Aging and fasting regulation of leptin and hypothalamic neuropeptide Y gene expression

HUA LI, MICHAEL MATHENY, NIHAL TÜMER, AND PHILIP J. SCARPACE
Geriatric Research, Education and Clinical Center, Department of Veterans Affairs Medical Center, Gainesville 32608-1197; and Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, Florida 32610

Li, Hua, Michael Matheny, Nihal Tümer, and Philip J. Scarpace. Aging and fasting regulation of leptin and hypothalamic neuropeptide Y gene expression. Am. J. Physiol. 275 (Endocrinol. Metab. 38): E405–E411, 1998.—To investigate the role of aging on the fasting-induced suppression of leptin gene expression and increase in hypothalamic neuropeptide Y (NPY) gene expression, we fasted or fed ad libitum male F-344xBN rats aged 3, 24, and 31 mo for 2 days. We examined leptin mRNA levels in retroperitoneal, inguinal, and epididymal white adipose tissue (WAT); serum leptin levels; and NPY mRNA levels in the hypothalamus. We found that leptin mRNA levels were increased from 3 to 24 mo and leveled off between 24 and 31 mo in both retroperitoneal WAT and inguinal WAT but were unchanged with age in epididymal WAT. Serum leptin levels increased with age, whereas hypothalamic NPY mRNA levels did not change with age. Fasting suppressed leptin gene expression in all three WATs and serum leptin. Moreover, this suppression of serum leptin and of leptin message in retroperitoneal WAT was less in aged rats. Conversely, fasting increased hypothalamic NPY message, again to a lesser extent in aged rats. In both fed (ad libitum) and fasted rats, there was a strong correlation between serum leptin and hypothalamic NPY mRNA levels in the young but not in either of the two aged groups. These data suggest that aged F-344xBN rats are leptin resistant and that the fasting regulation of serum leptin, leptin mRNA, and hypothalamic NPY mRNA is impaired in aged rats.

LEPTIN IS ONE REGULATOR of food intake and energy expenditure. It is the protein product of the Ob gene (32), which is synthesized by white adipose tissue (WAT). Leptin is an afferent signal molecule that interacts with the appetite and satiety centers in the brain to regulate body weight (17). In addition, leptin appears to be the signal that indicates the size of the fat depot in the body (32). When injected into mice or rats, leptin reduces food intake and increases energy expenditure, resulting in a loss of body weight (5, 10, 19, 22). Obesity results from an imbalance of food intake over energy expenditure. Leptin is involved in both of these processes.

Adults tend to gain weight and become obese as they age until early senescence, after which body weight declines (4, 8, 16). The F-344xBN rat strain is a useful model for human obesity because it demonstrates a steady increase in body fat into early senescence (25). In our recent study (14), we found that adiposity in F-344xBN rats increased with age, which could not be attributed to either increased food intake, impaired leptin gene expression, or impaired peripheral leptin production. In fact, serum leptin levels and WAT leptin mRNA levels were actually increased with age in these rats. It is not known, however, whether a physiological challenge such as fasting would expose any impairment in the dynamic regulation of leptin gene expression. Because fasting suppresses leptin message (15, 21, 30), we hypothesized that the fasting-induced suppression of leptin message may be impaired in aged rats.

Another goal of the present study was to investigate the relationship among leptin, fasting, and hypothalamic neuropeptide Y (NPY) message in aged rats. NPY is an important feeding stimulant in the brain (7, 13, 26). Two known factors regulating hypothalamic NPY message are leptin and fasting. Leptin inhibits NPY synthesis, and this may be the mechanism by which leptin reduces food intake (24, 27). In contrast, fasting increases hypothalamic NPY levels (18, 31) and in turn stimulates appetite. Although NPY is only one of many redundant pathways regulating food intake, it is a potent feeding stimulant. Because serum leptin increases with age in F-344xBN rats (14), one might expect NPY mRNA levels to be suppressed. If NPY levels were reduced with age, and other factors being equal, one might expect that less food would be consumed by aged F-344xBN rats. We were surprised to find that aged rats consumed the same amount of food as their young, lean counterparts (14). Thus the elevated serum leptin levels with age suggest that NPY mRNA levels in the hypothalamus of aged F-344xBN rats may be elevated, whereas the food consumption data suggest that NPY mRNA levels may be unchanged with age.

To answer these questions, we examined leptin mRNA levels in three WAT depots, serum leptin levels, and NPY mRNA levels in the hypothalamus after a 48-h fasting or ad libitum feeding in F-344xBN rats of three different ages: 3 mo, the age of sexual maturation; 24 mo, the age of peak leptin gene expression and leptin serum level (14); and 31-mo-old aged rats.

METHODS

Animals. Male F-344xBN rats aged 3, 24, and 31 mo (n = 16/age group) were obtained from Harlan Sprague Dawley (Indianapolis, IN). They were housed individually under a reversed light-dark 12:12-h cycle, with lights on from 6:30 PM to 6:30 AM, at a room temperature of 26°C, which is the thermoneutrality for these rats (23). Food (Purina rat chow) and water were provided ad libitum.

Eight rats of each age were either fed ad libitum or fasted for 48 h. Thus we had a factorial design with age as one factor (3, 24, and 31 mo) and feeding status (ad libitum vs. fasting) as the other. There were a total of six treatment groups, with
eight rats in each group except the 31-mo-old fasted group, which had only seven because one rat died before the experiment.

Average daily food intake was measured in all rats by the difference in weight between the amount of food provided and the amount remaining over a 2-day period before the fasting treatment. Daily food intake values were presented in terms of grams divided by the naso-anal length in millimeters times 25°C. Aliquots of the supernatant (serum) were rapidly frozen and kept at −70°C until analysis. Collected blood was allowed to clot (dot activator present in SST tubes) for 30 min and then centrifuged at 2,300 rpm for 10 min at 25°C. Aliquots of the supernatant (serum) were rapidly frozen in liquid nitrogen. 

The tissues were stored at −80°C in 0.9% saline. Epididymal WAT (EWAT), inguinal WAT (IWAT), and retroperitoneal WAT (RTWAT) and the hypothalamus (data not shown) were collected, weighed, and rapidly frozen in liquid nitrogen.

Effect of aging, feeding, and fasting on body weights. Body weight increased significantly from 3 to 24 mo and leveled off between 24 and 31 mo (Table 1). During the two-day ad libitum feeding period, weight gain (difference between pre- and post-ad libitum feeding or fasting weight) occurred in both 3-mo-old (4.8±0.7 g, P<0.0001, paired t-test) and 24-mo-old (4.3±0.6 g, P<0.0001) but not in 31-mo-old rats (−0.3±1.1 g). Thus F-344xBN rats continued to gain weight at 24 mo under ad libitum feeding conditions. In contrast, for the fasted animals, comparable weight loss was found across three ages (3 mo, 28.6±1.15 g, P<0.0001; 24 mo, 30.9±1.13 g, P<0.0001; 31 mo, 30.6±1.5 g, P<0.0001). As a result, the rats fed ad libitum had higher body weights than the 2-day-fasted rats (Table 1).

Tissue weights. The weights of EWAT, RTWAT, and IWAT all increased from 3 to 24 mo and leveled off between 24 and 31 mo (Table 1). In addition, RTWAT weight decreased with fasting at each rat age (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Body and tissue weights</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>3 mo</td>
</tr>
<tr>
<td>Fed ad libitum</td>
</tr>
<tr>
<td>Fasted</td>
</tr>
<tr>
<td>24 mo</td>
</tr>
<tr>
<td>Fed ad libitum*</td>
</tr>
<tr>
<td>Fasted*</td>
</tr>
<tr>
<td>31 mo</td>
</tr>
<tr>
<td>Fed ad libitum*</td>
</tr>
<tr>
<td>Fasted*</td>
</tr>
</tbody>
</table>

Data are means ± SE of 7–8 rats/group. EWAT, RTWAT, and IWAT: epididymal, retroperitoneal, and inguinal white adipose tissue, respectively. *P<0.0001, main effect of age for body wt and EWAT, RTWAT, and IWAT weights. †P<0.0001, main effect of feeding status for body wt. ‡P<0.02, main effect of feeding status for RTWAT weight.
The weights of the three WATs strongly correlated with the following obesity-related measures: serum leptin (r = 0.73–0.77, P < 0.0001), body weight (r = 0.92–0.96, P < 0.0001), and Lee index (r = 0.70–0.74, P < 0.001).

Adiposity. In both fed (ad libitum) and fasted animals, the Lee index increased between 3 mo (fed, 298.5 ± 2.2; fasted, 293.2 ± 2.9) and 24 mo (fed, 319.4 ± 2.3; fasted, 311.5 ± 2.6) and then leveled off between 24 and 31 mo (fed, 313.1 ± 2.8; fasted, 311.3 ± 1.5; P < 0.0001 for the main effect of age). These data are similar to our previous report indicating an increase in adiposity with age up to 24 mo of age (13). In addition, the Lee index was less in the fasted rats than in the rats fed ad libitum (P < 0.02 for the main effect of feeding status).

Daily food intake for animals fed ad libitum. As shown in Table 2 and similar to our previous study (14), the daily food intake was not significantly different among the ages (main effect for age, P < 0.16). When food intake was calculated independent of body mass by dividing by (body wt)0.67, there was a significant decrease in the 3- and 24-mo-old rats (P < 0.0001) and between the 3- and 31-mo-old rats (P < 0.0001) but not between the 24- and 31-mo-old rats.

Leptin mRNA in RTWAT. There was a significant increase in leptin gene expression from 3 to 24 mo and no change between 24 and 31 mo for controls fed ad libitum (Fig. 1). Fasting significantly suppressed leptin mRNA in all three age groups. Although the magnitude of the decrease was greater in the older ages, because baseline leptin mRNA levels (i.e., fed ad libitum) increased with age, the percent decrease in leptin mRNA induced by fasting was greater in the 3-mo-old (70 ± 11.2%) than in either the 24-mo-old (48 ± 5.3%, P < 0.04) or the 31-mo-old rats (58 ± 4.0%, P < 0.05). Moreover, in the fasted rats, similar to rats fed ad libitum, leptin gene expression was significantly greater in 24- and 31- compared with 3-mo-old rats (P < 0.0001). In contrast to the age-related increase in leptin mRNA, there was no change in β-actin mRNA level in RTWAT with age (Table 3). However, there was a significant decrease in β-actin mRNA level in RTWAT in the fasted rats.

Leptin mRNA in IWat. Similar to the results in RTWAT, leptin mRNA levels increased with age from 3 to 24 mo and leveled off between 24 and 31 mo for both ad libitum fed and fasted animals (Fig. 2). Leptin mRNA in IWat was suppressed by fasting. In particu-

Table 2. Daily food intake and food intake independent of body mass

<table>
<thead>
<tr>
<th>Age</th>
<th>Food Intake, g/day</th>
<th>Mass-Independent Food Intake, g·day⁻¹·kg⁻¹⁰·⁶⁷</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 mo (n = 12)</td>
<td>17.85 ± 0.73</td>
<td>44.97 ± 1.77</td>
</tr>
<tr>
<td>24 mo (n = 16)</td>
<td>17.18 ± 0.69</td>
<td>25.78 ± 1.22*</td>
</tr>
<tr>
<td>31 mo (n = 15)</td>
<td>15.67 ± 0.89</td>
<td>23.36 ± 1.29*</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = no. of animals in group. Note that food intake was measured for both rats fed ad libitum and fasted rats before fasting treatment. *P < 0.0001 for difference compared with value from 3-mo-old group.
mRNA levels in IWat with age (Table 3), whereas β-actin mRNA levels in IWat decreased with fasting.

Serum leptin levels. Because the effects of fasting and age on leptin mRNA levels were variable in the three WATs investigated and because the size of each WAT changed with age, for a better representation of the effect of fasting and age on whole body leptin synthesis, serum leptin was determined. Serum leptin levels increased with age (Fig. 4A). Fasting suppressed serum leptin levels at each age. Furthermore, the percent decreases in serum leptin in the 3-mo-old rats (80 ± 2.7%) were significantly greater than in either the 24-mo-old (45 ± 5.1%, *P < 0.0001) or the 31-mo-old rats (51 ± 5.0%, †P < 0.0001).

NPY mRNA levels in the hypothalamus. Despite the elevated serum leptin levels with age, there were no significant age-related differences in NPY mRNA levels (Fig. 4B). As expected, fasting significantly increased hypothalamic NPY levels at each age (*P < 0.0001 for main effect of feeding status). In addition, the percent...
increase in NPY mRNA levels was the greatest in the 3-mo-old (76 ± 21.0%) compared with the 24-mo-old (36 ± 12.1%, P < 0.05) or the 31-mo-old rats (31 ± 9.6%, P < 0.04). There was no change in β-actin mRNA levels in the hypothalamus with either age or fasting (Table 3).

Correlation between serum leptin and hypothalamic NPY mRNA with age. Under ad libitum fed conditions, aged rats had elevated leptin levels and unchanged NPY mRNA levels (Fig. 4). Similar results were observed for fasting conditions (Fig. 4). These data imply an impairment in the leptin suppression of hypothalamic NPY synthesis with age. To examine this further, the correlation between serum leptin and hypothalamic NPY was determined at each age in ad libitum fed and fasted rats. In the 3-mo-old rats, there was a strong inverse correlation (r = −0.70, P < 0.004; Fig. 5), whereas no significant correlation was found in either 24-mo-old (r = −0.15, P < 0.6) or 31-mo-old rats (r = −0.49, P < 0.07).

DISCUSSION

In the present study, we found that the fasting-induced suppression of leptin mRNA was variable with age in EWAT, RTWAT, and IWAT. In RTWAT, there was a decrease with age; in IWAT, there was an increase with age; and in EWAT, there was no consistent change with age. In addition, leptin mRNA levels were unchanged in EWAT with age, whereas in both RTWAT and IWAT, levels of leptin mRNA increased with age from 3 to 24 mo and were unchanged between 24 and 31 mo. These results were similar to the changes with age in the leptin mRNA in different WAT depots as reported previously (14). Because of these inconsistencies in the fasting regulation of leptin gene expression with age, we examined serum leptin levels, which reflect the sum of all peripheral leptin production. It was not surpris-
senescence (25). On the other hand, the ability to lose weight by fasting was similar across all ages.

In contrast to serum leptin levels, NPY levels in the hypothalamus were unchanged with age. This may explain why aged, more obese F-344xBN rats consume the same amount of food per day as their young, lean counterparts. Similar results in daily food consumption were found in the present study and in our recent study (14). These data are also similar to those of Gruenewald et al. (9) in that the daily food intake of middle-aged rats (12 mo) was not different from that of the old rats (24 mo). When food intake independent of body mass was considered, we found a decrease between 3 and 24 mo and no change between 24 and 30 mo. This was again in line with the findings of Gruenewald et al. However, in contrast to our finding, Gruenewald et al. found an age-related decrease in NPY gene expression.

There were several major methodological differences between the two reports, which may explain the differences in NPY mRNA levels with age. One of the main differences is the time at which rats were killed with respect to the light-dark cycle: we killed the rats 2 h after lights-off, whereas Gruenewald et al. presumably killed their rats in the light phase. NPY mRNA demonstrates a circadian rhythm with a sustained increase in the second one-half of the light phase and then a sharp decline around lights-off (1). With age, the most commonly reported change in circadian rhythm is a diminished amplitude, i.e., the difference between the peak and the trough of the rhythm (20). Thus it is possible that young and aged rats may have a difference in their NPY mRNA levels in the light phase, when NPY mRNA level is at its peak, but not during the dark phase, when NPY mRNA level is at its baseline. In another study (unpublished data) from this laboratory, when we killed the rats during the light phase, we found a decrease in NPY mRNA with age similar to that reported by Gruenewald et al. Because we are examining the effect of fasting on leptin mRNA and NPY mRNA and because the normal feeding period for rodents is in the dark phase, assessment of NPY mRNA during dark phase may be more appropriate. There are other differences between the two studies as well. Different rat strains were used: we used F-344xBN rats, and they used BN rats. Whereas our rats were individually housed at thermoneutral temperature (26°C), at which level both young and aged rats maintain minimal oxygen consumption (23), their animals were doubly housed at an unspecified temperature (presumably room temperature). These housing conditions may affect the animal’s energy balance and food intake, which may in turn influence NPY gene expression. Gruenewald et al. did not measure leptin mRNA or serum leptin levels; thus the relationship between NPY gene expression and serum leptin was not determined in that study.

With age, the increased serum leptin levels and unchanged hypothalamic NPY mRNA levels present a dilemma (24, 27). The inability of high peripheral leptin levels in aged rats to suppress NPY mRNA levels suggests that there is a leptin resistance with age. Further supporting this conclusion is the relationship between serum leptin and hypothalamic NPY mRNA levels within each age group of rats. There was a strong negative correlation between serum leptin and hypothalamic NPY mRNA in 3-mo-old rats, whereas the two aged groups demonstrated no significant correlation. Thus the aged rats demonstrated an impaired ability for leptin to suppress hypothalamic NPY message. One possibility for this impairment is that leptin levels in the cerebral spinal fluid do not parallel serum levels, such that leptin levels are not elevated in the cerebral spinal fluid with age. Another possibility is that there is impairment in the leptin receptor binding and/or the leptin signal transduction pathway (i.e., leptin resistance) with age that results in insufficient suppression of NPY.

In summary, the regulation of leptin gene expression, leptin serum levels, and hypothalamic NPY gene expression by fasting is impaired in aged rats. These data suggest that aged rats are leptin resistant, i.e., they have both increased adiposity and elevated serum leptin levels, whereas NPY mRNA levels in the hypothalamus are unchanged.

This research was supported by the Medical Research Service of the Department of Veterans Affairs (P. J. Scarpace) and National Institute on Aging Grant AG-11465 (P. J. Scarpace) and Training Grant AG-00196-08 (H. Li).

Address for reprint requests: P. J. Scarpace, Geriatric Research, Education and Clinical Center (182), Dept. of Veterans Affairs Medical Center, Gainesville, Florida 32608-1197.

Received 29 December 1997; accepted in final form 12 May 1998.

REFERENCES