Chronic Administration of the Glucagon-Like Peptide-1 Analog, Liraglutide, Delays the Onset of Diabetes and Lowers Triglycerides in UCD-T2DM Rats

Bethany P. Cummings,[1,2] Kimber L. Stanhope,[1,2] James L. Graham,[1,2] Denis G. Baskin,[3] Steven C. Griffen,[4] Cecilia Nilsson,[5] Anette Sams,[6] Lotte B. Knudsen,[5] Kirsten Raun,[5] and Peter J. Havel[1,2]

OBJECTIVE—The efficacy of liraglutide, a human glucagon-like peptide-1 (GLP-1) analog, to prevent or delay diabetes in UCD-T2DM rats, a model of polygenic obese type 2 diabetes, was investigated.

RESEARCH DESIGN AND METHODS—At 2 months of age, male rats were divided into three groups: control, food-restricted, and liraglutide. Animals received liraglutide (0.2 mg/kg s.c.) or vehicle injections twice daily. Restricted rats were food restricted to equalize body weights to liraglutide-treated rats. Half of the animals were followed until diabetes onset, whereas the other half of the animals were killed at 6.5 months of age for tissue collection.

RESULTS—Before diabetes onset energy intake, body weight, adiposity, and liver triglyceride content were higher in control animals compared with restricted and liraglutide-treated rats. Energy-restricted animals had lower food intake than liraglutide-treated animals to maintain the same body weights, suggesting that liraglutide increases energy expenditure. Liraglutide treatment delayed diabetes onset by 4.1 ± 0.8 months compared with control ($P < 0.0001$) and by 1.3 ± 0.8 months compared with restricted animals ($P < 0.05$). Up to 6 months of age, energy restriction and liraglutide treatment lowered fasting plasma glucose and A1C concentrations compared with control animals. In contrast, liraglutide-treated animals exhibited lower fasting plasma insulin, glucagon, and triglycerides compared with both control and restricted animals. Furthermore, energy-restricted and liraglutide-treated animals exhibited more normal islet morphology.

CONCLUSIONS—Liraglutide treatment delays the development of diabetes in UCD-T2DM rats by reducing energy intake and body weight, and by improving insulin sensitivity, improving lipid profiles, and maintaining islet morphology. ***Diabetes*** **59:2653–2661, 2010**

From the [1]Department of Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, Davis, California; the [2]Department of Nutrition, University of California, Davis, Davis, California; the [3]Research and Development Service, Department of Veterans Affairs Puget Sound Health Care System, Seattle, Washington, and the Department of Medicine, Division of Metabolism, Endocrinology, and Nutrition, University of Washington, Seattle, Washington; the [4]Department of Internal Medicine, University of California, Davis, Sacramento, California; [5]Biology and Pharmacology Management, Novo Nordisk, Maaloev, Denmark; and the [6]Diabetes Inflammation Department, Novo Nordisk, Gentofte, Denmark.

Corresponding author: Peter J. Havel, pjhavel@ucdavis.edu.

Received 22 October 2009 and accepted 30 June 2010. Published ahead of print at http://diabetes.diabetesjournals.org on 9 July 2010. DOI: 10.2337/db09-1564.

Targeting of glucagon-like peptide-1 (GLP-1) for the pharmaceutical treatment of type 2 diabetes has shown much promise, as demonstrated by the clinical success of GLP-1 agonists and dipeptidyl peptidase IV (DPP-IV) inhibitors (1,2). As an incretin hormone, GLP-1 potentiates glucose-induced insulin secretion, avoiding hypoglycemia observed with other pharmaceutical activators of insulin secretion such as sulfonylureas (3,4). GLP-1 has also been shown to reduce excess glucagon secretion, contributing to a reduction in hyperglycemia (1), and has been suggested to reduce inflammation (5). GLP-1 signaling increases satiety (6,7) and slows gastric motility and secretion, further contributing to a reduction in food intake (8). Furthermore, GLP-1 has been shown to increase β-cell differentiation, proliferation, and insulin synthesis and decrease β-cell apoptosis (9–11). Finally, administration of GLP-1 can improve insulin sensitivity by promoting peripheral glucose uptake and decreasing hepatic gluconeogenesis, independent of changes of pancreatic hormone secretion (12–16).

Early attempts to harness these antidiabetic effects through the production of GLP-1 analogs were complicated by the short half-life of endogenous GLP-1 (<2 min) because of rapid degradation by the ubiquitously expressed protease, DPP-IV (17). Thus, one method currently being pursued for the therapeutic targeting of GLP-1 is production of GLP-1 analogs that are resistant to degradation by DPP-IV. Liraglutide is a GLP-1 analog with an additional 16-carbon fatty acid and a small amino acid–based spacer that confers reversible binding of the agonist to albumin and increases resistance to DPP-IV activity, providing liraglutide with a half-life of approximately 13 h (18,19). These properties allow once-daily subcutaneous administration of liraglutide for the treatment of type 2 diabetes.

The efficacy of liraglutide for the treatment of type 2 diabetes has been demonstrated in a number of clinical studies, with patients showing significant decreases in body weight, glucose, A1C, and blood pressure (20–25). However, preclinical studies of the long-term use of chronic liraglutide administration for the prevention of type 2 diabetes have not been conducted. With the increasing prevalence of type 2 diabetes, preventive measures are urgently needed. Thus, this study investigated the metabolic effects of chronic liraglutide administration and the potential of liraglutide to prevent or delay the development of type 2 diabetes in pre-diabetic UCD-T2DM rats. The UCD-T2DM rat model develops polygenic adult-onset obesity and insulin resistance, without a monogenic deficit in leptin signaling, fol-

TABLE 1
Age of onset and incidence

	Control	Restricted	Liraglutide-treated
Animals treated until 6.5 months of age			
Age of onset (days)	127 ± 6	160 ± 6**	163 ± 12*
Incidence (%)	81	38	16
Diabetes-free days	79 ± 7	122 ± 4***	130 ± 3***
n	32	32	32
Animals treated until onset			
Age of onset (days)	156 ± 18	240 ± 22*	280 ± 25**
Incidence (%)	100	100	100
Diabetes-free days†	96 ± 18	179 ± 21*	207 ± 19***
n	16	16	16

Data are mean ± SEM unless otherwise indicated. $P < 0.01$ by one-way ANOVA; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with control by Bonferroni post-test. †Diabetes-free days starting at treatment initiation.

lowed by inadequate β-cell compensation and diabetes in both male and female animals (26). Thus, the UCD-T2DM rat more closely models the pathogenesis of type 2 diabetes in humans than other currently available models. Furthermore, UCD-T2DM rats demonstrate a later age of diabetes onset than other rodent models of type 2 diabetes, such as the ZDF rat, making them highly suitable for diabetes prevention studies (26,27).

RESEARCH DESIGN AND METHODS

Male UCD-T2DM rats from our colony were individually housed in hanging wire cages in the Department of Nutrition animal facility at the University of California, Davis, and maintained on a 1410 h light-dark cycle. Starting when rats were 2 months of age, male siblings were divided into three groups: control, food restricted, and liraglutide. Animals with a 2-month body weight >375 g were chosen for the study. Previous data from our laboratory have shown that animals weighing >375 g at 2 months of age have an average age of diabetes onset of 113 ± 5 days and a diabetes incidence rate of 98%, whereas animals weighing <375 g at 2 months of age have an average age of onset of 219 ± 10 days and a diabetes incidence rate of 89% (26). Body weights at the time of entry into the study were 398 ± 3, 399 ± 3, and 399 ± 3 g in control, food-restricted, and liraglutide groups, respectively ($n = 32$ per group). All animals received ground chow (no. 5012, Ralston Purina, Belmont, CA) in spill-resistant jars for accurate food intake measurements. Restricted animals were food restricted to 9% less energy per kg of body weight than was being consumed by the liraglutide-treated animal in the same cohort to equalize body weights between these two groups. Food intake and body weight were measured three times weekly. Control and food-restricted animals received subcutaneous Dulbecco's PBS injections (1 ml/kg body weight) twice daily (0800–1000 h and 1800–2000 h), and liraglutide animals received subcutaneous liraglutide injections (0.2 mg/kg body weight) twice daily. Animals received injections after the fasting blood draws and did not receive an injection on the day of euthanasia. Nonfasting blood glucose was monitored every week with a glucose meter (LifeScan One-Touch Ultra, Milpitas, CA) at 1300–1400 h using a lancet to collect a drop of blood from the tail. Diabetes onset was defined as a nonfasted blood glucose value above 11.1 mmol/l (200 mg/dl) on 2 consecutive weeks. Half of the animals in each group ($n = 16$) were killed for tissue collection at 6.5 months of age (short-term study). The remaining half ($n = 16$) continued treatment until the time of diabetes onset (long-term study). The experimental protocols were approved by the UC Davis Institutional Animal Care and Use Committee.

Monthly hormone and metabolic profiles. Blood samples were collected from rats in both the short-term and long-term treatment groups once monthly after an overnight (13-h) fast and placed into EDTA-treated tubes. The plasma was separated by centrifugation and assayed for glucose, insulin, glucagon, triglycerides (TG), cholesterol, and adiponectin. Plasma monocyte chemotactic protein-1 (MCP-1) was measured in plasma samples from 6-month-old rats. Plasma leptin and intercellular adhesion molecule (I-CAM) were measured in the final blood sample taken from 6.5-month-old rats from the short-term group. Twenty-four–hour urine samples were collected from 6-month-old rats in the short-term groups in sodium azide–treated flasks and assayed for glucose and albumin.

Plasma glucose, cholesterol, and urine glucose were measured using enzymatic colorimetric assays for glucose and cholesterol (Thermo DMA Louisville, CO). Insulin, leptin, glucagon, and adiponectin were measured with rodent/rat-specific radioimmunoassays (Millipore, St. Charles, MO). TG was measured with an enzymatic colorimetric assay (L-type TG H kit, Wako Chemicals, Richmond, VA). Plasma I-CAM was measured when the rats were 6 months of age with an ELISA (R&D, Minneapolis, MN). Plasma monocyte chemotactic protein-1 (MCP-1) levels were compared in 5× diluted plasma from 6-month-old rats using the Millipore Milliplex assay (RAT cytokine cat. no. RCYTO-80K). Urinary albumin excretion was measured using the Albumin Blue 580 fluorescence assay method described previously (28). A1C was measured using the direct enzymatic HbA1c kit (Diazyme, Poway, CA).

Body composition and liver and muscle triglyceride content. After 4.5 months of treatment (6.5 months of age), animals in the short-term groups were killed with an overdose of pentobarbital (200 mg/kg i.p.) after an overnight fast. Subcutaneous, mesenteric, retroperitoneal, and epididymal adipose depots and liver, heart, gastrocnemius muscle, and kidney were dissected, weighed, and flash-frozen in liquid nitrogen and stored at −80°C. Liver and skeletal muscle TG content were measured using the Folch method (29) for lipid extraction followed by spectrophotometric measurement of TG content (Thermo Electron, Louisville, CO). Rats were eviscerated and fat depots were returned to the carcass for body composition analysis, conducted as described by Bell and Stern (30). Briefly, carcass weight was determined, and the carcass was freeze-dried to determine total water weight, placed in a soxhlet ether extractor to determine total fat weight, and then placed in a muffle oven to determine total ash weight.

Islet immunohistochemistry and pancreatic insulin content. Pancreas samples were collected and immunostained, and insulin was extracted and analyzed as previously described (31).

Statistics and data analysis. Data are presented as mean ± SEM. Statistical analyses were performed using GraphPad Prism 4.00 for Windows, GraphPad Software, San Diego, CA. Body weight, food intake, and monthly fasting hormone and metabolite data were compared by two-factor (time and treatment) repeated-measures ANOVA followed by post hoc analysis with a Bonferroni multiple comparison test. Incidence data were analyzed by log-rank testing of Kaplan-Meier survival curves. Age of onset, tissue weights, tissue TG content, plasma leptin, plasma I-CAM, plasma MCP-1, and body composition were analyzed by one-factor ANOVA followed by post hoc analysis with Bonferroni multiple comparison test. Differences were considered significant at $P < 0.05$. For tissue weights and body composition data, animals that had become diabetic and had started to lose weight (at least 5% of peak weight) were not included in this dataset to avoid the confounding effects of weight loss on tissue TG content and adiposity. Five animals from the control group and one from the restricted group became diabetic at too early an age to allow collection of blood samples up to 6 months of age, and therefore they were included in incidence analysis, but excluded from longitudinal analyses.

RESULTS

Chronic liraglutide administration delays the onset of diabetes. Compared with control animals, the onset of diabetes was delayed in food-restricted and liraglutide-treated animals by 2.8 ± 0.7 and 4.1 ± 0.8 months of age, respectively. The mean ages of diabetes onset were 5.2 ± 0.6, 8.0 ± 0.7, and 9.3 ± 0.8 months in control, food-restricted, and liraglutide-treated animals, respectively (Table 1). By 6.5 months of age, only 5 of 32 liraglutide-

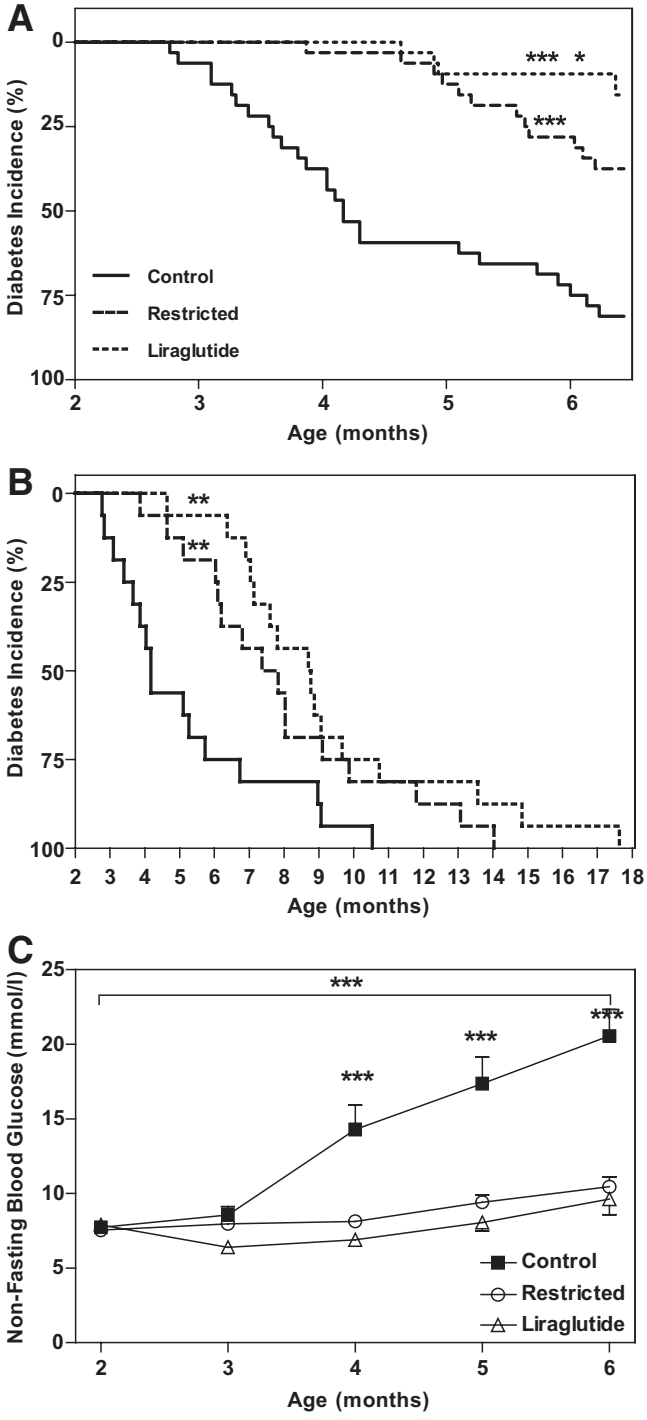

FIG. 1. Kaplan-Meier analysis of diabetes incidence in control, restricted, and liraglutide-treated animals up to 6 months (n = 32 per group) (A) and 12 months (n = 16 per group) (B) of age. ***P < 0.0001, **P < 0.01 compared with control; *P < 0.05 compared with the food-restricted group by log-rank test. Nonfasting blood glucose in control, restricted, and liraglutide-treated animals (C). ***P < 0.0001 by two-factor (time and treatment) repeated-measures ANOVA, ***P < 0.001 compared with restricted and liraglutide-treated animals by Bonferroni post-test. Control: n = 27; restricted: n = 31; liraglutide-treated animals: n = 32.

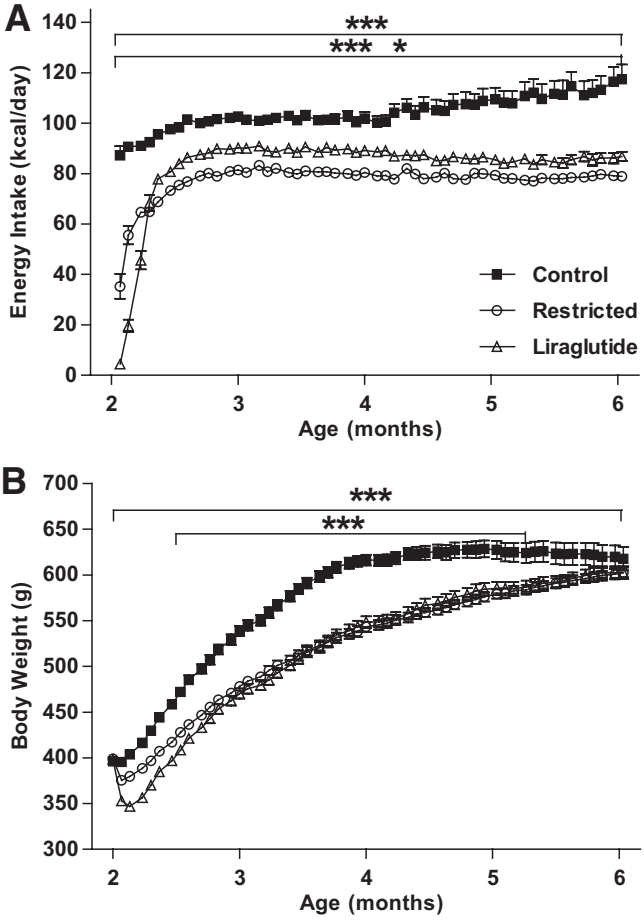

FIG. 2. Energy intake (A) and body weight (B) in control, restricted, and liraglutide-treated animals. ***P < 0.0001 by two-factor (time and treatment) repeated-measures ANOVA; ***P < 0.001 compared with restricted and liraglutide-treated animals; *P < 0.05 liraglutide-treated animals versus restricted by Bonferroni post-test. Control: n = 27; restricted: n = 31; liraglutide-treated animals: n = 32.

treated animals had become diabetic, whereas 12 of 32 food-restricted and 26 of 32 control animals were diabetic. Thus, within the first 4 months of treatment, liraglutide treatment significantly delayed diabetes onset compared with both the control and food-restricted groups (Fig. 1*A*). After 15 months of treatment, liraglutide and food restriction both significantly delayed diabetes onset compared with control animals ($P < 0.01$), but at this lower sample size and longer time course of treatment, the time of onset was not significantly different between food-restricted and liraglutide long-term treatment groups (Fig. 1*B*). Nonfasting blood glucose concentrations were significantly elevated in control animals compared with restricted and liraglutide-treated animals starting at 4 months of age (Fig. 1*C*).

Liraglutide treatment reduces energy intake and body weight. As expected, energy intake and body weight were significantly higher in control animals compared with restricted and liraglutide-treated animals throughout the study (Fig. 2). Energy intake was maintained 9% lower in restricted animals compared with liraglutide-treated animals to equalize body weights between these groups. Control animals began to lose weight at 6 months of age as the majority of these untreated animals had developed diabetes and were losing energy because of glucosuria and polyuria (Table 2). Diabetic animals developed moderate hyperphagia as previously reported, likely because of decreases of insulin and leptin (26).

Liraglutide lowers fasting glucose, A1C, insulin, and urinary albumin excretion. Fasting plasma glucose and A1C were elevated in control animals compared with food-restricted and liraglutide-treated animals, reflecting

TABLE 2
Urine volume (24-h), urine glucose, and albumin concentrations and excretion in 6-month-old rats

	Control	Restricted	Liraglutide-treated
Urine glucose (mmol/l)	294 ± 40	59 ± 33***	78 ± 41***
24-h urine volume (ml)	112 ± 19	15 ± 3***	29 ± 9***
24-h urine glucose output (mg)	7,201 ± 1,473	374 ± 273***	1,337 ± 823***
Urinary albumin excretion (mg/day)	14.6 ± 4.8	0.6 ± 0.1**	0.9 ± 0.2**
n	16	15	16

Data are means ± SEM unless otherwise indicated. $P < 0.001$ by one-way ANOVA; **$P < 0.01$, ***$P < 0.001$ compared with control by Bonferroni post-test.

the earlier age of onset in this group (Fig. 3*A* and *B*). Similar to urine glucose values, daily urine albumin excretion in 6-month-old rats was significantly higher in control animals compared with food-restricted and liraglutide-treated animals (Table 2).

Liraglutide treatment markedly lowered fasted plasma insulin compared with control and food-restricted animals starting 1 month into intervention and lasting throughout the 6-month sampling period, suggesting that the improved insulin sensitivity was not solely related to reduced body weight (Fig. 3*C*) ($P < 0.001$). Fasting insulin concentrations in the control group began to fall starting at 5 months of age as more of these animals developed diabetes. We previously reported that fasting plasma insulin concentrations decline progressively with the duration of diabetes in the UCD-T2DM rat model as a result of β-cell failure (26).

Liraglutide decreases body adiposity. In animals killed at 6.5 months of age, food-restricted and liraglutide-treated animals weighed significantly less than control animals and had smaller epididymal and mesenteric adipose depots (Table 3) ($P < 0.01$). Although food restriction resulted in a decrease of percent body fat content and an increase of the percent lean body mass compared with nondiabetic controls, liraglutide treatment further decreased percentage body fat and increased lean body mass compared with food-restricted animals (Table 3). Liraglutide-treated animals also had ~15% lower heart weights and higher percentage body mineral (bone ash) content compared with control animals.

Liraglutide lowers fasting TG and liver TG content. Fasting plasma TG concentrations were reduced ~50–60% in liraglutide-treated animals compared with control and food-restricted animals throughout the 6-month sampling period (Fig. 4*A*). Liver TG content was significantly lower in both food-restricted and liraglutide-treated animals at 6.5 months of age compared with control animals (Table 3). No significant differences in muscle TG content or plasma cholesterol were observed (Fig. 4*B*).

Liraglutide lowers plasma glucagon and leptin, maintains plasma adiponectin, and does not affect markers of inflammation. When the rats were 3 months of age, fasting plasma glucagon concentrations were significantly lower in liraglutide-treated animals compared with food-restricted and control animals (Fig. 4*C*). Fasting plasma leptin was significantly lower in liraglutide-treated animals compared with food-restricted and control animals at 6.5 months of age, after exclusion of animals that had become diabetic and had started to lose weight (control: 1,034 ± 168; restricted: 1,031 ± 344; liraglutide: 730 ± 237 pmol/l; $P < 0.05$; control: $n = 6$; restricted: $n = 14$; liraglutide: $n = 14$). Plasma adiponectin concentrations were maintained in restricted and liraglutide animals up to 6 months of age, whereas plasma adiponectin levels fell by 33 ± 12 pmol/l ($P < 0.05$) in 4-month-old rats compared with baseline concentrations in the control animals (Fig. 4*D*). Liraglutide treatment did not lower fasting plasma I-CAM concentrations in 6.5-month-old pre-diabetic animals compared with control animals; however, food restriction significantly lowered I-CAM concentrations (control: 286.2 ± 54.4; restricted: 202.1 ± 6.1, liraglutide: 355.9 ± 75.1 nmol/l; $P < 0.05$, control compared with restricted; control: $n = 6$; restricted: $n = 14$; liraglutide: $n = 14$). Plasma MCP-1 concentrations were lower in restricted and liraglutide-treated animals compared with control animals at 6 months of age (control: 3.0 ± 0.5; restricted: 1.8 ± 0.2; liraglutide: 2.0 ± 0.3 pmol/l; control: $n = 29$; restricted: $n = 27$; liraglutide: $n = 29$). However, plasma MCP-1 concentrations were not different between groups when diabetic animals were excluded (control: 2.2 ± 0.5; restricted: 1.8 ± 0.2; liraglutide: 2.0 ± 0.3 pmol/l; control: $n = 11$; restricted: $n = 23$; liraglutide: $n = 27$).

Liraglutide and energy restriction improve islet morphology and increase pancreatic insulin content. Immunostaining for insulin and glucagon was performed on a subset of nine animals per group to assess islet morphology. Figure 5 presents representative images of islets from control, food-restricted, and liraglutide-treated animals. Insulin staining appeared greater in islets from food-restricted and liraglutide-treated animals compared with control animals. This was expected since 8 of the 9 control animals had developed diabetes at the time of kill, whereas only 3 of 9 and 2 of 9 animals selected for immunostaining were diabetic prior to kill in the food-restricted and liraglutide-treated groups, respectively. Animals in the control group had been diabetic for 64 ± 13 days, whereas animals in the liraglutide and restricted groups had only been diabetic for 38 ± 3 and 26 ± 11 days, respectively. There were no visible differences noted between the islets from the one nondiabetic control animal and those from the nondiabetic restricted and liraglutide animals (data not shown). No significant visible differences in islet morphology were apparent between food-restricted and liraglutide-treated animals (Fig. 5). Pancreatic insulin content in the control animals was approximately one-third of that of food-restricted and liraglutide-treated animals (Table 3).

DISCUSSION

In the present study, we investigated the favorable metabolic effects of liraglutide administration (0.2 mg/kg body weight) and the potential of liraglutide to prevent the development of diabetes in pre-diabetic UCD-T2DM rats. Previous studies of liraglutide in rodents and humans have demonstrated reductions of food intake and body weight,

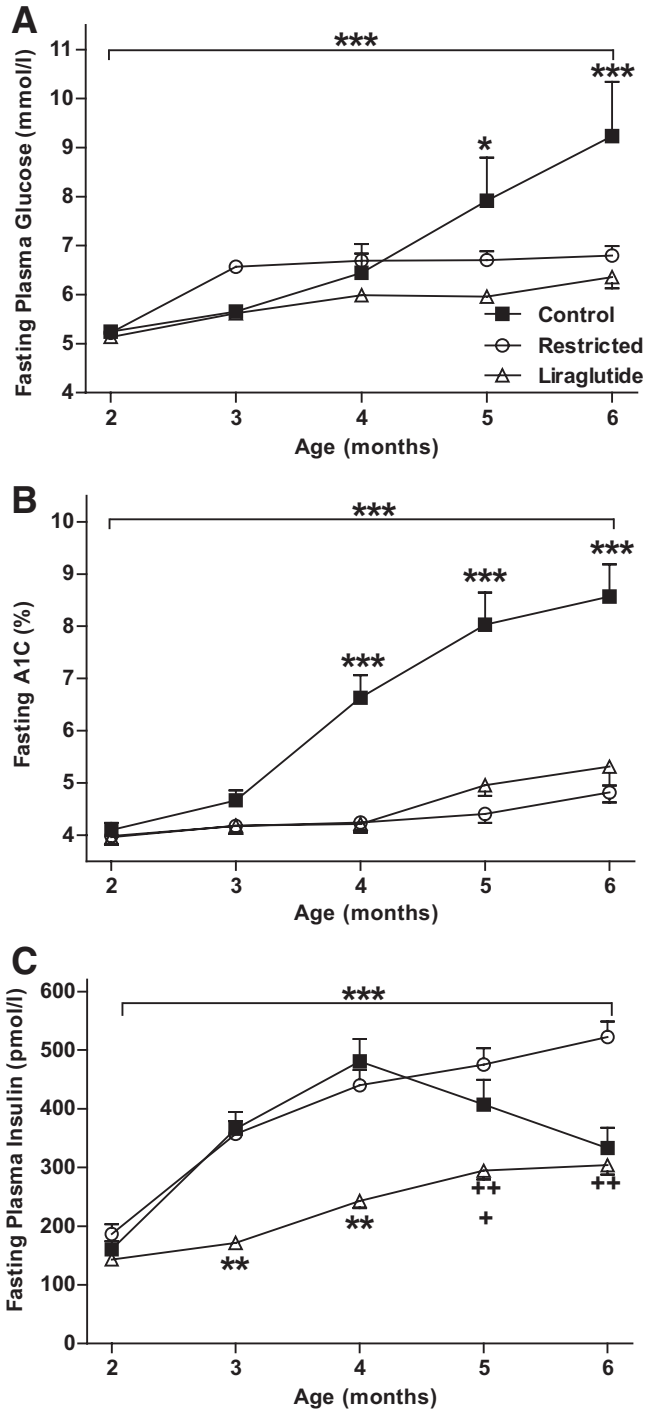

FIG. 3. Fasting circulating glucose (*A*), A1C (*B*), and insulin (*C*) in control, restricted, and liraglutide-treated animals. ***$P < 0.0001$ by two-factor (time and treatment) repeated-measures ANOVA; ***$P < 0.001$, *$P < 0.05$ compared with restricted and liraglutide-treated animals; **$P < 0.001$ compared with restricted and control; $^{++}P < 0.001$ compared with restricted; $^{+}P < 0.05$ compared with control by Bonferroni post-test. Control: $n = 27$; restricted: $n = 31$; liraglutide-treated animals: $n = 32$.

improvements in β-cell function, reduced glucagon concentrations, and subsequent improvements of insulin sensitivity and plasma glucose (20–25,32,33). In this study, we report that chronic liraglutide administration substantially delays the onset of diabetes as assessed by reductions of circulating glucose and A1C in UCD-T2DM rats compared with vehicle-treated control animals, and it modestly delays diabetes onset compared with food-restricted animals matched for body weight.

The large effects of energy restriction alone to delay the onset of diabetes in this study suggest that a substantial portion of the effects of chronic liraglutide administration on diabetes onset are a result of its effect to reduce food intake and body weight. Reductions of energy intake and body weight resulting from liraglutide administration are similar to that reported in other rodent studies (32,33). Food restriction is well known to lower glucose, A1C, and requirements for insulin (34); however, long-term compliance with voluntary food restriction regimens is rarely achieved. Treatment with liraglutide, a long-acting GLP-1 analog, has the benefit of achieving such reductions in food intake and body weight.

Although energy restriction played a key role in the effect of liraglutide to delay type 2 diabetes onset, differences between liraglutide-treated and food-restricted animals suggest additional antidiabetic actions of liraglutide treatment. First, similar body weights in the liraglutide and restricted groups, despite significantly lower food intake in the restricted group, suggests that liraglutide treatment increases energy expenditure. This has been previously suggested by the results of a study in which a 10% increase of energy expenditure was observed in rats treated with liraglutide (33). Furthermore, lower percentage of body fat mass in liraglutide-treated compared with food-restricted animals, despite similar body weight, is suggestive of a preferential increase of lipid oxidation with liraglutide treatment compared with energy restriction alone.

Second, substantially lower fasting plasma insulin concentrations in liraglutide-treated animals compared with food-restricted animals, despite similar plasma glucose concentrations, suggest that liraglutide administration improves insulin sensitivity, independent of effects on food intake and body weight. The mechanisms by which GLP-1 signaling improves insulin sensitivity remain incompletely understood. There is increasing evidence that GLP-1 may act to enhance peripheral insulin sensitivity and reduce hepatic gluconeogenesis independent of its effects on pancreatic hormones (35), which may be mediated by portal GLP-1 receptors that signal to the CNS through vagal afferent nerves and autonomic efferent signals to insulin target tissues such as adipose, liver, and muscle (36).

This improvement in insulin sensitivity may have contributed to the lower circulating TG concentrations in liraglutide-treated compared with both control and food-restricted animals. Liver TG content was also significantly lower in liraglutide-treated and food-restricted animals. Reductions of circulating and ectopic TG content are likely to contribute to the improvement in insulin sensitivity, as deposition of TG in the liver impairs insulin signaling through the production of lipid metabolites that reduce insulin receptor substrate-2 tyrosine phosphorylation (37). Additional studies are needed to investigate the mechanisms involved in the effects of liraglutide to lower circulating TG and to determine the extent to which this contributes to the liraglutide-induced improvement of insulin sensitivity and delay of diabetes onset.

Lower glucagon levels during the first 2 months of treatment in liraglutide-treated animals may have contributed to lower glucose levels, as glucagon promotes hepatic gluconeogenesis and the production of gluconeogenic substrates from other energy stores (38). This GLP-1–mediated improvement of α-cell function is in agreement with previous studies (1), and it is consistent with in-

TABLE 3
Body composition, tissue TG content, and pancreatic insulin content in 6.5-month-old rats

	Control	Restricted	Liraglutide-treated
Body weight (g)	647 ± 16	574 ± 7***	573 ± 11***
Epididymal fat depots (g)	12.0 ± 0.4	9.3 ± 0.4**	8.5 ± 0.5***
Retroperitoneal fat depots (g)	13.7 ± 0.3	12.5 ± 0.7	11.1 ± 0.7
Subcutaneous fat depot (g)	61 ± 3	51 ± 3	46 ± 2*
Mesenteric fat depot (g)	11.1 ± 0.5	8.2 ± 0.4**	8.0 ± 0.6**
Total white adipose tissue (g)	98 ± 4	81 ± 5	74 ± 4**
Heart (g)	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.1*
Kidney (g)	1.8 ± 0.1	1.7 ± 0.1	1.8 ± 0.1
Liver (g)	21.3 ± 0.9	17.4 ± 0.5***	17.1 ± 0.4***
Liver TG (mg/g liver)	23.0 ± 1.4	13.3 ± 1.7***	13.2 ± 1.1**
Skeletal muscle TG (mg/g muscle)	3.3 ± 0.4	4.2 ± 0.6	3.2 ± 0.3
Pancreatic insulin content (μg/g)	7 ± 3	21 ± 3*	25 ± 5**
% Body fat	23.9 ± 0.8	22.6 ± 0.9	20.0 ± 0.9*†
% Lean body mass	21.3 ± 0.1	21.4 ± 0.2	22.1 ± 0.2*†
% Body water	51.2 ± 0.7	52.2 ± 0.8	53.9 ± 0.7
% Body ash	3.6 ± 0.1	3.8 ± 0.1	3.9 ± 0.1**

Data are means ± SEM. Control: $n = 6$; restricted: $n = 14$; liraglutide-treated: $n = 14$. For pancreatic insulin content, control: $n = 10$; restricted: $n = 9$; liraglutide-treated: $n = 10$. One-way ANOVA; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with control; †$P < 0.05$ compared with restricted. Data exclude all animals that became diabetic and began losing weight.

creased hepatic gluconeogenesis observed in the GLP-1 receptor knockout mouse (39).

The lower plasma leptin concentrations in liraglutide-treated animals are likely the result of the decreased insulin levels observed in response to chronic liraglutide administration, as leptin production is regulated by insu-

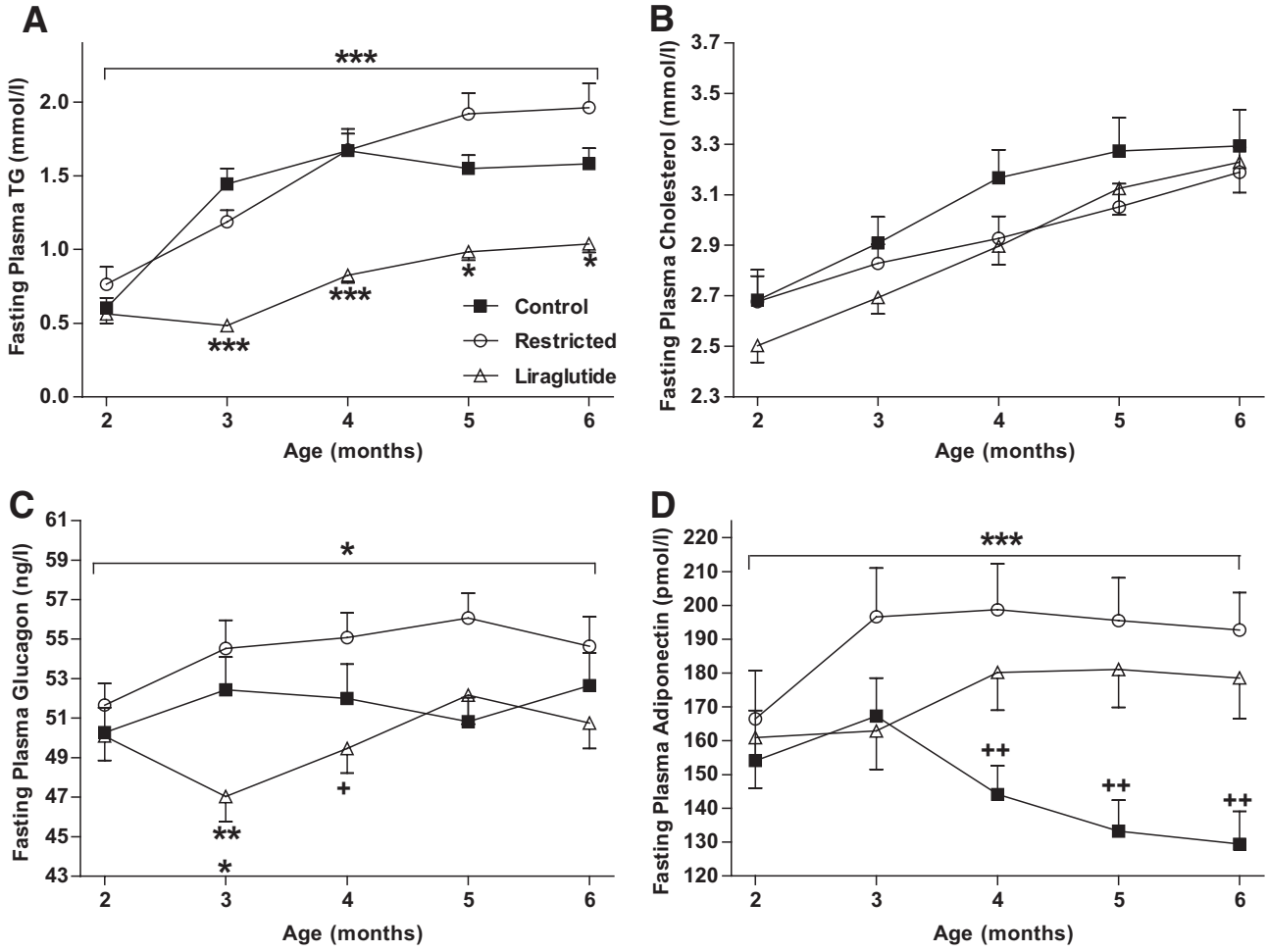

FIG. 4. Fasting plasma TG (*A*), cholesterol (*B*), glucagon (*C*), and adiponectin (*D*) in control, food-restricted, and liraglutide-treated animals. ***$P < 0.0001$, *$P < 0.05$ by two-factor (time and treatment) repeated-measures ANOVA; ***$P < 0.001$ compared with food-restricted and control; **$P < 0.001$ compared with food-restricted; *$P < 0.05$ compared with control; $^{+}P < 0.05$ compared with food-restricted; $^{++}P < 0.05$ compared with food-restricted and liraglutide-treated animals by Bonferroni post-test. Control: $n = 27$; restricted: $n = 31$; liraglutide-treated animals: $n = 32$.

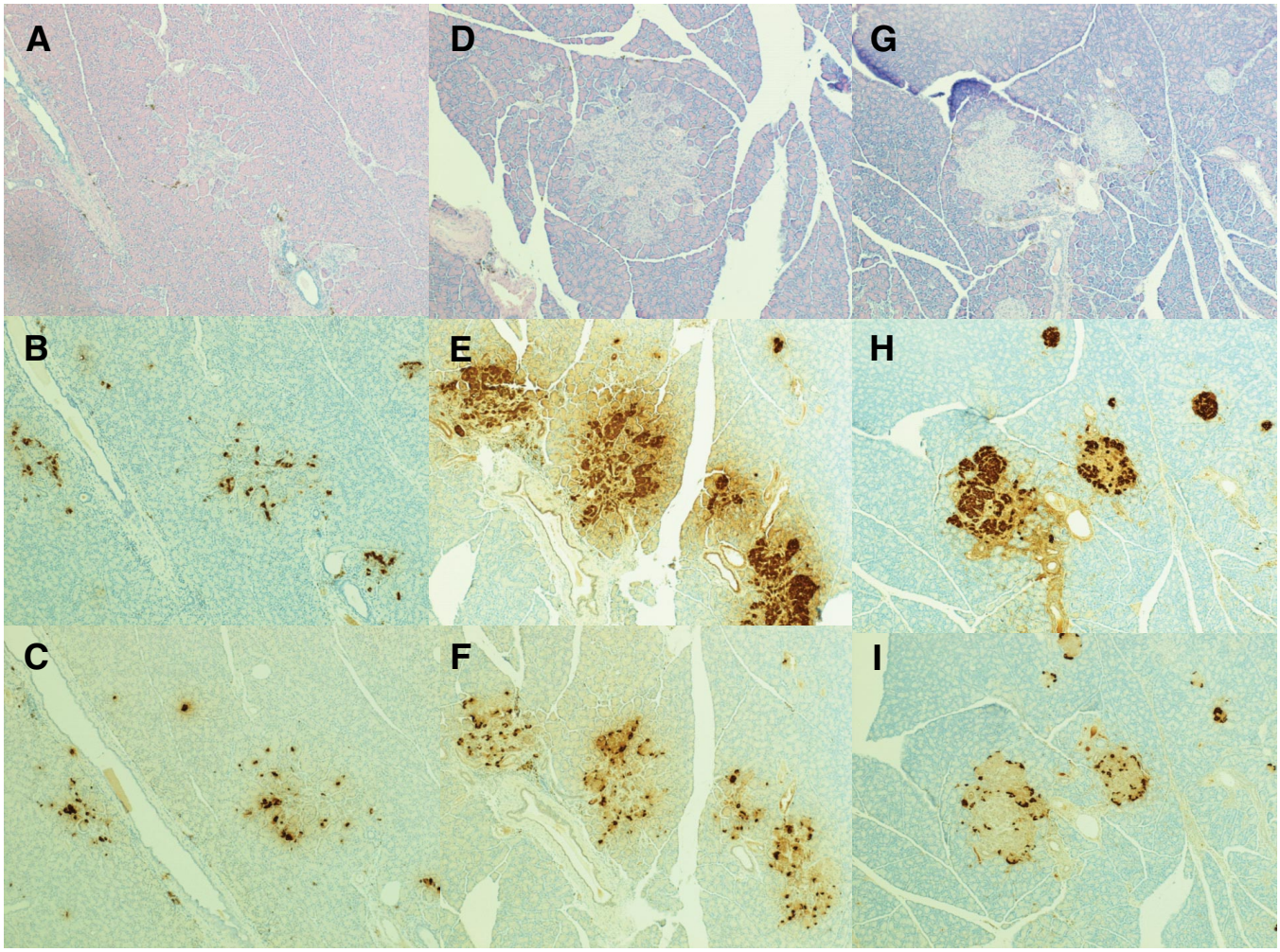

FIG. 5. Representative pancreas sections from control, food-restricted, and liraglutide-treated animals at 6 months of age. Hematoxylin and eosin staining for control (*A*), restricted (*D*), and liraglutide-treated animals (*G*) is shown. Anti-insulin immunostaining for control (*B*), restricted (*E*), and liraglutide-treated animals (*H*) is shown. Anti-glucagon immunostaining for control (*C*), restricted (*F*), and liraglutide-treated animals (*I*) is shown. (A high-quality digital representation of this figure is available in the online issue.)

lin-mediated glucose utilization in adipocytes (40). Lower adiposity in liraglutide-treated rats may also have contributed to lower leptin concentrations (41). Lower heart weights in liraglutide-treated animals compared with control animals appear consistent with the beneficial effects that GLP-1 signaling has been reported to have on cardiac function (1). Liraglutide did not have a significant effect on markers of inflammation in pre-diabetic rats; thus, the delay in diabetes onset does not appear to be influenced by inflammatory status.

Several parameters were similar between the food-restricted and liraglutide-treated groups, further indicating the importance of energy restriction and reduced adiposity in delaying the onset of diabetes in the UCD-T2DM rat model. Circulating levels of the insulin-sensitizing adipocyte hormone adiponectin (41) were elevated in both liraglutide-treated and food-restricted animals compared with control animals starting at 4 months of age. This is reflective of the later age of diabetes onset in these two treatment groups, as we have previously reported that adiponectin levels fall after the onset of diabetes in UCD-T2DM rats, potentially contributing to the progression of type 2 diabetes (26). Higher circulating adiponectin concentrations may also have contributed to the lower glucose levels in both the liraglutide-treated and food-restricted groups by maintaining insulin sensitivity.

Liraglutide treatment and energy restriction equally preserved islet morphology and pancreatic insulin content. Both treatments delayed the onset of diabetes such that none of the restricted or liraglutide animals had been diabetic for >1.5 months prior to tissue collection at 6.5 months of age. The deterioration of islet morphology and insulin staining during the progression of diabetes has been previously documented in the UCD-T2DM rat model (26), and it is clearly demonstrated in the control animals that had diabetes for >1 month. These observations are also consistent with those reported in pancreata from humans with type 2 diabetes (42). Thus, the preservation of islet morphology and pancreatic insulin content during food restriction or liraglutide administration in UCD-T2DM rats may be a result of slower weight gain and a consequent delay in the onset of hyperglycemia and possibly glucose toxicity. The effects of liraglutide administration and food restriction to delay the onset of diabetes and reduce hyperglycemia were also associated with markedly reduced urinary albumin excretion, indicating that delaying diabetes onset in UCD-T2DM rats delays the development of diabetic complications such as nephropathy.

In conclusion, both liraglutide treatment and energy

restriction delay the onset of type 2 diabetes in UCD-T2DM rats by slowing weight gain, obesity-associated insulin resistance, and islet dysfunction. Sustained liraglutide administration delayed the onset of diabetes compared with food-restricted, body weight–matched animals. This delay may be the result of improved insulin sensitivity and decreased circulating TG and glucagon concentrations seen with liraglutide administration relative to energy restriction alone. Thus, chronic liraglutide treatment is an effective weight management tool that delays the onset of type 2 diabetes. This is the first preclinical study investigating the long-term use of chronic liraglutide administration for the prevention of type 2 diabetes. Determining preventive indications for currently available and developing therapies is crucial in the attempt to reduce the growing worldwide burden of type 2 diabetes. Delaying the onset of type 2 diabetes and reducing hyperglycemia in predisposed individuals would have multiple benefits, including delaying the onset of diabetic complications, improving patient quality of life, and reducing health care expenses.

ACKNOWLEDGMENTS

This research was supported by Novo Nordisk, Bagsværd, Denmark. P.J.H.'s laboratory also receives or has received funding during the project period from the National Institutes of Health grants R01-HL075675, R01-HL09333, AT0-02599, AT0-02993, and AT0-03545. Immunohistochemistry and islet analysis work was supported by the National Institutes of Health Grant P30 DK-17047 through the Cellular and Molecular Imaging Core of the University of Washington Diabetes Endocrinology Research Center.

The immunohistochemistry studies were supported by the facilities at the Veterans Affairs Puget Sound Health Care System, Seattle, Washington. D.G.B. is Senior Research Career Scientist, Research and Development Service, Department of Veterans Affairs Puget Sound Health Care System, Seattle, Washington.

C.N., A.S., L.B.K., and K.R. are full-time employees of Novo Nordisk and are also minor stock owners as part of an employee offering program. Novo Nordisk markets liraglutide for treatment of type 2 diabetes. S.C.G. is currently employed by Bristol-Myers Squibb. P.J.H.'s laboratory received funding from Novo Nordisk to perform this project.

No other potential conflicts of interest relevant to this article were reported.

B.P.C. developed and characterized the UCD-T2DM rat model used for the study, designed and obtained funding for the study, conducted the experiments, analyzed data, wrote the manuscript, and edited the manuscript for intellectual content. K.L.S. developed and characterized the UCD-T2DM rat model used for the study, designed and obtained funding for the study, conducted the experiments, analyzed data, wrote the manuscript, edited the manuscript for intellectual content, and had primary responsibility for final content. J.L.G. developed and characterized the UCD-T2DM rat model used for the study, designed and obtained funding for the study, conducted the experiments, analyzed data, and edited the manuscript for intellectual content. D.G.B. analyzed data and edited the manuscript for intellectual content. S.C.G. developed and characterized the UCD-T2DM rat model used for the study and edited the manuscript for intellectual content. C.N. edited the manuscript for intellectual content. A.S. conducted the experiments, analyzed data, and edited the manuscript for intellectual content. L.B.K. and K.R. designed and obtained funding for the study and edited the manuscript for intellectual content. P.J.H. developed and characterized the UCD-T2DM rat model used for the study, designed and obtained funding for the study, analyzed data, edited the manuscript for intellectual content, and had primary responsibility for final content.

Parts of this study were presented in abstract form at the 69th Scientific Sessions of the American Diabetes Association, New Orleans, Louisiana, 5–9 June 2009.

The authors thank Sunhye Kim for extensive help with animal care, monitoring, and data collection; Joyce Murphy for excellent technical support with pancreatic immunohistochemistry; Noreene Shibata for body composition analysis; and Riva Dill for animal care assistance.

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