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Caloric restriction delays aging-induced cellular phenotypes in rhesus monkey skeletal muscle

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ABSTRACT

Sarcopenia is the age-related loss of skeletal muscle mass and function and is characterized by a reduction in muscle mass and fiber cross-sectional area, alterations in muscle fiber type and mitochondrial functional changes. In rhesus monkeys, calorie restriction (CR) without malnutrition improves survival and delays the onset of age-associated diseases and disorders including sarcopenia. We present a longitudinal study on the impact of CR on early stage sarcopenia in the upper leg of monkeys from ~16 years to ~22 years of age. Using dual-energy X-ray absorptiometry we show that CR delayed the development of maximum muscle mass and, unlike Control animals, muscle mass of the upper leg was preserved in CR animals during early phase sarcopenia. Histochemical analyses of vastus lateralis muscle biopsies revealed that CR opposed age-related changes in the proportion of Type II muscle fibers and fiber cross-sectional area. In contrast the number of muscle fibers with mitochondrial electron transport system enzyme abnormalities (ETS^{ab}) was not significantly affected by CR. Laser capture microdissection of ETS^{ab} fibers and subsequent PCR analysis of the mitochondrial DNA revealed large deletion mutations in fibers with abnormal mitochondrial enzyme activities. CR did not prevent stochastic mitochondrial deletion mutations in muscle fibers but CR may have contributed to the maintenance of affected fibers.

1. Introduction

Calorie restriction (CR) is the only dietary regimen that slows aging and extends lifespan in diverse species. The beneficial effects of CR extend to primates with improved health span and survival outcome for rhesus monkeys (Colman et al., 2009). In humans, CR improves indicators of cardiovascular health (Holloszy and Fontana, 2007) and, although controlled short-term CR studies have been conducted (Redman et al., 2008), analysis of its long-term effects on aging muscle at the cellular level has not been conducted. The rhesus monkey closely models human aging in many respects with the advantage of a rate of aging ~3 times that of humans (Colman et al., 2005). We have undertaken a longitudinal study of aging and the impact of CR in male rhesus monkeys (*Macaca mulatta*). One goal of this work is to determine the effects of CR on sarcopenia, the process of skeletal muscle aging. We have previously reported that CR preserves total muscle mass in rhesus monkeys (Colman et al., 2008). Herein we present the impact of CR on the early stages of sarcopenia through longitudinal examination of intact upper leg musculature using dual-energy X-ray absorptiometry (DXA) and at the cellular level using vastus lateralis (VL) biopsies.

In humans, sarcopenia has a significant impact on daily living for ~45% of adults over 60 years of age (Janssen et al., 2004) and is associated with both muscle fiber atrophy and fiber loss. Muscle fiber types are grouped based on dominant myosin isoform expression and metabolism, factors that contribute to the contractile properties of individual fibers. The impact of age is not equivalent among fiber types; unlike the slow-twitch myosin Type I fibers, the fast-twitch glycolytic myosin Type II fibers are vulnerable to age-associated atrophy and loss (Lexell et al., 1988). These negative phenotypes of aging skeletal muscle are conserved in the rhesus monkey (McKiernan et al., 2009).

Abbreviations: CR, calorie restriction; DXA, dual-energy X-ray absorptiometry; ETS^{ab}, electron transport system enzyme abnormalities; VL, vastus lateralis; mtDNA, mitochondrial DNA; COX, cytochrome c oxidase; SDH, succinate dehydrogenase; CSA, cross-sectional area; ESM, estimated skeletal muscle mass; LCM, laser capture microdissection.

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A proposed mechanism of muscle fiber loss involves age-dependent changes in mitochondrial DNA (Dirks et al., 2006; McKenzie et al., 2002). The mitochondrion is unique among cellular organelles in that it contains its own genome, a 16 kb double stranded circular DNA molecule encoding 22 tRNA and 13 polypeptides of the electron transport system (ETS). The activity of Complex IV of the ETS resides with the multimeric enzyme cytochrome c oxidase (COX) containing both nuclear and mitochondrial encoded subunits. MtDNA is susceptible to age-dependent mutations, including large deletion mutations that remove one or all of the COX subunits encoded by the mitochondrial genome (Gokey et al., 2004). Characteristic phenotypes of muscle cells that have a high concentration of mutant mitochondria are the absence of COX activity and an over-abundance of succinate dehydrogenase (SDH) activity. In rodents, ETS abnormal (ETS^{ab}) fibers often become atrophic within the region of the abnormality and some affected fibers break (Herbst et al., 2007), suggesting a possible mechanism for permanent fiber loss.

Lean mass loss in humans is estimated at 1–2% per year after the age of 50 (Hughes et al., 2001; Thomas, 2007). Rhesus monkeys begin to undergo muscle mass loss at ~16 years of age (Colman et al., 2005). As previously reported, age-dependent changes in muscle fibers of the vastus lateralis (VL) in monkeys between the ages of 16- and 22-years include decreases in the number of Type II muscle fibers, decreases in Type II muscle fiber cross-sectional area (CSA) and an increase in the number of ETS^{ab} muscle fibers (McKiernan et al., 2009). In the present study, we determine the impact of CR on the cellular phenotypes of early stage sarcopenia from 11 monkeys assessed at 3 year intervals; 6, 9 and 12 years from initiation of the CR diet and compare these results with those observed in Control monkeys.

2. Materials and methods

2.1. Animals and diet

The CR monkeys are part of an ongoing longitudinal study at the Wisconsin National Primate Research Center (WNPRC) (Colman et al., 2009; Kemnitz et al., 1993; Ramsey et al., 2000). The median life expectancy of rhesus monkeys in captivity is ~26 years with some of the monkeys in this colony living into their late 30s (Gresl et al., 2001). The animals at year 6 of the study had an average age of 15.8 y (15 to 21 y) and at the end, 21.8 y (21 to 27 y) representing their late middle years and into early old age. We have previously reported on the age-related changes in muscle from Control animals in this cohort (McKiernan et al., 2009) and, herein, report findings in the CR animals.

Briefly, 30 males, between 8- and 14-years of age, were monitored for baseline food intake and then randomized to either a Control (C, n = 15) or Calorie Restricted (CR, n = 15) diet. Food allotments for CR animals (Teklad diet 93131, enriched by 30% in vitamins and minerals) were reduced 10% per month for 3 months to reach a 30% CR. Control animals were fed ~20 g more than their average daily intake to assure *ad libitum* access to food (purified lactalbumin based diet containing 10% fat and 15% protein [Teklad #85387, Madison, WI]). VL biopsies were collected at time points 6-, 9-, and 12-years after introduction of the CR diet. Over the course of this study, 4 CR monkeys died and we report data from 11 of the remaining CR monkeys. All animal procedures were performed at the WNPRC under approved protocols from the Institutional Animal Care and Use Committee of the Graduate School of the University of Wisconsin, Madison.

2.2. Body composition

Body weight of each animal was assessed throughout the study. Appendicular lean mass and fat mass were assessed biannually using whole body DXA (Model DXP-L, GE/Lunar Corp., Madison, WI) scans as previously described (Colman et al., 2008). Estimated skeletal muscle mass (ESM) of the upper leg was determined by summing the lean mass from the thigh region of both limbs. Muscle mass loss for each individual animal was determined by dividing the upper leg lean mass at each time point by the maximum upper leg lean mass measured for that animal in the 12 years of the study. Fat mass, determined by DXA measurements, was used to calculate percent body fat (%BF = [fat mass/body weight] × 100).

2.3. Biopsy collection

Six, nine and twelve years post-initiation of the study, VL biopsies were performed immediately following DXA, alternating legs with successive biopsies. Biopsy tissue was bisected with one half of the sample flash frozen in liquid nitrogen and the other embedded in Optimal Cutting Temperature Medium (OCT, Sakura Inc., Torrance, CA) and frozen in liquid nitrogen. Samples were stored at −80 °C until use. Frozen muscle biopsies were sectioned using a cryostat. For each biopsy, 200 consecutive 10 μm-thick sections were cut and stored at −80 °C.

2.4. Histochemistry

Slide sections were stained with hematoxylin and eosin (Sheehan and Hrapchak, 1980) for muscle morphology and muscle fiber counts. Muscle fiber types were identified using standard immunohistochemical detection of the Type II isoform of myosin heavy chain (monoclonal antibody MY32, Sigma St. Louis, MO) followed by the 3,3′-Diaminobenzidine tetrachloride (DAB) reaction for visualization (Sheehan and Hrapchak, 1980). General muscle fiber atrophy was assessed using slides from the immunohistochemical analysis. Five 10× images per section were taken. The cross-sectional area (CSA) of Type I and Type II muscle fibers were measured using ImagePro. The CSA of at least 200 muscle fibers were measured for each type from each biopsy.

Histochemical staining for mitochondrial enzyme activities, COX and SDH were performed on air-dried sections according to Seligman et al. (1968) and Dubowitz (1985), respectively. Twenty-nine slide triplicates (the 2nd, 3rd and 4th, the 9th, 10th and 11th, etc.) from the 200 slide sections from each biopsy were stained for COX, SDH and both COX and SDH enzyme activity staining. Unique ETS^{ab} fibers were identified for each muscle sample and the percentage of ETS^{ab} fibers determined from the total number of cell present. To measure atrophy specifically associated with ETS^{ab} regions, fibers were followed along their length and the cross-sectional area was measured at 70 μm intervals. Cross-sectional area ratios were determined for 54 ETS^{ab} fibers (and 55 normal fibers) where the minimum CSA of the ETS abnormal region (or the minimum CSA of the normal fiber) was divided by the mean CSA of the normal region to give a minimum CSA ratio. Abnormal fibers with CSA ratios ± 2 standard deviations from the distribution of normal fiber CSA ratios were defined as atrophic or hypertrophic. The length of the ETS abnormality within a fiber was also measured.

2.5. Laser capture microdissection and Mt DNA amplification

Laser capture microdissection (LCM) was used to isolate 10 μm thick sections of ETS^{ab} and normal muscle fibers to determine the mitochondrial genotype. Frozen sections adjacent to those used for identification of ETS^{ab} phenotypes were stained for SDH activity, dehydrated in ethanol and cleared in xylenes. Muscle fibers of interest were microdissected using a PixCell II laser capture microscope (Arcturus Bioscience, Inc., Mountainview, CA, USA) as previously described (Gokey et al., 2004). Total DNA was extracted, primary PCR reactions were performed using mtDNA primers to amplify ~14,400 bp of the ~16,000 bp rhesus mitochondrial genome (1176 F:725R), followed by a nested amplification (3499 F:16245R). Specifics of techniques and sequencing of PCR products are as described before (McKiernan et al., 2009; Gokey et al., 2004).