

Original Article

Full title: The effect of encapsulated glutamine on gut peptide secretion in human volunteers

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Structured Abstract:

Context: Weight loss and improved blood glucose control after bariatric surgery have been attributed in part to increased ileal nutrient delivery with enhanced release of glucagon-like peptide 1 (GLP-1). Non-surgical strategies to manage obesity are required. The aim of the current study was to assess whether encapsulated glutamine, targeted to the ileum, could increase GLP-1 secretion, improve glucose tolerance or reduce meal size.

Methods: A single-centre, randomised, double blind, placebo-controlled, cross-over study was performed in 24 healthy volunteers and 8 patients with type 2 diabetes. Fasting participants received a single dose of encapsulated ileal-release glutamine (3.6 or 6.0g) or placebo per visit with blood sampling at baseline and for 4 hours thereafter. Glucose tolerance and meal size were studied using a 75g oral glucose tolerance test and ad libitum meal respectively.

Results: In healthy volunteers, ingestion of 6.0 g glutamine was associated with increased GLP-1 concentrations after 90 min compared with placebo (mean 10.6 pg/ml vs 6.9 pg/ml, $p=0.004$), increased insulin concentrations after 90 min (mean 70.9 vs 48.5, $p=0.048$), and increased meal size at 120 min (mean 542g eaten vs 481g, $p=0.008$). Ingestion of 6.0g glutamine was not associated with significant differences in GLP-1, glucose or insulin concentrations after a glucose tolerance test in healthy or type 2 diabetic participants.

Conclusions: Single oral dosing of encapsulated glutamine did not provoke consistent increases in GLP-1 and insulin secretion and was not associated with beneficial metabolic effects in healthy volunteers or patients with type 2 diabetes.

Abbreviations

AUC	Area under the curve
BMI	Body Mass Index
CaSR	Calcium sensing receptor
CV	Coefficient of variation
DPP-IV	Dipeptidyl peptidase-IV
GLP-1	Glucagon-like peptide-1
GPCR	G-protein coupled receptor
HV	Healthy volunteer
OGTT	Oral glucose tolerance test
T2DM	Type 2 diabetes mellitus
WHO	World Health Organisation

Introduction

Over several decades, population levels of obesity, defined as individuals having a body mass index of 30 kg/m^2 or more, have increased in many countries worldwide. The World Health Organisation now considers obesity to be one of the greatest public health challenges to be faced in the 21st century(1). Obesity and related conditions, such as type 2 diabetes mellitus (T2DM), are responsible for rising healthcare costs and a great burden of morbidity and mortality. Both obesity and T2DM can be successfully treated with bariatric surgery, although the precise mechanisms responsible for these beneficial effects remain poorly understood. One possibility is that bariatric surgery improves diabetes and aids weight loss by increasing nutrient delivery to the distal gut, which stimulates release of the satiety-promoting incretin hormone glucagon-like peptide 1 (GLP-1). Although GLP-1 mimetics have been given pharmacologically in the treatment of T2DM and obesity(2), an alternative would be to use agents which enhance endogenous GLP-1 production to harness similar benefits with potentially fewer adverse effects.

GLP-1 is produced in enteroendocrine L-cells in the mucosa of the ileum and colon in response to nutrient exposure. Many different proteins or amino acids have been found to stimulate GLP-1 release by interacting with the calcium-sensing receptor (CaSR)(3, 4), GPRC6A(5) or PEPT1(4). The amino acid glutamine is abundant in the human diet and a particularly effective stimulant of GLP-1 release in vitro because it not only generates an electrical signal in L-cells but also elevates intracellular cAMP levels(6). Oral ingestion of glutamine in human volunteers was associated with enhanced GLP-1 secretion and improved glucose tolerance, but a dose of at least 15g was required(7, 8), possibly due to the low stability of glutamine in gastric acid(9) and its absorption in the upper GI tract resulting in relatively low levels reaching the ileal L-cells. Encapsulation could circumvent this problem by facilitating targeted release of nutrients into parts of the intestine where the L-cells are most abundant.

The aim of the current study was to assess the effect of encapsulated glutamine on GLP-1 concentrations, glucose tolerance and meal size in healthy human volunteers and in individuals with T2DM.

Methods

Participant Recruitment

Healthy male and female volunteers (18-65 years old; body mass index (BMI) $18-35 \text{ kg/m}^2$) were recruited using advertisements in Addenbrooke's Hospital and the University of Cambridge. Patients with T2DM (18-65 years old; BMI of $18-40 \text{ kg/m}^2$) were identified through primary care, outpatient clinics and using advertisements. Participants with anaemia or other significant active diseases and those who were pregnant or breastfeeding were excluded. Patients with T2DM who had taken insulin or injectable GLP-1 agonists were excluded from the study. Patients who were taking metformin, sulphonylureas or dipeptidyl peptidase-IV (DPP-IV) inhibitors were asked to withhold these for 12, 24 and 72 hours prior to the test respectively.

Participants were provided with written information about the study and gave written informed consent prior to participation. The study was given ethical approval by the Norfolk & Norwich research ethics committee (Reference 12/EE/0389, 25/09/2012; ISRCTN 10757078).

Participants attended the clinical research facility in the morning following an overnight fast. The evening before each visit, participants had a standardised pasta meal (15% protein, 30% fat, 55% carbohydrate) designed to provide 33% of their daily calorie requirement based upon an estimation of their metabolic rate and activity levels(10).

Capsule Production

Capsule development and GMP manufacture was performed by Encap Drug Delivery Ltd (Livingston, UK). Each capsule contained 600mg of glutamine or 300mg microcrystalline cellulose (placebo). The capsules were manufactured with an enteric coating designed to promote capsule release 20 minutes after exposure to an alkaline environment.

Study Design

The capsules were tested in a series of blinded, controlled studies in human volunteers. The primary endpoint was the effect of the capsules upon circulating concentrations of total GLP-1 in venous blood. The secondary endpoints were safety, the effect of the capsules upon glucose tolerance, meal size and subjective feelings of hunger and fullness.

Fasting study: Initially, a single-blinded, non-randomised, placebo-controlled dose-ranging study in four healthy volunteers was performed to ascertain the optimal dose for further evaluation and provide preliminary safety data. The doses studied included placebo (10 capsules) and 0.6g, 1.8g, 3.6g and 6.0g glutamine supplemented where necessary with placebo capsules to preserve participant blinding. The remainder of the study used the two most promising doses, 3.6 and 6.0g Glutamine in comparison to placebo in a double-blind, controlled randomised design.

On each visit, venous blood was taken at baseline and at intervals following capsule ingestion for evaluation of GLP-1 concentrations. Participants recorded adverse events in a symptom diary and completed questionnaires evaluating hunger, fullness and nausea. On one visit, participants had a DXA scan. Ten healthy volunteers were recruited for evaluation of the primary endpoint in the fasting comparison between placebo, 3.6g Glutamine and 6.0g Glutamine.

Glucose tolerance and meal size in healthy volunteers and patients with T2DM: For evaluation of the effect on the capsules on glucose tolerance, nine healthy volunteers and eight patients with T2DM were recruited. The study visits were similar to the fasting study except that each participant had a 75g oral glucose tolerance test (OGTT) at 90 minutes after capsule ingestion.

Meal size in healthy volunteers: For evaluation of the effect of the capsules on meal size, ten healthy volunteers were recruited to attend the Clinical Research Facility on three occasions for an ad libitum breakfast given 120

minutes after capsule ingestion. The ad libitum meal consisted of muesli with chopped fruit, nuts and milk in a homogenous mixture designed to provide 15% protein, 30% fat and 55% carbohydrate. A portion of 1.5 kg (2400 kcals) was provided on each occasion and participants were advised to eat until they felt full. The total amount of food eaten was measured using a universal eating monitor.

Questionnaire Design

Hunger, fullness and nausea were assessed using a visual analogue scale which has been widely used in the medical literature(11).

Analytical Methodology

For analysis of total GLP-1, samples were taken into EDTA plasma tubes, placed on ice immediately after venesection and centrifuged (3500g at 4°C 10 minutes), aliquotted and frozen using dry ice within 15 minutes of venesection. Samples were stored at -80°C prior to batch analysis in duplicate of GLP-1 using the Mesoscale Discovery Total GLP-1 kit, a sandwich immunoassay with electrochemiluminescence detection which measures all endogenous forms of GLP-1. This method has a range of 1.4-1000 pg/ml and coefficients of variation (CVs) of 5-7%.

For biochemistry testing, serum samples were allowed to clot for 10 minutes then centrifuged, separated and frozen within 30 minutes of venesection and stored at -80°C prior to analysis. Glucose, alanine aminotransferase (ALT) and creatinine were measured in a clinically-accredited laboratory at Addenbrooke's hospital using a Siemens' Dimension analyser with CVs of <2% within the reference range. Thyroid stimulating hormone (TSH) was measured using a Bayer ADVIA Centaur immunoassay system with CVs of <6% within the reference range. HbA1c was measured on fresh whole blood using a Tosoh analyser with CVs of <5% within the reference range.

For measurement of insulin, samples were collected into lithium heparin plasma tubes, placed on ice, centrifuged (3500g at 4°C 10 minutes), aliquotted and frozen using dry ice within 15 minutes of venesection. Samples were stored at -80°C prior to batch analysis using the Diasorin Liaison, an immunoassay with chemiluminescent detection. This method has a range of 20-3470 pmol/l and intra-assay CVs of <5%.

Statistical Analysis

Based on previous work (7), using a paired study design, it was estimated that observing 8 subjects per comparison would have 90% power to detect an average difference of 6.0 pmol/l (19.8 pg/ml) of GLP-1. For each endpoint, 8-10 participants were recruited to allow for participant withdrawal. Plasma concentrations of hormones and analytes in the text and figures are shown as mean \pm SEM.

As this was a cross-over study, each participant was given placebo and active regimens in randomised order, using a pre-prepared protocol developed using a random number generation package. Paired t tests were used to compare plasma levels of GLP-1, glucose and insulin and reported levels of hunger, fullness and nausea according to a visual analogue scale. Peak GLP-1 was defined as the maximum concentration of GLP-1 for an individual on a single study

day. Peak GLP-1 was defined as the maximum concentration of GLP-1 for an individual on a single study day. Linear regression was used to assess the differences between GLP-1, glucose and insulin concentrations between groups. STATA/SE 13.1 was used for analysis.

Results

32 participants were recruited (24 healthy volunteers and 8 patients with T2DM). The baseline characteristics of the participants are listed in table 1. Most healthy volunteers were aged 22-30 years old with a body mass index of 20-25 kg/m² and around 25% body fat. As expected, the patients with T2DM were generally older and had a higher BMI, percentage fat mass and HbA1c compared to healthy volunteers. Most participants had normal haemoglobin, white cell count, ALT, TSH and creatinine at baseline.

Table 1: Baseline characteristics of study participants – characteristics shown as median (range). HV: healthy volunteers; T2DM: patients with type 2 diabetes mellitus. Data represent mean \pm SD.

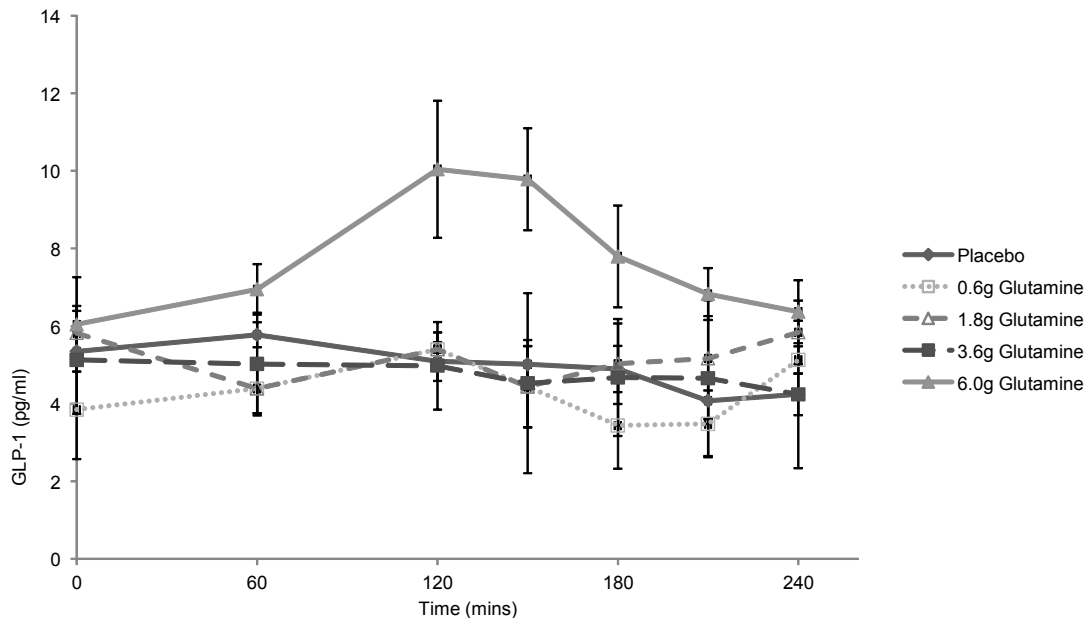
	HV: Assessment of GLP-1 concentrations n=10	HV: Assessment of glucose tolerance n=9	HV: Assessment of meal size§ n=10	T2DM: Assessment of glucose tolerance n=8
Gender	5 male, 5 female	4 male, 5 female	5 male, 5 female	4 male, 4 female
Age (years at enrolment)	31.3 (9.7)	36.2 (10.5)	33.9 (10.2)	57.9 (10.7)
Body Mass Index kg/m ²	22.9 (3.5)	25.0 (3.9)	23.4 (4.1)	32.5 (5.6)
Waist circumference cm	79.0 (14.0)	88.3 (11.5)	83.1 (15.5)	104.0 (16.8)
Hip circumference cm	94 (10)	102 (8)	96 (11)	120 (10)
DXA Percentage fat mass	23.8 (11.3)	29.3 (12.5)	29.1 (12.4)	41.8 (8.0)
Haemoglobin g/l	136 (13)	138 (14)	135 (15)	136 (16)
White Cell Count *10 ⁹ /l	5.1 (0.7)	5.3 (1.1)	4.8 (1.0)	6.7 (1.0)
Platelets *10 ⁹ /l	189 (25)	225 (52)	197 (30)	165 (82)
Creatinine umol/l	70.5 (11.8)	67.3 (11.0)	64.0 (8.9)	70.9 (10.4)
ALT IU/l	32.5 (23.8)	36.4 (25.9)	33.6 (27.6)	46.0 (23.3)
TSH U/l	2.0 (0.8)	2.1 (0.6)	2.0 (0.9)	1.9 (1.0)
HbA1c mmol/mol	36.0 (4.2)	36.9 (3.4)	36.4 (3.6)	57.6 (17.6)

§ Only 8 of these participants had blood taken and a DXA scan.

Dose ranging study: Assessment of the dose-related effects of glutamine capsules on circulating concentrations of total GLP-1 in fasting healthy volunteers

Four healthy volunteers were recruited to identify the optimal dose for further study. The highest dose, 6.0g Glutamine, was associated with a significant increase in peak GLP-1 concentration compared to placebo during (figure 1). Based on these results, the doses chosen for further study were 3.6g Glutamine and 6.0g Glutamine in comparison to placebo.

Figure 1: Results of the dose ranging study. 4 blinded healthy volunteers attended on 3-4 occasions for administration of increasing doses of glutamine. The doses chosen for further study were 3.6g and 6.0g in comparison to placebo. Capsules were administered at time 0, immediately after baseline blood samples were taken. Data represent mean \pm SEM.



Assessment of the effect of glutamine capsules on circulating concentrations of total GLP-1 in healthy volunteers

Ten healthy volunteers were recruited to assess the effect of glutamine capsules on GLP-1 concentrations (table 1; fig.2). Ingestion of 6.0g glutamine was associated with an increase in peak GLP-1 (mean \pm SEM: 7.5 ± 4.1 pg/ml placebo vs 10.3 ± 2.6 pg/ml with 6.0g glutamine; $p < 0.001$). The area under the curve (AUC) for GLP-1 (from 0-240 minutes) was not significantly different (1217 ± 154 vs 1484 ± 241 pg*min/ml for placebo and 6.0g Glutamine respectively). There was no significant difference in peak or AUC glucose, insulin, hunger, fullness or nausea between the regimens.

These results were different from those found during the dose-ranging study (fig. 1). Further examination of individual participants' data showed one group ($n=7$) with increased GLP-1 concentrations (responders) while other participants ($n=3$) showed no evidence of a GLP-1 response. In responders, the shape of the GLP-1 curve was heterogeneous with marked differences between participants. There was also marked intra-individual variation in fasting GLP-1 concentrations in some participants between each of the three visits which was not explained by altered diet or exercise patterns within 24 hours of the study visit.

Assessment of the effect of glutamine capsules on glucose tolerance in healthy volunteers

Nine healthy volunteers were recruited to assess the effect of glutamine capsules on glucose tolerance using an oral glucose tolerance test (OGTT; table 1; fig. 3). After ingestion of 6.0g Glutamine, participants had significantly higher concentrations of insulin (mean \pm SEM: 70.9 ± 13.4 vs 48.5 ± 7.8 pmol/l; $p=0.048$) and GLP-1 (10.6 ± 1.0 vs 6.9 ± 0.8

pg/ml; $p=0.004$) at 90 minutes, i.e. immediately prior to ingestion of the 75g glucose load. No significant difference was seen in the area under the curve before or after the OGTT for glucose, GLP-1, insulin, hunger, fullness or nausea.

The effect of encapsulated nutrients on concentrations of GLP-1, glucose and insulin in patients with type 2 diabetes.

Eight patients with T2DM were enrolled into the study and attended for two visits for the assessment of hormone concentrations and glucose tolerance using an OGTT given at 90 minutes (table 1; fig. 4) after placebo or 6.0g glutamine. There were no significant differences in peak concentrations of glucose, GLP-1 or insulin or in reported levels of hunger, fullness or nausea. Area under the curve before or after the OGTT for glucose, GLP-1 and insulin were similar between active and placebo treatment regimens.

Figure 2: Effect of encapsulated glutamine upon concentrations of total GLP-1, insulin and glucose, and subjective measurements of hunger, fullness and nausea in fasting subjects. Capsules were administered at time 0, immediately after baseline blood samples were taken. 10 participants attended on 3 occasions and took either placebo, 3,6g Glutamine or 6.0g Glutamine in a randomised, blinded, cross-over design. Data represent mean + SEM.

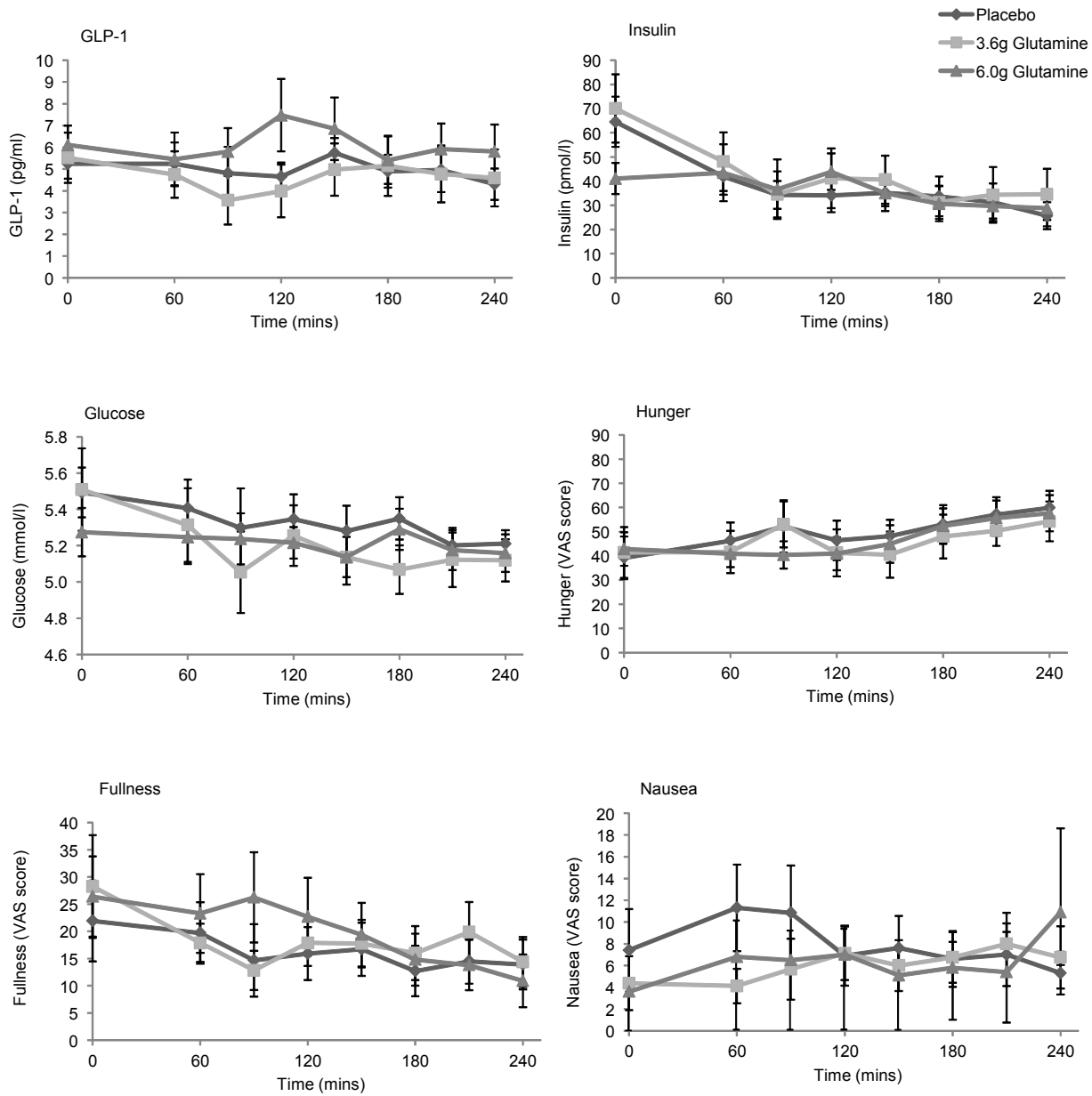


Figure 3: Effect of encapsulated glutamine upon concentrations of total GLP-1, insulin and glucose, and subjective measurements of hunger, fullness and nausea in healthy subjects before and after an oral glucose tolerance test (OGTT) which was given immediately after the 90 minute sampling. Capsules were administered at time 0, immediately after baseline blood samples were taken. 10 participants attended on 3 occasions and took placebo, 3,6g Glutamine or 6.0g Glutamine in a randomised, blinded, cross-over design. Data represent mean + SEM.

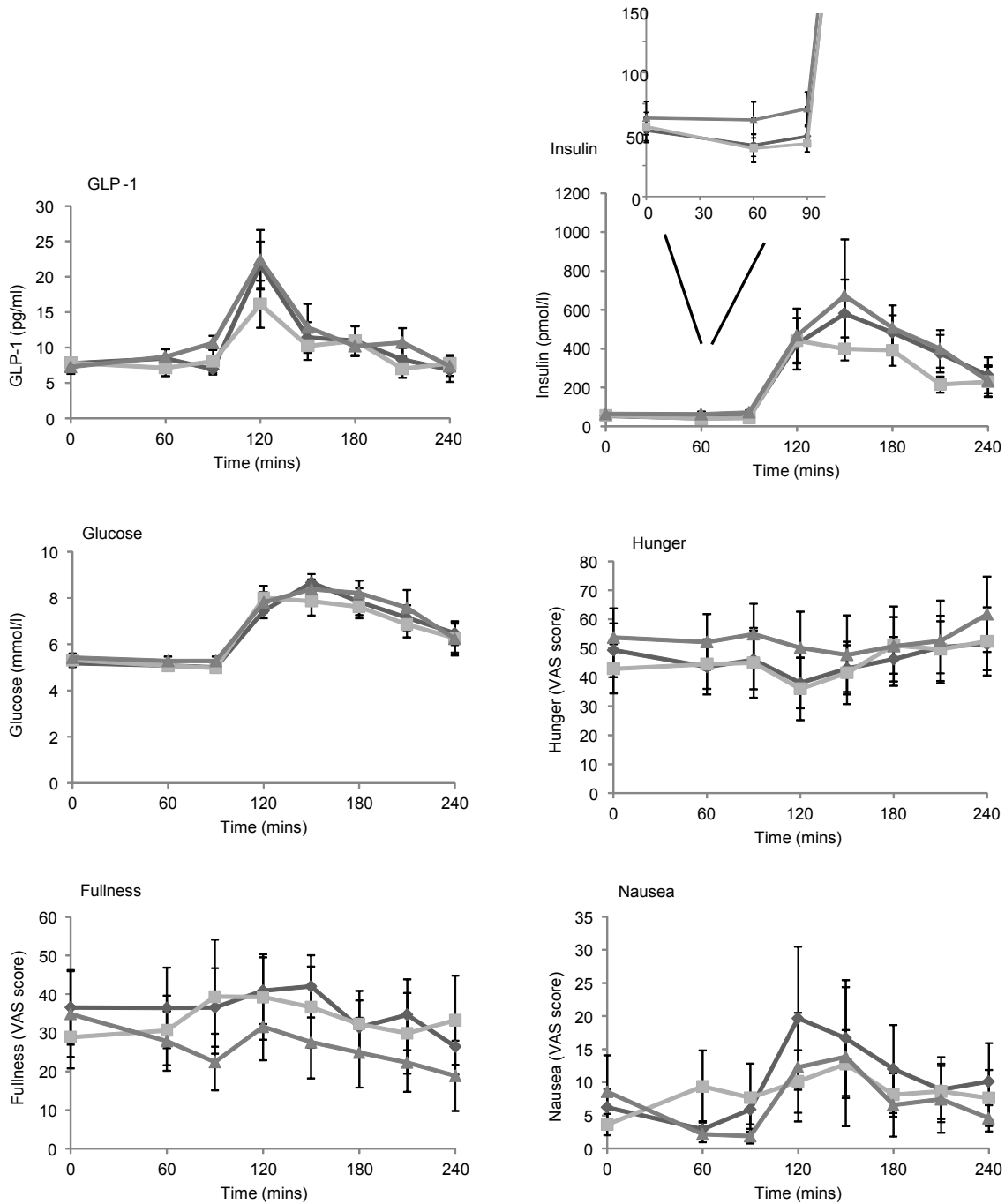


Figure 4: : Effect of encapsulated glutamine upon concentrations of total GLP-1, insulin and glucose, and subjective measurements of hunger, fullness and nausea in subjects with type 2 diabetes before and after an oral glucose tolerance test (OGTT) which was given immediately after the 90 minute sampling. Capsules were administered at time 0, immediately after baseline blood samples were taken. 8 participants attended on 2 occasions and took either placebo or 6.0g Glutamine in a randomised, blinded, cross-over design. Data represent mean \pm SEM.

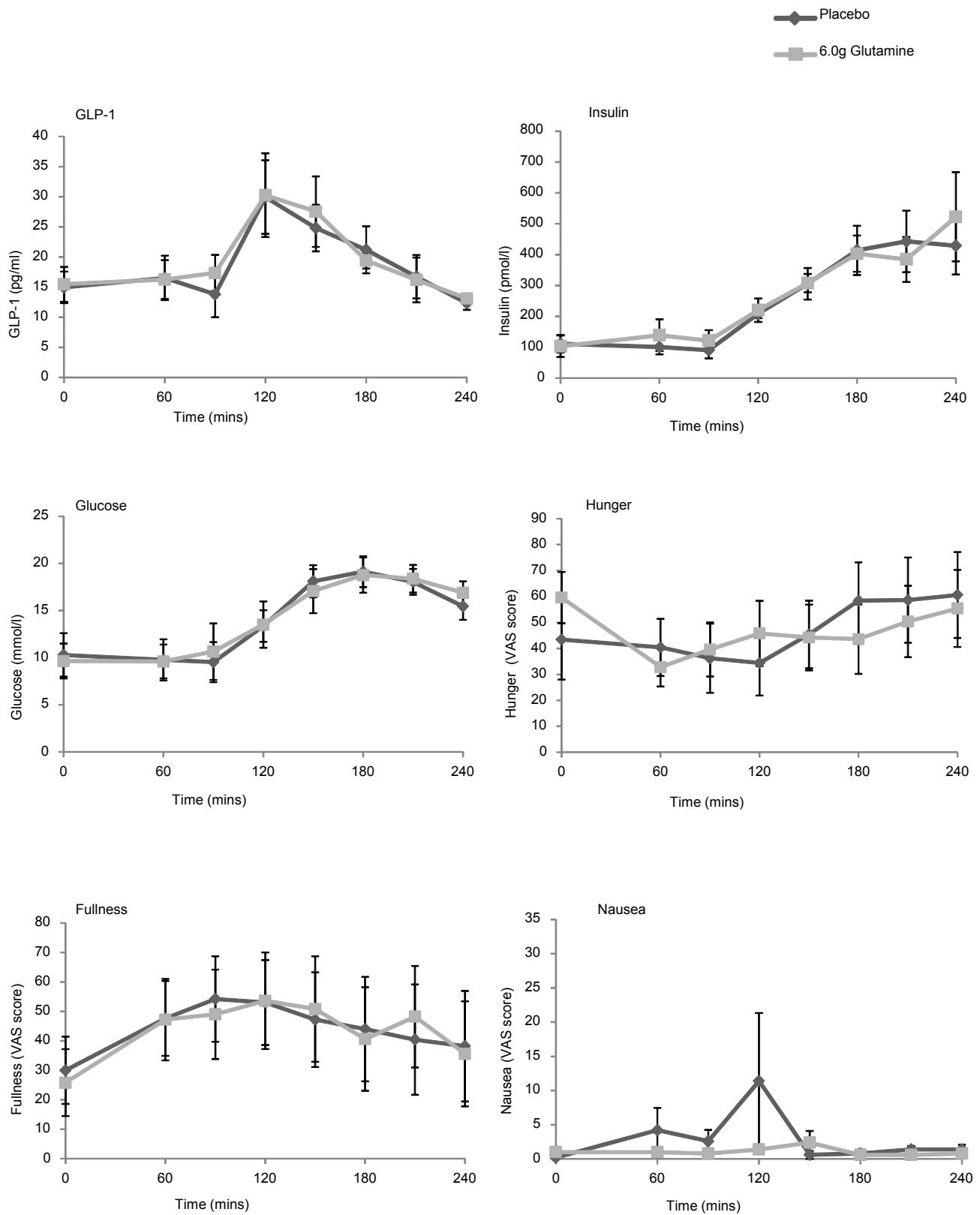
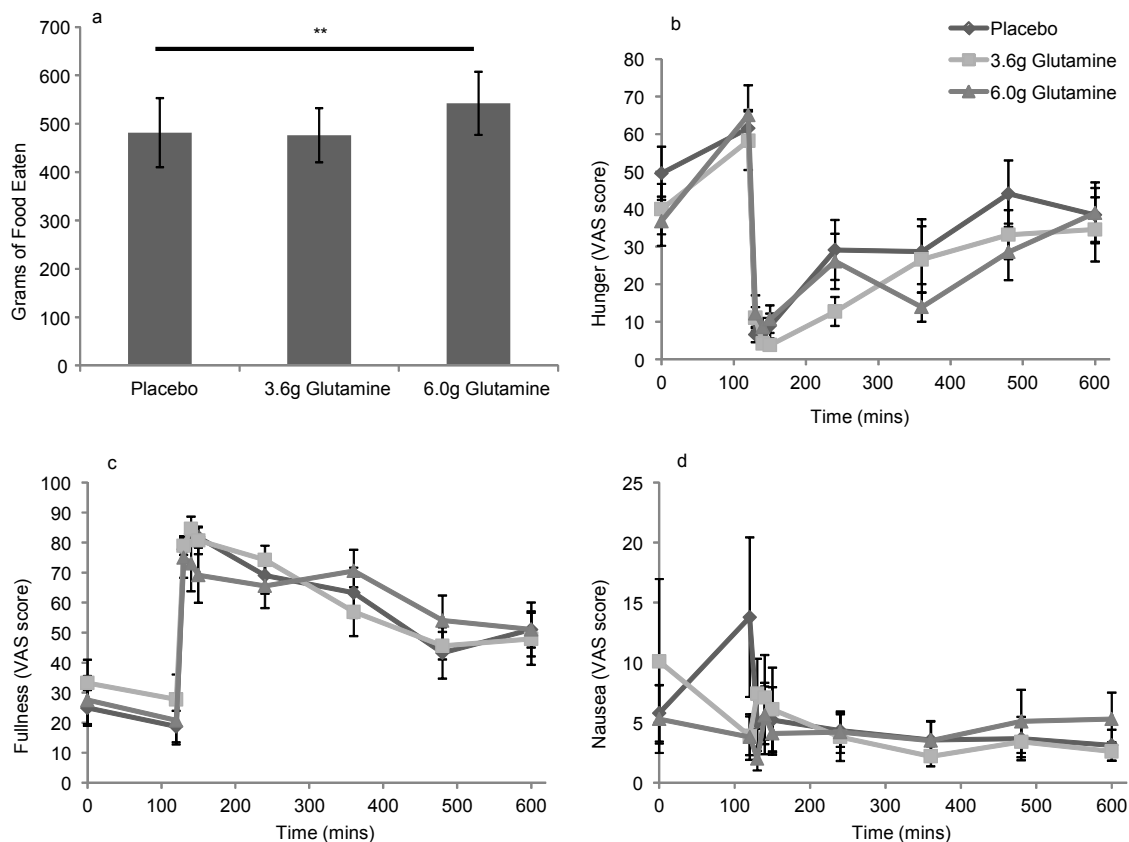


Figure 5: The effect of encapsulated glutamine on meal size, hunger, fullness and nausea in healthy volunteers. Capsules were administered at time 0, immediately after baseline blood samples were taken. An ad libitum meal was consumed 120 minutes later. a: Volunteers ate significantly more following ingestion of the 6.0g Glutamine capsules compared to placebo. b-d: Assessment of hunger, fullness and nausea during and after the ad libitum meal. Data are presented as mean \pm SEM. The symbol ** denotes significance at $p < 0.01$ respectively using paired t test analysis comparing the 6.0g Glutamine dose with placebo.



Assessment of the effect of glutamine capsules on meal size

Ten healthy volunteers were recruited to assess the effect of glutamine capsules on meal size (table 1). The group receiving 6.0g Glutamine ate significantly more food during the ad libitum meal compared to the placebo group (mean 542 ± 65 g eaten vs 481 ± 71 g, $p=0.008$; fig. 5) and had reduced hunger scores postprandially (reduced area under the curve from 120-480 minutes, $p=0.053$; and 0-480 minutes, $p=0.031$).

This finding was unexpected and contrary to the hypothesis. Unfortunately, no blood samples were taken from participants during the meal visit. However, participants had significantly higher hunger scores at 120min following ingestion of 6g glutamine, before beginning the meal. On linear regression analysis, hunger at 120 minutes was linearly related to the amount eaten when the model was adjusted for gender (coefficient 3.41, 95% confidence interval 0.12-6.7, $p=0.042$).

Assessment of the effect of glutamine capsules on hunger and satiety

Overall, there was no significant difference between peak or AUC for hunger, fullness or nausea for these variables between the three different regimens (figs 2-4) except during the meal study (fig 5). Hunger and fullness were linearly related and hunger scores tended to be lower where nausea scores were high (fig S1).

Discussion

This study demonstrates that up to 6g of encapsulated glutamine is unable to provoke a physiologically or clinically significant increase in plasma total GLP-1 concentrations in fasting healthy volunteers and is not associated with beneficial effects upon glucose tolerance or meal size. As evident by comparing the results shown in figures 1 and 2, some individuals showed small responses to capsule ingestion. However, the magnitude of these responses is likely to be inadequate to cause clinically beneficial effects.

This study used a double-blind, randomised, placebo-controlled, cross-over design to reduce bias and the effects of inter-individual variation. The encapsulated glutamine was tested in healthy individuals and in patients with type 2 diabetes. The capsules were designed to release their contents around 20 minutes after exposure to an alkaline medium, corresponding broadly to the upper-to-mid ileum. Gastrointestinal transit has not been measured and is likely to vary between individuals, but the capsules were all taken in the fasting state in order to minimise variation in transit time through the stomach. It is possible that not all capsules left the stomach at the same time, which might result in a different profile of glutamine exposure along the gastrointestinal tract and may in part explain inter-individual differences in response. Due to inter and intra-individual variations in gastrointestinal motility, gastric and intestinal secretion volumes and capsule opening profiles, it is not possible to know what concentration of glutamine was reached in the intestinal lumen. These factors might explain why the small GLP-1 responses to capsule ingestion observed in some subjects were not apparent in others. Theoretically, the dissolution of ten capsules simultaneously could trigger a sharp but short-lived GLP-1 response while more gradual dissolution of the capsules may give a more gradual GLP-1 release profile. It is likely, however, that the dose of glutamine was the limiting factor in this experiment. As ten capsules were required to give a dose of 6g glutamine, further dose increases were not considered feasible within this study design.

The study was powered according to published data and had 90% power to detect an average difference in GLP-1 of 6.0 pmol/l (20 pg/ml). However, as the difference in peak GLP-1 seen in the fasting state during this study was much lower than this, our study was not powered to detect such small effect sizes. It also remains unclear what levels of GLP-1 might be required to provoke a meaningful change in clinical endpoints such as glucose tolerance and weight reduction. In this study, in the fasting state, GLP-1 concentrations peaked at ~10 pg/ml, well below the concentrations expected following even a small meal(12). Indeed, GLP-1 concentrations rose much higher to 20-35 pg/ml after the OGTT. Bariatric surgery has been associated with postprandial total GLP-1 concentrations of 100-160 pmol/l (330-520 pg/ml)(13).

Glutamine is known to be associated with increased GLP-1 secretion and beneficial metabolic effects both in healthy volunteers and in patients with type 2 diabetes. Glutamine is known to elevate cAMP and cytosolic calcium in ileal L-cells and is likely to bind to the calcium sensing receptor (CASR) (14) and the sodium-dependent transporters such as SLC38A2 and SLC6A19(6). L-cells also express a range of alternative G-protein coupled receptors, which might be

targetable by dietary nutrients or high affinity ligands(15) As the EC50 for glutamine-triggered GLP-1 secretion in vitro was 0.2 mM(6), we speculate that the dose of glutamine delivered in the capsules was too low to activate L-cells along a large length of the small intestine. Previous work by Greenfield and colleagues has shown that 30g of oral glutamine in 300ml water can stimulate increases in GLP-1 and insulin in healthy and obese volunteers and patients with type 2 diabetes(7) with mean peak GLP-1 concentrations of around 22 pmol/l (72 pg/ml) in lean healthy volunteers. This has led to the hypotheses that glutamine could be developed therapeutically in patients with impaired glucose tolerance or diabetes (7). However, the mode of delivery in that study was challenging as 30g of glutamine is incompletely soluble in 300ml water and a 'swish and swallow' approach was required (7, 16). Furthermore, 30g glutamine has a calorie content of around 120 kcals and providing additional calories to patients with obesity or type 2 diabetes may exacerbate glucose tolerance through weight gain. The aim of the current approach was to identify if increases in gut hormone concentrations and beneficial metabolic effects could be harnessed using low doses of glutamine using an alternative delivery method which aimed to maximise the exposure of glutamine to lower intestinal L-cells. This approach was not, however, associated with an increase in circulating GLP-1 concentrations comparable to the magnitude of effects seen in Greenfield's study. Glucose concentrations were also not affected by glutamine ingestion in this study, a finding which is consistent with Greenfield's work (7). Chang and colleagues tested an alternative method of delivery involving intra-duodenal infusion of 7.5-15g glutamine (17). They found relatively small increases in GLP-1 (in healthy volunteers and patients with T2DM) and insulin (T2DM only) although their participants demonstrated higher baseline total GLP-1 concentrations than those seen in this study, possibly due to the different analytical methods used for GLP-1 analysis. Their finding of low GLP-1 responses to the 7.5g glutamine infusion is consistent with our results, and together both reports suggest that the dose of glutamine is limiting, even when administered directly into the small intestine.

One unexpected finding in our study was the increase in meal size which occurred following ingestion of glutamine in comparison to placebo. As GLP-1 is generally considered to be anorexigenic, and 6.0g glutamine has a caloric value of around 30 kcal, an association between glutamine and reduced meal size might have been expected rather than the converse. Although blood tests were not taken during the meal test, participants who received the 6.0g glutamine dose prior to an OGTT had slightly higher circulating concentrations of GLP-1 and insulin prior to the start of the OGTT compared to individuals who received placebo (fig. 3). As insulin is considered to be orexigenic(18), it is possible that the increased insulin may explain the increased meal size consumed by participants. It is possible, however, that other gut hormones or targets of glutamine might play a role.

No significant difference between concentrations of GLP-1, insulin or glucose after consumption of the glutamine capsules in comparison to placebo were found in subjects with type 2 diabetes. In patients with diabetes, responses may be complicated by altered background GLP-1 secretory patterns(19, 20), gastric emptying(21), or intestinal responsiveness to amino acids in patients taking metformin(22).

In conclusion, orally-administered glutamine capsules did not provoke a consistent increase in GLP-1 or insulin in human volunteers and was not associated with beneficial effects upon glucose tolerance or meal size. We speculate that the low dose of glutamine that could be administered in capsular form was limiting in this study, and suggest that the success of future capsule-based approaches to trigger GLP-1 release will be dependent, at least in part, on the choice of higher affinity ligands to receptors localised on L-cells. Further work is needed to identify if other nutritional products could function as more potent agonists to promote endogenous GLP-1 secretion and harness its clinical benefits.

Supplementary information: Safety Report

The assessment of safety was a secondary endpoint of the study and was assessed using a symptom diary and visual analogue scale for nausea. There were no serious adverse events during the study although a wide variety of symptoms were reported (table S1).

Table S1: Symptoms experienced according to treatment regimen given (individual participant identification number given in brackets).

Symptoms after Placebo	Symptoms after 6g Glutamine	Symptoms after 3.6g Glutamine
Dizzy, headache (P2)	Headache (P12)	Dizzy (P9)
Breathlessness & sensation of tachycardia (P2)	Headache (P13)	Headache, backache(P12)
Nausea (P13)	Uncomfortably full & abdominal pain (P14)	Constipation (P12)
Shakiness ?hypoglycaemia (P20)	Flatulence (P15)	
Nausea and vomiting (P21)	Abdominal discomfort & vomiting (P16)	
Light-headedness (P23)	Felt less hungry for several hours (P20)	

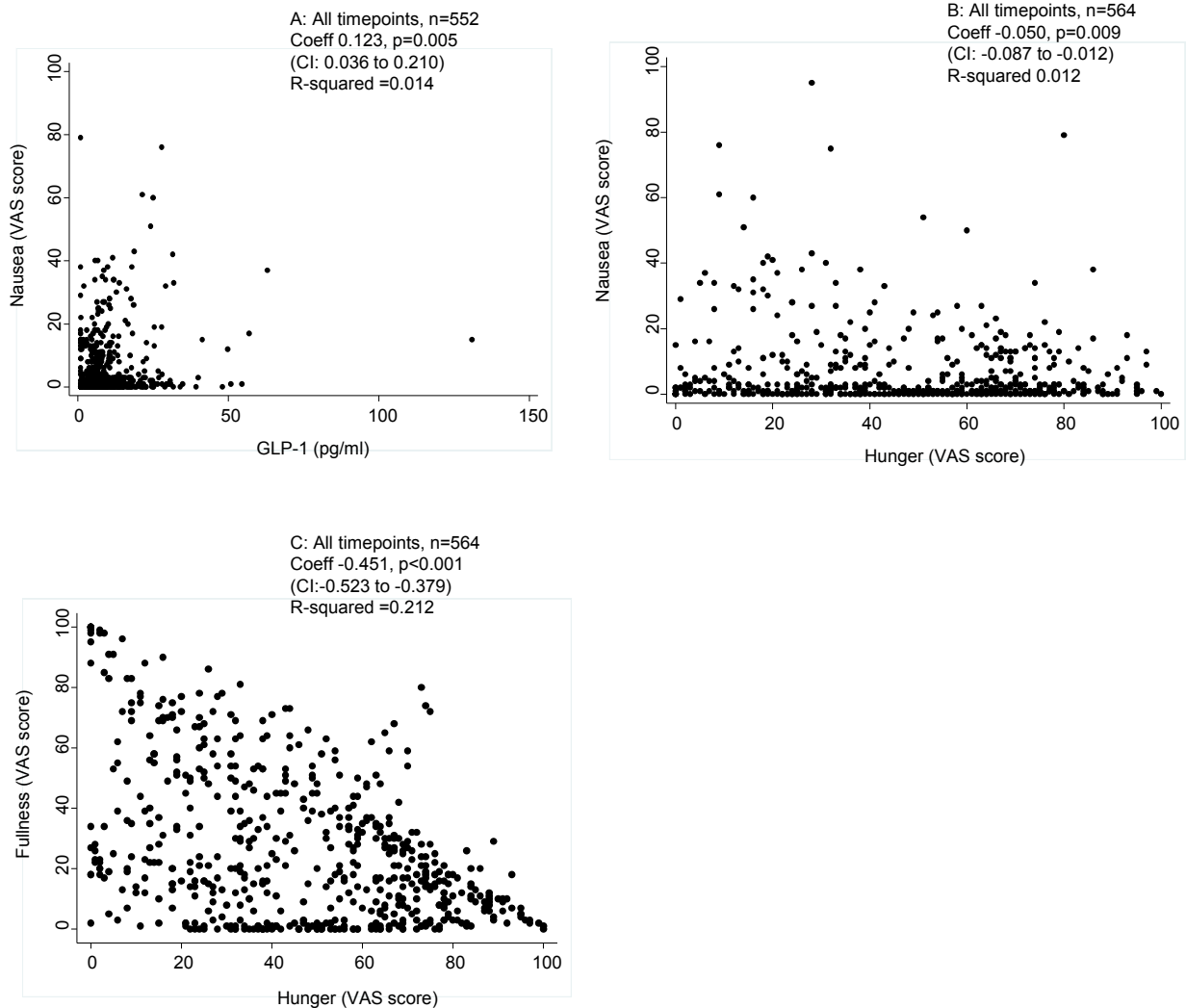
Results from the symptom diaries showed that side-effects were experienced following administration of all regimens (Table S1). The vast majority of symptoms were classified as mild (1-3/10 on a visual analogue scale).

Symptoms tended to fall within 3 broad categories:

- Symptoms related to the protocol, commonly associated with fasting. These included mild headache, dizziness and light-headedness and were broadly similar across all treatment groups.
- Gastrointestinal symptoms occurred including nausea, vomiting, constipation, flatulence and abdominal discomfort. Most symptoms within this category were mild. Two participants experienced vomiting episodes after placebo and 6g glutamine doses. Two participants specifically mentioned nausea on their symptom diary following ingestion of placebo capsules. One of these participants reported nausea after ingestion of the OGTT and the visit had to be abandoned after emesis of the intact capsules.
- Other events:
 - One participant experienced tremor after the test and attributed her symptoms to hypoglycaemia, although no testing facilities were available as she had left the hospital. The symptoms resolved quickly after eating and occurred after a placebo visit.
 - One participant experienced breathlessness and the sensation of tachycardia after her first visit. On assessment of her observations including saturations, clinical examination and electrocardiogram, no concerning abnormalities were identified. These symptoms occurred during a placebo visit and resolved quickly after reassurance.

When using the visual analogue scores, participants put a cross on a 100mm line to indicate how hungry, full or nauseated they felt at each timepoint. As expected, there was a small negative association between nausea and hunger, and a strong negative correlation between hunger and fullness (fig. S1). There was a small significant association between GLP-1 concentrations and nausea (fig. S1). Most participants experienced no nausea during the study.

Figure S1: Relationships between visual analogue scores and/or GLP-1 concentrations in all participants at all timepoints excluding the meal visits when blood was not taken. A: Most participants experienced no nausea during the test. There was a small but significant positive relationship between GLP-1 concentration and nausea scores. B: There was a small negative correlation between nausea and hunger. C: There was a strong negative correlation between hunger and fullness. Statistical analysis was performed using linear regression.



In conclusion, side effects occurred in both glutamine and placebo groups. Fasting was associated with dizziness, headache and light-headedness. Gastrointestinal symptoms were common in active and placebo groups with no particular evidence of clustering in the 6.0g glutamine group. However, there was a small but significant relationship between nausea and total GLP-1 concentrations.

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Duality of interest

Claire Meek receives salary funding from the European Union Seventh Framework Programme (FP7/2007-2013; grant agreement n° 266408) and from the Wellcome Trust Translational Medicine and Therapeutics Programme which is funded by the Wellcome Trust in association with Glaxo SmithKline. FMG is a member of the external scientific advisory board for BioKier, who develop encapsulated nutrients for the treatment of metabolic diseases.

CLM is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Contribution Statement

CLM designed the study, obtained regulatory approvals for the study, recruited volunteers, supervised capsule development and organised the participant visits. She also coordinated the safety reporting and provided medical cover for participants in case adverse events occurred, performed the data analysis and wrote the final report. HBL assisted with participant recruitment and statistical analysis and reviewed the final manuscript. BV assisted with participant recruitment and reviewed the final manuscript. AP assisted with study design, advised on regulatory matters and participant recruitment and reviewed the final manuscript. FR contributed to study design, data analysis and reviewed and revised the final manuscript with contributions to the discussion. FMG identified the study question and designed the study, contributed to the discussion and reviewed and revised the final manuscript.

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