Targeted Ablation of Glucose-dependent Insulinotropic Polypeptide-producing Cells in Transgenic Mice Reduces Obesity and Insulin Resistance Induced by a High Fat Diet*

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The K cell is a specific sub-type of enteroendocrine cell located in the proximal small intestine that produces glucose-dependent insulinotropic polypeptide (GIP), xenin, and potentially other unknown hormones. Because GIP promotes weight gain and insulin resistance, reducing hormone release from K cells could lead to weight loss and increased insulin sensitivity. However, the consequences of coordinately reducing circulating levels of all K cell-derived hormones are unknown. To reduce the number of functioning K cells, regulatory elements from the rat GIP promoter/gene were used to express an attenuated diphtheria toxin A chain in transgenic mice. K cell number, GIP transcripts, and plasma GIP levels were profoundly reduced in the GIP/DT transgenic mice. Other enteroendocrine cell types were not ablated. Food intake, body weight, and blood glucose levels in response to insulin or intraperitoneal glucose were similar in control and GIP/DT mice fed standard chow. In contrast to single or double incretin receptor knock-out mice, the incretin response was absent in GIP/DT animals suggesting K cells produce GIP plus an additional incretin hormone. Following high fat feeding for 21–35 weeks, the incretin response was partially restored in GIP/DT mice. Transgenic versus wild-type mice demonstrated significantly reduced body weight (25%), plasma leptin levels (77%), and daily food intake (16%) plus enhanced energy expenditure (10%) and insulin sensitivity. Regardless of diet, long term glucose homeostasis was not grossly perturbed in the transgenic animals. In conclusion, studies using GIP/DT mice demonstrate an important role for K cells in the regulation of body weight and insulin sensitivity.

Enteroendocrine (EE) cells are a complex population of rare, diffusely distributed hormone-producing intestinal epithelial cells (1–3). Peptides and hormones secreted by EE cells play important roles in many aspects of gastrointestinal and whole animal physiology (4–6). There are at least 16 different subtypes of EE cells based upon the major product(s) synthesized and secreted by individual cells (1). Several EE cell products, including GIP, glucagon-like peptide-1 (GLP-1), ghrelin, cholecystokinin, and peptide tyrosine, regulate food intake and/or degree of adiposity (7–11).

GIP is produced almost exclusively by K cells located in the proximal small intestine and is secreted immediately after ingestion of a meal (4, 5, 12, 13). GIP release is regulated by nutrients in the intestinal lumen but not by those in the blood (4, 6, 13, 14). Glucose (12, 15, 16), protein hydrolysates (17), specific amino acids (18), and fat (19) are major GIP secretagogues. Long term administration of a high fat diet increases intestinal GIP mRNA and peptide levels (12), as well as the circulating amount of plasma GIP (20, 21). There is a large body of biochemical and animal data suggesting that GIP signaling promotes the accumulation of fat (22–31). Obese humans also hyper-secrete GIP (32–36) suggesting that GIP may promote obesity in humans.

Originally, GIP was named “gastric inhibitory polypeptide” based upon its ability to inhibit gastric acid secretion and gastric emptying. However, it was later shown that these effects are observed only following administration of a supraphysiologic dose of GIP (13, 37). Thus, GIP was renamed “glucose-dependent insulinotropic polypeptide” to reflect its physiologic role in the potentiation of glucose-stimulated insulin release from pancreatic islet β-cells. However, mice lacking GIP receptors (GIPR−/−) exhibited only a subtle defect in glucose homeostasis (38) and were protected from the development of obesity and insulin resistance when placed on a high fat diet (21). Furthermore, blood sugar, water intake, hemoglobin A1c, triglyceride, free fatty acid, total cholesterol, low density lipoprotein cholesterol, and high density lipoprotein cholesterol levels were not significantly affected by the absence of GIP receptors. These observations could presumably be explained by GLP-1 compensation for the lack of GIPR signaling (39), although

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‡ The abbreviations used are: EE, enteroendocrine; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GIPR, GIP receptor; UTR, untranslated region; DT, diphtheria toxin A chain; RFP, red fluorescent protein.
GIP Regulates Obesity and Insulin Resistance

additional mechanisms could also contribute. Thus total inhibition of GIPR signaling reduces high fat diet-induced obesity and insulin resistance and is not associated with serious adverse consequences.

Based on the above information, it appears that reducing GIP action may have beneficial effects in terms of the development of obesity and insulin resistance. One way to inhibit GIP signaling is to inhibit hormone release from K cells. A potential advantage of this approach is that drugs may be able to target K cells from the intestinal lumen, rather than the blood thereby avoiding potential side effects associated with systemic delivery of antagonists to either GIP or the GIPR. Results from our laboratory have shown that many of the molecules that regulate GIP release appear to be distinct from those that control hormone release from other types of EE, endocrine, and excitatory cells (40–43). However, the consequences of eliminating or reducing hormone release of all hormones from K cells are unknown. They may differ from those seen after eliminating the GIPR, because K cells have also been reported to produce xenin, a hormone that may promote glucagon release, basal and glucose-stimulated insulin release, secretion from the exocrine pancreas, gut motility, and intestinal microcirculation (44–49). The physiologic importance of xenin or unknown hormones produced by K cells has not been established. The present study was therefore undertaken to define the metabolic consequences of eliminating K cells in mice and in particular to determine whether mice lacking K cells were protected from obesity induced by a high fat diet.

EXPERIMENTAL PROCEDURES

Design of Transgenic Constructs—Novel transgenic constructs are illustrated in Fig. 1. For GIP/RFP, the AGIP5−2 vector was generously provided by Dr. Rodger Liddle of Duke University (50) and contains the rat GIP promoter as well as a portion of the GIP structural gene. The initiator methionine in exon 2 of the GIP cDNA was converted to an Ncol site, and then a KpnI/Ncol fragment containing 3.1 kb of the rat GIP promoter through this initiator methionine was fused in-frame to the initiator methionine for RFP from DsRed 2-1 (Clontech, Mountain View, CA). A 1-kb fragment containing the SV40 3′-UTR was PCR-amplified from pGL2 basic and cloned downstream of the RFP stop codon. This fragment contains an intron, as well as splicing and polyadenylation signals, to ensure proper processing of the final primary transcript. For GIP/DT, the plasmid pIBI30−176 encodes an attenuated diphtheria toxin A chain (DT) and was generously provided by Dr. Ian Maxwell of the University of Colorado Health Science Center (51). An Ncol fragment from GIP/RFP was replaced with the DT cDNA so that DT, rather than RFP, was produced.

Production of Transgenic Mice—GIP/RFP and GIP/DT transgenic mice were produced on a C57BL/6J background through the Washington University School of Medicine Diabetes and Research Training Center Transgenic Core using standard pronuclear injection techniques. Genotyping was conducted on DNA isolated from tail biopsies using PCR and transgene-specific primers. Upstream and downstream primers for the GIP/RFP transgene are 5′-GAG TTC ATG CGC TTC AAG GT-3′ and 5′-CCC ATG GTC TTC TTC TGC AT-3′, respectively. Upstream and downstream primers for the GIP/DT transgene are 5′-CGC CAT GGA TCC TGA TGA TG-3′ and 5′-CCA TGG CTT CAC AAA GAT CGC CTG AC-3′, respectively. Animals were housed in a barrier facility under light-controlled conditions (12-h light and 12-h dark cycle) and given free access to food and water except as indicated for experimental manipulations. Group sizes are indicated in each figure. All experiments in this study were conducted using male mice and animal protocols approved by the Washington University Animal Studies Committee. Statistical analyses were conducted using the Student’s t test and/or analysis of variance.

Experimental Diets—Animals were continued on standard chow or switched to a high fat diet starting at 8 weeks of age. Standard chow (PicoLab Rodent Diet 20, Ralston Purina, St. Louis, MO) provided 3.08 kcal/g and 11.9% calories from fat. High fat “western” diet (TD.88137, Harlan Teklad, Madison, WI) provided 4.5 kcal/g and 42% calories from fat.

Immunohistochemistry—Small intestines were harvested, fixed, sectioned, and labeled using indirect immunofluorescence techniques as previously described (41, 42). Rabbit polyclonal antibodies to DsRed2 were obtained from Clontech. Some animals were injected with bromodeoxyuridine 90 min before they were sacrificed to label proliferating cells (52). To estimate the number of EE cells that co-express GLP-1 plus GIP, Swiss rolls of mouse small intestines from wild-type C57BL/6J mice were double-labeled using guinea pig anti-GIP plus rabbit anti-GLP-1 antibodies (42). The number of EE cells positive for GIP alone, GLP-1 alone, or GIP plus GLP-1 in random fields along the entire duodenal to ileal axis were then counted (42, 52). More than 100 EE cells positive for each increment were counted in the small intestine of each mouse.

Reverse Transcription-PCR—Procedures were essentially as previously described (40). Briefly, tissues were removed from mice and immediately snap frozen in liquid nitrogen. RNA was isolated from the indicated tissue or segment of the gut and reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Aliquots of cDNA were then amplified using the Applied Biosystems 7500 Fast system with the indicated TaqMan gene expression assay and normalized to the amount of β-actin mRNA present in the same sample. Assay numbers were: (a) GIP, Mm00433601_m1; (b) chromogranin A, Mm00514341_m1; (c) glucagon, Mm00801712_m1; (d) cholecystokinin, Mm00446170_m1; (e) somatostatin, Mm00436671_m1; (f) ghrelin, Mm00445450_m1; (g) secretin, Mm00441235_g1; (h) insulin 1, Mm01259683_g1; (i) amylin, Mm004394_m1; (j) gastrin-releasing peptide, Mm00612977_m1; (k) gastrin releasing peptide receptor, Mm00433860_m1; and (l) β-actin, 4352933E. RFP mRNA was assayed using a TaqMan assay custom designed by Applied Biosystems. Forward and reverse primers were 5′-AGC GCC TGA TGA ACT TCG A-3′ and 5′-GCC GAT GAA CTT CAC CTT GTA GAT-3′. Sequence of the 6-carboxyfluorescein-labeled probe was 5′-ACC CAG GAC TCC TCC-3′. Note that tissue-specific processing of preproglucagon generates glucagon-like peptides in intestinal L cells.

Metabolic studies were performed as outlined in the following sections. Sampling time points and concentrations of glu-
cose and insulin are similar to those used by other laboratories (e.g. Refs. 53–55).

Glucose Tolerance Tests—Animals were fasted for 16 h but given free access to water. Blood glucose levels were determined before and at the indicated time after administration of glucose by intragastric gavage (3 mg/g body weight) or by intraperitoneal injection (1 mg/g body weight).

Food Tolerance Tests—Animals were fasted for 16 h but given free access to water. Blood glucose levels were determined before and at the indicated time after animals were given the same type of food that they were previously fed.

Insulin Tolerance Tests—Animals were fasted for 5 h but given free access to water. Blood glucose levels were then determined before and at the indicated times following intraperitoneal injection of recombinant human insulin (0.5 unit/kg body weight).

Food Intake—Mice were switched to individualized housing and acclimated for 5 days before measurements were initiated (56). Total daily food intake was averaged over a 4- to 6-day period. Continuous food intake over a 24-h period was also assessed using the DietMax System (AccuScan Instruments Inc., Columbus, OH) at the University of Cincinnati Mouse Metabolic Phenotyping Center.

Intestinal Fat Absorption—Intestinal fat absorption was determined as part of the animals normal feeding regimen using a validated, non-invasive technique that does not require isotope analysis (57). Food and feces analyses were conducted at The University of Cincinnati Mouse Metabolic Phenotyping Center.

Energy Balance—Energy balance was assessed using the PhysioScan Oxygen Consumption/Carbon Dioxide Production System (AccuScan Instruments Inc.) at The University of Cincinnati Mouse Metabolic Phenotyping Center. Mice were placed in the PhysioScan chamber with food 3 h before the dark cycle and energy expenditure was recorded for 24 h. Food was removed the next evening, and the animals were fasted for 18 h while additional measurements were recorded.

Assays—Blood glucose concentrations were determined using MediSense Precision Xtra Blood Glucose Test Strips (Abbott Laboratories, Alameda, CA). HbA1c was determined on freshly collected blood using a Bayer Hemoglobin A1c reagent kit with the DCA 2000 Plus analyzer according to the manufacturer’s instructions (Bayer HealthCare LLC, Elkhart, IN). For determination of GIP, insulin, glucagon, and leptin, blood was added to chilled tubes. Plasma was then prepared and assayed for total GIP or insulin using enzyme-linked immunosorbent assays (Millipore) using an enzyme-linked immunosorbent assay according to the manufacturer’s protocol (Millipore). Because levels of active GLP-1 are very low following oral administration of 3 mg of glucose per g of body weight, mice were orally administered a high dose of glucose (6 mg/g body weight) (58) or 3 mg/g glucose plus intralipid (59) when this hormone was to be measured.

Nuclear Magnetic Resonance Imaging—Conscious mice were placed in a restraint tube and analyzed using an EchoMRI (EchoMedical Systems, Houston, TX) to estimate lean body mass, fat tissue mass, and water composition.

Dual-energy X-ray Absorptiometry—Dual-energy x-ray absorptiometry was conducted on anesthetized mice using a small animal densitometer (Lunar PIXImus, Madison, WI).

RESULTS

Regulatory Elements from the GIP Promoter and Gene Confer Transgene Expression to GIP-producing Cells in Vivo—DT-mediated ablation of GIP-producing cells requires DNA regulatory elements that confer proper transgene expression. It was previously reported that 2.5-kb of the rat GIP structural gene was isolated and fused in-frame to the initiator methionine encoding RFP. A second intron, a splice site, and polyadenylation signals are present in the 3′-UTR from SV40 Large T antigen. The resulting GIP/RFP construct encodes a chimeric mRNA transcript but not a chimeric RFP protein. Colors represent the GIP promoter (dark blue); exon 2 (light blue); intron 1 (orange) from the GIP gene (gray); RFP cDNA (red), the SV40 3′-UTR (light blue). To generate the GIP/DT construct, an NcoI fragment from the RFP cDNA was replaced with an NcoI fragment encoding the DT cDNA (green). The attenuated diphtheria toxin A chain without any additional amino acids was generated from the chimeric transcript.

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together, these results indicate that these regulatory elements from the rat GIP promoter and gene target reporters to the appropriate cells in vivo.

The GIP/DT Transgene Ablates Only GIP-producing Cells—Forced expression of an attenuated DT (51) is a well established strategy to ablate specific cell lineages in transgenic mice (61–63). It is important to note that this mutant DT exhibits greatly reduced toxicity compared with the wild-type toxin, which eliminates killing of cells adjacent to those targeted by the transgene as well as those that may exhibit very low levels of “leaky” promoter activity. This particular attenuated DT has been used to specifically ablate Paneth cells (64) and goblet cells (65) in the intestinal epithelium without killing adjacent cells or eliciting an immune response.

Transgenic mice were generated that express the GIP/DT transgene (Fig. 1). Transgenic and wild-type animals were born at expected frequencies and exhibit similar lifespans (not shown). Furthermore, GIP/DT mice did not exhibit any signs of ataxia or sluggish behavior indicating that they do not suffer from intestinal discomfort (not shown). Histochemical staining of intestines using hematoxylin and eosin, Alcian blue, periodic acid-Schiff, and phloxine/tartrazine (52) demonstrate that cellular morphology, crypt-villus architecture, and Paneth and goblet cell numbers are similar in wild-type and GIP/DT mice (not shown). The number of bromodeoxyuridine-labeled intestinal epithelial cells was also similar in both wild-type and GIP/DT mice (not shown). The number of bromodeoxyuridine-positive cells was confined to the mid-portion of the intestinal crypts. Thus, DT expression did not perturb cell proliferation. EE cell-derived hormones are each expressed in unique patterns along the proximal to distal axis of the gut (3–6) (see also Fig. 4). RNA was isolated from the stomach and sequential segments of the small intestines from wild-type and GIP/DT mice that had been fed standard chow. Liver served as a negative control. Real-time PCR assays were utilized to quantify transcript levels. GIP mRNA levels in wild-type mice were extremely high in the proximal small intestine and very low in the stomach and distal small intestine (Fig. 4B). Transcripts encoding GIP were greatly reduced in the intestines from GIP/DT animals. In the mouse, chromogranin A is produced by many types of EE cells but not by those that produce GIP (42). Chromogranin A transcripts were present at similar levels along the entire proximal to distal axis of intestines from wild-type and GIP/DT animals (Fig. 4C). Immunohistochemical studies (not shown) confirmed that GIP is present in singly dispersed EE cells in the proximal small intestine of wild-type mice but is undetectable in intestines from GIP/DT animals. In contrast, chromogranin A is present.
in normal numbers of EE cells in the small intestine of both wild-type and GIP/DT mice. Consistent with reduced GIP mRNA levels and GIP-producing cells, circulating GIP was detectable in plasma prepared from wild-type (86 ± 26 pg/ml), but not GIP/DT (<3.3 pg/ml) mice. To confirm that GIP action was abolished, the incretin response was measured in mice that had been maintained on standard chow. Fifteen minutes after administration of oral glucose (3 mg/g body weight) to fasted animals, plasma insulin levels increased 5-fold in wild-type and GIP/DT animals (Fig. 5A). Essentially identical results were obtained when mice were orally administered twice the dose of glucose or glucose plus intralipid (Table 1). Amylin is co-released with insulin from β-cells. Oral glucose-stimulated amylin release was also abolished in the GIP/DT mice fed standard chow (not shown). Thus, the absence of GIP-producing cells resulted in the complete loss of an incretin response (see below). Fasting plasma glucagon levels were similar in wild-type and GIP/DT mice and decreased comparably in response to oral glucose (Fig. 5B).

The complete lack of an incretin response raised the possibility that the GIP/DT transgene also ablated GLP-1-producing cells. K cells do not co-express cholecystokinin, somatostatin, substance P, serotonin, gastrin, or secretin (1, 4–6, 66). In contrast, a subset of EE cells in humans and pigs has been reported to produce both immunoreactive GIP and GLP-1 (67, 68). However, <3% of the EE cells in the mouse intestine were reported to co-express GIP plus GLP-1 (1). To confirm this latter observation, paraffin-embedded sections of intestines from wild-type C57BL/6J mice were stained for GIP and GLP-1. Consistent with the published data from an independent laboratory (1), co-staining for both incretins was observed in only 2.3 ± 0.2% of the EE cells (not shown). GLP-1 is produced by cell-specific processing of preproglucagon. As shown in Fig. 4D, there were no statistically significant differences in preproglucagon mRNA levels in the stomach or small intestine in wild-type versus GIP/DT mice. Similar numbers of GLP-1-immunoreactive cells were also observed in the small intestines from wild-type and GIP/DT mice (not shown). Next, GLP-1 release was measured before and 15 min after administration of oral nutrients. Fasting GLP-1 levels hovered around the lower limits of detection in wild-type and GIP/DT mice fed standard chow (Table 1). Fifteen minutes after administration of oral glucose or glucose plus intralipid to fasted mice, plasma GLP-1 increased to similar levels in wild-type and GIP/DT animals (Table 1). Gastrin-releasing peptide is produced by enteric neurons and is important for promoting oral glucose-stimulated GLP-1 release (58). The mRNA levels for gastrin-releasing peptide and its receptor were similar in intestinal samples from wild-type and GIP/DT mice (Fig. 4, E and F). GLP-1 action was also normal, because intraperitoneal administration of GLP-1 along with glucose improved glucose excursion from blood to similar extents in wild-type and GIP/DT mice (Fig. 6). Taken together, these observations indicate that GLP-1 production, release, and action are apparently normal in GIP/DT mice fed standard chow. Transcripts encoding somatostatin, cholecystokinin, secretin, and ghrelin were also similar in the stomach.
and nearly all intestinal segments from wild-type and GIP/DT mice (Fig. 8, G–J).

**Mice Lacking GIP-producing Cells Show Attenuated Weight Gain on a High Fat Diet**—Wild-type and GIP/DT mice were each randomized to 2 groups at 8 weeks of age. One group for each genotype (n = 7–9 mice per group) was switched to a high fat “western” diet and the other group was maintained on standard chow. As shown in Fig. 7, age-matched wild-type and GIP/DT mice maintained on standard chow exhibit similar body weights (30.2 ± 0.7 g versus 29.8 ± 1.0 g, respectively, at 35 weeks, p = 0.74). Wild-type and GIP/DT mice maintained on the high fat diet for 35 weeks weighed 55% (p < 0.001) and 11% (p < 0.01), respectively, more than those maintained on standard chow. Thus, on a high fat diet, GIP/DT mice gained five times less weight than wild-type littermates. Circulating leptin levels correlate well with body fat. Consistent with the body weight data, plasma leptin levels were similar in wild-type and GIP/DT mice fed standard chow (Fig. 5C). Following high fat feeding for 15 weeks, leptin levels increased nearly 20-fold in wild-type mice. This increase was markedly attenuated in the GIP/DT animals. Magnetic resonance imaging and dual-energy x-ray absorptiometry analyses (not shown) confirmed that the GIP/DT mice fed a high fat diet have decreased fat mass when compared with similarly fed wild-type animals. In contrast, lean and fat body masses were similar in aged-matched wild-type and GIP/DT mice that were maintained on standard chow. Thus animals lacking GIP-producing cells resist development of high fat diet-induced obesity.

**Mice Lacking GIP-producing Cells Exhibit Decreased Intake of High Fat Food and Increased Energy Expenditure**—Wild-type and GIP/DT mice maintained on standard chow consumed similar amounts of food per day (Fig. 8). Conversely, GIP/DT mice maintained on a high fat diet for 21 weeks consumed 16% less high fat food per day than did age-matched wild-type control animals. The pattern of food consumption over a 24-h period was similar in both groups of mice (not shown). The percentage of available fat that was absorbed from the high fat food was similar in both the GIP/DT and wild-type mice fed the high fat diet (99.4 ± 0.1 versus 98.7 ± 0.4, respectively, p = 0.15). As shown in Fig. 9, oxygen consumption, carbon dioxide production, and heat output were increased during the dark cycle in GIP/DT mice fed the high fat diet. These same parameters were similar in wild-type and GIP/DT mice during the light cycle, the period when animals exhibit reduced feeding and activity. In contrast, the respiratory quotient was similar in both lines of mice. Taken together, these results suggest that decreased food intake coupled with increased energy expenditure accounts for the reduced body weight of the GIP/DT mice fed a high fat diet.

**Mice Lacking GIP-producing Cells Do Not Develop Insulin Resistance on a High Fat Diet**—Insulin sensitivity was assessed to determine if the reduced weight gain in the GIP/DT mice was associated with changes in insulin action. Following intraperitoneal administration of insulin (0.5 unit/kg), glucose excursion from blood is essentially identical in wild-type and GIP/DT mice maintained on standard chow for up to 33 weeks (Fig. 10A and not shown). Following 9 weeks on a high fat diet, the wild-type mice exhibit a modest reduction in the glucose response to insulin when compared with GIP/DT animals (15% reduction in the area under the curve, p < 0.01, not shown). After 33 weeks of high fat feeding, glucose excursion from blood is markedly improved in the GIP/DT mice compared with wild-type animals (Fig. 10B). As an additional measure of insulin sensitivity, insulin to glucose ratios were determined in mice fed standard chow or high fat diets for 27 weeks. Random blood
Glucose levels were similar in all mice regardless of genotype or diet (Fig. 10C). However, the plasma insulin level, and thus, the insulin to glucose ratio, was elevated in wild-type animals maintained on the high fat diet (Fig. 10, D and E). Similar insulin to glucose ratios were observed following 5- and 16-h fasts (not shown). Thus, the GIP/DT mice did not develop high fat diet-induced insulin resistance.

Glucose Homeostasis Following Oral and Intraperitoneal Glucose Administration Is Nearly Normal in Mice Lacking GIP-producing Cells—Because GIP potentiates insulin secretion in response to oral nutrients, glucose homeostasis was assessed in the GIP/DT mice. On each diet, glucose excursion from blood was similar in wild-type and GIP/DT animals following intraperitoneal administration of glucose (Fig. 11, A and B). This is
consistent with data indicating that insulin and amylin mRNA levels are normal in the pancreas of GIP/DT mice (Fig. 4). However, regardless of genotype, animals fed the high fat diet exhibited reduced glucose clearance compared with mice fed standard chow. Importantly, glucose excursion did not worsen with age in the GIP/DT mice on either diet (not shown). In mice fed standard chow, plasma insulin levels both before and 2 min after intraperitoneal injection of glucose were essentially identical suggesting β-cell function was not perturbed in the GIP/DT mice (Fig. 12). 45 min after the glucose injection, plasma insulin levels were lower in the GIP/DT versus wild-type mice (p < 0.05). Because both of these groups exhibited similar glucose clearance from blood following intraperitoneal injection of glucose (Fig. 11A), the GIP/DT animals may have improved insulin sensitivity. In mice fed the high fat diet, plasma insulin levels were increased 45 min, but not 2 min, after intraperitoneal administration of glucose, regardless of genotype (Fig. 11A). Thus, high fat feeding resulted in delayed insulin release, but this effect was independent of the presence or absence of GIP-producing cells.

Blood glucose excursion in response to administration of oral glucose was also assessed in the same animals (Fig. 11, C and D). As expected for mice lacking an incretin effect, the GIP/DT mice fed standard chow exhibited a reduced rate of blood glucose clearance compared with similarly fed wild-type animals. Following high fat feeding for 31 weeks, the blood glucose excursion rate was also reduced in the GIP/DT versus wild-type animals but was not worse than that in the GIP/DT mice fed standard chow. Unlike the case for standard chow, the GIP/DT mice fed a high fat diet exhibited a modest increase in oral glucose-stimulated insulin release (Fig. 5A). Fasting plasma GLP-1 levels were similar in both wild-type and GIP/DT fed the high fat diet and higher than those observed in the mice fed standard chow (Table 1). However, following administration of oral nutrients, plasma GLP-1 levels were greater in the wild-type versus GIP/DT mice (Table 1). This suggests that, on a high fat diet, the partially restored incretin effect may not be due to increased GLP-1 release.

**Glucose Homeostasis Following Ingestion of Physiologic Meals Is Nearly Normal in Mice Lacking GIP-producing Cells**—Blood glucose excursion rates in response to administration of a single, high dose, oral glucose load may not reflect the response to ingestion of a mixed meal. Therefore, blood glucose levels were measured before and after fed animals were given free access to the same type of chow on which they had been previously maintained (Fig. 11, E and F). Although blood glucose excursion rates are reduced in the GIP/DT versus wild-type mice on either diet, blood glucose levels never exceed 275 mg/dl in the GIP/DT animals on either diet. A detailed analysis of insulin release following re-feeding was also conducted with mice on standard chow (Fig. 13). Blood glucose levels were only modestly increased in the GIP/DT mice. As expected, plasma insulin levels increased immediately after re-feeding wild-type mice. Consistent with the lack of an incretin effect in response to oral glucose (Fig. 5), physiologic re-feeding initially failed to elicit an increase in insulin release in the GIP/DT mice. However, as blood glucose levels became elevated, the GIP/DT mice released sufficient amounts of insulin to normalize blood glucose levels. To assess long term glucose homeostasis, HbA1c levels in blood were determined in animals that had been maintained on standard chow or high fat diets for 36 weeks. On standard chow, HbA1c levels of 3.7% were observed for both wild-type and GIP/DT mice. Following 36 weeks of high fat feeding, HbA1c levels were increased to 4.2% (p = 0.001) in the GIP/DT mice (Fig. 14). Thus, long term glucose homeostasis is only slightly perturbed in these animals. Furthermore, the decreased body weight in the GIP/DT mice fed a high fat diet is not due to diabetes.

**DISCUSSION**

**Identification of K Cell-specific Regulatory Elements**—In the mouse, the highest levels of GIP transcripts were present in the proximal small intestine, and much lower levels were expressed in the stomach and distal intestine (Figs. 2 and 4). It was previously reported that 2.5 kb of the rat GIP promoter fused to the human insulin gene confer tissue-specific and K cell-specific insulin expression in transgenic mice (60). However, Fig. 2A from this same report revealed that the highest levels of transgene-encoded insulin transcripts were observed in the stomach, not the duodenum, of the transgenic mice. Using a similar transgene, we observed this same pattern of mis-expression in
independently generated transgenic mice. Therefore additional regulatory elements required for proper GIP gene expression are located outside of this 2.5-kb promoter fragment. A new transgene (Fig. 1) was prepared that incorporated these additional regulatory elements conferred appropriate tissue-, region-, and cell-specific expression are located outside of this 2.5-kb promoter fragment. Within intron 1 of the GIP structural gene (50), this entire intron was maintained on standard chow or high fat food for 27 weeks. Blood was then collected from non-fasted wild-type and GIP/Dt mice between 10 a.m. and noon (C). Blood glucose and plasma insulin levels are shown in C and D, respectively. The insulin to glucose ratio (E) was calculated using glucose and insulin values from individual mice. Note that insulin levels and insulin to glucose ratios are elevated only in wild-type mice fed a high fat diet indicating that wild-type, but not GIP/Dt mice, are insulin resistant.

**FIGURE 10.** Insulin sensitivity is improved in GIP/Dt mice fed a high fat diet. A and B, insulin tolerance tests (ITT: wild-type (WT) and GIP/Dt (DT) mice fed standard chow (Chow; Panel A) or high fat food (HF; Panel B) for 33 weeks were fasted for 5 h and then administered human insulin by intraperitoneal injection (0.5 unit/kg body weight). Blood glucose levels were determined before (0 min) and at the indicated time following administration of insulin. Note that glucose clearance rates from blood are identical in wild-type and GIP/Dt mice fed standard chow, whereas insulin sensitivity is greatest in GIP/Dt mice fed high fat food. C–E, insulin to glucose ratios: mice were maintained on standard chow or high fat food for 27 weeks. Blood was then collected from non-fasted wild-type and GIP/Dt mice between 10 a.m. and noon (C). Blood glucose and plasma insulin levels are shown in C and D, respectively. The insulin to glucose ratio (E) was calculated using glucose and insulin values from individual mice. Note that insulin levels and insulin to glucose ratios are elevated only in wild-type mice fed a high fat diet indicating that wild-type, but not GIP/Dt mice, are insulin resistant.

Glucose homeostasis is similar in wild-type (WT) and GIP/Dt (DT) mice. Mice were fasted for 16 h. Blood glucose levels were determined before (time 0) and at the indicated time after animals were given intraperitoneal glucose (1 mg/g body weight; IPGTT), oral glucose (3 mg/g body weight; OGTT), or free access to standard chow (Chow) or high fat (HF) food (FTT; panels E and F). A and B, note that on standard chow, the GIP/Dt mice exhibited normal clearance of glucose from blood following administration of intraperitoneal glucose. Conversely, high fat feeding resulted in a similarly reduced rate of glucose clearance in both wild-type and GIP/Dt mice compared with parallel groups on a standard chow diet. C and D, note that GIP/Dt mice fed either standard chow or high fat food exhibited impaired glucose tolerance due to the lack of an incretin effect. However, high fat feeding worsened oral glucose tolerance only in the wild-type mice. E and F, note that, in contrast to administration of oral glucose, normal food intake results in only a modest increase in blood glucose levels. IPGTT, OGTT, and FTT were conducted following 30, 31, and 20 weeks, respectively, on a high fat diet.

**FIGURE 11.** Glucose homeostasis is similar in wild-type (WT) and GIP/Dt (DT) mice. Mice were fasted for 16 h. Blood glucose levels were determined before (time 0) and at the indicated time after animals were given intraperitoneal glucose (1 mg/g body weight; IPGTT), oral glucose (3 mg/g body weight; OGTT), or free access to standard chow (Chow) or high fat (HF) food (FTT; panels E and F). A and B, note that on standard chow, the GIP/Dt mice exhibited normal clearance of glucose from blood following administration of intraperitoneal glucose. Conversely, high fat feeding resulted in a similarly reduced rate of glucose clearance in both wild-type and GIP/Dt mice compared with parallel groups on a standard chow diet. C and D, note that GIP/Dt mice fed either standard chow or high fat food exhibited impaired glucose tolerance due to the lack of an incretin effect. However, high fat feeding worsened oral glucose tolerance only in the wild-type mice. E and F, note that, in contrast to administration of oral glucose, normal food intake results in only a modest increase in blood glucose levels. IPGTT, OGTT, and FTT were conducted following 30, 31, and 20 weeks, respectively, on a high fat diet.

**FIGURE 12.** Intraperitoneal glucose-stimulated insulin release is normal in mice lacking GIP-producing cells. Wild-type (WT) and GIP/Dt (DT) mice were fasted for 16 h. Blood was collected at the indicated time before (Fasting) or after intraperitoneal injection of glucose (1 mg/g body weight).
it is not known whether the additional critical regulatory elements reside within intron 1 or the additional 600 bp of the promoter, it is interesting to note that sequences located within intron 1 of the glucagon gene are also required for proper transgene expression in transgenic mice (70). Due to the importance of K cell-derived hormones for whole animal physiology, these novel regulatory elements will be extremely useful for generating additional transgenic mice that can be used to probe K cell function in vivo.

Ablation of GIP-producing Cells Eliminates the Incretin Response to Oral Glucose—Oral glucose-stimulated insulin release was essentially eliminated in the GIP/DT mice fed standard chow (Fig. 5A and Table 1) indicating that the total incretin response was abolished by ablating K cells. This observation was quite surprising, because GIPR−/− mice exhibited less than a 2-fold reduction in oral glucose-stimulated insulin release (38, 53, 55). In fact, 15 min after administration of oral glucose (3 mg/g body weight) to male GIPR plus GLP-1R double knock-out mice, plasma insulin levels still increased nearly 5-fold (53). This incretin effect was only slightly reduced compared with that observed in the single GIPPR or GLP-1R mice. In contrast, insulin release increased only 30–50% in the GIP/DT mice following administration of oral glucose (Fig. 5A). The absence of oral glucose-stimulated insulin release in GIP/DT mice fed standard chow did not result from a β-cell insufficiency, because glucose excursion from blood in response to intraperitoneal glucose was normal in these animals (Fig. 11A). Furthermore, insulin and amylin mRNA levels were similar in pancreatic RNA samples prepared from wild-type and GIP/DT mice (Fig. 4). Importantly, GLP-1 production, release, and action were not attenuated in the GIP/DT mice fed standard chow (Figs. 4 and 6 and Table 1). Taken together, these observations indicate that GIP is the major incretin in C57BL/6J mice and further raise the possibility that GIP/DT mice are lacking not only GIP, but also another major K cell-derived hormone that exhibits incretin-like activity or that is important for mediating the incretin response.

Xenin has been reported to be produced by a sub-population of K cells (44). It is important to note that this hormone is a cleavage product derived from the ubiquitously expressed α subunit of the coat protein (71) and, thus, could potentially arise from non-physiologic proteolysis of this protein. Supraphysiologic concentrations of xenin increased glucose-stimulated insulin release in perfused rat pancreas (45). In contrast to GIP, xenin is also released in response to sham feeding (72). Clearly, the physiologic importance for xenin has not been established. Preliminary results from our laboratory suggest that xenin is not an incretin for wild-type or GIP/DT mice. However, additional studies are required to determine if ablation of xenin or unknown molecules produced by K cells could explain the lack of an incretin response in the GIP/DT mice.

Ablation of GIP-producing Cells Reduces Diet-induced Obesity and Insulin Resistance—Numerous physiologic studies strongly suggested that GIP plays an important role in promoting high fat diet-induced obesity and insulin resistance. Studies using GIPR−/− mice provided genetic evidence in support of this hypothesis. Biochemical studies suggest that GIP promotes weight gain by increasing glucose uptake, heparin-releasable lipoprotein lipase activity, and fat storage by adipocytes (21). Two potential strategies to reduce GIP signaling, and thus obesity and insulin resistance, in vivo would be to 1) inhibit GIPR activity by administration of GIPR antagonists and 2) inhibit GIP production, release, and/or action. Results presented in this report indicate that, as in GIPR−/− mice, animals genetically engineered to lack GIP-producing cells also resist development of high fat diet-induced obesity and insulin resistance. Importantly, the complete absence of GIP plus K cell-derived xenin or other unknown hormones does not appear to result in serious adverse effects. Furthermore, in both model systems, elimination of the GIP-mediated incretin effect did not lead to severely impaired glucose homeostasis, because HbA1c levels were similar in wild-type and GIP/DT mice regardless of the diet (Fig. 14). This is particularly noteworthy, because the GIP/DT mice do not have a demonstrable incretin effect when maintained on a standard chow diet. These results can be explained by the fact that, even though the incretin effect is absent in the GIP/DT mice, once blood glucose levels increase, islet β-cells release insulin in direct response to glucose alone (Fig. 13). Taken together, these observations suggest reducing...
hormone production and release by K cells is a potential strategy to prevent and/or ameliorate diet-induced obesity and insulin resistance.

Common, as well as distinct, biochemical and physiologic pathways appear to be perturbed in GIPR<sup>−/−</sup> and GIP/DT mice. Loss of GIP signaling presumably explains the amelioration of obesity and insulin sensitivity in both model systems. The fact that the GIP/DT mice consume less high fat food per day and exhibits greater energy expenditure than similarly fed wild-type mice can most probably account for their reduced weight gain (Figs. 7–9). Although there are conflicting reports concerning high fat food intake in GIPR<sup>−/−</sup> versus wild-type mice (38, 55), our observations agree with those reported for long term diet related studies (55).

Several possible explanations exist that could account for the differences between the GIPR<sup>−/−</sup> and GIP/DT mice. First, mice lacking GIPRs still produce GIP and GIP degradation products, and these peptides could potentially interact with receptors other than the GIPR. Second, it is possible that novel peptides could interact with the GIPR and thus, the absence of GIPRs would also prevent the action of these hormones. Finally, the GIP/DT mice lack GIP, GIP degradation products, and these peptides could potentially interact with receptors other than the GIPR. Further, it is interesting to speculate that K cells may produce one or more important hormones in addition to GIP. Studies are currently underway to determine whether additional hormones produced by K cells may also play important roles in regulating obesity, insulin resistance, feeding behavior, and glucose homeostasis.

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