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Increased Glucose Metabolism and Insulin Sensitivity in Transgenic Skinny Mice Overexpressing Leptin

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Excess of body fat, or obesity, is a major health problem and confers a higher risk of cardiovascular and metabolic disorders such as diabetes, hypertension, and coronary heart disease. Leptin is an adipocyte-derived satiety factor that plays an important role in the regulation of energy homeostasis, and its synthesis and secretion are markedly increased in obese subjects. To explore the metabolic consequences of an increased amount of leptin on a long-term basis in vivo, we generated transgenic skinny mice with elevated plasma leptin concentrations comparable to those in obese subjects. Overexpression of leptin in the liver has resulted in complete disappearance of white and brown adipose tissue for a long period of time in mice. Transgenic skinny mice exhibit increased glucose metabolism accompanied by the activation of insulin signaling in the skeletal muscle and liver. They also show small-sized livers with a marked decrease in glycogen and lipid storage. The phenotypes are in striking contrast to those of recently reported animal models of lipoatrophic diabetes and patients with lipoatrophic diabetes with reduced amount of leptin. The present study provides evidence that leptin is an adipocyte-derived antidiabetic hormone in vivo and suggests its pathophysiologic and therapeutic implications in diabetes. *Diabetes* 48:1822–1829, 1999

Obesity is defined as increased mass of adipose tissue, conferring a higher risk of cardiovascular and metabolic disorders such as diabetes, hypertension, and coronary heart disease (1,2). However, the molecular basis for that association has remained to

be understood. The adipose tissue itself serves as the site of triglyceride storage and free fatty acids (FFA)/glycerol release in response to changing energy demands (1). It also participates in the regulation of a wide variety of energy homeostasis as an important endocrine organ that secretes a number of biologically active substances such as FFA, adiponin, angiotensinogen, and tumor necrosis factor- α (TNF- α) (1,3).

Leptin is an adipocyte-derived hormone with multiple regulatory potentials (4–6). It may act primarily as a blood-borne satiety factor that decreases food intake and increases energy expenditure, thereby leading to a significant reduction in body weight (7–9). The biological actions of leptin are thought to be mediated largely through interactions with its cognate receptor that is expressed in the hypothalamus (10). Several lines of evidence have also suggested that leptin may play important roles in the regulation of carbohydrate metabolism. Central administration of leptin is reported to increase glucose turnover and uptake and decrease hepatic glycogen storage, suggesting that the effect of leptin on glucose metabolism is mediated largely via the central mechanisms (11–14). On the other hand, several in vitro studies have shown that leptin can modulate insulin action in adipocytes, myocytes, and hepatocytes (15–18) and affect insulin secretion from pancreatic β -cells (19).

Numerous studies revealed that plasma leptin concentrations are elevated in several models of rodent obesity and human obesity in proportion to the degree of adiposity (20–23), suggesting that hyperleptinemia may be involved in the pathogenesis of obesity and its related disorders. On the other hand, plasma leptin concentrations are markedly decreased in patients with anorexia nervosa or those with lipoatrophic diabetes (24,25), suggesting that hypoleptinemia or leptin deficiency may play an important role in the pathogenesis of these disorders.

Because of the potent biological actions of leptin, its potential usefulness as a therapeutic agent for obesity and its related disorders has attracted the interest of many investigators. In obese rodents and humans, however, plasma leptin concentrations are elevated, suggesting the state of leptin resistance in obesity (20–22). It is not yet established whether the apparent resistance to leptin is mediated by increased amount of leptin in obesity and whether leptin is effective for a long period of time in vivo.

To elucidate the metabolic consequences of increased amount of leptin on a long-term basis in vivo, we have produced transgenic skinny mice with elevated plasma leptin con-

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BAT, brown adipose tissue; CSF, cerebrospinal fluid; FFA, free fatty acids; IR- β , insulin receptor β -chain; IRS-1, insulin receptor substrate-1; p85, 85-kD regulatory subunit of PI 3-kinase; PI 3-kinase, phosphatidylinositol 3-kinase; RIA, radioimmunoassay; SAP, serum amyloid P component; TNF- α , tumor necrosis factor- α ; WAT, white adipose tissue.

centrations comparable to those in obese subjects and examined glucose metabolism in these animals.

RESEARCH DESIGN AND METHODS

Generation of transgenic mice overexpressing leptin. The full-length mouse leptin cDNA was isolated by reverse transcription-polymerase chain reaction. A fusion gene comprising the human serum amyloid P component (SAP) promoter and mouse leptin cDNA coding sequences was designed so that the hormone expression might be targeted to the liver (26,27). The purified *HindIII-XhoI* fragment (10 µg/ml) was microinjected into the pronucleus of fertilized BDF 1 mice (Charles River Japan, Yokohama, Japan) eggs. The viable eggs were transferred into the oviducts of pseudopregnant female ICR mice (Japan CLEA, Osaka, Japan) using standard techniques. Transgenic founder mice were identified by Southern blot analysis of tail DNAs using the mouse leptin cDNA fragment as a probe. Transgenic mice were used as heterozygotes. Animals were maintained on standard rat food (CE-2, 352 kcal/100 g, Japan CLEA) on a 12 h light/12 h dark cycle. For some experiments, pair feeding of nontransgenic littermates to the food intake of transgenic mice was performed for 10–14 days. All experimental procedures were approved by the Kyoto University Graduate School of Medicine Committee on Animal Research.

Body weight and length and food intake measurements. Body weight was measured daily, beginning at 4 weeks of age. Body length was measured by manual immobilization and extension of the mouse to its full length, always by the same individual, and measurement of the nasal-anal length was in centimeters. Cumulative food intake was measured using 10-week-old male and female transgenic and nontransgenic littermates daily over a 2-week period. Mice were housed in individual metabolic cages for 1 week before measurement.

Histology. Sections (5 µm thick) from Bouin's fixed paraffin-embedded specimens were stained with hematoxylin and eosin, periodic acid Schiff (PAS), and Sudan IV, where applicable, and examined by light microscopy. The size of pancreatic islets was quantitated by a Macintosh computer (Apple, Cupertino, CA) combined with a microscope connected to a CCD camera (Olympus, Tokyo) as described (28). The size of the islets was determined by counting ~100 islets per mouse ($n = 4$).

Plasma leptin, glucose, triglyceride, and insulin measurements. Blood was sampled from the retro-orbital sinus of mice at 9:00 A.M. Plasma leptin concentrations in 15-week-old transgenic and pair-fed nontransgenic littermates were determined using the radioimmunoassay (RIA) for mouse leptin (Linc Research Immunoassay, St. Louis, MO). Plasma glucose, triglyceride, and insulin concentrations were determined by the glucose oxidase method with a reflectance glucometer (One Touch II; Lifescan, Milpitas, CA), the enzymatic kit (Wako Pure Chemical, Osaka, Japan), and the RIA with rat insulin standards (Linc Research Immunoassay), respectively.

Glucose and insulin tolerance tests. For glucose tolerance test, after an overnight fast, 20-week-old transgenic and nontransgenic littermates were treated with an intraperitoneal injection of 5.6 µmol/g glucose. For insulin tolerance test, mice were injected intraperitoneally with 3.0 µmol/g human regular insulin (Nobolin R; Novo Nordisk, Copenhagen, Denmark). Blood was sampled from the mouse tail vein before and 15, 30, and 60 min after the injection.

Western blot analysis and phosphatidylinositol 3-kinase activity assay. After an 8-h fast, 18- to 22-week-old mice were injected intraperitoneally with 45 µmol/g human regular insulin. The liver and gastrocnemius muscle were removed 3 and 5 min after insulin injection, respectively, and homogenized in ice-cold buffer containing 50 mmol/l HEPES (pH 7.5), 137 mmol/l NaCl, 1 mmol/l $MgCl_2$, 1 mmol/l $CaCl_2$, 2 mmol/l Na_3VO_4 , 10 mmol/l sodium pyrophosphate, 0.1 mg/ml aprotinin, 1 mmol/l phenylmethylsulfonyl fluoride, and 1% Nonidet P-40 (29). Homogenates were centrifuged, and supernatants were immunoprecipitated with an anti-insulin receptor substrate-1 (IRS-1) antibody (#06-248; Upstate Biotechnology, Lake Placid, NY). Supernatants or immunoprecipitated samples were used for SDS-PAGE and Western blot analysis using antibodies for insulin receptor β -chain (IR- β) (#sc-711 Santa Cruz Biotechnology, Santa Cruz, CA), IRS-1, and 85-kD regulatory subunit of phosphatidylinositol 3-kinase (PI 3-kinase) (p85) (Upstate Biotechnology, Lake Placid, NY) (29,30). Immunoprecipitates of IRS-1 were also assayed for PI 3-kinase activity using phosphatidylinositol as a substrate essentially as described (29,30).

Statistical analysis. Statistical significance was assessed by analysis of variance repeated measures analysis (Statview 4.01; Abacus Concepts) and Student's *t* test, where applicable. All values were expressed as means \pm SE.

RESULTS

Generation of transgenic mice overexpressing leptin.

A fusion gene comprising the human SAP promoter and mouse leptin cDNA coding sequences was designed so that the

hormone expression might be targeted to the liver (Fig. 1A). Several transgenic lines with differing copy number of the transgene were obtained.

Northern blot analysis identified a single mRNA species of 1.4 kb in size found abundantly in the liver from transgenic mice (Fig. 1B). No significant transgene expression was detected in the liver from transgenic mice before birth (data not shown). This is consistent with previous reports that the human SAP promoter is highly specific to the liver and is only active after birth (26,27). In 4-week-old transgenic mice carrying 30 copies of the transgene, hepatic transgene expression was ~10-fold greater than leptin mRNA expression in the epididymal white adipose tissue (WAT) from nontransgenic littermates, which was comparable to that from *ob/ob* mice (5) (Fig. 1B). At 15 weeks of age, plasma leptin concentrations were elevated significantly in transgenic mice in proportion to the transgene copy number (Fig. 1C). Plasma leptin concentrations in transgenic mice with 30 copies of the transgene were 60.6 ± 14.4 ng/ml ($n = 11$), which is ~12-fold higher than the concentrations in nontransgenic littermates (4.8 ± 0.6 ng/ml) ($n = 10$). Plasma leptin concentrations are roughly constant throughout the day and are unchanged with age or with possible age-related changes in the size of the liver. The plasma leptin concentrations attained in transgenic mice are comparable to or slightly higher than those in obese rodents and humans (20–23), suggesting that the phenotypic changes found in these animals are physiologically and pathophysiologically relevant. Transgenic mice overexpressing leptin were viable throughout adulthood with no appreciable complications. Here we analyzed the phenotypes of transgenic mice carrying 30 copies of the transgene.

Body weight and length of transgenic mice overexpressing leptin. During postnatal development, transgenic mice became markedly lean compared with nontransgenic littermates (Fig. 1D). No significant difference in body weight was observed between transgenic and nontransgenic littermates at birth. Body weights of 4-week-old male and female transgenic mice were 8.5 ± 0.3 and 8.3 ± 0.4 g, respectively, which were ~75% of those of nontransgenic littermates when fed ad libitum ($n = 16$) (Fig. 2A). At 17 weeks of age, transgenic mice gained weight ~20% less than nontransgenic littermates.

To determine whether transgenic mice overexpressing leptin exhibit alterations in linear growth, body length was measured in 17-week-old transgenic and nontransgenic littermates. No significant difference in linear growth (nasal-anal length) was noted between transgenic and nontransgenic littermates (10.2 ± 0.3 vs. 10.1 ± 0.1 cm, $n = 8$).

Food intake of transgenic mice overexpressing leptin. Ten-week-old male and female transgenic mice were monitored for food intake daily for a 2-week period. Cumulative food intake was markedly reduced in 10-week-old transgenic mice over 2 weeks (~70 and 75% in males and females, respectively, of those in nontransgenic littermates) ($n = 16$) (Fig. 2B). **Histology of adipose tissue in transgenic mice overexpressing leptin.** Transgenic mice overexpressing leptin developed no WAT postnatally. No visible adipose tissue was found in either subcutaneous, epididymal, mesenteric, or retroperitoneal fat depots from 17-week-old transgenic mice (Fig. 3A, left). At birth, the interscapular brown adipose tissue (BAT) was formed normally but disappeared by 3 weeks of age. No BAT was found in transgenic mice at 17 weeks of age

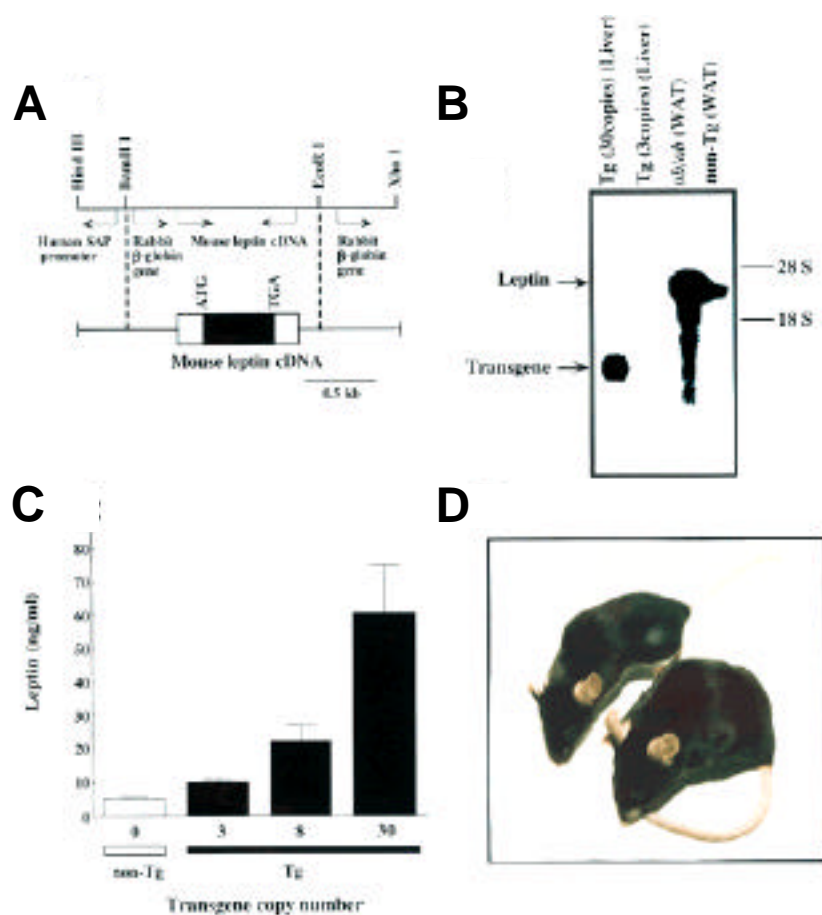


FIG. 1. Generation of transgenic mice overexpressing leptin. **A:** Schematic representation of the human SAP promoter/mouse leptin fusion gene. The coding region of mouse leptin cDNA is denoted by the closed box. **B:** Northern blot analysis of transgene expression in the liver from 4-week-old transgenic mice carrying 3 and 30 copies of the transgene and leptin mRNA expression in the epididymal WAT from 4-week-old nontransgenic littermates and *ob/ob* mice. Total RNA (20 μ g/lane) was analyzed. Tg, transgenic mice; non-Tg, nontransgenic littermates. **C:** Plasma leptin concentrations in 15-week-old male and female transgenic (Tg, ■) and nontransgenic (non-Tg, □) littermates. **D:** Gross appearance of a 24-week-old transgenic mouse with 30 copies of the transgene (Tg) and its nontransgenic littermate (non-Tg).

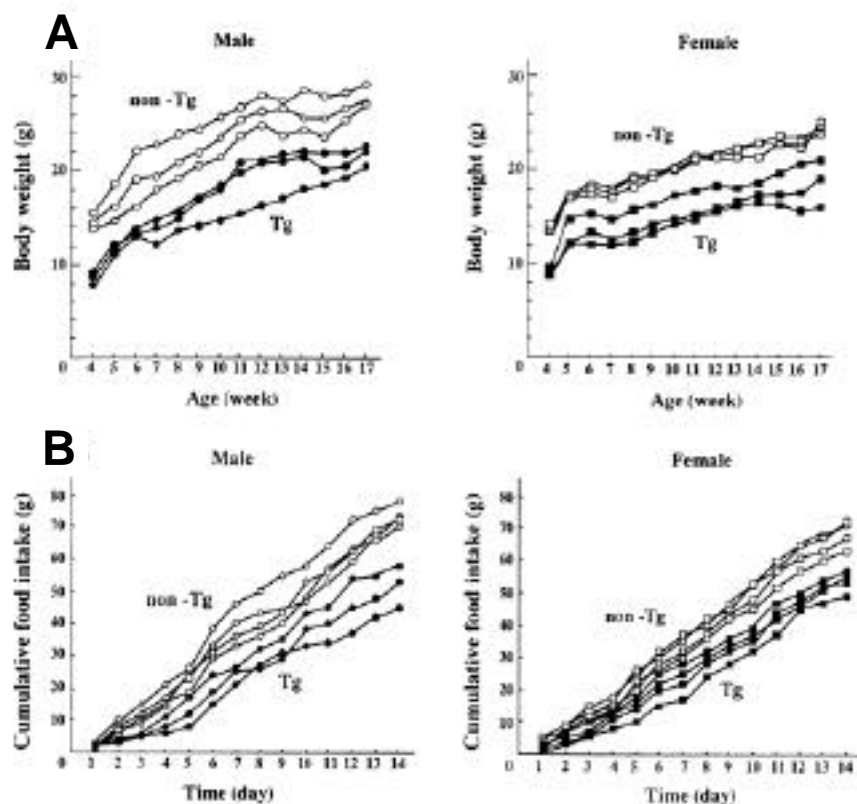


FIG. 2. Body weight gain and cumulative food intake of transgenic mice overexpressing leptin. **A:** Body weight gain of male and female transgenic (Tg, ● ■) and nontransgenic (non-Tg, ○ □) littermates. Each line represents the body weight gain of an individual mouse. **B:** Cumulative food intake of 10-week-old male and female transgenic (Tg, ● ■) and nontransgenic (non-Tg, ○ □) littermates. Each line represents the cumulative food intake of an individual mouse.

(Fig. 3A, right). Histologic analysis revealed no microscopic depots of adipose tissue even in periarticular, intramedullary, and periadrenal region sections from transgenic mice (Fig. 3B). No apparent WAT and BAT were found in transgenic mice overexpressing leptin at least up to 8 months after birth. We therefore call these animals transgenic skinny mice.

Glucose homeostasis in transgenic skinny mice. To elucidate the long-term effects of leptin on glucose metabolism, we measured plasma glucose, triglyceride, and insulin concentrations in transgenic skinny mice overexpressing leptin. No significant differences in plasma glucose concentrations were noted between 17-week-old transgenic skinny mice and pair-fed nontransgenic littermates at 9:00 A.M. (Fig. 4A). Plasma triglyceride and insulin concentrations were decreased significantly in transgenic skinny mice compared with pair-fed nontransgenic littermates ($P < 0.005$).

The size of pancreatic islets was markedly reduced in transgenic skinny mice compared with pair-fed nontransgenic littermates (Fig. 3C). The size of the pancreatic islet in transgenic skinny mice was ~40% of that in nontransgenic littermates. The marked reduction in the size of pancreatic islets may be a consequence of decreased insulin requirement caused by increased insulin sensitivity in transgenic skinny mice (see below).

Glucose and insulin tolerance tests were also performed using 20-week-old transgenic and pair-fed nontransgenic littermates. After intraperitoneal glucose injection, plasma glucose elevation was blunted significantly in transgenic skinny mice compared with nontransgenic littermates ($n = 5$, $P < 0.01$) (Fig. 4B). Plasma glucose concentrations returned to the baseline values as rapid as 60 min after the injection in transgenic skinny mice. When mice were injected with insulin, hypoglycemic response 15 min after the injection was exaggerated in transgenic skinny mice relative to pair-fed nontransgenic littermates ($n = 5$, $P < 0.01$) (Fig. 4C). These observations suggest increased glucose metabolism in transgenic skinny mice compared with nontransgenic littermates.

Insulin signaling for glucose utilization in the skeletal muscle from transgenic skinny mice. To elucidate the mechanism for increased glucose metabolism in transgenic skinny mice, insulin signaling for glucose utilization was examined in the skeletal muscle from 18- to 22-week-old transgenic and pair-fed nontransgenic littermates (Fig. 5, left). Western blot analysis revealed no significant differences in the amounts of IR- β , IRS-1, and p85 of PI 3-kinase in the gastrocnemius muscle between transgenic and pair-fed nontransgenic littermates (Fig. 5F-H, left). No appreciable differences in IRS-1-associated PI 3-kinase activity and associ-

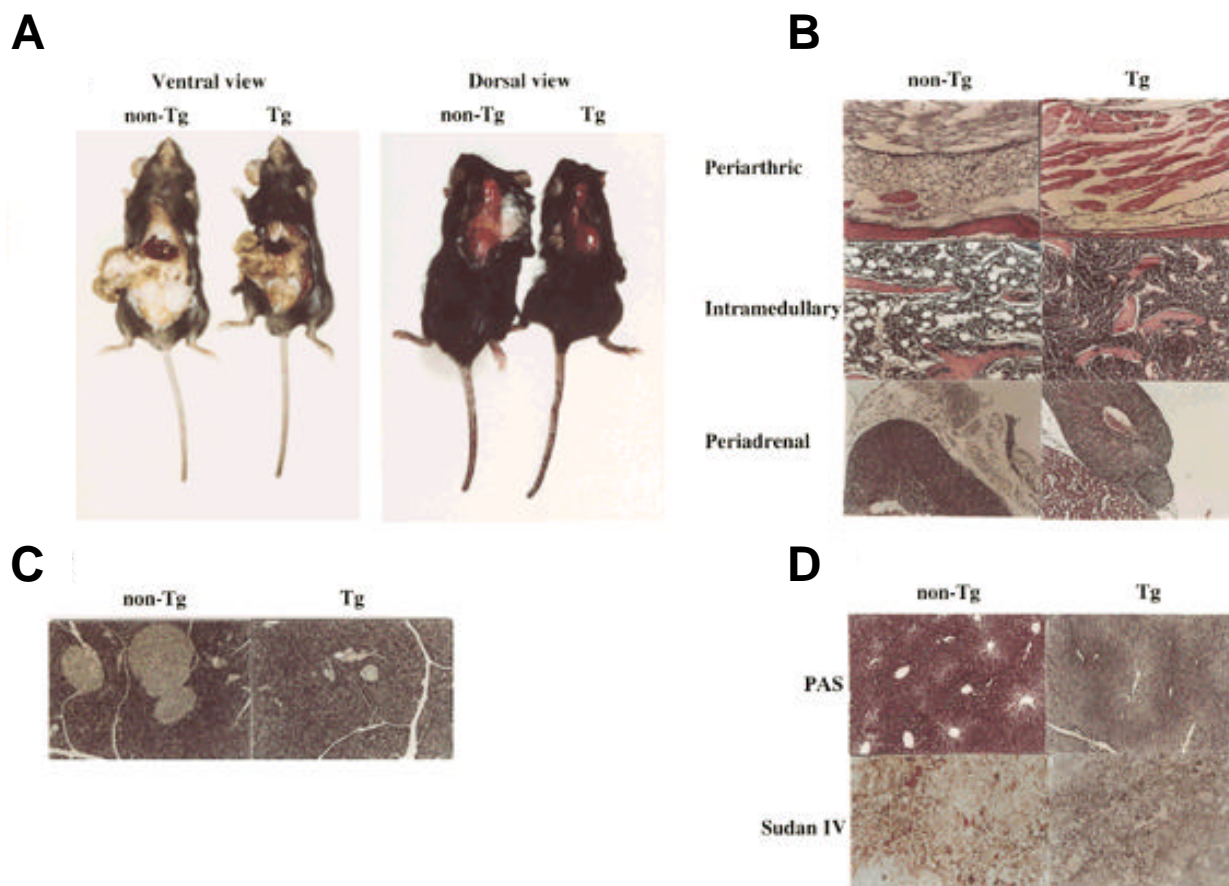


FIG. 3. Macroscopic inspection of transgenic skinny mice and microscopic analysis of the adipose tissue, pancreas, and liver. **A:** Macroscopic inspection of 17-week-old transgenic (Tg) and pair-fed nontransgenic (non-Tg) littermates. *Left:* ventral view. *Right:* dorsal view. **B:** Hematoxylin and eosin stainings of periarticular, intramedullary, and periadrenal region sections from 17-week-old transgenic (Tg) and pair-fed nontransgenic (non-Tg) littermates. Original magnification $\times 100$. **C:** Hematoxylin and eosin stainings of pancreatic islet sections from 17-week-old transgenic (Tg) and pair-fed nontransgenic (non-Tg) littermates. Original magnification $\times 100$. **D:** PAS and Sudan IV stainings of liver sections from 17-week-old transgenic (Tg) and pair-fed nontransgenic (non-Tg) littermates. Original magnification $\times 100$.

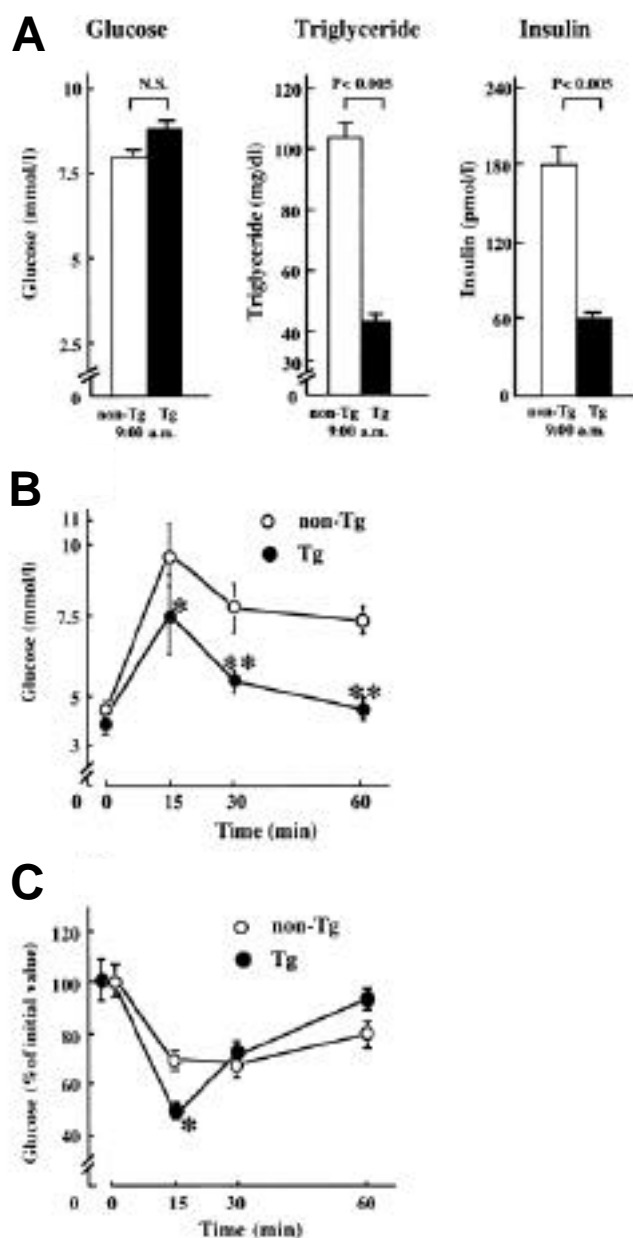


FIG. 4. Glucose homeostasis in transgenic skinny mice. **A:** Plasma glucose, triglyceride, and insulin concentrations in 17-week-old male transgenic (Tg, ■) and pair-fed nontransgenic (non-Tg, □) littermates, when fed ad libitum. **B:** Glucose tolerance test in 20-week-old male transgenic (Tg, ●) and pair-fed nontransgenic (non-Tg, ○) littermates. * $P < 0.05$ and ** $P < 0.01$ vs. nontransgenic littermates. **C:** Insulin tolerance test in 20-week-old male transgenic (Tg, ●) and pair-fed nontransgenic (non-Tg, ○) littermates. Plasma glucose concentrations were expressed as the percent of the initial values of transgenic and nontransgenic littermates. * $P < 0.01$ vs. nontransgenic littermates.

ation of IRS-1 with p85 were found in the skeletal muscle between transgenic and nontransgenic littermates without insulin treatment (Fig. 5A–C, left). After insulin stimulation, there was a significant increase in IRS-1-associated PI 3-kinase activity in transgenic skinny mice relative to pair-fed nontransgenic littermates (Fig. 5A and B, left) ($P < 0.005$). Insulin also increased the amount of PI 3-kinase co-precipitated with IRS-1 in the skeletal muscle from transgenic skinny mice relative to pair-fed nontransgenic litter-

mates (Fig. 5C, left) with a significant increase in tyrosine phosphorylations of IR- β and IRS-1 (Fig. 5D and E, left) ($P < 0.01$). These results indicate that insulin signaling for glucose utilization are increased in the skeletal muscle from transgenic skinny mice.

Hepatic lipid and glycogen metabolism in transgenic skinny mice. On macroscopic inspection, the size of the livers in transgenic skinny mice was smaller than that of nontransgenic littermates (Fig. 3A). The weight of the livers was decreased significantly in 17-week-old transgenic skinny mice compared with pair-fed nontransgenic littermates (852 ± 20 vs. $1,125 \pm 90$ mg, $n = 8$, $P < 0.001$). Histologic examinations revealed that the glycogen and triglyceride contents are markedly decreased in the liver from transgenic skinny mice (Fig. 3D).

Insulin signaling for glucose homeostasis was also examined in the liver from 18- to 22-week-old transgenic and pair-fed nontransgenic littermates (Fig. 5, right). Western blot analysis revealed no significant differences in the amounts of IR- β , IRS-1, and p85 of PI 3-kinase in the liver between transgenic and pair-fed nontransgenic littermates (Fig. 5F–H, right). No appreciable differences in IRS-1-associated PI 3-kinase activity and association of IRS-1 with p85 were found in the liver between transgenic and nontransgenic littermates without insulin treatment (Fig. 5A–C, right). After insulin stimulation, there was a significant increase in IRS-1-associated PI 3-kinase activity in the liver from transgenic skinny mice relative to pair-fed nontransgenic littermates (Fig. 5A and B, right) ($P < 0.005$). Insulin also increased the amount of PI 3-kinase co-precipitated with IRS-1 in the liver from transgenic skinny mice relative to pair-fed nontransgenic littermates (Fig. 5C, right) with a significant increase in tyrosine phosphorylations of IR- β and IRS-1 (Fig. 5D and E, right) ($P < 0.01$). These results indicate that insulin signaling for glucose homeostasis are increased in the liver from transgenic skinny mice.

DISCUSSION

The present study represents the first transgenic model system in which overexpression of a single hormone, leptin, can induce complete disappearance of its original site of production, the adipose tissue. Such phenotype might not occur if the transgene expression is targeted to the adipose tissue using adipocyte-specific promoters (31). Several transgenic or knockout mice with reduced mass of adipose tissue were reported, all of which should have no substantial amount of leptin (32–35). On the other hand, our transgenic skinny mice have no apparent adipose tissue due to overexpression of leptin and thus serve as a new genetic rodent model of reduced mass of adipose tissue for the elucidation of the roles of adipose tissue as well as the long-term effects of leptin in vivo.

In the present study, transgenic skinny mice exhibit increased glucose metabolism for a long period of time. Increased glucose metabolism in transgenic skinny mice may not be due to the lean phenotype because glucose metabolism is unchanged in severely underweight subjects (36). These observations, taken together, suggest that leptin can increase glucose metabolism independent of body weight change. The leptin-induced increase in glucose metabolism in transgenic skinny mice seems to be mostly via the central mechanisms, as suggested previously (11–14). However, leptin can also increase glucose metabolism via the periph-

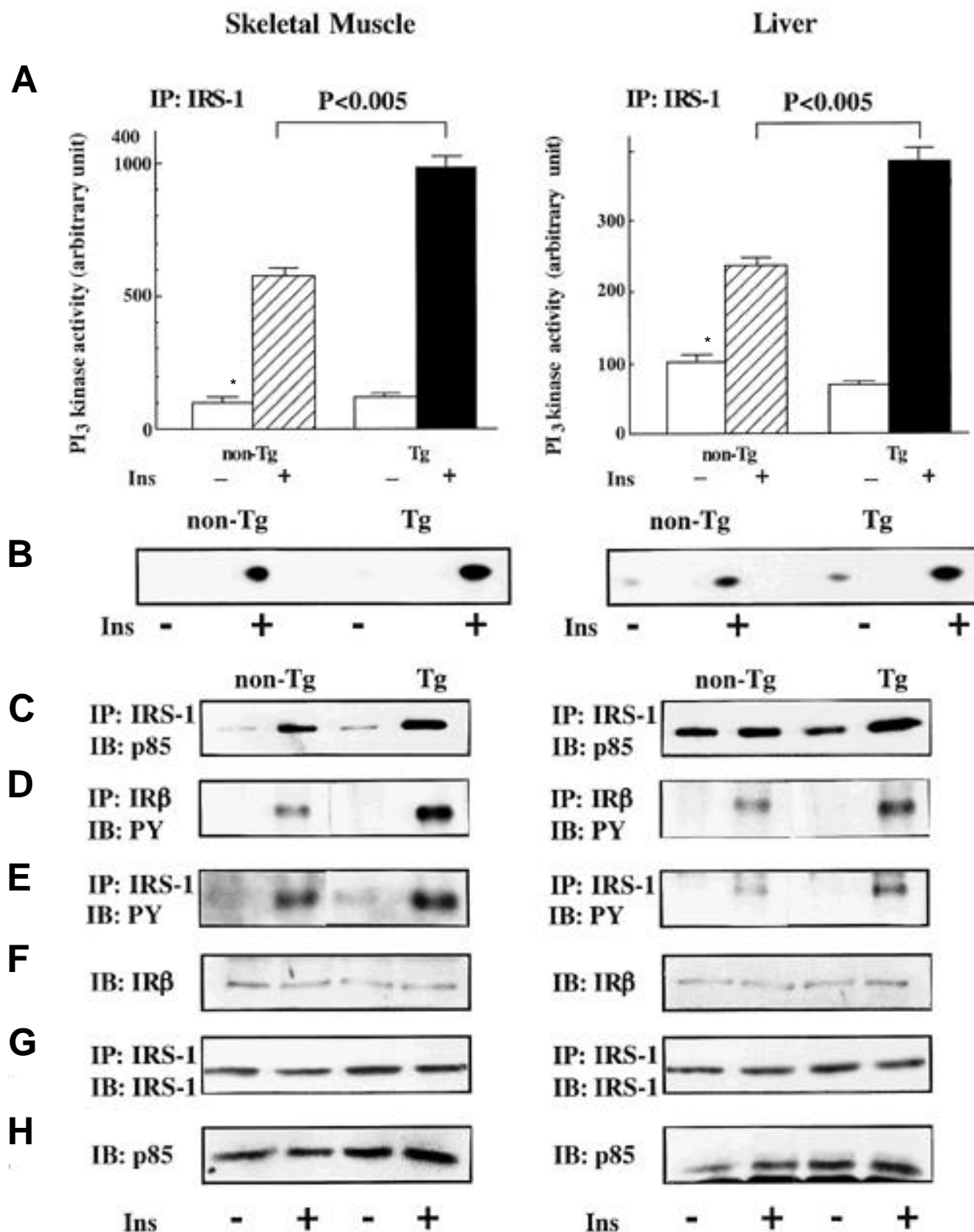


FIG. 5. Insulin signaling for glucose utilization in the skeletal muscle (*left*) and liver (*right*) from transgenic skinny mice. **A:** Insulin-stimulated activation of PI 3-kinase in the skeletal muscle (gastrocnemius muscle) and liver from transgenic and pair-fed nontransgenic littermates. Bar graphs marked with an asterisk showing basal IRS-1-associated PI 3-kinase activity in nontransgenic littermates are defined as 100%. Closed and hatched bars represent the values for transgenic and nontransgenic littermates treated with insulin, respectively. **B:** Autoradiographs of insulin-stimulated PI 3-kinase activity in the skeletal muscle and liver from transgenic and pair-fed nontransgenic littermates. **C:** Western blot analysis of amounts of IRS-1-associated p85 regulatory subunit of PI 3-kinase. **D:** Western blot analysis of amounts of phosphorylated tyrosine of IR-β. **E:** Western blot analysis of amounts of phosphorylated tyrosine of IRS-1. **F–H:** Western blot analysis of amounts of IR-β, IRS-1, and p85 regulatory subunit of PI 3-kinase. IP, immunoprecipitation; IB, immunoblotting; PY, phosphotyrosine; Tg, transgenic mice; non-Tg, pair-fed nontransgenic littermates.

eral mechanisms (15–18). In the present study, we also examined for the first time the long-term effect of leptin on insulin signaling for glucose homeostasis in the skeletal muscle and liver. The present study demonstrates that insulin signaling for glucose utilization is increased in the skeletal muscle from transgenic skinny mice. Furthermore, insulin signaling is also activated in the liver from transgenic skinny mice, which should suppress glycogenolysis and hepatic glucose output. Therefore, increased glucose metabolism in transgenic skinny mice is due at least in part to increased insulin sensitivity through the activation of insulin signaling in the skeletal muscle and liver. These findings are consistent with previous reports that leptin can enhance the insulin-mediated stimulation of glucose disposal and inhibition of hepatic glucose production (12–14). However, glucose metabolism might also be increased in transgenic skinny mice through an insulin-independent mechanism, as suggested previously (11).

Evidence has accumulated indicating that the adipose tissue plays an important role in the regulation of glucose homeostasis (1,2). There is a tight association between insulin resistance and adiposity; however, the mechanism for that association is not fully established. One hypothesis is that hypertrophied adipocytes secrete factors such as TNF- α that can cause insulin resistance (37). Thus, increased insulin sensitivity in transgenic skinny mice may be due to the absence of such adipocyte-derived factors that aggravate glucose homeostasis. On the other hand, it is known that a substantial amount of adipose tissue is necessary for normal glucose and lipid homeostasis, e.g., reduced mass of adipose tissue is often associated with severely insulin-resistant diabetes as in patients with lipotrophic diabetes (25) and in animal models for lipotrophic diabetes (33–35). Lipotrophic diabetes is characterized by an absence of adipose tissue, severe insulin resistance, hyperlipidemia, and hepatomegaly with a marked increase in hepatic lipid and glycogen storage (25,38). In lipotrophic diabetes, plasma leptin concentrations are markedly reduced because of the absence of body fat (25,33–35). However, transgenic skinny mice overexpressing leptin exhibit increased insulin sensitivity, decreased plasma triglyceride concentrations, and small-sized livers with a marked reduction of glycogen and triglyceride storage. These findings suggest that leptin can increase glucose and lipid metabolism with no apparent adipose tissue. If the adipose tissue produces humoral factors against diabetes, our data suggest that leptin may be such an adipocyte-derived anti-diabetic hormone. We therefore postulate that leptin deficiency may lead to abnormal glucose and lipid metabolism, which might contribute to the development of lipotrophic diabetes. In this regard, it is tempting to speculate that leptin treatment may improve abnormal glucose and lipid metabolism in lipotrophic diabetes. Previous studies using osmotic minipump or adenovirus-mediated gene transfer also show that chronic leptin administration induces disappearance of body fat (39–41). In these studies, however, there is no insight into the relationship between the leptin-induced disappearance of body fat and glucose metabolism.

A number of studies have demonstrated that plasma leptin concentrations are elevated in several models of rodent and human obesity, suggesting the state of leptin resistance in obesity (20–22). It has not been established whether the apparent resistance to leptin is mediated by increased amount of

leptin and whether leptin is effective for a long period of time in vivo. The present study demonstrates that leptin can exert its biological effects (decrease in food intake and body weight and increase in glucose metabolism) in normal weight animals for a long period of time, suggesting that constitutive activation of leptin receptor signaling does not necessarily induce leptin resistance. Boston et al. (42) showed that in yellow obese A^y/a mice, which are also resistant to leptin, deletion of the leptin gene by genetic crosses with ob/ob mice restores exogenously administered leptin sensitivity in A^y/a mice, suggesting that resistance to leptin results from desensitization of leptin signaling due to hyperleptinemia in these animals. Furthermore, a recent study (31) has demonstrated that exogenous leptin treatment can reduce food intake and body weight in obese ob/ob transgenic mice expressing leptin at a low dose, suggesting that leptin treatment is effective in obese subjects with a normal or relatively low amount of leptin. Collectively, these findings suggest leptin resistance may develop as a consequence of complex interactions between the increased amount of leptin and the obese phenotype. It was reported that the cerebrospinal fluid (CSF)/plasma leptin ratio is lower among obese human subjects with high plasma leptin concentrations, suggesting that reduced efficiency of brain leptin transport causes the apparent leptin resistance in human obesity (43,44). In this regard, it is interesting to know the CSF/plasma leptin ratio in transgenic skinny mice.

In conclusion, we succeeded in the generation of transgenic skinny mice overexpressing leptin. The present study provides evidence that leptin is an adipocyte-derived anti-diabetic hormone in vivo and suggests the pathophysiologic and therapeutic implications of leptin in diabetes. Our transgenic skinny mice will provide a new genetic model system with which to assess the roles of adipose tissue as well as the long-term effect of leptin in vivo.

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