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To cite this version:
Barry D. Kerr, Nigel Irwin, Finbarr P.M. O’Harte, Clifford J. Bailey, Peter R. Flatt, et al.. Fatty acid derivatised analogues of glucose-dependent insulinoactive polypeptide with improved antihyperglycaemic and insulinoactive properties. Biochemical Pharmacology, Elsevier, 2009, 78 (8), pp.1008. 10.1016/j.bcp.2009.05.037. hal-00514597

HAL Id: hal-00514597
https://hal.archives-ouvertes.fr/hal-00514597
Submitted on 3 Sep 2010

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Fatty acid derivatised analogues of glucose-dependent insulinotropic polypeptide with improved antihyperglycaemic and insulinotropic properties

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ABSTRACT

C-terminal acylation of Lys\textsuperscript{37} with myristic (MYR; tetradecanoic acid), palmitic (PAL; hexadecanoic acid) and stearic (octadecanoic acid) fatty acids with or without N-terminal acetylation was employed to develop long-acting analogues of the gluoregulatory hormone, glucose-dependent insulinotropic polypeptide (GIP). All GIP analogues exhibited resistance to dipeptidylpeptidase-IV (DPP-IV) and significantly improved \textit{in vitro} cAMP production and insulin secretion. Administration of GIP analogues to \textit{ob/ob} mice significantly lowered plasma glucose - GIP(Lys\textsuperscript{37}MYR), \textit{N}-AcGIP(Lys\textsuperscript{37}MYR) and GIP(Lys\textsuperscript{37}PAL) increased plasma insulin concentrations. GIP(Lys\textsuperscript{37}MYR) and \textit{N}-AcGIP(Lys\textsuperscript{37}MYR) elicited protracted glucose-lowering effect when administered 24h prior to an intraperitoneal glucose load. Daily administration of GIP(Lys\textsuperscript{37}MYR) and \textit{N}-AcGIP(Lys\textsuperscript{37}MYR) to \textit{ob/ob} mice for 24 days decreased glucose and significantly improved plasma insulin, glucose tolerance and beta-cell glucose responsiveness. Insulin sensitivity, pancreatic insulin content and triglyceride levels were not changed. These data demonstrate that C-terminal acylation particularly with myristic acid provides a class of stable, longer-acting forms of GIP for further evaluation in diabetes therapy.

Keywords: dipeptidylpeptidase-IV (DPP-IV); glucose-dependent insulinotropic polypeptide (GIP); GIP agonist; glucose homeostasis; insulin secretion.
1. Introduction

Glucose-dependent insulinotropic polypeptide (GIP) is a gluco-regulatory hormone released from K-cells in response to feeding [1]. Despite initial characterisation based on its ability to inhibit gastric acid secretion, GIP, together with its sister hormone, glucagon-like peptide-1 (GLP-1), are now widely recognised as incretin hormones [2]. GIP and GLP-1 account for the total insulinotropic arm of the enteroinsular axis following meal ingestion, thereby reducing postprandial hyperglycaemia [3]. In contrast to currently used non-endogenous drugs, both GIP and GLP-1 elicit their potent insulinotropic actions in a glucose-dependent manner and as such avoid unwanted hypoglycaemic episodes. While GLP-1 based therapies, for example, exenatide, have now reached the market and are currently being prescribed to type 2 diabetic patients, the progression of GIP-based molecules has been somewhat hindered due to several fundamental limitations [4].

Firstly, insensitivity of the beta-cell to the insulin-releasing actions of GIP in type 2 diabetes has been noted [5,6]. However, it has emerged that rather than representing a GIP specific defect, this is a readily reversible phenomenon that can be rectified by improved diabetes control using other glucose-lowering drugs [7,8]. Thus combination therapy or use of modified GIP analogues can be expected to overcome any beta-cell insensitivity. Indeed the insulin-releasing and anti-diabetic potential of several amino-terminally modified GIP analogues have been demonstrated both in animal models and in preliminary studies in patients with type 2 diabetes [9]. Secondly, the pharmacokinetic profile of the native GIP hormone is severely compromised through rapid proteolytic degradation with the enzyme dipeptidylpeptidase-IV (DPP-IV; EC 3.4.14.5) generating the inactive major degradation product GIP(3-42) [10]. Thirdly, GIP and its associated metabolites are quickly eliminated and cleared from the body primarily via the kidney [11].
Furthermore, present appreciation of the role of circulating GIP in clinical diabetes and particularly fat deposition is far from complete [4].

These intrinsic limitations can be overcome through two different approaches including the application of specific DPP-IV inhibitors or the development of structurally modified GIP molecules which are resistant to the actions of DPP-IV [12]. Indeed, a range of structurally modified analogues of GIP have been developed, several of which have improved antihyperglycaemic and insulinotropic properties [4]. Recently, we have shown that GIP conjugated at the C-terminus with a mini-polyethylene glycol residue exhibited prolonged and enhanced biological actions in vivo [13]. However, another approach to avoid rapid renal filtration and clearance from the body is fatty acid derivatisation. Studies in our laboratory have shown that conjugating a C-16 fatty acid to the epsilon side-chain of Lys at position 37 resulted in significant benefits in both glucose-lowering and insulin-releasing actions [14-18]. Fatty acid derivatisation prolongs the biological half-life of GIP by masking the DPP-IV cleavage sight, but also facilitates binding to serum proteins thus reducing renal clearance [15,16]. Indeed, this approach has been employed in the development of several GLP-1 based therapies, for example, NN2211, which has been shown to elicit a prolonged pharmacodynamic profile (half-life >12h) making it suitable for once-daily subcutaneous administration [19]. However, unlike GIP-based mimetics, prolonged administration of NN2211 and GLP-1 mimetics in general leads to gastrointestinal side-effects such as nausea due to a prolonged inhibitory effect on gastric emptying [9].

To date, only the biological efficacy of C-16 fatty acid derivatised GIP analogues have been reported [14-18]. Therefore, in the present study we characterised the effects of a range of fatty acid chain lengths (C-14, C-16 and C-18) with or without N-terminal acetylation on metabolic
stability and biological actions. Stability of GIP analogues to DPP-IV degradation, as well as in vitro cAMP production and insulin secretion were examined. Furthermore, acute and prolonged actions of selected analogues were assessed in ob/ob mice, a commonly employed animal model of type 2 diabetes displaying both beta-cell dysfunction and insulin resistance.

2. Materials and methods

2.1 Synthesis, purification and characterisation of GIP peptides

GIP peptides (see Table 1) were sequentially synthesised on an Applied BioSystems automated peptide synthesiser (ABI 432A Peptide Synthesiser; Warrington, Cheshire, UK) with a preloaded Fmoc-Gln(Trt)-Wang resin (typical loading 0.2-1.0 mmol/g; Sigma-Aldrich, Gillingham, Dorset, UK) using standard solid-phase Fmoc peptide chemistry [21]. Synthetic peptides were purified by semi-preparative HPLC followed by analytical HPLC and characterised using matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry as described previously [16].

2.2. Degradation of GIP peptides by DPP-IV

GIP peptides were incubated at 37°C in 50 mM triethanolamine-HCl (pH 7.8) with purified porcine dipeptidylpeptidase-IV (5 mU; Sigma-Aldrich, UK) for 0, 2, 4, 8 and 24 h (no albumin was present in the incubation buffer). Enzymatic reactions were terminated by addition of 15 μl of 10% (v/v) TFA/water. Reaction products were applied to a Vydac C-4 column (4.6 x 250mm; The Separations Group, Hesparia, California, USA) and intact peptide separated from the major degradation fragment GIP(3-42). Absorption was monitored at 206 nm using a SpectraSystem UV2000 detector (Thermoquest Ltd, Manchester, UK) and HPLC peak area data were used to calculate percentage intact peptide remaining at time points indicated during the incubation.
2.3. In vitro cAMP production and insulin secretion

The effects of GIP peptides on the stimulation of cAMP production and insulin secretion were measured using clonal pancreatic BRIN-BD11 cells as described previously [22]. For cAMP studies, BRIN-BD11 cells were seeded (100,000 cells per well) into 96-well plates (Nunc, Roskilde, Denmark) and washed with HBS buffer before being incubated with various concentrations of GIP peptides in the presence of 1 mM IBMX (Sigma-Aldrich, UK) for 20 min at 37°C. After incubation, medium was removed and the cells lysed prior to measurement of cAMP using an HTS Immunoassay Kit (Millipore, Watford, UK). For insulin-release studies, BRIN-BD11 cells were seeded (100,000 cells per well) into 24-well plates (Nunc, Roskilde, Denmark) and allowed to attach overnight at 37°C. Following a 40 min pre-incubation (1.1 mM glucose; 37°C), cells were incubated (20 min; 37°C) in the presence of 5.6 mM glucose with a range of peptide concentrations (estimated albumin concentration 76.5 μM). After 20 min incubation, buffer was removed from each well and aliquots (200 μl) stored at -20°C prior to measurement of insulin using radioimmunoassay.

2.4. Acute and persistent effects of GIP peptides on glucose-lowering and insulin release in vivo

Obese diabetic (ob/ob) mice and lean littermates from the Aston colony at 20-22 weeks of age were used to assess the in vivo biological properties of GIP peptides. The genetic background and characteristics of the colony used have been described in detail elsewhere [20]. Animals were age-matched, divided into groups and housed in an air-conditioned room at 22 ± 2°C with a 12 h light / 12 h dark cycle. Animals had free access to drinking water and normal laboratory chow (Trouw Nutrition, Cheshire, UK). All animal experiments were performed in accordance with the
UK Animals (Scientific Procedures) Act 1986. No adverse effects were observed following administration of any of the treatments.

In a first series of experiments, overnight fasted normal mice received an intraperitoneal (ip) injection of saline (0.9% (w/v) NaCl) or glucose (18 mmol/kg body weight) alone or in combination with GIP or N-AcGIP(Lys\textsuperscript{37}MYR) (each at 25 nmol/kg body weight). In a second set of experiments, overnight fasted \textit{ob/ob} mice received an ip injection of glucose alone (18 mmol/kg body weight) or in combination with GIP peptides (each at 25 nmol/kg body weight). In a third series of experiments, glucose (18 mmol/kg body weight; ip) was administered 24 and 48 h after injection of GIP peptides (each at 25 nmol/kg body weight) to \textit{ob/ob} mice. All test solutions were administered in a final volume of 8 ml/kg body weight.

2.5. Sub-chronic effects of once daily administration of GIP peptides in \textit{ob/ob} mice

Once-daily ip injections of N-AcGIP, GIP(Lys\textsuperscript{37}MYR) or N-AcGIP(Lys\textsuperscript{37}MYR) (each at 25 nmol/kg body weight) or saline vehicle (0.9% (w/v) NaCl) were administered at 16:00 h over a 24-day treatment period. Food intake, body weight, non-fasting plasma glucose and insulin levels were monitored at intervals of 2-4 days. Glucose tolerance (18 mmol/kg body weight; ip) and insulin sensitivity (50 U/kg body weight; ip) tests were performed at the end of the study. At termination, blood for measurement of circulating triglycerides was taken and pancreatic tissues excised and processed for measurement of insulin following extraction with 5 ml/g ice-cold acid ethanol.

2.6. Biochemical analyses

Blood samples were collected from the cut tip on the tail vein of conscious mice into chilled fluoride/heparin glucose micro-centrifuge tubes (Sarstedt, Numbrecht, Germany) at the
timepoints indicated in the figures. Samples were immediately centrifuged using a Beckman microcentrifuge (Beckman Instruments, Galway, Ireland) for 30 s at 13,000 x g. Plasma glucose was assayed by an automated glucose oxidase procedure [23] using a Beckman Glucose Analyser II (Beckman Instruments, Galway, Ireland). Plasma and pancreatic insulin was assayed by a modified dextran-coated charcoal radioimmunoassay as described previously [24]. Plasma triglyceride levels were measured using a Hitachi Automatic Analyser 912 (Boehringer Mannheim, Germany) as per manufacturer instructions.

2.7. Statistical analysis

Results are expressed as mean ± SEM and data compared using the unpaired Student’s t-test. Where appropriate, data were compared using repeated measures ANOVA or one-way ANOVA, followed by the Student-Newman-Keuls post-hoc test. Incremental area under the curve (AUC) analyses for plasma glucose and insulin were calculated using GraphPad Prism version 3.02. Groups of data were considered to be significantly different if p < 0.05.

3. Results


Following solid-phase peptide synthesis and HPLC purification, the molecular mass of each peptide was determined using MALDI-TOF MS (Table 1). The molecular masses resolved for GIP peptides were: 5194.2 Da, GIP(Lys\textsuperscript{37}MYR); 5236.4 Da, N-AcGIP(Lys\textsuperscript{37}MYR); 5219.1 Da, GIP(Lys\textsuperscript{37}PAL); 5263.3 Da, N-AcGIP(Lys\textsuperscript{37}PAL); 5249.8 Da, GIP(Lys\textsuperscript{37}STE); and 5292.8 Da, N-AcGIP(Lys\textsuperscript{37}STE). Peptides were purified to >95% purity and molecular masses correlated well with predicted theoretical molecular weights, thereby substantiating molecular identity. Also shown in Table 1, native GIP was rapidly hydrolysed in vitro by DPP-IV with an estimated half-
life of 2.2 h. In contrast, GIP analogues remained fully intact throughout the entire incubation period exhibiting half-lives of >24 h (Table 1).

3.2. In vitro cAMP production and insulin secretion

Native GIP stimulated intracellular cAMP production in a concentration-dependent manner in BRIN-BD11 cells with an EC_{50} value of 0.70 nM. GIP analogues followed a similar pattern of concentration-dependent stimulation of cAMP production but exhibited improved EC_{50} values (0.07 to 0.27 nM; Table 1). Consistent with effects on cAMP production, native GIP dose-dependently stimulated insulin secretion (1.5 to 2.1-fold; p < 0.05) in BRIN-BD11 cells compared to control (5.6 mM glucose alone) incubations (Fig. 1). Similarly, GIP analogues dose-dependently stimulated insulin secretion but with enhanced potency compared to control (2.8 to 4.0-fold; p < 0.001) and to GIP (1.5 to 2.1-fold; p < 0.01 to p < 0.001). No significant differences were observed in insulin-releasing potency between GIP analogues.

3.3. Acute effects of GIP peptides on glucose-lowering and insulin release in vivo.

The acute effects of native GIP and a fatty acid modified GIP analogue, N-AcGIP(Lys^{37}MYR), on glucose-lowering and insulin release in the absence and presence of a glucose load are shown in Fig. 2. When native GIP and N-AcGIP(Lys^{37}MYR) were administered in combination with saline vehicle to normal mice, no significant effects on plasma glucose and insulin concentrations were observed (Fig. 2A). However, in the presence of a glucose load (18 mmol/kg body weight), N-AcGIP(Lys^{37}MYR) significantly lowered plasma glucose (1.2 to 1.7-fold; p < 0.05 to p < 0.01) and increased plasma insulin concentrations (1.3 to 1.8-fold; p < 0.05 to p < 0.01) compared to GIP-treated animals (Fig. 2B).

Fig. 3 depicts the plasma glucose responses to ip administration of glucose alone or in combination with GIP peptides to ob/ob mice. Administration of glucose alone resulted in a rapid
and protracted rise in plasma glucose concentrations. The plasma glucose excursion following administration of native GIP was not significantly different to animals receiving glucose alone (control). However, administration of GIP analogues resulted in significantly reduced plasma glucose concentrations at all time points measured. This was corroborated by analysis of the area under the curve AUC (0-60 min) where GIP analogues exhibited significantly lower plasma glucose concentrations compared to glucose alone (1.2 to 1.4-fold; p < 0.05 to p < 0.001) and animals treated with GIP (1.2 to 1.4-fold; p < 0.05 to p < 0.001). The improved glucose-lowering action correlated with significantly elevated plasma insulin concentrations following administration of GIP(Lys\textsuperscript{37}MYR), N-AcGIP(Lys\textsuperscript{37}MYR) and GIP(Lys\textsuperscript{37}PAL) compared to control (1.2 to 1.8-fold; p < 0.05 to p < 0.001) and GIP-treated animals (1.2 to 1.8-fold; p < 0.05 to p < 0.001; Fig. 4). In contrast, N-AcGIP, N-AcGIP(Lys\textsuperscript{37}PAL), GIP(Lys\textsuperscript{37}STE) and N-AcGIP(Lys\textsuperscript{37}STE) did not demonstrate significantly improved insulinotropic actions under conditions used (Fig. 4).

3.4. Persistent effects of GIP peptides on glucose-lowering in ob/ob mice

As illustrated in Fig. 5, the glucose-lowering action of GIP(Lys\textsuperscript{37}MYR) and N-AcGIP(Lys\textsuperscript{37}MYR) is clearly evident when administered 24 h prior to an ip glucose load. GIP(Lys\textsuperscript{37}MYR) and N-AcGIP(Lys\textsuperscript{37}MYR) significantly lowered plasma glucose at 15 and 60 min post injection compared to control (1.2 to 1.3 fold; p < 0.01 to p < 0.001) and N-AcGIP treated mice (1.1 to 1.2 fold; p < 0.05 to p < 0.01). These results were supported by significantly decreased plasma glucose AUC values compared to control (1.2 fold; p < 0.05) and N-AcGIP treated mice (1.1 to 1.2 fold; p < 0.05 to p < 0.01). In contrast, GIP(Lys\textsuperscript{37}PAL), N-AcGIP(Lys\textsuperscript{37}PAL), GIP(Lys\textsuperscript{37}STE) and N-AcGIP(Lys\textsuperscript{37}STE) demonstrated similar glycaemic profiles to control animals (Fig. 5).
3.5. Effects of daily administration of N-AcGIP, GIP(Lys$^{37}$MYR) and N-AcGIP(Lys$^{37}$MYR) on bodyweight, food intake, non-fasting glucose and insulin concentrations in ob/ob mice

Daily administration of N-AcGIP, GIP(Lys$^{37}$MYR) and N-AcGIP(Lys$^{37}$MYR) for 24 days had no effect on body weight and food intake compared to saline-treated controls (Fig. 6A and 6B). Non-fasting plasma glucose concentrations were significantly lower in GIP(Lys$^{37}$MYR) (1.4 to 1.5-fold; $p < 0.05$ to $p < 0.01$) and N-AcGIP(Lys$^{37}$MYR) (1.5 to 1.7-fold; $p < 0.01$ to $p < 0.001$) treated mice compared to saline-treated controls from Day 20 onwards (Fig. 6C). Furthermore, N-AcGIP(Lys$^{37}$MYR) treated mice exhibited significantly reduced plasma glucose concentrations (1.4-fold; $p < 0.05$) compared to N-AcGIP treated animals. This reduced plasma glucose was correlated with significantly increased plasma insulin concentrations by Day 20 in GIP(Lys$^{37}$MYR) (1.4 to 1.9-fold; $p < 0.01$ to $p < 0.001$) and N-AcGIP(Lys$^{37}$MYR) (1.6 to 2.2-fold; $p < 0.01$ to $p < 0.001$) treated mice compared to saline controls and N-AcGIP treated animals (Fig. 6D).

3.6. Effects of daily administration of N-AcGIP, GIP(Lys$^{37}$MYR) and N-AcGIP(Lys$^{37}$MYR) on glucose tolerance and plasma insulin response to glucose in ob/ob mice

As shown in Fig. 7A, daily administration of GIP(Lys$^{37}$MYR) and N-AcGIP(Lys$^{37}$MYR) for 24 days resulted in an improved glycaemic response to an ip glucose load with significantly decreased plasma glucose concentrations compared to saline control (1.4 to 1.5 fold; $p < 0.001$) and N-AcGIP (1.3 to 1.4 fold; $p < 0.05$ to $p < 0.01$). The beneficial effects of GIP(Lys$^{37}$MYR) and N-AcGIP(Lys$^{37}$MYR) were particularly evident from plasma glucose AUC values (0-60 min), which was significantly reduced compared to saline control (1.4 and 1.5 fold, respectively; $p < 0.01$) (Fig. 7A). Glucose-mediated plasma insulin concentrations were significantly elevated (1.5 to 4.4 fold; $p < 0.01$ to $p < 0.001$) for both GIP(Lys$^{37}$MYR) and N-AcGIP(Lys$^{37}$MYR) compared to both saline and N-AcGIP treated animals (Fig. 7B).
3.7. Effects of daily administration of $N$-AcGIP, GIP(Lys$^{37}$MYR) and $N$-AcGIP(Lys$^{37}$MYR) on insulin sensitivity, pancreatic insulin content and circulating plasma triglyceride concentrations in $ob/ob$ mice

As shown in Table 2, plasma glucose AUC values were not significantly changed in any of the treatment groups following administration of exogenous insulin. Similarly, pancreatic insulin content and circulating plasma triglyceride concentrations were not significantly different between treated and control groups (Table 2).

4. Discussion

Renewed interest into the potential use of incretin hormones as antidiabetic therapies has emerged in recent years [4]. The glucose-dependent insulin secretion induced by GLP-1 and GIP offers benefits over currently used single action, non-endogenous drugs that stimulate insulin secretion at any glucose concentration. Lack of effect of GIP on gastric emptying offers a potential advantage compared with therapies based on GLP-1 [9]. However, circulating GIP is rapidly hydrolysed by the ubiquitous enzyme DPP-IV and efficiently cleared from the body through renal filtration [11]. The potential of C-16 fatty acid (PAL) conjugated GIP analogues has been explored recently with $N$-AcGIP(Lys$^{37}$PAL) appearing to be the most promising analogue [15,16]. The present study examined further the effect of fatty acid chain length, with or without N-terminal acetylation, on biological efficacy through assessing in vitro and in vivo antihyperglycaemic and insulinotropic properties.

Following confirmation of successful Fmoc peptide synthesis by MALDI-TOF MS, the susceptibility of GIP peptides to DPP-IV was examined. Unlike the native hormone which displayed an in vitro biological half life of approximately 2.2 h, GIP analogues remained fully
intact throughout the entire 24 h incubation (half-lives >24 h). While the conditions used for the
DPP-IV assay in the present study were not able to discriminate t_{1/2} between analogues, more
detailed examination of SAR would be of interest in future studies. However, despite the
probability of peptide adsorption during the longer incubation periods, this confirms in vitro
DPP-IV resistance of each GIP analogue tested. It therefore appears that N-terminal acetylation
and the addition of C14-C18 fatty acid residue close to the C-terminal region inhibits the action
of DPP-IV, perhaps by disrupting the GIP hydro-affinity for the enzyme, as reported previously
[16]. Interestingly, C14-C18 acylation without additional N-terminal acetylation also resulted in
stable GIP analogues most likely due to steric hindrance.

Broadly similar to previous studies, native GIP concentration-dependently stimulated cAMP
production with an EC_{50} value of 0.70 nM [16]. Likewise, GIP analogues stimulated cAMP
production in a concentration-dependent manner. However, analogues demonstrated up to a 10-
fold reduction in EC_{50} value relative to GIP. This suggests enhanced potency at the GIP receptor,
and is indicative of preserved receptor affinity with improved activation of the adenylate cyclase
transduction pathway. This increased potency is corroborated by GIP analogues significantly
inducing concentration-dependant stepwise increases in insulin secretion from BRIN-BD11 cells.
At the highest concentrations, GIP analogues evoked a notable improvement in insulin secretion
which quite likely reflects both increased potency and enhanced biological half-life [16]. Since
no significant degradation of GIP occurred during these in vitro incubations, these data suggest
that the combination of increased DPP-IV resistance and improved biological potency at the
 cellular level should provide analogues with greatly enhanced activity in vivo.

To assess the antihyperglycaemic and insulinotropic effects of GIP analogues in vivo, we
employed the ob/ob mouse model from the Aston colony [20]. On this genetic background, ob/ob
mice present with hyperphagia, marked obesity, moderate hyperglycaemia and severe hyperinsulinaemia representing a very robust model of type 2 diabetes and obesity. As in previous studies, native GIP had no significant effect on plasma glucose or the insulin response following an intraperitoneal glucose load [16,17] due to its short biological half-life following degradation by DPP-IV and efficient renal clearance. In contrast, all GIP analogues demonstrated improved glucose-lowering actions. However, only GIP(Lys\textsuperscript{37}MYR), N-AcGIP(Lys\textsuperscript{37}MYR) and GIP(Lys\textsuperscript{37}PAL) appeared substantially more effective and longer-acting in stimulating insulin release. Reasons underlying these differences are unclear but likely reflect alterations in beta-cell stimulation possibly due to changes in effective concentrations as a result of protein binding and various non beta-cell and extrapancreatic actions [4,16]. Nevertheless, the magnitude of improvements in the glycaemic and insulinotropic responses of GIP(Lys\textsuperscript{37}MYR), N-AcGIP(Lys\textsuperscript{37}MYR) and GIP(Lys\textsuperscript{37}PAL) is notable given the severe insulin resistance of the \textit{ob/ob} syndrome [20]. Importantly, when N-AcGIP(Lys\textsuperscript{37}MYR) was injected acutely together with saline in normal mice, no significant effects on glucose-lowering or insulin release were observed. However, when co-injected with a glucose load N-AcGIP(Lys\textsuperscript{37}MYR) significantly lowered plasma glucose and increased plasma insulin concentrations, thus demonstrating a potent glucose-dependent effect. From these acute studies it appears that DPP-IV resistant analogues of GIP can substantially overcome any possible defects in responsiveness of GIP in type 2 diabetes. The longer-acting glucose-lowering effects of GIP analogues were assessed through an intraperitoneal glucose tolerance in animals 24 h following peptide administration. Interestingly, only GIP(Lys\textsuperscript{37}MYR) and N-AcGIP(Lys\textsuperscript{37}MYR) elicited a persistent improvement in glycaemic control 24 h after administration. The reason why a persistent glucose-lowering effect was not observed with the palmitic acid GIP analogues is unclear [15,16], but could be due to differences in the conformation or activity of the analogue. The prolonged action of GIP(Lys\textsuperscript{37}MYR) and N-
AcGIP(Lys\textsuperscript{37}MYR) suggests a much more extended plasma half-life, reflecting an enhanced ability of this shorter C-14 chain to structurally orientate and bind more strongly to serum proteins preventing kidney filtration [11]. The development of a specific assay to assess plasma levels of these fatty acid GIP analogues would be necessary to provide more precise details of these kinetics.

Results of these acute in vivo studies provided a strong basis for the subsequent 24 day study. Daily intraperitoneal injection of N-AcGIP, GIP(Lys\textsuperscript{37}MYR) and N-AcGIP(Lys\textsuperscript{37}MYR) over this time had no apparent adverse or toxic effects. Bodyweight and food intake were similar to saline-treated controls confirming previous findings [15]. Importantly, GIP(Lys\textsuperscript{37}MYR) and N-AcGIP(Lys\textsuperscript{37}MYR), and to a lesser effect, N-AcGIP, significantly reduced plasma glucose levels from day 20 of the study, accompanied by significantly elevated plasma insulin levels. Glucose tolerance was markedly improved in animals treated with GIP(Lys\textsuperscript{37}MYR) and N-AcGIP(Lys\textsuperscript{37}MYR) and this was accompanied by significantly increased plasma insulin concentrations. This confirms the long-acting insulinotropic effects of these analogues and their ability to overcome the severe beta-cell defect in this model. Improvement in glucose homeostasis was independent of any change in insulin sensitivity which is in agreement with previous findings [14-18]. There were no observed changes in pancreatic insulin content or plasma triglyceride levels although a more prolonged treatment period may be necessary to see improvements in these parameters.

In conclusion, this study demonstrated that fatty acid derivatisation at position Lys\textsuperscript{37}, with or without N-terminal acetylation confers DPP-IV resistance and improved biological efficacy during in vitro and acute in vivo studies. Furthermore, derivatisation with a C-14 fatty acid offered a more protracted and markedly improved biological function over C-16 and C-18 fatty acids.
acid counterparts. These data represent an important step in the progression of fatty acid
derivatised GIP analogues as a potential therapy for type 2 diabetes. Further studies are required
to answer whether or not these analogues will be useful in human diabetes treatment.

Acknowledgements

These studies were supported by a Project Grant from Diabetes UK, the SAAD Trading and
Contracting Company and University of Ulster Strategic Research Funding. The authors wish to
thank Brett Greer and Pat Harriott (Queen’s University of Belfast, UK) for peptide synthesis and
Jenny Pineda and James Doherty for assistance in peptide purification.

References

[1] Brown JC. Enteroinsular axis. In Gut Peptides: Biochemistry and Physiology; Dockray,
pathophysiology of diabetes involves a defective amplification of late phase insulin
response to glucose by glucose-dependent insulinotropic polypeptide-regardless of


Table 1 In vitro characteristics of GIP peptides with and without N-terminal acetylation

<table>
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<th>Peptide</th>
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<td>GIP(Lys^{37}PAL)</td>
<td>5218.0</td>
<td>5219.1</td>
<td>&gt;24</td>
</tr>
<tr>
<td>N-AcGIP(Lys^{37}PAL)</td>
<td>5264.0</td>
<td>5263.3</td>
<td>&gt;24</td>
</tr>
<tr>
<td>GIP(Lys^{37}STE)</td>
<td>5247.0</td>
<td>5249.8</td>
<td>&gt;24</td>
</tr>
<tr>
<td>N-AcGIP(Lys^{37}STE)</td>
<td>5290.0</td>
<td>5292.8</td>
<td>&gt;24</td>
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</table>

**MALDI-TOF MS:** purified peptide samples were mixed with α-cyano-4 hydroxycinnamic acid, applied to the sample plate of a Voyager-DE BioSpectrometry Workstation and mass-to-charge (m/z) ratio vs relative peak intensity recorded. **DPP-IV degradation:** resistance of GIP and GIP analogues to degradation by DPP-IV was measured (n=4) following 0, 2, 4, 8 and 24 h incubations. Reaction products were subsequently analysed by HPLC and degradation calculated as a percentage of intact peptide relative to the major degradation fragment, GIP(3-42). **cAMP production:** EC_{50} values calculated are expressed as mean ± SEM of at least 3 independent experiments.
Table 2 Effects of daily administration of N-AcGIP, GIP(Lys$^{37}$MYR) and N-GIP(Lys$^{37}$MYR) on insulin sensitivity, insulin content and circulating triglycerides in ob/ob mice.

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<tr>
<th>Peptide treatment</th>
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<th>Insulin content (ng/g tissue)</th>
<th>Triglycerides (ng/dl)</th>
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<td>Saline vehicle</td>
<td>898.7 ± 80.0</td>
<td>3.9 ± 0.4</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>N-AcGIP</td>
<td>997.5 ± 72.6</td>
<td>4.0 ± 0.3</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>GIP(Lys$^{37}$MYR)</td>
<td>912.0 ± 34.2</td>
<td>4.0 ± 0.4</td>
<td>2.4 ± 0.3</td>
</tr>
<tr>
<td>N-GIP(Lys$^{37}$MYR)</td>
<td>902.3 ± 95.9</td>
<td>4.5 ± 0.5</td>
<td>2.1 ± 0.2</td>
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</tbody>
</table>

**Insulin sensitivity:** exogenous insulin (50 U/kg body weight; ip) was administered after daily treatment with GIP peptides (25 nmol/kg body weight) for 24 days. Plasma glucose AUC values for 0-60 min post injection were calculated. **Pancreatic insulin content and circulating triglycerides:** parameters were measured after daily treatment with GIP peptides for 24 days. Data are expressed as mean ± SEM for 8 mice.
**Legends to Figures**

Fig. 1 - Effects of GIP peptides on insulin secretion. Various concentrations of GIP peptides were incubated with BRIN-BD11 cells in the presence of 5.6 mM glucose for 20 min (n=8) and insulin release measured using RIA. Data are expressed as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 compared to glucose alone. Δp < 0.05, ΔΔp < 0.01, ΔΔΔp < 0.001 compared with GIP at the same concentration.

Fig. 2 - Acute effects of GIP and N-AcGIP(Lys\(^{37}\)MYR) on glucose-lowering and glucose-induced insulin release in normal mice. (A) Plasma glucose and insulin concentrations were measured prior to and after ip administration of saline alone (0.9% (w/v) NaCl) or in combination with GIP or N-AcGIP(Lys\(^{37}\)MYR) (each at 25 nmol/kg body weight). (B) Plasma glucose and insulin concentrations were measured prior to and after ip administration of glucose alone (18 mmol/kg bw) or in combination with GIP or N-AcGIP(Lys\(^{37}\)MYR) (each at 25 nmol/kg body weight). Plasma glucose and insulin AUC values for 0-60 min post injection are also included. Data are expressed as mean ± SEM for 6 mice. *p < 0.05, **p < 0.01, ***p < 0.001 compared to glucose alone. Δp < 0.05, ΔΔp < 0.01, ΔΔΔp < 0.001 compared with GIP treated mice. □, control; ○, GIP; ▲, N-AcGIP(Lys\(^{37}\)MYR).

Fig. 3 - Acute effects of GIP peptides on glucose-lowering in ob/ob mice. Plasma glucose concentrations were measured prior to and after ip administration of glucose alone (18 mmol/kg body weight) or in combination with GIP peptides (25 nmol/kg body weight). Plasma glucose AUC values for 0-60 min post injection are also included. Data are expressed as mean ± SEM for 6 mice. *p < 0.05, **p < 0.01, ***p < 0.001 compared to glucose alone. Δp < 0.05, ΔΔp < 0.01, ΔΔΔp < 0.001 compared with GIP treated mice. □, control; ○, GIP; ▲, N-AcGIP; ■,
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Fig. 4 - Acute effects of GIP peptides on insulin release in ob/ob mice. Plasma insulin concentrations were measured prior to and after ip administration of glucose alone (18 mmol/kg body weight) or in combination with GIP peptides (25 nmol/kg body weight). Plasma insulin AUC values for 0-60 min post injection are also included. Data are expressed as mean ± SEM for 6 mice. *p < 0.05, **p < 0.01, ***p < 0.001 compared to glucose alone. △p < 0.05, △△p < 0.01, △△△p < 0.001 compared with GIP treated mice. □, control; ○, GIP; ▲, N-AcGIP; ■, GIP(Lys$^{37}$MYR); ▽, N-AcGIP(Lys$^{37}$MYR); ▼, GIP(Lys$^{37}$PAL); ◇, N-AcGIP(Lys$^{37}$PAL); ●, GIP(Lys$^{37}$STE); △, N-AcGIP(Lys$^{37}$STE).

Fig. 5 - Persistence of glucose-lowering effects of GIP peptides in ob/ob mice. Plasma glucose concentrations were measured prior to and after ip administration of glucose alone (18 mmol/kg body weight) in animals injected 24 h previously with GIP peptides (25 nmol/kg body weight). Plasma glucose AUC values for 0-60 min post injection are also included. Data are expressed as mean ± SEM for 6 mice. *p < 0.05, **p < 0.01, ***p < 0.001 compared to glucose alone. △p < 0.05, △△p < 0.01 compared with N-AcGIP treated mice. □, control; ○, GIP; ▲, N-AcGIP; ■, GIP(Lys$^{37}$MYR); ▽, N-AcGIP(Lys$^{37}$MYR); ▼, GIP(Lys$^{37}$PAL); ◇, N-AcGIP(Lys$^{37}$PAL); ●, GIP(Lys$^{37}$STE); △, N-AcGIP(Lys$^{37}$STE).

Fig. 6 - Effects of daily administration of N-AcGIP, GIP(Lys$^{37}$MYR) and N-AcGIP(Lys$^{37}$MYR) on (A) body weight, (B) food intake, (C) non-fasting plasma glucose and (D) non-fasting plasma insulin in ob/ob mice. Parameters were measured prior to and 24 days during treatment with GIP.
peptides (25 nmol/kg body weight) or saline vehicle (0.9% (w/v) NaCl). Data are expressed as mean ± SEM for 8 mice. *p < 0.05, **p < 0.01, ***p < 0.001 compared to saline-treated group. △p < 0.05, △△p < 0.01, △△△p < 0.001 compared with N-AcGIP-treated mice. □, saline vehicle; ▲, N-AcGIP; ■, GIP(Lys^{37}MYR); ▼, N-AcGIP(Lys^{37}MYR).

Fig. 7 - Effects of daily administration of N-AcGIP, GIP(Lys^{37}MYR) and N-AcGIP(Lys^{37}MYR) on (A) glucose tolerance and (B) plasma insulin response to glucose in ob/ob mice. Tests were conducted after daily treatment with GIP peptides (25 nmol/kg body weight) or saline vehicle (0.9% (w/v) NaCl) for 24 days. Glucose (18 mmol/kg body weight; ip) was administered at time t=0. Plasma glucose and insulin AUC values for 0-180 min post injection are also included. Data are expressed as mean ± SEM for 8 mice. **p < 0.01, ***p < 0.001 compared to saline-treated group. △p < 0.05, △△p < 0.01, △△△p < 0.001 compared with N-AcGIP-treated mice. □, saline vehicle; ▲, N-AcGIP; ■, GIP(Lys^{37}MYR); ▼, N-AcGIP(Lys^{37}MYR).
**Figure 1**

![Graphs showing insulin secretion](image)

The graphs display the insulin secretion (ng/10^6 cells/20 min) in response to different peptides and glucose concentrations. Each graph compares the effects of 5.6 mM glucose, GIP, N-AcGIP, GIP(Lys^{37}MYR), and N-AcGIP(Lys^{37}MYR) at various log_{10} concentrations.

- **GIP** indicates the control group.
- **N-AcGIP** is the N-terminal acetyl modification of GIP.
- **GIP(Lys^{37}MYR)** and **N-AcGIP(Lys^{37}MYR)** show the effects of modified GIP.

Statistical significant differences are indicated by asterisks (*, **, ***), with *** indicating a p-value < 0.001.
Figure 2

A

**Figure A**

- **Plasma glucose (mM)**
  - Time: 0, 15, 30, 45, 60
  - Data points and error bars indicated

B

**Figure B**

- **Plasma glucose (mM)**
  - Time: 0, 15, 30, 45, 60
  - Data points and error bars indicated

- **Plasma insulin (ng/ml)**
  - Time: 0, 15, 30, 45, 60
  - Data points and error bars indicated

- **Plasma glucose AUC (mM.min)**
  - Data points and error bars indicated

- **Plasma insulin AUC (ng/ml.min)**
  - Data points and error bars indicated
Figure 3

**Graph 1:**
- **Y-axis:** Plasma glucose (mM)
- **X-axis:** Time (min)
- Lines represent different treatments: Control, GIP, N-AcGIP, GIP(Lys37MYR), N-AcGIP(Lys37MYR)
- AUC values are shown in the bar graph on the right.

**Graph 2:**
- **Y-axis:** Plasma glucose (mM)
- **X-axis:** Time (min)
- Lines represent different treatments: Control, GIP, N-AcGIP, GIP(Lys37PAL), N-AcGIP(Lys37PAL)
- AUC values are shown in the bar graph on the right.

**Graph 3:**
- **Y-axis:** Plasma glucose (mM)
- **X-axis:** Time (min)
- Lines represent different treatments: Control, GIP, N-AcGIP, GIP(Lys37STE), N-AcGIP(Lys37STE)
- AUC values are shown in the bar graph on the right.
Figure 4
Figure 5
Figure 6

A

Bodyweight (g)

Time (days)

B

Food Intake (g)

Time (days)

C

Plasma glucose (mM)

Time (days)

D

Plasma insulin (ng/ml)

Time (days)
Figure 7
Table 1 In vitro characteristics of GIP peptides with and without N-terminal acetylation

<table>
<thead>
<tr>
<th>Peptide</th>
<th>MALDI-TOF MS</th>
<th>Estimated half-life (h)</th>
<th>cAMP EC&lt;sub&gt;50&lt;/sub&gt; (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Theoretical (Da)</td>
<td>Experimental (Da)</td>
<td></td>
</tr>
<tr>
<td>GIP</td>
<td>4982.4</td>
<td>4982.2</td>
<td>2.2</td>
</tr>
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<td>N-AcGIP</td>
<td>5023.0</td>
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Figure 1
Figure 2

A

- Plasma glucose (mM)
  - 0 15 30 45 60
  - 0 2 4 6

B

- Plasma glucose (mM)
  - 0 15 30 45 60
  - 0 2 4 6

- Plasma insulin (ng/ml)
  - 0 15 30 45 60
  - 0 2 4 6 8 10

Legend:
- Saline alone
- Saline + GIP
- Saline + N-AcGIP (Lys37MYR)

Plasma glucose AUC (mM.min)
- 0 100 200 300

Plasma insulin AUC (ng/ml.min)
- 0 250 500 750

- Glucose alone
- Glucose + GIP
- Glucose + N-AcGIP (Lys37MYR)

Significance:
- * p < 0.05
- ** p < 0.01
- *** p < 0.001
-  p < 0.05 vs. saline
-   p < 0.01 vs. saline
-    p < 0.001 vs. saline
Figure 3

![Graphs and bar charts showing plasma glucose concentration and area under the curve (AUC) over time for different treatments.]

- **Graphs**: Time (min) vs. Plasma glucose (mM) for Control, GIP, N-AcGIP, GIP(Lys37MYR), N-AcGIP(Lys37MYR), GIP(Lys37PAL), N-AcGIP(Lys37PAL), and GIP(Lys37STE) treatments.

- **Bar Charts**: Plasma glucose AUC (mM.min) with significant differences indicated by asterisks (***, **, *) and error bars showing variation across treatments.

Legend:
- Control
- GIP
- N-AcGIP
- GIP(Lys37MYR)
- N-AcGIP(Lys37MYR)
- GIP(Lys37PAL)
- N-AcGIP(Lys37PAL)
- GIP(Lys37STE)
- N-AcGIP(Lys37STE)
Figure 4
Figure 5

![Graphs showing plasma glucose levels over time for different treatments.](image)

- **Graph 1:**
  - X-axis: Time (min) from 0 to 60
  - Y-axis: Plasma glucose (mM) from 0 to 50
  - Legend: Control, N-AcGIP, GIP(Lys37MYR), N-AcGIP(Lys37MYR)
  - Comparison of plasma glucose levels for different treatments over time.

- **Graph 2:**
  - X-axis: Time (min) from 0 to 60
  - Y-axis: Plasma glucose AUC (mM.min) from 0 to 3000
  - Legend: Control, N-AcGIP, GIP(Lys37PAL), N-AcGIP(Lys37PAL)
  - Comparison of plasma glucose AUC for different treatments over time.

- **Graph 3:**
  - X-axis: Time (min) from 0 to 60
  - Y-axis: Plasma glucose (mM) from 0 to 50
  - Legend: Control, N-AcGIP, GIP(Lys37STE), N-AcGIP(Lys37STE)
  - Comparison of plasma glucose levels for different treatments over time.

- **Graph 4:**
  - X-axis: Time (min) from 0 to 60
  - Y-axis: Plasma glucose AUC (mM.min) from 0 to 3000
  - Legend: Control, N-AcGIP, GIP(Lys37STE), N-AcGIP(Lys37STE)
  - Comparison of plasma glucose AUC for different treatments over time.
Figure 6

A

Bodyweight (g)

Time (days)

B

Food Intake (g)

Time (days)

C

Plasma glucose (mM)

Time (days)

D

Plasma insulin (ng/ml)

Time (days)
Figure 7

A

B

Plasma glucose (mM)

Time (min)

Plasma insulin (ng/ml)

Time (min)

Plasma glucose AUC (mM.min)

Saline
N-AcGIP
GIP(LysMYR37)
N-AcGIP(LysMYR37)

Plasma insulin AUC (ng/ml.min)

Saline
N-AcGIP
GIP(LysMYR37)
N-AcGIP(LysMYR37)
Graphical Abstract

C-terminal acylation particularly with myristic acid provides a class of stable, longer-acting forms of GIP for evaluation in diabetes therapy.

\[
\text{NH}_2-YAEGTFISYIAMDKIHQQDFVNWLLAQKGKNDWKAHINGQ-COOH
\]

represents: myristic, palmitic or stearic acid

*Albumin*