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 enteroendocrine L cells, is a potent insulinosotropic 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 ($1 \text{ mg/kg i.p.}$) 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 $28.5 \pm 5.0\%$ ($P < 0.05$). 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 adipose-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.
Cells were washed with Hank’s salt solution and incubated for 2 h 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.

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 gliadin-releasing peptide (GRP; Bachem, Torrance, CA). Peptides and small proteins in cell medium or cell extract were then collected by reversed-phase adsorption (C18 Sep-Pak; Bachem, Torrance, CA). Peptides and small proteins in cell medium or cell extract 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). 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 −20°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 NH2-terminal 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 −20°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 NH2-terminal

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 < 0.01 vs. control.
GLP-1 RIA, as above.

extraction medium (35

oral glucose. Blood was collected into Trasylol, EDTA, and Diprotin-A, and

by heart puncture before or 10, 30, and 60 min after administration of 6 g/kg

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Diet, North Brunswick, NJ) containing 10 or 45% kcal as fat, respectively.

either a low- or high-fat diet (no. D12450 or D12451, respectively; Research

rodent chow and water for a 1-week acclimation period. Mice then received

ratory. The animals were housed four per cage with ad libitum access to

assay (ELISA; Crystal Chem, Chicago, IL).

samples, insulin content was measured by enzyme-linked immunosorbent

ob/ob

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 NH2-terminal leptin receptor antibody; immunoreactive pro-

teins 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-

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B

FRIC

GLUTag

NCI-H716

C

FRIC

GLUTag

NCI

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) enteroendo-

crine 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 secre-

tion 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/l. In the GLUTag cell line, leptin significantly stimu-

lated 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 se-

cretion, 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) also signifi-

cantly stimulated GLP-1 secretion from the human NCI-H716 L

cell line, 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

NH2-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. 3A).

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. 3B). Double-immunofluores-

cent staining was also performed in GLUTag and NCI-H716

cell lines, in which most of the cells were immunopositive

Female 4-week-old C57BL/6 mice were obtained from The Jackson Labo-
ratory. 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. Illeum 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

FIG. 3. Expression of the leptin receptor by L cells. A: FRIC culture and

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teins 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 fluores-
cence microscopy (B), and in rat, mouse, and human paraffin-embedded

5-μm ileal sections, as visualized by fluorescence microscopy (C).
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 $234 \pm 107$ to $26,781 \pm 5,375\%$ 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. 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.
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 NH2-terminal leptin receptor antibody. As shown in Fig. 3C, 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. 7A) 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. 7B). 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. 7C 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. 7E 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 enteroendocrine cell (30), where it may play a role in 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
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 enterostatic 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-

![FIG. 5. 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.](image-url)
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 enteroen-docrine L cells through activation of the leptin receptor. These findings 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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