Life-long calorie restriction in Fischer 344 rats attenuates age-related loss in skeletal muscle-specific force and reduces extracellular space

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Payne, Anthony M., Stephen L. Dodd, and Christiana Leeuwenburgh. Life-long calorie restriction in Fischer 344 rats attenuates age-related loss in skeletal muscle-specific force and reduces extracellular space. J Appl Physiol 95: 2554–2562, 2003. First published September 12, 2003; 10.1152/japplphysiol.00758.2003.—The decline in muscle function is associated with an age-related decrease in muscle mass and an age-related decline in strength. However, decreased strength is not solely due to decreased muscle mass. The age-related decline in muscle-specific force (force/muscle cross-sectional area), a measure of intrinsic muscle function, also contributes to age-related strength decline, and the mechanisms by which this occurs are only partially known. Moreover, changes in the extracellular space could have a profound effect on skeletal muscle function. Life-long calorie restriction in rodents has shown to be a powerful anti-aging intervention. In this study, we examine whether calorie restriction is able to attenuate the loss of muscle function and elevations in extracellular space associated with aging. We hypothesize that calorie restriction attenuates the age-associated decline in specific force and increases in extracellular space. Measurements of in vitro contractile properties of the extensor digitorum longus (type II) and soleus (type I) muscles from 12-mo and 26- to 28-mo-old ad libitum-fed, as well as 27- to 28-mo-old life-long calorie-restricted male Fischer 344 rats, were performed. We found that calorie restriction attenuated the age-associated decline in muscle mass-specific force and strength-to-body mass ratio (mg/g) and strength-to-body mass ratio (N/kg) in the extensor digitorum longus muscle (P < 0.05) but not in the soleus muscle (P > 0.05). Importantly, muscle-specific force (N/cm²) in the extensor digitorum longus, but not in the soleus muscle, of the old calorie-restricted rats was equal to that of the young 12-mo-old animals. Moreover, the age-associated increase in extracellular space was reduced in the fast-twitch extensor digitorum longus muscle (P < 0.05) but not in the soleus muscle with calorie restriction. We also found a significant correlation between the extracellular space and the muscle-specific force in the extensor digitorum longus (r = −0.58; P < 0.05) but not in the soleus muscle (r = 0.38; P > 0.05). Hence, this study shows a loss of muscle function with age and suggests that long-term calorie restriction is an effective intervention against the loss of muscle function with age.

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The decrease in muscle strength with age presents a significant health problem estimated to cost society billions of dollars over the next few decades (15, 36, 37). By the year 2030, the elderly population will grow from 13 to ~20% of the total population, and it is estimated that $130 billion will be imposed by physical frailty (15, 36, 37). Age-related loss of strength contributes to disability and frailty in the elderly, leading to the inability to perform daily living tasks (2, 3, 5). The well-recognized age-related decrease in muscle mass contributes to the age-related decline in strength (16, 19, 28, 30, 39). Muscle mass declines at a greater rate with age than body mass (51) and fat-free mass (22, 55), reducing strength-to-weight ratio and possibly leading to disability and loss of independence (50, 53, 58).

Decreased strength is not, however, solely due to decreased muscle mass. The age-related decline in muscle-specific force [force/muscle cross-sectional area (CSA)], a measure of intrinsic muscle function, also contributes to age-related strength decline (17, 28, 30, 32, 51, 52). Evidence of this phenomenon has been found in vivo in humans by measuring isokinetic-specific (19, 28, 30) and isometric-specific (31, 56) force. Magnetic resonance imaging (18, 19) and computed tomography (38, 46) have revealed increased non-muscle tissue within quadriceps (19, 38), hamstrings (38), plantar flexors (18, 46), and spinalis (18) muscle groups with age. These changes would contribute to a decrease in specific force. In vitro investigations of rodent muscle have also shown decreased muscle-specific force with age (17, 29, 32). Mechanisms attributed to these findings include decreases in actin-myosin cross-bridge stability (29) and impairments in excitation-contraction coupling (9, 45), but many other potential mechanisms still require further investigation. Hence, understanding all mechanisms of sarcopenia could potentially permit the development of strategies and interventions that may attenuate the loss of skeletal muscle myocytes and sarcopenia associated with

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advancing age (48). It is well established that calorie restriction without malnutrition increases the mean and maximum lifespan of several mammalian species (49) and reduces age-related skeletal muscle myopathies, mitochondrial deletions, oxidative stress, and mitochondrial dysfunction (24, 26, 57). In addition, there are greater losses of type II fibers compared with type I fibers with age, and type II fibers show more mitochondrial deletions and mitochondrial damage (1, 7, 35, 54). Aiken and colleagues (1, 7, 35, 54) showed that specific muscle fibers (vastus lateralis) harboring mitochondrial deletions often display atrophy and increased steady-state levels of oxidative nucleic damage. They (1, 25) also showed that life-long calorie restriction was able to attenuate muscle fiber loss with age in the epitrochlearis midbelly muscle fibers (type II) but failed to do so in the soleus (type I) muscle. Moreover, increases in extracellular space and the formation of protein aggregates within this space with age may have direct impacts on skeletal muscle function (4, 18, 19, 38, 46). Specifically, age-related increases in extracellular space within a muscle may contribute to the decrease in specific force, in that a larger portion of the muscle CSA is not involved in force production. It is these findings that prompted the notion to examine the effects of calorie restriction on the function of specific muscle fibers and changes in the extracellular space of skeletal muscle with age.

We determined the effects of age on muscle mass-to-body mass ratio, muscle force-to-body mass ratio, and muscle-specific force in fast-twitch type II (extensor digitorum longus) and slow-twitch type I (soleus) muscles from 12-mo- to 28-mo-old ad libitum-fed as well as 27- to 28-mo-old life-long calorie-restricted Fischer 344 rats. In addition, we examined the effects of age on muscle extracellular space and the correlation of extracellular space to muscle-specific force. We hypothesized that aging would lead to decreased muscle mass-to-body mass and muscle force-to-body mass ratios and to decreased specific force and that the negative effects of age would be greater in the fast-twitch extensor digitorum longus muscle than in the slow-twitch soleus muscle. Moreover, we hypothesized that life-long calorie restriction would attenuate the age-associated decline in muscle function and attenuate the age-associated increase of the extracellular space to a greater extent in the extensor digitorum longus muscle than in the soleus muscle.

METHODS

Animals and diet. Young ad libitum-fed (n = 14, 12 mo), old ad libitum-fed (n = 10, 26–28 mo), and calorie-restricted old (n = 10, 27–28 mo) male Fischer 344 rats (National Institute of Aging colony, Harlan Sprague Dawley, Indianapolis, IN) were used. Calorie restriction was started at 3.5 mo of age (10% restriction), increased to 25% restriction at 3.75 mo, and maintained at 40% restriction from 4 mo throughout the individual animal’s life. Calorie-restricted animals were fed the NIH31-NIA fortified diet to ensure that they were not malnourished, whereas ad libitum-fed animals were given NIH31 rat diet. The rats were housed one per cage in a temperature- (18–22°C) and light-controlled environment with a 12:12-h light-dark cycle. After 2 wk of acclimation, one animal was randomly killed daily, after being anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt). All treatment of animals throughout this study conformed fully to the Guiding Principles in the Care and Use of Animals of the American Physiological Society and in addition received University of Florida institutional animal care committee approval.

Muscle contractile function. Soleus and extensor digitorum longus muscles from one leg were quickly removed and placed into ice-cold Krebs-Henseleit buffer containing (in mM) 1.2 MgSO4, 4.7 KCl, 1.2 KH2PO4, 118 NaCl, 25 NaHCO3, 1.8 CaCl2, and 2.0 D-glucose (Sigma Chemical, St. Louis, MO) bubbled with 95% O2-5% CO2 in preparation for contractile experiments. Soleus and extensor digitorum longus muscles were trimmed of surface fat and nerve while in ice-cold Krebs-Henseleit buffer. Tendons on each end of the soleus were placed between clips (Harvard Apparatus, Cambridge, MA). One clip was tied with silk string to a fixed brace, and the other was tied to the arm of a force transducer (Aurora Scientific, model 305B, Aurora, ON, Canada). Extensor digitorum longus muscles were trimmed of surface fat and nerve while in ice-cold Krebs-Henseleit buffer. Tendons on each end of the muscle were placed into a chamber of 37°C Krebs-Henseleit buffer bubbled with 95% O2-5% CO2 and positioned between two platinum electrodes.

Muscles were allowed to equilibrate to the temperature for 15 min at a slightly slack length. Optimal length (Lo) was determined with single twitch stimulations (see Stimulation protocols). The chamber was lowered, and muscle length was quickly measured twice from one myotendinous junction to the other. Three tetanic contractions were elicited, and their values were averaged for maximal tetanic force. Data were collected on a laboratory computer with LabView software (National Instruments, Austin, TX).

Specific tension is reported as force per physiological CSA (in N/cm2). CSA (in cm2) is calculated as muscle mass (g)/muscle length (cm)/muscle density (1.056 g/cm3). Fiber length was calculated as muscle length × 0.62 for soleus and muscle length × 0.40 for extensor digitorum longus (23, 47). After contractile experiments, muscles were trimmed of tendon and connective tissue, blood vessels, and exterior nerves and then quickly blotted dry on filter paper and weighed.

Stimulation protocols. All stimulations were delivered with a Grass S48 stimulator (Grass Instruments, Quincy, MA). Soleus muscles were stimulated with 0.5-ms square-wave pulses at 120 V for twitches. For tetanic contractions, stimulation trains were delivered for 1,000 ms at a frequency of 150 Hz. Extensor digitorum longus muscles were stimulated with 2.0-ms square-wave pulses at 120 V for twitches. For tetanic contractions, stimulation trains were delivered for 400 ms at a frequency of 200 Hz.

Histology. The soleus and extensor digitorum longus muscles from the contralateral leg were removed and placed into ice-cold Krebs-Henseleit buffer. After measurement of Lo in the muscle used for contraction, the contralateral muscle was placed onto a thin stainless steel spatula at the measured Lo, rapidly frozen in liquid nitrogen, and stored at −80°C until histological analysis. These muscles were cut into serial transverse sections 10 μm thick with a cryostat precooled to −20°C. Sections were placed onto slides and stained with hematoxylin and eosin (H&E). Sections were visualized with a light microscope and imaged with Sigma Scan software (SPSS, Chicago, IL). Two sections ~40,000 μm2 were randomly chosen within each muscle for determination of extracellular space fraction. Briefly, muscle fiber membranes were traced, and all areas within the membranes were added to
Table 1. Body mass and muscle mass of young (12-mo-old), old (26- to 28-mo-old), and calorie-restricted old (27- to 28-mo-old) male Fischer 344 rats

<table>
<thead>
<tr>
<th></th>
<th>Young (n = 15)</th>
<th>Old (n = 10)</th>
<th>%Change Old vs. Young</th>
<th>Old-CR (n = 10)</th>
<th>%Change Old-CR vs. Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass, g</td>
<td>416.3 ± 7.6</td>
<td>371.9 ± 16.2</td>
<td>↓11%</td>
<td>254.7 ± 9.0†</td>
<td>↓32%</td>
</tr>
<tr>
<td>EDL muscle mass, mg</td>
<td>144.1 ± 2.0</td>
<td>125.6 ± 7.8</td>
<td>↓13%</td>
<td>105.7 ± 4.1†</td>
<td>↓16%</td>
</tr>
<tr>
<td>Soleus muscle mass, mg</td>
<td>133.4 ± 4.1</td>
<td>116.0 ± 5.6</td>
<td>↓13%</td>
<td>89.5 ± 5.7†</td>
<td>↓23%</td>
</tr>
</tbody>
</table>

Values are means ± SE. EDL, extensor digitorum longus. Male Fischer 344 rats were calorie restricted starting at 3.5 mo of age (10% restriction), increased to 25% restriction at 3.75 mo, and maintained at 40% restriction from 4 mo throughout the individual animal’s life. Calorie-restricted (CR) animals were fed the NIH31-NIA fortified diet to ensure that they were not malnourished, whereas control animals (young and old) were fed NIH31 rat diet ad libitum. *P < 0.05, old vs. young; †P < 0.05, old-CR vs. old.

determine intracellular fraction. This was subtracted from the total area of the image to calculate extracellular fraction. Values from each section were averaged for each sample.

**Statistical analysis.** Group means for measured variables were analyzed with unpaired Student’s t-tests. Pearson’s product-moment correlation was performed to determine the relationship between maximal tetanic-specific tension and extracellular fraction. All statistical analyses were carried out with the Prism statistical package (GraphPad, San Diego, CA).

**RESULTS**

Body mass, muscle mass, and muscle mass-to-body mass ratio. When we compared the young (12-mo-old) vs. the old (26- to 28-mo-old) Fischer 344 rats, we found a significant decrease in body mass (11%) and a significant decrease in muscle mass in both soleus (13%) and extensor digitorum longus (13%) muscles (Table 1). Calorie-restricted rats had the lowest body mass of all animals, and the extensor digitorum longus and soleus muscle mass declined by 16 and 23%, respectively, compared with the old rats (Table 1). The muscle mass-to-body mass ratio (mg/g) remained unaltered with age in the extensor digitorum longus and soleus muscles (Fig. 1). However, the first finding of this study was that the extensor digitorum longus muscle mass-to-body mass ratio was increased in calorie-restricted old rats compared with old animals (Fig. 1). The change in the soleus mass-to-body mass ratio in the calorie-restricted rats was not significantly different from the old rats (P = 0.13).

Muscle force and force-to-body mass ratio. We further examined the maximal absolute tetanic muscle force and muscle force-to-body mass ratio (an indirect indication of strength-to-body weight ratio) in these two muscles. The frequency used to elicit maximal tetanic force was 150 Hz for the soleus muscle and 200 Hz for the extensor digitorum longus muscle. Absolute tetanic muscle force in the extensor digitorum longus muscle decreased by 27% with age (2.09 ± 0.31 N for young vs. 1.53 ± 0.29 N for old animals; P < 0.05) and by 17% in the soleus muscle (1.43 ± 0.12 N for young vs. 1.19 ± 0.08 N for old animals; P < 0.05). Because life-long calorie restriction resulted in significantly decreased muscle weights of the extensor digitorum longus (16%) and soleus (23%) muscles, we expected muscle force in both muscles to be significantly lower in old calorie-restricted rats compared with old ad libitum-fed rats. In contrast, the absolute tetanic muscle force in the extensor digitorum longus was unaltered (1.55 ± 0.25 N) in the old calorie-restricted group compared with the old rats, whereas force in the soleus muscle tended to decrease (0.98 ± 0.08 N), but this change was not statistically different (P = 0.07). Aging resulted in a significant decrease in muscle force-to-body mass ratio (N/kg) in both the soleus and the extensor digitorum longus muscles (Fig. 2). Moreover, in the extensor digitorum longus muscle, calorie restriction significantly increased muscle force-to-body mass ratio in old calorie-restricted compared with the old ad libitum-fed rats (Fig. 2). In the soleus, muscle force-to-body mass ratio...
force-to-body mass ratio in the extensor digitorum longus (*P < 0.05, Old vs. Young), whereas calorie restriction increased force-to-body mass ratio (Old-CR vs. Old, *P < 0.05, Old-CR vs. Old). Values are means ± SE.

Muscle function determined by specific force measurements. Muscle-specific force could be considered one of the most important parameters to determine muscle function. As expected, aging resulted in decreased muscle-specific force (N/cm²) in both the extensor digitorum longus and soleus muscles (Fig. 3). With age, the decreased specific force was due to muscle force decreasing to a greater extent than muscle CSA. In striking contrast, life-long calorie restriction was able to increase specific force in the extensor digitorum longus muscle but not in the soleus muscle (Fig. 3). Core fiber hypoxia is a concern when working with whole muscle preparations, since hypoxia can reduce maximal force output of a muscle and temperature can affect O₂ diffusion distance (47). However, our results for specific force (Fig. 3) in young control muscles are similar to published values (20, 47). We found that values for old calorie-restricted rats were not different from those for young control rats. Moreover, specific force in old ad libitum-fed controls was depressed from young controls; however, the size of the muscles was not different (Table 1). Therefore, we feel the difference seen in specific force was due to the effects of aging and not to whole muscle preparation in vitro. Thus these data strongly suggest that life-long calorie restriction has an adaptive beneficial effect on specific force in fast-twitch muscles.

Extracellular space fraction. Extracellular space fraction was determined histologically in extensor digitorum longus and soleus muscles (Fig. 4). The extracellular space was significantly increased with age in the soleus muscle (28%) and was increased to a much greater extent in the extensor digitorum longus (55%) muscle (Fig. 5). Life-long calorie restriction decreased the extracellular space by 45% in the extensor digitorum longus muscle (P < 0.05) and by only 12% (P > 0.05) in the soleus muscle (Fig. 5). We also determined the correlation between muscle-specific force and extracellular space and found a significant negative correlation between extracellular space fraction and muscle-specific force in the extensor digitorum longus muscle (r = −0.58; P < 0.05) but not in the soleus muscle (r = −0.38; P > 0.05). These data suggest that changes in the extracellular space with age may have a direct
impact on function in the extensor digitorum longus muscle but to a lesser extent on soleus muscle (see DISCUSSION).

DISCUSSION

Aging produces a well-recognized decline in muscle mass (16, 19, 28, 30, 38), with this decline being proportionally greater than the age-related decline in body mass (51) and fat-free mass (22, 55). Decline in muscle mass leads to loss of strength and independence in the elderly (2, 3, 5). Because muscle mass declines at a faster rate than other body tissue mass (22, 51), aging effectively produces individuals with lower strength-to-body mass ratio, which contributes to the inability to perform daily tasks (50, 53, 58). In this study, calorie restriction was able to increase the muscle mass-to-body mass and muscle force-to-body mass ratios in the fast-twitch type II fiber containing extensor digitorum longus muscle. These findings may implicate long-term calorie restriction without malnutrition as a potential means of improving quality of life. Indeed, higher lean mass-to-fat mass ratio (50) and lower percent body fat (53, 58) have been associated with better physical performance and daily task completion (i.e., less disability) in old men and women. Moreover, with age, there was an expected decrease in specific force in both the extensor digitorum longus and soleus muscles. The effect was greater in the extensor digitorum longus muscle (~20% decrease) compared with the soleus muscle (~13% decrease). Most importantly, calorie restriction was able to attenuate the age-associated decrease in specific force in the extensor digitorum longus muscle, the fiber type that undergoes greater loss in mass and function with age (27), which strongly suggests that this intervention may improve the intrinsic force-generating capacity of muscle fibers.

The extensor digitorum longus muscles in old calorie-restricted rats had ~16% lower muscle mass than their old ad libitum-fed control counterparts; however, absolute tetanic force was not different between the two groups. This can be explained by the increased specific force in the old calorie-restricted compared with old control rats. The increased specific force may also explain the increased muscle force-to-body mass ratio observed in the extensor digitorum longus. Decreased muscle-specific force contributes to the overall loss of strength with age (17, 28, 30, 32, 51, 52); therefore, interventions that maintain specific force with age warrant investigation (12). Calorie restriction increases muscle mass-to-body mass ratio and muscle-specific force (intrinsic muscle function), attenuating age-related changes that contribute to greater strength decline relative to body weight and contribute to disability in the elderly.

Several investigators have noted increases in the percentage of nonmuscle tissue within skeletal muscle with age (18, 19, 38, 46). Hatakenaka et al. (18), using histological and magnetic resonance techniques together, concluded that the increase in nonmuscle tissue was predominately due to an increase in extracellular material (which can include connective tissue, collagen, blood vessels, and interstitial fluid and/or space). The increase was found in human gastrocnemius and mouse spinalis muscles, predominately within muscles with fast-twitch (type II) fibers. The investigators attributed the increase in extracellular space fraction to age-related fiber atrophy, which has been shown to occur to a greater extent in fast-twitch

Fig. 4. Extracellular space in extensor digitorum longus (EDL) and soleus muscles. Hematoxylin and eosin stains were completed in extensor digitorum longus muscles from Young (A), Old (B), and Old-CR (C) Fischer 344 rats and in the soleus muscles from Young (D), Old (E), and Old-CR (F) Fischer 344 rats. Scale bar = 100 μm.
(type II) fibers than in slow-twitch (type I) fibers (27). Our findings are in agreement with their study, and we were able to demonstrate that life-long calorically restriction was able to reduce the age-associated rise in extracellular space in extensor digitorum longus muscle but not in the soleus muscle. Age-related increases in extracellular space within a muscle may contribute to the decrease in specific force, in that a larger portion of the muscle CSA is not involved in force production. After hindlimb suspension in rats, a preparation which causes marked fiber atrophy, Kandarian et al. (20) found an increased interstitial fluid volume in muscle. Specific force was also decreased after hindlimb suspension; however, when corrected for in the increased interstitial fluid volume (which contributes to increased extracellular space), specific force was unchanged. Similarly, muscle denervation leads to muscle fiber atrophy and increased extracellular fraction in the muscle CSA (13) as well as decreased specific force, which Finol et al. (14) speculated contributed to the denervation-induced decrease in specific force.

Furthermore, we found a statistically significant negative correlation between extracellular space fraction and specific force in extensor digitorum longus but not in the soleus muscle. Several studies strongly suggest that a variety of advanced age glycation end products (AGE) and lipoxidation end products (ALE) accumulate in tissues with age, specifically in long-lived proteins in the extracellular space (4, 8). Hence, AGE and ALE may accumulate on proteins in the extracellular space and impair muscle function. Moreover, others (43) have shown that AGE products derived from glucose have a profound effect on myosin function. Only one study (8) evaluated the effect of calorie restriction on glycation of blood proteins and accumulation of glyoxidation products, N-(carboxymethyl)lysine and pentosidine, in skin collagen. Life-long caloric restriction reduced the age-associated rise in glycation of hemoglobin, plasma proteins, and glycoxidation end products in skin collagen (8). Our findings of increased extracellular space with age are consistent with increased AGE and ALE products and suggest that these compounds may play a role in decreased muscle-specific force with age and that calorie restriction attenuates the age-related increase in extracellular space and decrease in specific force by reducing the levels of AGE and ALE. Future studies need to examine the possibility that advanced oxidation, lipid peroxidation, and glycation reactions of proteins correlate with the severity of loss in specific muscle function. Moreover, key mediators of inflammatory pathways, i.e., tumor necrosis factor-α and nuclear factor-κB, need to be examined with regard to a possible mechanism in sarcopenia and increases in extracellular space. Although increases in extracellular volume are associated with atrophy and “scarring” of skeletal muscle, no studies have been conducted to determine whether it may function to prevent concentric stretch injury to aging tissue or, alternatively, to improve lymph flow and vascularization. Future studies need to investigate this possibility and determine whether caloric restriction increases or decreases the tendency for muscle injury, which would have implications to the aging population.

Alterations in extracellular space are unlikely to be the only mechanism by which calorie restriction may benefit skeletal muscle function with age. Other key studies have shown that calorie restriction increases dihydropyridine-sensitive Ca\(^{2+}\) channels and ryanodine Ca\(^{2+}\)-release channels in old skeletal muscle compared with ad libitum-fed cohorts (32, 44) and have attributed this mechanism to the increased specific force found in muscles from calorie-restricted animals compared with ad libitum-fed animals (32). Besides an increase in specific force in extensor digitorum longus muscle, these investigators (32) also found that specific force was significantly increased in the soleus muscle with calorie restriction. Furthermore, other beneficial effects of calorie restriction, which could have an impact on protein function, could include reductions in DNA deletions, increases in proteasome function, and alterations in mitochondrial biogenesis and energy production (11, 40, 59). Indeed, calorie restriction initiated during late middle age has been shown to retard...
the age-associated fiber loss, decrease the number of skeletal muscle fibers showing mitochondrial enzyme abnormalities, and decrease the accumulation of mitochondrial DNA deletions (1). However, recently our group (11) demonstrated that ATP content and production in isolated mitochondria was decreased with age by ~50% in the gastrocnemius muscle. Surprisingly, no differences were observed in ATP content or rate of ATP production in isolated mitochondria of gastrocnemius muscle or heart in 26-mo-old life-long calorie-restricted rats compared with the 26-mo-old ad libitum-fed group. This may suggest that supply of ATP through aerobic sources by subsarcolemmal mitochondria is not a factor for improved muscle function.

We have observed that rats are more active during the actual feeding period but have not measured the spontaneous activity level. We have measured their basal metabolic rate and have not found differences between the ad libitum-fed and calorie-restricted group (unpublished observations). This is in agreement with published data of others who report no differences in basal metabolic rate between calorie-restricted and ad libitum-fed rats (21, 33, 34). Few studies have been conducted to investigate the exact activity levels during life-long calorie restriction. One study shows that spontaneous activity of calorie-restricted rats in their cages was higher compared with ad libitum-fed animals (reviewed in Ref. 33). This was measured as number of interruptions, that is, number of intersections of a light beam, and was recorded over a 24-h period (~4,000 counts/wk for the ad libitum-fed group and ~5,000 counts/wk for the calorie-restricted group). Although McCarter’s group (21, 34) concluded that physical activity was probably not an important factor in the action of dietary restriction on aging, it remains possible that the moderate increase in activity level could have an additional beneficial effect on muscle function.

The mechanisms causing the differential effects of the life-long calorie restriction on the extensor digitorum longus and soleus muscles are not entirely clear. Why is it that fast-twitch fibers are more affected at both functional and structural levels, whereas slow-twitch fibers are not? It is well established that the accumulation of mitochondrial DNA modification and mutations with age can increase free radical production, interfere with the synthesis of proteins and enzymatic pathways responsible for the transfer of electrons along the respiratory chain, and reduce the production of ATP (42). These processes, particularly the decreased energy production, have been implicated in the reduction of cell viability as well as the increase in cell necrosis and/or apoptosis with age (10, 41). It has been suggested that mitochondrial dysfunction, resulting from an increase in oxidative stress, may be involved in sarcopenia. Indeed, in skeletal muscle of old rats, there is strong evidence that specific muscle regions undergo atrophy, contain cytochrome c oxidase-negative fibers (indicative of mitochondrial dysfunction), have extensive mitochondrial DNA deletions, and have a significantly reduced number of nuclei (1). However, no one has investigated whether mitochondrial DNA deletions accumulate more in muscle containing less mitochondria (type II) compared with skeletal muscle, which has a higher number of mitochondria (type I). Future studies are needed to address this very important issue and to determine whether fiber-specific differences in the frequency of mitochondrial deletions, removal of mitochondria with deletions, and alteration in mitochondrial biogenesis could lead to differences in the effective removal of the number of mitochondria containing deleted species.

This study suggests multiple beneficial effects of life-long calorie restriction on muscle function. Calorie restriction was able to attenuate the age-associated declines in muscle mass-to-body mass ratio, strength-to-body mass ratio, and muscle-specific force and to reduce the extracellular space in the fast-twitch extensor digitorum longus muscle. Only some of these beneficial effects of calorie restriction were observed in the slow-twitch soleus muscle. Further studies are required to better understand the mechanisms by which caloric restriction preserves the loss of muscle function with age.

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DISCLOSURES

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