Attenuation of Sarcopenia by Dietary Restriction in Rhesus Monkeys

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Sarcopenia, the loss of muscle mass with normal aging, devastates quality of life—and related healthcare expenditures are enormous. The prevention or attenuation of sarcopenia would be an important medical advance. Dietary restriction (DR) is the only dietary intervention that consistently extends median and maximum life span, as well as health span in rodents. Evidence suggests that DR will have a similar effect in primates. Furthermore, DR opposes sarcopenia in rodents. We tested the hypothesis that DR will reduce age-related sarcopenia in a nonhuman primate. Thirty adult male rhesus monkeys, half fed a normal calorie intake and half reduced by 30% in caloric intake, were examined over 17 years for changes in dual-energy X-ray absorptiometry-estimated skeletal muscle mass. Body weight-adjusted skeletal muscle mass declined somewhat in both groups but was far more rapid in the control group. We have shown that moderate, adult-onset DR can attenuate sarcopenia in a nonhuman primate model.

Key Words: Dietary restriction—Sarcopenia—Monkey.

Sarcopenia is the loss of muscle mass with normal aging. After the age of 30, approximately 3%–8% of muscle mass is lost each decade and this rate of decline accelerates after the age of 60 (1,2). Approximately 45% of the older (> 60 years) United States population develops sarcopenia (3). This loss of muscle mass is related to disability, increased risk of injury, decreased quality of life, and increased healthcare expenditures. For the year 2000, it was estimated that 1.5% of total health care expenditures in the United States, totaling $18.5 billion, was attributable to sarcopenia (4). Therefore, the economic impact of even a modest reduction in sarcopenia would be appreciable.

While sarcopenia occurs in all populations, its prevention, delay, or attenuation is possible, for example, by increased physical activity and resistance training. Another potential way to influence sarcopenia is by altering the aging process. Dietary restriction (DR), or the healthy reduction of calorie intake, is the only dietary intervention that has repeatedly been shown to increase both median and maximal life span in rodents while opposing the development of a broad spectrum of age-associated biological changes (5–7). The ability of DR to increase life span is also seen in spiders, fish, water fleas, and other animals (5) including dogs (8), although its ability to increase life span in a primate species is still unclear.

Since 1989, we have been studying the effects of a moderate (30%), adult-onset DR in a nonhuman primate model (9,10). We chose a rhesus monkey (Macaca mulatta) model due to its similarity to humans in many aspects of its genetics, endocrinology, physiology, and aging course, in combination with an adequate degree of time compression (11). We have previously shown (12) that, similar to humans, rhesus monkeys lose significant skeletal muscle mass with advancing age. The goal of this study is to determine if DR can prevent this decline in skeletal muscle mass.

METHODS

Subjects and Design

The effects of aging and DR are being analyzed longitudinally in adult male rhesus monkeys, ranging in age from 8 to 14 years (average 9.3 years) at study onset. Details of the design and methodology have been previously reported (9,10,13). The average life span of rhesus monkeys at the Wisconsin National Primate Research Center is 27 years and the maximum life span is 40 years. This protocol was carried out with the approval of the Institutional Animal Care and Use Committee of the Graduate School of the University of Wisconsin, Madison.

The 30 rhesus monkeys used in this study were born at and have lived their entire lives at the Wisconsin National Primate Research Center. Prior to the start of this experiment, none of the animals had any clinical or experimental history that would be expected to affect muscle mass. All animals are individually housed to allow accurate assessment of daily food intake. Animals have extensive visual and auditory contact with each other and are provided with an enriched environment consisting of perches, branches, and small noninjurious toys that are rotated on a regular basis. The animals’ environment is maintained at an approximate temperature of 21°C and humidity of 50%–65%.
Room lighting is automatically controlled on a 12-hour light/12-hour dark schedule. All animals have 24-hour access to water and are supplied with food for approximately 6–8 hours per day.

Prior to the start of the study, animals were monitored for individual baseline food intakes of a purified diet (Teklad #85387 [lactalbumin based, 10% fat, 15% protein], Madison, WI) for 3 months. Animals were then randomized to either Control (C, n = 15) or Calorie Restricted (R, n = 15) groups based on their baseline food intakes. Food allotments for R animals were then reduced by 10% per month for 3 months to reach the intended 30% DR. C animals were fed 20% more than their average daily intake to assure ad libitum access to food for 6–8 hours per day. R animals were fed a diet (Teklad #93131, Madison, WI) enriched by 30% in vitamins and minerals to prevent malnutrition. Detailed methods for daily food intake monitoring have been previously reported (9).

Animals are monitored at least twice daily for general health by animal care and research staff and semiannually by veterinary staff. Since the onset of the study in 1989, 13 animals have died (8C, 5R). The remaining animals are in relatively good health as evidenced by normal complete blood count and serum chemistry values (with the exception of elevated triglyceride levels in the C animals). Additionally, some C animals began to show indications of altered glucose regulation (elevated basal insulin and glucose) by the 30-month assessment period (14), and this progressed to diabetes in two of the C animals. All animals had been included in the analysis until the time of their death or shortly before as detailed in the statistics section below. Data from years 6 through 17 of study were included in this analysis.

**Body Composition Analysis**

Dual energy X-ray absorptiometry (DXA) (Model DPX-L, GE/Lunar Corp., Madison, WI) was used to assess lean tissue mass of the total body and limbs and to estimate skeletal muscle mass. Following an overnight fast, animals were sedated with ketamine HCl (10 mg/kg, IM) and weighed. Animals were further administered a mixture of ketamine HCl and xylazine (7 mg/kg, 0.6 mg/kg xylazine, respectively, IM) for additional muscle relaxation and anesthesia maintenance. Upon scan completion, yohimbine (0.06 mg/kg, IV) was given to reverse xylazine. Approximate scan time was 20 minutes per animal.

Total body scans were acquired with the animal in the supine position and analyzed using Lunar pediatric software (version 1.5e for acquisition, version 4.0a for analysis) as previously described (13,15). Analyses were conducted on the total body and regions of interest (arms, legs). Regions of interest were defined based upon bony landmarks (i.e., upper and lower legs were divided at the interface of the femur and tibia). Estimated skeletal muscle mass (ESM) was determined by summing the lean tissue mass from the arms and legs. DXA coefficients of variation ([mean/standard deviation]/100) for sites evaluated in this study were as follows: total body lean mass 0.8%, ESM 1.6%, lean mass of legs 1.9%, lean mass of upper legs 3.7%, lean mass of lower legs 4.3%, and lean mass of arms 2.2% (12).

**Statistical Analyses**

Statistical comparisons of treatment groups across time were made by linear mixed models (LMM) using PROC MIXED in SAS (SAS Institute, Cary, NC; version 9.1). The model included a random intercept for each animal; the covariance structure was assumed to be autoregressive because of the serial nature of the multiple measures and was estimated using restricted maximum likelihood.

It is particularly important to assess whether lean mass differences observed between C and R animals were merely due to differences in body mass and age of the animal. To accomplish this, we used age and weight at the beginning of data collection as time-invariant covariates. Further, we used current weight at the time of the data collection to adjust for differences in mass in a manner that avoided the assumption of strict proportionality as is done when percent lean mass is expressed in ratio to total body weight. Data for animals that died during the course of the study were examined for outliers. The last few time points for five of these animals were excluded because of rapid loss of weight that we attributed to atrophy before death. After the exclusion of these data, analysis of the residuals indicated the normality assumptions of linear mixed models were tenable. In humans, physical activity is able to attenuate sarcopenia. Gross physical activity (Actiwatch, MiniMitter, Bend, OR) is not different between C and R monkeys and therefore does not explain our results.

**RESULTS**

As expected, body weight is lower in R compared to C animals (Figure 1A). For ESM, the results of the LMM showed that, after controlling for current weight and weight and age at the start of the experiment, the group by time interaction was statistically significant [F(22, 444) = 6.13, p < .0001]. That is, the treatment group differences in ESM varied significantly across time. Figure 1B shows that, through year 9, the two groups had approximately the same amount of adjusted ESM. At year 10, both groups of animals began to show a decline in adjusted ESM; however, the decline was much more rapid for the C animals. That is,
the R animals retained more ESM as they aged. Because the time by treatment group interaction was significant, treatment group comparisons at each time point were tested. With 22 dependent comparisons, a simulation-based adjusted alpha (16) was set at \( \alpha_{adj} = 0.006 \). These comparisons showed that there were no statistically significant differences in adjusted ESM until year 14 of the study. From year 14 to the end of the study, the R animals had significantly higher levels of adjusted ESM than the C animals.

**DISCUSSION**

We have shown that age-related loss of muscle mass, or sarcopenia, is delayed and possibly prevented in our rhesus monkey model of adult-onset, moderate DR. To our knowledge this is the first report of DR’s ability to maintain muscle mass in aging animals from a primate species. If translatable to humans, this result could have major positive effects on functionality and quality of life for elderly people, as well as on lowering healthcare expenditures.

The etiology of sarcopenia is not well understood and is most likely a multifactorial process. Many theories have been advanced including, but not limited to, changes at the cellular level such as a reduction in muscle cell number, decreased muscle twitch time and force, muscle fiber atrophy, and decreased satellite cell recruitment (17–20). Biochemical and metabolic changes in skeletal muscle related to mitochondrial DNA deletion mutations are also present with sarcopenia (21–23), and in rodents, these deletion mutations can be attenuated by DR (24). Additionally, central and peripheral nervous system innervation and decreased dietary protein intake are also likely to be involved in the progression of sarcopenia (17–20). More recent evidence points to the potential roles of apoptosis (25–31) and inflammation (18,19,32) in the etiology of sarcopenia.

The plethora of endocrine changes that occur during the aging process are also likely involved in the development and progression of sarcopenia. For example, several studies have shown that replacement of testosterone to young normal levels resulted in modest increases in muscle mass and muscle strength (33–36); however, not all studies report a positive effect of testosterone supplementation (37). Additionally, insulin has been shown to mediate muscle protein synthesis (38,39), and in the preinsulin era, diabetes was associated with severe muscle wasting.

The results for the rhesus monkey presented in this article are similar to previous findings in a rodent model. In the long-lived Fischer X Brown Norway F1 hybrid rat, ad libitum-fed animals lost significant muscle mass (dissected mass of vastus lateralis, rectus femoris, and soleus) between 21 and 36 months of age, and this loss was retarded but not prevented by DR (24). McKiernan and colleagues further found that significant muscle fiber loss and atrophy of type II muscle fibers contributed to sarcopenia in the aged ad libitum-fed animals, and that DR animals conserved muscle fiber number with advancing age; however, DR did not prevent type II muscle fiber atrophy (24).

Our results are from a noninvasive imaging technique that is not able to address issues of muscle fiber loss or atrophy. We are currently conducting studies that will address the effects of DR at the level of the individual muscle fiber. However, given our previous findings of sarcopenia in ad libitum-fed rhesus monkeys (12), both by in vivo DXA and ex vivo muscle mass, we are confident that results from the muscle fiber analysis will be consistent with the DXA findings presented here. Additionally, although there appears to be a clear relationship between muscle mass and muscle strength that further translates into functionality, techniques to test muscle strength in rhesus monkeys have yet to be developed. We continue to explore potential methods for this assessment. Despite these limitations, we have presented strong evidence of a beneficial effect of DR on a natural and universal process of physiological deterioration. These findings have significant implications for human health, quality of life, and healthcare expenditures.

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**REFERENCES**

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