ARTICLE

Early administration of the glucose-dependent insulinotropic polypeptide receptor antagonist (Pro^3)GIP prevents the development of diabetes and related metabolic abnormalities associated with genetically inherited obesity in *ob/ob* mice

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Abstract

Aims/hypothesis Ablation of gastric inhibitory polypeptide (GIP) receptor action is reported to protect against obesity and associated metabolic abnormalities. The aim of this study was to use prediabetic *ob/ob* mice to examine whether 60 days of chemical GIP receptor ablation with (Pro^3)GIP is able to counter the development of genetic obesity-related diabetes.

Materials and methods Young (5–7 weeks) *ob/ob* mice received once daily i.p. injections of either saline vehicle or (Pro^3)GIP (25 nmol kg^{-1} day^{-1}) over a 60 day period. Food intake, body weight and circulating glucose and insulin were measured at frequent intervals. At 60 days, glucose tolerance, response to native GIP, postprandial responses, insulin sensitivity, HbA$_{1c}$, circulating hormones and plasma lipids were assessed.

Results Body weight and food intake in (Pro^3)GIP-treated mice did not differ from *ob/ob* controls. GIP receptor blockade significantly improved non-fasting glucose ($p<0.001$), HbA$_{1c}$ ($p<0.05$), glucose tolerance ($p<0.001$), meal tolerance ($p<0.001$) and insulin sensitivity ($p<0.05$). Remarkably, (Pro^3)GIP treatment prevented the age-related development of diabetes, as none of these parameters differed significantly between treated *ob/ob* mice and normal age-matched lean controls. Circulating levels of glucagon, corticosterone, adiponectin and total cholesterol were unchanged by (Pro^3)GIP, while levels of triacylglycerol, LDL-cholesterol and resistin were decreased ($p<0.05$) compared with those in control *ob/ob* mice. Plasma and pancreatic insulin concentrations were generally lower after (Pro^3)GIP treatment than in control *ob/ob* mice ($p<0.01$), but plasma insulin levels remained substantially raised ($p<0.001$) compared with those observed in lean controls.

Conclusions/interpretation These data indicate that sustained GIP receptor antagonism provides an effective means of preventing the development of many of the metabolic abnormalities of obesity-driven diabetes.

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Abbreviations

DPPIV	dipeptidyl-peptidase IV
GIP	gastric inhibitory polypeptide
GLP-1	glucagon-like peptide 1

Introduction

The most widely accepted role of gastric inhibitory polypeptide (GIP), also known as glucose-dependent insulinotropic polypeptide, is potentiation of glucose-induced insulin secretion from pancreatic beta cells [1]. GIP is secreted from intestinal K cells in response to feeding and, together with glucagon-like peptide 1 (GLP-1), accounts for the full incretin effect [2]. GIP and GLP-1 exert these effects through binding to specific beta cell receptors, activating adenylate cyclase and other signal recognition pathways, leading to insulin exocytosis [3]. GIP and GLP-1 have also been reported to stimulate beta cell neogenesis, differentiation and proliferation whilst affording protection from cytokine attack and apoptosis [4–6]. These various attributes, plus reports of GLP-1-stimulated decreases of gastric emptying, glucagon secretion and appetite [7], have focused much attention on the potential of incretin hormones for the treatment of type 2 diabetes [8, 9].

Both GLP-1 and GIP are rapidly degraded in the circulation by the ubiquitous enzyme dipeptidyl-peptidase IV (DPPIV) [10], but problems of short half-life and pharmacokinetics have been overcome by the development of peptide mimetics possessing N-terminal modifications that make them resistant to DPPIV but able to activate incretin hormone receptors [8, 9, 11]. One such GLP-1 mimetic, exendin-4, has been approved for clinical use in the USA under the tradename Byetta (Amylin Pharmaceuticals, San Diego, CA), and a second palmitoylated derivative of GLP-1, liraglutide, is in late clinical development [6]. Similar exploitation of GIP is at the late preclinical stage, complicated to some extent by the debate that GIP but not GLP-1 action is specifically impaired in type 2 diabetes [12, 13]. This insensitivity appears most pronounced in response to continuous infusion rather than bolus injection of native GIP [14], possibly implicating receptor desensitisation. However, it is now accepted that there is no specific molecular defect of the GIP receptor in established diabetes, first-degree relatives or gestational diabetes [15–17]. Recognition that GLP-1 action is also impaired, to a lesser extent than GIP action, in type 2 diabetes [18], suggests that the diminished insulin response is linked to generalised beta cell secretory dysfunction, presumably stemming from beta cell glucose insensitivity [19].

Although further work is required to determine whether DPPIV-resistant GIP molecules can overcome beta cell insensitivity and eventually make it to the clinic, possibly in combination with GLP-1 mimetics to activate both arms of the enteroinsular axis, other less well-recognised actions of GIP may afford other unexpected therapeutic opportunities [11]. The GIP receptor is expressed in various extrapancreatic sites, including adipose tissue, bone, intestine, heart, stomach and brain [20]. In relation to this, GIP has been shown to inhibit gastric acid secretion [21], attenuate glucagon-stimulated glucose production [22], decrease hepatic insulin extraction [23] stimulate glucose uptake in muscle [24] and increase both fatty acid synthesis and lipoprotein lipase activity in adipocytes [25, 26]. The particularly potent stimulation of GIP secretion after high-fat feeding [27] suggests a role for GIP in fat metabolism [20]. Consistent with this, plasma GIP concentrations have been reported to be elevated in obesity-related type 2 diabetes [28] and animals with obesity-related diabetes [29]. Experimental evidence in mice also indicates that inhibition of GIP signalling by genetic knockout of the GIP receptor prevents diet-induced obesity through the preferential use of fat as an energy substrate [30]. Furthermore, it has been shown that short-term administration of the specific and stable GIP receptor antagonist (Pro^3)GIP [31] can reverse many of the established metabolic abnormalities in adult *ob/ob* mice [32].

In the present study we have employed young prediabetic *ob/ob* mice to determine whether prolonged 60 day chemical GIP receptor ablation using daily injections of (Pro^3)GIP is able to counter the development and progression of obesity-related diabetes in this commonly employed animal model. The results obtained indicate that sustained GIP receptor antagonism provides an effective means of preventing the development of many of the metabolic abnormalities of obesity-driven forms of diabetes.

Materials and methods

Animals Equal numbers of young male and female obese diabetic (*ob/ob*) mice derived from the colony maintained at Aston University (Birmingham, UK) [33] were used at 5–7 weeks of age. Animals were age-matched, divided into groups and housed individually in an air-conditioned room at 22 ± 2°C with a 12 h light:12 h dark cycle (08:00–20:00 h). Age-matched, normal, lean control mice from the same colony were used for comparative purposes as appropriate. The effects of repeated daily injection of (Pro^3)GIP in these lean control mice has previously been shown to result in mild impairment of glucose homeostasis [34]. Drinking water and a 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.

Synthesis, purification and characterisation of (Pro^3) GIP (Pro^3)GIP was sequentially synthesised on an Applied Biosystems (Foster City, CA) automated peptide synthesiser (Model 432 A), as reported previously [31]. (Pro^3)GIP was purified by reversed-phase HPLC on a Waters Millenium 2010 chromatography system (Software version 2.1.5) and

subsequently characterised using electrospray ionisation mass spectrometry (ESI-MS), as described elsewhere [31].

Experimental protocols for in vivo studies Over a 60 day period, the *ob/ob* mice received once daily i.p. injections (17:00 h) of either saline vehicle (0.9% [w/v] NaCl) or (Pro³)GIP (25 nmol/kg body weight). This dose of (Pro³) GIP was chosen on the basis of earlier studies showing blockade of glucoregulatory effects of exogenous and endogenous GIP in *ob/ob* mice [35, 36]. The once daily dosing regimen has also proved effective in longer-term studies [32, 34]. Food intake and body weight were recorded daily, whereas plasma glucose and insulin concentrations were monitored at intervals of 3–7 days. Blood for HbA_{1c} and plasma for measurement of cholesterol, triacylglycerol, glucagon, corticosterone and circulating adipokines were taken on day 60. Intraperitoneal glucose tolerance (18 mmol/kg body weight), metabolic response to native GIP (25 nmol/kg body weight) and insulin sensitivity (50 U/kg body weight) tests were performed at the end of the study period. Mice fasted for 18 h were used to examine the metabolic response to 15 min feeding on day 60. All acute experiments commenced at 10:00 h. In a separate series, pancreatic tissues were excised at the end of the 60 day treatment for measurement of insulin content following extraction with 5 ml/g of ice-cold acid ethanol (750 ml ethanol, 235 ml water, 15 ml concentrated HCl). Blood samples taken from the cut tip of the tail vein of conscious mice at the times indicated in the figures 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 Eppendorf tubes and stored at −20°C prior to analysis. Where indicated, identical experiments were performed using age-matched, normal, lean control mice. Non-fasting plasma glucose levels in the *ob/ob* mice were 10.9±0.8 vs 7.2±0.5 mmol/l in the lean controls at the beginning of the experiment ($p < 0.01$).

Biochemical analysis Plasma glucose was assayed by an automated glucose oxidase procedure [31] using a Beckman Glucose Analyzer II (Beckman Instruments). Plasma and pancreatic insulin were assayed by a modified dextran-coated charcoal radioimmunoassay [37]. Plasma triacylgly-cerol and cholesterol levels were measured using a Hitachi Automatic Analyser 912 (Boehringer Mannheim, Mannheim, Germany). HbA_{1c} was determined using a kit purchased from Chirus (Watford, UK). Glucagon, adiponectin and resistin were measured using radioimmunoassay kits from Linco Research (St Charles, MI, USA). Corticosterone was measured similarly using a kit from MP Biomedicals (Heidelberg, Germany). All analyses were carried out according to the manufacturers' instructions.

Statistical analyses Results are expressed as means±SEM. Data were compared using ANOVA, followed by a Student–Newman–Keuls post-hoc test. AUC analyses were calculated using the trapezoidal rule with baseline subtraction [31]. LDL-cholesterol was calculated using the Friedewald Equation [38]. Groups of data were considered to be significantly different if the *p* value was less than 0.05.

Results

Effects of (Pro³)GIP on food intake, body weight, non-fasting plasma glucose and insulin and HbA_{1c} concentrations The administration of (Pro³)GIP for 60 days had no effect on the food intake of *ob/ob* mice, and a pattern towards decreased food consumption was observed as the age-related hyperphagia declined (Fig. 1a). The food intake of *ob/ob* mice (Fig. 1) was consistently greater than that of lean controls (5.4±0.3 g mouse⁻¹ day⁻¹) ($p < 0.001$). At the start of the study the body weight of the *ob/ob* mice was 42.3±2.5 g compared with 36.8±1.2 g for lean controls ($p=0.067$). Although there was a general trend for reduced body weight gain in *ob/ob* mice treated with (Pro³)GIP, the difference between the two *ob/ob* groups, corresponding to 8.4% of the body weight of saline controls, failed to reach significance ($p=0.072$) over the study period (Fig. 1b). The corresponding weight gain of lean controls over this period was minimal (2.5±0.1 g). The plasma glucose concentrations of (Pro³)GIP-treated *ob/ob* mice remained stable throughout the study and were similar to those of lean controls at 60 days (Fig. 2a,c). In comparison, untreated *ob/ob* mice exhibited hyperglycaemia, and glucose concentrations were significantly raised in these mice compared with (Pro³)GIP-treated mice, from day 14 onwards. Consistent with this pattern, HbA_{1c} was only significantly elevated ($p < 0.05$) in the untreated *ob/ob* group at 60 days (Fig 2d). Plasma insulin had a tendency to be lower with (Pro³)GIP treatment, and on day 44 was significantly decreased ($p < 0.05$) compared with the levels observed control *ob/ob* mice (Fig. 2b). However, both groups exhibited quite marked hyperinsulinaemia ($p < 0.001$) compared with lean mice (Fig. 2e).

Effects of (Pro³)GIP on intraperitoneal glucose tolerance and response to native GIP Daily administration of (Pro³) GIP for 60 days resulted in significantly reduced ($p < 0.001$) plasma glucose concentrations at 0, 15, 30 and 60 min as compared with untreated *ob/ob* mice following i.p. glucose (Fig. 3a). This was corroborated by a significantly ($p < 0.001$) decreased 0–60 min AUC value (Fig. 3c). Indeed, the i.p. glucose tolerance of (Pro³)GIP-treated *ob/ob* mice at 60 days was similar to that of normal lean mice. As shown
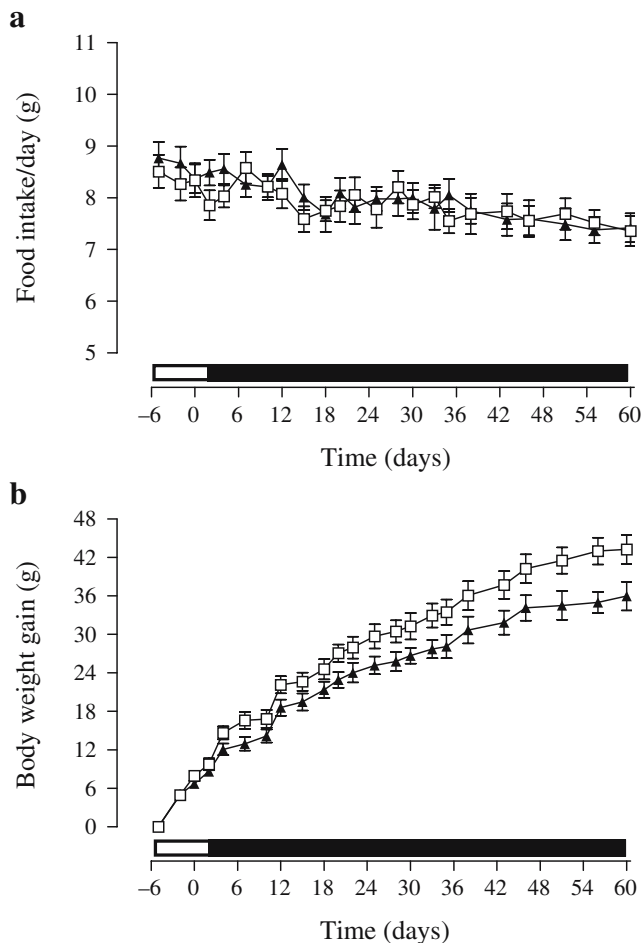
a

b

Fig. 1 Effects of daily (Pro³)GIP administration on food intake (**a**) and body weight (**b**) of *ob/ob* mice. Parameters were measured for 5 days prior to and for 60 days during (indicated by black bar) treatment with saline (*squares*) or (Pro³)GIP (25 nmol kg^{-1} day^{-1}; *triangles*). Food intake and body weight gain of normal lean control mice were 5.4±0.3 and 2.5±0.1 g, respectively. Values are means± SEM for groups of 7–8 mice

in Fig. 3b, corresponding plasma insulin concentrations were also significantly reduced ($p < 0.05$) in the (Pro³)GIP-treated group compared with the untreated *ob/ob* group, as was the 0–60 min AUC (Fig. 3d). However, insulin levels in the two groups were considerably greater ($p < 0.001$) than those observed in normal lean mice. Interestingly, a similar pattern was observed when *ob/ob* mice treated for 60 days were administered glucose together with native GIP (25 nmol/kg body weight), consistent with the antagonism of the action of GIP by extended (Pro³)GIP administration (data not shown).

Effects of (Pro³)GIP on metabolic response to feeding, insulin sensitivity and pancreatic insulin content The plasma glucose response to 15 min feeding was significantly lowered at 15, 30, 60 and 105 min ($p < 0.05$ to $p < 0.001$) in *ob/ob* mice treated for 60 days with (Pro³)GIP compared

with control *ob/ob* mice. This translated into a significantly ($p < 0.001$) decreased overall glycaemic excursion, despite similar food intakes (0.4–0.6 g mouse^{-1} 15 min^{-1}). Plasma insulin levels were not significantly different between the two groups of *ob/ob* mice. However, the glycaemic and insulinotropic responses of both groups were significantly different from those of normal lean mice (data not shown).

As shown in Fig. 4a, insulin sensitivity was significantly ($p < 0.05$) improved in *ob/ob* mice in terms of AUC measures and post-injection values following treatment with (Pro³)GIP for 60 days. The hypoglycaemic action of this dose of insulin was similar in (Pro³)GIP-treated *ob/ob* mice and normal lean controls (Fig. 4a). Compared with untreated *ob/ob* mice, (Pro³)GIP-treated *ob/ob* mice had a significantly decreased ($p < 0.01$) pancreatic insulin content, with values similar to normal lean mice at 60 days (Fig. 4b). Pancreatic weight was similar in all three groups.

Effects of (Pro³)GIP on circulating triacylglycerol and cholesterol As shown in Fig. 5a, in *ob/ob* mice, (Pro³)GIP treatment for 60 days significantly reduced ($p < 0.05$) circulating triacylglycerol concentrations. Total cholesterol levels were not significantly different between the two groups of *ob/ob* mice (Fig 5b). More detailed assessment revealed significantly reduced ($p < 0.05$) levels of LDL-cholesterol in (Pro³)GIP-treated *ob/ob* mice (Fig 5d). However, concentrations of the various lipids in the two groups were consistently greater than those observed in normal lean mice.

Effects of (Pro³)GIP on circulating glucagon, corticosterone and adipokines Once daily injection of *ob/ob* mice with (Pro³)GIP did not significantly affect circulating glucagon or corticosterone concentrations (Fig. 6a and b). Plasma adiponectin was also unchanged, but a small decrease ($p < 0.05$) of circulating resistin was observed following treatment with (Pro³)GIP (Fig. 6c and d). Concentrations of glucagon and corticosterone in both groups of *ob/ob* mice were substantially raised ($p < 0.001$) compared with those in normal lean mice.

Discussion

It has been speculated for decades that GIP plays a role in the aetiology of obesity [39]. More recently it has been shown that obese diabetic (*ob/ob*) mice have intestinal K cell hyperplasia, markedly elevated concentrations of intestinal (2.8-fold increase) and circulating GIP (15.1-fold increase) [29, 40, 41] and display a diminished insulinotropic response to native GIP [11]. Additionally, *ob/ob* mice genetically manipulated to knock out GIP receptor function displayed significant amelioration of adiposity [30]. These
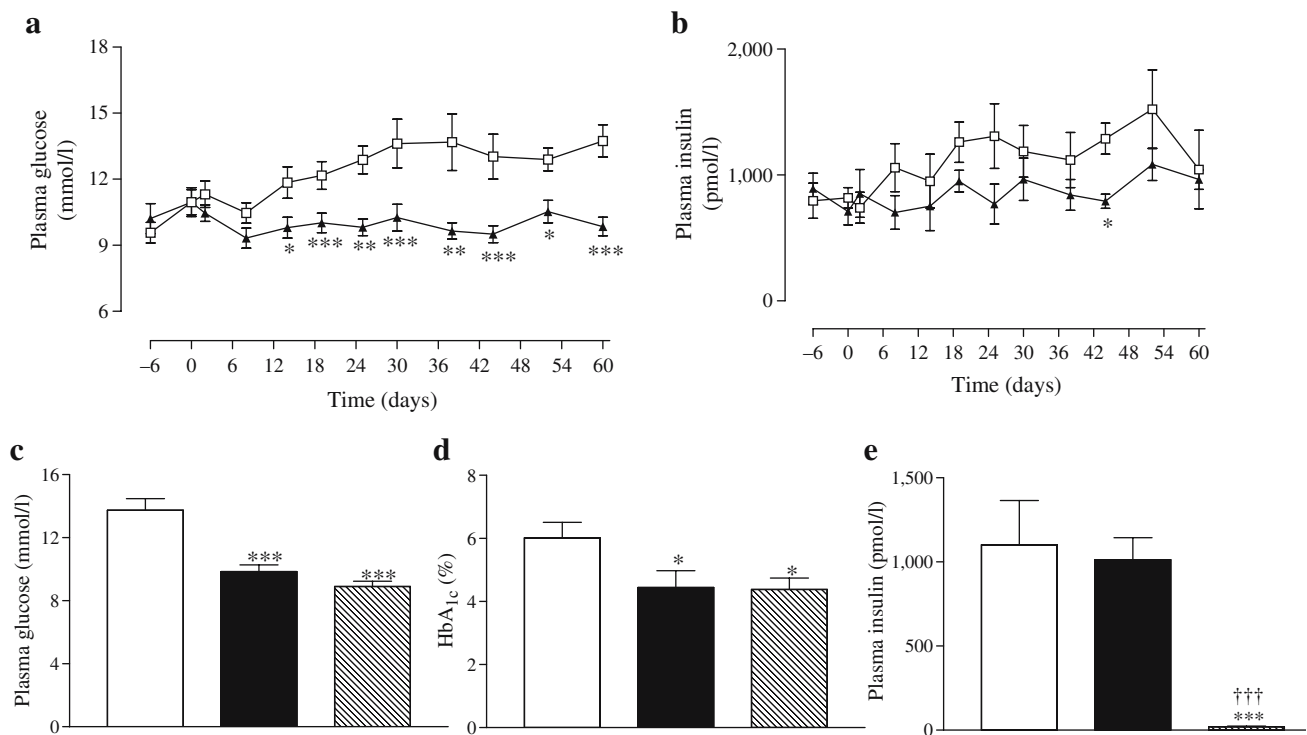
a

b

c

d

e

Fig. 2 a, b Effects of daily (Pro^3)GIP administration on non-fasting glucose profile (**a**) and insulin profile (**b**) of *ob/ob* mice. Parameters were measured for 5 days prior to and for 60 days during treatment with saline (*squares*) or (Pro^3)GIP (25 nmol kg^{-1} day^{-1}; *triangles*). **c**– **e** Final 60 day measures of glucose (**c**), HbA$_{1c}$ (**d**) and insulin (**e**) in (Pro^3)GIP-treated *ob/ob* mice (*black bars*), saline-treated *ob/ob* mice (*white bars*) and untreated lean control mice (*hatched bars*). HbA$_{1c}$ concentrations were assessed on day 60. Values are means±SEM for groups of 7–8 mice. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs saline-treated *ob/ob* mice; $^{†††}p<0.001$ vs (Pro^3)GIP-treated *ob/ob* mice
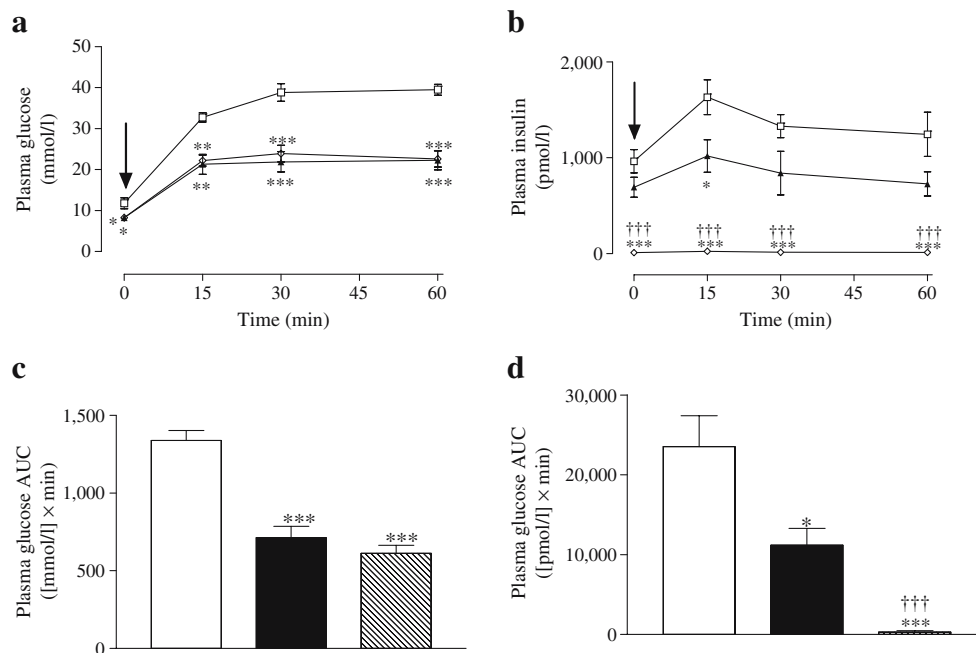
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Fig. 3 Effects of daily (Pro^3)GIP administration on glucose tolerance (**a**) and plasma insulin (**b**) response to glucose in (Pro^3)GIP-treated *ob/ob* mice (*triangles*), saline-treated *ob/ob* mice (*squares*) and untreated lean control mice (*diamonds*). Tests were conducted after daily treatment for 60 days. Glucose (18 mmol/kg body weight) was administered at the time indicated by the arrow. Plasma glucose (**c**) and insulin AUC values (**d**) for 0–60 min post-injection are also shown for the (Pro^3)GIP-treated *ob/ob* mice (*black bars*), saline-treated *ob/ob* mice (*white bars*) and untreated lean control mice (*hatched bars*). Values are means±SEM for groups of eight mice. *$p<0.05$, **$p<0.01$, ***$p<0.001$ vs saline-treated *ob/ob* mice; $^{†††}p<0.001$ vs (Pro^3)GIP-treated *ob/ob* mice

Fig. 4 **a** Effects of daily (Pro^3) GIP administration on plasma glucose in (Pro^3)GIP-treated *ob/ob* mice (25 nmol kg^{-1} day^{-1}, triangles), saline-treated *ob/ob* mice (squares) and untreated lean control mice (diamonds). Insulin (50 U/kg body weight) was administered by intraperitoneal injection at the time indicated by the arrow. **b** Plasma glucose AUC values. **c, d** Pancreatic weight (**c**) and insulin content (**d**) in (Pro^3)GIP-treated *ob/ob* mice (black bars), saline-treated *ob/ob* mice (white bars) and untreated lean control mice (hatched bars). Tests were conducted after daily treatment for 60 days. Values are means±SEM for groups of 7–8 mice. *p< 0.05, **p<0.01 vs saline-treated *ob/ob* mice; †p<0.05 vs (Pro^3) GIP-treated *ob/ob* mice
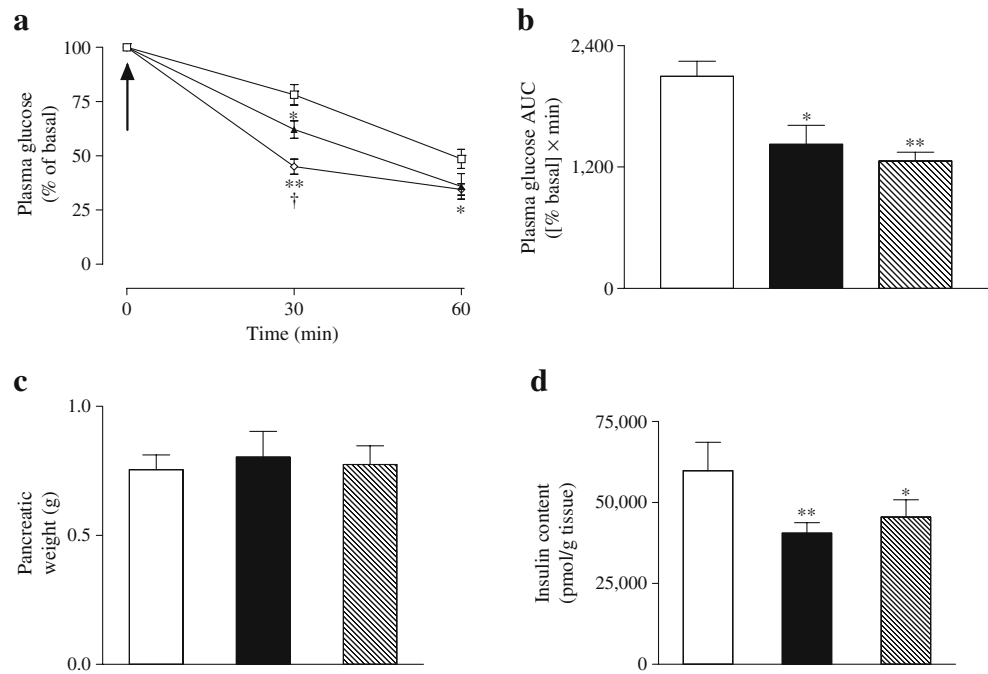
key observations, together with increasing awareness of the extrapancreatic actions of GIP, such as effects on lipid metabolism, have led to speculation that GIP receptor antagonism may offer a novel and potentially useful therapeutic approach to the treatment of obesity and associated metabolic abnormalities [11, 35].

In the present study, (Pro^3)GIP, a specific and stable GIP receptor antagonist [31], was utilised to assess whether chemical ablation of GIP signalling for 60 days in young prediabetic *ob/ob* mice could prevent the development of diabetes and associated metabolic features of the obesity-

driven syndrome. The *ob/ob* mouse model represents an extreme phenotype, being noted for marked hyperphagia, gross obesity and particularly severe insulin resistance, driven by leptin deficiency [42]. As such, the *ob/ob* model represents an appropriate but strong challenge to potential antihyperglycaemic agents. Consistent with other observations [30, 32, 34], GIP blockade had no effect on food intake, and mice continued to thrive throughout the study period. Obesity was already well established in the *ob/ob* mice, which were 5–7 weeks of age at commencement of the study, and body weights increased progressively

Fig. 5 Effects of daily (Pro^3) GIP administration on lipid profile in (Pro^3)GIP-treated *ob/ob* mice (black bars), saline-treated *ob/ob* mice (white bars) and untreated lean control mice (hatched bars). Parameters were measured after daily treatment with (Pro^3)GIP (25 nmol/kg body weight) or saline for 60 days. Values are means±SEM for groups of 7–8 mice. *p< 0.05 vs saline-treated *ob/ob* mice; ††p<0.01, †††p<0.001 vs (Pro^3)GIP-treated *ob/ob* mice
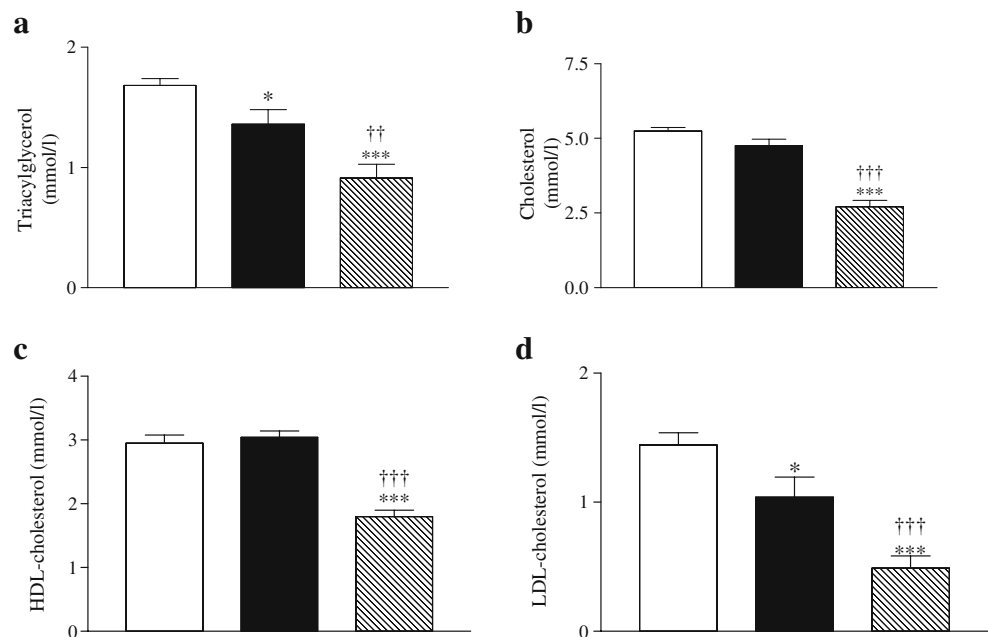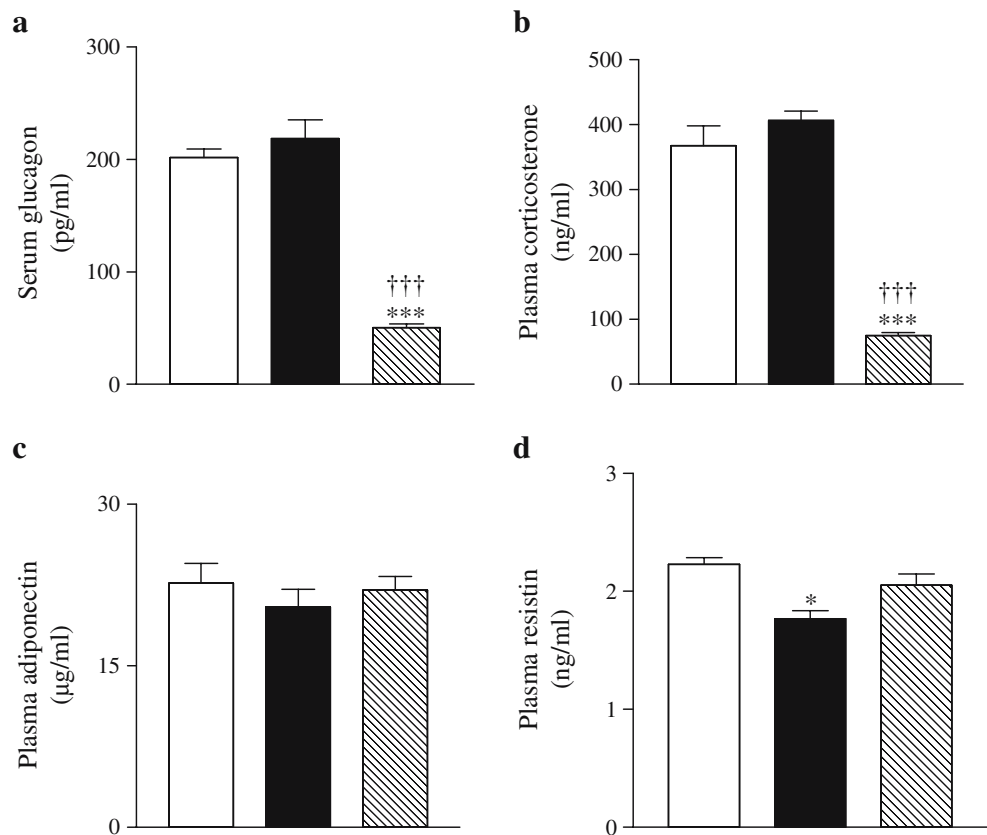
compared with lean controls. In contrast to the results of Miyawaki et al., obtained in a study that used GIP receptor knockout mice [30], interference of GIP signalling by daily injections of (Pro³)GIP did not significantly arrest the development of obesity as assessed by simple body weight or body weight gain measurements. Perhaps such a comparison is unfair given the narrow window of the present treatment compared with an 8 month study in *ob/ob* mice with lifelong knockout of the GIP receptor. Nevertheless, (Pro³)GIP did show an obvious and progressive trend towards decreased body weights over the present 60 day study. Further research is required to clarify such an effect, possibly involving more protracted observations with larger experimental groups and exploitation of dual-energy X-ray absorptiometry (DEXA) to accurately measure body fat mass. Notably, similar (Pro³)GIP treatment of mice fed high-fat and cafeteria diets resulted in significant decreases in body weight, tissue fat deposition and adipocyte size (unpublished results). Initiation of treatment in preweanling *ob/ob* mice before adipocyte proliferation, or consumption of a high-fat diet as opposed to the present carbohydrate-rich diet are also potentially interesting avenues for exploration.

Despite the absence of significant effects on the progression of obesity, the early administration of (Pro³) GIP to prediabetic *ob/ob* mice had many notable effects on metabolic features of the obesity-related diabetes syndrome. The GIP receptor antagonist significantly improved non-

fasting glucose, HbA$_{1c}$, i.p. glucose tolerance, meal tolerance and insulin sensitivity in *ob/ob* mice. Similar actions have also been noted with 11 days of (Pro³)GIP treatment in adult *ob/ob* mice [32]. However, more remarkably, GIP receptor blockade prevented the age-related development of diabetes in the present study, and none of the above mentioned parameters differed significantly between (Pro³)GIP-treated *ob/ob* mice and normal age-matched lean control mice. Furthermore, the present observations suggest that GIP antagonism may be particularly effective therapeutically in the early stages of diabetes.

Although (Pro³)GIP-treated *ob/ob* mice did not develop diabetes as judged against relevant parameters in normal lean mice, chemical GIP receptor blockade did not prevent the emergence of other characteristic features of the *ob/ob* syndrome. Plasma and pancreatic insulin concentrations were lower in treated than in untreated *ob/ob* mice, but plasma insulin concentrations were still substantially raised compared with those in lean control mice. This suggests the persistence of abnormalities in counter-regulatory mechanisms, such as those manifested by the observed elevations of glucagon and corticosterone, which were offset by moderate levels of beta cell hyperactivity. For example, although there is no doubt that insulin sensitivity was greatly improved by (Pro³)GIP administration, it is unlikely to have been totally normalised in *ob/ob* mice as judged by simple tests based on hypoglycaemic activity of injected

insulin. Circulating adipokines were not appreciably affected by (Pro³)GIP, with only a small possibly beneficial decrease in resistin observed. This does not indicate a significant GIPergic adipokine generation in *ob/ob* mice and questions their recently postulated role in alleviating insulin sensitivity in GIP receptor knockout mice [43]. The abnormal lipid profile also persisted in *ob/ob* mice treated with (Pro³)GIP, but there were significant improvements in triacylglycerol and LDL-cholesterol. The former is in accordance with the reported elevation of GIP concentrations in hypertriglyceridaemic subjects [44].

Given that GIP is classically recognised as an important insulin-releasing hormone and a major component of the enteroinsular axis [2], the beneficial antihyperglycaemic actions of GIP receptor blockade reported here and elsewhere [30, 32] are quite notable. In normal mice, genetic knockout or chemical antagonism of the GIP receptor clearly results in mild impairments of glucose homeostasis and insulin secretion [34, 45]. Similar effects were produced by acute as opposed to longer-term administration of (Pro³)GIP to adult *ob/ob* mice [36]. Viewed in simple terms, ablation of GIP receptor function in normal mice resulted in modest detrimental deficits in insulin release, as also observed when *ob/ob* mice were administered (Pro³) GIP acutely. However, in situations of obesity and insulin resistance, as exemplified by *ob/ob* mice, longer-term GIP receptor blockade resulted in significant amelioration of diabetes and associated metabolic features [30, 32]. Thus, substantial differences exist between GIP actions in obesity as opposed to normal physiology, emphasising an important pathophysiological role of GIP. Adipocyte GIP receptors may be especially important, as GIP has been shown to promote fatty acid uptake and lipogenesis [25, 26]. Since *ob/ob* mice exhibit leptin deficiency and low sympathetic activity [42], we cannot rule out additional effects, such as increased sympathetic activity and fatty acid oxidation. However, it is important to note that similar metabolic actions of (Pro³)GIP were observed in mice fed high-fat and cafeteria diets to induce obesity and insulin resistance (unpublished results). These various observations suggest that changes in fat deposition in tissues that influence insulin sensitivity, such as liver and muscle, may be particularly important.

Although the above conclusions are necessarily based on experimental studies in animal models, there are strong parallels with recent studies in humans undergoing surgical bypass for treatment of gross obesity and associated diabetes [46–49]. The similarity with our results of GIP receptor blockage in *ob/ob* mice suggest that GIP is key in this type of surgery and links obesity to insulin resistance and diabetes in man. Accordingly, antagonism of GIP action appears to offer considerable promise for the development of a new and effective therapeutic approach to obesity-related diabetes through alleviation of insulin resistance.

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