

# Oral glutamine increases circulating glucagon-like peptide 1, glucagon, and insulin concentrations in lean, obese, and type 2 diabetic subjects<sup>1-4</sup>

Jerry R Greenfield, I Sadaf Farooqi, Julia M Keogh, Elana Henning, Abdella M Habib, Anthea Blackwood, Frank Reimann, Jens J Holst, and Fiona M Gribble

## ABSTRACT

**Background:** Incretin hormones, such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), play an important role in meal-related insulin secretion. We previously demonstrated that glutamine is a potent stimulus of GLP-1 secretion in vitro.

**Objective:** Our objective was to determine whether glutamine increases circulating GLP-1 and GIP concentrations in vivo and, if so, whether this is associated with an increase in plasma insulin.

**Design:** We recruited 8 healthy normal-weight volunteers (LEAN), 8 obese individuals with type 2 diabetes or impaired glucose tolerance (OB-DIAB) and 8 obese nondiabetic control subjects (OB-CON). Oral glucose (75 g), glutamine (30 g), and water were administered on 3 separate days in random order, and plasma concentrations of GLP-1, GIP, insulin, glucagon, and glucose were measured over 120 min.

**Results:** Oral glucose led to increases in circulating GLP-1 concentrations, which peaked at 30 min in LEAN ( $31.9 \pm 5.7$  pmol/L) and OB-CON ( $24.3 \pm 2.1$  pmol/L) subjects and at 45 min in OB-DIAB subjects ( $19.5 \pm 1.8$  pmol/L). Circulating GLP-1 concentrations increased in all study groups after glutamine ingestion, with peak concentrations at 30 min of  $22.5 \pm 3.4$ ,  $17.9 \pm 1.1$ , and  $17.3 \pm 3.4$  pmol/L in LEAN, OB-CON, and OB-DIAB subjects, respectively. Glutamine also increased plasma GIP concentrations but less effectively than glucose. Consistent with the increases in GLP-1 and GIP, glutamine significantly increased circulating plasma insulin concentrations. Glutamine stimulated glucagon secretion in all 3 study groups.

**Conclusion:** Glutamine effectively increases circulating GLP-1, GIP, and insulin concentrations in vivo and may represent a novel therapeutic approach to stimulating insulin secretion in obesity and type 2 diabetes. *Am J Clin Nutr* 2009;89:106–13.

## INTRODUCTION

Nutritional therapy is a key intervention for reducing weight and improving glycemic control in type 2 diabetes. In addition to insulin resistance, impaired insulin secretion is a key pathogenic factor in the development of type 2 diabetes (1). Incretin hormones, such as glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), play a major role in determining the physiologic profile of insulin release after a meal (2, 3). The reported ability of GLP-1 to enhance glucose-dependent insulin release led to the development of several ther-

apeutic strategies to harness the GLP-1 system for the treatment of diabetes. Indeed, the GLP-1 receptor agonist, exenatide, and orally active dipeptidyl peptidase-IV (DPP-IV) inhibitors have entered clinical practice. An alternative therapeutic approach would be to stimulate the release of endogenous GLP-1. However, it remains unclear whether this would be feasible in individuals with diabetes, because diabetes itself has been associated with impaired GLP-1 responses to nutrient ingestion (4–7), albeit inconsistently (8, 9), in part due to the use of different assays. This approach would have other potential metabolic benefits, because the L-cells that produce GLP-1 also secrete GLP-2, which stimulates regeneration and repair of the intestinal epithelium (10); peptide YY, which may suppress appetite (11, 12); and oxyntomodulin, which is believed to reduce food intake in part via the GLP-1 receptor (13).

Understanding the pathways involved in GLP-1 release from L-cells is key to developing agents that enhance GLP-1 secretion in vivo. Experiments in humans and isolated perfused gut preparations in animals demonstrated that carbohydrate, fat, and protein are all involved in meal-stimulated GLP-1 release (14, 15). Extensive use has been made of the GLP-1-secreting murine cell line GLUTag, which is responsive to a range of sugars, amino acids, and fats. As in other endocrine cells, GLP-1 release is triggered by an elevation of intracellular calcium, either after membrane depolarization and the opening of voltage-gated calcium channels or by the release of calcium from intracellular stores (15). By screening GLUTag cells for GLP-1 release in response to a range of

<sup>1</sup> From the Cambridge Institute for Medical Research and Department of Clinical Biochemistry, University of Cambridge, Cambridge, United Kingdom (JRG, ISF, JMK, EH, AMH, AB, FR, and FMG), and the Department of Medical Physiology, University of Copenhagen, The Panum Institute, Copenhagen, Denmark (JJH).

<sup>2</sup> JRG and ISF contributed equally to this work.

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<sup>4</sup> Reprints not available. Address correspondence to FM Gribble, Cambridge Institute for Medical Research and Department of Clinical Biochemistry, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, United Kingdom. E-mail: fmg23@cam.ac.uk.

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amino acids and sugars, we previously identified glutamine as a highly effective stimulus (16). Glutamine was effective at physiologic concentrations (half-maximal effective concentration:  $\approx 0.5$  mM); the underlying mechanism involved both a triggering step associated with electrogenic amino acid uptake and an enhancing step downstream of the calcium signal (16).

Glutamine is widely available as a nutritional supplement. There is accumulating evidence that its addition to enteral and parenteral nutrition is beneficial for the maintenance of intestinal integrity (17–19). Glutamine is also administered orally at high doses to protect the gut during chemo- and radiotherapy (20, 21). The finding that glutamine triggers GLP-1 release from GLUTag cells raises the possibility that stimulation of GLP-1 and GLP-2 release from L-cells might contribute to its known physiologic effects in vivo.

The aim of the present study was to investigate whether glutamine increases GLP-1, GIP, glucagon, and insulin concentrations in human subjects.

## SUBJECTS AND METHODS

### Subjects

Twenty-four subjects were recruited via advertisements placed around the hospital campus. All nondiabetic obese subjects [body mass index (BMI; in  $\text{kg}/\text{m}^2$ )  $\geq 30$ ] were screened with a 75-g oral glucose tolerance test (OGTT) before inclusion in the study. Subjects were recruited into 3 groups of 8 subjects: healthy, lean (BMI  $< 25$ ) volunteers (LEAN); obese subjects with type 2 diabetes ( $n = 6$ ) or impaired glucose tolerance ( $n = 2$ ) (OB-DIAB); and obese control subjects with normal glucose tolerance (OB-CON) who were age- and BMI-matched to the OB-DIAB group. This sample size is consistent with that used previously in studies comparing nutrient-stimulated GLP-1 release in subjects with type 2 diabetes and healthy control subjects (8, 9). No subject in the OB-DIAB group was receiving oral hypoglycemic agents; 5 were diagnosed with impaired glucose tolerance or type 2 diabetes

at the screening OGTT. Diabetes was defined as a fasting plasma glucose concentration of  $\geq 7.0$  mmol/L and/or a plasma glucose of  $\geq 11.1$  mmol/L 120 min after receiving a 75-g glucose load. Impaired glucose tolerance was diagnosed as a plasma glucose concentration of 7.8–11.0 mmol/L 120 min after receiving a 75-g glucose load. Patients with mild degrees of hyperglycemia were specifically recruited to avoid any potentially confounding effects of glucotoxicity and oral hypoglycemic agents on GLP-1 release. Exclusion criteria were as follows: excessive alcohol intake; liver, kidney, or thyroid disease; anemia; treatment with weight loss or lipid-lowering agents; previous bowel surgery; and documented malabsorption. All studies were approved by the Local Regional Ethics Committee in Cambridge and were conducted in accordance with the principles of the Declaration of Helsinki. All subjects provided written informed consent.

### Study design

Subjects attended the Wellcome Trust Clinical Research Facility at Addenbrooke's Hospital between 0745 and 0800 on 3 separate occasions over a period of  $< 1$  mo, having fasted from 2200 the night before the study. Subjects were instructed not to walk or cycle to study visits. Studies were performed in a random order on separate days. A large-bore intravenous indwelling cannula was inserted into an antecubital vein on each visit for blood sampling. At the first visit, weight and height were measured with the subject wearing light street clothing. BMI was calculated as weight divided by height squared ( $\text{kg}/\text{m}^2$ ). Blood after fasting was drawn for glycated hemoglobin (Hb A<sub>1c</sub>), full blood count, and renal, liver and thyroid function tests. On each study day, 3 fasting baseline blood samples were taken for glucose, insulin, and GLP-1 at  $t = -10$ ,  $-5$ , and  $-1$  min. After collection of the last baseline sample, subjects ingested glucose (75 g, 0.42 mol, in 300 mL water), glutamine (30 g, 0.21 mol, in 300 mL water), or water (300 mL) over  $\approx 2$  min (last mouthful at  $t = 0$ ). We examined the effect of a maximum dose of glutamine known to be safely tolerated in humans (22) and compared

**TABLE 1**  
Demographic, anthropometric, and fasting metabolic characteristics of study participants<sup>1</sup>

	LEAN subjects	OB-CON subjects	OB-DIAB subjects	ANOVA <i>P</i> value
Sex (M/F)	6/2	7/1	8/0	—
Age (y)	30 $\pm$ 5.8 <sup>2</sup>	39 $\pm$ 9.8	38.5 $\pm$ 8	0.06
Weight (kg)	70.3 $\pm$ 8.6	106 $\pm$ 14.8 <sup>3</sup>	120.6 $\pm$ 24.2 <sup>3</sup>	$< 0.0001$
BMI ( $\text{kg}/\text{m}^2$ )	21.9 $\pm$ 2.2	34.5 $\pm$ 4.4 <sup>3</sup>	38.5 $\pm$ 6.5 <sup>3</sup>	$< 0.0001$
Fasting plasma glucose (mmol/L) <sup>4</sup>	4.6 $\pm$ 0.2	4.9 $\pm$ 0.2 <sup>5</sup>	6.2 $\pm$ 0.9 <sup>3,6</sup>	$< 0.0001$
Fasting plasma insulin (pmol/L) <sup>4</sup>	34 (25–47) <sup>7</sup>	103 (74–144) <sup>3</sup>	147 (82–266) <sup>3</sup>	$< 0.0001$
HOMA2-%S	160 (116–220)	53 (38–73) <sup>3</sup>	42 (33–55) <sup>3</sup>	$< 0.0001$
HOMA2-% $\beta$	85 (68–105)	153 (118–198) <sup>3</sup>	115 (79–167)	0.002
Hb A <sub>1c</sub> (%)	Not done	5.2 $\pm$ 0.3	6.3 $\pm$ 0.5 <sup>8</sup>	—
Fasting total GLP-1 (pmol/L) <sup>4</sup>	14.4 $\pm$ 1.5	13.0 $\pm$ 2.0	9.4 $\pm$ 2.9 <sup>3,9</sup>	0.006

<sup>1</sup> LEAN, healthy normal-weight volunteers; OB-DIAB, obese individuals with type 2 diabetes or impaired glucose tolerance; OB-CON, obese nondiabetic control subjects; HOMA2-%S, updated homeostasis model assessment used to estimate values of insulin sensitivity; HOMA2-% $\beta$ , updated homeostasis model assessment used to estimate values of  $\beta$  cell function; Hb A<sub>1c</sub>, glycated hemoglobin; GLP-1, glucagon-like peptide 1.

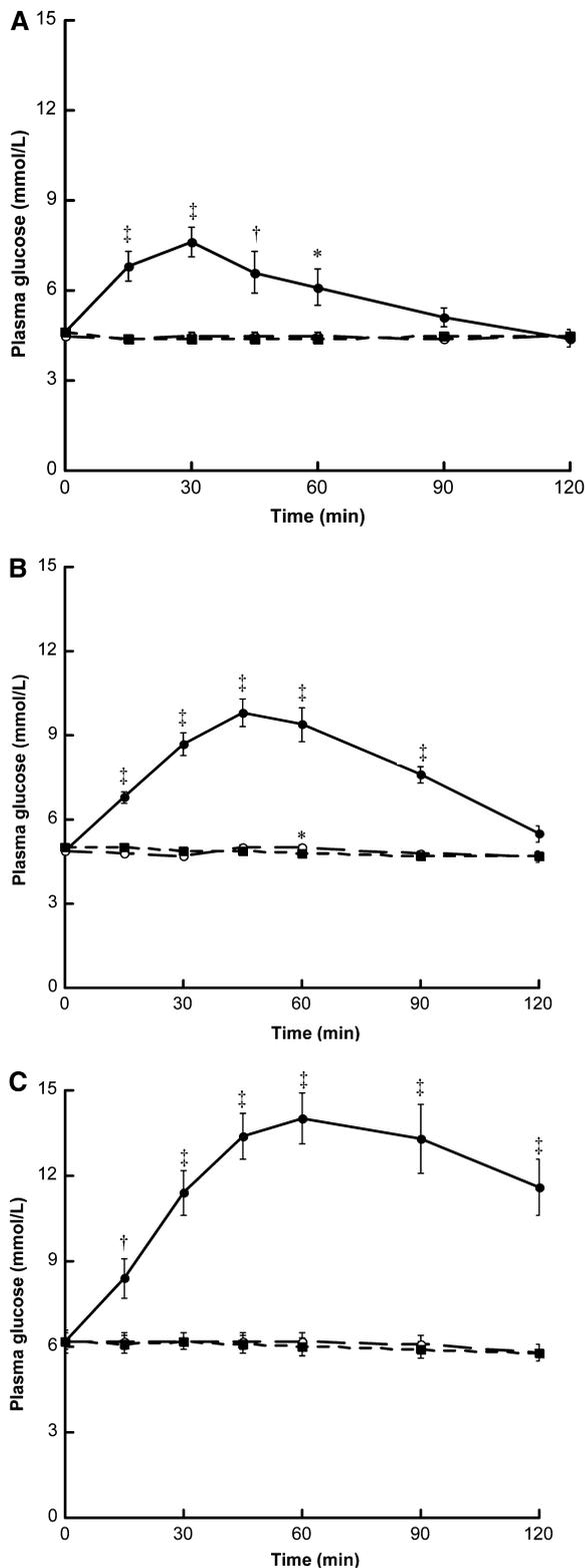
<sup>2</sup> Mean  $\pm$  SD (all such values).

<sup>3,5</sup> Significantly different from LEAN subjects (unpaired *t* test): <sup>3</sup>*P*  $< 0.001$ , <sup>5</sup>*P*  $< 0.01$ .

<sup>4</sup> Average of baseline samples from glucose, glutamine, and water study days.

<sup>6,8,9</sup> Significantly different from OB-CON subjects (unpaired *t* test): <sup>6</sup>*P*  $< 0.01$ , <sup>8</sup>*P*  $< 0.001$ , <sup>9</sup>*P*  $< 0.05$ .

<sup>7</sup> Geometric mean; 1 SD range in parentheses (all such values).



**FIGURE 1.** Plasma glucose concentrations after the ingestion of glucose (black circles), glutamine (white circles), and water (black squares) in (A) 8 lean subjects, (B) 8 obese nondiabetic control subjects, and (C) 8 obese individuals with type 2 diabetes or impaired glucose tolerance. Data are mean  $\pm$  SE. \* $P < 0.05$ , † $P < 0.01$ , and ‡ $P < 0.001$  compared with water (paired  $t$  test). There were no differences in baseline glucose concentrations within each group between visits.

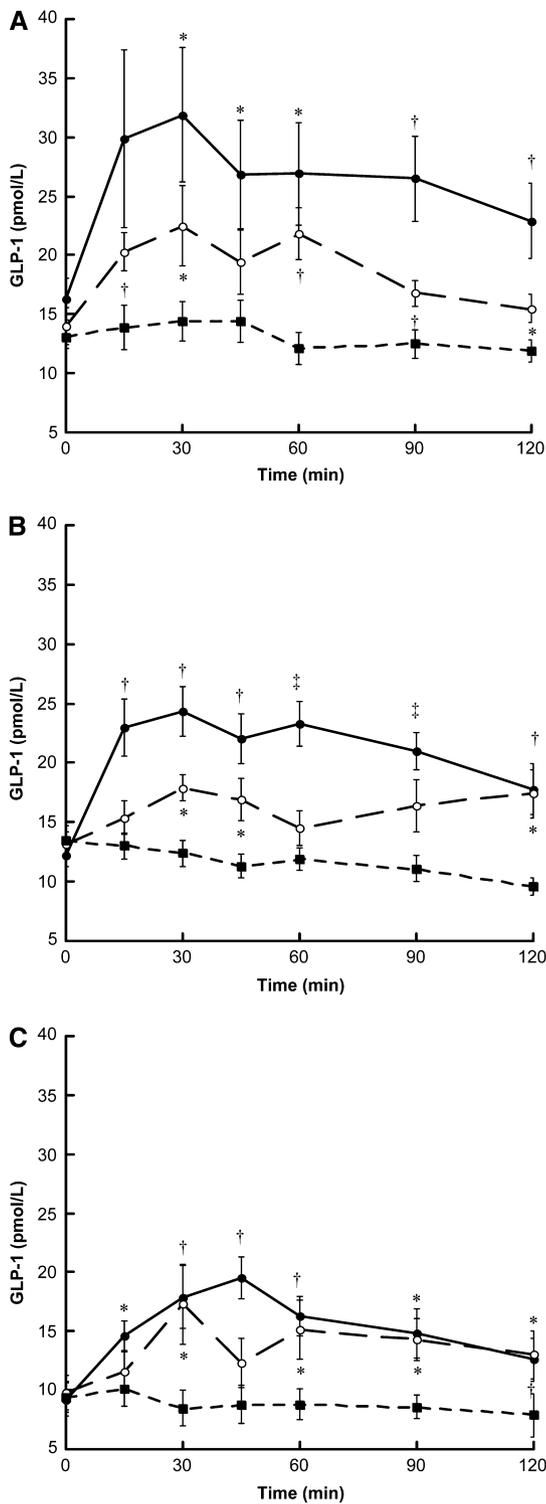
this with the effect of water (negative control) and of a dose of glucose previously reported to be a reliable stimulus of GLP-1 secretion (positive control) (23). Blood samples were collected at  $t = 15, 30, 45, 60, 90,$  and  $120$  min for plasma glucose and GLP-1. Plasma insulin was assayed at  $t = 15, 30, 60,$  and  $120$  min. Plasma GIP was measured at  $t = -10, -5, 15, 30, 60,$  and  $90$  min in a subset of 11 subjects from the OB-CON and OB-DIAB groups and covered a range of BMI values (reduced numbers due to limited plasma availability). Plasma glucagon was assayed at  $t = -10, -5, 15, 30, 60,$  and  $90$  min in 18 subjects from all 3 groups. The updated homeostasis model assessment (HOMA2) was used to estimate values of insulin sensitivity (HOMA2-%S) and  $\beta$  cell function (HOMA2-% $\beta$ ) as previously described (HOMA values not calculated in one OB-DIAB subject with fasting plasma insulin  $> 400$  pmol/L) (24). Subjects were provided with breakfast at the end of the study.

To determine the time course of the glutamine response and to ensure adequate absorption of oral glutamine, plasma glutamine concentrations were measured at all time points after glutamine ingestion in one LEAN subject. Plasma glutamine increased from  $728 \mu\text{mol/L}$  at baseline to  $1260 \mu\text{mol/L}$  at 15 min,  $2052 \mu\text{mol/L}$  at 30 min,  $2559 \mu\text{mol/L}$  at 60 min, and  $1863 \mu\text{mol/L}$  at 120 min. In all remaining subjects, plasma glutamine was assayed at the 60-min time point only.

#### Analytic methods

Blood for plasma glucose was collected in a fluoride oxalate tube; plasma glucose was assayed on the same day by using the glucose oxidase method. Insulin and glutamine were assayed in plasma collected in a tube containing lithium-heparin; plasma was frozen and stored at  $-80^\circ\text{C}$  until analysis. Insulin was quantified using a commercially available immunoassay (AutoDELFLIA Insulin Kit; Perkin Elmer, Wellesley, MA), which has a cross-reactivity with intact proinsulin of  $<0.1\%$  and a cross-reactivity with 32–33 split proinsulin of  $<0.44\%$ ; the intraassay CV was 3.5–4.5%. Glutamine was measured by cation exchange chromatography with postcolumn ninhydrin derivitization (Biochrom 30 amino acid analyzer; Biochrom Ltd, Cambridge, United Kingdom), with an intraassay CV of 3.2% and an interassay CV of 4%. Blood for GLP-1, GIP, and glucagon was collected into chilled EDTA-coated tubes, which were immediately centrifuged for 7 min at 3000 rpm. The plasma sample was snap-frozen and stored at  $-80^\circ$  until analysis. Total plasma GLP-1 concentrations were measured by radioimmunoassay after extraction of plasma with 70% ethanol (vol:vol, final concentration). Carboxy-terminal GLP-1 immunoreactivity was determined using antiserum 89390, which has an absolute requirement for the intact amidated carboxyl terminus of GLP-1 7–36 amide and cross-reacts  $< 0.01\%$  with carboxy-terminally truncated fragments and 89% with GLP-1 9–36 amide, the primary metabolite of DPP-IV-mediated degradation. The sum of the 2 components (total GLP-1 concentration) reflects the rate of secretion of the L-cell. Sensitivity was  $<1$  pmol/L, and intraassay CV was  $<5\%$  at 20 pmol/L (25). Total GIP was measured using a human GIP (total) enzyme-linked immunosorbent assay kit (LINCO Research, St Charles, MO), which cross-reacts 100% with human GIP(1–42) and GIP(3–42) and has a sensitivity of  $<2$  pmol/L and an intraassay CV of 7% at 4 pmol/L. Plasma glucagon concentrations were measured after extraction of plasma with 70% ethanol (vol:vol, final concentration).





**FIGURE 2.** Plasma glucagon-like peptide 1 (GLP-1) concentrations after the ingestion of glucose (black circles), glutamine (white circles), and water (black squares) in (A) 8 lean subjects, (B) 8 obese nondiabetic control subjects, and (C) 8 obese individuals with type 2 diabetes or impaired glucose tolerance. Data are mean  $\pm$  SE. \* $P < 0.05$ , † $P < 0.01$ , and ‡ $P < 0.001$  compared with water (paired  $t$  test). There were no differences in baseline GLP-1 concentrations within each group between visits.

The glucagon assay is directed against the carboxy terminus of the glucagon molecule (antibody code no. 4305) and therefore measures glucagon of mainly pancreatic origin. The de-

tection limit of the assay was 1 pmol/L, and the intra-assay CV was  $<6\%$ .

**Statistical methods**

Normally distributed data are expressed as mean  $\pm$  SD or SE. Because of their skewed distribution, plasma insulin and HOMA values were analyzed after  $\log_{10}$ -transformation and are presented as geometric mean (1 SD or SE range) after back-transformation. SE is presented except for the baseline data in **Table 1**. The incremental area under the curve was calculated using the trapezoidal rule, subtracting the baseline values extrapolated over 120 min from the total area under the curve. Results from different study days on the same subject were compared using paired  $t$  tests. Comparisons between study groups were made by analysis of variance (ANOVA) or regression; post hoc unpaired  $t$  tests were performed as appropriate. Correlations between variables were examined using Pearson’s correlation. Data were analyzed using Statview 5.0.1 (SAS Institute Inc, Cary, NC) or Microsoft Excel (Microsoft Corp, Redmond, WA). All  $P$  values were 2-sided.  $P < 0.05$  was considered statistically significant.

**RESULTS**

**Cohort characteristics**

Demographic, anthropometric, and fasting metabolic parameters of the study participants are shown in Table 1. OB-CON and OB-DIAB subjects were matched for sex, age, weight, and BMI. As expected, OB-DIAB subjects had higher fasting plasma glucose concentrations than nondiabetic subjects. OB-CON and OB-DIAB subjects had higher fasting insulin concentrations than LEAN subjects. Although LEAN subjects were more insulin-sensitive than OB-DIAB and OB-CON subjects, only the latter exhibited higher HOMA2-% $\beta$  relative to LEAN control subjects (Table 1).

Fasting GLP-1 concentrations in OB-DIAB subjects were significantly lower when compared with those in subjects without diabetes (Table 1). However, when compared across the whole study cohort by regression analysis, fasting GLP-1 concentrations were found to be inversely related to BMI ( $P = 0.003$ ) but not associated with fasting plasma glucose per se.

**Plasma glucose responses**

Plasma glucose responses after glucose, glutamine, and water ingestion are shown in **Figure 1**. As expected, mean plasma glucose concentrations increased rapidly above baseline after the consumption of oral glucose and peaked at 30 min in LEAN subjects ( $7.6 \pm 0.5$  mmol/L), 45 min in OB-CON subjects ( $9.8 \pm 0.5$  mmol/L), and 60 min in OB-DIAB subjects ( $14 \pm 0.9$  mmol/L). Plasma glucose concentrations after glutamine ingestion were comparable with those after water alone in all study groups.

**Plasma GLP-1 responses**

GLP-1 concentrations and total and incremental GLP-1 area under the curve after the ingestion of glucose, glutamine, and water are shown in **Figure 2** and **Table 2**. After the ingestion of water alone, GLP-1 concentrations remained at or below baseline throughout the 120-min sampling period in all study groups. In contrast, glucose induced a rapid increase in GLP-1 release;

**TABLE 2**

Total area under the curve (AUC) and incremental AUC (iAUC) for glucagon-like peptide 1 (pmol/L · 120 min) after glucose, glutamine, and water ingestion in 8 lean subjects (LEAN), 8 obese nondiabetic control subjects (OB-CON), and 8 obese individuals with type 2 diabetes or impaired glucose tolerance (OB-DIAB)<sup>1</sup>

	LEAN subjects	OB-CON subjects	OB-DIAB subjects	ANOVA <i>P</i> value
<b>AUC</b>				
Glucose	3192 ± 431 (0.01)	2550 ± 145 (<0.001)	1846 ± 185 (0.001) <sup>2,3</sup>	0.01
Glutamine	2245 ± 187 (<0.001)	1929 ± 169 (0.05)	1653 ± 207 (0.004)	0.11
Water	1548 ± 146	1396 ± 97	1054 ± 137 <sup>2</sup>	0.04
<b>iAUC</b>				
Glucose	1242 ± 385 (0.008)	1082 ± 102 (<0.001)	746 ± 159 (0.005)	0.37
Glutamine	560 ± 185 (0.01)	344 ± 199 (0.05)	480 ± 170 (0.04)	0.71
Water	-17 ± 89	-219 ± 82	-73 ± 86	0.25

<sup>1</sup> Data are mean ± SE (*P* compared with water, paired *t* test). ANOVA *P* value for comparison between subject groups.

<sup>2</sup> *P* < 0.05 compared with LEAN subjects (unpaired *t* test).

<sup>3</sup> *P* < 0.01 compared with OB-CON subjects (unpaired *t* test).

mean peak concentrations were observed at 30 min in LEAN subjects (31.9 ± 5.7 pmol/L) and OB-CON subjects (24.3 ± 2.1 pmol/L) and at 45 min in OB-DIAB subjects (19.5 ± 1.8 pmol/L) (Figure 2). Consistent with lower fasting GLP-1 concentrations in OB-DIAB subjects, the glucose-induced GLP-1 response (area under the curve) was significantly impaired in the OB-DIAB group compared with that in nondiabetic subjects (Table 2).

Thirty grams of oral glutamine were well tolerated by all subjects. Mean 60-min plasma glutamine concentrations were 1782 ± 191 μmol/L (range: 1287–2675 μmol/L) in LEAN subjects, 1584 ± 206 μmol/L (range: 1022–2866 μmol/L) in OB-CON subjects, and 1854 ± 241 μmol/L (range: 931–2723 μmol/L) in OB-DIAB subjects. Ingestion of glutamine resulted in elevation of circulating GLP-1 concentrations (Figure 2). In all groups, there was the suggestion of a biphasic response, with an initial peak at 30 min (22.5 ± 3.4, 17.9 ± 1.1, and 17.3 ± 3.4 in LEAN, OB-CON, and OB-DIAB subjects, respectively) and a second peak at 60–90 min. In the total cohort, both total and incremental GLP-1 responses to glutamine were significantly greater than those ob-

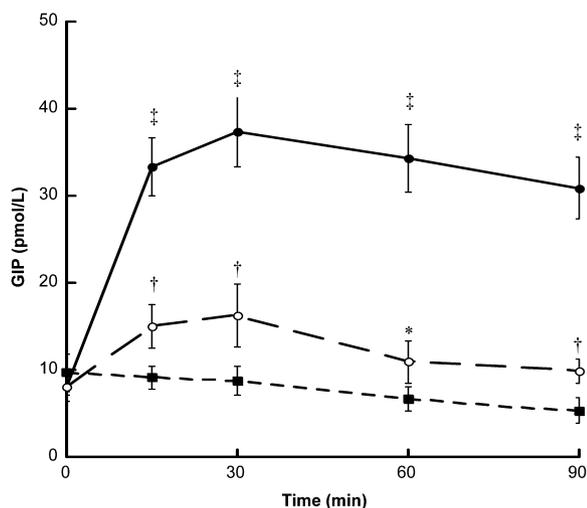
served after the ingestion of water alone (*P* < 0.0001). Of note, the magnitude of the glutamine-induced incremental GLP-1 response in OB-DIAB subjects was not significantly different from that observed in LEAN and OB-CON subjects (Table 2). It was also unrelated to the subject's age (*r* = -0.04, *P* = 0.85), weight (*r* = -0.19, *P* = 0.36), BMI (*r* = -0.09, *P* = 0.67), and fasting plasma glucose concentration (*r* = -0.30, *P* = 0.15).

### Plasma GIP responses

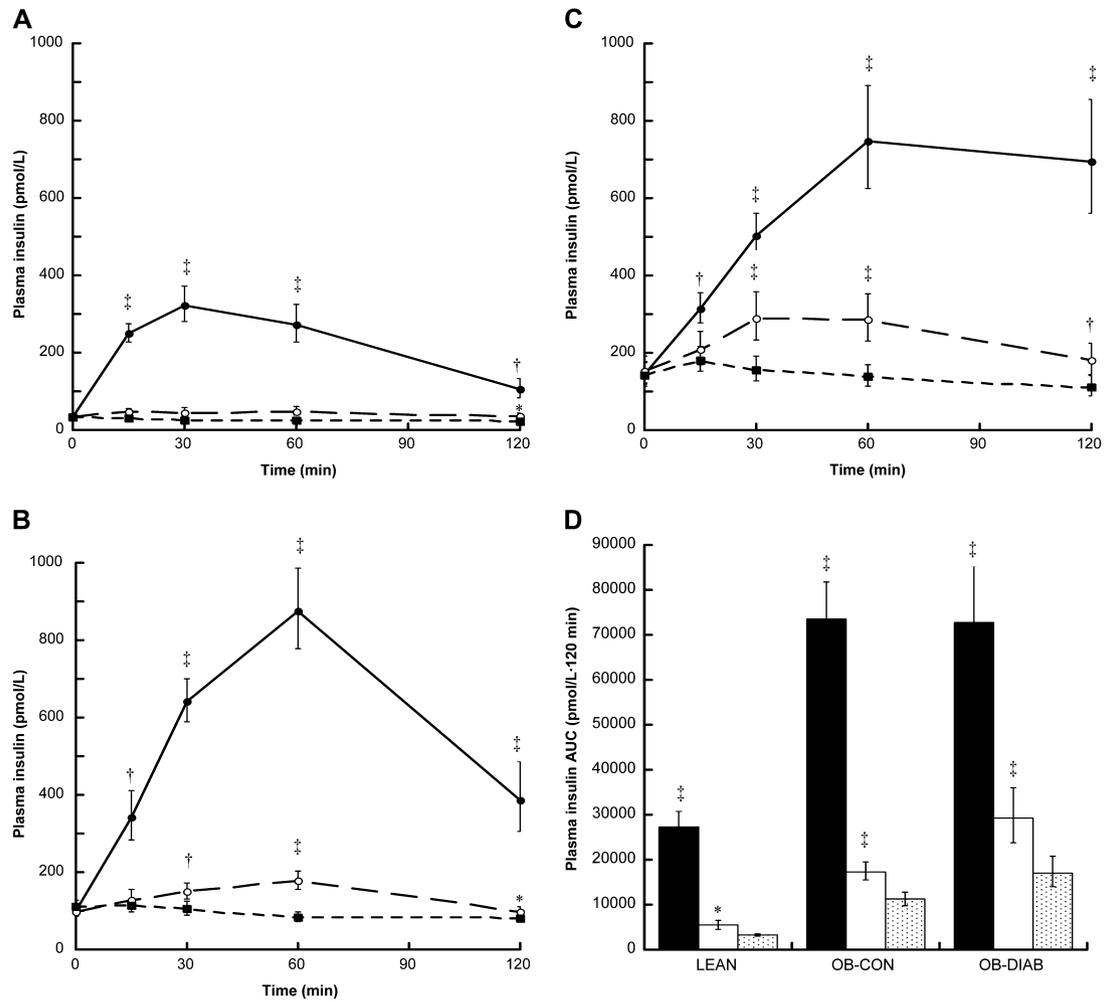
Plasma GIP concentrations were measured only in subjects from the LEAN and OB-CON groups because GIP concentrations are not significantly affected by diabetes (26). Fasting GIP concentration (8.6 ± 1.4 pmol/L) was not correlated with BMI. Glucose was a potent stimulus of GIP secretion (area under the curve: 2894 ± 276 compared with water, 686 ± 133 pmol/L, respectively; *P* < 0.001), with elevated concentrations detected as early as 15 min and peak concentrations of 37.4 ± 4.0 pmol/L at 30 min (Figure 3). Glutamine also triggered GIP secretion (peak: 16.3 ± 3.6 pmol/L at 30 min; area under the curve: glutamine, 1127 ± 216 pmol/L; *P* = 0.001 compared with water) but was markedly less effective than glucose.

### Plasma insulin responses

Insulin responses to glucose, glutamine, and water are shown in Figure 4. As expected, insulin concentrations increased markedly in all groups after the glucose load. The ingestion of glutamine was also associated with a significant increase in plasma insulin concentrations, particularly in the OB-CON and OB-DIAB groups. The absolute glutamine-induced insulin response was greatest in OB-DIAB subjects, intermediate in OB-CON subjects, and lowest in LEAN subjects (ANOVA, *P* < 0.0001 for comparison of log<sub>10</sub> insulin area under the curve across groups). A similar result was found when comparing the incremental area under the curve for insulin (ANOVA, *P* = 0.0013). Differences between the glutamine-induced and water-induced insulin responses (area under the curve) were significant when all subjects were considered together (*P* < 0.0001) and when each group of subjects was analyzed separately (Figure 4). Consistent with these results, the incremental area under the curve for insulin was greater after ingestion of glutamine than of



**FIGURE 3.** Plasma glucose-dependent insulinotropic polypeptide (GIP) concentrations after the ingestion of glucose (black circles), glutamine (white circles), and water (black squares) in 11 subjects (3 lean and 8 obese nondiabetic control subjects). Data are mean ± SE. \**P* < 0.05, †*P* < 0.01, and ‡*P* < 0.001 compared with water (paired *t* test).



**FIGURE 4.** Plasma insulin concentrations after the ingestion of glucose (black circles), glutamine (white circles), and water (black squares) in (A) 8 lean subjects, (B) 8 obese nondiabetic control subjects, and (C) 8 obese individuals with type 2 diabetes or impaired glucose tolerance. (D) Insulin area under the curve (AUC) after the ingestion of glucose (black bars), glutamine (white bars), and water (gray bars) in the 3 study groups. Data are geometric mean (1 SE range). \* $P < 0.05$ , † $P < 0.01$ , and ‡ $P < 0.001$  compared with water (paired  $t$  test). There were no differences in baseline insulin concentrations within each group between visits.

water in all groups (LEAN,  $P = 0.01$ ; OB-CON,  $P = 0.0002$ ; OB-DIAB,  $P = 0.007$ ).

**Plasma glucagon responses**

Fasting plasma glucagon concentrations were positively associated with BMI ( $P = 0.016$ ) but unrelated to the fasting plasma glucose concentration. Changes in glucagon after the ingestion of glucose, glutamine, and water are shown in **Figure 5**. Glutamine led to a marked early stimulation of glucagon release, with significant differences in the area under the curve between the glutamine and water visits observed in all 3 groups.

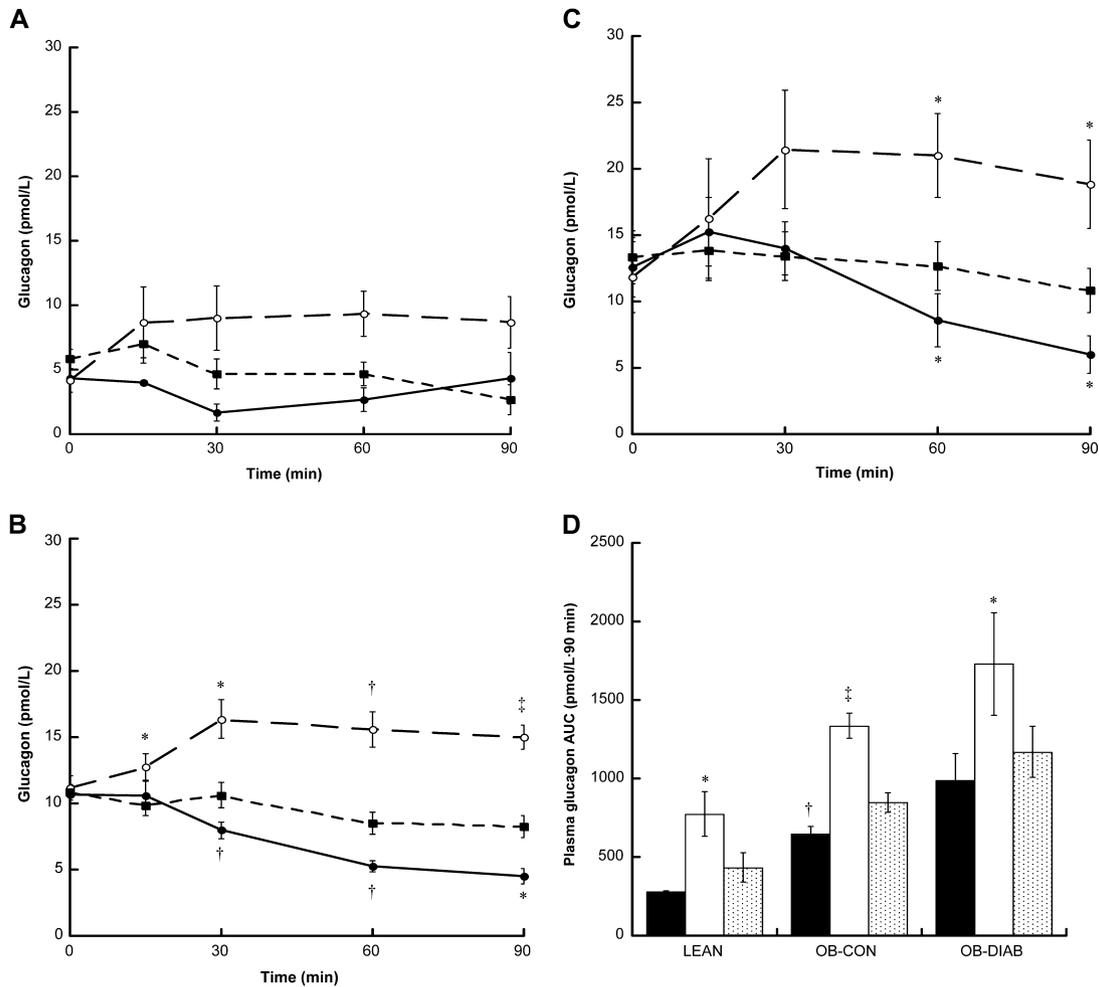
**DISCUSSION**

Consistent with our previous finding for the GLP-1-secreting cell line GLUTag that glutamine is an effective stimulus of GLP-1 secretion at physiologic concentrations (16), we show here that oral glutamine increases circulating GLP-1 concentrations in human subjects. Glutamine-triggered GLP-1 release was observed in subjects who were lean, obese, or who had type

2 diabetes, with no significant difference in the magnitude of the response between the 3 study groups. By contrast, glutamine was a relatively weak stimulus of GIP secretion, which is consistent with previous observations that GIP release is primarily triggered by the ingestion of carbohydrate and fat (26).

There are a number of reports that diabetes is associated with impaired GLP-1 release in response to glucose and, particularly, mixed meals (3–7). However, in one study, the incremental GLP-1 response to oral glucose was greater in patients with type 2 diabetes (9). In a more recent report, small intestinal administration of glucose led to comparable increases in plasma GLP-1 in patients with type 2 diabetes and control subjects (8). In our diabetic study group, we observed a lower GLP-1 response to glucose compared with the other groups. In part, this may be related to the finding of lower fasting GLP-1 concentrations in subjects with diabetes, which result from a negative association between fasting GLP-1 and BMI.

Interestingly, the GLP-1 response to glutamine was not significantly different in the diabetic group compared with the lean and obese control subjects, which suggests that it may be possible to circumvent the diabetes-associated GLP-1 secretory defect



**FIGURE 5.** Plasma glucagon concentrations after the ingestion of glucose (black circles), glutamine (white circles), and water (black squares) in (A) 3 lean subjects, (B) 8 obese nondiabetic control subjects, and (C) 7 obese individuals with type 2 diabetes or impaired glucose tolerance. (D) Glucagon area under the curve (AUC) after the ingestion of glucose (black bars), glutamine (white bars), and water (gray bars) in the 3 study groups. Data are mean  $\pm$  SE. \* $P < 0.05$ , † $P < 0.01$ , and ‡ $P < 0.001$  compared with water (paired  $t$  test). There were no differences in baseline glucagon concentrations within each group between visits.

with agents that target alternative pathways in the L-cells. The GLP-1 responses to glucose and glutamine could be divided into 2 phases: the first occurring with a peak at  $\approx 30$  min and a second extending for  $\leq 2$  h. Similar separation of GLP-1 release into early and late phases was reported previously in response to glucose or meal ingestion (4, 5, 14).

The mechanism by which glutamine stimulates GLP-1 release *in vivo* remains uncertain. In GLUTag cells, we previously showed that sodium-dependent glutamine uptake could itself act as a trigger for GLP-1 release (16). However, glutamine is also an important energy source for the gut and is metabolized via a range of pathways culminating in the production of carbon dioxide, lactate, proline, citrulline, alanine, ornithine, and glucose (27).

Glutamine is used extensively as a supplement to enteral and parenteral nutrition, because it is believed to maintain the integrity of the intestinal mucosa (17–19). It has also been shown to protect the gut from the toxic effects of radio- and chemotherapy, during which doses are 0.3–0.65 g/kg body weight (21, 22), similar to those used in the current study ( $\approx 0.25$ –0.4 g/kg). In agreement with previous dose evaluation studies (21), mean glutamine concentrations 60 min after ingestion were 1584–1854

$\mu\text{mol/L}$  in our study. Time course measurements have shown that oral glutamine doses of 0.35–0.65 g/kg result in peak concentrations at 30–60 min, with a subsequent fall to baseline over the following 180 min (21). These high doses, although somewhat unpalatable because of the limited solubility of glutamine, are well tolerated and do not cause dangerous concentrations of hyperammonemia (21). Because GLP-2 is coproduced with GLP-1 at a 1:1 molar ratio, it is tempting to speculate that part of the reported protective effect of glutamine on the gut mucosa is attributable to the release of GLP-2, the principal action of which is to stimulate the proliferation and repair of the intestinal epithelium (10).

After glutamine ingestion, insulin concentrations rose in parallel with GLP-1 in all study groups. Although this could reflect a direct effect of glutamine on pancreatic  $\beta$  cells, previous studies showed that glutamine is not a classical trigger of insulin release (28) but instead enhances secretion in the presence of other stimuli. However, further studies in humans that compare insulin concentrations in response to oral and intravenous glutamine administration with similar raised plasma glutamine concentrations are required to rule out a direct effect of circulating glutamine on the pancreatic  $\beta$  cell.



Glutamine was a potent stimulus for glucagon secretion in all groups. This likely explains the observation that plasma glucose concentrations were similar in the glutamine and water (control) arms of the study, despite stimulation of GLP-1 and insulin release after glutamine ingestion. Future studies are required to determine whether glutamine reduces postprandial glucose concentrations when ingested with a glucose load, which is a condition expected to inhibit glucagon release. The absence of an effect of raised insulin concentrations on plasma glucose may also be due to glutamine itself acting as a precursor for gluconeogenesis. Further studies will be required to ascertain whether it is possible to stimulate similar GLP-1 and insulin release with lower doses of glutamine that provide a smaller load of gluconeogenic precursors and do not stimulate glucagon release. Finally, the relatively small increase in insulin concentrations in LEAN subjects and prevailing insulin resistance in OB-CON and OB-DIAB subjects may also partly explain the absence of an effect on glucose concentrations.

Our study has limitations. First, the lack of an amino acid comparator means that we are unable to definitively conclude that the GLP-1 response to glutamine is specific to this amino acid. Although it is possible that this is a generalized amino acid effect, the *in vitro* data reported by Reimann et al (16) would argue against this suggestion. Second, because we specifically selected subjects with impaired glucose tolerance or mild type 2 diabetes, who had relatively intact  $\beta$  cell function, we are unable to comment on whether the effect of glutamine on postprandial insulinemia would be more or less pronounced in patients with a longer duration of diabetes and a greater impairment of insulin secretion.

In conclusion, together with the *in vitro* data on GLP-1 release from GLUTag cells, our results raise the possibility that nutritional supplementation with agents such as glutamine may increase GLP-1 release, enhance insulin secretion, and possibly improve glycemic control in patients with type 2 diabetes. Because patients often poorly adhere to the large numbers of medications, which are required to adequately treat chronic diseases such as type 2 diabetes, but are positively disposed toward nutritional therapies, this potential therapeutic approach to enhance insulin secretion deserves further exploration.

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## REFERENCES

- Porte D Jr. Banting lecture 1990:  $\beta$ -cells in type II diabetes mellitus. *Diabetes* 1991;40:166–80.
- Holst JJ, Deacon CF. Glucagon-like peptide 1 and inhibitors of dipeptidyl peptidase IV in the treatment of type 2 diabetes mellitus. *Curr Opin Pharmacol* 2004;4:589–96.
- Vilsboll T, Holst JJ. Incretins, insulin secretion and type 2 diabetes mellitus. *Diabetologia* 2004;47:357–66.
- Vilsboll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* 2001;50:609–13.
- Vilsboll T, Krarup T, Sonne J, et al. Incretin secretion in relation to meal size and body weight in healthy subjects and people with type 1 and type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2003;88:2706–13.
- Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7–36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest* 1993;91:301–7.
- Toft-Nielsen MB, Damholt MB, Madsbad S, et al. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 2001;86:3717–23.
- O'Donovan DG, Doran S, Feinle-Bisset C, et al. Effect of variations in small intestinal glucose delivery on plasma glucose, insulin, and incretin hormones in healthy subjects and type 2 diabetes. *J Clin Endocrinol Metab* 2004;89:3431–5.
- Orskov C, Jeppesen J, Madsbad S, Holst JJ. Proglucagon products in plasma of noninsulin-dependent diabetics and nondiabetic controls in the fasting state and after oral glucose and intravenous arginine. *J Clin Invest* 1991;87:415–23.
- Wallis K, Walters JR, Forbes A. Review article: glucagon-like peptide 2—current applications and future directions. *Aliment Pharmacol Ther* 2007;25:365–72.
- Batterham RL, Cohen MA, Ellis SM, et al. Inhibition of food intake in obese subjects by peptide YY3-36. *N Engl J Med* 2003;349:941–8.
- Batterham RL, Cowley MA, Small CJ, et al. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 2002;418:650–4.
- Stanley S, Wynne K, Bloom S. Gastrointestinal satiety signals III. Glucagon-like peptide 1, oxyntomodulin, peptide YY, and pancreatic polypeptide. *Am J Physiol Gastrointest Liver Physiol* 2004;286:G693–7.
- Herrmann C, Goke R, Richter G, Fehmann HC, Arnold R, Goke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma concentrations in response to nutrients. *Digestion* 1995;56:117–26.
- Reimann F, Ward PS, Gribble FM. Signaling mechanisms underlying the release of glucagon-like peptide 1. *Diabetes* 2006;55(suppl):S78–85.
- Reimann F, Williams L, da Silva Xavier G, Rutter GA, Gribble FM. Glutamine potently stimulates glucagon-like peptide-1 secretion from GLUTag cells. *Diabetologia* 2004;47:1592–601.
- van der Hulst RR, van Kreel BK, von Meyenfeldt MF, et al. Glutamine and the preservation of gut integrity. *Lancet* 1993;341:1363–5.
- Tremel H, Kienle B, Weilemann LS, Stehle P, Furst P. Glutamine dipeptide-supplemented parenteral nutrition maintains intestinal function in the critically ill. *Gastroenterology* 1994;107:1595–601.
- Reeds PJ, Burrin DG. Glutamine and the bowel. *J Nutr* 2001;131:2505S–8S(discussion 23S–4S).
- Anderson PM, Schroeder G, Skubitz KM. Oral glutamine reduces the duration and severity of stomatitis after cytotoxic cancer chemotherapy. *Cancer* 1998;83:1433–9.
- Ward E, Picton S, Reid U, et al. Oral glutamine in paediatric oncology patients: a dose finding study. *Eur J Clin Nutr* 2003;57:31–6.
- Ziegler TR, Benfell K, Smith RJ, et al. Safety and metabolic effects of L-glutamine administration in humans. *JPEN J Parenter Enteral Nutr* 1990;14(suppl):137S–46S.
- Ahren B, Larsson H, Holst JJ. Reduced gastric inhibitory polypeptide but normal glucagon-like peptide 1 response to oral glucose in postmenopausal women with impaired glucose tolerance. *Eur J Endocrinol* 1997;137:127–31.
- Levy JC, Matthews DR, Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 1998;21:2191–2.
- Orskov C, Rabenhøj L, Wettergren A, Kofod H, Holst JJ. Tissue and plasma concentrations of amidated and glycine-extended glucagon-like peptide I in humans. *Diabetes* 1994;43:535–9.
- Meier JJ, Nauck MA. Glucose-dependent insulinotropic polypeptide/gastric inhibitory polypeptide. *Best Pract Res Clin Endocrinol Metab* 2004;18:587–606.
- Wu G. Intestinal mucosal amino acid catabolism. *J Nutr* 1998;128:1249–52.
- Henquin JC, Dufrane D, Nenquin M. Nutrient control of insulin secretion in isolated normal human islets. *Diabetes* 2006;55:3470–7.