Prolonged hyperphagia with high-fat feeding contributes to exacerbated weight gain in rats with adult-onset obesity

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Abstract

The F344xBrown Norway (F344xBN) rat is a rodent model for late-onset obesity, whose weight gain with age parallels that observed in humans. This rat strain demonstrates a steady gain in adiposity into early senescence, followed by a decline beginning at 30 mo that continues into late life (15, 22). This increase in body fat with age cannot be accounted for by an increase in food intake (15). However, locomotor activity declines considerably with age (19) and potentially could contribute to the increase in obesity. Additionally, parallel to the increase in body fat with age, there is a rise in serum leptin levels (15, 22). This elevation in serum leptin with age should normally serve to lower body weight, but aged rats demonstrate reduced responsiveness to leptin, including an impaired anorexic response and little increase in energy expenditure, indicative of leptin resistance (6, 15).

We sought to address two hypotheses. First, because aged obese rats are leptin resistant (6, 15), these data predict that they will have a delayed normalization of caloric intake and exacerbated weight gain when provided a HF diet. Second, because both age and body weight impair locomotor activity, we hypothesized that both age and HF feeding would reduce voluntary wheel running. To evaluate these hypotheses, we chow or HF fed rats of various ages and examined caloric intake, body weight, and body composition over a 5-mo period, as well as voluntary wheel running.

MATERIALS AND METHODS

Animals. Male F344xBN rats of various ages were obtained from the National Institute on Aging. Rats were cared for in accordance with the principles of the “Guide to the Care and Use of Experimental Animals”. Rats were housed individually on a 12:12-h light-dark cycle (lights on from 0700 to 1900). Protocols were approved by the University of Florida Institutional Animal Care and Use Committee.

Experimental design. Six- (n = 5), 12- (n = 5), 18- (n = 5), 24- (n = 7), and 30-mo old rats (n = 5) were provided a HF diet (60% fat; 5.2 kcal/g D12492; Research Diets, New Brunswick, NJ) ad libitum for up to 5 mo, and additional 24-mo-old rats (n = 7) were fed a standard rat chow (15% fat; 3.3 kcal/g diet 2018; Harlan Teklad, Madison, WI) ad libitum. Body weight and food intake were recorded daily. A separate group of 3- (n = 32) and 30-mo-old (n = 23) rats was placed on either a standard chow or HF diet for 60 days and killed for assessment of serum leptin levels, hypothalamic protein tyrosine phosphatase 1B (PTP1B) levels, hypothalamic suppressor of cytokine

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signaling (SOCS-3) mRNA expression levels, brown adipose tissue (BAT) uncoupling protein 1 (UCP1) levels, and fat depot sizes.

Body composition. Body composition was determined before initiation of the experimental diets and periodically thereafter. Toward the end of the 150-day experimental period, physical performance tests and voluntary wheel running were assessed.

Additional groups of 3- (n = 36) and 30-mo-old rats (n = 24) on a chow diet were used to examine a 7-day dose response to peripheral leptin infusion.

**RESULTS**

**Body weight change with HF feeding.** Normally, when young rats are provided with a HF diet, they spontaneously divide into two groups, DIO prone and DR, based on the amount of weight gained (11). This phenomenon has been well described in the Sprague-Dawley rat strain (7, 11, 12) and recently described in the F344xBN rat strain (20). In the present study, the 3-mo-old rats fed the HF diet spontaneously divided into two distinct groups, consisting of the top 75% of weight gainers (DIO prone), and the lowest 25% (DR) (Fig. 1A). The latter were more statistically similar to the chow fed as measured by the maximized value of the log likelihood. Surprisingly, this phenomenon was not seen in the aged rats. All of the aged rats provided the HF diet gained similar and considerable amounts of weight, and thus aged, as opposed to young rats, are all susceptible to the weight-gaining effects of HF feeding (Fig. 1B).

This age-related susceptibility to HF feeding was also evident in the body weight gain across different rat ages. Particularly, age exacerbated the initial body weight gain seen with HF feeding (Fig. 2). Both the 6-mo-old young rats and the 24-mo-old mature rats steadily gained weight on the HF diet and continued to gain weight throughout the experimental period, but the weight gain in the 24-mo-old rats was significantly greater throughout the study period, except during the last 2 wk. This diet- and age-related weight gain was most pronounced in the oldest age group, especially during the initial phase of HF feeding. In the first 30 days of HF feeding, the 30-mo-old rats gained the most weight, but this increase in body weight reached a plateau by day 30 and at ~31 mo of age.

It should be noted that initial body weights, before initiation of HF feeding, were greater with increasing age (3 mo olds: 324.35 ± 3.68 g; 6 mo olds: 385.26 ± 11.28 g; 12 mo olds: 435.58 ± 14.24 g; 18 mo olds: 500.86 ± 13.47 g; 24 mo olds: 551.23 ± 9.47 g; 30 mo olds: 568.33 ± 9.17 g).** Caloric intake with HF feeding.** The age-related disproportion in initial weight gain suggests that this may be related to the hyperphagia normally observed after introduction of a HF diet. When 3-, 6-, and 30-mo-old rats were provided the HF diet, all ages experienced an immediate increase in daily caloric intake that gradually normalized to basal levels, or near basal level in the case of the oldest age group, over time (Fig. 3A). For ease in analyzing the data, the daily caloric intake was divided into two phases, with phase 1 representing the peak caloric intake on initiation of the HF diet, and phase 2 being the days necessary to normalize the elevated caloric intake back to levels before HF feeding. It is important to note that the animals of all ages consumed a similar amount of calories on the regular chow diet before the introduction of the HF diet.

The peak daily caloric intake with HF feeding increased with age (R² = 0.85, P = 0.009), as is evident in a comparison of phase 1 across ages (Fig. 3B). The 3-mo-old rats reached a peak caloric intake of 100 kcal. The 6-, 12-, and 18-mo-old rats all reached a peak of ~115 kcal, and the 24-mo-old rats reached a peak caloric intake of 125 kcal. The 30-mo-old

We used the maximized value of the log likelihood as a measure of model fit (larger values indicate better fit).
rats, the oldest age tested in this experiment, reached a maximal caloric intake of 142 kcal.

A similar trend was seen in phase 2 (Fig. 3C), where the days required to normalize caloric intake to basal levels increased with age (R² = 0.93, P = 0.002). In this F344xBN rodent strain, the 3-mo-old rats experienced a complete normalization of caloric intake by day 6. The 6-mo-old rats and the 12-mo-old rats required 9 and 17 days, respectively, whereas the 18- and 24-mo-old rats did not experience normalization until day 25. In contrast to the younger ages, the 30-mo-old rats were unable to normalize to pre-HF caloric intake, but stabilized at a new slightly elevated plateau by day 29.

Body composition. Absolute fat and lean body mass were greater in the 30-mo-old rats compared with the 3-mo-old rats at the beginning of the study (fat mass: 175.67 ± 4.38 vs. 73.56 ± 1.86 g, P < 0.001; lean mass: 339.78 ± 5.33 vs. 199.05 ± 4.17 g, P < 0.0001). By examining the change in body composition, the increase in fat and lean mass was significantly greater in both young and old HF-fed groups compared with their age-matched, chow-fed controls (Fig. 4). In addition, the HF-fed aged rats gained significantly more fat mass than the HF-fed young rats. Conversely, the HF-fed young rats gained significantly more lean mass than the HF-fed aged rats. This resulted in a significantly larger fat-to-lean mass ratio in the aged, HF-fed rats compared with all other age/diet groups (Fig. 4C). In contrast, the ratio of fat mass to lean mass was relatively constant over time in the chow-fed aged rats (Fig. 4C).

Serum leptin and adiposity. With HF feeding in young rats, by day 60, serum leptin levels rose greater than twofold compared with that in the chow-fed counterparts. Interestingly, after 60 days of HF feeding in the young, serum leptin reached the same levels as that in the aged chow-fed rats (Fig. 5A). Similar to the young, the HF-fed aged rats had serum leptin levels...
levels more than twofold greater than the aged chow-fed rats by day 60. Serum leptin levels at day 60 paralleled that of the amount of white adipose tissue (sum of perirenal, retroperitoneal, and epididymal white adipose tissues) present in each group at death (Fig. 5B). The young HF-fed and aged chow-fed rats had the same amount of white adipose tissue, whereas the young chow-fed rats had significantly less and the aged HF-fed rats had significantly more. This is consistent with previous reports that leptin circulates in proportion to whole body fat depots (1).

Wheel running and physical performance tests. From 2–5 mo after HF or chow feeding, three performance tests were administered: wheel running and two measures of forelimb grip strength. Each age group was provided access to running wheels for 4 consecutive days, but the rats were not provided any training or encouragement to run. The youngest chow-fed rats ran >1,000 m/day (Fig. 6). In contrast, the oldest chow-fed rats ran eightfold less, only 136 m/day during the same period. HF feeding also impacted wheel running, but not to the same degree as age. The youngest HF-fed rats ran twofold less than the corresponding chow fed. With increasing age and HF feeding, wheel running declined with the oldest HF-fed rats running only 81.6 m/day (Fig. 6).

Because wheel running may be dependent on muscle strength, we subjected the 24-mo-old rats to a grip strength and inclined plane test at approximately day 130 on the standard chow or HF diet. There was no significant difference in either grip test or ability to perform in the inclined plane test between the diet groups in the 24-mo-old rats (data not shown).

Hypothalamic measures of leptin action. We examined two factors that participate in leptin signaling, SOCS-3, a negative regulator of leptin signaling, and PTP1B, a phosphatase that dephosphorylates activated components in the leptin signaling cascade. Consistent with models of leptin resistance, both SOCS-3 mRNA levels and PTP1B protein levels were significantly increased in aged rats compared with young rats (Table 1). In addition, both hypothalamic SOCS-3 expression and PTP1B levels were significantly elevated with HF feeding (Table 1).

BAT UCP1 levels. The induction of UCP1 in BAT is a marker for enhanced thermogenesis in rodents and is often used as an indicator of energy expenditure. Consistent with our laboratory’s previous findings (20, 21), HF feeding increased UCP1 protein levels in BAT in young rats (Fig. 7). Surprisingly, the HF diet also induced increased UCP1 protein levels in BAT in the aged leptin-resistant rats.

Dose response to peripheral leptin infusion. Because previous data suggest that leptin resistance prolongs or prevents the renormalization of caloric intake following HF feeding, we evaluated the responsiveness to leptin before initiation of the HF feeding in both young and aged rats. The doses used were 0.03, 0.05, 0.07, 0.1, and 0.5 mg/day (0.1, 0.167, 0.233, 0.33, and 1.67 mg·kg⁻¹·day⁻¹, respectively) in the young rats and 0.05, 0.1, and 0.5 mg/day (0.091, 0.182, and 0.909 mg·kg⁻¹·day⁻¹, respectively) in the aged rats. In the young rats (initially 3 mo old and weighing 308.79 ± 3.19 g), all doses >0.03 mg/day of peripheral leptin significantly increased serum leptin levels compared with saline-infused rats (Table 2). The leptin-infused rats displayed a dose-dependent, anorectic, and body weight reduction in response to a peripheral leptin up to a dose of 0.07 mg/day, above which there was no additional effect (Fig. 7, A and B, respectively). The decrease in body weight for each of the leptin-infused groups was significantly greater than that of the saline-infused group. In addition, the reduction in cumulative food intake due to leptin infusion was greater with each leptin dose, except for the lowest dose compared with the saline-infused group. The anorectic and weight reduction responses to doses of 0.07, 0.1, and 0.5 mg/day were not statistically different from each other and appear to represent the maximum response to leptin.

In contrast, the aged rats (initially 30 mo old and weighing 553.39 ± 5.48 g) did not respond to even the largest dose of leptin. In particular, cumulative food consumption was un-

Fig. 3. A: daily caloric intake of 3- (circles), 6- (squares), and 30-mo-old (triangles) rats on a HF diet. Values are means ± SE of 3- (n = 9), 6- (n = 5), and 30-mo-HF-fed rats (n = 5). B: the peak caloric intake after the initiation of HF feeding. Values are means ± SE of 3- (n = 24), 6- (n = 5), 12- (n = 5), 18- (n = 5), 24- (n = 7), and 30-mo-old HF-fed (n = 5) rats. P < 0.0001 for difference with age by one-way ANOVA. *P < 0.05 for the difference between 3-mo-old rats and all other ages by post hoc analysis. **P value < 0.001 for the difference between the 30-mo-old rats and all other ages by post hoc analysis. C: the days required to normalize the elevated caloric intake following HF feeding. Values are means ± SE of 3- (n = 11), 6- (n = 5), 12- (n = 5), 18- (n = 4), 24- (n = 7), and 30-mo-old HF-fed (n = 5) rats. P < 0.0001 for difference with HF feeding by one-way ANOVA. **P value < 0.001 for the difference compared with 3-mo-old rats by one-way ANOVA and post hoc.
changed across leptin doses (Fig. 8B). All of the aged rats lost weight, likely in response to the detrimental effects of surgery (Fig. 8A). However, the leptin-infused rats did not differ from the saline-infused rats with respect to either body weight or food intake reduction, even though the doses of both 0.1 and 0.5 mg/day of leptin significant increased serum leptin compared with saline-infused rats (Table 2).

DISCUSSION

Rodents provided with a highly palatable caloric-dense diet initially consume an elevated level of calories, but within several weeks adjust their total food consumption, such that their diet becomes isocaloric to that of chow-fed companions (6, 13, 17). Leptin-resistant animals, however, display impaired normalization of caloric intake with HF feeding (21). Because aged obese rats are also leptin resistant (17), we predicted that aged rats would also fail to properly normalize caloric intake after exposure to HF feeding. The present investigation confirms this hypothesis by examining the physiological effects of a HF diet on rats of various ages between 3 and 33 mo of age and provides several salient findings.

First, our data are consistent with several previous studies indicating that aging increases the susceptibility to obesity and fat storage (9, 10). Iossa et al. (9) showed that young male Wistar rats that are naturally growing to maturity have the ability to store both proteins and lipids. However, as the rats age, from 1 to 6 mo old, the protein deposition eventually becomes almost nonexistent, and all excess energy consumed is stored as fat (9). They propose that this is one mechanism underlying age-associated obesity. Moreover, these adult rats were more prone to obesity when fed a HF diet than were younger counterparts (9, 10). It is reasonable that these trends toward obesity continue as rats age even further. Supporting this hypothesis is our demonstration that aged rats do not divide into DIO and DR, as previously and currently seen in young F344xBN rats (20). Whereas young rats are either susceptible or resistant to weight gain, all aged rats are susceptible to this negative effect of a HF diet. Moreover, body composition analysis indicates that the older rats gain a disproportionate amount of body fat compared with younger counterparts when provided a HF diet. Hence, these data support the concept that energy storage shifts toward fat deposition with aging, implicating one mechanism underlying age-related obesity. Moreover, aged F344xBN rats, in our case both 24- and 30-mo old rats, provided a HF diet, gain more weight than correspondingly fed young rats. Together, these
Fig. 5. A: serum leptin at day 60 in 3- and 30-mo-old rats on chow or HF diets. Ages represent the age of the animal when HF or chow feeding was begun. Assessments were determined 60 days later. Values are means ± SE of 3- (n = 9–10) and 30-mo-old (n = 9–13) rats. P < 0.0001 for the difference with HF feeding and difference with age by two-way ANOVA; P = 0.0041 for the interaction. *P < 0.05 for the difference between chow-fed young and aged rats by post hoc analysis. **P < 0.001 for the difference between HF-fed young and aged rats by post hoc analysis. †P < 0.05 for the difference between chow-fed and HF-fed young rats by post hoc analysis. ††P < 0.001 for the difference between chow-fed and HF-fed aged rats by post hoc analysis. B: white adipose tissue at death from 3- and 30-mo-old rats following chow or HF feeding. Ages represent the age of the animal when HF or chow feeding was begun. Assessments were determined 60 days later. Values are means ± SE of 3- (n = 10–12) and 30-mo-old (n = 8–14) rats. P < 0.0001 for the difference with HF feeding and difference with age by two-way ANOVA. *P < 0.001 for the difference between chow-fed young and aged rats by post hoc analysis. **P < 0.001 for the difference between HF-fed young and aged rats by post hoc analysis. †P < 0.05 for the difference between chow-fed and HF-fed young rats by post hoc analysis. ††P < 0.001 for the difference between chow-fed and HF-fed aged rats by post hoc analysis.

Data indicate that all aged rats, as opposed to only some young rats, are prone to develop obesity on a HF diet, and furthermore the degree of weight gain is greater in the older rats.

Second, our data indicate that the nature of the transient increase in caloric intake on initiation of HF feeding is dependent on age. Both the peak increase in caloric intake on initiation of HF feeding and the time to normalization increase with age. Moreover, this hyperphagia was a specific result of the HF diet: before initiation of the HF diet, all rats, regardless of age, consumed the same amount of chow diet, confirming earlier studies (8, 15, 22).

Thus the greater initial body weight gain in the 24- and 30-mo-old rats appears to be a consequence of this failure to normalize caloric intake after initiation of HF feeding, and we suggest the latter is a direct result of leptin resistance in these aged animals. Previous experiments demonstrated that the simultaneous administration of a leptin receptor antagonist along with HF feeding prevents the normalization of caloric intake after HF feeding, indicating that leptin receptor activity is necessary for this normalization (21). Older rats have reduced numbers of leptin receptors and diminished leptin signaling (16). In addition, in the present study, we found increases with age in both SOCS-3, a negative regulator of leptin signaling, and PTPIB, a phosphatase that dephosphorylates activated components in the leptin signaling cascade. These changes with age likely impair the native responses to the endogenous elevation in leptin triggered by HF feeding. We examined the status of leptin resistance at the point before initiation of the HF feeding by assessing the leptin dose-
response decrease in food consumption and body weight over the course of 7 days in young and aged rats. As expected, the young rats responded in a dose-response fashion, whereas there were no responses in the aged rats, thus confirming that, before initiating HF feeding, the aged rats were unresponsive to leptin. As such, these leptin-resistant animals display a delayed normalization of caloric intake on a HF diet, strongly suggesting that preexisting leptin resistance is causal to the exacerbated weight gain with age. It should be noted, however, that these measures of leptin responsiveness were examined only in the 3- and 30-mo-old rats. While they demonstrate impaired leptin responsiveness by 30 mo of age, we cannot dismiss the possibility that the leptin resistance may be fully manifested before this age. If this is the case, the leptin resistance may be only one factor in the progressive exacerbated weight gain with age to HF feeding.

Our data indicating that leptin receptor activity is necessary for the normalization of caloric intake predicts that any impaired normalization should be proportional to the degree of leptin resistance and possibly even never occur in aged rats that are fully leptin resistant. As such, these leptin-resistant animals display a delayed normalization of caloric intake on a HF diet, strongly suggesting that preexisting leptin resistance is causal to the exacerbated weight gain with age. It should be noted, however, that these measures of leptin responsiveness were examined only in the 3- and 30-mo-old rats. While they demonstrate impaired leptin responsiveness by 30 mo of age, we cannot dismiss the possibility that the leptin resistance may be fully manifested before this age. If this is the case, the leptin resistance may be only one factor in the progressive exacerbated weight gain with age to HF feeding.

Our data indicating that leptin receptor activity is necessary for the normalization of caloric intake predicts that any impaired normalization should be proportional to the degree of leptin resistance and possibly even never occur in aged rats that are fully leptin resistant. Our data support this prediction. The delay in normalization is proportional to advancing age, with the oldest group achieving only a partial normalization. Similarly, leptin resistance is greater in 30-mo-old rats compared with 18-mo-old ones (18). We suspect the partial normalization in the oldest age group represents some residual leptin receptor activity or compensation by another anorexic pathway.

Subsequent to the caloric normalization, rats of all ages continued to gain weight, suggesting that energy expenditure must be diminished. However, any such decrease in energy

Table 2. Serum leptin levels in young and aged rats on a chow diet after 7-day saline or leptin infusion

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<th>Dose (mg/day)</th>
<th>Young Rats</th>
<th>Aged Rats</th>
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<tbody>
<tr>
<td>Control</td>
<td>6.91±1.12</td>
<td>13.25±2.00</td>
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<tr>
<td>0.03</td>
<td>10.10±0.47</td>
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<tr>
<td>0.05</td>
<td>14.05±0.86*</td>
<td>19.56±1.34</td>
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<tr>
<td>0.07</td>
<td>19.75±1.38*</td>
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<tr>
<td>0.1</td>
<td>20.16±1.08*</td>
<td>25.38±2.86†</td>
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<tr>
<td>0.5</td>
<td>22.23±2.15*</td>
<td>24.62±2.26†</td>
</tr>
</tbody>
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Values are means ± SE in ng/ml; n = 7–9 rats/group. Young rats: P < 0.0001 for the difference with leptin infusion. *P < 0.01 for the increase in serum leptin levels compared with controls. Aged rats: P = 0.0008 for the difference with leptin infusion. †P < 0.01 for the increase in serum leptin levels compared with control values by post hoc analysis.
expenditure does not appear to be related to the thermic effect of food, because both the young and aged HF-fed rats responded equally with an increase in UCP1 protein level in BAT. In addition to thermogenesis, an important component of energy expenditure is physical activity levels. There is an inverse relationship between body weight and physical activity (19), and a decrease in locomotor activity, including volitional activity, may be an important contributor to age-related obesity. Voluntary wheel running is one form of volitional activity involving motivational, exploratory, muscular, age, and body size components (19). Data indicate that locomotor activity declines both with age and obesity (19). We hypothesized that both age and HF feeding would impact voluntary wheel running, and this hypothesis proved correct: wheel running activity declined with both age and HF feeding, the latter especially in young rats. It has previously been reported that Sprague-Dawley and SSB/P1Ras rats on a high-carbohydrate diet voluntarily run more than those fed a HF diet, but there was no comparison to rats on a standard chow diet (3). Another group showed that the introduction of sweet milk plus standard chow decreased voluntary wheel running in female rats, but not male rats (5). Research with hamsters indicated that aged hamsters run significantly less than young ones (4). These data are consistent with our findings that aged F344xBN rats run significantly less than young F344xBN rats on a standard chow diet. In addition, HF feeding can further reduce voluntary wheel running activity in young rats. Interestingly, the aging and HF feeding suppressive effect on voluntary wheel running do not appear to be independent; for instance, in the oldest age group that ran the least, HF feeding had little additional suppressive effect. Collectively, these data suggest that the propensity for inactivity with age may be one contributory factor in age-related obesity, and the inactivity with HF feeding may accelerate the rate of diet-induced obesity.

Perspectives and significance. Obesity is a worldwide epidemic in developed countries, and the lure of palatable HF food presents as much of a threat to the aging population as it does to the young in our modern society. Our data indicate that, with HF feeding, aged obese rats experience a greater hyperphagia and a delayed normalization in caloric intake, therefore resulting in an exaggerated weight gain. The mechanism appears to be a consequence of the preexisting leptin resistance present with age and obesity. Moreover, both age and HF feeding diminish volitional activity. Obesity in males and females over 70 yr of age dramatically increases by nearly two-thirds the number of remaining years spent disabled (14). Our data provide a negative link between a HF diet and physical activity. Collectively, aged rats experience greater negative effects of a HF diet, and that leads to both obesity and diminished physical activity, which, in turn, contribute to an ever escalating cycle of weight gain. If these results are applicable to humans, there may be a greater need for intervention in our aging community to combat this heightened susceptibility to HF-induced obesity and subsequent decrease in volitional activity.

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