Caloric restriction optimizes the proteasome pathway with aging in rat plantaris muscle: implications for sarcopenia

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Hepple RT, Qin M, Nakamoto H, Goto S. Caloric restriction optimizes the proteasome pathway with aging in rat plantaris muscle: implications for sarcopenia. Am J Physiol Regul Integr Comp Physiol 295: R1231–R1237, 2008. First published August 13, 2008; doi:10.1152/ajpregu.90478.2008.—To gain insight into the significance of alterations in the proteasome pathway for sarcopenia and its attenuation by calorie restriction, we examined protein oxidation and components of the proteasome pathway in plantaris muscle in 8-, 30-, and 35-mo-old ad libitum-fed (AL) rats; and in 8-, 35-, and 40-mo-old calorie-restricted (CR) rats. We hypothesized that CR rats would exhibit a lesser accumulation of protein carbonyls with aging and that this would be associated with a better maintenance of skeletal muscle proteasome activity and function with aging. Consistent with this view, whereas AL rats had a significant increase in protein carbonylation with aging, there was no such increase in CR rats. Protein levels of the ubiquitin ligases MuRF1 and MAFbx increased similarly with aging in both AL and CR rats. On the other hand, chymotrypsin-like activity of the proteasome increased with aging more gradually in CR rats, and this increase was paralleled by increases in the expression of the C2 subunit in both groups, suggesting that differences in activity were not related to differences in proteasome function with aging. Interestingly, the plot of muscle mass vs. proteasome activity showed that the oldest animals in both diets had a lower muscle mass than would be predicted by their proteasome activity, suggesting that other factors explain the acceleration of sarcopenia at advanced age. Since calorie restriction better protects skeletal muscle function than muscle mass with aging (Hepple RT, Baker DJ, Kaczor JJ, Krause DJ, FASEB J 19: 1320–1322, 2005), and our current results show that this protection of function is associated with a prevention of oxidative protein damage accumulation, we suggest that calorie restriction optimizes the proteasome pathway to preserve skeletal muscle function at the expense of modest muscle atrophy.

oxidative stress

AGING IS ASSOCIATED WITH A progressive decline in skeletal muscle mass and contractile function known as sarcopenia. Although the data thus far remain largely circumstantial, recent studies support the idea that declining skeletal muscle function is due in part to the accumulation of oxidatively damaged protein (11, 19, 28). In the context of sarcopenia, there are disparate views and evidence regarding age-related changes in protein degradation pathways. On the one hand, an accelerated rate of protein degradation (12, 13) is suggested to contribute to the age-related decline in skeletal muscle mass (44). On the other hand, an impaired removal of oxidatively damaged protein (17, 18, 26) is thought to lead to accumulation of oxidatively damaged protein and thus, impaired myocyte function (41). As would be expected with the conflicting views on the physiological relevance of protein degradation changes in aged skeletal muscles, whereas some data indicate an increase in the mRNA levels of the ubiquitin ligases in skeletal muscles with aging (suggesting augmented activation of the proteasome pathway) (12), other studies find no change (51) or a decline (suggesting impaired removal of oxidatively damaged protein by this pathway) (14, 16). It is noteworthy that no prior studies in aged skeletal muscle have examined ubiquitin ligase protein levels per se. Similarly, whereas some studies find a decreased proteasome biochemical activity and/or proteasome function (e.g., reduced proteasome activity relative to the amount of its protein components) in aged skeletal muscles (17, 26, 46), others find an increased proteasome activity with no change in proteasome function (4, 12). In addition, no prior studies have examined proteasome function specifically as it relates to the evolution of sarcopenia. Clearly, further study is required to help resolve these issues.

Calorie restriction markedly attenuates age-related declines in tissue function with aging. In the context of skeletal muscle, this is manifest as a significant attenuation of age-related impairment in contractile (25, 34, 39) and metabolic function (3, 15, 25) by calorie restriction. One of the primary mechanisms thought to underlie this protection by calorie restriction is an attenuation of the accumulation of oxidatively damaged protein with aging (31, 47, 48). Indeed, not only does calorie restriction reduce damage by decreasing ROS production (7, 29, 30) and increasing ROS scavenging (49), but calorie restriction has also been hypothesized to attenuate age-related changes in the proteasome pathway (21). Only two prior studies have examined the effect of calorie restriction on the proteasome pathway in aging skeletal muscle. The first study, by Radak et al. (43), observed that every other day feeding for 3.5 mo prevented the decline in proteasome activity observed in gastrocnemius muscle of 30-mo-old male Fischer 344 rats. Similar to this first investigation, the second study, by Selsby et al. (46), observed that 40% restriction of caloric intake (with maintained nutrition) beginning from the juvenile period also prevented the decline in proteasome activity observed in gastrocnemius muscle of 24- to 26-mo-old male Fischer 344 rats. However, it is noteworthy that the Fischer 344 rat exhibits little muscle atrophy with aging (45), leaving the significance of the observations to sarcopenia and its functional consequences unclear.

On the basis of the above considerations, we sought to determine how aging and calorie restriction affected the pro-
teasome pathway in skeletal muscle using a well-established model of sarcopenia, the Fischer 344 X Brown Norway F1-hybrid (F344BN) rat. We also sought to determine how these changes related to the nonlinear trajectory of muscle atrophy with aging, as a means of obtaining insight into the physiological relevance of changes in this pathway to sarcopenia. We reasoned that if an increased activation of the proteasome pathway is a cause of skeletal muscle atrophy with aging, one would expect an upregulation of this pathway with aging that is opposed by calorie restriction. Alternatively, if a decreased proteasome activity is a cause of impaired skeletal muscle function with aging, one would expect a downregulation and/or dysfunction of this pathway with aging that is opposed by calorie restriction. To this end, we examined changes in protein oxidation, ubiquitin ligase protein expression, proteasome activity, and subunit expression in plantaris muscles of ad libitum-fed (AL) and calorie-restricted (CR) male F344BN rats, and related these changes to plantaris muscle mass in AL vs. CR rats with aging. We hypothesized that CR rats would exhibit a lesser accumulation of protein carbonyls with aging and that this would be associated with a better maintenance of skeletal muscle proteasome activity and function with aging.

METHODS

Animals. Male F344BN rats were obtained from the National Institute on Aging (NIA; Baltimore, MD) in either AL (8-, 30-, 35-mo-old) or CR (8-, 35-, 40-mo-old) groupings. These ages were selected on the basis of survival curves (50) to permit absolute and relative age comparisons and to sample animals along the progression of sarcopenia (see DISCUSSION).

Calorie restriction was imposed at the NIA colony at 14 wk of age and gradually induced over a 2-wk period to reach levels that were 40% lower in the CR rats than AL rats. To maintain the nutritional requirements of these animals, the feed was supplemented (NIH 31/NIA fortified) to contain 20% more calories than the ad libitum-fed (AL) diet. To maintain nutritional homogenization in an extraction buffer containing 20 mM Tris, 5 mM EDTA, 5 mM dithiothreitol (pH 7.0), 300 mM NaCl, in a ratio of 1 part muscle to 7 parts homogenization buffer. Subsequently, Western blot analysis was done according to methods we have described previously (3). The relative amount of muscle ubiquitin ligases was measured using primary antibodies (1:1,000 dilution) directed against MuRF1 (Novus NB 100–1193) and MAFA (Santa Cruz SC-27645) incubated overnight at 4°C, with 20 μg or 15 μg of protein loaded per well, respectively. Following incubation with an horseradish peroxidase-conjugated secondary antibody (1:4,000; Pierce, Rockford, IL), blots were incubated with chemiluminescent solution (Pierce), and imaged using the Gel Doc Chemigenius 2 (Syngene, Frederick, MD). Densitometry was analyzed by the Gene Tools for Syngene software package (Syngene).

Statistics. Results are presented as means ± SE. Comparisons between groups for muscle mass, ubiquitin ligase content, proteasome activity, and C2 subunit content were made by one-way ANOVA with a Holm-Sidak post hoc test. The relationship between the chymotrypsin-like activity of proteasome and muscle mass in each dietary treatment was analyzed by linear regression. Muscle mass predicted by the regression equations was compared with the observed muscle mass in the 35- and 40-mo-old CR animals by paired t-test.

RESULTS

Body and plantaris muscle mass. As shown in Table 1, whereas there was an increase in body mass between 8 mo and 30 mo in AL rats, body mass subsequently declined close to 8-mo-old values by 35 mo of age. CR rats maintained their body mass constant between 8 and 40 mo of age. In AL rats there was a nonsignificant 6% decline in plantaris muscle mass between 8 and 30 mo (P = 0.066), but a significant 36% decline by 35 mo of age. In CR rats the amount of muscle atrophy was only 12% across the same absolute age range, but reached a similar level of atrophy (34% lower) by 40 mo of age.

Table 1. Body mass and muscle mass

<table>
<thead>
<tr>
<th>Group</th>
<th>No. Rats/Group</th>
<th>Body Mass, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 mo AL</td>
<td>8</td>
<td>423 ± 4*</td>
</tr>
<tr>
<td>8 mo CR</td>
<td>8</td>
<td>307 ± 5b</td>
</tr>
<tr>
<td>30 mo AL</td>
<td>8</td>
<td>558 ± 5c</td>
</tr>
<tr>
<td>35 mo AL</td>
<td>6</td>
<td>446 ± 18c</td>
</tr>
<tr>
<td>35 mo CR</td>
<td>7</td>
<td>323 ± 7a</td>
</tr>
<tr>
<td>40 mo CR</td>
<td>6</td>
<td>327 ± 6a</td>
</tr>
</tbody>
</table>

Values are means ± SE. AL, ad libitum fed; CR, caloric restricted. a,b,c Values sharing a superscript are not different from one another.

Assay for proteasome activity. The proteasome activity was evaluated by chymotrypsin-like activity as described (23). The cell extracts containing 60 μg protein were incubated for 30 min at 37°C in 50 μl of a solution containing 100 mM Tris·HCl (pH 8.0), 1 mM DTT, 5 mM MgCl2, 01 mM Suc-LLVY-MCA, 2 mg/ml ovalbumin, and 0.07% SDS. The reaction was terminated by 25 μl of 10% SDS and diluted by 2 ml of 0.1 M Tris·HCl (pH 9.0). The absorption was recorded at an emission wave length of 440 nm by excitation at 380 nm (F-3000 fluorometer; Hitachi, Tokyo, Japan). The enzyme activity was expressed as moles of the substrate cleaved per microgram protein per min.

Immunoblot for the C2 subunit. Relative proteasome amount was measured by Western blot analysis by using 5 μg of the muscle extract and an antibody against the C2 subunit as described (23).

Immunoblot for ubiquitin ligase content. An aliquot of crushed muscle was mechanically homogenized in an extraction buffer containing 20 mM Tris, 5 mM EDTA, 5 mM dithiothreitol (pH 7.0), 300 mM NaCl, in a ratio of 1 part muscle to 7 parts homogenization buffer. Subsequently, Western blot analysis was done according to methods we have described previously (3). The relative amount of muscle ubiquitin ligases was measured using primary antibodies (1:1,000 dilution) directed against MuRF1 (Novus NB 100–1193) and MAFA (Santa Cruz SC-27645) incubated overnight at 4°C, with 20 μg or 15 μg of protein loaded per well, respectively.
as seen in the 35-mo-old AL animals (Fig. 1). Thus, sarcopenia markedly accelerated at the oldest ages in both AL and CR animals, with the time course being delayed in proportion to the longer life span in CR rats.

Protein carbonyl measurement. Whereas AL animals demonstrated a significant increase in protein carbonyls in plantaris muscle between 8 and 35 mo of age, CR animals started at a similar point at 8 mo of age and exhibited no increase by 35 mo of age (Fig. 2).

Ubiquitin ligase protein expression. Both MAFbx (Fig. 3, top) and MuRF1 (Fig. 3, bottom) protein levels increased significantly with aging, and this effect was largely independent of diet. Indeed, at 35 mo of age there was no evidence that calorie restriction attenuated the age-related increase in either of the ubiquitin ligases examined, and for MuRF1 the levels reached by 40 mo of age surpassed those of both 35-mo-old AL and CR rats.

Proteasome activity and C2 subunit expression. The chymotrypsin-like activity of the proteasome demonstrated a significant age-related increase in both AL and CR rats; however, this took longer to evolve in the CR rats as the increase did not reach statistical significance until 40 mo of age in this group (Fig. 4, top). Protein levels of the C2 subunit of the proteasome were proportional to age-related changes in the chymotrypsin-like activity of the proteasome with aging in both groups (Fig. 4, bottom), suggesting a relatively constant proteasome function (i.e., relative molecular activity maintained) with aging and no effect of caloric intake.

Relationship between proteasome activity and muscle mass changes with aging. Fig. 5 shows plots of the plantaris muscle mass vs. the chymotrypsin-like activity of the proteasome in AL (Fig. 5, top) and CR (Fig. 5, bottom) rats. Using the regression equation for this relationship revealed that the observed muscle mass in 35-mo-old AL animals (210 ± 15 mg) was significantly less than that predicted from their proteasome activity (270 ± 8 mg; P < 0.05). Similarly, the observed muscle mass in 40-mo-old CR animals (158 ± 18 mg) was significantly less than that predicted from their proteasome activity (215 ± 6 mg; P < 0.05). This suggests that, in addition to an age-related increase in proteasome activity, other factors must be involved to explain the accelerated loss of muscle observed between 30 and 35 mo of age in the AL animals and between 35 and 40 mo of age in CR animals.

DISCUSSION

The purpose of this study was to evaluate the effects of aging and CR on the proteasome pathway in skeletal muscle and to relate these changes to the progression of muscle atrophy with aging as a means of obtaining insight into the physiological relevance of changes within this pathway for sarcopenia. We reasoned that if an upregulation of the proteasome pathway is a cause of skeletal muscle atrophy with aging, one would expect an upregulation of this pathway with aging that is...
opposed by calorie restriction. On the other hand, if a decrease in the activity of the proteasome is a cause of impaired skeletal muscle function with aging, one would expect a downregulation and/or dysfunction of this pathway with aging that is opposed by calorie restriction. Our results show that calorie restriction prevents the increase in protein carbonyl accumulation in plantaris muscle out to an age of 35 mo, which corresponds to senescence in the AL animals and an age where there is considerable muscle contractile dysfunction in this strain of rats (22, 33). There was also a marked increase in the expression of two key skeletal muscle ubiquitin ligases, MAFbx and MuRF1, with aging, but this effect was not opposed by caloric restriction. In fact, the levels of MuRF1 in 40-mo-old CR rats were the highest of any group. Proteasome activity, as indicated by the chymotrypsin-like activity, increased with aging in AL. Although this age-related increase also occurred in CR rats, the evolution of the increase was delayed in proportion to the extension of life span by caloric restriction. Changes in the chymotrypsin-like activity of the proteasome paralleled changes in protein levels of the C2 subunit, suggesting that differences in proteasome activity with aging and diet did not involve changes in proteasome function. Finally, plotting the plantaris muscle mass vs. the chymotrypsin-like activity of the proteasome revealed that the oldest animals in both diets had a lower muscle mass than would be predicted by their proteasome activity, implicating other factors in the acceleration of sarcopenia at advanced age. Thus, our results are consistent with the view that sarcopenia is, in part, due to an increased activation of the proteasome pathway and that this increase is temporally delayed in proportion to the extension of life span in CR animals. Notwithstanding this point, the increase in accumulation of oxidatively damaged proteins with aging in AL but not CR animals suggests that the increase in proteasome activity is insufficient to keep pace with the accumulation of oxidative damage with aging in AL and/or that caloric restriction better maintains the ability of the proteasome to specifically target and remove oxidatively damaged proteins with aging. This latter effect may play an important role in explaining how caloric restriction is able to preserve skeletal muscle function with aging.

Effect of aging and caloric restriction on skeletal muscle mass. We studied the plantaris muscle of male F344BN rats as a model of sarcopenia. The plantaris muscle is composed of primarily mixed fast-twitch muscle fibers (2), which are the fibers that exhibit the greatest degree of atrophy with aging in humans (32) and rat models of aging, such as the F344BN rat (9, 24). In addition, we examined the impact of a CR regimen that was imposed in juvenile animals such that the youngest age group studied, 8-mo-old CR animals, represented animals that had been on the 40% lower caloric intake (with maintained...
Nutrition for ~4 mo. Previous studies have shown that calorie restriction imposed in this fashion in male F344BN rats extends both mean and maximal life span by ~25% (50), and markedly slows the rate of age-related muscle atrophy and functional decline (3, 25). The current results showing that the calorie restriction regimen reduced the amount of muscle atrophy between 8 and 35 mo of age are consistent with these prior studies.

Use of three age groups in each dietary treatment in our study design not only permits examination of the factors underlying sarcopenia and its acceleration between late middle age (30 mo old in AL animals) and senescence (35 mo old in AL animals), but also permits absolute (e.g., 35-mo-old AL vs. 35-mo-old CR rats) and relative age comparisons (e.g., 30-mo-old AL vs. 35-mo-old CR rats; 35-mo-old AL vs. 40-mo-old CR rats) between dietary treatments [based upon survival curve data reported previously for this strain of rat; (50)]. As such, the design of our study permits insight into the factors that relate to the progression and acceleration of sarcopenia with advancing age, and the attenuation of sarcopenia by calorie restriction. Furthermore, this design permits one to determine whether the differences observed in calorie restriction represent a prevention of a given change (e.g., no change even out to 40 mo of age) or simply a delay of a given change that is proportional to the life span extension of calorie restriction.

Effect of aging and calorie restriction on ubiquitin ligase expression. The attachment of multiple ubiquitin molecules to proteins is an essential part of the targeting of such proteins for degradation by the proteasome (1). In skeletal muscle, this is accomplished through the action of two primary ubiquitin ligases, MAFbx [also known as atrogin-1 (20)] and MuRF1 (8). Two studies have observed an increased amount of ubiquitinated proteins in aged muscles (10, 12), an observation that would be consistent with an increased ubiquitin ligase activity with aging, whereas another study did not see an increase in ubiquitinated proteins in aged muscles (14). Similarly, whereas one study reported an increased mRNA expression of MAFbx and/or MuRF1 in aged skeletal muscle (12), other studies found no change (51) or a decreased mRNA expression of these ubiquitin ligases with aging (14, 16). It is noteworthy that protein levels of these ubiquitin ligases have not been previously examined in aging muscles. In this respect, our results show that protein levels of MAFbx and MuRF1 are markedly elevated with aging in plantaris muscle in AL animals and that this begins at an age where the degree of muscle atrophy is only modest (30 mo of age). Note that these changes in protein levels do not necessarily correspond to changes in mRNA expression as we have observed a decrease in MAFbx and an increase in MuRF1 mRNA expression in plantaris muscle from 33-mo-old male F344BN rats (Baker DJ, Manuel S-J, and Hepple RT, unpublished results). Thus, our results show that there is an augmented protein expression of the ubiquitin ligases in aged muscles, which likely contributes to the previously observed increase in ubiquitinated proteins with aging (10, 12). These findings contradict a recent assertion that upregulation of the proteasome pathway is not involved in sarcopenia (16). To our knowledge, no prior study has addressed the effect of calorie restriction on skeletal muscle ubiquitin ligase expression in aged skeletal muscle. Our results show that calorie restriction did not attenuate the age-related increase in expression of either MuRF1 or MAFbx. Indeed, MuRF1 expression was significantly higher in the 40-mo-old CR animals than in any other group. Thus, calorie restriction is associated with a similar, if not augmented, age-related increase in ubiquitin ligase expression in skeletal muscle.

Impact of aging and calorie restriction on proteasome activity and function in skeletal muscle. The proteasome is a multicatalytic enzyme complex involved in the degradation of cellular proteins. Since the proteasome plays a key role in the removal of oxidatively damaged proteins (1, 27, 36), its role in the biology of aging as it relates to the accumulation of oxidatively damaged proteins is thought to be particularly important (36). Indeed, oxidative stress is considered an important modulator of proteasome activity in skeletal muscle (5), and is implicated in the atrophy observed with models of muscle disuse, such as mechanical ventilation (35, 40).

The 26S proteasome consists of a 20S core subunit that houses the catalytic core containing the primary proteolytic active sites (i.e., trypsin-like, chymotrypsin-like, and peptidylglycine-hydrolyzing activities of the proteasome), and the 19S regulatory subunit involved in degradation of ubiquitinated proteins (1, 38). In the context of aging skeletal muscle, one study reported decreased chymotrypsin-like activity but not trypsin-like activity in soleus muscle of 26- to 28-mo-old male F344 rats (46) and two other studies reported unchanged trypsin-like, chymotrypsin-like, and peptidylglycine-hydrolyzing activities of the proteasome in both slow-twitch soleus muscle (26) and mixed fast-twitch muscles (17) of 30- to 37-mo-old male F344BN rats. Another study showed a biphasic response with increasing age in male and female LOU-C rats, with a progressive increase in trypsin-like, chymotrypsin-like, and peptidylglycine-hydrolyzing activities of the proteasome among 4, 24, and 29 mo of age, and a decline to 24-mo-old levels at 34 mo of age (4). In previous studies from our laboratory, we observed a trend to an increase in chymotrypsin-like activity of the proteasome in gastrocnemius muscles of 28-mo-old vs. 18-mo-old male F344/DuCrj rats (42). On the other hand, there was no change in chymotrypsin-like activity and a decrease in trypsin-like activity of the proteasome in gastrocnemius muscle of male F344/DuCrj rats between 10 and 30 mo of age (43). Thus, there is considerable variability in the observed changes in proteasome activity in aged skeletal muscles between studies. This variability may derive from several study-to-study differences, including strains of rats, ages, muscles studied, and proteasome isolation procedures (see below).

Interestingly, although their studies reported no change in proteasome activities with aging when normalized to muscle mass, Husom et al. (26) and Ferrington et al. (17) previously reported markedly lower specific activities of the proteasome in aged fast-twitch muscles of male F344BN rats when normalizing to the α6-subunit (17) or C2 subunit (26), suggesting impaired proteasome function with aging. The current results in the same sex and strain of rat and also in a primarily fast-twitch muscle (plantaris muscle) show an increase in the chymotrypsin-like activity of the proteasome between 8 and 35 mo of age in male F344BN rats. Furthermore, these changes in proteasome activity were paralleled by proportional increases in the protein content of the C2 subunit, suggesting no change in proteasome-specific activity. As such, our results contrast with those previously reported by Ferrington et al. (17) in mixed fast-twitch muscles in this strain of rat. Although the
results suggest that with advancing age other factors beyond an increase in proteasome activity account for the acceleration of sarcopenia at advanced age. It is also relevant that with aging not only is there marked muscle atrophy, but the function of the muscle mass remaining is also compromised. This dysfunction is thought to be due in part to accumulation of oxidatively damaged proteins (11, 19, 41). Our results are consistent with this view in that they show the profound skeletal muscle contractile and metabolic dysfunction we previously observed in distal hindlimb muscles of 36-mo-old AL F344BN rats (22) is associated with an elevated plantaris muscle protein carbonyl content (current results). Similarly, the lack of age-related increase in plantaris muscle carbonyl content with calorie restriction reported here is consistent with our prior results showing that calorie restriction completely protects skeletal muscle contractile and metabolic function in distal hindlimb muscles of 35-mo-old F344BN rats (25). Note that a lower production of ROS (6, 7) and higher endogenous antioxidant enzyme expression (47) likely lowers the rate of oxidatively damaged protein generation in calorie restriction. Thus, the fact that calorie restriction prevented the increase in protein carbons at 35 mo in AL suggests that CR animals are able to optimize the removal of oxidatively damaged proteins to keep pace with their generation, whereas this does not occur in AL animals. As such, our observations suggest an important role for appropriate activation of the proteasome with aging in explaining the means by which calorie restriction is able to preserve function in aged muscles (25, 34, 39).

GRANTS

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REFERENCES


Perspectives and Significance

The causes of sarcopenia are numerous and the degree of contribution of any one of the proposed causes may shift as muscle atrophy becomes more severe, particularly during the transition to a more accelerated rate of atrophy as occurs between late middle age and senescence. Therefore, to gain insight into the significance of changes in proteasome activity with aging on the progression of sarcopenia, in each dietary treatment we plotted the plantaris muscle mass vs. the chymotrypsin-like activity measured in the same muscles. As seen in Fig. 5 and described in RESULTS, muscle mass was significantly lower than would be predicted by the respective regression equations in 35-mo-old AL and 40-mo-old CR animals. These objectives that informed the experimental design in these studies were clearly different, in seeking to explain the disparate results, it is relevant that the study of Ferrington et al. not only had a much wider age range in their older animals (30- to 37-mo-old animals were combined, vs. 35-mo-old AL rats in the current study), but they also combined more than seven muscles in their analyses (17). Potential muscle-to-muscle variability would be obscured by combining so many muscles, and, since the amount of muscle atrophy seen between 30 and 35/36 mo of age in male F344BN rats is considerable (present results and Ref. 22), any variability in proteasome activity across this age range as muscle atrophy proceeds would also be obscured. Our data show that although an increase in chymotrypsin-like activity of proteasome occurs with increasing age in AL animals, this increase is relatively smaller than the muscle atrophy between 30 and 35 mo of age. Another difference between studies involves the method of proteasome isolation between studies. Specifically, whereas Ferrington et al. (17) employed a very rigorous column separation, yielding the 20S proteasome exclusively, we employed a more gentle centrifugation procedure that yields both 20S and 26S forms of the proteasome in the extract.

There is very little previous data concerning the impact of calorie restriction on proteasome activity and function in aged skeletal muscle. Previously, Selby et al. (46) reported that 40% calorie restriction beginning in the juvenile period augmented the trypsin-like activity in soleus muscle of 26- to 28-mo-old male F344 rats. In a prior study from our laboratory, we found that every other day feeding for 3.5 mo increased the trypsin-like activity of the proteasome in gastrocnemius muscle of 30-mo-old male F344/DuCrj rats (43). The current data found that 40% calorie restriction initiated in juvenile animals was associated with a slower age-related increase in chymotrypsin-like activity in plantaris muscle of male F344BN rats. Similar to the issues raised in the preceding section, study-to-study variability in muscle studied, rat strain used, calorie restriction regimen, and ages studied likely also contribute to the apparently inconsistent effect of calorie restriction between studies. Notwithstanding this point, our study suggests that differences in muscle proteasome activity with aging were not related to differences in function of the proteasome since differences in proteasome activity with aging and diet were paralleled by proportional differences in the content of the C2 subunit. Thus, the current data do not support an age- or calorie restriction-mediated effect on proteasome function per se in aging skeletal muscle.


