Role of Leptin in the Regulation of Glucagon-Like Peptide-1 Secretion

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Glucagon-like peptide-1 (GLP-1), released from intestinal endocrine L cells, is a potent insulinotropic hormone. GLP-1 secretion is diminished in obese patients. Because obesity is linked to abnormal leptin signaling, we hypothesized that leptin may modulate GLP-1 secretion. Leptin significantly stimulated GLP-1 secretion (by up to 250% of control) from fetal rat intestinal cells, a mouse L cell line (GLUTag), and a human L cell line (NCI-H716) in a dose-dependent manner (P < 0.05–0.001). The long form of the leptin receptor was shown to be expressed, and leptin induced the phosphorylation of STAT3 in the three cell types. The leptin receptor was also expressed by rodent and human intestinal L cells, and leptin (1 mg/kg i.p.) significantly stimulated GLP-1 secretion in rats and *ob/ob* mice. To determine the effect of leptin resistance on GLP-1 secretion, C57BL/6 mice were fed a high-fat (45%) or low-fat (10%) diet for 8 weeks. Mice on the high-fat diet became obese; developed glucose intolerance, hyperinsulinemia, and hyperleptinemia; and were leptin resistant. Mice on the high-fat diet also had twofold lower basal plasma GLP-1 and a diminished GLP-1 response to oral glucose, by 28.5 ± 5.0% (P < 0.05). These results show for the first time that leptin stimulates GLP-1 secretion from rodent and human intestinal L cells, and they suggest that leptin resistance may account for the decreased levels of GLP-1 found in obese humans. *Diabetes* 52:252–259, 2003

The hormone glucagon-like peptide-1 (GLP-1) is secreted from enteroendocrine L cells, which are localized in the distal ileum and colon (1), after nutrient ingestion (2–5). GLP-1 acts through a specific G-protein–coupled receptor to potently stimulate glucose-dependent insulin secretion (6–8). GLP-1 further reduces glycemia through inhibition of both glucagon secretion (9) and gastric emptying (10) and by stimulation of pancreatic β-cell proliferation and neogenesis (11,12). The GLP-1 receptor is also expressed in hypothalamic nuclei that are responsible for modulating feeding behavior (13,14), and central GLP-1 administration reduces food intake in rodents, whereas peripheral administration of GLP-1 promotes satiety and decreases body weight in humans (13,15). These pleiotropic actions of GLP-1 therefore offer great potential for the treatment of hyperglycemia in patients with type 2 diabetes (1,15).

It has recently been shown that plasma GLP-1 levels are reduced in obesity (16–19), a condition that is highly correlated to type 2 diabetes (20). The mechanisms leading to decreased GLP-1 secretion in obesity have not been elucidated. However, because plasma leptin levels are proportional to fat mass, we postulated the existence of an adipo-enteroendocrine interaction between leptin and GLP-1. Leptin, a product of the *ob* gene in white adipose tissue, activates hypothalamic circuits, leading to the inhibition of food intake (21,22). The leptin receptor gene encodes five alternatively spliced forms of mRNA (23,24). However, only the long form of the leptin receptor (Ob-Rb) contains an intracellular JAK-STAT signaling motif, and Ob-Rb appears to be responsible for the physiological actions of leptin (25–27). Although Ob-Rb is predominantly distributed in the ventral medial hypothalamic region known to be important in determining feeding behavior (21,28), it is now recognized that Ob-Rb is also expressed in several peripheral tissues, including the gut and the pancreas (29–31).

It has been shown that *ob/ob* mice, which are homozygous for a mutation of the *ob* gene, and *db/db* mice, which have a mutation in the leptin receptor, both exhibit hyperphagia and morbid obesity, leading to hyperinsulinemia and hyperglycemia (22,23). Although *ob/ob* and *db/db* mice serve as models of type 2 diabetes, human *ob* or *db* gene mutations are extremely rare, and no linkages with diabetes have been found (32,33). Nonetheless, most obese humans exhibit hyperleptinemia, and increased adiposity is believed to occur in association with the development of leptin resistance (21,32,33). One model of leptin resistance is the C57BL/6 mouse submitted to a high-fat diet (34). In contrast to the *ob/ob* and *db/db* mice, these mice develop impaired glucose tolerance but seldom progress to frank diabetes. We now demonstrate that leptin stimulates GLP-1 secretion from enteroendocrine L cells in vitro and in vivo and, furthermore, that leptin resistance in obese C57BL/6 mice is associated with impaired secretion of GLP-1.

RESEARCH DESIGN AND METHODS

Cell cultures. Fetal rat intestinal cell (FRIC) cultures were prepared as previously described in detail (35–37). The GLUTag enteroendocrine cell line was derived from a large bowel tumor in mice carrying a proglucagon promoter/Simian virus 40 large T antigen transgene, as described previously

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ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine solution; FRIC, fetal rat intestinal cell; GLP-1, glucagon-like peptide-1; GRP, gastrin-releasing peptide; PKC, protein kinase C; PMA, phorbol myristic acid; RIA, radioimmunoassay; TBST, Tris-buffered saline containing 1% Tween 20.

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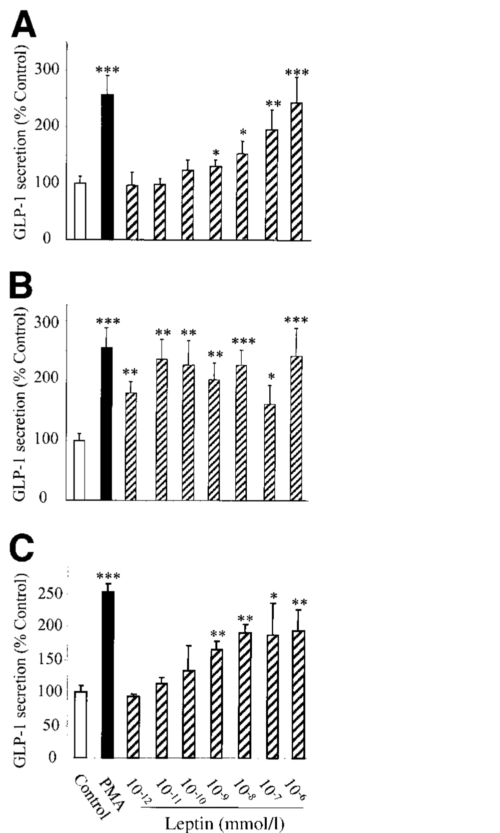

FIG. 1. GLP-1 secretion by FRIC cultures (*A*), the GLUTag cell line (*B*), and NCI-H716 cells (*C*) in response to a 2-h treatment with medium alone (negative control), PMA (1 μmol/l, positive control), and graded concentrations of recombinant leptin. GLP-1 secretion is expressed as a percentage of the control (n = 6–8). *P < 0.05, **P < 0.01, ***P < 0.001 vs. control.

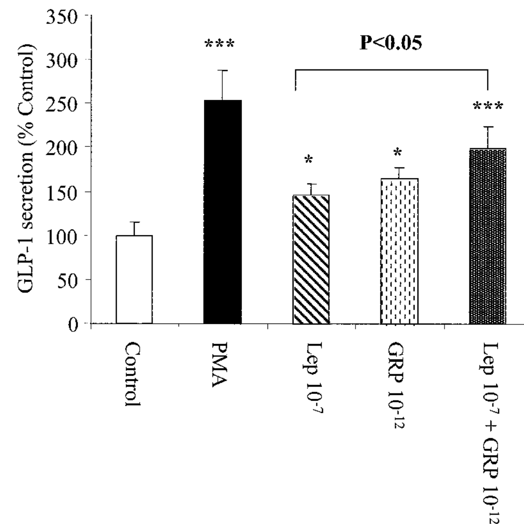

FIG. 2. GLP-1 secretion by GLUTag cells in response to a 2-h treatment with media alone (negative control), PMA (1 μmol/l, positive control), leptin (10^{-7} mol/l), GRP (10^{-12} mol/l), or a combination of leptin (10^{-7} mol/l) and GRP (10^{-12} mol/l). GLP-1 secretion is expressed as a percentage of the control (n = 6). Lep, leptin. *P < 0.05, ***P < 0.001 vs. control.

(38). Several days before each experiment, the cells were split into 24-well plates (for GLP-1 secretion experiments) or 6-well plates (for Western blot analysis) and were allowed to reach 80–90% confluence. Human enteroendocrine NCI-H716 cells were maintained in suspension culture as described by the American *Type Culture* Collection (Manassas, VA). At 2 days before the experiment, cells were seeded into 12-well culture plates coated with Matrigel as described by Reimer et al. (39). All three cell models have been reported to secrete GLP-1 in a regulated fashion (35–39).

In vitro secretion experiments. Cells were washed with Hank's balanced salt solution and incubated for 2 h with medium alone (containing 0.5% fetal bovine solution [FBS]) or with 1 μmol/l phorbol myristic acid (PMA), mouse recombinant leptin (Sigma, St. Louis, MO), or gastrin-releasing peptide (GRP; Bachem, Torrance, CA). Peptides and small proteins in cell medium or cell extracts were then collected by reversed-phase adsorption (C_{18} Sep-Pak; Waters, Milford, MA) as previously reported (35–38). We have demonstrated that this methodology permits >88% recovery of intact proglucagon-derived peptides, including GLP-1, from tissues and cell cultures (36). Extracts were stored at −20°C until assay.

Peptide extracts from FRIC cultures, GLUTag, and NCI cell lines were assayed for GLP-1 using an antiserum against the COOH-terminal of GLP-1(7-36)-amide (Affinity Research Products, Nottingham, U.K.) as described previously (35–38). Secretion was calculated as the total amount of GLP-1 in the medium, normalized for the total content of GLP-1 (i.e., medium plus cells).

Immunohistochemistry. FRIC, GLUTag, and NCI-H716 cells were grown in 8-well chamber slides (Nalge Nunc, Naperville, IL) overnight. The medium was removed, and the cells were washed with PBS and fixed in methanol at −10°C for 5 min. Pieces of distal ileum from rats and mice were fixed in neutral buffered formalin and embedded in paraffin, and 5-μm sections were prepared. Sections of human small intestine were obtained from Dr. S. Asa (University Hospital Network, Toronto, ON, Canada). After deparaffinization and hydration, fixed cells and tissue sections were incubated with 5% normal donkey serum (Jackson Immunoresearch Laboratories, West Grove, PA) for 30 min. Slides were then incubated with primary antisera for both GLP-1 (rabbit polyclonal antibody, used at 1:1,250 dilution) (35) and NH_2-terminal leptin receptor (goat polyclonal antibody K-20, used at 1:50 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. After three serial washes with PBS, slides were incubated with Cy3-conjugated donkey anti-rabbit IgG (used at 1:250 dilution; Jackson Immunoresearch Laboratories) and Cy2-conjugated donkey anti-goat IgG (used at 1:50 dilution; Jackson Immunoresearch Laboratories) for 1 h at room temperature. After rinsing with PBS, the sections were mounted and visualized using a fluorescence microscope. No immunostaining was observed when the primary or secondary antisera were omitted (not shown).

Western blot. FRIC, GLUTag and NCI-H716 cells were grown in 6-well plates until 80% confluent. Some cells were washed with PBS and then incubated for 2 h in medium without FBS, followed by incubation with leptin (100 nmol/l) for 0, 5, 15, 30, and 60 min in medium without FBS. Cells were then washed with PBS, lysed in 100 μl of SDS sample buffer (62.5 mmol/l Tris base [pH 6.8], 2% wt/vol SDS, 10% glycerol, 50 mmol/l dithiothreitol, 0.1% wt/vol bromphenol blue, and 2-mercaptoethanol) and placed on ice. Cell lysates were heated to 95–100°C for 5 min, cooled on ice, and microcentifuged for 5 min, and then proteins (20–40 μg) were resolved on 7.5% SDS-PAGE gels, transferred to nitrocellulose membranes, and subjected to immunoblot analysis.

Membranes were blocked for 1–3 h with 5% nonfat dry milk in Tris-buffered saline containing 1% Tween 20 (TBST). After washing three times with TBST, the blots were incubated overnight at 4°C with goat anti-leptin receptor antiserum K-20 (1:500; Santa Cruz Biotechnology), rabbit polyclonal anti–phospho-Stat3 (Tyr705) antibody (1:1,000; Cell Signaling Technology, Beverly, MA), or rabbit polyclonal anti-Stat3 antibody (1:1,000; Cell Signaling Technology). After three washes of 30 min with TBST, blots were incubated for 1 h at room temperature with anti-goat horseradish peroxidase–conjugated IgG (1:1,000) or anti-rabbit horseradish peroxidase–conjugated secondary antibody (1:2000; Cell Signaling Technology). The membranes were washed three times for 30 min and then probed using an ECL system (Amersham Pharmacia, Piscataway, NJ).

In vivo experiments. Male Wistar rats (250–300 g; Charles River Laboratories, St Constant, Canada) and 6-week-old female *ob/ob* mice (The Jackson Laboratory, Bar Harbor, ME) were fasted for 18 and 12 h, respectively. Mice were anesthetized with halothane and exsanguinated by heart puncture before or 15, 30, and 120 min after administration of 1 mg/kg i.p. recombinant leptin. Rats were anesthetized with sodium pentobarbital (60 mg/kg), and the carotid artery and jugular vein were cannulated. Blood samples (1.2 ml) were collected into 120 μl Trasylol (5,000 Kallikrein inactivating units/ml), EDTA (1.2 mg/ml), and Diprotin-A (0.1 mmol/l) before and after either intraperitoneal saline or recombinant leptin at 0.1 or 1 mg/kg. Plasma was separated from erythrocytes and stored at −20°C until assay. In rat experiments, erythrocytes were immediately resuspended in heparinized saline and reinjected via the jugular cannula. Plasma was extracted in ethanol and assayed using a radioimmunoassay (RIA) kit from Linco Research (St. Charles, MO). The assay uses an antibody specific for the NH_2-terminal end of bioactive GLP-1 and does not cross-react with the circulating degradation product, GLP-1(9-

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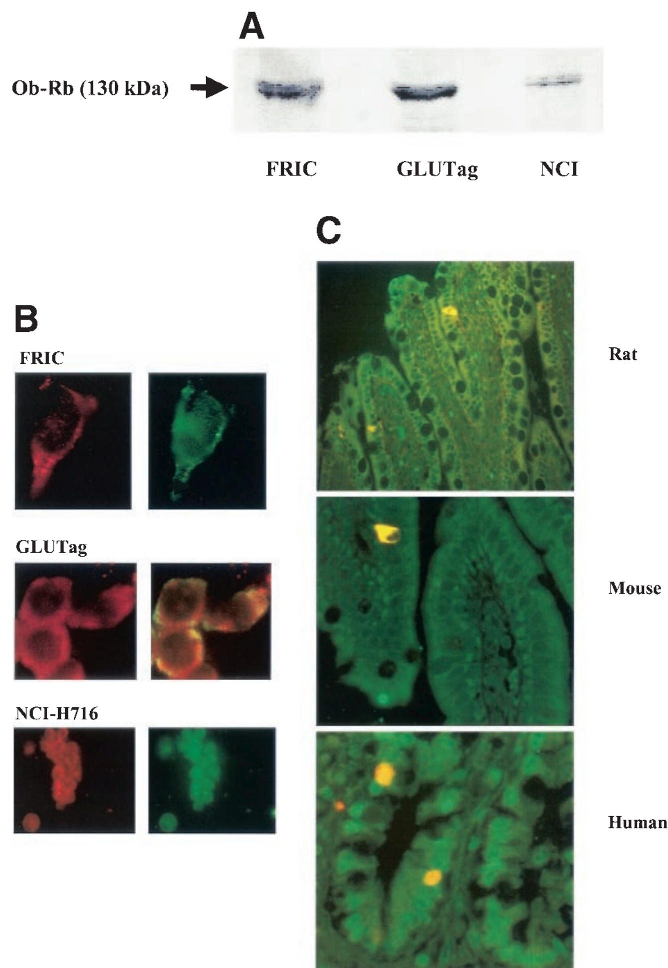

FIG. 3. Expression of the leptin receptor by L cells. *A*: FRIC culture and GLUTag and NCI-H716 cell extracts were analyzed by Western blot using an NH_2-terminal leptin receptor antibody; immunoreactive proteins with a relative molecular mass of 130 kDa were detected in the three cell types. *B* and *C*: Double-immunofluorescent staining for GLP-1 (red) and leptin receptors (green) in methanol-fixed FRIC cultures and GLUTag and NCI-H716 cell lines, visualized by fluorescence microscopy (*B*), and in rat, mouse, and human paraffin-embedded 5-μm ileal sections, as visualized by fluorescence microscopy (*C*).

36)-amide. The detection limit of the assay was 3 pmol/l. In *ob/ob* plasma samples, insulin content was measured by enzyme-linked immunosorbent assay (ELISA; Crystal Chem, Chicago, IL).

Female 4-week-old C57BL/6 mice were obtained from The Jackson Laboratory. The animals were housed four per cage with ad libitum access to rodent chow and water for a 1-week acclimation period. Mice then received either a low- or high-fat diet (no. D12450 or D12451, respectively; Research Diet, North Brunswick, NJ) containing 10 or 45% kcal as fat, respectively. Body weight was recorded regularly. After 8 weeks on the low- or high-fat diet, mice in each diet group were randomly divided for the following experiments. *1*) Mice were fasted for 12 h, anesthetized with halothane, and exsanguinated by heart puncture before or 10, 30, and 60 min after administration of 6 g/kg oral glucose. Blood was collected into Trasylol, EDTA, and Diprotin-A, and plasma was stored at −20°C until GLP-1 RIA. *2*) Mice were exsanguinated as above, and plasma leptin and insulin concentrations were measured by RIA and ELISA, respectively. Ileum and colon were resected and homogenized in extraction medium (35–37). Peptide and small proteins were then collected by reversed-phase adsorption, and extracts were assayed for COOH-terminal GLP-1 RIA, as above. *3*) Mice from each diet group were administered either saline or 10 mg/kg i.p. leptin once daily at the beginning of the dark phase for 3 days. Food consumption was measured at 2-h intervals and was normalized to kilocalories consumed, using the conversion factors 3.85 and 4.7 kcal/g for the 10 and 45% fat diets, respectively. All animal procedures were approved by the Animal Care Committee of the University of Toronto.

Data analysis. Peptide secretion is expressed as a percentage of control secretion (in vitro experiments) or as the change from basal values (in vivo experiments). Area under the curve for changes in hormone levels was determined using the trapezoidal rule. All data are expressed as the means ± SE. Statistical significance between experimental groups was assessed by ANOVA using "n − 1" post hoc custom hypothesis tests. Significance was assumed at the $P < 0.05$ level in these comparisons.

RESULTS

Leptin and GLP-1 secretion in vitro. The effect of leptin on GLP-1 secretion was studied in three cellular models known to synthesize and secrete GLP-1: FRICs in culture and mouse (GLUTag) and human (NCI-H716) enteroendocrine cell lines. Cells were incubated for 2 h with medium alone (negative control), PMA (1 μmol/l, a protein kinase C [PKC] activator that stimulates GLP-1 secretion in the three cell types) (35–39), or graded concentrations of leptin (Fig. 1). PMA stimulated GLP-1 secretion to 248 ± 28, 260 ± 38, and 252 ± 14% of the control ($P < 0.001$) in FRIC, GLUTag, and NCI-H716 cells, respectively. Leptin (10^{-9} to 10^{-6} mol/l) significantly stimulated GLP-1 secretion in a dose-dependent manner, from 129 ± 12 to 242 ± 45% of the control, respectively ($P < 0.05$ to $P < 0.001$, $n = 8$), in FRIC cultures, but it had no effect at 10^{-12} to 10^{-10} mol/1. In the GLUTag cell line, leptin significantly stimulated GLP-1 secretion at all concentrations, from 10^{-12} to 10^{-6} mol/l, reaching 241 ± 46% of control values ($P < 0.05$ to $P < 0.001$). Leptin (10^{-9} to 10^{-6} mol/l) also significantly stimulated GLP-1 secretion from the human NCI-H716 L cell line, to 164 ± 13 and 192 ± 34% of the control ($P < 0.05$ to $P < 0.01$), respectively, but had no effect at concentrations $<10^{-9}$ mol/l.

We, and others, have previously demonstrated that GRP is involved in nutrient-induced GLP-1 secretion (37,40,41), signaling via a PKC-dependent pathway (42). To determine whether there is an interaction between leptin and GRP signaling in the L cell, GLUTag cells were incubated with both leptin and GRP. As shown in Fig. 2, leptin (10^{-7} mol/l) and GRP (10^{-12} mol/l) significantly stimulated GLP-1 secretion, to 147 ± 12 and 165 ± 12%, respectively ($P < 0.05$ vs. the control). Incubating GLUTag cells simultaneously with leptin (10^{-7} mol/l) and GRP (10^{-12} mol/l) stimulated GLP-1 secretion to 199 ± 25% of control ($P < 0.001$); this effect was higher than the effects of each agent alone, reaching significance for leptin ($P < 0.05$), albeit not for GRP ($P = 0.11$), suggesting an additive effect of the two hormones.

Expression of leptin receptor by FRIC, GLUTag, and NCI cells. Consistent with the biological effectiveness of leptin in the three cell lines, leptin receptor protein was detected by immunoblotting of cell extracts, using an NH_2-terminal leptin receptor antibody that recognizes all leptin receptor isoforms. An immunoreactive band with a relative molecular mass of ~130 kDa corresponding to the long form of the leptin receptor (Ob-Rb) was detected in extracts from all cell types (Fig. 3*A*).

Double-immunofluorescent staining was also performed to localize the presence of the leptin receptor in L cells. Although only 1% of the cells in FRIC cultures are L cells, all cells immunopositive for GLP-1 in these cultures also showed expression of the leptin receptor, predominantly in the cell membrane (Fig. 3*B*). Double-immunofluorescent staining was also performed in GLUTag and NCI-H716 cell lines, in which most of the cells were immunopositive

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FIG. 4. Tyrosine phosphorylation of STAT3 by leptin in FRIC cultures and in GLUTag and NCI-H716 cell lines. Cells were exposed to 10^{-7} mol/l leptin and, at the indicated times, extracts of the three cell types were prepared and immunoblotted with rabbit polyclonal anti–Tyr705-phospho-STAT3 antibody or with anti-STAT3 antibody. One representative Western blot analysis and densitometric analysis of STAT3 phosphorylation for three experiments are shown for FRIC cultures (*A*), GLUTag cells (*B*), and NCI-H716 cells (*C*). Values are expressed as a percentage of the control value without leptin. *$P < 0.05$, **$P < 0.01$ vs. control.

for GLP-1, and also showed membrane expression of the leptin receptor.

Phosphorylation of STAT proteins after leptin treatment in FRIC, GLUTag, and NCI cells. Because phosphorylation-dependent activation of STAT3 is the major transduction pathway for leptin signaling (25–27), changes in the levels of phosphorylated STAT3 were investigated in FRIC, GLUTag, and NCI-H716 cells in response to 10^{-7} mol/l leptin, using a Tyr705-phospho-STAT3 antibody. As shown in Fig. 4, exposure of all three cell types to leptin resulted in an increase in the level of phosphorylated STAT3, reaching 234 ± 107 to 26,781 ± 5,375% of control ($P < 0.01$) after 30 min, and decreasing thereafter (Fig. 4). Total STAT3 levels did not change significantly in response to leptin treatment.

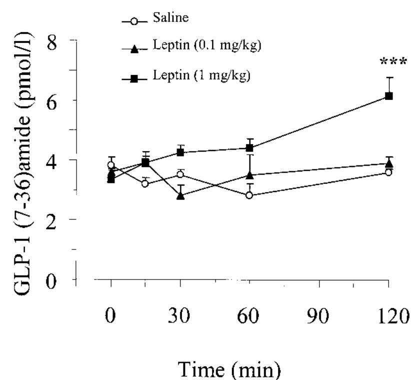

FIG. 5. Effect of leptin (0.1 and 1 mg/kg i.p.) or saline on bioactive GLP-1 secretion in fasted male Wistar rats ($n = 6$). ***$P < 0.001$ vs. basal.

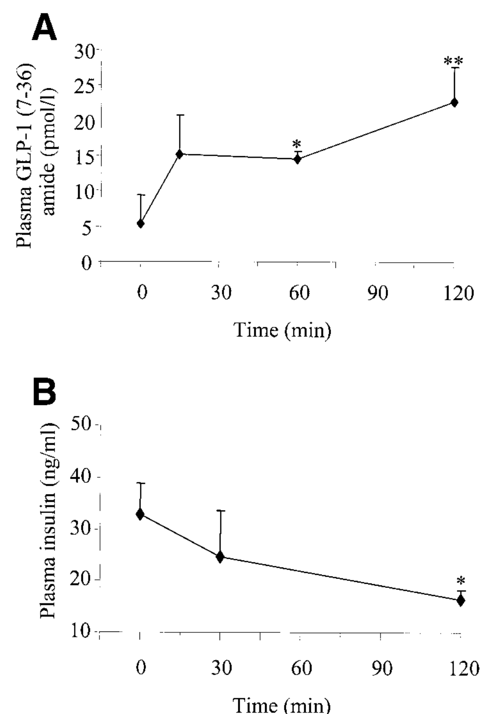

FIG. 6. Effect of leptin (1 mg/kg i.p.) on bioactive GLP-1 and insulin secretion in fasted *ob/ob* mice ($n = 6$). *$P < 0.05$, **$P < 0.01$ vs. basal.

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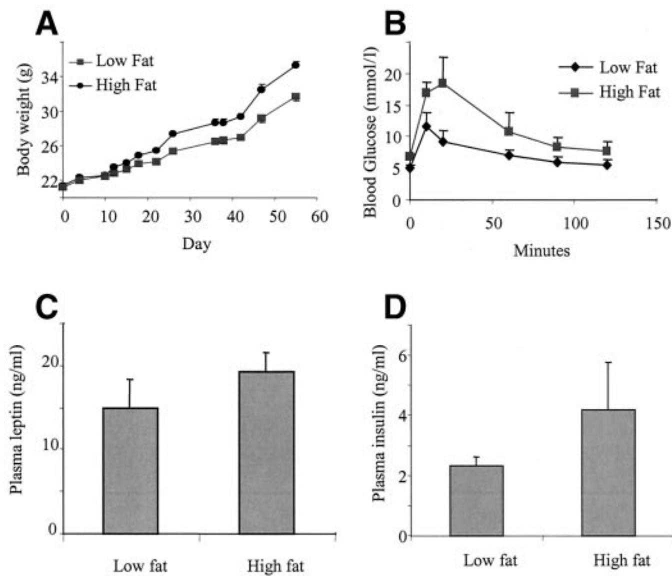

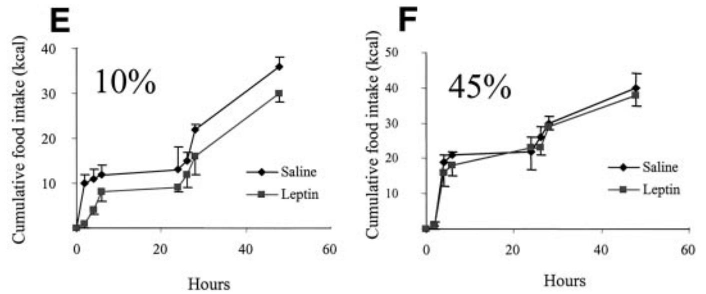

FIG. 7. Changes in body weight (n = 25) (A), oral glucose tolerance (n = 6) (B), leptin (n = 4) (C), insulin (n = 4) (D), and cumulative food intake (n = 6) (E and F) in C57BL/6 mice maintained for 8 weeks on a 10% (low) or 45% (high) fat diet. E and F: C57BL/6 mice were given saline or leptin (10 mg/kg i.p.) at 0, 24, and 48 h.

Expression of leptin receptor by rodent and human L cells. To establish the relevance of the effect of leptin on GLP-1 secretion in vivo, we determined whether rat, mouse, and human L cells express the leptin receptor by double-immunofluorescent staining, using an anti–GLP-1 antibody and an NH_2-terminal leptin receptor antibody. As shown in Fig. 3*C*, GLP-1–expressing L cells constitute ~1% of the epithelial cells of the distal small intestine in rodents and humans. The leptin receptor was found to be present on the membrane of all cells of the epithelium, including all rodent and human L cells.

Leptin and GLP-1 secretion in vivo. Leptin (1 mg/kg i.p.) significantly stimulated GLP-1 secretion by 1.8-fold after 120 min ($P < 0.001$) in fasted rats, whereas a dose of 0.1 mg/kg had no effect on GLP-1 secretion compared with intraperitoneal saline (Fig. 5). The effect of leptin on GLP-1 secretion was also studied in leptin deficient *ob/ob* mice (Fig. 6). Leptin (1 mg/kg i.p.) significantly stimulated GLP-1 secretion in these mice, reaching a fold increase of 4.7 ± 1.2 at 120 min ($P < 0.01$). At the same time, leptin significantly inhibited insulin secretion, by a fold decrease of 1.8 ± 0.6 ($P < 0.05$).

GLP-1 secretion in a model of leptin resistance. Four-week-old C57BL/6 mice were subjected to either a high-fat (45%) or a low-fat (10%) diet for 8 weeks. Mice on the high-fat diet became obese (38.1 ± 0.3 vs. 30.8 ± 0.5 g, $P < 0.001$) (Fig. 7*A*) and developed glucose intolerance (the delta area under the curve after oral glucose was increased to 605 ± 108 from 267 ± 79 mmol/l × 120 min, $P < 0.05$) (Fig. 7*B*). Mice on the high-fat diet also exhibited hyperinsulinemia (4.2 ± 1.5 vs. 2.3 ± 0.6 ng/ml) and hyperleptinemia (19.4 ± 2.3 vs. 14.9 ± 3 ng/ml) (Fig. 7*C* and *D*) as well as leptin resistance, as assessed by measuring food consumption (intraperitoneal injection of 1 mg/kg leptin reduced cumulative food intake by 16.3 ± 4.0% vs. saline, $P < 0.05$, in mice on the low-fat diet, whereas it had no effect in mice on the high-fat diet) (Fig. 7*E* and *F*). Interestingly, mice on the high-fat diet also had lower basal plasma GLP-1 levels compared with mice on the low-fat diet (2.1 ± 0.4 vs. 4.0 ± 0.5 pmol/l, $P < 0.05$) (Fig. 8), and they had a diminished GLP-1 response to oral glucose by 28.5 ± 5.0% at 10 min ($P < 0.05$). In contrast, the GLP-1 concentration of the ileal and colonic mucosa was 44 ± 5 and 38 ± 7% higher, respectively, in mice on the high-fat diet ($P < 0.05$).

DISCUSSION

GLP-1 is a potent insulinotropic and glucagonostatic hormone that also inhibits food intake and reduces body weight after long-term administration (1,15). Because of its pleiotropic actions in nutrient homeostasis, GLP-1 is now under investigation as a potential treatment for patients with type 2 diabetes. Interestingly, several studies have demonstrated that circulating GLP-1 levels are reduced in obese individuals, either with or without concomitant type 2 diabetes (16–19), and this impairment can be partially reversed by weight loss (18). These findings suggested that leptin may modulate GLP-1 secretion from the enteroendocrine L cells, and that leptin resistance in obesity may be linked to impaired GLP-1 secretion.

In the present study, we used several models of the L cell to investigate the effects and mechanism of action of leptin on GLP-1 secretion. Physiological concentrations of leptin were demonstrated to stimulate GLP-1 secretion from rat, mouse, and human L cells in a dose-dependent fashion. The presence of Ob-Rb, the long form of the leptin receptor, on the membrane of the L cell confirmed these findings, as did the demonstration that leptin treatment activated STAT signaling via phosphorylation of STAT3. Very recent studies have also demonstrated that Ob-Rb is present in rat, mouse, and human small intestinal enterocytes (30), where it may play a role in fat (43) and small-peptide (44) absorption. Leptin was also shown to stimulate the secretion of the gastrointestinal hormones gastrin (45) and cholecystokinin (46) in vivo in rats. The present study suggests an additional role for leptin in the intestine, to regulate glucose homeostasis through its effects on GLP-1 secretion. Additionally, GLP-1 is cosecreted with GLP-2, a related peptide that is derived with GLP-1 from the proglucagon prohormone in the L cell (47). GLP-2 is an intestinal tropic factor that enhances the digestion and absorption of nutrients (48,49). Thus, leptin

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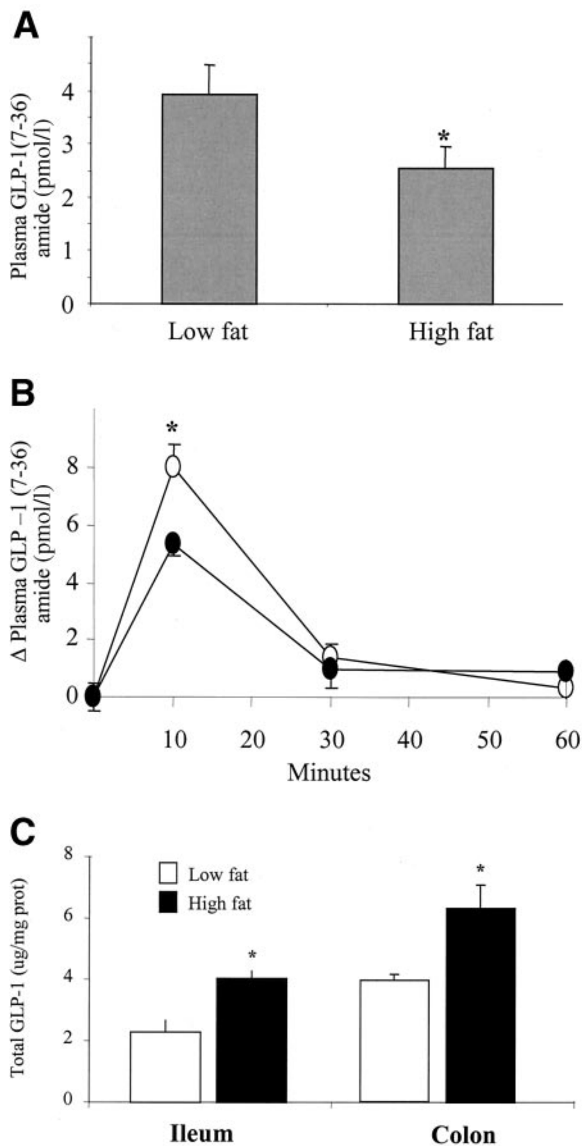

FIG. 8. Fasting plasma bioactive GLP-1 (*A*), increment in plasma GLP-1 after oral glucose (*B*), and GLP-1 concentration in ileal and colonic mucosa (*C*) in C57BL/6 mice after 8 weeks on a 10% (low) or 45% (high) fat diet ($n = 6$). *$P < 0.05$ vs. control.

may also serve to enhance intestinal function through effects to stimulate secretion of GLP-2 from the L cell.

Interestingly, the mouse cell line GLUTag was found to be sensitive to leptin at picomolar concentrations, in contrast to the FRIC and NCI-H716 cells, which were activated by leptin in the nanomolar range only. This may possibly be explained by species-specific differences between the three cell models, including expression levels of the Ob-Rb or, alternatively, the homogeneity of the single cell–clone GLUTag cells as compared with the more heterogenous FRIC and NCI-H716 cells.

Consistent with the results of the in vitro studies, injection of leptin into both normal rats and leptin-deficient *ob/ob* mice significantly stimulated GLP-1 secretion. Furthermore, in *ob/ob* mice, insulin secretion was markedly inhibited by leptin treatment. Previous studies have demonstrated that leptin has a direct effect on the pancreatic B cell to reduce both basal and GLP-1–stimulated insulin secretion (29,50,51). Thus, the observation that leptin increases GLP-1 release at the same time as it decreases insulin secretion appears contradictory. These findings suggest that the inhibitory effect of leptin on the β-cell is stronger than its stimulatory effect on the L cell. However, it must be noted that the insulinotropic effects of GLP-1 on the β-cell are glucose-dependent, such that insulin secretion is only enhanced by GLP-1 in the presence of hyperglycemia. Also, *ob/ob* mice are known to be very sensitive to exogenous leptin (21,22), consistent with the finding that an increase in GLP-1 secretion in response to leptin occurred earlier and was of greater magnitude as compared with rats. Finally, because leptin and GLP-1 have similar effects to reduce food intake after release into the peripheral circulation (13,15,21), leptin-induced GLP-1 secretion may be considered to potentiate the leptin signal. These findings are consistent with the hypothesis of an interaction between the gut, the endocrine pancreas, and the brain in the regulation of nutrient homeostasis (52).

Because most obese humans are not leptin deficient, but rather exhibit leptin resistance (21,32,53), we also examined a murine model of leptin resistance for changes in GLP-1 secretion. When submitted to a high-fat diet for 8 weeks, C57BL/6 mice developed insulin and leptin resistance in association with obesity. Interestingly, these mice also had lower basal GLP-1 levels, and their GLP-1 secretion in response to oral glucose was significantly diminished. Consistent with our finding that leptin stimulates GLP-1 secretion, we hypothesize that leptin was unable to maintain basal GLP-1 secretion or to potentiate food-induced GLP-1 secretion in these leptin-resistant mice. These findings therefore demonstrate that leptin resistance is associated with diminished GLP-1 secretion in obese mice, and they suggest that leptin is a physiological regulator of GLP-1 secretion under both basal and stimulated conditions. Furthermore, the data strongly implicates leptin resistance as a causative factor in the impaired GLP-1 secretion observed in obese humans (16–19).

Studies conducted in the GLUTag cell line suggested that leptin and the GLP-1 secretagogue GRP (37,40,41) exert additive effects on GLP-1 secretion. Similar studies have demonstrated synergistic effects of the monounsaturated fatty acid oleic acid and the enteroendocrine hormone glucose-dependent insulinotropic peptide in the regulation of GLP-1 secretion by GLUTag cells (48,54). In the in vivo setting, GLP-1 is rapidly secreted by L cells upon ingestion of a meal, and by fat and glucose in particular (3–5), as was also shown in the present study. Both direct effects of nutrients on the L cell and indirect effects involving endocrine and neuronal mediators, including GRP (3,40,48,54), have been shown to be involved in this secretion. As a consequence, GLP-1 release is biphasic, with the indirect effects occurring rapidly (15–30 min) and the direct effects taking place much later (60–120 min) after a meal (4,5). Although leptin is normally released from adipose tissue 60–120 min postprandially (55), recent studies have demonstrated that leptin is also synthesized by the gastric chief cells (31,56), and release of gastric leptin occurs 15 min after refeeding in a fasted rat (56). Thus, gastric leptin may be involved in the early indirect regulation of GLP-1 secretion by neuronal media-

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tors such as GRP, whereas leptin from the adipocyte may be more important in the maintenance of basal levels and the later postprandial phase of GLP-1 release.

High fat–fed, obese mice were found to have elevated concentrations of GLP-1 in both the ileum and colon. Previous studies have demonstrated that alterations in the fat composition of rodent chow, while maintaining fat content at 5%, do not alter GLP-1 concentrations in the ileum of rats (57), although dietary fat does stimulate GLP-1 release from the intestinal L cell (3,37,40,54). The mechanism(s) by which fat enhances the synthesis and secretion of GLP-1 remains to be determined.

In summary, the results of the present study show, for the first time, that leptin stimulates GLP-1 secretion in vivo in rodents and in vitro from rodent and human enteroendocrine L cells through activation of the leptin receptor. These finding provide evidence for the existence of an adipo-enteroendocrine axis involved in the regulation of nutrient homeostasis. Because leptin resistance was found to be associated with decreases in both basal and nutrient-stimulated GLP-1 secretion, these findings may provide an explanation for the impairment in GLP-1 release that has been observed in obese individuals.

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