GIP receptor antagonism reverses obesity, insulin resistance, and associated metabolic disturbances induced in mice by prolonged consumption of high-fat diet

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GIP receptor antagonism reverses obesity, insulin resistance, and associated metabolic disturbances induced in mice by prolonged consumption of high-fat diet. Am J Physiol Endocrinol Metab 293: E1746–E1755, 2007. First published September 11, 2007; doi:10.1152/ajpendo.00460.2007.—The gut hormone gastric inhibitory polypeptide (GIP) plays a key role in glucose homeostasis and lipid metabolism. This study investigated the effects of administration of a stable and specific GIP receptor antagonist, (Pro3)GIP, in mice previously fed a high-fat diet for 160 days to induce obesity and related diabetes. Daily intraperitoneal injection of (Pro3)GIP over 50 days significantly decreased body weight compared with saline-treated controls, with a modest increase in locomotor activity but no change of high-fat diet intake. Plasma glucose, glycated hemoglobin, and pancreatic insulin were restored to levels of chow-fed mice, and circulating triglyceride and cholesterol were significantly decreased. These changes were accompanied by significant improvement of insulin sensitivity, meal tolerance, and normalization of glucose tolerance in (Pro3)GIP-treated high-fat-fed mice. (Pro3)GIP concentrations peaked rapidly and remained elevated 24 h after injection. These data indicate that GIP receptor antagonism using (Pro3)GIP provides an effective means of countering obesity and related diabetes induced by consumption of a high-fat, energy-rich diet.

In addition to the classical β-cell target, the GIP receptor is expressed on various extrapancreatic tissues, including bone, intestine, heart, stomach, brain, and adipose tissue (46, 49). The significance of GIP action at these sites is largely unknown, but the particularly potent and prolonged stimulation of GIP secretion after high-fat feeding (35) draws attention to a possible key role in fat metabolism (50). This is additionally supported by studies demonstrating that high-fat feeding increased K cell hyperplasia and enhanced GIP gene expression and intestinal GIP content, resulting in elevated circulating GIP concentrations (2, 13, 15, 34). Obesity/diabetes in humans and animal models has also been shown (4, 14, 38, 47) to be associated with elevated circulating GIP and exaggerated K cell secretory responses. In tune with these observations, GIP mediates various anabolic effects on adipocytes at the cellular level, including stimulation of glucose uptake, lipoprotein lipase activity, fatty acid synthesis, and fatty acid incorporation (9, 27, 28, 31). Since GIP also inhibited glucagon and isoproterenol-induced lipolysis, these diverse cellular actions of GIP strongly favor enhanced fat deposition (8, 20).

The above scenario suggests that GIP has partly evolved as a hormone to efficiently promote fat storage and energy deposition in times of plenty. Consistent with this view, both normal and obese/diabetic ob/ob mice with genetic knockout of the GIP receptor are protected from diet-induced obesity (30). Furthermore, our previous studies in ob/ob mice have shown that short-term administration of the specific and stable GIP receptor antagonist (Pro3)GIP (16) can reverse or prevent many of the established metabolic abnormalities associated with obesity (18, 26). In the present study, normal mice fed high-fat diet for 160 days were used to examine whether prolonged GIP receptor antagonism using daily injections of (Pro3)GIP was able to reverse well-established diet-induced obesity and related metabolic abnormalities. The results demonstrate that chemical GIP receptor blockade was capable of promoting weight loss, improving insulin resistance, and reversing both glucose intolerance and diabetes.

MATERIALS AND METHODS

Animals. Young (8-wk-old) male National Institutes of Health Swiss mice (Harlan UK) were age-matched, divided into groups, and housed individually in an air-conditioned room at 22 ± 2°C with a 12:12-h light-dark cycle (0800–2000). Experimental animals had free access to drinking water and a high-fat diet (45% fat, 20% protein, and

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35% carbohydrate; %total energy of 26.15 KJ/g; Special Diets Service, Essex, UK). Age-matched control mice from the same colony had free access to standard rodent maintenance diet (10% fat, 30% protein, and 60% carbohydrate; %total energy of 12.99 KJ/g; Trouw Nutrition, Cheshire, UK) and were used for comparative purposes as appropriate. Prior to commencement of experimental studies, animals were maintained on a high-fat diet for 160 days. In addition, a further set of animals was maintained on high-fat diet for 112 days prior to measurement of circulating GIP and GLP-1 levels. On both occasions obesity and diabetes were clearly manifested as judged by body weight, plasma glucose, and glycated hemoglobin analyses. All animal experiments were carried out in accordance with the UK Animals
(Scientific Procedures) Act of 1986 and approved by the University of Ulster Animal Ethics Review Committee.

Experimental protocols. Mice previously fed a high-fat diet for 160 days received once daily intraperitoneal injections (1700) of either saline vehicle [0.9% (wt/vol) NaCl] or (Pro3)GIP (25 nmol/kg body wt; Sigma Genosys, Suffolk, UK) over an ensuing 50-day period. Food intake and body weight were recorded daily, and plasma glucose and insulin concentrations were monitored at intervals of 5–7 days. Blood for glycated hemoglobin and plasma for measurement of cholesterol, triglycerides, glucagon, corticosterone, and circulating adipokines were taken on day 50. Intraperitoneal glucose tolerance (18 mmol/kg body wt) and insulin sensitivity (50 U/kg body wt) tests were performed at the end of the study period. The metabolic response of 18-h-fasted mice to 15 min refeeding of high-fat diet (45% fat, 20% protein, and 35% carbohydrate) was also investigated. This test offers a more physiological insight into the actions of (Pro3)GIP compared with an oral glucose tolerance test and allows assessment of hormonal and metabolic responses to mixed high-fat meal. Similar observations were made using age-matched normal lean control mice as indicated.

In a separate series, pancreatic tissues were excised at the end of the 50-day treatment period, weighed, and snap-frozen for measurement of insulin content following extraction with 5 ml/g of ice-cold acid ethanol (750 ml of ethanol, 235 ml of water, 15 ml of concentrated HCl). Brown adipose tissue, white adipose tissue (perirenal, subcutaneous, and epididymal), liver, and skeletal muscle (gastrocnemius) were also excised, weighed, and stored for histology or analysis of triglyceride content following extraction (667 ml of chloroform, 332.5 ml of methanol, 0.5 ml of 0.1% H2SO4). For studies of incretin hormones, plasma samples were collected from nonfasted mice fed a high-fat diet for 112 days plus an additional 35 days of treatment with (Pro3)GIP or saline as above. Circulating (Pro3)GIP was estimated by cross-reacting COOH-terminally reactive GIP assay, using normal mice after acute ip injection of 25 nmol/kg (Pro3)GIP or using high-fat fed mice at intervals following the last in series of 40 daily 100 nmol/kg (Pro3)GIP injections. These two additional groups of long-term (Pro3)GIP-treated mice exhibited similar benefits in body weight and glycemic status, as documented in the main part of the study. In a final set of experiments, locomotor activity tests were performed in high-fat-fed mice after 4 wk of treatment with either 25 nmol/kg (Pro3)GIP or saline, as assessed from total distance traversed in an open field (120 × 120 cm surface area with 35-cm-high walls), calculated from measurement of line breaks (15 × 15 cm grid). Animals were given 3 min each in the arena and tested for 5 days (25).

Further details of the experimental protocols employed have been published elsewhere (18, 26).

Biochemical analysis. 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 Fig. 3. Effects of daily (Pro3)GIP administration on plasma glucose (A), insulin (B), and intact glucagon-like peptide-1 (GLP-1; C) responses to feeding in mice with diet-induced obesity. Observations were made in 18-h-fasted mice. D: fasting and fed concentrations of intact GLP-1 in mice with diet-induced obesity. Mice had previously been fed control or high-fat diet for 160 days. Tests were conducted after daily treatment with saline vehicle or (Pro3)GIP (25 nmol/kg body wt−1day−1) for an additional 50 days. The horizontal black bar indicates the time of feeding (15 min). Plasma glucose, insulin, and GLP-1 AUC values for 0–105 min are also shown. All values are means ± SE for 8 mice. A, B, and C: *P < 0.05 and **P < 0.01 compared with high-fat group. D: *P < 0.05 and **P < 0.01 compared with respective fed levels.

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Intra-assay coefficient of variation was 2%.

Corticosterone was measured similarly using a kit from MP Biomedicals (Heidelberg, Germany). Plasma and tissue triglyceride and cholesterol levels were measured using a Hitachi Automatic Analyser 912 (Boehringer Mannheim). Protein was determined using a DC protein kit (BioRad, Hercules, CA). All analyses were carried out according to instructions supplied by the various manufacturers.

(Pro3)GIP concentrations after administration of large doses of (Pro3)GIP were measured, after extraction with 70% ethanol (vol/vol, final concentration), by established radioimmunoassay for total GIP (29). The COOH-terminally directed GIP antiserum used (R 65) cross-reacts fully with human GIP, GIP-(3–42), and (Pro3)GIP. In this study, antiserum R 65 measured endogenous total GIP of nonfasted normal mice as 36.6 ± 7.9 pmol/l. It is assumed that concentrations (≤26,000 pmol/l) measured above this baseline level after injection of (Pro3)GIP primarily reflect exogenous peptide. (Pro3)GIP is not degraded by dipeptidyl peptidase IV, as established in previous studies (16). In extracts of mouse small intestine, GIP immunoreactivity reached similar concentrations as in human tissues diluted in parallel with human standards and coeluted with human GIP upon gel exclusion and HPLC analysis (45). Human GIP and 125I human GIP (70 MBq/mmol) were used for standards and tracer. In mouse plasma, the sensitivity was <5 pmol/l, intra-assay coefficient of variation was <6% at 20 pmol/l, and recovery of standard, added to plasma before extraction, was ~100% when corrected for losses inherent in the plasma extraction procedure.

Total GLP-1 concentrations in plasma were measured by radioimmunoassay after extraction of plasma with 70% ethanol (vol/vol, final concentration). Carboxy-terminal GLP-1 immunoreactivity was determined using antiserum 89390, which has an absolute requirement for the intact amidated carboxy terminus of GLP-1-(7–36) amide and cross-reacts <0.01% with carboxy-terminally truncated fragments and 89% with GLP-1-(9–36) amide. The sum of the two components (total GLP-1 concentration) reflects the rate of secretion of the L cell (32). Sensitivity was <5 pmol/l and intra-assay coefficient of variation <10%. Recovery of incretin peptides from plasma, before correction, was >96%. The assay has been validated for use in mouse material by HPLC analysis. Intact GLP-1 was measured by enzyme-linked immunosorbent two-site sandwich assay (47) using two monoclonal antibodies: GLP-1F5 as catching antibody and Mab26.1 as detecting antibody. GLP-1F5 is COOH-terminally directed, reacting around residue 26 of GLP-1 (i.e., PG 104), whereas Mab26.1 is NH2-terminally directed and requires the intact NH2 terminus of GLP-1. The assay uses unextracted plasma, which is collected and stored in the presence of a dipeptidyl peptidase IV inhibitor (valine-pyrrolidide, 0.01 mM final concentration added to the blood sample immediately after collection). GLP-1-(7–36) amide standards were prepared in human plasma that had been depleted of endogenous intact GLP-1 immunoreactivity (by overnight incubation at 37°C).

The assay has an absolute requirement for the free intact NH2 terminus of GLP-1 but reacts equally with GLP-1-(7–36) amide and GLP-1-(7–37), which are found in almost equal amounts in mouse material (47). The assay has a detection limit of <0.5 pmol/l. Intra-assay coefficient of variation was 2%.

Histology. Tissue for histology was fixed in 4% (wt/vol) paraformaldehyde-PBS and embedded in paraffin. Sections (6 μm) were cut, mounted on slides, stained with hematoxylin, and counterstained with eosin. Stained slides were viewed under a microscope (Nikon Eclipse E2000; Diagnostic Instruments, Sterling Heights, MI) attached to JVC camera Model KY-F55B (JVC, London, UK).

Statistics. Results are expressed as means ± SE. Data were compared using ANOVA followed by a Student-Newman-Keuls post hoc test. Area under the curve (AUC) analyses were calculated using the trapezoidal rule with baseline subtraction. Groups of data were considered to be significantly different if \( P < 0.05 \).

![Fig. 4. Effects of daily (Pro3)GIP administration on insulin sensitivity and pancreatic insulin content in mice with diet-induced obesity. Mice had previously been fed control or high-fat diet for 160 days. Observations were made after daily treatment with saline vehicle or (Pro3)GIP (25 mmol/kg body wt \( \cdot \) day \( \cdot \) 1) for an additional 50 days. A: glucose-lowering action of insulin (25 U/kg body wt) administered by ip injection at the time indicated by the arrow (0 min). B: AUC values for 0–60 min postinjection are also shown. C: pancreatic insulin content. All values are means ± SE for 8 mice. **P < 0.01 and ***P < 0.001 compared with control group. \( \Delta \)P < 0.05 and \( \Delta\Delta \)P < 0.01 compared with high-fat group.](http://ajpendo.physiology.org/)

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RESULTS

Effects of (Pro³)GIP on food intake, body weight, nonfasting glucose, glycated hemoglobin, cholesterol, and triglyceride levels. Compared with standard rodent diet (control), mice fed high-fat diet for the previous 160 days exhibited increased energy intake, body weight, and circulating glucose concentrations (Fig. 1). These parameters remained elevated throughout the study, and plasma cholesterol and triglycerides were also raised at 50 days. Administration of (Pro³)GIP had no effect on energy intake, but from day 8 onward (Pro³)GIP significantly (P < 0.05 to P < 0.001) lowered body weights by a modest 8% compared with high-fat-fed, saline-treated mice (60 ± 1 vs. 55 ± 1 g on day 50, respectively). Plasma glucose was correspondingly decreased (P < 0.05 to P < 0.01) from day 14 onward (10.7 ± 0.5 vs. 9.4 ± 0.4 mmol/l on day 50, respectively) and remained significantly lower than high-fat-fed controls throughout the study (Fig. 1). Plasma glucose levels were restored almost to those of mice on regular maintenance diets despite similar insulin concentrations in the three groups. Consistent with this pattern, glycated hemoglobin was significantly lower (18%, P < 0.05) after 50 days of treatment with (Pro³)GIP compared with saline-treated, high-fat-fed mice. This value was not significantly different from control mice on a standard rodent diet. (Pro³)GIP treatment for 50 days also completely reversed the detrimental effects of high-fat feeding on plasma cholesterol and triglyceride levels (P < 0.01 and P < 0.05, respectively; Fig. 1).

Effects of (Pro³)GIP on glucose tolerance. High-fat feeding induced elevated and impaired glycemic response glucose compared with mice fed a regular diet (Fig. 2). Basal plasma glucose levels were 8.2 ± 0.3, 10.5 ± 0.7, and 9.1 ± 0.4 mmol/l in the lean control, high-fat, and (Pro³)GIP high-fat groups, respectively. Daily administration of (Pro³)GIP for 50 days significantly reduced (P < 0.05) the overall glycemic excursion by 30% following intraperitoneal glucose in high-fat-fed mice (Fig. 2). Indeed, (Pro³)GIP-treated mice exhibited similar glycemic responses to mice fed standard rodent diet. Further extension of the observation period would likely emphasize the observed differences of glucose intolerance between (Pro³)GIP-treated and insulin-resistant high-fat control mice. Plasma insulin concentrations were not significantly different in the three groups (Fig. 2).

Effects of (Pro³)GIP on hormonal and metabolic responses to feeding. Because intraperitoneal glucose administration gives information on β-cell glucose sensitivity and insulin action without involvement of intestinal factors, the hormonal and metabolic responses to being fed a high-fat diet were examined. As shown in Fig. 3A, the overall glycemic response to 15 min of feeding (reflecting the carbohydrate content of the high-fat diet) was significantly decreased (45%, P < 0.05) in high-fat-fed mice treated with (Pro³)GIP compared with saline controls despite similar food intake (0.41 ± 0.03 vs. 0.45 ± 0.06 g·15 min⁻¹·mouse⁻¹, respectively). Furthermore, plasma insulin levels were significantly lowered in terms of postfeed-
decreased by (Pro3)GIP treatment, with values similar to nor-
pancreatic insulin content was significantly (39%, 
P < 0.01) and (Pro3)GIP-treated (51%, 
P < 0.05) animals (Fig. 3D). Intact GLP-1 levels of mice treated with (Pro3)GIP were not significantly different from saline-treated, high-fat-fed mice.

Effects of (Pro3)GIP on insulin sensitivity and pancreatic insulin. As shown in Fig. 4, the hypoglycemic action of insulin was significantly augmented (P < 0.05) by ≥29% in terms of postinjection values and AUC measures in high-fat-fed mice treated with (Pro3)GIP compared with saline controls. Although (Pro3)GIP significantly improved insulin resistance induced by high-fat diet, postinjection and overall AUC mea-
sures were still significantly (P < 0.01 to P < 0.001) impaired compared with mice on control diet (Fig. 4). Interestingly, pancreatic insulin content was significantly (39%, 
P < 0.01) decreased by (Pro3)GIP treatment, with values similar to nor-
mal lean mice at 50 days (Fig. 4C). Pancreatic weights were not significantly different in all three groups (data not shown).

Effects of (Pro3)GIP on circulating glucagon, corticoste-
rone, and adipokines. Compared with saline-treated, high-fat-
fed mice, daily (Pro3)GIP treatment significantly lowered cir-
culating glucagon concentrations by 33% (P < 0.05; Fig. 5). Plasma corticosterone was substantially raised by 229% (P < 0.001) by high-fat feeding; however, 50-day (Pro3)GIP treat-
ment completely reversed this effect, returning corticosterone to levels similar to those of mice on the standard diet (Fig. 5). Neither high-fat feeding nor (Pro3)GIP administration had any effect on plasma resistin or adiponectin levels (Fig. 5).

Effects of (Pro3)GIP on circulating incretin hormones. As shown in Fig. 5, plasma concentrations of intact GLP-1 were similar in high-fat-fed mice treated with daily (Pro3)GIP or saline. High-fat feeding significantly decreased (P < 0.05) total GLP-1 by 29% in mice treated with saline but not (Pro3)GIP. Figure 6 shows circulating (Pro3)GIP concentra-
tions after single injection in normal, untreated mice or 40-day (Pro3)GIP-treated high-fat-fed mice. In the former, a rapid peak was observed at 15 min that was followed by protracted decline with significant levels still recorded at 24 h (P < 0.05). The extended daily treatment group exhibited significantly raised baseline concentrations (235.8 ± 39.7 vs. 99.4 ± 15.0 pmol/l, P < 0.05), a similar prominent peak at 15 min (26,823.2 ± 1,631.0 pmol/l), and gradual decline such that concentrations were significantly increased at 24 h (186.4 ± 24.7 pmol/l, P < 0.05) compared with nadir (99.4 ± 15.0 pmol/l) at 72 h after final injection.

Effects of (Pro3)GIP on liver and adipose tissue weights. Compared with regular diet, high-fat feeding increased combined adipose tissue weight (173%, P < 0.001), being ac-
counted for by increases in subcutaneous, perirenal, and brown adipose tissue deposits (Fig. 7). Combined adipose tissue weights, brown adipose tissue deposits, and subcutaneous white adipose tissue deposits were significantly decreased 23–46% (P < 0.001, P < 0.01, and P < 0.01, respectively) by (Pro3)GIP treatment compared with saline-treated, high-fat-fed mice. Furthermore, the brown adipose tissue depot was re-
stored to levels observed in mice fed normal diet (Fig. 7).

Neither high-fat feeding nor (Pro3)GIP treatment had any significant effects on percentage of liver or epididymal white adipose tissue weights.

Effects of (Pro3)GIP on liver and muscle lipid content and morphology. High-fat feeding for 160 days significantly ele-
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cate liver triglyceride content by 25 and 21%, respectively (P < 0.001 and P < 0.05; Fig. 8). Daily (Pro3)GIP administration significantly reduced these levels (P < 0.05), although values were still significantly elevated (P < 0.05 and P < 0.001, respectively) compared with control mice (Fig. 8). Mice on high-fat diet for 160 days had larger epididymal adipocytes relative to control mice, whereas 50-day (Pro3)GIP treatment appeared to substantially decrease adipocyte size (Fig. 9, A–C). In addition, this (Pro3)GIP treatment appeared to partially restore liver morphology in high-fat-fed mice (Fig. 9, D–F).

Effects of (Pro3)GIP on locomotor activity. High-fat feeding had no significant effect on physical activity compared with control diet (73.9 ± 2.9 vs. 82.1 ± 4.7 line breaks/min, means ± SE, n = 6, respectively). However, mice treated with
(Pro³)GIP exhibited significantly greater locomotor activity (95.4 ± 6.2 line breaks/min) compared with mice on the regular (P < 0.05) but not high-fat diet (P < 0.05).

**DISCUSSION**

Consumption of high-fat diet resulted in increased energy consumption, progressive weight gain, and elevations of plasma glucose and glycated hemoglobin, leading to impaired insulin sensitivity and glucose intolerance by 160 days. Adipose tissue deposits were increased together with adipocyte hypertrophy and deposition of enhanced amounts of triglyceride in liver and muscle. Circulating cholesterol and triglyceride concentrations were also raised. This form of diet-induced obesity has been used extensively alongside genetic models (2, 15) and has close parallels with obesity, as found increasingly in humans consuming high-fat, energy-rich diets.

It is quite remarkable that simple dietary intervention can so readily induce overt diabetes in otherwise normal animals. Nevertheless, blockade of GIP action by daily administration of (Pro³)GIP was able to partially or fully reverse many of the parameters in these mice. Thus, despite continuing on the high-fat diet, attenuation of GIP signaling modestly but significantly decreased body weight and many of the associated metabolic abnormalities without change of energy intake. This clearly illustrates the important role of GIP as a critical link between overnutrition and development of obesity (16, 26). Recent studies have also shown that chemical blockade of GIP action counters the development of obesity/diabetes in high-fat-fed mice (19). However, the present study importantly demonstrates that antagonism of GIP, using (Pro³)GIP, can promote weight loss and ameliorate insulin resistance and diabetes in animals without genetic manipulation and in which diet-induced obesity and related traits are already well established.

Although previous short-term studies using (Pro³)GIP have shown similar improvement of diabetes in ob/ob mice without change of body weight (18, 26), interference in common biochemical mechanisms seem likely to underlie both actions. Knockout of the GIP receptor in ob/ob or normal mice fed a high-fat diet has been shown to decrease respiratory quotient without change in oxygen consumption, indicating preferential oxidation of fat as an energy source (23, 30). Interestingly, the change of respiratory quotient related specifically to the light phase, during which mice are usually inactive. Thus, increase of energy expenditure might partly reflect the increased locomotor activity noted in this and previous studies (23). However, such an effect is unlikely to be the sole mechanism underlying differences in energy expenditure following ablation of GIP signaling. Furthermore, transgenic mice with overexpression of GIP have been reported to display increased locomotor activity (5), which contrasts with these other findings.

Despite uncertainties of the exact role of GIP in lipid metabolism and energy balance, it is well established that
high-fat feeding, such as that used in the present study, will increase intestinal K cell hyperplasia, enhance GIP gene expression together with intestinal GIP content, and result in elevated basal and feeding-induced circulating GIP concentrations (2, 11, 13, 15). Through binding to adipocyte GIP receptors (46, 49), this will increase lipoprotein lipase activity and cleavage of triglyceride, resulting in GIP-stimulated fatty acid uptake and intracellular triglyceride deposition (9, 20, 27, 31). In preventing this scenario, GIP blockade will favor uptake and rapid oxidation of fatty acids by muscle and liver, which lack GIP receptors (46, 52). Thus, increased expression of genes for fatty acid transport and β-oxidation has been observed in muscle and liver together with lower respiratory quotient and higher fat oxidation in vivo (30, 52). Adipose tissue lipolysis will also be enhanced with (Pro3)GIP treatment through alleviation of GIP-mediated inhibition of effects of glucagon and sympathetic activation (20). As a result of these effects, triglyceride stores in adipose tissue, muscle, and liver were significantly decreased in (Pro3)GIP-treated, high-fat-fed mice, as similarly observed in GIP receptor knockout mice (23). Such actions induced by (Pro3)GIP may explain not only protection from excessive weight gain but also increased body weight loss in animals with diet-induced obesity. However, NMR or spectroscopy studies of total body fat distribution are needed to confirm these findings.

Our previous studies in ob/ob mice have shown that amelioration of insulin resistance is a key element underlying the improvement of glucose homeostasis following blockade of GIP action using (Pro3)GIP (18, 26). The two major abnormalities underlying insulin resistance are increased hepatic glucose output and decreased glucose uptake primarily by skeletal muscle (51). It is well established in both human and animal studies that consumption of a high-fat diet results in increased fat deposition not just in adipocyte stores but also in liver and skeletal muscle (51). Crucially, such changes have been shown to interfere with insulin action at both sites, giving rise to notable insulin resistance (21, 37, 39). Although (Pro3)GIP treatment resulted in a notable improvement of insulin sensitivity, we are unable at the present time to differentiate this effect within distinct tissues. However, by simply alleviating tissue triglyceride stores as observed in the present study, (Pro3)GIP administration can be expected to account for the noted improvements of insulin sensitivity, blood glucose, lipids, glycated hemoglobin, and glucose tolerance. Pancreatic insulin content was decreased as observed in similarly treated ob/ob mice along with β-cell sparing and restoration of islet architecture (18, 26). Insulin levels were also lower following test meal, reflecting decreased need as a result of improved insulin sensitivity together with reduced functional demand imposed by circulating glucose and lipids. However, as noted previously (26), treatment with (Pro3)GIP is unlikely to totally normalize insulin sensitivity, and as a consequence a degree of β-cell hyperactivity is needed for metabolic control.

Changes in circulating glucagon might theoretically contribute to the metabolic benefits of GIP blockade because of its established insulin counterregulatory actions (44). However, the observed decrease of glucagon was small, suggesting against a key involvement in the mechanism of (Pro3)GIP action. Similarly, any potential compensatory increase in circulating intact GLP-1 appears to be absent from the present results. Circulating adiponectin and resistin were also both unchanged, indicating dissociation from their purported effects on insulin sensitivity (3). This questions the proposed role of GIP in promoting circulating resistin concentrations (23). However, similar to GIP receptor knockout mice (30), endogenous GIP was unchanged by (Pro3)GIP, indicating absence of normal regulatory K cell feedback inhibition. Taking advantage of COOH-terminally directed GIP assay to monitor (Pro3)GIP, it was evident that this exogenous peptide remained in circulation for at least 4 h after a single one-off injection. Furthermore, repeated daily administration was associated with elevated (Pro3)GIP at least 24 h after the last injection, being reminiscent of delayed clearance of exendin-4 (40).

Perhaps the most interesting hormonal change was normalization of raised plasma corticosterone in high-fat-fed mice following GIP blockade. This is especially true given its actions to promote hepatic gluconeogenesis and antagonize insulin-stimulated glucose uptake in both skeletal muscle and adipose tissue (6). Significant benefits of adrenalectomy have been demonstrated previously in ob/ob mice (1). Corticosterone is also generated in most tissues by the action of the enzyme 11β-hydroxysteroid dehydrogenase-1 (42). Inhibition of 11β-hydroxysteroid dehydrogenase-1 ameliorated key metabolic features of diet-induced obesity in a manner similar to (Pro3)GIP treatment (24), inviting further studies on possible effects of (Pro3)GIP on corticosterone production and action.
In conclusion, this study has shown that blockade of GIP action using (Pro³)GIP in mice with established high-fat diet-induced obesity and diabetes results in significant weight loss, improvement of insulin resistance, and amelioration of diabetes. This is consistent with the view that GIP antagonists represent an interesting new approach to the treatment of obesity and metabolic disturbances (17, 48). Interestingly, possible parallels exist with the benefits of Roux-en-Y surgery in the treatment of gross obesity and associated diabetes in man (22, 36). In this procedure, nutrients surgically bypass the area of the small intestine, where most endocrine K cells are located, resulting in a deficiency of circulating GIP without accompanying malabsorption (22, 36). Interestingly, compensatory increase of GLP-1 has been reported in some but not all surgical studies, but it is not an important mediator of the beneficial effects of GIP receptor antagonism reported here.

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DISCLOSURES
N. Irwin, V. A. Gault, and P. R. Flatt are shareholders in Diabetica.

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