Senescent terminal weight loss in the male F344 rat

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Departments of 1Medicine, 2Pathology and 3Physiology, University of Texas Health Science Center, San Antonio 78229-3900; and 4Research Service and 5Geriatric Research, Education and Clinical Center, South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio, Texas 78229-4404

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Black, Bill J., Jr., C. Alex McMahan, Edward J. Masoro, Yuji Ikeno, and Michael S. Katz. Senescent terminal weight loss in the male F344 rat. Am J Physiol Regul Integr Comp Physiol 284: R336–R342, 2003. First published October 17, 2002; 10.1152/ajpregu.00640.2001.—Loss of weight, often of unknown cause and culminating in death, commonly occurs in humans at advanced ages. Rats that live to old ages, such as the Fischer 344 (F344) strain, also exhibit a terminal loss in body weight. A presently held hypothesis is that the terminal weight loss in the F344 rat model is due to reduced food intake because of an alteration in hypothalamic function resulting in early satiation. We report findings on terminal weight loss and food intake in male F344 rats fed ad libitum (AL group) or a life-prolonging dietary regimen in which caloric intake was restricted (DR group). Rats in both dietary groups that did not exhibit a terminal weight loss died at younger ages than those exhibiting the loss. Terminal weight loss in the AL group was not associated with decreased food intake; indeed, half of the rats in this group had increased food intake during the period of terminal weight loss. This finding is not in accord with the presently held hypothesis. In the DR group, terminal weight loss was associated with decreased food intake. Pathology (renal disease and neoplasms) did not explain the presence or absence of the association between reduced food intake and weight loss in either dietary group. The duration of the period of terminal weight loss was similar for the AL and DR groups. Apparently, restricting calories delays the occurrence but does not affect the duration of senescent terminal weight loss.

Loss of weight culminating in death commonly occurs in humans at advanced ages and is a hallmark of a geriatric syndrome termed failure to thrive (11). Although in some individuals the loss in body weight is due to age-associated disease, the cause of this weight loss in many elderly individuals is unknown (28).

Most rats that live to old ages also exhibit a terminal loss in body weight (5, 27, 33). Blanton et al. (4) proposed that the Fischer 344 (F344) rat is a good animal model for the study of the physiological basis of senescent terminal weight loss. They found the loss of weight to be associated with a decrease in food intake related to the consumption of shorter and smaller meals, rather than a decreased number of daily meals (5). These investigators hypothesized that senescent terminal weight loss is caused by neurochemical alterations in the hypothalamus resulting in dysregulation of food intake relative to the organism’s energy needs (4).

Since 1975, our laboratory has carried out life span studies on male F344 rats, and it was our impression that senescent weight loss often was not associated with decreased food intake. In the present study we have investigated this issue in three groups of rats studied in the 1980s and early 1990s for which detailed data are available on body weight, food intake throughout the life span, and pathological lesions at the time of spontaneous death. Two groups of rats were fed ad libitum, the method of feeding used by Blanton and associates (5).

Our study also included a group of rats in which food intake was restricted, starting at 6 wk of age, to ~60% of the daily intake of the rats fed ad libitum. This dietary regimen, which gerontologists refer to as dietary restriction (DR) or caloric restriction, is known to slow markedly the rate of aging in laboratory rodents (17). An unanswered question is whether the DR rats will have an abbreviated period of marked senescent deterioration or merely a delayed senescence. Therefore, in this communication we present an analysis of the terminal weight loss of the DR rats as a means of assessing the effects of an antiaging, life-prolonging intervention on the duration of marked senescent deterioration.

MATERIALS AND METHODS

Rat maintenance and dietary procedures. Specific pathogen-free (SPF), weaning (26–30 days of age), male F344 rats were purchased from Charles River Laboratories (Kingston, NY). The care and use of the rats were in accord with the guidelines of the University of Texas Health Science Center at San Antonio. The SPF status of the rats was maintained by immediate transfer of the animals to a barrier facility, where they were singly housed in plastic cages with wire...
A 12:12-h light-dark cycle was maintained in the barrier facility. The basic operation of the barrier facility has been described previously (32). The rats were monitored for SPF status on receipt and at 6-mo intervals thereafter, as described elsewhere (18); SPF status was maintained throughout the study.

All rats were fed a semisynthetic diet (diet A) ad libitum until 6 wk of age. At 6 wk of age, rats were randomly assigned to continue diet A ad libitum (AL group) or to receive a daily allotment of diet B (DR group), which provided 60% of the mean caloric intake of the AL group. The compositions of diets A and B (Table 1) were almost identical, except the amount of the vitamin mix was increased in diet B so that both groups of rats received a similar daily dietary intake of vitamins (18). The DR rats were given their daily food allotment ~1 h before the beginning of the dark phase of the light-dark cycle. The AL and DR rats were undisturbed, other than the activity required for the cleaning of cages, removal and replacement of food cups and water bottles, and measurement of body weight at 2-wk intervals.

One group of 60 AL rats was studied during 1983–1987 (18), and a second group of 40 AL rats was studied during 1987–1991 (24). These two groups were fed diet A and studied using the same experimental protocol. The 60 DR rats were studied concurrently with the 60 AL rats during 1983–1987.

Food intake in the AL group was measured during alternating 3- or 4-day intervals. The weight of the food at the start of the interval and the weight of the food remaining at the end of the interval were recorded. Occasionally, there was food spillage, which was detected as described elsewhere (32). The weight of food eaten was obtained by subtraction, and the average food intake during each day of the interval was calculated by dividing by 3 or 4 days. The weight of food eaten was converted to calories by multiplying by 4.1 kcal/g. The average food eaten during a time period was computed as the weighted mean daily consumption during all food measurement periods within the specified time period, with the number of days during the food measurement periods (i.e., 3 or 4) used as the weight. For total food consumption, the average food consumed per day was multiplied by the number of days.

Food intake in the DR group was measured daily. The weight of the food given and the weight of any remaining food were recorded. On most days, all food was consumed. The weight of the food eaten was obtained by subtraction if any food remained. The weight of the food eaten was converted to calories by multiplying by 4.04 kcal/g. The daily food consumption was summed for all days during a time interval to obtain the total consumption.

Analysis of pathological lesions. The rats were inspected at least twice daily (at the start and end of the light phase of the light-dark cycle). All rats died spontaneously and were immediately necropsied or briefly refrigerated before necropsy. Major organs were excised, fixed in 10% formalin, and examined histologically as described previously (18). The most prevalent pathologies were chronic nephropathy and neoplasia (leukemia plus a variety of solid tissue tumors).

The rats with chronic nephropathy were divided into two groups for statistical analysis: 1) those with grades 1–3 lesions, with the severity of the lesions increasing from minor (grade 1) to moderate (grade 3), and 2) those with very severe lesions referred to as grades 4 and E. The separation of rats with chronic nephropathy into two groups is based on an earlier cross-sectional study (16), in which it was found that serum creatinine and blood urea nitrogen levels were not elevated in rats with grades 1–3 lesions (indicating no major loss of renal function), were moderately elevated in rats with grade 4 lesions, and were markedly elevated in rats with grade E lesions (indicating renal failure).

### Table 1. Composition of diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Diet A</th>
<th>Diet B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein (vitamin free)</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td>Dextrin</td>
<td>43.65</td>
<td>42.19</td>
</tr>
<tr>
<td>Sucrose</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Corn oil</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Choline mix</td>
<td>2</td>
<td>3.33</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>0.2</td>
<td>0.33</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Fiber</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Values (g/100 g diet) are from Ref. 18. Fiber (Solka Floc) was obtained from James River (Berlin, NH).

### Statistical analysis

Survival curves were estimated using product limit estimates, and curves were compared using a Wilcoxon test (12). Food intake and weight during 30-day periods prior to weight decline were analyzed using analysis of variance for repeated measurements (29) with Bonferroni-adjusted comparisons among means. Means of characteristics of AL and DR rats and mean characteristics of groups of defined pathology were compared using analysis of variance (26). Values are means ± SE. Correlation coefficients were used to describe the associations of food intake and weight.

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loss. Correlation coefficients were compared using a z-transformation (26).

RESULTS

A similar fraction of rats in the two AL groups experienced a terminal weight decline (49 of 60 rats = 81.7% in 1983–1987 and 33 of 40 rats = 82.5% in 1987–1991, \( P = 0.9154 \)). Among those rats experiencing a terminal weight decline, life span (766.4 ± 12.0 and 789 ± 14.2 days in 1983–1987 and 1987–1991, respectively, \( P = 0.2233 \)), duration of decline (147.3 ± 10.2 and 133.2 ± 11.7 days in 1983–1987 and 1987–1991, respectively, \( P = 0.3722 \)), weight loss (152.2 ± 11.2 and 148.7 ± 13.1 g in 1983–1987 and 1987–1991, respectively, \( P = 0.8396 \)), weight loss relative to weight at start of decline (29.1 ± 2.0 and 27.1 ± 2.4% in 1983–1987 and 1987–1991, respectively, \( P = 0.5162 \)), and baseline food intake (55.8 ± 0.6 and 55.7 ± 0.8 kcal/day in 1983–1987 and 1987–1991, respectively, \( P = 0.9189 \)) were similar in the two groups, whereas age at the start of the decline (619.1 ± 11.9 and 656.1 ± 13.5 days in 1983–1987 and 1987–1991, respectively, \( P = 0.0458 \)) and weight at the start of the decline (519.8 ± 6.3 and 548.8 ± 5.7 g in 1983–1987 and 1987–1991, respectively, \( P = 0.0011 \)) were different.

The two groups of rats were similar for the majority of the variables, and, although statistically different for two variables, the differences were small. In the remainder of this report, we present results based on these two AL groups combined.

Terminal weight loss occurred in 82 of the 100 AL rats and 38 of the 60 DR rats. Survival curves are presented in Fig. 1. The rats in both dietary groups that did not exhibit a terminal loss in body weight died at younger ages than rats that did exhibit the loss (\( P = 0.0001 \) and 0.0021 in AL and DR groups, respectively). The remainder of this report is focused on the rats that underwent a terminal weight loss.

Timing, duration, and magnitude of the terminal weight decline in AL and DR rats are reported in Table 2. Terminal weight loss started at an older age in the DR group than in the AL group (\( P = 0.0001 \)). The duration of the weight loss was similar for both groups of rats (\( P = 0.6954 \)). Moreover, the duration varied considerably among animals of both dietary groups (48–325 and 42–289 days in AL and DR rats, respectively). The body weight before the beginning of the period of terminal weight loss was greater for the AL group than for the DR group (\( P = 0.0001 \)). The AL rats exhibited a greater absolute weight loss (\( P = 0.0001 \)) and weight loss relative to weight at the start of the decline (\( P = 0.0001 \)) than did the DR rats. These findings are similar to those obtained when only the AL and DR rats studied concurrently during 1983–1987 were considered (results not shown).

In Fig. 2, the terminal weight loss is plotted against total food intake relative to baseline. Of the 82 rats in the AL group, 41 had an increase in food intake above that of baseline and 41 had a decrease. In this group, there was no association of food intake with terminal loss of body weight (\( r = 0.08, P = 0.4566 \)). Of the 38 DR rats, 8 had the same food intake during terminal body weight loss as baseline (unlike the AL group, it was not possible for a rat in the DR group to have an intake of

![Fig. 1. Survival curves of ad libitum-fed (AL) and dietary-restricted (DR) rats. ● Rats exhibiting terminal weight loss; ○ rats not exhibiting terminal weight loss.](image-url)
food greater than baseline) and 30 had a decreased intake. In the DR group, there was an association between food intake and terminal loss of body weight: the less food the rat consumed, the greater the weight loss ($r = -0.88$, $P = 0.0001$).

The data were analyzed separately for the period of 42 days before death and the period >42 days before death to learn whether the relationship between food intake and loss of body weight was different from that in the period. In the AL group, weight loss was associated with reduced food intake for the period of 42 days before death ($r = -0.36$, $P = 0.0008$), whereas for the period >42 days, the more food consumed, the greater the weight loss ($r = 0.37$, $P = 0.0017$). Even for the period of 42 days before death, food intake was above baseline in 29 of 82 animals. For the DR group, the less food consumed, the greater the weight loss for both time periods ($r = -0.85$, $P = 0.0001$ for the period of 42 days before death and $r = -0.46$, $P = 0.0096$ for the period >42 days before death).

Results on terminal weight loss, food intake relative to baseline, and their association in relation to major pathology at the time of spontaneous death are presented in Table 3. The most prevalent pathologies were chronic nephropathy and neoplasia (leukemia plus a variety of solid tissue tumors). For the AL group, examination of the correlation coefficients did not reveal a significant association between food consumed and weight loss within any of the categories of pathology. In contrast, for the DR rats, the correlation coefficients indicated a significant association between food consumed and terminal weight loss within all categories of pathology. (Note that none of the DR rats exhibited grade 4 or E nephropathy.) Significant differences among categories of pathology were found in mean food intake relative to baseline and mean weight loss for both the AL and DR rats. In the AL rats, there was little difference in weight loss between animals with and without neoplasms (144.0 ± 15.4 and 153.8 ± 10.2 g with and without neoplasm, respectively, $P = 0.5976$), even though animals with neoplasms consumed less food relative to baseline ($351.8 ± 159.4$ and $185.0 ± 105.5$ kcal with and without neoplasm, respectively, $P = 0.0062$). As shown in Table 3, in the DR rats there were significant differences in food con-

Table 3. Mean weight loss and food consumed relative to baseline and correlation coefficients between weight loss and food consumed relative to baseline, by pathology and diet group

<table>
<thead>
<tr>
<th>Pathology</th>
<th>AL Rats</th>
<th>DR Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>Food consumed, kcal</td>
</tr>
<tr>
<td>Renal 1–3 Nonneoplasm</td>
<td>2</td>
<td>1,273.5 ± 1,862.9</td>
</tr>
<tr>
<td>Renal 1–3 Neoplasm</td>
<td>15</td>
<td>-554.3 ± 133.2</td>
</tr>
<tr>
<td>Renal 4–E Nonneoplasm</td>
<td>55</td>
<td>145.5 ± 88.8</td>
</tr>
<tr>
<td>Renal 4–E Neoplasm</td>
<td>10</td>
<td>-48.1 ± 375.8</td>
</tr>
<tr>
<td>Neoplasm</td>
<td>57</td>
<td>189.0 ± 101.4</td>
</tr>
<tr>
<td>Neoplasm (Leukemia)</td>
<td>16</td>
<td>-451.5 ± 240.7</td>
</tr>
<tr>
<td>Neoplasm (Other)</td>
<td>9</td>
<td>-174.7 ± 224.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Correlation coefficient significantly ($P < 0.05$) different from zero.
Terminal weight loss in both the AL and DR dietary groups tended to occur in rats that died at older ages; this finding is in agreement with the view of McDonald and associates (21) that terminal weight loss is a marker of senescence. Terminal weight loss appears to be an integral component of advanced senescence.

McDonald and associates (20) found that terminal loss of body weight in ad libitum-fed F344 rats is associated with a reduction in food intake. This group of investigators subsequently reported that the reduction in food intake results from a change in feeding pattern, specifically, smaller meals of shorter duration, but no reduction in the number of daily meals (5). They concluded that these findings indicated earlier satiation. They also reported that a loss of thermoregulation is associated with terminal weight loss (20). Blanton et al. (3, 4) proposed an involvement of the hypothalamus in the marked senescence that occurs before the end of life, with alterations in food intake, body weight, and thermoregulation serving as both markers and components of senescence. As an approach to uncovering the mechanism underlying the reduction in food intake, they administered neuropeptide Y intracerebroventricularly to rats undergoing terminal weight loss and to age-matched healthy rats not losing weight. The group without weight loss increased food intake, much as young rats do, whereas rats undergoing senescent weight loss exhibited a markedly blunted response. Neuropeptide Y is only one of many peptides that regulate food intake (e.g., amylin, cholecystokinin, leptin, opioids, orexins, and proopiomelanocortin) (10, 22, 25), which makes the exploration of one in isolation barely a beginning.

In many of our AL rats, food intake increased during some or most of the period of terminal weight loss. Even in the period of 42 days before death, when weight loss was significantly related to reduced food intake \( r = -0.36 \), the relationship was weak, accounting for only 13% \( r^2 = 0.13 \) of the variance in weight loss, and many rats (29 of 82 = 35.4%) increased their food intake above baseline. Clearly, early satiation due to changes in hypothalamic function cannot be the reason for terminal weight loss in many of these rats; the basis for their weight loss must be sought elsewhere. The wasting of dietary fuel due to altered gastrointestinal function and an increase in metabolic rate are the obvious candidates.

Although there are changes in gastrointestinal tract function with increasing age (8, 14), the extent of functional change does not appear to be sufficient to result in an energy deficit in any species studied, except the cat (13, 14). However, in the rat, as in most species, carefully conducted studies of macronutrient processing by the gastrointestinal tract in which animals undergoing terminal weight loss are compared with those of the same age not losing weight have not been performed. Until such studies are done, whether altered gastrointestinal function is involved in terminal weight loss in the rat will remain unanswered.

McDonald et al. (20) reported no change in metabolic rate during terminal weight loss; however, only eight rats were studied, and no information was provided on the food intake of the rats. It may be that those rats with a reduced food intake during terminal weight loss have no change in metabolic rate, whereas those with an increase in food intake exhibit an increased metabolic rate. McCarter and Palmer (19) found an increase in metabolic rate late in the life span of AL male F344 rats, but they did not provide information on food intake and body weight. An increase in metabolic rate during terminal weight loss could be due to the deterioration of neural regulation of energy balance. For example, there is evidence that leptin decreases food intake and increases metabolic rate (9); if, during the terminal period of life of some rats, the effect of leptin on food intake was blunted and its effect on metabolic rate enhanced, then a loss of body weight accompanied by an increase in food intake would be a possible outcome. Of course, this theoretical example is far too simple given the complex processes involved in the regulation of body weight (10). Moreover, a terminal increase in metabolic rate may have little to do with alterations in the neural regulation of energy balance. It is well known that mitochondrial deterioration occurs in all tissues during senescence (1); many believe that this deterioration of mitochondrial function is a cause of senescence. In this circumstance, a terminal increase in metabolic rate might be due to a generalized uncoupling of mitochondrial oxidative phosphorylation. However, before studies of any such mechanisms can be contemplated, metabolic rate must be measured during terminal weight loss, and the findings must show a subset of rats exhibiting an increased metabolic rate.

In our DR group, there was a significant association between weight loss and decreased food intake. However, 8 of the 38 rats in this group underwent terminal weight loss without a reduction in food intake. Moreover, the design of our DR study was such that the rats were prevented from increasing their food intake during any part of the period of terminal weight loss. Thus it is possible that the design of our study was responsible, at least in part, for the association between weight loss and decreased food intake.

The terminal weight loss in our rats did not relate to specific pathologies, which is in agreement with the findings of McDonald et al. (21). For the DR rats, the pattern of differences in food intake and weight loss (Table 3) between pathology classifications (renal 1–3, nonneoplasm vs. renal 1–3, neoplasm) was consistent with weight loss being associated with reduced food intake. That is, the larger the caloric deficit the greater the weight loss. However, as indicated by the correlation coefficients, the association between reduced food intake and loss in body weight is similar in those DR rats with and without neoplasms. In the AL rats, animals with and without neoplasms lost similar
amounts of weight, despite the fact that animals with neoplasms consumed significantly less food. In addition, for AL rats with neoplasms, the group with grades 1–3 renal lesions consumed less food yet lost less weight than the animals with grades 4 and E renal lesions. Thus, for the AL rats the differences in food intake and weight loss among pathology categories suggested no relationship between these two variables, a conclusion consistent with that obtained from the correlation coefficients. The findings in AL and DR rats indicate that a particular pathology, such as neoplasm, does not appear to be either necessary or sufficient for the existence of a relationship between reduced food intake and terminal loss in body weight. It may be that the relationship hypothesized by Blanton et al. (4) between reduced food intake and weight loss describes the association in a subgroup of animals. Our data do not dispute this; however, we are not able to identify such a subgroup on the basis of pathology.

It is striking that the length of the period of terminal decline in body weight is similar in the AL and DR groups. McDonald and associates (21) equate the period of terminal weight loss in rats with marked functional decline. Moreover, these authors refer to the terminal weight loss of rats as senescence. If this view is valid, then DR, an intervention that markedly slows the aging processes (17), delays the occurrence but does not decrease the length of time the rats experience senescent functional deterioration. Interestingly, the amount of weight loss, expressed in absolute or relative terms, is greater in the AL group; this may indicate that DR reduces the severity of functional decline. Such a conclusion may not be correct, however, in view of the data from a previous study on body composition of AL and DR rats. Our earlier work (2) showed that, during the period of life prior to the beginning of terminal weight loss, the body fat content of the AL rats averaged 17% of body weight, a value that was much greater than the 11% body fat content found in the DR rats. In addition, body fat was markedly decreased during terminal weight loss before a reduction in lean body mass was observed (2, 33). Thus it is possible that the loss of functionally important lean body mass during the terminal weight decline differs less between the two dietary groups than suggested by an examination of total mass lost.

The relevance, if any, of terminal weight loss in rats to senescent deterioration in other species including humans remains to be determined. McDonald and associates (21) suggested that the physiological decline occurring in senescent F344 rats is similar to the geriatric failure to thrive syndrome in humans. In humans, a decline in appetite and food intake with age, termed the anorexia of aging, is thought to predispose elderly individuals to the unintentional weight loss and malnutrition that are hallmarks of the failure to thrive syndrome (7, 11). The present study demonstrates for the first time that terminal weight loss in F344 rats fed ad libitum often occurs without reduced food intake. It is of interest in this regard that hormones, cytokines, and other factors thought to modulate weight changes in humans during senescence and in association with age-related diseases have been shown to suppress body weight and induce protein loss by metabolic processes unrelated to reductions in food intake (6, 9, 15, 30, 31). Any attempt to relate terminal weight loss in rats to the failure to thrive syndrome in senescent humans is further complicated by the fact that the clinical syndrome is imprecisely defined as the aggregate of weight loss and other interrelated impairments often attributable to specific comorbid conditions (23). Ultimately, however, identification of the dietary, metabolic, and pathological determinants of terminal weight loss in rats will likely provide insights into mechanisms of physiological decline occurring at the end of life in multiple mammalian species, including humans.

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