A novel, long-acting agonist of glucose dependent insulinotropic polypeptide (GIP) suitable for once daily administration in type 2 diabetes

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Abstract

Glucose-dependent insulinotropic polypeptide (GIP) is a gastrointestinal hormone with a potential therapeutic role in type 2 diabetes. Rapid degradation by dipeptidylpeptidase IV (DPP IV) has prompted the development of enzyme-resistant N-terminally modified analogs, but renal clearance still limits *in vivo* bioactivity. In this study, we report long-term antidiabetic effects of a novel, N-terminally protected, fatty acid derivatized analog of GIP, *N*-AcGIP(LysPAL^{37}) in obese diabetic (*ob/ob*) mice. Once daily injections of *N*-AcGIP(LysPAL^{37}) over a 14 day period significantly decreased plasma glucose, glycated hemoglobin and improved glucose tolerance compared to *ob/ob* mice treated with saline or native GIP. Plasma insulin and pancreatic insulin content were significantly increased by *N*-AcGIP(LysPAL^{37}). This was accompanied by a significant enhancement in the insulin response to glucose together with a notable improvement of insulin sensitivity. No evidence was found for GIP-receptor desensitization and the metabolic effects of *N*-AcGIP(LysPAL^{37}) were independent of any change in feeding or body weight. Similar daily injections of native GIP did not affect any of the parameters measured. These data demonstrate, the ability of once daily injections of N-terminally modified, fatty acid derivatized analogs of GIP, such as *N*-AcGIP(LysPAL^{37}), to improve diabetes control and to offer a new class of agents for the treatment of type 2 diabetes.

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Introduction

Glucose dependent insulinotropic polypeptide (GIP) secreted from intestinal K-cells in response to nutrient absorption is an important enteroinsular hormone capable of lowering blood glucose concentrations (Meier et al., 2002). In addition to enhancing insulin secretion, GIP has been shown to stimulate proinsulin gene transcription and translation (Wang et al., 1996), increase pancreatic β cell growth (Trumper et al., 2001), and inhibit pancreatic β cell apoptosis (Ehses et al., 2003). Since GIP also displays antihyperglycemic actions through extrapancreatic effects on muscle, adipose and liver tissue (Yip et al., 1998), increasing attention has been devoted to its possible antidiabetic potential (Gault et al., 2003a). Moreover, because the insulinotropic actions of GIP are glucose-dependent (Bailey and Flatt 1995), the risk of hypoglycemia, a major drawback with non-endogenous insulin-releasing drugs, is minimized (Collins 2002). Accordingly, an important opportunity currently exists to generate a safe and efficient GIP-based pharmaceutical agent for the treatment of type 2 diabetes (O'Harte et al., 2000; Hinke et al., 2002; O'Harte et al., 2002; Gault et al., 2002a; Gault et al., 2003b).

Despite its antihyperglycemic properties, rapid degradation of GIP in the circulation poses a major obstacle in the realization of any possible therapeutic potential for GIP. Thus, GIP is rapidly metabolized by the ubiquitous enzyme dipeptidylpeptidase IV (DPP IV) to release the N-terminal dipeptide Tyr[1]-Ala[2], giving rise to the major degradation fragment GIP(3-42) (Kieffer et al., 1995). This N-terminally truncated peptide lacks biological activity and possibly serves as a GIP receptor antagonist *in vivo* (Gault et al., 2002b). Therefore, it is anticipated that the development of DPP IV resistant analogs of GIP would not only extend the biological half-life of the peptide but also curtail production of GIP(3-42), thereby alleviating possible GIP receptor antagonism. In addition to inactivation by DPP IV, GIP is also subject to rapid renal clearance (Meier et al., 2004a), thereby imposing further limitations on biological half-life and potential for type 2 diabetes therapy.

In the past 6 years, a number of N-terminally modified analogs of GIP have been developed which exhibit profound resistance to DPP IV (O'Harte et al., 2000; O'Harte et al., 2002; Gault et al., 2002a; Gault et al., 2003b). Several of these, most notably those modified at Tyr[1] of GIP with an addition of an acetyl, glucitol, pyroglutamyl or Fmoc adduct, exhibit enhanced activity at the GIP receptor *in vitro* (O'Harte et al., 2002; Gault et al., 2002a; Gault et al., 2003b; O'Harte et al., 1998). As a result of degradation resistance and enhanced cellular activity, these analogs display enhanced and protracted antihyperglycemic and insulin-releasing activity when administered acutely to animals with genetically-inherited obesity-diabetes (O'Harte et al., 2000; O'Harte et al., 2002; Gault et al., 2002a; Gault et al., 2003b). To increase potency and generate a long-acting GIP analog, possibly suitable for once daily injection in diabetes, the problem of renal clearance needs to be overcome. We have designed, therefore, a novel analog of GIP, namely *N*-AcGIP(LysPAL[37]), based on the premise that fatty acid derivatization will counter renal clearance by promoting binding of the peptide to albumin. Such a strategy has been shown to prolong the half-life of insulin (Kurtzhals et al., 1995) and the sister incretin glucagon-like peptide-1 (GLP-1) (Knudsen et al., 2000; Kim et al., 2003; Green et al., 2004).

The present study was designed to examine the ability of long-term treatment with *N*-AcGIP(LysPAL[37]) to counter the glucose intolerance and related features exhibited by obese diabetic (*ob/ob*) mice, a commonly employed animal model of type 2 diabetes (Bailey and Flatt 1982). *N*-AcGIP(LysPAL[37]), native GIP or saline as control, were administered once daily by intraperitoneal injection for 14 days prior to evaluation of glucose homeostasis, pancreatic β cell function and insulin sensitivity. Furthermore, possible desensitization of GIP receptor action by prolonged exposure to elevated concentrations of *N*-AcGIP(LysPAL[37]) was examined. The results indicate significant antidiabetic potential for this second generation N-

terminally acetylated GIP analog containing a C-16 palmitate group linked to Lys at position

37.

Methods

Animals. Obese diabetic (*ob/ob*) mice derived from the colony maintained at Aston University, UK (23) were used at 14-17 weeks of age. Animals were housed in an air-conditioned room at $22\pm2°C$ with a 12 h light: 12 h dark cycle (08:00 – 20:00 h). Drinking water and standard rodent maintenance diet (Trouw Nutrition, Cheshire, UK) were freely available. All animal experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986. No adverse effects were observed following long-term administration of GIP or *N*-AcGIP(LysPAL[37]).

Synthesis, purification and characterization of GIP and *N*-AcGIP(LysPAL[37]). Native GIP was sequentially synthesized on an Applied Biosystems automated peptide synthesizer (Model 432 A, Foster City, CA, USA) using standard solid-phase Fmoc peptide chemistry as previously reported (O'Harte et al., 2002). *N*-AcGIP(LysPAL[37]) was sequentially synthesized in the same way but with the exception that the lysine residue at position 37 was conjugated to an Fmoc protected C-16 palmitate fatty acid. The synthetic peptides were judged pure by reversed-phase HPLC on a Waters Millenium 2010 chromatography system (Software version 2.1.5) and subsequently characterized using matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry as described previously (Gault et al., 2002c). The molecular masses of GIP and *N*-AcGIP(LysPAL[37]) were 4982.4 Da and 5267.7 Da, respectively. These were within 1-2 Da of the theoretical masses indicating successful peptide synthesis. Biological potency of both peptides was confirmed by acute *in vivo* tests (Fig. 1).

Effects of GIP and *N*-AcGIP(LysPAL[37]) in *ob/ob* mice. Initially, extended biological activity of *N*-AcGIP(LysPAL[37]) was examined in fed *ob/ob* mice in comparison with *N*-AcGIP and native GIP. Over a 14-day period, groups of *ob/ob* mice received once daily intraperitoneal

injections (17:00 h) of either saline vehicle (0.9%, w/v, NaCl), native GIP or *N*-AcGIP(LysPAL[37]) (both at 12.5 nmoles/kg body weight/day). This dose was chosen on the basis of preliminary experiments that showed significant antihyperglycaemic and insulinotropic activity of *N*-AcGIP(LysPAL[37]) over the range 6.25 – 25 nmoles/kg, when administered acutely with glucose (18 mmoles/kg) (Fig. 1). Food intake and body weight were recorded daily from 5 days before commencement of the treatment regimes. Plasma glucose and insulin concentrations (10:00h) were monitored at 2-6 day intervals. At 14 days, groups of animals were used to evaluate intraperitoneal glucose tolerance (18 mmoles/kg) and insulin sensitivity (50 U/kg). In a separate series, two experimental protocols were employed to examine the possibility of GIP receptor desensitization after 14 days treatment. Acute metabolic effects of the usual injection of either saline, GIP or *N*-AcGIP(LysPAL[37]) were monitored when administered together with glucose (18 mmoles/kg). In the second, acute effects of *N*-AcGIP(LysPAL[37]) given together with glucose were examined in all 3 groups of mice. All acute tests were commenced at 10:00 h. At the end of the 14-day treatment period, pancreatic tissues were excised for measurement of insulin following extraction with 5 ml/g ice-cold acid ethanol (75% ethanol, 2.35% H_2O, 1.5% HCl). Whole blood was taken for determination of glycated hemoglobin. All other blood samples were collected from the cut tip of the tail vein of conscious mice into chilled fluoride/heparin coated glucose microcentrifuge tubes (Sarstedt, Nümbrecht, Germany) at the times indicated in the Figures. Blood samples were immediately centrifuged using a Beckman microcentrifuge (Beckman Instruments, Galway, Ireland) for 30 s at 13,000 *g*. The resulting plasma was then aliquoted into fresh tubes and stored at -20°C prior to glucose and insulin determinations.

Biochemical analyses. Plasma glucose was assayed by an automated glucose oxidase procedure (O'Harte et al., 2002) using a Beckman Glucose Analyzer II. Plasma and pancreatic

insulin was assayed by dextran-charcoal RIA as described previously (O'Harte et al., 2002).

Glycated hemoglobin was determined using cation-exchange columns (Sigma, Poole, Dorset,

UK) with measurement of absorbance (415 nm) in wash and eluting buffers using a VersaMax

microplate spectrophotometer (Molecular Devices, Wokingham, Berkshire, UK).

Statistics. Results are expressed as mean ± SEM. Data were 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 areas

under plasma glucose and insulin curves (AUC) were calculated using a computer-generated

program employing the trapezoidal rule (O'Harte et al., 2002) with baseline subtraction. Groups

of data were considered to be significantly different if $p < 0.05$.

Results

Persistent glucose homeostatic effects of N-AcGIP(LysPAL^{37}). As shown in Figure 2, administration of N-AcGIP(LysPAL^{37}) decreased the glycaemic excursion and glucose levels for up to 24h after administration with glucose to fed *ob/ob* mice (32% reduction; $p < 0.05$). In comparison, the glucose homeostatic effects of N-AcGIP were relatively short lived (27% reduction at 1h; $p < 0.05$) and native GIP lacked any effect on circulating glucose at the time points studied. This supports a protracted biological half-life of N-AcGIP(LysPAL^{37}) and forms the basis of the once-daily injections.

Effects of N-AcGIP(LysPAL^{37}) on food intake, body weight, glycated hemoglobin and non-fasting plasma glucose and insulin concentrations. Administration of GIP or N-AcGIP(LysPAL^{37}) had no effect on food intake or body weight (Fig. 3a, b). Plasma glucose and insulin concentrations were also unchanged by treatment with native GIP for 14 days (Fig. 4a, b). In contrast, daily injection of N-AcGIP(LysPAL^{37}) resulted in a progressive lowering of plasma glucose, resulting in significantly ($p < 0.05$) lowered concentrations at 14 days (Fig. 4a). At this time, glycated hemoglobin was also significantly ($p < 0.01$) decreased in N-AcGIP(LysPAL^{37}) treated *ob/ob* mice (Fig. 4c). These changes were accompanied by a tendency towards elevated insulin concentrations, but these did not achieve statistical significance over the time frame studies (Fig. 4b).

Effects of N-AcGIP(LysPAL^{37}) on glucose tolerance. Consistent with effects on glycated hemoglobin, treatment of *ob/ob* mice for 14 days with N-AcGIP(LysPAL^{37}) resulted in a significant improvement in glucose tolerance (Fig. 5a). Plasma glucose concentrations throughout the test and the overall 0-60 min AUC values were decreased ($p < 0.01$ to $p < 0.001$). This was accompanied by increased insulin concentrations during the latter stages ($p <$

10

0.05) and a greater ($p < 0.01$) overall AUC insulin response (Fig. 5b). In contrast, daily administration of native GIP had no effect on glucose tolerance or the plasma insulin response to glucose compared with control *ob/ob* mice receiving saline injections for 14 days (Fig. 5).

Effect of *N*-AcGIP(LysPAL[37]) on insulin sensitivity. Insulin sensitivity of the 3 groups of mice after 14 days treatment is shown in Fig. 6. Compared with *ob/ob* mice receiving daily injections of saline or native GIP, *N*-AcGIP(LysPAL[37]) prompted a significant improvement of insulin sensitivity. Both the individual glucose concentrations and 0-60 min AUC values were significantly different ($p < 0.05$) from the other two groups. In contrast, daily treatment with native GIP did not affect the characteristic insulin resistance of *ob/ob* mice (Fig. 6).

Evaluation of GIP receptor desensitization. As shown in Fig. 7, treatment of *ob/ob* mice with *N*-AcGIP(LysPAL[37]) for 14 days did not prevent the ability of the peptide to significantly moderate the glycemic excursion ($p < 0.01$) and enhance plasma insulin concentrations ($p < 0.01$) when administered acutely with intraperitoneal glucose. In contrast, the responses of *ob/ob* mice to acute administration of native GIP were almost identical in mice receiving treatment with GIP or saline for 14 days (Fig. 7). To further substantiate the lack of GIP receptor desensitization following chronic treatment with *N*-AcGIP(LysPAL[37]), the acute effects of the analog, administered with glucose, were examined in each of the 3 groups after 14 days treatment with *N*-AcGIP(LysPAL[37]), native GIP or saline (Fig. 8). Apart from lower basal values in the former group, the glucose and insulin responses were identical with similar 0-60 min AUC measures for both plasma glucose and insulin concentrations.

Effects of *N*-AcGIP(LysPAL[37]) on pancreatic insulin content. Treatment of *ob/ob* mice for 14 days with native GIP or *N*-AcGIP(LysPAL[37]) did not affect pancreatic weight compared

with saline-treated controls (Fig. 9a). Similarly, pancreatic insulin content was similar in the

GIP and saline treated groups. However, daily administration of N-AcGIP(LysPAL^{37})

significantly increased ($p < 0.01$) insulin content compared with each of the other groups (Fig.

9b).

Discussion

In common with the sister incretin hormone GLP-1 (Meier and Nauck 2004), great effort has been devoted in recent years to generate stable long-acting analogs of GIP for potential use in the therapy of type 2 diabetes (Gault et al., 2003a; Gault et al., 2003c). This approach supposes that selected GIP analogs can overcome any defect of GIP action in diabetes. The prospect for this is encouraging in that the once postulated specific defect in GIP stimulation of insulin secretion in diabetes (Nauck et al., 1993) is now recognized to represent one aspect of a generalized pancreatic β cell dysfunction that extends to many secretagogues including incretin hormones (Kjems et al., 2003, Meier et al., 2003a). Additionally, DPP IV resistant N-terminally modified analogs of GIP have been shown to induce a protracted insulin response in both animals and humans with type 2 diabetes (O'Harte et al., 2000; Lindsay et al., 2002; O'Harte et al., 2002; Gault et al., 2002a; Gault et al., 2003b).

In the present study, we have examined the ability of a second generation N-terminally modified GIP analog to counter aspects of the *ob/ob* syndrome in mice (Bailey and Flatt 1982). The analog *N*-AcGIP was chosen for secondary modification by fatty acid derivatization to extend half-life and bioavailability by binding to albumin, thereby decreasing renal filtration. *N*-AcGIP has already been shown to exhibit profound DPP IV resistance, enhanced ability to stimulate cyclic AMP production and insulin secretion *in vitro* together with substantial antihyperglycemic and insulin releasing action in *ob/ob* mice *in vivo* (O'Harte et al., 2002). Further modification of GIP to carry a C-16 palmitate fatty acid at the ε-amino group of the naturally occurring Lys at position 37 has been shown to conserve these attributes (O'Harte et al., 2004). At the same time *N*-AcGIP(LysPAL[37]) exhibits extended action in acute tests making it suitable for exploration as a possible once daily treatment using animal models of diabetes.

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Administration of N-AcGIP(LysPAL^{37}) to young adult ob/ob mice by daily intraperitoneal injection resulted in a progressive lowering of plasma glucose concentrations and a significant decrease of glycated hemoglobin by 14 days. This was associated with a substantial improvement of glucose tolerance. Importantly food intake and body weight were unchanged during the study ruling out the possibility that improvement of glucose homeostasis was merely the consequence of body weight loss. These observations also indicate that N-AcGIP(LysPAL^{37}) was not associated with body weight gain and did not exert any untoward toxic actions affecting feeding over the study period. This is in harmony with recent studies showing that GIP, unlike the sister incretin GLP-1, does not inhibit gastric emptying (Meier et al., 2003b). Daily administration of native GIP to ob/ob mice for 14 days had no effect on any of the parameters measured, consistent with the very short half-life of the native GIP $in vivo$ (Holz et al., 1993).

As expected, a key component of the beneficial action of N-AcGIP(LysPAL^{37}) concerned the stimulation of insulin secretion. Thus although native GIP is a weak stimulus to insulin secretion in ob/ob mice at the age studied, plasma and pancreatic insulin concentrations were raised in ob/ob mice receiving the novel fatty acid derivatized analog. This is consistent with the action of GIP as a promoter of proinsulin gene expression (Wang et al., 1996) and exemplifies the increased potency reported for N-terminally modified GIP analogs in animal models of diabetes (O'Harte et al., 2000; Hinke et al., 2002; O'Harte et al., 2002; Gault et al., 2002a; Gault et al., 2003b). Furthermore, the insulin response to glucose was significantly enhanced in ob/ob mice receiving N-AcGIP(LysPAL^{37}). This ability to augment or restore pancreatic beta cell glucose responsiveness in diabetes has been similarly observed with GLP-1 (Holz et al., 1993). As with observations on glycemic control, none of these attributes were reproduced by daily injections of native GIP.

Results of insulin sensitivity tests conducted after 14 days treatment indicate that the improvement of diabetic status achieved in *ob/ob* mice with *N*-AcGIP(LysPAL^{37}) was not solely due to the potentiation of insulin secretion. Thus, these animals also exhibited a significant improvement of insulin sensitivity compared to the GIP or saline treated groups. Given that hyperinsulinemia is generally believed to down-regulate insulin receptor function, this suggests that *N*-AcGIP(LysPAL^{37}) may exert other compensatory effects. Further study is necessary to evaluate this aspect but possibilities include inhibition of counter-regulatory hormones and effects on extrapancreatic sites such as muscle, adipose tissue and liver (Yip et al., 1998).

Irrespective of knowledge of the full range of actions contributing to the antihyperglycemic effect of *N*-AcGIP(LysPAL^{37}), a currently envisaged problem of long-term treatment with stable analogs of GIP or GLP-1 concerns desensitization of hormone receptor action (Delmeire et al., 2004). Although this has been observed during prolonged exposure of pancreatic β cells to GIP during culture *in vitro* (Tseng et al., 1996), there was no evidence that treatment with *N*-AcGIP(LysPAL^{37}) for 14 days compromised the glucose lowering or insulin releasing actions of *N*-AcGIP(LysPAL^{37}) in any way. Thus the antidiabetic actions of *N*-AcGIP(LysPAL^{37}) were clearly evident when the analog was administered acutely together with glucose. Furthermore, the acute effects of *N*-AcGIP(LysPAL^{37}) in such experiments were identical in groups of *ob/ob* mice receiving either *N*-AcGIP(LysPAL^{37}), native GIP or saline injections for 14 days.

Such data clearly indicate that prolonged exposure to *N*-AcGIP(LysPAL^{37}) does not induce and possibly overcomes inherent GIP receptor desensitization in *ob/ob* mice. Given the high circulating concentrations of GIP in these obese-diabetic rodents (Flatt et al., 1983; Flatt et al., 1984), it is tempting to link β cell refractoriness to GIP evident in *ob/ob* mice and reported

in some individuals with clinical diabetes (Ebert et al., 1979) to simple receptor desensitization at the hands of inappropriate secretion and metabolism of GIP. This is supported by the recent observation that the insulin response to intravenous bolus injection of GIP was effectively preserved in patients with type 2 diabetes whereas continuous intravenous infusion induced a poor response (Meier et al., 2004b). However, present appreciation of the role of circulating GIP in clinical diabetes is far from complete. This is due to lack of specificity of radioimmunoassays for active as opposed to the predominant inactive metabolite GIP(3-42). However it is quite reasonable to consider that the latter truncated form of GIP as a receptor antagonist (Gault et al., 2002b) might actively contribute to the reported down-regulation of GIP receptor function on pancreatic β cells in diabetes.

In conclusion, these studies indicate that once daily injection of N-AcGIP(LysPAL^{37}) to *ob/ob* mice for 14 days results in a significant amelioration of diabetes and associated metabolic disturbances. Such effects are independent of changes in feeding and body weight. The antidiabetic actions appear to be mediated by enhancement of both pancreatic β cell function and insulin sensitivity, although other possible extrapancreatic actions may exist. Development of antibodies against analogs of naturally circulating peptides such as N-AcGIP(LysPAL^{37}) is also likely to be minimized by the minor structural changes undertaken, as evidenced by the use of insulin analogues (Bolli 2003) Overall, these novel observations provide strong encouragement for the development of long-acting fatty acid derivatized N-terminally modified analogs of GIP, such as N-AcGIP(LysPAL^{37}), for the once-daily treatment of type 2 diabetes.

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Footnotes

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Figure Legends

Fig. 1. Acute glucose homeostatic and insulin releasing effects of N-AcGIP(LysPAL^{37}) and native GIP. Glucose (18 mmoles/kg) was administered by intraperitoneal injection alone or in combination with 6.25, 12.5 or 25 nmoles/kg N-AcGIP(LysPAL^{37}) or 12.5 nmoles/kg native GIP at the time indicated by the arrow. Plasma glucose and insulin AUC values for 0-60 min post injection are shown in the right panels. Values are mean $\pm$ SEM for 8 mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to mice receiving glucose alone.

Fig. 2. Persistent glucose-homeostatic effects of N-AcGIP(LysPAL^{37}). Plasma glucose concentrations were measured prior to and after intraperitoneal injection of fed *ob/ob* mice with glucose (18 mmoles/kg body weight) alone or in combination with 12.5 nmoles/kg body weight N-AcGIP(LysPAL^{37}), N-AcGIP or native GIP. The arrow indicates the time after which access to food was withdrawn. Values represent means $\pm$ SEM for 6 mice. *$p < 0.05$ compared to mice injected with glucose alone.

Fig. 3. Effects of daily N-AcGIP(LysPAL^{37}) administration on food intake (A) and body weight (B). N-AcGIP(LysPAL^{37}) (12.5 nmoles/kg/day), native GIP (12.5 nmoles/kg/day) or saline vehicle (control) were administered for the 14-day period indicated by the horizontal black bar. Values are mean $\pm$ SEM for 8 mice.

Fig. 4. Changes of plasma glucose (A), insulin (B) and final glycated hemoglobin (C) after daily treatment of *ob/ob* mice with N-AcGIP(LysPAL^{37}) (12.5 nmoles/kg/day), native GIP

(12.5 nmoles/kg/day) or saline vehicle (control) for 14 days. Values are mean ± SEM for 8 mice. *$p < 0.05$, **$p < 0.01$ compared to control. $^{\Delta\Delta}p < 0.01$ compared to native GIP.

Fig. 5. Effects of daily *N*-AcGIP(LysPAL[37]) administration on glucose tolerance and plasma insulin response to glucose. Tests were conducted after 14 daily injections of either *N*-AcGIP(LysPAL[37]) (12.5 nmoles/kg/day), native GIP (12.5 nmoles/kg/day) or saline vehicle (control). Glucose (18 mmoles/kg) was administered by intraperitoneal injection at the time indicated by the arrow. Plasma glucose and insulin AUC values for 0-60 min post injection are shown in the right panels. Values are mean ± SEM for 8 mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to control. $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$, $^{\Delta\Delta\Delta}p < 0.001$ compared to native GIP.

Fig. 6. Effects of daily *N*-AcGIP(LysPAL[37]) administration on insulin sensitivity. Tests were conducted after 14 daily injections of either *N*-AcGIP(LysPAL[37]) (12.5 nmoles/kg/day), native GIP (12.5 nmoles/kg/day) or saline vehicle (control). Insulin (50 U/kg) was administered by intraperitoneal injection at the time indicated by the arrow. Plasma glucose AUC values from baseline for 0-60 min post injection are shown in the right panels. The starting values for saline, GIP and N-AcGIP(LysPAL[37]) were 16.3 ± 2.0, 15.7 ± 1.7, 13.9 ± 2.4 mmol/l, respectively. Values are mean ± SEM for 8 mice. *$p < 0.05$, **$p < 0.01$ compared to control. $^{\Delta}p < 0.05$ compared to native GIP.

Fig. 7. Retention of glucose homeostatic and insulin releasing activity of *N*-AcGIP(LysPAL[37]) and native GIP after daily injection for 14 days. Glucose (18 mmoles/kg) was administered by intraperitoneal injection alone or in combination with either *N*-AcGIP(LysPAL[37]) or native GIP (both at 25 nmoles/kg) at the time indicated by the arrow. Plasma glucose and insulin AUC

values for 0-60 min post injection are shown in the right panels. Values are mean $\pm$ SEM for 8

mice. $*p < 0.05$, $**p < 0.01$ compared to glucose alone. $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$ compared to

native GIP.

Fig. 8. Acute glucose homeostatic and insulin releasing effects of N-AcGIP(LysPAL[37]) after 14

daily injections of either N-AcGIP(LysPAL[37]) (12.5 nmoles/kg/day), native GIP (12.5

nmoles/kg/day) or saline vehicle (control). N-AcGIP(LysPAL[37]) (25 nmoles/kg) was

administered by intraperitoneal injection with glucose (18 mmoles/kg) at the time indicated by

the arrow. Plasma glucose and insulin AUC values for 0-60 min post injection are shown in the

right panels. Values are mean $\pm$ SEM for 8 mice. $*p < 0.05$, $**p < 0.01$ compared to mice

receiving control injections. $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$ compared to group receiving injections of

native GIP.

Fig. 9. Effects of daily N-AcGIP(LysPAL[37]) administration on pancreatic weight and insulin

content. Parameters were determined after 14 daily injections of N-AcGIP(LysPAL[37]) (12.5

nmoles/kg/day), native GIP (12.5 nmoles/kg/day) or saline vehicle (control). Values are mean $\pm$

SEM for 8 mice. $**p < 0.01$ compared to control. $^{\Delta\Delta}p < 0.01$ compared to native GIP.
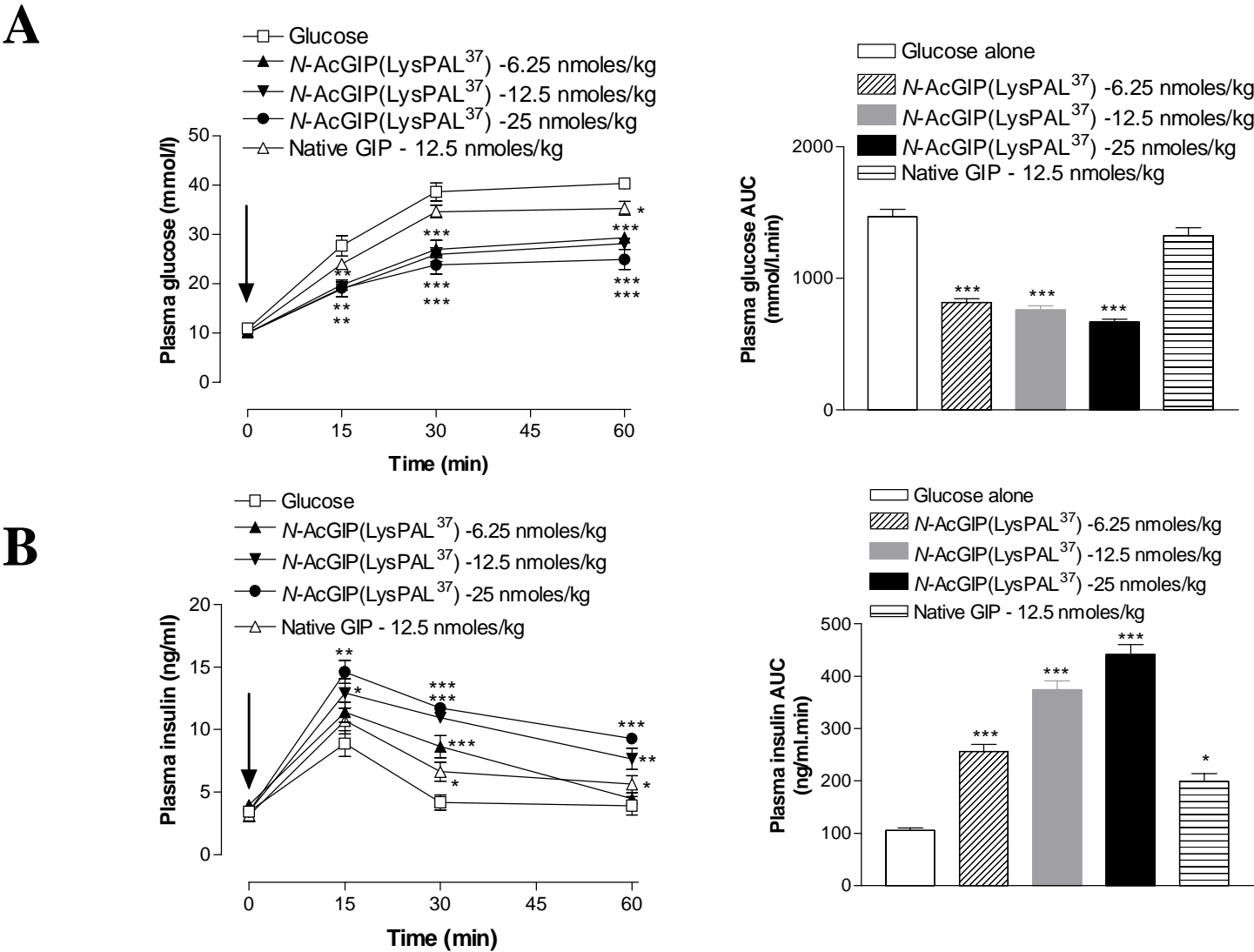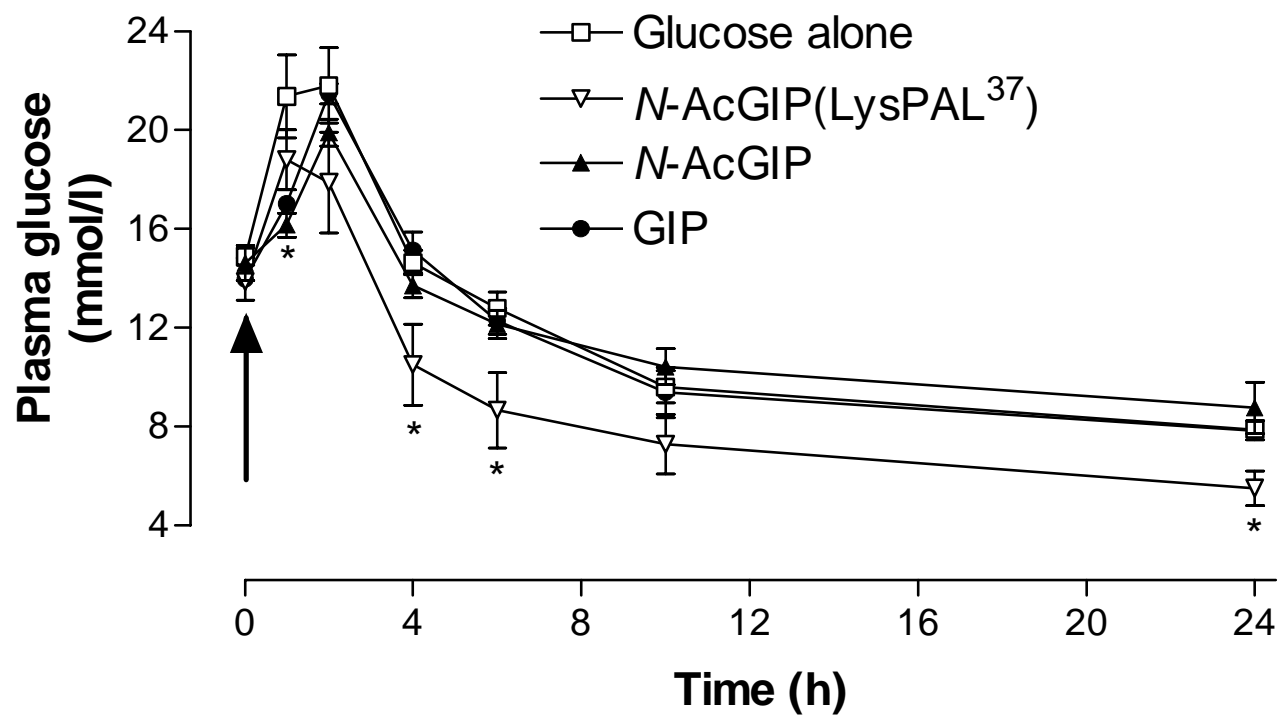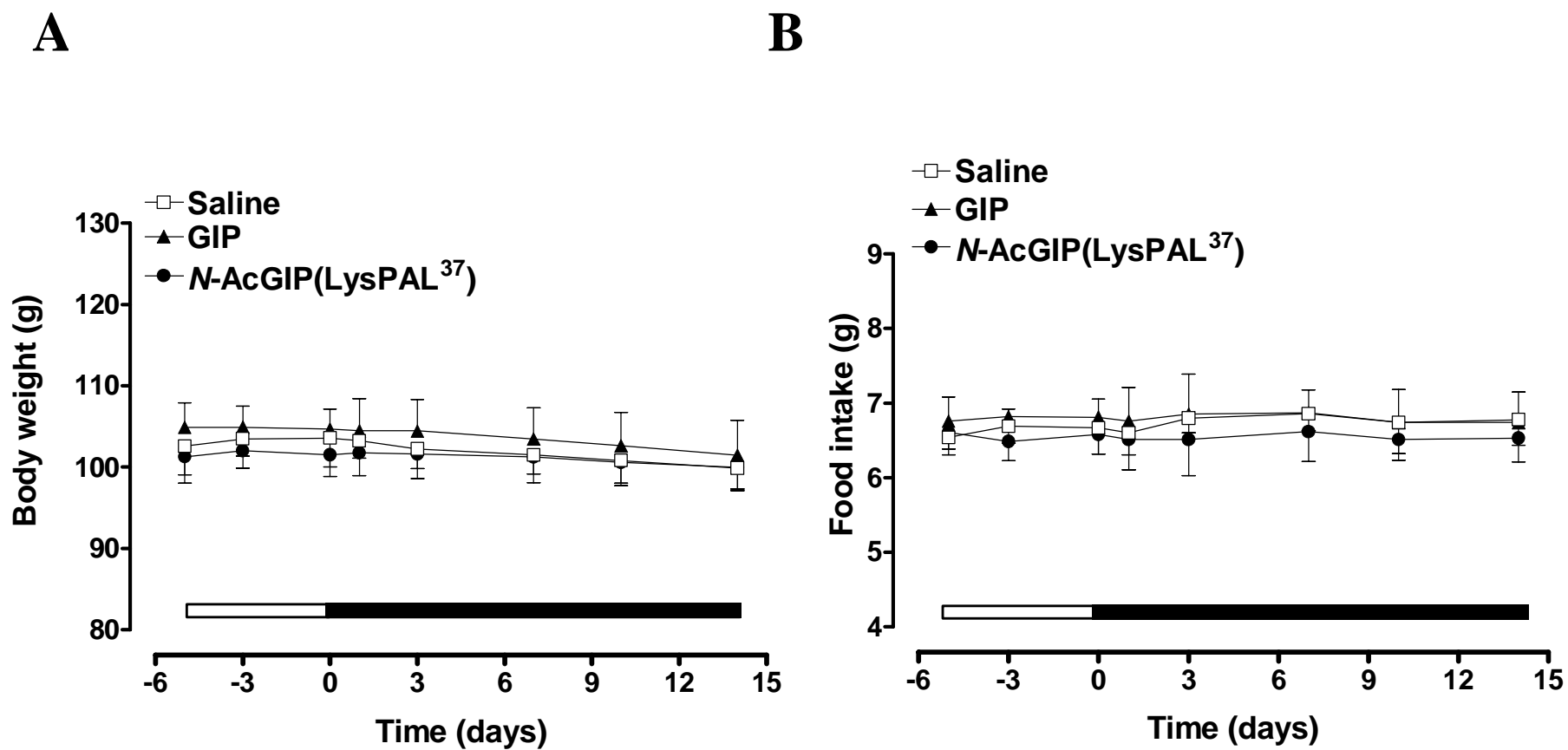
Fig. 1

A

Legend (glucose vs time plot):
- □ Glucose
- ▲ N-AcGIP(LysPAL^{37}) -6.25 nmoles/kg
- ▼ N-AcGIP(LysPAL^{37}) -12.5 nmoles/kg
- ● N-AcGIP(LysPAL^{37}) -25 nmoles/kg
- △ Native GIP - 12.5 nmoles/kg

Legend (glucose AUC bar chart):
- ☐ Glucose alone
- ▨ N-AcGIP(LysPAL^{37}) -6.25 nmoles/kg
- ▨ N-AcGIP(LysPAL^{37}) -12.5 nmoles/kg
- ■ N-AcGIP(LysPAL^{37}) -25 nmoles/kg
- ☰ Native GIP - 12.5 nmoles/kg

B

Legend (insulin vs time plot):
- □ Glucose
- ▲ N-AcGIP(LysPAL^{37}) -6.25 nmoles/kg
- ▼ N-AcGIP(LysPAL^{37}) -12.5 nmoles/kg
- ● N-AcGIP(LysPAL^{37}) -25 nmoles/kg
- △ Native GIP - 12.5 nmoles/kg

Legend (insulin AUC bar chart):
- ☐ Glucose alone
- ▨ N-AcGIP(LysPAL^{37}) -6.25 nmoles/kg
- ▨ N-AcGIP(LysPAL^{37}) -12.5 nmoles/kg
- ■ N-AcGIP(LysPAL^{37}) -25 nmoles/kg
- ☰ Native GIP - 12.5 nmoles/kg

Fig. 2

Fig. 3

A

B

Fig. 4

A — Plasma glucose (% change) vs Time (days); legend: Saline, GIP, N-AcGIP(LysPAL^{37})

B — Plasma insulin (% change) vs Time (days); legend: Saline, GIP, N-AcGIP(LysPAL^{37})

C — Glycated haemoglobin (%); legend: Saline, GIP, N-AcGIP(LysPAL^{37})

Fig. 5

Fig. 6

Fig. 7

Fig. 8

A

B

Fig. 9

A

B