Influences of calorie restriction and age on energy expenditure in the rhesus monkey

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Caloric restriction (CR) is known to retard the aging process, and a marker of aging is decreased energy expenditure (EE). To assess longitudinal effects of CR on EE in rhesus monkeys (Macaca mulatta), data from 41 males (M) and 26 females (F) subjected to 9 or 15 yr of CR were studied. EE and body composition of monkeys 11–28 yr of age were measured using indirect calorimetry and dual X-ray absorptiometry. Total EE (24-h EE) was divided into daytime (day EE), nighttime (night EE), and daytime minus nighttime (D–N EE). Mortality and morbidity were decreased in CR monkeys compared with control-fed (C) monkeys. However, resting EE explained only 13% of the difference. The effect of CR on energy expenditure has also been reported in other species (2, 25). This lower EE in excess of the decrease attributed to FFM has been termed metabolic adaptation or accommodation to CR (20).

The main aim of the present study was to determine the effects of long-term CR on the EE of 34 C (21 males and 13 females) and 33 calorie-restricted (20 males and 13 females) monkeys over a span of 7 yr. We also sought to determine whether CR influences the age-related changes in EE and whether gender differences occur in response to CR.

METHODS

Animals

Rhesus monkeys that were part of the CR study at the Wisconsin National Primate Research Center were studied. The study design was a combination of longitudinal and cross-sectional measures. EE was measured annually for 7 yr, corresponding to 9–15 yr of CR in the cohort of older monkeys (18) and 3–9 yr in cohort of younger monkeys. Data from any animals that died during this period were excluded. Monkeys were housed separately in stainless steel cages with inner dimensions of 89 cm wide × 86 cm deep × 86 cm high under controlled conditions of temperature and humidity. The rooms in which the cages were housed were located in a controlled environment building. The cages were monitored for 24 h for temperature, humidity, and lighting. The monkeys were fed a diet of 10% fat, 30% carbohydrate, and 60% protein and were fed ad libitum. The monkeys were housed in constant temperature rooms (21 ± 1°C) with a 12-h light/dark cycle (lights on at 0600 h and off at 1800 h). The rooms were monitored for temperature, humidity, and lighting.

Experimental Procedures

Respiratory gas exchange. EE was measured in a standard cage enclosed within a transparent metabolic chamber with dimensions of 75 cm wide × 86 cm deep × 86 cm high under controlled conditions of temperature and humidity. The chambers were housed in a constant temperature building. The cages were monitored for 24 h for temperature, humidity, and lighting. The monkeys were housed in constant temperature rooms (21 ± 1°C) with a 12-h light/dark cycle (lights on at 0600 h and off at 1800 h). The rooms were monitored for temperature, humidity, and lighting.

HUMAN AGING IS ASSOCIATED with morphological, physiological, and behavioral changes (31). An age-related decrease in energy expenditure (EE) is one of the measurable changes, such that total EE was shown to decrease by 1–2% with each decade increase in adult life (19). Some of this decrease was attributed to decreases in fat-free mass (FFM), but even after accounting for differences in FFM, resting metabolic rate (RMR) was significantly lower in the elderly compared with the younger individuals (24, 33, 34).

Calorie restriction (CR) slows the aging process and extends maximal life span in multiple species (37). The physiological effects of CR in rhesus monkeys include decreased body weight, lowered body temperature (21), and a decrease in the metabolic rate; dietary restriction; indirect calorimetry

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cm wide × 75 cm deep × 80 cm high. Chamber temperature was maintained at 21°C to maintain the animals at thermoneutrality. The animals were housed in these chambers 1 day before the start of measurements for acclimatization. Respiratory gas exchange was measured for 2 consecutive days. The chamber was placed in the room where other animals were housed to decrease the stress induced, possibly by the change in cage environment (27).

Filtered air was drawn into the chamber, and the flow rate, temperature, and humidity were measured. A portion of the exhaust air from the chamber was dried and analyzed for oxygen (S-3A O2 analyzer; Ametek, Pittsburgh, PA) and CO2 (CD-3 CO2 analyzer; H11003 cm wide). Outputs from the oxygen and CO2 analyzers, along with the flow rates, were recorded every 5 min into a Workbench Data analyzer; Ametek, Pittsburgh, PA) contents. The chamber was tested by burning ethanol from a lamp and the %recovery of oxygen and CO2 was used to calibrate the analyzer contents. The chamber was dried and analyzed for oxygen (S-3A O2 analyzer; Ametek) contents. The chamber was tested by burning ethanol from a lamp and the %recovery of oxygen and CO2 was used to calibrate the analyzer contents. The chamber was placed in the room where other animals were housed to decrease the stress induced, possibly by the change in cage environment (27).

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Total EE (24-h EE) was divided into daytime EE [day EE; measured from 6 AM to 6 PM (12 h)] and nighttime EE [night EE; measured from 6 PM to 6 AM (12 h)]. Energy expended in physical activity was approximated as the difference between daytime and nighttime EEs (D – N EE); however, this would also include the thermic effect of food.

Body composition. Whole body composition was measured every 6 mo using dual energy X-ray absorptiometry (DEXA; Model DPX-L; GE Lunar, Madison, WI). Briefly, animals were sedated with a mixture of ketamine HCl (10 mg/kg im) and xylazine (0.6 mg/kg im) for additional muscular relaxation and were scanned in supine position (5, 7).

### Statistical Analysis

Statistical comparisons of treatment groups across time were made by linear mixed models using Proc Mixed in Statistical Analysis System (version 9.1; SAS Institute). The model included a random effect for each animal and assumed an underlying variance-covariance structure because of multiple measures for each animal. When the time-by-treatment group interaction was statistically significant (P < 0.05), indicating that the treatment group differences varied significantly across time, treatment group comparisons at each time were tested by Fisher’s protected least significant difference procedure.

It is particularly important to assess whether EE differences observed between C and calorie-restricted animals during these 7 yr of measurement were due merely to differences in metabolic body mass. To accomplish this, we used fat mass (FM) and FFM as a covariate to adjust for differences in metabolic mass in a manner that avoided the assumption of strict proportionality, as is done when metabolic rate is expressed as the ratio of EE to body size. Approximate linearity of the relationships between EE and body mass measures over the interval of interest and similarity of slopes for the two treatment groups were verified. Similarly, to quantify the effect of aging, data were adjusted for FM and FFM differences using the Proc Mixed in SAS. Gender differences were analyzed in similar-aged monkeys, with only sex as a predictor of EE in the regression model.

### RESULTS

The mean age of all three groups of animals was 18.5 ± 3 yr (range of 11–28 yr). The CR and C animals differed significantly with respect to body weight and body composition in male and female monkeys, as shown in Table 1. Energy intake in the calorie-restricted animals was ~24% lower than controls in all three groups.

#### Twenty-Four-Hour EE in Males

The mean EE of the CR and C monkeys are as shown in Table 2.

### Table 1. Average primate characteristics over the course of the longitudinal study*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CR</th>
<th>C</th>
<th>CR</th>
<th>C</th>
<th>CR</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>13</td>
<td>8</td>
<td>8</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Age, yr</td>
<td>21 ±0.3*</td>
<td>21 ±0.3*</td>
<td>17 ±0.6b</td>
<td>17 ±0.6b</td>
<td>17 ±0.3f</td>
<td>17 ±0.3b</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>10 ±0.2c</td>
<td>14 ±0.3b</td>
<td>11 ±0.4f</td>
<td>15 ±0.5b</td>
<td>8 ±0.2#</td>
<td>9 ±0.2b</td>
</tr>
<tr>
<td>FFM, kg</td>
<td>8.5 ±0.1c</td>
<td>9.7 ±0.2b</td>
<td>9.1 ±0.2f</td>
<td>10.3 ±0.2b</td>
<td>6.3 ±0.1f</td>
<td>6.6 ±0.1a</td>
</tr>
<tr>
<td>FM, kg</td>
<td>1.7 ±0.1c</td>
<td>4.6 ±0.2b</td>
<td>2.0 ±0.2f</td>
<td>4.8 ±0.4b</td>
<td>1.6 ±0.1f</td>
<td>2.9 ±0.1#</td>
</tr>
<tr>
<td>Energy intake, kcal/day</td>
<td>548 ±81</td>
<td>720 ±13</td>
<td>640 ±11</td>
<td>840 ±26</td>
<td>474 ±81</td>
<td>621 ±16</td>
</tr>
</tbody>
</table>

Values are means ± SE. CR, calorie-restricted; C, controls; FM, fat mass; FFM, fat-free mass. * Annual assessment period corresponding to 3–9 yr of CR in younger males and females and 9–15 yr of restriction in older males; †‡statistically significant differences between CR and C monkeys within each of the 3 groups (P < 0.01); # different symbols indicate statistically significant differences between the 3 groups of C monkeys (P < 0.01); †‡different symbols indicate statistically significant differences between the 3 groups of CR monkeys (P < 0.01). Statistical differences were assessed using ANOVA followed by Fisher’s least significant difference multiple comparisons.

### Table 2. Components of mean EE in the restricted and C male monkeys averaged over longitudinal study

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24-h EE, kcal/day</th>
<th>Day EE, kcal/12 h</th>
<th>Night EE, kcal/12 h</th>
<th>D – N EE, kcal/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>568 ±14</td>
<td>334 ±10</td>
<td>234 ±6</td>
<td>100 ±10</td>
</tr>
<tr>
<td>C</td>
<td>630 ±22</td>
<td>360 ±16</td>
<td>270 ±8</td>
<td>90 ±13</td>
</tr>
<tr>
<td>P (age effects)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.10</td>
<td>0.61</td>
</tr>
<tr>
<td>P (CR effects)</td>
<td>0.002</td>
<td>0.08</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. EE, energy expenditure. All analyses were performed in Statistical Analysis System (SAS) using the Proc Mixed procedure. There was no statistically significant interaction between treatment and age for any of the components of total EE (24-h EE), daytime EE (day EE), nighttime EE (night EE), or daytime minus nighttime EE (D – N EE).
Day EE (kcal/12 h) in Males

Age effects. Day EE significantly decreased by 12 ± 2 kcal with each year increase in age (P < 0.0001). When adjusted for FM, day EE still decreased by 12 ± 2 kcal with each year increase in age (P < 0.0001). There was a significant decrease (P < 0.0001) in day EE even after adjusting for FM and FFM in these aging male monkeys.

CR effects. Day EE between the calorie-restricted and C monkeys trended toward significance with the mean difference of 26 ± 16 kcal (P = 0.08). After adjusting for FFM, the mean difference was 8 ± 16 kcal between the calorie-restricted and C monkeys (P = 0.13), with no significant interaction (P = 0.79) between age and treatment. Furthermore, the effects of CR were obliterated (P = 0.97) by adding FM to the model.

Night EE (kcal/12 h) in Males

Night EE was used as a proxy measure for the resting EE, assuming that the animal does not engage in any physical activity from 6 PM to 6 AM while the lights are turned off.

Age effects. Night EE tended to decrease by 1.4 kcal with each year increase in age (P = 0.10). Adjusting for FM and FM did not influence the effect of age on night EE (P = 0.65 and P = 0.96, respectively).

CR effects. Night EE was significantly different between the calorie-restricted and C monkeys (P < 0.0001), with a mean difference of 36 ± 8 kcal. Adjusting for FM, calorie-restricted monkeys had a lower night EE than C monkeys by 18 ± 2 kcal with marginal significance (P < 0.06), with no significant interaction between age and treatment (P = 0.72). The effect of CR on night EE was obliterated (P = 0.72) by adding FM to the model.

D − N EE in males

Night EE was subtracted from day EE to approximate of energy expended in physical activity during the day.

Age effects. D − N EE decreased by 10 kcal with each year increase in age (P < 0.001), accounting for most of the decrease in 24-h EE with age. Even after adjusting for FFM, the decrease in D − N EE was significant with age (10 ± 2 kcal/yr of age, P < 0.0001), indicating that the decrease in D − N EE with age is not solely due to decreases in FFM. When also adjusted for FM, D − N EE significantly (P < 0.0001) decreased with each year increase in age.

CR effects. There was no effect of CR on the physical activity of these monkeys (mean difference = 10 kcal, P = 0.61). Adjusting for FFM, physical activity EE did not differ between the CR and C monkeys (mean difference = −14 kcal, P = 0.63), with no significant interaction (P = 0.69) between age and treatment. CR did not have an effect on D − N EE, even after FM was added (P = 0.86) to the model.

The CR and C monkeys differed in their night EE, and a marginally significant difference remained after an adjustment for FFM differences was made. Nonetheless, this difference in night EE was insignificant after FM was added in the model. The total, day, or D − N EE did not differ with age between the CR and C monkeys.

Gender Effects (Younger Males and Females Only)

Younger male and female monkeys, which are of similar ages, significantly differed in their 24-h EE, as shown in Table 3. Twenty-four-hour EE was significantly higher in male than in female monkeys (mean difference = 140 kcal/day, P < 0.0001; Fig. 2). FFM-adjusted night EE remained significantly greater in the males (mean difference = 21 kcal, P < 0.0001), and this difference remained significant, even when adjusted for FM (mean difference = 18 kcal, P = 0.014) between the sexes.

CR effects. CR was associated with a statistically insignificant decrease in 24-h EE in the younger male (mean difference between CR and C = 34 kcal/day, P = 0.39) and female (mean difference between CR and C = 10 kcal/day, P = 0.96) monkeys.

Table 3. Components of mean energy expenditure in younger CR and C monkeys collected for 7 yr

<table>
<thead>
<tr>
<th>Treatment</th>
<th>24-h EE, kcal/day</th>
<th>Day EE, kcal/12 h</th>
<th>Night EE, kcal/12 h</th>
<th>D − N EE, kcal/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult males</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>619 ± 18</td>
<td>381 ± 14</td>
<td>246 ± 8</td>
<td>135 ± 13</td>
</tr>
<tr>
<td>C</td>
<td>653 ± 30</td>
<td>388 ± 23</td>
<td>281 ± 9</td>
<td>107 ± 19</td>
</tr>
<tr>
<td>Adult females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>499 ± 10</td>
<td>314 ± 7</td>
<td>190 ± 4</td>
<td>124 ± 7</td>
</tr>
<tr>
<td>C</td>
<td>492 ± 10</td>
<td>314 ± 2</td>
<td>207 ± 4</td>
<td>84 ± 7</td>
</tr>
<tr>
<td>P (gender effects)</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.6</td>
</tr>
<tr>
<td>P (CR effects)</td>
<td>0.39 0.7</td>
<td>0.0002 0.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. All analyses were performed in SAS using the Proc Mixed procedure. No statistically significant interaction between gender and age was observed in any of the components of 24-h EE, day EE, night EE, or D − N EE.
whereas we measured FFM, which includes a significant emphasis on the metabolically active portion of the body, also reflected an age-related decrease in cellular metabolic composition, observed an age-related decrease in resting EE that was due in part to decreases in FFM but suggested that it 

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degree in resting EE. We think that this is unlikely, however, because the Δ − N EE (a proxy for physical activity EE) decreased with age independent of changes in FFM and FM, which is in the opposite direction from the hypothetical nighttime change in physical activity that would be required to mask any underlying change in RMR.

The decrease in activity-related EE accounted for 80% of the total decrease in 24-h EE and thus was the most important contributor to our observed aging effect. It is interesting to compare this with the effects of aging on EE in healthy humans. Elia et al. (10) showed that, in aging humans, physical activity EE contributed only 46% of the decrease in 24-h EE and was independent of the decrease in RMR. An important difference between these studies, however, was that the humans were free-living individuals in whom the ratio of 24-h EE to RMR was about 1.5 (9), whereas our animals were caged and 24-h EE/night EE was only about 1.2. The higher 24-h EE to RMR ratio in humans may reflect a higher level of physical activity required by our daily existence, which might override and dilute out changes due to aging.

Again, however, Δ − N EE is only a proxy estimate of physical activity EE in that the Δ − N EE also includes the thermic effect of food (TEF) component within. In previous studies in this cohort, the TEF in these animals was determined to be −7% of total energy expended (TEF = Δ − N EE for 2 h postprandial − night EE for the same time period), which is very similar to the estimates (−10%) provided in other mammalian studies (12). Because energy intake also decreases slowly with age, the TEF contribution to the Δ − N EE would be expected to decrease. This, however, would not be sufficient to account for the age-related annual decrease in Δ − N EE, because 7 or even 10% of the annual decrease in energy intake would equal only 1–2 kcal/day. Nonetheless, aging is associated with a significantly lower physical activity EE even after adjusting for body composition changes.

**Effect of Age**

We partitioned our EE data into night EE, day EE, and the difference between day and night EE. We consider the night EE a measure of resting EE, and we did not detect an age-related decrease in night EE. This contrasts with human data, in which aging has been associated with a decline in RMR. This is often attributed to decreases in FFM in older individuals (3, 12, 23, 28, 30). Flynn et al. (11), in one of the largest longitudinal studies on the age-related effects on human body composition, observed an age-related decrease in resting EE that was due in part to decreases in FFM but suggested that it also reflected an age-related decrease in cellular metabolic activity (4). An important difference between our study and that of Flynn et al. was that Flynn et al. utilized total body potassium to approximate body cell mass and thus placed emphasis on the metabolically active portion of the body, whereas we measured FFM, which includes a significant amount of metabolically inactive tissue as well as tissues with high metabolic activities (25). A recent human study (35) indicates that the age-related decrease in resting EE, however, does not so much result from a decrease in the size of organ tissue per se but rather from a smaller fraction of organ tissue being composed of active cell mass. Unfortunately, the DEXA methodology used in our study cannot partition body composition in a manner that would allow us to investigate this issue further.

Another possible, but less likely, explanation for our not finding an age-related decline in night EE is that it is not strictly equivalent to resting EE. Because the animals generally sleep during this nighttime period, we used this measure as a proxy measure for resting EE. Physical activity was not monitored in the metabolic cages, however, and thus there is a small possibility that the absence of an age-related decrease in resting EE may have resulted from animals being active enough during the night to increase EE and cancel out an age-related decrease in resting EE. We think that this is unlikely, however, because the Δ − N EE (a proxy for physical activity EE) decreased with age independent of changes in FFM and FM, which is in the opposite direction from the hypothetical nighttime change in physical activity that would be required to mask any underlying change in RMR.

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**Gender Effects**

Age-matched males have higher metabolic rates than females. However, when basal metabolic rate (night EE) was adjusted for individual differences in FFM and FM, females were found to have lower nighttime metabolic rates compared with age matched males.

**DISCUSSION**

Using this nonhuman primate model of long-term CR, we have shown that 24-h EE decreased by about 1.6% annually in male monkeys, and most of this difference (−80%) was explained by a decrease in energy expended in physical activity with age. This rate of decrease, however, was not altered by CR in animals between the ages of 11 and 28 yr. Our results also confirmed that CR was associated with an −10% decrease in 24-h EE in the male monkeys compared with controls, with most of the difference (60%) due to a lower night EE in the CR males. Adjusting for FM did not obviate this decrease, but adjusting for FFM and FM obviated the effect of CR. Additionally, females had a lower 24-h EE compared with age-matched males, and night EE was significantly lower in females even after adjusting for body composition differences. FM had a significant effect on night EE independent of CR in younger and old male and female monkeys.

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**Gender Effects**

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Effect of CR

Our data in the calorie-restricted rhesus monkeys expands upon our prior cross-sectional findings of Blanc et al. (2) and Ramsey et al. (27), who published part of this data from this same cohort. Specifically, we have provided a longitudinal aspect to the data by analyzing the same group of monkeys for a period of 7 yr. Our findings suggest that CR in aging monkeys was associated with a decrease in 24-h EE and that most of this was due to changes in body composition. We have expanded these analyses of EE by fully breaking the data down into 24-h EE, night EE, and D − N EE. In so doing, we have shown that the night EE is lower in CR monkeys due to a lower FFM and FM. Additionally, FM has an independent effect on the night EE in younger and older male and female monkeys.

The adjustment for FM, however, does not appear to be a simple mass effect reflecting the physiological metabolic costs of the FM itself. In our regression models, the slope for FFM influencing night EE was 53 kcal/kg, whereas that for FM was 29 kcal/kg. Although the slope for FFM is in a range where one could account for the EE on the basis of the energy requirements of the tissue that makes FFM, the slope for FM is much too large for that option. We were concerned that the FM might be an artifact of the group differences in FM between CR and C but found that there was overlap in FM between these groups and that the slopes did not differ when analyzed within the treatment group. We could not rule out a possible statistical artifact resulting from the correlation between FM and FFM; however, it is unlikely that this would explain why we observed differences between CR and controls with inclusion of FFM in the model but not when FFM and FM were included. We speculate instead that the FM effect is an indirect biochemical effect that might be related to the effects of adipose tissue-derived hormones and cytokines on the metabolism of cells other than adipocytes.

Our central prediction was that CR would slow the rate of change in EE with aging. This prediction was based largely on the facts that mitochondrial proton leak is a major contributor to RMR and has been shown to decrease during CR in rodents (29, 32) and free radical production. Because a reduction in free radical production should, in turn, reduce damage to the mitochondria with age, we hypothesized that EE would decrease less with age in CR (1, 14). Consistent with this hypothesis, we found that CR induced a decrease in FFM-adjusted RMR, although as indicated above, about one-half of the difference could also be explained by the reduced core temperature and TEF observed in CR. In contrast, however, aging calorie-restricted and C monkeys did not differ in their rate of decrease in RMR with age, and thus for the ages of our animals studied to date, our finding was not consistent with our prediction.

We did not measure mitochondrial leak directly but instead assumed that the composition of the FFM was the same in the calorie-restricted and C animals. In this regard, our DEXA data, although not conclusive, were in agreement with previous rodent data with regard to a proportionally smaller loss of high energy-consuming organ mass under CR (36). In our present study, as well as those published previously (13, 15), the central FFM mass, as delineated by DEXA, constituted a greater percentage of body weight in the calorie-restricted animals compared with C animals. Nonetheless, the appendicular FFM (upper and lower limb FFM) and central FFM (total FFM minus appendicular FFM) constituted a similar percentage of total FFM in the calorie-restricted and C monkeys (appendicular FFM = 44%, central FFM = 56%). Because this is not a direct measure of metabolically active tissues, further measurement of organ masses and body cell mass to ascertain the longitudinal effects of CR on the metabolically active tissue mass is warranted.

Another limitation of our data is that the measurements of EE in the chamber may not be fully representative of the animals’ EE in their home cages. Difference in energy intake between home cage and chamber may influence EE differences in calorie-restricted and C monkeys, but we ascertained this difference to be insignificant in all three groups of monkeys (average difference in EE between calorie-restricted and C monkeys in all 3 groups was 8 kcal/day). Hence, thermic effect of meals should have been similar in both conditions (assuming that thermic effect of meals constitutes 10% of this difference, 0.8 kcal) and would not influence the difference in EE in the calorie-restricted and C monkeys.

In conclusion, this is the first analyses looking at the longitudinal effects on EE of aging rhesus monkeys. The energy requirements of aging male monkeys decrease with age independent of body composition changes, with a 1.6% decrease in total EE with each year increase in age that was attributed to the decreases in energy expended in physical activity. Comparisons between species are difficult but are based on life span differences; the age of monkeys used in this analysis (11–28 yr) can be estimated to correspond to ~20–60 human yr (6). CR was associated with a decrease in EE, but this difference was attributed to lower FFM and FM in the calorie-restricted animals. However, there was no influence of CR on the annual decreases in EE in this cohort of rhesus monkeys of 11–28 yr.

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