, reduces endogenous glucose release, and ameliorates 24-h glycaemia in type 2 diabetic patients. Diabetologia 2003; 46(suppl 2): A285.

TRIAL PERIOD	**DEVELOPMENT PHASE**
9 May 2001 to 11 February 2002	Phase II

OBJECTIVES

In type 2 diabetic subjects to assess the effect of liraglutide after treatment for 9 to 10 days on:

Primary objective:

- 24-hour plasma glucose profiles

Secondary objectives:

- insulin secretion
- fasting rates of endogenous glucose release (EGR), glycogenolysis (GLY), and glyconeogenesis (GNG)
- circulating glucagon profiles
- circulating free fatty acids (FFA) profiles
- gastric emptying rate
- pharmacokinetic profile in steady state

METHODOLOGY

- The trial was a single-centre, randomised, double-blind trial in subjects with type 2 diabetes. Liraglutide and placebo were injected subcutaneously (s.c.) for 9 to 10 days in a cross-over design. Previous treatment with oral hypoglycaemic agents (OHAs) was discontinued 2-3 weeks before each treatment period.
- The trial was comprised of seven visits: Visit 1 (screening to assess subject eligibility), Visit 2 (randomisation, initiation of treatment period 1), Visit 3 (experimental session 1), Visit 4 (telephone contact), Visit 5 (control, initiation of treatment period 2), Visit 6 (experimental session 2), and Visit 7 (follow-up).
- A period of 9-11 weeks was included between the two experimental sessions, as a relatively large volume of blood was drawn at each of the experimental sessions. Also, both OHA treatment and glycaemic control had to be re-established before experimental session 2 in order to have comparable conditions for the two experimental sessions.
- Each experimental session included a 2-day stay at the clinic to perform meal stimulation (3 fixed meals – breakfast, lunch, dinner – were served) and corresponding 24-hour profiles (Day 1), and to measure endogenous glucose release (EGR), gluconeogenesis (GNG), indirect calorimetry, and response to a hyperglycaemic clamp (Day 2).

NUMBER OF SUBJECTS PLANNED AND ANALYSED

In total, 14 subjects with type 2 diabetes were planned for enrolment. The subject disposition was as follows:

Type 2 Diabetic Subjects	N	(%)
Screened	17	
Screening Failures	4	
Randomised	13	
Exposed	13	
Withdrawals	0	(0%)
Completed	13	(100%)

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

Subjects with type 2 diabetes of both sexes with a body mass index (BMI) ≤35 kg/m^2, either diet or OHA treated. At randomisation, fasting plasma glucose had to be within 7-15 mmol/L (both inclusive).

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Liraglutide, once daily (in the morning) s.c. injection of 6 µg/kg (corresponding to 0.55 mg using the mean body weight of 91.4 kg found in this trial), corresponding to 1.2 µL/kg of the 5 mg/mL preparation, batch no. 317010, 317012

DURATION OF TREATMENT

9 to 10 days

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Placebo (vehicle of liraglutide), once daily (in the morning) s.c. injection (1.2 µL/kg), batch no. 317005

CRITERIA FOR EVALUATION – EFFICACY

Primary endpoint:

- 24-hour glucose profiles after three fixed meals

Secondary endpoints:

- insulin secretion
  - -24-hour profiles after three fixed meals
  - -first phase and maximal secretory capacity after a hyperglycaemic clamp and arginine bolus
- endogenous glucose release (EGR), glycogenolysis (GLY), and gluconeogenesis (GNG)
  - -EGR expressed in mg/kg/min using a labelled glucose method
  - -GNG expressed in mg/kg/min using a labelled water method
  - -indirect calorimetry
- glucagon and free fatty acids (FFA) profiles
  - -24-hour profiles after three fixed meals
- gastric emptying rate
  - -4-hour paracetamol profiles after two fixed meals
- pharmacokinetic profile in steady state
  - -30-hour profile (AUC, C_{max}, t_{max}, $t_{½}$)
- leptin
  - -4-hour profile after a fixed meal (dinner)
- pro-insulin
  - -4-hour profile after a fixed meal (breakfast)

CRITERIA FOR EVALUATION – SAFETY

Adverse events, hypoglycaemic episodes, vital signs and ECG, physical examination, haematology and biochemistry

STATISTICAL METHODS

- For the efficacy endpoints the null hypothesis was that the effects of treatment with liraglutide and placebo did not differ. The alternative hypothesis was that they differed. A 5% significance level was used.
- The primary endpoint (24-hour glucose profiles) analysis was based on a mixed model, assuming gaussian distributed residuals, with period and treatment as fixed factors and subject as a random factor. The analysis can be regarded as a paired t-test of the treatment effect allowing missing values and with adjustment for period. 95% confidence intervals for the mean differences between liraglutide and placebo were constructed. These confidence intervals were based on variance estimated in the mixed model.
- Secondary endpoints were analysed using the same technique.
- Pharmacokinetic endpoints were summarised.
- Adverse events were summarised by treatment, NN-ARD (Novo Nordisk Adverse Reaction Dictionary) system-organ class and preferred term, and described by summary statistics; number of subjects with event, percent exposed subjects with event, and number of events.
- Hypoglycaemic episodes were to be listed by treatment and subject.
- Abnormal ECGs, vital signs, biochemistry, and haematology data were listed individually and summarised by treatment and visit, and described by summary statistics.

DEMOGRAPHY OF TRIAL POPULATION

More than half the subjects were male (8 males vs. 5 females), mean age was 56.4 years, and mean duration of diabetes was 3 years. Mean BMI was 31.2 kg/m^2 and mean fasting plasma glucose at randomisation (after 2 weeks of wash-out) was 9.8 mmol/L, confirming the diabetic state of the subjects (fasting serum glucose >7.8 mmol/L, according to the 1997 ADA criteria).[17] Previous diabetes treatment was equally distributed among diet and OHA.

EFFICACY RESULTS

24 hour profiles:

- For the primary objective, 24-hour glucose profiles, liraglutide treatment provided 24-hour glycaemic control, as $glucose_{AUC(0-24)}$ was statistically significantly lower compared with placebo. No statistically significant difference was seen for fasting glucose (p=0.0782).
- No statistically significant differences could be demonstrated between the two treatments with regard to $AUC_{(0-24)}$, or fasting values of insulin and C-peptide.
- The overall glucagon level ($glucagon_{AUC(0-24)}$) was significantly inhibited after treatment with liraglutide.
- No statistically significant difference could be demonstrated for free fatty acids.
- No statistically significant difference could be demonstrated for insulin secretion rate (ISR).
- Fasting value of pro-insulin was statistically significantly lower after liraglutide treatment than after placebo.

Hyperglycaemic clamp:

- Treatment with liraglutide increased insulin levels throughout the entire hyperglycaemic clamp. In addition, first phase insulin response improved after liraglutide treatment.
- During the steady state part of the clamp, treatment with liraglutide increased mean levels of insulin, C-peptide, and pro-insulin, while glucagon and pro-insulin/insulin ratio were decreased.
- Maximum insulin and C-peptide concentrations after arginine infusion were statistically significantly increased after liraglutide treatment, compared with placebo. No statistically significant difference was seen for maximum concentration of pro-insulin, while the maximum glucagon concentration was statistically significantly lower after liraglutide treatment.

Other endpoints:

- β-cell function (insulin secretion, $ISEC_{HOMA}$) was statistically significantly increased after treatment with liraglutide, whereas no statistically significant differences were seen between treatments for insulin resistance ($IRES_{HOMA}$) or insulin sensitivity (ISEN).
- No statistically significant differences were found between the two treatments with regard to gastric emptying rate.
- Treatment with liraglutide resulted in a statistically significant lowering of endogenous glucose release, which could be contributed to a decrease in glycogenolysis, as no effect was seen on gluconeogenesis. No statistically significant effect was seen for the indirect calorimetry parameters.
- The 30-hour liraglutide plasma concentration profile confirmed previously obtained values for AUC, C_{max}, t_{max} (mean 10.3 hours), and $t_{½}$ (harmonic mean 18.1 hours).

SAFETY RESULTS

- The frequency of adverse events was increased during liraglutide treatment; 62% vs. 23% during placebo treatment.
- Adverse events reported by more than one subject during liraglutide treatment included headache (N=4), nausea (N=4), abdominal pain (N=2), and vomiting (N=2). Other adverse events were single events. When treated with placebo, no adverse event was reported by more than one subject.
- All adverse events were mild, except for a single moderate event of bursitis (unlikely related to trial product), of transient nature, and resolved without intervention.
- No serious adverse events, adverse event withdrawals, or hypoglycaemic episodes occurred during this trial.
- No clinically relevant changes were observed for vital signs, ECGs, or clinical laboratory tests.

CONCLUSIONS

Based on the data, results, and considerations presented in this report we conclude that 9-10 days treatment with liraglutide (6 μg/kg):

- provides significant 24-hour glycaemic control
- does not influence 24-hour insulin secretion rate profiles
- has the potential to improve β-cell sensitivity as measured by first phase insulin response and pro-insulin/insulin ratio
- decreases fasting endogenous glucose release due to a decrease of glycogenolysis
- inhibits 24-hour glucagon profiles
- does not slow gastric emptying rate at the tested dose
- demonstrated steady state pharmacokinetics as previously reported
- is well tolerated; adverse events (mainly headache and nausea) were mild and resolved spontaneously
- does not affect vital signs, ECG, or clinical laboratory assessments to any clinically relevant degree

Trail Design:

OHA discontinued	Trial medication			OHA resumed	OHA discontinued	Trial medication			OHA resumed
2-3 weeks	7-8 d	2d	3d	6-7 w	2-3 w	7-8 d	2d	3d	1-2 w
Visit 1	Visit 2	Visit 3 Exp.session 1			Visit 4 Tel contact	Visit 5	Visit 6 Exp session 2		Visit 7 (Tel contact)

Efficacy Analysis:

The statistical analysis of primary and secondary efficacy endpoints was based on a mixed model, assuming gaussian distributed residuals and with period and treatment as fixed factors and subject as a random factor:

Response = overall mean + subject effect + period effect + treatment effect + random error

The analysis was regarded as a paired t-test of the treatment effect allowing missing values and with adjustment for period.

Derivations

AUC

Throughout the statistical analyses AUCs were calculated using the trapezoidal rule. AUC and incremental AUC had a different baseline. The baseline for AUC was zero, whereas the baseline for incremental AUC was the value just prior to the period in question. Incremental AUC was calculated as the total area under the curve minus the area under the baseline value.

HOMA/Insulin Sensitivity

Insulin secretion (β-cell function) and insulin resistance were calculated by means of the Homeostasis model assessment (HOMA).

Insulin secretion, $ISEC_{HOMA}$, was derived as follows:

$ISEC_{HOMA}$ = 20×fasting serum insulin (μU/mL)/[fasting plasma glucose (mmol/L) – 3.5]

Insulin resistance, $IRES_{HOMA}$, was derived as follows:

$IRES_{HOMA}$ = fasting serum insulin (μU/mL)×fasting plasma glucose (mmol/L)/22.5

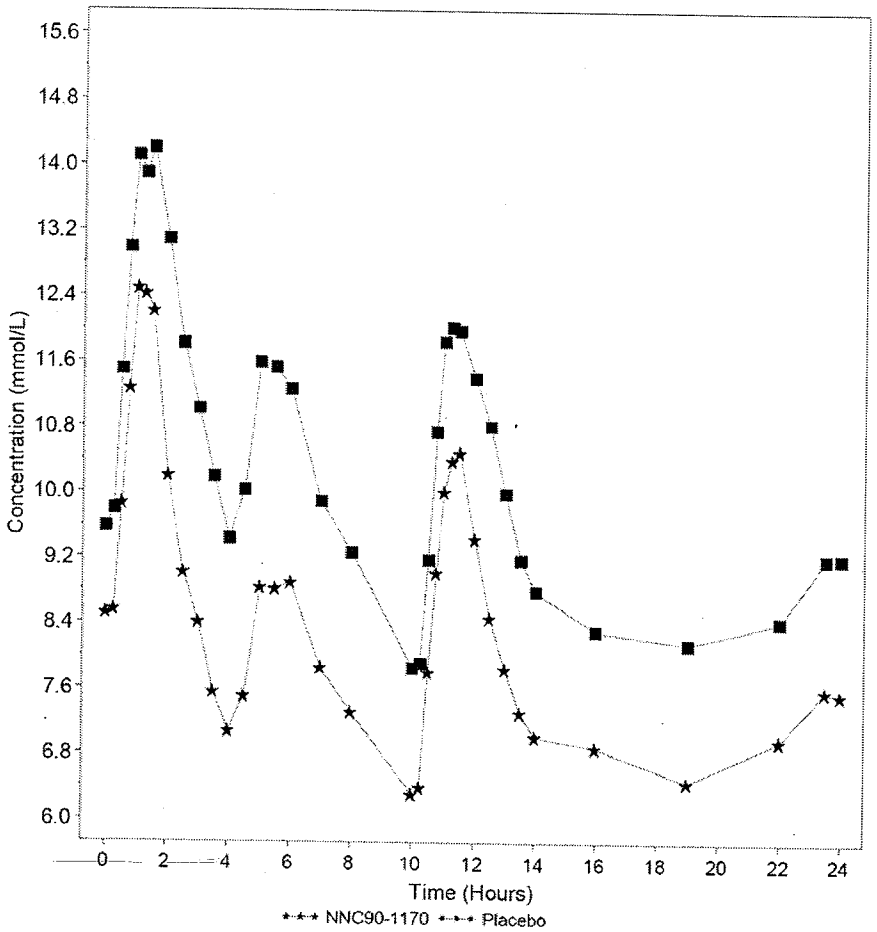
Insulin sensitivity, ISEN, was derived as follows:

ISEN = GIR/mean serum insulin (μU/mL),

Where, GIR is the glucose infusion rate during the hyperglycaemic clamp and mean serum insulin level was estimated from four observations taken at 10:45, 10:55, 11:05, and 11:15 during the hyperglycaemic clamp. The logarithm of insulin sensitivity was analyzed.

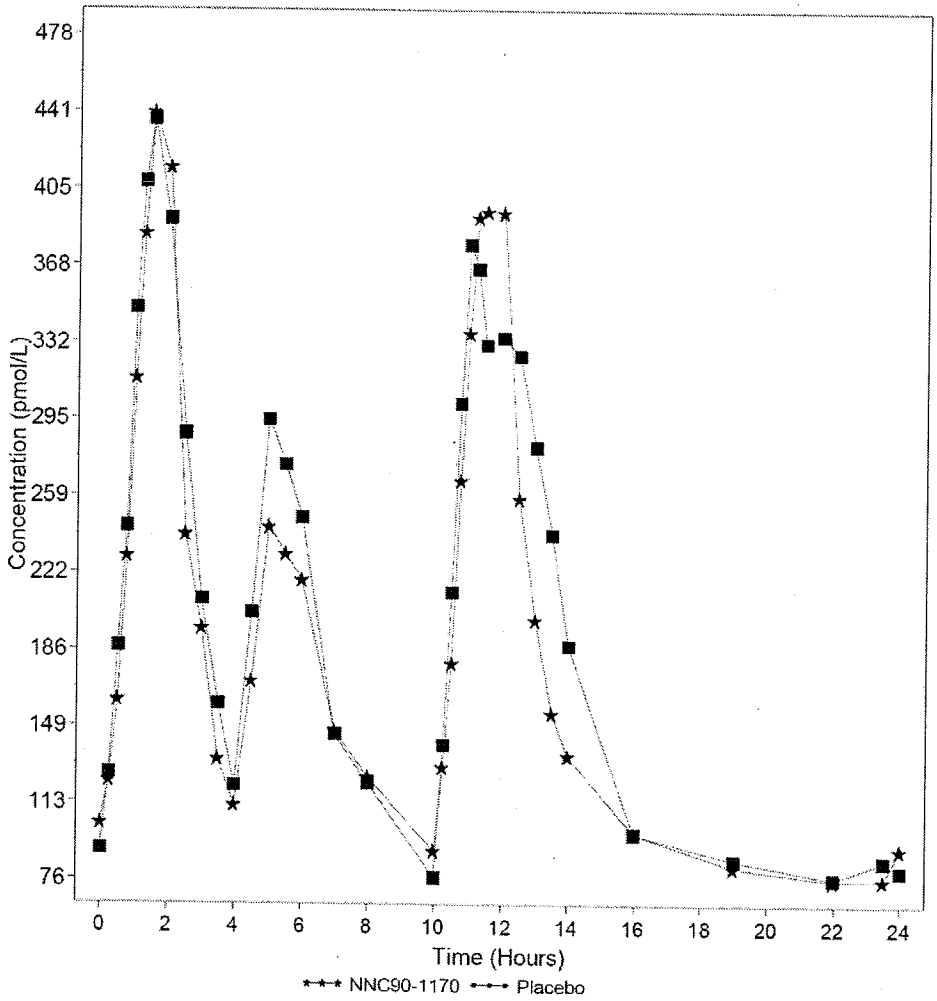
Plots for the Results:

24-hr glucose profile:



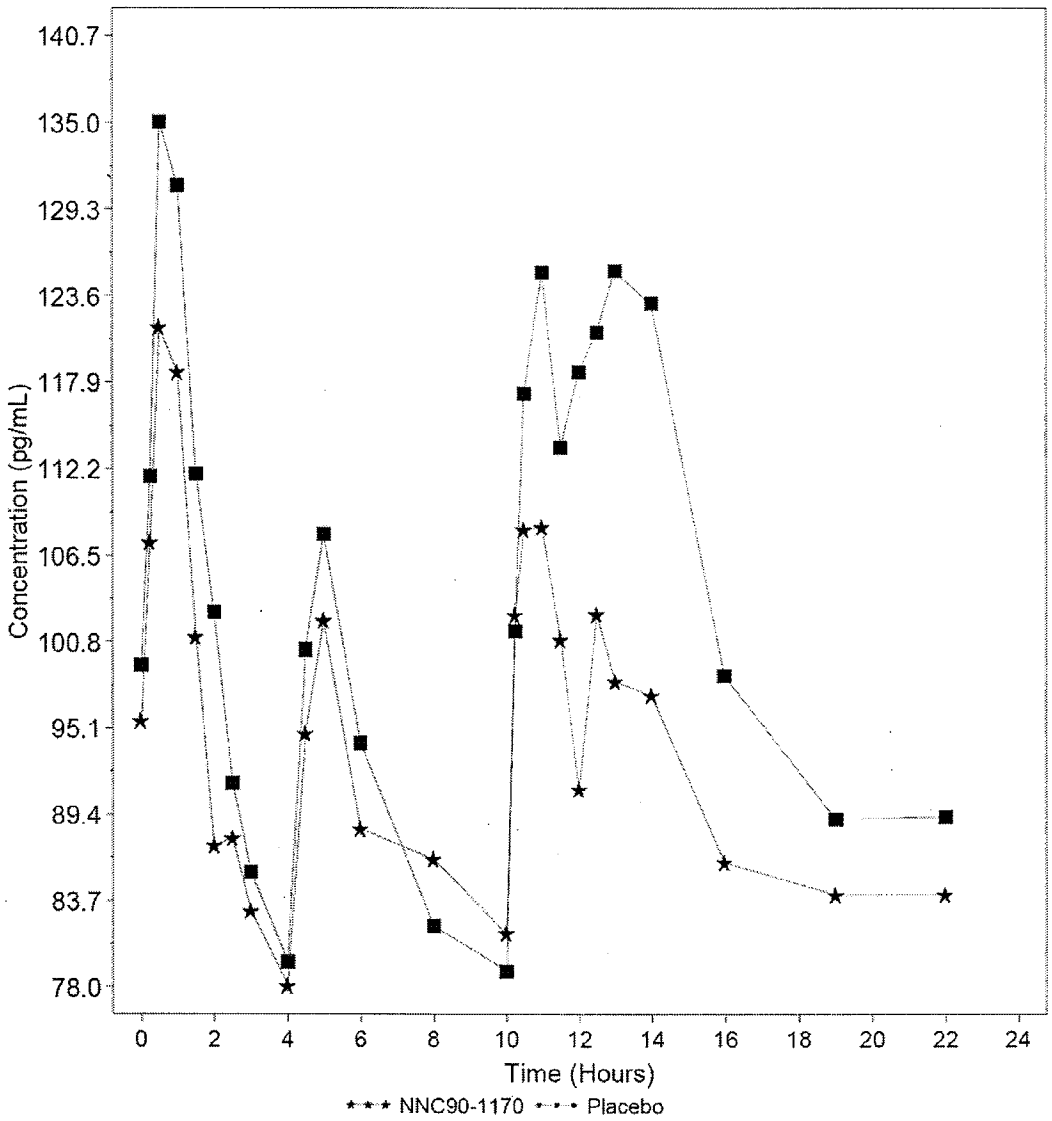
Start time for profile: Day 1, 8.00 am

24-hr Insulin profile



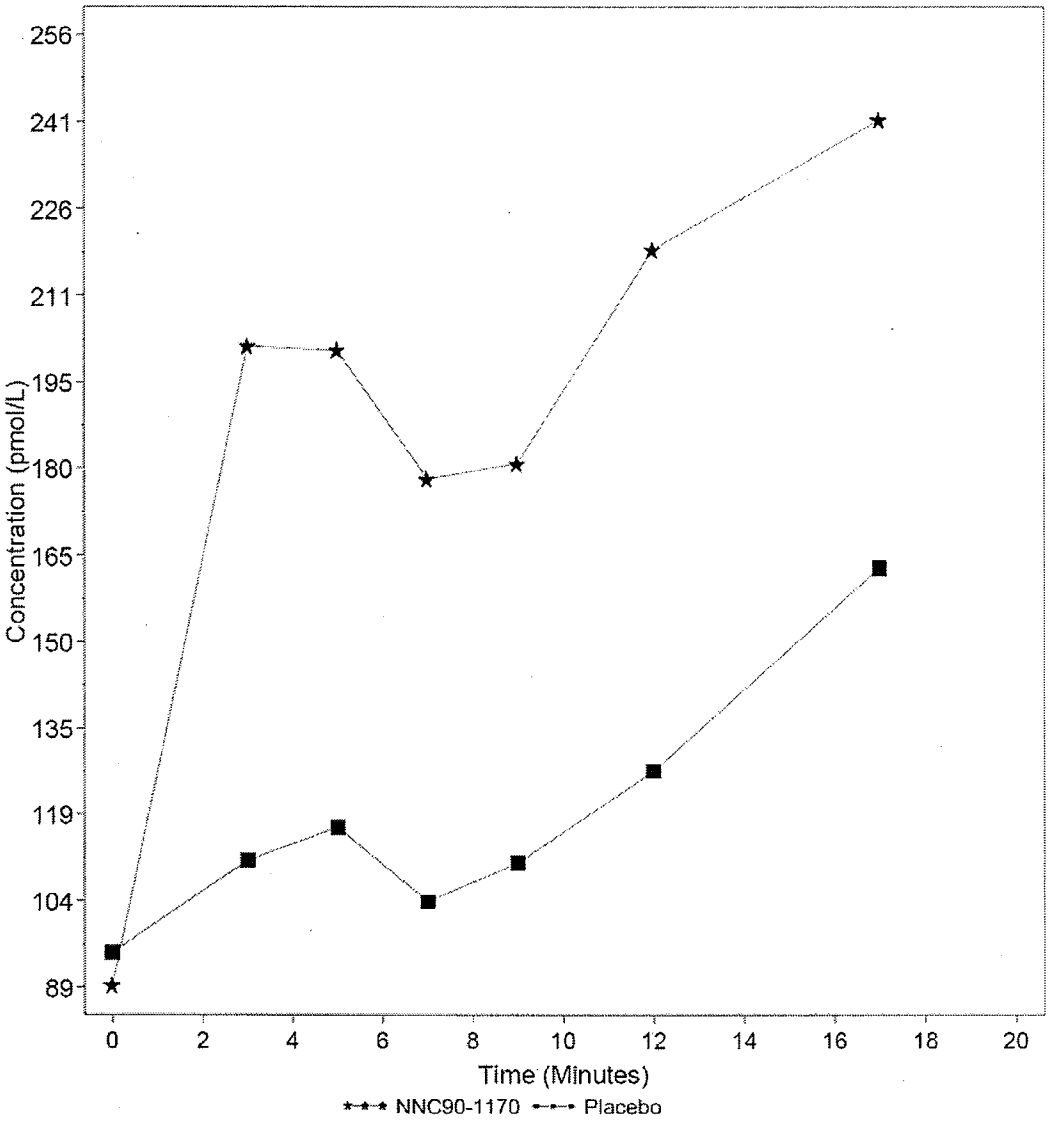
Start time for profile: Day 1, 8.00 am

24-hr Glucagon profile



Start time for profile: Day 1, 8.00 am

First Phase Insulin Secretion



Start time for profile: Day 2, 9.15 am

Sponsors Conclusions:

Based on the data, results, and considerations presented in this report we conclude that 9-10 days treatment with liraglutide (6 μg/kg):

- provides significant 24-hour glycaemic control
- does not influence 24-hour insulin secretion rate profiles
- has the potential to improve β-cell sensitivity as measured by first phase insulin response and proinsulin/insulin ratio
- decreases fasting endogenous glucose release due to a decrease of glycogenolysis
- inhibits 24-hour glucagon profiles
- does not slow gastric emptying rate at the tested dose
- demonstrated steady state pharmacokinetics as previously reported

- is well tolerated; adverse events (mainly headache and nausea) were mild and resolved spontaneously

Reviewer's Comment:

Overall, the study conduct and assessments were appropriate and the concentration data was supported by the analytical method. There were no major protocol deviations affecting the study outcome. The sponsor's conclusions are also reasonable from a clinical pharmacology perspective.

APPEARS THIS WAY
ON ORIGINAL

4.2.5 Single-Dose PD Study (NN2211-1224)

Title of Trial	Effect of liraglutide on hypoglycaemic counter-regulation during stepwise hypoglycaemic clamp in type 2 diabetic subjects. A double-blind, placebo-controlled, randomised, 2-period cross-over trial.
Trial ID	NN2211-1224
Development Phase	Phase 1
IND Number (US only)	Not applicable
Compound Name	Liraglutide (NNC 90-1170)
Indication	Diabetes Mellitus
Investigators	Dr Michael Nauck and Dr Marcus Hompesch
Trial Sites	Diabeteszentrum, Bad Lauterberg, Germany Profil Institut für Stoffwechselforshung, Neuss, Germany
Trial Initiated	02 May 2001
Trial Completed	14 December 2001
Sponsor	Novo Nordisk A/S Novo Allé, DK-2880 Bagsvaerd, Denmark.
International Medical Officer	Milan Zdravkovic, M.D., Ph.D. Novo Nordisk A/S
International Trial Manager	Bodil Elbroend, M.D. Novo Nordisk A/S
Local Trial Manager	Eva-Maria Maaßen-Quotschalla, Novo Nordisk Pharma GmbH
Statisticians	Judith L. Jacobsen, Ph.D., René Tabanera y Palacios, M.Sc. and Charlotte Hindsberger, Ph.D., Novo Nordisk A/S
Medical Writers	Ann Olling, M.Sc. and Trine Kruse, M.Sc., Novo Nordisk A/S
Report Dates	27 November 2003 30 August 2006

TITLE OF TRIAL

Effect of liraglutide on hypoglycaemic counter-regulation during stepwise hypoglycaemic clamp in type 2 diabetic subjects. A double-blind, placebo-controlled, randomised, 2-period cross-over trial.

INVESTIGATORS

Dr Michael Nauck and Dr Marcus Hompesch

TRIAL SITES

Diabeteszentrum, Bad Lauterberg, Germany (Trial site 1) and
Profil Institut für Stoffwechselforschung, Neuss, Germany (Trial site 2).

PUBLICATIONS

- Nauck, M., El-Quaghlidi, A., Hompesch, M., Jacobsen, J., Elbroend, B. No impairment of hypoglycemia counterregulation via glucagon with NN2211, a GLP-1 derivative, in subjects with type 2- diabetes. Diabetes, Vol. 52 (Supplement 1) p. A128 (2003).
- Nauck, M.A., El-Quaghlidi, A., Hompesch, M., Jacobsen, J., Elbroend, B. No impairment of hypoglycemia counterregulation via glucagon with long-acting GLP-1 derivative, NN2211, in subjects with Type 2-diabetes. Diabetotologia 46: (Supplement 2) p. A285 (2003)

TRIAL PERIOD	**DEVELOPMENT PHASE**
02 May 2001 – 14 December 2001	Phase 1

OBJECTIVES

Primary objective:

- To examine the effect of liraglutide on the hypoglycaemic counter-regulation.

Secondary objectives:

- To assess the safety and tolerability of liraglutide.
- Assessment of the pharmacokinetics of single subcutaneous doses of liraglutide in type 2 diabetic subjects.

METHODOLOGY

- A 2–centre, randomised, double-blind, cross-over trial in subjects with type 2 diabetes. Each subject received one subcutaneous injection of 7.5 µg/kg liraglutide and placebo in a random sequence.
- Each subject attended a screening visit, a treatment visit consisting of two treatment sessions on separate days, followed by a follow-up visit. At each treatment session around midnight, a single dose of liraglutide or placebo was administered subcutaneously and approximately 9 hours later, glucagon secretion was assessed for 240 minutes during a hypoglycaemic clamp by concentrations measurement. Insulin was infused continuously intravenously and at a constant rate. The clamp was conducted at four different plasma glucose levels (78, 66, 54 and 42 mg/dL corresponding to 4.3, 3.7, 3.0 and 2.3 mmol/L), which were achieved by variation of the glucose infusion.
- Insulin, glucose, cortisol, growth hormone, adrenaline, noradrenaline and C-peptide were measured for metabolic control.
- A continuous plasma profile of liraglutide was measured during both treatment days.

NUMBER OF SUBJECTS PLANNED AND ANALYSED

A total of 15 subjects were planned for enrolment to obtain 13 evaluable subjects. The subject disposition is shown below:

Subjects with Type 2 Diabetes	N	(%)
Screened	27	
Screening Failures	8	
Randomised	19	
Exposed (ITT)	19	(100%)
SC	11	
Withdrawals	3	(16%)
Adverse events	0	(0%)
Non-compliance	3	(16%)
Other	0	(0%)
Completed	16	(84%)

ITT = Intention To Treat
SC = Successful Clamp Analysis set

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

Subjects of either sex with type 2 diabetes and aged 30–65 years were planned for inclusion. Subjects were to be diet and/or OHAs treated for at least 3 months, with a body mass index (BMI) ≤ 38 kg/m^2 and HbA_{1c} ≤ 11%. Fasting plasma glucose was to be ≤ 12 mmol/L (≤ 8.88 mmol/L for trial site 2).

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Liraglutide; 5 mg/mL; 7.5 µg/kg (corresponding to a dose of 0.68 mg at a mean weight of 90.1 kg); subcutaneous injection; batch number 317012

DURATION OF TREATMENT

Two (2) single doses on 2 separate treatment days

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Placebo (liraglutide vehicle); subcutaneous injection; batch number 317005

CRITERIA FOR EVALUATION – EFFICACY

Efficacy endpoint: glucagon secretion measured as mean glucagon for each of the four 40-minute clamps at 78, 66, 54 and 42 mg/dL (4.3, 3.7, 3.0 and 2.3 mmol/L) glucose concentrations performed over the 0-240 minute period. Secondarily, insulin, glucose, cortisol, growth hormone, adrenaline, noradrenaline and C-peptide were measured as for glucagon secretion. Additionally, glucose infusion rate and insulin secretion rate were calculated.
Pharmacokinetic endpoints: AUC, C_{max}, t_{max}, $t_{½}$, CL/f and V_z/f derived from the liraglutide concentration time profiles.

CRITERIA FOR EVALUATION – SAFETY

Adverse events, hypoglycaemic episodes, haematology and biochemistry, vital signs, ECG and physical examination.

STATISTICAL METHODS

- Efficacy analyses were carried out on the basis of two analysis sets (intention to treat (ITT) and successful clamp (SC) analysis set).
- For the efficacy endpoints, the null hypothesis was that there was no difference in response between liraglutide and placebo, the alternative hypothesis was that the response of liraglutide and placebo differed. 95% confidence intervals for the mean differences between liraglutide and placebo were constructed for each clamp level.
- The primary endpoint (mean glucagon over the time intervals for the different clamp levels) was analysed using a mixed model, assuming Gaussian distributed residuals and with period, centre and treatment as fixed factors and subject as a random factor.
- The secondary endpoints (insulin, glucose, glucose infusion rate, cortisol, growth hormone, adrenaline, noradrenaline, C-peptide and insulin secretion rate) were analysed the same way as the primary endpoint.
- Additionally, a longitudinal analysis was performed for all efficacy parameters during the clamp period for the ITT analysis set and the SC analysis set. The mixed model uses subjects as random factor and visit, treatment, centre and clamp level as fixed factors.
- Pharmacokinetic parameters were summarised by descriptive statistics.
- Incidence of adverse events was presented by descriptive statistics: number of subjects with an event, percent of subjects exposed with an event and number of events. The incidence of hypoglycaemic episodes (minor and major) and the number of subjects experiencing hypoglycaemia were summarised by treatment and dose.
- Safety laboratory parameters, vital signs and ECG were summarised by visit and treatment using descriptive statistics.

DEMOGRAPHY OF TRIAL POPULATION

Nineteen (19) Caucasian subjects were randomised in this trial: 14 males and 5 females. The mean age was 54 years, mean BMI was 30 kg/m^2 and mean duration of diabetes was 7 years. The subjects were well controlled with a mean HbA_{1c} of 7.2% and a mean FPG of 8.1 mmol/L. Sixteen (16) subjects were OHA treated, whereas 3 subjects were diet-treated.

EFFICACY RESULTS

Primary endpoint

- Mean plasma glucagon increased by 1.5-fold with progressive hypoglycaemia and there was no statistically significant difference between treatment with liraglutide or placebo (p=0.7590).

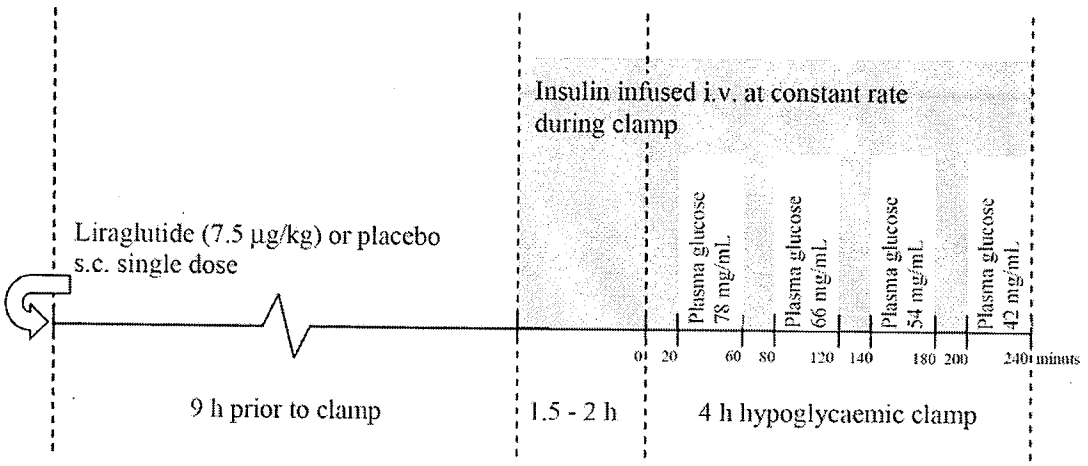
Secondary endpoints

- Insulin was infused throughout the clamp procedure to reach steady state levels with no statistically significant difference between treatments (p=0.9988).
- Mean glucose values for the ITT analysis set were statistically significantly different between treatment groups (p=0.0408). However, this difference was eliminated by exclusion of the 8 subjects who were unable to reach a satisfactory glucose level at the last clamp level (below 50 mg/dL or 2.8 mmol/L), p=0.6167. There was no statistically significant difference in the glucose infusion rate (GIR) between treatments (p=0.5489).
- Mean cortisol levels and mean noradrenaline levels, increased with progressing hypoglycaemia, with no statistically significant difference between treatments.
- Mean growth hormone levels increased with progressing hypoglycaemia in both treatment groups. However, there was an inhibition in increase for subjects treated with liraglutide as compared to placebo, and the difference was statistically significant (p=0.0320). Similar results were seen for adrenaline (p=0.0389).
- Mean C-peptide level and insulin secretion rates were higher for subjects treated with liraglutide as compared to subjects treated with placebo during the clamp and the difference was statistically significant (p<0.0001 and p=0.0026, respectively). The difference was most distinct at the highest glucose levels, which is in accordance with the glucose-dependent mode of action of liraglutide.
- The 84-hour liraglutide plasma concentration profile confirmed previously obtained values for AUC, C_{max}, t_{max} (mean 12.4 hours) and $t_{½}$ (harmonic mean 13.4 hours).

SAFETY RESULTS

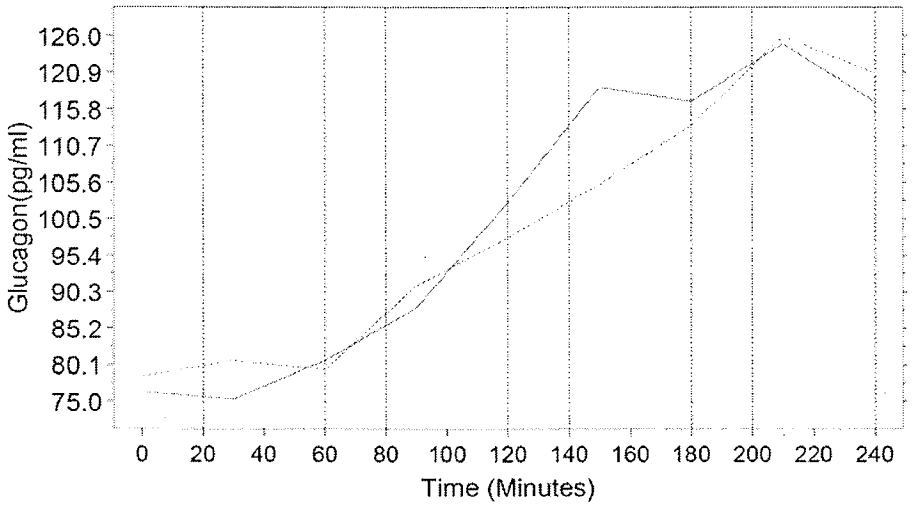
- The overall frequency and severity of treatment emergent adverse events were similar between the two treatments.
- No serious adverse events were reported.
- Relation to treatment was considered possible for 2 treatment emergent adverse events (nausea) reported in 2 subjects treated with liraglutide. No subjects treated with placebo reported treatment emergent adverse events probably or possibly related to trial product.
- No subjects were withdrawn due to the onset of adverse events.
- One (1) subject reported 2 events of symptomatic hypoglycaemic episodes. Both events were considered non-treatment related.
- No clinically relevant findings were observed for any of the clinical laboratory assessments, vital signs, ECG or physical examinations.

Trial Design:



A single s.c. dose of liraglutide 7.5 µg/kg or placebo was administered in the abdominal skin at midnight. Prior to dose administration, a pre-dose blood sample was drawn. During and following the clamp procedure, blood samples (2 mL) were drawn at 0, 2, 4, 6, 8, 10, 12, 14, 16, 24, 36 and 84 hours after dosing for pharmacokinetic assessment.

Mean Glucagon Profile for Liraglutide and Placebo



--- The mean profile for placebo
_ The mean profile for liraglutide

	Liraglutide (a)	Placebo (a)	Liraglutide-Placebo (b)
Baseline			
N	11	11	11
Mean (S.E.M.)	76.32 (8.40)	78.44 (8.01)	-2.630 (8.74)
95% C.I.			[-22.40 ; 17.14]
78 mg/dL			
N	11	11	11
Mean (S.E.M.)	77.97 (4.88)	80.04 (4.79)	-2.769 (3.78)
95% C.I.			[-10.47 ; 4.932]
p-value			0.4688
66 mg/dL			
N	11	11	11
Mean (S.E.M.)	95.40 (6.77)	94.49 (6.66)	0.932 (5.64)
95% C.I.			[-10.58 ; 12.44]
p-value			0.8700
54 mg/dL			
N	11	11	11
Mean (S.E.M.)	117.85 (8.25)	109.42 (7.21)	8.666 (4.80)
95% C.I.			[-1.118 ; 18.45]
p-value			0.0806
42 mg/dL			
N	11	11	11
Mean (S.E.M.)	120.80 (8.19)	123.34 (10.38)	-2.696 (6.35)
95% C.I.			[-15.64 ; 10.24]
p-value			0.6739

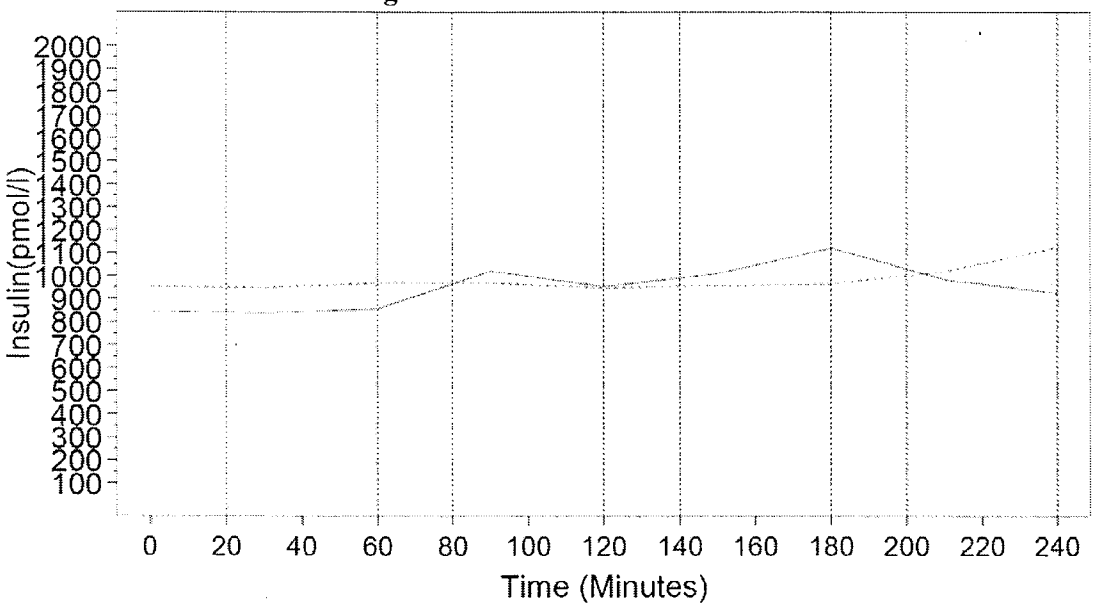
(a) Descriptive statistics
(b) The statistics are obtained from a mixed model with subjects as a random factor and visit, treatment and centre as fixed factors.
An asterisk indicates statistical significance.

Longitudinal analysis	
N	11
Mean (S.E.M.)	1.033 (3.36)
95% C.I.	[-5.607 ; 7.673]
p-value	0.7590

Centre effect p = 0.6933
Clamp effect p = 0.0000
Centre*treatment was not significant p =0.9185

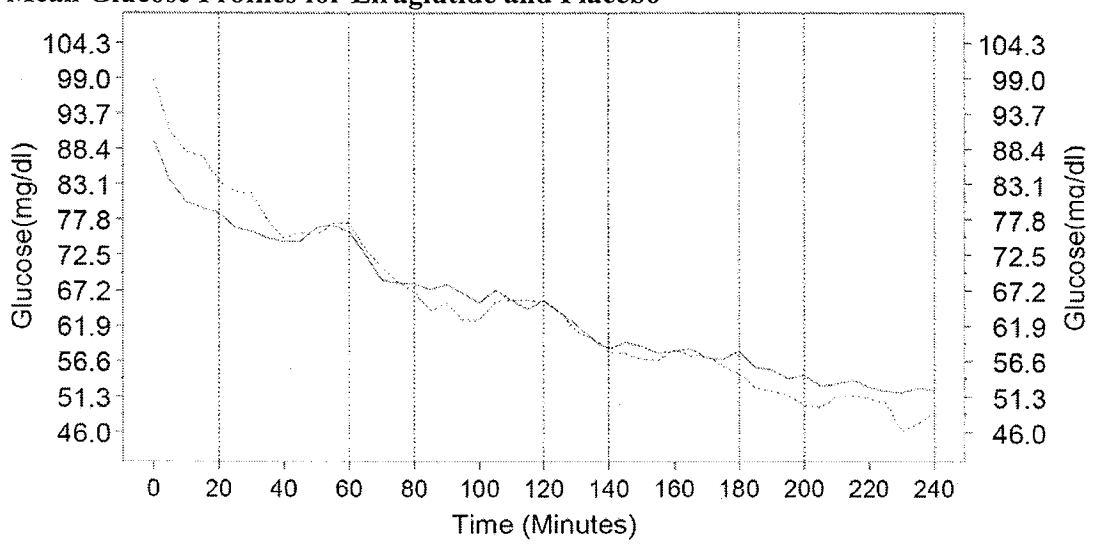
The statistics are obtained from a mixed model with subjects as a random factor and visit, treatment, centre and clamp level as fixed factors.
An interaction term centre*treatment was also investigated. If not significant it was removed from the model.

Mean Insulin Profile for Liraglutide and Placebo



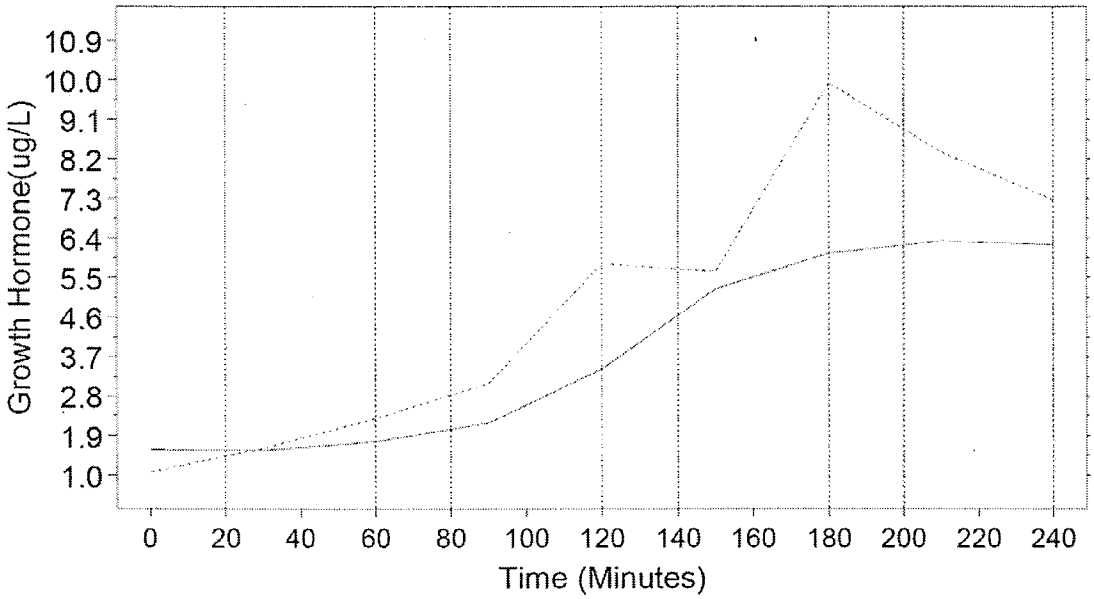
--- The mean profile for placebo
_ The mean profile for liraglutide

Mean Glucose Profiles for Liraglutide and Placebo



--- The mean profile for placebo
_ The mean profile for liraglutide

Mean Growth Hormone Profile for Liraglutide and Placebo



--- The mean profile for placebo
_ The mean profile for liraglutide

Summary of Pharmacokinetic Parameter Estimates

Gender	Subject	t_{max} (h)	C_{max} (pmol/L)	AUC (h·pmol/l)	AUC%extrap (%)	λ_z (1/h)	$t_{½}$ (h)	MRT (h)	CL/f (mL/h/kg)	V_z/f (mL/kg)
Female	106									
	108									
	111									
	206									
	216									
Female	Mean	10.9	11200	273400	5.4	0.056	12.4[a]	24.3[a]	8.39	169
	SD	3.18	7030	111400	11	0.018			3.43	91.6
Male	102									
	104									
	105									
	107									
	110									
	112									
	207[b]									
	209									
	210									
	211									
	212									
	213									
	214									
Male	Mean	13.0	12600	306600	18	0.050	13.9[a]	26.8[a]	8.97	198
	SD	3.31	10400	219100	21	0.014			4.06	111
Overall	Mean	12.4	12200	296800	15	0.052	13.4[a]	26.0[a]	8.80	190
	SD	3.32	9420	190700	19	0.015			3.79	104

a) harmonic mean
b) no PK parameters could be calculated due to no elimination phase

b(4)

This trial demonstrated that glucagon responses to hypoglycaemia were unaffected by liraglutide at a dose of 7.5 μg/kg (corresponding to a dose of 0.68 mg with a mean weight of 90.1 kg) and that glucagon suppression was reduced during increasing levels of hypoglycaemia, which correlates well with what was observed in healthy subjects. For both healthy subjects and subjects with type 2 diabetes, GLP-1 has been reported to suppress glucagon concentrations during hyperglycaemia and the glucose threshold for the glucagonostic action of GLP-1 is believed to be equivalent to normal fasting glucose concentrations.

Sponsor's overall conclusions

Based on the data, results and considerations presented in this study, liraglutide after a single s.c. dose of 7.5 μg/kg:

- does not affect the glucagon response to hypoglycaemia
- does not impair the overall hypoglycaemic counter-regulation response
- induces minor statistically significant differences for adrenaline and growth hormone
- (suppressed release relative to placebo)
- in accordance with the glucose-dependent stimulation of insulin secretion, ISR was borderline significantly increased at the two highest glucose levels (78 and 66 mg/dL), but not at the two lower glucose levels (54 and 44 mg/dL)
- was well tolerated; adverse events were mild and hypoglycaemic events were non-treatment related
- does not affect vital signs, ECG or clinical laboratory assessments to any clinically relevant Degree

Reviewer's Comments:

The study assessments and conclusions are reasonable from a clinical pharmacology perspective.

4.2.6 Multiple-Dose PD Study Appetite Suppression (NN2211-1589)

Trial Registration ID-number	**EudraCT number – EU only**
Not applicable	2006-000377-30
Title of Trial	
A 4 Week Double-Dummy Randomised, Double-Blind, Balanced Incomplete Latin Square Design Study to Evaluate the Effects of Liraglutide on Appetite in Subjects with Type 2 Diabetes Compared to Glimepiride and Placebo	
Investigators	
Principal and signatory investigator, Australia, Michael Horowitz, Professor, MD Principal investigator, Germany, Christoph Kapitza, MD	
Trial Sites	
Department of Medicine, Royal Adelaide Hospital, North Terrace, Adelaide, South Australia 5000, Australia Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany	
Publications	
Not applicable	
Trial Period	**Development Phase**
23 Nov 2005 to 26 Sep 2007	Phase 1

Objectives

Primary Objective:

- To assess the effects of subcutaneously administered liraglutide on energy intake in subjects with type 2 diabetes compared to glimepiride and placebo

Secondary Objectives:

- To assess the effects of liraglutide on macronutrient distribution in subjects with type 2 diabetes compared to glimepiride and placebo
- To assess the effects of liraglutide on satiation and satiety in subjects with type 2 diabetes compared to glimepiride and placebo
- To assess the effects of liraglutide on antral area and gastric emptying compared to glimepiride and placebo
- To assess relationships between the effects of liraglutide on appetite and antral area.
- To assess the effects of treatment on energy intake adjusted for sensations of appetite, hunger, fullness, satiety and nausea as measured by VAS-scales
- To assess the relationship between energy intake and mean antral area at baseline and immediately before intake of a buffet meal as well as between energy intake and change in antral area
- To assess the effects of liraglutide on postprandial metabolic and hormonal responses compared to glimepiride and placebo
- To assess the effects of liraglutide on resting energy expenditure compared to glimepiride and placebo
- To assess safety and tolerability of liraglutide during 4 weeks treatment
- To assess the effects of liraglutide on macronutrient distribution in subjects with type 2 diabetes compared to glimepiride and placebo

Methodology

- A double-dummy, randomised, double-blind two-centre study with balanced incomplete latin square design comparing the effect of liraglutide (1.8 mg), glimepiride and placebo on energy intake at an *ad libitum* buffet meal, duration of the meal, macronutrient distribution, appetite sensations and nausea, gastric distension (assessed by ultrasound measurements of antral area), gastric emptying (assessed by paracetamol concentrations) and metabolic and hormonal response.
- Subjects discontinued current oral anti-diabetic drug (OAD) treatment and were randomised to treatment group (a) liraglutide followed by placebo, (b) placebo followed by glimepiride or (c) glimepiride followed by liraglutide. The trial consisted of a screening visit to assess eligibility (Visit 1) performed 3-35 days before the

two treatment periods (Visits 2-5 and Visits 6-9) each lasting 4 weeks with 3-5 weeks wash-out in between. The total trial duration was up to 19 weeks.

- Liraglutide was given using a forced three step dose escalation (0.6, 1.2 and 1.8 mg): the dose increased each week until the subjects reached 1.8 mg. Glimepiride was given using a stepwise dose-escalation (1, 2, and 4 mg): the dose increased each week, however, the current dose level was maintained when the fasting blood glucose (FBG) was above or equal to 6 mmol/L but below 7 mmol/L. If the FBG was below 6 mmol/L the dose decreased to the next whole capsule down, lowest dosing being 1 mg.

Test Days 1 and 2 (Visit 5) and Test Days 1 and 2 (Visit 9)

After two weeks of treatment at the third liraglutide/glimepiride/placebo dose levels there were two testing days. Test Day 1 was a meal test that included a preload paradigm. The preload was a palatable liquid mixture of yoghurt, cream etc. (250 kcal ~ 1047 kJ) known to suppress subsequent food intake and suitable for ultrasound imaging. 1.0 gram of paracetamol was included in the preload. One hour later an *ad libitum* buffet-style meal was served. Gastric distension, gastric emptying, duration of eating, amount of energy consumed and sensations of appetite (hunger, fullness and satiety) and nausea were measured.

On Test Day 2 the resting energy expenditure was measured (after having fasted overnight) before an *ad libitum* buffet-style meal without a preload paradigm was served. Again, the duration of eating, amount of energy consumed, sensations of appetite (hunger, fullness and satiety) and nausea were measured.

Number of Subjects Planned and Analysed

Planned number of subjects was 42 randomised and at least 36 completing subjects. 116 subjects were screened and the 46 subjects that were randomised and exposed to trial products were included in the safety analysis set. Four (4) subjects withdrew from the trial due to adverse events (AEs), thus 42 subjects completed the trial. Four (4) subjects were excluded from the pharmacodynamic (PD) analysis set because of non-compliance or violation of an inclusion criterion. Therefore, the PD analysis set contained 42 subjects of which 38 subjects completed the trial.

Diagnosis and Main Criteria for Inclusion

Euthyroid male and female subjects aged 18 to 65 years (both inclusive) with type 2 diabetes either diet treated (6.5 % $\leq HbA_{1c} \leq$10.0 %) or in OAD monotherapy (6.5 % $\leq HbA_{1c} \leq$ 9.5 %), with a body mass index between 27 kg/m2 and 40 kg/m2, the fasting plasma glucose (FPG) in the range of 7-13 mmol/L and possible visualisation of the antrum by ultrasonography were included in the trial.

Test Product, Dose and Mode of Administration, Batch Number

- Liraglutide was supplied as 6.25 mg/mL (Formulation 3) and 6.0 mg/mL (Formulation 4) solutions and was administered in a three-step dose escalation scheme:
  - 0.625 mg, 1.25 mg and 1.875 mg, was provided in 3mL penfills for the NovoPen® 3 device in Australia (batch numbers: PQ50363 and RQ50536 PQ50365)
  - 0.6 mg, 1.2 mg and 1.8 mg, was provided in prefilled 3 mL FlexPen® devices in Germany and Australia (batch numbers: SP52281 and SP51132)
- The apparent difference in dose levels is due to a change in the way liraglutide content was declared, the dose levels were actually similar. The content of active liraglutide in Formulations 3 and 4 is equivalent and 0.6, 1.2 and 1.8 mg/day is thus used consistently in this synopsis and clinical trial report
- Glimepiride (Amaryl®, batch numbers D431 and E479) was administered as capsules in the morning. Dosing was based on individual glycaemic control to mitigate hypoglycaemia using a stepwise dose-escalation scheme of weekly dosing periods of 1, 2 and 4 mg
- Paracetamol (Benuron® and Herron® 500 mg tablets, batch numbers 618056 and 53783) was administered at two visits as two tablets (1 mg) for the assessment of gastric emptying.

Duration of Treatment

- Subcutaneous administration of liraglutide for four weeks
- Glimepiride administration as capsules administered orally for four weeks

- A single dose of 1.0 gram paracetamol on Visit 5 and 9, Test Day 1

The total trial duration for each individual subject was up to 19 weeks.

Reference Therapy, Dose and Mode of Administration, Batch Number

- Liraglutide placebo medium provided in 3mL penfills for the NovoPen® 3 device (0mg/mL, batch numbers PQ50297 and RQ50390) and in prefilled 3 mL FlexPen® devices (0mg/mL, batch numbers PQ50695, SP51130 and SP51129)
- Glimepiride placebo capsules: 0 mg, batch numbers PBBK034, PBBK039 and PBBK071

Criteria for Evaluation – Efficacy

- Energy intake
- Macronutrient intake
- Meal duration
- Appetite and nausea (VAS-ratings)
- Gastric distension (assessed by ultra sound antral area measurements)
- Gastric emptying (assessed by plasma paracetamol concentrations)
- Resting energy expenditure
- Change from baseline in efficacy variables: plasma glucose, insulin, glucagon, hormones (ghrelin, leptin, adiponectin GLP-1, GIP, peptide YY, CCK), anti-inflammatory markers (hsCRP, TNF, PAI-1, lipids (TC, LDL-C, VLDL-C, HDL-C, FFA), body weight and waist circumference

Criteria for Evaluation – Safety

Adverse events, episodes of hypoglycaemia, clinical laboratory tests (haematology, clinical chemistry, urinalysis), 12-lead ECG, physical examination and vital signs.

Statistical Methods

Primary Endpoint

Energy intake (kJ) at an *ad libitum* buffet-style meal that included a preload paradigm

Secondary Endpoints

Energy intake

Energy intake (kJ) at an *ad libitum* buffet-style meal without a preload paradigm

Macronutrient intake

- The relative amount of energy intake (kJ) in each of the macronutrient groups (protein, fat and carbohydrate) of an *ad libitum* buffet-style meal with a preload paradigm.
- The relative amount of energy intake (kJ) in each of the macronutrient groups (protein, fat and carbohydrate) of an ad *libitum* buffet-style meal without a preload paradigm.

Meal duration

- The duration of eating at the *ad libitum* buffet-style meal with a preload paradigm.
- The duration of eating at the *ad libitum* buffet-style meal without a preload paradigm.

VAS-ratings

- Fasting sensation of hunger, fullness, satiety and nausea prior to the preload period on the test day with the preload paradigm ($R_{0,\ preload}$). The -10 min rating is used as the fasting sensation.
- Average sensations of hunger, fullness, satiety and nausea during the preload period on the test day with the preload paradigm ($R_{average,\ preload}$).
- Sensations of hunger, fullness, satiety and nausea prior to the *ad libitum* buffet-style meal period including the preload paradigm on Test Day 1 ($R_{60,\ buffet\ meal,\ D1}$).
- Average sensations of hunger, fullness, satiety and nausea during the post *ad libitum* buffet-style meal period on Test Day 1 that included a preload paradigm ($R_{average,\ buffet\ meal,\ D1}$).
- Fasting sensation of hunger, fullness, satiety and nausea prior to the *ad libitum* buffet-style meal period on Test

Day 2 without a preload paradigm ($R_{0,\ without\ preload}$). The -10 min rating was used as the fasting sensation.

- Average sensations of hunger, fullness, satiety and nausea during the post *ad libitum* buffet-style meal period on Test Day 2 without a preload paradigm ($R_{average,\ buffet\ meal,\ D2}$).

Gastric distension (assessed by ultra sound antral area measurements)

- Fasting antral area prior to the preload period. The -10 minutes measurement was used as baseline.
- Average antral area during the preload period. The average was calculated as $AUC_{0\text{-}60min}$/60 min, where AUC was calculated by the trapezoidal method and the baseline value at 0 minute was identical to the measurement at -10 minutes.
- Antral area prior to the buffet meal period measured 60 minutes after the preload.
- Change in antral area from baseline (-10 minutes) to 60 minutes as indices of gastric emptying. The change was calculated in percentage, i.e. change= 100%*antral $area_{60min}$/antral $area_{-10min}$.
- T75%, time at which the antral area has decreased to 75% of the maximum antral area.
- T50%, time at which the antral area has decreased to 50% of the maximum antral area.

Gastric emptying (assessed by paracetamol concentrations)

- C_{max}, the maximum paracetamol concentration.
- t_{max}, the time to the maximum paracetamol concentration.
- $AUC_{0\text{-}60min}$, area under the paracetamol curve from time zero to 60 minutes, calculated by the trapezoidal method where the baseline value at 0 minutes was identical to the measurement at -10 minutes.
- $AUC_{0\text{-}300min}$, area under the curve from time zero to 300 minutes, calculated as described above.
- $AUC_{0\text{-}60min}/AUC_{0\text{-}300min}$ with AUCs calculated as described above.

Resting energy expenditure

- The resting energy expenditure (kJ) measured on the day without the preload.

Change from baseline in efficacy variables

The change from baseline to end of treatment period in the efficacy variables described below was calculated as the difference between the efficacy outcome measured at Visit 5 (Test Day 1) and Visit 2 for the first period and the difference between the efficacy outcome measured at Visit 9 (Test Day 1) and Visit 6 for the second period.

Change from baseline in efficacy variables: plasma glucose, insulin, glucagon, hormones (ghrelin, leptin, adiponectin GLP-1, GIP, peptide YY, CCK), anti-inflammatory markers (hsCRP, TNF□PAI-1, lipids (TC, LDL-C, VLDL-C, HDL-C, FFA), body weight and waist circumference

Statistical Analyses

Primary Analysis

The primary endpoint was assumed to follow a lognormal distribution and analysed using a linear normal model (ANOVA) that included effect of treatment, period and random effect of subject. The model was used to estimate

- the ratio between the energy intake (with a preload paradigm) after administration of liraglutide and the energy intake (with a preload paradigm) after administration of glimepiride.
- the ratio between the energy intake (with a preload paradigm) after administration of liraglutide and the energy intake (with a preload paradigm) after administration of placebo.

The comparison of liraglutide versus glimepiride and the comparison of liraglutide versus placebo were analysed jointly in the same model. The hypothesis that the ratios should be equal to 1 (corresponding to no treatment effect) was tested by a two-sided test at a 5% significance level.

Secondary Analyses

Efficacy variables that were assumed to follow a normal distribution were analysed in a linear normal model (ANOVA) including effect of treatment, period and a random effect of subject. In these cases the model was used to estimate

- the difference between the efficacy variable after administration of liraglutide and the efficacy variable after

administration of glimepiride
- the difference between the efficacy variable after administration of liraglutide and the efficacy variable after administration of placebo

The comparison of liraglutide versus glimepiride and the comparison of liraglutide versus placebo were analysed jointly in the same model. The hypothesis that the difference should be equal to 0 (corresponding to no treatment effect) was tested by a two-sided test at a 5% significance level.

- The energy intake (without a preload paradigm) and the relative amount of energy intake in each of the macronutrient groups were analysed by a model identical to the one used for the primary PD endpoint.
- Meal duration and all VAS-rating endpoints were analysed by the model described above.
- Antral Area: the fasting antral area prior to the preload period, the average antral area during the preload period and the antral area prior to the buffet meal period were analysed by a model corresponding to the one described for the primary PD endpoint with the addition of a centre effect. The change in antral area was analysed by the model described above with the addition of a centre effect. The time at which the antral area decreased to 75% of the maximum antral area and the time at which the antral area decreased to 50% of the maximum antral area are tabulated.
- Gastric Emptying: C_{max}, $AUC_{0\text{-}60min}$, $AUC_{0\text{-}300min}$ and $AUC_{0\text{-}60min}/AUC_{0\text{-}300min}$ were analysed by a model corresponding to the one described for the primary PD endpoint. The analysis of t_{max} was performed by use of non-parametric methods for paired samples. The median difference of t_{max} (glimepiride versus liraglutide and placebo versus liraglutide) was estimated together with a 95% confidence interval using the Hodges Lehmann estimator.
- The resting energy expenditure and the change in efficacy variables were analysed by the model described above.

Explorative Analyses

It was expected that some of the efficacy variables measured during the test days would be correlated. To explore this further a linear normal model (analysis of covariance, ANCOVA) that included effects of treatment, period, random effect of subject plus the effects of one or more covariates and potentially an interaction between treatment and the covariates was applied. The expected difference in the efficacy variable associated with a given difference in the covariate was estimated together with a 95% confidence interval. The effect of glimepiride and placebo compared with liraglutide was estimated in a manner similar to the one described under the ANOVA model (see above).

The following was analysed:

- The relationship between the energy intake and the fasting antral area prior to the preload period.
- The relationship between the energy intake and the antral area prior to the buffet meal period.
- The relationship between the energy intake and the change in antral area from -10 to 60 min.
- The relationship between the energy intake and the average antral area.
- The relationship between the energy intake and the average sensation of hunger, fullness, satiety and nausea during the preload period.
- The relationship between the 24 h resting energy expenditure and pulse

Safety

The assessment of safety parameters was based on descriptive statistics.

Demography of Trial Population

Twenty-seven (27) male and 19 females with type 2 diabetes were enrolled in the trial. Forty-four (44) subjects were white and two were of other origin. The subjects were between 38 and 65 years of age. Thirty-three (33) subjects were in OAD monotherapy treatment prior to the trial and 13 were diet treated. The mean HbA_{1c} was 7.4%. The subjects had a BMI between 27.0 and 39.9kg/m^2.

Efficacy Results

- **Energy Intake**
  - The estimated reduction of energy intake was 9% at the *ad libitum* buffet-style meal including a preload

paradigm after liraglutide treatment compared to both placebo and glimepiride (the ratios of the energy intake with 95% CIs were 0.91 [0.78; 1.06] and 0.91 [0.78; 1.07] respectively).
  - No statistically significant difference for the energy intake between liraglutide treatment versus placebo and glimepiride at the *ad libitum* meal including a preload paradigm was found.
  - The estimated reductions of energy intake were 9% and 15% at the *ad libitum* meal without a preload paradigm after liraglutide treatment compared to both placebo and glimepiride (the ratios of the energy intake with 95% CIs were 0.91 [0.76; 1.09] and 0.85 [0.70; 1.03] respectively).
- **Macronutrient Distribution, Duration of Eating and Sensations of Appetite (Hunger, Fullness and Satiety) and Nausea**
  - The duration of eating at the *ad libitum* buffet meal including a preload paradigm was shorter after treatment with liraglutide compared to placebo (estimated difference -4.3min, 95% CI [-6.9; -1.7]) but no significant difference was found between liraglutide and glimepiride treatment. No significant difference of the duration of eating at the meal without a preload paradigm was found between liraglutide and glimepiride or placebo treatment.
  - No statistically significant differences between liraglutide and glimepiride or placebo treatment were found with respect to the macronutrient composition.
  - A statistically significantly lower fasting sensation, $R_{0,\ no\ preload}$, of hunger was observed after liraglutide treatment compared to placebo and glimepiride (estimated difference -20 mm, 95% CI [-28.3; -11.7] and estimated difference -11.7 mm, 95% CI [-20.4; -2.92] respectively).
  - No statistically significant differences were observed for all other endpoints regarding appetite sensations and nausea between liraglutide and glimepiride or placebo treatments.
- **Gastric Distension (Antral Area Measured by Ultrasound)**
  - No statistically significant differences between any of the treatments were found for the endpoints derived from the antral images.
  - No statistically significant relationships were observed between the effects of liraglutide on appetite and antral area.
  - No statistically significant relationship between energy intake and any of the derived variables of antral area was found.
- **Gastric Emptying (Assessed by Paracetamol Concentrations)**
  - The mean paracetamol $AUC_{0\text{-}300min}$ of liraglutide treatment was significantly lower compared to placebo (estimated ratio 0.88, 95% CI [0.80; 0.96]) but not significantly different from glimepiride (estimated ratio 0.93, 95% CI [0.85; 1.03]).
  - The mean paracetamol $AUC_{0\text{-}60min}$ and C_{max} after liraglutide treatment were significantly lower compared with placebo and glimepiride (estimated ratios of $AUC_{0\text{-}60min}$: 0.62, 95% CI [0.52; 0.73] and 0.67, 95% CI [0.56; 0.80]; estimated ratios of C_{max}: 0.80, 95% CI [0.72; 0.89] and 0.85, 95% CI [0.76; 0.95].
  - The mean part of paracetamol exposure that appeared within the first postprandial hour ($AUC_{0\text{-}60min}/AUC_{0\text{-}300min}$) was significantly lower after liraglutide treatment compared with placebo and glimepiride (estimated ratios 0.70, 95% CI [0.62; 0.79] and 0.71, 95% CI [0.62; 0.81]).
  - Paracetamol t_{max} occurred on average 20 minutes later after liraglutide treatment compared with placebo and glimepiride.
- **Resting Energy Expenditure**
  - The estimated change in mean 24-hour resting energy expenditure was 576 kJ higher after liraglutide treatment compared with placebo (95% CI [-132; 1285]) and 270 kJ higher compared with glimepiride (95% CI [-483; 1022]) but the result was not statistically significant.
- **Body Weight and Waist Circumference**
  - Liraglutide significantly lowered the mean body weight 1-2 kg after a 4-week treatment period compared to placebo or glimepiride (estimated difference -1.31 kg, 95% CI [-2.06; -0.56] and -2.02 kg, 95% CI [-2.79; -

1.24]).
  - No significant difference in waist circumference was found after a 4-week treatment period.
- **Fasting Plasma Glucose, Hormones, Lipids and Anti-inflammatory Markers**
  - Liraglutide significantly lowered the mean fasting plasma glucose after a 4-week treatment period compared to placebo and glimepiride treatment (estimated difference -3.21 mmol/L, 95% CI [-4.11; -2.30] and -1.37 mmol/L, 95% CI [-2.29; -0.44])), however, there were no differences regardless of treatment for insulin and glucagon levels.
  - Liraglutide significantly suppressed the mean peptide YY concentration after a 4-week treatment period compared to placebo and glimepiride (estimated difference -34.0, 95% CI [-54.2; -13.8] and -37.4, 95% CI [-58.5; -16.3]).
  - A significant difference between liraglutide and placebo treatment was found in the change from baseline in mean adiponectin (estimated difference -1.23, 95% CI [-2.16; -0.29]) but not between liraglutide and glimepiride treatment (estimated difference -0.38, 95% CI [-1.34; 0.57]).
  - Liraglutide significantly lowered the concentration of hsCRP after the 4-week treatment period compared to glimepiride (estimated difference -3.13, 95% CI [-6.02; -0.24]) but not compared to placebo (estimated difference -2.35, 95% CI [-5.17; 0.48]).
  - No significant difference between liraglutide and placebo or liraglutide and glimepiride were found for concentration changes of ghrelin, leptin, GIP, lipids (TC, LDL-C, VLDL-C, HDL-C, FFA) and TNF□after a 4-week treatment period.

Safety Results

- TEAEs were reported by 54.8%, 67.7% and 40.0% of the subjects when treated with liraglutide, glimepiride and placebo respectively. The most frequently reported AEs for liraglutide and glimepiride were gastro-intestinal disorders such as nausea (16.1% and 12.9% respectively), diarrhoea (6.5%) and constipation (6.5%), and nervous system disorders as headache (19.4% and 22.6% respectively). The frequency of AEs of gastro-intestinal disorders appeared to be similar for liraglutide and glimepiride (29%) but less for placebo (13.3%). The most frequently reported AEs for placebo treatment were headache (13.3%), nasopharyngitis (13.3%), nausea (6.7%) and dizziness (6.7%).
- The majority of AEs were mild and moderate. The 11 severe AEs reported after treatment with liraglutide (4), glimepiride (4) and placebo (3) were gastro-intestinal and nervous system disorders.
- Four (4) subjects withdrew from the trial due to non-serious AEs. Three (3) subjects withdrew during liraglutide treatment due to diarrhoea, depressed mood and erythema, respectively, and 1 subject withdrew during glimepiride treatment because of nausea, anorexia and anxiety. All AEs leading to withdrawal were considered by the investigator to be possibly or probably related to trial products.
- No serious AEs were reported during liraglutide treatment but 5 serious AEs were reported by 2 subjects in glimepiride treatment. One (1) subject experienced abdominal distension, constipation, gastro-intestinal pain and vomiting on the same day and 1 subject experienced abdominal pain. For both subjects, the serious AEs (gastro-intestinal disorders) were assessed by the investigator to be possibly related to trial product.
- Eleven (11) hypoglycaemic episodes, all symptoms only, were reported. Three (3) episodes occurred during liraglutide treatment, 6 during glimepiride treatment and 1 during placebo treatment).
- No safety concerns were raised from vital signs or laboratory measurements.

Conclusions

- The estimated reduction of energy intake was 9% to 15% when treated with liraglutide compared to glimepiride or placebo, however, no statistically significant difference of the energy intake between liraglutide treatment versus placebo and glimepiride was found at the *ad libitum* meals.
- The duration of the *ad libitum* buffet-style meal including a preload paradigm was shorter after treatment with liraglutide compared to placebo whereas no difference was found between the macronutrient compositions of the meal regardless of treatment.

- A significant lower fasting sensation of hunger at the meal without a preload was observed after liraglutide treatment compared to placebo and glimepiride. No other effects of liraglutide on sensations of appetite (hunger, fullness and satiety) and nausea were found.
- All subjects enrolled in the trial reported low ratings of nausea during the entire period.
- No treatment effect was found regarding antral area (gastric distension).
- Liraglutide caused a minor delay in the postprandial rate of gastric emptying.
- No significant effect of treatment on energy intake adjusted for sensations of appetite was found.
- No relationships between energy intake and antral area were found.
- The change in resting energy expenditure was 576 kJ and 270 kJ higher after liraglutide treatment compared with placebo and glimepiride but no difference of the resting energy expenditure (24 hour) regardless of treatment was found.
- Liraglutide lowered the body weight by 1-2 kg compared to placebo or glimepiride. No change in waist circumference was found.
- Liraglutide lowered the fasting plasma glucose by 3.21 mmol/L (compared to placebo) and 1.37 mmol/L (compared to glimepiride). No differences were found in the levels of fasting insulin and glucagon regardless of treatment.
- Liraglutide suppressed the peptide YY concentration compared to placebo and glimepiride.
- No overall effect of liraglutide on ghrelin, GIP, leptin, lipids or TNFα was found.
- Liraglutide lowered the concentration of hsCRP (when compared to glimepiride) and the concentration of adiponectin (when compared to placebo), however, the result was inconclusive.
- No SAEs were reported during liraglutide treatment (5 SAEs reported by 2 subjects in glimepiride treatment). AEs were generally of mild or moderate severity. The frequency of AEs was higher during liraglutide and glimepiride treatment than during treatment with placebo. The most frequently reported AEs were related to the gastro-intestinal and the nervous system (primarily headache) regardless of treatment.

The trial was conducted in accordance with the Declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, October 2000. Last amended with Note of Clarification on Paragraph 29 by the WMA General Assembly, Washington 2002) and ICH Good Clinical Practice (1996).

4.2.7 BE-Evaluations (NN2211-1331, 1636, 1692, 1693)

NN2211-1331

Title: A randomized, single-blind, single-centre, two period, cross-over trial investigating the bioequivalence between completed Phase 2 and planned Phase 3 formulations of liraglutide in healthy subjects

Investigator and Study Center(s):
Dr. Med. Margarete Müller
AAI Deutschland GmbH & Co KG

Trial Sites
AAI Deutschland GmbH & Co KG
Wegenerstraße 13
89231 Neu-Ulm, Germany

Study Sponsor:
Novo Nordisk A/S
Novo Allé
2880 Bagsvaerd, Denmark

Bioanalytical Analysis:

b(4)

STUDY PERIOD: 28 May 2004 (Trial Initiated) to 12 July 2004 (Trial Completed)

Objective:
The primary objective of this study was to test for bioequivalence of two formulations, based on AUC(0-t) and Cmax, after a single subcutaneous (s.c.) dose of two formulations of liraglutide

The secondary objective of the study was to

- To estimate the relative bioavailability (Frel) between the two formulations of liraglutide based on AUC (0-t) and AUC (0-∞), and to estimate tmax and t½ of liraglutide.
- To test for equivalence of two formulations of liraglutide, based on AUC(0-∞).
- To evaluate the safety of two formulations of liraglutide.

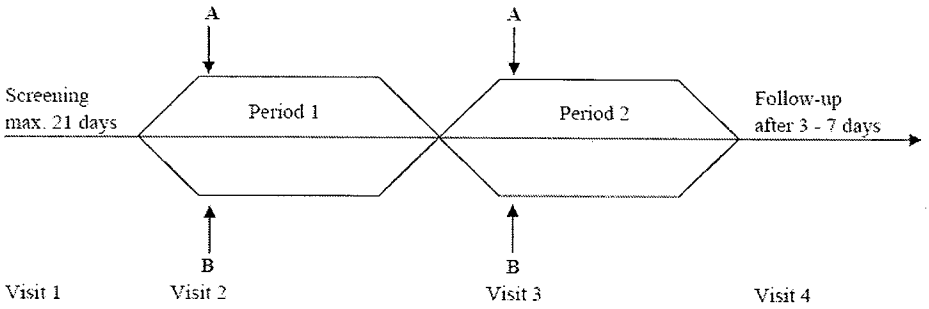
Rationale for the Trial:
The trial was performed in order to determine whether two formulations of liraglutide, from completed Phase 2 and planned Phase 3, are bioequivalent after single doses in healthy subjects. The change in formulation from Phase 2 to Phase 3 consisted of the switch of isotonic agent from mannitol to propylene glycol.

Study Design:

The trial was a randomised, single-blind, single-centre, two-period, cross-over trial designed to test for bioequivalence between the two formulations of liraglutide, i.e. formulation from completed Phase 2 studies and planned Phase 3 studies.

In this trial a s.c. administration of single doses of liraglutide were administered to healthy male and female subjects on two different occasions, separated by a two-week wash-out period. The total duration of the trial for the individual subject was up to 7 weeks.

Figure 1: Trial Design



Treatment A: Liraglutide 6.25 mg/mL (Phase 3 formulation), 1 mg s.c. in the abdomen
Treatment B: Liraglutide 5 mg/mL (Phase 2 formulation), 1 mg s.c. in the abdomen

The trial comprised the following visits:

- Visit 1 (Screening)
- Visit 2 (Dosing of first dose): in-house with 3 nights, within 3 weeks after Visit 1
- Visit 3 (Dosing of second dose): in-house with 3 nights, 14 days after first dose
- Visit 4 (Follow-up): within 3-7 days after completion of Visit 3

Blood samples for estimation of the liraglutide plasma concentrations were drawn at Visit 2 and 3; before dosing at -30 and -15 minutes, and at 2, 4, 6, 8, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 14.5, 15, 16, 24, 36, 48 and 60 hr after dosing.

Study Population:
Twenty two healthy volunteers were enrolled in this study. The mean age of the study population was 32.5 years (range 19 to 43 years). Table 1 below shows the demographics of the enrolled patients.

Table 1: Baseline Demographics of Study Population.

	N	Minimum	Median	Maximum	Mean	Std-Dev.
Age [years]	22	19	36	43	32.5	8.1
Body Weight [kg]	22	49.6	74.75	89.8	72.66	12.4
Height [m]	22	1.57	1.8	1.89	1.7	0.1
BMI [kg/m²]	22	19.6	23.75	27	23.73	2.4

Investigational Product and Dose Selection:

Liraglutide was supplied by Novo Nordisk A/S as 5 mg/mL and 6.25 mg/mL trial products, respectively, in 1.5 mL Penfill cartridges, a formulation of 5 mg/mL (pH = 7.4, mannitol) and a formulation of 6.25 mg/mL (pH = 7.7, propylene glycol), respectively. NovoFine®G30 needles (8mm) were used for administration.

The 1 mg liraglutide dose (corresponding to 200 µl= 20 click of Phase 2 formulation (5 mg/mL) and to 160 µl= 16 click of the Phase 3 formulation (6.25 mg/mL) was administered by the investigator subcutaneously in the abdomen by means of a NovoPen® and NovoFine®G30 needles.

Table 2: Batch number of product used in this trial

Trial Product	Dose	Batch Number	Expiry Date
Liraglutide 6.25 mg/mL (Phase 3 formulation)	1 mg corresponding to 160 µl (= 16 clicks)	NLDP016	09 June 2005
Liraglutide 5 mg/mL (Phase 2 formulation)	1 mg corresponding to 200 µl (= 20 clicks)	LLDP007	02 October 2005

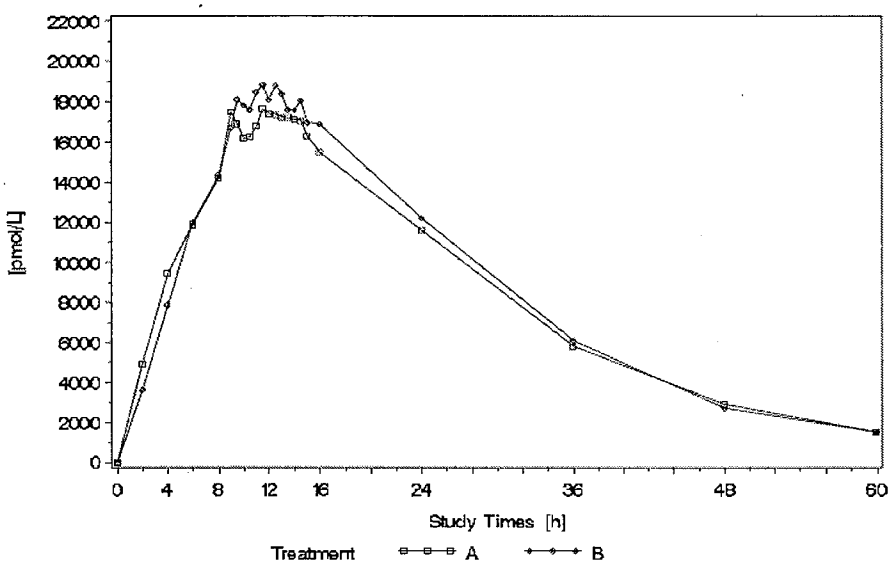
The chosen dose of 1 mg for this trial was the highest single dose, which was well tolerated in the previously performed single dose study (NN2211-1149) without causing unacceptable nausea and vomiting. The selected dose was not expected to result in hypoglycemic events. Furthermore, the dose was sufficient to give measurable plasma concentrations up to 60 hr post dosing.

Bioanalysis:
Quantitative assessment of liraglutide was done using enzyme immunoassays (ELISA). The lower limit of quantification of the assay was 18 pmol/L. The calibration curves were analyzed at liraglutide concentration range of 18 pmol/L to 4500 pmol/L. The precision of the assay, as determined from analysis of quality control samples ranged between [redacted]. The mean accuracy (% Bias) ranged between [redacted]. Between-batch precision (%CV) results of the calibration standards of liraglutide was less than or equal to 5.9 % and accuracy (%Bias) ranged from [redacted] b(4)

Data Analysis:
AUC (0-t), AUC (0-∞), Cmax, and t½ were compared statistically between treatments by analysis of variance (ANOVA) after logarithmic pre-transformation, HVD without transformation. The model included effects of subject, visit and treatment. Ratios between the two formulations were estimated with 90% confidence intervals. tmax was compared between treatments by the corresponding nonparametric methods. Bioequivalence could be declared if the confidence intervals for the ratios of AUC(0-t) and Cmax were fully contained within the limits (0.80, 1.25). Safety data were evaluated descriptively only.

Pharmacokinetics Results:
The mean plasma concentration time profile of liraglutide is shown in Figure-1. The mean plasma liraglutide concentration profiles were almost identical for the two trial products.

Figure 1: Mean Plasma profile of liraglutide by formulation-linear scale



A : Phase 3 formulation (n=21), B : Phase 2 formulation (n=22)

The summary of pharmacokinetic parameters of liraglutide from Phase 2 formulation and Phase 3 formulation is presented below in Table-3.

Table 3: Summary Statistics for the Pharmacokinetics Endpoints by Formulation.

Parameter	A : Phase 3 formulation (n=21)	B : Phase 2 formulation (n=22)
AUC(0-t) [h*nmol/L]	481 (25%)	489 (27%)
AUC(0-∞) [h*nmol/L]	508 (25%)	515 (26%)
C_{max} [nmol/L]	19.9 (23%)	20.5 (25%)
t_{max} [h] - median and range	12 (9-15)	13 (9.5-16)
$t_{½}$ [h]	12.2 (14%)	11.7 (21%)
HVD [h] - mean and %CV	21.6 (26%)	21.5 (20%)

The statistical results of the comparisons between Phase 2 formulation and Phase 3 formulation for the primary endpoints are summarized in Table 4.

Table 4: Statistical Analysis for the Pharmacokinetics Endpoints by Formulation

Parameter - Method	Ratio A / B with 90% Confidence Interval
AUC(0-t) - ANOVA(ln)	97.63% (92.03%, 103.55%)
AUC(0-∞) - ANOVA(ln)	98.03% (92.83%, 103.50%)
C_{max} - ANOVA(ln)	96.32% (89.18%, 104.01%)
t_{max} [h] - difference, nonparametric	-0.25h (-1h, +0.25h)
$t_{½}$ - ANOVA(ln)	104.96% (96.81%, 113.78%)
HVD - ANOVA	101.15% (92.51%, 109.79%)

The relative bioavailability Frel of the Phase 3 formulation compared to the Phase 2 formulation was estimated at 98%. Based on the pre-defined criteria based on AUC(0-t) and Cmax, but also based on AUC(0-∞), it can be concluded that the two formulations are bioequivalent.

Summary of pharmacokinetic results

- The relative bioavailability Frel of the Phase 3 formulation compared to the Phase 2 formulation was estimated at 97.63% based on AUC(0-t) with the 90% confidence interval (CI) ranging from 92% to 104%, and at 98.03% based on AUC(0-∞) (CI: 93% 104%). The between treatment ratio was 96.32% for Cmax (CI: 89%-104%). In all cases the confidence intervals were fully contained within the (80%, 125%)-acceptance range.
- Mean concentration-time profiles of both preparations were very similar with a slow absorption phase, reaching a maximum plasma concentration approximately 8 hours after dosing.
- The maximum concentration Cmax was estimated at 19898 pmol/L for the Phase 3 formulation (geometric LS mean, n=21) and at 20659 pmol/L for the Phase 2 formulation (n=22).
- The median tmax value was 12 (Phase 3 formulation) or 13 hours (Phase 2 formulation).
- The last sample, taken 60 hours after dosing, in the mean still contained about 1500 pmol/L. Terminal half-lives were estimated at approximately 12 hours.
- The mean half-value duration (HVD) was 21.8 h or 21.6 h, respectively.

Safety Conclusions

- As to adverse events, both treatments were safe and well tolerated. No serious adverse events were reported and none of the subjects withdrew due to an adverse event.
- In total, 48 treatment-emergent adverse events were reported, 16 events after Treatment A (liraglutide 6.25 mg/m, Phase 3 formulation) and 32 events after Treatment B (liraglutide 5mg/mL, Phase 2 formulation). Most of the adverse events were mild in intensity (28 events were mild, 16 were moderate and 4 were severe). Forty-one (41) adverse events were considered to be probably or possibly drug-related. As expected, the most frequently reported adverse events were gastrointestinal disorders.
- The following probably or possibly drug-related adverse events were reported: Nausea, vomiting, retching, stomach discomfort, gastrointestinal pain, upper abdominal pain, abdominal pain, abdominal distension, diarrhoea, malaise, headache, fatigue and dizziness.
- For laboratory tests, only single values outside the reference range were observed without obvious trend or pattern. Mean values of the laboratory parameters assessed remained quite stable during the course of the trial.
- Mean blood pressure and pulse remained stable after dosing of both treatments.
- Physical examinations were without clinically relevant findings.
- There were no indications that any of the ECG parameters were affected by one of the treatments.
- No episodes of hypoglycemia were observed.

Overall Conclusions

The results from this trial in healthy subjects demonstrated that:

- Based on the pre-defined criteria of AUC(0-t), Cmax, and AUC(0-∞), it can be concluded that the two formulations are bioequivalent.
- The relative bioavailability Frel of the Phase 3 formulation compared to the Phase 2 formulation was estimated at 98%.
- For both formulations equally, tmax was estimated at about 12 to 13 hours, HVD at about 22 hours and t½ at about 12 hours.
- There were no indications of any clinically relevant differences of the two formulations of liraglutide, with respect to safety, when administered as a subcutaneous single dose of 1 mg.

Reviewer's Comment:

The overall study design and data analysis seems reasonable except that the sponsors used a dose of 1.0 mg in this bioequivalence study, whereas the highest proposed dose is 1.8 mg. The sponsor's rationale for using a lower dose is the better tolerability profile expected from the lower dose due to decreased incidences of gastro-intestinal and hypoglycemic adverse events.

NN2211-1636

Title: A randomized, double-blind, single-centre, three-period, cross-over trial in healthy subjects investigating the bioequivalence between each of the two new liraglutide formulations at pH 7.9 and 8.15 and the planned Phase 3 formulation at pH 7.7. b(4)

Title of Trial	A randomised, double-blind, single-centre, three-period, cross-over trial in healthy subjects investigating the bioequivalence between each of the two new liraglutide formulations at pH 7.9 and 8.15 and the planned Phase 3 formulation at pH 7.7
Trial ID	NN2211-1636
Development Phase	Phase 1
IND Number (US only)	Not Applicable
Compound Name	Liraglutide
Indication	Diabetes mellitus
Investigator	[redacted]
Trial Site	[redacted]
Trial Initiated	13 January 2005
Trial Completed	30 March 2005
Sponsor	Global Development, Novo Nordisk A/S
Director, Medical & Science - Liraglutide	Milan Zdravkovic, MD, PhD Novo Nordisk A/S
International Trial Manager	Marianne Ekblom, PhD Novo Nordisk A/S
Local Trial Manager	Elie Antoun, Novo Nordisk Pharmaceuticals Pty Ltd
Statistician	Charlotte Hindsberger, PhD, Novo Nordisk A/S
Medical Writer	Julie Maubach Edmed, PhD, Novo Nordisk A/S
Report Date	24 August 2005

b(4)

This trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

TITLE OF TRIAL

A randomised, double-blind, single-centre, three-period, cross-over trial in healthy subjects investigating the bioequivalence between each of the two new liraglutide formulations at pH 7.9 and 8.15 and the planned Phase 3 formulation at pH 7.7

INVESTIGATOR

The signatory and principal investigator in this trial was Patrick Walker, MD, CMAX, Level 5, East Wing, Royal Adelaide Hospital, North Terrace, Adelaide, South Australia 5000, Australia

TRIAL SITE

The trial was conducted in one centre: CMAX, Level 5, East Wing, Royal Adelaide Hospital, North Terrace, Adelaide, South Australia 5000, Australia

PUBLICATIONS

None

TRIAL PERIOD	**DEVELOPMENT PHASE**
13 January 2005 until 30 March 2005	Phase 1

OBJECTIVES

Primary Objective:

- To test for bioequivalence between each of the two new liraglutide formulations at pH 7.9 and 8.15 and the planned phase 3 formulation at pH 7.7, based on $AUC_{(0-t)}$ and C_{max}, after a single subcutaneous (s.c.) dose.

Secondary Objectives:

- To estimate the relative bioavailabilities (F_{rel}) between the three formulations of liraglutide based on $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$, and to estimate t_{max} and $t_{½}$ of liraglutide.
- To evaluate the safety of three formulations of liraglutide.

In both the trial protocol and statistical analysis plan, liraglutide at pH 7.7 has been referred to as the planned phase 3 formulation. However, this Integrated Clinical Trial Report will refer to liraglutide at pH 7.7 only, as the formulation used in the phase 3 programme may be different.

METHODOLOGY

This was a single-centre, randomised, double-blind, three-period, cross-over trial in healthy subjects. All subjects were to receive 0.75 mg liraglutide s.c. at three different pH values; pH 7.7, 7.9, and 8.15 and on three different occasions. All doses were administered in the evening using a NovoPen® (3 mL) injection device. The trial included a screening visit followed by three dosing visits, spent in the trial facility and lasting four days each, and a follow-up visit. Each dose was followed by blood sampling (60-hour profiles) and was separated by a 14-day washout period. A number of safety parameters were investigated throughout the trial.

NUMBER OF SUBJECTS PLANNED AND ANALYSED

Forty-two subjects were screened, 24 subjects entered the trial, and 22 subjects completed the trial. All 24 subjects were included in both the pharmacokinetic and safety analyses.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION

Healthy subjects of both sexes, aged 18-50 years, and with a BMI between 18-27 kg/m^2 (both inclusive) were eligible for inclusion into the trial. Informed consent was obtained for each subject prior to the start of any trial-related activities.

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Liraglutide in 6.25 mg/mL solutions were delivered in pre-filled cartridges to be used in a disposable pen device (NovoPen®, 3 mL) at the following pH values: pH 7.7 (batch number PLDP002), 7.9 (batch number PLDP003), and 8.15 (batch number PLDP004). A single dose of 0.75 mg (corresponding to 120 µL) was administered subcutaneously in the abdomen of the participating subjects.

DURATION OF TREATMENT

Three single doses of liraglutide at different pH levels were administered in the trial. The total trial duration for each subject was up to 11 weeks.

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Not applicable.

CRITERIA FOR EVALUATION – PHARMACOKINETICS

The pharmacokinetic results were based on concentration-time curves of liraglutide after administration of liraglutide at pH 7.7, 7.9, and 8.15 in plasma up to 60 hours (22 time-points) after dose administration.

CRITERIA FOR EVALUATION – SAFETY

The safety evaluation was based on physical examination, vital signs, ECG, adverse events, thyroid ultrasonography, clinical laboratory assessments (haematology, clinical chemistry, urinalysis, and thyroid safety parameters), hypoglycaemic episodes, and bed-side glucose monitoring.

STATISTICAL METHODS

- *Primary pharmacokinetic analysis*

$AUC_{(0-t)}$ and C_{max} were determined after a single, subcutaneous dose of liraglutide at pH 7.7, 7.9, and 8.15. Comparisons of the three formulations were performed using a linear normal model (Analysis of Variance; ANOVA) for the log transformed values of $AUC_{(0-t)}$ and C_{max}, respectively. The model included effects of period, formulation and a random effect of subject. Using this model, the ratios of the two formulations with pH 7.9 and 8.15, respectively, and the formulation of pH 7.7, were estimated with 90% confidence intervals (CI). The estimated ratios and CIs were retransformed from the log values after analysis. Bioequivalence was defined as having the CI for both $AUC_{(0-t)}$ and C_{max} entirely contained within [0.80, 1.25].

- *Secondary pharmacokinetic analysis*

The relative bioavailabilities (F_{rel}) of the three formulations of liraglutide were calculated using $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$. Comparisons of $AUC_{(0-\infty)}$ and $t_{1/2}$ were performed for the different formulations as described for the primary endpoints. The analysis of t_{max} was performed by the use of non-parametric methods for paired samples. The difference in medians between the two formulations (pH 7.9 and 7.7 and pH 8.15 and 7.7) was estimated with a 90% CI using the Hodges-Lehmann estimator.

- *Safety*

All adverse events were listed by subject, including demographic information. MedDRA system organ class and MedDRA preferred term. Treatment emergent adverse events were additionally summarised by formulation. Clinical laboratory parameters, including thyroid safety parameters, plasma glucose, vital signs, and ECG, were summarised by formulation and/or sample time using descriptive statistics. Abnormal laboratory data was listed separately.

DEMOGRAPHY OF TRIAL POPULATION

The 24 participating subjects (13 males and 11 females) had a mean age of 25 years (range 18 to 45 years), mean weight of 70 kg (range 51 to 93 kg), and a mean BMI of 23 kg/m^2 (range 18 to 27 kg/m^2). Twenty-one subjects were white, two were Asian/Pacific Islanders, and one was referred to as other (Eurasian).

PHARMACOKINETIC RESULTS

- Bioequivalence was demonstrated for liraglutide for pH 7.9 and pH 7.7, and for pH 8.15 and pH 7.7 with respect to the primary endpoints, as the CI of the ratio of the corresponding values were entirely contained within the interval from 0.80 to 1.25 required for the demonstration of bioequivalence.

		pH 7.9 / pH 7.7	pH 8.15 / pH 7.7
AUC(0-t)(pmol*h/L)	Estimate	0.93	1.02
	Upper 90% limit	0.98	1.07
	Lower 90% limit	0.89	0.97
Cmax(pmol/L)	Estimate	0.93	1.02
	Upper 90% limit	1.00	1.09
	Lower 90% limit	0.88	0.96

- Additionally, bioequivalence was supported by the secondary endpoints $AUC_{(0-\infty)}$ and $t_{1/2}$, having CIs entirely contained within the interval from 0.80 to 1.25.
- Similar results for t_{max} were obtained with all three pH formulations.

		pH 7.9 / pH 7.7	pH 8.15 / pH 7.7
AUC(0-inf)(pmol*h/L)	Estimate	0.94	1.02
	Upper 90% limit	0.98	1.07
	Lower 90% limit	0.90	0.98
t1/2(h)	Estimate	1.01	0.98
	Upper 90% limit	1.10	1.07
	Lower 90% limit	0.93	0.90
		pH 7.9 - pH 7.7	pH 8.15 - pH 7.7
tmax(h)	Estimate	-0.75	-0.50
	Upper 90% limit	0.25	1.00
	Lower 90% limit	-2.00	-2.00

SAFETY RESULTS

- All three formulations of liraglutide were well tolerated when administered as a single 0.75 mg s.c. injection.
- There was one serious adverse event in the trial. This subject experienced vomiting of a moderate severity after the first liraglutide administration (pH 8.15) and was admitted to hospital, where he received intravenous fluids and anti-emetics. The subject fully recovered and was discharged from hospital two days after dosing. The relation to trial drug was considered probable by the principal investigator and the subject was withdrawn from the trial.
- A total of 63 treatment emergent adverse events experienced by 20 subjects were reported. The incidence of treatment emergent adverse events was similar following administration of all three liraglutide formulations (50, 61, and 42% at pH 7.7, 7.9, and 8.15, respectively). The most commonly reported events were gastrointestinal disorders (vomiting and nausea) and nervous system disorders (headache and dizziness), where relation to trial drug was possible or probable. Most of these events were mild in severity and subjects fully recovered within a few days.
- There were no clinically significant and/or consistent drug-related changes in vital signs, physical findings, thyroid safety parameters, or safety laboratory parameters after administration of either of the three liraglutide formulations.
- Overall, liraglutide at pH 7.7, 7.9, and 8.15 have comparable safety profiles.

CONCLUSIONS
The results of this trial in healthy subjects demonstrated: • Bioequivalence between the formulations of liraglutide at pH 7.9 and 7.7 with respect to $AUC_{(0-t)}$ and C_{max}. Additionally, bioequivalence was supported by the secondary endpoints $AUC_{(0-\infty)}$ and $t_{1/2}$, having CIs entirely contained within the interval from 0.80 to 1.25. t_{max} was similar for both formulations. • Bioequivalence between the formulations of liraglutide at pH 8.15 and 7.7 with respect to $AUC_{(0-t)}$ and C_{max}. Additionally, bioequivalence was supported by the secondary endpoints $AUC_{(0-\infty)}$ and $t_{1/2}$, having CIs entirely contained within the interval from 0.80 to 1.25. t_{max} was similar for both formulations. • One SAE relating to moderate vomiting was reported after liraglutide administration. • There were no clinically significant and/or consistent drug-related changes in vital signs, physical findings, thyroid safety or safety laboratory parameters after administration of either of the three liraglutide formulations.
The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Reviewer's Comment:

The objective of this trial was to test the bioequivalence (BE) between each of the two new liraglutide formulations at pH 7.9 and 8.15 and the planned phase 3 formulation at pH 7.7, based on AUC(0-t) and Cmax, after a single s.c. dose. In this BE study the sponsor used 0.75 mg dose of liraglutide, whereas the highest proposed dose is 1.8 mg. The sponsor's rationale for using a lower dose is the better tolerability profile expected from the lower dose due to decreased incidences of gastro-intestinal and hypoglycemic adverse events.

Quantitative assessment of liraglutide was done using enzyme immunoassays (ELISA). The lower limit of quantification of the assay was 18 pmol/L. The calibration curves were analyzed at liraglutide concentration range of [redacted] pmol/L. The precision of the assay, as determined from analysis of quality control samples ranged between [redacted]. The mean accuracy (% Bias) ranged between [redacted] Between-batch precision (%CV) results of the calibration standards of liraglutide was less than or equal to 8.6 % and accuracy (%Bias) ranged from [redacted]. Overall, the sponsor's study design and interpretation of pharmacokinetic and BE data was reasonable and acceptable.

b(4)

NN2211-1692 (Pivotal)

Title: A randomized, double-blind, single-centre, two-period, cross-over trial in healthy subjects investigating the bioequivalence between the Phase 3a formulation of liraglutide (formulation 4) and the planned Phase 3b formulation (final formulation 4).

Investigator and Study Center(s):

Edward Högestätt, M.D., Ph.D.,
Dept. of Clinical Chemistry and Pharmacology,
Lund University Hospital, Lund, Sweden

Trial Sites
Clinical Pharmacology, Phase I Unit, Lund University Hospital, Lund, Sweden

Study Sponsor:

Novo Nordisk A/S, Denmark

Bioanalytical Analysis: b(4)

Study Period: 29 January 2007 (Trial Initiated) to 16 April 2007 (Trial Completed)

Objective:
The primary objective of this study was to test for bioequivalence, after a single s.c. administration, of the phase 3a formulation of liraglutide (formulation 4) and the liraglutide formulation planned for phase 3b trials and subsequent marketing (final formulation 4).

The secondary objective of the study was to estimate the pharmacokinetics and to evaluate the safety of the two liraglutide formulations.

Brief Summary on Various Formulations Used in this NDA:

Through out the development of this product changes have been made within chemistry, manufacturing and control to give a more robust drug substance manufacturing process suitable for commercial production. In addition, the drug product formulation and manufacturing process has been gradually modified to give b(4)

of the liraglutide formulation 2, used in e.g. the trials NN2211- 1326, 1551 (phase 1) and 1310, 2072, 1499, 1571 (phase 2), a new formulation of liraglutide at pH 7.7 (formulation 3) was produced. The bioequivalence of liraglutide formulations 2 and 3 was demonstrated in trial NN2211-1331. The liraglutide formulation 3 was produced at three different pH (pH 7.7, 7.9 and 8.15) and their bioequivalence was demonstrated in trial NN2211-1636. The liraglutide formulation 3 (pH 7.7) was used in e.g. trials NN2211-1328, 1329, and 1334 (phase 1 and 2).

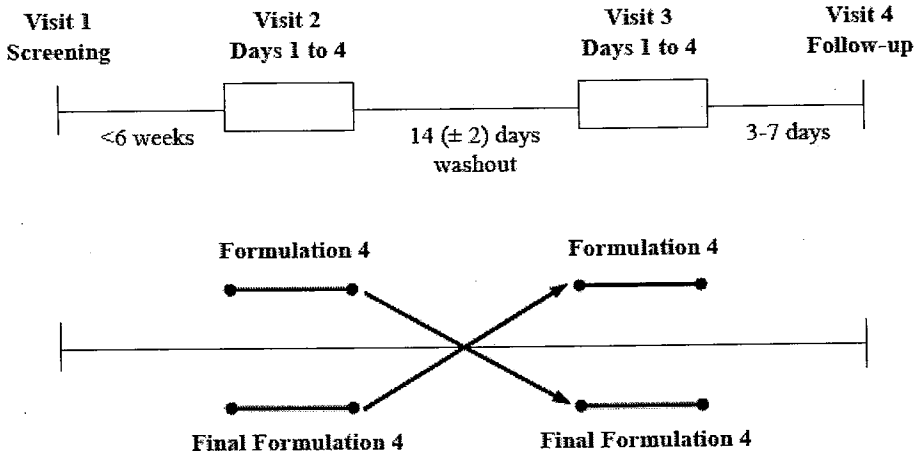
formulation 4 (pH 8.15). Formulation 4 was found to be bioequivalent with formulation 3 in trial NN2211-1693. Formulation 4 has been used in phase 3a trials in the EU and US.

In liraglutide final formulation 4, the drug substance manufacturing process has been optimised and the drug product manufacturing process has been up scaled from ———— . Final formulation 4 is the formulation planned to be used in phase 3b trials and the formulation planned to be marketed. b(4)

Study Design:
The current trial was a randomised, double-blind, single-centre, two-period, cross-over trial designed to test for bioequivalence between the phase 3a formulation of liraglutide (formulation 4) and the phase 3b formulation (final formulation 4).

In total, 22 healthy subjects were to be included in the trial. All subjects were to be treated with two formulations of liraglutide, formulation 4 and final formulation 4. The total duration of the trial for the individual subject was up to 10 weeks.

Figure 1: Trial Design



In this study each subject attended 4 visits:

- The first visit was a screening visit where the subject's eligibility was assessed.
- At Visits 2 subjects were admitted to the clinic for an in-house period, lasting for 3 days, when they were dosed with the liraglutide formulations. The Visit 2 (dosing of first formulation) was to take place within 6 weeks from the screening visit.
- Visit 3 (dosing of second formulation) was to take place 14 (± 2 days) from Visit 2.
- Finally, a follow-up visit (Visit 4) was to take place 3-7 days after finalization of Visit 3.

The trial was a randomized, double-blind, single-centre, two-period, cross-over trial where two single s.c. doses of liraglutide were administered to healthy male and female subjects in the evening on two different occasions, separated by a 14 (±2) day wash-out period. The liraglutide formulations, each in a dose of 0.72 mg, were given in the abdomen as a fixed volume of 120 µL

(12 clicks) from a FlexPen®. All subjects were administered liraglutide at approximately 9-10 pm. The rationale for evening administration was to utilize the pharmacokinetic profile of the drug with Cmax at 10-13 h after administration.

Blood samples for estimation of the liraglutide plasma concentrations were drawn at Visit 2 and 3; before dosing at -30 and -15 minutes, and at 2, 4, 6, 8, 9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 15, 16, 24, 36, 48 and 60 h after dosing.

Study Population:
Twenty one healthy volunteers were enrolled in this study. The mean age of the study population was 22 years (range 19 to 27 years). Table 1 below shows the demographics of the enrolled patients.

Table 1: Baseline Demographics of Study Population.

	All subjects N=21
Age (y)	
Mean (SD)	22 (2.1)
Min – Max	19 – 27
Sex (N (%))	
Male	19 (90.5%)
Female	2 (9.5%)
Ethnic origin (N (%))	
White	21 (100%)
Height (m)	
Mean (SD)	1.81 (0.08)
Min – Max	1.65 – 1.96
Weight (kg)	
Mean (SD)	74.3 (7.7)
Min – Max	61.8 – 89.5
BMI (kg/m^2)	
Mean (SD)	22.6 (1.7)
Min – Max	19.7 – 26.4

SD = standard deviation

Investigational Product and Dose Selection:
The trial products during the treatment period were provided by Novo Nordisk A/S as follows:

- Formulation 4: Liraglutide 6.0 mg/mL at pH 8.15, in pre-filled 3 mL cartridges dispensed in disposable pen device (———) for s.c. injection. b(4)

- Final formulation 4: Liraglutide 6.0 mg/mL at pH 8.15, in pre-filled 3 mL cartridges dispensed in disposable pen device (——— for s.c. injection.

The difference between the two formulations of the product was a slight modification of the drug substance manufacturing process to [redacted] upscale in drug product production from [redacted]. The products were indistinguishable from one another. There was a small difference between actual drug content in the two trial products, although the drug contents were within product specifications. The actual drug content in formulation 4 was 5.87 mg/mL and the actual drug content in final formulation 4 was 6.08 mg/mL. Since 120 µL of liraglutide was administered on each dosing, this corresponded to an actual dose of 0.7044 mg of liraglutide in formulation 4 and 0.7296 mg of liraglutide in final formulation 4. b(4)

Table 2: Batch number of product used in this trial

Trial Product	Dose	[redacted] Batch Number	Drug Product Batch Number (Bulk Batch Number)	Expiry Date
Formulation 4	120 µL of 6.0 mg/mL	SP51133	SQ50213	28 September 2007
Final Formulation 4	120 µL of 6.0 mg/mL	SP51755	SQ50360	4 May 2008

b(4)

The selected volume of 120 µL, corresponding to a dose of 0.72 mg, in the present trial was within the expected therapeutic window of treatment, was not expected to give hypoglycaemic episodes and was sufficient to give measurable plasma concentrations up to 60 hours post dosing.

Bioanalysis:
Quantitative assessment of liraglutide was done using enzyme immunoassays (ELISA). The lower limit of quantification of the assay was 18 pmol/L. The calibration curves were analyzed at liraglutide concentration range of 19 pmol/L to 5186 pmol/L. The precision of the assay, as determined from analysis of quality control samples ranged between [redacted]. The mean accuracy (% Bias) ranged between [redacted] %. Between-batch precision (%CV) results of the calibration standards of liraglutide was less than or equal to 10.9 % and accuracy (%Bias) ranged from -12.3 to 7.6 %.

b(4)

Data Analysis:
The primary endpoints were derived by the standard model-free, non-compartmental method. AUC (0-t) was calculated by the trapezoidal method and Cmax was the largest observed concentration during the 60 h post-dose blood sampling period. The comparison of the two formulations was done by using a linear normal model (analysis of variance, ANOVA) for the log-transformed values of AUC (0-t) and Cmax, respectively. The model included formulation and period as fixed effects and subject as a random effect. Bioequivalence between the two formulations was to be declared if the 90% CIs for the corresponding ratios of AUC(0-t) and Cmax were fully contained within the limits (0.80, 1.25).

Pharmacokinetics Results:
The mean plasma concentration time profile of liraglutide is shown in Figure-1&2. The mean plasma liraglutide concentration profiles were almost identical for the two trial products.

Figure 1: Mean Plasma profile of liraglutide by formulation-linear scale

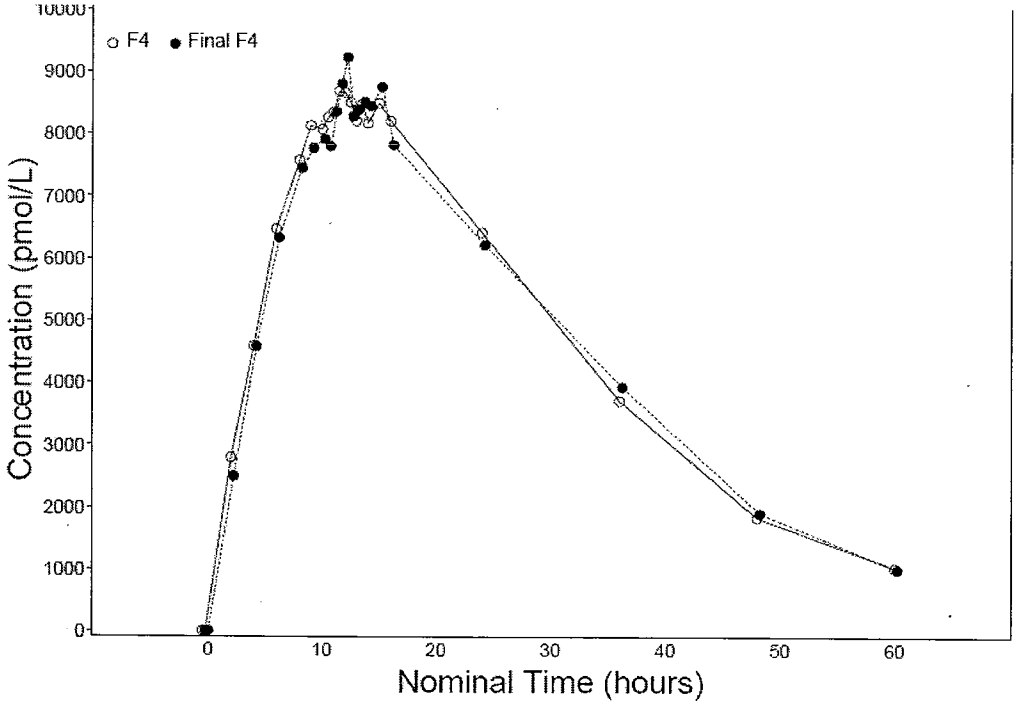
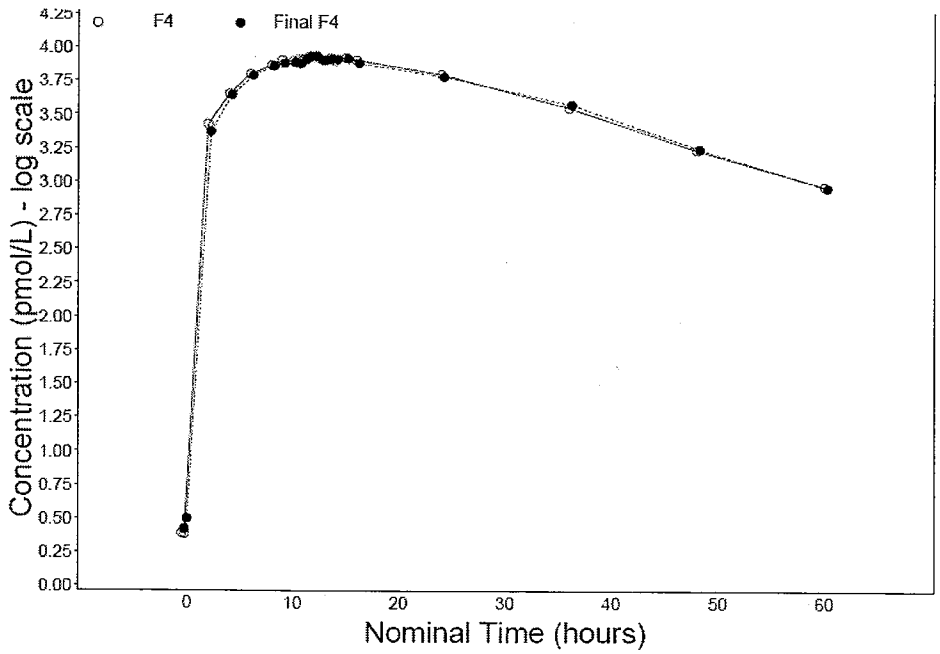


Figure 2: Mean Plasma profile of liraglutide by formulation-logarithmic scale.



The summary of pharmacokinetic parameters of liraglutide from formulation 4 and final formulation 4 is presented below in Table-3

Table 3: Summary Statistics for the Primary Pharmacokinetics Endpoints by Formulation.

	Formulation 4	Final Formulation 4	Final Formulation 4/ Formulation 4
$AUC_{(0-t)}$ (pmol × h/L)			
N	21	21	21
Mean	271944.7	271304.2	1.006
SD	44960.3	59414.8	0.193
Geometric Mean	268474.1	265463.1	0.989
Median	280023.5	243829.2	0.96
Min			
Max			
C_{max} (pmol × h/L)			
N	21	21	21
Mean	9771.1	10364.6	1.065
SD	1671.1	4320	0.402
Geometric Mean	9629.7	9798.8	1.018
Median	9960	9378	0.959
Min			
Max			

b(4)

The statistical results of the comparisons between formulation 4 and final formulation 4 for the primary endpoints are summarized in Table 4.

Table 4: Statistical Analysis for the Primary Pharmacokinetics Endpoints by Formulation

	Final Formulation 4/ Formulation 4
$AUC_{(0-t)}$	
Estimate	0.99
Lower 90% limit	0.92
Upper 90% limit	1.06
C_{max}	
Estimate	1.02
Lower 90% limit	0.91
Upper 90% limit	1.14

From the results in Table-4, formulation 4 and final formulation 4 were demonstrated to be bioequivalent because the 90% CIs for the corresponding estimated ratios of AUC(0-t) and Cmax were entirely contained within the limits (0.80, 1.25).

Due to the difference between actual drug content in the two trial products, a supplementary analysis was performed to correct for potential minor differences in the concentration of liraglutide in the two applied batches. The results of the comparisons between formulation 4 and final formulation 4 for AUC (0-t) and Cmax adjusted to actual drug content are summarized in Table 5 The results showed that bioequivalence could be demonstrated for the ratios of AUC (0-t)

and Cmax adjusted for actual drug content because the 90% CIs for the corresponding estimated ratios were entirely contained within the limits (0.80, 1.25).

Table 5: Statistical Analysis for the Primary Pharmacokinetics Endpoints by Formulation-Dose Adjusted

		Final Formulation 4 / Formulation 4
AUC(0-t)		
	Estimate	0.96
	Lower 90% limit	0.89
	Upper 90% limit	1.03
Cmax		
	Estimate	0.98
	Lower 90% limit	0.88
	Upper 90% limit	1.10

The results of secondary pharmacokinetic endpoints were AUC, tmax, t½ and λz and these endpoints are summarized for the pharmacokinetic analysis set in Table-6.

Table 6: Summary Statistics for the Secondary PK Endpoints of Liraglutide by Formulation

	Formulation 4	Final Formulation 4	Final Formulation 4/ Formulation 4
AUC (pmol × h/L)			
N	21	21	21
Mean	293481.1	292990	1.007
SD	57144.7	70791.1	0.203
Geometric Mean	288703.2	285480.3	0.989
Median	289149.3	272185.9	0.941
Min			
Max			
$t_{½}$ (h)			
N	21	21	21
Mean	13.24	13.31	1.022
SD	2.93	2.47	0.147
Harmonic Mean	12.7	12.9	1.001
Median	13.14	12.92	1.045
Min			
Max			
λ_z (1/h)			
N	21	21	21
Mean	0.055	0.054	0.999
SD	0.012	0.01	0.153
Geometric Mean	0.054	0.053	0.989
Median	0.053	0.054	0.957
Min			
Max			
	Formulation 4	**Final Formulation 4**	**Final Formulation 4 – Formulation 4**
t_{max}, h			
N	21	21	21
Mean	12.57	12.17	-0.405
SD	1.85	1.48	2.295
Median	12	12	0
Min			
Max			

b(4),

Summary of pharmacokinetic results

- Bioequivalence was demonstrated for liraglutide formulation 4 and liraglutide final formulation 4 with respect to the primary endpoints (AUC(0-t) and Cmax), because the 90% CIs of the corresponding ratios were entirely contained within the limits (0.80, 1.25). The estimated ratio for AUC(0-t) was 0.99 (CI: 0.92, 1.06) and for Cmax 1.02 (CI: 0.91, 1.14).
- In addition, similar results for tmax were obtained for the two formulations.
- Bioequivalence could also be demonstrated for AUC(0-t) and Cmax adjusted for actual drug content.

Safety Conclusions

- There were 34 adverse events reported by 17 subjects during the trial and all adverse events were assessed as TEAEs (treatment emergent adverse effect). Of the 34 TEAEs, 20 were assessed as possibly related to trial treatment (11 to formulation 4 and 9 to final formulation 4). The most commonly reported TEAEs related to the trial products were nausea and headache. All TEAEs were mild or moderate and equally distributed between the two formulations.
- No serious adverse events or deaths occurred during the trial.
- Four (4) clinically significant changes in laboratory values were recorded in 3 subjects during the trial. All of the changes were transient and required no action taken. No clinically significant changes in ECG or vital signs were recorded during the trial.
- Both liraglutide formulations were well tolerated and no safety concerns were raised.

Overall Conclusions

The results from this trial in healthy subjects demonstrated that:

- Bioequivalence between liraglutide formulation 4 and liraglutide final formulation 4.
- After a single administration of liraglutide formulation 4 or final formulation 4 both liraglutide formulations were well tolerated.

Reviewer's Comment:

The overall study design and data analysis seems reasonable except that the sponsors used a dose of around 0.7 mg in this bioequivalence study, whereas the highest proposed dose is 1.8 mg. The sponsor rationale for using lower dose is because the lower dose is well tolerated.

Revised Analysis to Address the DSI findings on Bioanalytical Method

Sponsor generated an updated dataset, where samples affected by inconsistent acceptance of analytical runs were excluded. This was followed by a review of the plasma concentration vs. time profiles based on the updated dataset for validity in terms of calculation of AUC0-t and Cmax (the primary endpoints). The criteria for including a profile for calculation of AUC0-t were: 1) minimum one sample $\leq$10 hours post-dose and 2) acceptable number and scattering of samples. The criterion for including a profile for calculation of Cmax was the presence of an acceptable number of samples around the maximal concentration. This resulted in 19 of the original 42 profiles being excluded for the calculation of AUC0-t and 17 profiles being excluded for the estimation of Cmax. Three profiles were accepted for calculation of AUC0-t, although the last sample was t = 48 hours (sample for t = 60 hours was missing). Mean profile (linear scale) based on the updated dataset is presented in Figure below:

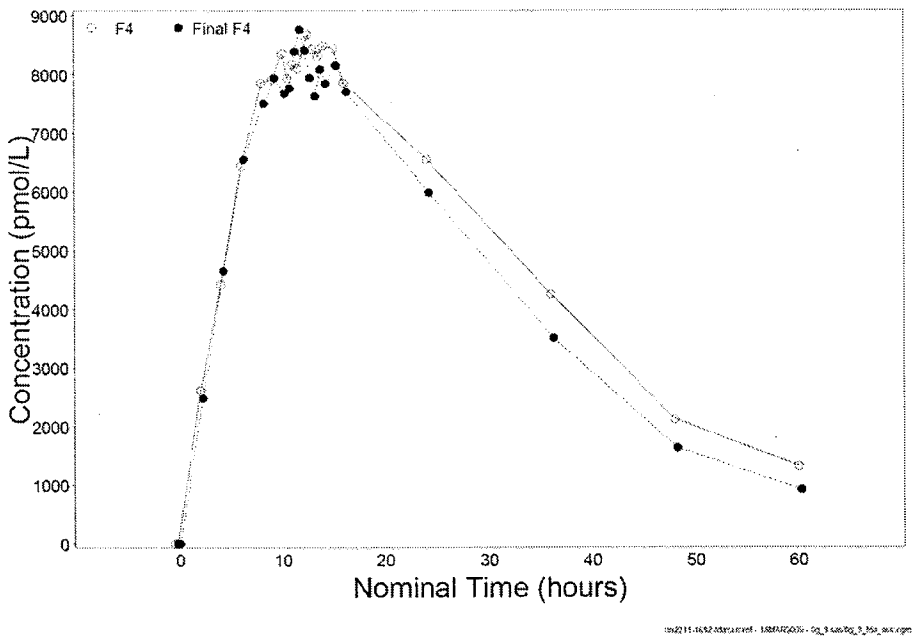


Figure 1–1 Mean Profiles (Linear Scale) of Liraglutide Concentration in Plasma by Formulation, Values used for AUC – Updated Dataset (Trial 1692)

Summary Statistics for AUC_{0-t} (h*pmol/L) – Trial 1692

	Original Analysis		Updated Analysis	
	Final F4	F4	Final F4	F4
N	21	21	12	11
Mean	271304	271945	256057	265692
SD	59415	44960	54223	50463
Median	243829	280024	240358	249830
GeoMean	265463	268474	251192	261851
Min				
Max				

b(4)

Summary Statistics for C_{max} (pmol/L) – Trial 1692

	Original Analysis		Updated Analysis	
	Final F4	F4	Final F4	F4
N	21	21	13	12
Mean	10365	9771	9333	9682
SD	4320	1671	1677	1351
Median	9378	9960	9158	9910
GeoMean	9799	9630	9201	9593
Min				
Max				

b(4)

Comparison between Formulations (Final Formulation 4 / Formulation 4) – Primary Endpoints – Trial 1692

		Original Analysis Final Formulation 4 / Formulation 4	Updated Analysis Final Formulation 4 / Formulation 4
AUC(0-t)			
	Estimate	0.99	1.01
	Lower 90% limit	0.92	0.91
	Upper 90% limit	1.06	1.12
Cmax			
	Estimate	1.02	0.97
	Lower 90% limit	0.91	0.88
	Upper 90% limit	1.14	1.07

Based on the results of revised analysis:
The updated analysis results from Trial 1692 showed bioequivalence between Formulation 4 and Final Formulation 4, which was in accordance with the original analysis results.

APPEARS THIS WAY
ON ORIGINAL

NN2211-1693

Title: A randomized, double-blind, single-centre, two-period, cross-over trial in healthy subjects investigating the bioequivalence between the Phase 2 formulation of liraglutide at pH 7.7 (formulation 3) and the Phase 3 formulation at pH 8.15 (formulation 4).

Investigator and Study Center(s):

Ulf Malmqvist, MD, Ph.D.,
Dept. of Clinical Chemistry and Pharmacology,
Lund University Hospital, Lund, Sweden

Trial Sites
Clinical Pharmacology, Phase I Unit, Lund University Hospital, Lund, Sweden

Study Sponsor:

Novo Nordisk A/S, Denmark

Bioanalytical Analysis of Pharmacokinetic Sample:

b(4)

Study Period: 11 April 2006 (Trial Initiated) to 27 June 2006 (Trial Completed)

Objective:
The primary objective of this study was to test for bioequivalence of the phase 3a formulation of liraglutide at pH 8.15 (formulation 4) and the earlier phase 2 formulation at pH 7.7 (formulation 3), based on AUC(0-t) and Cmax after a single sc dose.

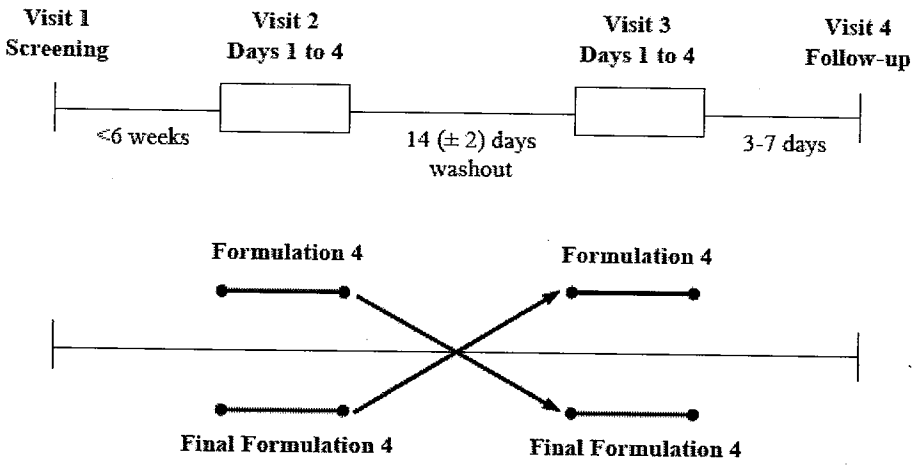
The secondary objective of the study was:

- To estimate Frel of the two liraglutide formulations based on AUC(0-t) and AUC, and to estimate tmax, t½ , and λz of liraglutide.
- To evaluate the safety of two formulations of liraglutide.

Study Design:
The current trial was a randomized, double-blind, single-centre, two-period, cross-over trial designed to test for bioequivalence between the phase 3a formulation of liraglutide at pH 8.15 (formulation 4) and the phase 2 formulation at pH 7.7 (formulation 3).

In total, 25 healthy subjects were screened for the trial and 22 subjects were randomized and treated with two formulations of liraglutide, formulation 3 at pH 7.7 and formulation 4 at pH 8.15.The total duration of the trial for the individual subject was up to 10 weeks.

Figure 1: Trial Design



In this study each subject attended 4 visits:

- The first visit was a screening visit where the subject's eligibility was assessed.
- At Visits 2 subjects were admitted to the clinic for in-house periods, lasting for 3 days, when they were dosed with the liraglutide formulations. The Visit 2 (dosing of first formulation) was to take place within 6 weeks from the screening visit.
- Visit 3 (dosing of second formulation) was to take place 14 (± 2 days) from Visit 2.
- Finally, a follow-up visit (Visit 4) was to take place 3-7 days after finalization of Visit 3.

The trial was a randomized, double-blind, single-centre, two-period, cross-over trial where two single s.c. doses of liraglutide were administered to healthy male and female subjects in the evening on two different occasions, separated by a 14 (±2) day wash-out period. In the evening of Day 1 at Visit 2, 120 µL of liraglutide formulation 3 or formulation 4 (corresponding to 0.7080 mg of formulation 3 and 0.7092 mg of formulation 4) was sc administered into the abdomen of the subjects. Thereafter, blood samples for pharmacokinetic analyses of plasma liraglutide concentrations were taken at 20 time points up to 60 h after dosing (Day 4).Blood samples for estimation of the liraglutide plasma concentrations were drawn at Visit 2 and 3; before dosing at -30 and -15 minutes, and at 2, 4, 6, 8, 9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 15, 16, 24, 36, 48 and 60 h after dosing.

Study Population:
Twenty two healthy volunteers were enrolled in this study. The mean age of the study population was 22.7 years (range 19 to 28 years). Table 1 below shows the demographics of the enrolled patients.

Table 1: Baseline Demographics of Study Population.

	All Subjects N=22
Age, yrs	
Mean (SD)	22.7 (2.4)
Min – Max	19 – 28
Sex, n (%)	
Male	19 (86.4%)
Female	3 (13.6%)
Ethnic origin, n (%)	
White	22 (100.0 %)
Height, m	
Mean (SD)	1.81 (0.08)
Min – Max	1.64 – 1.95
Weight, kg	
Mean (SD)	74.8 (8.4)
Min – Max	53.8 – 88.5
BMI, kg/m^2	
Mean (SD)	22.9 (1.8)
Min – Max	19.8 – 26.1
Systolic blood pressure, mmHg	
Mean (SD)	120.77 (7.62)
Min – Max	104.00 – 132.00
Diastolic blood pressure , mmHg	
Mean (SD)	68.86 (6.43)
Min – Max	59.00 – 84.00
Pulse, beats/min	
Mean (SD)	60.09 (9.13)
Min – Max	44.00 – 85.00

Investigational Product and Dose Selection:

The trial products during the treatment period were provided by Novo Nordisk A/S as follows:

- Formulation 3: Total liraglutide related protein of 6.25 mg/mL at pH 7.7, in pre-filled 3 mL cartridges dispensed in a disposable pen device ( ) for sc injection.
- Formulation 4: Liraglutide 6.0 mg/mL at pH 8.15, in pre-filled 3 mL cartridges dispensed in a disposable pen device ( )) for sc injection

b(4)

Formulation 4 was pH 8.15) and modifying the drug manufacturing process used for producing formulation 3, i.e. . Even though the strengths of formulation 3 and formulation 4 were different (as a consequence of different analysis methods), the amount of liraglutide in each dose of the two formulations used in this trial was demonstrated to be nearly the same (actual dose of 0.7080 mg of formulation 3 and 0.7092 mg of formulation 4, when analyzed by new method).

b(4)

Table 2: Batch number of product used in this trial

Trial Product	Dose	[redacted] Batch Number	Drug Product Batch Number (Bulk Batch Number)	Expiry Date
Formulation 3 (pH 7.7)	120 µL of 6.25 mg/mL	PP51138	PQ50365	11 September, 2006
Formulation 4 (pH 8.15)	120 µL of 6.0 mg/mL	[redacted]	RQ50576	30 March, 2007

b(4)

The rationale for using this dose for BE assessment is because of the fact minimize gastrointestinal related side effects. Also, this dose has shown to be sufficient in giving measurable plasma concentrations up to 60 h post dosing. The dose of 0.75 mg liraglutide was also within the expected therapeutic window of treatment. Hence, the selected dose in the present trial was within the expected therapeutic window of treatment and was not expected to give any side effects related to the gastrointestinal tract or any hypoglycaemic episodes.

Bioanalysis:

Quantitative assessment of liraglutide was done using enzyme immunoassays (ELISA). The lower limit of quantification of the assay was 18pmol/L. The calibration curves were analyzed at liraglutide concentration range of 17pmol/L to 4399pmol/L. The precision of the assay, as determined from analysis of quality control samples ranged between [redacted]. The mean accuracy (% Bias) ranged between [redacted] Between-batch precision (%CV) results of the calibration standards of liraglutide was less than or equal to 6.0 % and accuracy (%Bias) ranged from [redacted].

b(4)

Data Analysis:

The primary analysis in this trial was testing for bioequivalence between liraglutide formulation 3 and liraglutide formulation 4, based on AUC (0-t) and Cmax. Bioequivalence was to be declared if the adjusted 90% confidence intervals for the corresponding ratios were fully contained within the limits (0.80, 1.25). Secondary analyses in this trial were estimation of Frel of the two liraglutide formulations based on

AUC(0-t) and AUC, and estimations of tmax, t½ , and λz of liraglutide. The comparisons between formulations of AUC(0-t), AUC, Cmax and t½ was performed using a linear normal model (analysis of variance, ANOVA) based on the logarithmic transformed values. The model included effects of subject, period and formulation. Based on the statistical model, ratios between the two formulations with 90% confidence intervals were estimated. The subject effect was included as a random effect. Bioequivalence between the two formulations was to be declared if the 90% CIs for the corresponding ratios of AUC(0-t) and Cmax were fully contained within the limits (0.80, 1.25).

Pharmacokinetics Results:

The mean plasma concentration time profile of liraglutide is shown in Figure-1&2. All subjects received 0.7080 mg of formulation 3 and 0.7092 mg of formulation 4. Analyses of the pharmacokinetic endpoints have been adjusted for actual drug content. The mean response to the trial drug formulations demonstrated that the two formulations were bioequivalent.

Figure 1: Mean Plasma profile of liraglutide by formulation-linear scale

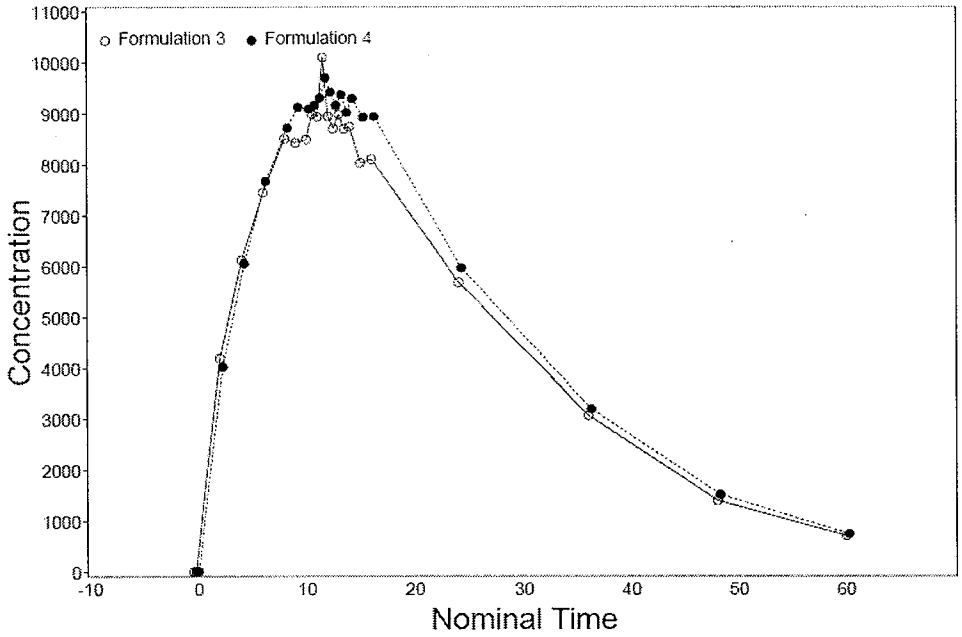
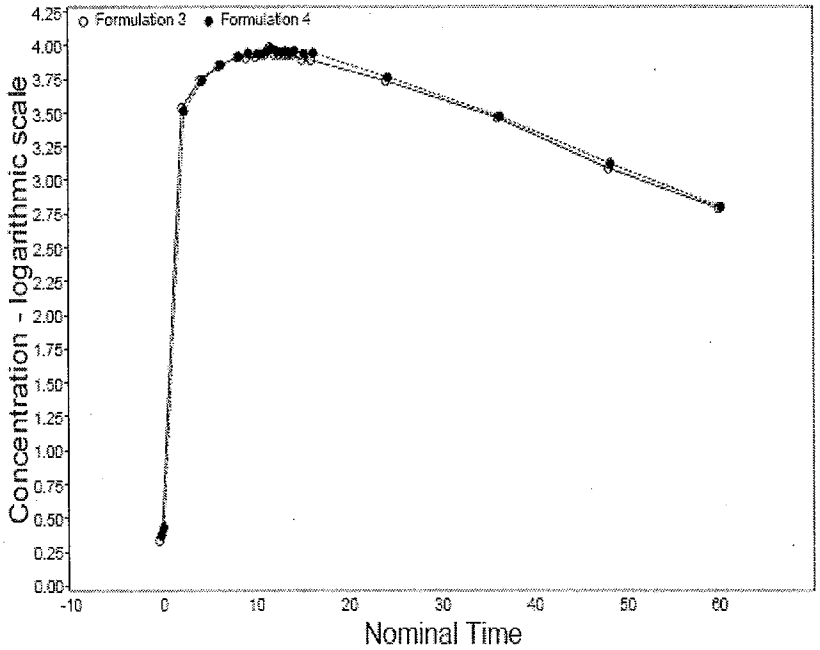


Figure 2: Mean Plasma profile of liraglutide by formulation-logarithmic scale.



The summary of pharmacokinetic parameters of liraglutide from formulation 3 and formulation 4 is summarized in Table-3

Table 3: Summary Statistics for the Primary Pharmacokinetics Endpoints by Formulation.

	Formulation 3	Formulation 4	Formulation 4/Formulation 3[1]
$AUC_{(0-t)}$, pmol × h/L			
N	21	22	21
Mean	260384.5	273841.2	1.072
SD	73141.5	65095.9	0.194
Geometric Mean	251371.1	266365.1	1.057
Median	242384.4	276006.8	1.021
C_{max}, pmol × h/L			
N	21	22	21
Mean	10888.6	11187.3	1.063
SD	3073.1	2664.5	0.261
Geometric Mean	10489	10875.4	1.035
Median	9748	10970.5	1.013

b(4)

b(4)

[1]The ratios corresponding to $AUC_{(0-t)}$ and C_{max} are adjusted for actual drug content.

The statistical results of the comparisons between formulation 3 and formulation 4 for the primary endpoints are summarized in Table 4.

Table 4: Statistical Analysis for the Primary Pharmacokinetics Endpoints by Formulation

	Formulation 4/Formulation 3
$AUC_{(0-t)}$	
Estimate	1.06
Lower 90% limit	1.00
Upper 90% limit	1.13
C_{max}	
Estimate	1.04
Lower 90% limit	0.95
Upper 90% limit	1.13

The analyses of the primary endpoints are adjusted for actual drug content.

From the results in Table-4, formulation 3 and formulation 4 were demonstrated to be bioequivalent because the 90% CIs for the corresponding estimated ratios of AUC(0-t) and Cmax were entirely contained within the limits (0.80, 1.25).

The results of secondary pharmacokinetic endpoints were AUC, tmax, t½ and λz and these endpoints are summarized for the pharmacokinetic analysis set in Table-5. The comparisons between the secondary pharmacokinetic endpoints for formulation 3 and formulation 4 are presented in Table 6.

Table 5: Summary Statistics for the Secondary PK Endpoints of Liraglutide by Formulation

	Formulation 3	Formulation 4	Formulation 4/Formulation 3[1]
AUC, pmol × h/L			
N	21	22	21
Mean	273202.2	287402.5	1.072
SD	80830.9	73555.2	0.184
Geometric Mean	262806	278547.7	1.058
Median	250193.7	292382.6	1.026
Min			
Max			
$t_{½}$, h			
N	21	22	21
Mean	11.53	11.4	0.996
SD	2.08	2.19	0.165
Median	11.25	10.71	0.95
Min			
Max			
Harmonic Mean	11.3	11	0.971
λ_z, 1/h			
N	21	22	21
Mean	0.062	0.063	1.029
SD	0.009	0.011	0.166
Geometric Mean	0.061	0.062	1.017
Median	0.062	0.065	1.052
Min			
Max			
	Formulation 3	**Formulation 4**	**Formulation 4 – Formulation 3**
t_{max}, h			
N	21	22	21
Mean	11.71	11.66	-0.048
SD	1.83	3.12	3.271
Median	11.5	11.5	0
Min			
Max			

[1]The ratio corresponding to AUC is adjusted for actual drug content.

b(4)

The bioequivalence seen for the primary endpoints was supported by the results of the secondary endpoints Frel (based on AUC), λz and t½, because the 90% confidence intervals for the corresponding values were entirely contained within the limits (0.80 to 1.25) (Table-6). In addition, similar results for tmax were obtained for the two formulations because the estimated differences were equal to zero.

Table 6: Statistical Analysis for the Secondary Pharmacokinetics Endpoints by Formulation

	Formulation 4/Formulation 3
AUC	
Estimate	1.06
Lower 90% limit	1.00
Upper 90% limit	1.12
$t_{½}$,	
Estimate	0.98
Lower 90% limit	0.92
Upper 90% limit	1.04
	Formulation 4 – Formulation 3
t_{max}	
Estimate	0.00
Lower 90% limit	-1.50
Upper 90% limit	1.00

The analyses of AUC is adjusted for actual drug content.

The result of λ_Z is not shown since the ratio is the inverse of the estimated ratio of $t_{½}$.

Summary of pharmacokinetic results

- Bioequivalence was demonstrated for liraglutide formulation 3 at pH 7.7 (phase 2 formulation) and formulation 4 at pH 8.15 (phase 3a formulation) with respect to the primary endpoints (AUC(0-t) and Cmax), because the 90% confidence intervals of the corresponding ratios were entirely contained within the limits (0.80, 1.25).
- In addition, bioequivalence was supported by the results of the secondary endpoints Frel (based on AUC), λz and t½, because the 90% confidence intervals for the ratios were entirely contained within the limits (0.80, 1.25). Similar results for tmax were also obtained with the two formulations because the estimated differences were equal to zero.

Safety Conclusions

- There were 25 AEs reported by 13 subjects during the trial. Of these were 22 TEAEs, of which 15 were classified as possibly related to study treatment (8 to formulation 4 and 7 to formulation 3). The most commonly reported AEs were nausea and headache. All AEs were mild or moderate.
- No serious adverse events or deaths occurred during the trial.
- No clinically significant changes in laboratory values, ECG or vital signs were recorded during the trial.
- Both liraglutide formulations were well tolerated and no safety concerns were raised.

Overall Conclusions

The results from this trial in healthy subjects demonstrated that:

- Bioequivalence between liraglutide formulation 4 (at pH 8.15) and liraglutide formulation 3 (at pH 7.7).

- No safety concerns were raised after a single administration of liraglutide formulation 4.

Reviewer's Comment:
The overall study design and data analysis seems reasonable except that the sponsors used a dose of around 0.7 mg in this bioequivalence study, whereas the highest proposed dose in 1.8 mg. The sponsor's rationale for using a lower dose is the better tolerability profile expected from the lower dose due to decreased incidences of gastro-intestinal and hypoglycemic adverse events.

APPEARS THIS WAY
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4.2.8 Relative Bioavailability Study (NN2211-1745)

Title: A randomized, open-label, single centre, three period cross-over trial in healthy subjects comparing the pharmacokinetic profiles after single dose administration of liraglutide at three different injection sites

Investigator and Study Center(s):
Dr. Christoph Kapitza, MD.
Profil Institut für Stoffwechselforschung GmbH
Hellersbergstraße 9, D-41460 Neuss
Germany

Trial Site
Profil Institut für Stoffwechselforschung GmbH, Neuss, Germany.

Study Sponsor:

Novo Nordisk A/S, Denmark

Bioanalytical Analysis of Pharmacokinetic Sample:

b(4)

STUDY PERIOD: 2 February 2007 (Trial Initiated) to 7 May 2007 (Trial Completed)

Objective:
The primary objective of this study was to compare the PK profile of liraglutide between administrations in the thigh versus the abdomen and between administrations in the upper arm versus the abdomen after a single s.c. dose, based on AUC0-∞.

The secondary objective of the study was:

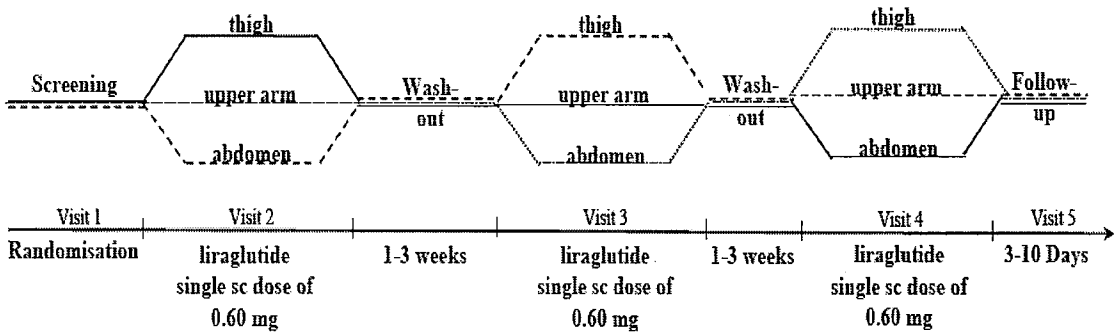
- To compare the PK profile of liraglutide between administrations in the upper arm versus the thigh after a single s.c. dose, based on AUC.
- To compare the PK profiles of liraglutide between administrations in the thigh versus the abdomen, the upper arm versus the abdomen and the upper arm versus the thigh after a single s.c. dose, based on Cmax, AUC0-t, tmax, t½, and λz of liraglutide.
- To estimate Frel of liraglutide between the three injection sites.
- To evaluate the safety of liraglutide administration by three different injection sites

Study Design:
The current trial was a randomized, open-label, single-centre, three periods, cross-over trial designed to was to compare the PK profile of liraglutide between administrations either in the abdomen, thigh or upper arm after a single s.c. dose.

In total, 25 healthy subjects were screened for the trial and 21 subjects were randomized and exposed to trial product. Single s.c. doses of liraglutide were planned to be administered in the

evening to 21 healthy male and female subjects on three different occasions, each separated by a 1-3 weeks wash-out period counting from time of dosing. The site of administration differed between the dosing days, either in the abdomen, thigh or upper arm. The dose was given as a fixed dose (0.60 mg) in a fixed volume (100 µl).

Figure 1: Trial Design



The total trial duration for the individual subject was up to 11 weeks. Each subject was to attend the clinic 5 times: At Visit 1 (screening visit) the subject's eligibility were assessed. Visit 2 (first dosing visit) was to be performed 1-3 weeks after Visit 1. At Visits 2-4 the subjects were to be dosed and were admitted to the clinic for an in-house stay each lasting for 3 days. Finally, Visit 5 was a follow-up visit to be scheduled 3-10 days after finalization of Visit 4.

Blood samples for determination of plasma concentrations of liraglutide were obtained before dosing at -30 and -15 minutes, and at 2, 4, 6, 8, 9, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5, 14, 15, 16, 24, 36, 48 and 60 h after dosing.

Study Population:

Twenty-five (25) healthy female or male subjects were screened of which 21 were randomized and exposed to trial product. Twenty (20) subjects completed the trial while 1 subject withdrew from the trial (withdrew informed consent after visit 3). The mean age of the study population was 38.6 years (range 22 to 49 years). Table 1 below shows the demographics of the enrolled patients.

Table 1: Baseline Demographics of Study Population.

Subjects Exposed	
N	21
Sex (N (%))	
Males	11 (52.4%)
Females	10 (47.6%)
Ethnic Origin (N (%))	
White	20 (95.2%)
American Indian or Alaska Native	1 (4.8%)
Age (y)	
N	21
Mean	38.6
SD	7.7
Median	41
Min	22
Max	49
Height (m)	
N	21
Mean	1.73
SD	0.09
Median	1.74
Min	1.56
Max	1.92
Weight (kg)	
N	21
Mean	73.5
SD	13.8
Median	72.6
Min	51.5
Max	98.3
BMI (kg/m**2)	
N	21
Mean	24.2
SD	2.4
Median	24.3
Min	19.1
Max	27.5

Percentages have been calculated with respect to subjects exposed

Investigational Product and Dose Selection:

Liraglutide was provided as a sterile solution contained in 3 mL ——— as described in Table 2. In this trial the lowest dose (0.60 mg liraglutide) of the phase 3a program (including 0.60, 1.20, and 1.80 mg liraglutide per day) has been chosen in order to reflect the future clinical treatment. This dose has previously been shown to be well tolerated and gave measurable concentrations of liraglutide in plasma.

b(4)

Table 2: Batch number of product used in this trial

Trial Product	Dose	Liraglutide in ——— Batch Number[1]	Liraglutide Product Batch Number[1]	Expiry Date
Liraglutide 3 mL ——— 6 mg/mL	0.60 mg	SP51132	SQ50212	24-Sep-2007

[1] The same batch was used for all subjects. The liraglutide drug product was contained within the liraglutide ———

Bioanalysis:
Quantitative assessment of liraglutide was done using enzyme immunoassays (ELISA). The lower limit of quantification of the assay was 18pmol/L. The calibration curves were analyzed at liraglutide concentration range of 16 pmol/L to 4400 pmol/L. The inter-assay precision of the assay, as determined from analysis of quality control samples ranged between 12.2% and 16.6%. The mean accuracy (% Bias) ranged between -8.6% to 10.1%. Between-batch precision (%CV) results of the calibration standards of liraglutide was less than or equal to 8.8% and accuracy (%Bias) ranged from -3.5 to 2.5 %.

Data Analysis:
The primary endpoint, AUC0-∞ for liraglutide from dosing to infinity for each injection site, was derived using standard model-free, non-compartmental methods. The comparison between the injection sites was performed by use of a linear normal model (ANOVA) for the log transformed values of AUC. The model included effects of period and injection site and a random effect of subject. In this model, the difference between the log transformed AUC for administration in the thigh and the log transformed AUC for administration in the abdomen as well as the difference between the log transformed AUC for administration in the upper arm and the log transformed AUC for administration in the abdomen were estimated together with the corresponding 90% confidence intervals. The estimated differences with confidence intervals were retransformed to the corresponding ratios with confidence intervals.
The secondary endpoints were derived using standard model-free, non-compartmental methods. AUC0-t was calculated by the trapezoidal method. The terminal rate, λz, was determined through linear regression with the logarithm to concentration as the response variable and time as the explanatory variable. Valid observations from the final part of the curve, which is approximately linear, were used for the analysis. The terminal half-life, t½, was calculated as log(2)/ λz. Frel was the estimated ratios of AUC0-∞ between the different injection sites. Statistical analysis of the secondary PK endpoints was performed for the PK analysis-set. The comparison between the AUC0-∞ for administration in the upper arm and AUC0-∞ for administration in the thigh was made in the same model and using the same criteria as described for the primary analysis.

Pharmacokinetics Results:
The mean plasma concentration time profile of liraglutide after single s.c dose at different injection sites is shown in Figure-1 & 2.

Figure 1: Mean Plasma profile of liraglutide by formulation-linear scale

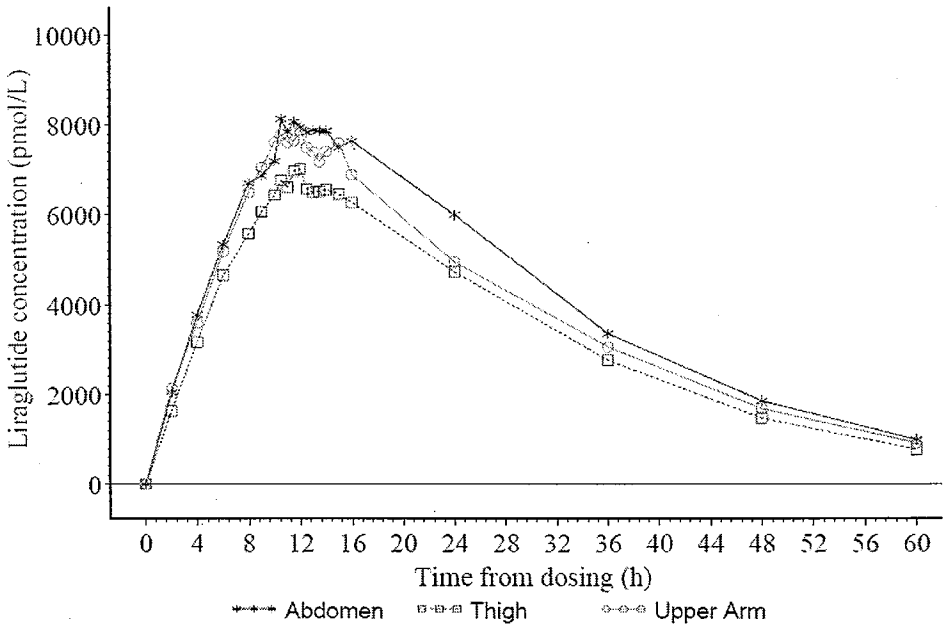
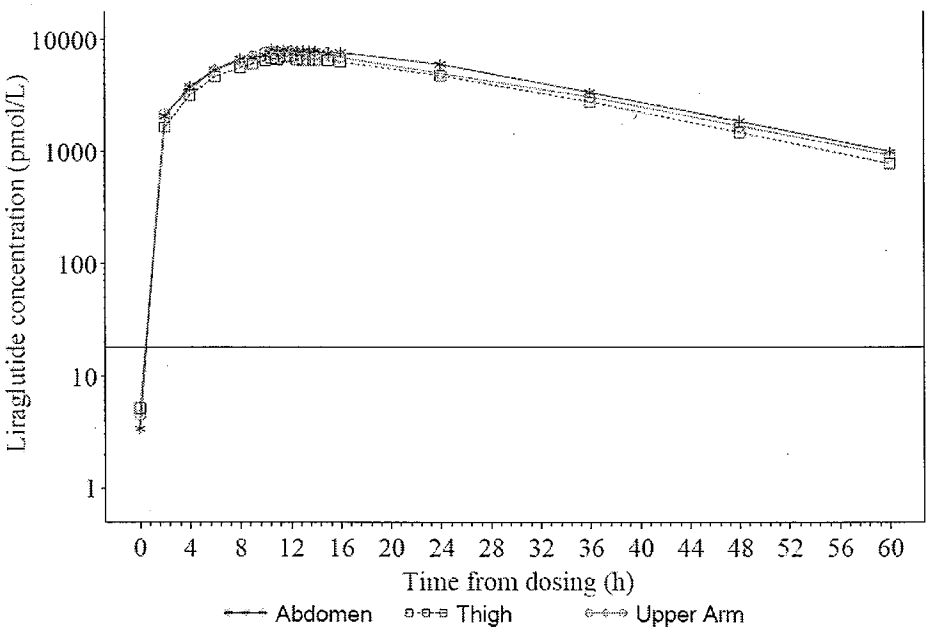


Figure 2: Mean Plasma profile of liraglutide by formulation-logarithmic scale.



Throughout the investigated time period (0-60 h after administration), the mean liraglutide concentration was lowest after injection in the thigh compared to injection in the abdomen or upper arm. The primary pharmacokinetic comparisons of AUC0-∞ for liraglutide between injections in the abdomen versus the upper arm and the thigh are presented in Table 3

Table 3: Summary Statistics for the Primary Pharmacokinetics Endpoints (AUC0-∞) by Injection Site.

	Abdomen	Thigh	Upper arm
AUC(0-inf)(pmol*h/L)			
N	21	21	19
Mean	271011	221125	249958
SD	72609	71453	65110
Geometric Mean	262488	210935	242292
Median	260911	204727	225017
Min			
Max			

b(4)

The secondary comparison of AUC0-∞ after injection of liraglutide in the upper arm versus the thigh is presented in the table 4 along with the primary PK endpoint.

From the results in Table-4, the 90% confidence interval of the ratio of AUC0-∞ between injection of liraglutide in the upper arm and the abdomen was included in the defined interval of 0.80 to 1.25 with an estimated relative bioavailability (Frel) of 90%. Comparison of the thigh versus the abdomen the lower limit of the 90% confidence interval was 0.76 (below 0.80) for the ratio of AUC0-∞ with an estimated Frel of 81%.

Table 4: Statistical Analysis for the Comparison between Injection Sites.

	Thigh / Abdomen	Upper arm / Abdomen	Upper arm / Thigh
AUC(0-inf)			
Estimate	0.81	0.90	1.11
Lower 90% limit	0.76	0.83	1.03
Upper 90% limit	0.86	0.96	1.19

Therefore, based on the defined equivalence criteria for the ratios of AUC0-∞, equivalence can be declared with respect to AUC0-∞ of liraglutide after injection in the upper arm and the abdomen, but not after injection in the thigh and the abdomen.

For the secondary comparison, the ratio of AUC0-∞ after injection of liraglutide in the upper arm versus the thigh were within the defined limits of the 90% confidence interval of 0.80 to 1.25 with an estimated Frel of 111% and thus equivalence with respect to AUC0-∞ can be declared between these two injection sites.

Summary statistics and statistical analysis for the secondary PK endpoints are presented by injection site in Tables 5 and 6, respectively.

Table 6: Statistical Analysis for the Secondary Pharmacokinetics Endpoints by Injection Site

	Thigh / Abdomen	Upper arm / Abdomen	Upper arm / Thigh
AUC(0-t)			
Estimate	0.81	0.88	1.09
Lower 90% limit	0.75	0.81	1.00
Upper 90% limit	0.88	0.96	1.18
Cmax			
Estimate	0.82	0.95	1.16
Lower 90% limit	0.74	0.85	1.03
Upper 90% limit	0.91	1.06	1.29
Lambdaz			
Estimate	1.03	0.95	0.92
Lower 90% limit	0.93	0.85	0.83
Upper 90% limit	1.14	1.05	1.03

	Thigh - Abdomen	Upper arm - Abdomen	Upper arm - Thigh
tmax (h)			
Estimate	0.00	0.25	0.00
Lower 90% limit	-1.00	-0.50	-0.50
Upper 90% limit	0.50	1.00	0.75

Results of $t_{1/2}$ are not shown since the estimated ratio is the inverse of the lambdaZ -ratio.

Summary of pharmacokinetic results

- The two injection sites upper arm and abdomen were equivalent with respect to AUC0-∞ for liraglutide, with a mean ratio of 0.90 (90% CI [0.83;0.96]), while the two injection sites thigh and abdomen could not be declared equivalent with respect to AUC0-∞ for liraglutide, with a mean ratio of 0.81 (90% CI [0.76;0.86])
- The two injection sites upper arm and thigh were equivalent with respect to AUC0-∞ for liraglutide, with a mean ratio of 1.11 (90% CI of [1.03;1.19])
- Results based on the primary analysis were supported by the secondary PK endpoints based on AUC0-t and Cmax (90% CI were within the defined limits for the upper arm versus the abdomen, but not for the thigh versus the abdomen).
- Based on t½ and λz, elimination of liraglutide was similar for all three injection sites and tmax for liraglutide was similar after injection in all three injection sites.
- Estimated Frel of liraglutide was 81% after injection in the thigh versus the abdomen, 90% after injection in the upper arm versus the abdomen and 111% after injection in the upper arm versus the thigh.

Safety Conclusions

No serious AEs or deaths were reported during the trial

- There were a total of 10 AEs reported by 5 subjects. Of these, 7 AEs were considered to be possibly related to trial products (6 events of nausea and 1 event of sensation of pressure in the head)
- Single dose administration of liraglutide was well tolerated after s.c. injection in the abdomen, thigh and upper arm

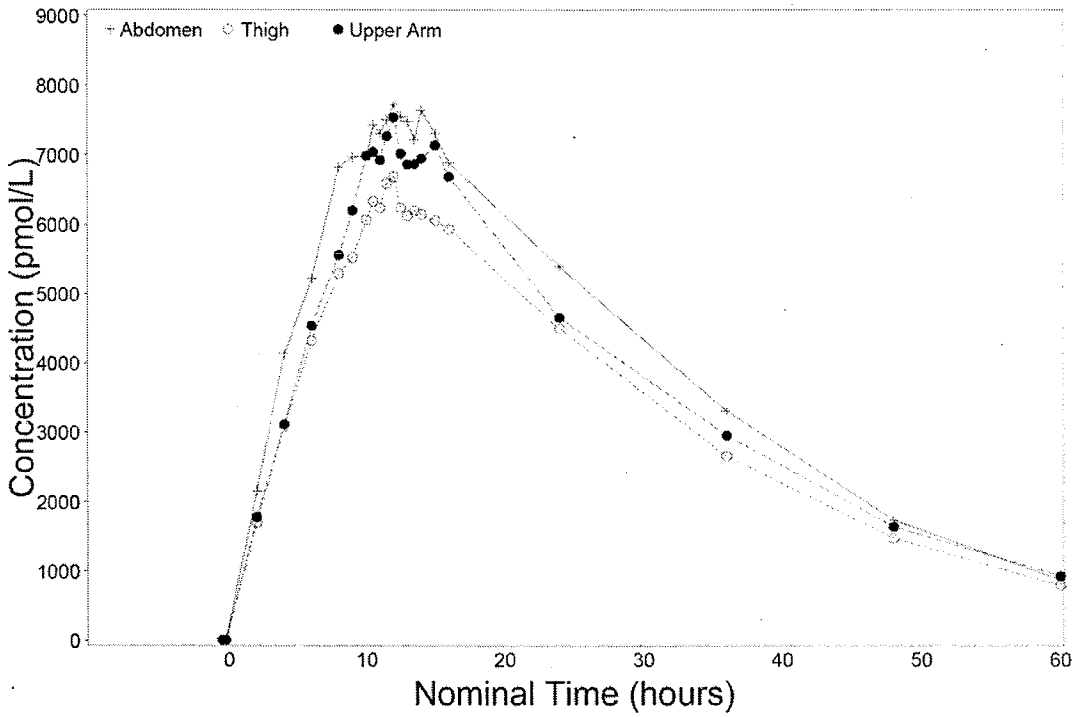
Overall Conclusions

The results from this trial in healthy subjects demonstrated that:

- Equivalence was demonstrated with respect to AUC0-∞ for liraglutide between injection in the upper arm and the abdomen, while equivalence could not be declared between injection in the thigh and the abdomen
- Equivalence with respect to AUC0-∞ for liraglutide was demonstrated between the upper arm and the thigh
- Results from the primary comparisons were supported by comparisons based on the secondary endpoints AUC0-t and Cmax tmax for liraglutide and elimination of liraglutide, based on t½ and λz, was similar between injection sites
- Frel for liraglutide was estimated to 81% for the thigh versus the abdomen, 90% for the upper arm versus the abdomen and 111% for the upper arm versus the thigh
- Single dose administration of liraglutide was well tolerated after s.c. injection in the abdomen, the thigh and the upper arm.

Reviewer's Comment:
The overall study design and data analysis seems reasonable. Equivalence was demonstrated between the upper arm and abdomen and also with upper arm and thigh. However, the injections site thigh and abdomen was not equivalent. However, sponsor has proposed that a 21 % lower mean reduction is not clinically meaningful. From a clinical pharmacology perspective, we agree to the sponsor's conclusion.

Revised Analysis to Address the DSI findings on Bioanalytical Method
Sponsor conducted re-evaluation of liraglutide plasma concentration raw data The primary endpoint in Trial 1745 was AUC0-∞ and the criteria for including a profile in the updated analysis were: 1) minimum one sample ≤10 hours post-dose, 2) at least 3 out of 4 possible samples in the 24–60 hour post-dose period (sampling schedule: 24, 36, 48 and 60 hours) and 3) acceptable number and scattering of samples. Of the 60 profiles available for re-evaluation, 42 profiles were accepted for AUC analysis while 18 profiles were rejected. Mean profile based on the updated dataset is presented below.



Summary Statistics for $AUC_{0-\infty}$ (h*pmol/L) – Trial 1745

	Abdomen	Thigh	Upper arm
Original Analysis			
AUC(0-inf)(pmol*h/L)			
N	21	21	19
Mean	271011	221125	249958
SD	72609	71453	65110
Geometric Mean	262488	210935	242292
Median	260911	204727	225017
Min			
Max			
Updated Analysis			
AUC(0-inf)(pmol*h/L)			
N	13	17	12
Mean	256312	214845	238695
SD	61294	70717	46341
Geometric Mean	249502	204880	234617
Median	260216	204717	221923
Min			
Max			

b(4)

Comparison between Injection Sites – Primary Endpoint – Trial 1745

	Thigh / Abdomen	Upper arm / Abdomen	Upper arm / Thigh
Original Analysis			
AUC(0-inf)			
Estimate	0.81	0.90	1.11
Lower 90% limit	0.76	0.83	1.03
Upper 90% limit	0.86	0.96	1.19
Updated Analysis			
AUC(0-inf)			
Estimate	0.78	0.87	1.10
Lower 90% limit	0.72	0.78	1.01
Upper 90% limit	0.86	0.96	1.21

Based on the results of revised analysis:

- Equivalence could not be demonstrated for thigh/abdomen as the 90% confidence interval for the ratio was not contained within the pre-specified [0.80; 1.25] interval. This was in accordance with the original analysis results.
- F_{rel} for liraglutide was estimated to 78% for the thigh versus the abdomen, 87% for the upper arm versus the abdomen and 110% for the upper arm versus the thigh

APPEARS THIS WAY
ON ORIGINAL

4.2.9 DDI Study (NN2211-1330)

Title: A Double-Blind, Two Period Cross-Over, Single Centre Trial in Healthy Subjects Investigating the Influence on the Pharmacokinetics of Ethinylestradiol and Levonorgestrel in an Oral Contraceptive Drug after Multiple Dose Administration of Liraglutide

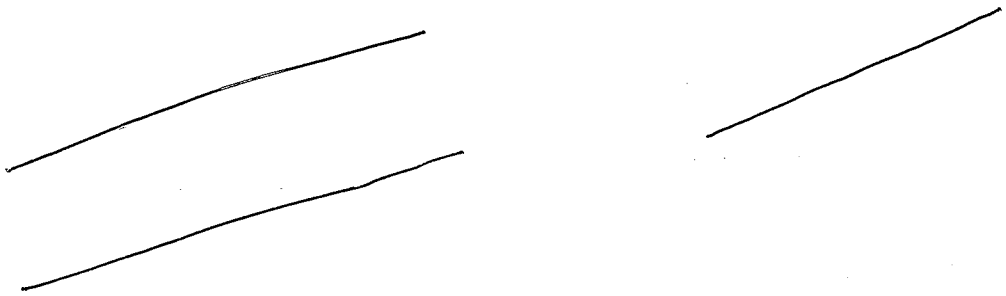
Investigator and Study Center(s):
Jan Vouis, MD,
Quintiles Phase I Unit,
Strandbodgatan 1, SE-753 23 Uppsala, Sweden

Trial Sites
Quintiles Phase I Unit, Strandbodgatan 1, SE-753 23 Uppsala, Sweden

Study Sponsor:

Novo Nordisk A/S, Denmark

Bioanalytical Analysis:



b(4)

STUDY PERIOD: 24 November 2006 (Trial Initiated) to 05 April 2007 (Trial Completed)

Objective:

The primary objective of this study was to determine if liraglutide at steady state changes $AUC_{0-\infty}$ of ethinylestradiol and levonorgestrel.

The secondary objectives of the study were:

- To investigate if liraglutide at steady state changes C_{max} and t_{max} of ethinylestradiol and levonorgestrel.
- To assess exposure of liraglutide during the single dose administration of the combination ethinylestradiol/levonorgestrel.
- To assess the safety after administration of liraglutide in combination with ethinylestradiol and levonorgestrel.

Study Design:

This was a single centre, randomized, double-blind, placebo-controlled, two-period cross-over trial comparing the influence of liraglutide and placebo on the pharmacokinetics (PK) of ethinylestradiol and levonorgestrel administered as a combination contraceptive drug.

The potential influence of liraglutide on the absorption of an orally administered contraceptive drug (Neovletta® ; 0.03 mg ethinylestradiol and 0.15 mg levonorgestrel) was investigated at steady state using the highest liraglutide/placebo dose (1.8 mg). A single dose of

Neovletta® was administered at the time of steady state of liraglutide 1.8 mg for the drug-drug interaction (DDI) investigations.

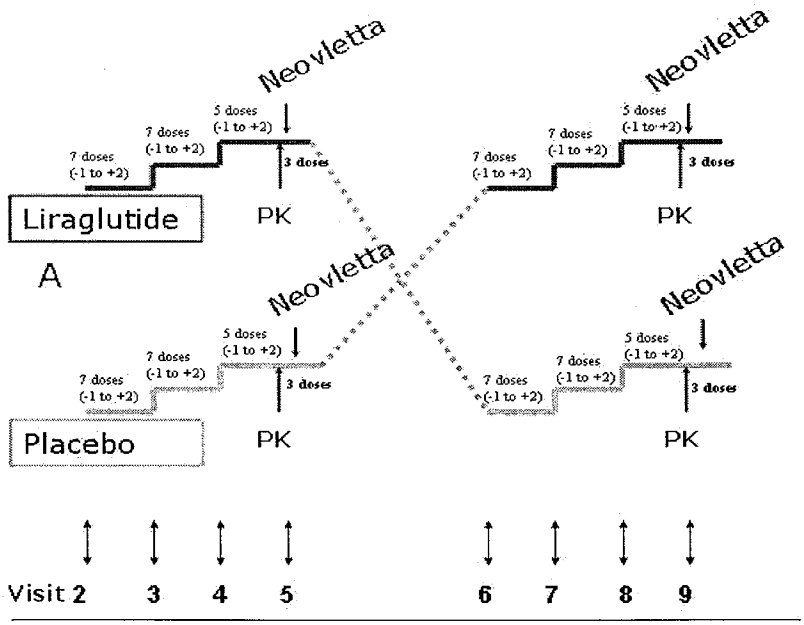


Figure 1: Trial Design

In this study each subject attended 10 visits:

- Visit 1 was a screening visit to assess their eligibility
- Visits 2-4 and 6-8: were for liraglutide/placebo dose increase. Randomization of the subjects was performed at Visit 2. Liraglutide/placebo was administered daily with weekly increase of dose (0.6 mg, 1.2 mg and 1.8 mg) for approximately 3 weeks in both cross-over periods.
- At Visits 5 and 9 administrations of Neovletta® and serial blood sampling for bioanalysis was done to investigate any drug-drug interaction. Visits 5 and 9 were of 4 days duration each and included one overnight stay at the trial site. One Neovletta® tablet was administered 7 h after administration of 1.8 mg liraglutide/placebo (steady state conditions). Fourteen (14) to 42 days were allowed between each cross-over period.
- Visit 10: Follow-up visit, performed 5-14 days after completing Visit 9.

On the day of the DDI investigation (Day 1 of Visit 5 and Visit 9), one single oral tablet of Neovletta® was administered 7 h after administration of 1.8 mg liraglutide or placebo. This timing was chosen so that liraglutide C_{max} was reached at approximately the same time as absorption of ethinylestradiol/levonorgestrel (both of which are rapidly absorbed), reaching C_{max} 1-2 h after administration.

Blood samples (7 mL) were drawn on the DDI visits (Visits 5 and 9) for determination of serum concentrations of ethinylestradiol and levonorgestrel. Thirteen samples were drawn on each visit; pre-dose (-15 min) and at 0.5, 1, 2, 3, 4, 6, 8, 12, 17, 24, 48 and 74 h post-administration of Neovletta® on Day 1 (the scheduled times in relation to administration of liraglutide/placebo were 6.45, 7.5, 8, 9, 10, 11, 13, 15, 19, 24, 31, 55 and 81 h). Blood samples (3 mL) for the determination of plasma concentrations of liraglutide were also drawn at the DDI visits 5 and 9. Nine blood samples were drawn during each of these visits; pre-dose (-15 min) and at 4, 6, 8, 10, 12, 15, 17 and 24 h post administration of liraglutide on Day 1.

Study Population:
Twenty one postmenopausal woman volunteers were enrolled in this study. The mean age of the study population was 58.3 years (range 51 to 71 years). Table 1 below shows the demographics of the enrolled patients.

Table 1. Baseline Demographics of Study Population.

Number of subjects	21
Sex (N (%))	
Male	0(0.00%)
Female	21(100.00%)
Race (N (%))	
White	21(100.00%)
Age (y)	
N	21
Mean	58.3
SD	4.9
Median	58
Min	51
Max	71
Weight at Baseline (Kg)	
N	21
Mean	68.81
SD	10.83
Median	67.3
Min	53.3
Max	91.1
Height (m)	
N	21
Mean	1.670
SD	0.062
Median	1.66
Min	1.56
Max	1.77
Baseline BMI (kg/m2)	
N	21
Mean	24.62
SD	3.25
Median	23.8
Min	19.8
Max	29.5

Bioanalysis:
Quantitative assessment of serum ethinylestradiol and levonorgestrel concentration was done employing a validated GC/MS method (Project code OX006 and PX006). Samples were

extracted using a liquid-liquid extraction procedure using toluene. Extraction was followed by two clean-up steps, resulting in a final dichloromethane extract. After a two step derivatization, 1-2μL of the derivatized samples was injected into the GC/MS system. GC/MS measurements were performed in the chemical ionization mode (negative ions) using ammonia as reagent gas. The calibration curves were analyzed at ethinylestradiol concentrations ranging from 2.5 pg/mL to 500 pg/mL. The lower limit of quantification (LOQ) for ethinylestradiol was 2.5 pg/mL. Between-batch precision (%CV) results for QC samples prepared at low, medium, and high QC concentrations of ethinylestradiol was less than or equal to 6.06% and accuracy (%Bias) ranged from -0.51 to 1.55 %. Between-batch precision (%CV) results of the calibration standards of ethinylestradiol was less than or equal to 5.80 % and accuracy (%Bias) ranged from -4.61 to 6.04 %. For levonorgestrel the calibration curves were analyzed at ethinylestradiol concentrations ranging from 50 pg/mL to 25000 pg/mL. The lower limit of quantification (LOQ) for levonorgestrel was 50 pg/mL. Between-batch precision (%CV) results for QC samples prepared at low, medium, and high QC concentrations of levonorgestrel was less than or equal to 4.46% and accuracy (%Bias) ranged from -7.65 to 5.03 %. Between-batch precision (%CV) results of the calibration standards of levonorgestrel was less than or equal to 3.85 % and accuracy (%Bias) ranged from -8.92 to 4.98 %.

Quantitative assessment of liraglutide was done using enzyme immunoassays (ELISA). The lower limit of quantification of the assay was 18 pmol/L. The calibration curves were analyzed at liraglutide concentration range of 19 pmol/L to 5186 pmol/L. The precision of the assay, as determined from analysis of quality control samples ranged between 11.8% and 21.8%. The mean accuracy (% Bias) ranged between -7.7% to 0.9%. Between-batch precision (%CV) results of the calibration standards of liraglutide was less than or equal to 6.0 % and accuracy (%Bias) ranged from -11.8 to 3.8 %.

Data Analysis:

The PK analysis set consisted of all exposed subjects with at least one evaluable PK profile of ethinylestradiol or levonorgestrel, who fulfilled the inclusion and exclusion criteria and who did not violate the protocol in a manner judged to affect the PK results. The analysis of the PK endpoints was based on the PK analysis set.

The endpoints were derived from serum ethinylestradiol and levonorgestrel concentrations or plasma liraglutide concentrations and actual times by the non-compartmental method using model 200 for extravascular administration of WinNonlin Professional, Version 4.1.b.

λz was determined using at least three time points. Valid observations from the final part of the curve (which was approximately linear) were used for the analysis and the start and end times that were used to define the elimination phase were common time points for all subjects. At the database release (DBR) meeting (before unblinding) it was decided to use measurements from 17 h and onwards for estimation of ethinylestradiol λz and from 12 h and onwards for estimation of levonorgestrel λz.

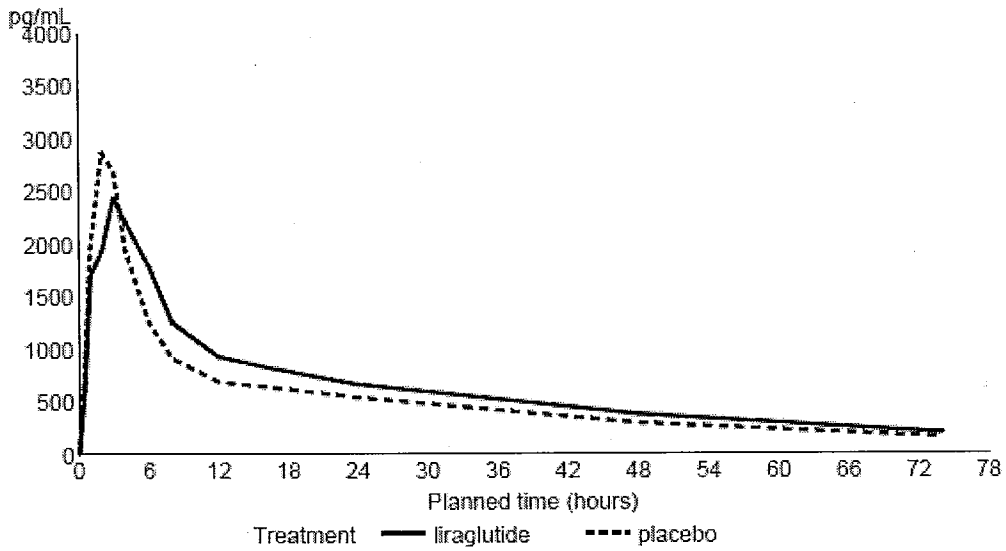
For both ethinylestradiol and levonorgestrel, the liraglutide treatment and the placebo treatment was declared equivalent with respect to primary endpoint ($AUC_{0-\infty}$) if the 90% confidence interval (CI) for the corresponding ratios of $AUC_{0-\infty}$were fully contained within the limits (0.80, 1.25). The comparison between liraglutide and placebo treatments was performed for ethinylestradiol and levonorgestrel separately by use of a linear normal model (ANOVA) for the log transformed values of $AUC_{0-\infty}$, respectively. The model included effects of period and treatment and a random effect of subject. From this model, the ratio between the levels corresponding to liraglutide and placebo was estimated together with their 90% CIs. The

estimated ratios and CIs were retransformed from the corresponding estimated differences in means of the log transformed values together with their CIs.

Pharmacokinetics Results:

The effect of liraglutide on pharmacokinetics of ethinylestradiol and levonorgestrel is presented in Figure 2A and 2B, respectively. The effect of liraglutide on the absorption of an orally administered contraceptive drug (Neovletta®) was investigated at highest steady state dose of liraglutide (1.8 mg). The oral contraceptive administered after 7 hours of liraglutide administration resulted in mean plasma ethinylestradiol and plasma levonorgestrel concentration time profiles that were characterized by a reduced C_{max} and T_{max}

Figure 2: Mean Plasma ethinylestradiol (2A) and levonorgestrel (2B) following single dose administration of oral contraceptive (Neovletta; 0.03mg ethinylestradiol, 0.15 mg levonorgestrel).



The effect of liraglutide on the primary PK parameter (AUC0-∞) of ethinylestradiol and levonorgestrel is summarized in Table 2 and 3, respectively. $AUC_{0-\infty}$ was not calculated if the extrapolated part was more than 20% of the total AUC. This was observed with 3 ethinylestradiol profiles and 18 levonorgestrel profiles.

Table 2. Summary Statistics for AUC0-∞of Ethinylestradiol and Levonorgestrel by Treatment

	liraglutide	placebo	Indvidual ratio liragl./plac.
Number of subjects	21	21	21
$AUC_{0-\infty}$ (h*pg/mL) ethinylestradiol			
N	20	19	18
Mean	856.25	776.12	1.06
SD	225.54	187.97	0.17
Geometric mean	825.16	755.27	1.05
$AUC_{0-\infty}$ (h*pg/mL) levonorgestrel			
N	12	12	10
Mean	63152.80	55230.51	1.22
SD	25495.64	17071.50	0.26
Geometric mean	57938.50	52853.13	1.20

Table 3. Statistical Comparison between Treatments (Liraglutide/Placebo) for Ethinylestradiol and Levonorgestrel

		liraglutide/placebo
ethinylestradiol		
$AUC_{0-\infty}$	N	21
	Estimate	1.057
	Lower 90% limit	0.988
	Upper 90% limit	1.131
levonorgestrel		
$AUC_{0-\infty}$	N	14
	Estimate	1.182
	Lower 90% limit	1.040
	Upper 90% limit	1.343

After statistical analysis for ethinylestradiol, equivalence was demonstrated with respect to AUC0-∞ as the 90% CI for the estimated ratio of AUC0-∞ (liraglutide/placebo treatment) was within the pre-specified limits for equivalence, i.e. within 0.80 to 1.25.
However, for levonorgestrel, equivalence was not demonstrated with respect to AUC0-∞ as the 90% CI for the estimated ratio of AUC0-∞ (liraglutide/placebo treatment) was outside the pre-specified limits for equivalence. The estimated ratio of AUC0-∞ (liraglutide/placebo treatment) was 1.18 and the 90% CI was 1.04 to 1.34; i.e. the levonorgestrel AUC0-∞ was 18% higher during liraglutide treatment.

A summary of secondary PK parameters of ethinylestradiol and levonorgestrel is shown in Table 4 and Table 5, respectively. The mean C_{max} and T_{max} values reduced in the presence of liraglutide treatment.

Statistical analysis showed (Table 6 & 7) that with respect to C_{max}, for ethinylestradiol and levonorgestrel, equivalence was not demonstrated when given during liraglutide and placebo treatment. C_{max} was 12% and 13% lower for ethinylestradiol and levonorgestrel, respectively, during liraglutide treatment compared to placebo (ratio 0.88 (90% CI [0.79; 0.97]) for ethinylestradiol and ratio 0.87 (90% CI [0.75; 1.00]) for levonorgestrel).

Table 4. Summary Statistics for the Secondary PK Endpoints of Ethinylestradiol

	liraglutide	placebo
AUC(0-48h) (h*pg/mL)		
N	21	21
Mean	752.29	706.52
SD	171.42	146.26
Geometric mean	732.51	692.43
Cmax (pg/mL)		
N	21	21
Mean	51.50	56.62
SD	17.68	12.18
Geometric mean	48.78	55.38
tmax (h)		
N	21	21
Median	3.00	2.00
Min	2.00	2.00
Max	12.00	3.00
t1/2 (h)		
N	21	20
Mean	14.88	18.45
SD	6.20	23.06
Harmonic mean	13.03	12.64
Lambda (1/h)		
N	21	20
Mean	0.05	0.05
SD	0.02	0.02
Geometric mean	0.05	0.05
CL/F (L/h)		
N	20	19
Mean	37.92	40.79
SD	12.13	9.58
Geometric mean	36.36	39.72
Vz/F (L)		
N	20	19
Mean	720.52	764.53
SD	193.82	264.93
Geometric mean	695.99	728.44

Table 5. Summary Statistics for the Secondary PK Endpoints of Levonorgestrel

	liraglutide	placebo
AUC(0-t) (h*pg/mL)		
N	21	21
Mean	47150.05	39527.76
SD	24259.96	17448.27
Geometric mean	41408.84	36117.70
Cmax (pg/mL)		
N	21	21
Mean	3072.90	3264.29
SD	1791.07	1352.92
Geometric mean	2603.68	2989.58
tmax (h)		
N	21	21
Median	3.00	2.00
Min	1.00	1.00
Max	8.00	4.00
t1/2 (h)		
N	21	21
Mean	32.56	34.56
SD	9.82	13.08
Harmonic mean	29.95	30.62
Lambda (1/h)		
N	21	21
Mean	0.02	0.02
SD	0.01	0.01
Geometric mean	0.02	0.02
CL/F (L/h)		
N	12	12
Mean	2.86	2.97
SD	1.44	0.95
Geometric mean	2.59	2.84
Vz/F (L)		
N	12	12
Mean	113.73	116.05
SD	68.32	57.91
Geometric mean	97.10	104.47

Table 6. Comparison between Treatments (Liraglutide/Placebo), Secondary PK Endpoints of Ethinylestradiol

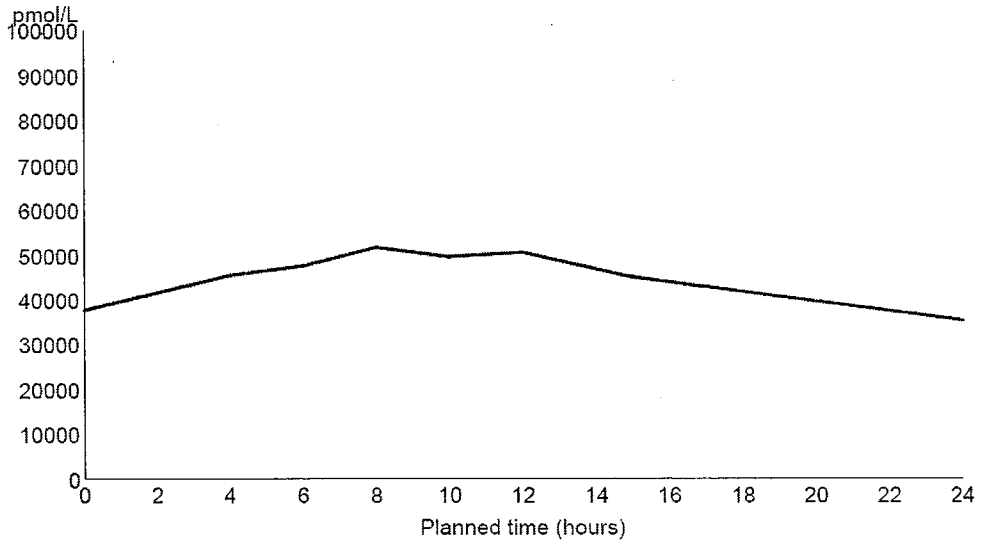
		liraglutide/placebo
AUC (0-48h)	N	21
	Estimate	1.055
	Lower 90% limit	1.001
	Upper 90% limit	1.112
Cmax	N	21
	Estimate	0.876
	Lower 90% limit	0.789
	Upper 90% limit	0.972
t1/2	N	21
	Estimate	0.981
	Lower 90% limit	0.785
	Upper 90% limit	1.225
Vz/F	N	21
	Estimate	0.960
	Lower 90% limit	0.865
	Upper 90% limit	1.066

Table 7. Comparison between Treatments (Liraglutide/Placebo), Secondary PK Endpoints of Levonorgestrel

		liraglutide/placebo
AUC(0-t)	N	21
	Estimate	1.145
	Lower 90% limit	1.055
	Upper 90% limit	1.243
Cmax	N	21
	Estimate	0.867
	Lower 90% limit	0.754
	Upper 90% limit	0.998
t1/2	N	21
	Estimate	0.962
	Lower 90% limit	0.896
	Upper 90% limit	1.033
Vz/F	N	14
	Estimate	0.854
	Lower 90% limit	0.759
	Upper 90% limit	0.961

Mean plasma concentration profile of liraglutide at steady state is shown in Figure 3.
All subjects had quantifiable plasma concentrations of liraglutide at all sampling time points during the 24 h sampling period at steady state. Liraglutide median tmax was 8 h. Mean AUCτ, Cmax and CL/F of liraglutide were 1063092 pmol*h/L, 54542 pmol/L and 0.47 L/h, respectively.

Figure 11–3 Mean Liraglutide 1.8 mg Profile at Steady State



Summary of pharmacokinetic results

- Equivalence was demonstrated with respect to the primary endpoint AUC0-∞ of ethinylestradiol when given during liraglutide and placebo treatment while equivalence was not demonstrated for the primary endpoint AUC0-∞ of levonorgestrel. AUC0-∞ for levonorgestrel was 18% higher during liraglutide treatment (ratio 1.18 (90% CI [1.04; 1.34])).

- For ethinylestradiol and levonorgestrel, equivalence was not demonstrated with respect to C_{max} when given during liraglutide and placebo treatment. C_{max} was 12% and 13% lower for ethinylestradiol and levonorgestrel, respectively, during liraglutide treatment compared to placebo (ratio 0.88 (90% CI [0.79; 0.97]) for ethinylestradiol and ratio 0.87 (90% CI [0.75; 1.00]) for levonorgestrel).

- T_{max} was delayed by 1.0 h for both ethinylestradiol and levonorgestrel during liraglutide treatment compared to during placebo.

- Mean AUCτ, Cmax and T_{max} for 1.8 mg liraglutide at steady state in combination with a single dose of Neovletta® were 1063092 pmol*h/L, 54542 pmol/L and 9.4 h, respectively.

Reviewer's Comment:

The present study evaluated the effect of liraglutide (at steady state) on pharmacokinetics of oral contraceptive (Neovletta®). Based on PK analysis the median T_{max} was delayed by 1.0 h for both ethinylestradiol and levonorgestrel during liraglutide treatment compared to during placebo. This is in contrast with T_{max} delay of 1.5 hours that is being reported by the sponsor. Also, C_{max} was found to be 12% and 13% lower for ethinylestradiol and levonorgestrel, respectively, during liraglutide treatment compared to placebo.

4.2.10 DDI Study (NN2211-1608)

Title: A Two-way Cross-Over, Placebo-Controlled Interaction Trial in Two Parts (in Healthy Subjects), Studying Liraglutide's Potential Influence on the Absorption Pharmacokinetics of Lisinopril, Atorvastatin, Griseofulvin and Digoxin, and Liraglutide's Potential Influence on Intragastric pH.

Investigator and Study Center(s):
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Quintiles Phase I Unit,
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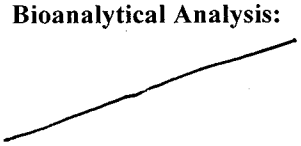
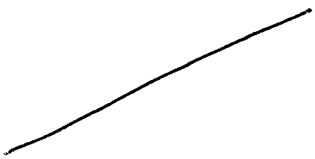
Trial Sites
Quintiles Phase I Unit, Strandbodgatan 1, SE-753 23 Uppsala, Sweden
Quintiles Hermelinen, Varvsgatan 53, SE-972 33 Luleå, Sweden

Study Sponsor:

Novo Nordisk A/S, Denmark

Bioanalytical Analysis:





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Study Period: 29 May 2006 (Trial Initiated) to 20 April 2007 (Trial Completed)

Objective:
The primary objective of this study was to determine if liraglutide at steady state changes $AUC_{0-\infty}$ of atorvastatin, lisinopril, griseofulvin and digoxin.

The secondary objectives of the study were:
- To investigate if liraglutide at steady state changes C_{max} and t_{max} of atorvastatin, lisinopril, griseofulvin and digoxin
- To investigate if liraglutide changes intragastric pH
- To estimate the pharmacokinetics of liraglutide after a single dose and at steady state
- To assess the safety after administration

Trial Rationale: The purpose of this trial was to investigate whether administration of liraglutide causes a change of the absorption pharmacokinetics of four drugs with a range of different solubility and permeability properties i.e. atorvastatin and griseofulvin (Class II drugs), lisinopril (Class III drug) and digoxin (Class IV drug).

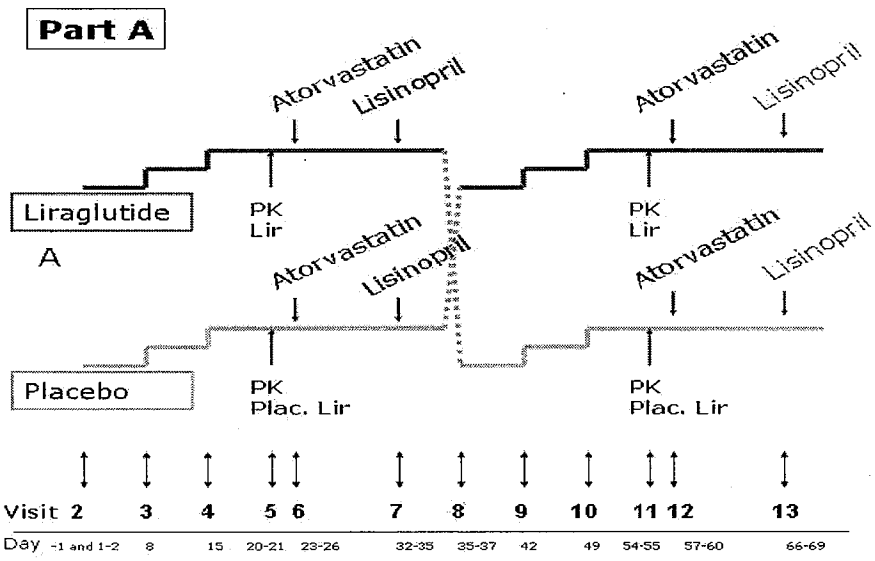
Study Design:
The trial was a randomized, double-blinded, placebo-controlled, two-way cross-over trial with two Parts (A and B) comparing the influence from liraglutide or placebo on the absorption pharmacokinetics of 40 mg atorvastatin and 20 mg lisinopril (Part A), 500 mg griseofulvin and 1 mg digoxin (Part B) and on intragastric pH (Part B). Volunteers in good general health were included in Part A, n = 42 or Part B, n = 28.

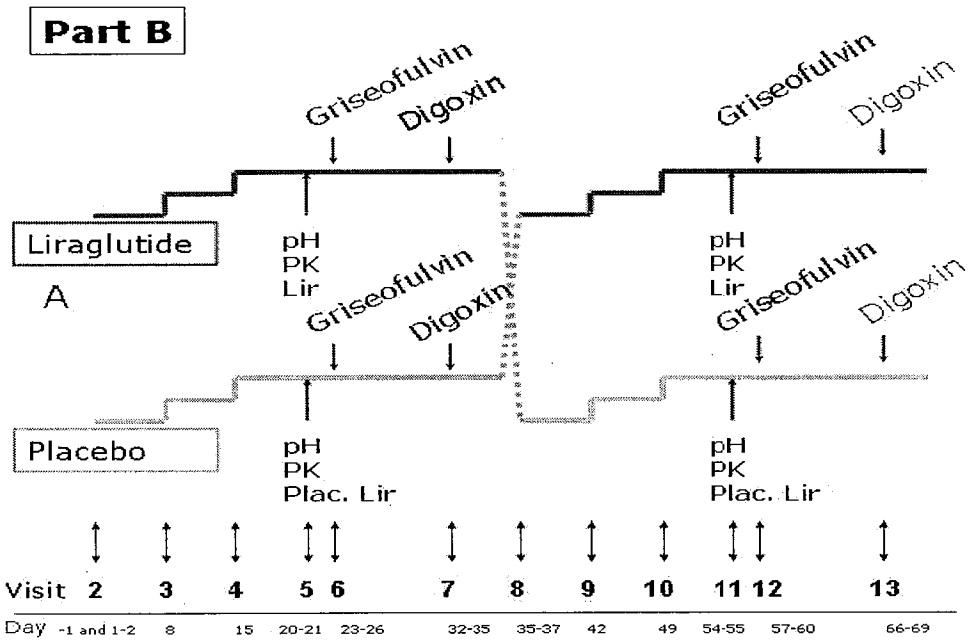
Each subject attended 14 visits:

- Visit 1: a screening visit to assess eligibility for inclusion in the trial
- Visits 2-4 and 8-10: three visits each during the liraglutide/placebo dose increase. The randomisation of the subjects and initiation of administration of liraglutide or placebo were performed at Visit 2
- Visits 5 and 11: two visits for pH measurements (in Part B) and liraglutide pharmacokinetics (in Part A and Part B)
- Visits 6, 7, 12 and 13: four in-house visits of 4 days each when the DDI investigations were performed
- Visit 14: an End of Trial Visit 7-14 days after the completion of Visit 13

Liraglutide/placebo was administered daily in the morning with weekly increasing dose (0.6 mg, 1.2 mg and 1.8 mg) for 35 days each in random order in both cross-over periods.
In Part A one single dose of 40 mg atorvastatin and one single dose of 20 mg lisinopril were administered, and in Part B one single dose of 500 mg griseofulvin and one single dose of 1 mg digoxin were administered. Sufficient wash-out periods of 9 days were allowed between the drug administrations. The dose increase of the second part started immediately after the completion of Visit 7. The total duration of the trial for each individual subject was up to 15 weeks. The DDI investigations took place after the subject received either 1.8 mg liraglutide at steady state or placebo. The administration of the interacting drugs was timed so Cmax of liraglutide would coincide with the absorption peak of the co-administered drugs.

Figure 1: Trial Design





The investigation of intragastric pH took place after the subject received either liraglutide at steady-state or placebo on Day 20 in each cross-over period (Visits 5 and 11). Liraglutide/placebo was administered 1 h after the start of measurement of pH, which was then continued to be measured for a further 23 h.

Study Population:
Seventy (70) subjects were randomized of whom 42 were allotted to participate in Part A (exposure to atorvastatin and lisinopril) and 28 in Part B (exposure to griseofulvin and digoxin as well as gastric pH analysis). Table 1 and 2 below shows the demographics of the enrolled patients.

Table 1: Baseline Demographics of Study Population Part A

Number of subjects	42
Sex (N (%))	
Male	29(69.05%)
Female	13(30.95%)
Ethnic origin (N (%))	
Other	1(2.38%)
White	41(97.62%)
Age at screening (Years)	
N	42
Mean	28.7
SD	8.2
Median	26
Min	18
Max	49
Weight (kg)	
N	42
Mean	76.16
SD	10.86
Median	77.3
Min	53.5
Max	101
Height (m)	
N	42
Mean	1.769
SD	0.085
Median	1.76
Min	1.59
Max	1.97
BMI (kg/(m^2))	
N	42
Mean	24.34
SD	2.99
Median	23.65
Min	19.5
Max	30.1

Table 2: Baseline Demographics of Study Population Part B

Number of subjects	28
Sex (N (%))	
Male	27(96.43%)
Female	1(3.57%)
Ethnic origin (N (%))	
White	28(100.00%)
Age at screening (Years)	
N	28
Mean	25.8
SD	4.8
Median	24.5
Min	18
Max	42
Weight (kg)	
N	28
Mean	75.58
SD	8.22
Median	73.9
Min	60.4
Max	93.8
Height (m)	
N	28
Mean	1.800
SD	0.052
Median	1.805
Min	1.68
Max	1.9
BMI (kg/(m^2))	
N	28
Mean	23.32
SD	2.38
Median	22.95
Min	19.6
Max	28.3

Bioanalysis: Quantitative assessment of atorvastatin was determined by a previously validated LC-MS/MS method (validation report Q-26078). The LOQ of atorvastatin was 0.200 ng/mL and a ULOQ of 60.0 ng/mL with a 500 µL sample. Between-batch precision (%CV) results for QC samples prepared at low, medium, and high QC concentrations of atorvastatin was less than or equal to 4.7% and mean accuracy (%Bias) ranged from -4.2% to 0.2 %. Between-batch precision (%CV) results of the calibration standards of atorvastatin was less than or equal to 8.80 % and accuracy (%Bias) ranged from -4.5 to 2.8 %.

Quantitative assessment of griseofulvin was determined by a previously validated HPLC method (validation report Q-26282). The LLOQ of griseofulvin was 0.100 µg/mL and a ULOQ of 5.0 µg/mL. Between-batch precision (%CV) results for QC samples prepared at low, medium, and high QC concentrations of griseofulvin was less than or equal to 3.2% and mean accuracy (%Bias) ranged from -3.5% to -3.0 %. Between-batch precision (%CV) results of the calibration standards of griseofulvin was less than or equal to 3.2 % and accuracy (%Bias) ranged from -2.0 to 3.0 %.

Quantitative assessment of Lisinopril was determined by a previously validated LC-MS/MS method (validation report Q-26079). The LLOQ of griseofulvin was 0.5 ng/mL and a ULOQ of

150 ng/mL. Between-batch precision (%CV) results for QC samples prepared at low, medium, and high QC concentrations of Lisinopril was less than or equal to 4.1% and mean accuracy (%Bias) ranged from -3.5% to 1.7 %. Between-batch precision (%CV) results of the calibration standards of Lisinopril was less than or equal to 7.5 % and mean accuracy (%Bias) ranged from -0.7 to 2.0 %.Quantitative assessment of Digoxin was determined by a previously validated chemiluminescent immunometric assay (validation report Q-26251). The LLOQ of Digoxin was 0.64 nmol/L and a ULOQ of 10 nmol/L. Between-batch precision (%CV) results for QC samples prepared at low, medium, and high QC concentrations of Digoxin was less than or equal to 7.9% and mean accuracy (%Bias) ranged from -5.3 % to -8.2% .

Data Analysis:
Primary endpoint AUC0-∞ for atorvastatin, lisinopril and griseofulvin and AUC0-72h for digoxin were derived from serum or plasma concentrations and actual times by the standard model-free, non-compartmental method, using Model 200 for extravascular administration of WinNonlin Professional, Version 4.1.b (Pharsight Corporation, Mountain View, CA, USA).

The comparison between the treatments (liraglutide and placebo) was performed for atorvastatin, lisinopril, griseofulvin and digoxin separately by use of a linear normal model (ANOVA) for the log transformed values of AUC0-∞(AUC0-72h for digoxin), respectively. The model included effects of period and treatment and a random effect of subject. From this model, the ratio between the levels corresponding to liraglutide and placebo was estimated together with their 90% CIs. The estimated ratios and CIs were retransformed from the corresponding estimated differences in means of the log transformed values together with their CIs. The liraglutide treatment and the placebo treatment were declared equivalent in AUC0-∞ (or AUC0-72h) for a given drug if the 90% CI for the ratio between the two treatments was fully contained within the interval (0.8, 1.25).

Pharmacokinetics Results:
Mean concentration versus time curves for atorvastatin, lisinopril, griseofulvin and digoxin are presented in Figure 2, Figure 3, Figure 4 and Figure 5, respectively.

Figure 2: Mean Plasma Concentration Time Profile for Atorvastatin in Presence and Absence of Liraglutide

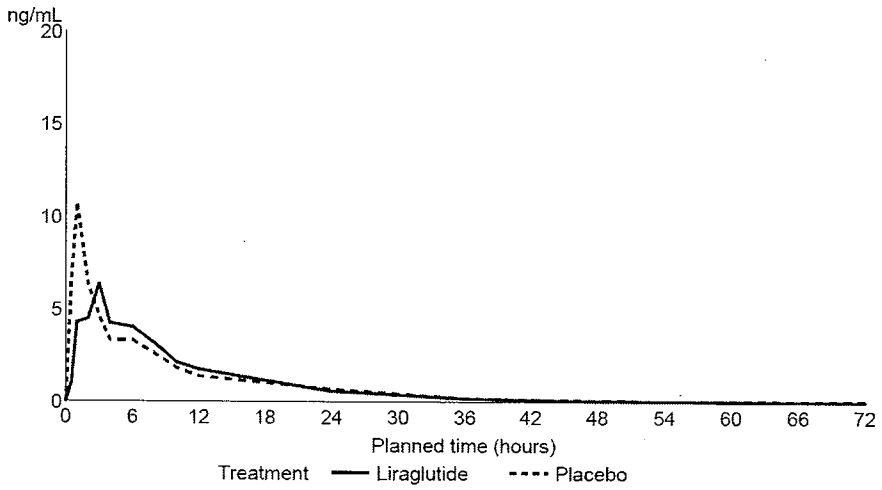


Figure 3: Mean Plasma Concentration Time Profile for Lisinopril in Presence and Absence of Liraglutide

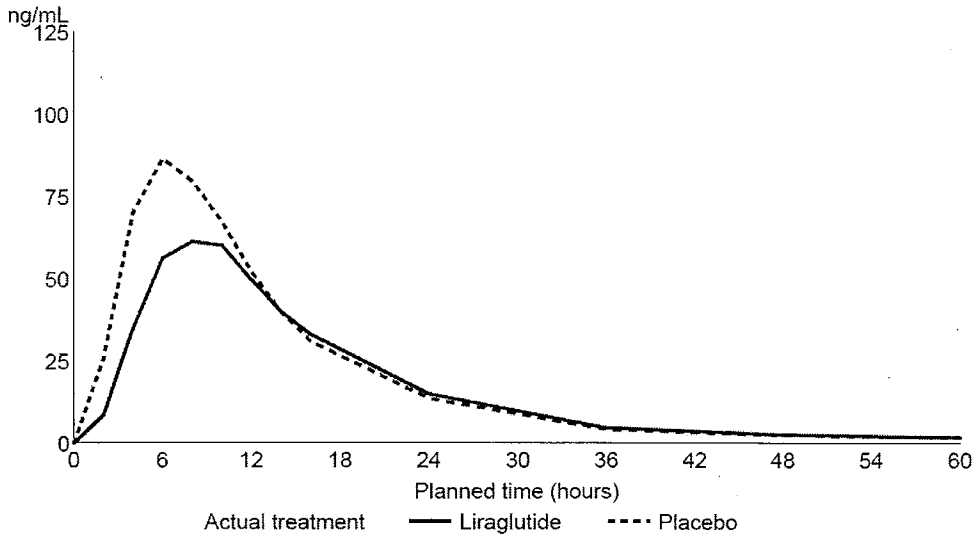


Figure 4: Mean Plasma Concentration Time Profile for Griseofulvin in Presence and Absence of Liraglutide

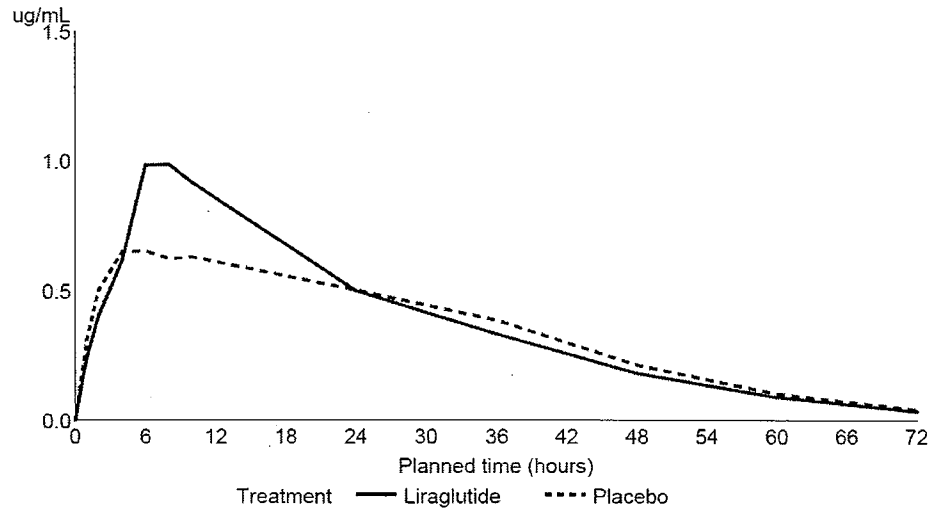
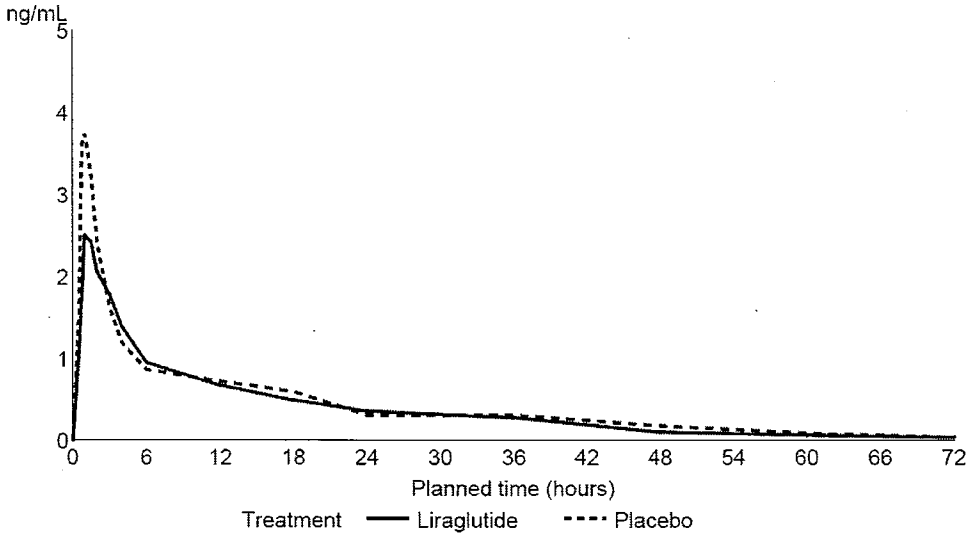


Figure 5: Mean Plasma Concentration Time Profile for Digoxin in Presence and Absence of Liraglutide



The effect of liraglutide on the primary PK parameter (AUC0-∞) of Atorvastatin, Lisinopril and Griseofulvin, and AUC0-72h of Digoxin is summarized in Table 3 and 4. $AUC_{0-\infty}$ was not calculated if the extrapolated part was more than 20% of the total AUC.

Table 3: Summary Statistics for AUC0-∞ of Atorvastatin and Lisonopril by Treatment

	Liraglutide	Placebo
Number of subjects	39	42
AUC (h*ng/mL) atorvastasin		
N	38	41
Mean	62.37	65.68
SD	25.78	24.45
Geometric mean	57.81	61.62
Median	54.64	66.83
Min		
Max		
AUC (h*ng/mL) lisonopril		
N	38	38
Mean	1086.43	1272.04
SD	429.44	467.88
Geometric mean	1006.30	1188.47
Median		
Min		
Max		

b(4)

Table 4: Summary Statistics for AUC0-∞ of Griseofulvin and AUC0-72 Digoxin by Treatment

	Liraglutide	Placebo
Number of subjects	27	26
AUC (h*ug/mL) griseofulvin		
N	19	21
Mean	32.56	29.62
SD	8.05	7.86
Geometric mean	31.75	28.68
Median	28.61	27.82
Min		
Max		
AUC(0-72h) (h*ng/mL) digoxin		
N	26	25
Mean	28.22	31.78
SD	16.83	15.89
Geometric mean	23.69	28.32
Median	25.58	30.31
Min		
Max		

b(4)

The results from the primary statistical analyses of Part A and Part B are presented in Table 5 and Table 6, respectively. For atorvastatin and griseofulvin, equivalence was demonstrated with respect to AUC0-∞ as the 90% confidence interval for the estimated ratio of AUC0-∞ (liraglutide/placebo treatment) was within the pre-specified limits for equivalence, i.e. within 0.80 to 1.25.

Table 5: Statistical Comparison between Treatments (Liraglutide/Placebo) for Atorvastatin and Lisonopril.

		liraglutide/placebo
atorvastatin		
$AUC_{0-\infty}$	N	42
	Estimate	0.946
	Lower 90% limit	0.886
	Upper 90% limit	1.010
lisonopril		
$AUC_{0-\infty}$	N	40
	Estimate	0.849
	Lower 90% limit	0.747
	Upper 90% limit	0.966

Table 6: Statistical Comparison between Treatments (Liraglutide/Placebo) for Griseofulvin and Digoxin.

Table 11–6 Comparison of $AUC_{0-\infty}$ of Griseofulvin and AUC_{0-72h} of Digoxin

		liraglutide/placebo
griseofulvin		
AUC	N	22
	Estimate	1.096
	Lower 90% limit	1.013
	Upper 90% limit	1.185
digoxin		
AUC(0-72h)	N	27
	Estimate	0.843
	Lower 90% limit	0.722
	Upper 90% limit	0.984

For lisinopril and digoxin, however, equivalence could not be demonstrated for AUC0-∞ or AUC0-72h, respectively, when the drug was given at liraglutide steady state conditions compared to during placebo treatment. The AUC0-∞ for lisinopril was 15% smaller at liraglutide treatment than during placebo and AUC0-72h for digoxin was 16% smaller at liraglutide treatment compared to during placebo.

A summary of secondary PK parameters of Atorvastatin, Lisinopril and Griseofulvin, and Digoxin is summarized in tables below.

Table 7: Pharmacokinetic parameters for Atorvastatin

	Liraglutide	Placebo
Number of subjects	39	42
Cmax (ng/mL)		
N	39	42
Mean	8.30	14.19
SD	4.41	9.36
Geometric mean	7.41	12.05
tmax (h)		
N	39	42
Median	3.00	1.00
Min		
Max		
thalf (h)		
N	39	42
Mean	7.47	9.03
SD	2.13	2.60
Harmonic mean	6.95	8.24

b(4)

Table 8: Pharmacokinetic parameters for Lisinopril

	Liraglutide	Placebo
Number of subjects	38	38
Cmax (ng/mL)		
N	38	38
Mean	64.41	87.59
SD	27.69	35.58
Geometric mean	58.51	80.30
tmax (h)		
N	38	38
Median	8.00	6.00
thalf (h)		
N	38	38
Mean	20.63	20.26
SD	8.11	4.63
Harmonic mean	18.50	19.30

b(4)

Table 9: Pharmacokinetic parameters for Griseofulvin

	Liraglutide	Placebo
Number of subjects	27	26
Cmax (ug/mL)		
N	27	26
Mean	1.07	0.75
SD	0.40	0.18
Geometric mean	0.99	0.73
tmax (h)		
N	27	26
Median	6.00	6.00
thalf (h)		
N	27	26
Mean	16.75	16.59
SD	6.22	10.41
Harmonic mean	15.07	14.35

b(4)

Table 10: Pharmacokinetic parameters for Digoxin

	Liraglutide	Placebo
Number of subjects	26	25
Cmax (ng/mL)		
N	26	25
Mean	3.29	4.67
SD	1.55	2.03
Geometric mean	2.92	4.32
tmax (h)		
N	26	25
Median	1.50	1.00
Min		
Max		

b(4)

Table 11: Statistical Comparison between Treatments (Liraglutide/Placebo) for Atorvastatin and Lisonopril.

		liraglutide/placebo
atorvastatin		
Cmax (ng/mL)	N	42
	Estimate	0.619
	Lower 90% limit	0.533
	Upper 90% limit	0.720
t1/2 (h)	N	42
	Estimate	0.826
	Lower 90% limit	0.769
	Upper 90% limit	0.887
lisonopril		
Cmax (ng/mL)	N	40
	Estimate	0.730
	Lower 90% limit	0.630
	Upper 90% limit	0.846
t1/2 (h)	N	40
	Estimate	0.982
	Lower 90% limit	0.901
	Upper 90% limit	1.070

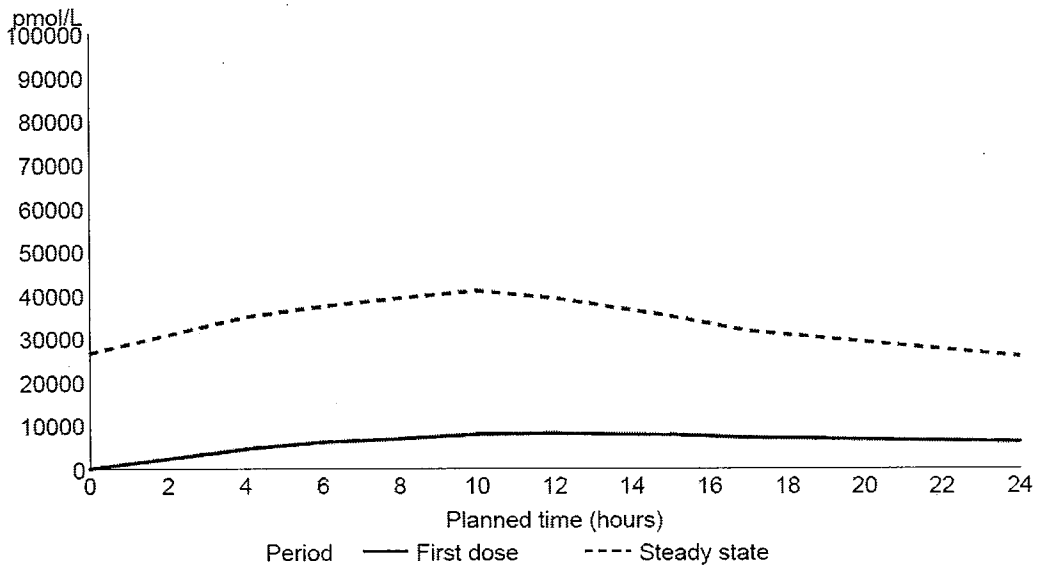
Table 12: Statistical Comparison between Treatments (Liraglutide/Placebo) for Griseofulvin and Digoxin.

		liraglutide/placebo
griseofulvin		
Cmax	N	27
	Estimate	1.369
	Lower 90% limit	1.243
	Upper 90% limit	1.507
t1/2	N	27
	Estimate	1.043
	Lower 90% limit	0.912
	Upper 90% limit	1.194
digoxin		
Cmax	N	27
	Estimate	0.691
	Lower 90% limit	0.602
	Upper 90% limit	0.794

For atorvastatin, equivalence was not demonstrated for Cmax (38% lower) than the placebo group. For lisinopril, equivalence was not demonstrated for Cmax (27% lower) than the placebo group. For griseofulvin, equivalence was not demonstrated for Cmax (37% higher). For digoxin, equivalence was not demonstrated for Cmax (31% lower).

Mean plasma concentration profiles of liraglutide are shown in Figure 6

Figure 6: Plasma Concentration Profile of Liraglutide.



Descriptive statistics for liraglutide pharmacokinetic parameters are displayed in Table 13. The ratio of mean dose-adjusted AUCτ at steady state and AUC0-24 after the first dose was approximately 1.8.

Table 13: Pharmacokinetic Parameters for Liraglutide

	First dose	Steady state
Number of subjects	67	65
Cmax (pmol/L)		
N	67	65
Mean	8839.75	44663.05
SD	2694.22	10524.41
Geometric mean	8500.23	43464.33
Median	8625.00	44837.00
Min		
Max		
tmax (h)		
N	67	65
Mean	13.33	8.97
SD	3.68	3.25
Geometric mean	12.84	9.24
Median	12.00	10.00
Min		
Max		
AUC* (h*pmol/L)		
N	67	65
Mean	147910.94	809120.72
SD	40249.31	186605.27
Geometric mean	143417.83	789093.25
Median	145720.00	803166.89
Min		
Max		
CL/F		
N	.	65
Mean	.	0.62
SD	.	0.14
Geometric mean	.	0.61
Median	.	0.60
Min	.	
Max	.	

b(4)

Efficacy Conclusions

- Atorvastatin and griseofulvin, equivalence was demonstrated with respect to AUC0-∞ when the drugs were given at liraglutide steady state conditions compared to during placebo treatment.

- Lisinopril and digoxin, equivalence was not demonstrated with respect to AUC0-∞ (lisinopril) and AUC0-72h (digoxin) when the drugs were given at liraglutide steady state conditions compared to during placebo treatment. The AUC0-∞ for lisinopril was 15%

lower and the AUC0-72h for digoxin was 16% lower at liraglutide steady state conditions compared to during placebo treatment.

- Atorvastatin, lisinopril, griseofulvin and digoxin, equivalence was not demonstrated with respect to Cmax when the drugs were given at liraglutide steady state conditions compared to during placebo treatment. Cmax for atorvastatin, lisinopril and digoxin were 38% lower, 27% lower and 31% lower respectively. The Cmax for griseofulvin was 37% higher when administered at liraglutide steady state conditions.

- Atorvastatin, lisinopril and digoxin, median tmax was delayed by 2 h, 2 h and 0.5 h at liraglutide steady state conditions compared to during placebo treatment. For griseofulvin, tmax was not affected by treatment.

- Median intragastric pH showed no statistically significant difference between liraglutide steady state conditions and placebo treatment for the entire period as well as during the supine, postprandial and meal periods. Further, there was no difference in the fraction of intragastric pH values above 4 during the entire period, pre-drug, post-prandial or supine periods. For the meal period, the fraction of measured pH values above 4 was lower at liraglutide steady state conditions than during placebo treatment (P = 0.044).

- An expected increase in AUCτ and Cmax was shown at steady state compared to single dose. The ratio of mean dose-adjusted AUCτ at steady state and AUC0-24h after the first dose was 1.8. Liraglutide tmax occurred 4 h earlier at steady state than at first dose (estimated median difference was -4 h (90% CI: [-5.5; -3.0]).

Overall Conclusions

- The exposure (AUC) of single dose griseofulvin or atorvastatin was equivalent at steady state levels of liraglutide and during placebo treatment.
- The lower Cmax and delayed tmax for the oral drugs when given concomitantly with steady state liraglutide was as expected reflecting a slight delay in gastric emptying.
- No significant overall effect of liraglutide on intragastric pH was recorded.
- Steady state pharmacokinetics for liraglutide showed increased AUCτ and Cmax and earlier tmax compared to single dose pharmacokinetics. The ratio between dose-adjusted AUCτ at steady state and AUC0-24h after the first dose was approximately 1.8, indicating accumulation of liraglutide.
- No safety concerns were raised.

Reviewer's Comment:

Overall the study design and the data analysis seem reasonable. However, it was noted that detailed bionalytical validaltion reports of atorvastatin, digoxin, lisinopril and griseofulvin were not included in the submission.

The effect of liraglutide on various co-administered drug observed from this study is summarized below:

Co-administered Drug	Cmax	AUC
Atorvastatin (40 mg)	↓38 %	↔
Digoxin (1 mg)	↓31 %	↓16 %

Lisinopril (20 mg)	↓27 %	↓15 %
Griseofulvin (500 mg)	↑37 %	↔

↔ **No change**

APPEARS THIS WAY
ON ORIGINAL

4.2.11 Pharmacokinetics in Elderly (NN2211-1327)

Study Details:

Title of Trial	An open label, single dose trial with two groups comparing the pharmacokinetics of liraglutide in young versus elderly subjects of both sexes
Trial ID	NN2211-1327
Development Phase	Phase 1
IND Number (US only)	Not applicable
Compound Name	Liraglutide
Indication	Diabetes mellitus
Investigators	Georg Golor, M.D., Ph.D. (principal investigator) [redacted] b(4)
Trial Site	[redacted]
Trial Initiated	19 April 2004
Trial Completed	16 June 2004
Sponsor	Global Development, Novo Nordisk A/S
International Medical Officer	Milan Zdravkovic, M.D., Ph.D. Novo Nordisk A/S
International Trial Managers	Birgitte Bentz Damholt, Ph.D., and Marianne Ekblom, Ph.D. Novo Nordisk A/S
Local Trial Manager	Ulrike Petry, Novo Nordisk Pharma GmbH, Germany
Statisticians	[redacted] b(4) Poul C. Pedersen, M.Sc., Novo Nordisk A/S
Medical Writer	Trine Kruse, M.Sc., Novo Nordisk A/S
Report Date	07 April 2005

Objective:

The primary objective of the study was to compare the pharmacokinetic exposure of liraglutide (NNC 90-1170), AUC(0-t), where t is the time of the last quantifiable concentration, after a single subcutaneous injection in young versus elderly healthy subjects.

The secondary objectives were; to compare the pharmacokinetic parameters Cmax, tmax, AUC(0-∞), CL/F, Vz/F, and t½ of liraglutide after a single subcutaneous injection in young versus elderly subjects; to compare the pharmacokinetic parameters AUC(0-t), Cmax, tmax, AUC(0-∞), CL/F, Vz/F, and t½ of liraglutide after a single subcutaneous injection in male versus female subjects, and to evaluate the safety of liraglutide in young and elderly, male and female subjects.

Methodology:

- The trial was an open label, single centre, and single dose trial between two groups (young and elderly) of healthy subjects.

- The young group was comprised of subjects between 18–45 years of age and the elderly group comprised subjects aged 65 years and above. Each age group consisted of an equal number of males and females, and the age distribution (male vs. female) within each age group was to be roughly matched.

- The trial consisted of three visits; Visit 1 (screening), Visit 2 (dosing; days 1–4 in-house stay at the clinic), and Visit 3 (follow-up). The subjects were dosed with a single dose of 1 mg liraglutide in the evening, administered as a s.c. injection in the abdomen. Dosing took place at approximately 21:00 hours and blood samples for pharmacokinetic evaluation were drawn from pre-dose to 60 hours post-dose.

Number of Subjects (Planned and Analyzed):

A total of 32 subjects were planned to be enrolled in the trial; 16 subjects per age group – 8 males and 8 females. The subject disposition is provided below:

	Young	Elderly	Total
Screened	16 (100.0 %)	16 (100.0 %)	32 (100.0 %)
Safety population	16 (100.0 %)	16 (100.0 %)	32 (100.0 %)
PK population	16 (100.0 %)	16 (100.0 %)	32 (100.0 %)
Completers	16 (100.0 %)	16 (100.0 %)	32 (100.0 %)

PK: pharmacokinetics

INCLUSION CRITERIA, DOSE

Healthy male or female subjects; aged between 18–45 years or above 65 years; good general health; body mass index (BMI) between 18–30 kg/m^2, both inclusive. Liraglutide (NNC 90-1170), 5 mg/mL formulation (Batch no. LLDP007) was utilized and 1 mg was administered as a single subcutaneous (s.c.) injection by a NovoPen® 1.5.

Pharmacokinetic Assessment:

A 60-hour, 24-point plasma profile of liraglutide to determine AUC_{0-t}, C_{max}, t_{max}, $AUC_{0-\infty}$, CL/F, V/F, and t½ after a single s.c. dose of liraglutide.

Safety:

Hematology, biochemistry (incl. safety plasma glucose), urinalysis, physical examination, vital signs, electrocardiogram (ECG), adverse events, and hypoglycaemic episodes were assessed for the safety.

Statistical Methods:

The primary endpoint was AUC_{0-t} – the area under the liraglutide plasma concentration curve from time 0 to last quantifiable concentration. The equivalence criterion was defined as the interval [0.80; 1.25]. The null hypothesis to be tested was that the ratio between the age groups

was outside the [0.80; 1.25] interval. This hypothesis was rejected and the two age groups declared equivalent if a 90% confidence interval for the ratio was fully contained within the interval.

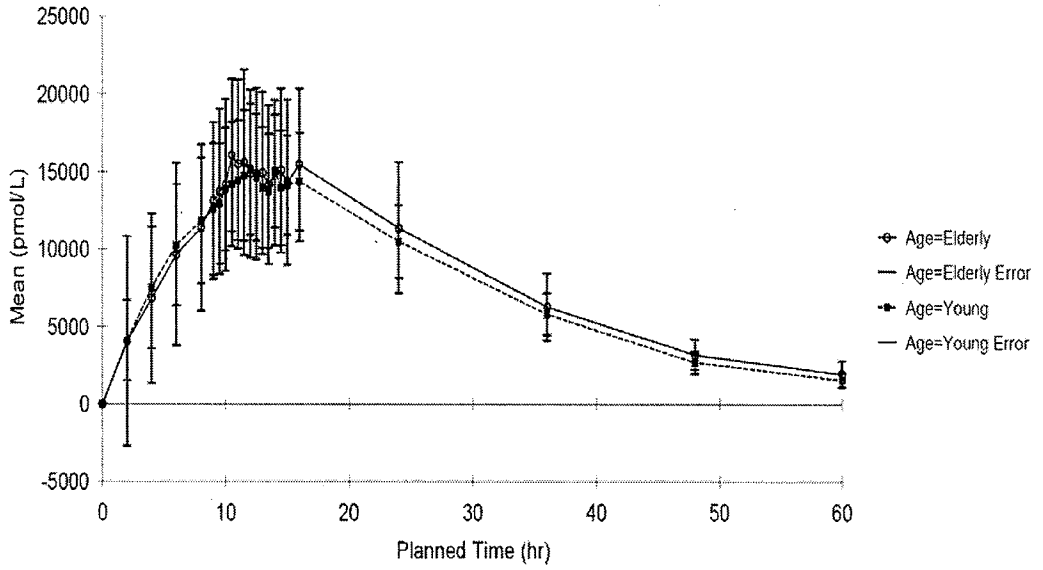
The comparison 'young versus elderly' as well as 'male versus female' was performed for AUC_{0-t} and for the secondary endpoints $AUC_{0-\infty}$, CL/F, V/F, C_{max}, and t½ using a linear normal model (analysis of covariance, ANCOVA) based on the logarithmic transformed values. The model included fixed effects of age group and of sex, and included log(body weight) as a covariate. Based on the statistical model, ratios of 'elderly versus young' and 'female versus male' with 90% confidence intervals were estimated on the original linear scale by re-transforming the corresponding estimates for the differences 'elderly minus young' and 'female minus male' on the log-scale.

The analysis of t_{max} was done by use of non-parametric methods; the difference in medians between age groups as well as between genders with 90% confidence intervals was estimated using the Hodges-Lehman estimator. Throughout the analyses a two-sided significance level of 5% was used for descriptive p-values and a 90% confidence level was applied (only applicable to the ANCOVA for pharmacokinetic parameters). No multiplicity adjustment was performed.

Adverse events were summarized by age group and overall, by system-organ class and MedDRA preferred term, severity, and relation to trial product. Other safety assessments (clinical laboratory parameters, vital signs, ECG) were presented by descriptive statistics and change from pre-dose to follow-up, where appropriate.

Pharmacokinetic Results:

Pharmacokinetics was evaluated in a subject population, where mean age in the young age group was 33.0 years (range 21–45 years) and mean BMI was 24.3 kg/m^2 (range 20.6–28.0 kg/m^2). In the elderly age group, mean age was 69.2 years (range 65–83 years) and mean BMI was 25.7 kg/m^2 (range 20.7–30.7 kg/m^2). In both age groups, the male subjects weighed more than the female subjects. Both age groups consisted of 8 male and 8 female subjects. The mean (±SD) profiles of liraglutide concentrations (linear scale) after a single s.c. dose of 1 mg – by age group are presented below:



	Statistics	AUC(0-inf) [h*pmol/L]	Cmax [pmol/L]	Tmax [h]	T1/2 [h]	CL/F [L/h]	Lambda z	Vz/F [L]
Gender: Overall								
Age: Young	N	16	16	16	16	16	16	16
	Mean	465268.669	17193.3	12.729	12.860	0.596	0.054	11.042
	SD	95395.421	4429.8	1.866	1.203	0.121	0.005	2.417
	Geom. Mean	456213.118	16654.1		12.806	0.584	0.054	10.796
	Geom. CV%	61.939	62.8		60.934	61.939	60.934	62.134
	Min							
	Median	438786.672	17405.5	12.508	12.970	0.608	0.053	10.674
	Max							
Age: Elderly	N	16	16	16	16	16	16	16
	Mean	502989.679	18494.9	11.625	14.074	0.577	0.052	11.732
	SD	131162.398	4915.3	3.186	3.313	0.204	0.011	4.664
	Geom. Mean	484150.956	17856.1		13.739	0.551	0.050	10.914
	Geom. CV%	63.458	63.1		62.179	63.458	62.181	65.474
	Min							
	Median	501944.509	17796.0	11.500	13.279	0.532	0.052	10.094
	Max							

b(4)

- AUC_{0-t} was declared equivalent in young and elderly subjects as assessed from the 60-hour liraglutide plasma profile obtained after a single 1 mg dose of liraglutide.

$AUC_{(0-t)}$ (h*pmol/L)			
young (N=16) mean (SD)	elderly (N=16) mean (SD)	estimate (ratio)	90% CI
435363.5 (89627.8)	460276.9 (128486.8)	0.94	[0.84;1.06]

- No statistically significant differences were found between the other pharmacokinetic parameters, Cmax, tmax, $AUC_{0-\infty}$, CL/F, V/F, and t½, after administration of a single dose of liraglutide to young and elderly subjects.

- There appeared to be a difference between male and female subjects based on the time-concentration profiles and the corresponding derived parameters. However, when adjusting for body weight (which was a significant covariate), there were no statistically significant differences between the pharmacokinetic parameters, AUC(0-t), Cmax, tmax, AUC(0-∞), CL/F, Vz/F, and t½,after administration of a single dose of liraglutide to male and female subjects.

- tmax (overall mean) was found to be 12.2 hours and t½ (overall mean) was 13.5 hours. Both results were in accordance with previously reported results.

Safety Results:

- Adverse events were reported by 7 (44%) young subjects (6 female and 1 male) and by 1 (6%) elderly, female subject. Most events were of moderate severity, none of the events were considered severe, and all subjects recovered from the adverse events. The most frequently reported adverse events were headache (4 events), vomiting (4 events), and nausea (3 events).

- All adverse events, except one episode of 'stomach discomfort' were judged by the investigator to be possibly or probably related to the trial product.

- No serious adverse events were reported and no subject withdrew due to an adverse event.

- Adverse events related to the gastro-intestinal body system occurred at time of Cmax.

- No clinically relevant changes were found for clinical laboratory tests, vital signs, ECG, or safety plasma glucose.

Sponsor's Conclusions:

- Liraglutide AUC_{0-t} is equivalent in young and elderly subjects after a single 1 mg dose.

- No statistically significant or clinically relevant differences in the exposure or pharmacokinetic parameters of liraglutide were found between young and elderly subjects.

- There appeared to be a difference between male and female subjects based on the time-concentration profiles and the corresponding derived parameters. However, when adjusting for body weight (which was a significant covariate), there were no statistically significant differences between male and female subjects.

- The adverse event profile was as previously described, although adverse events seemed to be most frequent in young, female subjects.

- No clinically relevant findings were seen for clinical laboratory tests, vital signs, ECG, or safety plasma glucose.

Assay Performance:

The inter-assay precision (coefficient of variation) of quality control samples ranged between 11.5 % and 17.4 %.

There was no marked inaccuracy in the results from these quality control samples: mean inaccuracies: -13.7 % (n=34) to 9.4 % (n=34).

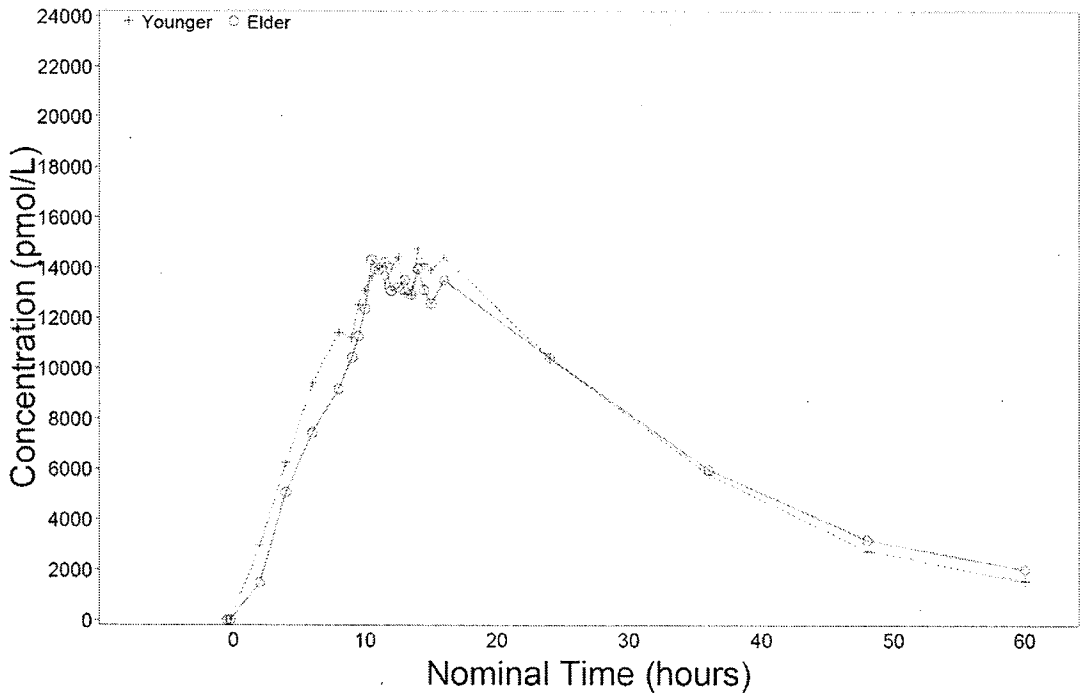
Reviewer's Comments:

Overall, the study conduct and assessments were appropriate. There were no major protocol violations affecting the study outcome. The sponsor's conclusions are also reasonable from a clinical pharmacology perspective.

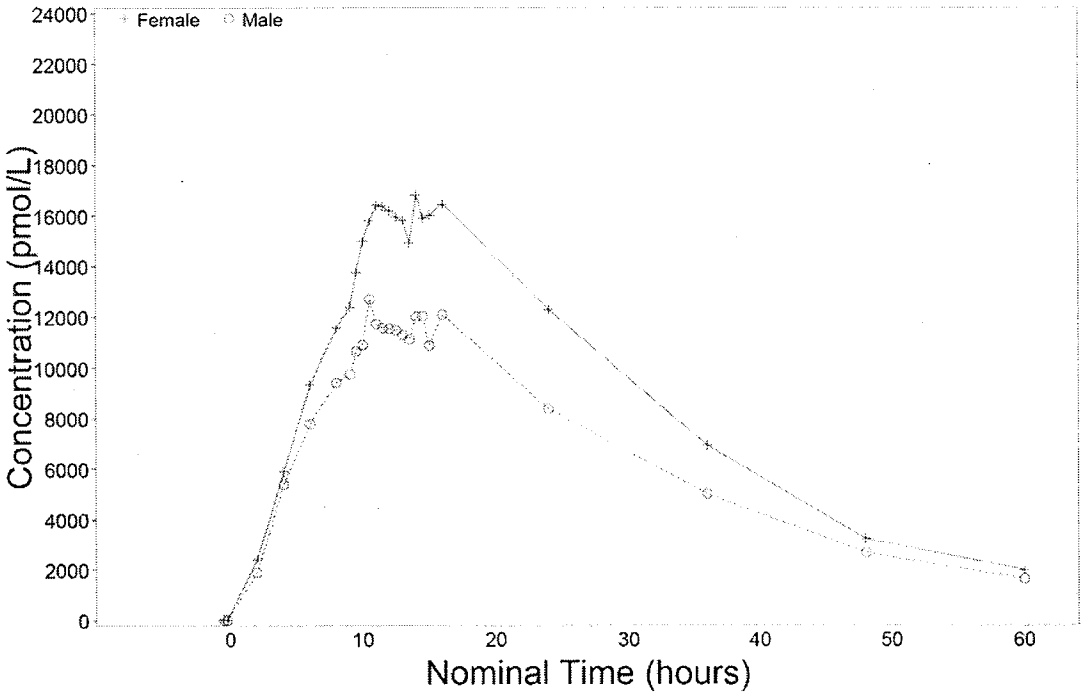
Revised Analysis to Address the DSI findings on Bioanalytical Method

Sponsor conducted re-evaluation of liraglutide plasma concentration raw data from this study. The primary endpoint in Trial 1327 was AUC0-t and the criteria for including a profile in the updated analysis were: 1) minimum one sample ≤10 hours post-dose and 2) acceptable number and scattering of samples. Of the 32 profiles available for re-evaluation, 26 profiles were accepted for AUC analysis while 6 profiles were rejected. Mean profiles by age and gender group based on the updated dataset are presented below.

(A) By Age group



(B) By Gender



Comparison between Age Groups and Gender – Primary Endpoint – Trial 1327

	Elderly / Young	Female / Male
Original Analysis		
AUC(0-t)		
Estimate	0.94	1.08
Lower 90% limit	0.84	0.93
Upper 90% limit	1.06	1.26
Updated Analysis		
AUC(0-t)		
Estimate	0.91	1.08
Lower 90% limit	0.80	0.92
Upper 90% limit	1.03	1.28

Based on the revised analysis:

- Equivalence was declared between age groups based on the updated analysis as the 90% confidence interval for the ratio (AUC0-t) was contained within the pre-specified [0.80; 1.25] interval.
- For gender, equivalence could not be declared as the 90% confidence interval for the ratio (AUC0-t) was not contained within the pre-specified [0.80; 1.25] interval. Both these results were in agreement with the original analysis results.

4.2.12 Renal Impairment Study (NN2211-1329)

Title of Study: A single-centre, open-label, trial investigating the pharmacokinetics and the tolerability of liraglutide in subjects with normal renal function and in subjects with impaired renal function

Study Site: [redacted] b(4)

Studied Period: 1 Sep 2005 to 20 Mar 2006

Primary Objective:

- to assess whether dose adjustment is required for subjects with renal impairment by investigating the pharmacokinetics of liraglutide after a single s.c. dose in subjects with normal renal function and in subjects with various degrees of renal impairment

Secondary Objectives:

- to estimate renal clearance (CLR) of liraglutide in subjects with normal renal function and in subjects with various degrees of renal impairment
- to estimate the plasma protein binding of liraglutide in plasma samples from subjects with normal renal function and in subjects with various degrees of renal impairment
- to examine the disposition of liraglutide in subjects with end stage renal disease (ESRD) on continuous ambulatory peritoneal dialysis (CAPD)
- to evaluate the safety after a single dose of liraglutide in subjects with normal renal function and in subjects with various degrees of renal impairment

Methodology: This was a single-centre, open-label, parallel group, single dose trial in healthy subjects and in subjects with renal impairments grouped according to their creatinine clearance or whether they received CAPD. The trial consisted of a Screening Visit to assess eligibility (Visit 1), Visit 2 where relevant inclusion and exclusion criteria were re-checked and which took place within 21 days of Visit 1, Visit 3 lasting 3 days and included administration of liraglutide and immediately followed Visit 2. Visit 4 was the last day of liraglutide plasma sampling (72 h after dosing).

Blood samples for plasma liraglutide were drawn at the following time-points: - 30 and - 15 minutes prior to dosing and at 2h, 4h, 6h, 8h, 9h, 9.5h, 10h, 10.5h, 11h, 11.5h, 12h, 12.5h, 13h, 13.5h, 14h, 15h, 16h, 21h, 24h, 36h, 48h, 60h and 72h after dosing at Visit 2. The pharmacokinetic endpoints were derived from the liraglutide time concentration curves.

Number of Subjects (Planned and Analyzed): 35 subjects were screened and 30 subjects were enrolled into the trial. 30 subjects were exposed to liraglutide and completed the entire trial period. All 30 exposed subjects were included in both the pharmacokinetic and the safety populations. 30 subjects were exposed to liraglutide; 22 males and 8 females with at least one female per renal group. All enrolled subjects were white, except for two males of Maori and Asian Pacific Islander origin, respectively. The subjects were between 31 and 82 years of age

(mean age 57.0 years) with the group of healthy subjects being younger than all the renal impairment groups. The renal function groups were balanced with respect to weight and BMI, although the mean and median weight was lower in the subjects with severe renal impairment (mean weight 71.7 kg in the severe group versus more or equal to 82.2 kg in the other renal function groups). The mean BMI across the groups was 27.9 kg/m^2 (range: 22.8 to 37.4).

Diagnosis and Main Criteria for Inclusion: Male and female subjects aged between 18 to 85 years (both inclusive) who were either healthy or had renal impairments as defined by creatinine clearance (using the Cockcroft & Gault formula). End-stage renal disease subjects were enrolled if receiving CAPD. The subjects' health status, further to their renal impairment, was assessed at screening and included physical examination, vital signs, medical history, ECG and clinical laboratory tests. The body mass index of enrolled subjects was to be below 40.0 kg/m^2.

b(4)

Test Product, Dose/Strength/Concentration, Mode of Administration and Lot Numbers: Liraglutide was supplied by Novo Nordisk A/S as a 6.25 mg/mL solution (batch number PP51138). The solution was supplied in a prefilled disposable pen device, ——— (3 mL). The trial product was administered using NovoFine® needles (30G), also supplied by Novo Nordisk A/S. The dosage administered was 0.75 mg injected subcutaneously into the thigh of trial subjects.

Criteria for Evaluation

Pharmacokinetics:

The primary objective of the trial was to investigate whether healthy subjects and subjects with various degrees of renal impairment were equivalent with respect to $AUC_{0-\infty}$ after a single dose of liraglutide.

Equivalence could be demonstrated if the 90% confidence interval for the ratio of $AUC_{0-\infty}$ for the group comparison healthy/severely renally impaired was within the pre-defined limits of [0.70, 1.43]. The same equivalence criterion was used for the other group comparisons.

Secondary pharmacokinetic endpoints were derived from the liraglutide time concentration curves and included AUC_{0-t}, CL/F, C_{max}, λz, $t_{½}$, t_{max}, V/F and CLR, CLPD.

The secondary endpoints were also estimated for the unbound fraction of liraglutide.

An ANOVA of the log transformed endpoints adjusted for renal group, age and log(weight) was performed. The ANOVA performed for the unbound fractions of liraglutide were furthermore adjusted for concentration and an interaction between concentration and renal group with a random effect of subject.

Regression analyses of log($AUC_{0-\infty}$), log(secondary endpoints) and log(unbound fraction) corrected for age, log(weight) and log(creatinine clearance) were also performed. An explorative regression analysis was made to investigate a potential association between the primary endpoint, $AUC_{0-\infty}$, and the following covariates; AAG, LDL, VLDL, HDL, and albumin.

Safety: Safety was evaluated based on assessments of adverse events, physical examinations, vital signs, ECGs and laboratory tests.

Statistical Methods:

Efficacy

- The primary objective of the trial was to investigate whether healthy subjects and subjects with various degrees of renal impairment were equivalent with respect to AUC(0-∞) after a single dose of liraglutide.

- The trial was carried out in accordance with available guidelines (EMEA and FDA) on the conduct of trials in subjects with renal impairment. Equivalence could be demonstrated if the 90% confidence interval for the ratio of $AUC_{0-\infty}$ for the group comparison healthy/severely renally impaired was within the pre-defined limits of [0.70, 1.43]. The same equivalence criterion was used for the other group comparisons.

- Secondary pharmacokinetic endpoints were derived from the liraglutide time concentration curves and included AUC_{0-t}, CL/F, C_{max}, λz, $t_{½}$, t_{max}, V/F and CLR, CLPD.

- The secondary endpoints were also estimated for the unbound fraction of liraglutide.

- An ANOVA of the log transformed endpoints adjusted for renal group, age and log(weight) was performed. The ANOVA performed for the unbound fractions of liraglutide were furthermore adjusted for concentration and an interaction between concentration and renal group with a random effect of subject.

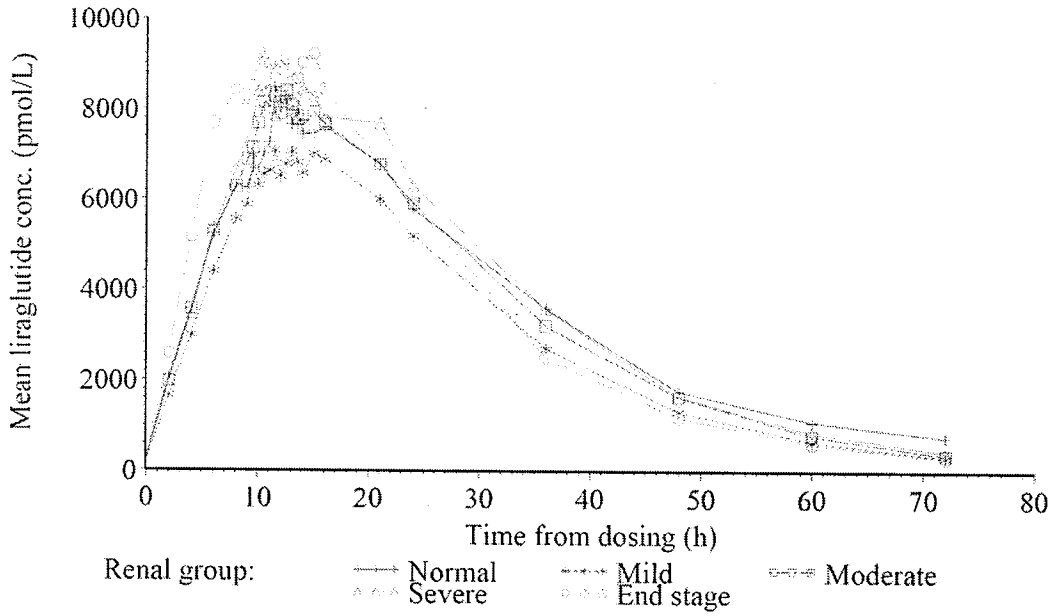
- Regression analyses of log($AUC_{0-\infty}$), log(secondary endpoints) and log(unbound fraction) corrected for age, log(weight) and log(creatinine clearance) were also performed.

- An explorative regression analysis was made to investigate a potential association between the primary endpoint, $AUC_{0-\infty}$, and the following covariates; AAG, LDL, VLDL, HDL, and albumin.

Safety

- The assessment of safety parameters was based on descriptive statistics

Pharmacokinetic Results:



- Equivalence was not demonstrated between the group of subjects with severe renal impairment and healthy subjects with respect to the primary endpoint $AUC_{0-\infty}$ and the clearance was higher in severe renal impairment (estimated ratio 0.73 and CI [0.57, 0.94]).
- However, no clear association was observed between degree of renal impairment and liraglutide $AUC_{0-\infty}$.

Safety Results:

Liraglutide was well tolerated by subjects in all renal groups. No serious adverse events were reported. The most frequently occurring treatment emergent adverse events were headache (8 events reported by 6 subjects), vomiting (5 events reported by 4 subjects) and nausea (4 events reported by 4 subjects). The treatment related gastro-intestinal adverse events were, however, mild or moderate in character and with a duration of 1 to 2 days. There was no trend for a greater number of adverse events reported in subjects with various grades of renal impairment compared to healthy subjects, although the group of subjects with end-stage renal disease on CAPD experienced more events of vomiting compared to the other renal function groups. However, this was not matched with a greater exposure in these subjects.

Sponsor's Conclusions:

- Subjects with type 2 diabetes who also suffer from renal impairment, including subjects with end stage renal disease, should use standard treatment regimens for liraglutide and be dosed according to their glycaemic control.
- The categorical and continuous analyses were not in agreement and therefore it cannot be concluded that reduced renal function has an impact on the liraglutide pharmacokinetics.
- No conclusion as to excretion of intact liraglutide in urine and dialysis fluid can be made due to lack of documentation for the stability of the samples during storage.

- The analysis of the unbound fraction of liraglutide did not indicate increased unbound liraglutide concentrations with renal impairment, although the data were highly variable.
- Liraglutide was well tolerated in all renal groups and no safety concerns were raised.

Reviewer's Comment:

Assay Performance

The plasma sarnples (749) were analyzed in 37 assay runs, accepted according to the predefined acceptance criteria. Eleven assay runs were rejected (eight due to calibration failure and three due to QC- failure). 311 samples were re-analyzed due to various causes (valid reasons were provided for reanalysis).

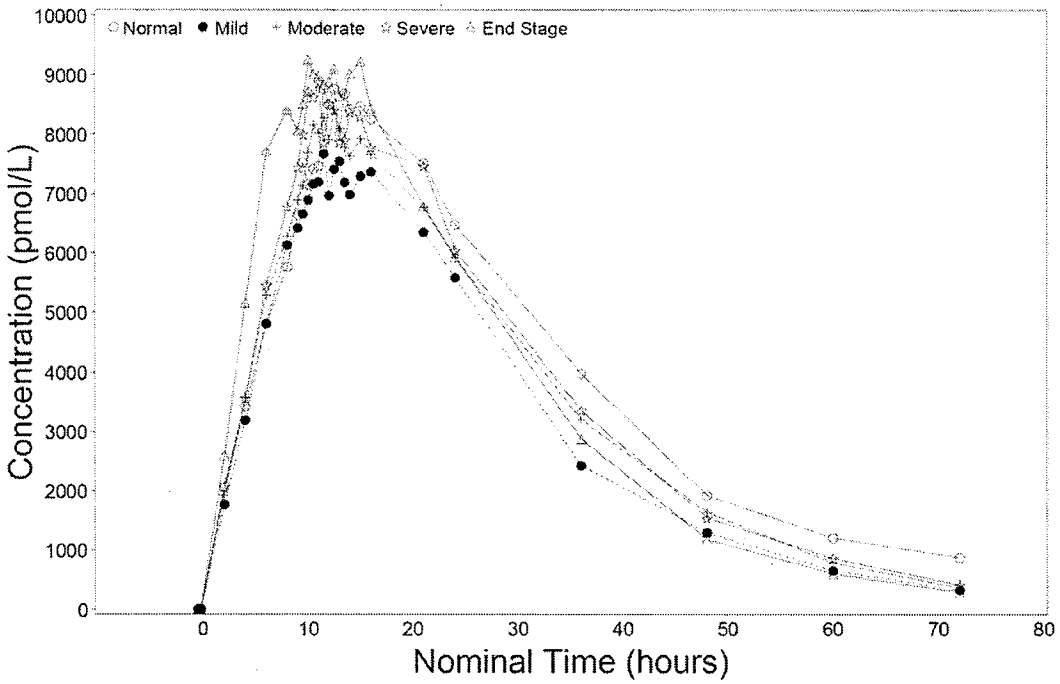
The inter-assay precision (coefficient of variation) of quality control samples ranged between 6.1 % and 17.9 %.

There was no marked inaccuracy in the results from these quality control samples: mean inaccuracies: -13.2 % (n=8) to 8.7% (n=8).

Study

Overall, the study conduct and assessments were appropriate. There were no major protocol violations affecting the study outcome. The sponsor's conclusions are also reasonable from a clinical pharmacology perspective.

Revised Analysis to Address the DSI findings on Bioanalytical Method
Sponsor conducted re-evaluation of liraglutide plasma concentration raw data from this study. The primary endpoint in Trial 1329 was AUC0-∞ and the criteria for including a profile in the updated analysis were: 1) minimum one sample $\leq$10 hours post-dose, 2) at least 3 out of 6 possible samples in the 20–72 hour post-dose period (sampling schedule: 20, 24, 36, 48, 60 and 72 hours) and 3) acceptable number and scattering of samples. Of the 30 profiles available for re-evaluation, 27 profiles were accepted for AUC analysis while 3 profiles were rejected. Mean profile based on the updated dataset is presented below.



Summary Statistics for $AUC_{0-\infty}$ (h*pmol/L) – Trial 1329

	Renal group				
	Normal	Mild	Moderate	Severe	End Stage
Original Analysis					
AUC(pmol*h/L)					
N	6	6	7	5	6
Mean	274339.2	219830.0	256658.3	273568.1	265429.4
SD	71389.4	76562.7	63160.2	61355.9	104227.0
Geometric Mean	264710.7	209400.6	249804.3	267781.1	248729.7
CV	26.0	34.8	24.6	22.4	39.3
Median	277843.9	204390.5	257009.5	295145.6	241195.0
Min					
Max					
Updated Analysis					
AUC(pmol*h/L)					
N	5	5	7	4	6
Mean	297622.8	234715.4	256641.3	268172.3	265426.1
SD	36062.5	83260.3	63147.2	69465.1	104224.9
Geometric Mean	295964.7	223095.8	249790.0	261344.4	248726.8
CV	12.1	35.5	24.6	25.9	39.3
Median	281414.2	250466.3	257008.0	266039.6	241193.5
Min					
Max					

b(4)

Comparison between Renal Groups – Primary Endpoint – Trial 1329

	Mild/Normal	Moderate/Normal	Severe/Normal	End stage/Normal
Original Analysis				
AUC				
Estimate	0.67	0.86	0.73	0.74
Lower 90% limit	0.54	0.70	0.57	0.56
Upper 90% limit	0.85	1.07	0.94	0.97
Updated Analysis				
AUC				
Estimate	0.65	0.81	0.71	0.70
Lower 90% limit	0.51	0.65	0.55	0.53
Upper 90% limit	0.83	1.01	0.92	0.91

The results from the revised analysis were in agreement with the original analysis.

4.2.13 Hepatic Impairment Study (NN2211-1328)

Title of Trial

A single-centre, open-label trial investigating the pharmacokinetics and the safety profile after a single dose of liraglutide in subjects with hepatic impairment and in subjects with normal hepatic function

Signatory and Principal Investigator

Professor Janusz Cianciara, Clinic of Hepatology and Acquired Immunodeficiencies,
Institute of Infectious and Parasitic Diseases of Medical Academy of Warsaw, Poland

Trial Site

Clinic of Hepatology and Acquired Immunodeficiencies,
Institute of Infectious and Parasitic Diseases of Medical Academy of Warsaw
37, Wolska Street
01-210 Warsaw
Poland

Publications

Flint A, Nazzal K, Jagielski P, Segel S, Zdravkovic M. Influence of Hepatic Impairment on Pharmacokinetics of the Long-Acting Human GLP-1 Analogue Liraglutide. Abstract presented at the 67th Scientific Sessions, American Diabetes Association, Chicago, US. 22-26 June 2007.

Trial Period	**Development Phase**
9 Mar 2006 to 26 Jun 2006	Phase 1

Objectives

Primary Objective

- To investigate the pharmacokinetics of liraglutide after a single dose in subjects with mild, moderate and severe degrees of hepatic impairment compared with subjects with normal hepatic function.

Secondary Objectives

- To compare the safety profile after a single dose of liraglutide in subjects with mild, moderate, or severe hepatic impairment and subjects with normal hepatic function.
- To compare the plasma protein binding of liraglutide in plasma samples from subjects with mild, moderate or severe hepatic impairment and subjects with normal hepatic function.

Methodology

- A single-centre, open-label, parallel group, single dose trial in healthy subjects and in subjects with hepatic impairments grouped according to their Child-Pugh classification.
- The trial consisted of a Screening Visit to assess eligibility (Visit 1), a 96 hour in-house visit including administration of liraglutide (Visit 2) within four weeks of Visit 1and a Follow-up Visit (Visit 3) up to two weeks after Visit 2.
- Plasma sampling was performed for 72 hours after administration of liraglutide.

Number of Subjects Planned and Analysed

- 25 subjects were screened and 24 subjects were randomised and exposed to liraglutide.
- One subject did not attend Visit 3 and was withdrawn from the trial.
- 23 subjects completed the entire trial period.
- All 24 exposed subjects were included in both the pharmacokinetic and safety populations.

Diagnosis and Main Criteria for Inclusion

Male and female subjects aged between 18 to 75 years (both inclusive) who were either healthy or had stable hepatic impairment classified as Child-Pugh grade A (mild), B (moderate) or C (severe) as assessed by the investigator. The subjects' health status, further to their hepatic impairment, was assessed at screening and included physical examination, vital signs, medical history, ECG and clinical laboratory tests. The body mass index of enrolled subjects was to be between 18.5 to 40.0 kg/m^2 (both inclusive).

Test Product, Dose and Mode of Administration, Batch Number

- Liraglutide was supplied by Novo Nordisk A/S as a 6.25 mg/mL solution (batch number PP51138). The solution was supplied in a prefilled disposable pen device, FlexPen® (3 mL). The trial product was administered using NovoFine® needles (30G), also supplied by Novo Nordisk A/S.
- The dosage administered was 0.75 mg injected subcutaneously into the thigh of trial subjects.

Duration of Treatment

One single administration of liraglutide was administered during Visit 2, a visit which lasted 96-hours. The total trial duration for each individual subject was up to 7 weeks.

Reference Therapy, Dose and Mode of Administration, Batch Number

No reference therapy was used.

Criteria for Evaluation – Efficacy

Blood samples for plasma liraglutide were drawn at the following time-points: 15 and 30 minutes prior to dosing and at 2h, 4h, 6h, 8h, 9h, 10h, 11h, 12h, 13h, 14h, 15h, 16h, 21h, 24h, 36h, 48h, 60h and 72h after dosing at Visit 2. The pharmacokinetic endpoints were derived from the liraglutide time concentration curves.

Urine was collected in the time period 10 to 21 hours after dosing and used for estimating renal clearance of liraglutide.

Criteria for Evaluation – Safety

Safety parameters included adverse events, episodes of hypoglycaemia, clinical laboratory tests (haematology, clinical chemistry and urinalysis), 12-lead ECG, physical examination and vital signs.

Statistical Methods

Efficacy

- The primary endpoint of the trial was to investigate whether healthy subjects and subjects with hepatic impairments classified according to Child-Pugh grades A (mild), B (moderate) and C (severe) were equivalent with respect to $AUC_{(0-\infty)}$ after a single dose of liraglutide.
- The trial was carried out in accordance with available guidelines (EMEA and FDA) on the conduct of trials in subjects with hepatic impairments. Equivalence could be demonstrated if the 90% confidence interval for the ratio of $AUC_{(0-\infty)}$ for the group comparison healthy/severely hepatically impaired subjects was within the pre-defined limits of [0.70, 1.43]. The same equivalence criterion was applied for the other group comparisons.
- Secondary pharmacokinetic endpoints were derived from the liraglutide time concentration curves and included $AUC_{(0-t)}$, CL/F, C_{max}, λ_z, $t_{\frac{1}{2}}$, t_{max}, V_z/F and CL_R.
- All endpoints were also estimated for the unbound fraction of liraglutide.
- An ANOVA of the log transformed endpoints adjusted for hepatic group, age, gender and log(weight) was performed. Regression analyses of $log(AUC_{(0-\infty)})$ and log(unbound fraction) corrected for age, sex, log(weight), log(albumin) and log(portal vein diameter) were also performed. Interaction between concentration and hepatic group and a random effect of subject were accounted for in the analyses of the unbound fraction of liraglutide.
- An explorative regression analysis was made to investigate a potential association between the primary endpoint, $AUC_{(0-\infty)}$, and the following covariates; bilirubin, ASAT, ALAT, LDL, VLDL, HDL, AAG, PTT and liver diagnosis (i.e. viral or alcoholic hepatic impairment).

Safety

- The assessment of safety parameters were based on descriptive statistics.

Demography of Trial Population

24 subjects (6 subjects per group) were exposed to liraglutide; 14 males and 10 females and at least two females per hepatic group. The groups were well balanced with respect to weight (mean: 76.7 kg, range: 49.3 to 111.5) and BMI (mean: 27 kg/m^2, range: 19.7 to 34.8). All enrolled subjects were white and between 21 and 61 years of age (mean age 47.8 years). The subjects in the groups of moderate and severe hepatic impairment were generally older than the subjects in the normal and mild hepatic impairment groups (53.2 and 49.8 years versus 43.8 and 44.5 years respectively).

Efficacy Results

- Equivalence with respect to $AUC_{(0-\infty)}$ was not demonstrated between the groups of severely hepatically impaired subjects and healthy subjects (estimated ratio of 0.56 with a 90% confidence interval of [0.39, 0.81]), with hepatically impaired subjects having a lower exposure to liraglutide.

	Hepatic group		
	Mild/Normal	Moderate/Normal	Severe/Normal
AUC			
Estimate	0.77	0.87	0.56
Lower 90% limit	0.53	0.60	0.39
Upper 90% limit	1.11	1.25	0.81

- Equivalence with respect to $AUC_{(0-\infty)}$ was not demonstrated between any of the other groups of hepatically impaired subjects and healthy subjects either (estimated ratios and 90% confidence intervals of 0.77 [0.53, 1.11] and 0.87 [0.60, 1.25] for mild/normal and moderate/normal, respectively).
- The group comparisons of subjects with severe, moderate and mild hepatic impairment versus healthy subjects were found to be equivalent with respect to λ_Z (inverse $t_{1/2}$) (severe/normal comparison had an estimated ratio of 1.18 with a 90% confidence interval of [1.02, 1.36], but not for any of the other analysed secondary pharmacokinetic endpoints.
- No clear association between the unbound fraction of liraglutide and hepatic group was seen. However, the group of subjects with severe hepatic impairment did not have a higher unbound fraction compared to the group of subjects with normal hepatic function.

Safety Results

- Three adverse events were reported, of which two were treatment emergent nausea and headache, experienced by two different subjects in the moderate hepatic impairment group. These events were thought possibly or probably related to liraglutide administration and both subjects recovered within one day.
- No serious adverse events were reported.
- No safety concerns were raised during the trial and liraglutide was well tolerated at the dose given in all subjects.

Conclusions

- Equivalence with respect to $AUC_{(0-\infty)}$ was not demonstrated between the groups of severely hepatically impaired and healthy subjects, with the exposure to liraglutide being lower in the group of subjects with severe hepatic impairment. Equivalence with respect to $AUC_{(0-\infty)}$ was not demonstrated for the hepatic group comparisons mild/normal or moderate/normal either.
- The group comparisons of subjects with severe, moderate and mild hepatic impairment versus healthy subjects were found to be equivalent with respect to λ_Z (inverse $t_{1/2}$) (severe/normal comparison had an estimated ratio of 1.18 with a 90% confidence interval of [1.02, 1.36], but not for any of the other analysed secondary pharmacokinetic endpoints.
- No clear association between the unbound fraction of liraglutide and hepatic group was seen. The group of subjects with severe hepatic impairment did not have a higher unbound fraction compared to the group of healthy subjects.
- Liraglutide was well tolerated in all hepatic groups and no safety concerns were raised.
- Subjects with type 2 diabetes who also suffer from hepatic insufficiency should use standard treatment regimens for liraglutide and be dosed according to their glycaemic control.

The trial was conducted in accordance with the Declaration of Helsinki and ICH Good Clinical Practice.

Sponsor's Conclusions:

- Equivalence with respect to $AUC_{0-\infty}$ was not demonstrated between the groups of severely hepatically impaired subjects and healthy subjects (estimated ratio of 0.56 with a 90% confidence interval of [0.39, 0.81]), with hepatically impaired subjects having a lower exposure to liraglutide.
- Equivalence with respect to $AUC_{0-\infty}$ was not demonstrated between any of the other groups of hepatically impaired subjects and healthy subjects either (estimated ratios and

90% confidence intervals of 0.77 [0.53, 1.11] and 0.87 [0.60, 1.25] for mild/normal and moderate/normal, respectively).

- The group comparisons of subjects with severe, moderate and mild hepatic impairment versus healthy subjects were found to be equivalent with respect to λ_Z (inverse $t_{1/2}$) (severe/normal comparison had an estimated ratio of 1.18 with a 90% confidence interval of [1.02, 1.36], but not for any of the other analyzed secondary pharmacokinetic endpoints.
- No clear association between the unbound fraction of liraglutide and hepatic group was seen. However, the group of subjects with severe hepatic impairment did not have a higher unbound fraction compared to the group of subjects with normal hepatic function.

Reviewer's Comment:

Assay Performance

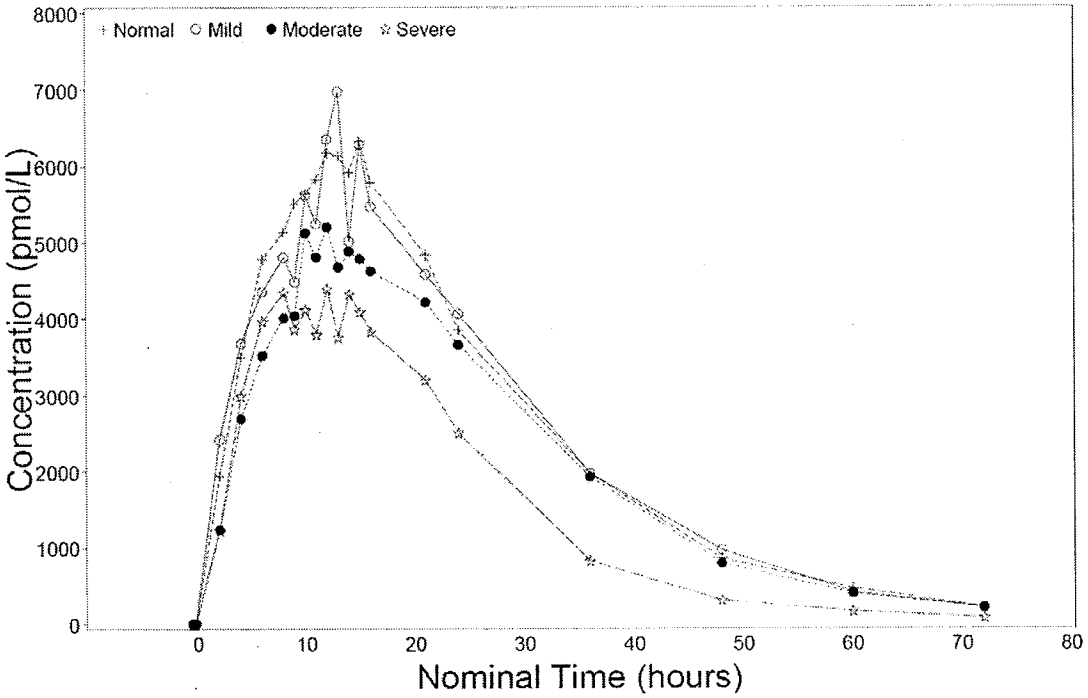
The plasma samples (480) were analyzed in 21 assay runs, accepted according to acceptance criteria. Two assay runs were rejected due to QC-failure. 93 samples were re-analyzed due to various causes. The inter-assay precision (coefficient of variation) of the quality control samples ranged between 4.5 % and 15.5 %. The mean inaccuracies of the quality control samples ranged between -11.2 % (n=12) and 8.3 % (n=30).

Study

Overall, the study conduct and assessments were appropriate and the concentration data was supported by the analytical method. There were no major protocol violations affecting the study outcome. The sponsor's conclusions are also reasonable from a clinical pharmacology perspective.

Revised Analysis to Address the DSI findings on Bioanalytical Method

Sponsor conducted re-evaluation of liraglutide plasma concentration raw data from this study. The primary endpoint in Trial 1328 was AUC0-∞ and the criteria for including a profile in the updated analysis were: 1) minimum one sample ≤10 hours post-dose, 2) at least 3 out of 5 possible samples in the 20–60 hour post-dose period (sampling schedule: 20, 24, 36, 48 and 60 hours) and 3) acceptable number and scattering of samples. Of the 24 profiles available for re-evaluation, 22 profiles were accepted for AUC analysis while 2 profiles were rejected. Mean profile based on the updated dataset is presented below.



Summary Statistics for $AUC_{0-\infty}$ (h*pmol/L) – Trial 1328

	Hepatic group			
	Normal	Mild	Moderate	Severe
Original Analysis				
AUC(pmol*h/L)				
N	6	6	6	6
Mean	179641.3	149812.0	154614.5	105158.3
SD	43154.1	70006.5	47939.1	40842.5
Geometric Mean	174831.3	138109.8	147762.5	97846.1
CV	24.0	46.7	31.0	38.8
Median	193425.4	129439.6	163200.8	108238.2
Min				
Max				
Updated Analysis				
AUC(pmol*h/L)				
N	6	4	6	6
Mean	179677.8	178419.1	154586.2	105939.6
SD	43189.9	69852.5	47934.0	39851.8
Geometric Mean	174860.9	168781.5	147736.5	99029.6
CV	24.0	39.2	31.0	37.7
Median	193539.1	165113.3	163118.9	108236.7
Min				
Max				

b(4)

Comparison between Hepatic Groups – Primary Endpoint – Trial 1328

	Hepatic group		
	Mild/Normal	Moderate/Normal	Severe/Normal
Original Analysis			
AUC			
Estimate	0.77	0.87	0.56
Lower 90% limit	0.53	0.60	0.39
Upper 90% limit	1.11	1.25	0.81
Updated Analysis			
AUC			
Estimate	0.89	0.86	0.58
Lower 90% limit	0.57	0.60	0.40
Upper 90% limit	1.40	1.25	0.85

Based on the revised analysis:

- Equivalence could not be demonstrated for any of the comparisons as none of the 90% confidence intervals for the ratios were contained within the pre-specified [0.70; 1.43] interval, which was in agreement with the original analysis results.
- The point estimates and confidence intervals obtained with the updated analysis were also in agreement with the original analysis.

4.2.14 Single-Dose PK Study in Healthy Japanese (NN2211-1326)

TITLE OF TRIAL A randomised, double-blind, single-centre, placebo-controlled, ascending single s.c. dose, sequential group study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of liraglutide (NNC 90-1170) in healthy Japanese male subjects	
INVESTIGATOR Shin Irie, MD Head of Kyushu Clinical Pharmacology Research Clinic	
TRIAL SITE Kyushu Clinical Pharmacology Research Clinic 13-16, Jigyo 2-chome, Chuo-ku, Fukuoka, 810-0064, Japan	
PUBLICATIONS None	
TRIAL PERIOD 17 December 2002 (FSFV) to 28 March 2003 (LSLV)	**DEVELOPMENT PHASE** Phase 1
OBJECTIVES Primary: To assess the safety and tolerability after single s.c. doses of liraglutide from 2.5 to 17.5 µg/kg, at maximum, in healthy Japanese male subjects Secondary: To assess the pharmacokinetic (PK) profile after single s.c. doses of liraglutide To assess the effect of liraglutide on insulin, glucose and glucagon levels	
METHODOLOGY This was a Phase I, randomised, double-blind, placebo-controlled, ascending single-dose, sequential group study. Four ascending dose levels were studied in four dose groups of 8 subjects per group (active : placebo = 6 : 2 ratio). Subjects received a single s.c. dose after breakfast, starting with the lowest dose of 2.5 µg/kg up to 15 µg/kg, as the highest planned dose of 17.5 µg/kg was not administered. All doses were administered subcutaneously using a NovoPen® III injection device. Subjects stayed in the study site from Day 0 to Day 4, 72 hours after the administration of liraglutide, and returned for a post-trial visit 7–21 days after Day 1.	
NUMBER OF SUBJECTS PLANNED AND ANALYSED A total of 40 healthy Japanese male subjects, aged 20–45 years and BMI of 18–27 were planned for enrolment in this trial, 8 subjects at each of the five dose levels. Altogether, 92 male healthy subjects were screened. During the conduct of this trial, since the results from the first four dose levels suggested that the 15 µg/kg dose was the maximum tolerated single dose due to the occurrence of gastrointestinal adverse events, the 17.5 µg/kg dose group was cancelled, thus, a total of 32 subjects were randomised and completed the trial. 60 subjects were only screened and not enrolled in the trial due to cancellation of the planned highest dose level of 17.5 µg/kg. These subjects were recorded as screening failures in this trial. No replacements were required.	
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION Healthy Japanese male subjects, aged 20 to 45 years, with a BMI of 18–27 kg/m^2	
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Liraglutide (NNC 90-1170) 5 mg/mL solution, 1.5 mL Penfill® cartridge, s.c. administration Batch No.: LLDP007	
DURATION OF TREATMENT Single dose administration.	
REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Matching placebo solution, 1.5 mL Penfill® cartridge, s.c. administration Batch No.: LLDP005	
CRITERIA FOR EVALUATION – PHARMACOKINETICS AND PHARMACODYNAMICS	

The concentration of plasma liraglutide was measured after a single dose over 72 hours. Liraglutide was determined by a specific ELISA assay. From the concentration profiles the following PK endpoints were derived:

- Area under the plasma liraglutide curve from time 0 to infinity after injection, $AUC_{0-\infty}$
- Maximum plasma liraglutide concentration, C_{max}
- Time to maximum plasma liraglutide concentration, t_{max}
- Terminal phase elimination rate-constant, λ_z
- Mean residence time, MRT
- Terminal elimination half-life, $t_{1/2}$
- Apparent clearance, CL/F
- Apparent volume of distribution, V_z/F

Pharmacodynamic evaluation comprised the following endpoints:

- Average glucose level 11 hours after liraglutide administration, AUC_{0-11}(glucose)/11 hours
- Average glucose level 11-24 hours after liraglutide administration, AUC_{11-24}(glucose)/13 hours
- Average glucose level 11 hours after liraglutide administration, AUC_{0-11}(insulin)/11 hours
- Average glucose level 11-24 hours after liraglutide administration, AUC_{11-24}(insulin)/13 hours
- Average glucose level 11 hours after liraglutide administration, AUC_{0-11}(glucagon)/11 hours
- Average glucose level 11-24 hours after liraglutide administration, AUC_{11-24}(glucagon)/13 hours

CRITERIA FOR EVALUATION – SAFETY

Safety was addressed by the following primary endpoints:

- Physical examination
- Body weight
- Vital signs (blood pressure and pulse rate)
- ECG
- Clinical laboratory assessments (haematology, biochemistry including FPG and urinalysis)
- Safety blood glucose at 3, 6, 9, 12 and approximately 72 hours post-dose
- Adverse events

STATISTICAL METHODS

Subject characteristics (age, height, weight, BMI) and vital signs (pulse, blood pressure) were summarised by descriptive statistics including N (counts), mean, minimum, median, maximum and standard deviation (SD). The PK (except $t_{1/2}$ and CL/F) and pharmacodynamic (PD) parameters, and haematological and biochemical laboratory parameters were summarised by descriptive statistics including N (counts), mean, minimum, median, maximum and SD. Additionally, geometric mean and geometric CV% were provided for PK parameters $AUC_{0-\infty}$, C_{max}, MRT, CL/F, V_z/F, $t_{1/2}$, λ_z and for PD parameters. Urinalysis was given by frequency tables including counts (N) and percentages (%). The PK endpoints $AUC_{0-\infty}$, C_{max}, λ_z, MRT and V_z/F were transformed using log transformation prior to the statistical analysis. The remaining PK parameters were not transformed. No statistical analysis of the PK endpoints, $t_{1/2}$ and CL/F were carried out since all inference about $t_{1/2}$ and CL/F could be made from the inference about the equivalent parameters λ_z and AUC, respectively. The model for the endpoints is as follows: The response for each subject is the sum of an overall mean, a fixed dose effect (categorical variable) and a random error.

An analysis of variance (ANOVA) was carried out for PK parameters and estimated population means for each dose level with corresponding 95% confidence intervals (CIs) were calculated. The CIs for the log-transformed parameters were transformed back to get CIs on the original data scale. T_{max} was presented by descriptive statistics including N, mean, minimum, median, maximum and standard deviation and no further analyses were performed. Dose proportionality was addressed for the endpoints $AUC_{0-\infty}$ and C_{max} and by performing a regression analysis of a log-transformed parameter on log-transformed dose. An estimate of the slope of the regression line and corresponding 95% CIs were calculated.

The statistical analysis of the PD endpoints was based upon average levels for glucagon, glucose and for insulin. The model for the PD endpoints is as follows: The response in each subject is the sum of an overall mean, a fixed dose effect (categorical variable), a fixed group effect, baseline as a covariate and a random error. The analysis was similar as for the PK endpoints with log transformation prior to the statistical analysis. An analysis of covariance (ANCOVA) was done.

For clinical laboratory parameters, the dose-response relationship for the change from baseline was separately

investigated non-parametrically by using the Jonckheere-Terpstra test (trend analysis).
Throughout the analyses a significance level of a two-sided 5% was used and no multiplicity adjustment was performed.

DEMOGRAPHY OF TRIAL POPULATION

The subjects enrolled were 20 to 27 years old (mean: 22.1 ±1.7 years), had a BMI between 19.0 and 24.2 kg/m^2 (mean: 21.1 ±1.4 kg/m^2) and were free of any significant concomitant disease.

PHARMACOKINETIC AND PHARMACODYNAMIC RESULTS

PHARMACOKINETICS

The PK parameters after a single dose of liraglutide are summarised in the table below:

Dose [µg/kg]	Statistics	C_{max} [pmol/L]	t_{max} [h]	$AUC_{0-\infty}$ [h·pmol/L]	$t_{1/2}$ [h]	CL/F [L/h/kg]	V_z/F [L/kg]	λ_z [1/h]	MRT [h]
2.5	Mean	3,129.0	7.51 [a]	64,005.2	10.13	0.01070	0.1573	0.06865	19.32
	SD	834.6		11,724.3	0.81	0.00185	0.0363	0.00504	1.94
5	Mean	4,857.8	11.00 [a]	134,242.7	11.03	0.01002	0.1590	0.06335	22.27
	SD	720.6		14,563.8	1.18	0.00105	0.0175	0.00676	2.60
10	Mean	12,267.3	10.00 [a]	295,248.0	11.35	0.00961	0.1558	0.06135	21.18
	SD	2,601.8		74,748.6	0.87	0.00281	0.0380	0.00497	2.59
15	Mean	18,378.0	10.00 [a]	447,940.0	10.88	0.00921	0.1440	0.06402	21.23
	SD	2,937.9		83,133.1	0.90	0.00181	0.0270	0.00527	2.94

[a] Median

- Liraglutide was quantifiable from 0.5 to 72 hours after administration and was not quantified in any plasma sample from placebo-dosed subjects.
- Absorption of liraglutide was slow and the maximum plasma concentrations were reached after 7.5 to 11 hours (medians) across all dose groups.
- Liraglutide was cleared from plasma with a terminal elimination half-life of approximately 10 to 11 hours for all dose groups.
- The linear regression analysis was consistent with dose proportionality for the PK endpoints $AUC_{0-\infty}$ and C_{max}. The PK parameters CL/F, V_z/F, $t_{1/2}$, λ_z and MRT were approximately constant over the dose range of 2.5 to 15 µg/kg liraglutide.

PHARMACODYNAMICS

- For the 15 µg/kg dose group, reliable PD data only from four out of six subjects were included in the analysis as the remaining two subjects consumed a very small evening meal due to GI adverse events.
- On average, the release of insulin concentrations after a meal stimulus, being served 11 hours after administration of liraglutide, appeared to be attenuated in particular after the highest dose of 15 µg/kg. However, no significant dose effect was observed in the ANCOVA for the derived AUC_{11-24} value for insulin. Also no significant effect was seen for the plasma concentrations and the derived AUC_{0-11} values during the fasting period from pre-dose until 11 hours post-dose.
- Attenuation in peak glucose concentrations after the evening meal appeared to occur in a dose-dependent fashion. This was supported by a significant dose effect in the ANCOVA carried out on AUC_{11-24} values for glucose. No significant effects or tendency of difference were observed for the 0-11-hour fasting period, although the mean glucose level appeared to be lower in the 15 µg/kg dose group in comparison to the other treatment groups.
- Suppressions in mean glucagon levels after the evening meal were most pronounced in the 10 and 15 µg/kg dose groups. The AUC_{11-24} value for glucagon was significantly different for the different doses of liraglutide in the ANCOVA. The results suggested a tendency of difference for a dose effect in the ANCOVA for AUC_{0-11} values, as plasma concentrations of the 10 and 15 µg/kg dose groups appeared to be markedly lower during the 11-hour fasting interval when compared to placebo or the lower dose groups.

SAFETY RESULTS

- Nine treatment-emergent adverse events were reported from four (12.5%) out of 32 subjects during this study, two

AEs in one subjects exposed to 2.5 µg/kg of liraglutide and seven AEs in three subjects exposed to 15 µg/kg of liraglutide. All AEs were of mild severity and resolved with no remedial treatment. No subject was withdrawn due to an AE and there were no fatal, serious or other clinically relevant AEs.

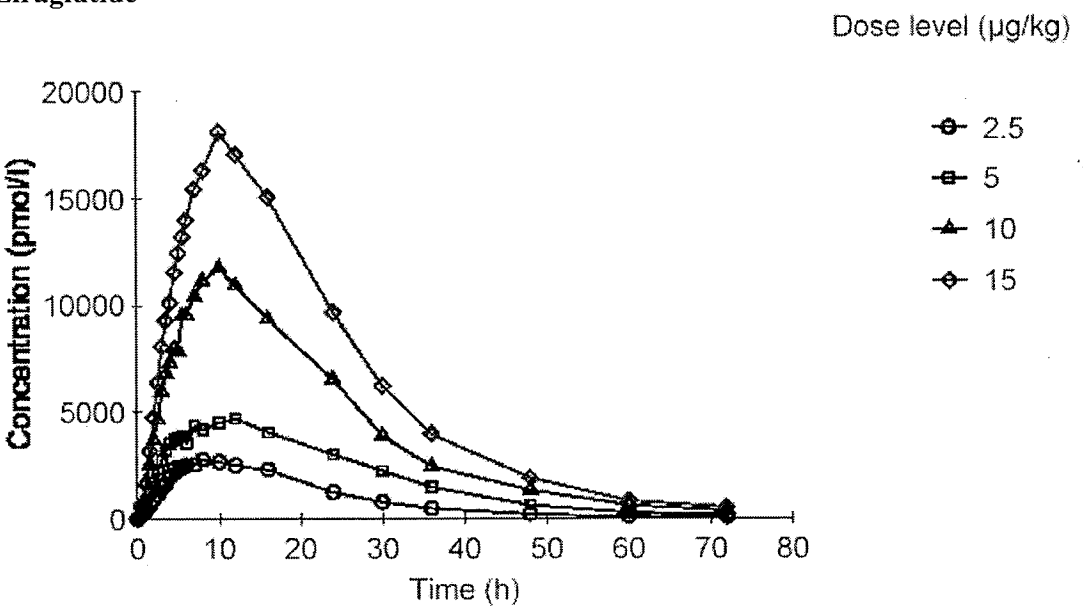
- Liraglutide was administered at doses of 2.5 to 15 µg/kg and was generally well tolerated over the dose range of 2.5 to 10 µg/kg. At the 15 µg/kg dose level, three of six subjects exposed to liraglutide reported gastrointestinal side effects of mild severity, which comprised nausea and vomiting and were considered by the investigator to be probably related to the trial product. Based on these results, the progression to the next higher dose level (17.5 µg/kg or placebo) was cancelled according to the recommendation given by the Trial Safety Review Group.
- Total cholesterol and phosphate tended to decrease to a higher degree in liraglutide groups than placebo group; there were statistically significant differences or a tendency of significant difference between the dose groups for the changes from Day 1 (baseline) to Day 4 and from baseline to Visit 3 (post trial). A decrease from Day 1 to Day 4 was seen for the parameters magnesium and albumin, whereby the changes appeared to be more pronounced with incremental dose. A tendency of statistically significant result for the changes in pH values over the tested dose range at the Visit 3. For the other haematological, biochemical and urinary parameters, there were no dose-dependent changes over time over the dose tested suggested by the two-sided Jonckheere-Terpstra test. There were no individual clinically significant abnormalities observed for any of the laboratory parameters assessed; no clinical laboratory adverse event (CLAE) was reported. Safety glucose assessments did not indicate hypo- or hyperglycaemia.
- Vital signs (systolic and diastolic blood pressure, pulse rate) showed a tendency towards lower values during the first 8–12 hours after trial product administration. There were no dose-related changes in vital signs assessed.
- There was no abnormal ECG evaluation in this study.
- The subjects' mean body weights tended to be slightly lower after dosing, when compared to the corresponding baseline values, without pointing to dose- or treatment-related changes.

CONCLUSIONS

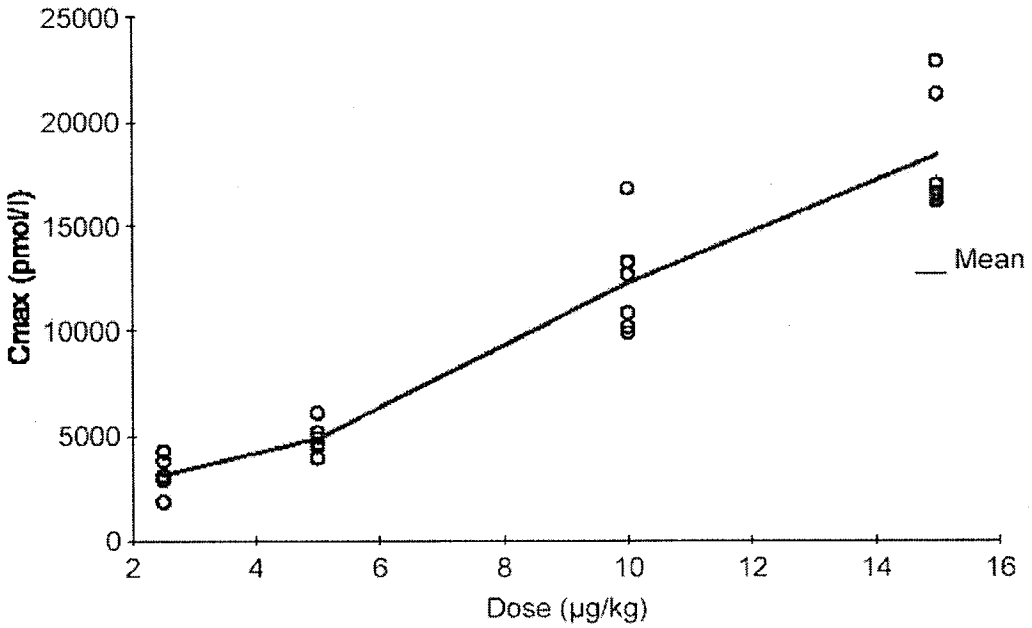
- In healthy Japanese subjects, liraglutide was generally well tolerated over the dose range of 2.5 to 10 µg/kg, but less at the highest dose level of 15 µg/kg, where three of six subjects exposed to liraglutide reported gastrointestinal side effects (nausea and vomiting) of mild severity. In conclusion, it was suggested that the maximum tolerated single dose in healthy Japanese male subjects might be 15 µg/kg.
- No subject was withdrawn due to an AE and there were no fatal, serious or other clinically relevant AEs. All AEs resolved with no remedial treatment.
- The total cholesterol and phosphate tended to decrease to a higher degree in liraglutide groups than placebo groups while no apparent dose-response relationships were seen, and a tendency of statistically significant result was seen for the changes in some haematological, biochemistry and urinary parameters from baseline (Day 1) to Day 4 or Visit 3 over the tested dose range including placebo, no major safety concerns were raised from clinical laboratory tests.
- There were no dose-related changes in vital signs and ECG evaluation.
- Overall safety profiles were consistent with those from previous reports and no major safety concerns were raised from this trial.
- Absorption of liraglutide was slow and the maximum plasma concentrations were reached after 7.5 to 11 hours (medians) across all dose groups, and cleared from plasma with a terminal elimination half-time of approximately 10 to 11 hours for all dose groups. The linear regression analysis showed dose proportionality for the PK endpoints $AUC_{0-\infty}$ and C_{max}. The PK parameters CL/F, V_z/F, $t_{1/2}$, λ_z and MRT were approximately constant over the dose range of 2.5 to 15 µg/kg of liraglutide.
- Comparing with the data from previous trial (NN2211-1149), overall PK profiles in healthy Japanese subjects seem to be comparable to those in healthy Caucasian subjects.
- Key PD findings obtained from this trial was dose-depending attenuation of post-prandial glucose assessed by AUC_{11-24}, and there seemed to be only a small effect of liraglutide on post-prandial glucagon but seemingly little effect on post-prandial insulin in healthy subjects by a single dose of a dose range administered in this trial. Pharmacodynamic profiles should be further investigated in Japanese subjects with type 2 diabetes.

The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

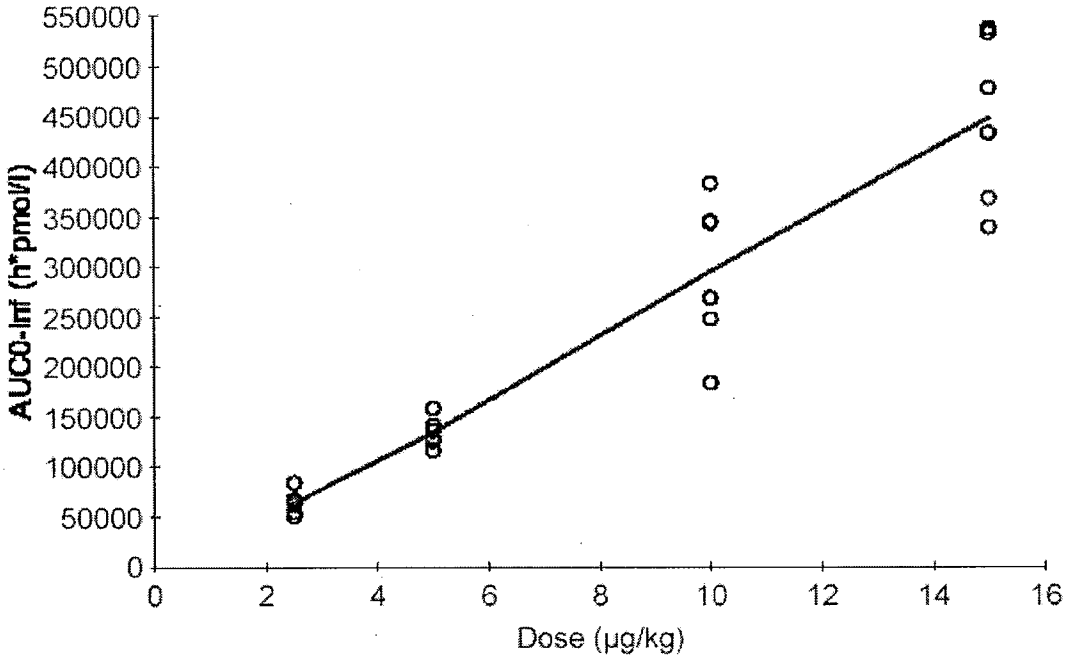
Mean (Arithmetic) Plasma Concentration-Time Profiles Following Single Dose of Liraglutide



C_{max} Values versus Dose of Liraglutide



Values of AUC_{0-} Versus Dose of Liraglutide



Reviewers Comments: The study assessments and conclusions appear reasonable.

4.2.15 Multiple-Dose PK Study in Healthy Japanese (NN2211-1551)

TITLE OF TRIAL A randomised, double-blind, single-centre, placebo-controlled, 21-day multiple s.c. doses, dose escalation study to assess the safety, tolerability, pharmacokinetics and pharmacodynamics of liraglutide (NNC 90-1170) in healthy Japanese male subjects	
INVESTIGATOR(S) Shin Irie, MD, PhD, head of Kyushu Clinical Pharmacology Research Clinic	
TRIAL SITE(S) Kyushu Clinical Pharmacology Research Clinic 13-16, Jigyo 2-chome, Chuo-ku, Fukuoka, 810-0064, Japan	
PUBLICATIONS None	
TRIAL PERIOD 11September 2003 to 13 March 2004	**DEVELOPMENT PHASE** Phase 1
OBJECTIVES Primary objective: To assess the safety and tolerability after multiple *s.c.* doses of liraglutide in healthy Japanese male subjects Secondary objectives: - To assess the pharmacokinetic profile after multiple *s.c.* doses of liraglutide - To assess the effect of liraglutide on serum glucose level - To assess the effect of liraglutide on calcium homeostasis [calciton, ionised calcium (Ca^{2+}), parathyroid hormone (PTH), Vitamins D and B_{12} levels in serum and urinary calcium and phosphorus]	
METHODOLOGY This was a single-centre, randomised within dose group, double-blind, placebo-controlled, dose-escalation trial with sequentially-ascending multiple *s.c.* doses of liraglutide at three dose levels in healthy Japanese male subjects. In each dose group, eight subjects were administered multiple *s.c.* doses; six subjects received liraglutide and two subjects received placebo. In all three dose groups liraglutide or placebo was injected once daily for 21 days. One dose group was with 5 µg/kg/day treatment for 21 days, and the second dose group was with 5 µg/kg/day for 7 days followed by 10 µg/kg/day for 14 days. The third dose group, the highest dose group of liraglutide, was titrated from 5 µg/kg/day to 15 µg/kg/day using a weekly 5 µg/kg/day dose increase for 3 weeks. All *s.c.* doses were administered with a NovoPen® III injection device. Screening (Visit 1) was performed at 28 to 3 days prior to Day 1 (first dosing) of Visit 2; all subjects stayed at the trial site from Day –2 to post last dosing day (PLDD) 3, *i.e.* for a 26-day period. They returned for a follow-up visit (Visit 3) 7 to 21 days after the last dose of the trial product.	
NUMBER OF SUBJECTS PLANNED AND ANALYSED Number of subjects planned: 24 subjects, 8 per dose group (six on liraglutide, two on placebo) Number of subjects screened: 62 Number of subjects randomised: 24 Number of subjects exposed: 24 Number of subjects completed and analysed: 24 Number of withdrawals: 0	
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION Healthy Japanese male subjects, aged 20 to 45 years, with a body mass index (BMI) of between 18 and 27 kg/m^2	

TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Test product:	Liraglutide 5 mg/mL solution, 1.5 mL Penfill® cartridge
Doses:	
Dose-group 1:	5 µg/kg body weight for 21 days, once daily
Dose-group 2:	5 µg/kg body weight for 7 days + 10 µg/kg body weight for 14 days, both once daily
Dose-group 3:	5 µg/kg body weight for 7 days + 10 µg/kg body weight for 7 days + 15 µg/kg body weight for 7 days, all once daily
Mode of administration:	*s.c.* administration into a lifted skin fold of the abdominal wall on a line between the umbilicus and the anterior superior iliac spine
Batch No.:	LLDP007

DURATION OF TREATMENT

In all three dose groups liraglutide or placebo was injected once daily for 21 days.

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Test product:	Placebo (liraglutide [INN; NNC 90-1170] vehicle) solution 1.5 mL Penfill® cartridge
Doses:	Calculated matching clicks as liraglutide treatment in each dose group
Mode of administration:	*s.c.* administration into a lifted skin fold of the abdominal wall on a line between the umbilicus and the anterior superior iliac spine
Batch No.:	LLDP005

CRITERIA FOR EVALUATION – PHARMACOKINETICS & PHARMACODYNAMICS

Pharmacokinetic (PK) endpoints derived from 72-hour plasma liraglutide profile:

$AUC_{0\text{-}24h}$ (last dosing day), C_{max} (last dosing day), t_{max} (last dosing day), C_{trough} from Day 2 to the day before last dosing, dose-corrected R_{ac} [dose-corrected ratios of $AUC_{0\text{-}inf}$ (last dosing day)/$AUC_{0\text{-}24h}$ (last dosing day), $AUC_{0\text{-}24h}$ (last dosing day)/$AUC_{0\text{-}24h}$ (Day 1), C_{max} (last dosing day)/C_{max} (Day 1)], λ_Z, MRT, $t_{1/2}$, CL/F, V_Z/F (all after last dosing)

Pharmacodynamic (PD) endpoints derived from 24-hour serum glucose profile on Day -1 (baseline) and last dosing day:

$AUC_{0\text{-}16h}$ (glucose)/16h, $AUC_{0\text{-}24h}$ (glucose)/24h, $AUC_{1\text{-}3h,4\text{-}6h,11\text{-}13h}$ (glucose)/6h, incremental $AUC_{1\text{-}3h}$ (glucose)

CRITERIA FOR EVALUATION – SAFETY

Adverse events (AEs), Clinical laboratory assessments (haematology, biochemistry including lipids, fasting plasma glucose [FPG] and urinalyses), Urinary volume and electrolyte excretion, Vitamins D and B_{12} in serum, Antibodies against liraglutide, Body weight, Vital signs (blood pressure and pulse), electrocardiogram (ECG), 24-hour profiles of calcitonin, Ca^{2+} and PTH, Glucose monitoring.

STATISTICAL METHODS

Pharmacokinetics: Descriptive statistics were presented for the PK endpoints except for t_{max}, which included: N, mean, geometric mean, min, median, max, SD, geometric CV%. For t_{max} only N, mean, min, median, max and SD were presented. Individual plasma liraglutide PK endpoints were listed by active dose and subject number, *i.e.* excluding placebo-treated subjects. All PK endpoints except t_{max} and C_{trough} were presented as scatter plots of endpoint versus dose. The PK endpoints $AUC_{0\text{-}24h}$ (last dosing day), C_{max} (last dosing day), C_{trough} (only Days 7, 14 and 21), R_{ac}, λ_Z, MRT and V_Z/F were transformed using a log transformation prior to statistical modelling. The remaining PK endpoints were not analysed further. The model for the endpoints could be stated as follows: the response for each subject is the sum of an overall mean, a fixed dose effect and a random measurement error. An analysis of variance, following the above-mentioned model, was carried out and estimated population means (least square means) for each dose level with corresponding 95% confidence intervals (CIs) were calculated. The CIs for the log-transformed variables were transformed back to get CIs on the original data scale. Contrasts on the log scale were calculated using the lowest dose as the reference point, corresponding to ratios on the original scale.

Pharmacodynamics: Descriptive statistics were presented for the PD endpoints, which included: N, mean, geometric mean, min, median, max, SD, geometric CV%. Individual endpoints were listed by active dose and subject number. Furthermore, scatter plots of PD endpoints [$AUC_{0\text{-}16h}$ (glucose)/16h, $AUC_{0\text{-}24h}$ (glucose)/24h and $AUC_{1\text{-}3h,4\text{-}6h,11\text{-}13h}$ (glucose)/6h] versus PK endpoint [$AUC_{0\text{-}24h}$ (last dosing day)] or logarithm of PD endpoints versus logarithm of PK endpoint were shown for all subjects except subjects who were treated with placebo. The statistical

modelling of PD endpoints except FPG was similar to the one described for the PK endpoints. The endpoints were log transformed prior to the statistical analysis and estimation of contrasts with placebo treatment as reference was made. Model for the PD endpoints: the response in each subject is the sum of an overall mean, a fixed dose effect, a fixed group effect, baseline as a covariate and a random error. The corresponding PD parameters on Day –1 (baseline) were used as covariates in the statistical analysis. No statistical analysis of FPG was performed.

Safety: All AEs were listed by subject, including demographic information, treatment group, date and time of onset, outcome, date and time of outcome, severity, changes to the trial product due to AE, relation to the trial product, Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC), MedDRA preferred term (PT), lowest level term (LLT) and term reported by the Investigator. Laboratory assessments, urinary pH, urinary volume, urinary electrolytes, serum vitamins D and B_{12}, vital sings, body weight and ECG were summarised descriptively by treatment group. Antibodies against liraglutide were summarised as frequency table. Sixteen- and 24-hour average levels derived from 24-hour profiles of serum calcitonin, Ca^{2+} and PTH were analysed in the same way as PD endpoints.

Liraglutide was detected in plasma samples from Subject 02 on Days 10 to 16, although this subject was randomised to receive placebo. Therefore, an additional statistical analysis for PD and safety endpoints was performed without data from Subject 02 for assessments after the time of dosing on Day 9.

DEMOGRAPHY OF TRIAL POPULATION

A total of 24 healthy Japanese male subjects entered the study. They were on average 24.7 (±2.1) years old, had a BMI of 21.23 (±1.95) kg/m^2, with a weight of 63.47 (±5.76) kg and a height of 1.729 (±0.048) m. There was no relevant difference in the demographic data between the different dose groups.

Descriptive Statistics of Demographics

	N	Age [years]	Height [m]	Weight [kg]	BMI [kg/m^2]
Placebo	6	23.8 (2.3)	1.723 (0.038)	65.42 (5.80)	22.07 (2.31)
Constant-dose	6	25.5 (2.0)	1.767 (0.040)	64.18 (6.85)	20.52 (1.70)
Escalation 1	6	24.7 (1.4)	1.710 (0.027)	60.62 (4.45)	20.75 (2.08)
Escalation 2	6	24.8 (2.6)	1.717 (0.067)	63.67 (6.11)	21.60 (1.77)

PHARMACOKINETIC & PHRMACODYNAMIC RESULTS

- Liraglutide was measurable in plasma from all liraglutide treated subjects and quantifiable from 1 to 24 hours on Day 1 and from 0 to 72 hours following the last dosing on Day 21. In addition, liraglutide was quantifiable in all trough plasma samples during the treatment period.
- Liraglutide was detected in the plasma from Subject 02 on Days 10 to 16, although this subject was randomised to receive placebo. It is suspected that liraglutide was administered to the subject once on Day 9 by mistake.
- C_{max}(last dosing day) and $AUC_{0\text{-}tau}$(last dosing day) increased proportionally to the dose administered.
- T_{max}, $t_{1/2}$, MRT, CL/F and V_Z/F were approximately constant within the dose range of 5 to 15 µg/kg/day.
- R_{ac}s were calculated to 1.4–1.6 within the dose range of 5 to 15 µg/kg/day.
- The glucose profiles over time reflected the meal-related alterations in this healthy population.
- Compared to a 24-hour baseline profile of serum glucose on Day –1, the meal-induced increases in glucose levels after the three-week liraglutide treatment were reduced without any apparent differences for the three liraglutide treatment groups.
- In the ANCOVA, the liraglutide treatment effect was found in the AUCs of glucose levels compared to placebo. A 9–16% difference in derived endpoints of liraglutide 5 µg/kg and 15 µg/kg treatment groups was obtained, while the analysis did not reveal any significant difference for the 10 µg/kg treatment group. The variability of data was high.

SAFETY RESULTS

- Three TEAEs of mild severity were reported by two subjects during the study. Two TEAEs (rash and alanine aminotransferase increased) occurred in placebo treatment group, one TEAE (nasopharyngitis) was reported by a subject treated with a constant dose of liraglutide. No AE was observed in the two dose escalation groups. No

serious and no significant AE occurred. None of the subjects was withdrawn due to an AE and no rescue medication was necessary.

- There was no treatment- or dose-related change in safety laboratory parameters, except for an approximately 12–18% decrease in total cholesterol concentrations compared to baseline across all treatments.
- There was no apparent effect of liraglutide on vital signs across all treatment regimens including placebo.
- All subjects showed a normal 12-lead ECG during the trial.
- There was no relevant change in body weight during the 21-day treatment regimen with liraglutide compared to placebo.
- The only calcium related finding that was observed in this study was a tendency towards a lowering in PTH levels. However, this was not accompanied by changes in calcium or phosphorus excretion in the urine, nor consistent changes in Ca^{2+} levels, suggesting limited significance of the finding.
- Vitamins D and B_{12} levels did not show any systematic trend during this trial for any treatment.
- Assessment of urine volume and urinary electrolyte excretion did not reveal any relevant change during the 21-day treatment with liraglutide compared to placebo.
- No liraglutide antibodies were detected in the serum of any subject.

CONCLUSIONS

Safety

- In this study in 24 healthy Japanese male subjects, a constant-dose of 5 µg/kg and stepwise escalated doses of 10 and 15 µg/kg as a three-week treatment regimen were well tolerated.
- Three TEAEs of mild severity were reported by two subjects during the study. Two TEAEs (rash and alanine aminotransferase increased) occurred in placebo treatment group, one TEAE (nasopharyngitis) was reported by a subject treated with a constant dose of liraglutide. No AE was observed in the two dose escalation groups. No serious and no significant AE occurred. None of the subjects was withdrawn due to an AE and no rescue medication was necessary.
- There was no treatment- or dose-related change in safety laboratory parameters, except for an approximately 12–18% decrease in total cholesterol concentrations compared to baseline across all treatments.
- The only calcium related finding that was observed in this study was a tendency towards a lowering in PTH levels. However, this was not accompanied by changes in calcium or phosphorus excretion in urine, or consistent changes in Ca^{2+} levels, suggesting limited significance of the finding.
- The three-week liraglutide treatment did not reveal any clinically relevant impact on vital signs data, 12-lead ECG, vitamins D and B_{12} levels, as well as urine volume and urinary electrolyte excretion data compared to placebo.
- No antibodies against liraglutide were detected in the serum of any subject.

Pharmacokinetics

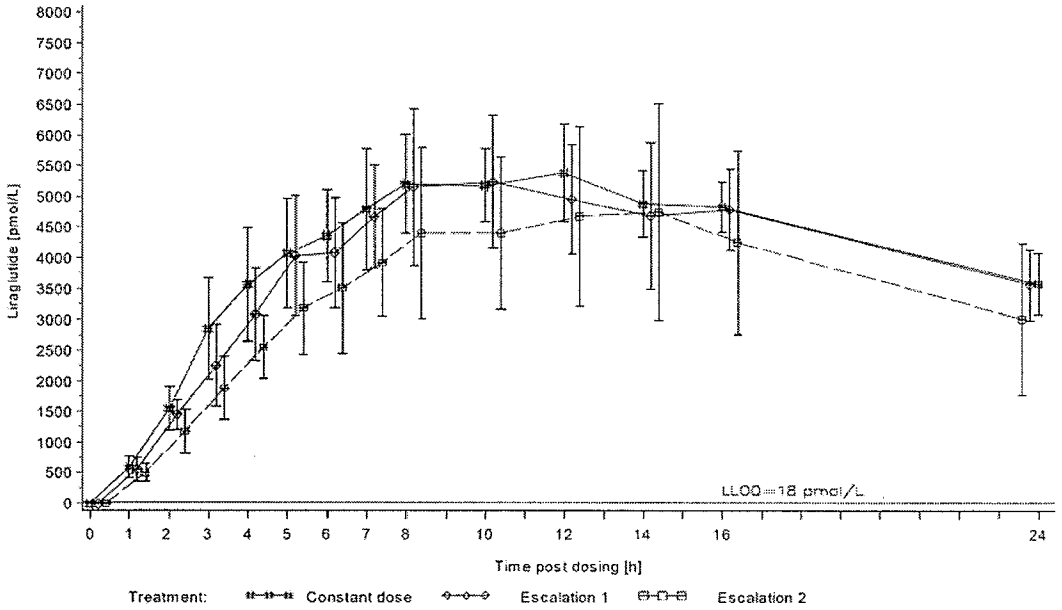
- C_{max} and AUC increased proportionally to the dose administered.
- T_{max}, $t_{1/2}$, CL/F, V_Z/F and MRT were approximately constant over the dose range of 5 to 15 µg/kg/day.
- R_{ac}s were calculated to 1.4–1.6 within the dose range of 5 to 15 µg/kg/day.

Pharmacodynamics

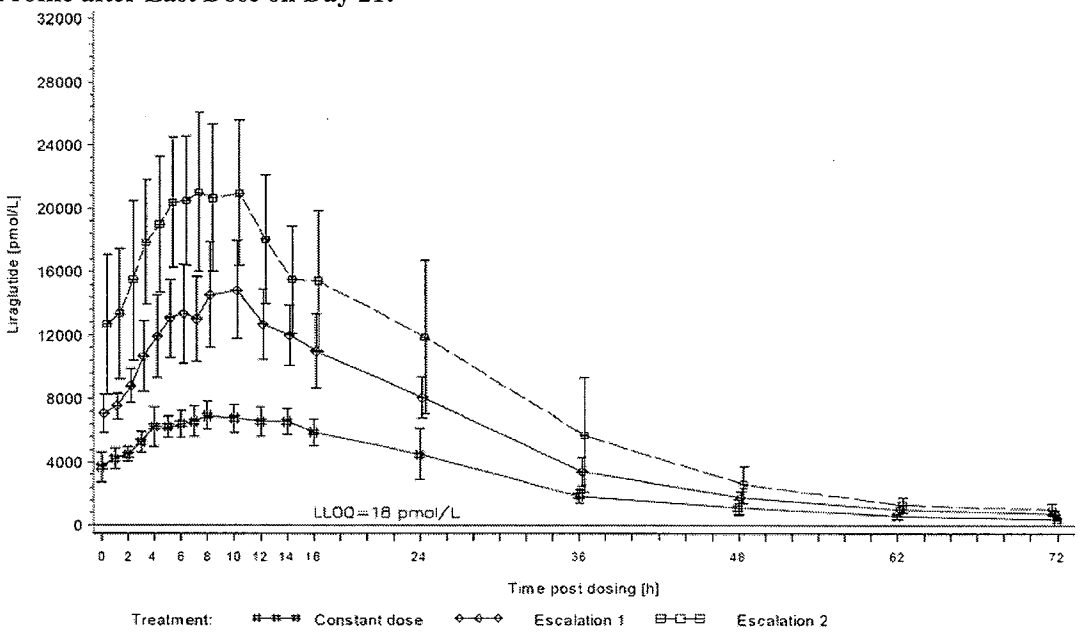
- Compared to a 24-h baseline profile of serum glucose on Day –1, the meal-induced increases in glucose levels after the three-week liraglutide treatment were reduced without any apparent differences for the three liraglutide treatment groups.
- In the ANCOVA, a 9–16% difference in derived glucose endpoints of liraglutide 5 µg/kg and 15 µg/kg treatment groups compared to placebo was obtained, while the analysis did not reveal any significant difference for the liraglutide 10 µg/kg treatment group compared to placebo.

The trial was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

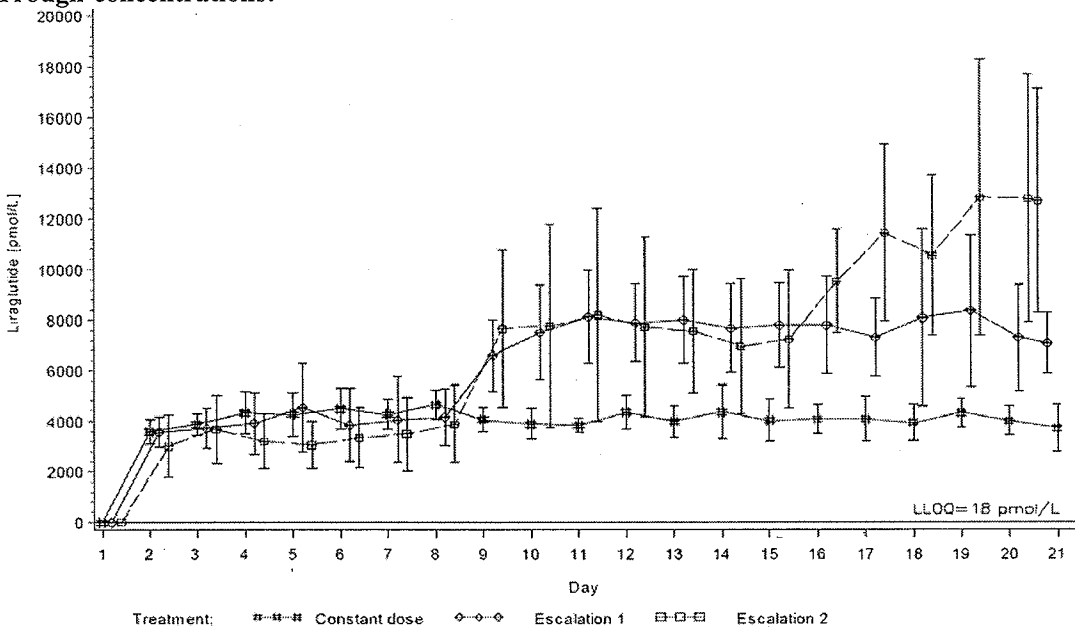
Single Dose Day 1 Profile:



Profile after Last Dose on Day 21:



Trough-concentrations:



Reviewers Comments: The study assessments and conclusions appear reasonable.

4.2.16 Multiple-Dose PKPD Study in Type 2 Japanese (NN2211-1591)

TITLE OF TRIAL A randomised, double-blind within dose group, single-centre, placebo controlled, parallel 2-different dose group, 14-day multiple s.c. doses study to assess the safety, pharmacokinetics and pharmacodynamics of liraglutide (NNC 90-1170) in subjects with type 2 diabetes	
INVESTIGATOR Shin Irie, MD, PhD, Head of Kyushu Clinical Pharmacology Research Clinic	
TRIAL SITE Kyushu Clinical Pharmacology Research Clinic, 13-16, Jigyo 2-chome, Fukuoka, 810-0064, Japan	
PUBLICATIONS None	
TRIAL PERIOD 03 December 2003 (FSFV) – 8 March 2004 (LSLV)	**DEVELOPMENT PHASE** Phase 1
OBJECTIVES Primary objective: • To assess the safety after 14-day multiple s.c. doses of liraglutide in Japanese subjects with type 2 diabetes, and compare the safety at doses 5 µg/kg and 10 µg/kg. Secondary objectives: • To assess the pharmacokinetic (PK) profile after multiple s.c. doses of liraglutide in Japanese subjects with type 2 diabetes. • To assess the effect of liraglutide on serum insulin and plasma glucose levels.	
METHODOLOGY This was a single-centre, randomised, double-blind within dose group, placebo-controlled, multiple-dose (s.c.), parallel-group trial in Japanese subjects with type 2 diabetes. The constant-dose group received 5µg/kg for two weeks, whereas the dose-escalation group received 5 µg/kg for the first week and 10 µg/kg for the second week. Within each dose group, six subjects received liraglutide and two subjects received placebo (ratio of active : placebo = 6 : 2). All doses were administered using a NovoPen® III injection device. Subjects stayed in the trial site from Day −3 to post last dosing day (PLDD) 3, i.e. 72 hours after the last administration of NNC 90-1170 and returned for a follow-up visit 7-31 days after last dosing.	
NUMBER OF SUBJECTS PLANNED AND ANALYSED Number of subjects planned: 16 subjects, 8 per treatment group Number of subjects screened: 17 Number of subjects randomised: 16 Number of subjects exposed: 15 (One subject withdrew for private reasons after randomisation before any exposure to trial product.) Number of subjects completed and analysed: 15 (constant-dose: 6, dose-escalation: 5, placebo: 4 subjects) Number of withdrawals: 1	
DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION Japanese male and female subjects with type 2 diabetes, aged 20 to 64 years, with a body mass index of ≥ 18.5 and ≤ 30.0 kg/m^2, and with HbA_{1c} of 6.0 to 9.0%.	
TEST PRODUCT, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER Test product: Liraglutide (NNC 90-1170) 5 mg/mL solution, 1.5 mL Penfill® cartridge, Batch No.: LLDP007 Doses: Constant-dose group: 5 µg/kg body weight once daily Dose-escalation group: 5 µg/kg for seven days followed by 10 µg/kg body weight, both periods once daily Mode of administration: s.c. administration into a lifted skin fold of the abdominal wall on a line between umbilicus and the anterior superior iliac spine	

DURATION OF TREATMENT

14 consecutive days.

REFERENCE THERAPY, DOSE AND MODE OF ADMINISTRATION, BATCH NUMBER

Test product: Placebo (liraglutide vehicle) solution 1.5 mL Penfill® cartridge, Batch No.: LLDP005
Doses: Calculated number of clicks to match the 5 or 10 µg/kg doses.
Mode of administration: s.c. administration into a lifted skin fold of the abdominal wall on a line between umbilicus and the anterior superior iliac spine

CRITERIA FOR EVALUATION – PHARMACOKINETICS AND PHARMACODYNAMICS

Pharmacokinetic (PK) variables: Plasma level of liraglutide for 24 hour after the first dosing (Day 1) and for 72 hours after the last dosing (Day 14).
Pharmacodynamic (PD) variables: Serum insulin and plasma glucose levels for 24 hours at baseline (Day -1) and after the last dosing (Day 14), fasting plasma glucose (FPG) from Day -1 through post-last-dosing-day (PLDD) 3.

CRITERIA FOR EVALUATION – SAFETY

Physical examination, body weight, vital signs (blood pressure and pulse rate), ECG, clinical laboratory assessment (haematology and biochemistry including FPG), glucose monitoring and adverse events (AEs), vitamins D and B_{12}, liraglutide antibody, calcitonin, ionised calcium, parathyroid hormone (PTH), urinary volume, electrolyte excretion

STATISTICAL METHODS

PK and PD endpoints:

PK endpoints derived from liraglutide profiles were $C_{max,\,ss}$, $t_{max,\,ss}$ and $AUC_{0\text{-}24h,\,ss}$ (Day 14); C_{trough} from Day 2 to the day of last dosing day; R_{ac}, such as dose-corrected ratio of $AUC_{0\text{-}inf}/AUC_{0\text{-}24h}$ (Day 14), $AUC_{0\text{-}24h}$ (Day 14)/$AUC_{0\text{-}24h}$ (Day 1), C_{max} (Day 14)/C_{max} (Day 1); λ_Z, $t_{1/2}$, MRT, CL/F = Dose/$AUC_{0\text{-}24h}$ (Day 14) and $V_{d,\,ss}/F$ after last dosing on Day 14; $V_{d,\,ss}/F=(CL/F)/\lambda_Z$.

PD evaluation comprised of the following endpoints:

$AUC_{0\text{-}16h}$ (insulin)/16 hours; $AUC_{0\text{-}24h}$ (insulin)/24 hours; [$AUC_{1\text{-}3h}$ (insulin)+$AUC_{4\text{-}6h}$ (insulin)+$AUC_{11\text{-}13h}$ (insulin)]/6 hours; incremental AUC(insulin) ($AUC_{1\text{-}3h,\,4\text{-}6h,\,11\text{-}13h}$); $AUC_{0\text{-}16h}$ (glucose)/16 hours; $AUC_{0\text{-}24h}$ (glucose)/24 hours; [$AUC_{1\text{-}3h}$ (glucose)+$AUC_{4\text{-}6h}$ (glucose)+$AUC_{11\text{-}13h}$ (glucose)]/6 hours; incremental AUC(glucose) ($AUC_{1\text{-}3h,\,4\text{-}6h,\,11\text{-}13h}$); FPG.

Pharmacokinetics: The statistical analysis for the PK endpoints was based on summary statistics, which, except for t_{max}, included: N, mean, geometric mean, min, median, max, SD, geometric CV%. For t_{max} only N, mean, min, median, max, and SD were presented. Individual plasma liraglutide PK endpoints were listed by active dose and subject number, i.e. excluding placebo-treated subjects, as an End-of-Text selected listing. All PK endpoints were presented as scatter plots of endpoint versus dose. The PK endpoints $AUC_{0\text{-}24h}$, C_{max}, C_{trough}, R_{ac}, λ_z, MRT and V_d/F were transformed using a log transformation prior to statistical modelling. The remaining PK endpoints were not analysed further. The model for the endpoints could be stated as follows: The response for each subject is the sum of an overall mean, a fixed dose effect and a random measurement error. An analysis of variance, following the above-mentioned model, was carried out and estimated population means (least square means) for each dose level with corresponding 95% confidence intervals (CIs) were calculated. The CIs for the log-transformed variables were transformed back to get CIs on the original data scale. Contrasts on the log scale were calculated using the lowest dose as the reference point, corresponding to rates on the original scale.

Pharmacodynamics: The statistical analysis for the PD endpoints was based on summary statistics, which included: N, mean, geometric mean, min, median, max, SD, geometric CV%. Individual endpoints were listed by active dose and subject number as an End-of-Text selected listing. Furthermore, scatter plots of PD endpoints [$AUC_{0\text{-}16h}$/16 hours, $AUC_{0\text{-}24h}$/24 hours and ($AUC_{1\text{-}3h}$+$AUC_{4\text{-}6h}$+$AUC_{11\text{-}13h}$)/6 hours for serum insulin and plasma glucose] versus PK endpoint [$AUC_{0\text{-}24h}$ (last dosing day)] or logarithm of PD endpoints versus logarithm of PK endpoint were shown for all subjects.

The statistical modelling of PD endpoints except FPG was similar to the one described for the PK endpoints. The endpoints were log transformed prior to the statistical analysis and estimation of contrasts with placebo treatment as reference was made. Model for the PD endpoints: The response in each subject was the sum of an overall mean, a fixed dose effect, a fixed group effect, baseline as a covariate and a random error. The corresponding PD parameters on Day –1 (baseline) were used as covariates in the statistical analysis. No statistical analysis of FPG was performed.

Safety: Adverse events with a date of onset preceding the date of the first administration of study drug that resolved before that day or that continued into the treatment phase without worsening in terms of intensity and/or relationship to study medication was considered as non-treatment emergent adverse events (non-TEAE). All other adverse events were considered and reported as treatment emergent adverse events (TEAE).
Adverse events were summarised by dose group, MedDRA (The Medical Dictionary for Regulatory Activities) system organ class and preferred term, severity and relation to trial product, and described by summary statistics: number of subjects with event, percent exposed subjects with event, and number of events. Haematology and biochemistry data, serum vitamins D and B_{12}, urinary electrolytes and volume, ECG, vital signs, body weight and antibody against liraglutide were summarised by dose group and visit/time, and described summary statistics.
Sixteen- and 24-hour average levels derived from 24-hour profiles of serum calcitonin, Ca^{2+} and PTH were analysed in the same way as PK and PD endpoints.

DEMOGRAPHY OF TRIAL POPULATION

Altogether, nine male and six female Japanese subjects with type 2 diabetes were included into the treatment phase of the study. The following table shows the descriptive statistics of the treated subjects.

Descriptive statistics of the study population. Mean (SD)

	N	Age [years]	Height [cm]	Weight [kg]	Body Mass Index [kg/m^2]
Total	15	57.00 (6.12)	161.5 (9.8)	64.23 (10.75)	24.50 (2.87)
Constant dose	6	57.00 (6.39)	162.7 (7.8)	67.37 (8.59)	25.35 (1.57)
Dose escalation	5	54.00 (7.25)	165.4 (9.4)	64.00 (12.24)	23.20 (2.57)
Placebo	4	60.75 (1.71)	155.0 (11.8)	59.83 (13.07)	24.85 (4.58)
Male	9	57.33 (6.36)	167.4 (4.2)	69.94 (6.44)	24.99 (2.68)
Female	6	56.50 (6.28)	152.7 (9.0)	55.67 (10.51)	23.77 (3.24)

PHARMACOKINETIC AND PHARMACODYNAMIC RESULTS

- In Japanese subjects with type 2 diabetes mellitus, dose escalation to 10 µg/kg showed a dose dependent increase in liraglutide AUCs and C_{max} values compared with the constant 5 µg/kg group.
- Absorption rate, as indicated by a t_{max} of 9 – 12 hours, and elimination rate with a $t_{1/2}$ of around 14 hours closely resembled previous PK results.
- The accumulation ratios Day 14/Day 1 for AUC and C_{max} of approximately 1.6 to 1.8 were consistent with elimination kinetics and did not show relevant differences between the two liraglutide treatment groups. Three dose-corrected accumulation ratios (R_{ac}), $AUC_{0\text{-}inf,\ ss}/AUC_{0\text{-}24h,\ ss}$, $AUC_{0\text{-}24h,\ ss}/AUC_{0\text{-}24h,\ Day\ 1}$ and $C_{max,\ ss}/C_{max,\ Day\ 1}$ were comparable in the two liraglutide treatment groups.
- Liraglutide plasma concentrations generally showed a long plateau phase with small within-individual changes between at least eight and 16 hours post-dose.
- For both liraglutide treatment groups a decrease in FPG concentrations from baseline during the 14 days of treatment was found, which seemed different from that in placebo group.
- In both liraglutide treatment groups, the postprandial plasma glucose curve was generally shifted one hour earlier after 14-day treatment, compared to baseline and placebo group.
- Three average plasma glucose level endpoints, $AUC_{0\text{-}16h}/16$, $AUC_{0\text{-}24h}/24$ and $AUC_{(1\text{-}3+4\text{-}6+11\text{-}13h)}/6$ (corresponding to after breakfast, lunch and dinner), in the two liraglutide treatment groups were statistically significantly lower than the placebo group, while no significant difference was observed in the incremental AUC.
- Three average serum insulin level endpoints, $AUC_{0\text{-}16h}/16$, $AUC_{0\text{-}24h}/24$ and $AUC_{(1\text{-}3+4\text{-}6+11\text{-}13h)}/6$ were statistically significantly higher in the dose-escalation group, but not in the constant-dose group compared with placebo group.
- The incremental $AUC_{1\text{-}3h,\ 4\text{-}6h,\ 11\text{-}13h}$ for serum insulin in the dose-escalation group was approximately twice to three times as high as in the constant-dose group, though statistically significant results were not found.

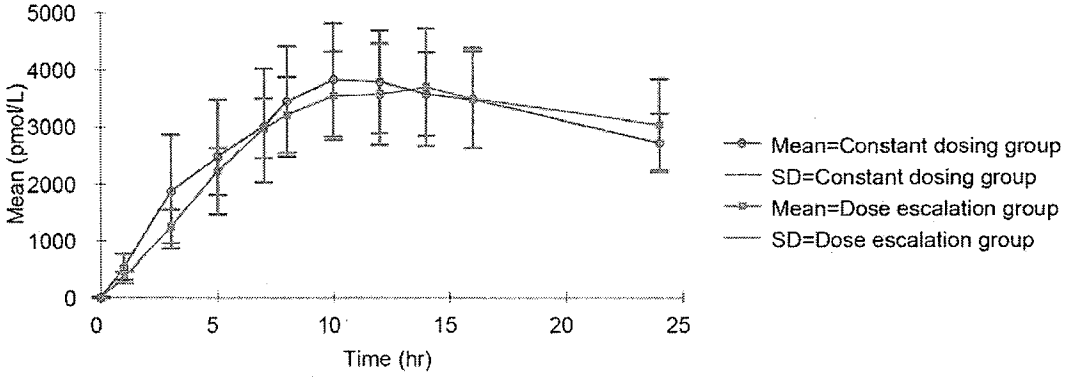
SAFETY RESULTS

- Overall six AEs were reported by four subjects during the study, with three of these six AEs as TEAEs occurring in three subjects in the active treatment groups: one (constipation) in the constant-dose group and two (constipation and a skin depigmentation in the face) in the dose-escalation group. The skin depigmentation in the face was considered unlikely related to liraglutide. The two constipations were considered related to treatment with liraglutide. These symptoms were both of mild severity and lasted for approximately one and three days, respectively. In both cases magnesium oxide was given as rescue treatment. No serious AE occurred; none of the subjects were withdrawn due to an AE.
- No vomiting or nausea was reported either in the constant-dose group or in dose-escalation group.
- Except for reduced total cholesterol and FPG concentrations, both treatment regimens showed no changes in routine laboratory parameters of haematology and biochemistry from baseline to post-last-dosing day 1 (PLDD 1).
- Total cholesterol levels were reduced by approximately 20% on PLDD 1 from the baseline in both liraglutide treatment groups, which seems to be different from the change in the placebo group. However, there were no differences between the two liraglutide treatment groups.
- There was no apparent influence of liraglutide on SBP and DBP during the 14-day treatment in both dose regimens; however, the pulse rate was elevated in the higher dose period with 10 µg/kg (second week) compared with the lower dose (first week) in dose-escalation group.
- Normal ECG findings were recorded for all subjects at each assessment after screening.
- Body weight was generally reduced during 14-day treatment period in both in the two liraglutide treatment groups and the placebo group. No clear differences were seen among the treatment groups.
- There were no significant differences in either serum calcitonin or ionised calcium. While there was a tendency towards a lowering in the liraglutide treatment groups for PTH, there was not statistically significant difference between treatments for both $AUC_{0\text{-}16h}/16$ and $AUC_{0\text{-}24h}/24$. A borderline significant treatment effect was found for $AUC_{0\text{-}24h}/24$, carried by the dose-escalation group where a significant lower level of PTH was seen.
- Vitamins D and B_{12} showed normal values from Day −1 to PLDD 1 for all subjects in all treatment groups. No differences between the two liraglutide treatment groups and placebo group were observed.
- A tendency towards a minor increase in urinary volume was seen in the two liraglutide treatment groups while urinary volume was slightly decreased in placebo group. No clinically significant changes in urinary calcium and phosphorus excretion were observed.
- None of the subjects developed liraglutide antibodies.

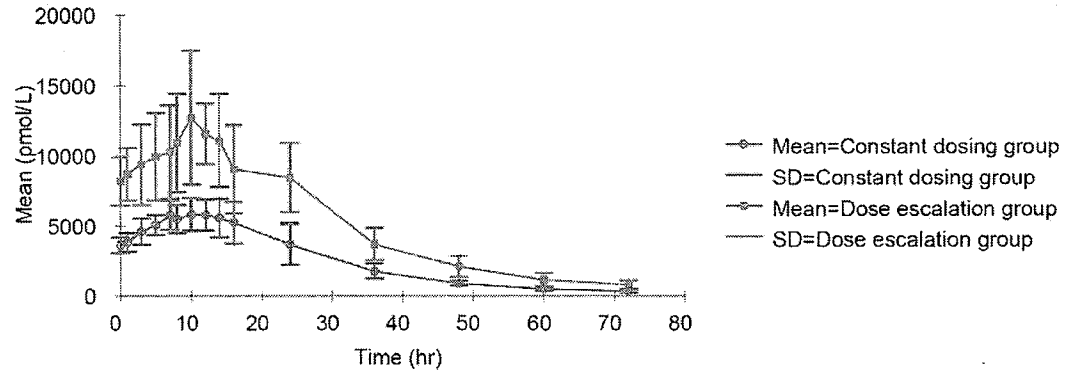
CONCLUSIONS

- Overall, 14-day treatment of liraglutide at doses of 5 and 10 µg/kg was safe and well tolerated in Japanese type 2 diabetic subjects.
- No vomiting or nausea were reported in the dose titration design with the initial low dose of 5 µg/kg and a weekly increment of 5 µg/kg in subjects with type 2 diabetes.
- The only calcium related finding that was observed in this study was a tendency towards a lowering in PTH levels. However, this was not accompanied by changes in calcium or phosphorous excretion in the urine, or consistent changes in ionised calcium levels, suggesting limited significance of the finding.
- Dose escalation from 5 to 10 µg/kg showed a dose dependent increase in liraglutide AUCs and C_{max} values. A t_{max} of 9 – 12 hours and a $t_{1/2}$ of around 14 hours closely resembled previous PK results.
- The accumulation ratios Day 14/Day 1 for AUC and C_{max} of approximately 1.6 to 1.8 were consistent with elimination kinetics and did not show relevant differences between the two dose groups. The three dose-corrected accumulation ratios (R_{ac}) were comparable in the two liraglutide treatment groups.
- The long plateau concentrations with small within-individual changes at least between eight and 16 hours post-dose may be supportive for evening dosing, in order to achieve more prominent reduction on meal-induced increases in glucose concentrations during the main meal times.
- Liraglutide provided significant 24-hour glycaemic control and a decrease in FPG. The postprandial plasma glucose curve was generally shifted one hour earlier after 14-day treatment. Average meal-related glucose level was decreased, though meal-related increment in glucose was not different from placebo.
- Liraglutide significantly increased 24-hour serum insulin in dose-escalation group. Meal-related insulin secretion also increased, though the difference was not statistically significant from placebo.

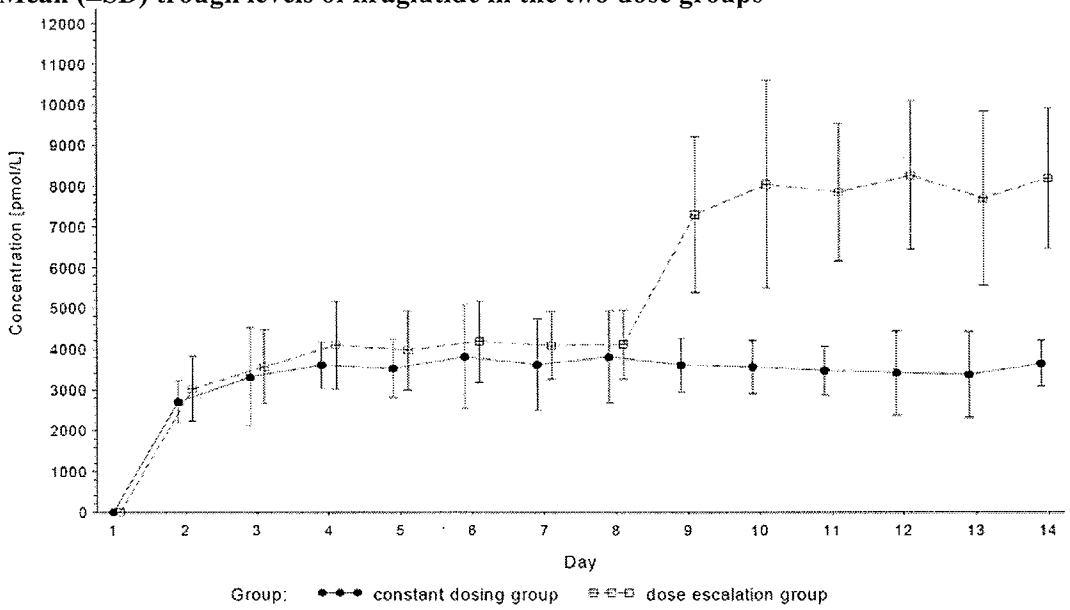
Mean (±SD) Liraglutide Concentrations on Day 1:



Mean (±SD) Liraglutide Concentrations on Day 14



Mean (±SD) trough levels of liraglutide in the two dose groups



Summary of PK Parameters Day 14:

Parameter	Statistics	Constant-dose group (N=6)	Dose-escalation group (N=5)
$t_{max, ss}$	Median	9.0	12.0
[h]	Min/max		
$C_{max, ss}$	Arith. mean	6113.3	13202.4
[pmol/L]	SD	1062.9	4438.3
$C_{trough\ Day\ 7}$	Arith. mean	3618.7	4085.4
[pmol/L]	SD	1118.2	820.0
$C_{trough\ Day\ 14}$	Arith. mean	3636.8	8190.0
[pmol/L]	SD	561.1	1716.2
$AUC_{0-24h, ss}$	Arith. mean	119024.8	237346.2
[h·pmol/L]	SD	26978.2	63093.7
$AUC_{inf, ss}$	Arith. mean	188838.8	394005.8
[h·pmol/L]	SD	44434.5	100221.4
$t_{1/2}$	Geom. mean	13.92	14.44
[h]	CV%	11.45	12.88
MRT	Arith. mean	25.67	27.78
[h]	SD	1.97	4.11
CL/F	Arith. mean	0.928	0.682
[L/h]	SD	0.341	0.353
$V_{d, ss}/F$	Arith. mean	18.95	14.65
[L]	SD	7.35	7.98
$AUC_{0-inf, ss}/AUC_{0-24h, ss}$	Arith. mean	1.587	1.670
	SD	0.074	0.169
$AUC_{0-24h, ss}/AUC_{0-24h, Day\ 1}$	Arith. mean	1.777	1.798
	SD	0.521	0.409
$C_{max, ss}/C_{max, Day\ 1}$	Arith. mean	1.573	1.698
	SD	0.360	0.274

b(4)

Sponsor's Summary of findings:

Cmax, ss and AUC0-24h, ss values of liraglutide exposure on Day 14 dose-proportionally increased with an increase of dose from 5 µg/kg to 10 µg/kg. All observed values of Cmax, ss in the dose-escalation group were higher than those in the constant-dose group. Median tmax, ss of 9 hours in the 5 µg/kg constant-dose group was achieved 3 hours earlier than in the 10 µg/kg dose escalation group.

In accordance with the slightly higher dose-related AUC in the dose-escalation group, both CL/F and the apparent volume of distribution, Vd, ss/F were somewhat lower compared with the constant dose group. Elimination kinetics did not differ between the two groups and was characterized by an average elimination half-life of approximately 14 hours. The mean dose-corrected accumulation ratio of the last dosing day of approximately 1.6 was also similar between the two dose groups and well corresponded with the elimination half-life.

Reviewer's Comments: The study assessments and conclusions appear reasonable.

4.3 PHARMACOMETRICS REVIEW

OFFICE OF CLINICAL PHARMACOLOGY: PHARMACOMETRIC REVIEW

1 SUMMARY OF FINDINGS

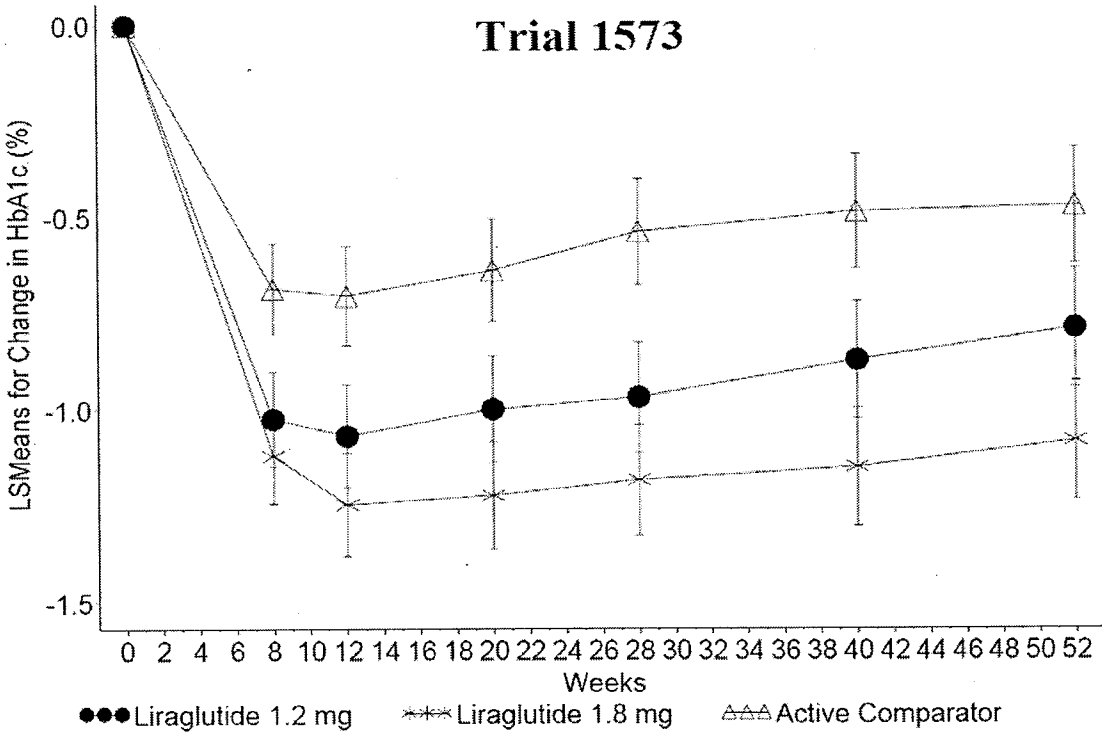
1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 What is the exposure-response relationship for liraglutide in type 2 diabetic patient population?

The change in HbA1c versus time profile from Phase 3 study showed that the maximal mean reduction in HbA1c from baseline is achieved by week 12 (*Figure 1*), thus allowing the comparison of week 12 data among Phase 3 and Phase 2 monotherapy trial, the latter were of 12 to 14-weeks duration.

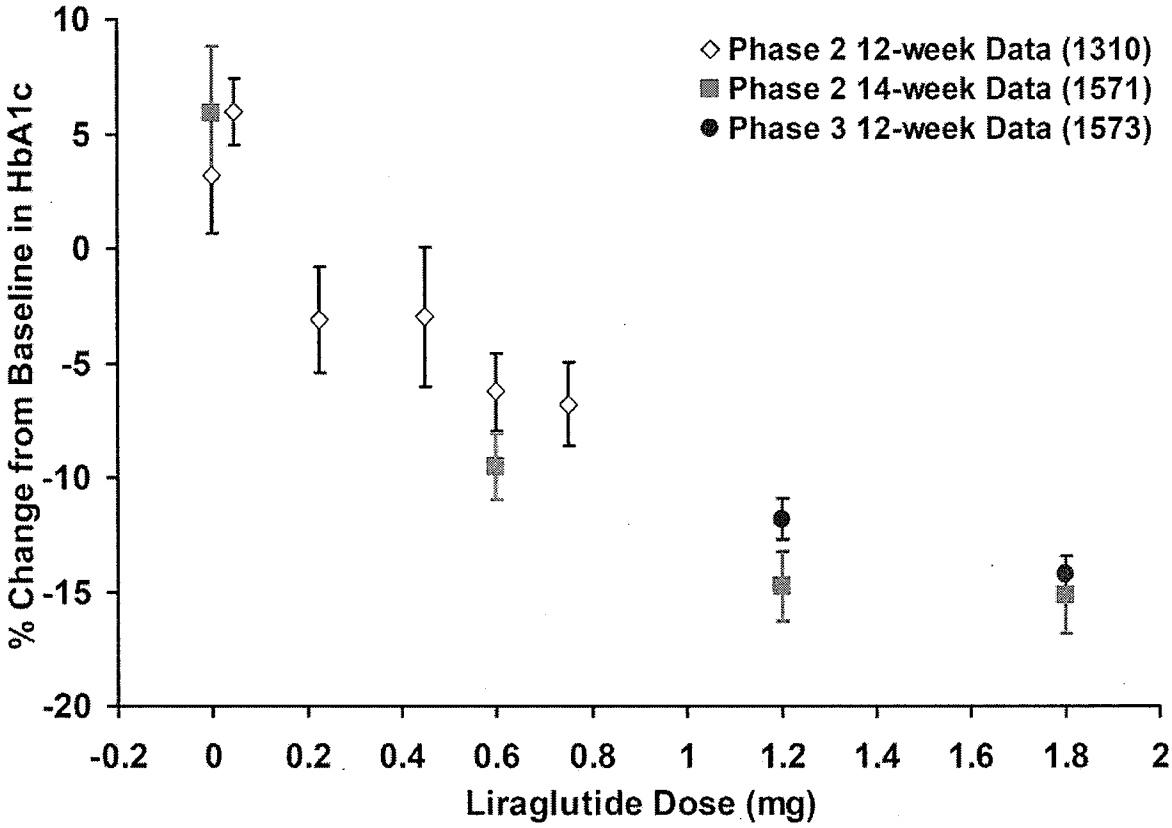
Figure 1. Time course of change from baseline in HbA1c from the 52-week Phase 3 confirmatory trial (1573)



Graphically, the response with 0.6 mg was in reasonable proximity to half-the maximal response (*Figure 2*). Graphical analysis of pooled dose-response data from Phase 2 and Phase 3 studies showed that the liraglutide treatment is associated with a dose dependent

reduction in HbA1c from baseline (see *Figure 2*). The maximal effect is achieved at 1.2 mg dose with a numerical advantage of 1.8 mg over 1.2 mg with regards to maximal HbA1c reduction.

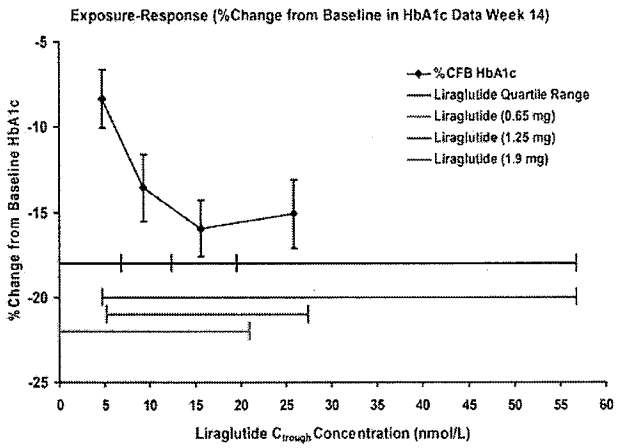
Figure 2. Dose dependent increase in effectiveness of liraglutide based on Mean(±SE) %change from baseline in HbA1c from 12-week Phase 2 trial (1310), 14-week Phase 2 trial (1571), and 12-week data from the 52-week Phase 3 confirmatory trial (1573)



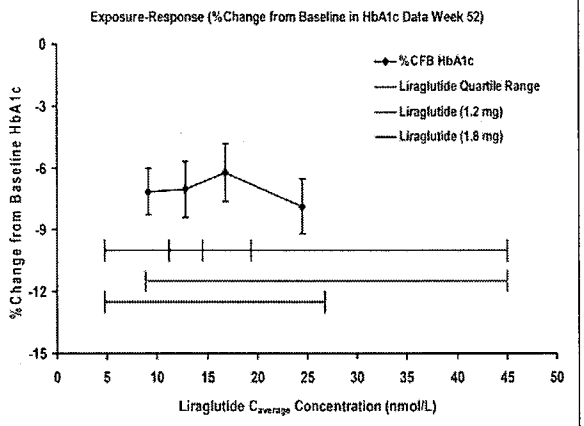
Further the graphical evaluation of exposure-response data revealed that %change from baseline in HbA1c decreased with increasing liraglutide concentration (see *Figure 3*). There was a considerable overlap in the exposures for 1.2 mg and 1.8 mg doses.

Figure 3. Concentration-Response relationship of liraglutide in (a) Phase 2 exploratory (1571) trial and Phase 3 confirmatory (1573) trial PK/PD data

(a) *Mean(SE) %change in HbA1c from baseline versus mean liraglutide C_{trough} of each quartile range* ***(Phase 2)***



(b) *Mean(SE) %change in HbA1c from baseline versus mean liraglutide Caverage concentrations of each quartile range* ***(Phase 3)***

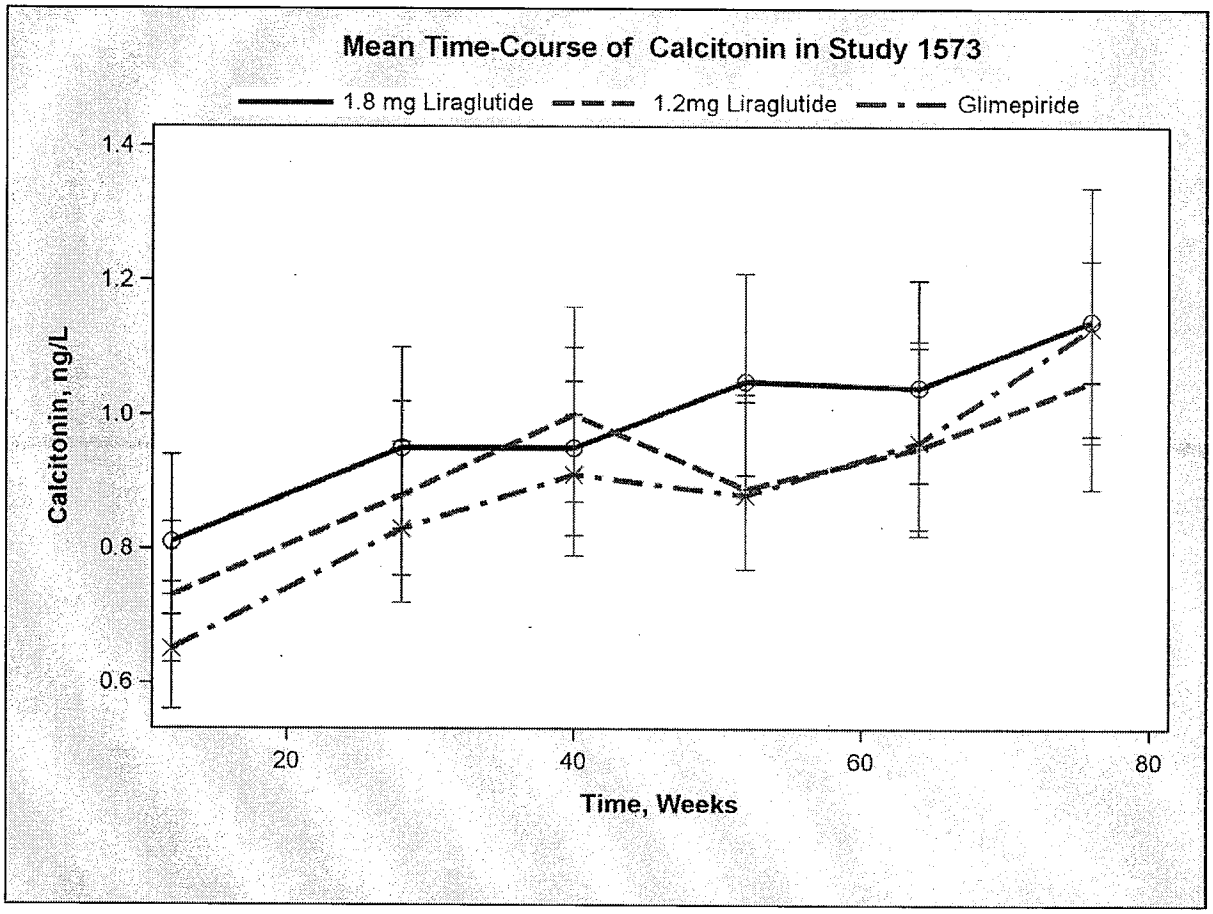


The exploratory PK/PD analysis using fasting plasma glucose (FPG) data from Trial 1571 also revealed that the liraglutide concentrations resulting from the doses 0.6 mg and above exceeded the predicted EC_{50} value of ~4 nmol/L estimated from the analysis of liraglutide-FPG relationship. This was consistent with the graphical analysis of dose-response and exposure-response presented above.

1.1.2 What is the liraglutide dose or exposure-calcitonin relationship (safety) with regards to effects on Thyroid C-cells in type 2 diabetic patient population?

The mean Calcitonin versus time profile from Phase 3 monotherapy trial showed that there was a gradual increase in Calcitonin for liraglutide and active comparator. However, among the liraglutide treatment arms, dose-response was not consistent at all the time points. Although in general mean Calcitonin levels appeared to be higher for 1.8 mg dose in comparison to 1.2 mg dose, there was considerable overlap in 95% CI at all time points (*Figure 4*). Further, the add-on to metformin trial (Study 1572) also did not reveal a consistent increase in calcitonin levels and dose levels of 0.6, 1.2 and 1.8 mg were indistinguishable with regards to the serum Calcitonin levels at all the time points (see Appendix *5.3*).

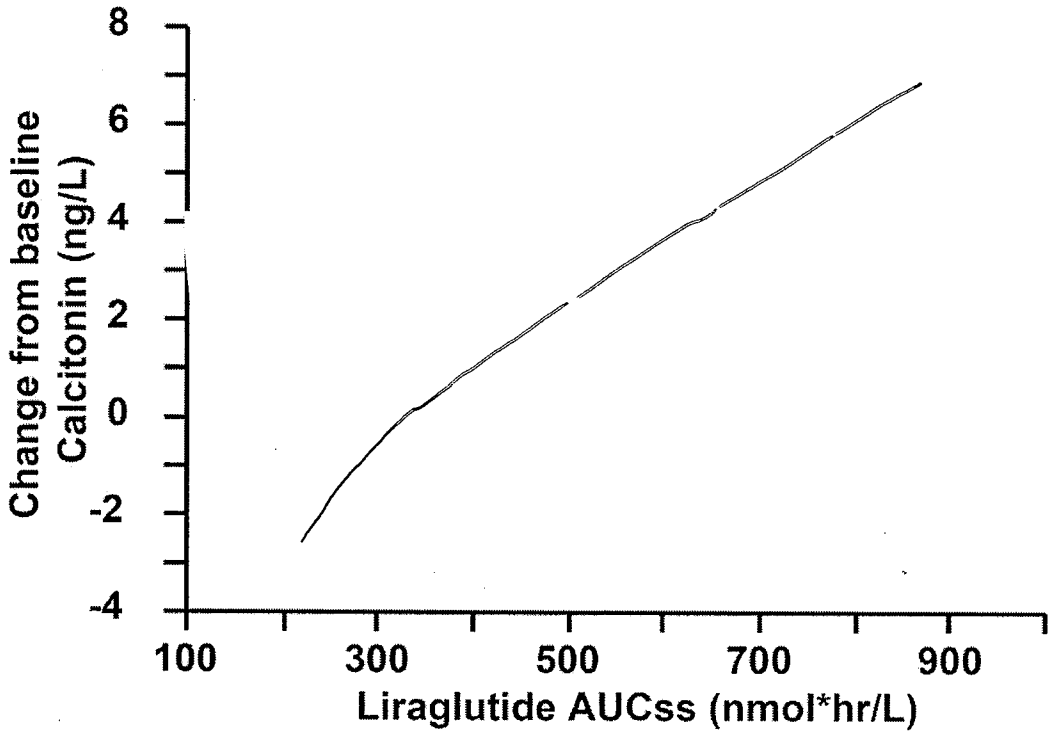
Figure 4. Time course of Calcitonin from the 52-week Phase 3 confirmatory trial (1573)



[Note: The LS mean estimates are from a repeated measurements analysis for normal censored data with time, treatment, gender and treatment by time interaction as fixed effects and subject as random effect. Error bars represent 95% confidence intervals around the LS mean. Source: Sponsor's Table 3-5 Page 187 Report 2.7.4 Summary of Clinical Safety.pdf]

Further, the graphical evaluation shows a flat relationship between change from baseline in Calcitonin at week 26 and steady-state liraglutide exposure (see *Figure 5*).

Figure 5. Flat steady-state liraglutide AUC-change from baseline in Calcitonin relationship at week 26 from the 52-week Phase 3 confirmatory trial (1573)



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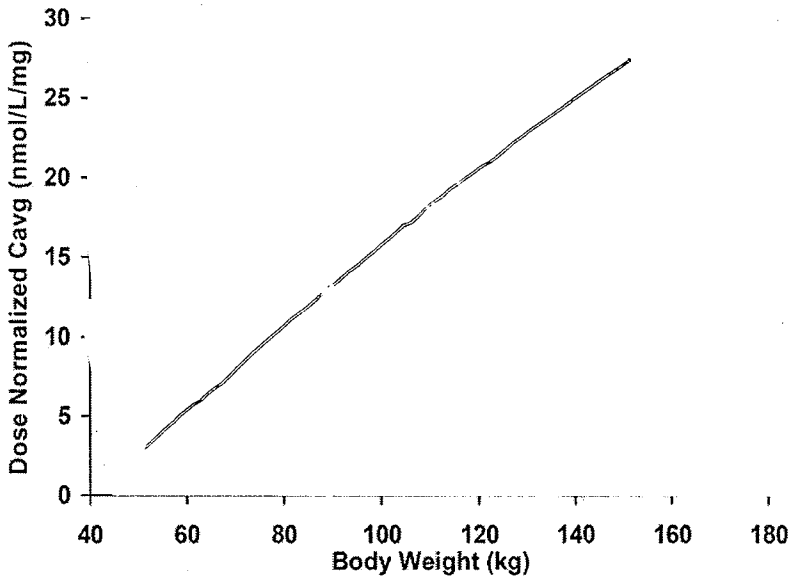
1.1.3 What is the influence of body weight, gender and race on PK of Liraglutide in type 2 diabetic patient population?

Body weight was found to be a significant predictor of the apparent clearance of liraglutide, as shown in *Figure 6*. The clearance changed from 0.6 L/hr to 1.8 L/h over a body weight range of 40 to 160 Kg. Body weight also explained 5% points of the inter-subject variability when applied as covariate on clearance (reduced from 36% to 31%). Exposures of 160 kg patient with reference to median weight of 90 kg were around 40% lower and expected Cavg was 9 nmoL/L and 13 nmol/L using 1.2 mg and 1.8 mg dose, respectively. However, no dose adjustment based on bodyweight is being proposed. (See exposure-response for further details).

Figure 6. Liraglutide exposure decreases with increase in body weight



b(4)

Females were found to have 34% lower liraglutide clearance than males in the population However, GENDER only explained 3% point of the inter-individual variability in body-weight adjusted clearance (reduced from 31% to 28%) and therefore, is not a significant predictor of the clearance. The effect of race (Blacks versus Others) was not evident from the data as well and couldn't be confirmed in reviewer's analysis (*Figure 10*).

1.1.4 Should liraglutide dose be adjusted based on body weight?

In patients with body weight 160 kg the expected C_{avg} is around 9 nmoL/L and 13 nmol/L using 1.2 mg and 1.8 mg dose, respectively. However, the liraglutide concentration-response (%change from baseline HbA1c) suggests that maximum effect is achieved at or above 7 nmol/L liraglutide concentration (which is the lowest limit of 2nd quartile) (see *Figure 3*). This was consistent for the Phase 3 data where the observed concentrations resulting from 1.2 mg and 1.8 mg liraglutide doses ranged from 5 nmol/L to 45 nmol/L (see *Figure 3b*). Hence, it can be inferred that the proposed doses provide adequate liraglutide exposures over the body weight range of 40-160 kg, and does not warrant for any weight based dose adjustment in this population.

1.1.5 Are the labeling claims based on population analysis of liraglutide in type 2 diabetic patient population justified?

Yes, based on the results of the population PK analysis, sponsor's proposal of no dose adjustment based on age (elderly population), gender and race is justified. These covariates do not affect the liraglutide pharmacokinetics in a clinically meaningful way.

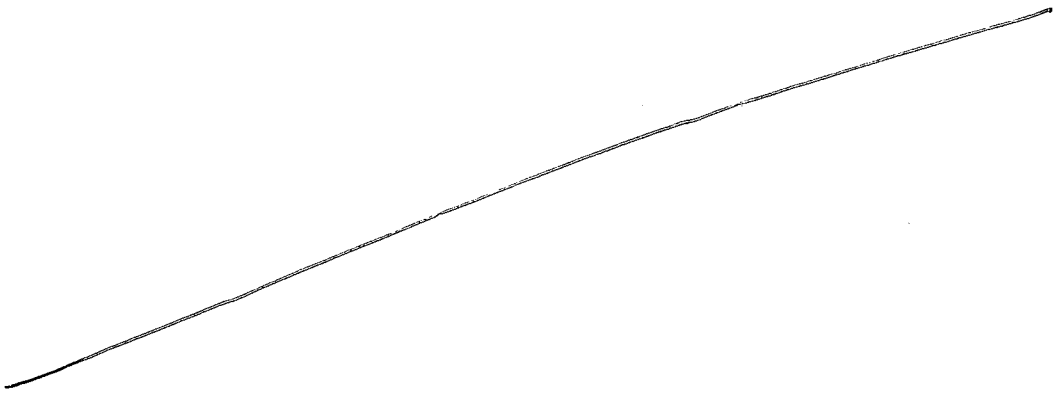
1.2 Recommendations

The sponsor's proposed doses are acceptable from clinical pharmacology perspective. The labeling statements based on the population PK analysis as proposed by the sponsor are acceptable except as noted below in the recommendations.

1.3 Label Statements

Labeling statements to be removed are shown in ~~red strikethrough font~~ and suggested labeling to be included is shown in underline blue font.

Proposed Text:



b(4)

2 PERTINENT REGULATORY BACKGROUND

The sponsor, Novo Nordisk, submitted an original NDA on May 23, 2008 for liraglutide, a GLP-1 analog intended for treatment as an adjunct therapy to diet and exercise to improve glycemic control in subjects with type 2 diabetes mellitus. Liraglutide is developed for once-daily administration as:

- Monotherapy
- Combination therapy with one or more oral antidiabetic drugs (metformin, sulphonylureas or a thiazolidinedione) when previous therapy does not achieve adequate glycaemic control.

For all patients the proposed dosing regimen is that liraglutide should be initiated with a dose of 0.6 mg for at least one week, after which the dose should be increased to 1.2 mg. Based on clinical response and after at least one week the dose can be increased to 1.8 mg to achieve maximum efficacy.

3 RESULTS OF SPONSOR'S ANALYSIS

The phase 3a study, NN2211-1573, was used to capture liraglutide concentration data and develop a population pharmacokinetic (PK) model. This model was used to explore the inter-subject variability observed in liraglutide plasma concentrations and to determine if covariates within the population explain some of this variability. The covariate analysis was performed using forward addition of all covariates that were significant at the 5% level, and backward elimination of covariates not significant at the 0.1% level. The covariates assessed were dose, body weight, body mass index (BMI), age, gender, race, ethnicity and time.

According to sponsor's analysis:

- A one-compartment model with first order absorption and elimination, with the absorption parameter, Ka, fixed to 0.0704 h^{-1} best described the available pharmacokinetic data. Both clearance (CL/F) and volume of distribution (Vd/F) and their variability could be estimated. The CL/F population mean estimate was 0.0126 L/h/kg with a 30.8 % CV and the population mean estimate of Vd/F was 0.373 L/kg with a 106.8 % CV between subjects. However, a covariance structure on these parameters did not significantly improve the fit or estimation of variability.
- The residual error was best described with a proportional error model and was estimated to be 40.1 %.
- The population mean estimate of CL/F was similar to that obtained from phase 1 data, while the Vd/F population mean estimate was higher than that obtained from phase 1 data and may be due to misspecification of Ka.
- The full covariate model contained both gender and the four races as significant covariates. The mean population estimates of CL/F for Other Females, Other Males, Asian Females, Asian Males, Black Females, Black Males, White Females and White Males were, 0.0098, 0.0150, 0.0102, 0.0158, 0.0098, 0.0116, 0.0116 and 0.0149 L/h/kg, respectively, (CL/F in Other Females, Asian Females, Black Females, Black Males and White Females were 34.1%, 31.5%, 34.5%, 22.1% and 22.1% lower, respectively, compared to White Males. While the CL/F for Other Males and Asian Males were 0.7% and 6.04% higher, respectively, compared to White Males), with a single inter-subject variability on CL/F of 28% CV. The mean population estimate of Vd/F for both genders and all race groups was 0.378 L/kg, with an inter-subject variability of 92.3% CV. The residual error was estimated to be 40.1% CV.
- The final model was a reduced form of full covariate model, so that only two race groups remained (Black and Non Black), this, the final model, was found to be more parsimonious than the full covariate model containing four race groups. Neither the estimates of Vd/F or inter-subject variability on CL/F or Vd/F changed as the model was reduced.
- The estimated gender difference in CL/F between Blacks and Non Blacks differed somewhat. The Black Females had a 15% lower CL/F compared with Black

Males, while Non Black Females had a 23% lower CL/F compared with Non Black Males. However, Blacks appear to have a lower CL/F in general compared to Non Blacks, 0.0975 and 0.0116 L/h/kg for Black Females and Black Males, compared with 0.0115 and 0.015 L/h/kg for Non Black Females and Non Black Males, respectively. Compared to the CL/F of Non Black Males, the CL/F of Non Black Females, Black Females and Black Males were 23.3%, 35.0% and 22.7% lower respectively. The large difference between Black Females and Non Black Males is a consequence of the additive effect of gender and race.

- Though a statistically significant difference in the population mean estimates of CL/F between genders and race was found there is a substantial overlap between the individual values in the groups and the difference in CL/F between males and females and race appears small in comparison with the over all inter-subject variability. The mechanism causing the difference in CL/F has not been discerned.

Please refer to the following link for details on the population analysis report in EDR. **\\CDSESUB1\EVSPROD\NDA022341\0016**

Reviewer's Comments:

Sponsor conducted a well detailed population pharmacokinetic analysis. However, sponsor used weight normalized dose and concluded in their report that that weight and BMI did not influence PK when evaluated as covariate. Sponsors inference was incorrect as use of weight normalization stems from the assumption that weight is an inherent covariate with a linear relationship. Though, the use of weight normalization of dose could be a reasonable approach of discerning effect of additional covariates, it does not permit the estimation of effect size of body weight per se. Sponsors used 6 digit numerical ID in the input data and NONMEM rounds off the last two digits to nearest zero, potentially leading to ambiguous IDs in the output table with multiple subjects having similar IDs. However, the population analysis results were not affected by this issue. However, the following were noted:

(1) The available data only allowed reasonable estimation of clearance (CL/F) as most of the data were collected at or around trough (Time after Dose = 24h) and not around the T_{max}.

(2) Model consistently under-predicted the higher concentrations in the data set (see Figure 7) and thus did not adequately estimate the volume of distribution (V/F), due to the limitations of available data as stated above, though sponsor mentioned that it could be due to misspecification of absorption rate constant (Ka). In this reviewer's opinion, the other reason is that sponsor's assumption of no accumulation could be incorrect, as several of these higher concentrations were observed to occur between 20-30 h post-dose, which is not consistent with the expected peak and trough with Q24 h dosing. (For each of the visit when a sample was collected, the system was reset using EVID=4 in the dataset forcing no relationship of the observations to the previous event in a subject).

Considering that the sponsor's themselves acknowledge this limitation and focused their conclusions around CL and the factors affecting the CL of liraglutide, this issue is not investigated further and is accepted as a limitation of the model.

4 REVIEWER'S ANALYSIS

4.1 Introduction

The population pharmacokinetic analysis was repeated with nominal doses to determine the effect of body weight and other covariates (Gender and Race) on liraglutide pharmacokinetics as proposed by sponsor in their report.

Additional population pharmacodynamic analysis was conducted using the data from exploratory Phase 2 trial to assess the exposure-response relationship and how it supports the Phase 3 dose selection. Graphical analysis of dose and exposure-response with regards to efficacy (HbA1c reduction) and thyroid related safety (Calcitonin) from Phase 2/3 trials was also conducted. The details of this analysis are presented in section 4.4.2.

4.2 Objectives

Analysis objectives are:

- To determine the influence of weight and other covariates on liraglutide PK.

4.3 Methods

4.3.1 Data Sets

Reviewer's population PK analysis was performed using a modified data-set that was revised to include nominal doses and serial ID numbers instead of 6 digit numerical ID used by the sponsor. If a subject ID contains >5 digits in the input data, NONMEM will output 6 digits but rounds off the last two digits to nearest zero, potentially leading to ambiguous IDs in the output table with multiple subjects having similar IDs. However, the population analysis results were not affected by this issue.

Exploratory PKPD analysis dataset was created using the liraglutide concentration, fasting plasma glucose and HbA1c data collected during the 14 week exploratory Phase 2 study where, the liraglutide concentrations at steady-state and the fasting plasma glucose levels were co-measured at week 0, 2, 4, 6, 8 and 14.

Data sets used are summarized in Table 1.

Table 1. Analysis Data Sets

Study Number	Name	Link to EDR
NN1129-1573	newfinalv4.csv	NA
NN1129-1571	Liragpdr.csv	NA

4.3.2 Software

Data-set for the analysis was prepared using SAS v 9.1. NONMEM Version VI was used for the analysis and run using Wings for NONMEM VI on an IBM Thinkpad laptop computer T60, equipped with a Compaq Visual Fortran compiler. The diagnostic and other plots were generated using S-plus script.

4.3.3 Models

The base model of the sponsor (one-compartment model with first-order absorption and first-order elimination with IIV on CL and V) was used. Graphical analysis of the base model output (goodness-of-fit plots and Eta-covariate plots) was used to evaluate the adequacy of the model and selection of covariates for further evaluation. Allometric model with centering on median weight was used for evaluating body weight. Categorical covariates were tested using proportional model.

4.4 Results

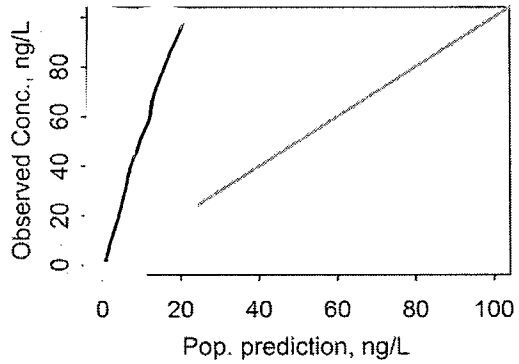
4.4.1 Population pharmacokinetics

Structural Model

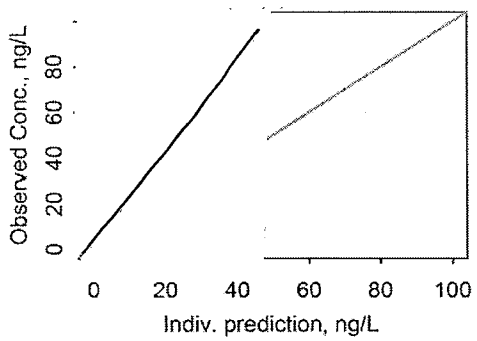
A one-compartment model with first-order elimination reasonably describes the population PK of liraglutide after multiple oral administration of s.c. doses of liraglutide in type 2 diabetic subjects titrated to 1.2 and 1.8 mg doses.

Figure 7. Diagnostic plots from the base model

(a) Observed versus population predicted (black dots) [trend line ( ) and line of unity (—)]

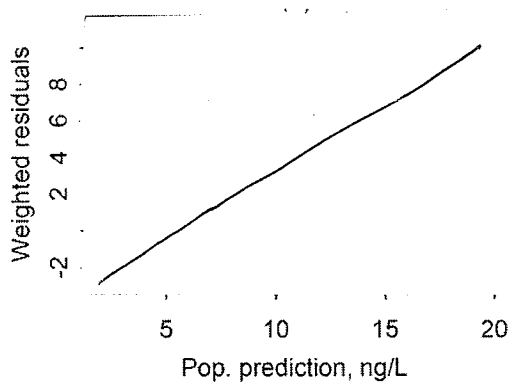


(b) Observed versus Individual predicted (black dots) [trend line ( ) and line of unity (—)]

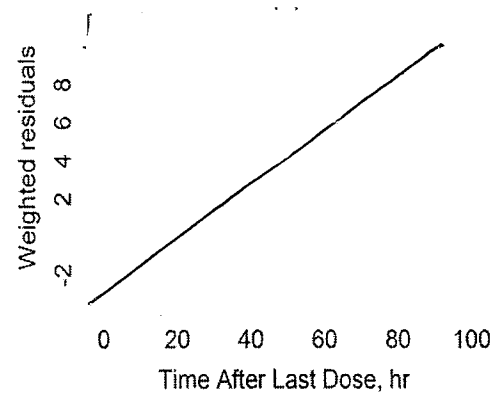


b(4)

(c) Weighted residual versus population predicted (black dots) [trend line ( ) and reference line at y=0 (—)]



(d) Weighted residual versus time after last dose (black dots) [trend line ( ) and reference line at y=0 (—)]

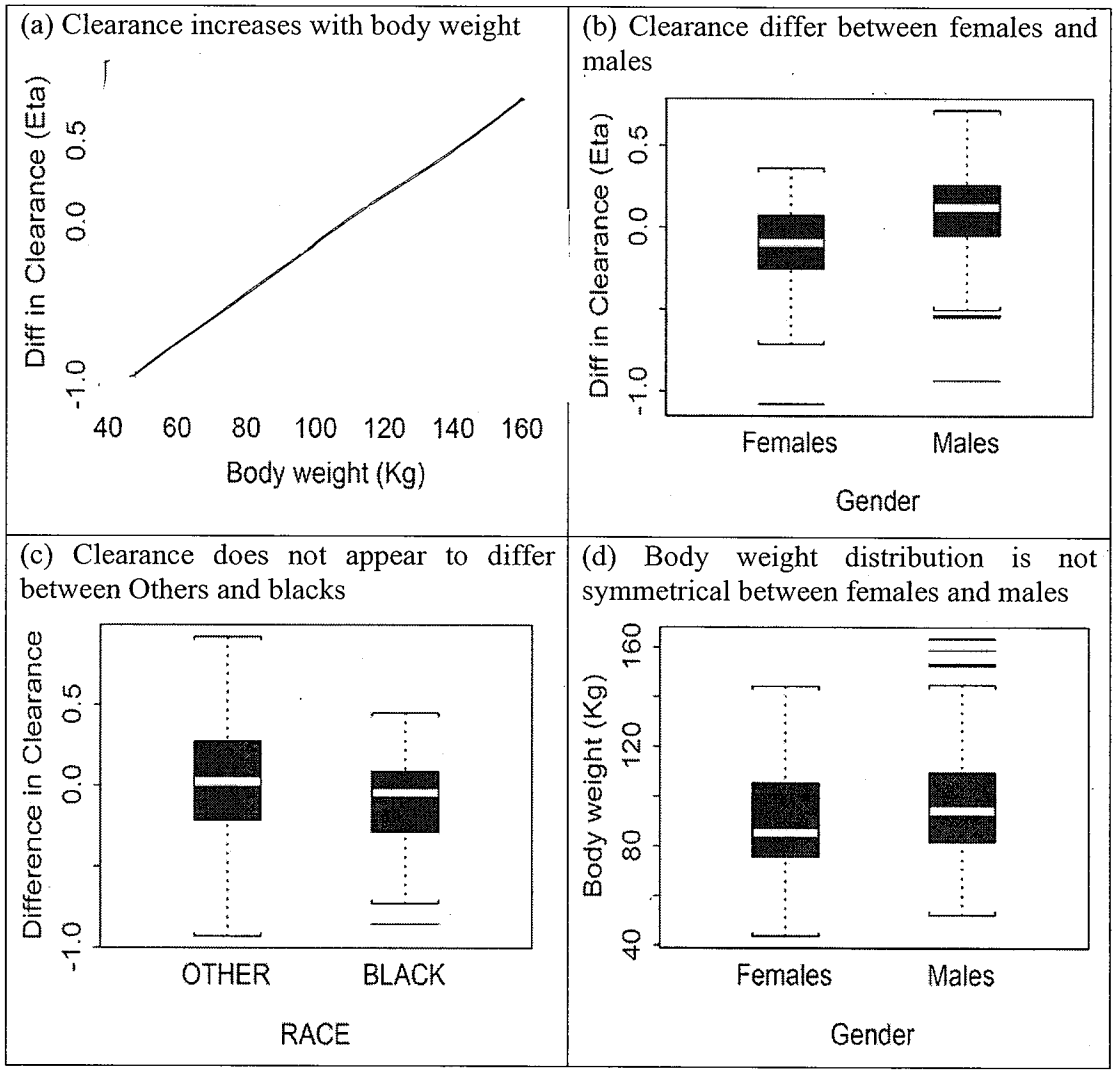


b(4)

Covariate Analysis

Eta-Covariate plots from the base model revealed that body weight, gender and race are likely predictors of the between-subject variability in the apparent oral clearance of liraglutide as shown in *Figure 8* below, though the body weight distribution differed slightly between females and males. It was also seen that body weight is correlated with body mass index (BMI) and gender. Hence body weight was the first covariate tested to explain the variability in clearance of liraglutide.

Figure 8. Body weight is a significant predictor of liraglutide clearance (Note: From Base Model)



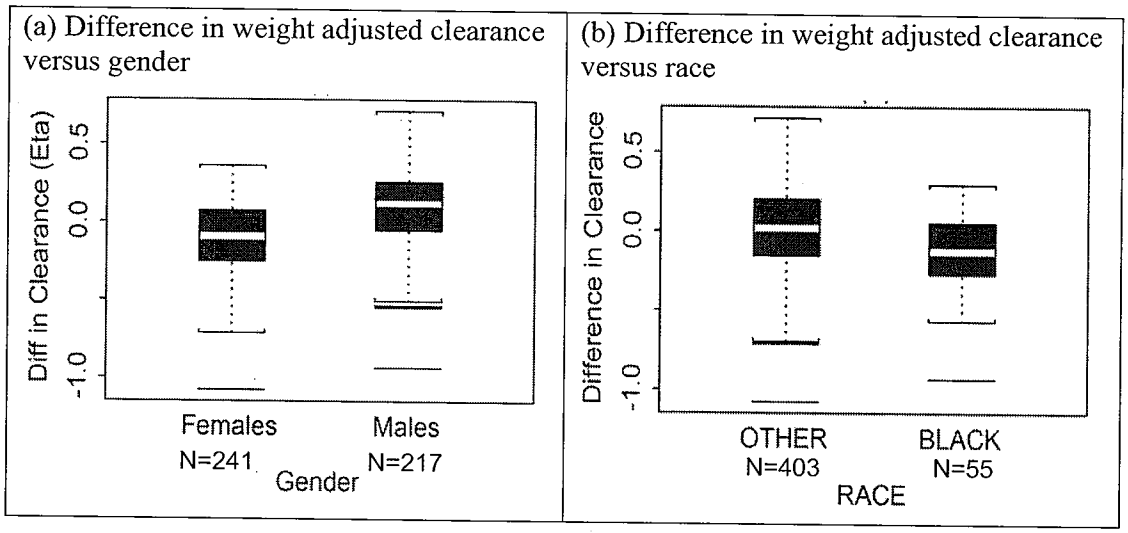
Body weight was found to influence the clearance (CL) of liraglutide and CL increased with body weight. When applied as a covariate, body weight explained 5% points of the inter-individual variability in clearance (reduced from 36% to 31%) and the final allometric relationship is presented in the equation below:

$$CL(L/h) = 1.11 * \left(\frac{WT}{90}\right)^{0.79}$$

Where, 1.11 L/h is the estimated population clearance at a median body weight (WT) of 90 Kg in the study population.

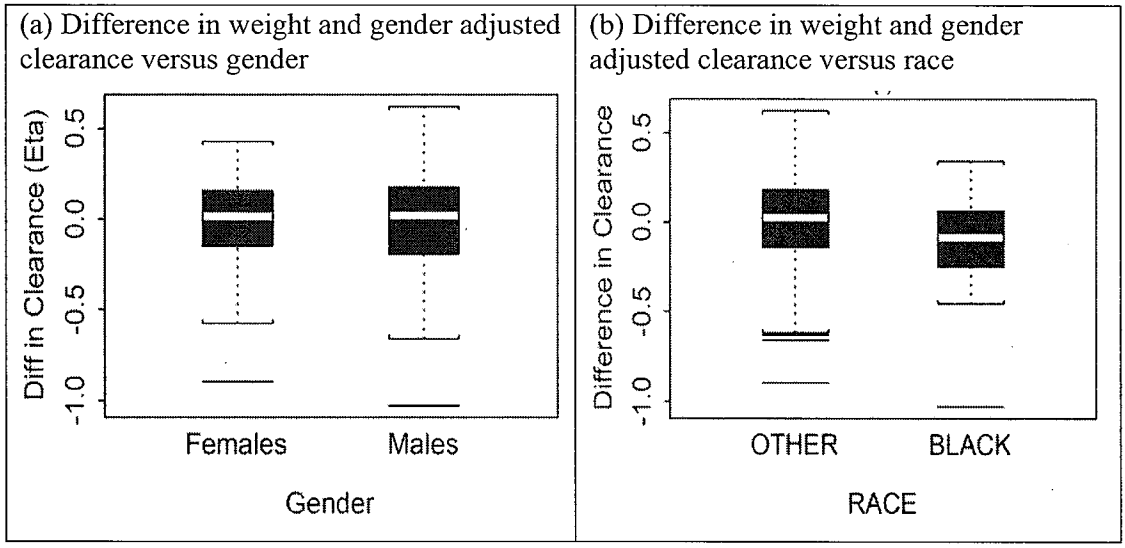
When clearance was adjusted for body weight (as a covariate), relationship of Gender was still evident on inter-individual variability (represented by Eta's in the model) with CL of liraglutide as shown in *Figure 9* below. This was in agreement to the observation in the sponsor's analysis

Figure 9. Difference in weight adjusted clearance is apparent by gender but not by race.



When applied as a covariate, GENDER further explained 3% points of the inter-individual variability in clearance (reduced from 31% to 28%) and revealed that females have 34% lower clearance than males in the population, though there was no biologically plausible reason for this difference. Moreover, this difference does not appear to be clinically meaningful considering the concentration-response relationship of liraglutide (*Figure 3b*). However, RACE effect on CL, as proposed by sponsor, did not appear convincing from the graphical analysis due to the fact that difference in body weight adjusted clearance overlapped between the two groups (see *Figure 10*) and blacks only represented 12% of the population to assess any meaningful differences. Further, the effect of RACE could not be confirmed in the reviewer's analysis. When applied as a covariate on weight and gender adjusted clearance, the estimate of relative difference among the two race groups was 0.3% and covariance step was not executed. The significance of RACE as an influential covariate was further not supported by the small increase in OFV of ~0.4 units when this covariate was tested.

Figure 10. Difference between individual and population predicted clearance is not apparent for race after adjusting CL for WT and GENDER



4.4.2 Exploratory PK/PD and Graphical Exposure-Response Analyses

Exploratory PK/PD:

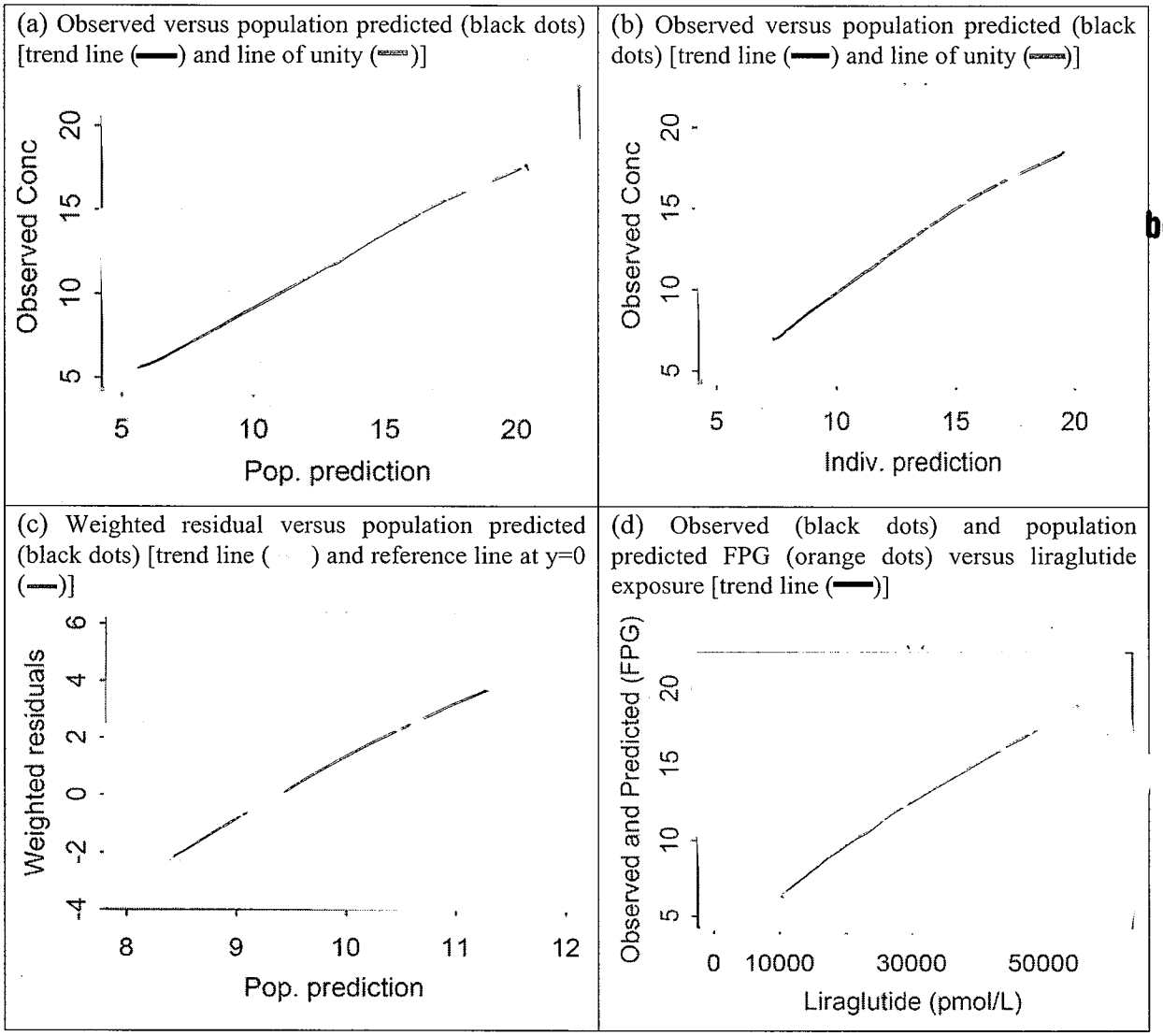
The liraglutide concentration, fasting plasma glucose and HbA1c data collected during the 14 week exploratory Phase 2 study was utilized to assess the exposure-response relationship and the effect of covariates. The liraglutide concentrations at steady-state and the fasting plasma glucose levels were measured at week 0, 2, 4, 6, 8 and 14. Based on the graphical assessment of data, the following model was used to explore the exposure-response relationship.

$$Y = E_0 * \left(1 - \left[\frac{E_{MAX} * LIRA}{EC_{50} + LIRA}\right]\right) + ERR(1),$$

Where, E_0 is FPG when liraglutide concentration is 0, E_{MAX} is the maximum proportional change in FPG from E_0, EC_{50} is the concentration eliciting 50% of the E_{MAX}, LIRA is liraglutide concentration and ERR(1) is the residual error. The data from week 4 and onwards was utilized as the response (FPG) data indicated to have achieved steady-state permitting the use of a direct-response relationship.

The model reasonably described the exposure-response data as evident from the model diagnostic plots (see *Figure 11*). The results from the base model showed that E_0 was 11.9 mmol/L (RSE 2.2%) with an inter-subject variability of 17%; (RSE 17%), E_{MAX} of 0.35 (RSE 10.3%) with an inter-subject variability of 53% (RSE 278%) and EC_{50} was estimated to be 3530 pmol/L (RSE 47%) with an inter-subject variability of 69% (RSE 178%).

Figure 11. Diagnostic plots from the base PD model

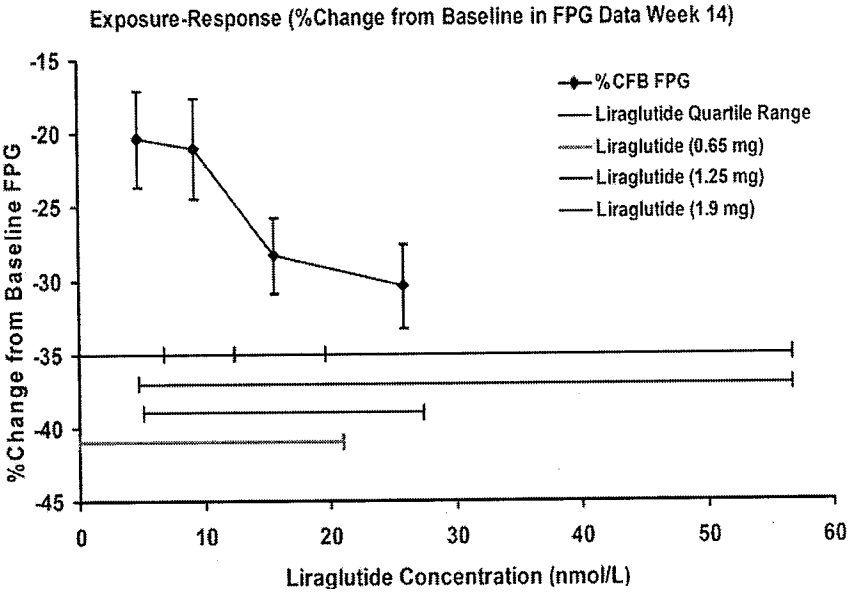


b(4)

b(4)

Although the available data did not permit a very precise estimate of inter-subject variability in E_{MAX} (RSE 128% on ω^2_{EMAX}) and EC_{50} (RSE 178% on ω^2_{EC50}), it does provide reasonable information that the there was around 35% maximum reduction in FPG, and 50% of this maximal response was achieved with an EC_{50} of 3530 pmol/L (~4 nmol/L) liraglutide concentration. Exposure-response was also evident from the visual assessment of mean %change in FPG versus concentration (means of the four quartiles) from the Phase 2 Trial data, presented in *Figure 12* below. The Phase 2 results were, however, supportive of the Phase 3 dose selection.

Figure 12. Mean (SE) %change from baseline versus mean liraglutide in each quartile range (Phase 2)



The graphical assessment of E_0, E_{MAX} and EC_{50} versus covariates (WT, BMI, AGE, BASE) only revealed E_0 and BASE (baseline FPG) correlation as expected, which was addressed by the mathematical model and hence not explored further. Also the PK/PD analysis was attempted with HbA1c but was not successful, most likely due to lack of data as the HbA1c data was measured only at week 8 and 14.
Some additional plots from the Phase 2 data are presented in *Appendix 5.2*.

Exposure-Response (Efficacy) Assessment:

The dose-response was analyzed graphically from the pooled data (week 12/14) from Phase 2 and 3 monotherapy trial. The exposure-response with regards to efficacy (HbA1c) was also evaluated for Phase 2 and Phase 3 studies.

Exposure-Response (Safety) Assessment:

Liraglutide caused Thyroid C-cell tumors in mice and rats in long-term toxicity studies at or above equivalent human exposure. This finding was associated with dose dependent increase in incidence of tumor and an increase in serum Calcitonin levels, which is a hormone secreted from C-cells. The serum Calcitonin was also measured in the 52 week monotherapy trial 1573 (including its extension phase) where liraglutide concentration was also measured for population PK analysis. The serum Calcitonin was also measured in other efficacy trials as part of thyroid safety investigation following long-term liraglutide administration.
The Sponsor compared the Calcitonin data from all trials at week 26 in their analysis as majority of efficacy trials were of 26 week duration. For the individual studies, the Calcitonin data was also analyzed using a repeated measurement analysis for normal censored data, where the logarithm of calcitonin was the (censored) response. The calcitonin data was characterized by a large percentage of the results being below the

lower limit of quantification (LLOQ). To obtain a quantitative estimate of the trends over time and treatment these observations were considered censored results in the sponsor's analysis, as the information was only that the observation was less than 0.7 ng/L. This was incorporated into the statistical model by adding the information into the likelihood function; in statistical terms the contribution to the likelihood function for the censored observations corresponds to the distribution function taken at 0.7 ng/L. Separate estimation and pair-wise comparisons of treatment effect were made at all visits where calcitonin was measured to enable an evaluation of the trends over time. The statistical model included an interaction effect between treatment and time to ensure independent estimates for each time-point. The analyses per time-point were made irrespectively of whether the interaction effect was statistically significant. This analysis revealed an overall trend of dose-dependent increase in the serum Calcitonin. In the reviewer's analysis, the Calcitonin data from monotherapy trial (1573) was evaluated for dose-response using the sponsor's results from repeated measurement analysis. The liraglutide concentration-response was evaluated for change from baseline in calcitonin at week 26 to determine if it corresponds to the dose-response reported by the sponsor. In addition, the time-course of serum Calcitonin for the combination trial with metformin (1572) was also evaluated.

5 APPENDIX

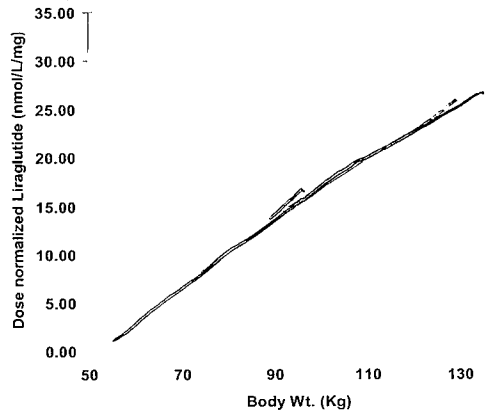
5.1 Listing of Analyses Codes and Output Files

File Name	Description	Location in \\cdsnas\pharmacometrics\
finalv4.xpt	Sponsor's NONMEM data set for population PK analysis	NDA22341_Liraglutide\Datasets
Nonmemformat.sas	Reviewer's SAS code for dataset preparation	NDA22341_Liraglutide\Datasets
Newfinalv4.csv	Reviewer's NONMEM data set for population PK analysis	NDA22341_Liraglutide\Datasets
Newfinalv5.csv	Reviewer's NONMEM data set for graphical exposure-response assessment with baseline, change from baseline and %change from baseline in HbA1c at week 52 information added to newfinalv4	NDA22341_Liraglutide\Datasets
Lirag1.ctl and Lirag2.ctl	Reviewer's NONMEM base model code for population PK analysis	NDA22341_Liraglutide\Controlstream
Lirag3.ctl	Reviewer's NONMEM model code for population PK analysis with WT as covariate on CL	NDA22341_Liraglutide\Controlstream
Lirag4.ctl* **(Final model)**	Reviewer's NONMEM model code for population PK analysis with WT and GENDER as covariate on CL	NDA22341_Liraglutide\Controlstream
Lirag5.ctl	Reviewer's NONMEM model code for population PK analysis with WT, GENDER and RACE as covariate on CL	NDA22341_Liraglutide\Controlstream
Lirag6.ctl	Reviewer's NONMEM model code for population PK analysis with WT and RACE as covariate on CL	NDA22341_Liraglutide\Controlstream
Lirag7.ctl	Reviewer's NONMEM model code for population PK analysis with WT and GENDER as covariate on CL (same as Lirag4.ctl) with response data included in the output for plotting purpose	NDA22341_Liraglutide\Controlstream
LiragN.df (N= 1 to 7)	NONMEM output files generated by Wings for NONMEM for respective Control stream files	NDA22341_Liraglutide\Output
liraN.ssc (N=1 to 7)	S-plus script used to generate plot	NDA22341_Liraglutide\Plots
PlotsliragN.pdf (N=1 to 7)	S-plus generated plots	NDA22341_Liraglutide\Plots
Lirapdr.csv (PD dataset prepared	Reviewer's Additional PD analysis datset, controlstream, output, s-plus	NDA22341_Liraglutide\Additional

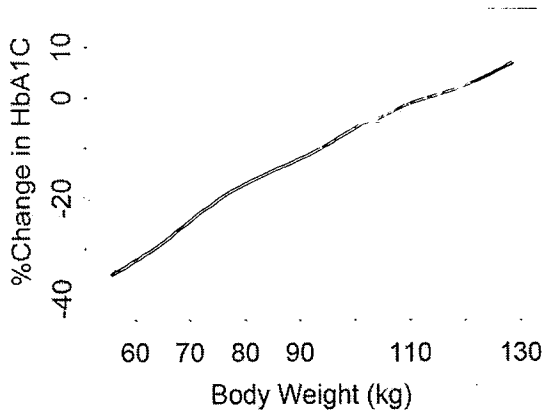
using JMP), lirapd.ctl, Liragpd.df, Liragpd.ssc Liragpd.pdf	script and plot files, respectively	

5.2 Exploratory PK/PD Plots from Phase 2 Trial 1571

(a) Impact of body weight on observed dose normalized liraglutide concentrations

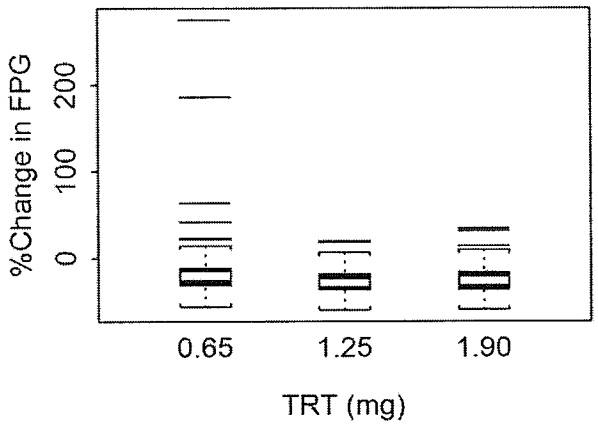


(b) Impact of body weight on observed liraglutide response (%CFB in HbA1c)

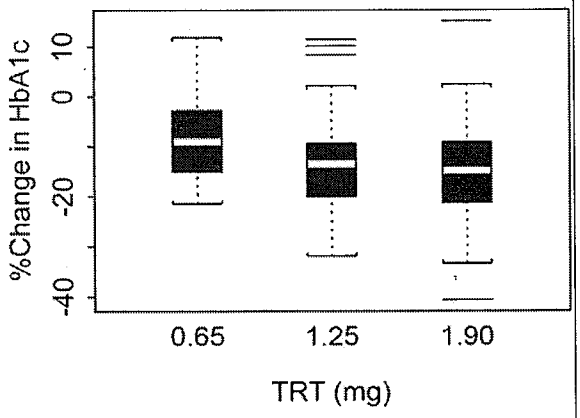


b(4)

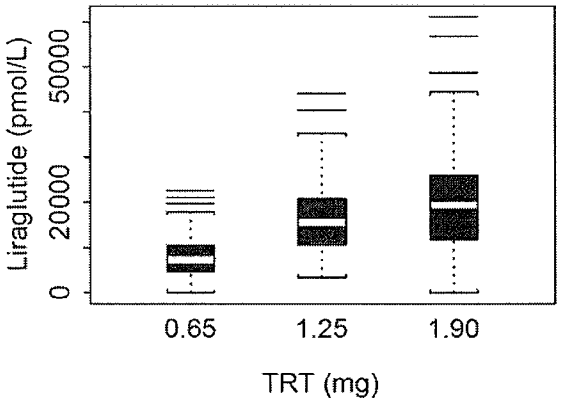
(c) Dose-dependent increase in effectiveness of liraglutide (%CFB in FPG)



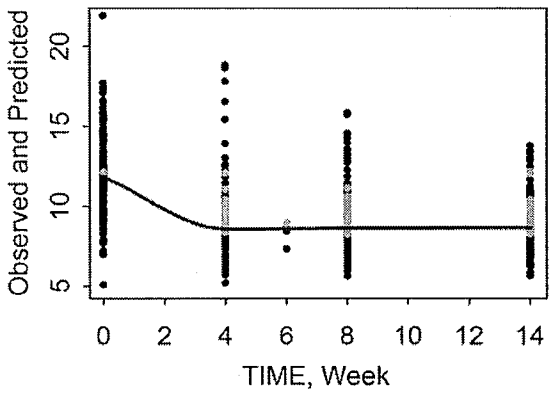
(d) Dose-dependent increase in effectiveness of liraglutide (%CFB in HbA1c)



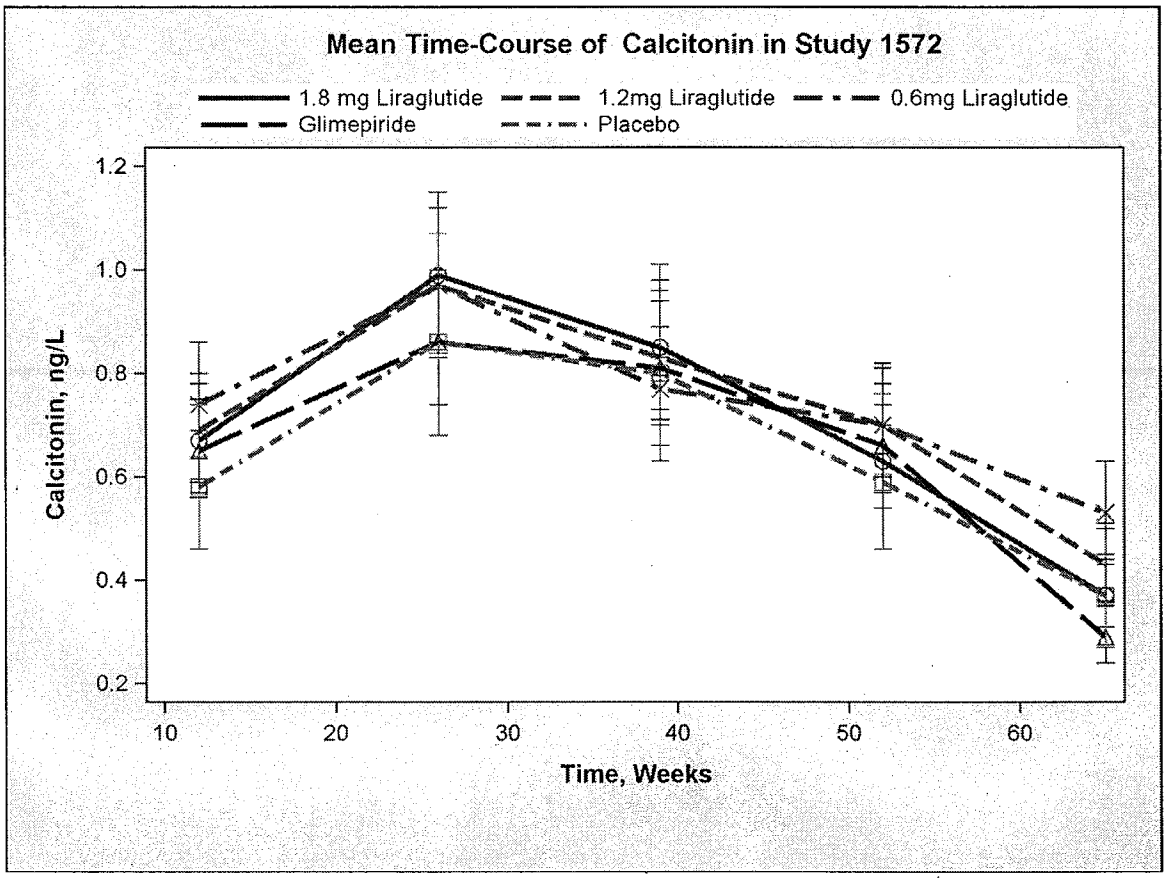
(e) Dose-dependent increase in liraglutide exposure



(f) FPG versus time [observed (•) and model predicted(◦)]



5.3 Calcitonin time course in Trial 1572



[Note: The LS mean estimates are from a repeated measurements analysis for normal censored data with time, treatment, gender and treatment by time interaction as fixed effects and subject as random effect. Error bars represent 95% confidence intervals around the LS mean. Source: Sponsor's Table 3-6 Page 188 Report 2.7.4 Summary of Clinical Safety.pdf]

4.4 OCP FILING MEMO

Office of Clinical Pharmacology *New Drug Application Filing and Review Form*			
General Information About the Submission			
	Information		Information
NDA Number	22-341	Brand Name	Victoza® (Proposed)
OCP Division (I, II, III, IV, V)	DCP II	Generic Name	Liraglutide
Medical Division	DMEP	Drug Class	
OCP Reviewer	Manoj Khurana, Ph.D.	Indication(s)	An adjunct therapy to diet and exercise to improve glycemic control in subjects with type 2 diabetes.
OCP Pharmacometrics Reviewer	Primary: Manoj Khurana, Ph.D. Secondary: TBD	Dosage Form	Injection
OCPB Team Leader	Sally Choe, Ph.D.	Dosing Regimen	Once Daily
Date of Submission	May 23, 2008	Route of Administration	Subcutaneous
Estimated Due Date of OCP Review	December 23, 2008	Sponsor	NOVO NORDISK
PDUFA Due Date	March 23, 2009	Priority Classification	Standard
Division Due Date			

Clin. Pharm. and Biopharm. Information				
	"X" if included at filing	**Number of studies submitted**	**Number of studies reviewed**	**Critical Comments If any**
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	**X**			
Tabular Listing of All Human Studies	**X**			
HPK Summary	**X**			
Labeling	**X**			
Reference Bioanalytical and Analytical Methods	**X**			
I. Clinical Pharmacology	**X**			
Mass balance:	**X**	1	1	**NN2211-**1699#
Isozyme characterization:	**X**	5	5	NN205145, NN206480, NN206665, NN207147, NN207312
Blood/plasma ratio:				
Plasma protein binding:	**X**	2	2	NN200152, NN201223
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	**X**	2	2	NN2211-1149# (Dose Escalation) NN2211-1326 (PKPD in healthy Japanese Subjects)
multiple dose:	**X**	2	2	**NN2211-**1694, 1551, 1591 (PKPD in healthy Japanese Subjects)
Patients-				
single dose:	**X**	3	3	**NN2211-**1219, 2063, 1224

multiple dose:	X	2	2	NN2211-1332 NN2211-1591 (PKPD in T2DM Japanese Subjects)
Dose proportionality -				
fasting / non-fasting single dose:	X	1	1	NN2211-1189
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:	X	2	2	**NN2211-** 1330#, 1608#
In-vitro:	X	3	3	NN980278, NN201224, NN203029
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:	X	1	1	NN2211-1327#
renal impairment:	X	1	1	NN2211-1329# (Trial also included 3 subjects with type 2 diabetes)
hepatic impairment:	X	1	1	NN2211-1328# (Trial also included 5 subjects with type 2 diabetes)
PD:				
Phase 1:	X	5	5	**NN2211-** 1219#, 1224#, 1589#, 1698#, 2063#
Phase 2:	X	1	1	NN2211-1332#
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:	X	1	1	NN2211-1644# (TQT)
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:	X	1	1	NN2211-1573
II. Biopharmaceutics				
Absolute bioavailability:	X			NN2211-1149
Relative bioavailability -				
solution as reference:	X	1	1	NN2211-1745# (Comparing different sites of injection)
alternate formulation as reference:	X	2		NN2211-1464 (Pulmonary) NN2211-1898 (Intranasal)
Bioequivalence studies -				
traditional design; single / multi dose:	X	4	4	**NN2211-** 1331#, 1636#, 1692#, 1693#
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				

III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		41	39	

Filability		
	"X" if yes	Comments
Application filable?	X	**Comments to the Sponsor:** Please submit the following datasets to support the population analysis conducted under NN2211-1573: • All datasets used for model development and validation should be submitted as a SAS transport files (*.xpt). A description of each data item should be provided in a Define.pdf file. Any concentrations and/or subjects that have been **excluded from the analysis** should be flagged and maintained in the datasets. • Model codes or control streams and output listings should be provided for all major model building steps, e.g., base structural model, covariates models, final model, and validation model. These files should be submitted as ASCII text files with *.txt extension (e.g.: myfile_ctl.txt, myfile_out.txt). • A model development decision tree and/or table which gives an overview of modeling steps. For the population analysis reports we request that you submit, in addition to the standard model diagnostic plots, individual plots for a representative number of subjects. Each individual plot should include observed concentrations, the individual predication line and the population prediction line. In the report, tables should include model parameter names and units. For example, oral clearance should be presented as CL/F (L/h) and not as THETA (1). Also provide in the summary of the report a description of the clinical application of modeling results.
Submission in Brief: See the details below.	**Reviewer's Comments:** **DSI inspection will be requested for the study NN2211-1692 trial site and its analytical site.** **Consult to IRT for review of TQT.** **Consult to PM group for the population analysis conducted under NN2211-1573.**	

#Studies submitted with Analysis Data sets

Submission in Brief:

Sponsor, Novo Nordisk has submitted the new drug application (NDA) 22-341 for liraglutide. Liraglutide (Arg34Lys26-(N-ε-(γ-Glu (N-α-hexadecanoyl)))-GLP-1[7-37]), a new molecular entity, is once-daily human GLP-1 analogue, in which lysine at position 34 has been replaced with arginine, and palmitic acid has been attached via a glutamoyl spacer to lysine at position 26. Liraglutide was discovered and is being developed by Novo Nordisk.

A comprehensive list of completed or ongoing clinical pharmacology and clinical trials is provided below:

Clinical Pharmacology Trials (Single-dose trials)

Healthy subjects:
1149, 1327, 1328*, 1329*, 1331, 1636, 1692, 1693, 1699, 1745, 1464 (pulmonary), NN9233-1898 (intranasal)

Subjects with type 2 diabetes:
1219, 1224, 2063

Japanese healthy subjects:
1326

Therapeutic Exploratory Trials (Intermediate-term trials)

Subjects with type 2 diabetes:
1310, 1571, 1499, 1333, 2072

Japanese subjects with type 2 diabetes:
1334

Non-diabetic, obese subjects:
NN8022-1807 (minus ext)

Ongoing Trials

1796, 1797, 1799
1573 extension
1572 extension
1700 and 1701 (Japanese phase 3a trials)
NN8022-1807 (obesity) extension

Clinical Pharmacology Trials (Short-term trials)

Healthy subjects:
1189*, 1330, 1608, 1644

Subjects with type 2 diabetes:
1332, 1589, 1698

Japanese healthy subjects:
1551 and 1694

Japanese subjects with type 2 diabetes:
1591

Therapeutic Confirmatory Trials (Long-term trials)

Subjects with type 2 diabetes:
1573 (52 wks + extension) #
1572 (26 wks + extension) #
1436 (26 wks)
1574 (26 wks)
1697 (26 wks)

Total: 38 trials excluding ongoing trials (i.e. trials where final statistical analyses were not yet available)
*included 5, 3 and 4 subjects with type 2 diabetes, respectively
Two reports are available for each of Trials 1573 and 1572; one covering all data from the double-blind main part of the trials and one covering all data from double-blind and open-label extension period until 21 Feb 2008. The latter reports only describe subjects who entered the open-label extension part of the trial.

Figure 1–1 Grouping of Liraglutide Clinical Trials

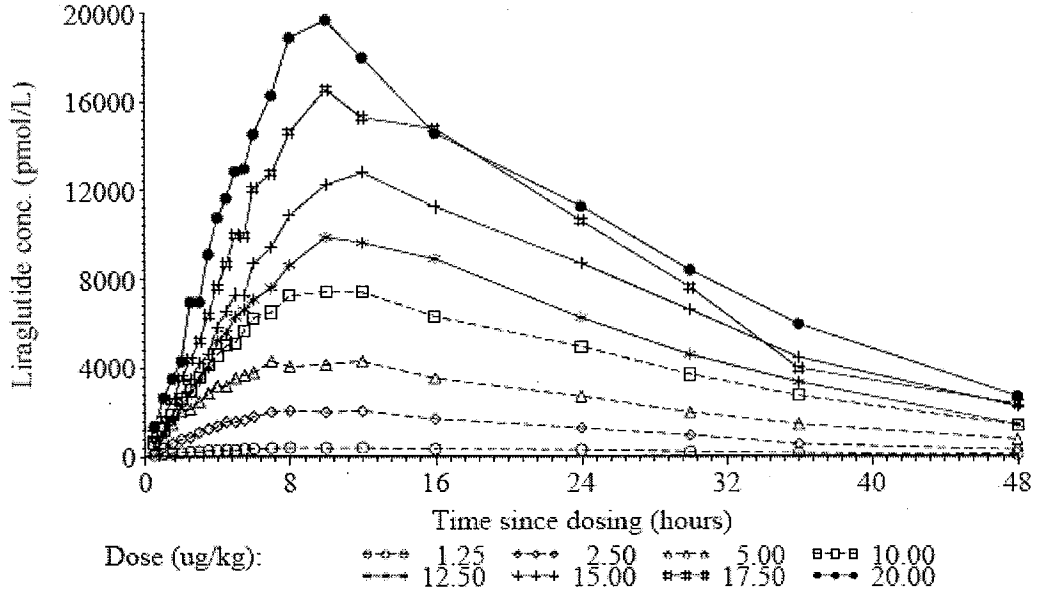
The clinical pharmacology program included 26 clinical pharmacology trials. These comprised:

- 19 trials in healthy subjects (including bioequivalence trials, trials in elderly subjects, subjects with renal or hepatic impairment and Japanese subjects),
- 7 trials in subjects with type 2 diabetes (including one trial in Japanese subjects).

The program was supported by evidence from five Phase 2 trials, a population pharmacokinetic analysis from the therapeutic confirmatory Trial 1573, and from ten in vitro studies performed with human biomaterials, i.e. cells, recombinant enzymes, plasma or plasma proteins.

The pharmacokinetic data demonstrated slow absorption of liraglutide (tmax = 10–13 hours) and a half-life of approximately 13 hours (10–18 hours), suitable for the intended once daily dosing. In addition, pharmacokinetics was demonstrated to be dose proportional for both single and

multiple doses. The absolute bioavailability was demonstrated to be 55% based on intravenous (i.v.) and subcutaneous (s.c.) administration of 5 µg/kg. The relative bioavailability was slightly lower in the thigh compared with the abdomen and the upper arm based on s.c. administration of 0.60 mg.



Mean Plasma Liraglutide Profiles following Single-dose Administration in Healthy Subjects – Trial 1149

Investigation of the metabolism of liraglutide in healthy subjects (Trial 1699) indicated that liraglutide is endogenously metabolised and that neither renal nor biliary excretion are major routes of clearance. The effect of liraglutide to slow gastric emptying did not affect the absorption of orally administered medical products to any clinically significant degree. At steady state concentrations, liraglutide did not alter the rate and extend of absorption of orally administered paracetamol, atorvastitin, griseofulvin, lisinopril, digoxin and oral contraceptives (ethinylestradiol and levonorgestrel) to a clinically relevant degree.

Mode of action trials in subjects with type 2 diabetes demonstrated glucose lowering as well as insulin secretion effects of liraglutide after a single s.c. dose (10 µg/kg). Furthermore, at a s.c. dose of 7.5 µg/kg, liraglutide did not impair glucagon response nor the general hypoglycaemic counterregulation response and liraglutide was no longer insulinotropic at hypoglycaemic plasma glucose concentrations. It has been shown, that liraglutide provides 24-hour glycaemic control and restores β-cell responsiveness to increasing blood glucose concentrations.

The six reported Phase 2 trials showed significant effects on glycaemic control (measured by HbA1c and fasting serum glucose) after 5–14 weeks of treatment. In addition, the trials showed that treatment with liraglutide was associated with weight loss. The Japanese Phase 2 trial (Trial 1334) showed similar results on glycaemic control, whereas no effect was seen on body weight. Preliminary results from the ongoing Phase 3a trials with completed clinical phase showed robust effects on glycaemic control (measured by HbA1c and fasting plasma glucose) after 6 months of treatment with liraglutide in various treatment regimens with liraglutide doses of 0.6 mg, 1.2 mg or 1.8 mg per day in combination with sulphonylureas, metformin or

thiazolidinediones in subjects with type 2 diabetes. Results from the Phase 3a trials confirmed the weight loss observed in the Phase 2 trials.

Attachment 1: Tabular Listing of Clinical Studies

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1331 DE	BE	Single-centre, randomised, single-blind, cross-over trial	Single s.c. doses of 1 mg liraglutide formulation 2 and formulation 3	22 (13/9)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1636 AU	BE	Single-centre, randomised, double-blind, cross-over trial	Single s.c. doses of 0.75 mg liraglutide formulation 3 at pH 7.7, 7.9 and 8.15	24 (13/11)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1692 SE	BE	Single-centre, randomised, double-blind, cross-over trial	Single s.c. doses of 0.72 mg liraglutide formulation 4 and formulation 4 with final manufacturing process for drug substance	21 (19/2)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1693 SE	BE	Single-centre, randomised, double-blind, cross-over trial	Single s.c. doses of 0.71 mg liraglutide formulation 3 and formulation 4	22 (19/3)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1745 DE	PK and BA	Single-centre, randomised, open-label, cross-over trial	Three single s.c. doses of 0.6 mg liraglutide in the abdomen, thigh and upper arm, respectively	21 (11/10)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1149 GB	PK, PD and BA	Single-centre, randomised, double-blind, placebo-controlled, parallel-group, dose-escalation trial	Eight single s.c. doses of 1.25, 2.5, 5, 10, 12.5, 15, 17.5 or 20 µg/kg liraglutide or one single i.v. dose of 5 µg/kg liraglutide	72 (72/0) (54 liraglutide, 18 placebo)	Healthy	Single-dose	Complete; Full; Module 5.3.3.1
NN2211-1699 NL	PK	Single-centre, open-label trial	Single s.c. dose of 0.75 mg radiolabelled liraglutide	7 (7/0)	Healthy	Single-dose	Complete; Full; Module 5.3.3.1

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1189 GB	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, parallel-group, dose-escalation trial	Initial single s.c. dose, and subsequent once daily s.c. doses with dose-escalation of 1.25, 5, 7.5, 10 and 12.5 µg/kg liraglutide	Healthy: 30 (30/0) (20 liraglutide, 10 placebo) Type 2: 4 (4/0) (2 liraglutide, 2 placebo)	Healthy and type 2	Single dose followed by 7 days	Complete; Full; Module 5.3.3.1
NN2211-1327 DE	PK	Single-centre, open-label, parallel-group trial	Single s.c. dose of 1 mg liraglutide	32 (16/16) (young: 16 (8/8); elderly: 16 (8/8))	Healthy, young and elderly	Single dose	Complete; Full; Module 5.3.3.3
NN2211-1329 NZ	PK	Single-centre, open-label, parallel-group trial	Single s.c. dose of 0.75 mg liraglutide	30 (22/8) divided in 5 groups according to renal function (healthy: 27; type 2 diabetes: 3)	Healthy, with normal renal function or renal impairment	Single dose	Complete; Full; Module 5.3.3.3
NN2211-1328 PL	PK	Single-centre, open-label, parallel-group trial	Single s.c. dose of 0.75 mg liraglutide	24 (14/10) divided in 4 groups according to hepatic function (healthy: 19; type 2 diabetes: 5)	Healthy, with normal hepatic function or hepatic impairment	Single dose	Complete; Full; Module 5.3.3.3
NN2211-1330 SE	DDI	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial	Once daily s.c. doses of 1.8 mg liraglutide (titrated in weekly steps of 0.6 mg). Single p.o. dose of 0.03 mg ethinylestradiol plus 0.15 mg levonorgestrel (i.e. one tablet of Neovletta®)	21 (0/21)	Healthy, post-menopausal women	3 weeks and single dose DDI	Complete; Full; Module 5.3.3.4

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1608 SE	DDI	Two-centre, randomised, double-blind, placebo-controlled, two-way cross-over trial in two parts (A and B)	Liraglutide: Once daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg) Part A: Single p.o. doses of 40 mg atorvastatin and 20 mg lisinopril Part B: Single p.o. doses of 500 mg griseofluvin and 1 mg digoxin	42 (19/13) in Part A and 28 (27/1) in Part B	Healthy	4 weeks and single dose DDI	Complete; Full; Module 5.3.3.4
NN2211-1644 US	QTc	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial followed by open-label moxifloxacin (positive control)	Liraglutide: Once daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg). Moxifloxacin: Single p.o. dose of 400 mg	58 (29/29)	Healthy	7 weeks	Complete; Full; Module 5.3.4.1
NN2211-1698 DE	PD and DDI	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial	Liraglutide: Once daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg. Paracetamol: Single p.o dose 1 g	18 (14/4)	Type 2	3 weeks	Complete; Full; Module 5.3.4.2
NN2211-1589 AU and DE	PD	Two-centre, randomised, double-blind, balanced incomplete latin square design trial. Placebo and active control (glimepiride)	Liraglutide: Once daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg). Glimepiride: Once daily p.o. dose of 1-4 mg Paracetamol (gastric emptying): Single p.o dose of 1 g	46 (27/19)	Type 2	4 weeks	Complete; Full; Module 5.3.4.2

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1332 DK	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial	Once daily s.c. doses of 6 µg/kg liraglutide for 9-10 days	13 (8/5)	Type 2	9-10 days	Complete; Full; Module 5.3.4.2
NN2211-1219 DK	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, single-dose, cross-over trial	Single s.c. dose of 10 µg/kg liraglutide	11 (6/5)	Type 2	Single dose	Complete; Full; Module 5.3.4.2
NN2211-2063 US	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial	Single s.c. dose of 7.5 µg/kg liraglutide	Type 2: 10 (6/4) Healthy: 10 (5/5) (not dosed)	Type 2 and healthy	Single-dose	Complete; Full; Module 5.3.4.2
NN2211-1224 DE	PK and PD	Two-centre, randomised, double-blind, placebo-controlled, cross-over trial	Single s.c. dose of 7.5 µg/kg liraglutide	19 (14/5)	Type 2	Single-dose	Complete; Full; Module 5.3.4.2
NN2211-1571 DK, FR, NL, SK	Efficacy and safety	Multi-centre, multi-national randomised, double-blind, placebo-controlled, parallel-group trial	Once daily s.c. doses of 0.65, 1.25 or 1.9 mg liraglutide for 14 weeks	Type 2: 163 (99/64) Healthy: 12 (9/3) (not dosed) Liraglutide 0.65 mg: 40 Liraglutide 1.25 mg: 42 Liraglutide 1.90 mg: 41 Placebo: 40	Type 2 and healthy	14 weeks	Complete; Full; Module 5.3.5.1
NN2211-1310 DK, NO, SE, GB	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind (open-label glimepiride), parallel-group trial. Placebo and active control (glimepiride)	Liraglutide: Once daily s.c. doses of 0.045, 0.225, 0.45, 0.60, or 0.75 mg. Glimepiride: Once daily p.o. dose of 1-4 mg	190 (127/63) Liraglutide 0.045 mg: 26 Liraglutide 0.225 mg: 24 Liraglutide 0.45 mg: 27 Liraglutide 0.60 mg: 30 Liraglutide 0.75 mg: 28 Glimepiride: 26 Placebo: 29	Type 2	12 weeks	Complete; Full; Module 5.3.5.1

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1499 AT, AU, CZ, DK, FR, DE, PL, GB	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind (metformin and glimepiride open-label), parallel-group trial Active control (metformin and glimepiride)	Liraglutide: Once daily s.c. doses of 2.0 mg (titrated in weekly steps of 0.5 mg) Metformin: b.i.d., p.o. doses of 1 g Glimepiride: Once daily p.o. doses of 2-4 mg	144 (91/53) Liraglutide 36 Liraglutide+metformin: 36 Metformin: 36 Metformin and glimepiride: 36	Type 2	5 weeks	Complete; Full; Module 5.3.5.1
NN2211-1573 US, MX	Efficacy, safety and popula-tion PK	Multi-centre, randomised, double-blind, parallel-group trial Active control (glimepiride)	Liraglutide: Once daily s.c. doses of 1.2 mg or 1.8 mg (titrated in weekly steps of 0.6 mg) Glimepiride: Once daily p.o. 8 mg/day	745 (371/374) Liraglutide 1.2 mg: 251 Liraglutide 1.8 mg: 246 Glimepiride: 248	Type 2	52 weeks	Complete; Full; Module 5.3.5.1
NN2211-1573 extension 1 US, MX	Efficacy and safety	Multi-centre, multi-national, randomised, open-label, parallel-group extension trial Active control (glimepiride)	Liraglutide: Once daily s.c. doses of 1.2 mg or 1.8 mg Glimepiride: Once daily p.o. 8 mg/day	440 (223/217) Liraglutide 1.2 mg: 149 Liraglutide 1.8 mg: 154 Glimepiride: 137	Type 2	Cut off date 21 Feb 2008 ~18 months	Ongoing; Full; Module 5.3.5.1
NN2211-1572 AR, AU, BE, BG, DE, DK, ES, GB, HR, HU, IE, IN, IT, NL, NO, NZ, RO, RU, SE, SK, ZA	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind, parallel-group trial (open-label extension) Placebo and active control (glimepiride)	Liraglutide: Once-daily s.c. doses of 0.6, 1.2 or 1.8 mg (titrated in weekly steps of 0.6 mg) in combination with metformin Metformin: b.i.d., p.o., 2 g/day Glimepiride: q.d., p.o., 4 mg/day	1087 (633/454) Liraglutide 0.6 mg + metformin: 242 Liraglutide 1.2 mg + metformin: 240 Liraglutide 1.8 mg + metformin: 242 Metformin: 121 Metformin+glimepiride: 242	Type 2	26 weeks	Complete; Full; Module 5.3.5.1

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1572 extension 1 AR, AU, BE, BG, DE, DK, ES, HR, HU, IE, IN, IT, NL, NO, NZ, RO, RU, SE, SK, ZA	Efficacy and safety	Multi-centre, multi-national, randomised, open-label, parallel-group extension trial Placebo and active control (glimepiride)	Liraglutide: Once-daily s.c. doses of 0.6, 1.2 or 1.8 mg in combination with metformin Metformin: b.i.d., p.o., 2 g/day Glimepiride: q.d., p.o., 4 mg/day	780 (453/327) Liraglutide 0.6 mg + metformin: 184 Liraglutide 1.2 mg + metformin: 178 Liraglutide 1.8 mg + metformin: 174 Metformin: 61 Metformin +glimepiride: 183	Type 2	Cut off date 21 Feb 2008 ~18 months	Ongoing; Full; Module 5.3.5.1
NN2211-1436 AR, AU, BG, HR, CZ, FI, FR, HK, IN, IL, IT, KR, MY, PH, PL, RO, ZA, CH, TW, TH, TR	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind, parallel-group trial Placebo and active control (rosiglitazone)	Liraglutide: Once-daily s.c. doses of 0.6, 1.2 or 1.8 mg (titrated in weekly steps of 0.6 mg) in combination with glimepiride Glimepiride: q.d., p.o., 4 mg/day Rosiglitazone; q.d., p.o., 4 mg/day	1040 (513/527) Liraglutide 0.6 mg + glimepiride: 233 Liraglutide 1.2 mg + glimepiride: 228 Liraglutide 1.8 mg + glimepiride: 234 Glimepiride: 114 Glimepiride+rosiglitazone: 231	Type 2	26 weeks	Complete; Full; Module 5.3.5.1
NN2211-1574 US, CA	Efficacy and safety	Multi-centre, randomised, double-blind, parallel-group trial Placebo control	Liraglutide: Once-daily s.c. doses of 0.6, 1.2 or 1.8 mg (titrated in weekly steps of 0.6 mg) in combination with metformin Metformin: b.i.d., p.o., 2 g/day Rosiglitazone: b.i.d., p.o., 8 mg/day	530 (295/235) Liraglutide 1.2 mg + metformin+rosiglitazone: 177 Liraglutide 1.8 mg + metformin+rosiglitazone: 178 Metformin+rosiglitazone: 175	Type 2	26 weeks	Complete; Full; Module 5.3.5.1

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1697 AR, AT, DK, ES, FI, FR, GB, IN, IT, ME, NL, NO, PH, PL, RS, RU, SK, ZA	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind, parallel-group trial Placebo and active control (insulin glargine)	Liraglutide: Once-daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg) in combination with glimepiride and metformin Glimepiride: q.d., p.o., 4 mg/day Metformin: b.i.d., p.o., 2 g/day Insulin glargine (100 IU/mL): q.d., s.c., individual titration	576 (325/251) Liraglutide 1.8 mg + glimepiride+metformin: 230 Glimepiride+metformin: 114 Insulin glargine+glimepiride + metformin: 232	Type 2	26 weeks	Complete; Full; Module 5.3.5.1
NN2211-1694 JP	PK and PD	Randomised, double-blind within dose group, placebo-controlled, dose escalation, multiple s.c. dose trial	Once-daily s.c. doses of 15, 20 or 25 µg/kg liraglutide, titrated in week steps of 5 µg/kg	24 (24/0) (18 liraglutide, 6 placebo)	Healthy	5 weeks	Complete; Full; Module 5.3.5.4
NN2211-1551 JP	PK and PD	Randomised, double-blind, placebo-controlled, dose escalation trial	Once-daily s.c. doses of 5, 10 or 15 µg/kg liraglutide titrated in weekly steps of 5 µg/kg	24 (24/0) (18 liraglutide, 6 placebo)	Healthy	3 weeks	Complete; Full; Module 5.3.5.4
NN2211-1326 JP	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, dose escalation trial	Single s.c dose of 2.5, 5, 10 or 15 µg/kg liraglutide	32 (32/0) (24 liraglutide, 8 placebo)	Healthy	Single dose	Complete; Full; Module 5.3.5.4
NN2211-1591 JP	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, parallel-group	Once daily s.c. doses of 5 or 10 µg/kg liraglutide	15 (9/6) (11 liraglutide, 4 placebo)	Type 2	14 days	Complete; Full; Module 5.3.5.4
NN2211-1334 JP	Efficacy and safety	Multi-centre, randomised, double-blind, placebo-controlled, parallel-group	Once-daily, s.c. doses of 0.1, 0.3, 0.6, or 0.9 mg	226 (151/75) (180 liraglutide, 46 placebo)	Type 2	14 weeks	Complete; Full; Module 5.3.5.4

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-2072 US	Efficacy and safety	Multi-centre, randomised, double-blind, parallel-group trial Active control (metformin)	Liraglutide: Once daily s.c. doses of 0.045, 0.225, 0.45, 0.60, or 0.75 mg for 12 weeks Metformin: b.i.d. p.o., 1 g/day	210 (84/126) (176 liraglutide, 34 metformin) Liraglutide 0.045 mg: 37 Liraglutide 0.225 mg: 35 Liraglutide 0.45 mg: 33 Liraglutide 0.60 mg: 34 Liraglutide 0.75 mg: 37 Metformin: 34	Type 2, obese	12 weeks	Complete; Full; Module 5.3.5.4
NN2211-1333 DK	Efficacy and safety	Single-centre, randomised, double-blind, placebo-controlled, parallel-group trial	Once daily s.c. doses of 0.6 mg for 8 weeks	33 (12/21) (21 liraglutide, 12 placebo)	Type 2, obese	8 weeks	Complete; Full; Module 5.3.5.4
NN2211-1464 GB	BA (pulmo-nary)	Single-centre, randomised, cross-over trial	Single inhalation dose of 6, 12 and 24 µg/kg. Single s.c. dose of 6 µg/kg	32 (32/0) (30 liraglutide, 2 placebo)	Healthy	Single dose	Complete; Full; Module 5.3.5.4
NN8022-1807 DK, SE, FI, GB, NL, BE, ES, CZ	Efficacy and safety	Multi-centre, randomised, double-blind (orlistat open-label) trial Placebo and active (orlistat) control	Liraglutide: Once daily s.c. doses of 1.2, 1.8, 2.4, or 3.0 mg titrated in weekly steps of 0.6 mg Orlistat: t.i.d. p.o. doses of 120 mg	564 (135/429) Liraglutide 1.2 mg: 95 Liraglutide 1.8 mg: 90 Liraglutide 2.4 mg: 93 Liraglutide 3.0 mg: 93 Orlistat: 95	Healthy, obese	20 weeks	Complete; Full; Module 5.3.5.4
NN9233-1898 US	BA (intra-nasal)	Single-centre, randomised, double-blind, placebo-controlled trial	Single intranasal dose of 2.5, 5 and 10 mg. Single s.c. dose of 0.6 mg	12 (12/0) (9 liraglutide; 3 placebo)	Healthy	Single dose	Complete; Full; Module 5.3.5.4

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/s/

Manoj Khurana
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Wei Qiu
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Office of Clinical Pharmacology

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	22-341	Brand Name	Victoza® (Proposed)
OCP Division (I, II, III, IV, V)	DCP II	Generic Name	Liraglutide
Medical Division	DMEP	Drug Class	
OCP Reviewer	Manoj Khurana, Ph.D.	Indication(s)	An adjunct therapy to diet and exercise to improve glycemic control in subjects with type 2 diabetes.
OCP Pharmacometrics Reviewer	Primary: Manoj Khurana, Ph.D. Secondary: TBD	Dosage Form	Injection
OCPB Team Leader	Sally Choe, Ph.D.	Dosing Regimen	Once Daily
Date of Submission	May 23, 2008	Route of Administration	Subcutaneous
Estimated Due Date of OCP Review	December 23, 2008	Sponsor	NOVO NORDISK
PDUFA Due Date	March 23, 2009	Priority Classification	Standard
Division Due Date			

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology	X			
Mass balance:	X	1	1	**NN2211**-1699#
Isozyme characterization:	X	5	5	NN205145, NN206480, NN206665, NN207147, NN207312
Blood/plasma ratio:				
Plasma protein binding:	X	2	2	NN200152, NN201223
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	2	2	NN2211-1149# (Dose Escalation) NN2211-1326 (PKPD in healthy Japanese Subjects)
multiple dose:	X	2	2	**NN2211**-1694, 1551, 1591 (PKPD in healthy Japanese Subjects)
Patients-				
single dose:	X	3	3	**NN2211**-1219, 2063, 1224

multiple dose:	X	2	2	NN2211-1332 NN2211-1591 (PKPD in T2DM Japanese Subjects)
Dose proportionality -				
fasting / non-fasting single dose:	X	1	1	NN2211-1189
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:	X	2	2	**NN2211-** 1330#, 1608#
In-vitro:	X	3	3	NN980278, NN201224, NN203029
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:	X	1	1	NN2211-1327#
renal impairment:	X	1	1	NN2211-1329# (Trial also included 3 subjects with type 2 diabetes)
hepatic impairment:	X	1	1	NN2211-1328# (Trial also included 5 subjects with type 2 diabetes)
PD:				
Phase 1:	X	5	5	**NN2211-** 1219#, 1224#, 1589#, 1698#, 2063#
Phase 2:	X	1	1	NN2211-1332#
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:	X	1	1	NN2211-1644# (TQT)
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:	X	1	1	NN2211-1573
II. Biopharmaceutics				
Absolute bioavailability:	X			NN2211-1149
Relative bioavailability -				
solution as reference:	X	1	1	NN2211-1745# (Comparing different sites of injection)
alternate formulation as reference:	X	2		NN2211-1464 (Pulmonary) NN2211-1898 (Intranasal)
Bioequivalence studies -				
traditional design; single / multi dose:	X	4	4	**NN2211-** 1331#, 1636#, 1692#, 1693#
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				

III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies		41	39	

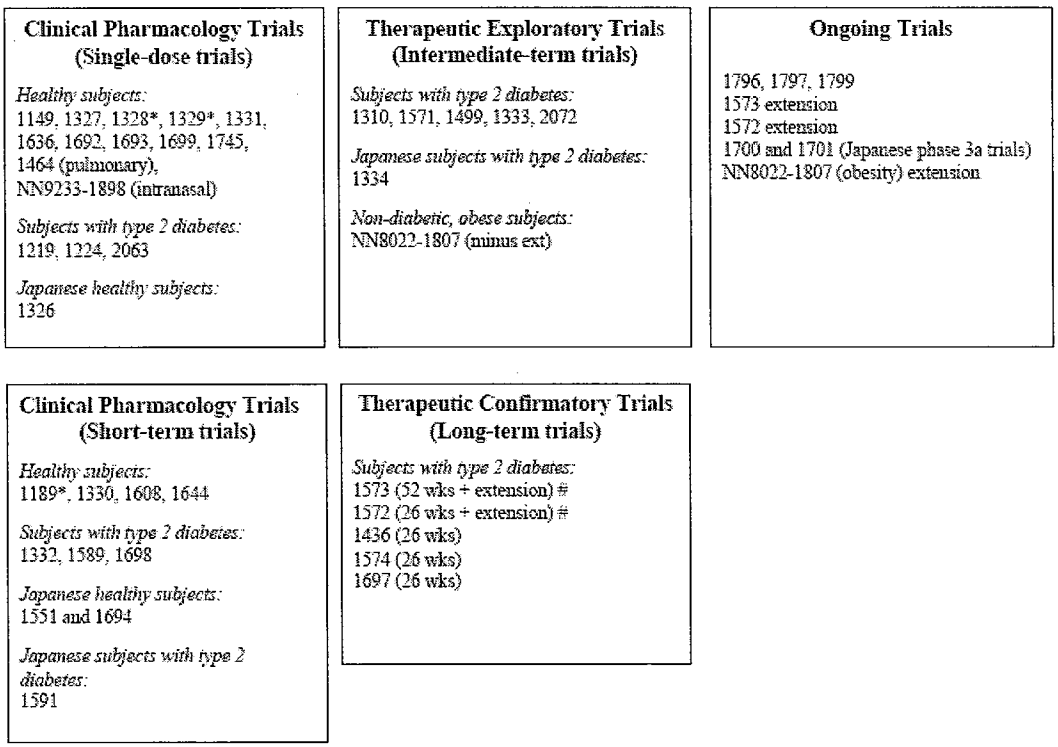
Filability		
	"X" if yes	Comments
Application filable?	X	**Comments to the Sponsor:** Please submit the following datasets to support the population analysis conducted under NN2211-1573: • All datasets used for model development and validation should be submitted as a SAS transport files (*.xpt). A description of each data item should be provided in a Define.pdf file. Any concentrations and/or subjects that have been **excluded from the analysis** should be flagged and maintained in the datasets. • Model codes or control streams and output listings should be provided for all major model building steps, e.g., base structural model, covariates models, final model, and validation model. These files should be submitted as ASCII text files with *.txt extension (e.g.: myfile_ctl.txt, myfile_out.txt). • A model development decision tree and/or table which gives an overview of modeling steps. For the population analysis reports we request that you submit, in addition to the standard model diagnostic plots, individual plots for a representative number of subjects. Each individual plot should include observed concentrations, the individual predication line and the population prediction line. In the report, tables should include model parameter names and units. For example, oral clearance should be presented as CL/F (L/h) and not as THETA (1). Also provide in the summary of the report a description of the clinical application of modeling results.
Submission in Brief: See the details below.	**Reviewer's Comments:** **DSI inspection will be requested for the study NN2211-1692 trial site and its analytical site.** **Consult to IRT for review of TQT.** **Consult to PM group for the population analysis conducted under NN2211-1573.**	

#Studies submitted with Analysis Data sets

Submission in Brief:

Sponsor, Novo Nordisk has submitted the new drug application (NDA) 22-341 for liraglutide. Liraglutide (Arg34Lys26-(N-ε-(γ-Glu (N-α-hexadecanoyl)))-GLP-1[7-37]), a new molecular entity, is once-daily human GLP-1 analogue, in which lysine at position 34 has been replaced with arginine, and palmitic acid has been attached via a glutamoyl spacer to lysine at position 26. Liraglutide was discovered and is being developed by Novo Nordisk.

A comprehensive list of completed or ongoing clinical pharmacology and clinical trials is provided below:



Total: 38 trials excluding ongoing trials (i.e. trials where final statistical analyses were not yet available)
*included 5, 3 and 4 subjects with type 2 diabetes, respectively
Two reports are available for each of Trials 1573 and 1572; one covering all data from the double-blind main part of the trials and one covering all data from double-blind and open-label extension period until 21 Feb 2008. The latter reports only describe subjects who entered the open-label extension part of the trial.

Figure 1–1 Grouping of Liraglutide Clinical Trials

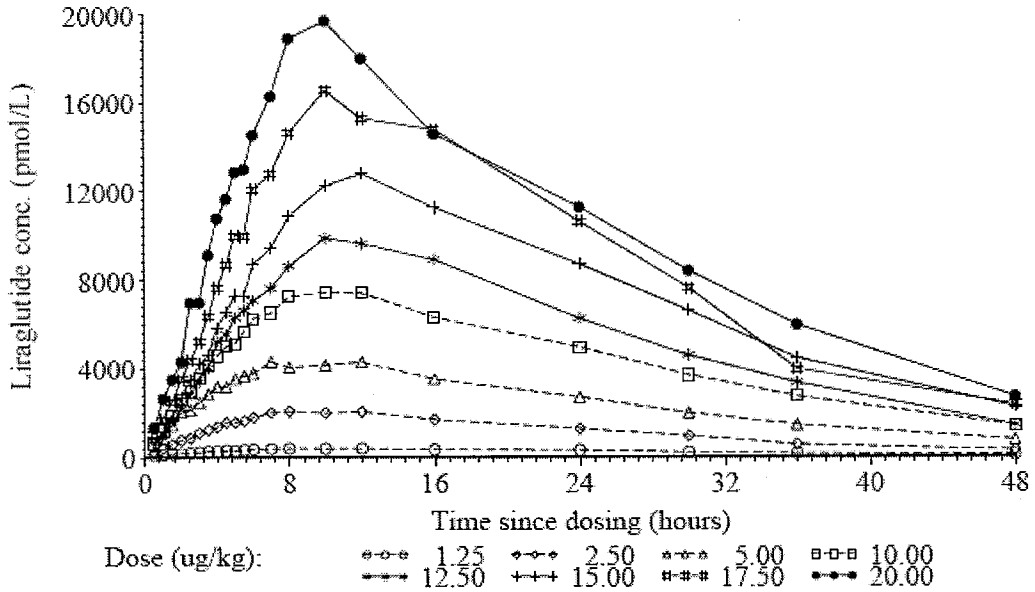
The clinical pharmacology program included 26 clinical pharmacology trials. These comprised:

- 19 trials in healthy subjects (including bioequivalence trials, trials in elderly subjects, subjects with renal or hepatic impairment and Japanese subjects),
- 7 trials in subjects with type 2 diabetes (including one trial in Japanese subjects).

The program was supported by evidence from five Phase 2 trials, a population pharmacokinetic analysis from the therapeutic confirmatory Trial 1573, and from ten in vitro studies performed with human biomaterials, i.e. cells, recombinant enzymes, plasma or plasma proteins.

The pharmacokinetic data demonstrated slow absorption of liraglutide (tmax = 10–13 hours) and a half-life of approximately 13 hours (10–18 hours), suitable for the intended once daily dosing. In addition, pharmacokinetics was demonstrated to be dose proportional for both single and

multiple doses. The absolute bioavailability was demonstrated to be 55% based on intravenous (i.v.) and subcutaneous (s.c.) administration of 5 µg/kg. The relative bioavailability was slightly lower in the thigh compared with the abdomen and the upper arm based on s.c. administration of 0.60 mg.



Mean Plasma Liraglutide Profiles following Single-dose Administration in Healthy Subjects – Trial 1149

Investigation of the metabolism of liraglutide in healthy subjects (Trial 1699) indicated that liraglutide is endogenously metabolised and that neither renal nor biliary excretion are major routes of clearance. The effect of liraglutide to slow gastric emptying did not affect the absorption of orally administered medical products to any clinically significant degree. At steady state concentrations, liraglutide did not alter the rate and extend of absorption of orally administered paracetamol, atorvastitin, griseofulvin, lisinopril, digoxin and oral contraceptives (ethinylestradiol and levonorgestrel) to a clinically relevant degree.

Mode of action trials in subjects with type 2 diabetes demonstrated glucose lowering as well as insulin secretion effects of liraglutide after a single s.c. dose (10 µg/kg). Furthermore, at a s.c. dose of 7.5 µg/kg, liraglutide did not impair glucagon response nor the general hypoglycaemic counterregulation response and liraglutide was no longer insulinotropic at hypoglycaemic plasma glucose concentrations. It has been shown, that liraglutide provides 24-hour glycaemic control and restores β-cell responsiveness to increasing blood glucose concentrations.

The six reported Phase 2 trials showed significant effects on glycaemic control (measured by HbA1c and fasting serum glucose) after 5–14 weeks of treatment. In addition, the trials showed that treatment with liraglutide was associated with weight loss. The Japanese Phase 2 trial (Trial 1334) showed similar results on glycaemic control, whereas no effect was seen on body weight. Preliminary results from the ongoing Phase 3a trials with completed clinical phase showed robust effects on glycaemic control (measured by HbA1c and fasting plasma glucose) after 6 months of treatment with liraglutide in various treatment regimens with liraglutide doses of 0.6 mg, 1.2 mg or 1.8 mg per day in combination with sulphonylureas, metformin or

thiazolidinediones in subjects with type 2 diabetes. Results from the Phase 3a trials confirmed the weight loss observed in the Phase 2 trials.

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Attachment 1: Tabular Listing of Clinical Studies

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1331 DE	BE	Single-centre, randomised, single-blind, cross-over trial	Single s.c. doses of 1 mg liraglutide formulation 2 and formulation 3	22 (13/9)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1636 AU	BE	Single-centre, randomised, double-blind, cross-over trial	Single s.c. doses of 0.75 mg liraglutide formulation 3 at pH 7.7, 7.9 and 8.15	24 (13/11)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1692 SE	BE	Single-centre, randomised, double-blind, cross-over trial	Single s.c. doses of 0.72 mg liraglutide formulation 4 and formulation 4 with final manufacturing process for drug substance	21 (19/2)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1693 SE	BE	Single-centre, randomised, double-blind, cross-over trial	Single s.c. doses of 0.71 mg liraglutide formulation 3 and formulation 4	22 (19/3)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1745 DE	PK and BA	Single-centre, randomised, open-label, cross-over trial	Three single s.c. doses of 0.6 mg liraglutide in the abdomen, thigh and upper arm, respectively	21 (11/10)	Healthy	Single-dose	Complete; Full; Module 5.3.1.2
NN2211-1149 GB	PK, PD and BA	Single-centre, randomised, double-blind, placebo-controlled, parallel-group, dose-escalation trial	Eight single s.c. doses of 1.25, 2.5, 5, 10, 12.5, 15, 17.5 or 20 µg/kg liraglutide or one single i.v. dose of 5 µg/kg liraglutide	72 (72/0) (54 liraglutide, 18 placebo)	Healthy	Single-dose	Complete; Full; Module 5.3.3.1
NN2211-1699 NL	PK	Single-centre, open-label trial	Single s.c. dose of 0.75 mg radiolabelled liraglutide	7 (7/0)	Healthy	Single-dose	Complete; Full; Module 5.3.3.1

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1189 GB	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, parallel-group, dose-escalation trial	Initial single s.c. dose, and subsequent once daily s.c. doses with dose-escalation of 1.25, 5, 7.5, 10 and 12.5 µg/kg liraglutide	Healthy: 30 (30/0) (20 liraglutide, 10 placebo) Type 2: 4 (4/0) (2 liraglutide, 2 placebo)	Healthy and type 2	Single dose followed by 7 days	Complete; Full; Module 5.3.3.1
NN2211-1327 DE	PK	Single-centre, open-label, parallel-group trial	Single s.c. dose of 1 mg liraglutide	32 (16/16) (young: 16 (8/8); elderly: 16 (8/8))	Healthy, young and elderly	Single dose	Complete; Full; Module 5.3.3.3
NN2211-1329 NZ	PK	Single-centre, open-label, parallel-group trial	Single s.c. dose of 0.75 mg liraglutide	30 (22/8) divided in 5 groups according to renal function (healthy: 27; type 2 diabetes: 3)	Healthy, with normal renal function or renal impairment	Single dose	Complete; Full; Module 5.3.3.3
NN2211-1328 PL	PK	Single-centre, open-label, parallel-group trial	Single s.c. dose of 0.75 mg liraglutide	24 (14/10) divided in 4 groups according to hepatic function (healthy: 19; type 2 diabetes: 5)	Healthy, with normal hepatic function or hepatic impairment	Single dose	Complete; Full; Module 5.3.3.3
NN2211-1330 SE	DDI	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial	Once daily s.c. doses of 1.8 mg liraglutide (titrated in weekly steps of 0.6 mg). Single p.o. dose of 0.03 mg ethinylestradiol plus 0.15 mg levonorgestrel (i.e. one tablet of Neovletta®)	21 (0/21)	Healthy, post-menopausal women	3 weeks and single dose DDI	Complete; Full; Module 5.3.3.4

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1608 SE	DDI	Two-centre, randomised, double-blind, placebo-controlled, two-way cross-over trial in two parts (A and B)	Liraglutide: Once daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg) Part A: Single p.o. doses of 40 mg atorvastatin and 20 mg lisinopril Part B: Single p.o. doses of 500 mg griseofluvin and 1 mg digoxin	42 (19/13) in Part A and 28 (27/1) in Part B	Healthy	4 weeks and single dose DDI	Complete; Full; Module 5.3.3.4
NN2211-1644 US	QTc	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial followed by open-label moxifloxacin (positive control)	Liraglutide: Once daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg). Moxifloxacin: Single p.o. dose of 400 mg	58 (29/29)	Healthy	7 weeks	Complete; Full; Module 5.3.4.1
NN2211-1698 DE	PD and DDI	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial	Liraglutide: Once daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg. Paracetamol: Single p.o dose 1 g	18 (14/4)	Type 2	3 weeks	Complete; Full; Module 5.3.4.2
NN2211-1589 AU and DE	PD	Two-centre, randomised, double-blind, balanced incomplete latin square design trial. Placebo and active control (glimepiride)	Liraglutide: Once daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg). Glimepiride: Once daily p.o. dose of 1-4 mg Paracetamol (gastric emptying): Single p.o dose of 1 g	46 (27/19)	Type 2	4 weeks	Complete; Full; Module 5.3.4.2

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1332 DK	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial	Once daily s.c. doses of 6 µg/kg liraglutide for 9-10 days	13 (8/5)	Type 2	9-10 days	Complete; Full; Module 5.3.4.2
NN2211-1219 DK	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, single-dose, cross-over trial	Single s.c. dose of 10 µg/kg liraglutide	11 (6/5)	Type 2	Single dose	Complete; Full; Module 5.3.4.2
NN2211-2063 US	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, cross-over trial	Single s.c. dose of 7.5 µg/kg liraglutide	Type 2: 10 (6/4) Healthy: 10 (5/5) (not dosed)	Type 2 and healthy	Single-dose	Complete; Full; Module 5.3.4.2
NN2211-1224 DE	PK and PD	Two-centre, randomised, double-blind, placebo-controlled, cross-over trial	Single s.c. dose of 7.5 µg/kg liraglutide	19 (14/5)	Type 2	Single-dose	Complete; Full; Module 5.3.4.2
NN2211-1571 DK, FR, NL, SK	Efficacy and safety	Multi-centre, multi-national randomised, double-blind, placebo-controlled, parallel-group trial	Once daily s.c. doses of 0.65, 1.25 or 1.9 mg liraglutide for 14 weeks	Type 2: 163 (99/64) Healthy: 12 (9/3) (not dosed) Liraglutide 0.65 mg: 40 Liraglutide 1.25 mg: 42 Liraglutide 1.90 mg: 41 Placebo: 40	Type 2 and healthy	14 weeks	Complete; Full; Module 5.3.5.1
NN2211-1310 DK, NO, SE, GB	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind (open-label glimepiride), parallel-group trial. Placebo and active control (glimepiride)	Liraglutide: Once daily s.c. doses of 0.045, 0.225, 0.45, 0.60, or 0.75 mg. Glimepiride: Once daily p.o. dose of 1-4 mg	190 (127/63) Liraglutide 0.045 mg: 26 Liraglutide 0.225 mg: 24 Liraglutide 0.45 mg: 27 Liraglutide 0.60 mg: 30 Liraglutide 0.75 mg: 28 Glimepiride: 26 Placebo: 29	Type 2	12 weeks	Complete; Full; Module 5.3.5.1

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1499 AT, AU, CZ, DK, FR, DE, PL, GB	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind (metformin and glimepiride open-label), parallel-group trial Active control (metformin and glimepiride)	Liraglutide: Once daily s.c. doses of 2.0 mg (titrated in weekly steps of 0.5 mg) Metformin: b.i.d., p.o. doses of 1 g Glimepiride: Once daily p.o. doses of 2-4 mg	144 (91/53) Liraglutide 36 Liraglutide+metformin: 36 Metformin: 36 Metformin and glimepiride: 36	Type 2	5 weeks	Complete; Full; Module 5.3.5.1
NN2211-1573 US, MX	Efficacy, safety and popula-tion PK	Multi-centre, randomised, double-blind, parallel-group trial Active control (glimepiride)	Liraglutide: Once daily s.c. doses of 1.2 mg or 1.8 mg (titrated in weekly steps of 0.6 mg) Glimepiride: Once daily p.o. 8 mg/day	745 (371/374) Liraglutide 1.2 mg: 251 Liraglutide 1.8 mg: 246 Glimepiride: 248	Type 2	52 weeks	Complete; Full; Module 5.3.5.1
NN2211-1573 extension 1 US, MX	Efficacy and safety	Multi-centre, multi-national, randomised, open-label, parallel-group extension trial Active control (glimepiride)	Liraglutide: Once daily s.c. doses of 1.2 mg or 1.8 mg Glimepiride: Once daily p.o. 8 mg/day	440 (223/217) Liraglutide 1.2 mg: 149 Liraglutide 1.8 mg: 154 Glimepiride: 137	Type 2	Cut off date 21 Feb 2008 ~18 months	Ongoing; Full; Module 5.3.5.1
NN2211-1572 AR, AU, BE, BG, DE, DK, ES, GB, HR, HU, IE, IN, IT, NL, NO, NZ, RO, RU, SE, SK, ZA	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind, parallel-group trial (open-label extension) Placebo and active control (glimepiride)	Liraglutide: Once-daily s.c. doses of 0.6, 1.2 or 1.8 mg (titrated in weekly steps of 0.6 mg) in combination with metformin Metformin: b.i.d., p.o., 2 g/day Glimepiride: q.d., p.o., 4 mg/day	1087 (633/454) Liraglutide 0.6 mg + metformin: 242 Liraglutide 1.2 mg + metformin: 240 Liraglutide 1.8 mg + metformin: 242 Metformin: 121 Metformin+glimepiride: 242	Type 2	26 weeks	Complete; Full; Module 5.3.5.1

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1572 extension 1 AR, AU, BE, BG, DE, DK, ES, HR, HU, IE, IN, IT, NL, NO, NZ, RO, RU, SE, SK, ZA	Efficacy and safety	Multi-centre, multi-national, randomised, open-label, parallel-group extension trial Placebo and active control (glimepiride)	Liraglutide: Once-daily s.c. doses of 0.6, 1.2 or 1.8 mg in combination with metformin Metformin: b.i.d., p.o., 2 g/day Glimepiride: q.d., p.o., 4 mg/day	780 (453/327) Liraglutide 0.6 mg + metformin: 184 Liraglutide 1.2 mg + metformin: 178 Liraglutide 1.8 mg + metformin: 174 Metformin: 61 Metformin +glimepiride: 183	Type 2	Cut off date 21 Feb 2008 ~18 months	Ongoing; Full; Module 5.3.5.1
NN2211-1436 AR, AU, BG, HR, CZ, FI, FR, HK, IN, IL, IT, KR, MY, PH, PL, RO, ZA, CH, TW, TH, TR	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind, parallel-group trial Placebo and active control (rosiglitazone)	Liraglutide: Once-daily s.c. doses of 0.6, 1.2 or 1.8 mg (titrated in weekly steps of 0.6 mg) in combination with glimepiride Glimepiride: q.d., p.o., 4 mg/day Rosiglitazone; q.d., p.o., 4 mg/day	1040 (513/527) Liraglutide 0.6 mg + glimepiride: 233 Liraglutide 1.2 mg + glimepiride: 228 Liraglutide 1.8 mg + glimepiride: 234 Glimepiride: 114 Glimepiride+rosiglitazone: 231	Type 2	26 weeks	Complete; Full; Module 5.3.5.1
NN2211-1574 US, CA	Efficacy and safety	Multi-centre, randomised, double-blind, parallel-group trial Placebo control	Liraglutide: Once-daily s.c. doses of 0.6, 1.2 or 1.8 mg (titrated in weekly steps of 0.6 mg) in combination with metformin Metformin: b.i.d., p.o., 2 g/day Rosiglitazone: b.i.d., p.o., 8 mg/day	530 (295/235) Liraglutide 1.2 mg + metformin+rosiglitazone: 177 Liraglutide 1.8 mg + metformin+rosiglitazone: 178 Metformin+rosiglitazone: 175	Type 2	26 weeks	Complete; Full; Module 5.3.5.1

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-1697 AR, AT, DK, ES, FI, FR, GB, IN, IT, ME, NL, NO, PH, PL, RS, RU, SK, ZA	Efficacy and safety	Multi-centre, multi-national, randomised, double-blind, parallel-group trial Placebo and active control (insulin glargine)	Liraglutide: Once-daily s.c. doses of 1.8 mg (titrated in weekly steps of 0.6 mg) in combination with glimepiride and metformin Glimepiride: q.d., p.o., 4 mg/day Metformin: b.i.d., p.o., 2 g/day Insulin glargine (100 IU/mL): q.d., s.c., individual titration	576 (325/251) Liraglutide 1.8 mg + glimepiride+metformin: 230 Glimepiride+metformin: 114 Insulin glargine+glimepiride + metformin: 232	Type 2	26 weeks	Complete; Full; Module 5.3.5.1
NN2211-1694 JP	PK and PD	Randomised, double-blind within dose group, placebo-controlled, dose escalation, multiple s.c. dose trial	Once-daily s.c. doses of 15, 20 or 25 µg/kg liraglutide, titrated in week steps of 5 µg/kg	24 (24/0) (18 liraglutide, 6 placebo)	Healthy	5 weeks	Complete; Full; Module 5.3.5.4
NN2211-1551 JP	PK and PD	Randomised, double-blind, placebo-controlled, dose escalation trial	Once-daily s.c. doses of 5, 10 or 15 µg/kg liraglutide titrated in weekly steps of 5 µg/kg	24 (24/0) (18 liraglutide, 6 placebo)	Healthy	3 weeks	Complete; Full; Module 5.3.5.4
NN2211-1326 JP	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, dose escalation trial	Single s.c dose of 2.5, 5, 10 or 15 µg/kg liraglutide	32 (32/0) (24 liraglutide, 8 placebo)	Healthy	Single dose	Complete; Full; Module 5.3.5.4
NN2211-1591 JP	PK and PD	Single-centre, randomised, double-blind, placebo-controlled, parallel-group	Once daily s.c. doses of 5 or 10 µg/kg liraglutide	15 (9/6) (11 liraglutide, 4 placebo)	Type 2	14 days	Complete; Full; Module 5.3.5.4
NN2211-1334 JP	Efficacy and safety	Multi-centre, randomised, double-blind, placebo-controlled, parallel-group	Once-daily, s.c. doses of 0.1, 0.3, 0.6, or 0.9 mg	226 (151/75) (180 liraglutide, 46 placebo)	Type 2	14 weeks	Complete; Full; Module 5.3.5.4

Trial ID Country	Type of study	Trial design and type of control	Test drugs and route of administration	Subjects exposed (M/F)	Healthy or type 2 diabetes	Duration of treatment	Study status Type of report Location
NN2211-2072 US	Efficacy and safety	Multi-centre, randomised, double-blind, parallel-group trial Active control (metformin)	Liraglutide: Once daily s.c. doses of 0.045, 0.225, 0.45, 0.60, or 0.75 mg for 12 weeks Metformin: b.i.d. p.o., 1 g/day	210 (84/126) (176 liraglutide, 34 metformin) Liraglutide 0.045 mg: 37 Liraglutide 0.225 mg: 35 Liraglutide 0.45 mg: 33 Liraglutide 0.60 mg: 34 Liraglutide 0.75 mg: 37 Metformin: 34	Type 2, obese	12 weeks	Complete; Full; Module 5.3.5.4
NN2211-1333 DK	Efficacy and safety	Single-centre, randomised, double-blind, placebo-controlled, parallel-group trial	Once daily s.c. doses of 0.6 mg for 8 weeks	33 (12/21) (21 liraglutide, 12 placebo)	Type 2, obese	8 weeks	Complete; Full; Module 5.3.5.4
NN2211-1464 GB	BA (pulmo-nary)	Single-centre, randomised, cross-over trial	Single inhalation dose of 6, 12 and 24 µg/kg. Single s.c. dose of 6 µg/kg	32 (32/0) (30 liraglutide, 2 placebo)	Healthy	Single dose	Complete; Full; Module 5.3.5.4
NN8022-1807 DK, SE, FI, GB, NL, BE, ES, CZ	Efficacy and safety	Multi-centre, randomised, double-blind (orlistat open-label) trial Placebo and active (orlistat) control	Liraglutide: Once daily s.c. doses of 1.2, 1.8, 2.4, or 3.0 mg titrated in weekly steps of 0.6 mg Orlistat: t.i.d. p.o. doses of 120 mg	564 (135/429) Liraglutide 1.2 mg: 95 Liraglutide 1.8 mg: 90 Liraglutide 2.4 mg: 93 Liraglutide 3.0 mg: 93 Orlistat: 95	Healthy, obese	20 weeks	Complete; Full; Module 5.3.5.4
NN9233-1898 US	BA (intra-nasal)	Single-centre, randomised, double-blind, placebo-controlled trial	Single intranasal dose of 2.5, 5 and 10 mg. Single s.c. dose of 0.6 mg	12 (12/0) (9 liraglutide; 3 placebo)	Healthy	Single dose	Complete; Full; Module 5.3.5.4

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/s/

Manoj Khurana
9/25/2008 05:31:24 PM
BIOPHARMACEUTICS

Sally Choe
9/29/2008 08:33:28 AM
BIOPHARMACEUTICS

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-341

OTHER REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research
Division of Drug Marketing, Advertising, and Communications

PRE-DECISIONAL AGENCY MEMO

Date: October 26, 2009

To: John Bishai – Regulatory Project Manager
Division of Metabolism and Endocrinology Products (DMEP)

From: Sam Skariah – Regulatory Review Officer
Kendra Jones – Regulatory Review Officer
Division of Drug Marketing, Advertising, and Communications (DDMAC)

Subject: DDMAC draft labeling comments
NDA #22-341 Victoza (liraglutide [rDNA origin] injection) solution

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DDMAC has reviewed the proposed product labeling (PI) and MedGuide for Victoza (liraglutide [rDNA origin] injection) solution (Victoza) submitted for consult on October 5, 2009.

The following comments are provided regarding the October 5, 2009 version of the proposed PI, located in the eRoom.

Thank you for the opportunity to comment on this label.

44 Page(s) Withheld

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√ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Other Reviews- 1

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/s/

SAMUEL M SKARIAH
10/26/2009

Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Surveillance and Epidemiology

Date:	October 20, 2009
To:	Mary Parks, MD Director Division of Metabolism and Endocrinology Products
Thru:	Carlos M. Mena-Grillasca, R.Ph., Team Leader Denise Toyer, PharmD, Deputy Director Carol Holquist, R.Ph., Director Division of Medication Error Prevention and Analysis
From:	Walter Fava, R.Ph., Safety Evaluator Division of Medication Error Prevention and Analysis
Subject:	Label and Labeling Review
Drug Name(s):	Victoza (Liraglutide) Injection 18 mg/3 mL multiple dose prefilled pen
Application Type/Number:	IND: 061040 NDA: 022341
Applicant/sponsor:	Novo Nordisk, Inc.
OSE RCM #:	2009-1336

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CONTENTS

1 INTRODUCTION

This review is written in response to a request from the Division of Metabolism and Endocrinology Products to assess the labels and labeling of Victoza (Liraglutide), to identify areas that could lead to medication errors.

2 REGULATORY HISTORY

b(4)

The Applicant originally submitted labels and labeling and working samples of [redacted] DMEPA expressed our concerns with this proposal and the potential for confusion and medication errors that might arise. On August 24, 2009 DMEPA met with the Division of Metabolism and Endocrinology Products (DMEP) to recommend that the Applicant market only one prefilled pen capable of delivering all three doses (0.6 mg, 1.2 mg, and 1.8 mg). DMEP concurred with our recommendation. On August 26, 2009, DMEP communicated these safety concerns to the Applicant and requested that the Applicant market only one multi-dose pen that delivers the three doses. On August 28, 2009, the Applicant agreed to market only the multi-dose pen that delivers all three doses as recommended.

3 MATERIALS REVIEWED

The Division of Medication Error Prevention and Analysis (DMEPA) used principles of Human Factors and Failure Mode and Effects Analysis (FMEA) in our evaluation of the labels and labeling submitted. For this product the Applicant submitted revised labels and labeling for DMEPA review on September 30, 2009. Revised physician insert labeling was submitted on September 4, 2009. The Applicant also submitted a Usability Study on August 11, 2009 (see Appendix A for images):

- Retail Pen Labels ('0.6/1.2/1.8 mg')
- Retail Carton Labeling ('0.6/1.2/1.8 mg')
- Pen Labels for Physician Samples ('0.6/1.2/1.8 mg')
- Carton Labeling for Physician Samples ('0.6/1.2/1.8 mg')
- Medication Guide and Patient Instructions for Use (no image)
- Prescribing Information (no image)
- Usability Study (no image)

A working model of the pen was also provided by the Applicant and was used in our evaluation of the labels and labeling. The retail multidose pen will be available in two pens per carton and three pens per carton packaging configurations. The physician sample pen will be packaged one pen per carton.

Our evaluation noted areas where information in the labels and labeling can be improved upon to provide increased readability and minimize the potential for confusion. We provide our recommendations in Section 4.

4 RECOMMENDATIONS

We request the following recommendations be communicated to the Applicant prior to approval.

4.1 COMMENTS TO THE DIVISION

A. Pen Design

During the October 7, 2009 wrap-up meeting, we discussed with the Division that the pen design does not have a lock-out mechanism to prevent patients/caregivers from administering doses other than 0.6 mg, 1.2 mg, and 1.8 mg. After considering that the instructions for use explain how to dial prescribed doses correctly, and the fact that the pen does not deliver doses greater than 1.8 mg per dose, the Division did not believe this to be a substantial safety issue.

B. Package Insert Labeling

1. Remove all trailing zeros throughout the package insert. Trailing zeros are listed as a dangerous dose designation on the Institute of Medicine's 'List of Error-Prone Abbreviations, Symbols, and Dose Designations', because they can lead to ten-fold dosing errors if the decimal is not seen (e.g. revise Section 8.1, '...1.0 mg/kg/day liraglutide...' to read '...1 mg/kg/day liraglutide...'). When placed in the approved labeling of products, this terminology tends to used by practitioners in prescribing. In June 2006, FDA launched an educational campaign with ISMP to educate healthcare practitioners not to use dangerous abbreviations or dose designations in their prescribing. As part of this campaign, FDA agreed not to allow such dangerous abbreviations and dose designations in the approved labeling of products because these carry over to prescribing habits. Thus, DMEPA requests the Division not allow such abbreviations be approved as part of labeling.
2. Revise the abbreviation, 'sc' used throughout the labeling to read 'subcutaneous' to clearly identify the route of administration being referenced. The abbreviation 'sc' is also listed on ISMP's 'List of Error-Prone Abbreviations, Symbols, and Dose Designations', because it can be mistaken as 'SL' (sublingual).
3. Revise all sections of labeling where dose statements are presented without units of measure (e.g., revise statement in Section 12.2, '...1.8, 1.2, and 0.6 mg Victoza treatment...' to include the units with the presentation of the strength '...1.8 mg, 1.2 mg, and 0.6 mg treatment...').
4. Revise Section 3, 'Dosage Forms and Strengths' to include a statement, 'Each pen delivers 30 doses of 0.6 mg, 15 doses of 1.2 mg, or 10 doses of 1.8 mg'.
5. Delete all references to 0.6/1.2/1.8 mg, as this presentation omits units of measure and does not clearly convey dosing information.
6. Relocate the statement in Section 16, 'How Supplied/Storage Handling' which reads, '*Each Victoza pen is for use by a single patient. A Victoza pen should never be shared between patients even if the needle is changed*' to Sections 2 and 5, 'Dosage and Administration' and 'Warnings and Precautions' respectively.
7. Section 16, 'How Supplied/Storage Handling' revise the presentation of the packaging configuration to read:

   'Victoza is a sterile injectable solution packaged in the following pen delivery systems available as:

   Two pens per carton (NDC 0169-4060-12)

   - 3 mL disposable multiple dose prefilled pen containing 18 mg liraglutide (6 mg/mL), each pen providing 30 doses of 0.6 mg, or 15 doses of 1.2 mg, or 10 doses of 1.8 mg.

   Three pens per carton (NDC 0169-4060-13)

   - 3 mL disposable multiple dose prefilled pen containing 18 mg liraglutide (6 mg/mL), each pen providing 30 doses of 0.6 mg, or 15 doses of 1.2 mg, or 10 doses of 1.8 mg.

C. Patient Instructions for Use and Medication Guide

The following comments were forwarded to the Division of Risk Management Patient Labeling Team to be incorporated into their final review of the Patient Instructions for Use and Medication Guide:

1. **Patient Instructions for Use**

   a. Increase the size of the graphic image of the pen in the introduction section. As currently presented, the graphic is small and uses small font to label each pen component, making it difficult to read. Also, increase the font size used to label each pen part to make each labeled part easier to identify.

   b. Include terminology identifying the threaded cartridge tip where the needle is attached to the pen in the diagram of the labeled pen parts.

2. **Medication Guide**

   a. Begin section entitled, 'Delivering the Dose', with the first sentence reading, 'Use injection technique shown by your healthcare professional'.

   b. Revise instruction # 1 under 'Delivering the Dose' to read, 'Press down on the center of the dose button to inject and keep the dose button pressed down until 0 mg in the dose display window lines up with the pointer'.

   c. Revise instructions under 'Delivering the Dose' reminding patients not to cover the dose display window with their fingers while injecting their dose so that they can see when the dose counter reaches '0'.

   d. Revise the instruction, 'Unscrew the needle' to explain to patients/caregivers how to safely unscrew the needle.

   e. Include instructions in the section entitled, 'How should I store Victoza' telling patients to write down the start date or expiration date since the product is only good for 30 days after initial use.

4.2 COMMENTS TO THE APPLICANT

A. Pen Label (Retail and Physician Samples)

1. Include a statement on the principle display panel that the pen is for single patient use only.

2. Include the total drug content statement, '18 mg/3 mL (6 mg/mL)' following the dosage form statement in accordance with USP requirements.

3. Revise the dose statement, '0.6/1.2/1.8 mg' appearing to the right of the proprietary name to read, 'Pen delivers doses of 0.6 mg, 1.2 mg or 1.8 mg', and relocate this statement to appear on the principle display panel after the total drug content and concentration statement. As currently presented, '0.6/1.2/1.8 mg' lacks the units of measure following each dose and may be misinterpreted to mean that the pen is a combination product that contains three different active ingredients.

4. If space permits, revise the statement on the retail pen label, 'Each prefilled pen contains 6 mg/mL and will deliver 10 doses of 1.8 mg', to read 'Each prefilled pen contains 6 mg/mL and will deliver 30 doses of 0.6 mg or 15 doses of 1.2 mg or 10 doses of 1.8 mg'. Likewise revise the corresponding statement on the physician sample label accordingly.

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√ Draft Labeling (b4)

_____ Draft Labeling (b5)

_____ Deliberative Process (b5)

Withheld Track Number: Other Reviews- 2

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/s/

WALTER L FAVA
10/20/2009

CARLOS M MENA-GRILLASCA
10/21/2009

DENISE P TOYER
10/21/2009

CAROL A HOLQUIST
10/28/2009

10/14/09

Department of Health and Human Services
Public Health Service
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Surveillance and Epidemiology

Date:	October 13, 2009
To:	Mary Parks, M.D., Director, Division of Metabolic and Endocrine Drug Products, OND
Thru:	Gwen Zornberg, M.D., Team Leader, Division of Epidemiology, OSE
Thru:	Solomon Iyasu, M.D., Director, Division of Epidemiology, OSE
From:	Diane K. Wysowski, Ph.D., Epidemiologist, Division of Epidemiology, OSE
Subject:	Review of Protocol for "Medullary Thyroid Carcinoma Surveillance Study: a Case-Series Registry"
Drug Name(s):	Liraglutide injection (Victoza)
Submission Number:	0039
Application Type/Number:	NDA 22-341
Applicant/sponsor:	Novo Nordisk
OSE RCM #:	2009-1562

CONTENTS

EXECUTIVE SUMMARY

Liraglutide injection is an analogue of glucagon-like peptide (GLP-1) with 97% amino acid homology to human GLP-1, indicated for treatment of type 2 diabetes mellitus. The drug's sponsor, Novo Nordisk, is currently seeking approval of a new drug application for liraglutide, but concern exists about a potential safety problem because of an increase in C-cell tumors of the thyroid gland found during the preclinical test phase in mice and rats. In addition, an increased frequency of C-cell hyperplasia, a dose-related trend in calcitonin levels (a biomarker of medullary thyroid cancer), and a numerical imbalance in papillary thyroid cancer were found in patients exposed to liraglutide during phase 3 clinical trials. As a result, Novo Nordisk has proposed to conduct postmarketing studies to determine if patients exposed to liraglutide have increased frequencies of medullary thyroid cancer (MTC) and protocols have been submitted to the FDA.

The protocol entitled "Medullary Thyroid Carcinoma Surveillance Study: a Case-Series Registry" was sent by the Division of Metabolic and Endocrine Products (DMEP) to the Division of Epidemiology for review. A synopsis of the study and a critique follow in the text below. In brief, the researchers propose to identify MTC cases from established cancer registries and comprehensive cancer centers for inclusion in an MTC case series registry. They also plan to monitor the incidence of MTC for a —— period after liraglutide marketing, compare it with the national (background) incidence, and interview cases about possible risk factors including liraglutide exposure. b(4)

Some limitations and concerns include the following:

- the contractor and staff are not identified and experience performing similar studies are not provided;
- nonparticipation of cancer registries, patients, and physicians could seriously limit sample size and missing reports of MTC associated with liraglutide exposure would underestimate risk;
- difficulty may be encountered in detecting and interpreting changes in MTC national background incidence;
- identifying deceased patients and obtaining exposure information from proxies may be problematic;
- absence of controls could lead to difficulty interpreting results;
- data collected may not be representative of MTC cases; and
- no commitment is made to publish the results.

The protocol should acknowledge these problems and, where remediable, offer possible solutions. Some recommendations are provided in the text below.

The most important limitation of this MTC case series registry might be nonparticipation of cancer registries, patients, and physicians leading to missing cases and possible underascertainment of liraglutide risk. Missing cases in the MTC case series registry

might be supplemented by timely reporting of MTC in liraglutide-exposed patients to the FDA's Adverse Event Reporting System (AERS) and case reports in the medical literature. Consequently, the product information and the Medication Guide should prominently display the FDA's MedWatch contact information.

Of note, a similar "case series study established to monitor at least 40% of osteosarcomas occurring annually in men and women older than 40 years old who reside in the United States" (1) to detect an association of osteosarcoma with teripatide (Forteo), apparently has not detected any cases, although two cases of osteosarcoma following teripatide exposure have been reported in the medical literature (1, 2). The first case involved a "postmenopausal woman in her 70s with a complex past medical history" initially reported to a Lilly sales representative (2). The second teripatide-exposed osteosarcoma case was a 67-year-old man with a history of radiation therapy who used teripatide two months before his diagnosis of osteosarcoma, according to clinicians at the University of Texas M.D. Anderson Cancer Center (1). The two cases were among the "more than 430,000 persons who have received teripatide for treatment of severe osteoporosis" (1).

1 BACKGROUND

Glucagon-like peptide (GLP-1) is an incretin hormone secreted by the L-cells in the lower gut which stimulates endogenous insulin secretion in a physiologic, glucose-dependent manner. Liraglutide is an analogue of GLP-1 with 97% amino acid homology to human GLP-1. This injectable drug, indicated for treatment of type 2 diabetes mellitus, stimulates insulin secretion, lowers inappropriately high glucagon secretion in a glucose-dependent manner, and improves beta-cell function.

The new drug application for liraglutide injection is currently being reviewed in the Division of Metabolic and Endocrine Drug Products (DMEP). Concern exists about a potential safety problem because of an increase in C-cell tumors of the thyroid gland found during the preclinical test phase in mice and rats. In addition, according to a review by Karen Mahoney, M.D., DMEP, an increased frequency of C-cell hyperplasia, a dose-related trend in calcitonin levels (a biomarker of medullary thyroid cancer), and a numerical imbalance in papillary thyroid cancer were found in patients exposed to liraglutide during phase 3 clinical trials. Furthermore, a small increase in major cardiovascular events occurred with liraglutide compared to placebo. Dr. Mahoney also noted imbalances not favoring liraglutide for gastrointestinal adverse events, pancreatitis, serious neoplasm events (besides nonmalignant neoplasms and papillary thyroid), thyroid neoplasms, serious hypoglycemic events, injection site reactions, immunogenicity events including urticaria, hepatobiliary events, increased heart rate, animal fetal anomalies, and nonserious adverse events of dizziness and fatigue. She also noted liraglutide antibody formation with possible predispostion to infections and musculoskeletal pain, slowing of gastric emptying with pharmacokinetic effects on other drugs, and a potential for medication errors and off-label use/abuse. Dr. Mahoney does not recommend approval at this time.

In response to these adverse events, Novo Nordisk stated that, "Several potential rare safety signals described in the liraglutide new drug application may require further assessment through post marketing surveillance of a large patient group," and the

company submitted to the FDA a risk evaluation and mitigation strategy (REMS). Also appended were two protocols to evaluate the post-marketing safety of liraglutide.

One protocol involved a prospective active surveillance program to monitor the annual incidence of medullary thyroid cancer (MTC) and to establish a registry of incident MTC cases in adults in the U.S. to characterize their medical histories and risk factors including history of treatment with liraglutide.

DMEP sent a consult request to the Division of Epidemiology, Office of Surveillance and Epidemiology, to evaluate this protocol. A synopsis of the study and its evaluation follow.

2 MATERIALS REVIEWED

The protocol entitled "Medullary Thyroid Carcinoma Surveillance Study: a Case-Series Registry" was reviewed.

3 RESULTS

3.1 Synopsis of study

This protocol describes plans for an active surveillance program and case-series registry to identify incident cases of MTC that occur in the U.S. and describe the characteristics of those cases. This post-approval active surveillance program for MTC will be established to further evaluate whether there is an association between treatment with liraglutide and the occurrence of MTC in humans. The study will be conducted for up to [redacted] after the approval of liraglutide. The protocol states that there are two objectives: b(4)

- To systematically monitor the annual incidence of MTC in the U.S. through the North American Association of Central Cancer Registries (NAACCR) to identify any possible increase related to the introduction of liraglutide into the U.S. market
- To establish a registry of incident cases of MTC in adults in the U.S. in order to characterize their medical histories and possible risk factors, including history of liraglutide

Although not specifically stated in the protocol, it appears that the MTC surveillance program and case-series registry will be conducted under a contract to [redacted] b(4)

Cases will be identified from state/regional population-based cancer registries through the NAACCR. Cancer registries that have an average of at least 10 reported cases of MTC per year and meet the NAACCR's standards for data collection and timeliness will be invited to participate in the surveillance program. In areas where a population-based registry is unable or unwilling to participate, comprehensive cancer center registries may be directly invited to participate. According to the protocol, 40 states have longitudinal MTC data and at least 14 states will be asked to participate in the MTC case series registry, representing a total of 1789 (75.3%) of the 2375 MTC cases reported historically from 2001-2005.

NAACCR monitors the annual incidence rates of various cancer sites including MTC, and incidence rates from 2001 until the time of the U.S. market introduction of liraglutide will serve as a baseline. Based on a personal communication from the NAACCR, the protocol reported that the age-adjusted rate of MTC for the period 2001 through 2005 in the U.S. was 0.2 per 100,000. Annual rates from all population-based cancer registries included in NAACCR will be documented for the 10-year period after liraglutide approval. Trends by age and gender will be examined to identify any possible increases.

MTC cases will be identified using the specific histologic criteria for classification of MTC based on the International Classification of Diseases for Oncology, 3rd edition (ICD-O-3) codes.

Each participating cancer registry will be asked to identify all cases of MTC that occur as soon as possible after diagnosis (based on the registry's specific standard operating procedures). A contract will be established with each cancer registry willing to participate in the MTC case series registry and compensation will be provided to each cancer registry for identifying the patients and physicians to be recruited. Each participating registry or cancer center will be required to obtain Institutional Review Board or Ethics Committee approval of the protocol prior to inclusion of cases in the MTC registry.

Protocols for recruiting patients into the MTC case series registry will be developed based on the requirements of the reporting cancer registry. There are two scenarios that each participating cancer registry or cancer center can use for recruiting patients: direct invitation of patients or recruitment through the diagnosing physician. In cancer registries that allow direct patient contact, the participating cancer registry will be asked to send a written invitation to the patient to participate in the MTC case series registry and contact the patient directly to explain the study and to request consent to release his/her name to a Study Coordinating Center (SCC). The patient's physician as indicated in the cancer registry would then be notified of the MTC registry as a courtesy.

After patient informed consent is obtained by the cancer registry staff, patient identifying data will be transferred to the SCC staff who will contact the patient by telephone, further explain the study, and confirm his/her consent to participate. Data provided by the cancer registry about the incident MTC case will include demographic information, clinical data about the cancer, and vital status including date of death, if deceased. The telephone interview will be conducted by a trained interviewer using a standard questionnaire to obtain additional information from the patient (or his/her proxy) including: additional demographic factors, family history of cancer (including history of MEN 2A or MEN 2B, history of familial MTC, history of RET proto-oncogene mutations), results of RET proto-oncogene testing, comorbid conditions (type 2 diabetes mellitus, previous history of cancer), diabetes medication exposures (including liraglutide and other GLP-1 agonists) with dose and duration of use, other medication exposures, events leading to diagnosis (e.g., calcitronin screening, thyroid nodule, thyroid ultrasound, thyroid scan, fine needle aspiration, surveillance related to family history), radiation exposures, lifestyle factors such as smoking and alcohol use, environmental exposures from occupational history and radioiodine exposure, and nuclear fallout.

If the patient is unable to provide sufficient medical history and risk factor data, he/she will be asked to identify his/her primary care physician and oncologist and a release of information form will be sent to the patient to allow the SCC to contact the physician(s) and obtain information. If the patient is unable to participate in the phone interview, he/she will be asked to indicate a family member or caregiver familiar with his/her medical history who may be contacted to participate. Patients who complete the interview will be compensated with a payment of $25.

For state or regional cancer registries that are unable to directly contact a patient, the patient's physician identified in the cancer registry records will be asked to provide the required information for the MTC case series registry or to directly recruit the patient. The cancer registry will send a written invitation to the physician to participate. If the physician agrees, he/she will be provided with a written invitation to send to the patient asking him/her to call the SCC and enroll in the study.

If the physician is unwilling to contact the patient, he/she will be asked to provide the required data directly without specific patient identifiers. The SCC will send data collection forms to the physician requesting the patient's initials, gender, and year of birth. Alternatively, the physican will be directed to the study website for entry of data online.

If allowed by the cancer registry, the physician will be contacted to complete a data collection form for any patient who is deceased.

Incident MTC cases will come from each reporting NAACCR registry or comprehensive cancer center supplied in the NAACCR format. According to the protocol, the basic NAACCR dataset will be provided for each case so that the characteristics of patients not included in the MTC case series registry (those who do not consent to the interview) can be compared with those who are included.

Using data supplied by NAACCR for 2001 through 2005, and assuming the 14 areas having large numbers of cases of MTC continue to have them, then approximately 358 patients will be identified per year for posible inclusion in the MTC registry. However, the actual number of participating patients is expected to be "somewhat smaller."

The demographic and clinical characteristics of all identified cases of MTC will be summarized using descriptive statistics. Characteristics of cases included in the MTC case series registry will be compared to those not included. Comparisons will be done within the originating cancer registry and the overall NAACCR database.

The hypothesis of intensified screening for identification of MTC cases will be explored in the analysis.

Descriptive statistics will be used to characterize potential risk factors, including drug exposures, radiation exposure, lifestyle factors, environmental exposures and other characteristics (including family history of MEN syndromes or familial MTC history). For patients with diabetes, liraglutide exposure will be characterized by dose and duration prior to the diagnosis of MTC.

The MTC case series registry will be approved by the required central or local Institutional Review Board (IRB) prior to any registry activities being initiated by a reporting cancer registry.

A registry data monitoring committee will be established to review the data from the MTC registry over time amd make recommendations to Novo Nordisk regarding its conduct. The Data Monitoring Committee in consultation with Novo Nordisk and the FDA will be responsible for making the determination to initiate a case-control study if warranted.

Data on the progress of the study will be provided to the FDA at 12 months after the approval of liraglutide and annually. The reports will include an evaluation of the effectiveness of surveillance in meeting the objective of successfully obtaining data for a substantial proportion of the incident cases of MTC in adults in the U.S. A final study report will be provided to the FDA within 6 months of completion of the study.

3.2 Reviewer's comments

1) Contractor and staff not identified and previous experience with similar studies not provided

b(4)

The protocol used — stationary with the acronym — at the top of each page, but it did not state what organization and staff will conduct the study and the staff's qualifications. From an internet search, it seems likely that ——— ———, a contractor used by pharmaceutical companies to perform epidemiological research. Protocols should include the name of the contractor and staff credentials and mention any previous experience with similar studies.

Of note, a similar "case series study established to monitor at least 40% of osteosarcomas occurring annually in men and women older than 40 years old who reside in the United States" (1) to detect an association of osteosarcoma with teripatide (Forteo), apparently has not detected any cases, although two cases of osteosarcoma following teripatide exposure have been reported in the medical literature (1, 2). The first case involved a "postmenopausal woman in her 70s with a complex past medical history" initially reported to a Lilly sales representative (2). The second teripatide-exposed osteosarcoma case was a 67-year-old man with a history of radiation therapy who used teripatide two months before his diagnosis of osteosarcoma, according to clinicians at the University of Texas M.D. Anderson Cancer Center (1). The two cases were among the "more than 430,000 persons who have received teripatide for treatment of severe osteoporosis" (1).

2) Participation of cancer registries and comprehensive cancer center registries

The study's objective, to define the incidence of MTC in the U.S., would be seriously compromised if there is poor participation of cancer registries and comprehensive cancer center registries. The protocol states that cancer registries that have at least 10 reported cases of MTC per year and meet the NAACCR standards for data collection and timeliness will be invited to participate. It also stated that in areas where a population-based registry is unable or unwilling to participate, comprehensive cancer center registries may be directly invited to participate. At least 14 states will be asked to participate in the MTC case series registry, representing a total of 1789 (75.3%) of the 2375 cases reported historically from 2001-2005. If even a few of the 14 states refuse participation, the rate might drop to around 50%.

The protocol does not state whether similar case series registries have been undertaken previously using NAACCR data, and what the participation rate was or can be expected

to be. This information should have been included in the protocol. If this is the first time that the NAACCR is engaging in this type of study, this should have been stated.

Sensitivity analyses showing various participation rates should have been presented.

The protocol states that compensation will be provided to each registry for the work involved in identifying and recruiting patients and physicians. The question arises whether the compensation will be incentive enough to enhance cancer registry participation rates. Pilot testing might be performed to determine if the amount offered will be enough to enhance participation.

3) Participation of patients

This study and its objectives will be significantly compromised if there is poor enrollment/participation of patients. Many studies do not achieve desired participation rates when patients are contacted for consent to enroll in a study and to provide and release personal medical information over the telephone. The protocol should have stated the number of telephone call back attempts (with varying times of day) that will be made before the patient is counted as a non-respondent.

The protocol should have stated the expected range of patient participation rates and the resulting sample sizes. Patients will be offered an incentive of $25 to complete the telephone interview. Pilot testing might be performed to determine if the amount offered is enough to enhance participation.

4) Participation of physicians

In situations where a state or regional cancer registry is unable to directly contact a patient, the cancer registry staff will ask the patient's physician identified in their records to provide the desired patient information for the MTC case series registry or to directly recruit the patient. If physicians fail to recruit patients or provide patient information, the study will be seriously compromised.

When allowed by the cancer reporting registry, physicians will be contacted to complete a data collection form for any patient who is deceased. The protocol should state what proportion of cancer registries will allow this contact, and provide a range for the expected participation rate in completing the data collection form.

5) Sample size, missing reports, and reporting to AERS

Sample size might be low since, as stated above, it will depend on the joint participation of cancer registries, physicians, and patients. Sensitivity analyses showing various assumptions for participation rates, relative risks, and latency periods for the development of MTC should have been presented.

Because MTC is rare, missing even a couple of cases of MTC in liraglutide-exposed patients could lead to serious underascertainment of risk.

Missing cases in the MTC case series registry might be supplemented by timely reporting of MTC in liraglutide-exposed patients to the FDA's Adverse Event Reporting System (AERS). Consequently, the product information and the Medication Guide should prominently display the FDA's MedWatch contact information.

6) Possible difficulty in detecting a change in MTC incidence and interpretation of any change

If liraglutide use is relatively low, it may not be possible to detect a change in MTC incidence even if the drug causes MTC.

Furthermore, if the national (background) incidence of MTC changes during the study period compared with the baseline period, it may be difficult to determine that the change is due to liraglutide. The protocol should acknowledge problems with the interpretation of ecological data.

7) Lag time between diagnosis and cancer registry registration

From data accumulated by the NAACCR, the protocol should have stated what the average lag time is between the date of diagnosis and the reporting of MTC to the cancer registry.

8) Detection of double counting of patients

Since there might be overlap between data accumulated by NAACCR and comprehensive cancer center registries, the cancer registry and Study Coordinating Center staffs should be aware of, and try to avoid, any double counting of patients.

9) Identifying deceased patients and obtaining exposure information

The protocol should state if patients diagnosed with MTC at death are included in the cancer registries. If possible, the proportion of patients diagnosed at death with MTC should be provided.

Obtaining anti-diabetic exposure information about deceased patients may be difficult, and misclassification of exposure is a potential problem.

10) Collecting additional data on thyroid conditions

The Study Coordinating Center will collect additional demographic data, medical history, and exposure information by telephoning the patient or his/her proxy. In addition to the list of information requested, I suggest that history of other thyroid conditions be added including hypothyroidism and hyperthyroidism. I also suggest that weight and height information be added to the list of lifestyle factors.

The protocol should specifically state if all anti-diabetic medications including insulin will be requested from the patient and his/her proxy.

The protocol should state the relevant time period for data collection (e.g., ever antidiabetic use, use in the past five years, etc.).

11) Use of proxies to obtain data and probability of missing information

Unless the proxy is the spouse of the patient with MTC, he/she is unlikely to know the answers for much of the information requested. Consequently, use of proxies is likely to be associated with more missing and lower quality data.

12) Interpretation of data due to absence of controls

Since no control data will be collected, causality assessment will likely be problematic. The Data Monitoring Committee, in consultation with Novo Nordisk and the FDA, will

decide if a case-control study is warranted. In anticipation of the need for such a study, the protocol should state what controls might be appropriate.

13) Representativeness of data

Since the data on MTC will not be a total count of cases nor a scientific sample from cancer registries, they may not be a reliable estimate of the incidence of MTC in the U.S. The protocol should acknowledge this. Performing demographic comparisons between cases included and not included may help determine the representativeness of those included.

14) Publication of data

The protocol states that a final study report will be provided to the FDA within 6 months of the completion of the study.

Novo Nordisk and ———— should commit to a plan to publish the data to make publicly available more information on MTC incidence and potential etiology as well as procedural and methodological issues involved in setting up a case series registry for a rare event.

4 SUMMARY

Because of an increase in C-cell tumors of the thyroid gland in mice and rats during the liraglutide preclinical test phase, and an increased frequency of C-cell hyperplasia, a dose-related trend in calcitonin levels, and a numerical imbalance in papillary thyroid cancer in patients exposed to liraglutide injection during phase 3 clinical trials, Novo Nordisk has proposed to conduct postmarketing studies to determine if patients exposed to liraglutide have increased frequencies of MTC. As a result, the protocol entitled "Medullary Thyroid Carcinoma Surveillance Study: a Case-Series Registry" was reviewed. b(4)

In brief, the researchers plan to identify MTC cases from established cancer registries and comprehensive cancer centers for inclusion in an MTC case series registry. They also plan to monitor the incidence of MTC for a —— period after liraglutide marketing, compare it with the national (background) incidence, and interview cases about possible risk factors including liraglutide exposure. b(4)

A number of limitations are provided in the text above. The most important limitation of this MTC case series registry might be nonparticipation of cancer registries, patients, and physicians leading to missing cases and possible underascertainment of liraglutide risk.

Missing cases in the MTC case series registry might be supplemented by timely reporting of MTC in liraglutide-exposed patients to the FDA's Adverse Event Reporting System (AERS). Consequently, the product information and the Medication Guide should prominently display the FDA's MedWatch contact information.

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Public Health Service
Food and Drug Administration
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Office of Surveillance and Epidemiology

Date:	October 7, 2009
To:	Mary Parks, M.D., Director, Division of Metabolic and Endocrine Drug Products, OND
Through:	Solomon Iyasu, M.D., Director, Division of Epidemiology, OSE
Through:	Gwen Zornberg, M.D., Team Leader, Division of Epidemiology, OSE
From:	Diane K. Wysowski, Ph.D., Epidemiologist, Division of Epidemiology, OSE
Subject:	Review of Protocol for "A Health Care Database Study Using i3 Aperio to Evaluate Safety of Liraglutide"
Drug Name(s):	Liraglutide injection (Victoza)
Submission Number:	0039
Application Type/Number:	NDA 22-341
Applicant/sponsor:	Novo Nordisk
OSE RCM #:	2009-1563

CONTENTS

EXECUTIVE SUMMARY

Liraglutide is a human glucagon-like peptide-1 (GLP-1) receptor agonist developed as a treatment for type 2 diabetes mellitus. Following its launch in the U.S., Novo Nordisk plans active adverse drug event surveillance by comparing liraglutide with other anti-diabetic agents using the i3 Aperio database. The company also plans to use the database for longitudinal follow-up to five years of patients exposed to liraglutide and comparison drugs for specific outcomes including the primary outcome, thyroid cancer, and secondary outcomes, pancreatitis, serious hypoglycemia, and cardiovascular diagnoses.

The protocol that presents the company's plans was reviewed and a number of issues were identified and are discussed in detail below. The most important include: difficulty interpreting results when only drugs within the same class are compared, misclassification of outcomes, possible inadequate sample size and statistical power, incomplete mortality data, possible selection bias, misclassification of exposures, the absence of a specific ICD code for medullary thryoid cancer necessitating access to medical and histological records to determine the type of cancer, possible inability to obtain medical records for validation purposes, missing information about potentially important confounders, and difficulty interpreting multiple tests of significance.

The most important limitation is likely to be an insufficient sample size and statistical power to adequately evaluate an association between liraglutide and the primary outcome, thyroid cancer and particularly the more lethal and rarer medullary thyroid cancer.

In general, after several years of operation, the i3 Aperio database is not known for its ability to identify new serious adverse drug events. Epidemiologists within FDA who have used the i3 Aperio database have not found it to be particularly useful in this respect.

1 BACKGROUND

Glucagon-like peptide (GLP-1) is an incretin hormone secreted by the L-cells in the lower gut which stimulates endogenous insulin secretion in a physiologic, glucose-dependent manner, and liraglutide is an analogue of GLP-1 with 97% amino acid homology to human GLP-1. This injectable drug stimulates insulin secretion and lowers inappropriately high glucagon secretion in a glucose-dependent manner and improves beta-cell function. It is developed for treatment of type 2 diabetes mellitus.

The new drug application for liraglutide injection is currently being reviewed in the Division of Metabolic and Endocrine Drug Products (DMEP). Concern exists about a potential safety problem because of an increase in C-cell tumors of the thyroid gland found during the preclinical test phase in mice and rats. In addition, according to a review by Karen Mahoney, M.D., DMEP, an increased frequency of C-cell hyperplasia, a dose-related trend in calcitonin levels (a biomarker of medullary thyroid cancer, the

human equivalent of C-cell carcinoma in rodents), and a numerical imbalance in papillary thyroid cancer were found in patients exposed to liraglutide during phase 3 clinical trials. Furthermore, a small increase in major cardiovascular events occurred with liraglutide compared to placebo. Dr. Mahoney also noted imbalances not favoring liraglutide for gastrointestinal adverse events, pancreatitis, serious neoplasm events (besides nonmalignant neoplasms and papillary thyroid), thyroid neoplasms, serious hypoglycemic events, injection site reactions, immunogenicity events including urticaria, hepatobiliary events, increased heart rate, animal fetal anomalies, and nonserious adverse events of dizziness and fatigue. She also noted liraglutide antibody formation with possible predispostion to infections and musculoskeletal pain, slowing of gastric emptying with pharmacokinetic effects on other drugs, and a potential for medication errors and off-label use/abuse. Dr. Mahoney does not recommend approval at this time.

In response to the adverse events observed in liraglutide clinical trials, Novo Nordisk stated that, "Several potential rare safety signals described in the liraglutide new drug application may require further assessment through post marketing surveillance of a large patient group" and the company submitted to the FDA a risk evaluation and mitigation strategy (REMS). Also appended were two protocols to evaluate the post-marketing safety of liraglutide.

One protocol involved a prospective cohort study of adult diabetic patients using a large healthcare database from a U.S. managed care population. According the company, the aim of the study is "to describe and monitor the safety profile of liraglutide and compare incidence of adverse events with other similar anti-diabetic medications." The study will commence after the drug's market approval in the U.S. and will use data of the i3 Aperio system. It is planned to continue for five years.

DMEP sent a consult request to the Division of Epidemiology, Office of Surveillance and Epidemiology, to evaluate this protocol. A synopsis of the study and its evaluation follow.

2 MATERIALS REVIEWED

The protocol entitled "A Health Care Database Study Using i3 Aperio to Evaluate Safety of Liraglutide" (trial ID: NN2211-3784) was reviewed.

3 RESULTS

3.1 Synopsis of study

This safety surveillance study will use the i3 Aperio database to compare liraglutide with other anti-diabetic drugs for a wide range of safety outcomes.

The primary outcome of interest is:

- thyroid cancer

Secondary outcomes include:

- serious hypoglycemia that results in medical care
- pancreatitis

- macro-vascular conditions (myocardial infarction, ischemic heart disease, coronary artery bypass grafting, PTCA, other surgeries, lower limb amputation, stroke, and heart failure)
- micro-vascular conditions (blindness, retinopathy, nephropathy, neuropathy, peripheral vascular disease) and
- thyroid events

The i3 Aperio data originate from the Ingenix National Health Informatics (NHI) database, a proprietary data environment with longitudinal health care information containing claims and health plan enrollment data dating back to 1993 that offers linkages among patient and physician survey data, pharmacy and medical claims, medical record data, socioeconomic measures, and clinical laboratory results. For year 2006, data relating to more than 14 million individuals with both medical and pharmacy benefit coverage are available. The patient population is geographically diverse across the United States and is updated frequently.

For the NHI, medical claims data are collected from all available health care sites (inpatient hospital, outpatient hospital, emergency room, physician's office, surgery center, etc.) for virtually all types of provided services, including specialty, preventive, and office-based treatments. Each facility service record contains information on up to nine diagnoses recorded with the the International Classification of Diseases, Ninth Revision (ICD-9-CM) diagnosis codes, and up to six procedures recorded with ICD-9-CM procedure codes, Current Procedural Terminology (CPT), or Health Care Financing Administration (HCFA) Common Procedure Coding System (HCPCS) codes. The NHI data do not include drugs administered in a hospital.

Each individual provider service record contains information on up to four diagnoses recorded with ICD-9-CM codes, and one procedure code recorded using CPT or HCPCS codes. Incorporation of medical claims data in the NHI database requires about six months to capture 95% of the data.

Pharmacy service claims are typically submitted electronically by the pharmacy at the time prescriptions are filled. Pharmacy claims data are included in the NHI database within approximately six weeks of payment of the underlying claim.

Laboratory test results are available "for subpopulations of the research database." Results included are typically from "blood-borne tests." Standard Logical Observation Identifier Names and Codes (LOINC) are used to define specific tests and results.

The main inclusion criteria for study subjects will be health plan membership with pharmacy benefit, ≥18 years old, and a diagnosis of type 2 diabetes "treated with one or more oral antidiabetic drugs" for the previous three months using any of the following drugs or combinations: liraglutide, metformin, exenatide, sulfonylureas, sitagliptin, rosiglitazone, and pioglitazone. Subjects will be required to have six months of continuous health plan enrollment before the date of their first pharmacy claim for an antidiabetic drug to allow time to evaluate baseline conditions of study subjects.

Data accrual will start after liraglutide approval in the U.S. and health plan members receive dispensings of the drug. Follow-up will be for at least three years with options of

extending to five years or more. The frequency of generating interim reports will be determined after consultation with regulatory agencies. For the i3 Aperio system, the shortest interval for generating interim reports is every three months.

Claims data will be extracted from NHI by Drug Safety staff for the study population and will be prepared for the i3 Aperio format and loaded into the i3 Aperio data system. Through a comprehensive level subscription, de-identified data sets will be transferred to Novo Nordisk for further analysis.

All patients exposed to liraglutide meeting the inclusion criteria will be included. The protocol states that "Approximately 5,000 active subjects exposed to liraglutide in the database per year and approximately 25,000 subjects exposed to liraglutide in 5 years are expected." Subjects exposed to metformin, exenatide, sulfonylureas, sitagliptin, rosiglitazone, and pioglitazone are already in the database. Based on propensity score matching, the same numbers exposed to these comparators will be included in 1:1 ratio. So the total sample size will be approximately 150,000 in 5 years."

Data used for propensity score matching that may be associated with the decision to prescribe therapy with liraglutide as opposed to a comparator drug include age, sex, geographic region, measures of health care utilization, inpatient or physician diagnosis codes, procedures performed, and categories of drugs dispensed during the six months before the date of drug initiation. The comparison cohorts will be liraglutide versus each of the following: exenatide, sitagliptin, metformin, rosiglitazone or pioglitazone, and a sulfonylurea. For study subjects in each comparison, a propensity score is then estimated for each member of the liraglutide or comparator initiator cohort using a logistic regression model with group status (liraglutide or comparator) as the outcome and all identified predictors of therapy as independent variables. Further details of the propensity score matching and analysis process are provided within the protocol.

The six months before drug initiation including the drug initiation date itself comprise the baseline period. The two cohorts (liraglutide initiators and comparator initiators) are followed indefinitiely as long as the patient is an active health plan member regardless of persistency in antidiabetic drug and switching between different antidiabetic agents. Outcome events occur after baseline from one day through the entire open-ended follow-up period after the drug initiation date. Treatment-emergent events are defined as those not found for the same patient during the baseline period. Patients' claims are followed for one year or until the end date of the current claims data or until the disenrollment date whichever is earliest.

With a subscription to i3Aperio, Novo Nordisk will have access to a standard set of baseline and outcome tables. The tables and analytic tools are accessed through a web-based interface (www.i3aperio.com) and controlled through account specific user names and passwords. For each outcome event, the magnitude of the difference in frequency of the event between liraglutide and comparator initiator cohorts stratified by baseline attributes will be presented in the tables as a relative risk estimate with nominal 95% confidence bounds. According to the protocol, "Poisson regression analysis, Kaplan-Meier estimation, and Cox proportional hazards regression analysis will be conducted to estimate adjusted rates, relative risks, cumulative hazards, and hazard ratios."

The i3 Aperio system also presents a score that summarizes the magnitude and statistical uncertainty of the association between the event and the drug exposure (liraglutide or comparator). Scores are defined as zero if the number of patients with the emergent code is equal to the expected number (based on the total number of events and the total number of liraglutide and comparator drug initiators). Scores are a positive value if the event is more frequent in the liraglutide cohort than the comparator cohort, and a negative value if the event is more frequent in the comparator cohort than in the liraglutide cohort.

In addition to the standard analyses, a stratified analysis can be conducted based on user-defined baseline attributes (e.g., age, sex, geographical region, baseline diagnosis, procedures, and therapeutic drug class). Users also may restrict emergent diagnoses, procedures, and dispensed drug classes to the events that occurred during intervals (1-7 days, 8-29 days, 30-89 days, and 90-365 days) from the first dispensing of the drug of interest.

For 25 or fewer events, the web-based i3 Aperio system allows the user to view the de-identified claims history of specific patients to provide further clinical perspective. In addition, the i3 Drug Safety staff has developed "a robust process" to obtain medical records for review from medical care providers after ethical review by an Institutional Review Board for privacy protection considerations. However, a separate contract is needed to conduct medical record reviews.

Novo Nordisk will communicate results to the FDA and regulatory authorities "at agreed time intervals." The protocol also states that Novo Nordisk commits to communicating or otherwise making available for public dislosure (publication in a scientific journal, abstract submission with a poster or oral presentation at a scientific meeting, etc.) of results of studies regardless of outcome.

3.2 Reviewer's comments

1) Interpretation of results when comparing only drugs within a class

To determine a drug's unique profile of adverse events, it is useful to not only compare drugs within the same therapeutic class, but also to compare those that are not within the same therapeutic class. Drugs within the same therapeutic class often have similar adverse event profiles and, therefore, no important adverse event differences are found; however, differences are more likely found when comparing drugs that are not in the same therapeutic class. Therefore, one cannot conclude that a drug does not have an adverse event based on a comparison with other drugs in the same class, but only that no large differences exist among the drugs in frequencies of the adverse event. As a result, it would be useful if the i3 Aperio system also allowed for comparison of liraglutide with other chronically used drugs outside its therapeutic class (e.g., antihypertensives or cholesterol-lowering drugs).

2) Rule out or provisional diagnoses and misclassification of outcome

While i3 Aperio might be useful as a safety surveillance tool, it would not provide definitive results because it is expected that a large proportion of diagnoses will be "rule out" or provisional diagnoses with misclassification of outcomes. A recent study by i3 Drug Safety staff that concerned validation by medical records of acute pancreatitis diagnosis codes indicated that the predictive value positive was 49% (1). In the i3 Aperio

study, the primary outcome of interest, thyroid cancer, and most secondary outcomes including pancreatitis, myocardial infarction, ischemic heart disease, stroke, heart failure, retinopathy, nephropathy, neuropathy, and peripheral vascular disease would require validation by medical records.

3) Absence of ICD code for medullary thyroid cancer

There is no International Classification of Diseases (ICD) code specific for medullary thyroid cancer. Consequently, any thyroid cancers that are identified in this study would require that medical and histological records be obtained to identify the type of cancer. This should be stated explicitly in the protocol.

4) "Thyroid events" as an outcome

The protocol should specify what "thyroid events," in addition to thyroid cancer, are of interest and would be analyzed using i3 Aperio.

5) Sample size and statistical power

The protocol states that "Approximately 5,000 active subjects exposed to liraglutide in the database per year and approximately 25,000 subjects exposed to liraglutide in 5 years are expected." Subjects exposed to metformin, exenatide, sulfonylureas, sitagliptin, rosiglitazone, and pioglitazone are already in the database. Based on propensity score matching, the same numbers exposed to these comparators will be included in 1:1 ratio. So the total sample size will be approximately 150,000 in 5 years."

The protocol does not provide any basis for its estimation of 5,000 subjects exposed to liraglutide per year, 25,000 over 5 years, and the sample size for all anti-diabetics of 150,000 in 5 years. The company should provide some basis for its exposure estimates. Also, it should provide estimates of the range of exposure to liraglutide and perform calculations using these ranges to estimate statistical power for the ability to detect differences in drugs for thyroid cancer incidence. Based on the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) data for 2002-2006 (2), the age-adjusted annual incidence rate of invasive thyroid cancer for all ages, both sexes, and all races was 9.6 per 100,000 population, and for individuals < 65 years old, it was 8.7 per 100,000 population, or about 1 per 11,500 population (2). Furthermore, medullary thyroid cancer, the human equivalent of C-cell carcinoma in rodents and the greatest concern because of its case-fatality rate, accounts for a fairly small proportion of thyroid cancer overall, estimated at 1.6% to 5%. According to a separate protocol submitted by Novo Nordisk concerning active surveillance of medullary thyroid cancer with a personal communication from the North American Association of Central Cancer Registries, the age-adjusted rate in the U.S. for the period 2001 through 2005 was 0.2 per 100,000. Consequently, unless exposure to liraglutide and the risk of thyroid cancer in liraglutide-exposed patients is high and the latency period for thyroid cancer is relatively short, very few cases of thyroid cancer and probably no cases of medullary thyroid cancer will be identified over the five-year study period.

Besides thyroid cancer, other rare outcomes also would be unlikely to be detected.

6) Representativeness and generalizability of the findings

Since i3 Aperio uses data from the Ingenix National Health Informatics (NHI) database of medical claims from mostly employed individuals who are generally ≤ 65 years of age, the findings would be most applicable to this group.

7) Lack of complete mortality data

Deaths that occurred in a hospital affiliated with Ingenix NHI would result in a claim in the database; however, if a death occurred outside of an affiliated hospital (as often is the case) and without the plan's coverage, no claim would be filed and neither the fact of death nor the cause of death would be identified in the NHI or in the i3 Aperio systems. The sponsor might be able to remedy this by accessing the Nationl Death Index of the National Center for Health Statistics to identify the fact and causes of death of included patients, especially those who are lost to follow-up.

8) Inclusion/exclusion of patients taking insulin

Although the protocol states that type 2 diabetes subjects who are ≥18 years of age and treated with one of more oral antidiabetic drugs for the last 3 months and satisfy the enrollment criteria can be included in the study, it does not specify if patients who use insulin concomitantly with the study drugs will be included or excluded. A statement should be made regarding whether concomitant insulin will be an inclusion or exclusion criterion, and, if included, how concomitant insulin use data will be analyzed (e.g., by stratification or adjustment).

9) Selection bias and injectable antidiabetic agents

In analyses, liraglutide, an injectable antidiabetic agent, will be compared 1:1 with mostly oral antidiabetic agents. Since it's likely that persons using an injectable product have more serious diabetes, analyses should be presented to show that propensity score matching takes account of increased severity of diabetes in liraglutide-exposed patients ie., by comparing the drugs at baseline and after propensity score matching. Also, the analyses should provide the number of patients that were not able to be matched and were excluded from the analyses.

10) "Intent to treat analysis" and exposure misclassification

The protocol states that "Although patients may switch from one drug to the other after the first dispensing of a drug of interest, the principle of intent to treat analysis will be followed, such that each patient is assigned to a cohort according to the first dispensing of a drug of interest." Further it states that "The two cohorts (liraglutide and comparator initiators) are followed indefinitely as long as the patient is an active health plan member, regardless of persistency in antidiabetic drug and switching between different antidiabetic drugs." Consequently, since discontinuation and switching of antidiabetic agents is expected, exposure misclassification over time is likely, resulting in problems with interpretation of positive findings.

The protocol should discuss the rationale for an intent to treat analysis as compared with a time to event analysis that takes discontinuation, switching, and duration of medication use into account.

11) Possible inability to obtain medical records for validation purposes

Although the protocol states that i3 Drug Safety staff has been successful in obtaining medical records to validate diagnoses, it does not state what their usual success rate is. This should have been stated, since in some studies the rate of obtaining medical records has been as low as 50%.

12) Lack of information on testing for balance following propensity score matching

The i3 Aperio system should show statistically significant differences between liraglutide and the comparator drug at baseline and after propensity score matching to show the effect of the matching process. The number of individuals who could not be matched and remain outside of the analyses should be provided.

13) Missing information for potentially important confounders

The protocol acknowledges that "given the potentially wide range of outcomes of interest to be evaluated, there may be important confounders for certain outcomes that may not be measured and adequately controlled for in the design and analysis." Important confounders that would be likely missing over time in claims data include cigarette smoking, body mass index, alcohol use, illegal drug use, non-prescription drug use, etc.

14) Latency of claims data

While pharmacy claims data are included in the database within about six weeks of payment of the underlying claim and laboratory tests are generally added within six weeks of the test, six months is required to capture 95% of medical claims data. Since the study is planned to be ongoing for five years, this does not appear to be an important limitation.

15) Difficulty interpreting multiple tests of significance

Because a wide range of outcomes will be compared between liraglutide and comparator drugs, a number of outcomes may achieve statistical significance based on chance alone. Consequently, acknowledgment of this issue should be made in the protocol's methods section.

16) "Track record" of the i3 Aperio database

In general, after several years of operation, the i3 Aperio database is not known for its ability to identify new serious adverse drug events. Using i3 Aperio as a search term in PubMed, I was able to find only two published studies in which i3 Aperio was used (3,4), and in both studies adverse events were not identified or confirmed. Epidemiologists who have used the i3 Aperio database at the FDA for exploratory analyses stated that they have not found it to be particularly useful in this respect.

4 SUMMARY

Following the launch of liraglutide in the U.S., Novo Nordisk plans active adverse drug event surveillance by comparing liraglutide with other anti-diabetic agents using the i3 Aperio database. The company also plans to use the database for longitudinal follow-up to five years of patients exposed to liraglutide and comparison drugs for specific outcomes such as thyroid cancer, pancreatitis, serious hypoglycemia, and cardiovascular diagnoses.

The protocol that details their plans was reviewed and a number of issues are discussed in detail above. The most important include: difficulty interpreting results when only drugs within the same class are compared, misclassification of outcomes, possible inadequate sample size and statistical power, incomplete mortality data, possible selection bias, misclassification of exposures, the absence of a specific ICD code for medullary thryoid cancer necessitating access to medical and histological records to determine the type of cancer, possible inability to obtain medical records for validation purposes, missing information about potentially important confounders, and difficulty interpreting multiple tests of significance.

The most important limitation is likely to be an insufficient sample size and statistical power to adequately evaluate an association between liraglutide and the primary outcome, thyroid cancer, and particularly the more lethal and rarer medullary thyroid cancer.

In general, after several years of operation, the i3 Aperio database is not known for its ability to identify new serious adverse drug events. Epidemiologists who have used the i3 Aperio database at the FDA for exploratory data analyses have not found it to be particularly useful in this respect.

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