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Cellular adaptation contributes to calorie restriction-induced preservation of skeletal muscle in aged rhesus monkeys

Susan H. McKiernan [a,b,*], Ricki J. Colman [a], Erik Aiken [b], Trent D. Evans [c], T. Mark Beasley [d], Judd M. Aiken [e], Richard Weindruch [c,f], Rozalyn M. Anderson [c,f,*]

[a] Wisconsin National Primate Research Center, University of Wisconsin, Madison, WI 53715, United States
[b] Department of Kinesiology, University of Wisconsin, Madison, WI 53706, United States
[c] Department of Medicine, School of Medicine and Public Health, University of Wisconsin, Madison, WI 53706, United States
[d] Department of Biostatistics, University of Alabama, Birmingham, AL 35294, United States
[e] Department of Agriculture, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2M8
[f] Geriatric Research, Education and Clinical Center, William S. Middleton Memorial Veterans Hospital, University of Wisconsin, Madison, WI 53705, United States

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ABSTRACT

We have previously shown that a 30% reduced calorie intake diet delayed the onset of muscle mass loss in adult monkeys between ~16 and ~22 years of age and prevented multiple cellular phenotypes of aging. In the present study we show the impact of long term (~17 years) calorie restriction (CR) on muscle aging in very old monkeys (27–33 yrs) compared to age-matched Control monkeys fed *ad libitum*, and describe these data in the context of the whole longitudinal study. Muscle mass was preserved in very old calorie restricted (CR) monkeys compared to age-matched Controls. Immunohistochemical analysis revealed an age-associated increase in the proportion of Type I fibers in the VL from Control animals that was prevented with CR. The cross sectional area (CSA) of Type II fibers was reduced in old CR animals compared to earlier time points (16–22 years of age); however, the total loss in CSA was only 15% in CR animals compared to 36% in old Controls at ~27 years of age. Atrophy was not detected in Type I fibers from either group. Notably, Type I fiber CSA was ~1.6 fold greater in VL from CR animals compared to Control animals at ~27 years of age. The frequency of VL muscle fibers with defects in mitochondrial electron transport system enzymes (ETS^{ab}), the absence of cytochrome c oxidase and hyper-reactive succinate dehydrogenase, were identical between Control and CR. We describe changes in ETS^{ab} fiber CSA and determined that CR fibers respond differently to the challenge of mitochondrial deficiency. Fiber counts of intact *rectus femoris* muscles revealed that muscle fiber density was preserved in old CR animals. We suggest that muscle fibers from CR animals are better poised to endure and adapt to changes in muscle mass than those of Control animals.

1. Introduction

Sarcopenia is one of a number of geriatric syndromes that contributes to morbidity in the aged (Cruz-Jentoft et al., 2010). While not directly responsible for mortality, grave impairment to health and well-being is associated with significant muscle mass loss. Sarcopenia is simply defined as the age-related loss of skeletal muscle mass and size. A refined definition of sarcopenia has developed over the years to include the loss of strength and/or function (Rosenberg, 1997; Morley et al., 2001; Roubenoff, 2001). Recent studies in mice and humans have pointed to the importance of skeletal muscle in overall metabolic homeostasis including glucoregulatory function (Lira et al., 2010). This raises the possibility that sarcopenia has a systemic impact and places renewed emphasis on understanding the factors that contribute to it and how to prevent it.

Calorie restriction (CR), the reduction of caloric intake without malnutrition, delays aging in diverse species and prevents the onset of numerous age-associated pathologies (McCay et al., 1935; Weindruch and Walford, 1982). CR improves survival and reduces morbidity in rhesus monkeys (*Macaca mulatta*), demonstrating that CR's anti-aging effect is conserved in primates (Colman et al., 2008). Rhesus monkeys have a lifespan of several decades and share many human characteristics including the spectrum of age-associated diseases, the onset of sarcopenia in middle age (~16 yrs for rhesus

Abbreviations: CR, calorie restriction; VL, vastus lateralis; RF, rectus femoris; DEXA, dual energy X-ray absorptiometry; ESM, estimated skeletal muscle mass; CSA, cross-sectional area; COX, cytochrome c oxidase; SDH, succinate dehydrogenase; mtDNA, mitochondrial DNA; ETS^{ab}, electron transport system enzyme abnormalities where COX is absent and SDH hyper-reactive in muscle fibers.

* Corresponding author at: University of Wisconsin, Dept, Kinesiology, 2000 Observatory Drive, Madison, WI 53706, United States. Tel.: +1 608 365 0470.
E-mail addresses: shmckier@wisc.edu (S.H. McKiernan), rmanderson5@wisc.edu (R.M. Anderson).

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monkeys) and the trajectory of muscle mass decline. Importantly, CR significantly reduces sarcopenia in rhesus monkeys (Colman et al., 2005) and delays aging-induced cellular phenotypes in skeletal muscle in adult animals 16–22 yrs of age (McKiernan et al., 2011).

The constituent fibers of skeletal muscle are heterogeneous in terms of metabolism and functional capacity (Bassel-Duby and Olson, 2006). Type I fibers are more reliant on oxidative metabolism and express a specialized isoform of the structural protein myosin that is associated with endurance. Type II fibers, also known as "fast twitch", tend to rely less on oxidative metabolism and express distinct myosin isoforms that are associated with greater contractile force and velocity. We have previously shown fiber type distribution shifts with age in rhesus monkey *vastus lateralis* (VL), where the proportion of Type I fibers is higher in muscle from ~22 year old Control (fed an *ad libitum* diet) monkeys (McKiernan et al., 2009) compared to muscle from ~22 year old CR (caloric intake restricted by 30% for ~12 years) monkeys (McKiernan et al., 2011). This shift was not simply due to the loss of Type II fibers, but an actual increase in the absolute number of Type I fibers (McKiernan et al., 2004). In addition, cross sectional area of Type II fibers declined with age, coincident with the decline in estimated muscle mass (McKiernan et al., 2009). In CR animals Type II fiber atrophy was not observed at ~22 years of age (McKiernan et al., 2011).

The mitochondrion is unique among cellular organelles in that it carries genomic material encoding several genes essential to oxidative phosphorylation. Mitochondrial DNA (mtDNA) deletion mutations occur stochastically in aging muscle tissue, but once established the dysfunctional mitochondria replicate and eventually expand intracellularly along a muscle fiber (Cao et al., 2001; Gokey et al., 2004). These deletion mutations lead to defects in the activities of Electron Transport System enzymes (ETS^{ab}) which can be detected histologically (Herbst et al., 2007). The age-dependent accumulation of mitochondrial deletion mutations in skeletal muscle of the rat contributes to muscle fiber loss and is linked to dysfunctional cellular phenotypes and muscle fiber atrophy, breakage and loss (Herbst et al., 2007).

The extent to which the beneficial effects of CR on sarcopenia are still observed in old age is unknown. The average lifespan for rhesus monkeys in captivity is about 27 yrs (Colman et al., 1998). In this study we investigated the impact of age and diet on the remaining aged animals from our longitudinal study. The monkeys in this now smaller cohort were on average 27 years old for both the Control and CR groups. CR animals had been on the 30% reduced calorie diet for an average of 17 years. At the whole body level we determined body weight, percent body fat and muscle mass of the upper legs. Cellular aspects of muscle aging were measured in biopsy sample of the VL muscle from both Control and CR monkeys. Age and diet associated changes in muscle composition were determined, including the relative proportion of Type I and Type II fibers, and the degree of fibrotic infiltration. To measure the extent of muscle fiber atrophy, Type I and Type II fiber cross-sectional area (CSA) were measured. The percentage of fibers that presented abnormal mitochondrial enzyme activities were determined and further analyzed for localized fiber atrophy. To determine the impact of age and diet on muscle fiber loss we used whole *rectus femoris* (RF). The RF has a similar fiber type composition to VL. Finally, we conducted statistical analyses of data collected over the entire longitudinal study. This included previously published data from the same animals at ~16 years, ~18 years and ~22 years of age (McKiernan et al., 2009, 2011), as well as the ~27 year data reported here.

We show that CR continues to confer a positive impact on rhesus monkey skeletal muscle in old animals of ~27 yrs. Fiber loss and fiber atrophy are attenuated in CR animals compared to age-matched Controls. The impact of both age and CR is fiber type specific. Type I fibers significantly increase in cross-sectional area in aging CR animals, an increase that may offset the eventual atrophy of Type II fibers. The incidence of fibers with ETS^{ab} was the same between diet groups, however, ETS^{ab} fibers from CR monkeys were resistant to atrophy. Our data suggest muscle fibers from old Control and CR animals are distinct, and that adaptation of Type I muscle fibers plays a role in conserving skeletal muscle mass.

2. Materials and methods

2.1. Animals and diets

All animal procedures were performed at the Wisconsin National Primate Research Center (WNPRC) under approved protocols from the Institutional Animal Care and Use Committee of the Graduate School of the University of Wisconsin, Madison. This work is part of an ongoing longitudinal study on aging and CR in non-human primates at the WNPRC (Colman et al., 2008; Kemnitz et al., 1993; Ramsey et al., 2000). Briefly, 30 male rhesus monkeys (*M. mulatta*) between 8- and 14-years of age, were monitored for baseline food intake and randomly assigned to either Control (n = 15) or CR (n = 15) diets. Control animals were provided *ad libitum* access to food (purified lactalbumin based diet containing 10% fat and 15% protein [Teklad #85387, Madison, WI]). For CR animals (Teklad diet #93131, enriched by 30% in vitamins and minerals), a 30% restriction was applied in 10% increments over a 3-month period at the outset of the study.

Although the study began with 15 monkeys in the Control group and 15 in the CR group, here we report data from nine Control and 11 CR monkeys with average age of ~27ys for both groups (26–33 yrs) and average time on the study of 17 yrs. Numerical values calculated for the longitudinal analyses reported here were not equivalent to our previous reports (McKiernan et al., 2009, 2011) in that data shown here includes these current animals only and is presented in years of age and not years on the study. Five of the Control monkeys died between 2003 and 2008. Age and cause of death were: 30.13 y — peritonitis/septicemia; 25.93 y — accidental; 26.11 y renal and cardiac disease; 25.91 y — adenocarcinoma; 27.66y — adenocarcinoma. Of the 11 CR monkeys two died; 25.76 y — disseminated intravascular coagulation and 25.92y — peritonitis. Age, length of time in the study, as well as whole body and muscle characteristics of these animals were consistent with the live monkeys in the study.

2.2. Body composition

Body weight, appendicular lean mass and fat mass were measured biannually using whole body dual energy X-ray absorptiometry (DEXA) (Model DXP-L, GE/Lunar Corp., Madison, WI). Estimated skeletal muscle mass (ESM) of the upper leg was determined by summing the lean mass from the thigh region of both limbs. Upper leg muscle mass for each individual animal was determined and expressed as a proportion of its upper leg lean mass at ~27 years of age compared to its maximum upper leg lean mass attained during the study. On average, maximum upper leg muscle mass was realized at 16 years of age for the Control monkeys and at 18 years of age for the CR monkeys [8]. DEXA measurement of fat mass was used to calculate percent body fat (%BF = [fat mass/body weight] × 100).

2.3. Tissue collection and processing

Upper leg skeletal muscle biopsies of the *vastus lateralis* (VL) were collected at year 19 of the study. The specimen was bisected and one half was flash frozen in liquid nitrogen. The other half was 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.