Co-infusion of low-dose glucagon-like peptide-1 (GLP-1) and glucagon in man results in a reduction in food intake

Short running title:

GLP-1 and glucagon reduce food intake in man

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ABSTRACT

Obesity is a growing epidemic and current medical therapies have proven inadequate. Endogenous satiety hormones provide an attractive target for the development of drugs that aim to cause effective weight loss with minimal side-effects. Both glucagon and glucagon-like peptide 1 (GLP-1) reduce appetite and cause weight loss. Additionally, glucagon increases energy expenditure. We hypothesised that the combination of both peptides, administered at doses that are individually sub-anorectic, would reduce appetite, while GLP-1 would protect against the hyperglycaemic effect of glucagon.

In this double-blind crossover study, sub-anorectic doses of each peptide alone, both peptides in combination, or placebo, were infused into 13 human volunteers for 120 minutes. An *ad libitum* meal was provided after 90 minutes and calorie intake determined. Resting energy expenditure was measured by indirect calorimetry at baseline and during infusion.

Glucagon or GLP-1, given individually at sub-anorectic doses, did not significantly reduce food intake. Co-infusion at the same doses led to a significant reduction in food intake of 13%. Furthermore, the addition of GLP-1 protected against glucagon-induced hyperglycaemia and an increase in energy expenditure of 53 kcal/day was seen on co-infusion. These observations support the concept of GLP-1 and glucagon dual agonism as a possible treatment for obesity and diabetes.

INTRODUCTION

Glucagon is a counter-regulatory hormone, secreted at high levels during hypoglycaemia and fasting. It promotes glycogenolysis and gluconeogenesis, as well as hepatic fatty acid β-oxidation and ketogenesis(1). The glucagon receptor is expressed in a broad range of tissues, including the liver, kidney, adipose tissue, pancreas, heart, brain, gastrointestinal tract and adrenal glands(2). Its effects may therefore be more widespread than just control of glucose metabolism and it is increasingly recognised that glucagon plays a key role in general energy homeostasis. Glucagon has been shown to potently increase satiety and acutely reduce food intake in humans(3). Additionally, glucagon has the ability to significantly increase energy expenditure during infusion in man(4;5), and has been reported to promote non-shivering thermogenesis in brown adipose tissue in rodents(6). Appetite inhibition classically results in defence of body weight by a reduction of energy expenditure(7;8). The increased energy expenditure in association with anorexia induced by glucagon thus potentially enhances its usefulness as an anti-obesity therapy.

The prohormone for glucagon, proglucagon, is processed to glucagon-like peptide-1 (GLP-1) in the gut. GLP-1 is secreted post-prandially in response to direct stimulation of mucosal L-cells by nutrients within the gut lumen, and indirectly via neuronal pathways within the enteric nervous system. GLP-1 binds to the G-protein coupled GLP-1 receptor found in pancreatic islet cells as well as brain, heart and lung tissue(9) and exerts an incretin effect, stimulating glucose-dependent insulin release by β cells(10). Acute intravenous injection of GLP-1 has shown it also reduces appetite and calorie intake(11), an effect that has been observed in lean, obese and type 2 diabetic volunteers. As a result, GLP-1 is capable of achieving a modest reduction in body weight(12). GLP-1 also causes nausea and delayed gastric emptying which limits the dose that can be used clinically(13). At present, GLP-1 analogues, such as exenatide and liraglutide, are licensed for the treatment of type 2 diabetes, improving glucose control and resulting in mild weight loss(14).

Obesity is a growing global epidemic. By 2015, projections suggest that 4 billion adults will be overweight and over 700 million will be obese(15). It is therefore clear that new

strategies are urgently needed to tackle obesity. Both glucagon and GLP-1 are apparently involved in physiological regulation of appetite and are consequently attractive targets for the development of drugs for weight loss. GLP-1 analogues produce only a small weight loss in diabetic subjects or obese patients(14). Moreover, glucagon would be expected to cause hyperglycaemia, an undesired effect especially in diabetics. Dual administration of glucagon and GLP-1, or analogues thereof, could provide additional benefit over GLP-1 analogues alone. We have previously shown that the gut hormone oxyntomodulin (OXM), which is an agonist at both the glucagon and GLP-1 receptors, is able to reduce body weight and increase energy expenditure without causing hyperglycaemia in man(16). Interestingly, the anorectic effect of OXM is abolished in *Glp1r*$^{-/-}$ mice(17), suggesting that OXM exerts its effect on food intake via the GLP-1 receptor. However, it is a relatively weak agonist for the GLP-1 receptor, being less potent by two orders of magnitude compared to GLP-1(18). This calls into question the mechanism of OXM's anorectic effect, as OXM is secreted post-prandially at concentrations which are in the same order as GLP-1 itself(19). We propose that this unexpectedly strong inhibition of appetite by OXM might be due to its combined action on both the glucagon and GLP-1 receptor. Others have demonstrated that dual agonism at both the GLP-1 and glucagon receptors augments appetite reduction and weight loss in rodents, and improves glucose homeostasis in animal models of diet-induced obesity and diabetes(20; 21). This approach could combine the appetite suppressive effects of GLP-1 and glucagon with the energy expenditure increasing effects of glucagon. Recent work by our group has also demonstrated that the combination of glucagon and GLP-1 does indeed increase energy expenditure, and that the hyperglycaemic effects of glucagon are counterbalanced by the action of GLP-1 in humans(5).

We hypothesised that the combination of glucagon and GLP-1 might enhance the reduction of food intake over that observed when the respective hormones are given alone. The current study was therefore designed to demonstrate the acute effects of intravenous infusion of GLP-1 and glucagon when given at low doses, both alone and in combination, on food intake(22), glucose homeostasis and energy expenditure in humans. This approach was taken, instead of using OXM, as it gave us the flexibility to determine the subanorectic doses of GLP-1 and glucagon individually.

RESEARCH DESIGN AND METHODS

This study was reviewed and approved by the West London Research Ethics Committee (10/H0707/80) and carried out according to the principles of Good Clinical Practice and the Declaration of Helsinki.

Sixteen non-diabetic, overweight volunteers with a mean BMI of 27kg/m^2 (range 24-32.9) were recruited by advertisement. All participants underwent health screening including medical history, physical examination, biochemical and haematological testing and 12-lead electrocardiogram. Any abnormal eating behaviour was assessed using the Dutch Eating Behaviour Questionnaire(23), and the SCOFF questionnaire(24). Female volunteers were premenopausal, with regular menstrual cycles and not taking hormonal contraceptives. Smokers were excluded. Informed and written consent were obtained. Of the initial 16 recruited, 3 were excluded from final analysis, prior to unblinding, due to abnormal eating behaviour not picked up by the standard questionnaires. One participant ate less than the minimum required 300 kcal at the acclimatisation visit, one participant did not finish eating within the allotted time(20 minutes) and the third demonstrated progressive aversion to his chosen meal throughout the course of the study as demonstrated by visual analogue scores (VAS). Participants' demographics are described in Table 1.

Study Design: An initial dose finding phase was undertaken in order to establish a sub-anorectic dose of glucagon. We utilised a known sub-anorectic dose of GLP-1(11; 25). Following the dose finding phase, participants attended for 5 study visits. The first visit was an unblinded acclimatisation visit, during which participants were infused with placebo alone (Gelofusine; B. Braun, Crawley, UK) in order to become familiar with study protocol. The four subsequent studies were conducted in a double blind, four-way crossover, randomised controlled manner at least two days apart. Infusions consisted of: a) placebo (Gelofusine: B. Braun, Crawley, UK), b) GLP-$1_{7\text{-}36\ \text{amide}}$ (0.4 pmol/kg/min; Clinalfa Basic, Bachem, Switzerland), c) glucagon (2.8 pmol/kg/min); Novo Nordisk, Crawley, UK), or d) combined GLP-1 and glucagon at the above doses. Gelofusine was employed as the vehicle for hormone infusions in order to minimize adsorption of peptides to infusion lines and syringes(26).

Study Protocol: Volunteers attended the research centre at 0830h, having fasted from 2200h the night before, and refrained from alcohol and strenuous exercise for the preceding 24 hours. The study room was kept at a consistent temperature of 21°C, which was centrally controlled.

The study commenced at -60 min with the placement of two venous cannulae, one for blood sampling and one for intravenous hormone infusion. Following cannulation, volunteers were encouraged to relax, and were seated in reclining chairs. They were permitted to watch TV or listen to music. After 30 minutes (-30 min), they were placed under an indirect calorimeter canopy (Gas Exchange Monitor; GEM Nutrition; Daresbury,UK). Prior to measurement of energy expenditure, the calorimeter was calibrated with 'zero'(0.000%O_2, 0.000%CO_2) and 'span'(20%O_2, 1.125%CO_2) gases (BOC Gases, Surrey,UK). Indirect calorimetry measurement was performed as described previously(5) for 30 minutes, allowing time for initial stabilisation of readings, with the last 10 minutes of measurements used for analysis. Resting energy expenditure (REE), respiratory quotient (RQ), carbohydrate and fat oxidation rates were calculated from the VO_2 and VCO_2 measured at one minute intervals, adjusted for urinary nitrogen excretion(27; 28). After 30 minutes of calorimetry, the canopy was removed, and infusion of hormones was commenced (0 min). The infusion was initially ramped in order to rapidly achieve a steady state plasma concentration of hormone. Ramping was carried out at four times the nominal infusion rate for 5 minutes, then twice the nominal infusion rate for a further 5 minutes, and then reduced to the nominal rate for the remaining 120 minutes. At 40 min, further 30 minute measurements of REE and substrate oxidation rates were made, still in the fasting phase. At 90 min an *ad libitum* meal of known specific calorific value was served (spaghetti bolognese 188 kcal/100 g, chicken tikka masala 178 kcal/100 g, or macaroni cheese 194 kcal/100 g; Sainsbury's Supermarkets Ltd,UK). Participants had tasted their chosen meal during the acclimatisation visit and all deemed it to be palatable. The same meal was served at all five visits. Participants were allowed 20 minutes to eat, instructed to eat until comfortably full, and then stop. The hormone infusion continued for 120 minutes in total, and was terminated 10 minutes after the end of the meal. Participants remained in the study room for 60 minutes following termination of the infusion. At 180 min the cannulae were removed, and the participant emptied their

bladder. Urinary volume was measured in order to calculate urinary nitrogen excretion for estimation of protein oxidation. The participant was then discharged home.

During the study, pulse and blood pressure were measured at -60, -30, 0, 40, 70, 90, 120, 150 and 180 min. At these times, blood samples were also taken for measurement of glucose, insulin, glucagon and active GLP-1. Glucose and insulin levels were measured by the Department of Chemical Pathology, Imperial College Healthcare NHS Trust (CV <5% and <10% respectively across the working range). Samples for active GLP-1 and glucagon were collected in lithium heparin tubes containing 1000 kallikrein inhibitor units. Active GLP-1 and total ghrelin were measured using commercially available ELISA kits (Millipore, Livingston, UK) according to the manufacturer's instructions (CV <7% and 8% respectively), as was acylated ghrelin (Biovendor, Brno, Czech Republic) (CV <7%). Glucagon and total PYY were assayed according to established immunoassay protocols by 'in-house' radioimmunoassay (10; 29) (CV <10% and <15% respectively). At each of the above time points, a VAS was completed by the participant to assess nausea and satiety.

Statistical Analysis. Statistical analysis was carried out using GraphPad Prism 5.0d (GraphPad Software, San Diego, CA). Two-way repeated measures ANOVA with Bonferroni post hoc test was used to compare differences in glucose, insulin, PYY levels, blood pressure, pulse and VAS. One-way repeated measures ANOVA with Newman-Keuls and Bonferroni post hoc tests was used to compare food intake, change in REE and substrate oxidation rates between groups. Area under the curve was calculated using the trapezoidal rule and differences between treatment groups were compared using one-way repeated measures ANOVA with Tukey's post hoc test. Paired Student's t-test was used to compare ghrelin levels at baseline and during infusion. Data are reported ± SEM unless otherwise stated.

RESULTS

Baseline plasma active GLP-1 levels at -30 min were 4-5pmol/l. In the experimental groups receiving GLP-1 alone or GLP-1 with glucagon, levels rose to 11-16pmol/l at 70 min post-infusion (Figure 1A). Although active GLP-1 plasma levels appeared to be lower during the combination infusion compared to the GLP-1 only infusion, $AUC_{GLP\text{-}1}$ was not significantly different during the infusion period (0-120 min Supplementary Figure 1A). Mean plasma glucagon levels at -30 min were 14-19pmol/l, rising to 147-73pmol/l at 70 min in those groups receiving glucagon infusion (Figure 1B).

Plasma glucose and serum insulin responses to placebo, glucagon, GLP-1 or combination infusions are shown in Figure 2. In the placebo group, glucose and insulin remained constant during infusion and as expected, rose in response to the meal served at 90 min. Glucagon infusion caused a rise in glucose from 4.8 ±0.08mmol/l to a peak of 6.5 ±0.3mmol/l at 40 min with a corresponding rise in insulin to 31.2 ±3.8mU/l. GLP-1 infusion reduced plasma glucose during infusion from 4.9 ±0.1mmol/l to 4.1 ±0.3mmol/l at 40 min, with serum insulin levels similar to that observed in the placebo arm. Following glucagon and GLP-1 co-administration, $AUC_{glucose}$ was similar to that seen with placebo and significantly lower than with glucagon alone (Fig 2C). There was a significant increase in insulin during the glucagon/GLP-1 co-infusion of greater magnitude than seen with GLP-1 or glucagon alone (Fig 2D). Post-meal, where calorie intake differed between treatment groups, glucose and insulin levels were not significantly different between groups.

As expected, glucagon alone and GLP-1 alone, at the doses given, did not significantly reduce food intake. However, glucagon and GLP-1 co-infusion significantly reduced food intake by 13% at the study meal compared to placebo ($p<0.05$) which was also a significantly greater reduction than seen during infusion of glucagon alone ($p<0.05$) or GLP-1 alone ($p<0.05$) (mean energy intake at study meal: 1086 ±110.1 kcal [placebo], 1086 ±96.9 kcal [glucagon], 1052 ±81.3 kcal [GLP-1] and 879 ±94.2 kcal [combined glucagon+GLP-1] – Fig 3).

Neither the palatability of the buffet meal nor other satiety-related VAS responses were altered significantly by any of the infusions (Fig 4A,4C,4D). The nausea score significantly increased post-meal (120 min) during the combined infusion of glucagon and GLP-1 (Fig 4B). Three participants reported mild nausea after the combined infusion and two participants vomited following glucagon infusion. In all cases, this occurred post-meal between 120 and 160 min.

There were no significant differences in baseline REE between groups: 1336 ±65.8 kcal/day[placebo], 1314 ±53.0 kcal/day[glucagon], 1330 ±71.9 kcal/day[GLP-1] and 1341 ±56.6 kcal/day[combined glucagon+GLP-1] p=0.7275. The mean within-subject coefficient of variation was 4.1 ±1.3%. Following infusion, there was a trend towards higher REE in response to glucagon alone and glucagon/GLP-1 co-administration by a mean of 66.8 and 52.5 kcal/day respectively (Fig 5A).

RQ values and carbohydrate oxidation rates at baseline were similar in all treatment groups and RQ did not change following GLP-1 infusion. A significant increase in RQ and carbohydrate oxidation was observed with both the glucagon and combination infusions (Fig 5B,5C). Glucagon alone and in combination with GLP-1 significantly reduced fat oxidation rates (Fig 5D). Protein oxidation rate was calculated over the entire study period for each infusion and none of the treatment arms were significantly different from placebo (data not shown). There were no significant changes in pulse, systolic or diastolic blood pressure (Supplementary Figure 2) with any of the treatment groups.

Infusion of GLP-1 or glucagon alone did not affect total or acylated ghrelin levels. However, co-infusion led to a significant fall in both total (p<0.05) and acylated ghrelin (p<0.05) (Fig 6A,6B). Plasma PYY levels were unaffected by GLP-1 and glucagon, individually or in combination (Fig 6C).

DISCUSSION

This study shows that dual infusion of GLP-1 and glucagon reduces food intake significantly whereas the same low doses of glucagon and GLP-1, when administered separately, do not exert a similar anorectic effect.

The dose of glucagon used in this study (2.8 pmol/kg/min) was established in a dose-finding study to be subanorectic. It is higher than the dose used previously by Geary et al. (0.84 pmol/kg/min)(3) which was demonstrated to reduce food intake. Our intention was to examine the effect of raised pre-prandial levels of glucagon and GLP-1 on food intake in a fasting state. In contrast, Geary et al's study studied the effect of elevated post-prandial levels of glucagon after consumption of 500 g of tomato soup. The soup would be expected to stimulate anorectic gut hormone secretion (e.g. PYY, GLP-1) and suppress ghrelin secretion, explaining the differences in glucagon doses.

Three subjects receiving the combination infusion experienced nausea. This nausea only became apparent post-prandially, with no significant nausea occurring during the 90 minutes of infusion before the meal was served. The nausea is therefore unlikely to explain the reduction in food intake that was noted. GLP-1 based therapies for diabetes can cause nausea(14), as can glucagon(30). However, the doses used in this study were far smaller than those administered in these clinical situations. The post-prandial nausea seen in this study may instead be accounted for by a delay in gastric emptying triggered by glucagon and GLP-1(31; 32).

Despite the significant reduction in food intake with co-infusion, no differences were observed in perceived hunger and satiety scores, Palatability of the meal was reduced with co-infusion, although this difference did not reach statistical significance. This discrepancy between perceived appetite and energy intake highlights the importance of measuring energy intake in an *ad libitum* meal, a more robust endpoint than visual analogue scores, which can be affected by other factors such as age, gender and physical activity(33).

Multiple pathways are responsible for the food intake reduction observed with both hormones. The hypothalamus expresses both glucagon and GLP-1 receptors(34; 35) and intracerebroventricular glucagon and GLP-1 are both capable of reducing food intake(36; 37), suggesting that peripheral glucagon and GLP-1 could exert a direct effect on the hypothalamus after crossing the incomplete blood-brain barrier at the median eminence. A second mechanism might be via activation of vagal afferents to the brainstem, as vagotomy attenuates the anorectic effect of glucagon and GLP-1 following peripheral administration(38; 39). A third mechanism for food intake reduction might be via indirect effects on other gut hormones. Co-administration of GLP-1 (0.8 pmol/kg/min) and glucagon (14 pmol/kg/min) causes a significant reduction in circulating levels of the orexigenic hormone ghrelin(5). The current study corroborates these findings at a lower dose of GLP-1 (0.4 pmol/kg/min) and a far lower dose of glucagon (2.8 pmol/kg/min). In our study, neither GLP-1 nor glucagon, alone or in combination, affected plasma PYY levels. Naslund et al.(40) demonstrated a small inhibitory effect of GLP-1 on PYY secretion, where an infusion of 0.75 pmol/kg/min reduced post-prandial PYY levels by 4-5 pmol/L. In contrast, our study examined fasting PYY levels and utilized a smaller dose of GLP-1. Thus it appears that GLP-1 and glucagon, at the doses used here, can modulate ghrelin secretion but not PYY.

The hyperglycaemic effect of glucagon is undesirable in patients with diabetes or impaired glucose tolerance, albeit the greatest element results from a one-off stimulation of glycogenolysis which would be expected to decline with time. Co-administration with GLP-1 attenuates this hyperglycaemia, consequent on enhanced insulin release and glucose disposal(5). Both GLP-1 and glucagon act directly on the β cell to release insulin and in addition, the hyperglycaemia itself is a stimulus for insulin release(41). GLP-1's insulinotropic effects are dependent on the prevailing glucose level(10) which accounts for the relatively small insulinotropic response observed during GLP-1 infusion alone, as glucose levels tend to decline after the start of the infusion (Figure 2A). The insulinotropic response with the co-infusion is much larger in amplitude due to the triple effect of GLP-1, glucagon and hyperglycaemia. The insulin level during the co-infusion is sustained even when glucose returns to ≤5 mmol/L at 70 minutes (Figure 2A and B) because glucagon continues to exert an insulinotropic effect independent of the prevailing

glucose level(42). Therefore, the addition of GLP-1 to glucagon in the doses used for our co-infusion is able to neutralize the undesirable hyperglycaemic effect of glucagon alone.

Moreover, the reduction in food intake seen with the combination infusion is likely to contribute to the attenuated postprandial glycaemic response. The post-meal glucose response to GLP-1 alone is attenuated compared to placebo. However, the rise in insulin is delayed with infusion of GLP-1, suggesting that this is not an incretin effect. This phenomenon may be related to delayed gastric emptying with GLP-1. Analysis of the glucose and insulin response to the meal is complex as the subjects ate different amounts. Further studies examining the effect of glucagon and GLP-1 combination on the glucose and insulin response to a standardized calorie load are warranted in order to formally assess the effects on carbohydrate tolerance, particularly in diabetic patients who may have compromised β cell reserve.

Consistent with our previous study, we demonstrated an increase in REE of ~50 kcal/day in both the glucagon alone and combination infusion groups, although this did not reach statistical significance. We also found a rise in RQ, rise in carbohydrate oxidation rate, and fall in fat oxidation rate with glucagon alone and combination infusion, likely to be related to the relative substrate availabilities of glucose versus free fatty acid(5). The fact the rise in REE did not reach statistical significance was not unexpected in this current study since the dose of glucagon used was a fifth that of our previous study(5). We speculate that the chronic sustained effects of this small increase in REE would have an important impact on body weight in the long term when combined with the food intake reduction. The mechanism behind the increase in REE mediated by glucagon remains unclear. This phenomenon could be mediated by increased thermogenesis in brown adipose tissue(6) and/or by futile substrate cycling(43). These effects may be direct, via tissue glucagon receptor (e.g. in brown adipose tissue) or indirect, via an increase in catecholamines(44).

We also found a rise in RQ, a rise in carbohydrate oxidation rate, and a fall in fat oxidation rate with glucagon alone consistent with our previous study, and likely to be related to the relative substrate availabilities of glucose versus free fatty acids(5). In contrast, GLP-1 alone caused a small reduction in carbohydrate oxidation and a small

increase in fat oxidation consistent with previous studies(45). Interestingly, co-infusion caused an increase in RQ, increase in carbohydrate oxidation and decrease in fat oxidation with magnitudes approximately double seen with glucagon alone. This phenomenon is consistent with our observation of a similar increase in RQ, increase in carbohydrate oxidation and decrease in fat oxidation with the combination compared to glucagon alone, although there was no significant statistical difference at the higher doses used in our previous study(5). It is possible that the combination of GLP-1 with glucagon may increase carbohydrate oxidation through a combined stimulation of glycolysis and glycogenolysis and this requires further study.

In conclusion, this study reports that co-administration of glucagon and GLP-1, at doses which are individually sub-anorectic, significantly reduces food intake in humans. Furthermore, the co-administration of GLP-1 ameliorates the hyperglycaemia of glucagon. These data are consistent with findings seen with acute infusion of OXM (16), suggesting that the anorectic and energy expenditure effects of oxyntomodulin can be explained by co-stimulation of both the GLP-1 and glucagon receptors. These observations provide support for the further development of GLP1/glucagon receptor co-agonists as a therapeutic approach for obesity. This study has only examined the acute effects of GLP-1/glucagon co-agonism and further chronic studies need to be performed in humans to establish a therapeutically useful anorectic effect without exerting nausea as well as maintaining euglycaemia. Establishing these effects will be the key to therapeutic exploitation.

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The authors declare that there is no duality of interest associated with this manuscript.

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TABLE 1 Demographics of study participants. M, Male; F, Female.

Volunteer	BMI (kg/m^2)	Age	Sex
F1	24.0	25	M
F2	24.2	41	M
F3	24.8	32	M
F4	26.3	40	F
F5	26.6	33	M
F6	26.8	21	F
F7	26.8	40	F
F8	26.3	35	M
F9	32.9	28	F
F10	29.4	23	M
F11	27.6	39	M
F12	28.5	32	M
F13	27.4	22	M
Mean	27.0	31.6	9M, 4F

FIGURE LEGENDS

Figure 1: Plasma active GLP-1 (A) and glucagon (B) levels after hormone infusion. Mean ± SEM plasma levels plotted. Infusion denoted by grey bar and meal served at the arrow.

Figure 2: The effects of glucagon and GLP-1, alone and in combination, on plasma glucose (A and C) and serum insulin (B and D). A and B: Mean ± SEM plasma glucose and serum insulin levels. Infusion denoted by grey bar and meal served at the arrow. *p<0.05, **p<0.01, ****p<0.0001 compared with placebo. C: Area under the curve for glucose levels from 0 to 90 min (from the start of the infusion until just before the meal is served). ####p<0.0001 compared with placebo; ††p<0.01 compared with glucagon. D: Area under the curve for insulin levels from 0 to 90 min. $$$$$p$<0.0001 compared with placebo; %p<0.01 compared with glucagon; &p<0.0001 compared with GLP-1.

Figure 3: **Energy intake at the study meal at 90 min.** A: Mean ± SEM absolute energy intake; *p<0.05 compared with placebo, #p<0.05 compared with glucagon, †p<0.05 compared with GLP-1. B: Energy intake as a percentage change of the placebo visit for individual volunteers.

Figure 4: Subjective rating of satiety (A), nausea (B), hunger (C) and palatability (D) as measured by Visual Analogue Score (VAS) response. For A-C, scores are depicted as change from baseline value (millimetres). **p<0.01 compared with placebo. For D, an absolute value (millimetres) is given.

Figure 5: The effects of glucagon and GLP-1, alone and in combination, on resting energy expenditure (A), Respiratory Quotient (B), Carbohydrate oxidation (C), and Fat oxidation (D). Mean absolute change for each parameter (± SEM) between baseline and infusion phases plotted. *p< 0.05, **p< 0.01, ***p< 0.001 compared with placebo; #p< 0.05, ##p< 0.01, ###p< 0.001 compared with glucagon; †††p<0.001 compared with GLP-1.

Figure 6: The effects of glucagon and GLP-1, alone and in combination, on Total ghrelin (A), acylated ghrelin (B) and PYY levels (C) A and B: Mean ± SEM plasma total and acylated ghrelin levels at baseline (0 min) and during infusion (90 min)*p<0.05. C: Mean ± SEM plasma PYY levels at 0, 40, 70 and 90 min.

Supplementary Figure 1; Plasma active GLP-1 (A) and glucagon (B) levels after hormone infusion. Area under the curve from 0 to 120 min for (A) active GLP-1 levels. \$$p$<0.05, \$\$$p$<0.01 compared with placebo; &p<0.05, &&p<0.01 compared with glucagon; and (B) glucagon levels. ###p<0.001 compared with placebo; †††p<0.001 compared with glucagon; †††p<0.001 compared with GLP-1.

Supplementary Figure 2: Heart rate (A), Systolic (B) and Diastolic (C) blood pressure. Two way repeated measures ANOVA showed no significant differences between treatment groups.

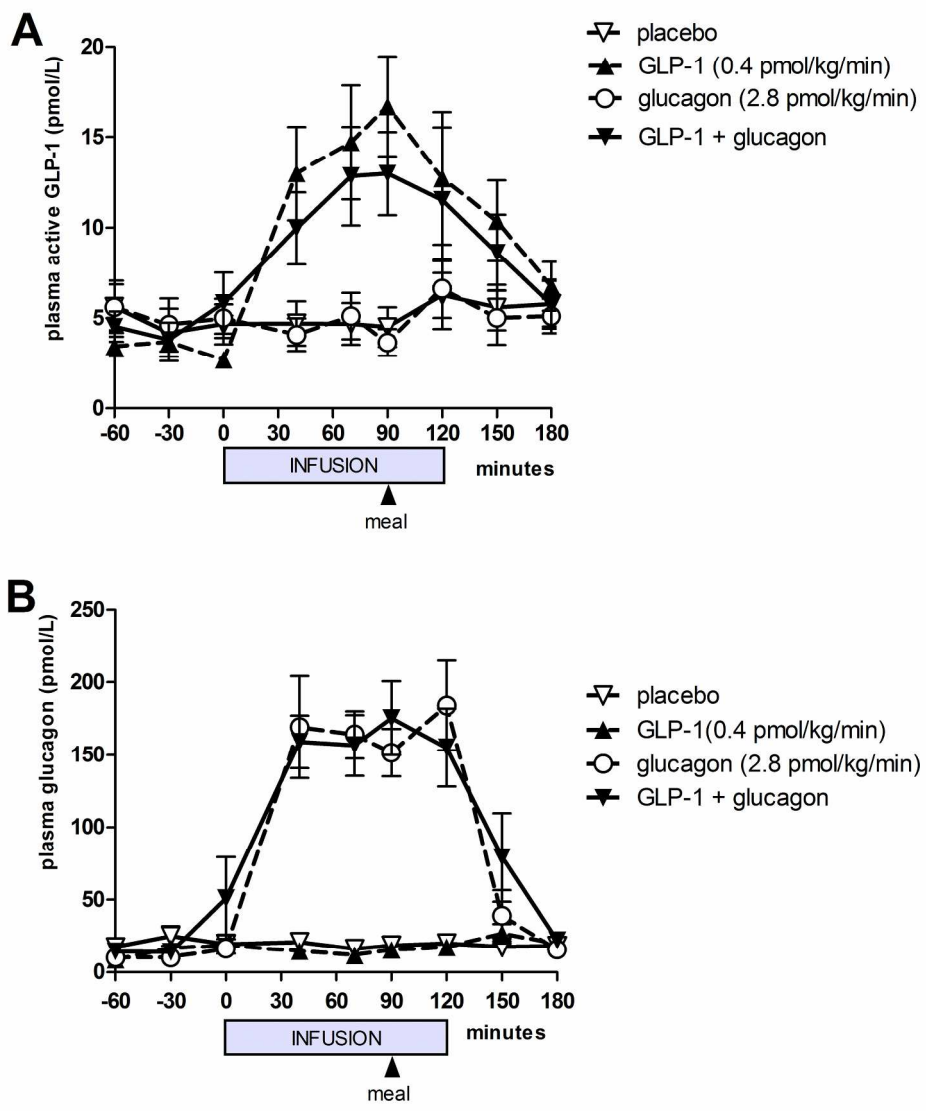

Figure 1
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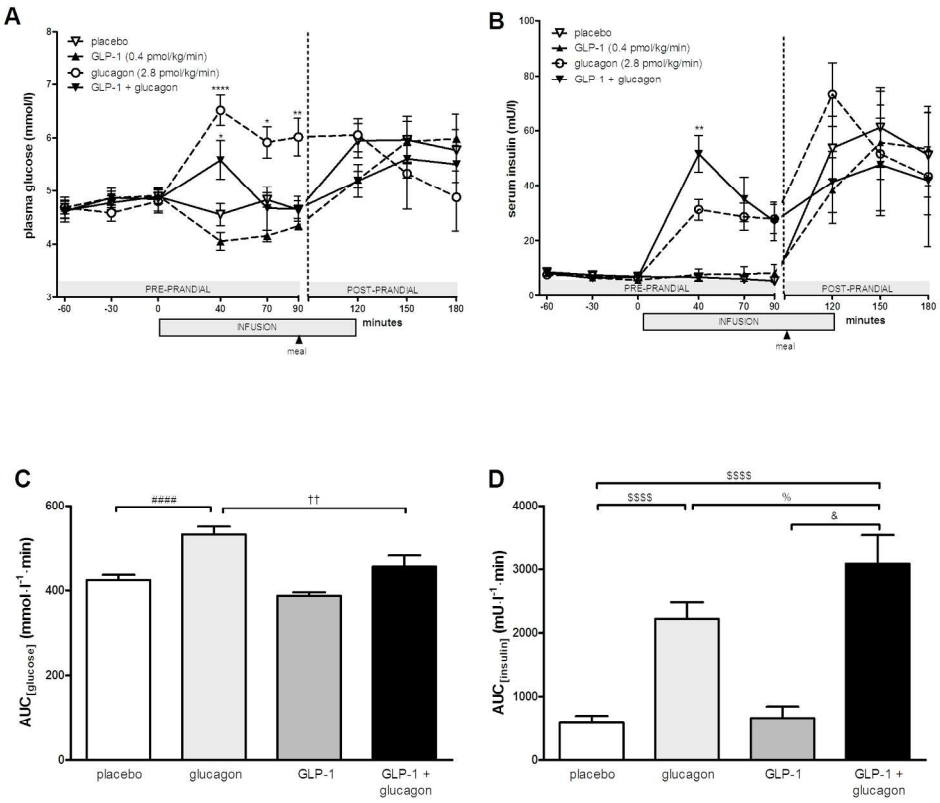

Figure 2
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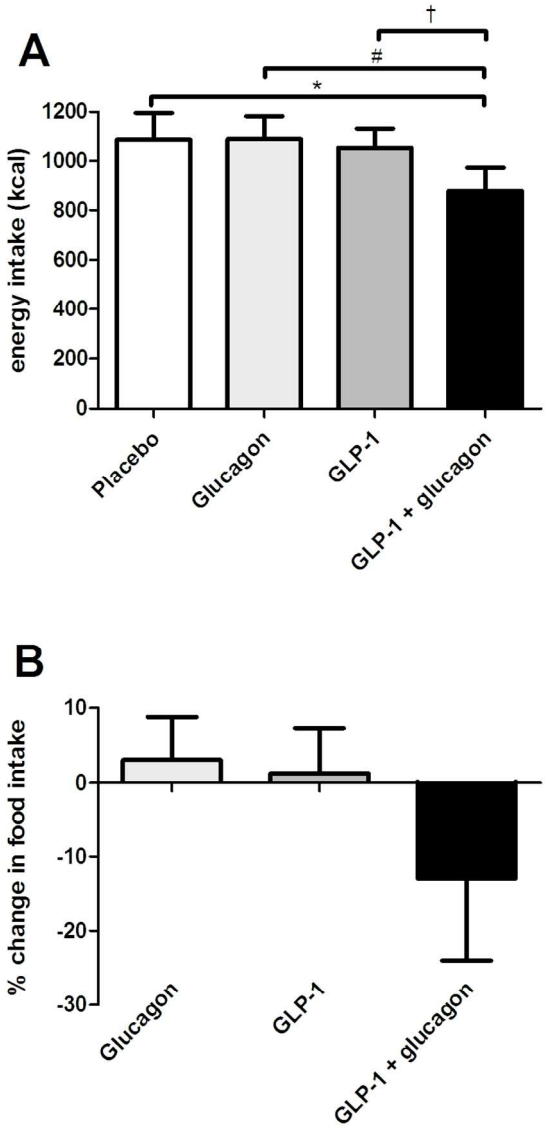

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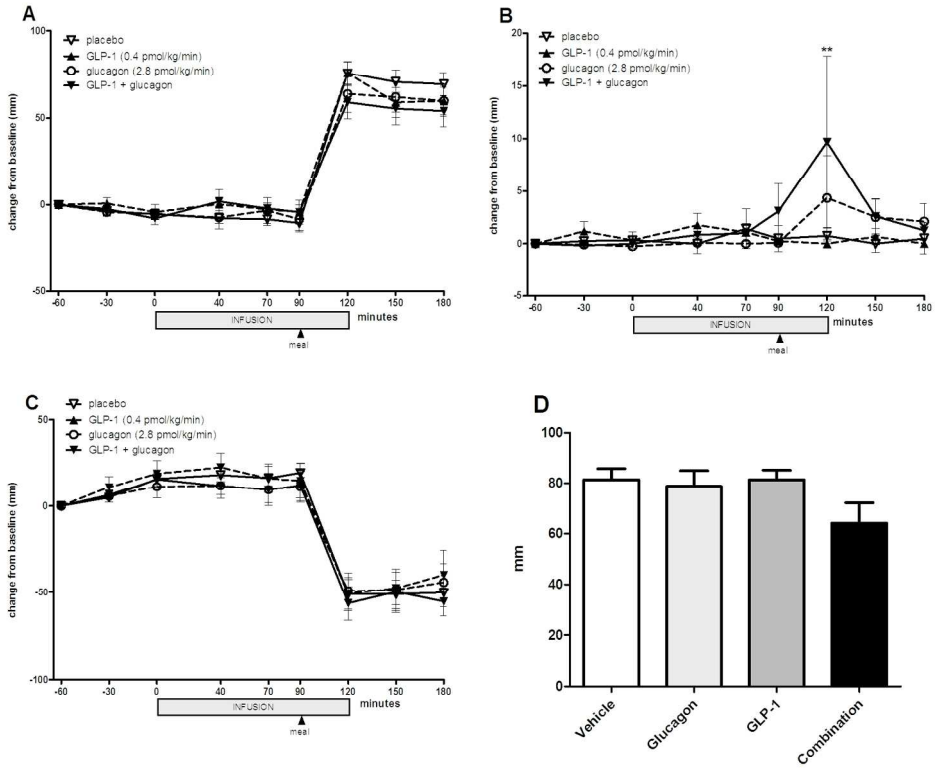

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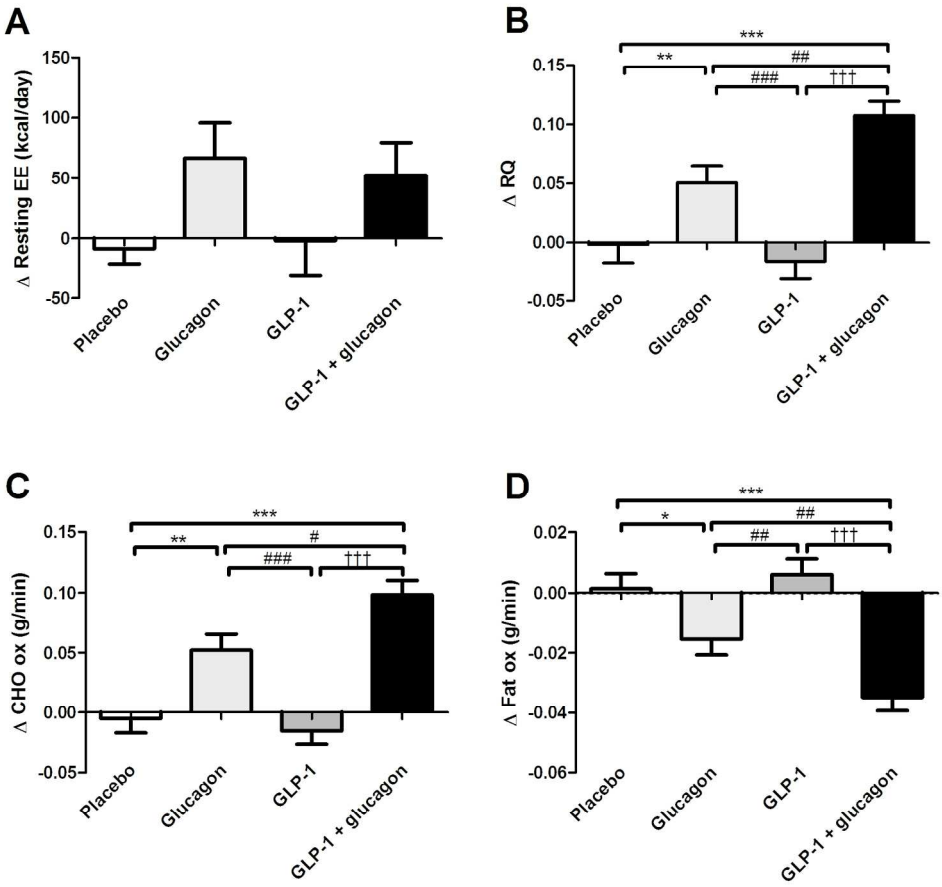

Figure 5
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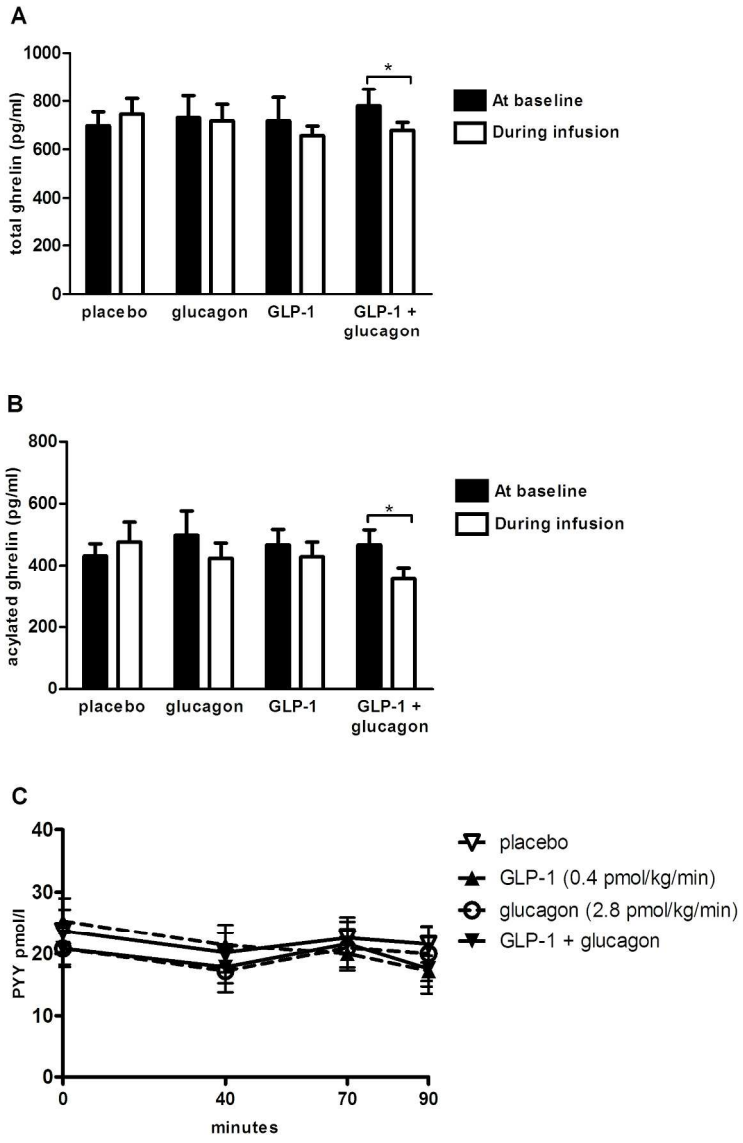

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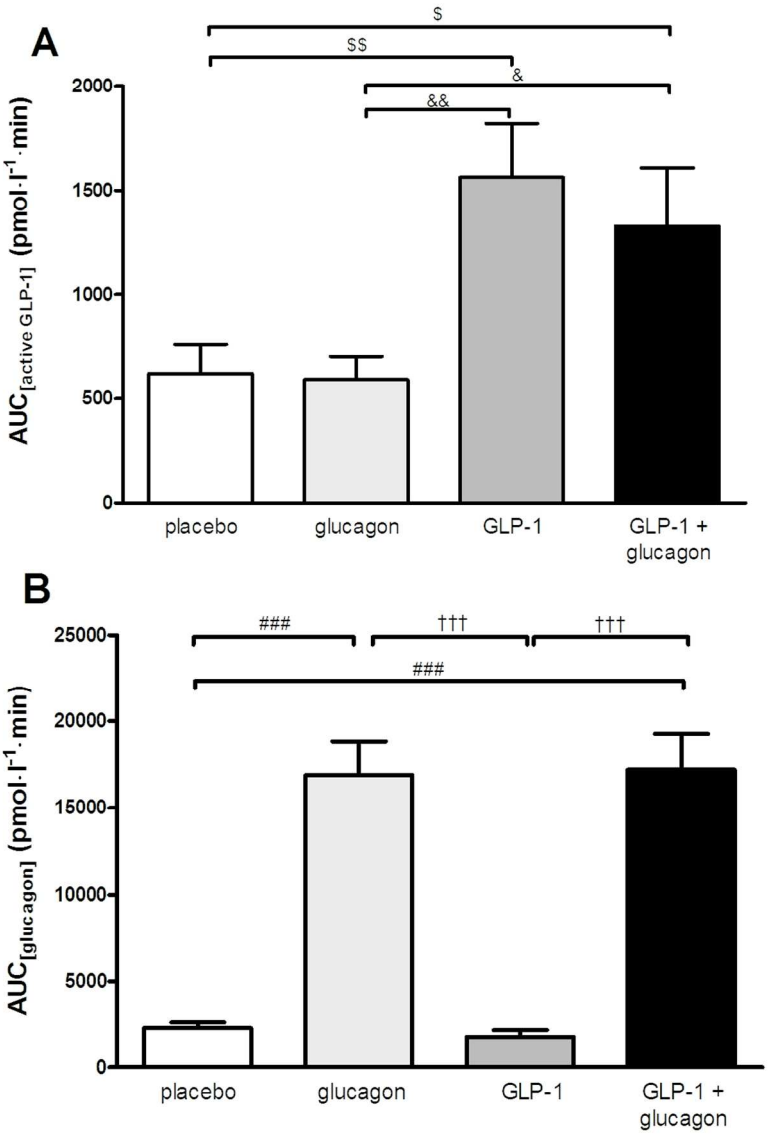

Supplemental Figure 1
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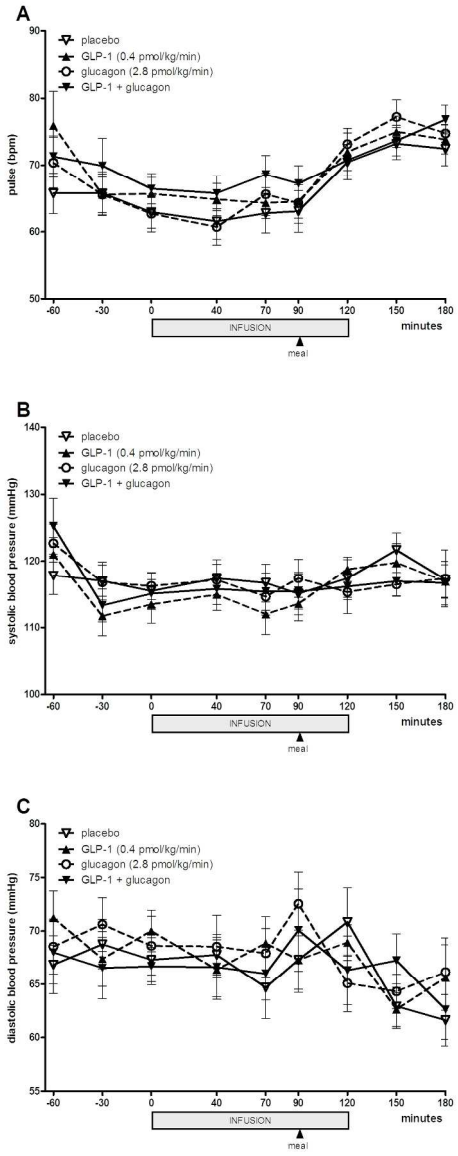

Supplemental Figure 2
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