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Myosin Heavy Chain Isoform Expression in the *Vastus Lateralis* Muscle of Aging African Green Vervet Monkeys

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Abstract

Non-human primates (NHP) represent an emerging animal model for the study of physical function, and provide opportunities for exploration of relationships of muscle biomolecular changes with age. One such primate model, the African green vervet monkey, has been used extensively in biomedical research but little is known regarding skeletal muscle composition, expression of myosin heavy chain (MHC) isoforms, and changes with age. In the present study we examined the effects of age on *vastus lateralis* (VL) muscle fiber-type composition, fiber cross-sectional area (CSA), and MHC isoforms expressed in 4 young and 4 older adult vervet monkeys. Proteomics analysis, using a human and nonhuman primate protein database, showed five MHC isoforms (I, IIA, IIX, IIB, and IIB′) expressed in female vervet VL muscle, which matched the human MHC isoforms. Fast type II fibers predominated and no pure type IIB or IIB′ containing fibers were detected. Hybrid fibers containing IIB/IIB′ MHC decreased in the old vervets. The CSA of both type I and type II fibers was significantly smaller in older vervet while type IIA fibers showed the most severity of atrophy. The decrease of fast MHC and atrophy of muscle fiber with aging recapitulate observations in human VL muscle. These findings, along with its homology of MHC between the vervet and human suggested that the vervet monkey may be a suitable preclinical model for understanding the cellular and molecular basis of sarcopenia and for developing new interventions to ameliorate the impact of disorders that affect skeletal muscle structure and function.

Keywords

myosin heavy chain; aging; non-human primate; vervet

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1. Introduction

Skeletal muscle atrophy and decline in force with aging, termed *sarcopenia*, limit daily living activities and contribute to morbidity and mortality in older adults (Faulkner and others 2007; Marzetti and Leeuwenburgh 2006). Many cellular and molecular factors contribute to skeletal muscle dysfunction with aging (Delbono 2003; Dutta and Hadley 1995) and vary widely based on genetic background, nutritional status, environmental factors, and physical activity, among others (Dennis and others 2008; Walker and others 2011; Welle and others 2003; Zhang and others 1998). As their genomes are similar to humans', nonhuman primates (NHP) would seem an excellent preclinical model for examining the mechanisms of aging tissue and interventions aimed at ameliorating detrimental effects on skeletal muscle function and physical performance. Further, under the same dietary and habitat conditions, they would present fewer potential confounding factors than typical human research studies. Emerging data suggests that nonhuman primates experience alterations in physical function with age, such that while overall activity levels are similar, walking speed and the amount of time spent leaping and jumping are considerably reduced in older animals (Shively and others 2011).

Relatively little is known regarding NHP skeletal muscle myosin heavy chain (MHC) composition and how it changes with aging. One study examined the effects of microgravity on single muscle fiber function in young rhesus monkeys (Fitts and others 2000), and two reported the influence of dietary interventions on gene transcription in aging rhesus monkeys (Kayo and others 2001; McKiernan and others 2011). The vervet monkey represents a very useful NHP model for biomedical research (Freimer and others 2007; Jasinska and others 2007), and their use in translational research as an alternative to the rhesus macaque has grown over the past ten years (Bacopoulos and others 1980; Palmour and others 1997). Nonetheless, their muscle fiber-type composition, expression of specific MHC isoforms, and the effects of age have not been reported. The present study was designed to assess the impact of age on the biomolecular characteristics of vervet muscle to provide a foundation for future studies on aging of the neuromuscular system.

Our research focused on the *vastus lateralis* (VL) muscle, the most widely used muscle in human research due to its superficial location, which facilitates surgical dissection or needle biopsy to obtain samples for in vitro studies (Kohn and Myburgh 2006; Staron and others 2000). The extensive knowledge regarding fiber- type composition of the human VL (Larsson 1978; Larsson and others 1978) provides an excellent reference for assessment of vervet monkey VL MHC composition, and cross-comparisions between the two will provide a useful translational reference base for future studies of the mechanisms underlying human sarcopenia. We quantified and compared muscle fiber-type composition, myofiber CSA, and MHC isoforms expressed in single VL fibers from young (8–11 year-old) and old (21–26 year-old) female vervet monkeys. Muscle was examined using immunohistochemistry, biochemistry, and mass spectrometry based proteomics analysis, allowing in depth assessment of the effects of age on the VL muscle of vervet monkeys. The results suggest that age related changes in vervet VL muscle are not only present but are similar to that observed in human, suggesting these NHP may be useful as a preclinical model of sarcopenia in biomedical research.

2. Methods

2.1. Animals

Skeletal muscle was collected from 8 female African green vervet monkeys (*Chlorocebus aethiops sabaeus*), 4 young (8–11 years, corresponding to humans in their 30s) and 4 old (21–26 years, corresponding to humans in their 70s and older), at the Wake Forest

University Primate Center. All had free access to standard commercial monkey chow (Purina LabDiet) and water. For inclusion in this study, animals had to be in good health, with normal posture and locomotion patterns, and not known to be pregnant or to have dependents younger than 9 months. All procedures involving monkeys were conducted in accordance with state and federal laws, Department of Health and Human Services standards, and guidelines established by the institutional Animal Care and Use Committee.

2.2. Skeletal Muscle Preparation

A cylindrical piece of the vastus lateralis (VL) muscle (5mm long and 5mm thick) was dissected immediately after monkeys were euthanized. To minimize intramuscular variability, all samples were obtained from the middle region of the right superficial VL. It was rinsed in a 4°C relaxing solution containing 7.0 mM EGTA, 14.5 mM creatine phosphate, 20.0 mM imidazole, 4 mM Mg^{2+}-ATP, and 1 mM free Mg^{2+} to avoid muscle contracture, and small longitudinal bundles containing approximately 30–50 fibers were dissected. Each bundle was transferred to a skinning solution containing 50% glycerol and 50% relaxing solution at 4°C for 24 hours and subsequently stored at −20°C before single fiber isolation. Another cylindrical piece of VL muscle (10mm long and 5mm thick), adjacent to the previous area, was embedded in OCT (Tissue-Tek, Torrance, CA), quickly frozen in liquid nitrogen, and stored at −80°C for ATPase staining.

2.3. ATPase Staining

Frozen muscle sections (12 μm) were mounted on glass slides for ATPase staining as described (Round and others 1980). Briefly, slides were incubated in a 0.01 M ATP solution containing 0.1 M glycine/NaCl with 0.75 M $CaCl_2$, pH 10.2, at 37°C for 15 min and then in 2% $CoCl_2$ for 5 min. Color was developed in ammonium sulfide solution (1:50) for 30 seconds. Slides were mounted on glycerin jelly, observed under a microscope, and imaged using TSView 7.1 (Tucsen Imaging Technology, Elmsford, NY). Images of multiple muscle fields were taken at 40X magnification and composed as a single cross-section using Adobe Photoshop CS3 (New York, NY). Number and cross-sectional area (CSA) were analyzed in at least 100 fibers of each category per muscle visualized at 100x magnification.

2.4. Single Muscle Fiber MHC Composition

MHC isoforms were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Single fibers were dissected from muscle bundles, exposed to sampling buffer containing 62.5 mM Tris, 2% SDS, 10% glycerol, 5%-mercaptoethanol, and 0.001% bromophenol blue, boiled for 10 min to reduce and denature the proteins, centrifuged, and the supernatant was frozen at −80°C. SDS-PAGE for MHC detection was done as described (Choi and others 2011; Wu and others 2000). Gel slabs (0.75 mm thick) consisted of 8% separating gel and 4% stacking gel (49:1 acrylamide-bisacrylamide). All gels were made from the same stock solutions, and all chemicals were of electrophoresis grade. Electrophoresis was performed using a vertical slab gel unit (Bio-Rad Mini-Protean Tetra II, Bio-Rad Laboratories) run at 140 V for 7.5 h at 4°C. Separating gels were silver-stained to visualize the MHC bands (Shevchenko and others 1996) using the Silver Stain Plus Kit (Bio-Rad Laboratories). Vervet skeletal muscle MHC standards were prepared from pooled VL biopsy samples and run on each gel to verify separation of all MHC isoforms. Images of silver-stained gels were obtained using an AGFA Duoscan HiD scanner (AGFA Corporation, Ridgefield Park, NJ).

2.5. Mass Spectrometry Analysis of Myosin Heavy Chain

SDS-PAGE gel bands presumably containing myosin proteins, as described above (2.4), were cut, washed, and destained by adding 30 mM potassium ferricyanide and 100 mM

sodium thiosulfate, following a p rotocol previously described (Gharahdaghi and others 1999). In-gel trypsin digestion was performed according to standard protocols. The resulting tryptic peptides were further separated and analyzed by nano-LC (Dionex Ultimate 3000 System) coupled to a Thermo electrospray ionization linear trap quadrupole (ESI LTQ) mass spectrometer. A typical gradient was run for 30 min from 0 to 100% solvent (80% ACN, 20% H_2O, and 0.1% formic acid). Solvent A consisted of 5% ACN, 95% H_2O, and 0.1% formic acid. The flow rate was set at 200 nL/min on a PepMap 100, C18 (Dionex; catalog no.160321). The ESI LTQ mass spectrometer was operated in data-dependent mode. Peptides were identified using the Mascot search engine and the UniprotKB protein database (human and nonhuman primate taxonomies) in Protein Discoverer 1.2. The results were filtered to maintain a protein false discovery rate (FDR) of <1%.

2.6. Data Analysis

Variables are expressed as mean ± standard deviation (SD). Data were analyzed using two-way ANOVA. Post-hoc analysis was performed using a t-test with the Bonferroni adjustment method. The alpha level was set at $p = 0.05$.

3. Results

3.1. Muscle Fiber-type Composition of Vervet Monkeys

Muscle fiber-type composition was examined in VL muscle cross-sections from 3 young and 3 old monkeys. The ATPase staining method at pH 10.2 allowed us to differentiate three muscle fiber types, type I (light), type IIA (intermediate), and type IIX/B (dark) (Figures 1A &B), similar to the fiber identification described in neck muscles of rhesus monkeys (Richmond and others 2001). Figure 1C shows the percent of fiber types represented in the vervet VL muscle. The analysis of 1,200 fibers from each monkey showed that type IIA fibers predominate followed by IIX/B and I ($p < 0.05$) in both young and old monkeys. No statistically significant difference between proportions of fiber types in young and old monkeys was observed.

3.2. Muscle Fiber Cross-sectional Area

Table 1 shows the CSA of type I, IIX/B, and IIA fibers analyzed in the VL muscle of the vervet monkeys. In both young and old monkeys, type IIA fibers exhibit the largest CSA; type I the smallest CSA; while type IIX/B was intermediate. The CSA of the three fiber subtypes, type I, IIX/B, and IIA, was, respectively, 30%, 31%, and 38% smaller in the old compared to the young monkeys ($p < 0.05$).

3.3. Identification of Myosin Heavy Chain Isoforms by Mass Spectrometry

Five MHC bands were detected in pools of VL samples using SDS-PAGE and silver staining (Figure 2A, lane a). Bands 1 and 2 and 3–5 were cut separately for protein identification using mass spectrometry. Table 2 shows various MHC isoforms detected from these five bands in the UniprotKB protein database. The literature commonly terms myosin-1, -2, -4, and -7 as *MHC IIX, IIA, IIB, and I* (Richmond and others 2001; Tonge and others 2010; Weiss and Leinwand 1996). Figure 3 shows examples of MS/MS spectra for unique peptides identified in each of these proteins. Based on the FDR filtering and the protein sequence coverage (Table 2) the top two bands correspond to MHC IIA and IIX. Myosin-4 was detected in bands 3 and 4 in the silver-stained gel, which suggests that vervet monkey VL muscle has two IIB isoforms. The bottom band 5 corresponds to MHC I.

3.4. Single Fiber-Type Distribution According to Myosin Heavy Chain Composition

As type IIA and IIX bands run very near each other in the gel, quantifying them separately was difficult, and to avoid any bias, we combined them. SDS-PAGE/silver staining was used to examine 498 and 456 single muscle fibers from 4 young and 4 old monkeys, respectively. MHCs were divided into five groups: pure type I (1), type IIA or IIX (2), a IIA +IIX hybrid (3), a IIA+IIX^{-} hybrid in which type IIA predominated (4), and others, including I+IIA, IIA+IIB, IIA+IIX+IIB, I+IIA+IIX+IIB, IIA+IIX+IIB′ (5). Figures 2Ad–i show various hybrids detected in the single muscle fibers. Figure 2B shows the relative quantities of pure and hybrid MHC in the fibers analyzed from young and old monkeys. Fibers expressing pure type I and type IIA or IIX and type IIA+IIX hybrids were observed more frequently in old (7.0%, 42%, 16%) than young (3.0%, 29%, 11%) monkeys. Hybrid IIA+IIX^{-} and other hybrid fibers were found less frequently in old (26% and 9%, respectively) than young (29%, 29%) monkeys. Hybrid fibers containing type IIA+IIX+IIB or IIA+IIX+IIB′ were observed most frequently in the *other hybrid fibers* category in young monkeys.

4. Discussion

4.1. The Vervet Monkey as a Preclinical Model for Human Sarcopenia

To our knowledge, this study is the first to determine the effects of age on skeletal muscle fiber-type composition in non-human primates in general and in African Green vervet monkeys in particular. Muscle fiber-types and their physiological function have been studied in rhesus and other monkey strains (Acosta and Roy 1987; Petter and Jouffroy 1993; Rahnert and others 2010; Raleigh and others 1992; Smith and others 2006; Sokoloff and others 2007), but no data on MHC composition in aging subjects have been reported. Due to the critical shortage of rhesus monkeys for biomedical research, vervets have been recognized as an alternative; they are less expensive, exhibit fewer diseases (Everard and others 1991), are similar in behavior and physiology (Disotell 2000; Raleigh and others 1992; Ziegler and Bercovitch 1990), and about equally related genetically to humans (Page and others 1999; Page and Goodman 2001; Raaum and others 2005). The genetic similarity in MHC isoforms between vervet monkeys and human beings found in this study makes this model particularly relevant for examinations of the cellular and molecular basis of sarcopenia, facilitating its use as a preclinical model to test interventions aimed at ameliorating the impact of aging muscle deficits and muscle disorders. Rodents have been extensively used for aging studies due to their short life span and lower cost than larger mammals (Rice and others 2005; Wineinger and others 1995). However, the NHP model has significant advantages over rodents, including the homogeneous composition of the myosin isoform and other variables described in Table 3.

In addition, reports on muscle fiber-type composition associated with aging in human are inconsistent in part due to interpersonal differences in physical activity, nutritional status, pharmaceutical use, smoking, alcohol use, and genetic background (Walker and others 2011). The vervet monkeys used in this study were all females, eating the same diet, living in similar indoor and outdoor enclosures during development, and they were never exposed to alcohol or tobacco and not subjected to any physical training regimen. Thus, we could measure the effects of aging on muscle without many of the factors that commonly mar human research.

4.2 Vervet Monkey Muscle Fiber Composition

Specific fiber subtypes are not uniformly distributed in most muscles (Petter and Jouffroy 1993). ATPase staining showed that type I fibers constitute 6–7% of VL muscle, which is similar to the ~11% reported in Cynomolgus monkeys (Acosta and Roy 1987). A whole

muscle cross-sectional study found less than 5% of this fiber subtype in the VL muscle of Microcebus-Murinus, a small NHP (Petter and Jouffroy 1993). In comparison, type I fiber comprises 20–50% of young adult human VL muscle (Andersen 2003; Staron and others 2000), a higher percentage than in vervets. However, type IIA is the predominant fast fiber in the VL muscle of vervets (~71%) and humans (~40%) (Andersen 2003; Staron and others 2000). In addition, 20% of vervet VL consists of type IIX or type IIB fibers, much less than in the small rodents frequently used in aging muscle studies. Type IIB represents 70% of all fibers in the VL of rats (Armstrong and Phelps 1984; Eng and others 2008) and 83% in mice (Allen and others 2001). A low proportion of type IIB was detected in the vervet VL muscle, and it was found only in combination with other MHC isoforms (see single fiber results). These results support the concept that vervet VL muscle composition is closer to humans' than rodents'.

4.3. Identification of Hybrid Myofibers in African Green Vervet Monkeys

Hybrid myofibers expressing two or more MHC isoforms represent stable phenotypes in normal skeletal muscle (Caiozzo and others 2003; Stephenson 2001). The current study found that 68% of fibers in the VL muscle of young vervet monkeys were hybrids, which is higher than the value reported in human (Andersen 2003; Staron and others 2000). Hybrids differ from pure fibers in their contractile properties and may play an important role in skeletal muscle diversity and plasticity. The relative proportion of hybrid fibers varies significantly from muscle to muscle among different species, possibly explaining differential adaptation to physical demands (Korfage and others 2005). The high proportion of hybrid fibers in the vervet VL muscle suggest a higher capacity for fine gradation in force and movement.

Type IIA MHC is the most frequently observed and present in almost all MHC combinations in hybrid fibers, consistent with our histochemical analysis. Pure type IIB or IIB′ has not been found in single muscle fibers, appearing only in combination with other MHC isoforms. Type IIB MHC was found in both young and old vervet muscles, but IIB′ was found only in the young. Whether these two "IIB" are the same MHC with different post-translational modifications or two different MHC isoforms sharing a protein sequence is unknown. Other techniques are expected to answer this question.

4.4. Effects of Aging on Fiber Composition in Vervet Monkeys

We did not detect any significant change in fiber type composition with aging in vervet monkeys. Similarly, studies in whole human muscle demonstrated that aging does not affect type I and type II fiber composition (Lexell 1995; Lexell and Downham 1991). In contrast, a switch from fast to slow fibers has been reported in aging rats (Larsson and others 1993). Conclusions from the present study are limited by the number of monkeys examined and the muscle sample size.

The histological technique used here does not distinguish type IIX/IIB from other hybrid fibers; single muscle fiber SDS-PAGE provides more information on MHC composition (Pette and others 1999). This technique showed a minor increase in type I MHC in old compared to young vervets (7.0% vs. 3.0%) but a 13% increase in pure fibers containing either IIA or IIX MHC in old vervets. Note that hybrid fibers comprised 68% and 51% of total fibers in young and old vervets, respectively. MHC IIA plus IIX was the most frequent combination, with almost equal proportions or IIA predominating. Hybrid fibers with equal IIA and IIX increased 5% with aging, while IIA+IIX^{-} fibers decreased 3%, which suggests an increase in type IIX MHC expression in VL muscle over time.

Other hybrid fibers, including I+IIA, IIA+IIB, IIA+IIX+IIB, I+IIA+IIX+IIB, and IIA+IIX +IIB′, were significantly reduced in old (9.0%) compared to young (28%) monkeys. Younger vervets have type IIB and/or IIB′ MHC, which are rarely detected in old monkeys. MHC isoform transformation has been proposed as a muscle fiber adaptation mechanism in the elderly (Andersen 2003; Stephenson 2001). We found that the proportion of type I and type II fibers in vervet VL muscle did not change dramatically with aging, while the distribution of type II MHC isoforms and hybrid populations did. Compared to ATPase histological staining, electrophoretic separation of MHC isoforms in single fibers achieved more detailed information on MHC expression in hybrid fibers.

4.5. Effects of Aging on Fiber Cross-sectional Area (CSA)

The VL fiber CSA in vervets was IIA>IIX/B>I, with type IIA having the largest. However, type IIA exhibits a larger CSA than IIX; IIA>I>IIX in men's and I>IIA>IIX in women's VL (Chalmers and Row 2011; Staron and others 2000). Type I fiber CSA is the largest in women but smallest in female vervets. However, in both humans and vervets, IIA is bigger than IIX; in mice and rats, IIX/B fibers usually have the largest CSA (Delp and Duan 1996; Sartorius and others 1998).

Decreased skeletal muscle mass and strength positively correlate with decreased muscle fiber type-specific CSA (Staron 1997; Verdijk and others 2010) particularly in type II fibers in humans (Lexell 1995). The current study found that the CSA of both type I and type II fibers decreased with aging in the vervet. The larger the fiber CSA in the young, the more severe the atrophy recorded with aging (38% atrophy in IIA, 31% in IIX/B and 30% in I). Old vervets' type I fiber but older humans' type II shows preferential atrophy (Aniansson and others 1986; Lexell and others 1988). However, most studies excluded women. One study including both sexes showed 25% atrophy in type I fiber compared to 57% atrophy in type II fibers (Andersen 2003). The vervet monkeys included in this study were all female. Note that one study showed that muscle fiber atrophy with aging was more severe in women than men (Coggan and others 1992). Further research is required to elucidate gender-related effects on muscle atrophy with aging in vervets. The decreased muscle fiber size in old monkeys may account for the whole muscle atrophy and associated loss of function. Unfortunately, the effects of aging on muscle function have not been reported for monkeys in general and African green vervet monkeys in particular.

5. Conclusion

We found five MHC isoforms matching human MHC isoforms in the VL muscle of African green vervet monkeys: I, IIA, IIX, IIB, and IIB′. Type IIA predominates, and IIB expression decreases, while IIX increases with aging. We propose that MHC transformation and decreased CSA in old vervets may play an important role in functional decline and adaptation with aging. We propose the vervet monkey as a valuable model for determining the mechanisms and treatment of human sarcopenia.

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References

Acosta L Jr, Roy RR. Fiber-type composition of selected hindlimb muscles of a primate (cynomolgus monkey). Anat Rec. 1987; 218:36–141.

Allen DL, Harrison BC, Sartorius C, Byrnes WC, Leinwand LA. Mutation of the IIB myosin heavy chain gene results in muscle fiber loss and compensatory hypertrophy. Am J Physiol Cell Physiol. 2001; 280:C637–645. [PubMed: 11171584]

Andersen JL. Muscle fibre type adaptation in the elderly human muscle. Scand J Med Sci Spor. 2003; 13:40–47.

Aniansson A, Hedberg M, Henning GB, Grimby G. Muscle morphology, enzymatic activity, and muscle strength in elderly men: a follow-up study. Muscle Nerve. 1986; 9:585–591. [PubMed: 3762579]

Armstrong RB, Phelps RO. Muscle fiber type composition of the rat hindlimb. Am J Anat. 1984; 171:259–272. [PubMed: 6517030]

Bacopoulos NG, Redmond DE, Baulu J, Roth RH. Chronic haloperidol or fluphenazine: effects on dopamine metabolism in brain, cerebrospinal fluid and plasma of Cercopithecus aethiops (vervet monkey). J Pharmacol Exp Ther. 1980; 212:1–5. [PubMed: 6766186]

Caiozzo VJ, Baker MJ, Huang K, Chou H, Wu YZ, Baldwin KM. Single-fiber myosin heavy chain polymorphism: how many patterns and what proportions? Am J Physiol-Reg I. 2003; 285:R570–R580.

Chalmers GR, Row BS. Common errors in textbook descriptions of muscle fiber size in nontrained humans. Sport Biomech. 2011; 10:254–268.

Choi SJ, Lim JY, Nibaldi EG, Phillips EM, Frontera WR, Fielding RA, Widrick JJ. Eccentric contraction-induced injury to type I, IIa, and IIa/IIx muscle fibers of elderly adults. Age (Dordr). 2012; 34:215–226. [PubMed: 21431924]

Coggan AR, Spina RJ, King DS, Rogers MA, Brown M, Nemeth PM, Holloszy JO. Histochemical and enzymatic comparison of the gastrocnemius-muscle of young and elderly men and women. J Gerontol. 1992; 47:B71–B76. [PubMed: 1573181]

Delbono O. Neural control of aging skeletal muscle. Aging Cell. 2003; 2:21–29. [PubMed: 12882331]

Delp MD, Duan C. Composition and size of type I, IIA, IID/X, and IIB fibers and citrate synthase activity of rat muscle. J Appl Physiol. 1996; 80:261–270. [PubMed: 8847313]

Dennis RA, Przybyla B, Gurley C, Kortebein PM, Simpson P, Sullivan DH, Peterson CA. Aging alters gene expression of growth and remodeling factors in human skeletal muscle both at rest and in response to acute resistance exercise. Physiol Genomics. 2008; 32:393–400. [PubMed: 18073271]

Disotell, TR. Molecular systematices of the Cercopithecidae. Cambridge: Cambridge University Press; 2000.

Dutta C, Hadley EC. The significance of sarcopenia in old age. J Gerontol A Biol Sci Med Sci. 1995; 50:1–4. [PubMed: 7493199]

Eng CM, Smallwood LH, Rainiero MP, Lahey M, Ward SR, Lieber RL. Scaling of muscle architecture and fiber types in the rat hindlimb. J Exp Biol. 2008; 211:2336–2345. [PubMed: 18587128]

Everard CO, Baulu J, Carrington DG, Korver H, Terpstra WJ. Retention of leptospiral agglutinins and long-term response to administration of monoclonal antibodies in vervet monkeys (Cercopithecus aethiops) on Barbados. Eur J Epidemiol. 1991; 7:396–402. [PubMed: 1915792]

Faulkner JA, Larkin LM, Claflin DR, Brooks SV. Age-related changes in the structure and function of skeletal muscles. Clin Exp Pharmacol P. 2007; 34:1091–1096.

Fitts RH, Desplanches D, Romatowski JG, Widrick JJ. Spaceflight effects on single skeletal muscle fiber function in the rhesus monkey. Am J Physiol Regul Integr Comp Physiol. 2000; 279:R1546–1557. [PubMed: 11049835]

Freimer NB, Service SK, Ophoff RA, Jasinska AJ, Mckee K, Villeneuve A, Belisle A, Bailey JN, Breidenthal SE, Jorgensen MJ, Mann JJ, Cantor RM, Dewar K, Fairbanks LA. A quantitative trait locus for variation in dopamine metabolism mapped in a primate model using reference sequences from related species. P Natl Acad Sci USA. 2007; 104:15811–15816.

Gharahdaghi F, Weinberg CR, Meagher DA, Imai BS, Mische SM. Mass spectrometric identification of proteins from silver-stained polyacrylamide gel: a method for the removal of silver ions to enhance sensitivity. Electrophoresis. 1999; 20:601–605. [PubMed: 10217175]

Jasinska AJ, Service S, Levinson M, Slaten E, Lee O, Sobel E, Fairbanks LA, Bailey JN, Jorgensen MJ, Breidenthal SE, Dewar K, Hudson TJ, Palmour R, Freimer NB, Ophoff RA. A genetic linkage

map of the vervet monkey (Chlorocebus aethiops sabaeus). Mamm Genome. 2007; 18:347–360. [PubMed: 17629771]

Kayo T, Allison DB, Weindruch R, Prolla TA. Influences of aging and caloric restriction on the transcriptional profile of skeletal muscle from rhesus monkeys. Proc Natl Acad Sci USA. 2001; 98:5093–5098. [PubMed: 11309484]

Kohn TA, Myburgh KH. Electrophoretic separation of human skeletal muscle myosin heavy chain isoforms: the importance of reducing agents. J Physiol Sci. 2006; 56:355–360. [PubMed: 17014746]

Korfage JA, Koolstra JH, Langenbach GE, van Eijden TM. Fiber-type composition of the human jaw muscles--(part 2) role of hybrid fibers and factors responsible for inter-individual variation. J Dent Res. 2005; 84:784–793. [PubMed: 16109985]

Larsson L. Morphological and functional characteristics of the ageing skeletal muscle in man. A cross-sectional study. Acta Physiol Scand Suppl. 1978; 457:1–36. [PubMed: 281113]

Larsson L, Biral D, Campione M, Schiaffino S. An age-related type IIB to IIX myosin heavy chain switching in rat skeletal muscle. Acta Physiol Scand. 1993; 147:227–234. [PubMed: 8475750]

Larsson L, Sjodin B, Karlsson J. Histochemical and biochemical changes in human skeletal muscle with age in sedentary males, age 22--65 years. Acta Physiol Scand. 1978; 103:31–39. [PubMed: 208350]

Lexell J. Human aging, muscle mass, and fiber type composition. J Gerontol A Biol Sci Med Sci. 1995; 50:11–16. [PubMed: 7493202]

Lexell J, Downham DY. The occurrence of fibre-type grouping in healthy human muscle: a quantitative study of cross-sections of whole vastus lateralis from men between 15 and 83 years. Acta Neuropathol. 1991; 81:377–381. [PubMed: 2028741]

Lexell J, Taylor CC, Sjostrom M. What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. J Neurol Sci. 1988; 84:275–294. [PubMed: 3379447]

Marzetti E, Leeuwenburgh C. Skeletal muscle apoptosis, sarcopenia and frailty at old age. Exp Gerontol. 2006; 41:1234–1238. [PubMed: 17052879]

McKiernan SH, Colman RJ, Lopez M, Beasley TM, Aiken JM, Anderson RM, Weindruch R. Caloric restriction delays aging-induced cellular phenotypes in rhesus monkey skeletal muscle. Exp Gerontol. 2011; 46:23–29. [PubMed: 20883771]

Page SL, Chiu CH, Goodman M. Molecular phylogeny of old world monkeys (Cercopithecidae) as inferred from gamma-globin DNA sequences. Mol Phylogenet Evol. 1999; 13:348–359. [PubMed: 10603263]

Page SL, Goodman M. Catarrhine phylogeny: noncoding DNA evidence for a diphyletic origin of the mangabeys and for a human-chimpanzee clade. Mol Phylogenet Evol. 2001; 18:14–25. [PubMed: 11161738]

Palmour RM, Mulligan J, Howbert JJ, Ervin F. Of monkeys and men: vervets and the genetics of human-like behaviors. Am J Hum Genet. 1997; 61:481–488. [PubMed: 9326311]

Pette D, Peuker H, Staron RS. The impact of biochemical methods for single muscle fibre analysis. Acta Physiol Scand. 1999; 166:261–277. [PubMed: 10468663]

Petter A, Jouffroy FK. Fiber-type population in limb muscles of microcebus-murinus. Primates. 1993; 3:181–196.

Raaum RL, Sterner KN, Noviello CM, Stewart CB, Disotell TR. Catarrhine primate divergence dates estimated from complete mitochondrial genomes: concordance with fossil and nuclear DNA evidence. J Hum Evol. 2005; 48:237–257. [PubMed: 15737392]

Rahnert JA, Sokoloff AJ, Burkholder TJ. Sarcomeric myosin expression in the tongue body of humans, macaques and rats. Cells Tissues Organs. 2010; 191:431–442. [PubMed: 19907142]

Raleigh MJ, Brammer GL, McGuire MT, Pollack DB, Yuwiler A. Individual differences in basal cisternal cerebrospinal fluid 5-HIAA and HVA in monkeys. The effects of gender, age, physical characteristics, and matrilineal influences. Neuropsychopharmacology. 1992; 7:295–304. [PubMed: 1282317]

Rice KM, Linderman JK, Kinnard RS, Blough ER. The Fischer 344/NNiaHSd X Brown Norway/BiNia is a better model of sareopenia than the Fischer 344/NNiaHSd: a comparative analysis of

muscle mass and contractile properties in aging male rat models. Biogerontology. 2005; 6:335–343. [PubMed: 16463110]

Richmond FJR, Singh K, Corneil BD. Neck muscles in the rhesus monkey. I Muscle morphometry and histochemistry. J Neurophysiol. 2001; 86:1717–1728. [PubMed: 11600634]

Round JM, Matthews Y, Jones DA. A quick, simple and reliable histochemical method for ATPase in human muscle preparations. Histochem J. 1980; 12:707–710. [PubMed: 6160131]

Sartorius CA, Lu BD, Acakpo-Satchivi L, Jacobsen RP, Byrnes WC, Leinwand LA. Myosin heavy chains IIa and IId are functionally distinct in the mouse. J Cell Biol. 1998; 141:943–953. [PubMed: 9585413]

Shevchenko A, Wilm M, Vorm O, Mann M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem. 1996; 68:850–858. [PubMed: 8779443]

Shively CA, Willard SL, Register TC, Bennett AJ, Pierre PJ, Laudenslager ML, Kitzman DW, Childers MK, Grange RW, Kritchevsky SB. Aging and physical mobility in group-housed Old World monkeys. Age (Dordr). 2011 (Epub ahead of print).

Smith JC, Goldberg SJ, Shall MS. Myosin heavy chain and fibre diameter of extrinsic tongue muscles in rhesus monkey. Arch Oral Biol. 2006; 51:520–525. [PubMed: 16325759]

Sokoloff AJ, Yang B, Li H, Burkholder TJ. Immunohistochemical characterization of slow and fast myosin heavy chain composition of muscle fibres in the styloglossus muscle of the human and macaque (Macaca rhesus). Arch Oral Biol. 2007; 52:533–543. [PubMed: 17210117]

Staron RS. Human skeletal muscle fiber types: delineation, development, and distribution. Can J Appl Physiol. 1997; 22:307–327. [PubMed: 9263616]

Staron RS, Hagerman FC, Hikida RS, Murray TF, Hostler DP, Crill MT, Ragg KE, Toma K. Fiber type composition of the vastus lateralis muscle of young men and women. J Histochem Cytochem. 2000; 48:623–629. [PubMed: 10769046]

Stephenson GM. Hybrid skeletal muscle fibres: A rare or common phenomenon? Clin Exp Pharmacol P. 2001; 28:692–702.

Tonge DP, Jones SW, Bardsley RG, Parr T. Characterisation of the sarcomeric myosin heavy chain multigene family in the laboratory guinea pig. Bmc Mol Biol. 2010; 11:52. [PubMed: 20587025]

Verdijk LB, Snijders T, Beelen M, Savelberg HHCM, Meijer K, Kuipers H, van Loon LJC. Characteristics of muscle fiber type are predictive of skeletal muscle mass and strength in elderly men. J Am Geriatr Soc. 2010; 58:2069–2075. [PubMed: 21054286]

Walker DK, Dickinson JM, Timmerman KL, Drummond MJ, Reidy PT, Fry CS, Gundermann DM, Rasmussen BB. Exercise, amino acids, and aging in the control of human muscle protein synthesis. Med Sci Sports Exerc. 2011; 43:2249–2258. [PubMed: 21606874]

Weiss A, Leinwand LA. The mammalian myosin heavy chain gene family. Annu Rev Cell Dev Bi. 1996; 12:417–439.

Welle S, Brooks AI, Delehanty JM, Needler N, Thornton CA. Gene expression profile of aging in human muscle. Physiol Genomics. 2003; 14:149–159. [PubMed: 12783983]

Wineinger MA, Sharman RB, Stevenson TR, Carlsen RC, Mcdonald RB. Peripheral-nerve and muscle function in the aging fischer-344 brown-norway rat. Growth Develop Aging. 1995; 59:107–119.

Wu YZ, Baker MJ, Crumley RL, Caiozzo VJ. Single-fiber myosin heavy-chain isoform composition of rodent laryngeal muscle: modulation by thyroid hormone. Arch Otolaryngol Head Neck Surg. 2000; 126:874–880. [PubMed: 10889000]

Zhang C, Liu VW, Addessi CL, Sheffield DA, Linnane AW, Nagley P. Differential occurrence of mutations in mitochondrial DNA of human skeletal muscle during aging. Hum Mutat. 1998; 11:360–371. [PubMed: 9600454]

Ziegler, TE.; Bercovitch, FB. Socioendrocrinology of Primate Reproduction. New York: Wiley-Liss; 1990.

Highlights

- Vervet monkeys and humans' vastus lateralis muscle expresses similar MHC isoforms
- Type I and type II fibers exhibit significant atrophy in vervet muscle with aging
- The vervet monkey may be a suitable preclinical model for understanding *sarcopenia*

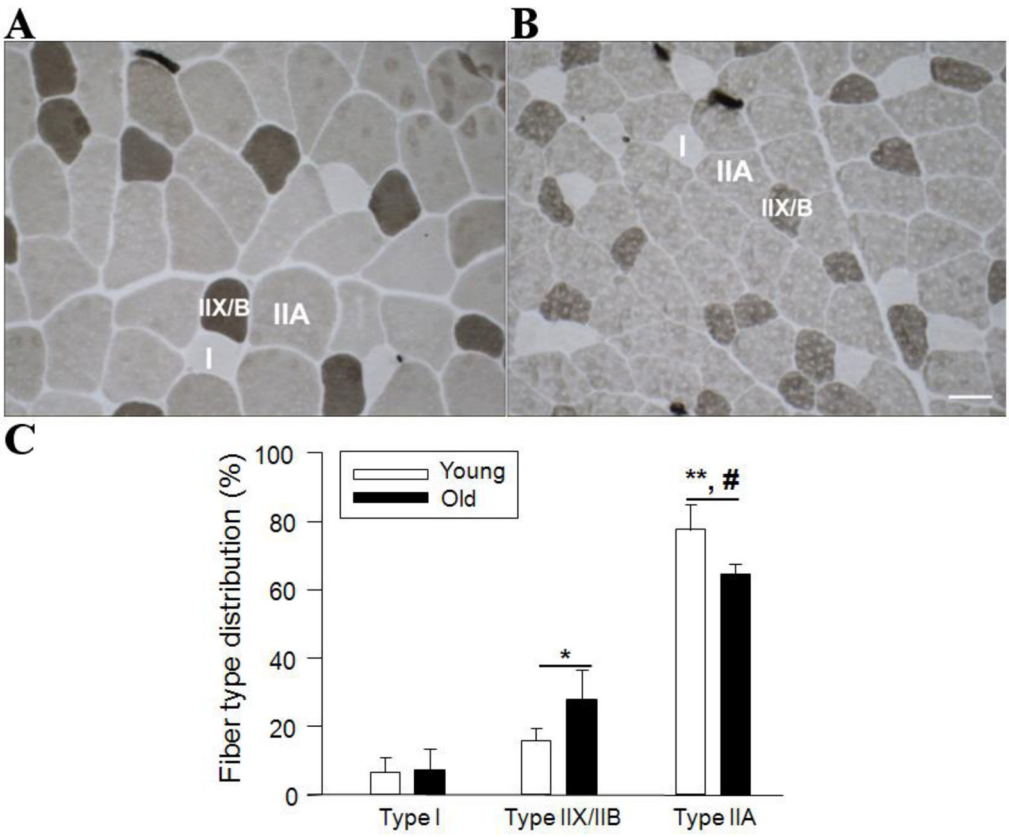

Figure 1. ATPase staining of *vastus lateralis* muscle from young and old vervet monkeys

Type I (light), type IIA (intermediate), and type IIX/B (dark) fibers are represented in VL muscle from both young (**A**) and old (**B**) vervets. Scale bar = 50μm. **C.** Fiber-type composition in VL muscle from young and old vervets according to the ATPase-staining procedure illustrated in A and B. Statistical analysis compares type IIA with type IIX/B ($P < 0.01$, #) or type I ($P < 0.01$, **), and type IIX/B with type I ($P < 0.05$, *). No significant differences between young and old monkeys were found for any fiber type (analysis of the fiber type composition was based on 1,200 fibers from each monkey).

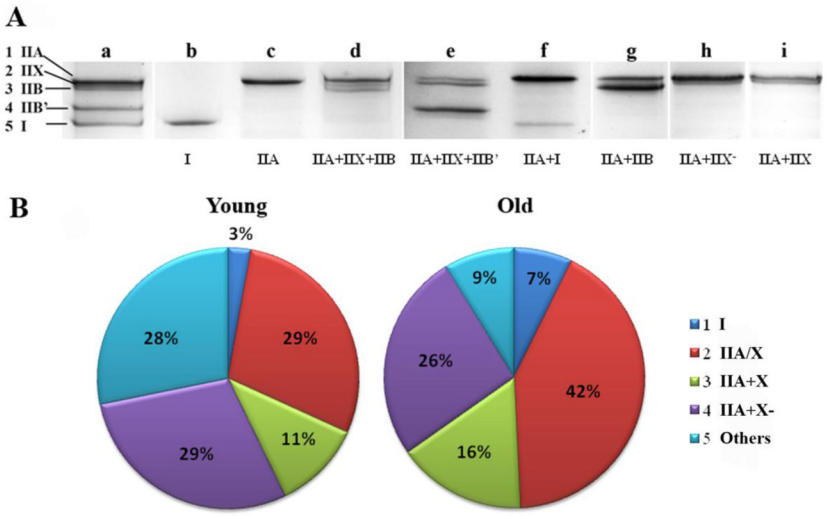

Figure 2. A. Electrophoretic and silver-stained SDS-PAGE separation of myosin heavy chain (MHC) isoforms in single *vastus lateralis* (VL) muscle fibers from vervet monkeys

Lane a corresponds to a standard prepared from a pool of vervet VL samples; it includes the five MHC isoforms (IIA, IIX, IIB, IIB′, and I) identified as pure or expressed in combination in single fibers. Lanes b-i illustrate myofibers expressing one or many MHCs. **B.** Quantification of myofibers containing type I, IIA/IIX, IIA+IIX, IIA+IIX^{-}, or multiple MHC combinations in VL muscles from young (498 single fibers) and old (456 single fibers) monkeys.

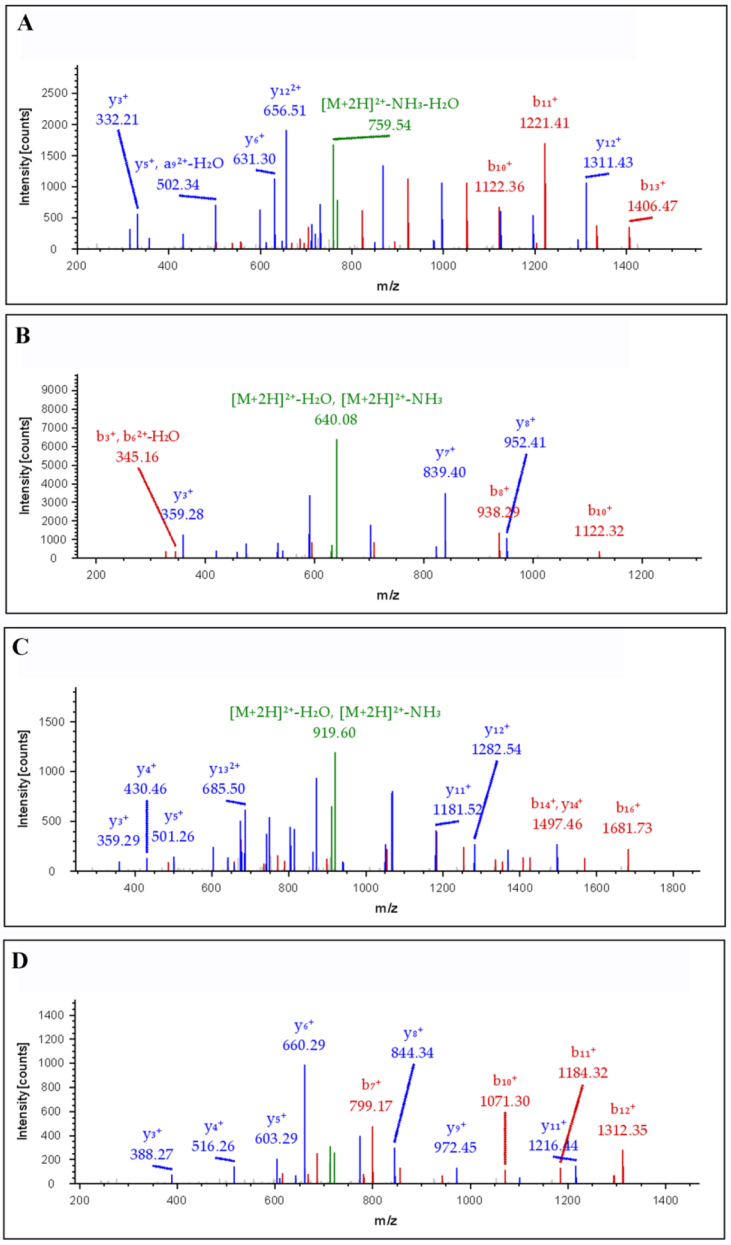

Figure 3. Representative MS/MS spectra of tryptic peptides from the identified proteins in Table 2

A. Peptide Sequence: LQDAEEHVEAVNAK belonging to protein Myosin-1 (Accession number: P12882); Mascot IonScore: 96. **B.** Peptide Sequence: DTQIHLDDALR belonging to protein: Myosin-2 (Accession number: Q9UKX2); Mascot IonScore: 84. **C.** Peptide Sequence: DLEESTLQHEATAAALR belonging to protein Myosin-4 (Accession number: P12882); Mascot IonScore: 108. **D.** Peptide Sequence: IEDEQALGSQLQK belonging to protein Myosin-7 (Accession number: P12883); Mascot IonScore: 113.

Table 1

Vastus Lateralis

Muscle Fiber Cross-sectional Area in Young and Old Monkeys

Monkeys	CSA (μm^2)		
	TYPE I	**TYPE IIX/B**	**TYPE IIA**
Young (n=3)	2106 ± 251	2974 ± 167 *b*	6409 ± 172 *b,c*
Old (n=3)	1483 ± 33*a*	2060 ± 327 *a,b*	3975 ± 382 *a,b,c*
Percentage of change	−30%	−31%	−38%

CSA, cross-sectional area; VL, *vastus lateralis*

Values are means ± standard deviation

*a*Significant difference ($P < 0.05$) *vs* young;

*b*Significant difference ($P < 0.05$) *vs* type I;

*c*Significant difference ($P < 0.05$) *vs* type IIB/X

Table 2

SDS-PAGE band	Protein IDs	Protein name	Sequence Coverage
1&2	P12882	Myosin-1 OS=Homo sapiens GN=MYH1 PE=1 SV=3 - [MYH1_HUMAN]	19.24
	Q9Y623	Myosin-4 OS=Homo sapiens GN=MYH4 PE=1 SV=2 - [MYH4_HUMAN]	15.58
	Q9UKX2	Myosin-2 OS=Homo sapiens GN=MYH2 PE=1 SV=1 - [MYH2_HUMAN]	14.58
	P11055	Myosin-3 OS=Homo sapiens GN=MYH3 PE=1 SV=3 - [MYH3_HUMAN]	9.02
3	Q9Y623	Myosin-4 OS=Homo sapiens GN=MYH4 PE=1 SV=2 - [MYH4_HUMAN]	25.99
	P12882	Myosin-1 OS=Homo sapiens GN=MYH1 PE=1 SV=3 - [MYH1_HUMAN]	20.42
	Q9UKX2	Myosin-2 OS=Homo sapiens GN=MYH2 PE=1 SV=1 - [MYH2_HUMAN]	18.13
	P13535	Myosin-8 OS=Homo sapiens GN=MYH8 PE=1 SV=3 - [MYH8_HUMAN]	14.71
	P11055	Myosin-3 OS=Homo sapiens GN=MYH3 PE=1 SV=3 - [MYH3_HUMAN]	11.29
4	Q9Y623	Myosin-4 OS=Homo sapiens GN=MYH4 PE=1 SV=2 - [MYH4_HUMAN]	20.37
5	P12883	Myosin-7 OS=Homo sapiens GN=MYH7 PE=1 SV=5 - [MYH7_HUMAN]	7.55
	Q9Y623	Myosin-4 OS=Homo sapiens GN=MYH4 PE=1 SV=2 - [MYH4_HUMAN]	3.30

Table 3

Myosin isoforms, muscle atrophy and function in vervet, human, and rat

	Species		
	Vervet	**Human**	**Rat**
Myosin	Homologous to human myosin-1, -2, -4 & -7	MHC-1, -2, -4 & -7 in the skeletal muscle of adult (Andersen and Schiaffino 1997)	Homologous to human perinatal/myosin-8 (Feghali and Leinwand 1989)
BW	−24%	−13~26% per decade over 65 years old (Dziura and others 2004; Flynn and others 1989; Kenny and Prestwood 2000; Williamson 1993)	No significant decrease (Altun and others 2007)
CSA	↓ Type II > Type I	↓ Type II > Type I	No significant decrease (Schuenke and others 2011)
Physical performance	Slow walking speed (Shively and others 2011)	Slow gait speed (Cesari and others 2005)	Difficult to estimate due to different posture

BW: body weight; CSA: cross-sectional area