Elimination of Glucagon-Like Peptide 1R Signaling Does Not Modify Weight Gain and Islet Adaptation in Mice With Combined Disruption of Leptin and GLP-1 Action

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Leptin and glucagon-like peptide 1 (GLP-1) exhibit opposing actions in the endocrine pancreas. GLP-1 stimulates insulin biosynthesis, secretion, and islet growth, whereas leptin inhibits glucose-dependent insulin secretion and insulin gene transcription. In contrast, GLP-1 and leptin actions overlap in the central nervous system, where leptin has been shown to activate GLP-1 circuits that inhibit food intake. To determine the physiological importance of GLP-1 receptor (GLP-1R)–leptin interactions, we studied islet function and feeding behavior in *ob/ob*:GLP-1R$^{-/-}$ mice. Although GLP-1R actions are thought to be essential for glucose-dependent insulin secretion, the levels of fasting glucose, glycemic excursion after glucose loading, glucose-stimulated insulin, and pancreatic insulin RNA content were similar in *ob/ob*:GLP-1R$^{+/+}$ versus *ob/ob*:GLP-1R$^{-/-}$ mice. Despite evidence linking GLP-1R signaling to the regulation of islet neogenesis and proliferation, *ob/ob*:GLP-1R$^{-/-}$ mice exhibited significantly increased islet numbers and area and an increase in the number of large islets compared with GLP-1R$^{+/+}$ or $^{-/-}$ mice ($P <$ –0.01 to 0.05). Similarly, growth rates and both short- and long-term control of food intake were comparable in *ob/ob*:GLP-1R$^{+/+}$ versus *ob/ob*:GLP-1R$^{-/-}$ mice. Furthermore, leptin produced a similar inhibition of food intake in GLP-1R$^{-/-}$, *ob/ob*:GLP-1R$^{+/+}$, and *ob/ob*:GLP-1R$^{-/-}$ mice. These findings illustrate that although leptin and GLP-1 actions overlap in the brain and endocrine pancreas, disruption of GLP-1 signaling does not modify the response to leptin or the phenotype of leptin deficiency in the *ob/ob* mouse, as assessed by long-term control of body weight or the adaptive β-cell response to insulin resistance in vivo. *Diabetes* 49:1552–1560, 2000

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CNS, central nervous system; GLP-1, glucagon-like peptide 1; GLP-1R, GLP-1 receptor; ICV, intracerebroventricular; IPGTT, intraperitoneal glucose tolerance test; NPY, neuropeptide Y; OGTT, oral glucose tolerance test.

Glucose-dependent insulin secretion is tightly regulated to maintain plasma glucose within a narrow physiological range under alternating conditions of fasting and nutrient excess. Multiple positive and negative regulatory signals converge on the islet β-cell that are ultimately integrated and used to either stimulate or suppress insulin secretion as required. Nutrient ingestion stimulates the secretion of gut-derived hormones termed incretins, such as glucagon-like peptide 1 (GLP-1) and gastric inhibitory polypeptide that potentiate glucose-stimulated insulin secretion (1,2). GLP-1 may also regulate blood glucose indirectly via the inhibitory effects on gastric emptying and hypothalamic feeding centers (3–5). GLP-1 may also produce conditioned taste aversion and its anorexic actions may be partially caused by the activation of hypothalamic stress pathways (6–9).

A combination of experiments using GLP-1 receptor (GLP-1R) antagonists and studies of glucose homeostasis in GLP-1R$^{-/-}$ mice implicate an essential role for GLP-1 signaling in the control of β-cell function and glucose homeostasis (10–13). In contrast, the central importance of GLP-1 for regulation of satiety and body weight is less clear. The administration of GLP-1 to human subjects via peripheral infusion (5), or to rodents via intracerebroventricular (ICV) injection (4), induces satiety and reduces food intake and body weight, respectively. Conversely, chronic inhibition of GLP-1 signaling in the central nervous system via ICV infusion of the GLP-1 receptor antagonist exendin (9–39) increases food intake and body weight in rats (14). However, surprisingly, mice with complete disruption of GLP-1 receptor expression are lean, eat normally, and do not develop obesity with aging or after several months of high fat intake (12,15).

The adipose-derived hormone leptin is a key nutrient sensor that regulates food intake, body weight, and insulin secretion (16,17). Leptin levels increase in the nutrient-repleted state, leading to inhibition of feeding behavior via a complex network of hypothalamic regulatory signals (17). In contrast to the lean phenotype of GLP-1R$^{-/-}$ mice, disruption of the genes encoding leptin or the leptin receptor results in hyperphagia, obesity, and diabetes characterized by insulin resistance and islet hyperplasia (18). Whereas GLP-1 stimulates β-cell function and insulin biosynthesis, leptin inhibits glucose- and GLP-1–stimulated insulin secretion and insulin

gene transcription (1,2,19,20). The potential importance of leptin for β-cell function has led to the use of the term *adipoinsular axis* for description of the homeostatic network linking nutritional reserves, leptin, and the islet β-cell (21).

The lack of obesity in GLP-1R$^{-/-}$ mice has prompted the suggestion that compensatory regulatory signals that inhibit food intake may be upregulated in the absence of GLP-1 signaling (22). In this regard, neuropeptide Y (NPY)$^{-/-}$ mice respond to food deprivation, eat normally, and do not exhibit disturbances of body weight regulation, suggesting that other orexigenic factors compensate for NPY deficiency in vivo (23). Alternatively, the anorexic actions of GLP-1 may not reflect specific actions on hypothalamic feeding centers but may be indirectly attributable to GLP-1 effects on hypothalamic stress pathways that mediate the response to visceral illness (8,9,12,22,24).

Both NPY and GLP-1 have been demonstrated to reside downstream of leptin action in the central nervous system (CNS) pathways regulating food intake (25,26). Exendin (9–39) inhibits the leptin-induced reduction of food intake and weight loss (26), and leptin upregulates brainstem proglucagon RNA and hypothalamic GLP-1 content in rats in vivo (27). Similarly, leptin activates c-*fos* expression in a subset of rat brainstem neurons in the nucleus of the solitary tract (28). Furthermore, leptin and GLP-1 signaling pathways also interact in the islet β-cell, where leptin and GLP-1 exert opposing actions in the control of insulin secretion and insulin gene transcription (19,20,29). To ascertain the physiological importance of GLP-1R–leptin interactions, we studied glucose homeostasis, islet function, body weight gain, and feeding behavior in *ob/ob*:GLP-1R$^{-/-}$ mice.

RESEARCH DESIGN AND METHODS

Animals. All mice were maintained in the Toronto General Hospital Animal Facility and raised on a normal rodent diet under a 12-h dark/light cycle (lights on at 6:00 A.M. and off at 6:00 P.M.). All animal experiments were carried out under a protocol approved by the Toronto General Hospital Animal Care Committee. GLP-IR$^{-/-}$ mice were maintained in the CD1 genetic background and were mated with *ob/ob* mice after the *ob/ob* mice were treated with leptin for 4–6 weeks to reverse sterility, as previously described (30,31). F1 generation heterozygote mice (*OB/ob*:GLP-IR$^{+/-}$) were mated to generate mice homozygous for mutations at both the leptin and GLP-1R loci (*ob/ob*:GLP-IR$^{-/-}$). *ob/ob*:GLP-IR$^{+/+}$ littermates were used as genetic controls during all experiments. The GLP-IR genotype was assessed using genomic DNA from tail snips and Southern blot analysis, as previously described (12). The wild-type (*OB/OB*) and mutant (*ob/ob*) genotypes were ascertained by polymerase chain reaction amplification with primers: 5′-TGTCAAGATGGACCAGACTC-3′ and 5′-ACTGGTCGAGGCAGGGAGCA-3′ followed by digestion with *Dde*I under conditions described previously (32). *ob/ob* Mice were obtained from Charles River (Montreal, PQ, Canada).

Attempts to mate the GLP-IR$^{-/-}$ with *ob/ob* mice to generate double-homozygote mutant mice were complicated by repeated observations that male or female double mutant *ob/ob*:GLP-1R$^{-/-}$ mice were not fertile, even when leptin treatments were maintained each day for a period of up to 12 weeks. Accordingly, *ob/ob* mice were leptin-treated (30,31) and mated with GLP-IR$^{-/-}$ mice to generate F1 heterozygote *OB/ob*:GLP-1R$^{+/-}$ mice. These heterozygote mice were fertile and several mice were used as founders to produce all of the double-homozygote *ob/ob*:GLP-IR$^{-/-}$ and *ob/ob*:GLP-IR$^{+/+}$ mice used in our studies. Several rounds of heterozygote matings were required to generate enough double mutant mice for use in our experiments.

Glucose tolerance and plasma insulin determinations. Age- and sex-matched mice were administered glucose by oral gavage or intraperitoneal injection, as previously described (12,15,33,34). As *ob/ob* and GLP-IR$^{-/-}$ mice were generated in 2 different genetic backgrounds (C57BL/6 and CD1, respectively), we included all parental genetic strains as controls for the analysis of the *ob/ob*:GLP-1R$^{-/-}$ mouse, including hybrid wild-type CD1:C57BL/6 and *ob/ob*:GLP-1R$^{+/+}$ controls. After an oral glucose challenge, blood glucose was slightly higher in the C57/BL6$^{+/+}$ strain compared with CD1$^{+/+}$ mice (data not shown). Mice were fasted for 16–18 h before each glucose challenge experiment, after which glucose (1.5 mg/g body wt) was administered through a gavage tube or via intraperitoneal injection. Blood was withdrawn from a tail vein at 0, 10, 20, 30, 60, 90, and 120 min (7 samples per mouse), and blood glucose was determined using a One Touch Basic Glucometer (Lifescan, Canada, Burnaby, BC, Canada). Insulin levels were measured in venous blood samples in duplicate with an Insulin ELISA kit (Crystal Chem, Chicago), with mouse insulin as a standard.

Feeding studies. For the 24-h feeding study, mice were fasted for a period of 16 h overnight before the start of each experiment. Mice were placed into individual cages containing preweighed rodent food and food consumption was assessed at 2, 4, 8, and 24 h after the start of the experiment. For the 48-h feeding study, each mouse was weighed and placed into an individual cage containing preweighed rodent food. All mice received a subcutaneous injection of saline (0.5 ml) just before the start of each dark-cycle feeding (4–5 P.M.) and food intake was measured for 2 consecutive 24-h periods. After the first 48-h control cycle, fresh rodent food was placed into all of the cages and the identical groups of mice were treated with a subcutaneous injection of leptin (4 μg/g body wt) before the start of dark-cycle feeding (2 injections, 24 h apart) and food consumption was measured for an additional 2 consecutive 24-h periods. The recombinant mouse leptin was a gift from the Amgen Corporation (Thousand Oaks, CA). Plasma leptin was measured in 14 male and 14 female GLP-1R$^{-/-}$ and control CD1 mice (10–14 weeks old). Mouse blood was obtained by tail bleeding between 9 A.M. and 1 P.M. A volume of 50 μl blood was collected into microfuge tubes containing 5 μl of 20% EDTA. The blood was centrifuged at 4°C for 10 min to separate mouse plasma (20μl). The plasma was stored at –70°C. Plasma levels were measured using a mouse Leptin ELISA kit (Crystal Chem).

Islet histology. Quantitative evaluation of pancreatic tissue was performed by an individual blinded to the genetic background of the slides using a Leitz Laborlux microscope (Wetzar, Germany) with a color video camera (JVC TK-1280U) connected to a computer monitor and Leica Q500MC software (Leica, Cambridge, U.K.). The microscope was calibrated at 4× and 10× magnification, and the same microscope was used for all tissue analysis. For evaluation of islet size distribution, islets were designated as single (<300 μm^2), small (300–5,000 μm^2), medium (5,000–20,000 μm^2), or large (>20,000 μm^2). The number and size of islets, the area of each islet (μm), and the total pancreas area (μm) were determined for each section, as previously described (35). Total islet area was expressed as a percent of total pancreas area. Immunostaining for insulin and glucagon was carried out, as previously described (36,37).

Northern blot analysis. Total RNA was isolated and Northern blot analysis was performed, as previously described (38,39). Rat insulin and mouse proglucagon cDNA probes were ^{32}P-labeled and blots were analyzed using a Storm 840 (Molecular Dynamics, Sunnyvale, CA) with ImageQuant 5.0 software.

Statistics. Results are expressed as means ± SE. Statistical significance was calculated by analysis of variance and the Student's *t* test with INSTAT 1.12 (Graph-Pad Software, San Diego, CA). A P value < 0.05 was considered to be statistically significant.

RESULTS

To ascertain whether dysregulation of leptin physiology may contribute to the phenotype represented by mild glucose intolerance and normal body weight and feeding behavior observed in GLP-1R$^{-/-}$ mice, we initially assessed levels of circulating leptin in both fasted and fed GLP-1R$^{-/-}$ mice. Although leptin levels were comparable in female $^{+/+}$ and GLP-1R$^{-/-}$ mice, the circulating levels of plasma leptin were significantly higher in both the fasted and fed state in male GLP-1R$^{-/-}$ mice (Fig. 1) (P < 0.003–.007, GLP-1R$^{-/-}$ vs. $^{+/+}$ mice). The weights of inguinal, perirenal, and gonadal fat pads were comparable in $^{+/+}$ versus GLP-1R$^{-/-}$ female mice (data not shown). Although no differences in weights of perirenal and gonadal fat pads were detected in male $^{+/+}$ versus $^{-/-}$ mice, inguinal fat pads were significantly heavier in male GLP-1R$^{-/-}$ vs. $^{+/+}$ mice (0.47 ± 0.05 vs. 0.27 + 0.03 g, GLP-1R$^{-/-}$ vs. $^{+/+}$ mice; n = 12, P < 0.01). Because leptin opposes the action of GLP-1 at the pancreatic β-cell (21), and both leptin and GLP-1 inhibit food intake via overlapping central mechanisms, the findings of elevated levels of circulating leptin, taken together with previous experiments demonstrating increased leptin sensitivity in

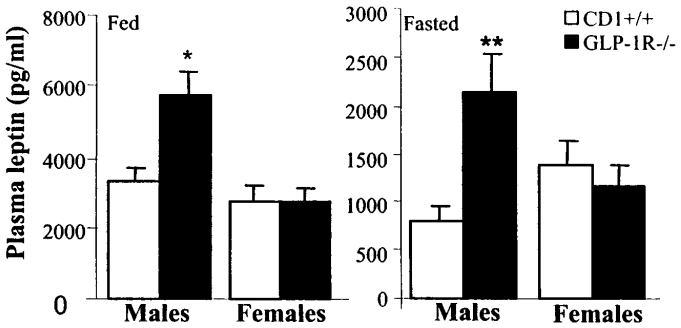

FIG. 1. Plasma leptin in fed and fasted age- and sex-matched 10–14 week old GLP-1R$^{-/-}$ and control$^{+/+}$ mice. *n* = 14 mice per group. **P* < 0.03, ***P* < 0.007, GLP-1R$^{-/-}$ vs. $^{+/+}$ mice.

GLP-1R$^{-/-}$ mice (33), raised the possibility that the phenotypes of the GLP-1R$^{-/-}$ mouse may be modified in part by enhanced leptin action. Consistent with this possibility, the degree of glucose intolerance is substantially greater in male compared with female GLP-1R$^{-/-}$ mice (12). To ascertain the potential contributions of leptin and GLP-1 to the phenotype of the GLP-1R$^{-/-}$ and *ob/ob* mouse, respectively, we generated *ob/ob*:GLP-IR$^{-/-}$ mice containing mutations in the genes for leptin and the GLP-1 receptor.

The adult *ob/ob* mouse exhibits hyperinsulinemia, insulin resistance, and markedly abnormal glycemic excursion after glucose challenge (40). Because GLP-1 has been shown to play an essential role in insulin biosynthesis, β-cell signaling, and insulin secretion (1,10–13), we hypothesized that *ob/ob*:GLP-1R$^{-/-}$ islets might exhibit defective upregulation of insulin biosynthesis or secretion in the setting of insulin resistance. Glycemic excursions were markedly abnormal in all obese mouse strains after glucose loading (Fig. 2*A* and *B*). Surprisingly, the extent of glucose intolerance was comparable in *ob/ob*:GLP-1R$^{+/+}$ versus *ob/ob*:GLP-1R$^{-/-}$ mice after oral glucose tolerance test (OGTT) or intraperitoneal glucose tolerance test (IPGTT) (Fig. 2*A* and *B*). All obese mouse strains exhibited marked increases in the levels of plasma insulin compared with lean$^{+/+}$ controls (Fig. 2*C*). However, the levels of glucose-stimulated insulin were comparable in *ob/ob*:GLP-1R$^{+/+}$ versus *ob/ob*:GLP-1R$^{-/-}$ mice after both oral and intraperitoneal glucose challenge (Fig. 2*C*). Consistent with previous findings, the levels of pancreatic insulin mRNA transcripts were markedly upregulated in the *ob/ob* mouse strains (Fig. 2*D*). Despite the ascribed importance of GLP-1R signaling for insulin gene transcription, biosynthesis, and secretion (1,2,10,11,41), the levels of insulin mRNA transcripts were also markedly elevated in *ob/ob*:GLP-1R$^{-/-}$ mice and comparable to levels detected in pancreatic RNA from *ob/ob* and *ob/ob*:GLP-1R$^{+/+}$ mice (Fig. 2*D*). These results clearly indicate that the loss of GLP-1R signaling does not significantly attenuate the β-cell insulin response to insulin resistance in the double mutant *ob/ob*:GLP-1R$^{-/-}$ mouse.

Although GLP-1 was originally characterized as an incretin, GLP-1 also stimulates islet cell proliferation and increases β-cell mass in normal and diabetic rodents (42–44). Because *ob/ob* islets undergo hyperplasia as part of the adaptive response to insulin resistance and hyperglycemia (40,45), we examined whether disruption of GLP-1R signaling might compromise the development of islet hyperplasia in *ob/ob*:GLP-1R$^{-/-}$ mice. Both the number of islets and islet area were significantly increased in *ob/ob*:GLP-1R$^{-/-}$ compared with GLP-1R$^{-/-}$ mice (Fig. 3*A*–*C*). No significant difference in these parameters was observed in *ob/ob*:GLP-1R$^{+/+}$ versus *ob/ob*:GLP-1R$^{-/-}$ mice (Fig. 3*B* and *C*). Although the number of large islets was significantly greater in *ob/ob*:GLP-1R$^{-/-}$ mice vs. GLP-1R$^{-/-}$ mice ($P < 0.05$ for GLP-1R$^{-/-}$ vs. *ob/ob*:GLP-1R$^{-/-}$) (Fig. 3*D*), no difference was detected in islet number, islet size, or the relative number of small, medium, and large islets in *ob/ob*:GLP-1R$^{+/+}$ vs. *ob/ob*:GLP-1R$^{-/-}$ mice (Fig. 3*B*–*D*). These findings demonstrate that elimination of the GLP-1R signaling system does not compromise the adaptive islet hyperplastic response to insulin resistance in the *ob/ob* genetic background.

Both leptin and GLP-1 activate receptors in hypothalamic feeding centers leading to the inhibition of food intake in both rodents and human subjects (17,46). Although mice with mutations in the leptin gene (*ob/ob*) or leptin receptor (*db/db*) exhibit significant abnormalities in the regulation of food intake and body weight, GLP-1R$^{-/-}$ mice are surprisingly lean, eat normally, and are resistant to the development of obesity after high-fat feeding (12,15). To determine the consequences of the combined loss of the inhibitory inputs of both GLP-1 and leptin, we assessed food intake and weight gain in *ob/ob*:GLP-1R$^{+/+}$ and *ob/ob*:GLP-IR$^{-/-}$ mice.

Both male and female GLP-1R$^{-/-}$ mice remained lean and actually gained significantly less weight, compared with CD1$^{+/+}$ control mice, by 6 months of age (Fig. 4*A*, *B*–$P < -0.05$ to 0.001 for male and female CD1$^{+/+}$ versus GLP-1R$^{-/-}$ mice, respectively). Furthermore, elimination of both leptin and GLP-1 action did not result in significantly greater weight gain in obese male or female *ob/ob*:GLP-IR$^{-/-}$ mice compared with *ob/ob*:GLP-IR$^{+/+}$ mice studied up to 6 months of age (Fig. 4*C* and *D*). Because both leptin and GLP-1 inhibit food intake, we analyzed feeding behavior over a 24-h time period in GLP-1R$^{+/+}$, $^{-/-}$, *ob/ob*:GLP-1R$^{+/+}$, and *ob/ob*:GLP-IR$^{-/-}$ mice. Although food intake was comparable in CD1$^{+/+}$ and GLP-IR$^{-/-}$ mice at 2 h, GLP-1R$^{-/-}$ mice ate significantly more food between 2 and 4 h ($P < 0.05$); however, this difference was not sustained over a 24-h period (Fig. 5). Similarly, although *ob/ob*:GLP-1R$^{-/-}$ mice ate significantly more food compared with *ob/ob* and *ob/ob*:GLP-1R$^{+/+}$ mice during the 8- to 24-h time period, no significant difference in cumulative food intake was observed in *ob/ob*:GLP-1R$^{+/+}$ versus *ob/ob*:GLP-1R$^{-/-}$ mice over a 24-h time period (Fig. 5*C*).

The *ob/ob* and GLP-1R$^{-/-}$ mice are sensitive to exogenous leptin administration, and recent experiments suggest that the inhibitory actions of leptin on appetite may be mediated in part via the CNS GLP-1 system (26,27). We examined whether *ob/ob*:GLP-1R$^{-/-}$ mice exhibited enhanced sensitivity to acute leptin administration over a 48-h time period. Leptin was administered (4 μg/g body wt) at the start of the dark-cycle feeding (5:00 P.M.), as previously described (25). Leptin treatment produced a significant reduction in food intake in GLP-IR$^{-/-}$, *ob/ob*, and both *ob/ob*:GLP-IR$^{+/+}$ and *ob/ob*:GLP-IR$^{-/-}$ mice (Fig. 6). Reanalysis of the data, after the normalization of food intake per gram of body size, revealed no significant difference amongst any obese groups in the relative response to leptin administration (data not shown). These results demonstrate that GLP-1R signaling is not required for the inhibitory response to leptin, and mice with mutations in both the lep-

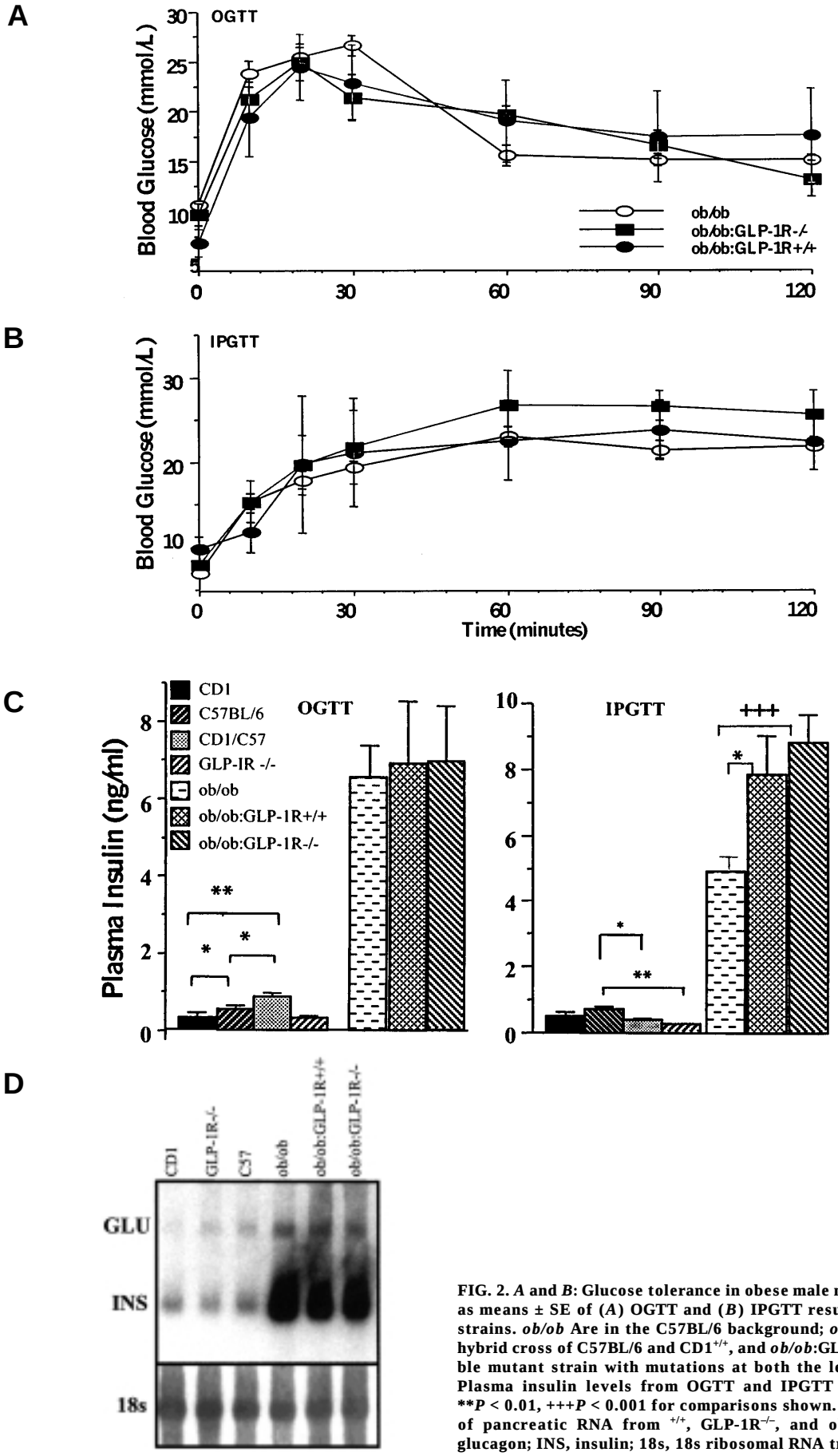

FIG. 2. ***A*** **and** ***B*****: Glucose tolerance in obese male mice. Values are expressed as means ± SE of (*A*) OGTT and (*B*) IPGTT results for obese male mouse strains. *ob/ob* Are in the C57BL/6 background; *ob/ob*:GLP-1R$^{+/+}$ mice are a hybrid cross of C57BL/6 and CD1$^{+/+}$, and *ob/ob*:GLP-1R$^{-/-}$ represent the double mutant strain with mutations at both the leptin and GLP-1R loci. *C*: Plasma insulin levels from OGTT and IPGTT experiments. *$P < 0.05$, **$P < 0.01$, +++$P < 0.001$ for comparisons shown. *D*: Northern blot analysis of pancreatic RNA from $^{+/+}$, GLP-1R$^{-/-}$, and obese mice strains. GLU, glucagon; INS, insulin; 18s, 18s ribosomal RNA transcripts.**

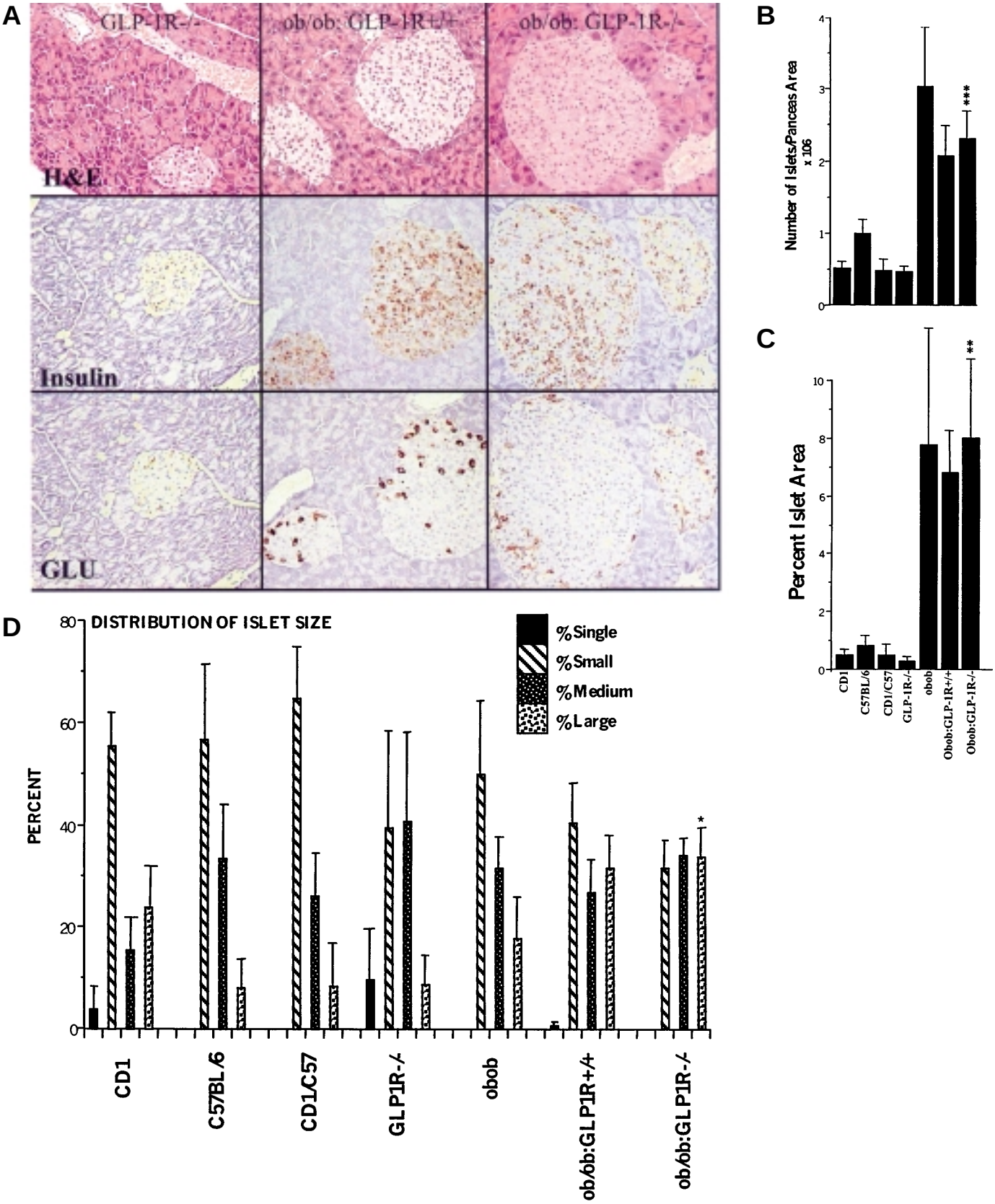

FIG. 3. ***A*: Islet hyperplasia and increased islet size in *ob/ob* mice. The top panels represent histological sections stained with hematoxylin and eosin (H&E). The middle and bottom panels represent histological sections stained with antisera directed against insulin and glucagon, respectively. Magnification × 400. *B*: Islet number in lean and obese mice. The number of islets, relative to total pancreatic area examined, is shown. ***$P < 0.002$ for *ob/ob*:GLP-1R$^{-/-}$ versus GLP-1R$^{-/-}$ mice. *C*: Relative islet area in lean versus obese mice. The percent islet area relative to total pancreatic area examined is shown. **$P < 0.02$ for *ob/ob*:GLP-1R$^{-/-}$ versus GLP-$^{1R-/-}$ mice. *D*: Number of single, small, medium, and large islets in lean and obese mice strains. *$P < 0.05$ for GLP-1R$^{-/-}$ versus *ob/ob*:GLP-1R$^{-/-}$ mice.**

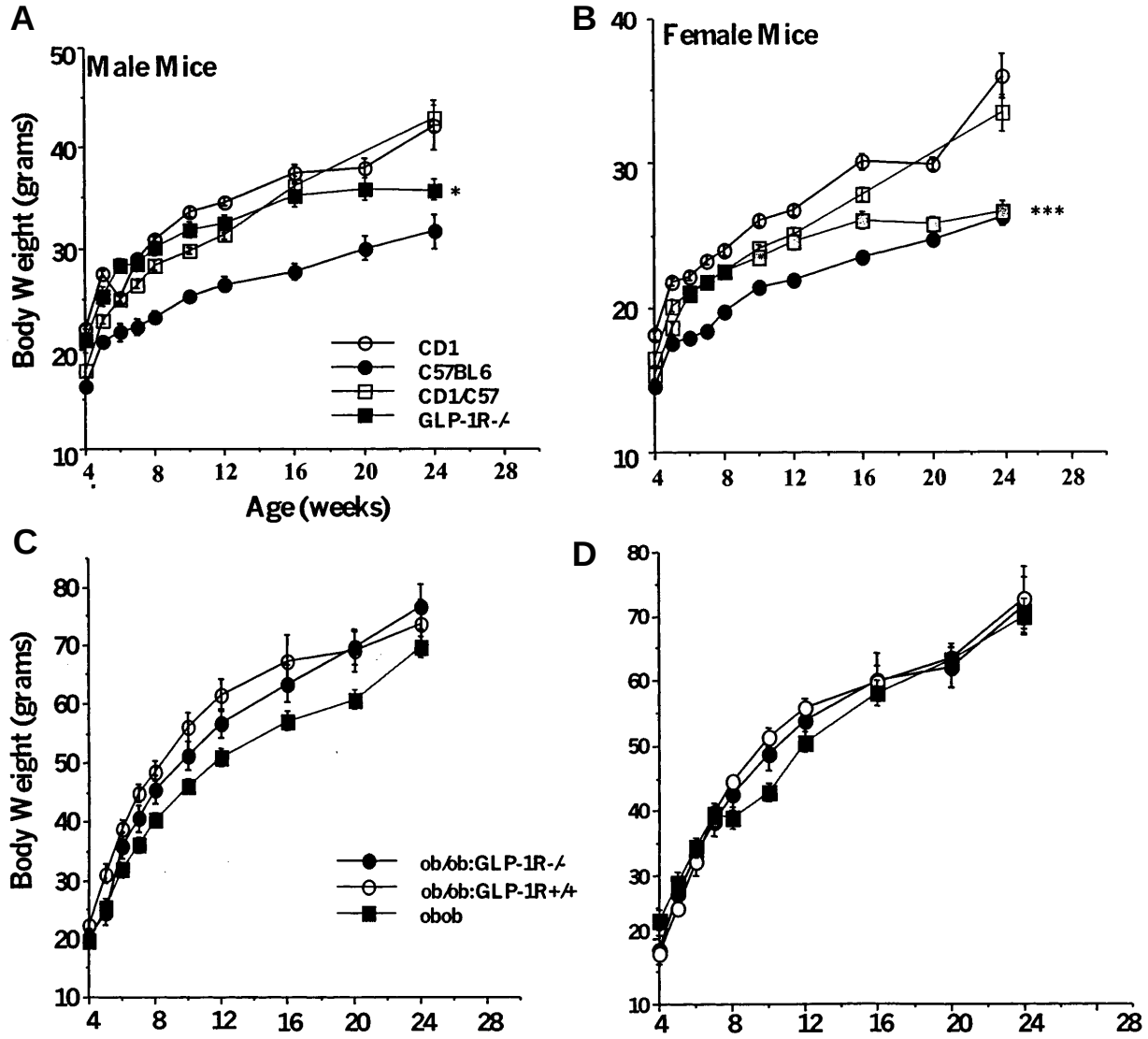

FIG. 4. Growth curves of mice from 4 to 24 weeks of age: male (*A*) and female (*B*) lean control and GLP-1R$^{-/-}$ mice and male (*C*) and female (*D*) obese mice. Values are expressed as body weight in grams, means ± SE, *n* = 5–6 for obese strains and *n* = 5–10 for control lean and GLP-1R$^{-/-}$ mouse strains. ***$P < 0.001$, *$P < 0.05$, CD1 versus GLP-1R$^{-/-}$ mice.

tin and GLP-1R genes do not exhibit additive or markedly enhanced sensitivity to exogenous leptin in vivo at the pharmacological doses of leptin used in our studies.

DISCUSSION

Several lines of evidence suggest that leptin and GLP-1 subserve interrelated actions in the regulation of metabolic functions via opposing and complementary effects on insulin secretion and satiety control, respectively. The sensitivity of GLP-1 secretion to acute nutrient ingestion, taken together with its effects on satiety, gastric emptying, and glucose-stimulated insulin secretion, is in keeping with the notion that GLP-1 functions as a short-term regulator of nutrient assimilation (1). Consistent with the transient effects of GLP-1 on the β-cell, the levels of bioactive GLP-1 increase and fall rapidly after food ingestion, in part because of the inactivation by the enzyme dipeptidyl peptidase IV (47,48). In contrast, the levels of circulating leptin are highly correlated with total body weight and adipose tissue mass, which is in keeping with a role for leptin as an indirect sensor of long-term energy stores and nutrient abundance (49).

A number of studies support a role for leptin in the regulation of β-cell function and insulin secretion (21). The long form of the leptin receptor is expressed on rodent and human islet β-cells (50–52), and leptin inhibits glucose-stimulated insulin secretion and insulin gene expression (19,20,50,52). As the GLP-1R$^{-/-}$ β-cell exhibits defects in signal transduction and glucose-stimulated insulin secretion (12,13), we speculated that the *ob/ob*:GLP-1R$^{-/-}$ β-cell might also exhibit defects in insulin biosynthesis and/or secretion, further exacerbating the degree of glucose intolerance and the response to insulin resistance in *ob/ob* mice. Nevertheless, we did not observe a further deterioration in glucose homeostasis or in levels of glucose-stimulated insulin secretion in

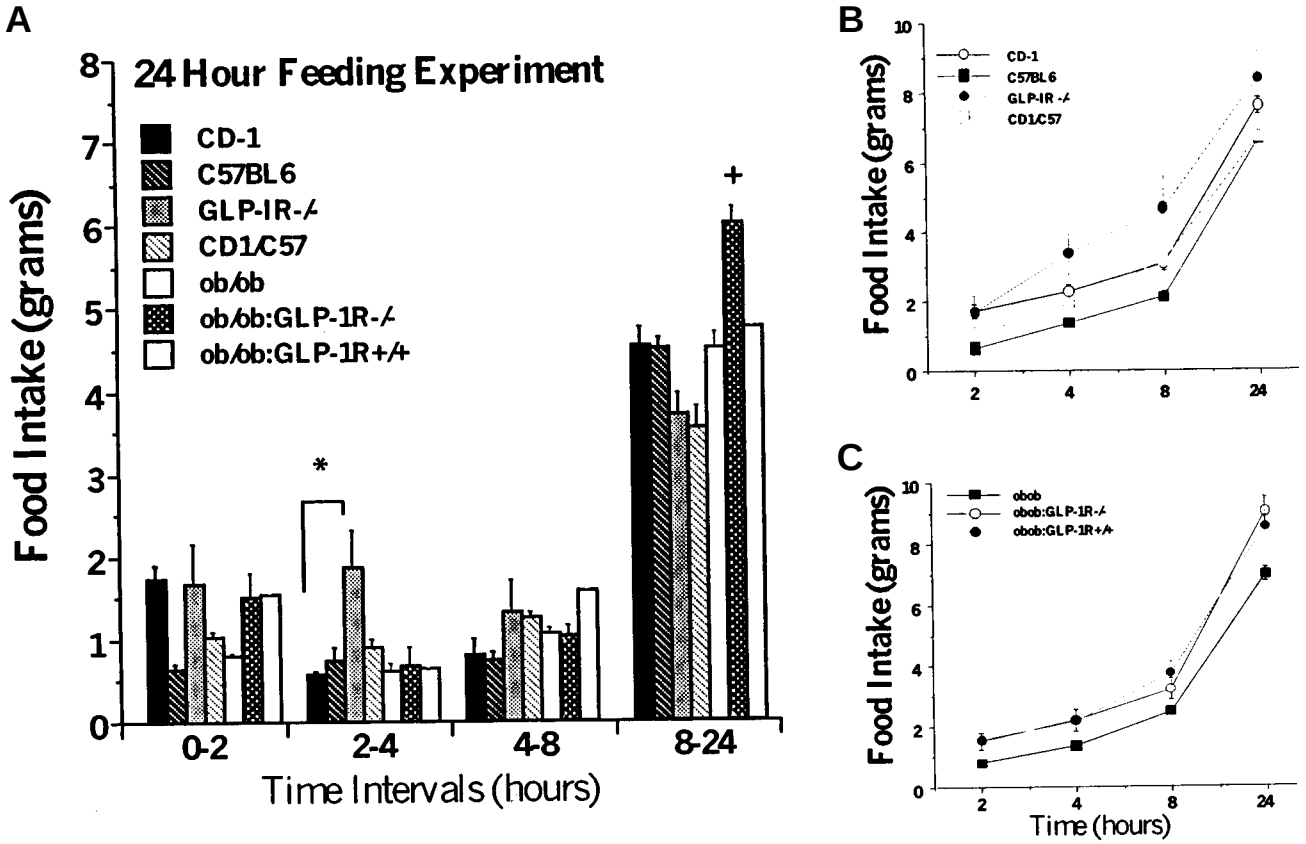

FIG. 5. The 24-h feeding experiments in lean and obese mice. *A*: Food intake during specific time intervals during the 24-h feeding period in lean and obese mice. Values are expressed as the means ± SE, *n* = 4–6 per group. **P* < 0.05, GLP-1R$^{-/-}$ versus CD1; +*P* < 0.05, *ob/ob*:GLP-1R$^{-/-}$ versus *ob/ob*:GLP-1R$^{+/+}$ mice. *B*: Cumulative food intake for lean control and GLP-1R$^{-/-}$ mice over 24 h. No significant difference was observed for CD1 versus GLP-1R$^{-/-}$ mice. *C*: Cumulative food intake for obese mice during 24 h. No significant differences were observed for *ob/ob*:GLP-1R$^{-/-}$ versus *ob/ob*:GLP-1R$^{+/+}$ mice. Baseline food intake in $^{+/+}$ and *ob/ob* mice strains has previously been shown to be comparable over short experimental time periods in similar experiments (58).

ob/ob:GLP-1R$^{-/-}$ mice. Indeed, levels of circulating insulin, pancreatic insulin, and proinsulin RNA were upregulated yet comparable in *ob/ob*:GLP-1R$^{+/+}$ versus *ob/ob*:GLP-1R$^{-/-}$ mice. These findings demonstrate that although the GLP-1 receptor is an essential determinant of glucose-stimulated insulin secretion and intracellular β-cell signaling in normal mice (12,13), basal GLP-1 signaling is not essential for the β-cell to significantly upregulate insulin synthesis and secretion in the setting of leptin deficiency, obesity, hyperglycemia, and insulin resistance.

Several lines of evidence suggest that GLP-1R signaling regulates islet proliferation and islet neogenesis. Incubation of quiescent rat islet cells with GLP-1–stimulated cell proliferation and immediate early gene expression (42,53), and both exendin-4 and GLP-1 promoted differentiation of pancreatic exocrine cells to a β-cell phenotype in vitro (54). Similarly, β-cell replication was increased in mice after GLP-1 administration, and exendin-4 significantly improved glucose tolerance and stimulated islet cell neogenesis in rats after subtotal pancreatectomy (44,55). Because *ob/ob* mice exhibit marked islet hyperplasia as part of the adaptive response to obesity and insulin resistance (45), we speculated that islet proliferation might be impaired in *ob/ob* mice with disrupted GLP-1R signaling.

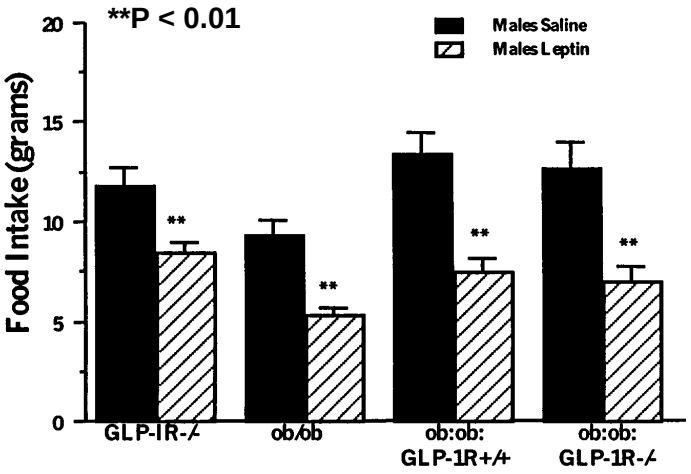

FIG. 6. Leptin sensitivity in GLP-1R$^{-/-}$ and obese mice strains. Leptin injections (4 μg/g body weight) were performed just before the start of dark phase eating (4–5 P.M. daily). The results are shown for a 48-h feeding experiment in male mice. Similar results were obtained for studies in female mice (data not shown). Values are expressed as means ± SE. ***P* < 0.01 for saline- versus leptin-treated mice.

Surprisingly, our data clearly show that significant islet hyperplasia develops in the absence of GLP-1R signaling and the extent of islet proliferation was comparable in both *ob/ob*:GLP-1R+/+ and *ob/ob*:GLP-1R$^{-/-}$ mice. Hence, GLP-1R signaling is not limiting for and may be upstream of growth regulatory signaling pathways that stimulate islet hyperplasia in response to leptin deficiency and insulin resistance.

The overlapping inhibitory actions of leptin and GLP-1 on CNS satiety centers, the finding of increased leptin sensitivity in GLP-1R$^{-/-}$ mice (33), and the increased leptin levels in male GLP-1R$^{-/-}$ mice have raised the possibility that simultaneous interruption of both leptin and GLP-1 action might lead to significantly increased food intake and weight gain above that seen in the *ob/ob* mouse alone. Surprisingly, however, no significant change in body weight was observed in male or female *ob/ob*:GLP-1R$^{-/-}$ mice compared with *ob/ob* and *ob/ob*:GLP-1R$^{+/+}$ mice, and total food intake measured over a 24-h period was comparable in *ob/ob*:GLP-1R$^{+/+}$ and *ob/ob*:GLP-1R$^{-/-}$ mice. These findings, taken together with our observation that GLP-1R$^{-/-}$ mice consume more food during a short-defined early time period, are consistent with previous suggestions that GLP-1 may function as a short-term transient regulator of food intake. For example, GLP-1 reduces food intake several hours after ICV administration, but has no effect on food intake and body weight over longer time periods (6,56). In contrast, the inhibitory action of leptin on food intake is more sustained (6), and hence interruption of leptin action, as exemplified by the *ob/ob* and *db/db* mouse, is associated with increased food intake and the development of obesity.

The initial observation that ICV GLP-1 inhibits food intake in rats (4) was followed by studies showing that daily administration of the GLP-1R antagonist exendin (9–39) increased food intake and body weight in rats (14). Subsequent experiments demonstrated that GLP-1 administration also produces conditioned taste aversion (6,7). These findings raised the possibility that the temporary reduction in food intake observed after ICV GLP-1 might reflect a role for GLP-1 signaling in the CNS response to visceral stress. Consistent with this hypothesis, GLP-1–positive neurons express c-fos after exposure of rats to lithium chloride or lipopolysaccharide (7,8), and the GLP-1 antagonist exendin (9–39) blocks the lithium chloride–mediated suppression of food intake (9,24).

Accordingly, the physiological importance of GLP-1 for the control of feeding and body weight gain versus mediation of interoceptive stress signaling remains somewhat controversial (22). Our findings that GLP-1R$^{-/-}$ mice are lean (12,15) and that the disruption of GLP-1R signaling in the *ob/ob* mouse has no additive impact on feeding behavior or weight gain clearly demonstrate that the GLP-1R is not an essential regulator of food intake and body weight in mice in vivo. Furthermore, despite the anatomical and functional evidence linking leptin and GLP-1 circuits in the CNS (26–28,57), GLP-1R signaling is not required for the acute response to leptin administration or the phenotypic manifestations of leptin deficiency in the mouse in vivo. Similarly, upregulation of insulin gene expression and the development of hyperinsulinemia and islet hyperplasia are not dependent on basal GLP-1R signaling. Whether these findings are attributable to the upregulation of related regulatory networks in the islets and CNS that compensate for the absence of GLP-1 will require further investigation.

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