



The effect of glucagon-like peptide-1 on energy expenditure and substrate metabolism in humans

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OBJECTIVE: To investigate the effects of a near-physiological peripheral glucagon-like peptide-1 (GLP-1) infusion, during and after a breakfast of fixed energy content, on resting energy expenditure, substrate oxidation and metabolism and the desire to eat specific types of food in humans.

DESIGN: A placebo-controlled, randomized, blinded, cross-over study. Infusion (GLP-1, 50 pmol/kg × h or saline) was started simultaneously with initiation of the test meals.

SUBJECTS: 20 healthy, normal weight (body mass index 20.3–25.7 kg/m²) men of 20–31 y of age.

MEASUREMENTS: Energy expenditure and substrate oxidations were measured before and for 4 h after standard breakfast (20% of calculated daily energy requirements, 50% of energy from carbohydrates, 37% of energy from fat and 13% of energy from protein) using a ventilated hood system. Visual analogue scales were used throughout the experiment to assess the desire to eat specific types of food and the palatability of the test meals. Blood was sampled throughout the day for analysis of plasma hormone and substrate concentrations.

RESULTS: Diet-induced thermogenesis (DIT) was lower (47%) on the GLP-1 infusion than on the saline infusion ($P < 0.0001$). This was due to a lower carbohydrate oxidation ($P < 0.01$). No differences in fat oxidation or total 4 h protein oxidation were observed. All hormone and substrate profiles except non-esterified fatty acids (NEFA) and cholecystokinin (CCK) were significantly suppressed (GLP-2 completely suppressed) during the GLP-1 infusion, whereas profiles of NEFA and CCK differed in time course during the two treatments (treatment × time effect), $P < 0.0001$. GLP-1 infusion also suppressed the desire to eat all food types following the breakfast (treatment effect: $P < 0.05$).

CONCLUSION: Peripheral GLP-1 decreased DIT and carbohydrate oxidation, probably secondary to a delayed absorption of nutrients, since substrate and hormone concentrations in plasma were suppressed during GLP-1 infusion. Endogenous secretion of GLP-1 and GLP-2 was completely suppressed by GLP-1 infusion. Finally, the desire to eat any type of food was decreased by exogenous administered GLP-1.

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Introduction

Glucagon-like peptide-1 (GLP-1) is secreted from the intestinal L-cells, which are abundant in the mucosa of the ileum and colon in humans.^{1,2} The secretion of GLP-1 from the gut increases rapidly in response to ingestion of carbohydrates, lipids and mixed meals.^{3–6}

Gut-released GLP-1 is known to be a potent insulinotropic hormone,^{3,7} and the effects of the upper intestine hormone glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 are additive and together can account for the incretin effect in humans.⁸ GLP-1 also inhibits glucagon secretion.⁹ This combination makes GLP-1 very interesting from

a pharmacological point of view as an alternative to insulin treatment of non-insulin-dependent diabetes mellitus (NIDDM) patients.^{10,11} Peripheral GLP-1 also influences gastrointestinal secretion and motility in humans. In physiological doses it inhibits meal-induced gastric acid secretion and gastric emptying.^{12–15}

Overweight and obesity are caused by a long-term imbalance of energy intake and energy expenditure. GLP-1 has been shown to affect the regulation of appetite and food intake. In animals intracerebroventricular (i.c.v.) injections of GLP-1 have been shown to result in pronounced decreased food intake.^{16–18} More recently peripheral intravenous infusions in humans have been seen to result in lower energy intake, decreased feelings of hunger and increased satiety.^{19–22} However, it is yet to be determined whether peripheral GLP-1 has a direct effect on the brain, by crossing the blood–brain barrier, or an indirect effect on appetite due to a slower rate of gastric emptying and/or neural signaling. Also, it is not known if the decreased feeling of

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hunger is due to a decreased desire to eat specific food types or to an overall decrease in hunger feelings.

Low concentrations of GLP-1 (or the co-secreted proglucagon and enteroglucagon) are observed in obese subjects,^{23–25} which makes the pharmacological interest even greater, as many NIDDM patients are also overweight or obese. Moreover, Western populations in general have become increasingly overweight and obese, and the term pandemic has been used to characterize this development during the past few years.²⁶

The effect of GLP-1 on energy expenditure, representing the other side of the energy balance equation, has only been investigated in one animal and one human study.^{27,28} In rats a single i.c.v. infusion of GLP-1 resulted in an increased O₂ consumption in lean rats while it was unchanged in obese animals.²⁷ Shalev and co-workers observed an increased resting energy expenditure during peripheral GLP-1 administration in humans during a hyperglycaemic clamp,²⁸ presumably due to the stimulation of insulin release. How GLP-1 administration during and after a meal affects energy expenditure in humans is still unknown.

The aim of the present study was to determine the effects in humans of peripheral GLP-1 infusion during and after an energy-fixed breakfast on resting energy expenditure, substrate oxidation and metabolism, and the desire to eat specific types of food.

Subjects and methods

Subjects

Twenty healthy male subjects, 20–31 y of age, normal-weight (body mass index, BMI: 20.3–25.7 kg/m², body fat 9.7–19.5%), non-smokers, non-athletes, and with no history of obesity or diabetes, participated in the study. All subjects gave written consent after the experimental protocol had been explained to them. One subject was excluded from the data analysis due to a severe headache on the second test day (GLP-1 infusion). The study was approved by the Municipal Ethical Committee of Copenhagen and Frederiksberg and is in accordance with the Helsinki-II declaration.

Diets

The test meal (breakfast) was of fixed size and energy content. It consisted of yoghurt, bread, butter, cheese, jam, kiwi-fruit, orange juice and water. Total available energy content of the meal was calculated to be 20% of each subject's individual energy requirements,²⁹ adjusted to the nearest 0.5 MJ. The distribution of energy was 50 energy% (E%) carbohydrates, 37 E% fat, 13 E% protein and 2.2 g/MJ dietary fibre. During the 2 days preceding each test day, the subjects followed a weight-maintaining standardized diet (same energy distribution as the test meals) consisting of ordinary food items. The diet was estimated to meet

each subject's individual energy requirements, 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. Food not consumed in the first pre-experimental period was returned to the department for weighing and registration. This amount of food was then deducted from the standardized diet for 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.³⁰

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.

Experimental protocol

The subjects were tested on two different occasions in a placebo-controlled, randomized, blinded, cross-over design. The two test days were separated by at least 3 weeks and by not more than 7 weeks.

On the test day, the subjects arrived at the department in the morning, having used the least strenuous means of transportation available. 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 previously³¹) 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. A venflon catheter (Viggo, Sweden, Gothenberg) was inserted in an antecubital arm vein. After another 20 min rest basal energy expenditure was measured for 45 min. Subsequently a fasting blood sample was taken. At 9:45 am breakfast was served and this was consumed within 15 min. Exactly the same time was spent on each individual subject on the two test days. Postprandial energy expenditure was measured for 4 h. Blood samples were taken 15, 30, 45, 60, 90, 120, 150, 180, 210 and 240 min after the start of consuming the breakfast. Ratings for appetite and desires for specific food types 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.³² VAS were used to assess satiety, hunger, fullness, prospective food consumption and the desire for something sweet, something salty, something fatty and something savoury. These ratings were recorded from immediately before the breakfast and throughout a 4 h period after the meal.

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 meal. An automatic pump (Infusomat, B.Braun) provided a steady-state infusion rate of 50 pmol/kg body weight/h for 4 h.

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 hour, the time spent, the type of activity, and the amount of water consumed was noted and repeated on the second test day. Energy expenditure was measured continuously during the day, interrupted by 10 min breaks from wearing the hood every hour. During the breaks the subjects remained resting in the supine position.

Energy expenditure

Energy expenditure (EE) was measured by indirect calorimetry using an open-air-circuit ventilated hood system.³³ 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. EE and oxidation of carbohydrate (C-OX), fat (F-OX), and protein (P-OX) were calculated from the gas exchange and urinary measurements using the formulas of Elia and Livesey:³⁴

$$\text{Energy expenditure (EE total) (kJ)} = 15.913 \times \text{O}_2(l) + 5.207 \times \text{CO}_2(l) - 4.646 \times N \text{ (g)} \quad (1)$$

$$\text{Non-protein EE (EEnp) (kJ)} = \text{EE total} - \text{P-OX} \quad (2)$$

$$\begin{aligned} \text{Diet-induced thermogenesis (DIT) (kJ)} \\ = (\text{Postprandial EE} - \text{Basal EE}) \text{ kJ/min} \times 240 \text{ min} \end{aligned} \quad (3)$$

$$\begin{aligned} \text{DIT (\%)} = (((\text{Postprandial EE} - \text{Basal EE}) \text{ kJ/min} \\ \times 240 \text{ min}) / \text{kJ in test meal}) \times 100 \end{aligned} \quad (4)$$

$$\begin{aligned} \text{Non-protein respiratory quotient (RQnp)} \\ = (\text{CO}_2(l) - 4.97 \times N \text{ (g)}) / (\text{O}_2(l) - 5.95 \times N \text{ (g)}) \end{aligned} \quad (5)$$

$$\text{Protein oxidation (P-OX) (kJ)} = 116 \times N \text{ (g)} \quad (6)$$

$$\text{P-OX (g)} = \text{P-OX (kJ)} / 18.56 \quad (7)$$

$$\begin{aligned} \text{Carbohydrate oxidation (C-OX) (\%)} = 21.12 \times 100 \\ \times (\text{RQnp} - 0.710) / (21.12 \times (\text{RQnp} - 0.710) \\ + (19.61 \times (1 - \text{RQnp}))) \end{aligned} \quad (8)$$

$$\text{C-OX (kJ)} = \text{EEnp} \times \text{C-OX(\%)} \quad (9)$$

$$\text{C-OX (g)} = \text{C-OX (kJ)} / 17.52 \quad (10)$$

$$\begin{aligned} \text{Fat oxidation (F-OX) (kJ)} = \text{EEnp} - \text{C-OX (kJ)} \\ \end{aligned} \quad (11)$$

$$\text{F-OX (g)} = \text{F-OX (kJ)} / 39.40 \quad (12)$$

Laboratory analyses

Blood was drawn without stasis through the indwelling antecubital cannula into iced syringes. The blood was centrifuged for 10 min at 3000 g and 4°C . Non-esterified fatty acids (NEFA) were extracted immediately and stored at -20°C until determination. Quantification of NEFA was done by an enzymatic colorimetric method (Wako NEFA test kit, NEFA C, ACS-ACOD Method, Code No. 994-75409 E). Serum triglyceride concentration (TG) was determined as described by Wahlefeld.³⁵ Plasma glucose and lactate were collected in fluoride-EDTA prepared tubes and analysed by standard enzymatic methods.^{36,37}

The syringes for hormone samples contained EDTA 6 $\mu\text{mol/l}$ and aprotinin (500 KIU/l, final concentrations). After centrifugation, plasma for hormone analyses 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.*³⁸ The tracer was human insulin, monoiodinated in position A14 (donated by Novo Nordisk A/S, Bagsvaerd, DK). The glucagon RIA was directed against the carboxy terminus of the glucagon molecule (antibody code 4305). The plasma concentrations of GLP-1 were measured against standards of synthetic GLP-1 7–36 amide using antiserum code 89390, which is specific for the amidated carboxy terminus of GLP-1. There was no ($< 0.1\%$) cross-reaction of GLP-1 and glucagon in the glucagon and GLP-1 assays.³⁹

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 $> 70 \text{ MBq/nmol}$.⁴⁰ The antiserum is directed against the N-terminus of GLP-2 and therefore measures only fully processed GLP-2 of intestinal origin. Sensitivity was below 5 pmol/l, and intra assay 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.⁴¹ Plasma cholecysto-

kinin (CCK) concentrations were measured by RIA using specific antibodies (antiserum code 92128), as detailed elsewhere.⁴²

Urinary nitrogen concentration (*N*) was measured by the method of Dumas⁴³ using a nitrogen analyzer (NA 1500, Carlo Erba Strumentazione, Milano, Italy).

Statistical analyses

All results are given as means (\pm standard error of the mean, s.e.m.). Fasting and 4 h mean values, incremental areas under the profile curves (net AUC) and total energy expenditure and substrate oxidation were compared using a paired *t*-test, while the postprandial response curves were compared by parametric analysis of variance (ANOVA) using repeated, paired measures with time and treatment as factors. Correlation analyses were performed on differences between the net AUCs during the treatments (net AUC saline – net AUC GLP-1). This was done to account for the data being paired. The level of significance was set at $P < 0.05$. The Statistical Analysis Package, version 6.11 (SAS Institute, Cary, NC, USA) was used for the statistical calculations.

Results

The results regarding plasma GLP-1, insulin, glucagon, blood glucose and subjective appetite sensations have been published in a separate paper,¹⁹ but will be summarized in this section.

GLP-1

During the saline infusion the plasma concentration of GLP-1 increased in response to the test meal as expected, while the GLP-1 infusion increased the concentration from a fasting level of 10 pmol/l to 60–90 pmol/l ($P < 0.0001$, Figure 1).

Energy expenditure and substrate oxidation

Basal energy expenditure (BEE) averaged 4.55 ± 0.10 kJ/min before the saline infusion and 4.52 ± 0.09 kJ/min before the GLP-1 infusion (n.s.). After the test meal energy expenditure increased for both types of infusion, but significantly less during the GLP-1 infusion ($P < 0.0001$, Figure 2). Diet-induced thermogenesis (DIT) averaged 201 ± 9 kJ/4 h ($6.5 \pm 0.3\%$) during the saline infusion and 106 ± 10 kJ/4 h ($3.4 \pm 0.3\%$) during the GLP-1 infusion ($P < 0.0001$, Table 1). At the end of the 4 h measurement period the incremental thermogenic response remained at a higher level on the saline infusion (NaCl, 5.04 ± 0.10 kJ/min; GLP-1, 4.87 ± 0.09 kJ/min, $P < 0.01$) than on the GLP-1 infusion.

Total protein oxidation during the experiment averaged 16.5 ± 0.7 g for the saline infusion and 17.0 ± 0.9 g for the GLP-1 infusion (n.s.; Table 1).

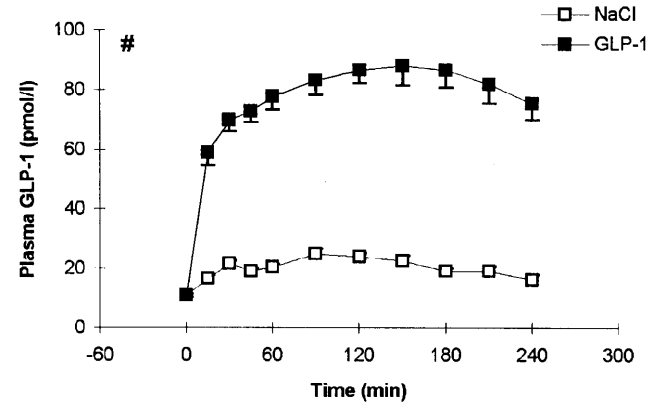


Figure 1 Plasma concentrations of glucagon-like peptide-1 (GLP-1) during GLP-1 (filled squares) and saline (open squares) infusions in 19 healthy, normal-weight male subjects. Data are means (\pm s.e.m.). ANOVA: treatment, time and interaction treatment \times time effect, $P < 0.0001$. #Previously published.¹⁹

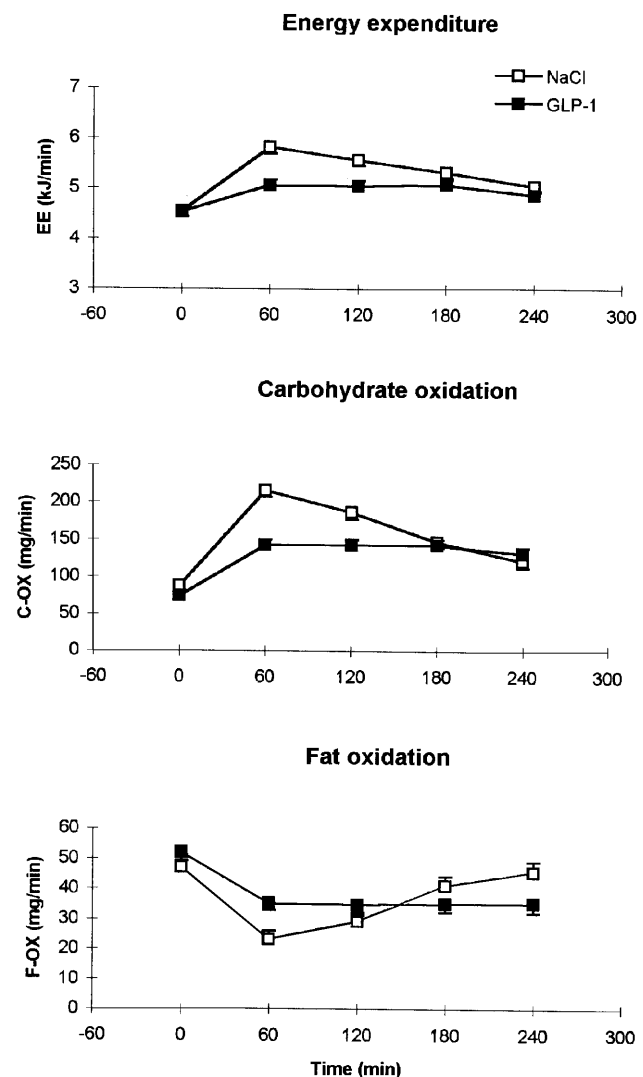


Figure 2 Energy expenditure (EE) and substrate oxidations during GLP-1 (filled squares) or saline (open squares) infusions in 19 healthy, normal-weight male subjects. Data are means (\pm s.e.m.). ANOVA: time and interaction treatment \times time effect, $P < 0.0001$; treatment effect—EE, $P < 0.0001$; carbohydrate oxidation (C-OX), $P < 0.01$; fat oxidation (F-OX), $P = 0.587$.

Carbohydrate oxidation increased postprandially during both infusions, but significantly less during the GLP-1 infusion (treatment \times time effect, $P < 0.0001$; Figure 2). Net carbohydrate oxidation averaged 18.1 ± 1.1 g during the saline infusion and 14.1 ± 1.0 g during the GLP-1 infusion ($P < 0.01$; Table 1). Postprandial fat oxidation decreased during both infusions compared with fasting levels (Figure 2). The decrease was initially larger during the saline infusion, but it reverted more quickly to fasting levels during saline infusion than during the GLP-1 infusion (treatment \times time effect, $P < 0.0001$; Figure 2). Net fat oxidation averaged -2.9 ± 0.5 g during the saline infusion and -3.6 ± 0.5 g during the GLP-1 infusion (n.s.; Table 1).

Plasma substrates

Figure 3 shows the plasma concentrations of glucose, lactate, NEFA and triglycerides (TG). There were no differences between the two treatments for basal concentrations of any of the parameters.

Plasma glucose peaked 30 min after the breakfast and returned to basal level within an hour in the saline experiment. During the GLP-1 infusion the concentration decreased during the first hour and then slowly increased to reach basal level at 180 min (time \times treatment effect, $P < 0.0001$).

Plasma lactate concentrations peaked 1 h after initiation of the test meal during both infusions, but the increase was larger on the saline day than during the GLP-1 test (time \times treatment effect, $P < 0.0001$).

Plasma concentrations of NEFA decreased significantly during both infusions. Minimum concentration was observed an hour after initiation of the test meal during the saline infusion, and 2 h later during the GLP-1 infusion (treatment \times time effect, $P < 0.0001$).

Plasma concentrations of TG were almost identical during the first hour after initiation of the test meal, but during the last 3 h the concentrations were larger during the saline infusion compared to the GLP-1 infusion (time \times treatment effect, $P < 0.0001$).

Plasma hormones

There were no differences between the two treatments in basal concentrations of any of the hormones. All breakfast-induced hormonal responses were reduced with GLP-1 compared to saline infusion (treatment \times time effect, $P < 0.0001$; Figure 4).

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 infusion. Both glucagon and GLP-2 responses were totally depressed throughout the whole test period during the GLP-1 infusion. Responses of GIP and CCK were lower and slower in their course on the GLP-1 test compared with saline test. The maximum concentration for CCK may even not have been reached at the end of experiment. As a consequence, there was no difference in net AUC for CCK between the two trials, while all other net AUCs are smaller during the GLP-1 infusion ($P < 0.0001$).

Appetite and desire for specific types of food

The infusion of GLP-1 compared with the saline infusion significantly decreased ratings of 'hunger' and 'prospective food consumption', and increased ratings of 'satiety' and 'fullness', postprandially ($P < 0.05$).¹⁹ No differences were observed in the fasting state. The same pattern was seen for the subjective ratings of desire to eat specific types of food. In the fasting state there were no differences between the treatments, but postprandially subjects felt significantly less desire to eat all types of food during the infusion of GLP-1 compared to the saline infusion (Figure 5).

Correlation analyses

The differences in DIT were significantly correlated to differences in NEFA ($r = 0.46$, $P < 0.05$) and TG ($r = 0.50$, $P < 0.05$). The differences in fat oxidations were significantly correlated to NEFA ($r = 0.50$, $P < 0.05$). Negative relations were found between differences in net AUC of NEFA and the desire to eat something salty ($r = -0.54$, $P < 0.05$).

Table 1 Total and meal-induced 4 h energy expenditure and substrate oxidations

		Total		Meal-induced	
		NaCl	GLP-1	NaCl	GLP-1
Energy expenditure	(kJ)	1423 \pm 27	1325 \pm 26***	201 \pm 9	106 \pm 10***
	(%)			6.5 \pm 0.3	3.4 \pm 0.3***
Carbohydrate oxidation	(g)	41.8 \pm 1.9	34.0 \pm 1.6**	18.1 \pm 1.1	14.1 \pm 1.0*
	(kJ)	732 \pm 33	596 \pm 28**	317 \pm 20	246 \pm 17*
Fat oxidation	(g)	9.8 \pm 0.7	10.5 \pm 0.7	-2.9 \pm 0.5	-3.6 \pm 0.5
	(kJ)	386 \pm 26	414 \pm 26	-115 \pm 21	-140 \pm 18
Protein oxidation	(g)	16.5 \pm 0.7	17.0 \pm 0.9	—	—
	(kJ)	307 \pm 12	315 \pm 17	—	—

Mean \pm s.e.m. Differences between the treatments by paired *t*-test: * $P < 0.01$; ** $P < 0.001$; *** $P < 0.0001$.

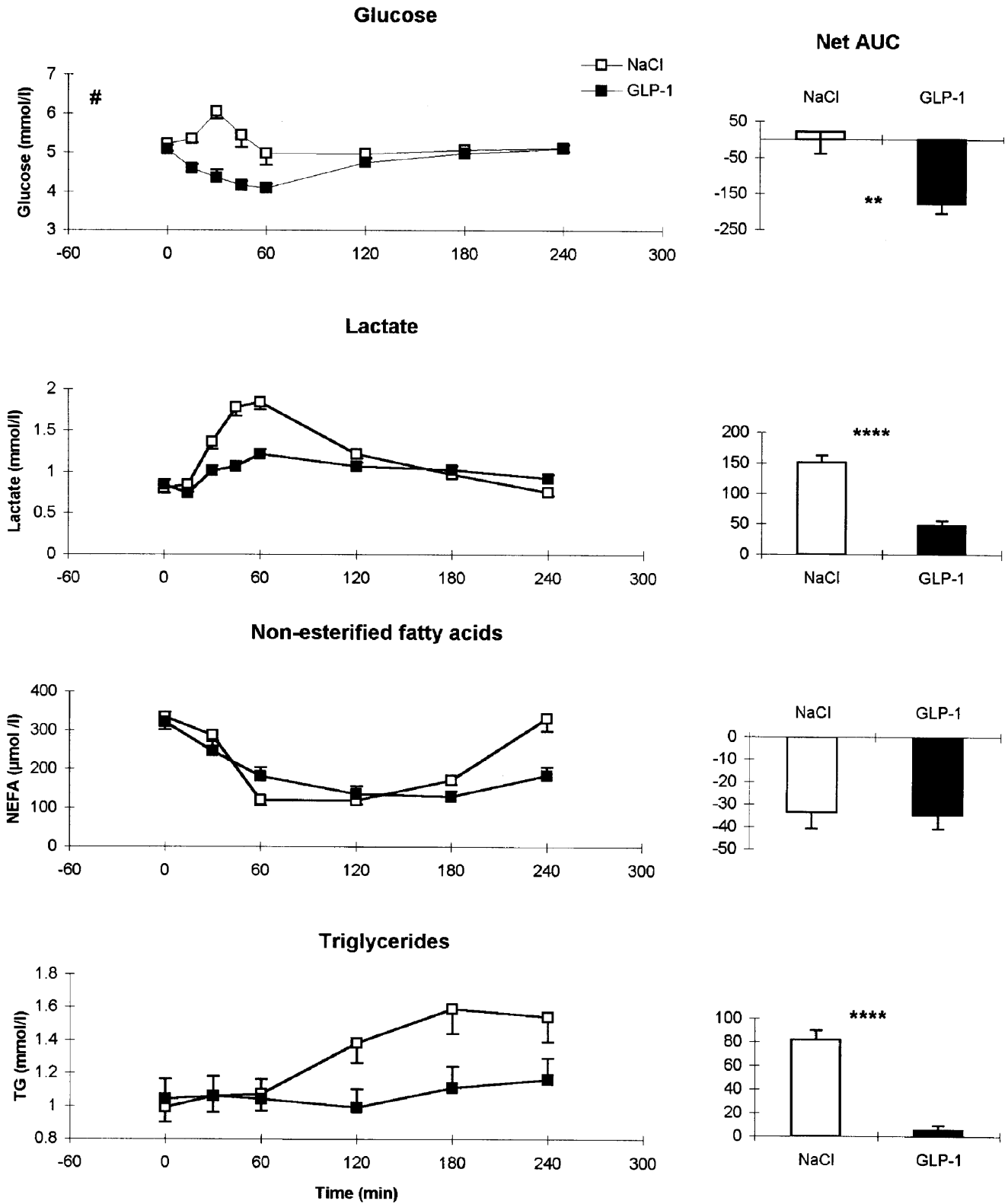


Figure 3 Plasma glucose, lactate, non-esterified fatty acid (NEFA) and triglyceride (TG) concentrations during GLP-1 (filled squares) or saline (open squares) infusions in 19 healthy, normal-weight, male subjects. Data are means (\pm s.e.m.). Left panel: ANOVA, time and interaction treatment \times time effect, $P < 0.0001$; treatment effect—glucose, $P < 0.0001$; lactate, $P < 0.001$; NEFA, $P = 0.248$; TG, $P < 0.05$. Right panel: paired t -test—glucose, $P < 0.01$; lactate and TG, $P < 0.001$; NEFA, $P = 0.903$. #Previously published.¹⁹

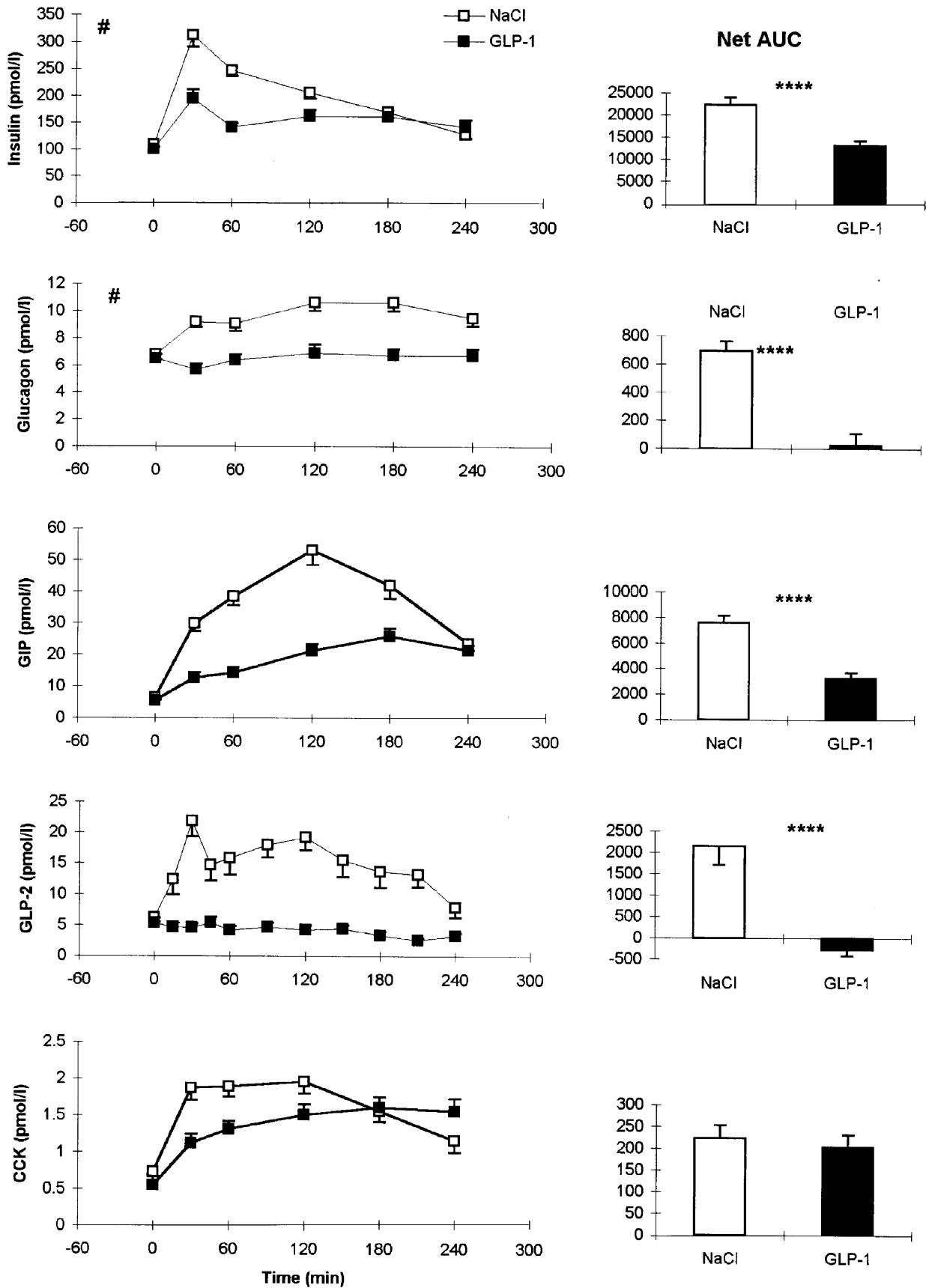


Figure 4 Plasma insulin, glucagon, gastric inhibitory peptide (GIP), glucagon-like peptide-2 (GLP-2) and cholecystokinin (CCK) concentrations during GLP-1 (filled squares) or placebo (open squares) infusions in 19 healthy, normal-weight, male subjects. Data are means (\pm s.e.m.). Left panel: ANOVA, time and interaction treatment \times effect, $P < 0.0001$; treatment effect—glucagon, GIP and GLP-2, $P < 0.0001$; insulin and CCK, $P < 0.01$. Right panel, paired t -test—insulin, glucagon, GIP and GLP-2, $P < 0.0001$; CCK, $P = 0.492$. #Previously published.¹⁹

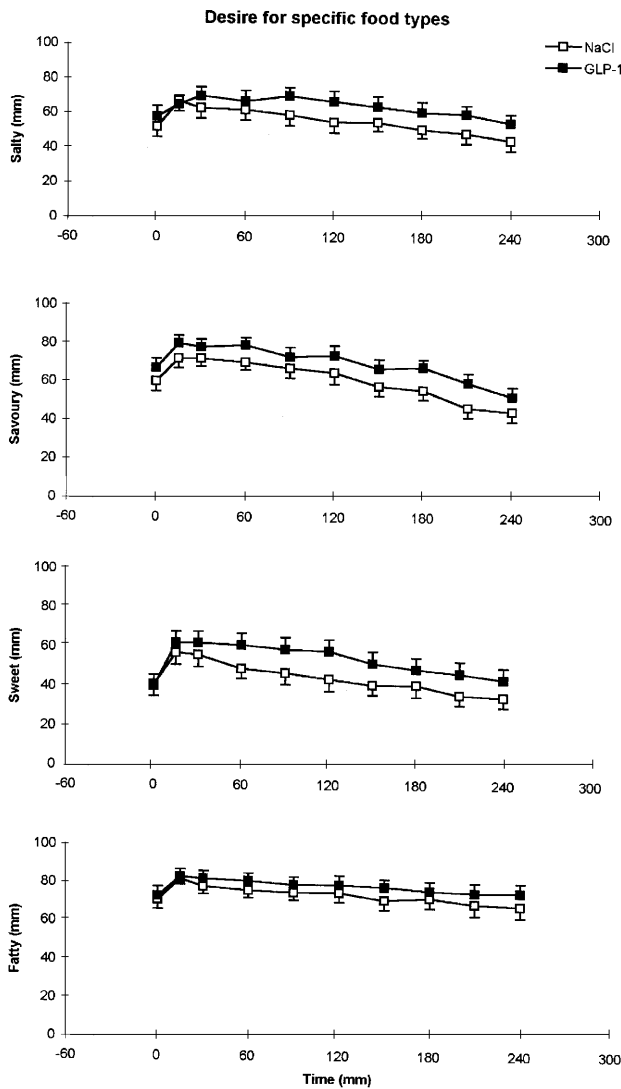


Figure 5 Mean ratings of the day for the desire of a specific type of food during GLP-1 (filled squares) and saline (open squares) infusions in 19 healthy, normal weight male subjects. VAS = 100 mm equals 'No desire at all'. Data are means (\pm s.e.m.). ANOVA: time effect, $P < 0.0001$; treatment effect—salty and sweet, $P < 0.01$; savoury, $P < 0.0001$; fatty, $P < 0.05$; treatment \times time interaction—sweet, $P < 0.01$; salty, savoury, fatty, n.s.

Discussion

In the present study GLP-1 administration to healthy humans resulted in a decreased diet-induced thermogenesis and carbohydrate oxidation during a 4 h postprandial period. Taken together with plasma profiles of substrates and hormones, these findings are easily explained by a retarded absorption of nutrients caused by GLP-1. It has been shown that GLP-1 inhibits gastric emptying rate dose-dependently in healthy subjects.^{13,15} Using a GLP-1 infusion of 50 pmol/kg \times h the rate of gastric emptying was approximately 50% of control levels.¹⁵ We did not actually measure the rate of gastric emptying, but the fact that blood glucose decreased in spite of ingestion of a test meal and that the concentration of insulin was

lower despite the insulinotropic effects of GLP-1 during the GLP-1 infusion supports the possibility of inhibition of gastric emptying. The presumed effect of GLP-1 administration on gastric emptying is also supported by the depressed profiles of lactate and TG. It seems that the effect on carbohydrate metabolism is greatest during the first hour of the infusion, while the fat metabolism is affected most during the last 2 h (120–240 min), presumably reflecting the nature and timing of lipid absorption and metabolic pathways.^{44–46}

Our finding of a decreased diet-induced thermogenesis at first sight contrasts with the results of Hwa²⁷ and Shalev.²⁸ Hwa used rats and i.c.v. injections of GLP-1. However, this discrepancy could be due to the fact that central and peripheral doses of exogenous GLP-1 probably do not involve the same functional pathways and mechanisms. On the other hand, large doses of GLP-1 administered intravenously *in vivo* in rats produced an increase in blood pressure and heart rate, and therefore presumably also in energy expenditure. This effect was not abolished by blocking the α - or β -adrenergic receptors.⁴⁷ Together with the results of Hwa *et al*,²⁷ this could be an indication of differences between species.

In humans, Shalev and coworkers also found an increase in energy expenditure induced by peripheral GLP-1 infusion in healthy young men.²⁸ They used the hyperglycaemic clamping technique, which resulted in high levels of plasma insulin. The effects on energy expenditure were abolished when the GLP-1 induced insulin secretion was blocked by somatostatin. This may explain our results, as plasma insulin concentrations in our study were higher during placebo infusion than during GLP-1 infusion. On closer inspection it seems that energy expenditure might be more dependent on plasma insulin levels than on the GLP-1 infusion itself.

Previously, GLP-1 has been found to decrease whole-body protein breakdown, possibly induced by the insulinotropic effect of GLP-1.²⁸ This was, however, not measured in relation to a meal. In the present study, no difference in protein oxidation was observed between the treatments. Due to the potent lowering effect of GLP-1 on gastric emptying, a significantly lower postprandial insulin response is seen during the GLP-1 infusion compared with saline. This probably explains the lack of effect on protein oxidation. Because of its insulinotropic and blood glucose lowering effects, GLP-1 is presently being considered as a potential therapeutic agent in the treatment of NIDDM.^{7,48,49} As many patients with NIDDM are also obese it has been an extremely interesting finding that peripherally administered GLP-1 also inhibits sensations of hunger and energy intake.^{19–22} Inhibition of gastric emptying may *per se* cause a limitation of energy intake. This may be due to either neural or endocrine signalling pathways, perhaps associated with distension of the stomach.⁵⁰ However, if these positive effects on glucose metabolism and appetite

are at the same time accompanied by a decrease in energy expenditure, the benefits for obese patients may not be as great as first thought. However, in the present study the observed decrease in diet-induced thermogenesis may only partly counteract our earlier findings of the effects of GLP-1 on appetite and energy intake.¹⁹ In the present study the decrease in energy expenditure was only 100 kJ (~7%), whereas the subsequent decrease in *ad libitum* energy intake was 500 kJ (~12%). The increase in satiety ratings was 11%. Thus, GLP-1 seems to have a stronger acute effect on energy intake than on energy expenditure and may therefore still be interesting as a means of regulating body weight. These results suggest that the effect of GLP-1 on appetite regulation is due at least in part, to a central mechanism. This is supported by the findings of Gutzwiller and co-workers.²¹ They observed a dose-dependent reduction in appetite and energy intake at lunch after GLP-1 infusion in fasted subjects. Thus, gastric emptying or activation of other gastro-intestinal receptors did not play a major role in their setup. The exact functions and regulatory mechanisms of peripheral GLP-1 in appetite regulation are therefore still open to debate.

GLP-1 and GLP-2 are co-secreted from the intestinal L-cells in equimolar amounts.⁵¹ The total depression of plasma GLP-2 during the exogenous infusion of GLP-1 in the present study indicates that the endogenous secretion of GLP-1 and GLP-2 is completely arrested. Thus, if endogenous secretion is arrested the plasma concentration of GLP-1 solely reflects what is infused. This total suppression of endogenous secretion could be due to lack of exposure of the L-cells to nutrients or to a blocking of other secretory signals (hormonal or neuronal). Presuming that the rate of gastric emptying is considerably decreased during an infusion of GLP-1,^{13,15} it seems possible that the exposure to nutrients is much lower during GLP-1 infusion than during control experimental conditions. A fast duodenal absorption of the small amounts released from the stomach would also add to the low degree of exposure to nutrients in the more distal part of the intestine. On the other hand Nauck and colleagues⁵² found that the sparse L-cells found in the proximal part of the intestine may play a larger role than first thought, perhaps due to the much larger exposure to nutrients here than more distally in the intestine.

GLP-1 administration also depressed plasma concentrations of all other hormones. This is probably again a result of a decreased rate of gastric emptying and a lower level of nutrients in the small intestine.

During the GLP-1 infusion a generally decreased desire to eat any specific type of food was observed. This is interesting because the demonstrated effects of GLP-1 infused intravenously in humans on sensations of appetite (decreased hunger and increased satiety)^{19–22} now can be ascribed to an overall effect, rather than being accounted for by one or two specific types of food. Further, these results are in line with

observations by Näslund *et al.*²⁰ Nausea has been reported with higher doses of GLP-1,^{53,54} whereas doses similar to the infusion in the present study have been without side effects.^{21,22,54} The subjects were not specifically asked about nausea, but nobody spontaneously expressed complaints or presented indications of an altered state of well-being during either infusion. Further, the palatability ratings of the meals did not show any significant differences between the two experimental protocols. This supports the conclusion that the effect of GLP-1 on appetite and the desire to eat specific types of food is a true effect rather than food aversion.

In conclusion, peripherally infused GLP-1 in healthy male subjects decreased diet-induced thermogenesis and carbohydrate oxidation, most probably secondary to a retarded absorption of nutrients. However, the effect was insufficient to fully counteract previously reported decreased *ad libitum* energy intake during the GLP-1 infusion. Endogenous secretion of GLP-2 was completely depressed, and most hormones and substrate concentrations in plasma were also suppressed during the GLP-1 infusion. Finally, the desire to eat any type of food was decreased. GLP-1 therefore seems to have an overall appetite suppressing effect.

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