DIET RESTRICTION AND AGE ALTERS SKELETAL MUSCLE CAPILLARITY IN B6C3F1 MICE

Keith, N¹, Bronson, RT², Lipman, RD², Ding, W², Lamont, L³, Cosmas, AC⁴, and Manfredi, TG³

¹ Bower-Suhrheinrich School of Education and Human Services, University of Southern Indiana 47712

² Jean Mayer USDA Human Research Center on Aging at Tufts University, Boston, MA 02111,

³ Exercise Science, University of Rhode Island, Kingston, RI 02881-0810,

⁴ School of Allied Health Professions, University of Connecticut, Storrs, CT 06269-2034

ABSTRACT

The effects of 40% diet restriction on skeletal muscle fiber area, capillary density (CD) and capillary to fiber ratio (C/F) were compared in 12, 24 and 30-month-old female B6C3F1 female hybrid mice. We hypothesized that diet restriction (DR) would retard the aging effects observed in skeletal muscle, in particular DR would pose opposite effects on skeletal muscle capillarity and fiber area. Samples were prepared for light microscopic examination by standard methods and for morphometric analysis using NIH-image software. There was a significant effect of age on muscle fiber area ($p < 0.05$). The age-associated decrease in fiber area between 12 and 30 months of age was greater ($p < 0.05$) in the ad libitum (AL) (37.7%) animals as compared to the diet restricted (DR) mice (29.2%). Diet had a significant effect on CD ($p < 0.05$) and C/F ($p < 0.05$). This finding suggests that the lower capillarity in the older DR mice may have been due to their larger muscle fibers. The results of this study support the contention that diet restriction delays the progression of age-associated muscle atrophy.

INTRODUCTION

Aging is characterized by an exponential increase in the number of catabolic reactions that result in an accelerated rate of degenerative changes within multiple organ systems. A number of these age-related degenerative alterations have been reported to occur within skeletal muscle and include a decrease in muscle weight and fiber area (12), an increase in capillary density (3) and a decrease in mitochondrial mass (11). In contrast to these aging effects on skeletal muscle, physical activity has been reported to increase capillarity and mitochondrial mass in man and animals (4, 11, 19).

*To whom all correspondence should be addressed:
Thomas Manfredi
Exercise Science
25 West Independence Way, Room 110
University of Rhode Island
Kingston, RI 02881-0810
(401) 874-5439
Fax: (401) 874-5630 (c/o Dr. Thomas Manfredi)
E-mail: manfredi@uri.edu

It has been known for quite some time that diet restriction alters lean body mass (3) and that maintaining lean body mass throughout life delays the onset of age-related debilitating conditions such as osteoporosis and arthritis (1,16). Current research confirms an age-associated increase in free radical susceptibility that appears to be attenuated by diet restriction, resulting in an increase in muscle antioxidant enzyme activities and a reversal in the decrease in mitochondrial electron transport activity in aging rats (13) and mice (9). Furthermore, nutritional regulation in the form of diet restriction has been demonstrated to have significant advantageous effects on aging including a reduction in the number of lesions (2,5) and a reduced sensitivity to carcinogen induced tumor formation (16,18). Data from studies on weaning to old age indicate that diet restriction (DR) delayed the age-related decline in skeletal muscle fiber area that is associated with a lower capillary/fiber ratio (3). This study extends those observations by examining the effects of age and diet restriction on specific morphological characteristics of skeletal muscle.

MATERIALS AND METHODS

Animal Care

The animals used for this study were 35 B6C3F1 hybrid female mice born and raised at the National Center for Toxicological Research in Jefferson, AR as part of the NIA Biomarkers of Aging Program. All mice were matched for age, gender, and genotype. The animals were housed in autoclaved, filtered individual cages at 23°C and 45% relative humidity with fifteen air changes of 100% fresh filtered air each hour and a 12/12 light/dark cycle. All mice were observed daily for disease. The mice were fed autoclaved chow PMI 5021 (Richmond, IN). At 14 weeks of age, the mice were divided into two cohorts that were fed ad libitum or dietary restricted feed, which comprised 60% of the caloric intake of the age-matched AL animals (5,21). The animals were maintained at the USDA Human Nutrition Research Center on Aging at Tufts University (Boston, MA), where the study was reviewed and approved by the Animal Care and Use Committee.

Tissue Preparation and Analysis

During sacrifice, each animal was deeply anesthetized with tribromoethanol (Avertin), exsanguinated, perfused with physiologic saline and fixed by intracardiac perfusion with Bouin's fixative. Gastrocnemius muscle samples were fixed in 3.0% glutaraldehyde in 1 molar cacodylate buffer, rinsed in buffer and post fixed in 1% osmium tetroxide. Following dehydration in ethyl alcohol, the samples were embedded in LX-112. Blocks, which were oriented for cross sectioning, were sectioned using a Dupont Sorvall Porter Blum MT-2B ultramicrotome at approximately 1 μ and stained with methylene blue, azure blue, following by staining with basic fuchsin. This combination of dyes stained muscle fibers blue, nuclei purple and connective tissue bright red. Approximately 100 fibers per animal were examined using a Leitz light microscope interfaced with a JVC RGB color video camera connected to a Mac IIci computer equipped with a Data Translation frame grabbing board. The cross sectional area of individual fibers was measured using NIH image 1.55 software. Muscle fiber areas were measured using a set scale for spatial calibration. Sections with oblique oriented fibers were rejected. Direct counts of composite images were measured for each muscle and the means combined to yield a mean fiber area for each animal.

Muscle fiber number/mm², muscle area in μ m², capillary number per muscle fiber number (C/F=capillary to fiber ratio) and capillary density (CD=number of capillaries/mm²) were measured using the criteria established by Maxwell et al. (14). Capillaries and fibers were counted using a microscope lens fractionated into an exact area calibrated with a slide micrometer. Structures bisecting one vertical and one horizontal perimeter of the frame were included.

A two-way analysis of variance (ANOVA) for independent samples was used to test significance of differences between dietary intervention and age. When *f* ratios were significant, post hoc comparisons of means were analyzed using Fishers simple effects test. Muscle fiber and capillary data were analyzed by linear regression.

RESULTS

Body Weight and Muscle Fiber Morphometry

As observed in tables 1 and 2, when compared to the ad libitum (AL) fed animals, the diet restricted (DR) group had a significantly lower body weight (*p*<0.0001). Diet restriction delayed the atrophic effect of age on muscle fiber area to some degree (*p*<0.05), although there was a significant increase in muscle fiber number/mm² in both AL and DR mice with age (*p*<0.05). Correlation coefficients of *r*=-0.438, 0.432 and 0.668 were computed between age and fiber area, age and fiber number/mm², and fiber area and fiber number/mm². Figures 1 and 2 show the age regression for AL and Dr groups. Slopes for age regression do not appear to differ dramatically regarding muscle fiber area.

Capillarity

Table 2 indicates that diet restriction significantly delayed the age-associated increase in capillary density (*p*<0.05) and decrease in capillary to fiber ratio (*p*<0.05). At 30 months of age, the difference in capillary density between the AL and DR animals was significant (*p*<0.011). As illustrated in Figures 3 and 4, age regression for capillary density and capillary to fiber ratios showed dramatic differences in the AL and DR animals. The age related decline in capillary density was associated with an increase in fiber area (*r* = 0.601) and fiber number/mm² (*R* = 0.826).

Table 1: Effect of Diet Restriction and Age on Skeletal Muscle Morphometry

Group	Age mo.	B. Wt. G.	Fib area μ m ²	Fib/mm ²	Cap/mm ²	Cap/fib
AL (N = 6)	12	39.1 $\pm$ 6.57	1526 $\pm$ 432	446 $\pm$ 189	664 $\pm$ 335	1.25 $\pm$ 0.386
AL (N = 5)	24	35.9 $\pm$ 8.90	1285 $\pm$ 557	738 $\pm$ 246	1163 $\pm$ 405	1.34 $\pm$ 0.169
AL (N = 6)	30	36.1 $\pm$ 3.50	951 $\pm$ 267	921 $\pm$ 192	1178 $\pm$ 314	1.13 $\pm$ 0.422
DR (N = 6)	12	24.4 $\pm$ 1.47	1441 $\pm$ 605	525 $\pm$ 203	700 $\pm$ 264	1.14 $\pm$ 0.127
DR (N = 6)	24	26.9 $\pm$ 1.22	1370 $\pm$ 521	732 $\pm$ 431	816 $\pm$ 627	0.88 $\pm$ 0.234
DR (N = 6)	30	28.5 $\pm$ 1.02	1020 $\pm$ 349	613 $\pm$ 216	641 $\pm$ 185	0.910 $\pm$ 0.152

Table 2: Two-Way ANOVA (*p* Values) Showing Effects of Age and Diet Restriction on Skeletal Muscle Morphometry

Source	Body Wt.	Fiber Area	Fib/mm ²	Cap/mm ²	Cap/fib
DIET (A)	.000	.887	.374	.034	.017
AGE (B)	.961	.043	.022	.139	.299
AB	.161	.891	.166	.177	.349

DISCUSSION

The results of this study may provide insight into previous diet restriction studies, which reported a reduced incidence of tumor (5), and the decreased incidence of age-related biological markers in skeletal muscles of diet restricted mice (6,7,8). Boreham et al. (3) who examined fast and slow twitch muscle fibers in rats subjected to chronic diet restriction and our earlier studies on B6C3F1 mice (7) demonstrated that diet restriction reduced the occurrence of these age-related markers of muscle damage. In this study, we did not quantify muscle damage, therefore it is difficult to state with confidence if our DR mice showed less evidence of fiber related damage.

No significant change in body weight occurred in either the diet restricted or ad libitum fed groups between 24 and 30 months of age. However, between 12 and 30 months the body weight of the ad libitum fed mice decreased by approximately 8 percent in comparison to

Figure 1. Age Regression for AL and DR Groups: Fiber Area

Figure 2. Age Regression for AL and DR Groups: Fiber Number

Figure 3. Age Regression for AL and DR Groups: Capillary Density

Figure 4. Age Regression for AL and DR Groups: Capillary:Fiber Ratio

a 14 percent increase in the diet restricted mice (table 1). In a related study using male Wistar rats between the ages of 5 and 11 weeks, Yamaguchi (22) reported a delay in growth related increases in body weight in the diet restricted animals while muscle weight and fiber area continued to increase. The results of this study supported Yamaguchi's data in that there was a greater age associated decrease in fiber area (37.7%) in the ad libitum fed mice as compared to the diet restricted group (29.2%). These differences, associated with fewer age-related lesions in the diet restricted animals (5) suggest that diet restriction may be an important factor in the morphology and metabolic characteristics of skeletal muscle. Chronic diet restriction appears to reduce the age-associated decline in skeletal muscle fiber cross section area, thereby affecting capillary density and capillary to fiber ratio without compromising its metabolic efficiency.

Ripoll and his associates (17) reported a growth related decrease in capillary density and an increase in capillary to fiber ratio in young rat gastrocnemius and soleus muscles. In a similar study, Maxwell (14) using older rats, found no relationship between fiber cross sectional area and capillary to fiber ratio. Our findings ($r = 0.072$ correlation between between fiber area and C/F ratio) support Maxwell's contention that no correlation exists between fiber cross sectional areas and capillary to fiber ratio. However, the disparity among these studies may be attributed to the age of the animals examined. Changes in capillary density with age may represent a vascular response to alterations in the cross sectional area of muscle fibers. Furthermore, as suggested by Sillau and Banchero (20), capillary to fiber ratio may be an indicator of skeletal muscle growth.

Unlike the oxidative challenge of intense exercise training which has been reported to result in an increase in capillary numbers (4,19), diet restriction may not provide an intense enough metabolic challenge to promote angiogenesis. However, the absence of capillary growth as a result of diet restriction does not appear to compromise muscle fiber oxygen supply and metabolic efficiency. Myoglobin concentration and the ratio of perfused to non-perfused capillaries may represent satisfactory compensatory mechanisms for the reduction in capillarity. Several studies have concluded that chronic diet restriction alters energy metabolism in animals (10,15). Duffy and his colleagues (10) in a study comparing old B6C3F1 diet restricted and ad libitum fed mice, reported an increase in spontaneous motor activity in the diet restricted animals even though both groups had similar daily oxygen consumption values, suggesting a relationship between metabolic efficiency and diet restriction. Generally, the amount of spontaneous activity is inversely related to body weight and this relationship may have been a factor in this study. Since muscle fiber area increases with physical activity and declines as physical activity levels decrease, the greater muscle fiber area in the diet restricted mice could be attributed to their higher activity levels. Continued research di-

rected toward diet restriction, physical activity and aging will undoubtedly provide relevant information regarding longevity and age-related metabolic disorders.

REFERENCES

1. Aliola, JF, Cohn, SH, Ostuni, JH, Cane, R, and Ellis, K: Prevention of involutional bone loss by exercise. *Ann. Of Intern. Med.*, 89:356-358, 1978
2. Beattie, L.B. and Louis, VY: Nutrition and health in the elderly. In *Clinical Aspects of Aging*. Baltimore, MD: Williams & Wilkins, 1989.
3. Boreham, CAG, Watt, PW, Williams, PE, Merry, BJ, Goldspink, G, and Goldspink, DF: Effect of aging and chronic dietary restriction on the morphology of fast and slow muscles of the rat. *J. Anat.*, 157: 111-125; 1988.
4. Brodal, P, Ingjer, F, and Hermansen, C: Capillary supply of skeletal muscle fibers in untrained and endurance-trained man. *Am. J. Physiol.*, 232:H705-H712; 1977.
5. Bronson, RT, and Lipman, RD: Reduction rate of occurrences of age related lesions in dietary restricted laboratory mice. *Growth Dev. Aging.*, 55:169-184; 1991.
6. Cartee, GD, Keitzke, EW, and Briggs-Tung, C: Adaptation of muscle glucose transport with caloric restriction in adult, middle-age, and old rats. *Am. J. Physiol.*, May; 266 (5pt 2): R1443-1447; 1994.
7. Cosmas, AC, Ding, W, Bronson, RT, Lipman, RD, Lee, HY, and Manfredi, TG: Age and diet alter skeletal muscle tubular aggregates. *AGE*, April; 18:2, 69-78; 1995.
8. Dean, DJ and Cartee, GD: Brief dietary restriction increases skeletal muscle glucose transport in old Fischer 344 rats. *J. Gerontol., A Biol. Sci. Med. Sci.* May; 51 (3): B208-B213; 1996.
9. Desai, VG, Weindrich, R, Hart, RW, and Feuers, RJ: Influences of age and dietary restriction on gastrocnemius electron transport system activities in mice *Arch. Biochem. Biophys.*, Sept. 1; 333(1): 145-151; 1996.
10. Duffy, PH, Feuers, RJ, and Hart, RW: Effect of chronic caloric restriction on circadian regulation of physiological and behavioral variables in old male B6C3F1 mice. *Chrono. Biol. Internat.*, 7:4, 291-303; 1990.
11. Edington, DW, Cosmas, AC, and McCafferty, WB, Exercise and longevity: Evidence for a threshold age: *J. Gerontol.*, 27:3, 342-343, 1972.
12. Holloszy, JO, Chen, M, Cortee, GD, and Young, JO: Skeletal muscle atrophy in old rats: Differentiated changes in the three fiber types. *Mech. Age Dev.*, 60:199-213; 1991.

13. Luhtala, TA, Roacker, EB, Paugh, T, Feuers, RJ, and Weindruch, R: Dietary restriction attenuates age-related increased in rat skeletal muscle antioxidant enzyme activities. *J. Gerontol.*, Sept; 49 (5):B231-B238; 1994.
14. Maxwell, LC White, TP, and Faulkner, JA: Oxidative capacity, blood flow, and capillarity of skeletal muscles. *J. Appl. Physiol.*, 49(4):627-633; 1980.
15. McCarter, RJ: Age related changes in skeletal muscle function. *Aging, (Milanno)*. Mar;2(1); 27-38; 1990.
16. Nolan, GA: Effects of various restricted dietary regimens on growth, health, and longevity of albino rats. *Kuhnau Journal (ed): Proclamation of the 7th International Congress of Nutrition*. 5:35-38; 1972.
17. Ripoll, E, Sillau, AH, Banchemo, N. Changes in the capillarity of skeletal muscle in the growing rat. *Pflugers Archiv.*, 380:153-158; 1979.
18. Rogers, AE: Diet and carcinogens. *Carcinogenesis*, 14 (11): 2205-2217; 1993.
19. Schmidt-Nielsen, K. and Pennycuik, P: Capillary density in mammals in relation to body size and oxygen consumption. *Amer. J. Physiol.*, 200(4):746-750; 1961.
20. Sillau, AH, and Banchemo, N: Effect of maturation on capillary density, fiber size and composition in rat skeletal muscle. *Proc. Soc. Exp. Biol. Med.*, 154:461-466; 1977.
21. Witt, WM, Brand, D, Attwood VG, and D. Soave, D: A nationally supported study on caloric restriction of rodents. *Lab animals.*, 18, 37-43; 1989.
22. Yamaguchi, A, Horio, Y, Kunihiro, S, and Katsuta, S: The effect of malnutrition on the size and proportion of muscle fiber types during growth. *J. Anat.*, 182:29-36; 1993.