Prandial subcutaneous injections of glucagon-like peptide-1 cause weight loss in obese human subjects

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Recombinant glucagon-like peptide-1 (7–36)amide (rGLP-1) was recently shown to cause significant weight loss in type 2 diabetics when administered for 6 weeks as a continuous subcutaneous infusion. The mechanisms responsible for the weight loss are not clarified. In the present study, rGLP-1 was given for 5 d by prandial subcutaneous injections (PSI) (76 nmol 30 min before meals, four times daily; a total of 302·4 nmol/24 h) or by continuous subcutaneous infusion (CSI) (12·7 nmol/h; a total of 304·8 nmol/24 h). This was performed in nineteen healthy obese subjects (mean age 44·2 (SEM 2·5) years; BMI 39·0 (SEM 1·2) kg/m^2) in a prospective randomised, double-blind, placebo-controlled, cross-over study. Compared with the placebo, rGLP-1 administered as PSI and by CSI generated a 15 % reduction in mean food intake per meal ($P=0.02$) after 5 d treatment. A weight loss of 0·55 (SEM 0·2) kg ($P<0.05$) was registered after 5 d with PSI of rGLP-1. Gastric emptying rate was reduced during both PSI ($P<0.001$) and CSI ($P<0.05$) treatment, but more rapidly and to a greater extent with PSI of rGLP-1. To conclude, a 5 d treatment of rGLP-1 at high doses by PSI, but not CSI, promptly slowed gastric emptying as a probable mechanism of action of increased satiety, decreased hunger and, hence, reduced food intake with an ensuing weight loss.

Glucagon-like peptide-1: Obesity: Gastric emptying: Weight loss

Glucagon-like peptide-1 (GLP-1 (7-36)amide) exerts an antidiabetogenic effect in man by stimulating insulin release, lowering glucagon secretion, inhibiting gastric emptying and probably also by enhancing glucose utilisation (Gutniak *et al.* 1992; Nauck *et al.* 1993b). The peptide is considered as an important incretin (Kreymann *et al.* 1987; Nauck *et al.* 1993a). In recently published studies GLP-1 improved glycaemia in diabetic patients of both type 1 and type 2. These properties of the peptide make GLP-1 a potential drug in the treatment of diabetes mellitus, especially type 2 (Gutniak *et al.* 1992; Nauck *et al.* 1993b).

Since GLP-1 immunoreactive nerve fibres have been found in areas of the brain that receive afferents from the gastrointestinal tract and control food intake (Kreymann *et al.* 1989; Larsen *et al.* 1997), and the peptide is a potent inhibitor of gastric emptying (Näslund *et al.* 1999b), it is expected to influence eating behaviour. Indeed, GLP-1 has been shown to be a potent inhibitor of food intake in both animals and human subjects. In animals, the central administration of GLP-1 inhibits food intake and the administration of the GLP-1 receptor antagonist exendin (9-39) increases food intake (Turton *et al.* 1996). In human subjects, intravenous administration of GLP-1 has been shown to decrease food intake and hunger feelings in normal-weight subjects (Flint *et al.* 1998; Gutzwiller *et al.* 1999b), obese subjects (Näslund *et al.* 1998, 1999a), and patients with non-insulin-dependent diabetes mellitus (Gutzwiller *et al.* 1999a). In a recent meta-analysis with data from 115 subjects, energy intake was decreased by 11·7 % during GLP-1 infusion (Verdich *et al.* 2001). In a study in patients with non-insulin-dependent diabetes mellitus, appetite was reduced during 48 h of continuous infusion of GLP-1 (Toft-Nielsen *et al.* 1999) and recent data suggest that 6 weeks of subcutaneous (SC) infusion of GLP-1 reduces body weight in patients with non-insulin-dependent diabetes mellitus (Zander *et al.* 2002). No studies of the long-term effects of GLP-1 on food intake and body weight have been performed in obese, but otherwise healthy, subjects.

One factor limiting the therapeutic potential of GLP-1 is its short half-life. Our group has reported a very rapid

absorption of the peptide from SC tissue (Gutniak *et al.* 1994). Since intravenous studies have shown that continuous delivery of GLP-1 affects satiety and the greatest effect was observed 3–4 h after a meal, it is plausible that an injection in connection with a meal could have a similar effect to a continuous infusion.

The objectives of the present study were: first, to determine if 5 d of recombinant GLP-1 (7-36)amide (rGLP-1) administration influences eating behaviour and food intake in obese healthy volunteers as studied with prandial SC injections (PSI) and continuous SC infusion (CSI); second, to investigate the effect of these treatments on body weight; third, to determine the effect of the two treatment regimens on gastric emptying as studied using the paracetamol (acetaminophen)-absorption technique (Näslund *et al.* 2000).

Material and methods

Subjects

The inclusion criteria were healthy men or post-menopausal women (as men and post-menopausal women have been shown to have similar rates of gastric emptying; Grybäck *et al.* 2000) between 18 and 65 years of age. The subjects should consume at least four solid meals/d and demonstrate a stable weight not varying more than $\pm 5\%$ during the last month. Nineteen subjects (seventeen men, BMI $40{\cdot}0 \pm 1{\cdot}3$ kg/m^2, age $43{\cdot}4 \pm 3{\cdot}0$ years) were recruited for the study. Of these, fifteen (thirteen men, BMI $39{\cdot}4 \pm 1{\cdot}3$ kg/m^2, age $43{\cdot}0 \pm 3{\cdot}0$ years) completed all four 5 d treatments. The two women were 51 and 55 years old with BMI of 38 and 36 kg/m^2, respectively.

Study protocol

The study was performed according to good clinical practice guidelines and was approved by the Karolinska Institutet regional ethics committee. The study design was a randomised cross-over, double-blind, placebo-controlled study where subjects received either rGLP-1 or saline CSI or rGLP-1 or saline PSI for five continuous days.

In this way, each subject was examined on four occasions with 9 d separating each treatment period (Fig. 1).

Dose selection and drug administration

The dose of rGLP-1 was chosen to ensure therapeutic levels of the peptide. In previous studies (Gutniak *et al.* 1992; Flint *et al.* 1998; Gutzwiller *et al.* 1999b), GLP-1 has been given as intravenous infusions ranging from 45 to 75 pmol/kg per h in order to achieve plasma levels ranging between 60 and 200 pmol/l. In other studies where the peptide was given as SC injections (Gutniak *et al.* 1994; Nauck *et al.* 1996; Todd *et al.* 1997) of 0·4 to 1·5 nmol/kg, peak levels of GLP-1 ranging between 100 and 550 pmol/l were obtained. According to clinical experience and a dose–response study of Ritzel *et al.* (1995), plasma levels above 150 pmol/l should be avoided because of possible side effects; mainly vomiting and dizziness. In the present study, the doses of 120 pmol/kg per h for the SC infusion and 0·8 nmol/kg for the SC injection would be expected to raise plasma GLP-1 concentrations to 100–200 pmol/l, which is the optimal therapeutic range according to up-to-date clinical experience. rGLP-1 (BioNebraska Inc., Lincoln, NB, USA) was administered as a CSI at a dose of 120 pmol/kg per min with a MiniMed pump (MiniMed Inc., Northridge, CA, USA) or as PSI at 0·8 nmol/kg given 30 min before four main meals/d. As a control, normal saline (Natriumklorid Pharmacia 9 mg/ml; Pharmacia & Upjohn, Stockholm, Sweden) was used. Based on our previous studies (Näslund *et al.* 1999a), with intravenously administered GLP-1, a power analysis was performed, where with a power of 80 % we expected to be able to detect a 870 kJ change in food intake and 6·5 mm change in hunger visual analogue scale (VAS) rating between the placebo and GLP-1.

Study visits

Screening visit. The subjects were informed regarding the study protocol and screening regarding the inclusion and
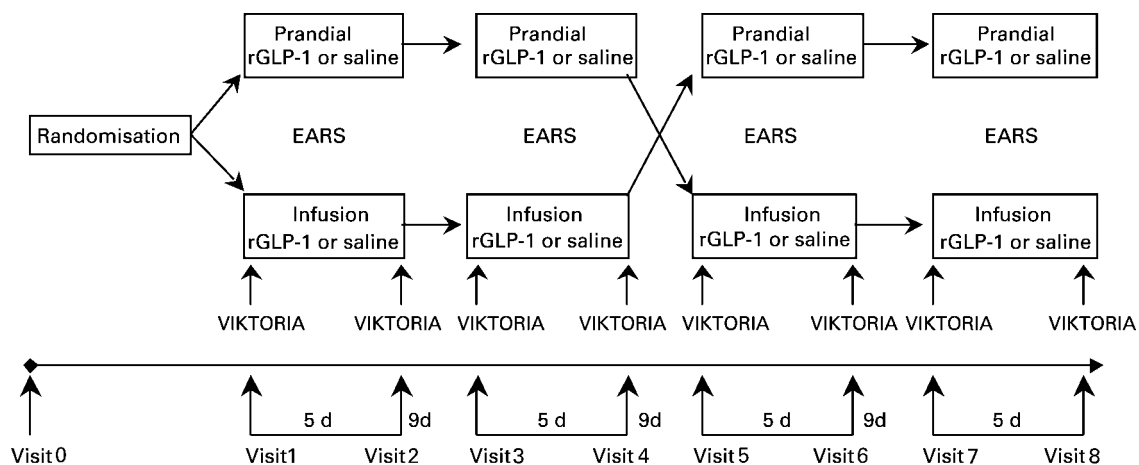
Fig. 1. Schematic presentation of the randomised cross-over, double-blind, prospective placebo-controlled study design where subjects received either recombinant glucagon-like peptide-1 (rGLP-1) or saline as a continuous subcutaneous infusion or rGLP-1 or saline as prandial subcutaneous injections for 5 d in a row, with a wash-out period of 9 d in between. EARS, electronic visual analogue scales; VIKTORIA, universal eating monitor.

exclusion criteria. Baseline anthropometric data were obtained, as was baseline blood chemistry.

Visit 1. After a 9 h fast, refraining from alcohol and exercise for 24 h, the subjects came to the clinic at 07.15 hours. Baseline data were obtained and the subjects were instructed regarding the SC infusion and prandial injections. At 30 min before an energy-fixed breakfast of 2·4 MJ (16 % energy as protein, 36 % energy as carbohydrate and 48 % energy as fat), either the CSI or PSI was begun.

A test meal was served on a universal eating monitor (VIKTORIA) at 12.00 hours. The VIKTORIA has been described in detail elsewhere (Barkeling *et al.* 1990). In short, the VIKTORIA consists of a plate, with a homogeneous meal in abundance, which is placed on a scale connected to a computer. The total food intake, meal duration and eating rate are measured by the VIKTORIA. The test meal was an industrially produced Swedish hash with a standard energy content of 0·7 MJ (170 kcal)/100 g consisting of diced meat, onions and potatoes, mixed and fried (Oxpytt; Nestlé AB, Bjuv, Sweden). The energy percentages were: protein, 16; carbohydrate, 36; fat, 48. The subjects were instructed to eat freely until completely full and satisfied. One glass (200 ml) of plain water and one glass with the dissolved paracetamol (see later) were given with the meal.

Feelings of hunger, satiety, thirst and nausea were assessed by the use of VAS 15 min before, 60, 120, 180, and 300 min after food intake on a hand-held computer (Psion 3Mx; Psion plc, London, UK) using EARS software (BioPsychology Group, School of Psychology, University of Leeds, Leeds, UK) for electronic recording. The same recording equipment was used for ambulatory recordings of hunger, satiety, thirst and nausea at home; before and after meals on days 2, 3 and 4 in-between the study meals on days 1 and 5.

Gastric emptying rate was assessed by utilising paracetamol absorption from the intestine after oral ingestion as a marker (Näslund *et al.* 2000). Plasma concentrations of paracetamol were measured at 0, 15, 30, 60, 90, 120 and 180 min after the ingestion of 2 g paracetamol with the test meal. Plasma samples were stored at − 20°C until the analysis of paracetamol by fluorescence-immunoassay (IMX; Abbott Laboratories, Chicago, IL, USA). The assay had a CV of 5 %. The gastric-emptying profile was estimated after the conversion of paracetamol plasma concentration values to accumulated values, i.e. total absorption of the drug. In this way a gastric-emptying curve from 100 to 0 % adapted to a third-degree polynomial was obtained for each subject. The gastric half-emptying time (T50) was defined as the period from the end of food intake with paracetamol until 50 % of gastric emptying was achieved (Näslund *et al.* 2000). The obtained values for paracetamol absorption during treatment with rGLP-1 were expressed in relation to paracetamol absorption during treatment with saline.

Blood samples were also obtained for the analysis of plasma concentrations of glucose, glucagon, insulin and GLP-1; 15 min before food intake, at the start of food intake and at 15, 30, 60, 90, 120, 150, 180, 240, and 300 min after food intake. Blood samples were collected in EDTA tubes placed on ice. The samples were

centrifuged at 4°C for 10 min at 3000 rpm. Plasma was collected and stored at − 20°C for analysis in one series.

Glucagon was assessed with an assay directed against the C-terminus of the glucagon molecule (antibody no. 4305) and therefore measures glucagon of mainly pancreatic origin. The detection limit of the assay is 1 pmol/l, with a CV of 5 %.

Insulin was analysed using a sandwich enzyme immunoassay (Insulin Kit K6219; DACO, Copenhagen, Denmark). The assay cross-reacts to 0·3 % with pro-insulin but not with C-peptide. The detection limit of the assay is 3 mU/l and the CV is 8 %.

Glucose was analysed using an enzyme assay (mutarotase and glucose dehydrogenase) with reagents from Boeringer-Mannheim GmbH (Mannheim, Germany) using a Hitachi 917 automatic analyser (Hitachi, Tokyo, Japan).

GLP-1 concentrations in plasma were measured by radioimmunoassay after the extraction of plasma with 70 % ethanol (v/v, final concentration). C-terminal GLP-1 immunoreactivity, comprising both intact, surviving GLP-1 (7-36)amide and the primary metabolite, and therefore reflecting the total amount of rGLP-1 administered, was determined using antiserum 89390 which has an absolute requirement for the intact amidated C-terminus of GLP-1 (7-36)amide and cross-reacts less than 0·01 % with C-terminally truncated fragments and 89 % with GLP-1 (9-36)amide (Ørskov *et al.* 1994). Sensitivity for the assay is below 5 pmol/l and the CV is below 10 %.

The subjects then left the clinic for further ambulatory treatment at home and returned on day 5 for visit 2.

Visits 2, 3, 4, 5, 6, 7, and 8

Visits 2 to 8 were identical to visit 1 with the difference that the administration route (CSI or PSI) or drug (rGLP-1 or saline) was changed in a randomised manner. Subjects were instructed to lead their 'normal' life during the study weeks (Fig. 1).

Statistical analysis

All data were analysed according to the intention to treat (ITT) (n 19). A per protocol analysis was made for the change in weight for the subjects who completed the study (n 15) (Tukey–Kramer HSD test correcting for multiple comparisons). Data are presented as means and standard errors of the mean as appropriate.

The results were tested for normality of distribution. The two-tailed Student's t test was carried out in normally distributed samples and the Mann–Whitney U test was performed where the data were not normally distributed. All statistical tests used a significance level of $P < 0.05$. Within-treatment group analysis was conducted using the t test in an ANOVA setting. Between-treatment group analysis was conducted using a two-way ANOVA model. A two-way repeated-measures ANOVA model was used to compare the result profiles over time. Average blood glucose, insulin, and paracetamol concentrations were calculated as areas under the curve using the trapezoidal rule. All clinical measurements were analysed using a mixed-model ANOVA. The model included fixed effects

for sequence, treatment and period and a random effect for patients. The mean square error from the ANOVA and *t* distribution were used in these calculations. A two-way repeated-measures ANOVA model was used to compare the result profiles over time for the EARS VAS.

For the statistical evaluation of gastric emptying with rGLP-1 or saline, either administered as PSI or as a CSI, we used a two-way ANOVA and *t* tests with Bonferroni *post hoc* test adjustment.

Results

Weight

The ITT analysis could not demonstrate any change in weight before and after the 5 d treatment period. However, per protocol analysis demonstrated that BMI decreased during PSI rGLP-1 treatment over the 5 d period by 0·18 (SEM 0·08) kg/m^2 (0·55 (SEM 0·20) kg) ($P<0·05$). In contrast, BMI (kg/m^2) increased slightly during the placebo PSI, as well as CSI treatments with the placebo and rGLP-1 by 0·15 (SEM 0·07) ($P<0·05$), 0·17 (SEM 0·09) ($P<0·05$) and 0·12 (SEM 0·05) ($P<0·05$), respectively. There was a significant difference in weight change between PSI rGLP-1 and the other three experimental conditions after correcting for multiple comparisons.

A linear regression analysis between change in weight and change in amount of food eaten during the test meal on day 1 and day 5 with the placebo and rGLP-1 PSI treatment showed a positive correlation (R^2 0·35; $P=0·02$) with decreased food intake along with decreased weight.

Vital functions

Systolic and diastolic blood pressure in the placebo experiments was not different from the values during rGLP-1 treatment. No acute or chronic haemodynamic effects were observed. No pulse reaction was noticed in any of the experiments. No effects on respiratory functions were noticed.

Food intake

There were no significant differences between the effects of rGLP-1 administered as PSI or CSI compared with saline on the amount of food eaten. However, there was a trend towards a reduction in food intake seen on day 5 with PSI treatment ($-62·5$ (SEM 6·2) g *v.* saline; $P=0·07$).

Taking CSI and PSI treatments together to analyse the effect of rGLP-1 treatment on food intake there was no difference during the placebo (392·3 (SEM 42·0) g) and during rGLP-1 treatment (362·9 (SEM 41·4) g) on day 1. On day 5 the effect of rGLP-1 was more pronounced and intake of the test meal was significantly reduced from 379·7 (SEM 38·5) g with the placebo to 323·6 (SEM 39·9) g with rGLP-1 ($P=0·02$).

Visual analogue scale ratings of hunger, satiety, thirst and nausea

Hunger. There were no significant differences between the effects of rGLP-1 administered as PSI or as CSI compared with saline on hunger ratings 300 min after the meal.

Taking CSI and PSI treatments together to analyse the effect of rGLP-1 treatment on hunger ratings, the ITT analysis on day 1 showed a significant suppression of VAS ratings for hunger before the meal and at 60 min from 55·0 (SEM 7·0) to 5·8 (SEM 3·4) mm in the placebo and from 49·8 (SEM 6·0) to 10·3 (SEM 4·2) mm in rGLP-1 experiments ($P=0·01$). After the meal, at 300 min, the hunger ratings were similar; 43·0 (SEM 5·9) mm in the placebo *v.* 37·3 (SEM 4·6) mm in rGLP-1 (NS) experiments. Correspondingly, the VAS values on day 5 showed a suppression of hunger ratings from 60 (SEM 5·1) to 7·5 (SEM 3·8) mm in the placebo and from 54·5 (SEM 5·9) to 9·5 (SEM 3·5) mm in the rGLP-1-treated subjects before and 60 min after the meal ($P=0·03$). At 300 min the values were 44·5 (SEM 5·9) *v.* 49·0 (SEM 5·8) mm for the placebo and rGLP-1 treatment, respectively (NS).

Satiety. There were no significant differences between the effects of rGLP-1 administered as a CSI compared with saline on satiety ratings 300 min after the meal. In contrast, on day 1 satiety ratings were 7·2 (SEM 0·9) mm ($P=0·03$) higher during the PSI treatment with rGLP-1 compared with saline. No significant differences were found on day 5 during PSI treatment.

Taking CSI and PSI treatments together to analyse the effect of rGLP-1 treatment on satiety, the ITT analysis of VAS ratings for satiety (fullness) showed a similar pattern as hunger with increased values between before the meal and 60 min after the meal during saline (34·3 (SEM 5·0) and 77·3 (SEM 4·8) mm, respectively; $P<0·05$) and rGLP-1 administration (37·8 (SEM 5·2) and 77·0 (SEM 4·2) mm, respectively; $P<0·05$). An increase in satiety was only observed on day 1 with rGLP-1 treatment compared with saline (5·6 (SEM 0·7) mm *v.* saline; $P=0·03$).

Thirst. There were no significant differences in the ratings of thirst with rGLP-1 treatment or the placebo, administered by either route at any point after the meal.

Nausea. There were no differences in feelings of nausea during treatment with rGLP-1 compared with saline. Feelings of nausea that were not related to the test meal were recorded as an adverse event.

Visual analogue scale at home

No differences could be observed during at-home recordings of feelings of hunger, satiety, thirst and nausea between the rGLP-1 and placebo groups.

Gastric emptying

Calculations of T50 resulted in a significantly prolonged T50 with rGLP-1 compared with the placebo. The T50 increased from 47·0 (SEM 2·3) min with the placebo to 67·9 (SEM 2·6) min with rGLP-1 administered as PSI on day 1 ($P<0·001$), and correspondingly from 44·1 (SEM 2·2) min with the placebo to 69·4 (SEM 3·9) min on day 5 ($P<0·001$). When rGLP-1 was administered as a CSI, the T50 was prolonged on day 1 from 46·9 (SEM 2·3) min with the placebo to 54·6 (SEM 3·3) min with rGLP-1 treatment ($P<0·05$), but not on day 5 (correspondingly 44·4 (SEM 1·9) *v.* 46·0 (SEM 2·1) min; NS) (Fig. 2).
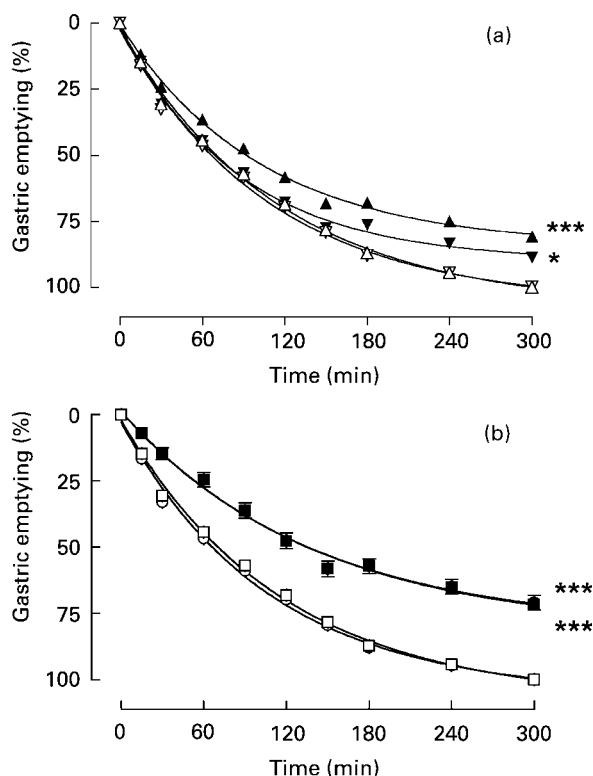
Fig. 2. Gastric emptying time as measured by the paracetamol technique in eighteen obese subjects. They were treated with a continuous subcutaneous infusion (a) of saline (placebo) or recombinant glucagon-like peptide-1 (rGLP-1) for 5 d (($\triangle$), placebo day 1; (∇), placebo day 5; ($\blacktriangle$), rGLP-1 day 1; ($\blacktriangledown$), rGLP-1 day 5) or subcutaneous prandial injections (b) of saline or GLP-1 for 5 d (($\square$), placebo day 1; ($\bigcirc$), placebo day 5; ($\blacksquare$), rGLP-1 day 1; ($\bullet$), rGLP-1 day 5). Values are means, with standard errors of the mean represented by vertical bars. * $P<0.05$, *** $P<0.001$; both v. placebo.

Plasma glucagon-like peptide-1 concentrations

In the PSI groups, the basal plasma level in the placebo group was 15·0 (SEM 1·2) pmol/l with a peak of 28·9 (SEM 2·3) pmol/l occurring 30 min after the start of the meal. With rGLP-1 injections, plasma concentrations rose from 25·8 (SEM 2·7) to 269·4 (SEM 22·1) pmol/l at 15 min after the meal and returned to the basal levels within 180 min. In the CSI groups, a minor rise of GLP-1 was observed from basal values (measured 4 h after the start of the infusion pump) of 49·7 (SEM 5·9) pmol/l to a peak concentration of 88·7 (SEM 13·1) pmol/l at 15 min after the meal (Fig. 3).

Plasma glucose, insulin and glucagon concentrations

Plasma glucose and glucagon concentrations were decreased and plasma insulin increased after PSI of rGLP-1. No significant alterations in plasma glucose, insulin or glucagon concentrations were seen during the CSI of rGLP-1 (Fig. 3).

Adverse events

Feelings of sickness and abdominal pain were the most frequently reported side effects. rGLP-1 treatment resulted in a significant increase only in the frequency of feeling of sickness, compared with the placebo in the PSI experiments ($P=0.05$). However, when all the adverse events were evaluated together, a significantly increased frequency was observed in the PSI experiments but not under CSI treatment. No effects of rGLP-1 were observed on blood pressure or heart rate (Table 1).

Discussion

The present study demonstrates that PSI of rGLP-1 during 5 d in obese subjects results in a weight loss of 0·5 kg during this time period. With a CSI of the same amount of rGLP-1, no significant weight loss occurred over a corresponding period. The reduction in weight was associated with a significant slowing of gastric emptying and a trend towards a reduced food intake, but no change of feelings of hunger and satiety. The PSI treatment was generally well tolerated by the subjects and resulted in an improved metabolic profile with balanced postprandial glucose and insulin concentrations.

Other studies have shown that SC administration of GLP-1 for up to 6 weeks reduces weight (Zander *et al.* 2002), yet the present study is the first to demonstrate this effect with PSI in direct association with meals. Previous studies over short time periods (4–8 h) have demonstrated that GLP-1 inhibits food intake and reduces feelings of hunger, as verified by a recent meta-analysis (Verdich *et al.* 2001). In the present study we did not find any effect of either PSI or CSI of rGLP-1 on feelings of hunger, but a trend towards a reduction in food intake during PSI of rGLP-1. One reason for this may be that since the present study was designed as an ambulatory at-home study, VAS estimations were chosen to be made at 60 min intervals. Hence, the first hunger rating was measured 60 min after the meal in the present study compared with immediately after the cessation of food intake in previous studies on obese subjects (Näslund *et al.* 1999a). Due to this choice of assessment we found fewer variations in the hunger ratings, and trough values were most probably not recorded.

Similarly, there is a question whether the dose of rGLP-1 administered during the CSI experiments was high enough to result in effects on eating behaviour (Zander *et al.* 2002). In the present study, the mean plasma GLP-1 concentration before the meal in the CSI group was 65 pmol/l. This may not be sufficient to suppress food intake, as previous studies suggest that concentrations of about 100 pmol/l before food intake are needed in order to achieve a reduction in food intake (Näslund *et al.* 1999a). Also, a newly published meta-analysis showed a clear dose–response relationship between the reduction of food intake and plasma GLP-1 concentrations (Verdich *et al.* 2001). Furthermore, as suggested in this meta-analysis, obese subjects may have a lower sensitivity to GLP-1 and may require higher doses in order to obtain effects on satiety.

The results with regards to gastric emptying are in accordance with previously published papers on the effect of GLP-1 on gastric emptying (Nauck *et al.* 1997; Näslund *et al.* 1999b). During PSI the gastric emptying
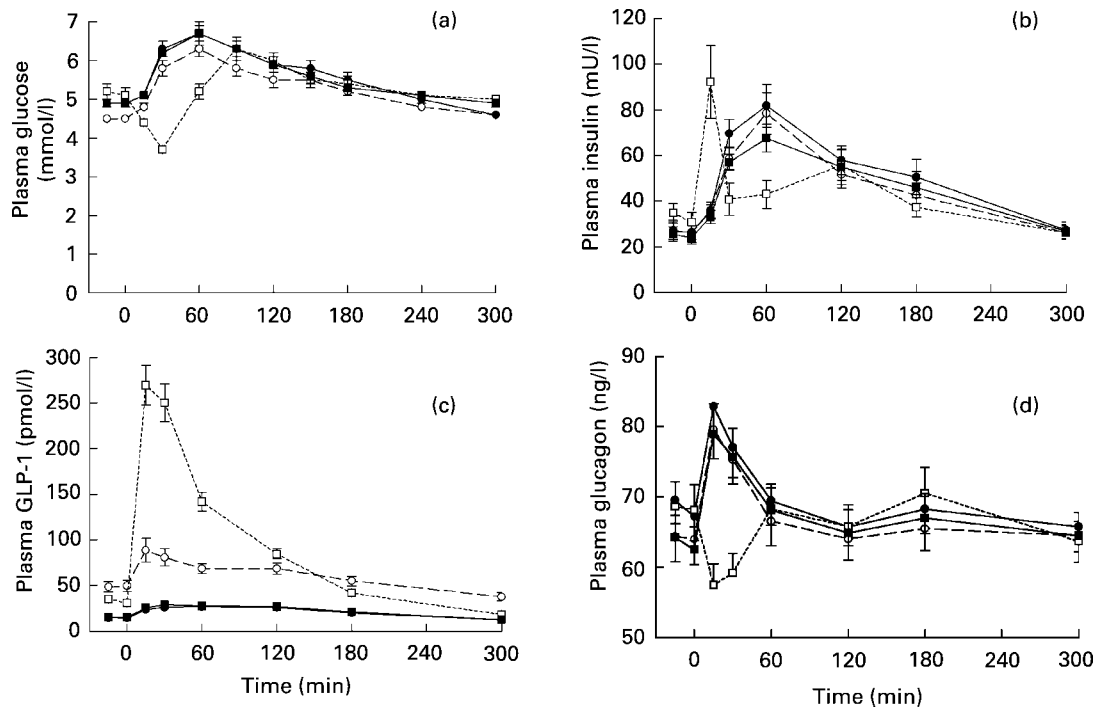
Fig. 3. Plasma glucose (a), insulin (b), C-terminal glucagon-like peptide-1 (GLP-1; (c)) and glucagon (d) concentrations in eighteen obese subjects receiving either saline or recombinant GLP-1 (rGLP-1) as a continuous subcutaneous infusion (CSI) or prandial subcutaneous injections (PSI) for 5 d. (■), Placebo PSI; (●), placebo CSI; (□), rGLP-1 PSI; (○), rGLP-1 CSI. Values are means, with standard errors of the mean represented by vertical bars.

was equally inhibited on day 1 and day 5. Yet, during CSI no significant inhibition of gastric emptying was found on day 5. However, on this day, plasma GLP-1 concentrations were also lower.

A delay in gastric emptying may be one mechanism for GLP-1 to exert its effects on food intake and the observed weight loss. Distension of the stomach with increasing feelings of fullness subsequently leading to prolonged postprandial satiety and reduced food intake may be one potential mechanism for the GLP-1 action on weight loss (Flint *et al.* 1998). In the present study, during PSI of rGLP-1,

increased plasma concentrations were elevated already at the start of the meal. This would favour the view that high GLP-1 levels already at the onset of food intake should reliably decrease gastric emptying and increase the prandial satiety period (Gutniak *et al.* 2001). As shown in another study from our group, GLP-1 has to be timely administered at the onset of a meal to cause the inhibition of gastric emptying (Gutniak *et al.* 2001). Another mechanism by which GLP-1 may influence food intake is directly in the central nervous system. Ørskov *et al.* (1996) identified peripherally injected [125I]GLP-1 in the subfornical organ and periphery

Table 1. Frequency of adverse events in prandial injection (PSI) and continuous infusion (CSI) of placebo or recombinant glucagon-like peptide-1 (rGLP-1)*

Adverse event	PSI		CSI		P value†	
	Placebo	rGLP-1	Placebo	rGLP-1	PSI	CSI
Abdominal pain	1	4	2	3	NS	NS
Feeling of sickness	2	8	2	1	0·05	NS
Nausea	4	3	1	6	NS	NS
Dizziness	0	1	0	2	NS	NS
Headache	0	1	0	1	NS	NS
Respiratory infection	0	0	2	0	NS	NS
Sweating	0	0	0	1	NS	NS
Polyuria	2	1	0	0	NS	NS
Thirst	0	0	1	0	NS	NS
Mouth dryness	0	0	2	0	NS	NS
Taste alteration	0	1	0	0	NS	NS
Visual defect	0	1	0	0	NS	NS
Complication to CSI or PSI	0	1	2	2	NS	NS
Total events	9	21	12	16	0·02	NS
Total subjects	7	19	11	12	0·01	NS

* For details of procedures, see p. 440
† Significance between placebo and rGLP-1.

of the area postrema. These areas are reported to project to nuclei in the central nervous system involved in the regulation of food intake (Shapiro & Miselis, 1985). Furthermore, damage to the nucleus arcuatus and circumventricular organ by neonatal monosodium glutamate treatment has been shown to diminish the inhibitory effect of GLP-1 on food intake (Tang-Christensen *et al.* 1998). Thus, both a peripheral effect mediated via the vagus nerve in concert with a central effect may contribute to the reduction in body weight observed in the present study with PSI rGLP-1 administration.

On day 5 during CSI, rGLP-1 infusion plasma concentrations were lower than those on day 1. The reasons for this are not clear, but indicate an increased plasma clearance, either due to an increased metabolic rate, renal excretion or increased volume of distribution of GLP-1. The underlying pharmacokinetic mechanisms for such observations are yet to be revealed. These findings may also account for the lack of effect by the CSI of rGLP-1 on body weight and food intake. This also pinpoints the problem of delivering natural GLP-1 to human subjects. GLP-1 has been shown to be very stable in the absence of plasma (Mojsov *et al.* 1987). Yet, in plasma or SC tissue the half-life is very short. After about 2 min most of the peptide is cleaved by the enzyme dipeptidyl-peptidase IV (Mentlein *et al.* 1993). It is possible that the dipeptidyl-peptidase IV activity was enhanced in the area of SC tissue where the needle for the CSI of rGLP-1 was placed, thus resulting in decreasing plasma concentrations over the 5 d period. This also demonstrates the problems of peptide delivery in the clinical setting. Clearly the results of rGLP-1 on food intake in the present study were dependent on the plasma concentration of GLP-1 achieved. With the present study design a sufficiently high plasma concentration was only achieved with the PSI mode of delivery. PSI of rGLP-1 resulted in a slight weight loss and improvement of metabolic parameters, yet this was also associated with an increase in adverse events which may be the result of the high peak of plasma GLP-1 seen during PSI. There is a need for a mode of delivery that can achieve sufficiently high plasma concentrations to influence food intake and metabolic parameters without the sharply increased plasma GLP-1 levels seen during the PSI administration seen in the present study. Studies employing stable GLP-1 analogues are underway (Larsen *et al.* 2001) and similarly the natural GLP-1 analogue, exendin-4, has been shown to reduce energy intake in human volunteers (Edwards *et al.* 2001).

To conclude, a 5 d treatment of rGLP-1 at high doses by PSI promptly slowed gastric emptying as one possible mechanism of action of increased satiety, decreased hunger and, hence, reduced food intake with an ensuing weight loss. This finding is in contrast to the CSI of the same total dose of rGLP-1, which does not reach the plasma levels needed to achieve the inhibition of gastric emptying and, hence, has no effects on satiety and hunger, as well as food intake and body weight. The prandial injections were not associated with any severe adverse events. Thus, it is concluded that slowed gastric emptying presents an avenue for how GLP-1 reduces food intake and causes weight loss.

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