Therapeutic Impact of Leptin on Diabetes, Diabetic Complications, and Longevity in Insulin-Deficient Diabetic Mice

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OBJECTIVE—The aim of the current study was to evaluate the long-term effects of leptin on glucose metabolism, diabetes complications, and life span in an insulin-dependent diabetes model, the Akita mouse.

RESEARCH DESIGN AND METHODS—We cross-mated Akita mice with leptin-expressing transgenic (LepTg) mice to produce Akita mice with physiological hyperleptinemia (LepTg:Akita). Metabolic parameters were monitored for 10 months. Pair-fed studies and glucose and insulin tolerance tests were performed. The pancreata and kidneys were analyzed histologically. The plasma levels and pancreatic contents of insulin and glucagon, the plasma levels of lipids and a marker of oxidative stress, and urinary albumin excretion were measured. Survival rates were calculated.

RESULTS—Akita mice began to exhibit severe hyperglycemia and hyperphagia as early as weaning. LepTg:Akita mice exhibited normoglycemia after an extended fast even at 10 months of age. The 6-h fasting blood glucose levels in LepTg:Akita mice remained about half the level of Akita mice throughout the study. Food intake in LepTg:Akita mice was suppressed to a level comparable to that in WT mice, but pair feeding did not affect blood glucose levels in Akita mice. LepTg:Akita mice maintained insulin hypersensitivity and displayed better glucose tolerance than did Akita mice throughout the follow-up. LepTg:Akita mice had normal levels of plasma glucagon, a marker of oxidative stress, and urinary albumin excretion rates. All of the LepTg:Akita mice survived for >12 months, the median mortality time of Akita mice.

CONCLUSIONS—These results indicate that leptin is therapeutically useful in the long-term treatment of insulin-deficient diabetes. *Diabetes* **60:2265–2273, 2011**

Leptin is an adipocyte-derived hormone that is involved in the regulation of food intake and energy expenditure (1). We previously created transgenic mice that overexpress leptin under the control of the liver-specific promoter (LepTg) (2). The plasma leptin level is stable in LepTg mice and similar to that in obese rodents and humans, suggesting that the phenotypic changes found in these animals are physiologically relevant (3,4). LepTg mice provide a unique experimental system to investigate the chronic in vivo effects of leptin. LepTg mice exhibit increased glucose metabolism, which is accompanied by the activation of insulin signaling in skeletal muscle and the liver (2). These findings indicate that leptin acts as an antidiabetic hormone. We have demonstrated the efficacy of leptin in various mouse models of diabetes (5–8).

Akita mice are an animal model of diabetes caused by pancreatic β-cell failure. Endoplasmic reticulum stress induced by misfolded proinsulin is responsible for the β-cell dysfunction and destruction in Akita mice. Male Akita mice start to develop hyperglycemia as early as weaning, when 71% decrease of β-cell mass is present. Plasma insulin levels in the mice are reduced to 41% of the control mice at 7 weeks of age. The blood glucose levels in male Akita mice increase irreversibly up to 700 mg/dL at 10 weeks of age, and about half the male Akita mice die of extreme hyperglycemia within the 1st year of life (9). Thus, the Akita mouse is a suitable model for evaluating the therapeutic impact of interventions on the onset, progression, and prognosis of diabetes.

In the current study, to clarify how and to what extent chronic leptin therapy affects the long-term course of diabetes, we genetically crossed LepTg and Akita mice to create a unique mouse model of nonobese diabetes with elevated plasma leptin level. Using this mouse diabetes model, we investigated the chronic lifelong effects of leptin on diabetes, diabetic nephropathy, and longevity in Akita mice.

RESEARCH DESIGN AND METHODS

Animals. Generation of LepTg mice was reported previously (2). Briefly, a fusion gene comprising the human serum amyloid P component promoter upstream of the mouse leptin cDNA coding sequences was designed to target hormone expression to the liver (2,8). The highest expressing transgenic line was used in this study (2). The genotype for LepTg mice was determined by PCR (5′-GCTGGTTGTTGTGCTGTCTC-3′; 5′-CAGGCTGGTGAGGACCTGTT-3′). B6-Ins2Akita ($Ins2^{WT/C96Y}$; referred to hereafter as Akita) mice were purchased from Japan SLC (Shizuoka, Japan). Presence of the Akita mutation was verified by absence of an Fnu4HI restriction site in the PCR product of the *Ins2* gene (5′-TGCTGATGCCCTGGCCTGCT-3′; 5′-TGGTCCACATATGCACATG-3′). Both LepTg and Akita mice were on the same C57BL/6 J background. Hemizygous male LepTg mice were cross-mated with female heterozygous Akita mice. Male F1 mice were used in this study. Mice were maintained in a temperature-, humidity-, and light-controlled room and allowed free access to standard diet (F-2 diet; Oriental BioService, Kyoto, Japan).

The care of the animals and all experimental procedures were conducted in accordance with the guidelines for animal experiments of Kyoto University and were approved by the Animal Research Committee of Kyoto University.

Metabolic parameters measurements. Levels of leptin (Mouse Leptin ELISA, Millipore, St. Charles, MO), glucose (Glutest Neo Super, Sanwa, Nagoya, Japan, or Glucose C2-test, Wako, Osaka, Japan), HbA$_{1c}$ (DCA2000 analyzer, Bayel-Sankyo, Tokyo, Japan), insulin (Ultra-Sensitive PLUS Mouse Insulin Kit, Morinaga, Yokohama, Japan), glucagon (Glucagon EIA Kit, Yanaihara, Shizuoka, Japan), triglyceride (TG; Triglyceride E-test, Wako), nonesterified fatty acid (NEFA; NEFA C-test, Wako), β-hydroxybutyrate (Precision Xtra, Abbott, Bedford, MA), and thiobarbituric acid reactive substances (TBARS; TBARS
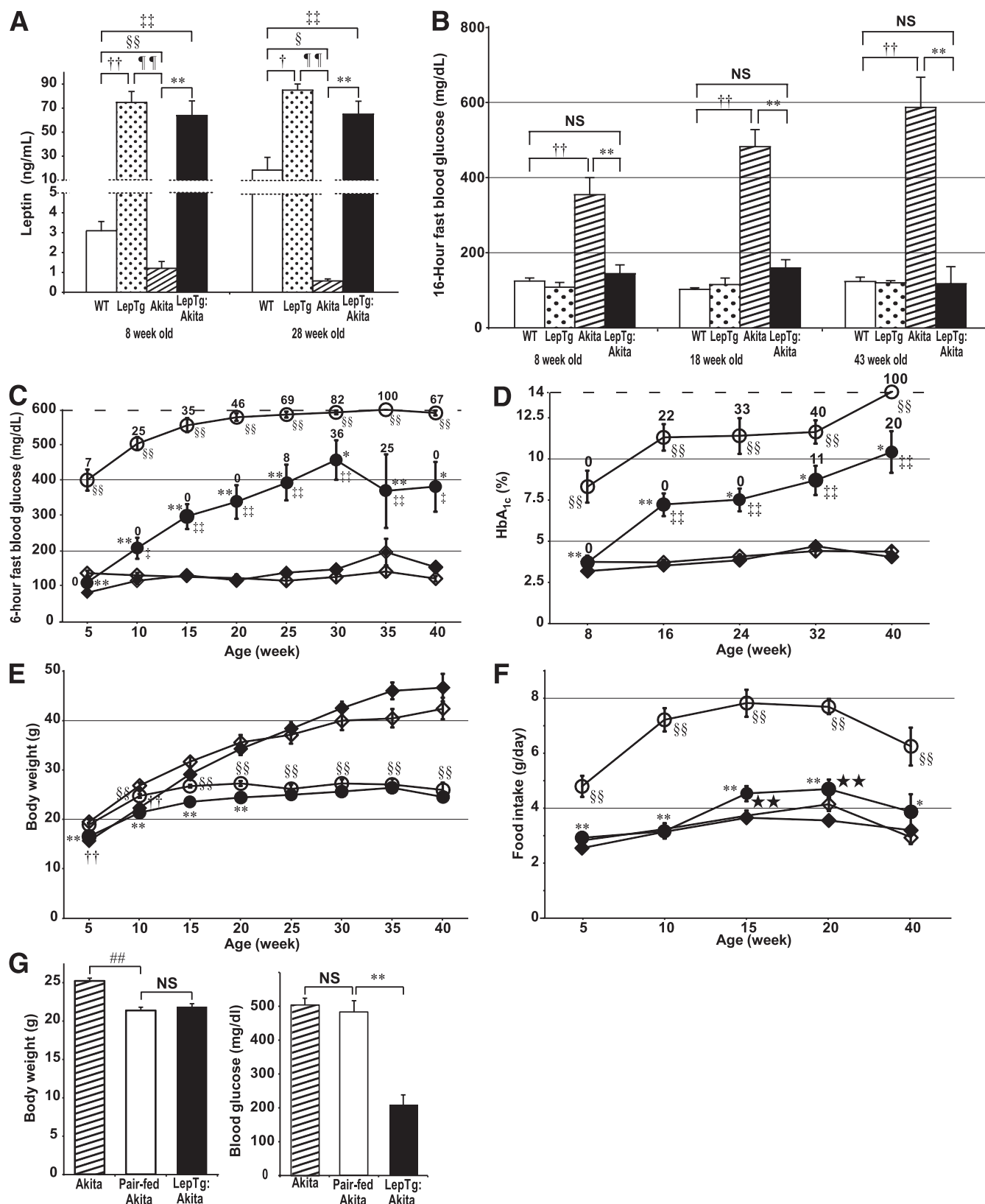
FIG. 1. Time course of changes in plasma leptin, blood glucose, HbA1c, body weight, and food intake. *A*: Plasma leptin levels of WT, LepTg, Akita, and LepTg:Akita mice at 8 and 28 weeks of age ($n \geq 4$ in each group). *B*: Sixteen-hour fasting blood glucose levels of WT, LepTg, Akita, and LepTg: Akita mice at 8, 18, and 43 weeks of age ($n \geq 4$ in each group). *C*: Time course of 6-h fasting blood glucose concentrations of WT ($\Diamond$), LepTg ($\blacklozenge$), Akita ($\bigcirc$), and LepTg:Akita ($\bullet$) mice ($n \geq 11$ in each group, except $n = 5$ for data of 40 weeks of age). Since the glucometer has a detection limit up to 600 mg/dL, values above the detection limit were treated as 601 mg/dL. Dashed line indicates detection limit of 600 mg/dL. The numbers along the curves indicate the percent of samples above detection limit. *D*: Time course of glycated hemoglobin (HbA1c) levels of WT ($\Diamond$), LepTg ($\blacklozenge$), Akita ($\bigcirc$), and LepTg:Akita ($\bullet$) mice ($n \geq 4$ in each group). Since the analyzer has a detection limit up to 14%, values above the detection limit were treated as 14.1%. Dashed line indicates detection limit of 14%. The numbers along the curves indicate the percent of samples above detection limit.

Assay Kit, Cayman, Ann Arbor, MI) were measured. Percent body fat was measured by Latheta LTC-100 (ALOKA, Tokyo, Japan). For glucose tolerance tests (GTTs), after 12-h fast, the mice were injected with 1.0 g/kg i.p. glucose. For insulin tolerance tests (ITTs), after a 6-hour fast, the mice were injected with 0.5 units/kg i.p. human insulin (Novo Nordisk, Bagsvaerd, Denmark).

Pair-feeding experiment. Akita mice were given the amount of food consumed by ad libitum–fed LepTg:Akita mice on the previous day. A pair-feeding study was conducted from 8 through 11 weeks of age. Body weights and blood glucose concentrations were measured at the end of the period.

Pancreatic hormone secretion and content. After 12-h fast, the mice were injected with 3.0 g/kg i.p. glucose. Blood samples were obtained from the retro-orbital venous sinus using heparin-coated glass capillaries. For hormone content, pancreata were homogenized in acid ethanol.

Histology. Pancreata were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were immunostained with the following antibodies: guinea pig anti-insulin antibody (Dako, Glostrup, Denmark), rabbit antiglucagon antibody (Dako), and Alexa488 anti–guinea pig antibody and Alexa546 anti-rabbit antibody (both from Molecular Probes, Eugene, OR). Kidney tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Periodic acid Schiff (PAS) was used to stain 1-μm sections. Mesangial area was determined by the presence of PAS-positive and nuclei-free area in the mesangium. Measurement of the mesangial area of more than 22 glomeruli randomly selected in each mouse was performed with a computer-assisted microscopy (Keyence, Osaka, Japan).

Albumin in urine. A metabolic cage was used to collect 24-h urine. Urinary albumin concentration was measured using Albuwell M (Exocell, Philadelphia, PA). Blood pressure was measured by the indirect tail-cuff method.

Survival rates. Survival data were analyzed by Kaplan-Meier analysis, and comparisons between genotypes were done by the log-rank test.

Statistical analyses. Data are expressed as means ± SE. Comparison between or among groups was by Student t test, Mann-Whitney U test, or ANOVA with Scheffé F test. $P < 0.05$ was considered statistically significant.

RESULTS

Generation of LepTg:Akita mice. Plasma leptin levels were measured periodically (Fig. 1A). At 8 weeks of age, the plasma leptin levels in Akita mice declined to 39% of the levels in WT mice (3.1 ng/mL for WT vs. 1.2 ng/mL for Akita; $P < 0.01$). Five months later, at 28 weeks of age, an age-dependent increase in plasma leptin levels in WT mice and decrease in Akita mice were observed (18.8 ng/mL for WT vs. 0.58 ng/mL for Akita; $P < 0.05$). The transgenic expression of leptin was associated with markedly and stably increased plasma leptin levels in both LepTg and LepTg:Akita mice (74.8 ng/mL for LepTg and 64.2 ng/mL for LepTg:Akita at 8 weeks of age and 85.1 ng/mL for LepTg and 65.3 ng/mL for LepTg:Akita at 28 weeks of age).

Time course of changes in blood glucose and HbA_{1c} levels. Blood glucose levels were measured after a 16-h fast at 8, 18, and 43 weeks of age (Fig. 1B). Akita mice showed hyperglycemia with blood glucose levels >300 mg/dL even after the 16-h fast at 8 weeks of age, and this hyperglycemia worsened progressively with time. By contrast, the glucose levels remained <160 mg/dL and were indistinguishable between LepTg:Akita and WT mice at all times studied.

Six-hour fasting blood glucose levels, which correlate closely with the daily averaged blood glucose level, and HbA_{1c} levels were followed for ~10 months (Fig. 1C and D) (10). In Akita mice, 6-h fasting blood glucose levels were >500 mg/dL after 10 weeks of age, and more than half of the mice had blood glucose levels >600 mg/dL after
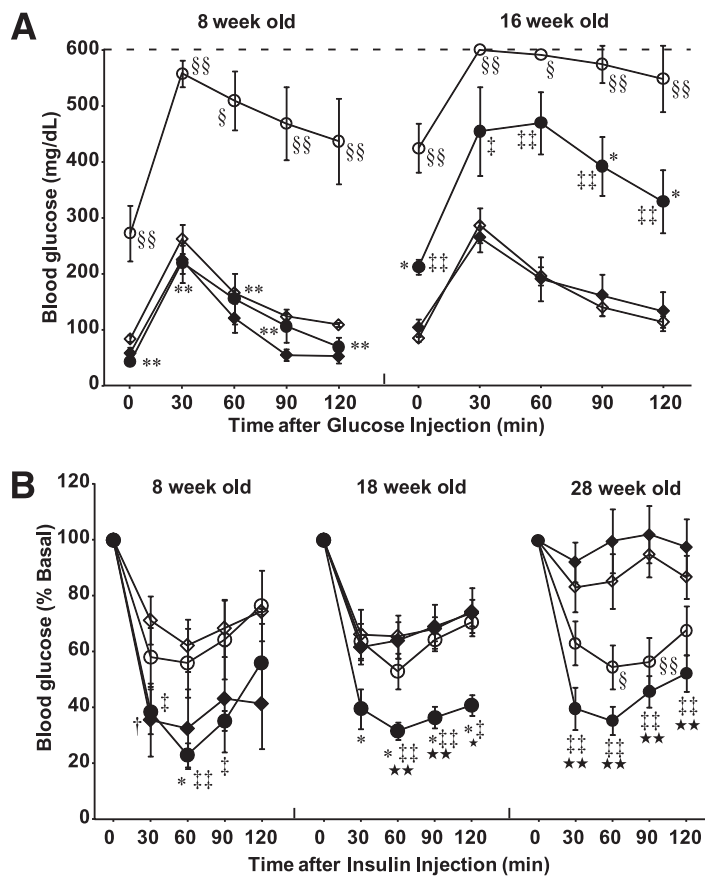
FIG. 2. GTTs and ITTs. *A*: GTTs of WT ($\diamond$), LepTg ($\blacklozenge$), Akita (open circles), and LepTg:Akita (closed circles) mice at 8 and 16 weeks of age. Blood glucose levels are shown at indicated times after glucose injections (1 g/kg body wt i.p.; $n \geq 4$ in each group). *B*: ITTs of WT ($\diamond$), LepTg ($\blacklozenge$), Akita ($\bigcirc$), and LepTg:Akita ($\bullet$) mice at 8, 18, and 28 weeks of age. Percent changes in blood glucose levels are shown at indicated times after injection of insulin (0.5 units/kg body wt i.p.; $n \geq 4$ in each group). Dashed line indicates detection limit of 600 mg/dL. Data are expressed as means ± SE. §$P < 0.05$, §§$P < 0.01$ for WT vs. Akita, ‡$P < 0.05$, ‡‡$P < 0.01$ for WT vs. LepTg:Akita, ★$P < 0.05$, ★★$P < 0.01$ for LepTg vs. LepTg:Akita, *$P < 0.05$, and **$P < 0.01$ for Akita vs. LepTg:Akita.

15 weeks of age (Fig. 1C). The blood glucose levels were lower in LepTg:Akita mice than in WT mice at 5 weeks of age and increased gradually after 10 weeks of age but remained <400 mg/dL at 40 weeks of age (Fig. 1C). Average 6-h fasting blood glucose levels were 552.4 ± 24.1 mg/dL in Akita, 321.0 ± 39.6 mg/dL in LepTg:Akita, 136.3 ± 11.8 mg/dL in LepTg, and 128.7 ± 3.0 mg/dL in WT mice from 5 to 40 weeks of age. Thus, the average blood glucose level in LepTg:Akita mice was ~58.1% of that in Akita mice.

HbA_{1c} levels changed in a similar pattern to the 6-h fasting blood glucose levels (Fig. 1D). By 8 weeks of age, Akita mice had markedly elevated HbA_{1c} levels (8.4 ± 1.0%), whereas HbA_{1c} levels were the same in LepTg:Akita mice (3.7 ± 0.1%) as in WT mice (3.8 ± 0.4%) at that age. Of Akita mice >16 weeks of age, >22% had HbA_{1c} levels above the detection limit (14%). The HbA_{1c} levels of LepTg:

E: Time course of body weight changes of WT ($\diamond$), LepTg ($\blacklozenge$), Akita ($\bigcirc$), and LepTg:Akita ($\bullet$) mice ($n \geq 11$ in each group, except $n = 5$ for data of 40 weeks of age). *F*: Time course of 24-h food intake of WT ($\diamond$), LepTg ($\blacklozenge$), Akita ($\bigcirc$), and LepTg:Akita ($\bullet$) mice ($n \geq 11$ in each group, except $n = 4$ for data of 40 weeks of age). *G*: Body weight of Akita, pair-fed Akita, and LepTg:Akita mice at the end of 3 weeks of pair feeding ($n = 4$ in each group). *H*: Six-hour fasting blood glucose concentrations of Akita, pair-fed Akita, and LepTg:Akita mice at the end of 3 weeks of pair feeding ($n = 4$ in each group). Data are expressed as means ± SE. In *A–F*, †$P < 0.05$, ††$P < 0.01$ for WT vs. LepTg, §$P < 0.05$, §§$P < 0.01$ for WT vs. Akita, ‡$P < 0.05$, ‡‡$P < 0.01$ for WT vs. LepTg:Akita, ¶¶$P < 0.01$ for LepTg vs. Akita, ★★$P < 0.01$ for LepTg vs. LepTg:Akita, *$P < 0.05$, and **$P < 0.01$ for Akita vs. LepTg:Akita. In *G* and *H*, ##$P < 0.01$ for Akita vs. pair-fed Akita and **$P < 0.01$ for LepTg:Akita vs. pair-fed Akita.
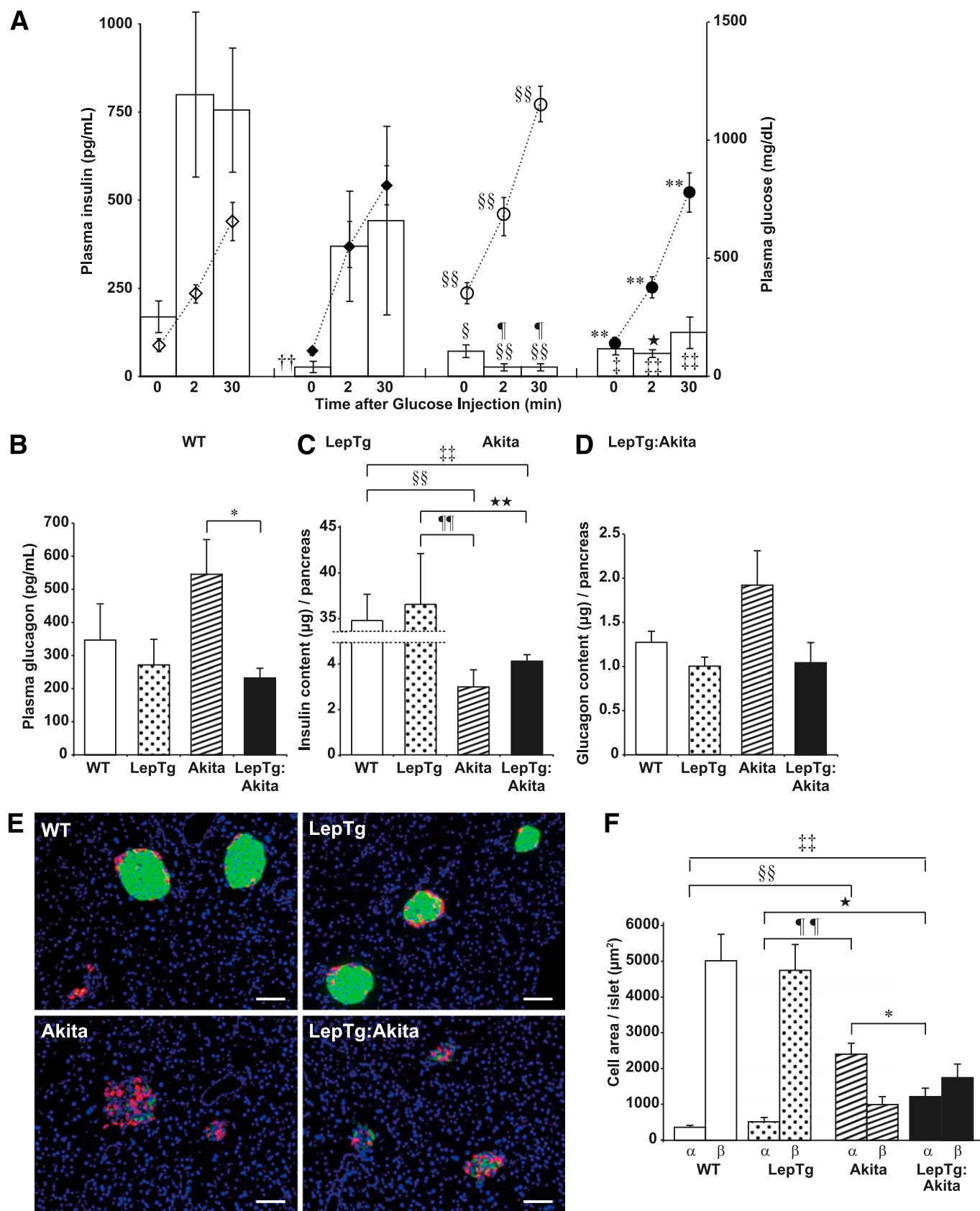
FIG. 3. Glucose-stimulated insulin secretion, plasma glucagon levels, and pancreatic hormone contents. *A*: Plasma insulin (open bars) and glucose (black lines) concentrations after glucose (3 g/kg i.p.) injection in WT, LepTg, Akita, and LepTg:Akita mice at 8 weeks of age ($n \geq 4$ in each group). *B*: Plasma glucagon concentration in ad libitum–fed WT, LepTg, Akita, and LepTg:Akita mice at 22 weeks of age ($n \geq 4$ in each group). *C* and *D*: Pancreatic insulin (*C*) and glucagon (*D*) content measured in acid-ethanol extracts of homogenized pancreas from WT, LepTg, Akita, and LepTg: Akita mice at 18 weeks of age ($n \geq 5$ in each group). *E*: Double immunofluorescent stainings against insulin (green) and glucagon (red) in pancreatic sections from WT, LepTg, Akita, and LepTg:Akita mice at the age of 18 weeks. Scale bar indicates 50 μm. *F*: α-Cell and β-cell areas per islet

Akita mice were elevated compared with WT mice; however, they remained >4% lower than those of Akita mice, even at 40 weeks of age (Fig. 1D). Average HbA$_{1c}$ levels were 11.4 ± 0.9% in Akita, 7.5 ± 1.1% in LepTg:Akita, 3.9 ± 0.3% in LepTg, and 4.1 ± 0.9% in WT mice from 5 to 40 weeks of age.

Time course of body weight and food intake. The body weight of all groups of mice increased gradually from birth (Fig. 1E). LepTg:Akita mice weighed significantly less than did Akita mice from 5 to 20 weeks of age. Akita mice lost body weight after 20 weeks of age. Percent body fat at 18 weeks of age was 27.6 ± 1.9, 29.9 ± 0.5, 2.1 ± 0.5, and 1.0 ± 0.4% for WT, LepTg, Akita, and LepTg:Akita mice, respectively.

The food intake of Akita mice was nearly double that of the other three groups of mice during the observation period (Fig. 1F). Food intake did not differ significantly between the WT, LepTg, and LepTg:Akita mice at any time during the study.

Pair-feeding experiments. To investigate whether leptin's ability to decrease food intake is the main reason for its efficacy in improving diabetes, Akita mice were pair fed to achieve the ad libitum food intake of LepTg:Akita mice for from 3 weeks to 8 weeks of age. Although pair feeding reduced the body weight of Akita mice to that of LepTg:Akita mice (Fig. 1G), it did not significantly improve the blood glucose levels in the Akita mice (Fig. 1H).

Glucose tolerance and insulin sensitivity. GTTs were performed to evaluate glucose metabolism further (Fig. 2A). At 8 weeks of age, Akita mice had reduced glucose tolerance; however, LepTg:Akita mice exhibited normal glucose tolerance similar to that of the LepTg and WT mice of the same age. At 16 weeks of age, Akita mice developed more severe glucose intolerance than that at 8 weeks of age. Although glucose tolerance was impaired in 16-week-old LepTg:Akita mice compared with that of LepTg and WT mice, it was better than that of 8- and 16-week-old Akita mice. These results indicate that hyperleptinemia significantly improved glucose tolerance during the progressive course of diabetes in Akita mice.

ITTs were performed to determine whether the improved glucose tolerance observed in LepTg:Akita mice was associated with insulin sensitivity (Fig. 2B). At 8 weeks of age, both LepTg and LepTg:Akita mice showed similar, exaggerated hypoglycemic responses to insulin relative to WT and Akita mice (Fig. 2C). At 18 weeks of age, the effect of insulin was blunted in LepTg mice compared with that in LepTg:Akita mice and was comparable with those in WT and Akita mice. At 28 weeks of age, glucose responses to insulin in LepTg and WT mice were severely impaired compared with those in Akita and LepTg:Akita mice. Insulin sensitivities in LepTg and WT mice deteriorated in parallel with advancing age and increasing body weight. In contrast, insulin sensitivities in Akita mice did not deteriorate with age, and the enhanced sensitivity in LepTg:Akita mice did not change at all during the course of our study.

Secretion and production of insulin and glucagon. Insulin secretion in response to a maximal glucose challenge was assessed. Plasma insulin levels were measured after an injection of glucose (3 g/kg body wt i.p.) (Fig. 3A).

The fasting insulin levels were similar in LepTg, Akita, and LepTg:Akita mice at 8 weeks of age, and all were significantly lower than in WT mice at the same age.

However, the fasting plasma insulin-to-glucose ratio was about three times higher in LepTg:Akita mice than in Akita mice. Both WT and LepTg mice showed an acute insulin response to glucose, Akita mice had virtually no response, and LepTg:Akita mice maintained a slow and slight response.

Plasma glucagon concentration was measured in ad libitum–fed mice at 22 weeks of age (Fig. 3B). Despite marked hyperglycemia, the glucagon level in Akita mice was nearly twice that in WT and LepTg mice. By contrast, LepTg:Akita mice had a normal plasma glucagon level, equivalent to that in the WT and LepTg mice.

Total pancreatic insulin contents of the Akita and LepTg:Akita mice decreased similarly to about one-tenth of those in the WT and LepTg mice (Fig. 3C). The pancreatic glucagon content of the Akita mice was twice that of the WT and LepTg mice; the glucagon content of the LepTg:Akita was half that in Akita mice (Fig. 3D).

Immunohistochemical examination of the pancreas revealed that Akita mice had profoundly abnormal islet histology with few active β-cells and a higher proportion of α-cells compared with WT and LepTg mice (Fig. 3E and F). LepTg:Akita mice had fewer β-cells, but α-cell hyperplasia was suppressed relative to Akita mice (Fig. 3E and F). These characteristics agree with the plasma hormone levels (Fig. 3A and B) and pancreatic hormone contents (Fig. 3C and D).

Lipids and ketones. Transgenic leptinemia did not significantly affect plasma levels of TG, NEFA, and β-hydroxybutyrate in WT mice (Fig. 4A–C). Akita mice had lower levels of both NEFA and β-hydroxybutyrate, possibly reflecting their lower adipose mass (Fig. 4B and C). None of the Akita mice developed ketonuria as determined by a urine ketone dipstick test (data not shown). In the Akita mice, leptin significantly decreased plasma TG levels by half (LepTg:Akita 37.4 ± 11.6 vs. Akita 89.4 ± 13.6 mg/dL; P < 0.05) (Fig. 4A) but did not change plasma NEFA or β-hydroxybutyrate levels (Fig. 4B and C).

Systemic oxidative stress. The plasma level of TBARS was examined as a marker of systemic oxidative stress (Fig. 4D). Akita mice exhibited the highest TBARS levels (35.1 ± 4.9 μmol/L) of the four genotypes at 18 weeks of age; the plasma TBARS levels were similar in WT, LepTg, and LepTg:Akita mice (WT 12.7 ± 1.3, LepTg 16.3 ± 1.8, and LepTg:Akita 17.8 ± 2.3 μmol/L).

Diabetic nephropathy. The renoprotective effects of leptin were investigated in Akita mice (Fig. 5A). Akita mice developed overt albuminuria at 12 weeks of age, and urinary albumin excretion was >200 μg/day during the follow-up period. By contrast, the increase in albuminuria was largely attenuated in LepTg:Akita mice throughout the follow-up period.

The increase in mesangial matrix (defined as mesangial area) observed in Akita mice was prevented completely in LepTg:Akita mice at 22 weeks of age (Fig. 5B and Table 1). Systolic and diastolic blood pressure and heart rates did not differ significantly between the four groups of mice (Table 1).

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in WT, LepTg, Akita, and LepTg:Akita mice at 18 weeks of age (n ≥ 4 in each group). Data are expressed as means ± SE. ††P < 0.01 for WT vs. LepTg, §§P < 0.01 for WT vs. Akita, ‡P < 0.05, ‡‡P < 0.01 for WT vs. LepTg:Akita, ¶P < 0.05, ¶¶P < 0.01 for LepTg vs. Akita, ★P < 0.05, ★★P < 0.01 for LepTg vs. LepTg:Akita, *P < 0.05, and **P < 0.01 for Akita vs. LepTg:Akita. (A high-quality digital representation of this figure is available in the online issue.)
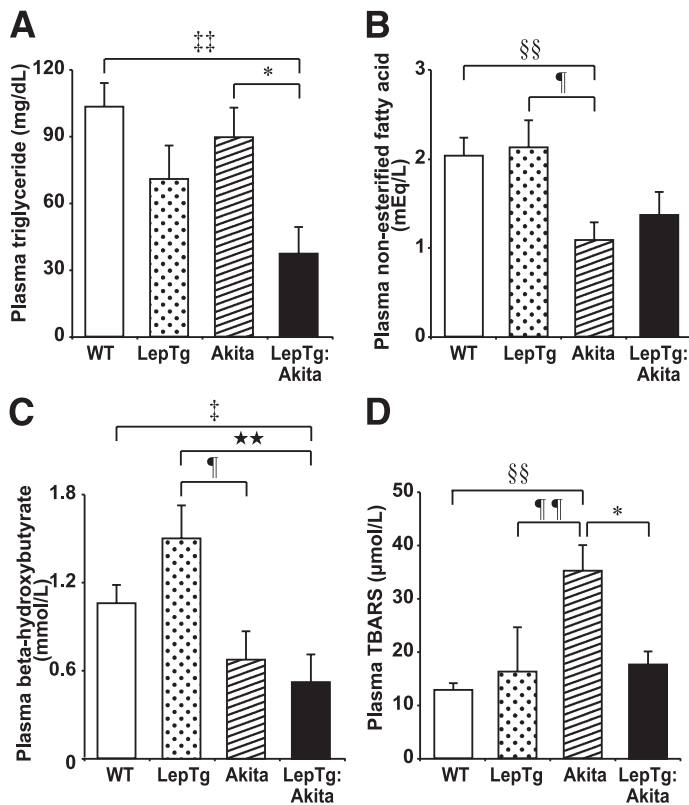
FIG. 4. Plasma levels of TG, NEFA, β-hydroxybutyrate, and TBARS. *A* and *B*: Fasting plasma levels of TG (*A*) and NEFA (*B*) concentrations in WT, LepTg, Akita, and LepTg:Akita mice at 18 weeks of age (*n* ≥4 in each group). *C*: Fasting plasma levels of β-hydroxybutyrate concentrations in WT, LepTg, Akita, and LepTg:Akita mice at 11 weeks of age (*n* ≥4 in each group). *D*: Fasting plasma levels of TBARS concentrations in WT, LepTg, Akita, and LepTg:Akita mice at 18 weeks of age (*n* ≥4 in each group). Data are expressed as means ± SE. §§$P < 0.01$ for WT vs. Akita, ‡$P < 0.05$, ‡‡$P < 0.01$ for WT vs. LepTg:Akita, ¶$P < 0.05$, ¶¶$P < 0.01$ for LepTg vs. Akita, ★★$P < 0.01$ for LepTg vs. LepTg:Akita, and *$P < 0.05$ for Akita vs. LepTg:Akita.

Survival rate. As shown in Fig. 6, the first Akita mouse died at 27 weeks of age, and half of the mice died within 40 weeks. Before death, Akita mice exhibited rough coats and decreased activity. Blood analysis showed liver and kidney dysfunction and extreme hyperglycemia ~1,000 mg/dL without overt ketone body production.

By contrast, the life span was significantly longer in LepTg:Akita mice than in Akita mice (*n* ≥ 14, $P < 0.01$), and none of the LepTg:Akita mice died during the observation period of 1 year (Fig. 6). Considering that the median survival time of male C57BL/6 mice is ~120 weeks (The Jackson Laboratory, Bar Harbor, ME), the survival rate did not appear to be affected significantly in LepTg:Akita mice.

DISCUSSION

The current study demonstrates that transgenic expression of leptin raised its plasma concentration to the level observed in morbidly obese individuals, markedly reduced mortality, and prolonged the survival time in Akita mice. The extension of life span in LepTg:Akita mice was accompanied by various beneficial effects on the course of diabetes.

We have pursued the therapeutic potential of leptin as an antidiabetic agent using transgenic skinny mice and propose that leptin could be used therapeutically in the treatment of diabetes of different etiology and pathophysiology (2,5–8,11–13). Leptin effectively improves glucose and lipid metabolism in streptozotocin (STZ)-induced insulinopenic diabetic mice (7) and in mildly obese mice fed a high-fat diet and administered low-dose STZ (6). Transgenic overexpression of leptin can delay the onset of insulin resistance and diabetes in KKAy mice at younger ages, when they are of normal weight (8). These findings suggest that leptin alone is effective in treating diabetes without obesity-induced leptin resistance. We also found that transgenic overexpression of leptin can rescue insulin resistance and diabetes in a mouse model of lipoatrophic diabetes, showing that leptin should be effective in the treatment of lipoatrophic diabetes (5). Therapeutic leptinemia can be achieved clinically by subcutaneous injection of recombinant human leptin (11,12). Leptin has been used in the treatment of human diabetes in patients with leptin deficiency and lipodystrophy (11–14).

Leptin delayed the onset and progression of diabetes in Akita mice. Hyperglycemia after a 16-h fast was prevented completely for >10 months in LepTg:Akita mice. The onset of the increase in the 6-h fasting blood glucose and HbA$_{1c}$ levels was also delayed for at least several weeks. Good metabolic control was achieved after the onset of diabetes in LepTg:Akita mice. There are several possible explanations for this glucose-lowering effect of leptin. This study demonstrated that the constitutive hyperleptinemia (approximately 10 times higher than control) strongly and stably increased insulin sensitivity in Akita mice. Leptin increases the effects of insulin in suppressing hepatic glucose production and stimulating muscular glucose uptake (2). The mechanisms through which leptin regulates insulin signaling are not understood completely, although reduction in ectopic fat deposition, especially in the liver and muscle, and activation of AMP-activated protein kinase in the peripheral tissues via stimulation of the hypothalamic-autonomic nervous system are important components (6,15,16). Increased mitochondrial biogenesis and oxygen consumption in skeletal muscle and adipose tissue may also contribute to the increase in glucose disposal independent of insulin action (15,17,18). Previous studies also demonstrated the antidiabetic effects of leptin in insulin-deficient diabetic rodents (19,20).

This study showed no attenuation of the biological effects of leptin (increase in insulin sensitivity and decrease in food intake) in Akita mice throughout the long follow-up. Most of the obese patients have elevated plasma leptin levels (21,22), implying leptin resistance for weight control (23). The basis for such leptin resistance is not understood enough, although such resistance coexists with hyperinsulinemia and insulin resistance (24). This context suggests that leptin has potent therapeutic effects on insulin-deficient diabetes with minimum insulin intervention (7). We and others reported that exogenously administered leptin can normalize hyperglycemia in STZ-induced diabetes, when fed plasma insulin levels were >0.10 ng/mL (7,25). The glycemic control of LepTg:Akita mice worsened gradually when the plasma insulin levels became extremely low (<0.10 ng/mL). These results show that the threshold of plasma insulin level is ~0.10 ng/mL, above which leptin can prevent hyperglycemia.

Akita mice, which have low plasma insulin and leptin levels, have increased food intake. Transgenic hyperleptinemia prevented hyperphagia in Akita mice. Although short-term food restriction did not affect hyperglycemia in Akita mice, continuous reduction in food intake might
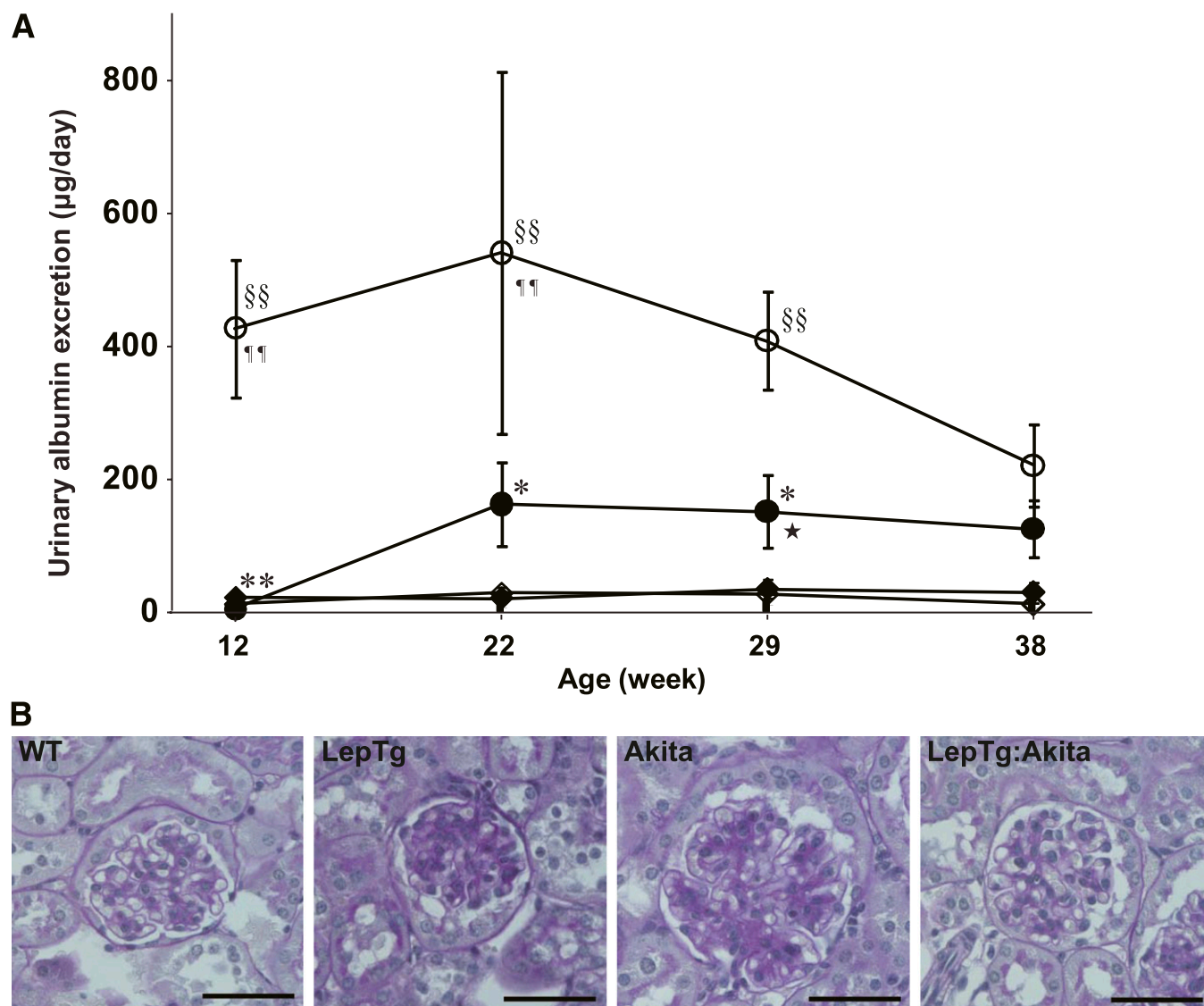
FIG. 5. Urinary albumin excretion and histology of glomeruli. *A*: Time course of urinary albumin excretion of WT ($\diamond$), LepTg ($\blacklozenge$), Akita ($\circ$), and LepTg:Akita ($\bullet$). Data are expressed as means ± SE ($n \geq 4$ in each group). §§$P < 0.01$ for WT vs. Akita, ¶¶$P < 0.01$ for LepTg vs. Akita, ★$P < 0.01$ for LepTg vs. LepTg:Akita, *$P < 0.05$, and **$P < 0.01$ for Akita vs. LepTg:Akita. *B*: PAS-staining of representative glomeruli from WT, LepTg, Akita, and LepTg:Akita mice at 22 weeks of age. Scale bar indicates 50 μm. (A high-quality color representation of this figure is available in the online issue.)

play a role in the antidiabetic effect of leptin. In insulin-deficient diabetic animals, adiposity and plasma leptin levels decrease and food intake increases concomitantly (26). In our previous report, leptin administration reversed hyperphagia by correcting the imbalance in the hypothalamic neuropeptide expression in STZ-administered mice (7). Upregulation of orexigenic neuropeptides (neuropeptide Y and agouti-related peptide) and downregulation of anorexigenic neuropeptides (proopiomelanocortin) were observed in the hypothalamus of insulin-deficient diabetic mice (7,27). Leptin and insulin are crucial signals that convey "adiposity negative feedback" information to the hypothalamus. Our previous and present results indicate that leptin is useful for preventing diabetic hyperphagia.

Glucose-stimulated insulin secretion and pancreatic insulin content were markedly lower in both LepTg:Akita and Akita mice compared with WT and LepTg mice; however, both the plasma concentration and pancreatic content of glucagon decreased to the normal level in LepTg:Akita mice. Leptin is reported to suppress the

synthesis and secretion of insulin by pancreatic β-cells (28,29). Our results did not reveal any negative effect of leptin on β-cell function in Akita mouse but showed that systemic hyperleptinemia plays a role in restoring the insulin-glucagon balance to proper equilibrium, which is lost in Akita mice. Wang et al. (30) reported recently that like insulin, leptin suppresses glucagon secretion in NOD mice. Our current data suggest that the antiglucagon and insulin-sensitizing effects of leptin on glucoregulatory hormones are therapeutically useful actions.

We found that chronic overexpression of leptin effectively prevented the development of diabetic nephropathy in Akita mice, as we have also demonstrated in lipoatrophic diabetic A-ZIP/F1 mice (31). Leptin suppressed the induction of massive albuminuria and the expansion of mesangial matrix in the glomeruli of Akita mice. Increased urinary albumin excretion and mesangial matrix accumulation are well-established features of diabetic nephropathy. Akita mice manifest the typical renal injury observed in human diabetic nephropathy that is associated

TABLE 1
Renal characteristics of 22-week-old F1 mice

	WT	LepTg	Akita	LepTg:Akita
Albuminuria (μg/day)	32.0 ± 9.4	23.5 ± 13.1	544.6 ± 272.7	165.9 ± 62.1*
Urine volume (mL/day)	1.7 ± 0.4	0.9 ± 0.1	27.4 ± 4.6	7.3 ± 2.2**
Mesangial area (μm^2)	1,210 ± 50	1,278 ± 50	2,025 ± 76	1,222 ± 67**
Body weight (g)	37.1 ± 1.7	38.4 ± 1.3	26.2 ± 0.9	25.0 ± 0.5
Kidney weight (g)	0.21 ± 0.03	0.18 ± 0.02	0.26 ± 0.04	0.21 ± 0.01
sBP (mmHg)	96.8 ± 0.3	93.5 ± 1.9	98.5 ± 4.1	107.7 ± 1.3
dBP (mmHg)	44.0 ± 0.5	42.3 ± 3.8	47.6 ± 3.9	52.2 ± 4.7
Heart rate (bpm)	714 ± 18	726 ± 7	611 ± 32	730 ± 30

Values are expressed as the mean ± SE ($n = 4$). sBP, systolic blood pressure; dBP, diastolic blood pressure. *$P < 0.05$. **$P < 0.01$, LepTg:Akita vs. Akita.

with renal hypertrophy, glomerular hypertrophy, mesangial expansion, and overt proteinuria (32). The nephropathy in Akita mice is more similar to that seen in human patients with diabetes than is the nephropathy in chemically induced diabetic mice (32). Although several reports suggest that leptin exerts profibrotic action in the kidney, which has caused concern about possible pathogenic roles of leptin in obesity-related glomerulopathy (31,33), the present results clearly show that leptin prevented renal injury in Akita mice. It is likely that leptin is beneficial for nephropathy in patients with insulin-dependent diabetes.

Reduced insulin action in diabetes elevates plasma TG levels by decreasing lipoprotein lipase activity and increasing hormone-sensitive lipase activity. We demonstrated previously a significant reduction in plasma VLDL-TG level in LepTg mice (34). Leptin suppresses the activities of liver lipogenic enzymes (35,36). In LepTg:Akita mice, decreased levels of plasma lipids may also result from dwindling body fat stores (orthotopic and ectopic) because of augmented effects of leptin. Since hypertriglyceridemia is reported to be an independent cardiovascular risk factor in patients with glucose intolerance (37), our observation of the TG-lowering effects of leptin may be useful in preventing and treating diabetic cardiovascular complications.

Lipid peroxidation is a well-established mechanism of cellular injury as a diabetes complication and is used as an indicator of oxidative stress. Increased oxidative stress also participates in the development and progression of diabetes and its complications (38). LepTg:Akita mice maintained normal levels of plasma TBARS, in contrast to Akita mice. Increased levels of serum TBARS were reported in patients with peripheral arterial disease, ischemic heart disease, hypertension, and diabetes (39). Our finding that leptin relieved systemic oxidative stress in Akita mice is of interest because TBARS level does not depend only on the blood glucose or lipid level but reflects the complex net redox balance (40,41).

Various interventions have been reported to improve the metabolic profiles in Akita mice (42,43). Targeted disruption of the transcription factor C/EBP homologous protein gene or C/EBP-β gene alleviates endoplasmic reticulum stress in pancreatic β-cells and improves hyperglycemia in Akita mice (42). Intracerebroventricular administration of adeno-associated viral vector expressing leptin also attenuates hyperglycemia in Akita mice (43). However, it is unclear whether those interventions are directly applicable to the human therapeutic settings. Therapeutic leptinemia in LepTg mice is induced by transgenic overexpression. Leptinemia can be achieved clinically by subcutaneous injection of leptin, as shown in leptin-replacement therapy

(11,12,44). Whether leptin affects the immunological processes of type 1 diabetes remains to be established. Leptin, a cytokine-like hormone, is suggested to be involved in linking nutritional status and immune response (45). Leptin administration accelerates autoimmune diabetes in NOD mice, and the incidence of diabetes is significantly reduced in NOD mice and BB/Wor rats with Ob-R mutation (46–48). However, another study that assessed NOD mice with defective leptin signaling (Ay, db/db, and ob/ob) has shown that leptin is not essential for the development of autoimmune diabetes (49). Whether the beneficial effects of leptin in Akita mice can be translated to type 1 diabetes in humans will be important to determine.

In conclusion, the current study demonstrates that leptin has a therapeutic impact on the onset and progression of glucose intolerance, diabetes complications, and longevity in a mouse model of insulin-deficient nonobese diabetes. These data offer proof of concept that leptin may be useful as a long-term therapeutic agent for treating human diabetes.
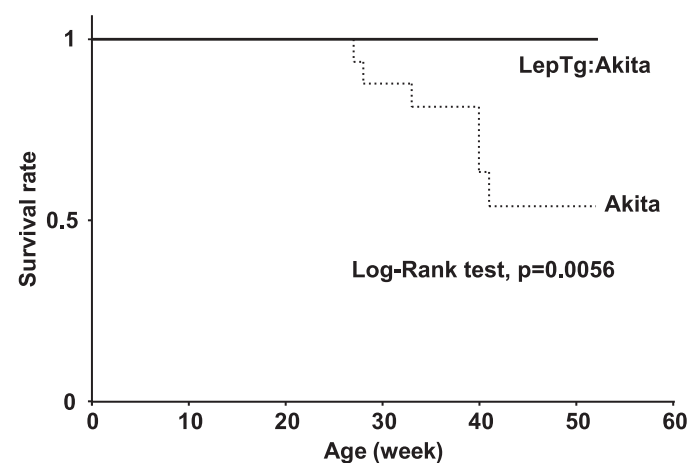
FIG. 6. Survival rates. Survival curves of Akita (dotted line) and LepTg: Akita (solid line) mice ($n \geq 11$ in each group). Survival rate of Akita mice markedly decreased relative to LepTg:Akita mice ($P < 0.01$) and was ~50% at 52 weeks of age.

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