Gastric inhibitory polypeptide and effects of glycation on glucose transport and metabolism in isolated mouse abdominal muscle

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Abstract

This study investigates the effects of gastric inhibitory polypeptide (GIP) and glycated GIP (glucitol adduct of GIP) on glucose uptake and metabolism in muscle. Glycated GIP (molecular mass 5147.2 Da) was purified by HPLC following *in vitro* incubation under hyperglycaemic reducing conditions (24 h at pH 7.4). GIP (10^{-10} – 10^{-8} mol/l) significantly stimulated (1.4- to 1.5-fold, $P<0.001$) 2-deoxy-D-[1- 3 H]glucose uptake in abdominal muscle pieces from 3- to 5-week-old lean mice compared with control incubations (without GIP). This stimulatory effect on glucose uptake at 10^{-10} – 10^{-9} mol/l was decreased by 13–20% following glycation of the peptide ($P<0.05$). GIP (10^{-9} and 10^{-8} mol/l) induced a stepwise 1.4- to 1.7-fold increase ($P<0.01$, $P<0.001$ respectively) in [14 C]glucose oxidation compared with controls. This

effect on glucose oxidation was diminished by 32% with 10^{-8} mol/l glycated GIP ($P<0.05$). GIP (10^{-9} and 10^{-8} mol/l) induced a 1.4- to 1.8-fold increase in [14 C]glucose incorporation into muscle glycogen (glycogenesis) compared with controls. Glycated GIP (10^{-8} mol/l) exhibited a 41% decrease in glycogenic activity ($P<0.01$). GIP (10^{-10} – 10^{-8} mol/l) stimulated lactate production in isolated abdominal muscle (1.2- to 1.3-fold, $P<0.05$); however glycated GIP did not exert a significant effect. This study demonstrates for the first time that GIP promotes glucose uptake, glucose oxidation and glycogenesis in muscle tissue. Furthermore, modification of GIP through glycation diminishes its biological effectiveness.

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Introduction

Incretins are endogenous peptides released from the gastrointestinal tract into the circulation following a meal, which potentiate glucose-stimulated insulin secretion. Currently there are two well established incretin hormones, gastric inhibitory polypeptide (GIP; glucose-dependent insulinotropic polypeptide) and glucagon-like peptide-1(7–36)amide (tGLP-1). The forty-two amino acid GIP was initially isolated from porcine intestine for its ability to inhibit gastric acid secretion (Brown *et al.* 1970), but its most important physiological role resides in its insulinotropic activity (Morgan *et al.* 1988). GIP acts under mild or moderate hyperglycaemic conditions through specific receptors on the pancreatic B-cell (Wheeler *et al.* 1995) to stimulate insulin secretion and enhance proinsulin gene expression (Andersen *et al.* 1978).

GIP secretion is stimulated from the endocrine K cells of the duodenum and jejunum by the active transport of macronutrients rather than just their presence in the gut lumen (Sykes *et al.* 1980). The most important stimuli for GIP release are carbohydrate (Sykes *et al.* 1980), fat and triglycerides (Krarup *et al.* 1985, Kwasowski *et al.* 1985) and proteins (Flatt *et al.* 1991). Oral glucose is absorbed from the small intestine through an active sodium-

dependent transport mechanism resulting in a sharp rise in plasma immunoreactive GIP and insulin concentrations (Cataland *et al.* 1974). The secretory response to fats is greater and more prolonged than glucose and may reflect the fact that fat slows gastric emptying and thus provides an extended stimulus for GIP release (Penman *et al.* 1981, Pedersen 1994). Ingestion of protein alone is a relatively weak stimulus for GIP release, although nearly all amino acids possess some GIP-releasing activity (Flatt *et al.* 1991).

Studies of the biological activity of GIP have identified a number of extrapancreatic target tissues including stomach, adipose tissue and liver. GIP can inhibit gastric acid secretion in man but only at supra-physiological concentrations (Maxwell *et al.* 1980, Nauck *et al.* 1992). However, GIP has a number of other potentially important actions which mirror some of the effects of insulin. These include the stimulation of glucose uptake in adipocytes, increased synthesis of fatty acids and activation of lipoprotein lipase in adipose tissue (Eckel *et al.* 1979, Oben *et al.* 1991, Morgan 1996). Both exogenous and endogenous GIP promote plasma triglyceride clearance in response to oral fat loading, indicating that fat-induced GIP secretion plays a physiological role in lipid metabolism in adipose tissue (Ebert *et al.* 1991). In liver, GIP has been shown to enhance insulin-dependent inhibition of

glycogenolysis (Elahi *et al.* 1986). GIP also reduces both glucagon-stimulated lipolysis in adipose tissue as well as hepatic glucose production (Hartmann *et al.* 1986).

The other major incretin hormone, tGLP-1, has been reported to have potent stimulatory effects on glucose oxidation, glycogen synthesis and lactate formation in rat skeletal muscle (Villanueva-Peñacarrillo *et al.* 1994) mediated through specific tGLP-1 receptors (Delgado *et al.* 1995). The present study was carried out to investigate the possible influence of GIP on glucose uptake and metabolism in skeletal muscle. Since recent studies in our laboratory have demonstrated that peptide hormones such as proinsulin and insulin can be glycosylated during the stages of synthesis and storage in secretory cells (Abdel-Wahab *et al.* 1996a, 1997), we have also investigated whether GIP can be glycosylated. A recent study demonstrated that modification of tGLP-1 through glycosylation reduces its effectiveness at promoting glucose uptake and utilization in isolated muscle tissue (O'Harte *et al.* 1997) and in this context it was of interest to examine the efficacy of the glycosylated form of the other major incretin hormone, GIP, on muscle glucose metabolism. The present study demonstrates for the first time that GIP promotes glucose uptake and metabolism in isolated muscle from mice and that glycosylation of GIP (glucitol adduct of GIP) reduces its biological activity in muscle *in vitro*.

Materials and Methods

Reagents

HPLC grade acetonitrile was obtained from Rathburn (Walkersburn, Scotland). Sequencing grade trifluoroacetic acid (TFA) was obtained from Aldrich (Poole, Dorset, UK). All water used in these experiments was purified using a Milli-Q Water Purification System (Millipore Corporation, Millford, MA, USA). Human insulin (*E. coli* recombinant) and sodium cyanoborohydride were obtained from Sigma (Poole, Dorset, UK). Human GIP was obtained from the American Peptide Company (Sunnyvale, CA, USA). Radiolabelled 2-deoxy-D-[1-³H]glucose, D-[U-¹⁴C]glucose and L-[1-¹⁴C]glucose were obtained from Amersham Life Science (Amersham, Bucks, UK).

Preparation of glycosylated GIP

Human GIP (50 µg) was incubated with 220 mmol/l D-glucose together with a 1000-fold molar excess of NaBH₃CN (dissolved in 10 mmol/l NaOH) in 10 mmol/l sodium phosphate buffer pH 7.4 for 24 h at 37 °C (total volume 1.0 ml). Control (non-glycosylated) GIP was prepared in the same manner except for the exclusion of glucose from the incubation mixture. The reaction was stopped by addition of 0.5 mol/l acetic acid (30 µl) and the sample mixture applied to a (4.6 × 250 mm) Vydac 218TP54

(C18) analytical column (The Separations Group, Hesperia, CA, USA) and eluted at a flow rate of 1.0 ml/min. The mobile phase consisted of solvent A: 0.12% (v/v) TFA in water; solvent B consisted of 0.1% (v/v) TFA in 70% acetonitrile, 29.9% water. Elution profiles were monitored at 206 nm and peptide peaks collected by hand. GIP and glycosylated GIP were separated using linear gradients of 0% to 31.5% acetonitrile over 10 min, followed by 31.5% to 45.5% acetonitrile over 20 min. Two major peaks were collected eluting after 16.7 min and 16.9 min corresponding to glycosylated GIP and GIP respectively. Fractions of glycosylated GIP were pooled, taken to dryness using a Speed-Vac (AES 1000 Savant, Life Sciences International, Runcorn, Cheshire, UK) and reconstituted in 0.12% (v/v) TFA/water. Glycosylated GIP was purified to homogeneity by repeated HPLC runs on a (4.6 × 150 mm) Supelcosil (C8) analytical column (Supelco Inc., Poole, Dorset, UK). GIP and glycosylated GIP were quantified by comparison of peak areas obtained from reverse-phase (RP)-HPLC with a standard curve using known concentrations of GIP.

Electrospray ionization mass spectrometry (ESI-MS)

Samples of GIP and glycosylated GIP for ESI-MS were dissolved in 100 µl 0.1% (v/v) TFA/water and applied to the LC/MS using gradient elution HPLC (C18 column, 150 × 2.0 mm). Samples (10 µl injection) were applied at a flow rate of 0.2 ml/min, using a linear gradient from 0% to 90% acetonitrile over 10 min and holding at 90% acetonitrile for 10 min. Mass spectra were recorded on a Finnigan Mat LCQ benchtop mass spectrometer (Finnigan Mat, Hemel Hempstead, Herts, UK) and spectra were obtained from the quadrupole ion trap mass analyzer. Spectra were collected using full ion scan mode (400–1850 m/z mode) and UV absorbance (280 nm).

Animals

Male lean mice aged between 3 and 5 weeks were obtained from the Behavioural and Biomedical Research Unit, University of Ulster, Coleraine. The animals were housed in an air-conditioned room at 22 ± 2 °C and were fed a standard rodent maintenance diet from weaning (3 weeks) (Specialist Diet Services, Witham, Essex, UK). Food and tap water were available *ad libitum*. Animals were killed by cervical dislocation and the abdominal muscle quickly excised. The muscle was dissected into 10 pieces of approximately 10 mg each (accurately weighed) and used for either glucose uptake or metabolism studies.

Glucose uptake studies

Glucose uptake was monitored using tritiated 2-deoxy-D-[1-³H]glucose (0.1 µCi, specific activity 11 000 mCi/mmol), and non-metabolizable L-[1-¹⁴C]glucose (0.1 µCi,

specific activity 55 mCi/mmol) to correct for 2-deoxy-D-[1-³H]glucose uptake in the extracellular space. Incubations were performed in mini-scintillation vials (Fisons Scientific, Leicester, UK) containing 5 µl of each radioisotope and 1.0 ml Krebs Ringer bicarbonate buffer (KRBB, 118 mmol/l NaCl, 5 mmol/l KCl, 25 mmol/l NaHCO₃, 1.18 mmol/l MgSO₄·7H₂O, 1.17 mmol/l KH₂PO₄, 2.0 mmol/l sodium pyruvate, 1.28 mmol/l CaCl₂) and 2% (w/v) bovine serum albumin (BSA). Vials were stoppered (Subaseal, Gallencamp, Loughborough, Leics, UK) and placed in a shaking water bath (92 cycles/min) at 30 °C and gassed (95% O₂:5% CO₂) for 20–25 min until the incubation medium reached pH 7.4. Test peptide (insulin or GIP, 10 µl of 100-fold concentration) and a freshly obtained pre-weighed piece of abdominal muscle were then added. Vials were stoppered and gassed (95% O₂:5% CO₂) for the initial 2 min of incubation. After a further 28 min incubation without further gassing, tissue pieces were removed, blotted thoroughly and hydrolysed in 0.5 ml 1 mol/l NaOH at 85 °C for 60 min. Samples were cooled and HiSafe II scintillation fluid (5.0 ml, Fisons, Loughborough, UK) added and ¹⁴C/³H counted for 10 min using a dual count program (Wallac 1409 Liquid Scintillation Counter; Wallac Oy, Turke, Finland). Values for glucose uptake are expressed as d.p.m. uptake/mg wet weight muscle/h.

Glucose oxidation studies

Glucose oxidation was determined by measuring ¹⁴CO₂ production during muscle incubations. An aliquot (5 µl) of D-[U-¹⁴C]glucose (specific activity 298 mCi/mmol) corresponding to 0.5 µCi radioactivity was added to mini-scintillation vials containing 1.0 ml KRBB supplemented with BSA 2% (w/v) and 10 mmol/l glucose. An inner tube containing a narrow strip of filter paper (Whatman No. 1) was placed in each vial which was then stoppered. Vials were placed in a shaking water bath at 37 °C and gassed with 95% O₂:5% CO₂ for 20–25 min until the incubation medium reached pH 7.4. Test peptide (insulin or GIP, 10 µl of 100-fold concentration) was added to each vial followed by a weighed piece of muscle tissue. The vials were stoppered, returned to the shaking water bath and gassed for an initial 2-min period. After a total of 60 min incubation, an aliquot (100 µl) of 1 mol/l NaOH was injected through the rubber stopper directly onto the filter paper and the vials placed on ice for 5 min before removing the muscle pieces. The vials were again stoppered and 100 µl 3 mol/l perchloric acid were added through the stopper into the incubation mixture. The samples were incubated at room temperature for 60 min and the filter paper subsequently removed to a scintillation vial and 5 ml HiSafe II scintillation fluid added prior to counting the ¹⁴C for 10 min (Wallac 1409 Liquid Scintillation Counter). Values for glucose oxidation

are expressed as nmol CO₂ produced/mg wet weight muscle/h.

Glycogen production studies

Following completion of the glucose oxidation studies, muscle was removed from the vials and blotted before transfer to a fresh scintillation vial containing 1 ml 1 mol/l KOH. These vials were capped and heated at 85 °C for 30 min to hydrolyse the tissue. The hydrolysate was cooled and 2 ml 95% ethanol added with mixing. After 30 min at room temperature the samples were centrifuged at 2000 g for 10 min (Beckman model J-6B centrifuge) and the supernatant discarded. The pellet was re-extracted twice with 95% ethanol as above and the supernatants discarded. Sulphuric acid was added (1 ml, 1 mol/l) to the pellet in a screw-cap vial and heated to 85 °C for 2 h. Following cooling, approximately 0.6 ml 5 mol/l NaOH was added to neutralize the solution (pH 7.0). Scintillation fluid (5 ml HiSafe II) was added to an aliquot (0.5 ml) of the neutralised solution and ¹⁴C counted for 10 min (Wallac 1409 Liquid Scintillation Counter). The glycogen production data are expressed as nmol glucose equivalents/mg wet weight muscle/h.

Lactate assay

The solution containing perchloric acid from the glucose oxidation studies (stored at –20 °C) was neutralised (pH 7.0) with 5 mol/l NaOH and appropriate dilutions used for the lactate determinations. Lactate analysis was performed using a colorimetric LDH-based enzymatic assay (Boehringer Mannheim, Mannheim, Germany, kit 256773). NAD reagent buffer (2.1 ml containing 4.6 mmol/l NAD, 63 mmol/l glutamic acid, 0.5 mol/l Na₂HCO₃ adjusted to pH 10.0 with 2 mol/l NaOH) was added to a known volume of neutralised sample which was mixed and divided into two 1.0 ml fractions in separate cuvettes. LDH/GPT enzyme solution (10 µl) or (NH₄)₂SO₄ (10 µl) was added to the test or blank sample respectively and, after inverting, both samples were left at 25 °C for 15 min. The absorbance of test versus blank samples was recorded at 340 nm on a spectrophotometer. The difference in absorbance readings was directly proportional to the amount of lactate in the sample. The concentration of lactate was calculated using the Beer-Lambert equation ($a = \epsilon \cdot c \cdot l$) where the molar extinction coefficient for NADH is 6.31×10^3 . Lactate production was expressed as nmoles lactate/mg wet weight muscle/h.

Statistical analysis

Results are expressed as means ± S.E.M. and values were compared using the Student's unpaired *t*-test. Groups of data were considered to be significantly different if $P < 0.05$.

Figure 1 HPLC separation of GIP and glycated GIP on a Vydac C18 column (4.6 × 250 mm). Glycated and non-glycated fractions were separated using linear gradients of 0% to 31.5% acetonitrile over 10 min followed by 31.5% to 45.5% over 20 min. Two major peaks were collected manually, purified to homogeneity by repeated HPLC chromatography and the molecular masses determined by ESI-MS. The observed mass for each species is indicated in parentheses.

Results

Figure 1 shows a typical RP-HPLC trace obtained for the separation of GIP and glycated GIP on a Vydac C18 column. Following purification by RP-HPLC (Supelcosil C8), the molecular masses of GIP (M_r 4984.0) and glycated GIP (M_r 5147.2) were determined by ESI-MS. The modified peptide contained a single glucitol adduct (+163.2 Da) corresponding to monoglycated GIP, which was used for subsequent glucose metabolism studies.

Table 1 shows the effects of human insulin on glucose uptake over the concentration range tested (10^{-9} – 10^{-7} mol/l). Insulin stimulated glucose uptake in isolated mouse abdominal muscle pieces ($n=6$) compared with control samples ($n=10$, no added insulin) in a dose-dependent manner. Furthermore, results for glucose oxidation indicate that insulin stimulates ($n=17$) this process above control values ($n=17$) in a dose-dependent fashion at 10^{-8} mol/l and 10^{-7} mol/l.

Figure 2A shows the effects of GIP and glycated GIP on glucose uptake in isolated abdominal muscle. The stimulatory effect of 10^{-8} mol/l insulin is included for comparison. GIP stimulated glucose uptake ($P<0.001$) over the concentration range tested (10^{-10} – 10^{-8} mol/l) compared with controls (no additions). Glycation of GIP significantly reduced its biological activity by 13–20% compared with GIP.

Table 1 Effect of insulin (10^{-9} – 10^{-7} mol/l) on glucose uptake and oxidation in isolated abdominal muscle pieces from mice. Results are expressed as means ± S.E.M. for pairs of muscle from 3 to 5-week-old male mice ($n=6$ –10 for glucose uptake; $n=17$ for glucose oxidation)

Insulin concentration (mol/l)	Glucose uptake (d.p.m./mg tissue/h)	Glucose oxidation (nmol/ CO_2 /mg tissue/h)
Control (no insulin)	283 ± 14	0.343 ± 0.042
10^{-9}	396 ± 55*	0.486 ± 0.059
10^{-8}	482 ± 48**	0.552 ± 0.063**
10^{-7}	663 ± 76***	0.631 ± 0.057***

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with control incubations without added insulin.

Figure 2B shows the effects of non-glycated and glycated peptides on glucose oxidation in isolated mouse abdominal muscle. GIP stimulated glucose oxidation over the concentration range 10^{-9} – 10^{-8} mol/l in a dose-dependent manner compared with controls. Glycation of GIP significantly reduced its biological activity by 32% ($P<0.05$) compared with non-glycated peptide at 10^{-8} mol/l. Furthermore, glycated GIP (10^{-9} – 10^{-8} mol/l) failed to stimulate glucose oxidation above control values.

Figure 3A shows the effects of GIP and glycated GIP on glycogen production in isolated abdominal muscle. GIP stimulated glycogenesis over the concentration range (10^{-9} – 10^{-8} mol/l) compared with controls ($P<0.01$). Glycation of GIP significantly reduced its biological activity by 41% ($P<0.01$) compared with the non-glycated peptide at 10^{-8} mol/l. Moreover, glycated GIP at 10^{-9} and 10^{-8} mol/l failed to stimulate glycogen production above controls.

Figure 3B shows the effects of GIP and glycated GIP on lactate production. GIP stimulated lactate production in isolated abdominal muscle over the concentration range tested (10^{-10} – 10^{-8} mol/l) compared with controls. Glycation of GIP showed a tendency towards reduced biological activity compared with non-glycated peptide, but this reduction was not statistically significant. Glycated GIP at 10^{-8} mol/l failed to stimulate lactate production above controls, whereas 10^{-9} mol/l glycated GIP was stimulatory.

Discussion

Although the insulin-releasing action of GIP makes a major contribution to its metabolic role, direct effects of GIP at extrapancreatic sites, including stomach, liver and adipose tissue, have been suggested to contribute to the overall action of the hormone on post-prandial nutrient homeostasis (Fehmann *et al.* 1995). Our observations that

Figure 2 Effects of 10^{-8} mol/l insulin and GIP and glycated GIP (10^{-10} to 10^{-8} mol/l) on (A) glucose uptake and (B) glucose oxidation in isolated mouse abdominal muscle. Results are expressed as means $\pm$ S.E.M. for pairs of muscle from 3 to 5-week-old mice (glucose uptake: insulin $n=19$, GIP $n=19$, glycated GIP $n=10$; glucose oxidation: insulin $n=14$, GIP $n=15$, glycated GIP $n=10$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with control incubations (without added insulin). $\Delta P<0.05$ compared with GIP at the same concentration.

GIP stimulates glucose transport, oxidation, glycogenesis and lactate formation in mouse abdominal muscle support this view. These findings are paralleled by observations of

Figure 3 Effects of 10^{-8} mol/l insulin and GIP and glycated GIP (10^{-10} to 10^{-8} mol/l) on (A) glycogenesis and (B) lactate production in isolated mouse abdominal muscle. Results are expressed as means $\pm$ S.E.M. for pairs of muscle from 3 to 5-week-old mice (glycogenesis: insulin $n=13$, GIP $n=13$, glycated GIP $n=9$; lactate production: insulin $n=14$, GIP $n=15$, glycated GIP $n=10$). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared with control incubations (without added insulin). $\Delta \Delta P<0.01$ compared with GIP at the same concentration.

Villanueva-Peñacarrillo *et al.* (1994), who found that the other major incretin hormone, tGLP-1, stimulated glycogenic events in rat soleus muscle.

Physiological circulating GIP concentrations in the rat range from approximately 5×10^{-12} to 10^{-9} mol/l depending on diet (Hampton *et al.* 1983). Our results on the actions of 10^{-10} and 10^{-9} mol/l GIP on glucose

uptake, oxidation, glycogenesis and lactate formation indicate that GIP exerts effects on muscle within the range of reported circulating physiological peptide concentrations. Receptors for GIP have been described in a number of tissues including human insulinomas (Maletti *et al.* 1986) as well as in endocrine pancreas, stomach, proximal small intestine, adipose tissue, adrenal gland, brain and pituitary (Usdin *et al.* 1993, Moens *et al.* 1996) but not to our knowledge in skeletal muscle. Kaplan and Vigna (1994) demonstrated saturable ^{125}I -GIP binding to sections of rat brain but were unable to show any radiolabelled-GIP binding to other rat tissues including rat skeletal muscle. Furthermore, others have failed to show any effect of GIP either in the presence or absence of insulin on 2-deoxy-D-glucose uptake in cultured ovine muscle cells (Roe *et al.* 1995). Taken together these findings tend to suggest that rat skeletal muscle does not contain specific receptors to GIP.

In contrast, our findings suggest that the action of GIP on glucose metabolism in isolated muscle may be as effective as equimolar concentrations of insulin. However, it should be noted that such effects of GIP are probably not recorded in the complete absence of insulin since isolated muscle preparations will potentially contain some residual insulin which could be involved in receptor binding and glycogenic effects. Interestingly, it has been reported (Villanueva-Peöacarrillo *et al.* 1994) that the direct effect of tGLP-1 on glycogen synthesis in skeletal muscle was similar to the action of insulin, involving an increase in glycogen synthase *a* activity but no change in glycogen phosphorylase *a* activity (Marchand-Brustel & Freychet 1981). Other observations with cultured BC3H-1 myocytes suggest that tGLP-1 may stimulate degradation of glycosyl-phosphatidylinositols into inositolphosphoglycans and diacylglycerol which act as second messengers in muscle tissue (Galera *et al.* 1996). Further studies are required to evaluate possible involvement of these mechanisms in the actions of GIP in skeletal muscle.

In addition to demonstrating significant glycogenic actions of GIP on muscle for the first time, the present study also indicates that the structurally modified monoglycated peptide (i.e. glucitol adduct of GIP) has a reduced ability to promote glucose uptake and metabolism in this tissue. Other recent studies have shown that the insulin-releasing potency of GIP on clonal BRIN-BD11 cells is also modified by glycation (Abdel-Wahab *et al.* 1996b). Structure-function studies have indicated that the insulinotrophic effectiveness of GIP is associated with Tyr¹, Ala², Gly⁴, Asp⁹, Ser¹¹ as well as C-terminal portions of the GIP molecule (Maletti *et al.* 1986, Schmidt *et al.* 1986, Pedersen *et al.* 1990, Suzuki *et al.* 1992). Potential sites of glycation within GIP include the amino-terminal Tyr¹ residue as well as five ϵ -amino groups at Lys residues 16, 30, 32, 33 and 37. Post-translation modification of GIP by glycation at the NH₂-terminal or C-terminal regions of the peptide could account for the reduction in biological

activity observed here. However, preliminary data obtained from attempted primary sequence determination by automated Edman degradation indicates that the glycated GIP is not modified at the amino terminus (F M O'Harte, J M Conlon & P R Flatt, unpublished observations) which contrasts with previous findings on the identification of the site of glycation within the insulin B-chain (O'Harte *et al.* 1996). Consequently, further studies are required to identify the exact locus of glycation within the human GIP molecule.

Recent studies in our laboratory have established that proinsulin and insulin are glycated in the pancreatic B-cells in diabetes (Abdel-Wahab *et al.* 1996a) and that glycation impairs the glucose lowering activity of insulin in both experimental animals and man (Flatt *et al.* 1994, Hunter *et al.* 1996). The present *in vitro* observations with glycated GIP raise questions concerning possible implications for the physiology of GIP and role in the pathophysiology of non-insulin-dependent diabetes mellitus. Further studies are required to determine the extent of glycation of GIP in the glucose-rich environment of the intestinal endocrine K cell and the significance of extrapancreatic metabolic actions of GIP.

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References

- Abdel-Wahab YHA, O'Harte FPM, Ratcliff H, McClenaghan NH, Barnett CR & Flatt PR 1996a Glycation of insulin in the islets of Langerhans of normal and diabetic animals. *Diabetes* **45** 1489–1496.
- Abdel-Wahab YHA, O'Harte FPM, Gray AM & Flatt PR 1996b Glycation of GLP-1(7–36)amide but not GIP reduces its insulinotropic action *in vitro*. *Diabetologia* **39** A23.
- Abdel-Wahab YHA, O'Harte FPM, Barnett CR & Flatt PR 1997 Characterization of insulin glycation in insulin-secreting cells maintained in tissue culture. *Journal of Endocrinology* **152** 59–67.
- Andersen DK, Elahi D, Brown JC, Tobin JD & Andres R 1978 Oral glucose augmentation of insulin secretion. Interactions of gastric inhibitory polypeptide with ambient glucose and insulin levels. *Journal of Clinical Investigation* **62** 152–161.
- Brown JC, Mutt V & Pederson RA 1970 Further purification of a polypeptide demonstrating enterogastrone activity. *Journal of Physiology* **209** 57–64.
- Cataland S, Crockett SE, Brown JC & Mazzaferri EL 1974 Gastric inhibitory polypeptide (GIP) stimulation by oral glucose in man. *Journal of Clinical Endocrinology and Metabolism* **39** 223–228.
- Delgado E, Luque MA, Alcántara AI, Trapote MA, Clemente F, Galera C, Valverde I & Villanueva-Peñacarrillo ML 1995 Glucagon-like peptide-1 binding to rat skeletal muscle. *Peptides* **16** 225–229.

- Ebert R, Nuack M & Creutzfeldt W 1991 Effects of exogenous and endogenous gastric inhibitory polypeptide on plasma triglyceride responses in rats. *Hormone and Metabolic Research* **23** 517–521.
- Eckel RH, Fujimoto WJ & Brunzell JD 1979 Gastric inhibitory polypeptide enhances lipoprotein lipase activity in cultured pre-adipocytes. *Diabetes* **28** 1141–1142.
- Elahi D, Meneilly GS, Minaker KL, Rowe JW & Andersen DK 1986 Regulation of hepatic glucose production by gastric inhibitory polypeptide in man. In *Proceedings of the Sixth International Conference on Gastrointestinal Hormones, Vancouver, BC*, p 18. Ottawa: National Research Council of Canadian Research Journals.
- Fehmann H-C, Göke R & Göke B 1995 Cell and molecular biology of the incretin hormones glucagon-like peptide-1 and glucose-dependent insulin releasing polypeptide. *Endocrine Review* **16** 390–410.
- Flatt PR, Kwasowski P, Howland RJ & Bailey CJ 1991 Gastric inhibitory polypeptide and insulin responses to orally administered amino acids in genetically obese hyperglycaemic (ob/ob) mice. *Journal of Nutrition* **121** 1123–1128.
- Flatt PR, Abdel-Wahab YHA, Wilson AM, Barnett CR, O'Harte FPM & Swanson-Flatt SK 1994 Site-specific defects and glucose toxicity in pancreatic B-cell dysfunction of non-insulin dependent diabetes mellitus. In *Frontiers of Insulin Secretion and Pancreatic B-cell Research*, pp 481–489. Eds PR Flatt & S Lenzen. London: Smith-Gordon & Co. Ltd.
- Galera C, Clemente F, Alcántara A, Trapote MA, Perea A, López-Delgado MI, Villanueva-Peñacarrillo ML & Valverde I 1996 Inositolphosphoglycans and diacylglycerol are possible mediators of the glycogenic effect of GLP-1(7–36)amide in BC3H-1 myocytes. *Cell Biochemistry and Function* **14** 43–48.
- Hampton SM, Kwasowski P, Tan K, Morgan LM & Marks V 1983 Effect of pre-treatment of a high fat diet on the gastric inhibitory polypeptide and insulin responses to oral triolein and glucose in rats. *Diabetologia* **24** 278–281.
- Hartmann H, Ebert R & Creutzfeldt W 1986 Insulin-dependent inhibition of hepatic glycogenolysis by gastric inhibitory polypeptide (GIP) in perfused rat liver. *Diabetologia* **29** 112–114.
- Hunter SJ, Wiggam MI, Boyd AC, O'Harte FPM, Ennis CN, Gamble R, Sheridan B, Barnett CR, McNulty H, Bell PM & Flatt PR 1996 Glycation of human insulin reduces its biological potency as assessed by euglycaemic clamps in normal man. *Diabetic Medicine* **13** (Suppl 7) S17 (Abstract).
- Kaplan AM & Vigna SR 1994 Gastric inhibitory polypeptide (GIP) binding sites in rat brain. *Peptides* **15** 297–302.
- Krarup T, Holst JJ & Larsen KL 1985 Responses and molecular heterogeneity of IR-GIP after intraduodenal glucose and fat. *American Journal of Physiology* **249** E195–E200.
- Kwasowski P, Flatt PR, Bailey CJ & Marks V 1985 Effect of fatty acid chain length and saturation on gastric inhibitory peptide release in obese hyperglycaemic (ob/ob) mice. *Bioscience Reports* **5** 701–705.
- Maletti M, Portha B, Hoa DHB, Carlquist M & Rosselin G 1986 Evidence of functional gastric inhibitory polypeptide (GIP) receptors in human insulinoma. Binding of synthetic human GIP 1–31 and activation of adenylate cyclase. *Diabetes* **36** 1336–1340.
- Marchand-Brustel YM & Freychet P 1981 Regulation of glycogen synthase activity in the isolated mouse soleus muscle. Effect of insulin, epinephrine, glucose and anti-insulin receptor antibodies. *Biochimica et Biophysica Acta* **677** 13–22.
- Maxwell V, Shulkes A, Brown JC, Solomon TE, Walsh JH & Grossman MI 1980 Effect of gastric inhibitory polypeptide on pentagastrin-stimulated acid secretion in man. *Digestive Diseases and Science* **25** 113–116.
- Moen K, Heimberg H, Flamez D, Huypens P, Quartier E, Ling Z, Pipeleers D, Gremlich S, Thorens B & Schuit F 1996 Expression and functional activity of glucagon, glucagon-like peptide 1, and glucose-dependent insulinotropic peptide receptors in rat pancreatic islet cells. *Diabetes* **45** 257–261.
- Morgan LM 1996 The metabolic role of GIP: physiology and pathology. *Biochemical Society Transactions* **24** 585–591.
- Morgan LM, Flatt PR & Marks V 1988 Nutrient regulation of the entero-insular axis and insulin secretion. *Nutrition Research Review* **1** 79–97.
- Nauck MA, Bartels E, Orskov C, Ebert R & Creutzfeldt W 1992 Lack of effect of synthetic human gastric inhibitory polypeptide and glucagon-like peptide-1(3–36)amide infused at near physiological concentrations on pentagastrin-stimulated gastric acid secretion in normal human subjects. *Digestion* **52** 214–221.
- Oben J, Morgan L, Fletcher J & Marks V 1991 Effect of the entero-pancreatic hormones gastric inhibitory polypeptide and glucagon-like peptide-1(7–36)amide on fatty acid synthesis in explants of rat adipose tissue. *Journal of Endocrinology* **130** 267–272.
- O'Harte FPM, Höjrup P, Barnett CR & Flatt PR 1996 Identification of the site of glycation of human insulin. *Peptides* **17** 1323–1330.
- O'Harte FPM, Gray AM, Abdel-Wahab YHA & Flatt PR 1998 Effects of non-glycated and glycated glucagon-like peptide-1(7–36) amide on glucose metabolism in isolated mouse abdominal muscle. *Peptides* **18** (In Press).
- Pedersen RA 1994 Gastric inhibitory polypeptide. In *Gut Peptides: Biochemistry and Physiology*, pp 217–259. Eds JH Walsh & GJ Dockray. New York: Raven Press Ltd.
- Pedersen RA, Mochizuki T, Yanaihara C, Yanihara N & Brown JC 1990 Dissociation of the somatostatinotropic and insulinotropic actions of GIP by fragments of GIP_[1–30]-NH₂ and GIP_[1–30]OH. *Digestion* **46** 86.
- Penman E, Wass JAH, Medbank S, Morgan LM, Lewis J, Besser GM & Rees LH 1981 Response of circulating somatostatin to nutritional stimuli in normal subjects. *Gastroenterology* **81** 692–699.
- Roe JA, Baba ASH, Harper JMM & Buttery PJ 1995 Effects of growth factors and gut regulatory peptides on nutrient uptake in ovine muscle cell cultures. *Comparative Biochemistry and Physiology* **110** 107–114.
- Schmidt WE, Siegel EG, Ebert R & Creutzfeldt W 1986 N-terminal tyrosine-alanine is required for the insulin releasing activity of glucose-dependent insulinotropic polypeptide. *European Journal of Clinical Investigation* **16** A9.
- Suzuki S, Kawai K, Ohashi S, Watanabe Y & Yamashita K 1992 Comparison of the insulinotropic activity of the glucagon-superfamily peptides in rat pancreas perfusion. *Hormone and Metabolic Research* **24** 458–461.
- Sykes S, Morgan LM, English J & Marks V 1980 Evidence for preferential stimulation of gastric inhibitory polypeptide secretion in the rat by actively transported carbohydrates and their analogues. *Journal of Endocrinology* **85** 201–207.
- Usdin TB, Mezey E, Button DC, Brownstein MJ & Bonner TI 1993 Gastric inhibitory polypeptide receptor, a member of the secretin vasoactive polypeptide receptor family, is widely distributed in peripheral organs and the brain. *Endocrinology* **133** 2861–2870.
- Villanueva-Peñacarrillo ML, Alcántara AL, Clemente F, Delgado E & Valverde I 1994 Potent glycogenic effect of GLP-1(7–36)amide in rat skeletal muscle. *Diabetologia* **37** 1163–1166.
- Wheeler MB, Gelling RW, McIntosh CHS, Georgioi J, Brown J & Pedersen RA 1995 Functional expression of the rat pancreatic islet glucose-dependent insulinotropic polypeptide receptor: ligand binding and intracellular signalling properties. *Endocrinology* **136** 4629–4639.

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