Am J Physiol Endocrinol Metab 306: E748–E755, 2014.
First published January 28, 2014; doi:10.1152/ajpendo.00681.2013.

Partial ablation of leptin signaling in mouse pancreatic α-cells does not alter either glucose or lipid homeostasis

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Submitted 12 December 2013; accepted in final form 20 January 2014

Tudurí E, Denroche HC, Kara JA, Asadi A, Fox JK, Kieffer TJ. Partial ablation of leptin signaling in mouse pancreatic α-cells does not alter either glucose or lipid homeostasis. *Am J Physiol Endocrinol Metab* 306: E748–E755, 2014. First published January 28, 2014; doi:10.1152/ajpendo.00681.2013.—The role of glucagon in the pathological condition of diabetes is gaining interest, and it has been recently reported that its action is essential for hyperglycemia to occur. Glucagon levels, which are elevated in some diabetic models, are reduced following leptin therapy. Likewise, hyperglycemia is corrected in type 1 diabetic mice treated with leptin, although the mechanisms have not been fully determined. A direct inhibitory effect of leptin on mouse and human α-cells has been demonstrated at the levels of electrical activity, calcium signaling, and glucagon secretion. In the present study we employed the Cre-*loxP* strategy to generate *Lepr*$^{flox/flox}$ *Gcg-cre* mice, which specifically lack leptin receptors in glucagon-secreting α-cells, to determine whether leptin resistance in α-cells contributes to hyperglucagonemia, and also whether leptin action in α-cells is required to improve glycemia in type 1 diabetes with leptin therapy. Immunohistochemical analysis of pancreas sections revealed Cre-mediated recombination in ~43% of the α-cells. We observed that in vivo *Lepr*$^{flox/flox}$ *Gcg-cre* mice display normal glucose and lipid homeostasis. In addition, leptin administration in streptozotocin-induced diabetic *Lepr*$^{flox/flox}$ *Gcg-cre* mice restored euglycemia similarly to control mice. These findings suggest that loss of leptin receptor signaling in close to one-half of α-cells does not alter glucose metabolism in vivo, nor is it sufficient to prevent the therapeutic action of leptin in type 1 diabetes.

diabetes; leptin signaling; leptin therapy

THE INSULIN-COUNTERACTING hormone glucagon, secreted from pancreatic α-cells, plays a crucial role in regulating blood glucose levels. While insulin is secreted after meal ingestion and stimulates glucose uptake (11, 35), glucagon is secreted in conditions of fuel demand and promotes hepatic glucose production by activating glycogenolysis and gluconeogenesis (19, 44). Hence, the finely tuned secretion and action of these two antagonistic hormones maintain glycemia within physiological levels. Some humans with diabetes (31), as well as diabetic animal models, including alloxan-treated dogs (32), leptin-deficient *ob/ob* mice (17), leptin receptor-deficient *db/db* mice (26), nonobese diabetic NOD mice (47), and streptozotocin (STZ)-treated mice (14), display hyperglucagonemia, a condition that worsens hyperglycemia. Treatments including neutralizing antibodies against glucagon (6, 7, 41) or glucagon receptor blocking agents (12, 23) have been reported to improve glucose homeostasis in diabetic models (8). Moreover, glucagon action is required for diabetes to develop in insulin-deficient mice (27). Remarkably, glucagon receptor knockout (*GcgR*$^{-/-}$) mice displayed normoglycemia and glucose tolerance after receiving a double dose of STZ that destroyed the majority of their insulin-secreting β-cells (27). Thus, a better understanding of the mechanisms that regulate glucagon secretion from pancreatic α-cells may aid in the development of therapeutic strategies to control glucagon release and thereby lower blood glucose levels in individuals with diabetes.

An endogenous molecule that can inhibit glucagon release is the adipose-derived hormone leptin. Leptin is well-known for reducing food intake and increasing energy expenditure upon activation of its receptor in the hypothalamus (38). However, leptin receptors are expressed in additional tissues, including α- and β-cells of the endocrine pancreas (24, 42). Leptin directly acts on pancreatic β-cells to suppress insulin synthesis and release (25, 39, 40). In addition to its action on β-cells, leptin also directly inhibits glucagon secretion from α-cells (42). Moreover, leptin therapy has been shown to reduce glucagon levels in rodent models of type 1 diabetes, which may contribute to the improvements in glycemia (14, 47). In addition, leptin replacement has been reported to decrease circulating glucagon levels in *ob/ob* mice (13). Thus, leptin regulation of glucagon secretion through direct action on α-cells might contribute to leptin-mediated control of glucose homeostasis.

Despite the hyperleptinemia displayed by obese rodents and humans, leptin often fails to cease the progress of obesity (9, 18, 45). Such a failure of leptin to suppress appetite is considered evidence for leptin resistance in the hypothalamus (3, 34). We have previously shown that the specific disruption of leptin receptors in β-cells results in several features, including increased body weight and adiposity, hyperinsulinemia, impaired glucose-stimulated insulin secretion, and insulin resistance (10). These observations suggest that leptin resistance in β-cells may contribute to obesity-related diabetes. Likewise, impaired leptin signaling in glucagon-secreting α-cells may contribute to the development of hyperglucagonemia in this disease. To explore this hypothesis we generated mice that specifically lack leptin receptors in pancreatic α-cells, referred to as *Lepr*$^{flox/flox}$ *Gcg-cre* mice. We have analyzed the phenotype of this model at the level of both glucose and lipid metabolism. In addition, we induced diabetes in *Lepr*$^{flox/flox}$ *Gcg-cre* mice by administration of STZ and tracked fasting blood glucose levels after leptin treatment to determine whether leptin action on glucagon-secreting α-cells is involved

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in the improvement of glycemia observed during leptin treatment of type 1 diabetes.

MATERIALS AND METHODS

Ethics statement. All procedures with animals were approved by the University of British Columbia Animal Care Committee and carried out in accordance with the Canadian Council on Animal Care guidelines.

Animals. B6.Cg-Tg(Gcg-cre)1Herr/Mmnc (referred to as *Gcg-cre*) mouse embryos were purchased from the Mutant Mouse Regional Resource Center and implanted into C57BL/6 surrogate mice at the Centre for Disease Modeling (Vancouver, Canada). *Gcg-cre* mice were crossed with *Lepr*$^{flox/flox}$ mice (30) to generate *Lepr*$^{flox/+}$ and *Lepr*$^{flox/+}$ *Gcg-cre* mice, which were subsequently crossed to generate *Lepr*$^{flox/flox}$ littermate controls and *Lepr*$^{flox/flox}$ *Gcg-cre* mice for our studies. In addition, *Lepr*$^{flox/flox}$ *Gcg-cre* mice were crossed with homozygous reporter *mT/mG* (33) to finally generate *Lepr*$^{flox/flox}$ *Gcg-cre;mT/mG* for Cre recombination immunohistochemistry analysis. The primers employed for genotyping the Gcg-cre transgene are 5′-CAG ATG AGA AAT TTA TAT TGT CAG CG-3′ (forward) and 5′-TCC ATG GTG ATA CAA GGG AC-3′ (reverse), which give an amplicon of ~300 bp. In our studies we employed both males and females. All mice were housed with a 12:12-h light-dark cycle and had ad libitum access to chow diet (2918; Harlan Laboratories, Madison, WI) and water.

Islet isolation, dispersion, and cell culture. Pancreases were injected with Hanks' balanced salt solution (HBSS) containing 1,000 U/ml of type XI collagenase (Sigma-Aldrich, St. Louis, MO) by the duct before their removal and digested at 37°C for 12 min. Islets were then washed with iced-cold HBSS and handpicked under a microscope. One hour after recovery at 37°C and 5% CO_2, freshly isolated islets were dispersed into single cells by trypsin enzymatic digestion and cultured in RPMI containing 10% FBS, 100 IU/ml penicillin, 0.1 mg/ml streptomycin, and 1% Glutamax 100X.

PCR. To assess *Lepr*^{flox} recombination, tissues from 6-wk-old *Lepr*$^{flox/flox}$ and *Lepr*$^{flox/flox}$ *Gcg-cre* female mice were harvested and homogenized in salt homogenization buffer (0.4 M NaCl, 10 mM Tris·HCl, pH 8.0, 2 mM EDTA, 1% SDS, and 0.25 mg/ml proteinase K), and DNA was extracted as previously described (1). For PCR reactions, 2 μl of genomic DNA were added to a 20-μl reaction containing 1× Accuprime Buffer II (Invitrogen, Carlsbad, CA), 1 U/μl Accuprime Taq polymerase (Invitrogen), and 1 μM of each primer Lepr105 (reverse): 5′-ACA GGC TTG AGA ACA TGA ACA C-3′ and 15369 (forward): 5′-TGG CTT TTA AGC TCT GCA GTC-3′ (Integrated DNA Technologies).

Immunohistochemical analysis. Pancreata were harvested and fixed in 4% paraformaldehyde overnight. After fixation, pancreases were washed in 70% ethanol and embedded in paraffin for subsequent sectioning. Primary antibodies against glucagon (1:1,000; Sigma-Aldrich), enhanced green fluorescent protein [EGFP, 1:500 (Clontech) or 1:500 (Life Technology)], and insulin (1:200; Cell Signaling) were used for an overnight incubation at 4°C. For secondary antibodies, goat anti-mouse Alexa 594 (1:1,000; Life Technology), goat anti-mouse Alexa 488 (1:1,000; Life Technology), goat anti-rabbit Alexa 488 (1:1,000; Life Technology), or goat anti-rabbit 594 (1:1,000; Life Technology) were added and incubated for 1 h at room temperature. Whole pancreas sections was scanned using the ImageXpress Micro Imaging System, and images were stitched together and analyzed using MetaXpress Software (Molecular Devices, Sunnyvale, CA).

Plasma analyte measurements. Blood glucose, body weight, and plasma analytes were typically measured after 4 h fasting unless specified otherwise. Random-fed samples were collected at 11:00 P.M., 5 h into the dark cycle. Blood glucose was monitored from the saphenous vein via a One Touch Ultra Glucometer (Life Scan, Burnaby, Canada). Plasma glucagon levels were measured by a Glucagon RIA (Millipore), plasma insulin levels were determined by an Insulin Mouse Ultrasensitive enzyme-linked immunosorbent assay (ELISA) (ALPCO Diagnostics, Salem, NH), and plasma leptin levels were measured using a mouse leptin ELISA (Crystal Chem, Downers Grove, IL). Free fatty acids (HR Series NEFA HR Kit; Wako Chemical), cholesterol (Cholesterol E kit; Wako Chemical, Richmond, VA), triglycerides (Serum Triglyceride kit; Sigma-Aldrich), and β-hydroxybutyrate (β-Hydroxybutyrate LiquiColor Test; Stanbio) were also measured from plasma.

Oral glucose tolerance test and glucose-stimulated insulin secretion. Mice were fasted for 4 h and then given an oral glucose gavage (2 g/kg body wt) of a 30% glucose solution. Blood was sampled from the saphenous vein and measured for glucose or insulin before (0 min) and at different time points. Blood glucose levels and plasma insulin levels were measured as described above.

STZ treatment and leptin therapy. Mice received a single dose of 160 mg/kg body wt of STZ (Sigma-Aldrich) prepared in acetate buffer, pH 4.5, by intraperitoneal administration on *day 0*. Seven days later, when diabetes had been confirmed (fasting blood glucose ≥16 mmol/l on two consecutive days), STZ-leptin mice received 20 μg/day mouse recombinant leptin (PeproTech, Rocky Hill, NJ) dissolved in H_2O via Alzet 7-day pumps (DURECT) implanted subcutaneously. STZ-vehicle control mice received pumps loaded with H_2O only.

Statistical analysis. Data are expressed as means ± SE. Statistical analysis was performed using Student's *t*-test or two-way repeated-measures ANOVA (GraphPad Prism; GraphPad Software, La Jolla, CA). *P* values ≤0.05 were considered significant.

RESULTS

Generation of mice with specific disruption of leptin receptors in pancreatic α-cells. To generate mice with α-cell-specific disruption of leptin signaling we employed the Cre-*loxP* strategy (Fig. 1*A*). *Lepr*$^{flox/flox}$ mice carry *loxP* sites flanking the exon 17 of the leptin receptor, which encodes the Janus kinase (JAK) binding site of the long isoform of Lepr-b (30). Excision of the exon 17 by Cre-mediated recombination at the *loxP* sites results in an allele with an altered 3′-terminus (*Lepr*$^{\Delta 17}$) (30) no longer encoding tyrosine-985 and tyrosine-1138 [sites of JAK-mediated tyrosine phosphorylation (4)]. Therefore, mice homozygous for the *Lepr*$^{\Delta 17}$ allele lack leptin-stimulated JAK/signal transducer and activator of transcription (STAT) signaling. *Lepr*$^{flox/flox}$ mice were crossed with *Gcg-cre* mice, which express Cre recombinase under the rat proglucagon promoter, conferring α-cell-specific expression of Cre (21), to generate both *Lepr*$^{flox/flox}$ *Gcg-cre* and *Lepr*$^{flox/flox}$ control mice.

We next analyzed Cre recombination in the α-cells of *Lepr*$^{flox/flox}$ *Gcg-cre* mice by means of PCR and immunostaining. First, genomic DNA was extracted from several tissues of *Lepr*$^{flox/flox}$ *Gcg-cre* mice and their control *Lepr*$^{flox/flox}$ littermates. With the primers used we were able to detect the recombined amplicon of the leptin receptor gene (411 bp) only in islet samples from *Lepr*$^{flox/flox}$ *Gcg-cre* mice, not in brain or gut samples where there is also proglucagon promoter activity, and the nonrecombined wild-type amplicon (823 bp) was detected in all the samples tested in *Lepr*$^{flox/flox}$ and *Lepr*$^{flox/flox}$ *Gcg-cre* mice (Fig. 1*B*). The nonrecombined amplicon present in *Lepr*$^{flox/flox}$ *Gcg-cre* islets is likely in part due to the presence of β-cells, which are the most prominent cell population within the islets. In addition to assessing leptin receptor gene recombination by PCR, we also crossed *Lepr*$^{flox/flox}$ *Gcg-cre* mice with reporter *mT/mG* mice. In this double-fluorescent reporter model, a chicken β-actin core promoter with a CMV enhancer (pCA) drives a *loxP*-flanked coding

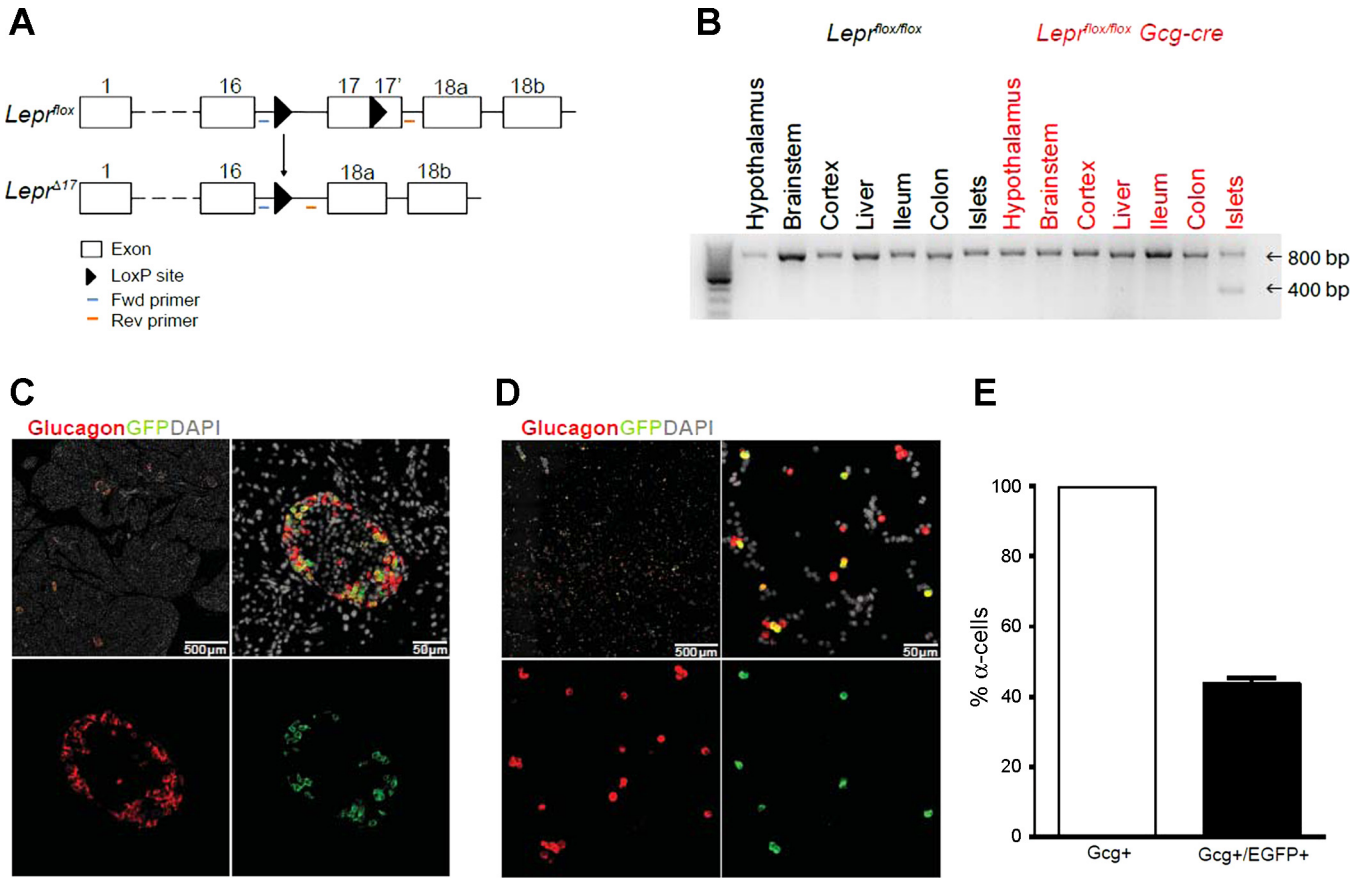

Fig. 1. Disruption of the long isoform of the leptin receptor in α-cells. *A*: schematic diagram of *Lepr*^{flox} and *Lepr*$^{\Delta 17}$ alleles. *B*: genomic DNA was extracted from several tissues and used as template for PCR. The primers indicated in *A* generate a 411-bp amplicon for the *Lepr*$^{\Delta 17}$ and a 823-bp amplicon for the *Lepr*^{flox}. Arrows on the *right* show the migration of molecular weight. *Lepr*$^{flox/flox}$ *Gcg-cre;mT/mG* pancreas section (*C*) and *Lepr*$^{flox/flox}$ *Gcg-cre;mT/mG* islet dispersed cells (*D*) stained for glucagon (red), enhanced green fluorescent protein (EGFP, green), and DAPI (gray). Scale bar = 500 (low magnification) and 50 (high magnification) μm. *E*: quantification of the percentage of *Lepr*$^{flox/flox}$ *Gcg-cre;mT/mG* α-cells that are EGFP positive as a result of Cre recombination. A total of 1,482 α-cells from 3 animals were analyzed.

sequence of membrane-targeted tandem dimer Tomato (mT), which results in mT expression. Upon Cre recombination, the mT sequence is excised allowing the pCA promoter to drive expression of membrane-targeted EGFP (mG) (33). Hence, by analyzing the pancreata of *Lepr*$^{flox/flox}$ *Gcg-cre;mT/mG* mice we were able to determine whether Cre expression is confined to the α-cell population and the extent of Cre recombination within the α-cells. Immunostaining was carried out in pancreas sections (Fig. 1*C*) and also in dispersed islet cells (Fig. 1*D*), for accurate quantification. The immunohistochemical analysis revealed that EGFP immunoreactivity was detectable in the membrane of ~43% of the glucagon-positive cells (Fig. 1*E*), which demonstrated that the *Gcg-cre* mice used in this study permitted only a partial recombination. EGFP immunoreactivity was otherwise only detected in very rare non-α-cells.

Glucose and lipid homeostasis is not altered in Lepr$^{flox/flox}$ *Gcg-cre mice.* Glucagon plays an essential role in maintaining blood glucose levels within a physiological range. Leptin exerts a direct inhibitory effect on α-cells at the levels of electrical activity, calcium signaling, and glucagon secretion in mouse and human isolated islets (42). To assess whether partial ablation of leptin signaling in glucagon-secreting α-cells could lead to abnormalities in glucose homeostasis we performed a series of in vivo studies in *Lepr*$^{flox/flox}$ *Gcg-cre* mice and in control *Lepr*$^{flox/flox}$ littermates. First, we monitored 4 h fasting body weight and blood glucose levels in age-matched female and male *Lepr*$^{flox/flox}$ *Gcg-cre* and *Lepr*$^{flox/flox}$ mice and did not observe differences between genotypes (Fig. 2*A*). Similarly, no differences between genotypes were detected in glycemia (Fig. 2*B*) or insulin (Fig. 2*C*) secretion when the same animals underwent an oral glucose tolerance test.

Aside from its well-known effects on glucose metabolism, glucagon plays an important role in regulating lipid metabolism, since it inhibits triglyceride synthesis and secretion, and stimulates fatty acid oxidation in mouse hepatocytes (28). However, there are some discrepancies in studies carried out in humans (20). In the present study we also measured plasma cholesterol, triglycerides, and free fatty acids and did not find differences between *Lepr*$^{flox/flox}$ *Gcg-cre* mice and their control *Lepr*$^{flox/flox}$ littermates (Fig. 3).

Given that leptin stimulation decreases glucagon secretion in mouse islets (42), plasma samples were analyzed for glucagon in the overnight-fasted and random-fed states (Fig. 4*A*) in both *Lepr*$^{flox/flox}$ *Gcg-cre* and *Lepr*$^{flox/flox}$ mice. No differences in circulating glucagon levels were detected between genotypes during either state. In addition, *Lepr*$^{flox/flox}$ *Gcg-cre* mice had unaltered fasted plasma insulin levels (Fig. 4*B*). Analysis of fed plasma leptin levels revealed a trend toward increased leptin

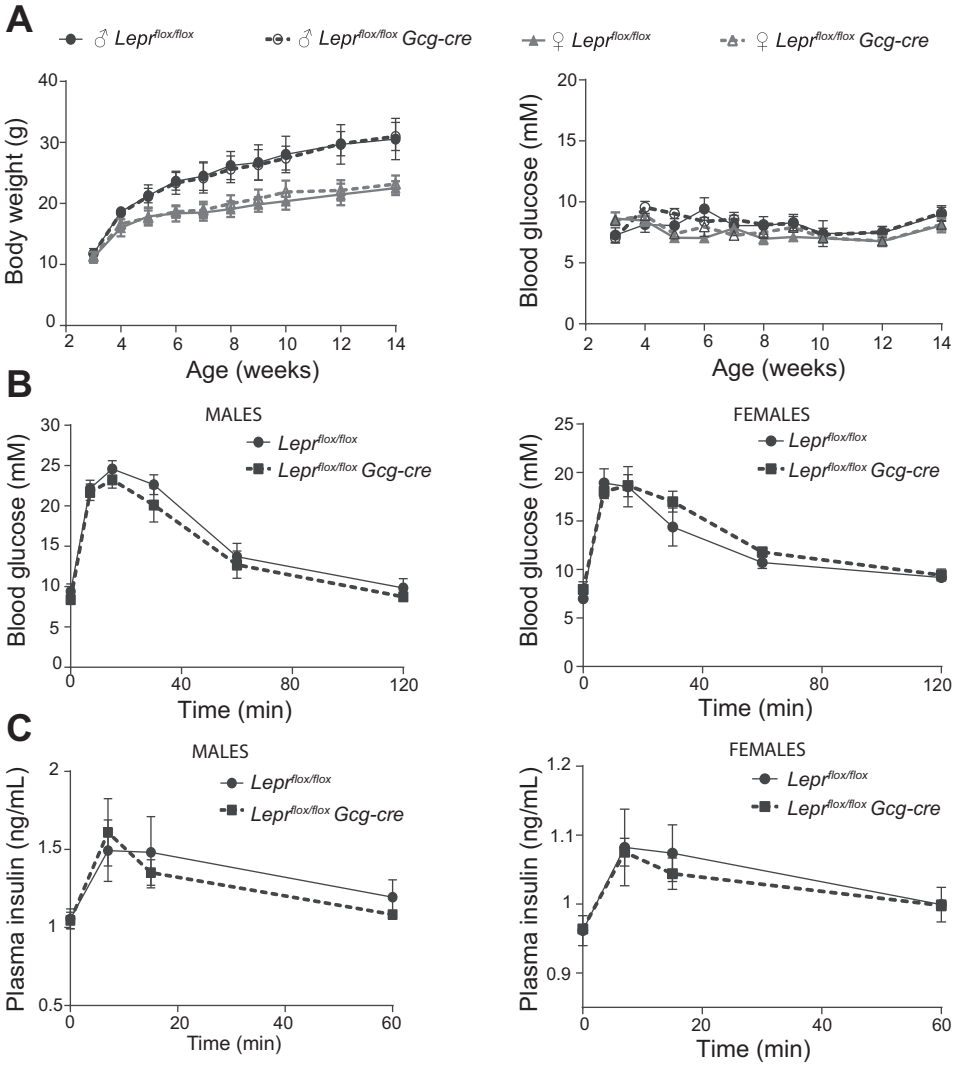

Fig. 2. Attenuation of leptin signaling in α-cells does not alter glucose metabolism. *A*: body weight and fasting blood glucose in males (circles) and females (triangles) over the duration of the study (n = 5–6 mice/group). Oral glucose tolerance test (n = 5–6 mice/group) (*B*) and glucose-stimulated insulin secretion (n = 4 for males and n = 3 for females) (*C*) were performed in 6-wk-old males and females in response to a glucose load of 2 g/kg body wt after a 4-h fast. Data are expressed as averages ± SE and were analyzed using two-way repeated-measures ANOVA.

concentrations in *Lepr$^{flox/flox}$ Gcg-cre* male mice compared with control males, although this did not reach statistical significance (Fig. 4*C*). It is plausible that elevated plasma leptin levels along with the remaining population of α-cells with intact leptin signaling maintain normal glucose homeostasis in *Lepr$^{flox/flox}$ Gcg-cre mice*.

STZ-induced diabetic Lepr$^{flox/flox}$ Gcg-cre mice have a normal response to leptin therapy. Leptin therapy has been shown to reverse hyperglycemia in STZ-induced diabetic mice (14, 15) although the underlying mechanism remains unknown. In addition, leptin treatment in STZ-diabetic mice notably decreases circulating glucagon levels (14), which may mediate glucose lowering. To determine whether the beneficial effect of leptin on glucose metabolism in STZ-treated mice occurs through a direct inhibitory action of leptin on the α-cells we administered a single high dose of STZ in *Lepr$^{flox/flox}$ Gcg-cre* and *Lepr$^{flox/flox}$* mice. Following development of hyperglycemia, both *Lepr$^{flox/flox}$ Gcg-cre* and *Lepr$^{flox/flox}$* control mice received 20 μg/day leptin, and an additional group of *Lepr$^{flox/flox}$* control mice received vehicle (diabetic control group), via subcutaneous miniosmotic pumps. As expected from previous observations (14, 15), leptin ameliorated hyperglycemia in the leptin-treated control *Lepr$^{flox/flox}$* mice within a few days, whereas vehicle-treated *Lepr$^{flox/flox}$* mice remained hyperglycemic for the study duration (Fig. 5*A*). Leptin therapy also ameliorated hyperglycemia in *Lepr$^{flox/flox}$ Gcg-cre* mice. Interestingly, however, there was a trend toward higher blood glucose levels under leptin treatment in the *Lepr$^{flox/flox}$ Gcg-cre* group compared with leptin-treated *Lepr$^{flox/flox}$* mice, although this did not reach statistical significance. We also analyzed plasma glucagon levels in *Lepr$^{flox/flox}$* and *Lepr$^{flox/flox}$ Gcg-cre* mice before and 7 days after the implantation of leptin/vehicle pumps (Fig. 5*B*). Leptin similarly reduced plasma glucagon levels in both groups, whereas glucagon levels remained elevated in diabetic vehicle-treated *Lepr$^{flox/flox}$* mice. Likewise, leptin treatment also reduced the plasma levels of the ketone body β-hydroxybutyrate in a similar manner in both *Lepr$^{flox/flox}$* and *Lepr$^{flox/flox}$ Gcg-cre* mice (Fig. 5*C*).

DISCUSSION

In the present study we aimed to unravel the in vivo metabolic consequences of disrupting leptin signaling in glucagon-secreting α-cells and to determine whether leptin action

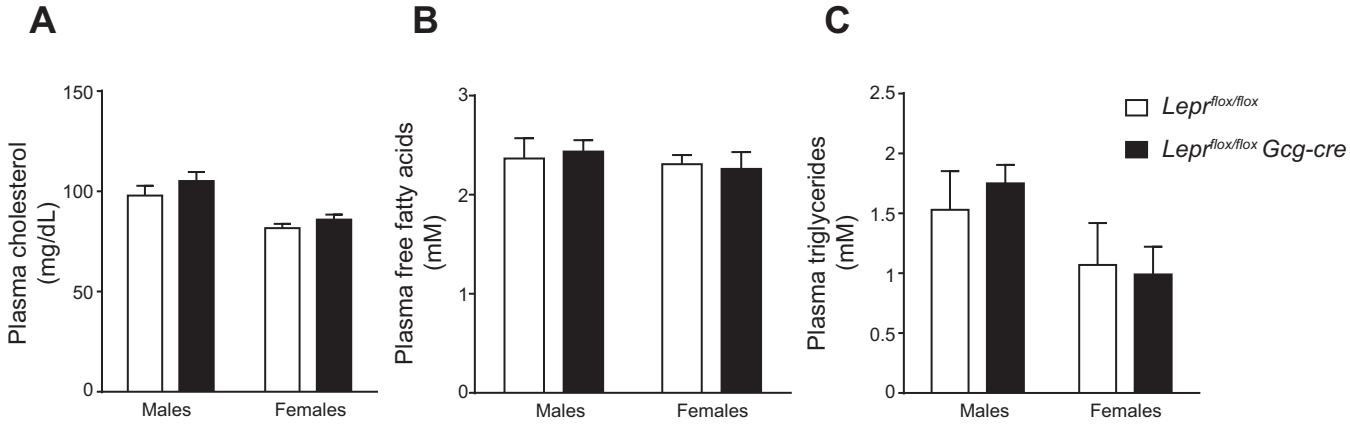

Fig. 3. Lipid metabolism remains unaltered after partial ablation of leptin receptors in α-cells. Overnight fasting plasma cholesterol (*A*), free fatty acids (*B*), and triglycerides (*C*) were measured in 14-wk-old *Lepr*$^{flox/flox}$ *Gcg-cre* males and females and their control *Lepr*$^{flox/flox}$ littermates. Data are expressed as averages ± SE and were analyzed using Student's *t*-test; n = 5–6 mice in each group.

in this cell population contributes to the metabolic improvement observed in type 1 diabetic models following leptin treatment. For this purpose, we employed the Cre-*loxP* strategy and crossed *Lepr*$^{flox/flox}$ mice, which have *loxP* sites flanking the exon 17 of the leptin receptor gene (30), with *Gcg-cre* mice, which express Cre recombinase under the glucagon promoter (21). Thus, the resulting *Lepr*$^{flox/flox}$ *Gcg-cre* mice specifically lack the long isoform of the leptin receptor in α-cells. Whereas short isoforms of the leptin receptor are also expressed in glucagon-secreting α-cells (42), these receptors are believed to have little effect on signaling, whereas they are involved in processes, including leptin internalization, clearance, or transport through the blood-brain barrier (5, 43). Similarly to other studies, where PCR is employed to detect recombination in specific tissues, we showed recombination of the leptin receptor in islet samples from *Lepr*$^{flox/flox}$ *Gcg-cre* mice. In contrast, we did not observe recombination of the leptin receptor in brain stem, hypothalamus, or gut samples, tissues known to possess proglucagon promoter activity (16, 22). In addition, to determine the extent of Cre recombination in the α-cell population, we crossed our model with the double-fluorescent reporter strain *mT/mG*, such that Cre recombination could be analyzed by detection of EGFP immunoreactivity in cell membranes. Unexpectedly, the immunohistochemical analysis of *Lepr*$^{flox/flox}$ *Gcg-cre;mT/mG* islet cells showed recombination in only ~43% of the α-cells. Although some studies that employed the same *Gcg-cre* mice reported recombination in ~70% of the glucagon-secreting cells (2, 36), others reported just 13% of Cre-mediated recombination (46). The reason for obtaining different degrees of Cre recombination is not known but may be due to strain differences.

Like leptin action on β-cells, evidence suggests that leptin may inhibit glucagon secretion by direct action on α-cells. Mouse models that lack leptin or leptin signaling, *ob/ob* and *db/db* mice, respectively, exhibit high circulating glucagon levels (17, 26), which, in the *ob/ob* model, can be reduced by leptin administration (13). Pancreatic α-cells express leptin receptors (42), and we and others have seen a decrease in glucagon secretion (14, 42, 47) as well as a reduction in glucagon expression (29) as a result of leptin treatment. Together, these results suggest that direct leptin action on glucagon suppression plays an important role in regulating glucose homeostasis. Hence, despite having a partial conditional knockout, we studied whether *Lepr*$^{flox/flox}$ *Gcg-cre* mice might display altered glucose metabolism. We found that glucagon levels in *Lepr*$^{flox/flox}$ *Gcg-cre* mice did not differ from those in

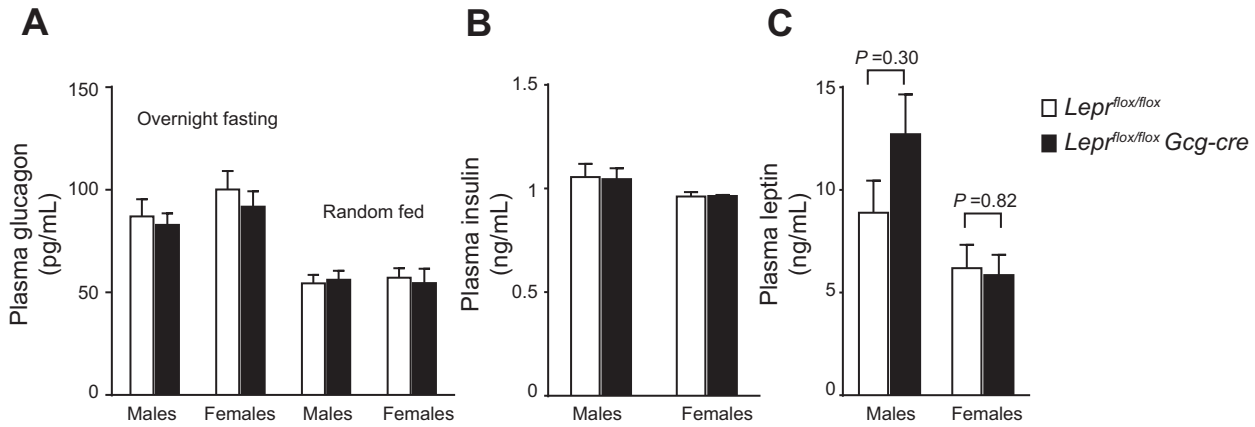

Fig. 4. Plasma glucagon, insulin, and leptin levels do not change in *Lepr*$^{flox/flox}$ *Gcg-cre* mice. Overnight and random-fed plasma glucagon (*A*), 4 h fasting plasma insulin (*B*), and fed plasma leptin (*C*) were measured in 14-wk-old *Lepr*$^{flox/flox}$ *Gcg-cre* males and females and their control *Lepr*$^{flox/flox}$ littermates. Data are expressed as averages ± SE and were analyzed using Student's *t*-test; n = 5–6 mice in each group.

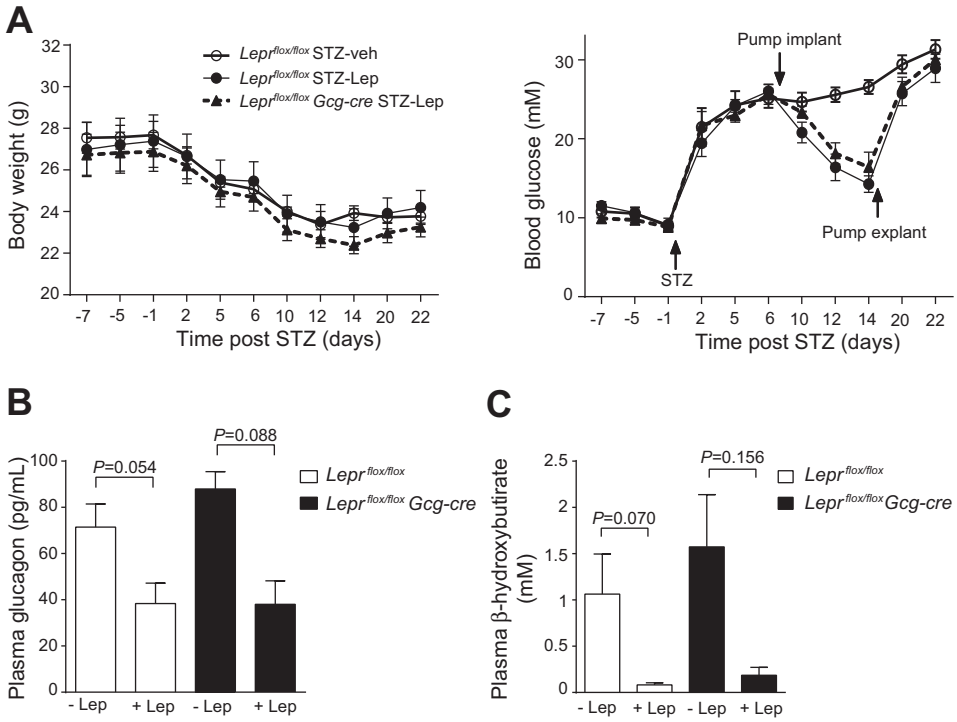

Fig. 5. Leptin therapy in streptozotocin (STZ)-treated *Lepr*$^{flox/flox}$ *Gcg-cre* mice ameliorates hyperglycemia. *A*: 4 h fasting body weight and blood glucose in *Lepr*$^{flox/flox}$ mice (n = 4, solid line) and *Lepr*$^{flox/flox}$ *Gcg-cre* mice (broken line) before and after treatment with leptin (20 μg/day, n = 6) or H_2O (n = 6) via subcutaneous osmotic pumps. Four hour fasting plasma glucagon (*B*) and β-hydroxybutyrate (*C*) were measured before (*day 6*) and after (*day 14*) leptin therapy (n = 6 and n = 5 *Lepr*$^{flox/flox}$ and *Lepr*$^{flox/flox}$ *Gcg-cre*, respectively, before leptin treatment, and n = 5 and n = 3 *Lepr*$^{flox/flox}$ and *Lepr*$^{flox/flox}$ *Gcg-cre*, respectively, after leptin treatment). Data are expressed as averages ± SE and were analyzed using two-way repeated-measures ANOVA or Student's *t*-test.

the control *Lepr*$^{flox/flox}$ group, and we believe that is most probably due to the still abundant number of α-cells that express functional leptin receptors in this model. Consequently, we did not observe alterations in fasting blood glucose levels or glucose tolerance. Likewise, we did not observe differences in other plasma metabolites, including insulin, leptin, cholesterol, FFA, and triglycerides.

Leptin therapy ameliorates hyperglycemia and hyperglucagonemia in models of type 1 diabetes (14, 47), yet the mechanism by which this phenomenon occurs remains unknown. Although insulin has an inhibitory action on glucagon secretion (37), most likely insulin-secreting β-cells are not the target tissue by which leptin exerts its favorable effects in type 1 diabetic rodents. First, leptin is known to inhibit insulin release from β-cells (25, 39, 40), and second, in models of type 1 diabetes, the majority of β-cells are destroyed. The liver was also considered a key candidate in mediating the antidiabetic effects of leptin; however, we already ruled out this tissue as a direct mediator of the therapeutic actions of leptin (14). The fact that hyperleptinemia leads to a reduction in the circulating glucagon levels (14, 47), which in turn might reduce hepatic glucose production, points to the α-cells as key candidates for leptin to mediate beneficial effects in the context of diabetes. We induced diabetes in *Lepr*$^{flox/flox}$ *Gcg-cre* mice and studied the effects of leptin treatment in this model that lacks leptin receptor signaling in ~43% of α-cells. Following treatment with leptin at a dose of 20 μg/day via miniosmotic pumps, both STZ-treated *Lepr*$^{flox/flox}$ *Gcg-cre* and control *Lepr*$^{flox/flox}$ mice displayed reduced glucagon levels and improved glycemia. The trend toward lower glucose levels in the *Lepr*$^{flox/flox}$ control group could reflect the partial ablation of leptin receptors in the *Lepr*$^{flox/flox}$ *Gcg-cre* α-cells. Therefore, although the model here employed does not permit clear conclusions, it remains possible that direct leptin signaling in α-cells contributes to leptin-mediated glucose lowering in type 1 diabetes. Alternatively, the suppressive effects of leptin therapy on hyperglucagonemia in diabetes may be mediated through indirect action on α-cells, for example, through the brain.

Together these results indicate that deletion of leptin receptors in approximately one-half of the α-cell population is not sufficient to determine whether lack of leptin signaling in glucagon-secreting α-cells may lead to hyperglucagonemia and possible alterations in glucose and lipid metabolism. Similarly, the model employed in this study does not allow for determining whether direct leptin action in the α-cells may mediate beneficial effects in the context of type 1 diabetes. Given the major hurdle in achieving Cre recombination in the α-cells of *Lepr*$^{flox/flox}$ *Gcg-cre* mice, we highlight the importance of developing more efficient *Gcg-cre* driver models and emphasize that it is critical to thoroughly analyze the efficiency of Cre recombination in the target tissue when the Cre-*loxP* strategy is employed.

ACKNOWLEDGMENTS

We thank Streamson Chua of Columbia University for providing *Lepr*$^{flox/flox}$ mice and Robert K. Baker and Shannon O'Dwyer from the University of British Columbia for technical assistance.

GRANTS

This work was supported by the Canadian Institutes of Health Research and Canadian Diabetes Association (CDA). T. J. Kieffer is grateful for a senior scholarship from the Michael Smith Foundation for Health Research (MSFHR). E. Tudurí was supported by MSFHR and CDA postdoctoral fellowships. H. C. Denroche received a studentship from the National Science and Engineering Research Council of Canada.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: E.T., H.C.D., and T.J.K. conception and design of research; E.T., H.C.D., J.A.K., and J.K.F. performed experiments; E.T., H.C.D., and A.A. analyzed data; E.T., H.C.D., and T.J.K. interpreted results of experiments; E.T. prepared figures; E.T. drafted manuscript; E.T., H.C.D., and T.J.K. edited and revised manuscript; E.T., H.C.D., J.A.K., A.A., J.K.F., and T.J.K. approved final version of manuscript.

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