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PAPER

The effect of physiological levels of glucagon-like peptide-1 on appetite, gastric emptying, energy and substrate metabolism in obesity

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OBJECTIVE: Peripheral infusions of glucagon-like peptide-1 (GLP-1) in humans have been shown to inhibit gastrointestinal motility and decrease hunger and energy intake. However, these investigations used supraphysiological doses. The objective of this study was to investigate the effects of a GLP-1 infusion in a physiological dose on appetite sensations, energy intake, gastric emptying, energy and substrate metabolism.
METHODS: Eighteen obese men participated in the placebo-controlled, randomized, single-blinded, cross-over study with infusion of GLP-1 or saline. Resting metabolic rate (RMR) and substrate oxidations were measured by ventilated hood before and after an energy-fixed breakfast. Gastric emptying was measured using paracetamol as a marker. Visual analogue scales were used to assess appetite sensations, thirst and comfort throughout the experiment and palatability of the test meals. Blood was sampled for analysis of hormones (GLP-1, GLP-2, glucose-dependent insulinotropic polypeptide (GIP), insulin, glucagon), and substrates (glucose, lactate, non-esterified fatty acids (NEFA), triacylglycerol (TAG)). *Ad libitum* energy intake at lunch was registered.
RESULTS: Following the breakfast, GLP-1 infusion suppressed ratings of hunger and prospective food consumption ($P < 0.05$), whereas all other subjective ratings and *ad libitum* energy intake were unaffected. RMR, carbohydrate oxidation and gastric emptying rate were lower during the GLP-1 infusion compared with the saline infusion ($P < 0.001$, $P < 0.05$, $P < 0.0001$, respectively). All plasma hormone and substrate profiles, except NEFA, were significantly reduced by GLP-1 ($P < 0.0001$).
CONCLUSION: It is concluded that GLP-1 in physiological concentrations powerfully reduces the rate of entry of nutrients into the circulation by a reduction of gastric emptying rate in obese subjects. The effect of GLP-1 on appetite and food intake may be beneficial in weight reduction.
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Keywords: resting metabolic rate; thermogenesis; oxidation; energy intake; satiety; hunger; glucagon; insulin; GIP; GLP-1; GLP-2

Introduction

Glucagon-like peptide-l (GLP-1) is produced in the intestinal L-cells[1,2] and secreted in response to ingestion of carbohydrates, lipids and mixed meals.[3–6] It has been shown to affect the regulation of appetite and food intake. Thus, intravenous GLP-1 infusions in humans have been demonstrated to result in decreased sensations of hunger and increased satiety, and in lower energy intake.[7–10] Also a lower intake of fluid after an intravenous (i.v.) GLP-1 infusion was seen in normal-weight human subjects.[10] Furthermore, in physiological doses, GLP-1 inhibits meal-induced gastric acid secretion and gastric emptying rate.[11–14] Hereby GLP-1 might contribute to a prolonged sensation of satiety. Investigations on the effect of GLP-1 on energy expenditure, representing the other side of the energy balance equation, are very sparse and inconsistent.[15–17] In rats, a single intracerebroventricular (i.c.v.) infusion of GLP-1 resulted in an increased O_2 consumption in lean animals while it was

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unchanged in obese animals.[15] In normal-weight human subjects the effect of exogenous peripheral infusions of GLP-1 during and after meal intake on O_2 consumption seems to parallel the retarded absorption and processing of nutrients.[16,17] However, the effect of i.v. GLP-1 on resting metabolic rate has not yet been investigated in obese subjects.

The previous studies performed on the effect of GLP-1 on appetite and energy expenditure in humans have primarily used infusions in the supraphysiological range and normal-weight subjects. The aim of the present study was, therefore, to determine the effects of a lower dose of peripheral GLP-1 than previously used (resulting in a physiological elevation of the plasma concentration) infused during and after an energy-fixed breakfast on appetite, resting metabolic rate, substrate oxidation and metabolism, gastric emptying and subsequent energy and water intake in obese humans.

Subjects and methods

Subjects

Eighteen obese healthy men with body mass index (BMI) 33.7 ± 0.6 kg/m^2 (mean$\pm$s.e.m.; range 30.2–39.8 kg/m^2), 33.9 ± 0.7% body fat (range 30.4–40.9%), age 42.6 ± 2.1 y (range 21–57 y), non-smokers, and with no history of diabetes, participated in the study. Fasting blood glucose concentrations averaged 5.2 ± 0.1 mmol/l (range 4.3–6.5 mmol/l), and none of the subjects had any glucose or protein in the urine. All subjects gave written consent after the experimental protocol had been explained to them. The study was approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg and is in accordance with the Helsinki-II declaration.

Design

The subjects were tested on two different occasions in a placebo-controlled, randomized, single-blinded, cross-over design. Half of the subjects had GLP-1 on the first test day and half had saline, and vice versa on the second test day. The two test days were separated by at least three weeks and by no more than eight weeks.

Experimental protocol

On the test day, the subjects arrived at the department at 8:00 am having used the least strenuous means of transportation. They had fasted for a minimum of 12 h from the evening before. After voiding and weighing, body composition (measured by bioelectrical impedance as described previously[18]) and height were measured. The subjects then rested in the supine position with a slight elevation of the head on a bed covered with an antidecubitus mattress. Two Venflon catheters (Viggo, Sweden, Gothenborg) were inserted in the antecubital arm veins. After another 20 min rest, resting metabolic rate was measured for 45 min. Subsequently, a fasting blood sample was taken. At 9:45 am, breakfast was served and consumed within 15 min. Exactly the same time was spent on the two test days for each individual subject. Postprandial resting metabolic rate was measured for 4 h, and blood samples were taken 15, 30, 45, 60, 90, 120, 150, 180, 210, 240 and 270 min after the start of the breakfast and again after the *ad libitum* lunch, served at 2:15 pm (300 min). Ratings of subjective sensations were made on 100 mm visual analogue scales (VAS) with the text expressing the most positive and the most negative rating anchored at each end.[19] VAS were used to assess satiety, hunger, fullness, prospective food consumption, the desire to eat something sweet, something salty, something fatty and something savoury, thirst and comfort. These ratings were recorded immediately before and throughout a 4.5 h period after breakfast and after lunch. Ratings of palatability (appearance, smell, taste, after-taste and overall palatability) were made immediately after each of the test meals. The *ad libitum* energy intake at lunch was registrered, and so was the *ad libitum* water intake on the first test day.

During the postprandial measurements, the subjects could listen to the radio or watch TV/video (light entertainment). Water consumption and toilet visits were allowed when necessary. The exact hours, the time spent, the type of activity, and the amount of water consumed were noted and repeated on the second test day. Resting metabolic rate was measured continuously during the day interrupted by 10 min breaks from wearing the hood every hour. During these breaks the subjects still rested on the bed.

Infusion

For the GLP-1 infusion, commercially available synthetic, human GLP-1(7-36 amide) of GMP quality was purchased from Saxon Biochemicals (Hannover, Germany). The peptide was dissolved in a 0.9% saline solution, also containing 1% human serum albumin, guaranteed to be free of hepatitis B surface antigen and human immunodeficiency virus (HIV) antibody (Albumin Nordisk, Novo Nordisk, Gentofte, Denmark), subjected to sterile filtration, checked for sterility and kept at -20°C until use. The infusion (GLP-1 or saline) was started simultaneously with initiation of the test meals (energy-fixed breakfast and *ad libitum* lunch). An automatic pump (Infusomat, B. Braun) provided a steady-state infusion rate of 45 pmol/kg fat-free mass (FFM)$\times$h. The infusion was stopped 30 min before lunch to allow plasma levels to return to baseline in order to mimic the normal physiological plasma profile.[6]

Test meals and diets

The test meals were a breakfast of fixed size and energy content, consisting of yoghurt, bread, butter, cheese, jam, kiwi-fruit, orange juice and water, and an *ad libitum* lunch, consisting of minced meat, pasta, corn, carrots, green pepper and cream. Water intake at lunch was also *ad libitum* on the first test day, and this amount was replicated on the second

test day. Total available energy content of the breakfast was calculated to be 20% of each subject's individual energy requirements,[20] adjusted to the nearest 0.5 MJ. The distribution of energy was 50 energy-percent (E%) carbohydrates, 37 E% fat and 13 E% protein in both meals.

Prior to each test day, the subjects followed a weight-maintaining standardized diet (50 E% carbohydrate, 37 E% fat, 13 E% protein, 2.5 g/MJ dietary fibre) consisting of ordinary food items, estimated to meet each subject's individual energy requirements,[20] adjusted to the nearest 0.5 MJ. The food was prepared at the department and delivered free of charge. All meals for 2 days were supplied and the subjects were instructed to adhere strictly to the diet. If they were not able to consume all the food in the first pre-experimental period, they were instructed to bring the left-overs to the department for weighing and registration. This amount of food was then deducted from the standardized diet in the second pre-experimental period. The subjects were also instructed to abstain from alcohol and strenuous physical activity for the 2 days prior to the test days in order to ensure equally filled glycogen stores and similar macronutrient balance on the test days.[21] The computer database of foods from the National Food Agency of Denmark (Dankost 2) was used in the calculations of energy and nutrient composition of the test meals and diets.

Resting metabolic rate

Resting metabolic rate (RMR) was measured by indirect calorimetry using an open-air-circuit, ventilated hood system.[22] Ventilation through the system was determined by a Hastings mass flowmeter (type HFM 201-100). CO_2 was measured by a Servomex 1490 infrared analyser and O_2 by a Servomex 1100A paramagnetic analyser. RMR and oxidation of carbohydrate (C-OX), fat (F-OX), protein (P-OX) and diet-induced thermogenesis (DIT) were calculated from the gas exchange and urinary measurements using the formulas of Elia and Livesey.[23]

$$\text{RMR(kJ)} = 15.913 \times \text{l O}_2 + 5.207 \times \text{l CO}_2 - 4.646 \times \text{N(g)}$$

$$\text{Non} - \text{protein RQ (RQnp)} = (4.97 \times \text{N(g)} \times \text{l CO}_2/(5.95 \times \text{N(g)}) \times \text{l O}_2)$$

$$\text{P-OX(kJ)} = 116 \times \text{N(g)}$$

$$\text{C-OX(\%)} = 21.12 \times (\text{RQnp} - 0.710)/(21.12 \times (\text{RQnp} - 0.710 + (19.61 \times (1 - \text{RQnp}))$$

$$\text{C-OX(kJ)} = (\text{RMR} - \text{P-OX})/100 \times \text{C-OX(\%)}$$

$$\text{F-OX(kJ)} = \text{RMRnp} - \text{C-OX(kJ)}$$

$$\text{DIT(\%)} = ((\text{postprandial RMR} - \text{basal RMR})(\text{kJ/min} \times 240\,\text{min})/\text{kJ in test meal}) \times 100$$

Laboratory analyses

Blood was drawn without stasis through the indwelling antecubital cannula into iced syringes. The blood was centrifuged for 10 min at 3 000 *g* and 4°C, and non-esterified fatty acids (NEFA) were immediately extracted from plasma, and the extract was stored at −20°C until determination. Quantification of NEFA was performed by an enzymatic colorimetric method (Wako NEFA test kit, NEFA C, ACS-ACOD Method, code no. 994-75409 E). Serum triacylglycerol concentration (TAG) was determined by enzymatic methods.[24] Blood for glucose and lactate were collected in EDTA-tubes prepared with flouride, and plasma analysed by standard enzymatic methods.[25,26]

For hormone analysis the syringes contained EDTA (6 μmol/l) and aprotinin (500 kIU/l, final concentrations), and after centrifugation, plasma was kept frozen at −20°C. Insulin concentrations in plasma were measured against standards of human insulin by radioimmunoassay (RIA) according to the principles described by Albano *et al*.[27] The tracer was human insulin, monoiodinated in positon A14 (a gift from Novo Nordisk A/S, Bagsvaerd, Denmark). The glucagon RIA was directed against the carboxy terminus of the glucagon molecule (antibody code 4305).

Plasma samples were assayed for GLP-1 using RIAs specific for the carboxy- (or C-) terminal end of the molecule, which measures the intact and the cleaved (= inactive) part of the molecule. C-terminal immunoreactivity was determined using antiserum 89390[28] which has an absolute requirement for the intact amidated carboxy-terminus of GLP-1^{7-36} amide and cross-reacts less than 0.01% with carboxy-terminally truncated fragments and 89% with GLP-1^{9-36} amide. For the assay, the intraassay coeffecient of variation was $<6\%$. Plasma samples were extracted with 70% ethanol (vol/vol, final concentrations) before assay, giving recoveries of 75%.[29]

GLP-2 concentrations in ethanol-extracted plasma were measured using a new radioimmunoassay employing antiserum code no. 92160 and standards of human GLP-2 (proglucagon 126-158, a gift from Novo Nordisk A/S) and monoiodinated Tyr-12 GLP-1, specific activity >70 MBq/nmol.[30] The antiserum is directed aganist the N-terminus of GLP-2 and therefore measures only fully processed GLP-2 of intestinal origin. Sensitivity was below 5 pmol/l, and intraassay coefficient of variation at 60 pmol/l was 6%. Glucose-dependent insulinotropic polypeptide (GIP) was determined by RIA on plasma extracted with ethanol as previously described.[31]

Paracetamol was analysed by fluorescence polarization immunoassay technology according to the AxSYM System Operations Manual from Abbott Laboratories (Diagnostic Division Abbott Park, IL, USA).

Urinary nitrogen concentration (N) was measured by the method of Dumas[32] using a nitrogen analyser (NA 1500, Carlo Erba Strumentazione, Milano, Italy).

Statistical analyses

All results are given as means (± standard error of the mean, s.e.m.). Fasting and 4 h mean values, incremental areas

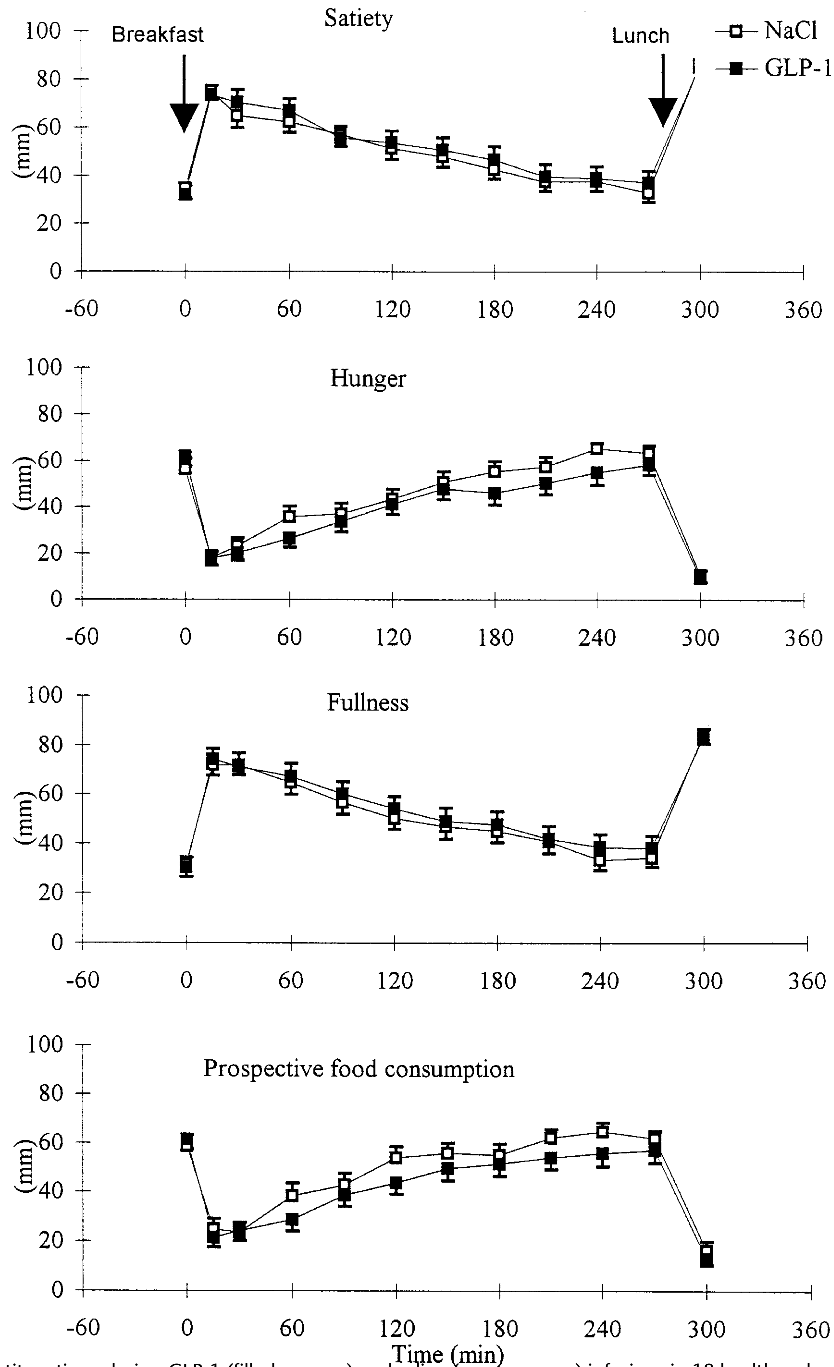

Figure 1 Subjective appetite ratings during GLP-1 (filled squares) and saline (open squares) infusions in 18 healthy, obese male subjects. VAS = 100 mm equals 'I cannot eat another bite' (satiety), 'I have never been more hungry' (hunger), 'I am totally full' (fullness), and 'I can eat a lot' (prospective food consumption). Data are means (±s.e.m.). ANOVA—time effect, $P<0.0001$; treatment effect, hunger, prospective food consumption, $P<0.05$ (*); satiety, $P=0.224$; fullness, $P=0.353$; interaction time×treatment effect, NS. Arrows indicate meals. Time and duration of the infusion was 0–240 min and 270–300 min.

under the profile curves (net AUC), total energy expenditure and substrate oxidations, energy intake and palatability were compared using a paired *t*-test. The postprandial response curves were compared by parametric analysis of variance (ANOVA) using mixed linear models with repeated measures. Time, treatment and period were included in the model as fixed factors, and subjects were included as random effect. The autocorrelation between repeated observations within each subject and treatment was modeled using spatial power structure, which is an autoregressive structure where the

actual difference in time between two observations are taken into account.[33,34] *Ad libitum* water intake at lunch on the first test day (on the second test day the amount was fixed in order to repeat the intake on the first test day, regardless of treatment with GLP-1 or saline) was compared between the subjects receiving the saline infusion ($n=9$) *vs* the subjects receiving GLP-1 ($n=9$) using an unpaired *t*-test.

The level of significance was set at $P<0.05$. The Statistical Analysis Software, version 6.12 (SAS Institute, Cary, NC, USA) was used in the statistical calculations.

Results

GLP-1

Infusion of GLP-1 at 45 pmol/kg FFM×h resulted in an elevation of C-terminal plasma GLP-1 concentrations from 10.0 ± 0.5 pmol/l in the fasting state to 50.4 ± 3.1 pmol/l 60 min after initiation of breakfast and infusion. Correspondingly, the concentrations were 10.7 ± 0.9 pmol/l in the fasting state and 20.3 ± 1.4 pmol/l during saline infusion. The plateau of postprandial GLP-1 concentrations were approximately 50 *vs* 20 pmol/l during GLP-1 and saline infusion, respectively ($P<0.001$). Thus, the infusion of GLP-1 resulted in a 2.5-fold elevation of C-terminal plasma GLP-1 concentrations compared with saline infusion.

Appetite, thirst and comfort

On the saline day, subjects initially felt a greater desire to eat something savoury than on the GLP-1 test day ($P<0.05$). No other differences between treatments were observed in the fasting state in any of the parameters. Postprandially, decreased ratings of hunger and prospective food consumption were seen during the GLP-1 infusion compared with saline ($P<0.05$, Figure 1). No other subjective mean appetite ratings (Figure 1) nor mean ratings of thirst and comfort differed between the two treatments. On the second test day, subjects initially felt less thirsty and had a greater desire to eat something savoury than on the first test day regardless of treatment type (period effect: $P<0.01$ and $P<0.05$, respectively).

Palatability, energy and water intake

No differences were seen in palatability ratings (appearance, smell, taste, aftertaste and overall palatability) of the breakfast, whereas the appearance of the lunch was found to be more attractive during the GLP-1 infusion ($P<0.05$). All palatability ratings were above medium. The energy intake at breakfast, estimated to amount to 20% of 24 h energy requirements, was on average 2.63 ± 0.05 MJ. At the *ad libitum* lunch there was no difference in the energy intake between the saline test (2.92 ± 0.23 MJ) and the GLP-1 test (2.83 ± 0.28 MJ). Neither did the *ad libitum* water intake at lunch on the first test day differ between the subjects receiving different treatments (GLP-1 or saline; $P=0.823$, $n=9$). On the second test day the water intake was not *ad libitum*, but instead a reproduction of the intake on the first test day.

Gastric emptying

Gastric emptying was delayed during the GLP-1 infusion as reflected by a reduced absorption rate of paracetamol (Figure 2, treatment effect $P<0.0001$). Peak concentration was lower ($P<0.01$), time to peak concentration appeared later ($P<0.05$), and net AUC was reduced by 32% ($P<0.0001$) during the GLP-1 infusion.

Resting metabolic rate and substrate oxidation

RMR did not differ before the saline infusion and the GLP-1 infusion (N.S.). After the test meal, RMR increased for both types of infusion, but significantly less after the GLP-1 infusion (Figure 3; $P<0.0001$). Thus, diet-induced thermogenesis (DIT) averaged 172 ± 10 kJ/4h (6.5 ± 0.4%) during the saline infusion and 112 ± 11 kJ/4h (4.3 ± 0.4%) during the GLP-1 infusion ($P<0.001$, Figure 3, right panel). During the second test day, RMR was lower than on the first test day (period effect: $P<0.01$). Total protein oxidation during the experiment averaged 16.0 ± 1.6 g for the saline infusion and 13.1 ± 0.6 g for the GLP-1 infusion (NS). Carbohydrate oxidation increased postprandially during both infusions, but significantly less during the GLP-1 infusion ($P<0.0001$). Net carbohydrate oxidation averaged 20.6 ± 1.6 g during the saline infusion and 15.9 ± 1.4 g during the GLP-1 infusion ($P<0.05$, Figure 3, right panel). The opposite was seen for postprandial fat oxidation, which decreased during both infusions, but significantly less during the GLP-1 infusion ($P<0.05$, Figure 3). Net fat oxidation averaged -4.8 ± 0.8 g during the saline infusion and -4.2 ± 0.7 g during the GLP-1 infusion (NS; Figure 3, right panel).

Plasma substrates

Figure 4 shows the plasma concentrations of glucose, lactate, NEFA and TAG. There were no differences in basal concentrations between the two treatments for any of the substrates.

Mean concentrations of blood glucose peaked 30–45 min after the breakfast and returned to basal level after 3 h during the saline infusion, whereas the concentration was maintained at basal level throughout the GLP-1 infusion ($P<0.0001$). During the 30 min intermission of the GLP-1 infusion, a significant increase in glucose occurred ($P<0.05$), which continued when the infusion was resumed during the lunch ($P<0.0001$).

Mean plasma lactate concentrations peaked 45–60 min after initiation of the test meal during both infusions, but the increase was larger on the saline day compared to the GLP-1 day ($P<0.0001$).

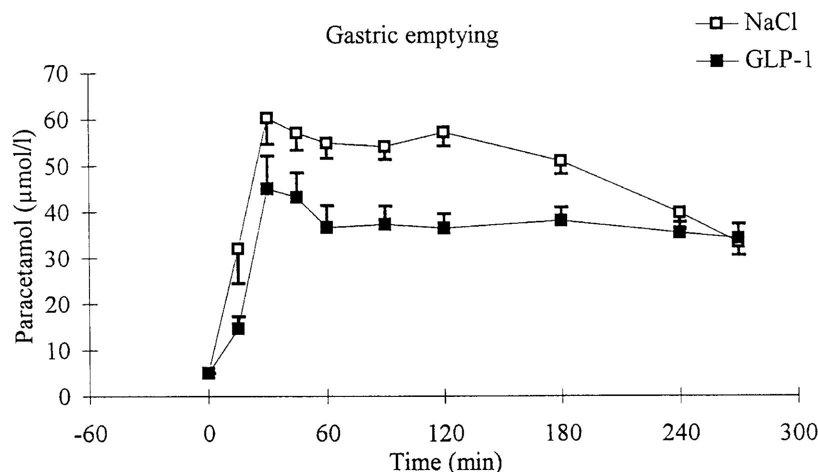

Figure 2 Gastric emptying. Plasma concentrations of paracetamol during GLP-1 (filled squares) or saline (open squares) infusions in 18 healthy, obese male subjects. Data are means (±s.e.m.). ANOVA—time effect, $P<0.0001$; treatment effect, $P<0.0001$; and interaction treatment×time effect, NS.

Mean plasma concentrations of NEFA decreased significantly during both infusions. The profiles were equal during the first 3 h, while the concentrations were higher during the saline infusion from 240 min to 300 min ($P<0.0001$).

Mean plasma concentrations of TAG were almost identical during the first hour after onset of the test meal, but during the last 3 h of the infusion the concentrations were higher during the saline infusion compared to the GLP-1 infusion ($P<0.0001$).

The net AUCs of glucose, lactate and TAG were smaller during the GLP-1 infusion than saline, while there was no difference in net AUC of NEFA (Figure 4, right panel).

Plasma hormones (insulin, glucagon, GIP, GLP-2)

There were no differences between the two treatments in fasting concentrations of insulin, GIP and GLP-2, while glucagon initially was higher on the saline day ($P<0.05$, Figure 5). All breakfast-induced hormonal responses were reduced with GLP-1 compared to saline infusion ($P<0.0001$, Figure 5).

Mean insulin concentrations peaked 30 min after the beginning of the test meal during both infusions, but the response was attenuated during GLP-1 infusion compared with saline (saline, 801±96; GLP-1, 375±52 pmol/l; $P<0.0001$). After 120 min no differences were observed between treatments. Likewise, glucagon profiles for the first 4 h of infusion were shaped equally, but concentrations were maintained at a lower level during the GLP-1 infusion (treatment effect $P<0.0001$). The breakfast-induced increase in GIP was lower on the GLP-1 test ($P<0.0001$), and the GLP-2 response was totally suppressed during the GLP-1 infusion compared with the saline test ($P<0.0001$). During the 30 min break of infusion, the concentration of glucagon, GIP and GLP-2 decreased or stayed the same on the saline day but increased on the GLP-1 day. All net AUCs were smaller during the GLP-1 infusion ($P<0.05$, Figure 5, right panel).

Discussion

The present study demonstrated that peripherally administered GLP-1 resulting in an elevation of plasma concentrations within the physiological range decreased ratings of hunger and prospective food consumption, and reduced gastric emptying and diet-induced thermogenesis in healthy obese men. In addition, all plasma hormone and substrate profiles, except NEFA, were significantly reduced by GLP-1. Thus, it is clear that GLP-1 is an important physiological regulator of digestion and/or absorption.

The reduced ratings of hunger and prospective food consumption during the infusion of GLP-1 confirm previous findings in lean and obese subjects.[7–10] On the other hand, satiety and fullness during the day, and energy intake at the *ad libitum* lunch were unaffected by the GLP-1 infusion. These observations may be related to the chosen dose, which is 30–40% lower than infusion rates used in most previous studies of obese and normal-weight males.[7–9] This lower rate of infusion resulted in plasma GLP-1 concentrations of about 50 pmol/l, which represents the upper limits of postprandial concentrations seen in normal-weight subjects given an appetizing high-energy mixed meal (range 32–76 pmol/l).[6] Gutzwiller and colleagues found a dose dependent reduction in energy intake in normal-weight men using three rates of GLP-1 infusion (22.5, 45 and 69 pmol/kg×h).[10] With the lowest rate no effect was seen in either appetite ratings or energy intake compared to the control situation, and with the medium rate, energy intake was slightly decreased, whereas only the highest rate affected both appetite ratings and energy intake.[10] Thus, the dose used in the

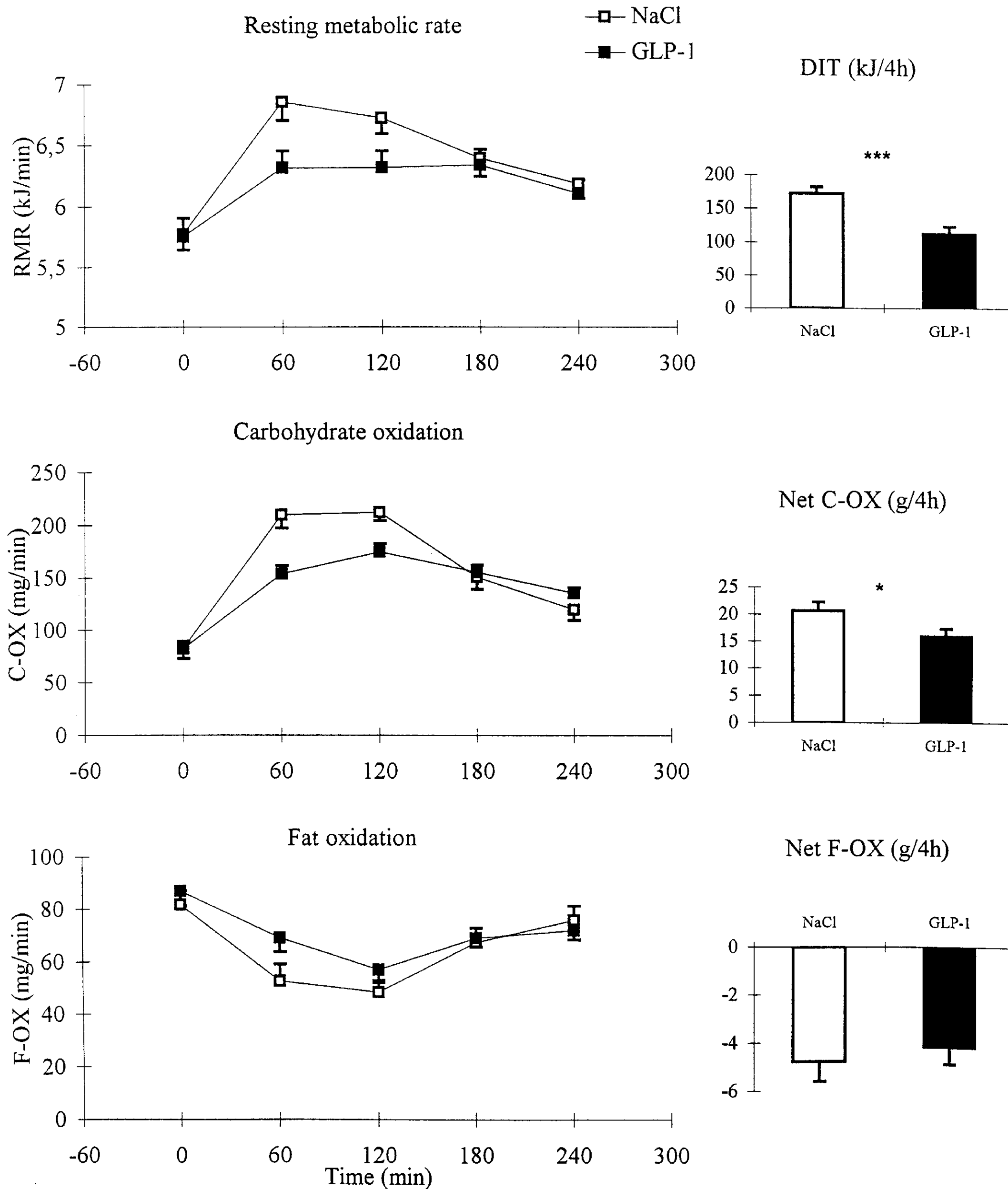

Figure 3 Resting metabolic rate (RMR) and substrate oxidations during GLP-1 (filled squares) or saline (open squares) infusions in 18 healthy, obese male subjects. Data are means (± s.e.m.). Left panel: ANOVA—time effect, $P<0.0001$; treatment effect, RMR, $P<0.0001$; carbohydrate oxidation (C-OX), $P<0.05$; fat oxidation (F-OX), $P=0.403$; interaction treatment×time effect, RMR, C-OX, $P<0.0001$; F-OX, $P<0.05$. Right panel: paired *t*-test—DIT, $P<0.001$ (***); C-OX, $P<0.05$ (*); F-OX, $P=0.541$.

present study could explain the less pronounced effects on appetite parameters compared with previous studies.

Also the timing of GLP-1 administration may influence its effects on appetite and energy intake. Thus, with meals taken at the beginning of a GLP-1 infusion, no differences in energy intake were observed, whereas energy intake was reduced after 4 and 8 h of infusion.[7–9] In order to mimic a normal pre-lunch plasma concentration of GLP-1, we arrested the infusion for half an hour, thereby possibly obtaining a situation comparable to the breakfast situation, thus blunting an effect of GLP-1 on *ad libitum* energy intake.

Further, differences between subject groups (normal-weight *vs* obese subjects) with regard to (1) responsiveness to GLP-1, (2) the degree of influence by the laboratory setting using overtly observed food intake surrounded by 'obesity experts', and lastly (3) the variation of appetite ratings (greater variation or lower reproducibility of VAS scores observed in obese subjects, unpublished observations

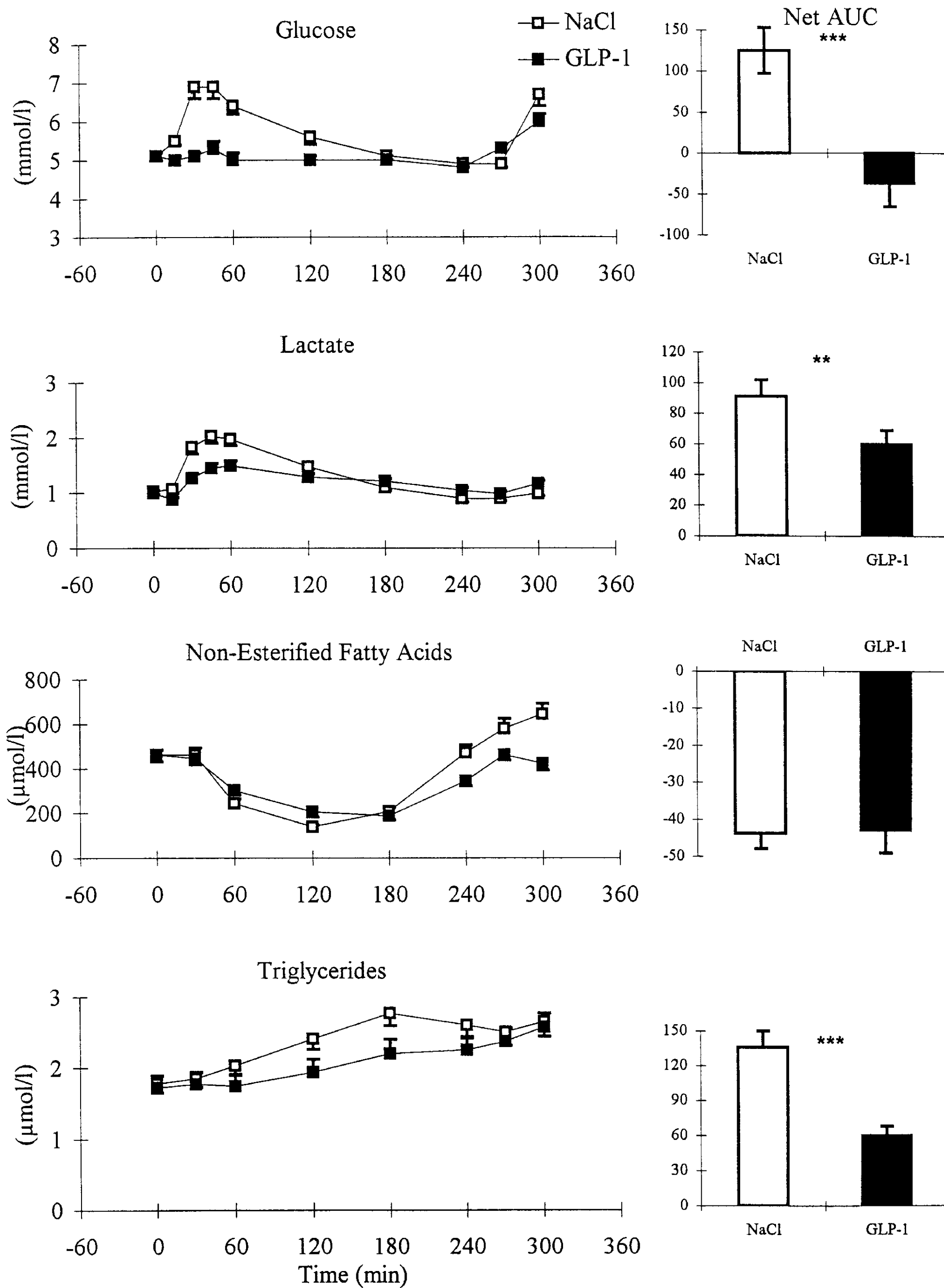

Figure 4 Plasma glucose, lactate, non-esterified fatty acid (NEFA) and triacylglycerol (TAG) concentrations during GLP-1 (filled squares) or saline (open squares) infusions in 18 healthy, obese male subjects. Data are means (±s.e.m.). Left panel: ANOVA—time and interaction treatment×time effect, $P<0.0001$; treatment effect, glucose, $P<0.0001$; lactate, $P<0.001$; NEFA, $P<0.01$; TAG, $P<0.05$. Right panel: paired *t*-test—glucose, $P<0.001$; lactate, $P<0.01$ (**); NEFA, $P<0.857$; TAG, $P<0.001$ (***).

by Flint *et al*) may be important factors to take into consideration when evaluating the effect of GLP-1 on appetite regulation in obese subjects. In rodents, it has been demonstrated that i.c.v. administration of GLP-1 inhibits water intake,[36] and inhibition of fluid intake was also observed in normal-weight human subjects using peripheral GLP-1 infusion.[10] In the present study there was no difference in water consumption between the two treatments. Again the low dose of GLP-1 may be the reason. This hypothesis is supported by the results of Gutzwiller *et al*,[10] who found a

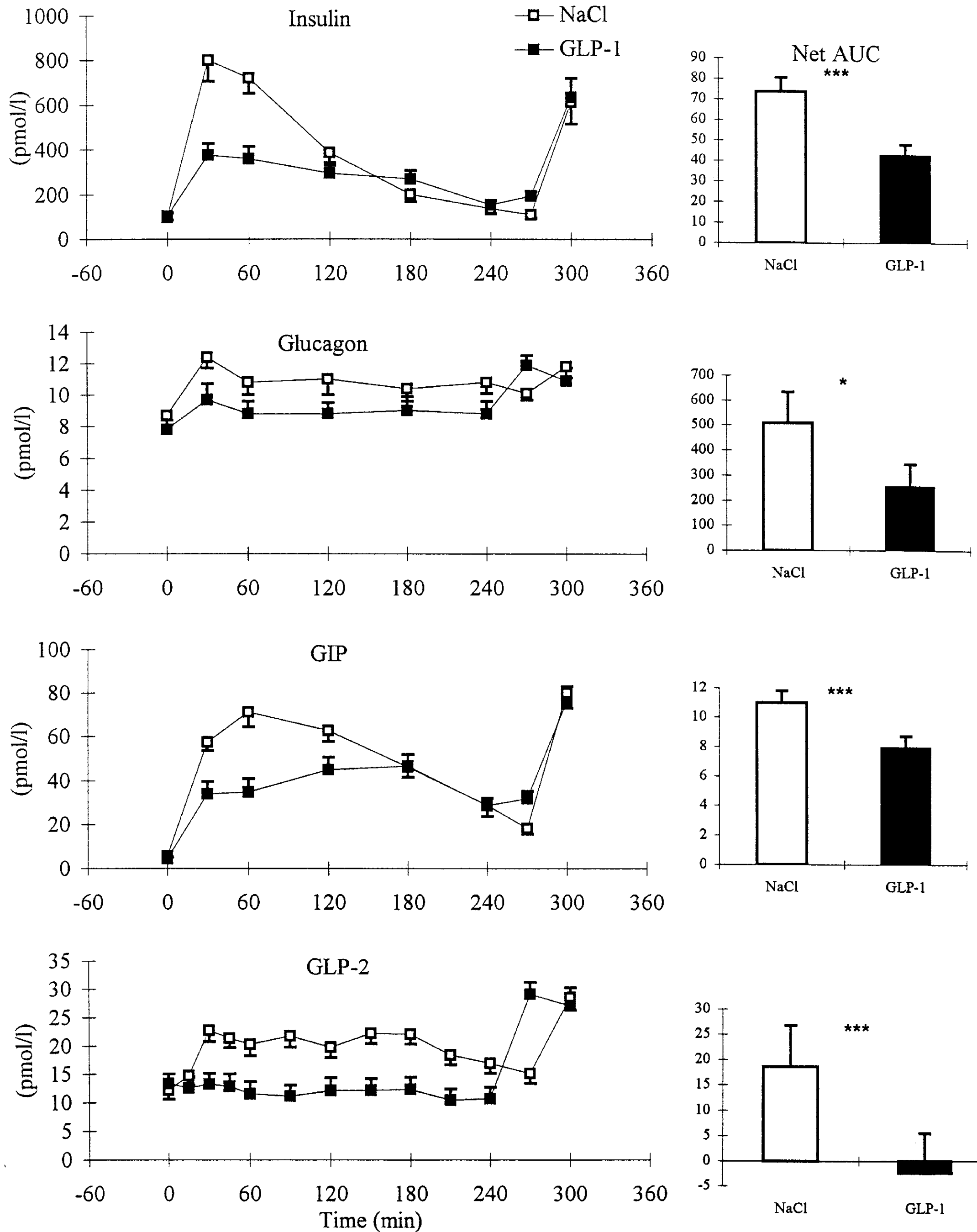

Figure 5 Plasma insulin, glucagon, glucose-dependent insulinotropic polypeptide (GIP), and glucagon-like peptide-2 (GLP-2) concentrations during GLP-1 (filled squares) or saline (open squares) infusions in 18 healthy, obese male subjects. Data are means ($\pm$s.e.m.). Left panel ANOVA—time and interaction treatment×time effect, $P<0.0001$; treatment effect, insulin, GLP-2, $P<0.0001$; glucagon, GIP, $P<0.05$. Right panel: paired *t*-test—insulin, GLP-2, $P<0.0001$ (****); GIP, $P<0.001$ (***); glucagon, $P<0.05$ (*).

significant difference in fluid intake from control conditions only when medium or high doses were used. However, these results may be influenced by the use of an energy containing drink.

Along the lines of other human studies our results did not reveal any side effects of the administration of GLP-1.[9,10,35]

All aspects of palatability were rated better than medium during both infusions, and there were no differences between treatments, except for the appearance of the lunch, which was rated better during the GLP-1 infusion. Moreover, no difference was observed in the ratings of comfort between the treatments.

In accordance with our previous results in normal-weight men,[17] GLP-1 administration resulted in a decreased diet-induced thermogenesis (DIT) in obese healthy humans. Taken together with the suppressed plasma profiles of all substrates and hormones but NEFA, which was higher during the GLP-1 infusion, this finding can be explained by a retarded delivery of nutrients caused by GLP-1. It has previously been shown that GLP-1 inhibits gastric emptying rate dose-dependently in healthy subjects.[14] In the present study, using a GLP-1 infusion of 45 pmol/kg FFM×h (or approximately 30 pmol/kg×h), the rate of gastric emptying was approximately 68% of control levels. This reduction is comparable with the results of Nauck *et al*,[14] who found the half-time of gastric emptying to be approximately 60% longer on a GLP-1 infusion of 24 pmol/kg×h compared with saline. The decrease in gastric emptying was of the same magnitude as the decrease in DIT (65% during GLP-1 compared with saline). As Shalev *et al*[16] showed, a GLP-1 stimulated insulin secretion is responsible for much of the effect on energy expenditure, the lower DIT in the present study is easily explained by the lower concentration of insulin with GLP-1 compared to saline infusion. This is in line with the view that plasma insulin parallels the cellular uptake and thermogenic metabolic processing of nutrients.

The total absence of a plasma GLP-2 response during the infusion of GLP-1 in the present study indicates that the endogenous secretion of GLP-1 was blocked. Thus, the plasma concentration of GLP-1 solely reflects what was infused. This total suppression of endogenous secretion could be due to a lack of exposure of the L-cells to nutrients or to a blocking of other secretory signals (hormonal or neuronal). Because of the inhibition of gastric emptying during the infusion of GLP-1, the exposure of the gut to nutrients was much lower than during control conditions. This is also reflected in the lower GIP response during the GLP-1 infusion.

The mechanism by which larger doses of peripheral GLP-1 inhibit appetite and food intake is not yet clear. Several possible pathways have been proposed and discussed thoroughly in recent publications.[7–10,37] Briefly, numerous GLP-1 receptors are present in the brain stem and hypothalamus, areas known to be important in appetite regulation.[37,38] In rodents, a potent effect on food intake has been shown when GLP-1 was administered i.c.v.[36,39,40] Further, in rats circulating GLP-1 is known to be able to enter the area postrema and the subfornical organ, areas where the density of GLP-1 receptors is high.[41] In humans the potent effect on gastric secretion and emptying has received great attention in relation to appetite regulation, but also interactions between GLP-1 and other mechanisms have been proposed to exist. In animals conflicting results on leptin interactions with GLP-1 have been obtained.[42,43] In humans, however, there is no evidence of interactions between GLP-1 and leptin, cholecystokinin or somatostatin.[10,44–46] On the other hand, the inhibition of gastric secretion by GLP-1 has been shown to depend on an intact n. vagus.[47] Satiety signaling via the parasympathetic system either by direct interaction with sensory fibers or indirectly via a decrease in gastric motility could be involved.[7–10] The latter could cause a prolonged period of gastric distension and a more sustained delivery of nutrients to the small intenstine. Both effects are known to result in increased sensations of satiety.[48,49]

In knock-out mice lacking the GLP-1 receptor, no effect of GLP-1 administration on food intake was seen compared with wild-type mice.[50] Nor was any phenotypical differences (eg body weight) observed in the knock-out mice, questioning the key role of GLP-1 in regulation of food intake or suggesting a redundancy of mechanisms in the regulation of appetite and body weight. Taken together with the somewhat weak results on appetite and the minor decrease in DIT in the present study using a GLP-1 infusion within the physiological range, it seems too early to conclude on the importance of GLP-1 as a long-term regulator of appetite, food intake and body weight. However, the short-term effect on appetite seems to be related to GLP-1's pronounced effect on gastric emptying, and the subsequent delay in postprandial delivery of nutrients from the gut.

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References

1 Varndell IM, Bishop AE, Sikri KL, Uttenthal LO, Bloom SR, Polak JM. Localization of glucagon-like peptide (GLP) immunoreactants in human gut and pancreas using light and electron microscopic immunocytochemistry. *J Histochem Cytochem* 1985; **33**: 1080–1086.
2 Eissele R, Göke R, Willemer S, Harthus H-P, Vermeer H, Arnold R, Göke B. Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur J Clin Invest* 1992; **22**: 283–291.
3 Kreymann B, Williams G, Ghatei MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet* 1987; **ii**: 1300–1303.
4 Elliott RM, Morgan LM, Tredger JA, Deacon S, Wright J, Marks V. Glucagon-like peptide-1 (7-36) amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J Endocrinol* 1993; **138**: 159–166.
5 Qualmann C, Nauck MA, Holst JJ, Ørskov C, Creutzfeldt W. Glucagon-like peptide-1 (7-36 amide) secretion in response to luminal sucrose from the upper and lower gut. *Scand J Gastroenterol* 1995; **30**: 892–896.
6 Ørskov C, Wettergren A, Holst JJ. Secretion of the incretin hormones glucagon-like peptide-1 and gastric inhibitory polypeptide correlates with insulin secretion in normal man throughout the day. *Scand J Gastroenterol* 1996; **31**: 665–670.

7 Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J Clin Invest* 1998; **101**: 515–520.
8 Näslund E, Gutniak M, Skogar S, Rössner S, Hellström PM. Glucagon-like peptide-1 (GLP-1) increases the period of postprandial satiety and slows gastric emptying in obese humans. *Am J Clin Nutr* 1998; **68**: 525–530.
9 Näslund E, Barkeling B, King N, Gutniak M, Blundell JE, Holst JJ, Rössner S, Hellström PM. Energy intake and appetite are suppressed by glucagon-like peptide-1 (GLP-1) in obese men. *Int J Obes Relat Metab Disord* 1999; **23**: 304–311.
10 Gutswiller JP, Göke B, Drewe J, Hildebrand P, Ketterer S, Handschin D, Winterhalder R, Conen D, Beglinger C. Glucagon-like peptide-1: a potent regulator of food intake in humans. *Gut* 1999; **44**: 81–86.
11 Schjoldager B, Mortensen P-E, Christiansen J, Ørskov C, Holst J. GLP-1 (glucagon-like peptide 1) and truncated GLP-1, fragments of human proglucagon, inhibit gastric acid secretion in man. *Dig Dis Sci* 1989; **35**: 703–708.
12 O'Halloran DJ, Nikou GC, Kreymann B, Ghatei MA, Bloom SR. Glucagon-like peptide-1 (7-36)-NH_2: a physiological inhibitor of gastric acid secretion in man. *J Endocrinol* 1990; **126**: 169–173.
13 Wettergren A, Petersen H, Ørskov C, Christiansen J, Sheikh SP, Holst JJ. Glucagon-like peptide-1 7-36 amide and peptide YY from the L-cell of the ileal mucosa are potent inhibitors of vagally induced gastric acid secretion in man. *Scand J Gastroenterol* 1994; **29**: 501–505.
14 Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Ørskov C, Ritzel R, Schmiegel WH. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol* 1997; **273**(Endocrinol Metab 36): E981–E988.
15 Hwa JJ, Ghibaudi L, Williams P, Witten MB, Tedesco R, Strader CD. Differential effects of intracerebroventricular glucagon-like peptide-1 on feeding and energy expenditure regulation. *Peptides* 1998; **19**: 869–875.
16 Shalev A, Holst JJ, Keller U. Effects of glucagon-like peptide 1 (7-36 amide) on whole-body protein metabolism in healthy man. *Eur J Clin Invest* 1997; **27**: 10–16.
17 Flint A, Raben A, Holst JJ, Astrup A. The effect of glucagon-like peptide-1 on energy expenditure and substrate metabolism in humans. *Int J Obes Relat Metab Disord* 2000; **24**: 288–298.
18 Raben A, Mygind E, Saltin B, Astrup A. Lower activity of oxidative key enzymes and smaller fiber areas in skeletal muscle of post-obese women. *Am J Physiol Endocrinol Metab* 1998; **275**: E487–494.
19 Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, validity and power of visual analogue scales in assessment of subjective appetite sensations in single meal test studies. *Int J Obes Relat Metab Disord* 2000; **24**: 38–48.
20 Klausen B, Toubro S, Astrup A. Age and sex effects on energy expenditure. *Am J Clin Nutr* 1997; **65**: 895–907.
21 Costill DL. Carbohydrates for exercise: dietary demands for optimal performance. *Int J Sports Med* 1988; **9**: 1–18.
22 Fellows IW, Macdonald IA. An automated method for the measurement of oxygen consumption and carbon dioxide excretion in man. *Clin Phys Physiol Meas* 1985; **6**: 349–355.
23 Elia M, Livesey G. Energy expenditure and fuel selection in biological systems: the theory and practice of calculations based on indirect calorimetry and tracer methods. In: Simopoulus AP (ed). *Metabolic control of eating, energy expenditure and the bioenergetics of obesity*. World Review of Nutrition and Diet, Vol 70. Karger: Basel; 1992. pp 68–131.
24 Wahlefeld AW. Triglycerides. Determination after enzymatic hydrolysis. In: Bergmayer HU (ed). *Methods of enzymatic analysis*, 2nd ed. Academic Press: New York; 1974.
25 Deeg R, W Kraemer, J Ziegenhorn: Kinetic determination of serum glucose by use of the hexokinase/glucose-6-phosphate dehydrogenase method. *J Clin Chem Clin Biochem* 1980; **18**: 49–52.
26 Noll F. L-(+)-Lactate. Determination with LDH, GPT and NAD. In: Bergmayer HU (ed). *Methods of enzymatic analysis*, 2nd ed. Academic Press: New York; 1974.
27 Albano JDM, Ekins RP, Maritz G, Turner RC. A sensitive precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. *Acta Endocrinol* 1972; **70**: 487–509.
28 Ørskov C, Rabenhøj A, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide 1 in humans. *Diabetes* 1994; **43**: 535–539.
29 Ørskov C, Jeppesen J, Madsbad S, Holst JJ. Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. *J Clin Invest* 1991; **87**: 415–423.
30 Hartmann B, Johnsen AH, Ørskov C, Holst JJ. Structure, analysis and secretion of human glucagon-like peptide-2 (GLP-2). (Abstract). *Diabetes* 1996; **45**(Suppl 2): 300A.
31 Krarup T, Madsbad S, Moody AJ, Regeur L, Faber OK, Holst JJ, Sestoft L. Diminished immunoreactive gastric inhibitory polypeptide response to a meal newly diagnosed type 1 (insulin-dependent) diabetics. *J Clin Endocrinol Metab* 1983; **56**: 1306–1312.
32 Kirsten WJ, Hesselius GU. Rapid, automatic, high capacity Dumas determination of nitrogen. *Microchem J* 1983; **28**: 529–547.
33 Diggle PJ, Liang K-Y, Zegler SL. *Analysis of longitudinal data* Clarendon Press: Oxford; 1994.
34 Senn S. *Cross-over trials in clinical research*. Wiley: New York; 1993.
35 Long SJ, Sutton JA, Amaee WB, Giouvanoudi A, Spyrou NM, Rogers PJ, Morgan LM. No effect of glucagon-like peptide-1 on short-term satiety and energy intake in man. *Br J Nutr* 1999; **81**: 273–279.
36 Tang-Christensen M, Larsen PJ, Göke R, Fink-Jensen A, Jessop DA, Møller M, Sheikh SP. Central administration of GLP-1 (7-36) amide inhibits food and water intake in rats. *Am J Physiol* 1996; **271** (Regulatory Integrative Comp Physiol 40): R848–R856.
37 Holst JJ. Enteroglucagon. *A Rev Physiol* 1997; **59**: 257–271.
38 Larsen PJ, Tang-Christensen M, Holst JJ, Ørskov C. Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience* 1997; **77**: 257–270.
39 Schick RR, vorm Walde T, Zimmermann JP, Schusdziarra V, Classen M. Glucagon-like peptide 1-a novel brain peptide involved in feeding regulation. In: Ditschuneit H, Gries FA, Hauner H, Schusdziarra V, Wechsler JG (eds). *Obesity in Europe*. Libbey & Company; 1993. pp 363–367.
40 Turton MD, O'Shea D, Gunn I, Beak SA, Edwards MB, Meeran K, Choi SJ, Taylor GM, Heath MM, Lambert PD, Wilding JPH, Smith DM, Ghatei MA, Herbert J, Bloom SR. A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 1996; **379**: 69–72.
41 Ørskov C, Poulsen SS, Møller M, Holst JJ. Glucagon-like peptide 1 receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide 1. *Diabetes* 1996; **45**: 832–835.
42 Goldstone AP, Mercer JG, Gunn I, Moar KM, Edwards CM, Rossi M, Howard JK, Rasheed S, Turton MD, Small C, Heath MM, O'Shea D, Steere J, Meeran K, Ghatei MA, Hoggard N, Bloom SR. Leptin interacts with glucagon-like peptide-1 neurons to reduce food intake and body weight in rodents. *FEBS Lett* 1997; **415**: 134–138.
43 Van Dijk G, Thiele TE, Donahey CK, Campfield IA, Smith FJ, Burn P, Bernstein IL, Woods SC, Seeley RJ. Central infusions of leptin and GLP-1-(7-36) amide differentially stimulate c-FLI in the rat brain. *Am J Physiol* 1996; **271**: R1096–R1100.
44 Drewes C, Nauck MA, Horn R, Holst J, Schmiegel W, Brabant G. A liquid mixed meal or exogenous glucagon-like peptide-1 (GLP-1) do not alter plasma leptin concentrations in healthy volunteers. *Acta Diabetol* 1997; **34**: 230–234.

45 Shalev A, Vosmeer S, Keller U. Absence of short-term effects of glucagon-like peptide-1 and of hyperglycemia on plasma leptin levels in man. *Metabolism* 1997; **46**: 723 – 725.
46 Todd JF, Wilding JPH, Edwards CMB, Khan FA, Ghatei MA, Bloom SR. Glucagon-like peptide-1 (GLP-1): a trial of treatment in non-insulin-dependent diabetes mellitus. *Eur J Clin Invest* 1997; **27**: 533 – 536.
47 Imeryuz N, Yegen BC, Bozkurt A, Coskun T, Villanueva-Penacarrillo ML. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am J Physiol* 1997; **273**: G920 – 927.
48 Sepple CP, Read NW. Gastrointestinal correlates of the development of hunger in man. *Appetite* 1989; **13**: 183 – 191.
49 Read N, French S, Cunningham K. The role of the gut in regulating food intake in man. *Nutr Rev* 1994; **52**: 1 – 10.
50 Scrocchi LA, Brown TJ, MacLusky N, Brubaker PL, Auerbach AB, Joyner AL, Drucker DJ. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nature Med* 1996; **2**: 1254 – 1258.