Clinical Endocrinology (2009) **71**, 500–506 doi: 10.1111/j.1365-2265.2008.03510.x

ORIGINAL ARTICLE

# No increased risk of hypoglycaemic episodes during 48 h of subcutaneous glucagon-like-peptide-1 administration in fasting healthy subjects

Susanne Lerche*, Liselotte Soendergaard*, Joergen Rungby*, Niels Moeller†, Jens Juul Holst‡, Ole E. Schmitz* and Birgitte Brock*

*Institute of Pharmacology, University of Aarhus, University Park 1240, 8000 Aarhus C, Denmark, †Medical Research Laboratory M, Aarhus University Hospital, Aarhus Hospital, Noerrebrogade 44, 8000 Aarhus C, Denmark, ‡Department of Biomedical Sciences, University of Copenhagen, Panum Institute, Blegdamsvej 3B, 2200 Copenhagen, Denmark

## Summary

**Objective** It is uncertain whether the ability to avoid hypoglycaemia during fasting is preserved, and the risk of reactive hypoglycaemia after an oral glucose stimulus following a prolonged fasting period is increased at augmented glucagon-like peptide-1 (GLP-1) levels.

**Design** A randomized, double-blind placebo-controlled cross-over study in eight healthy men to assess the safety, in terms of hypoglycaemia, of a continuously infused pharmacological dose of native GLP-1 during long-term fasting. After an overnight fast the fasting period continued for 48 h and was followed by a 3-h oral glucose tolerance test (OGTT). GLP-1(7-36 amide) or placebo was continuously infused subcutaneously and titrated to a dose of 4·8 pmol/kg per min.

**Results** Two subjects in the GLP-1 group and one subject in the placebo group were withdrawn due to protocol specified plasma glucose (PG) ≤ 2·8 mM and neuroglycopaenic symptoms.

The infusion of GLP-1 resulted in pharmacological levels of intact GLP-1. During the fasting period PG, insulin and C-peptide levels declined and glucagon, GH and free fatty acid (FFA) levels increased with no differences between GLP-1 and placebo. During OGTT circulating levels of insulin and C-peptide were higher with GLP-1 infusion. However, PG was similar during GLP-1 *vs.* placebo infusions. GLP-1 infusion increased norepinephrine and cortisol levels during OGTT.

**Conclusion** The counter-regulatory response during 48 h of subcutaneous GLP-1 infusion was preserved despite long-term fasting with no apparent increased risk of hypoglycaemic episodes. No reactive hypoglycaemia was observed when the fast was followed by an OGTT. Thus use of long-acting GLP-1 analogues may not increase the risk of hypoglycaemia.

Correspondence: Susanne Lerche, Institute of Pharmacology, University of Aarhus, The Bartholin Building, University Park 1240, 8000 Aarhus C, Denmark. Tel.: +458-9492071; Fax: +458-6128804; E-mail: lerche@ki.au.dk

(Received 21 August 2008; returned for revision 23 October 2008; finally revised 9 November 2008; accepted 11 December 2008)

## Introduction

Glucagon-like peptide-1 (GLP-1) is a potent insulinotrophic and glucagonostatic hormone secreted from entero-endocrine L-cells in the gastrointestinal mucosa in response to meal ingestion.[1] Several studies have demonstrated a beneficial glucose lowering effect in patients with type 2 diabetes,[2–5] and additionally GLP-1 may also play a vital role in the central (gut-to-brain) control of energy homeostasis.[6] Moreover, GLP-1 exerts several extra-pancreatic effects including inhibition of gastric emptying and possibly important protective effects in the brain and the heart.[7,8] *In vivo*, the stimulatory effect of GLP-1 on insulin secretion and the inhibitory effect on glucagon secretion are only observed at or above fasting glucose levels.[9] The counter-regulatory response to acute hypoglycaemia is preserved when infusing relevant doses of GLP-1,[10,11] and the suppression of glucagon by GLP-1 does not occur at PG ≤ 3·7 mmol/l.[10] Indeed, short-term infusion of different doses of GLP-1 did not induce hypoglycaemia in fasting healthy subjects.[12] However, another study revealed a possible risk of hypoglycaemia after a single subcutaneous pharmacological dose after fasting.[13]

In the fasting state hypoglycaemia is averted because of a shift in metabolism towards increased counter-regulation accompanied by reduced insulin secretion and sensitivity.[14] Endogenous GLP-1 production is low in the fasting state.[1] It is not known whether the ability to sufficiently compensate hypoglycaemia during long-term fasting is preserved, when pharmacological GLP-1 levels are present.

The reactive hypoglycaemia seen in gastrectomy patients following an oral glucose load may be due to an abnormally high endogenous GLP-1 response resulting in inappropriate hyperinsulinaemia.[15] Accordingly, a combination of a high-dose subcutaneous bolus administration of GLP-1 and an intravenous glucose bolus, imitating the glucose and hormone profiles of the gastrectomy

patient, resulted in hypoglycaemia in healthy subjects.[16] In type 2 diabetes patients, however, this combination could not induce hypoglycaemia.[17] It still remains uncertain whether an oral glucose stimulus after a long-term (48 h) fasting period accompanied by constant high GLP-1 levels would lead to reactive hypoglycaemia.

To date, the effect on glycaemia and counter-regulatory response of GLP-1 administration during fasting has never been explored after more than 12 h of fasting. Given the production of long-acting slow-release (LAR) GLP-1 analogues where high levels of GLP-1 are present in plasma for several days, questions arise as to whether the glucose dependency of GLP-1 and the counter-regulatory response is preserved during a long-term fasting challenge. Furthermore, the risk of reactive hypoglycaemia following an oral glucose load post-fasting is of great significance, and therefore, bearing this in mind, this potential risk is important to address.

Thus, the aim of this study was to assess the safety of a pharmacologically relevant continuously infused subcutaneous dose of native GLP-1 with respect to glycaemia and counter-regulatory response during a long-term fast followed by an oral glucose load in order to mimic the effect of a longer fasting period followed by resumption of food intake.

## Subjects, research design and methods

### Subjects

Eight nonsmoking, healthy males were included in the study. Mean age was 24 ± (SD) 2 years. Body mass index was 24·4 ± 0·7 kg/m$^2$. They had no history of diabetes or cardiovascular disease and received no medication. All had a normal physical examination. The study was conducted in accordance with the Declaration of Helsinki and the local Ethics Committee of the County in Aarhus approved the protocol. All participants received both oral and written information and signed an approved informed consent form before entering the study.

The study was monitored by the Good Clinical Practice Unit at Aarhus University Hospital, Denmark.

### Study design

The study was a randomized, double-blind, placebo controlled cross-over study. Thus each subject was studied twice in random order with GLP-1 and placebo infusion. The study periods were separated by an interval of a minimum of 10 weeks and a maximum of 16 weeks. Both periods commenced at 09·00 h after an overnight fast (11 h). The subjects did not exercise during the 24 h prior to the sessions. Subjects were placed in a bed and a cannula was inserted for blood sampling purposes.

Using a portable insulin pump, GLP-1 or placebo was infused for 51 h.

After 48 h of GLP-1 infusion and 59 h of fasting subjects received 75 g of oral glucose dissolved in 250 ml of water and a 3-h oral glucose tolerance test (OGTT) was performed.

The subjects were permitted to drink water *ad libitum* during fasting.

### Randomization

The randomization was performed by the pharmacy at Aarhus University Hospital where also the randomization list was kept during the study. Sealed envelopes containing information about the code were kept unbroken in the case report form. The code was first broken after all data analyses were completed.

### Hormone infusion

The subjects received either subcutaneous synthetic GLP-1(7-36 amide) or placebo at a rate of 1·6 pmol/kg*min (0–3 h), 3·2 pmol/kg per min (4–6 h) and finally 4·8 pmol/kg*min (7–48 h + 3 h of OGTT). The final dose of 4·8 pmol/kg*min was chosen as it has previously been shown to significantly improve glycaemic control in type 2 diabetes patients.[3]

Recombinant human GLP-1(7-36 amide) was a kind gift from BioNebraska Inc. (Lincoln, NE). Testing before use showed it to be sterile and free from bacterial endotoxins.

The concentration of GLP-1(7-36 amide) was 1 mg/ml and the solution was stored frozen at –20 °C. It was dissolved in a sterile buffer containing 600 mg of acetic acid, 50·7 g of mannitol and sterile water added up to 1000 g and had a pH of 4·5. The placebo solution consisted of the buffer. The test solution was infused using an Accu-Check® D-TRONplus insulin pump (Roche®).

### Blood sampling

Plasma glucose (PG) was measured in duplicate every hour as well as when symptoms of hypoglycaemia were observed. Blood for determination of insulin, C-peptide, glucagon, GLP-1 (total and intact), GH, free fatty acids (FFA), cortisol and ghrelin was drawn every 4 h. Blood for measuring epinephrine and norepinephrine was drawn every 8 h during the fasting period. During the OGTT, blood for measuring PG, insulin, C-peptide, glucagon, GLP-1 (total and intact), GH, FFA, cortisol, ghrelin, epinephrine and norepinephrine was drawn at –15, 0, 15, 30, 45, 60, 90, 120, 150 and 180 min.

### Assays

PG was measured immediately after sampling on a Beckman glucose analyser (Beckman, Palo Alto, CA). All other blood samples were stored at –20 °C (C-peptide at –80 °C) until assay.

The assays used for measuring serum insulin, serum C-peptide, serum FFA, serum GH, serum cortisol, plasma epinephrine and plasma norepinephrine have been described previously.[11] S-ghrelin was measured with an in-house assay.[18]

The assay for intact GLP-1 is an enzyme-linked immunosorbent assay using unextracted plasma, which was collected and stored in the presence of a dipeptidyl peptidase IV (DPP IV) inhibitor (valine-pyrrolidide, 0·01 mM final concentration added to the blood sample immediately after collection).[19,20] Total GLP-1 was analysed using a C-Terminal radioimmunoassay for amidated GLP-1.[21] Glucagon was measured using a previously described assay.[22]

### *Hypoglycaemia and safety*

The subjects were closely observed during both study periods. The heart rhythm was monitored during sleep in order to detect initial adrenergic effects of hypoglycaemia. At a PG ≤ 2·8 mmol/l and/or adrenergic symptoms subjects were withdrawn from the study period. Furthermore, subjects were withdrawn with the occurrence of neuroglycopaenic symptoms independent of PG level.

### *Statistics*

The primary endpoint was PG during the fasting period and during the OGTT.

The data were analysed using a linear mixed effects model with subject and all interactions involving subject, that is the interaction between subject and time and the interaction between subject and treatment, as random effects. Treatment (GLP-1 *vs.* placebo), time, and the interaction between the two were included in the analysis as fixed effects. Data at time = 0 were compared with data at time = 48 h using the Student's paired *t*-test. Hypoglycaemic events during fasting were compared using McNemar's $\chi^2$ square test. OGTT data were analysed by calculating AUCs of substrates and hormones by means of the trapezoidal rule and compared by a Student's paired *t*-test. Differences were considered significant at $P < 0{\cdot}05$. Normally distributed data are presented as mean ± SEM. The statistical software was STATA, StataCorp LP, TX.

This was intended as an exploratory study and thus the number of subjects was not derived from a specific power calculation.

## Results

Two subjects in the GLP-1 group and one subject in the placebo group were withdrawn from a study period due to hypoglycaemic events. However, all three subjects completed their second study period. The withdrawals were after 25 and 29 h (GLP-1) and 25 h (placebo) infusion at PG nadirs of 2·7, 2·8 and 2·8 mM respectively. All three subjects experienced neuroglycopaenic symptoms giving rise to nonscheduled glucose measurements. The difference in number of hypoglycaemic events during fasting, GLP-1 *vs.* placebo, was not statistically significant ($P = 0{\cdot}10$).

Plasma levels of intact GLP-1 increased during the first 4 h of titration (Fig. 1). The average concentration of intact GLP-1 was in the pharmacological range, 30·5 ± 2·0 *vs.* 5·6 ± 0·9 pmol/l (GLP-1 *vs.* placebo, mean ± SEM, $P = 0{\cdot}004$) from 4 to 51 h. No significant difference was observed between GLP-1 and placebo in levels of PG, insulin, C-peptide, FFA, glucagon, GH, cortisol, ghrelin (data not shown), epinephrine and norepinephrine ($P > 0{\cdot}10$) (Fig. 2). During the fasting period PG declined in a comparable manner [4·9 ± 0·09 to 3·7 ± 0·2 (GLP-1) *vs.* 5·0 ± 0·1 to 3·7 ± 0·1 (placebo) mmol/l, $P = 0{\cdot}003$]. Accordingly levels of insulin ($P = 0{\cdot}0006$) and C-peptide ($P = 0{\cdot}0001$) decreased with fasting time, whereas the glucagon level increased ($P = 0{\cdot}0001$) as did the levels of GH, ($P = 0{\cdot}0001$) and FFA ($P = 0{\cdot}0001$). Cortisol levels revealed a circadian pattern with peaks at 0, 24 and 48 h of fasting, this being similar during GLP-1 and placebo ($P = 0{\cdot}88$).

During the OGTT circulating insulin ($P = 0{\cdot}01$) and C-peptide ($P = 0{\cdot}02$) levels increased with GLP-1 infusion (Fig. 3). However, PG was similar with GLP-1 and placebo infusion ($P = 0{\cdot}59$). No

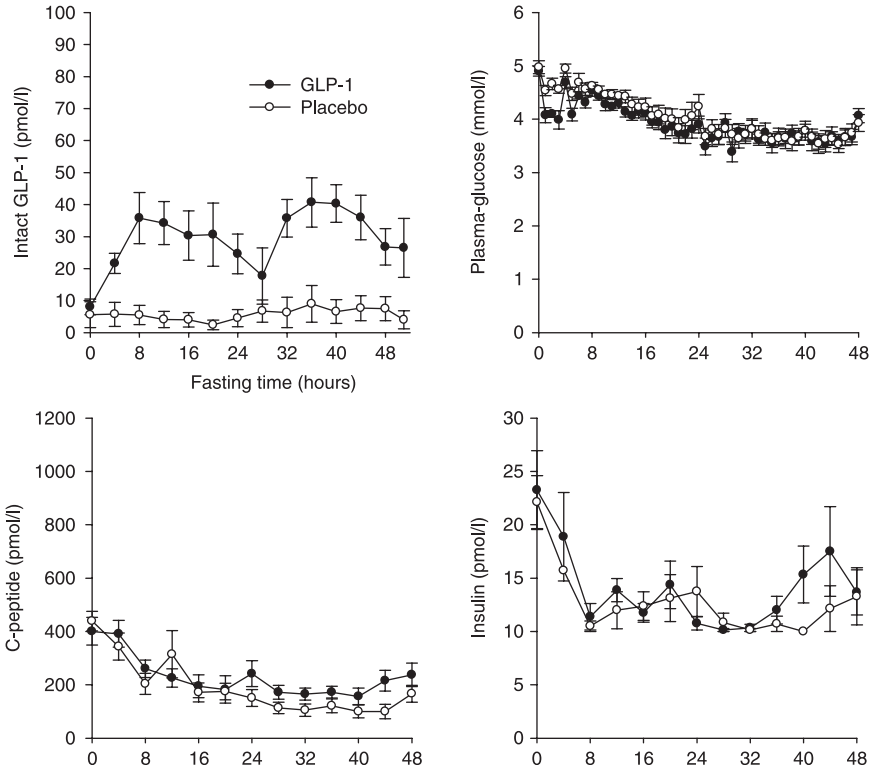


**Fig. 1** Concentration and time course of intact GLP-1, plasma glucose, C-peptide and insulin during fasting together with 48 h of subcutaneous GLP-1 (black dot) and placebo infusion (white dot). For intact GLP-1 $P = 0{\cdot}0004$, and for PG, insulin and C-peptide, $P > 0{\cdot}10$ GLP-1 *vs.* placebo. Data are mean ± SEM. Time = 0 equals clock time 09·00 h.

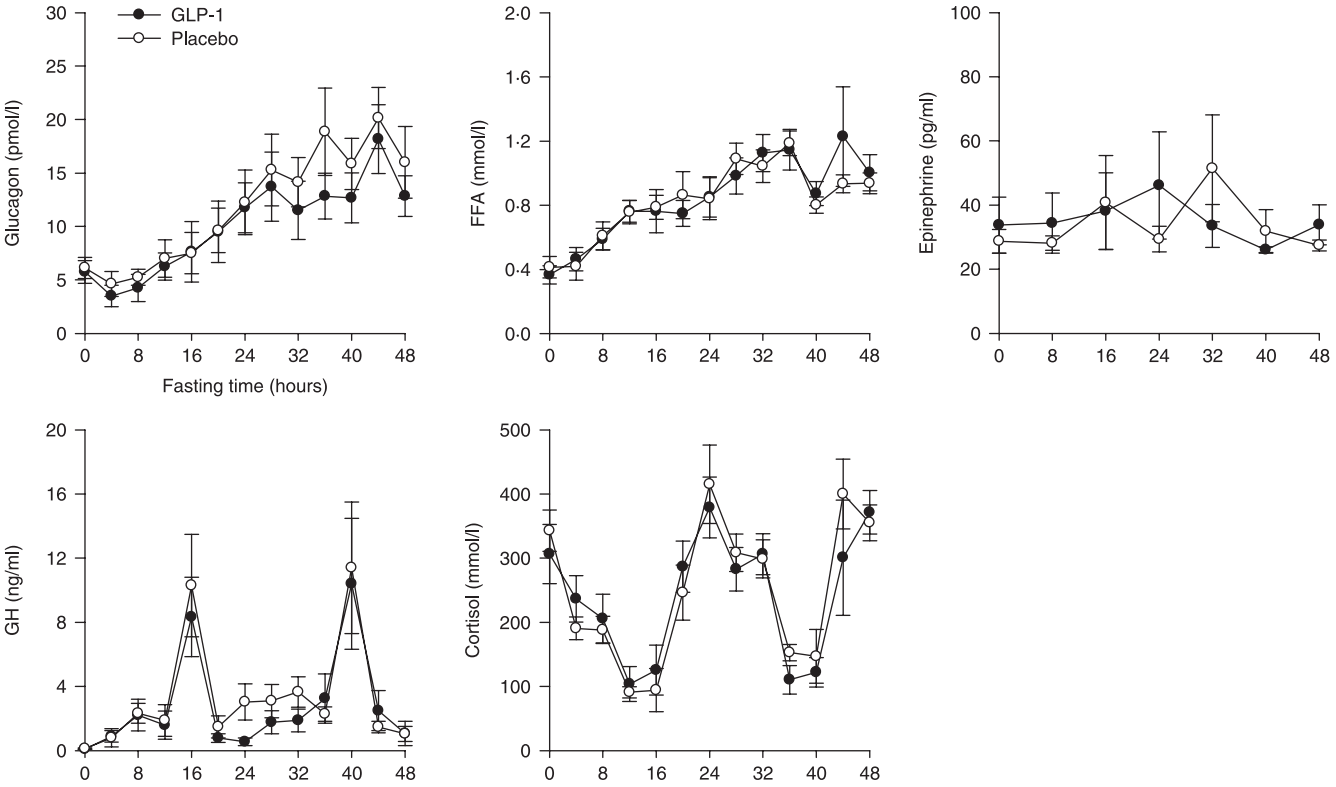


**Fig. 2** Concentration and time course of glucagon, GH, FFA, epinephrine and cortisol during fasting together with 48 h of subcutaneous GLP-1 (black dot) and placebo infusion (white dot), $P > 0{\cdot}30$ in all, GLP-1 *vs.* placebo. Data are mean ± SEM. Time = 0 equals clock time 09·00 h.

episodes of reactive hypoglycaemia were observed. Epinephrine levels did not change during the OGTT (data not shown). However, the norepinephrine level was significantly higher with GLP-1 *vs.* placebo during the OGTT ($P = 0{\cdot}04$) (Fig. 4). The cortisol level during OGTT was significantly higher with GLP-1 infusion, $P = 0{\cdot}046$. During the OGTT glucagon was measured only at times 0 and 180 min. No significant difference GLP-1 *vs.* placebo was observed at these single time points (data not shown) even though glucagon levels during GLP-1 infusion were below placebo levels.

No gastrointestinal discomfort was observed in this study.

## Discussion

During fasting conditions the absence of food intake results in a decrease in PG level, which in turn causes a decline in insulin level and an increase in glucagon and GH levels. Initially glucose supply depends on glycogenolysis. However, glycogen stores decrease rapidly and the body progressively depends on gluconeogenesis from amino acids and FFA.[23] During a longer fasting period (> 24–60 h) FFA becomes the most important source of fuel. The decrease in insulin : glucagon ratio stimulates FFA oxidation in the liver and accelerates production of ketone bodies which are metabolized in muscle, adipose tissue and most importantly in the brain.[23,24] Thus, the body can survive for around 2 months without any food intake.[14]

The GH level increases during fasting and contributes significantly to the increase in lipolysis and presumably the reduced insulin sensitivity during fasting.[25] During fasting GLP-1 might be expected to antagonize the shift in metabolic control due to its insulinotrophic effect and inhibition of glucagon secretion.

The three hypoglycaemic events in the current study occurred after 25 (GLP-1 and placebo) and 29 h (GLP-1) of infusion corresponding to 36 and 40 h of fasting. This correlates to exhaustion of liver glycogen stores which is predicted to occur after approximately 24–48 h.[26] This explanation is further supported by the fact that the risk of hypoglycaemia appeared to be similar during GLP-1 *vs.* placebo infusion.

No difference between GLP-1 *vs.* placebo was observed in hormone and metabolite profiles during fasting and they were exactly as predicted from previous fasting studies.[27] PG initially declined rapidly and thereafter plateaued with fasting time resulting in a decline in C-peptide and insulin levels with a similar course. The glucagon and GH levels increased with fasting time, supporting glucose output from the liver and increasing lipolytic activity as illustrated by the increase in FFA level.

Glucagon and epinephrine are initial key factors in the defence against hypoglycaemia and epinephrine may also be of importance in stimulating lipolysis during fasting,[28] but in the current study epinephrine levels did not alter during the fasting period. This may be due to the fact that mean PG levels after 35–59 h of fasting and 24–48 h of infusion, remained in the vicinity of approximately 3·7 mmol/l, which is not a sufficiently powerful stimulus for an adrenergic response in the presence of an adequate glucagon response and high GH levels.

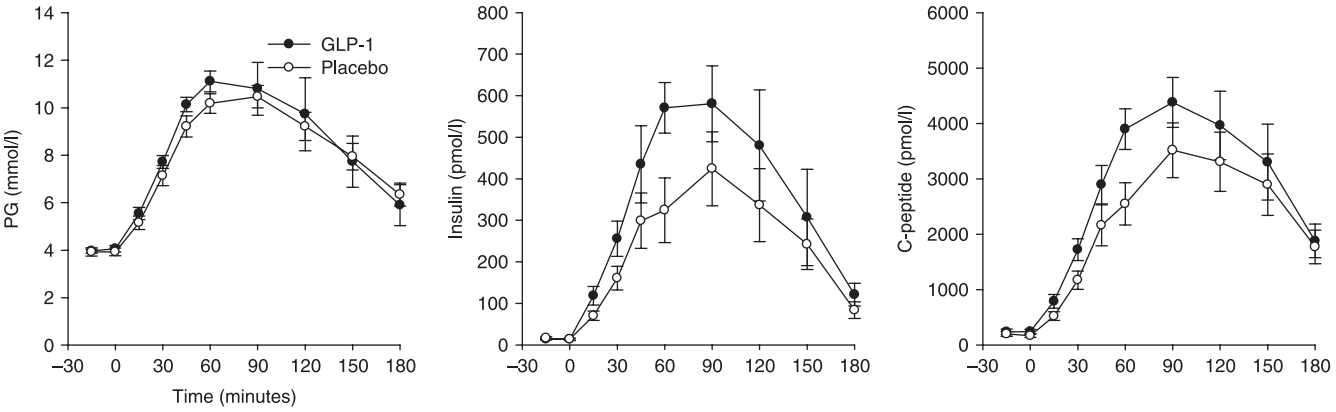


**Fig. 3** Concentration and time course of PG, Insulin and C-peptide during a 3-h OGTT. The data are post fasting for 59 h and during subcutaneous GLP-1 (black dot) and placebo infusion (white dot). For PG $P = 0{\cdot}59$, insulin $P = 0{\cdot}01$ and C-peptide $P = 0{\cdot}02$ GLP-1 *vs.* placebo. Data are mean ± SEM. Time = 0 equals clock time 09·00 h.

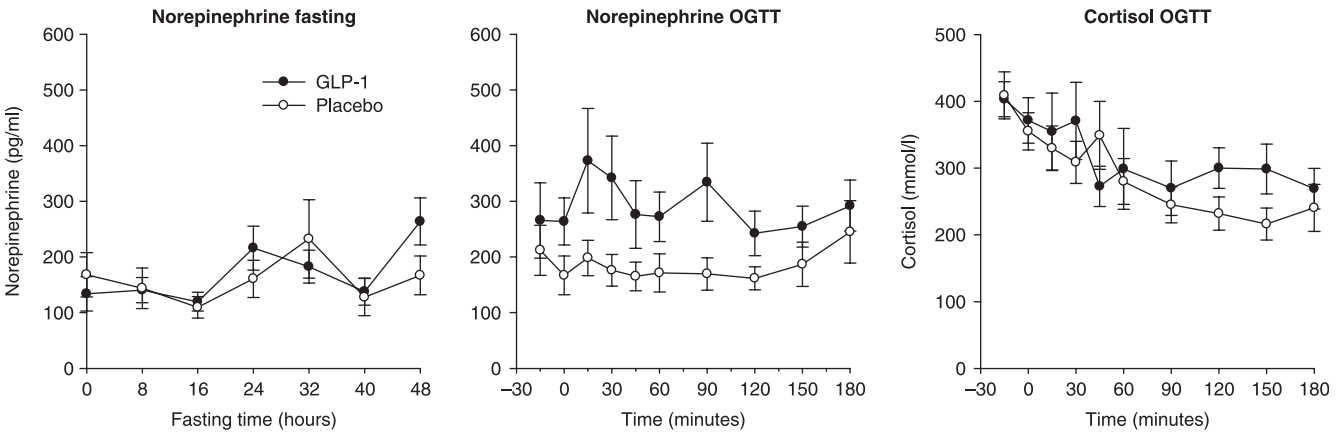


**Fig. 4** Concentration and time course of norepinephrine during fasting and during a 3-h OGTT post fasting and cortisol during OGTT post fasting. Data are during subcutaneous GLP-1 (black dot) and placebo infusion (white dot). For norepinephrine (OGTT) $P = 0{\cdot}04$ and for cortisol (OGTT) $P = 0{\cdot}046$ GLP-1 *vs.* placebo. Data are mean ± SEM. Time = 0 equals clock time 09·00 h.

Cortisol levels, however, exerted a classical circadian secretion during fasting with low levels during night time and high levels during daytime and were similar during GLP-1 *vs.* placebo infusions. GH also exhibited circadian variation but, contrary to cortisol, with high levels during night time and low levels during daytime. The circadian rhythm of the counter regulatory hormones may increase the susceptibility to hypoglycaemia during the night.[29]

Not all subjects completed both fasting periods. Eight subjects entered the study and thus completed the protocol. However, three of these subjects did experience a hypoglycaemic event leading to premature termination of one of their study periods. Hence the results after approximately 30 h of infusion and 41 h of fasting including the OGTT show data from six subjects in the GLP-1 group and seven subjects in the placebo group.

This may be a reflection of the power in the study being too low and thus implicates a type 2 error in the analysis. The difference in the number hypoglycaemic events was not significant ($P = 0{\cdot}10$). However, it may on the other hand reflect a trend towards an increased risk of hypoglycaemia with GLP-1 infusion. Still, hypoglycaemia was unexpectedly observed during placebo treatment and no difference in the hormone levels was observed between the two treatment arms. A study with a larger number of subjects would address this problem.

In the current study we demonstrate that during a long-term fast the effect of GLP-1 on insulin and glucagon secretion still appeared to be glucose dependent and the counter-regulatory response to fasting was preserved. No increased risk of hypoglycaemic events with GLP-1 as compared to placebo was apparent.

Dumping syndrome is a major cause of morbidity after gastrectomy.[30] Symptoms are closely related to an increased GLP-1 response that results in inappropriate hyperinsulinaemia leading to a risk of reactive hypoglycaemia.[31–33]

An oral glucose load following a prolonged fasting period with pharmacologically elevated plasma levels of GLP-1 may represent a situation with similarities to the dumping syndrome and may therefore be associated with an increased risk of reactive hypoglycaemia.

In our study, no reactive hypoglycaemia was observed during the OGTT despite a GLP-1 induced increase in insulin levels. This conflicting observation may be ascribed to the fact that the reactive

hypoglycaemia in dumping results from a rapid increase in GLP-1 secretion, whereas the GLP-1 concentration in the current study was constantly elevated.

A limitation to the current study is that gastric emptying rate was not measured. It is well known that even minor variations in gastric emptying may have a profound effect on insulin responses and postprandial glycaemia.[34] A recent study concluded that despite augmented rises in insulin secretion, the glucose-lowering effect of GLP-1 was markedly reduced when the deceleration of gastric emptying was antagonized.[35]

The contribution of OGTT glucagon levels to the observed insulin resistance can only be speculated upon due to sparse measurements. Still, most likely the glucagon level was lower with GLP-1 *vs.* placebo during the OGTT and thus may not explain the altered resistance.

The norepinephrine level was significantly higher with GLP-1 infusion *vs.* placebo during the OGTT with a possible peak 15 min after the glucose load. A recent study in patients with dumping syndrome confirmed this connection between a high GLP-1 level and a high norepinephrine level.[15]

A link may exist between peripheral GLP-1 and central catecholamine autonomic control areas,[36] and the central GLP-1 system may be a regulator of sympathetic outflow.[37,38] Furthermore, norepinephrine levels are stimulated by high PG levels.[39] In the current study this glucose-induced increase in plasma norepinephrine level was significantly higher with GLP-1 rather than with placebo during the OGTT indicating that high pharmacological levels of GLP-1 may further stimulate release of norepinephrine in the presence of high PG levels.

Cortisol levels were also significantly higher during OGTT with GLP-1 infusion but similar during fasting, thus our data suggest that GLP-1 also may stimulate release of cortisol when high glucose levels are present. This increase in cortisol is consistent with previous observations[40,41] and indicates an activation of hypothalamic neuroendocrine neurones by GLP-1.[42]

As cortisol impairs insulin action,[43] the increased cortisol levels could have eliminated the insulin sensitizing effect of GLP-1. Accordingly we speculate that the resistance to high insulin levels observed in this study during OGTT may be due to the increased norepinephrine and cortisol levels set off by GLP-1 infusion. To this extent further investigations are required in order to clarify the significance of these associations between GLP-1, norepinephrine and cortisol levels in particular regarding type 2 diabetic patients.

In conclusion, the counter-regulatory response during 48 h of subcutaneous GLP-1 infusion was preserved during fasting with no apparent increased risk of hypoglycaemic episodes. Moreover no reactive hypoglycaemia was observed after a glucose load following the fasting period. These observations may be of relevance when utilizing potential future long acting GLP1 analogues where pharmacological concentrations of GLP-1 are present in plasma for several days.

## Acknowledgements

The study was supported by grants from The Novo Nordisk Foundation and The Danish Diabetes Foundation, Denmark.

Mrs Joan Hansen, Mrs Elsebeth Hornemann and the staff at the Medical Research Laboratorium M, Aarhus University Hospital are thanked for their outstanding technical assistance.

ClinicalTrials. Gov Identifier: NCT00285896

**Grant support:** The study was supported by grants from The Novo Nordisk Foundation and The Danish Diabetes Foundation, Denmark.

**Disclosure statement:** The authors have nothing to disclose.

## References

1 Holst, J.J. (2004) On the physiology of GIP and GLP-1. *Hormone and Metabolic Research*, **36**, 747–754.
2 Nauck, M.A. (2004) Glucagon-like peptide 1 (GLP-1) in the treatment of diabetes. *Hormone and Metabolic Research*, **36**, 852–858.
3 Zander, M., Madsbad, S., Madsen, J.L. *et al.* (2002) Effect of 6-week course of glucagon-like peptide 1 on glycaemic control, insulin sensitivity, and β-cell function in type 2 diabetes: A Parallel-Group Study. *Lancet*, **359**, 824–830.
4 Vilsboll, T. & Holst, J.J. (2004) Incretins, insulin secretion and type 2 diabetes mellitus. *Diabetologia*, **47**, 357–366.
5 Ahren, B. & Schmitz, O. (2004) GLP-1 receptor agonists and DPP-4 inhibitors in the treatment of type 2 diabetes. *Hormone and Metabolic Research*, **36**, 867–876.
6 Knauf, C. Cani, P.D., Perrin, C. *et al.*(2005) Brain glucagon-like peptide-1 increases insulin secretion and muscle insulin resistance to favor hepatic glycogen storage. *Journal of Clinical Investigation*, **115**, 3554–3563.
7 Ahren, B. (2004) GLP-1 and extra-islet effects. *Hormone and Metabolic Research* **36**, 842–845.
8 Vella, A., Rizza, R.A. (2004) Extrapancreatic effects of GIP and GLP-1. *Hormone and Metabolic Research* **36**, 830–836.
9 Holst, J.J. (2007) The physiology of glucagon-like peptide 1. *Physiological Review*, **87**, 1409–1439.
10 Nauck, M.A., Heimesaat, M.M., Behle, K. *et al.* (2002) Effects of glucagon-like peptide 1 on counterregulatory hormone responses, cognitive functions, and insulin secretion during hyperinsulinemic, stepped hypoglycemic clamp experiments in healthy volunteers. *Journal of Clinical Endocrinology and Metabolism*, **87**, 1239–1246.
11 Degn, K.B., Brock, B., Juhl, C.B. *et al.* (2004) Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. *Diabetes*, **53**, 2397–2403.
12 Qualmann, C., Nauck, M.A., Holst, J.J. *et al.* (1995) Insulinotropic actions of intravenous glucagon-like peptide-1 (GLP-1) [7-36 amide] in the fasting state in healthy subjects. *Acta Diabetologica*, **32**, 13–16.
13 Edwards, C.M., Todd, J.F., Ghatei, M.A. *et al.* (1998) Subcutaneous glucagon-like peptide-1 (7-36) amide is insulinotropic and can cause hypoglycaemia in fasted healthy subjects. *Clinical Science*, **95**, 719–724.
14 Cahill, G.F. Jr, Herrera, M.G., Morgan, A.P. *et al.* (1966) Hormone–fuel interrelationships during fasting. *Journal of Clinical Investigation*, **45**, 1751–1769.
15 Gebhard, B., Holst, J.J., Biegelmayer, C. *et al.* (2001) Postprandial GLP-1, norepinephrine, and reactive hypoglycemia in dumping syndrome. *Digestive Diseases and Sciences*, **46**, 1915–1923.
16 Toft-Nielsen, M., Madsbad, S. & Holst, J.J. (1998) Exaggerated secretion of glucagon-like peptide-1 (GLP-1) could cause reactive hypoglycaemia. *Diabetologia*, **41**, 1180–1186.

17 Vilsboll, T., Krarup, T., Madsbad, S. *et al.* (2001) No reactive hypoglycaemia in type 2 diabetic patients after subcutaneous administration of GLP-1 and intravenous glucose. *Diabetic Medicine*, **18**, 144–149.
18 Gormsen, L.C., Nielsen, C., Gjedsted, J. *et al.* (2007) Effects of free fatty acids, growth hormone and growth hormone receptor blockade on serum ghrelin levels in humans. *Clinical Endocrinology*, **66**, 641–645.
19 Vilsboll, T., Krarup, T., Sonne, J. *et al.* (2003) Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism*, **88**, 2706–2713.
20 Wilken, M., Larsen, F.S., Buckley, D. *et al.* (1999) New highly specific immunoassays for glucagon-like peptide-1 (GLP-1) (Abstract). *Diabetologia* **42** (Suppl. 1), A.196.
21 Orskov, C., Rabenhoj, L., Wettergren, A. *et al.* (1994) Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes*, **43**, 535–539.
22 Holst, J.J. (1982) Evidence that enteroglucagon (II) is identical with the C-terminal sequence (residues 33–69) of glicentin. *Biochemical Journal*, **207**, 381–388.
23 Norrelund, H. (2005) The metabolic role of growth hormone in humans with particular reference to fasting. *Growth Hormone and IGF Research*, **15**, 95–122.
24 Hasselbalch, S.G., Knudsen, G.M., Jakobsen, J. *et al.* (1995) Blood-brain barrier permeability of glucose and ketone bodies during short-term starvation in humans. *American Journal of Physiology*, **268**, E1161–E1166.
25 Bjorkman, O. & Eriksson, L.S. (1985) Influence of a 60-h fast on insulin-mediated splanchnic and peripheral glucose metabolism in humans. *Journal of Clinical Investigation*, **76**, 87–92.
26 Rothman, D.L., Magnusson, I., Katz, L.D. *et al.* (1991) Quantitation of hepatic glycogenolysis and gluconeogenesis in fasting humans with 13C NMR. *Science*, **254**, 573–576.
27 Hojlund, K., Wildner-Christensen, M., Eshøj, O. *et al.* (2001) Reference intervals for glucose, β-cell polypeptides, and counterregulatory factors during prolonged fasting. *American Journal of Physiology, Endocrinology and Metabolism*, **280**, E50–E58.
28 Klein, S., Holland, O.B. & Wolfe, R.R. (1990) Importance of blood glucose concentration in regulating lipolysis during fasting in humans. *American Journal of Physiology*, **258**, E32–E39.
29 Jones, T.W., Porter, P., Sherwin, R.S. *et al.* (1998) Decreased epinephrine responses to hypoglycemia during sleep. *New England Journal of Medicine*, **338**, 1657–1662.
30 Carvajal, S.H. & Mulvihill, S.J. (1994) Postgastrectomy syndromes: dumping and diarrhea. *Gastroenterology Clinics of North America*, **23**, 261–279.
31 Kreymann, B., Williams, G., Ghatei, M.A. *et al.* (1987) Glucagon-like peptide-1, 7-36: a physiological incretin in man. *Lancet*, **2**, 1300–1304.
32 Miholic, J., Orskov, C., Holst, J.J. *et al.* (1991) Emptying of the gastric substitute, glucagon-like peptide-1 (GLP-1), and reactive hypoglycemia after total gastrectomy. *Digestive Diseases and Sciences*, **36**, 1361–1370.
33 Goldfine, A.B., Mun, E.C., Devine, E. *et al.* (2007) Patients with neuroglycopenia post gastric bypass surgery have exaggerated incretin and insulin secretory responses to mixed meal. *Journal of Clinical Endocrinology and Metabolism*, **92**, 4678–4685.
34 O'Donovan, D.G., Doran, S., Feinle-Bisset, C. *et al.* (2004) Effect of variations in small intestinal glucose delivery on plasma glucose, insulin, and incretin hormones in healthy subjects and type 2 diabetes. *Journal of Clinical Endocrinology and Metabolism*, **89**, 3431–3435.
35 Meier, J.J., Kemmeries, G., Holst, J.J. *et al.* (2005) Erythromycin antagonizes the deceleration of gastric emptying by glucagon-like peptide 1 and unmasks its insulinotropic effect in healthy subjects. *Diabetes*, **54**, 2212–2218.
36 Yamamoto, H., Kishi, T., Lee, C.E. *et al.* (2003) Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. *Journal of Neuroscience*, **23**, 2939–2946.
37 Yamamoto, H., Lee, C.E., Marcus, J.N. *et al.* (2002) Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *Journal of Clinical Investigation*, **110**, 43–52.
38 Bharucha, A.E., Charkoudian, N., Andrews, C.N. *et al.* (2008) Effects of glucagon-like peptide-1, yohimbine, and nitrergic modulation on sympathetic and parasympathetic activity in humans. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, **295**, R874–R880.
39 Rassam, A.G., Martin, P. III, Burge, M.R. *et al.* (2002) The mechanism of glucose-induced catecholamine stimulation. *Metabolism*, **51**, 761–764.
40 Vella, A., Shah, P., Basu, R. *et al.* (2001) Effect of glucagon-like peptide-1 (7-36)-amide on initial splanchnic glucose uptake and insulin action in humans with type 1 diabetes. *Diabetes*, **50**, 565–572.
41 Vella, A., Shah, P., Reed, A.S. *et al.* (2002) Lack of effect of exendin-4 and glucagon-like peptide-1-(7,36)-amide on insulin action in non-diabetic humans. *Diabetologia*, **45**, 1410–1415.
42 Ryan, A.S., Egan, J.M., Habener, J.F. *et al.* (1998) Insulinotropic hormone glucagon-like peptide-1-(7-37) appears not to augment insulin-mediated glucose uptake in young men during euglycemia. *Journal of Clinical Endocrinology and Metabolism*, **83**, 2399–2404.
43 Dinneen, S., Alzaid, A., Miles, J. *et al.* (1993) Metabolic effects of the nocturnal rise in cortisol on carbohydrate metabolism in normal humans. *Journal of Clinical Investigation*, **92**, 2283–2290.