Energy Restriction-Induced Changes in Body Composition Are Age Specific in Mice1–3

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3Supplemental Figures 1 and 2 are available with the online posting of this paper at jn.nutrition.org.
4Abbreviations used: Ad-C, 12-mo-old control group; Ad-ER, 12-mo-old energy-restricted group; Ad-EX, 12-mo-old exercise trained group; Aged-C, 24-mo-old control group; Aged-ER, 24-mo-old control group; BMD, bone mineral density; DXA, dual energy X-ray absorptiometry; ER, energy restriction.
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Abstract
Restricting energy intake while supplying adequate micronutrients slows aging and extends maximal lifespan, whereas loss of body weight with exercise training does not. Our goal was to test the hypothesis that weight loss via energy restriction (ER) alters body composition in a way that is: 1) distinct from exercise-induced weight loss; and 2) conserved regardless of the age at which ER is initiated. An experimental model was developed where matched losses in weight could be induced with 6 mo of ER (~55% of ad libitum energy intake) or voluntary exercise on a running wheel in adult (12 mo) male C57BL/6 mice and a similar amount of ER-induced weight loss could be induced in aged mice (24 mo). Using dual-energy X-ray absorptiometry, we determined that ER and exercise in the 12-mo-old mice caused nearly identical changes in the amount and distribution of adipose tissue in the 12-mo group, with 70–75% of overall weight loss due to fat loss. Decreased prostate and epididymal fat weights were similar with ER and exercise, and heart weight was unaffected by either intervention. In contrast to the adult mice, in aged mice, ER caused primarily a loss of lean body mass including the heart, with no decreased prostate or fat pad weight. Bone mineral density was decreased by ER but not exercise in the adult mice, an effect not seen in the aged mice. Our data refute the hypothesis that ER causes a unique change in body composition that is conserved across age and suggest that fat loss may not be an essential component of the anti-aging effects of ER. J. Nutr. 137: 2247–2251, 2007.

Introduction
Restricting energy intake while supplying adequate micronutrients slows aging, retards the development of age-associated diseases, and extends maximal lifespan in all species studied to date (1–3). The molecular and physiological effects of energy restriction (ER)7 occur within a few weeks of starting an ER diet and appear to be qualitatively similar whether the ER diet is initiated during young adulthood or late in life (4–6). Not surprisingly, there is intense interest in understanding the mechanisms by which ER exerts these unique effects. It has been suggested that the anti-aging phenotype may be directly linked to the profound changes in body composition that occur in animals placed on a ER diet (7,8). The view that loss of white adipose tissue is central to the anti-aging effects of ER is supported by the recognition of adipose tissue as an endocrine organ that plays a central role in age-associated diseases such as insulin resistance (9,10). Of particular interest are fat deposits directly linked to pathologies, including ectopic fat in organs and abdominal adipose (11,12). However, loss of body weight with exercise training does not reproduce the anti-aging effects of ER (13–15). One possible explanation is that weight loss secondary to ER occurs in an anatomically distinct pattern that is necessary, and possibly sufficient, to extend maximal lifespan.

Accordingly, our goal was to test the hypotheses that weight loss via ER alters body composition in a way that is: 1) distinct from exercise-induced weight loss; and 2) conserved regardless of the age at which ER is initiated. To test these hypotheses, an experimental model was developed where matched losses in body weight could be induced with 6 mo of either ER or voluntary exercise in young adult (12 mo of age) C57BL/6 mice and a similar amount of ER-induced weight loss could be induced in aged mice (24 mo old). The pattern of change in body composition was then compared across ages and weight loss modalities using dual-energy X-ray absorptiometry (DXA) and direct measurement of selected organ weights measured at sacrifice.

Methods

Animals. Male C57BL/6 mice were purchased at 5 (n = 30) and 17 mo of age (n = 24). Mice were housed individually and consumed a standard
rodent diet ad libitum [AIN-93M (16), 15.1 kJ/g, TestDiet] during a
1-mo acclimatization period. The composition of the AIN-93M diet is presented elsewhere (16). Baseline ad libitum energy intake during the final week of acclimatization was 59.4 ± 1.3 kJ/d in the 6-mo olds and 67.4 ± 2.9 kJ/d in the 18-mo olds (2.1 kJ·d⁻¹·g body weight⁻¹ in both age groups). At 6 and 18 mo of age, mice were randomized into a total of 5 experimental groups for the 6-mo experimental period (see Supplemental Fig. 1). All experimental procedures were approved by a University of Wisconsin-Madison Animal Care and Use Committee and adhered to the NRC’s Guide for the Care and Use of Laboratory Animals.

**Experimental groups.** The 6-mo-old adult mice were divided into 3 groups, 10 mice per group. Group 1 (Ad-C) was a control group that had energy intake adjusted in an attempt to maintain body weight near baseline levels. For this control group, energy intake was initially 90% of the mean ad libitum energy intake and was steadily decreased to 75% of ad libitum intake by the end of the experimental period. Group 2 (Ad-EX) received an equal amount of food (pair-fed) as the control group and was given 24-h access to running wheels (Mini-Mitter) equipped with wheel revolution counters allowing the distance run to be calculated. Group 3 (Ad-ER) was restricted in energy intake to the mean ad libitum energy intake and was steadily decreased to 75% of baseline levels. For this control group, energy intake was initially 90% of energy intake adjusted in an attempt to maintain body weight near control group levels. Adult and aged ER groups received a modified version of the AIN-93M diet enriched by 67% in vitamins and minerals to ensure that ER mice received approximately the same amounts of micronutrients as the control groups (16).

**Results**

At the start of the experimental period, the 6-mo-old mice \( (n = 30) \) weighed 29.0 ± 0.1 g and the 18-mo-old mice \( (n = 24) \) weighed 33.0 ± 0.1 g. Despite modest ER, the Ad-C group significantly increased body weight \( (~5\%) \) during the course of 6 mo (Supplemental Fig. 2A). In contrast, body weight in the Aged-C group did not change significantly during the 6-mo experimental period. During the course of the 6-mo period, 2 of the Aged-C, but interestingly none of the Aged-ER mice, died. The adult and aged ER groups demonstrated very similar timing and magnitude of weight loss. (Supplemental Fig. 2A). Importantly, the magnitude of weight loss in the 2 ER groups was comparable, facilitating comparisons between the 2 age groups. The Ad-EX group that was pair-fed with the Ad-C group ran 5426 ± 560 m/d during wk 1 of the experimental period. This approximate amount of running was maintained for the first 3 mo of the experimental period, with a subsequent gradual decline in running (Supplemental Fig. 2B). During the final week of the experimental period, mice ran 3291 ± 218 m/d. At the end of the 6-mo experimental period (12 mo of age), the body weight of the Ad-EX mice was less than the control group \( (P < 0.05) \) but not different from the Ad-ER group. Thus, the goal of achieving similar degrees of weight loss via ER and voluntary exercise was accomplished (Table 1).

**Body composition.** Body composition was assessed in all mice prior to killing using DXA as previously described (17–19). Mice were anesthetized for DXA using 1.5% to 2.0% isoflurane inhalation anesthetic administered via nose cone. Following anesthesia induction, mice were placed on the scanner bed in the prone position with the limbs and tail stretched away from the body. One scan per mouse, requiring 4 min, was performed and analyzed with PIXImus software (2.10, GE/Lunar). Total body analysis for lean body mass and fat mass required no region of interest placement. Abdominal, hind limb, and femur regions of interest were placed for determination of regional body composition. PIXImus accuracy was tested by comparison of scan values to values derived from proximate biochemical analysis of soft tissue composition determined by extraction.

Mice were killed via cervical dislocation. The heart was removed, lightly blotted, and weighed, as was the right epididymal fat pad. The prostate gland was then dissected and weighed.

**Data analysis.** Age and diet effects were tested by 2-way ANOVA. The Ad-EX group was excluded from this analysis. One-way ANOVA with Tukey’s post hoc and \( t \) tests were then used to examine treatment effects in the adult and aged mice, respectively. All data are presented as means ± SEM, with an \( \alpha \) level of 0.05 considered significant.

**TABLE 1** Body weight, body fat, and regional fat content in adult and aged mice that consumed a control diet, an ER diet, or performed voluntary exercise for 6 mo ¹

<table>
<thead>
<tr>
<th>P-values (2-way ANOVA)</th>
<th>C</th>
<th>ER</th>
<th>EX</th>
<th>Age</th>
<th>Diet</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
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</tr>
<tr>
<td>Adult</td>
<td>30.5 ± 0.6ᵃ</td>
<td>24.4 ± 0.3ᵇ</td>
<td>25.2 ± 0.4ᵇ</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.21</td>
</tr>
<tr>
<td>Aged</td>
<td>32.1 ± 1.4ᵃ</td>
<td>28.0 ± 0.5ᵇ</td>
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<tr>
<td>Fat (whole body), %</td>
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<tr>
<td>Adult</td>
<td>21.5 ± 1.1ᵃ</td>
<td>13.4 ± 0.9ᵇ</td>
<td>13.8 ± 1.1ᵇ</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aged</td>
<td>14.5 ± 1.7ᵃ</td>
<td>12.8 ± 0.9ᵇ</td>
<td>–</td>
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<td></td>
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<tr>
<td>Fat (abdomen), %</td>
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</tr>
<tr>
<td>Adult</td>
<td>20.9 ± 1.5ᵃ</td>
<td>10.2 ± 1.1ᵇ</td>
<td>10.8 ± 1.3ᵇ</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aged</td>
<td>12.9 ± 2.2ᵃ</td>
<td>9.9 ± 0.9ᵇ</td>
<td>–</td>
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<td></td>
<td></td>
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<tr>
<td>Fat (hind limb), %</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>24.1 ± 1.0ᵃ</td>
<td>14.9 ± 0.7ᵇ</td>
<td>15.5 ± 1.0ᵇ</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aged</td>
<td>16.7 ± 2.3ᵃ</td>
<td>15.5 ± 0.8ᵇ</td>
<td>–</td>
<td></td>
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</tbody>
</table>

¹ Values are means ± SEM, \( n = 10 \) except Aged-ER, \( n = 12 \). Within each age group, means without a common letter differ. Adult, 12 mo; aged, 24 mo; C, control diet; EX, performed voluntary exercise for 6 mo.
groups (21.5 ± 0.7 g) was not significantly different, with no tendency for exercise training to preserve lean body mass. In stark contrast to the 12-mo-old mice, in the aged mice, the lower body weight of the Aged-ER group relative to the Aged-C group was attributable primarily to a lower lean body mass, with only ~38% of the lower body weight due to less body fat.

To identify the anatomical location of the changes in fat and lean body mass, abdominal and hind limb composition were studied in more detail in parallel with the whole body measurements. Weight loss in the Aged-ER mice did not cause a significant decrease in percent fat of the body, abdomen, or hind limbs. Thus, the weight loss in all 3 of these regions was a proportional loss of lean and fat. In contrast, the Ad-ER and Ad-EX mice lost predominantly fat in both the abdomen and leg as indicated by decreased percent fat (Table 1). Interestingly, the effects of weight loss via ER and exercise were indistinguishable in terms of the amount and anatomical distribution of lean and fat mass.

Consistent with body composition assessed with DXA, the weight of tissues high in fat (epididymal fat pad, prostate gland) were significantly decreased in the Ad-ER and Ad-EX mice relative to the Ad-C group, whereas heart weight was not significantly (P = 0.7) affected by weight loss in these groups (Table 2). Also consistent with the DXA data, the aged mice showed the opposite pattern, with loss of heart mass, but no significant loss of epididymal or prostate tissue in the Aged-ER group.

In the 12-mo-old mice, ER but not exercise significantly decreased bone mineral density (BMD) (Table 2). As expected, BMD was significantly lower in the Aged-C than in the Ad-C group. In the aged mice, ER did not decrease BMD as it had in the adults.

**Discussion**

The growing recognition that adipose tissue is a dynamic endocrine organ has added support to the view that loss of fat is central to ER-induced retardation of aging (7,8). However, loss of fat with chronic exercise training does not recapitulate the ER-induced retardation of aging (13,15). One possible explanation for this is that ER causes changes in the amount and distribution of fat that differ in some critical way from other weight loss modalities. This possibility would be consistent with the extensive literature demonstrating that not only the amount but also the location of fat is critical to health (11,12). To examine this possibility, we developed a mouse model where matched amounts of weight loss could be induced with 6 mo of either ER or exercise training. Using this model in adult mice, we found that ER and exercise caused nearly identical changes in adipose tissue amount and distribution. This finding allowed us to make several conclusions. First, it indicates that the failure of exercise to mimic the anti-aging effects of ER is not due to a failure to remodel body composition appropriately. Second, it indicates that ER-like changes in the amount and distribution of adipose tissue are not sufficient to elicit an anti-aging phenotype. Thus, even a pharmacological weight loss treatment capable of decreasing fat and organ weights in the exact pattern of ER would not necessarily retard aging. Instead, our data support the approach of identifying factors that can recapitulate the changes in gene expression induced by ER (4,5). The model we have established of matched weight loss with ER and exercise should be useful in this regard, because it allows changes in gene expression present in ER but not exercise to be efficiently identified.

Data from several recent studies demonstrate that ER-induced retardation of aging and extension of lifespan occurs when ER is initiated in aged, as well as in younger, adult mice (4,20). Although the amount of lifespan extension is less when ER is initiated late in life, the molecular and physiological effects of ER appear to be qualitatively similar regardless of the age at which ER is initiated (4,6,20). Therefore, to determine whether common aspects of body composition could be identified that might be critical to the anti-aging effect of ER, we compared ER-induced changes in body composition in young adult and aged mice. Our data indicate that 6 mo of ER causes fundamentally different changes in body composition in adult and aged mice. Specifically, we found that ER-induced weight loss in adult mice is primarily due to loss of fat mass, whereas in aged mice it is due primarily to loss of lean mass. This was true when body composition was assessed with DXA and when weighing individual organs directly.

The finding that ER-induced changes in body composition are highly age-specific suggests that a particular pattern of change in body composition may not be necessary for anti-aging effects of ER to be manifest. Of particular interest is the fact that a decrease in percent body fat did not occur in the aged ER mice. However, our study was not designed to specifically measure any

**TABLE 2** Tissue weights and femoral BMD in adult and aged mice that consumed a control diet, an ER diet, or performed voluntary exercise for 6 mo.

<table>
<thead>
<tr>
<th></th>
<th>C</th>
<th>ER</th>
<th>EX</th>
<th>P-values (2-way ANOVA)</th>
<th>Age</th>
<th>Diet</th>
<th>Interaction</th>
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</thead>
<tbody>
<tr>
<td><strong>Prostate gland, mg</strong></td>
<td></td>
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</tr>
<tr>
<td>Adult</td>
<td>47 ± 4</td>
<td>33 ± 2</td>
<td>31 ± 2</td>
<td>0.30</td>
<td>&lt;0.01</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Aged</td>
<td>38 ± 4</td>
<td>34 ± 2</td>
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<tr>
<td><strong>Epididymal fat pad, mg</strong></td>
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<tr>
<td>Adult</td>
<td>405 ± 24</td>
<td>155 ± 15</td>
<td>186 ± 19</td>
<td>0.43</td>
<td>&lt;0.01</td>
<td>0.06</td>
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<tr>
<td>Aged</td>
<td>281 ± 62</td>
<td>175 ± 26</td>
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<tr>
<td><strong>Heart, mg</strong></td>
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<tr>
<td>Adult</td>
<td>114 ± 4</td>
<td>109 ± 6</td>
<td>110 ± 2</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
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<tr>
<td>Aged</td>
<td>151 ± 4</td>
<td>117 ± 4</td>
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<tr>
<td><strong>Femoral BMD, mg/cm²</strong></td>
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<tr>
<td>Adult</td>
<td>48.5 ± 0.5</td>
<td>44.9 ± 0.8</td>
<td>48.0 ± 0.4</td>
<td>&lt;0.01</td>
<td>0.93</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Aged</td>
<td>40.0 ± 1.7</td>
<td>42.9 ± 1.1</td>
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</tbody>
</table>

*Values are means ± SEM, n = 10 except Aged-ER, n = 12. Within each age group, means without a common letter differ. Adult, 12 mo; aged, 24 mo; C, control diet; EX, performed voluntary exercise for 6 mo.*
anti-aging outcome variables such as lifespan. Because we have no direct evidence of an anti-aging effect of our ER treatment, our data are consistent with but do not prove that the anti-aging effects of ER can occur without significant fat loss. However, our ER protocol was modeled after those known to cause anti-aging effects in mice (4,6,20,21). Additionally, there are several indirect pieces of evidence contained within our data consistent with an anti-aging effect of our ER treatment in the aged mice. For example, whereas there were 2 deaths in the Aged-C group during the 6 mo experimental period (a mortality rate consistent with published survival curves in this strain of mice), there were no deaths in the Aged-ER group (22). An additional suggestion of an anti-aging effect in our mice comes from the observation that ER seemed to prevent the age-associated loss of BMD in the mice. These pieces of indirect evidence all suggest that our ER protocol would be expected to have an anti-aging effect in the aged mice, even in the absence of a significant loss of fat.

Several other aspects of our experimental design merit mention. First, we assessed body composition in vivo using DXA. Although this technique has been shown to be very accurate and reproducible in C57BL/6 mice, we cannot rule out the possibility that some small effects of age or treatments went undetected (23). Second, we chose a cross-sectional experimental design as opposed to a longitudinal design, because it allowed us to make between-group comparisons in age-matched mice. This age matching was necessary, because body weight changes substantially in C57BL/6 mice during the 6-mo periods of interest, making it difficult to separate treatment effects from those of aging when a longitudinal design is used (22). Additionally, the cross-sectional design allowed between-group comparisons of variables such as organ weights that can only be made in terminal experiments. Another consideration of our experimental design was that we chose to restrict energy intake in our control and exercise groups. Limiting the amount of food given to the control groups in studies of ER is common practice, primarily because rodents consuming food ad libitum gain a substantial amount of weight during late middle age (21,24). Thus, if ad libitum-fed animals are used as the reference group, it is impossible to separate anti-obesity effects of ER from the anti-aging effects that occur when nonobese animals are energy restricted. The degree of ER in the control and pair-fed exercise groups would be anticipated to provide some small degree of anti-aging effect (25,26). However, the dose-response relationship between ER and lifespan extension is such that much larger anti-aging effects would be expected in the ER groups (25,26). The exercise group was energy restricted to the same degree as the control mice, because mice consuming feed ad libitum increase energy intake when given access to a running wheel and do not generally lose weight (27,28). Thus, energy restricting the exercise group was necessary, because our goal was to study weight loss, not attenuation of weight gain. Use of voluntary wheel running as the exercise modality imposed several limitations, most notably that the amount of running, and hence weight loss, was not under our control. However, the mice ran enough to lower body weight ~17%, a substantial amount. Another limitation of voluntary exercise is that as rodents age, they chose to exercise less (13,29). This declined voluntary running with age (see Supplemental data) prevented us from studying an aged exercise group, because they would be expected to perform an inadequate amount of voluntary exercise. Although we could have used forced treadmill running to induce weight loss in the adult and aged mice, the stressful and unnatural nature of this type of exercise could have complicated data interpretation.

In summary, the major findings of this study were that ER-induced changes in body composition are indistinguishable from those that occur with exercise training and are fundamentally different in adult and aged mice that should be experiencing qualitatively similar anti-aging effects. Our data strongly refute the hypothesis that ER-induced weight loss causes a unique change in body composition that is conserved across age and emphasize the need to identify the molecular adaptations unique to ER.

**Literature Cited**


