Early-onset calorie restriction conserves fiber number in aging rat skeletal muscle[1]

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SPECIFIC AIMS

Sarcopenia is the age-related loss of muscle mass and function. It is a complicated multifactorial process with no clear etiology. Muscle mass loss with age has a significant impact on the physiological and social well-being of the aged. Calorie restriction (CR) has been shown to retard many of the deleterious effects of aging in rodent and nonhuman primate models. We hypothesize that CR would significantly affect progressive muscle mass loss with age. The specific aims of this work were to examine the effects of aging on skeletal muscle in a long-lived hybrid rat strain by analysis of muscle weight, muscle fiber number, and muscle fiber atrophy using three different muscles and to determine the influence of early-onset CR on that process in young, intermediate aged, and very aged animals.

PRINCIPAL FINDINGS

1. Significant sarcopenic changes occurred in the skeletal muscle of the hybrid rat between 21 and 36 months of age. Calorie restriction retarded muscle mass loss but did not prevent it

The vastus lateralis, rectus femoris and soleus muscles of 5- 18- 21-, and 36-month-old ad libitum (AL) fed Fischer × Brown Norway F1 hybrid rats were dissected and weighed. The same muscles from 21- and 36-month-old calorie restricted (CR) rats placed on a 40% reduced calorie diet at 4 months of age were also dissected and weighed. All AL muscles underwent significant muscle mass loss between 21 and 36 months of age (**Fig. 1**). There was also a significant decrease in muscle mass for the vastus lateralis and rectus femoris muscles examined in the CR rats between 21 and 36 months (Fig. 1). There was no significant loss of muscle mass in the CR soleus muscles. The percent change (loss) in muscle mass between 21 and 36 months was significantly greater in the AL rats compared with the CR; 60% loss for AL vastus lateralis, 38% for CR; 53% loss for AL rectus femoris and 39% for CR; 40% loss between 21 and 36 months for AL soleus, 10% for CR.

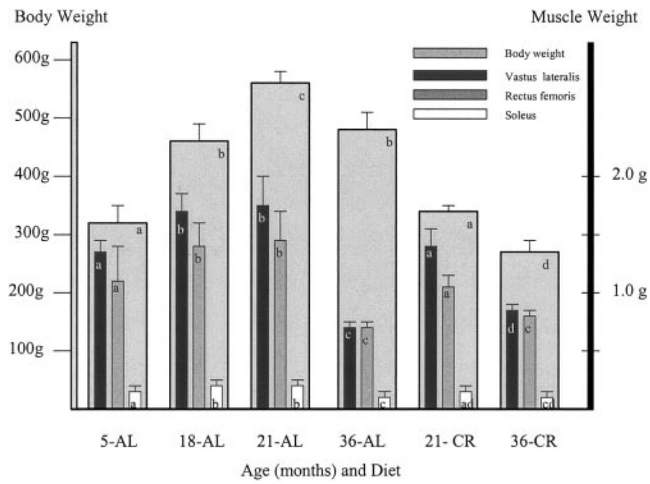

Figure 1. The effect of age and diet on body weight and muscle weight in the FBN rat. The body weight scale is on the left axis, the mean body weight is represented by the large gray bars. Muscle weight scale is on the right axis, mean muscle weight is represented by black (vastus lateralis), dark gray (rectus femoris), and white (soleus) bars. Age in months, and diets (AL=ad libitum and CR=calorie restriction) are on the x-axis. Different letters within like colored bars were significantly different ($P<0.01$). Five to seven animals are represented at each age and diet.

2. Significant muscle fiber loss contributed to sarcopenia in aged AL fed rat skeletal muscle. CR rat muscles conserved muscle fiber number with age

Individual muscles were bisected at the midbelly, flash frozen in optimal cutting temperature medium and then sectioned using a cryostat. Sections were stained with hematoxylin and eosin and composite images of the stained sections were digitally reproduced. Fibers were counted at the midbelly by annotating each fiber using Adobe Photoshop, and counts were tabulated using ImagePro software. The mean fiber number for 5-month AL vastus lateralis muscles was 12,043 ± 1429, 10,426 ± 714 for rectus femoris, and 2419 ± 309 for soleus. Muscle fibers were maintained through 18 and

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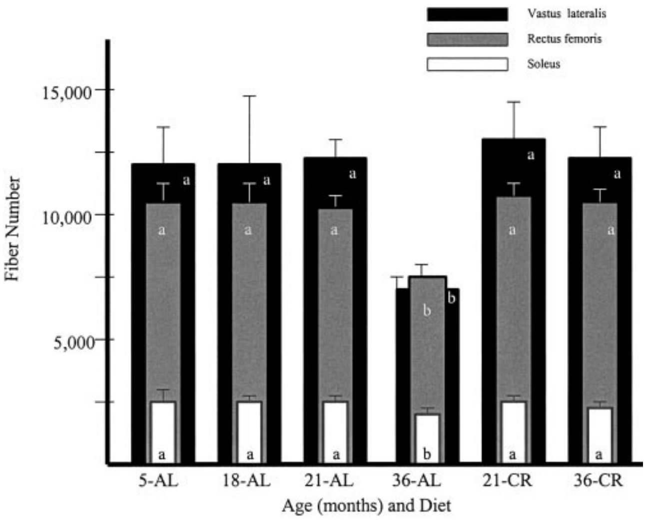

Figure 2. Fiber number for each muscle (counts taken from the midbelly of the muscle) at each age and diet examined. Age in months and diets (AL=ad libitum and CR=calorie restriction) are on the x-axis. Like bars with different letters were significantly different ($P<0.05$).) Five to seven animals are represented at each age and diet.

21 months of age. Significant muscle fiber loss occurred between 21 and 36 months among all three muscles of the AL fed animals; fiber number for 36-month vastus lateralis was 6993 ± 583, for rectus femoris 7424 ± 517, and for soleus 1997 ± 287. Calorie-restricted rats retained fiber number with age, no fiber loss between 21 and 36 months of age (**Fig. 2**).

3. Significant fiber atrophy occurred in Type II muscle fibers with age, Type I fibers were resistant to fiber atrophy. CR did not prevent Type II muscle fiber atrophy

Muscle fiber diameter was measured using the lesser fiber diameter method defined as the maximum diameter across the lesser aspect of the muscle fiber. Six hundred measurements were taken from muscles at each time point. Significant fiber atrophy was observed in the Type II predominant muscles, vastus lateralis (44% loss in fiber diameter), and rectus femoris (29% loss in fiber diameter) between 21 and 36 months of age in AL rats. There was also significant fiber atrophy in the vastus lateralis and rectus femoris muscles between 21 and 36 months of age in the CR rats. No fiber atrophy was observed in the Type I soleus muscle from either CR or AL rats.

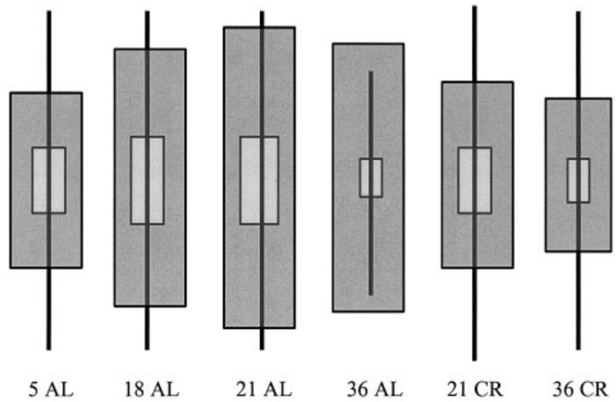

Figure 3. Illustration of the effects of age (months) and diet (ad libitum and calorie restricted) on Type II predominant muscles of the male FBN hybrid rat. Relative body weight changes (gray bars), muscle weight changes (light gray bars), and fiber number changes (vertical black bars).

CONCLUSIONS

This work supports the hypothesis that calorie restriction significantly retards the physiological deterioration that occurs in muscles with age. CR reduced muscle mass loss by retaining muscle fiber number. By using both 21- and 36-month old FBN rats maintained on 40% CR since 4 months of age and comparing them to age-matched ad libitum fed rats, we determined that *1)* muscle mass loss does occur among AL and CR rats, *2)* the degree of loss was significantly less in the CR group, *3)* fiber loss significantly contributed to muscle mass loss in the AL fed group, and *4)* no fiber loss occurred in the CR group (**Fig. 3**).

A significant outcome of CR on aging muscle is that it appears to counter the processes that induce fiber loss, conserving fiber number. The process by which CR supports muscle integrity is unclear. A body of evidence suggests cellular and molecular oxidative damage contribute to the aging process, especially in nonreplicative tissues such as heart, brain, and skeletal muscle and that CR decreases macromolecular damage, increases protein turnover and enhances the function of oxygen free radical scavengers. Since oxygen free radical production occurs primarily in the mitochondria, we hypothesize that mitochondrial DNA deletion mutations induce electron transport system enzyme abnormalities that are associated with intrafiber atrophy and fiber breakage. We continue to pursue this hypothesis and the effect of calorie restriction on sarcopenia in the aging rat. FJ