Insulin-like growth factor (IGF)-1 signaling might partly mediate effects of caloric restriction (CR), an experimental intervention for increasing longevity in mammals. The present study evaluated effects of recombinant human (rh)IGF-1 infusion on adipokine levels in CR and transgenic (Tg) dwarf rats with the reduced growth hormone–IGF-1 axis, which shared similar body weight and food intake. At 9 months of age, each rat received a continuous infusion of rhIGF-1 for 14 days, and rats received an injection of glucose after overnight fasting. Infusion of rhIGF-1 had metabolic effects in all rat groups although it did not affect insulin sensitivity in any of the groups. In addition, plasma adiponectin was decreased to the control group levels and plasma leptin was further reduced in CR and Tg rats. The similarity of phenotypes and adipokine responses to rhIGF-1 between CR and Tg rats supports a role for reduced IGF-1 signaling in the CR effect.

A moderately restricted calorie intake with an adequate supply of essential nutrients, referred to as caloric restriction (CR), slows down aging processes and prolongs life span in animals (1,2). Although the findings have been confirmed repeatedly in many laboratories, our understanding of the pathways that mediate the effect of CR is incomplete. Recent discoveries of longevity assurance genes emphasize the importance of reduced growth hormone (GH)–insulin-like growth factor (IGF)-1 signaling with a decrease in serum insulin levels (3,4). In CR rodents, plasma or serum IGF-1 levels are decreased during most of their life span (5–7). Serum insulin concentrations are also diminished concomitantly with enhanced insulin sensitivity (8–10).

We previously reported a transgenic (Tg) dwarf rat strain, in which the GH–IGF-1 axis is moderately suppressed by overexpression of the antisense GH gene, that significantly lives 7%–10% longer than do nontransgenic wild-type (W) rats (11). Subsequent studies revealed that these Tg rats, even if fed ad libitum (AL), have phenotypes similar to those of W-CR rats. Tg-AL rats have a similar body size and food intake as W-CR rats. The plasma IGF-1 levels are moderately reduced in both groups of rats as compared to W-AL rats (12). In W-CR and Tg-AL rats, glucose tolerance is normal or slightly improved, whereas the glucose-induced insulin response is lower, indicating activation of insulin-independent mechanisms as well as enhanced insulin sensitivity for glucose disposal (13). These findings led to our hypothesis that a reduction of the GH–IGF-1 axis is involved in the effect of CR on glucose metabolism and insulin sensitivity, and we concluded that the Tg rat strain is a good tool to analyze the effects of CR (13).

The present study was designed to investigate the potential role of the GH–IGF-1 axis in the CR effect. We administered recombinant human (rh)IGF-1 to W-CR, W-AL, and Tg-AL rats and assessed their responses, because data on heterozygous IGF receptor knockout mice (14) suggest that IGF-1 signaling in peripheral tissues is more important than GH signaling with respect to longevity. In those mice, the plasma IGF-1 concentrations are increased (14), probably because of an increase in GH signaling by the feedback mechanism that is elicited by the attenuation of peripheral IGF-1 signaling.

We focused on the CR-induced regulation of plasma adiponectin and leptin levels. Both adipokines are synthesized and secreted mostly from white adipose tissue (15,16), although they are regulated differentially. Plasma adiponectin levels are decreased in obese animals (15,16); plasma leptin levels, however, are proportionate to body fat content. Increased plasma adiponectin is associated with insulin-sensitizing, anti-diabetic, and anti-atherosclerotic effects (15,16). In contrast, elevated leptin is correlated to insulin resistance (15,16). In CR rodents, which have reduced plasma or serum IGF-1 concentration, plasma adiponectin levels are increased (17–19), whereas leptin levels are decreased (20,21). These alterations in plasma adipokine levels in CR rodents might be induced, at least in part, by the suppression of IGF-1 signaling.

**MATERIALS AND METHODS**

**Experimental Animals**

The present study was performed in accordance with the provisions of the Ethics Review Committee for Animal Experimentation at Nagasaki University. The rats used in the present study, and their care, are described in detail
and plasma samples were prepared and stored at 

After decapitation, trunk blood was collected, and serum

rats (W-CR and W-AL rats) were fasted overnight and injected intraperitoneally with D-glucose (1.0 g/kg body weight, 50% solution) or saline before being killed. Tg rats received saline injections before being killed were used for this calculation.

Quantitative Insulin Sensitivity Check Index (QUICKI)

To estimate insulin sensitivity, QUICKI (23) was calculated using fasting serum insulin (I0) and glucose (G0) according to the following formula; QUICKI = 1/\log(I0) + \log(G0). In the present analysis, insulin and glucose data in rats that received saline injections before being killed were used for this calculation.

Real-Time Reverse Transcription-Polymerase Chain Reaction

GH–mRNA was analyzed using quantitative real-time polymerase chain reaction (PCR) to evaluate possible negative feedback due to rhIGF-1 administration. Total RNA was extracted from the anterior pituitary gland using ISOGEN (Nippon Gene Inc., Toyama, Japan) and treated with DNase I (Promega, Madison, WI) to eliminate residual genomic DNA. According to the manufacturer’s protocol, extracted RNA was reverse-transcribed with a TaqMan Reverse Transcription Reagents kit (Applied Biosystems, Tokyo, Japan) and amplified using Platinum Quantitative PCR SuperMix-UDG (Invitrogen, Carlsbad, CA) with TaqMan MGB probes and primers (Applied Biosystems). Primers and probes for the GH and cyclophilin genes were designed based on the complete cDNA sequences of rat GH (NCBI accession No. U62779) and cyclophilin (NCBI accession No. M19533) with the software Primer Express (Applied Biosystems). Sequences for these primers and probes were as follows: GH sense, 5'-CCCCACCCGCAAGG; GH antisense, 5'-CAGCGGAGGCGAGAAT; GH TaqMan MGB probe sequence, 5'-FAM-CCAGCGAGAAGCGAAGCAAT; GH anti-sense, 5'-CAGCGGAGGCGAGAAT; GH TaqMan MGB probe sequence, 5'-FAM-CCAGCGAGAAGCGAAGCAAT.
RESULTS

Statistics

Data were presented as means ± standard error. The present data were analyzed by one-, two-, or three-factor analysis of variance (ANOVA) for the main effect of the rat group (RAT; W-AL, W-CR, or Tg-AL), administration of rhIGF-1 (IGF-1; + or −), injection of glucose (GLC; + or −), or their combinations. The interactions (RAT × IGF-1, RAT × GLC, IGF-1 × GLC, RAT × IGF-1 × GLC) were also analyzed. Fisher’s protected least significant difference (PLSD) test was performed as a post hoc test for multiple comparisons. Human IGF-1 and rat insulin concentrations were analyzed after logarithmic transformation of data to stabilize variance. The level of significance was set at a p value of less than .05; if p values were less than .10, it was noted. All statistical analyses were performed using StatView 5.0 software (SAS Institute Inc., Cary, NC).

Table 1. Effect of rhIGF-1 on Body Weight, Food Intake, and Fat Content

<table>
<thead>
<tr>
<th>Parameters</th>
<th>W-AL</th>
<th>W-CR</th>
<th>Tg-AL</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Increase in body weight*</td>
<td>−2.1 ± 0.2</td>
<td>3.4 ± 1.8</td>
<td>−2.2 ± 0.4</td>
</tr>
<tr>
<td>Cumulative food intake (g)³</td>
<td>136.5 ± 2.0</td>
<td>95.0 ± 2.0</td>
<td>92.9 ± 6.1</td>
</tr>
<tr>
<td>Perirenal fat (g)⁻³</td>
<td>3.08 ± 0.13</td>
<td>2.01 ± 0.11</td>
<td>2.22 ± 0.11</td>
</tr>
</tbody>
</table>

Notes: Data represent mean ± standard error (n = 9 or 10).

*Percent increase in body weight means the body weight change (%) between Day 0 (before operation) and 13.

³Cumulative food intake represents the amount of food ingested between Day 8 and 13.

⁻Perirenal fat denotes the weight change (g) which is normalized by 100 g body weight between Day 0 and 13.

†p < .0001 compared with each rat group with vehicle administration.

‡p < .0001 compared with W-AL and Tg-AL with rhIGF-1 administration.

§p < .002 compared with W-AL with vehicle administration.

Rats received subcutaneous infusions of rhIGF-1 or vehicle for 2 weeks (see “Materials and Methods” for details). rhIGF-1 = Recombinant human insulin-like growth factor-1; W-AL = nontransgenic wild-type rats fed ad libitum throughout the experiment; W-CR = nontransgenic wild-type rats fed a 30% calorie-restricted diet from 6 weeks of age; Tg-AL = transgenic dwarf rats fed ad libitum throughout the experiment.

Tg-AL rats consumed 30% less food than W-AL rats (Tg-AL, 16.0 ± 1.7 g/day; W-AL, 22 ± 1.9 g/day) before the infusion, thus the food intake of the Tg-AL rats was similar to that provided for W-CR rats (16.2 g/day). The food intake was slightly reduced after the minipump insertion surgery, and returned to presurgical levels within 2 days after the operation. The cumulative food intake for the last 6 days of the experiment was 29% lower in Tg-AL rats compared to W-AL rats (Table 1: RAT, p < .0001). The amount of food consumed by W-CR rats was similar to that consumed by Tg-AL rats. Infusion of rhIGF-1 did not significantly affect the food intake in any group of rats, although the food intake seemed to be slightly decreased (3%) in W-AL rats and slightly increased (6%) in Tg-AL rats. Infusion of rhIGF-1 did not significantly affect the food intake in any group of rats.

The perirenal fat tissue weight, when normalized by body weight, was lower in W-CR and Tg-AL rats than in W-AL rats (Table 1: RAT, p < .0001); there was no difference between W-CR and Tg-AL rats. The fat tissue weight was significantly decreased in W-AL rats infused with rhIGF-1, but not in W-CR or Tg-AL rats (IGF-1, p < .0001; RAT × IGF-1, p < .05).

Serum Human and Rat IGF-1 and GH-mRNA Levels in the Anterior Pituitary Gland

Human IGF-1 was detected in the serum in rhIGF-1–infused groups, and the levels of human IGF-1 were below the detection limit in vehicle-infused groups (Figure 1a). The serum concentrations of human IGF-1 did not significantly differ among the three rat groups. There was also no difference between the glucose-injected and saline-injected groups (GLC, p = .088).

The serum concentrations of rat IGF-1 were lowest in Tg-AL, next lowest in W-CR, and highest in W-AL rats (Figure 1a: RAT, p < .0001). Groups infused with rhIGF-1 had significantly lower rat IGF-1 concentrations, as compared to the vehicle-infused groups (IGF-1, p < .0001). There was a slight increase in IGF-1 concentration after the glucose injection, but it was not statistically significant (GLC, p = .0692).

Consequently, the total IGF-1 concentration, human and rat IGF-1 combined, was not increased in rhIGF-1-infused
groups when they were killed; on the contrary, the total concentration was slightly decreased in these groups (IGF-1, \( p < .05 \)). The total concentration of IGF-1 was greatest in W-AL rats (RAT, \( p < .002 \)), but there was no statistical difference between W-CR and Tg-AL rats. Glucose-injected groups had slightly increased total IGF-1 concentrations, particularly vehicle-infused, but not rhIGF-1-infused groups (IGF-1 \( \times \) GLC, \( p < .05 \)).

The GH-mRNA expression levels in anterior pituitary tissues were lowest in Tg-AL, next lowest in W-CR, and highest in W-AL rats (Figure 1b: RAT, \( p < .0001 \)). The expression levels were significantly reduced in rhIGF-1-infused groups (IGF-1, \( p < .0001 \)). There was no difference between the glucose-injected and saline-injected groups.

**QUICKI and Glucose-Induced Insulin Response**

QUICKI was greater in W-CR and Tg-AL rats than in W-AL rats (Figure 2a: RAT, \( p < .0005 \)); there was no significant difference between W-CR and Tg-AL rats. Administration of rhIGF-1 slightly increased QUICKI, but the increase was not significant (IGF-1, \( p = .0773 \)).

Serum glucose levels increased similarly in all rat groups after glucose load (Figure 2b: GLC, \( p < .0001 \)). Neither rat group nor rhIGF-1 administration significantly affected the serum glucose profile. The serum insulin concentrations were significantly increased after glucose load (Figure 2c: GLC, \( p < .0001 \)). As a whole, the serum insulin concentrations were lower in W-CR and Tg-AL rats than in W-AL rats (RAT, \( p < .0001 \)). The glucose-induced insulin response was lower in W-CR and Tg-AL rats compared with W-AL rats (RAT \( \times \) GLC, \( p < .05 \)). The serum insulin concentrations in Tg-AL rats did not differ from those in W-CR rats. There was no significant effect of rhIGF-1 on the insulin response.

**Plasma Adiponectin and Leptin Levels**

The plasma adiponectin concentrations were greater in W-CR and Tg-AL rats than in W-AL rats (Figure 3: RAT, \( p < .0001 \)), but did not differ between W-CR and Tg-AL rats. Infusion of rhIGF-1 significantly decreased plasma adiponectin levels in all three rat groups (IGF-1, \( p < .0001 \)). After rhIGF-1 infusion, the higher adiponectin levels in W-CR and Tg-AL rats returned almost to the W-AL levels. Glucose injection did not significantly affect plasma adiponectin concentrations as a whole; in W-AL rats, the plasma adiponectin response to glucose injection seemed to be slightly different from that in W-CR and Tg-AL rats (RAT \( \times \) IGF-1 \( \times \) GLC, \( p < .05 \)).

The plasma leptin concentrations were highest in W-AL, next in Tg-AL, and lowest in W-CR rats (Figure 4: RAT, \( p < .0001 \)). Administration of rhIGF-1 reduced plasma leptin levels by 32% in all three rat groups (IGF-1, \( p < .0003 \)). Glucose injection slightly elevated plasma leptin levels, particularly in the vehicle-groups, but not in rhIGF-1 groups (IGF-1 \( \times \) GLC, \( p < .05 \)).

**DISCUSSION**

The present study demonstrated that W-CR and Tg-AL rats had similar phenotypes for body weight, food intake, fat content, insulin sensitivity, and adipokines at baseline, although the GH–IGF-1 axis was slightly more suppressed in Tg-AL rats. The responses to rhIGF-1 infusion in these rats were also similar. These findings suggest the importance of the GH–IGF-1 axis in the CR effect.

In this study, we attempted to augment IGF-1 signaling in peripheral tissues in W-CR and Tg-AL rats. Infusion of a physiologic dose of rhIGF-1 elicited negative feedback regulation, thus the total serum IGF-1 concentrations did not increase compared with vehicle infusion in all three rat groups. Similar negative feedback regulation was reported.
in other studies (24–26). The present data, however, suggested that the net bioactivity of IGF-1 in peripheral tissues was increased by rhIGF-1 infusion, because body weight was significantly increased as compared to vehicle-treated groups in all three rat groups. In addition, perirenal fat weight was reduced in W-AL rats. These results are known metabolic effects exerted by IGF-I (27). Furthermore, the negative feedback suppression of the intrinsic GH–IGF-1 axis alone indicates an increase in IGF-1 signaling in animals, probably due to an increase in the free fraction of IGF-1 (28).

The present study confirmed increased plasma adiponectin levels in rats whose GH–IGF-1 axis was suppressed either by genetic manipulation or by CR, as reported in other animal models (17–19,29,30). This finding might be linked to the increased insulin sensitivity through the reduced fat
content in Tg-AL and W-CR rats. It is intriguing that rhIGF-1 infusion returned the augmented plasma adiponectin levels in Tg-AL and W-CR rats to the W-AL baseline value without any change in the fat content or insulin sensitivity. Because plasma adiponectin levels decrease at the early phase of type 2 diabetes in rhesus monkeys (31) and lower levels are predictive for the subsequent development of diabetes in humans (32), further analyses are needed to evaluate the long-term effect of rhIGF-1 on insulin sensitivity in Tg-AL or W-CR rats. The present data, however, suggest that exogenous rhIGF-1 modulates plasma adiponectin levels independent of insulin sensitivity or fat content in these CR and Tg rats.

It is sometimes difficult to distinguish the direct effect of IGF-1 from the effect of GH, because intrinsic GH and IGF-1 change concomitantly. Most rodent studies indicate that reduced GH signaling is associated with an increase in plasma adiponectin levels (29,30). The present data indicate that endogenous GH signaling was decreased in all rat groups due to negative feedback regulation induced by the rhIGF-1 infusion. The plasma adiponectin concentration was, however, decreased in those rats. As the metabolic effects of rhIGF-1 were noted, the findings suggest that a decrease in GH signaling alone cannot elevate the plasma adiponectin level in rats (i.e., a reduction in peripheral IGF-1 signaling is needed to increase the plasma adiponectin levels). In vitro studies suggest that IGF-1, GH, or GH-induced IGF-1 suppresses adiponectin synthesis or secretion in adipose tissue (33,34). Incubation of 3T3-L1 adipocyte cultures with IGF-1 or insulin, but not GH, downregulates the expression of adiponectin—mRNA in culture cells (33). In contrast, an in vitro human adipose tissue study demonstrated that incubation of small pieces of adipose tissue with rhGH decreased adiponectin levels in culture medium (34). In this in vitro study, however, did not exclude the possibility that rhGH induces the expression of IGF-1 within adipose tissue, i.e., rhGH treatment decreased the synthesis and secretion of adiponectin through an increase in IGF-1 in adipose tissue. It is indeed reported that GH positively regulates IGF-1 expression in adipose tissue in vivo (35). Thus, in the present study, it was thought that an increment in the net bioactivity of IGF-1 led to suppression of the synthesis and secretion of adiponectin in adipose tissue.

A considerable amount of IGF-1 is produced in adipose tissue. Although IGF-1 receptors have been demonstrated in adipose tissue in vitro (36), the in vivo evidence is poor (37). Although IGF-1 binds to insulin receptors [but with a 100-times-lower affinity (38)], there is no evidence for the involvement of insulin receptors in IGF-1 signaling. Therefore, mechanisms by which IGF-1 downregulates the synthesis and secretion of adiponectin in adipose tissue should be further investigated.

Plasma leptin levels increase in parallel with fat storage in the body (16). The present study confirmed that W-AL rats, with a greater fat content, had higher plasma leptin levels as compared with W-CR and Tg-AL rats. Plasma leptin levels are also regulated acutely by nutritional or hormonal status. Intraperitoneal injection of glucose or insulin upregulates leptin gene expression in normal, nonobese mice within 30 minutes (39); in obese mice, this acute response is attenuated. In the present study, the acute response of leptin to glucose was observed in all three rat groups. Administration of rhIGF-1 suppressed the acute response as well as the total plasma adiponectin levels in the three rat groups. Normal rats fed AL have reduced serum leptin levels after rhIGF-1 administration that correlates with a reduction in fat mass and serum insulin concentrations (40). Previous reports also indicate a positive role for insulin in the regulation of leptin expression in adipose tissues (41,42). In the present study, W-AL rats, but not W-CR or Tg-AL rats, had a reduced fat mass after rhIGF-1 infusion; serum insulin levels were not reduced in any rat group. These findings suggest the presence of an insulin-independent mechanism of leptin synthesis and secretion in adipose tissue, particularly in W-CR and Tg-AL rats.

Hypophysectomy reduces the leptin mRNA expression levels in rat adipose tissue (43). Exogenous administration of rhGH in hypophysectomized rats restores the serum IGF-1 levels, but does not affect the reduced gene expression of leptin (43). Infusion of rhIGF-1 in hypophysectomized rats, however, further decreases leptin gene expression, although serum IGF-1 concentrations only increase slightly (43). In addition, IGF-1 mRNA in the fat pads in hypophysectomized rats returned to normal levels following rhGH administration, but not by rhIGF-1 injection (43). A series of experiments by Boni-Schnetzler and colleagues (40,43) suggest that factors other than endogenous GH and IGF-1 are associated with the regulation of leptin mRNA expression in hypophysectomized rats. Nonetheless, their experiments (40,43) indicate that exogenous rhIGF-1 suppresses the synthesis of leptin in adipose tissue independent of endogenous IGF-1 status, thus the finding is consistent with the present study. As mentioned in the discussion of adiponectin, it remains to be elucidated how exogenous rhIGF-1 acts on adipose tissue, which does not substantially express IGF-1 receptors in vivo.

Summary

The similarity of phenotypes including the response to rhIGF-1 between W-CR and Tg-AL rats supports the role for the GH–IGF-1 axis in the CR effect. Although we did not exclude a role for GH, either directly or indirectly via IGF-1, the present study emphasizes the involvement of IGF-1 signaling in the CR effect, particularly the CR-induced alterations in plasma adiponectin levels. However, the precise mechanisms by which IGF-1 regulates synthesis and secretion of adipokines in CR animals remains to be elucidated.

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Address correspondence to Haruyoshi Yamaza, PhD, DDS, Pathology & Gerontology, Nagasaki University Graduate School of Biomedical Sciences, 12-4 Sakamoto 1-chome, Nagasaki City 852-8523, Japan. E-mail: hyamaza@net.nagasaki-u.ac.jp
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