Transgenic Overexpression of Leptin Rescues Insulin Resistance and Diabetes in a Mouse Model of Lipoatrophic Diabetes

Ken Ebihara, Yoshihiro Ogawa, Hiroaki Masuzaki, Mitsuyo Shintani, Fumiko Miyanaga, Megumi Aizawa-Abe, Tatsuya Hayashi, Kininori Hosoda, Gen Inoue, Yasunao Yoshimasa, Oksana Gavrilova, Marc L. Reitman, and Katsuwa Nakao

Lipoatrophic diabetes is caused by a deficiency of adipose tissue and is characterized by severe insulin resistance, hypoleptinemia, and hyperphagia. The A-ZIP/F-1 mouse (A-ZIPTg/+ mouse) is a model of severe lipoatrophic diabetes and is insulin resistant, hypoleptinemic, hyperphagic, and shows severe hepatic steatosis. We have also produced transgenic “skinny” mice that have hepatic overexpression of leptin (Leptg/+ mouse) and no adipocyte triglyceride stores, and are hypophagic and show increased insulin sensitivity. To explore the pathophysiological and therapeutic roles of leptin in lipoatrophic diabetes, we crossed Leptg/+ and A-ZIPTg/+ mice, producing doubly transgenic mice (Leptg/+;A-ZIPTg/+ mouse) virtually lacking adipose tissue but having greatly elevated leptin levels. The Leptg/+;A-ZIPTg/+ mouse were hypophagic and showed improved hepatic steatosis. Glucose and insulin tolerance tests revealed increased insulin sensitivity, comparable to Leptg/+ mice. These effects were stable over at least 6 months of age. Pair-feeding the A-ZIPTg/+ mice to the amount of food consumed by Leptg/+;A-ZIPTg/+ mice did not improve their insulin resistance, diabetes, or hepatic steatosis, demonstrating that the beneficial effects of leptin were not due to the decreased food intake. Continuous leptin administration that elevates plasma leptin concentrations to those of Leptg/+;A-ZIPTg/+ mice also effectively improved hepatic steatosis and the disorder of glucose and lipid metabolism in A-ZIP/F-1 mice. These data demonstrate that leptin can improve the insulin resistance and diabetes of a mouse model of severe lipoatrophic diabetes, suggesting that leptin may be therapeutically useful in the long-term treatment of lipoatrophic diabetes. Diabetes 50:1440–1448, 2001

Obesity, defined as increased adipose tissue mass, is associated with insulin resistance and type 2 diabetes (1). Adipose tissue’s major function is to store energy and release free fatty acids (FFAs) and glycerol as needed by the body. Adipose tissue also functions in the hormonal regulation of energy homeostasis (e.g., secreting tumor necrosis factor-α and leptin) (1).

Leptin is an adipocyte-derived hormone that plays a major role in the regulation of energy expenditure and food intake (2,3). In obese subjects, plasma leptin concentrations are elevated in proportion to the degree of adiposity (4–6), suggesting that leptin is a signal informing the body of the size of its adipose stores. Leptin deficiency in humans causes insulin resistance, whereas leptin deficiency in mice causes both insulin resistance and diabetes (7,8).

We have recently generated transgenic mice overexpressing leptin under the control of the liver-specific human serum amyloid P component promoter (9). The hyperleptinemia of these “skinny” mice causes disappearance of lipid from adipose tissue and provides a unique experimental system to investigate the long-term in vivo effects of chronic hyperleptinemia (9–12). The skinny mice have increased glucose metabolism and increased insulin sensitivity in both skeletal muscle and liver (9). These findings support the concept that leptin acts as an antidiabetic hormone in vivo (13–15), thereby suggesting its potential usefulness for the treatment of diabetes.

Lipoatrophic diabetes is caused by a deficiency of adipose tissue and is characterized by severe insulin resistance (16). Recently, mutations in the lamin A/C gene were identified as causing one form of lipodystrophy, Dunnigan’s familial partial lipodystrophy (17–19). However, the molecular mechanisms by which a paucity of fat causes diabetes remain to be elucidated. Plasma leptin concentrations are markedly reduced in both patients with lipoatrophic diabetes and rodent models of this disease (20–23). Given the antidiabetic effect of leptin, it is possible that leptin deficiency plays a role in the pathogenesis of lipoatrophic diabetes and that leptin may be useful for treatment of lipoatrophic diabetes (9). Recently, Shimomura et al. (23) developed a lipoatrophic mouse by expressing, in adipose tissue, a constitutively active form of the sterol regulatory element–binding protein-1c (aP2-
nSREBP-1c mice). Leptin treatment reversed the insulin resistance and diabetes of the aP2-nSREBP-1c mice (24). On the other hand, Gavrilo and colleagues (22,25) reported that leptin has a minimal effect in a more severely adipose-deficient mouse model of lipotoxic diabetes (A-ZIP/F-1 mice), which express, in adipose tissue, a protein that inactivates basic-zipper transcription factors. Whether leptin can be useful for treatment of severe lipoatrophic diabetes is not known, and if this effect exists, whether it lasts in the long term has not been tested.

In this study, we have genetically crossed the transgenic skinny and A-ZIP/F-1 mice to produce doubly transgenic mice virtually lacking adipose tissue but having greatly elevated leptin levels from birth onward. This report shows that transgenic overexpression of leptin rescues the insulin resistance and diabetes of A-ZIP/F-1 mice. The anti-diabetic effect was also observed with continuous leptin administration that elevated plasma leptin concentrations of A-ZIP/F-1 mice to those attained by transgenic overexpression of leptin. Collectively, the data of this study suggest that leptin may be therapeutically useful in the long-term treatment of severe lipoatrophic diabetes.

RESULTS

Physical appearance of transgenic mice. Figure 1A shows the appearance of ++/+ LepTg/+ A-ZIPTg/+ and LepTg/+A-ZIPTg/+ mice at 15 weeks of age. As reported (9), LepTg/+ mice were thinner than ++/+ mice, and the A-ZIPTg/+ animals had distended abdomens (22). The gross appearance of the LepTg/+A-ZIPTg/+ mice was similar to that of the LepTg/+ mice and not the A-ZIPTg/+ animals. The body length of A-ZIPTg/+ mice (88.1 ± 0.05 mm, n = 10) was longer than that of LepTg/+ or LepTg/+A-ZIPTg/+ mice (83.4 ± 0.04 mm and 84.0 ± 0.18 mm, n = 10, respectively). After removal of the skin, no subcutaneous WAT was visible in LepTg/+ or A-ZIPTg/+ and LepTg/+A-ZIPTg/+ mice, in contrast to the ample WAT visible in the ++/+ controls. The amount of interscapular BAT was reduced in LepTg/+ and A-ZIPTg/+ and LepTg/+A-ZIPTg/+ mice (Fig. 1B).

Transgene expression and plasma leptin concentrations. Northern blot analysis of liver mRNA revealed a significant amount of leptin message in the LepTg/+ and LepTg/+A-ZIPTg/+ mice but not in the ++/+ and A-ZIPTg/+ animals (Fig. 1C). Plasma leptin concentrations were significantly elevated in the LepTg/+ and LepTg/+A-ZIPTg/+ mice (31 and 52 ng/ml, respectively) relative to the ++/+ animals (7.6 ng/ml) (Fig. 1D). In contrast, leptin concentrations were significantly reduced in A-ZIPTg/+ mice (1.8 ng/ml).

Body weight and food intake. Figure 1E shows the growth curves for the transgenic mice. At 3 weeks of age, there was no significant difference in body weight among the four genotypes. However, by 4 weeks of age and thereafter, the LepTg/+ were significantly smaller than the ++/+ controls. In contrast, starting at week 8, the A-ZIPTg/+ mice were heavier than the ++/+ controls. The body weight of the LepTg/+A-ZIPTg/+ mice was comparable to that of LepTg/+ mice. Food intake was significantly reduced in the LepTg/+ mice and increased in the A-ZIPTg/+ mice compared with the ++/+ controls (Fig. 1F). Like the LepTg/+ mice, the LepTg/+A-ZIPTg/+ mice ate less than the ++/+ controls. Thus, both the body weight and food intake of the LepTg/+A-ZIPTg/+ mice are indistinguishable from those of the LepTg/+ mice and different from the A-ZIPTg/+ mice. This suggests that the A-ZIPTg/+ phenotype is suppressed by the overexpression of leptin.

Organ weights and histology. Table 1 presents the organ weights of 15-week-old ++/+ LepTg/+ A-ZIPTg/+ and LepTg/+A-ZIPTg/+ mice. The epididymal fat pads were markedly smaller in the LepTg/+A-ZIPTg/+ and LepTg/+A-ZIPTg/+ mice compared with the ++/+ mice (P < 0.05, n = 5). The liver was smaller in the LepTg/+ and LepTg/+A-ZIPTg/+ mice (P <
0.05) but enlarged in A-ZIPTg/+ mice ($P < 0.05$) relative to +/+ controls. The liver color was lighter in the A-ZIPTg/+ mice but darker in the LepTg/+ mice, as compared with the +/+ controls. The LepTg/+:A-ZIPTg/+ liver color was indistinguishable from that of +/+ livers (data not shown).

Microscopic examination revealed no subcutaneous WAT in the LepTg/+, A-ZIPTg/+, and LepTg/+:A-ZIPTg/+ mice (Fig. 2A). The interscapular BAT from the A-ZIPTg/+ mice resembled WAT, with large lipid droplets in the adipocytes (Fig. 2B). In contrast, the BAT from LepTg/+ mice was more eosinophilic than the +/+ BAT and had smaller lipid droplets. The appearance of the BAT from the LepTg/+:A-ZIPTg/+ mice was intermediate between that of A-ZIPTg/+ and +/+ mice. Livers from A-ZIPTg/+ mice

**TABLE 1**

Anatomical characteristics of F1 mice

<table>
<thead>
<tr>
<th>Mouse types</th>
<th>Body weight (g)</th>
<th>epiWAT (g)</th>
<th>BAT (g)</th>
<th>Liver (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/+</td>
<td>23.2 ± 0.6</td>
<td>0.062 ± 0.004</td>
<td>0.089 ± 0.005</td>
<td>1.22 ± 0.04</td>
</tr>
<tr>
<td>LepTg/+</td>
<td>18.3 ± 0.6 (0.79)</td>
<td>0.006 ± 0.001 (0.10)</td>
<td>0.035 ± 0.001 (0.39)</td>
<td>0.89 ± 0.03 (0.73)</td>
</tr>
<tr>
<td>A-ZIPTg/+</td>
<td>26.6 ± 1.0 (1.15)</td>
<td>0.012 ± 0.001 (0.19)</td>
<td>0.070 ± 0.003 (0.79)</td>
<td>3.38 ± 0.18 (2.77)</td>
</tr>
<tr>
<td>LepTg/+:A-ZIPTg/+</td>
<td>18.6 ± 0.3 (0.80)</td>
<td>0.006 ± 0.001 (0.10)</td>
<td>0.023 ± 0.001 (0.26)</td>
<td>0.82 ± 0.01 (0.67)</td>
</tr>
<tr>
<td>Pair-fed A-ZIPTg/+</td>
<td>18.8 ± 0.5 (0.81)</td>
<td>0.005 ± 0.001 (0.08)</td>
<td>0.033 ± 0.003 (0.37)</td>
<td>1.01 ± 0.08 (0.83)</td>
</tr>
</tbody>
</table>

Data are the means ± SE ratio. The ratio is the value expressed as a fraction of the +/+ value.
showed marked hepatic steatosis (Fig. 2C). Lipid accumulation in the LepTg/+ mouse was markedly reduced, though not completely normal. The size of the pancreatic islets was increased in A-ZIPTg/+ mice but reduced in LepTg/+ and LepTg/+;A-ZIPTg/+ mice (Fig. 2D). No appreciable difference in islet size was observed between LepTg/+ and LepTg/+;A-ZIPTg/+ mice.

**Glucose and lipid metabolism.** Fed plasma glucose concentrations were 98 mg/dl in the LepTg/+ mice, which was significantly lower than the 139 mg/dl of the +/+ mice (Fig. 3A). The A-ZIPTg/+ mice were hyperglycemic (302 mg/dl). However, transgenic overexpression of leptin completely normalized the A-ZIPTg/+ glucose levels (98 mg/dl in the LepTg/+;A-ZIPTg/+ mice). Plasma insulin concentrations were lower in the LepTg/+ mice (2.2 pmol/l) than in +/+ mice (15.6 pmol/l) (Fig. 3B). Insulin concentrations were markedly elevated in A-ZIPTg/+ mice (604.0 pmol/l), but completely reduced by leptin overexpression (8.2 pmol/l in LepTg/+;A-ZIPTg/+ mice).

Plasma triglyceride and FFA concentrations were significantly reduced in LepTg/+ mice and elevated in A-ZIPTg/+ mice relative to +/+ controls (Fig. 3C and D). Leptin overexpression reduced the triglyceride and FFA concentration in A-ZIPTg/+ mice to the level of +/+ mice but not quite to the level of the LepTg/+ mice.

Glucose and insulin tolerance tests revealed increased insulin sensitivity in LepTg/+ mice and insulin resistance in A-ZIPTg/+ mice (Fig. 3E and F). Leptin overexpression in the LepTg/+;A-ZIPTg/+ mice caused the insulin sensitivity of the A-ZIPTg/+ group to increase to the level of the LepTg/+ mice.

Taken together, these results demonstrate that hepatic leptin overexpression effectively compensates for the lack of adipose tissue of the A-ZIPTg/+ mice, preventing hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and increased FFA levels.

**Pair-feeding experiments.** We next investigated whether leptin’s action to decrease food intake is the reason for its efficacy in preventing diabetes. To do this, we pair-fed A-ZIPTg/+ mice to the amount of food consumed by LepTg/+;A-ZIPTg/+ mice for 12 weeks. The body weights of LepTg/+;A-ZIPTg/+ and pair-fed A-ZIPTg/+ mice were similar and 30% less, respectively, than that of the ad libitum–fed A-ZIPTg/+ mice (Table 1). No histological differences in the WAT, BAT, liver, or pancreas were found between pair-fed A-ZIPTg/+ and ad libitum–fed A-ZIPTg/+ mice (Fig. 2A–D). In addition, pair-feeding did not improve the plasma glucose, insulin, triglyceride, or FFA concentrations of the A-ZIPTg/+ mice (Fig. 4A–D). Finally, pair-feeding did not markedly improve either the glucose or insulin tolerance tests of the A-ZIPTg/+ mice (Fig. 4E and F). Thus, the improved phenotype of the LepTg/+;A-ZIPTg/+ mice caused by leptin is not simply due to the reduced food intake.

**Leptin injection experiments.** We explored whether exogenous administration of leptin that elevates plasma
FIG. 3. Glucose and lipid metabolism of 15-week-old +/+ LepTg/+ A-ZIPTg/+ and LepTg/+ A-ZIPTg/+ mice. Plasma glucose (A), insulin (B), triglyceride (C), and FFA (D) concentrations are shown (n = 8 per group). Blood samples were obtained from fed mice at 9:00 A.M. *P < 0.05 vs. +/+ mice. Glucose (E) and insulin (F) tolerance tests of +/+ (●), LepTg/+ (●), A-ZIPTg/+ (□), and LepTg/+ A-ZIPTg/+ (■) mice (n = 8 per group). *P < 0.05 vs. +/+ mice.
leptin concentrations to those of LepTg/+;A-ZIPTg/+ mice can improve lipoatrophic diabetes of A-ZIP/F-1 mice. To do this, we examined the metabolic effect of continuous leptin administration in A-ZIP/F-1 mice. At the third and sixth days of the experiment, continuous leptin administration through two osmotic minipumps increased plasma leptin concentrations in A-ZIP/F-1 mice to 57 and 54 ng/ml, respectively, roughly comparable to those in LepTg/+;A-ZIPTg/+ mice.
ZIPTg/+ mice (Fig. 5A). Body weight reduction in leptin-treated A-ZIP/F-1 mice was significantly greater than that in vehicle-treated group after the 6-day infusion (−2.30 ± 0.53 vs. 1.93 ± 0.10 g, n = 5, P < 0.01). Food intake of leptin-treated A-ZIP/F-1 mice was also decreased significantly relative to the vehicle-treated group throughout the experiment (3.48 ± 0.18 vs. 5.85 ± 0.11 g/day, n = 5, P < 0.01). Liver weight of leptin-treated A-ZIP/F-1 mice was markedly reduced relative to that of the vehicle-treated group at the end of experiment (1.52 ± 0.09 vs. 3.00 ± 0.54 g, n = 5, P < 0.01). Figure 5B shows the representative histological sections of livers from leptin- and vehicle-treated A-ZIP/F-1 mice. Lipid accumulation in the A-ZIP/F-1 mice liver was markedly reduced by leptin treatment, although the lipid droplets had not completely disappeared after the 6-day infusion. Hyperglycemia of A-ZIP/F-1 mice was improved by continuous leptin treatment but not by vehicle treatment (203 and 423 mg/dl in the leptin- and vehicle-treated groups, respectively) (Fig. 5C). Plasma insulin concentrations of A-ZIP/F-1 mice were normalized by leptin treatment (4.3 and 667.7 pmol/l in the leptin- and vehicle-treated groups, respectively) (Fig. 5D). Plasma triglyceride and FFA concentrations were also significantly reduced in the leptin-treated group relative to the vehicle-treated group (Fig. 5E and F).

Taken together, these results demonstrate that continuous leptin administration that elevates plasma leptin concentrations of A-ZIP/F-1 mice to those of LepTg/+ mice can effectively improve the disorders of glucose and lipid metabolism in A-ZIP/F-1 mice.

**DISCUSSION**

Deficiency of adipose tissue causes insulin resistance, diabetes, and dyslipidemia, although the underlying mechanisms are not known. In this study, we have bred A-ZIP/F-1 with LepTg/+ mice to study the effects of chronic hyperleptinemia in the near-total absence of adipose tissue. In LepTg/+::A-ZIPTg/+ mice, plasma leptin concentrations become elevated soon after birth and remain markedly elevated (~50 ng/ml) throughout the animal’s life. Transgenic overexpression of leptin rescues insulin resistance, diabetes, and much of the hepatic steatosis in A-ZIP/F-1 mice. This lack of insulin resistance, diabetes, and hepatic steatosis in LepTg/+::A-ZIPTg/+ is observed in both sexes and at up to 6 months of age (K.E., Y.O., H.M., F.M., M.S., M.A.-A., and K.N., unpublished data). This study demonstrates that leptin is useful for treatment of severe lipoatrophic diabetes and provides the first evidence for leptin as a long-term therapeutic agent for lipoatrophic diabetes.

Using the aP2-nSREBP-1c mouse model of lipoatrophic diabetes, Shimomura et al. (24) showed that leptin infusion for 12 days that achieved a concentration of ~3 ng/ml effectively treated the insulin resistance, diabetes, and hepatic steatosis of the mice (Table 2). In contrast, when leptin was infused into A-ZIP/F-1 mice, only moderate improvement in glucose and insulin levels occurred, despite increasing the plasma leptin concentrations by 5 ng/ml (25) (Table 2). The difference between these two studies could be attributable to the smaller amount of remaining fat in A-ZIP/F-1 mice (25). Indeed, surgical implantation of

**TABLE 2**

Leptin, fat mass, and insulin sensitivity in experimental mouse models

<table>
<thead>
<tr>
<th>Animals</th>
<th>Treatments</th>
<th>Leptin</th>
<th>Fat</th>
<th>Insulin sensitivity</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-ZIP/F-1</td>
<td>—</td>
<td>−</td>
<td>−</td>
<td>Decreased</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>+</td>
<td>−</td>
<td>Decreased</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>+ +</td>
<td>−</td>
<td>Normalized</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Fat transplantation</td>
<td>+</td>
<td>+</td>
<td>Normalized or increased</td>
<td>26</td>
</tr>
<tr>
<td>aP2-nSREBP-1c</td>
<td>—</td>
<td>−</td>
<td>±</td>
<td>Decreased</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>+</td>
<td>±</td>
<td>Normalized</td>
<td>24</td>
</tr>
<tr>
<td>ob/ob</td>
<td>—</td>
<td>−</td>
<td>+ +</td>
<td>Decreased</td>
<td>27</td>
</tr>
<tr>
<td>LepTg/+::A-ZIPTg/+</td>
<td>−</td>
<td>+ +</td>
<td>−</td>
<td>Normalized or increased</td>
<td>28</td>
</tr>
</tbody>
</table>
normal adipose tissue reverses the insulin resistance, diabetes, and hepatic steatosis of A-ZIP/F-1 mice while achieving plasma leptin concentrations of only \( \sim 3 \) ng/ml (26) (Table 2). Fat transplantation also decreases hepatic and muscular triglyceride content and restores the insulin stimulation of glucose utilization and the suppression of hepatic glucose production (27). Taken together, these findings suggest that an adequate mass of adipose tissue is required for the antidiabetic effects of physiological doses of leptin. With no substantial amount of leptin, fat transplantation may not be sufficient for recovery from insulin resistance and diabetes in A-ZIP/F-1 mice or lipoatrophic patients, given that leptin-deficient ob/ob mice are profoundly diabetic, though less severely so than A-ZIP/F-1 mice (22,25,28) (Table 2). This study demonstrates that even with minimal residual adipose tissue, either transgenic overexpression of leptin or exogenous leptin administration that achieves a plasma leptin concentration of \( \sim 50 \) ng/ml can rescue insulin resistance and diabetes in A-ZIP/F-1 mice, suggesting that adipose tissue itself is not required for the antidiabetic effects of chronic hyperleptinemia. Clinically, it is important to note that the efficacy of leptin treatment or fat transplantation in lipoatrophic diabetes may depend on how much adipose tissue remains or how much leptin is supplied.

The mechanisms through which leptin exerts its anti-diabetic actions are not known with certainty. The data suggest that leptin acts primarily through the hypothalamus, and thus most of its effects on insulin sensitivity are indirect (13–15). High doses of leptin cause a dramatic loss of visible adipose tissue, presumably by increasing energy expenditure and decreasing food intake. However, pair-feeding showed that decreased food intake does not explain the antidiabetic effects of leptin in LepTg/+:A-ZIPtg/+ mice. One possible explanation for the insulin sensitization by leptin is that the reduced levels of triglycerides and FFAs, both in the circulation and in body tissues (e.g., liver and muscle), is etiologic (29,30). Tissue triglyceride levels correlate with insulin resistance, being elevated in both lipoatrophic diabetes and obesity. It is not known whether the tissue triglycerides themselves cause insulin resistance or whether their levels serve as a surrogate for other molecules (e.g., fatty acyl-CoAs or malonyl-CoA) that may be causing insulin resistance. However, in LepTg/+A-ZIPtg/+ mice, the improvement in hepatic steatosis and circulating triglyceride and FFA concentrations is moderate relative to the reversal of insulin resistance and diabetes. This is consistent with findings of a relatively modest improvement in triglyceride and FFA concentrations but an impressive improvement in insulin resistance after fat transplantation into A-ZIP/F-1 mice (26). These observations suggest that additional mechanisms besides fat depletion of tissues may be involved in the antidiabetic effect of leptin in lipoatrophic diabetes.

In conclusion, this study demonstrates that transgenic overexpression of leptin can rescue insulin resistance and diabetes in a mouse model of severe lipoatrophic diabetes. Therefore, LepTg/+A-ZIPtg/+ mice provide the first experimental model demonstrating that leptin can be useful as a long-term therapeutic agent for severe lipoatrophic diabetes.

ACKNOWLEDGMENTS

We thank Shigeo Yura for discussion and Mayumi Nagamoto for technical assistance. The authors also acknowledge Yoshiko Isa and Yoko Nakajima for secretarial assistance. This work was supported in part by research grants from the Japanese Ministry of Education, Science, Sports, and Culture; the Japanese Ministry of Health and Welfare; the Yamanouchi Foundation for Research on Metabolic Disorders; the Inamori Foundation; the Naito Foundation; the Uehara Memorial Foundation; the Kato Memorial Trust for Nanbyo Research; the ONO Medical Research foundation; and “Research for the Future (RFTF)” of the Japanese Society for the Promotion of Science (JSPS-RFTF 96100204 and 98100801).

REFERENCES


