Title: Structural and molecular study of the supraspinatus muscle of modern humans (Homo sapiens) and common chimpanzees (Pan troglodytes)

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ABSTRACT

Objectives: To analyze the muscle architecture and the expression pattern of the myosin heavy chain (MyHC) isoforms in the supraspinatus of Pan troglodytes and Homo sapiens in order to identify differences related to their different types of locomotion.

Materials and methods: We have analyzed nine supraspinatus muscles of Pan troglodytes and ten of Homo sapiens. For each sample, we have recorded the muscle fascicle length (MFL), the pennation angle, and the physiological cross-sectional area (PCSA). In the same samples, by real-time quantitative polymerase chain reaction, we have assessed the percentages of expression of the MyHC-I, MyHC-IIa, and MyHC-IIx isoforms.

Results: The mean MFL of the supraspinatus was longer (P=0.001) and the PCSA was lower (P<0.001) in Homo sapiens than in Pan troglodytes. Although the percentage of expression of MyHC-IIa was lower in Homo sapiens than in Pan troglodytes (P=0.035), the combination of MyHC-IIa and MyHC-IIx was expressed at a similar percentage in the two species.

Discussion: The longer MFL in the human supraspinatus is associated with a faster contractile velocity, which reflects the primary function of the upper limbs in Homo sapiens – the precise manipulation of objects – an adaptation to bipedal locomotion. In contrast, the larger PCSA in Pan troglodytes is related to the important role of the supraspinatus in stabilizing the glenohumeral joint during the support phase of knuckle-walking. These functional differences of the supraspinatus in the two species are not reflected in differences in the expression of the MyHC isoforms.

1. INTRODUCTION

The rotator cuff (Fig. 1) is formed by the supraspinatus, subscapularis, infraspinatus and teres minor muscles and acts as the main stabilizer of the glenohumeral joint (Ashton and Oxnard, 1963). This function is especially important in the hominoid primates (gibbons, orangutans, gorillas, common chimpanzees, bonobos, and modern humans) since the anatomy of their glenohumeral joint prioritizes movement over stability (Aiello and Dean, 1990). The common chimpanzees (Pan troglodytes) and the bonobos (Pan paniscus) are the hominoid primates that are phylogenetically closest to modern humans (Homo sapiens). However, despite sharing a similar anatomical pattern in their glenohumeral joint (Gebo, 2014), chimpanzees and humans have anatomical and functional differences in their shoulders as a result of their different types of locomotion. While humans use strictly bipedal walking and reserve their upper extremities almost exclusively for holding, carrying and manipulating objects, chimpanzees combine different types of arboreal locomotion, including vertical climbing, brachiation, and quadrupedal walking, with different types of terrestrial locomotion, such as knuckle-walking (Doran, 1992; Hunt, 1992; Oishi, Ogihara, Endo, Ichihara and Asari, 2009). Because of these differences in locomotion, the supraspinatus muscle plays different roles in chimpanzees and humans. Several electromyographic studies have shown that in Homo sapiens, the supraspinatus acts together with the deltoid muscle to elevate the upper extremity in the scapular plane by abducting the glenohumeral joint and also acts as a stabilizer of the glenohumeral joint (Inman, Saunders and Abbott, 1944; Mathewson, Kwan, Eng, Lieber and Ward, 2014). In Pan troglodytes, the supraspinatus also elevates the upper extremity in the scapular plane during arm elevation (Tuttle and Basmajian, 1978a; Larson and Stern, 1986) and in the swing phase of brachiation and vertical climbing (Larson and Stern, 1986; Larson and Stern, 2013). In both species, during the

elevation of the upper extremity, the supraspinatus depresses the humeral head, compensating for its tendency to move upward in response to the deltoid (Larson and Stern, 1986; Larson and Stern, 2013; Larson, 2015). In addition, in Pan troglodytes, the supraspinatus plays an important role in knuckle-walking, during the early swing and support phases (Tuttle and Basmajian, 1978b; Larson and Stern, 1987; Larson and Stern, 2013). In the support phase, the supraspinatus is especially important, as it stabilizes the glenohumeral joint and enables it to resist the shear stress resulting from the non-lineal arrangement of the scapula and the humerus (Larson and Stern, 2013).

The functional characteristics of skeletal muscles can be analyzed by studying their internal architecture, since the length, the arrangement, and the number of muscle fibers can be related to force-producing capacity and contractile velocity (Ward et al., 2006). The muscle fascicle length (MFL) is related to the number of sarcomeres in series and to contractile velocity (Carlson, 2006), while the physiological cross-sectional area (PCSA) of a muscle is related to the number of parallel sarcomeres and to force-producing capacity (Carlson, 2006). PCSA is calculated based on muscle volume, MFL, and pennation angle in unipennate, bipennate and multipennate muscles (Michilsens, Vereecke, D'Aout and Aerts, 2009). The architecture of the supraspinatus in hominoid primates is more closely related to force production than to contractile velocity, due to its role in stabilizing the glenohumeral joint; it is a bipennate muscle, or circumpennate according to some authors (Thompson, 2013), with relatively short fibers and a relatively large PCSA in comparison with other muscles (Ward et al., 2006).

The functional characteristics of skeletal muscles can also be analyzed by studying the expression patterns of the myosin heavy chain (MyHC) isoforms (Bottinelli and Reggiani, 2000). The main MyHC isoforms that are expressed in the skeletal muscles of mammals are MyHC-I, MyHC-IIa, and MyHC-IIx (Sciote and Morris, 2000). The

MyHC-I isoform is expressed primarily in type I fibers, characterized by a slow contractile velocity, low force-producing capacity and high resistance to fatigue (Kohn, Curry and Noakes, 2011; Schiaffino and Reggiani, 2011). The MyHC-IIa and MyHC-IIx isoforms are expressed mainly in type IIa and type IIx fibers, respectively (Kohn et al., 2011; Schiaffino and Reggiani, 2011). The type IIx fibers are associated with higher contractile velocity, greater force-producing capacity, and lower resistance to fatigue, while type IIa fibers are associated with intermediate contractile velocity, force-producing capacity, and resistance to fatigue. Nevertheless, this relation between the expression of the MyHC isoforms and the functional and metabolic characteristics of the muscles, which has generally been described in humans, has been brought into question by several studies in other species. For example, the skeletal muscles of the black wildebeest, the fallow deer, and the springbok have a large number of type IIx fibers with an oxidative capacity similar to that of type I and type IIa fibers. This phenomenon enables the animals to run at high speeds for long periods of time to flee from their predators (Kohn et al., 2011; Curry, Hohl, Noakes and Kohn, 2012).

The comparison of the architecture of two different muscles (higher contractile velocity in a muscle with longer fibers and greater force production in a muscle with a larger PCSA) is only useful if the two muscles have equivalent biochemical properties (Carlson, 2006). Therefore, although the expression of the MyHC isoforms may be subject to constraints imposed by muscle architecture, muscle innervation, or genetic factors (Kohn et al., 2011), their expression is nonetheless a crucial parameter in cross-species studies of muscle properties.

We have analyzed the muscle architecture and the expression patterns of the MyHC isoforms in supraspinatus muscles of Pan troglodytes and Homo sapiens. Our primary objective was to identify differences related to types of locomotion. Specifically,

we hypothesized that the architecture and the MyHC isoform expression in the supraspinatus would reflect the greater force-producing capacity of the muscle in Pan troglodytes – related to its role in the stabilization of the glenohumeral joint during knuckle-walking and in the abduction of the glenohumeral joint during the elevation of the upper limb in the scapular plane during arboreal locomotion. Although the supraspinatus plays a similar role in the abduction of the glenohumeral joint in Homo sapiens, since the upper limb of Pan troglodytes is heavier, the supraspinatus must exert a greater force during its elevation (Aiello and Dean, 1990). We further hypothesized that the muscle architecture and the expression of the MyHC isoforms would reflect the greater contractile velocity of the supraspinatus in Homo sapiens – related to the primary role of the upper limb in the precise manipulation of objects. In addition, by using the same muscle samples for both the architectural and molecular studies, we would be able to determine if these differences were related primarily to the muscle structure, to the MyHC isoforms, or to both factors.

2. MATERIALS AND METHODS

2.1. Sample collection

The supraspinatus muscles from nine Pan troglodytes and ten Homo sapiens were analyzed in this study. The nine common chimpanzees (four males and five females) were adult specimens from different Spanish zoos and had died from causes unrelated to the present study. The samples were dissected at the Anatomic Museum of the University of Valladolid (Valladolid, Spain). The ten humans (five males and five females) came from the Body Donation Service of the University of Barcelona (Barcelona, Spain) and were dissected at the Human Anatomy and Embryology Unit of the University of Barcelona.

The median age of the human specimens was 83.7 years (range, 69-97). Any human specimen with pathologies in the shoulder region, including inflammation or degeneration of the rotator cuff tendons, muscle atrophy, injuries to the long head of the biceps brachii, arthrosis, or fractures, was excluded from the study. All specimens were cryopreserved at -20ºC without fixation until dissection.

The same investigator (JMP) dissected the upper extremities of all the specimens. Once the supraspinatus muscle had been identified, the adipose tissue and the muscle fascia were removed. The muscle was then disinserted from the supraspinatus fossa of the scapula and the greater tubercle of the humerus and weighed to determine the muscle mass (MM) in grams. Finally, the supraspinatus was longitudinally sectioned along the line of its internal tendon to expose its bipennate structure (Fig. 2) and photographs were taken of the internal structure with a Canon Eos-50 digital camera. In addition, 0.5-cm^3 samples of the central area of the muscle were cryopreserved in saline solution for later molecular analysis.

2.2. Architectural analysis

The photographs of the bipennate structure of the supraspinatus were analyzed with ImageJ, an open-source image processing program designed for scientific multidimensional images (https://imagej.nih.gov/ij). For each muscle, the length and pennation angle of ten different fascicles were measured, five on each side of the internal tendon, and the ten measurements were used to calculate the mean MFL and the mean pennation angle (Θ) for the muscle. Using these mean values, the PCSA for each muscle was calculated with the following formula (Kikuchi, Takemoto and Kuraoka, 2012):

$$PCSA = (MM \times \cos \Theta) / (\rho \times MFL)$$

where ρ = muscle density (1.06 g/cm^3).

Since the supraspinatus muscles of Pan troglodytes are larger than those of Homo sapiens, in order to compare the two species, the MFLs and PCSAs were normalized assuming geometric similarity (Michilsens et al., 2009). As the body mass in kilograms of the individuals was not available, the normalized values were calculated based on the MM of the supraspinatus. Thus, MFLs were normalized to $MM^{1/3}$ and PCSAs were normalized to $MM^{2/3}$ (Michilsens et al., 2009).

2.3 RNA isolation and cDNA synthesis

RNA was extracted from the muscle samples using the commercial RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. A NanoDrop 1000 Spectrophotometer was used to determine the concentration, purity and amount of RNA.

cDNA was synthesized with the TaqMan Reverse Transcription Reagent Kit (Applied Biosystems, Foster City, CA). Reverse transcription was performed using 330 ng of total RNA in 10 µl of RT Buffer, 22 ml of 25 mM magnesium chloride, 20 µl dNTPs, 5 µl Random Hexamers, 2 µl RNAse Inhibitor, 2.5 µl MultiScribe Reverse Transcription and RNA sample plus RNAse-free water, for a final volume of 100 µl, in the following thermal cycler conditions: 10 min 25ºC, 48 min 30 ºC and 5 min 95 ºC.

2.4 Gene expression and quantification by RT-qPCR

RT-qPCR was performed according to a standard protocol (Potau et al., 2011) to obtain the expression patterns of the MyHC isoforms. Finally, the percentage of expression of the mRNA transcripts of each of the MyHC isoforms was calculated relative to the mRNA transcripts of all the MyHC isoforms (%MyHC-I, %MyHC-IIa and %MyHC-IIx).

2.5 Statistical analyses

The non-parametric Mann-Whitney U test was used to compare the parameters analyzed between Homo sapiens and Pan troglodytes. All statistical analyses were performed with SPSS 22 and statistical significance was set at $P<0.05$.

2.6. Ethical note

The research complied with protocols approved by the Institutional Animal Care and Use Committee of the University of Barcelona and adhered to the legal requirements of Spain.

3. RESULTS

Table 1 shows the main results of the study. The architectural analysis showed that the supraspinatus is bipennate both in Pan troglodytes and in Homo sapiens. While the MM of the supraspinatus was greater in chimpanzees than in humans (80 ± 30.1 g vs 37 ± 8.6 g; P=0.003), the MFL was longer in humans than in chimpanzees, both in absolute values (5.28 ± 0.97 cm vs 3.80 ± 0.47 cm; P=0.001; U=8,000; N=19) and in normalized values (1.58 ± 0.27 vs 0.90 ± 0.08; P<0.001; U=4,000; N=19). The pennation angle was higher in chimpanzees than in humans (23.3 ± 2.6º vs 14.4 ± 2.5º; P<0.001; U=0,000; N=19). The PCSA was also higher in chimpanzees than in humans, both in absolute values (17.87 ± 6.1 cm^2 vs 6,64 ± 1.7 cm^2; P<0.001; U=2,000; N=19) and in normalized values (0.96 ± 0.09 vs 0.60 ± 0.14; P=0.001; U=6,000; N=19).

The percentages of expression of the mRNA transcripts of the MyHC-I isoform were similar in chimpanzees and humans: 34.5% ± 3.7% in Pan troglodytes vs 36.6% ± 1.8% in Homo sapiens (P=0.182; U=28,000; N=19). The percentages of the MyHC-IIx

isoform were also similar in the two species: 29.1% ± 6.7% in Pan troglodytes vs 29.6% ± 1.4% in Homo sapiens (P=0.549; U=37,000; N=19). In contrast, there was a significant difference between the two species in the percentage of the MyHC-IIa isoform: 36.4% ± 4.9% in Pan troglodytes vs 33.8% ± 1.6% in Homo sapiens (P=0.035; U=19,000; N=19). However, the two fast isoforms (MyHC-IIa and MyHC-IIx) in combination were expressed at a similar percentage in the two species: 65.5% ± 3.7% in Pan troglodytes vs 63.4% ± 1.8% in Homo sapiens (P=0.182; U=28,000; N=19).

4. DISCUSSION

As part of the rotator cuff, the supraspinatus muscle plays a dual role in the stabilization and the abduction of the glenohumeral joint during the elevation of the upper limb in hominoid primates (Ashton and Oxnard, 1963). The stabilizing function is more evident in Pan troglodytes than in Homo sapiens since it is crucial to the support phase of knuckle-walking (Tuttle and Basmajian, 1978b; Larson and Stern, 1987). Furthermore, the supraspinatus in Pan troglodytes needs a greater force-producing capacity due to the greater weight of the upper limb in this species and to its use in arboreal locomotion. In contrast, the upper limb in Homo sapiens is generally used for the precise manipulation of objects and is less rarely elevated. This functional difference can explain the larger MM, pennation angle, and PCSA that we have observed in the supraspinatus of our Pan troglodytes and the longer MFL in our Homo sapiens (Fig. 3).

Our findings on the PCSA of the supraspinatus are in line with those of other studies. The mean PCSA of 17.87 cm^2 in our nine Pan troglodytes is similar to the 16.81 cm^2 reported by Mathewson et al. (2014) in one specimen. Other small studies have reported PCSAs of 17.7 cm^2 in one female (Carlson, 2006), a mean of 23.4 cm^2 in three

males and one female (Oishi et al., 2009), and 6.92 cm^2 in one female (Kikuchi et al., 2012). In contrast, smaller mean PCSAs have been reported for Homo sapiens: 7.51 cm^2 in an unspecified number of individuals (Mathewson et al., 2014) and 6.65 cm^2 in ten individuals (Ward et al., 2006), which are in line with the 6.64 cm^2 in the present study.

The high mean PCSA (23.4 cm^2) for Pan troglodytes reported by Oishi et al. (2009) may be a result of the greater mean mass of the supraspinatus in their specimens (145.9 g), while the low PCSA (6.92 cm^2) reported by Kikuchi et al. (2012) may be due to the lower mass in their specimen (33.76 g). In the present study, the supraspinatus muscles with the highest mass also had the highest PCSA (PT03: 125 g, PCSA 24.36 cm^2; PT04: 123 g, PCSA 29.50 cm^2) and the sample with the lowest mass (PT08: 26 g) also had the lowest PCSA (7.89 cm^2).

In line with reports by other investigators, we found that MFL was significantly longer in Homo sapiens than in Pan troglodytes, both in absolute and normalized values. Mathewson et al. (2014) reported a MFL of 4.35 cm in one specimen of Pan troglodytes and a mean MFL of 5.65 cm in an unspecified number of specimens of Homo sapiens. Kikuchi et al. (2012) reported a MFL of 4.06 cm in a female Pan troglodytes, which is similar to the mean of 3.80 cm in our specimens, but they did not compare this with Homo sapiens. The longer muscle fascicles in the supraspinatus of Homo sapiens reflect a faster contractile velocity (Carlson, 2006). Other anatomic adaptations in Homo sapiens that permit greater contractile velocity and precision of shoulder movement include a reduced size of the muscles of the rotator cuff in comparison with other hominoid primates (Potau et al., 2009).

We observed no significant differences in the percentages of expression of the MyHC isoforms in the supraspinatus of Pan troglodytes compared to that of Homo sapiens (Fig. 4). In both species, the expression pattern of the MyHC isoforms was typical

of powerful phasic muscles (Larson and Moss, 1993; Harridge et al., 1998), with the percentage of expression of the slow MyHC-I isoform well below 50% (34.5% in Pan troglodytes and 36.6% in Homo sapiens) and the expression of the fastest MyHC-IIx isoform at 29.1% in Pan troglodytes and 29.6% in Homo sapiens. This expression pattern, typical of the supraspinatus muscle in hominoid primates (Potau et al., 2011), reflects the bipennate architecture of the supraspinatus in both species and is related to its function of elevating the upper extremity in the scapular plane by abducting the glenohumeral joint (Inman et al., 1944; Tuttle and Basmajian, 1978a; Larson and Stern, 1986). The only difference that we observed between Homo sapiens and Pan troglodytes was a slight but significant increase in expression of the MyHC-IIa isoform in Pan troglodytes, which was balanced by a lower – but not significantly so – expression of the MyHC-I isoform. This slightly higher expression of one of the fast isoforms in the supraspinatus may be related to the important role of this muscle during the swing phase of vertical climbing and brachiation (Larson and Stern, 1986; Larson and Stern, 2013) and to the larger size of the upper extremity in Pan troglodytes. The lower percentage of expression of the MyHC-IIa isoform and the higher expression of the MyHC-I isoform in Homo sapiens seems to be at odds with the longer muscle fascicles observed in our human specimens, both in absolute and normalized values. Long muscle fascicles are related to a higher contractile velocity, but the MyHC-I isoform is expressed primarily in muscles with a low contractile velocity, while the MyHC-IIa isoform is generally expressed in muscles with a high contractile velocity (Kohn et al., 2011). This apparent incongruity may be due to the constraints placed by the muscle architecture on the intrinsic contractile properties of the muscle, which can be determined by the functional use of the muscle (Kohn et al., 2011; Curry et al., 2012). Furthermore, under certain conditions, type IIa muscles are subject to hypertrophy in humans, and the higher percentage of expression of the MyHC-

IIa isoform in Pan troglodytes may well be due to an increase in the mass of the supraspinatus, which would lead to a higher PCSA and a more highly developed force-producing capacity (Fry et al., 2014).

One of the major limitations of our study is the older age of our human specimens, due to the fact that they were obtained from the Body Donation Service. This older age may well have affected the expression of the MHC isoforms, since a higher expression of the MyHC-I isoform and a lower expression of the MyHC-II isoforms have previously been reported in other muscles, such as the vastus lateralis (Short et al., 2005; Toth, Matthews, Tracy and Previs, 2005).

In conclusion, the architecture of the supraspinatus muscle purportedly reflects the different functions of this muscle in Homo sapiens and Pan troglodytes. In line with findings from previous studies (Mathewson et al., 2014), the supraspinatus of the common chimpanzees had a higher PCSA, which is related to its function as a stabilizer of the glenohumeral joint in the support phase of knuckle-walking, while the supraspinatus of the humans had a longer MFL, which reflects its adaptation to the primary function of the upper extremity in manipulating objects. In contrast, the two species shared a similar expression pattern of the MyHC isoforms, which could be related to the main role of the supraspinatus in hominoid primates. The fact that we used the same muscles for the architectural and the molecular analyses, as well as the relatively large number of samples included in our study, leads us to suggest that muscle architecture may well explain the functional differences between chimpanzee and human supraspinatus muscles better than the expression patterns of the MyHC isoforms. Similar studies with other species of hominoid and non-hominoid primates with different types of locomotion will clarify whether this phenomenon applies to the supraspinatus of other primates and to muscles with other functions.

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FIGURE LEGENDS

Figure 1. Dissection of the dorsal muscles of the rotator cuff in a modern human. 1 = supraspinatus muscle, 2 = infraspinatus muscle, 3 = teres minor muscle.

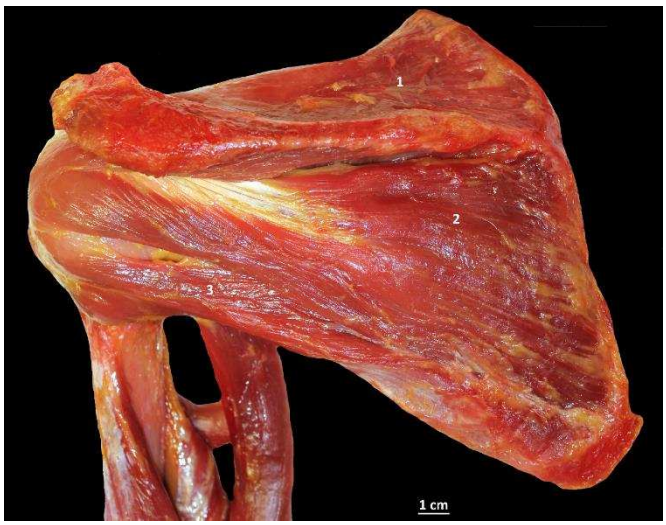

Figure 2. Cross-section of the supraspinatus muscle of a Pan troglodytes, showing its bipennate architecture.

Figure 3. Absolute and normalized mean values of MFL and PCSA in Homo sapiens and Pan troglodytes.

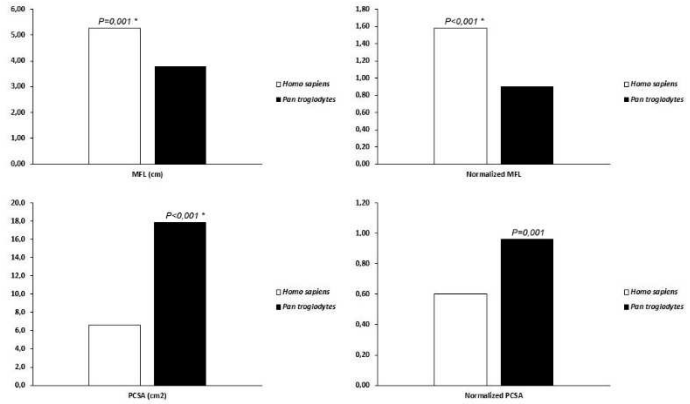

Figure 4. Percentages of expression of the MyHC isoforms in Homo sapiens and Pan troglodytes.

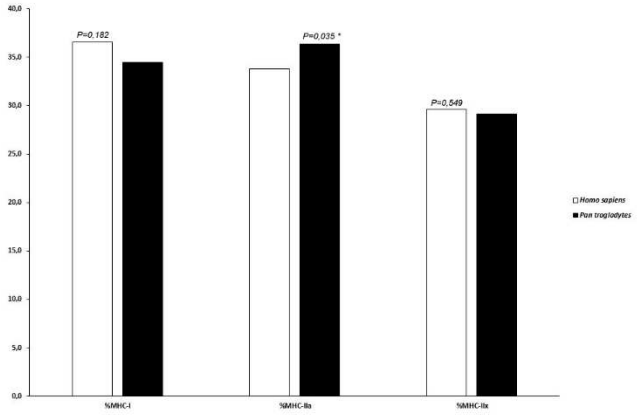

Table 1. Architecture of the supraspinatus muscle and percentages of expression of the mRNA transcripts of the MyHC isoforms in nine Pan troglodytes (PT) and ten Homo sapiens (HS).

M = male; F = female; NA = not available; MM = muscle mass (in grams); MFL = muscle fascicle length (in cms); $MFL/MM^{1/3}$ = normalized muscle fascicle length; MFA = muscle fascicle angle (in º); COS = cosine; PCSA = physiological cross-sectional area (in cm^2); $PCSA/MM^{2/3}$ = normalized physiological cross-sectional area; MyHC = myosin heavy chain; SD = standard deviation. Asterisks indicate statistical significance.

SAMPLE	SEX	AGE (years)	MM	MFL	MFL/MM$^{1/3}$	MFA	COS MFA	PCSA	PCSA/MM$^{2/3}$	%MyHC-I	%MyHC-IIa	%MyHC-IIx	%MyHC-II
PT01	M	NA	75	4.26	1.01	21.0	0.93	15.50	0.87	31.1	41.0	27.9	68.9
PT03	M	17	125	4.42	0.88	23.8	0.91	24.36	0.97	35.2	37.6	27.2	64.8
PT04	M	14	123	3.67	0.74	21.2	0.93	29.50	1.19	38.2	31.7	30.1	61.8
PT07	M	43	65	3.45	0.86	25.7	0.90	16.05	0.99	35.0	41.2	23.8	65.0
PT02	F	NA	70	4.04	0.98	19.9	0.94	15.42	0.91	32.7	36.3	30.9	67.3
PT05	F	26	86	4.13	0.94	22.5	0.92	18.13	0.93	36.3	38.2	25.5	63.7
PT06	F	25	75	3.76	0.89	28.1	0.88	16.62	0.93	31.6	26.2	42.2	68.4
PT08	F	40	26	2.90	0.98	22.3	0.93	7.89	0.90	29.3	35.2	35.5	70.7
PT09	F	28	73	3.60	0.86	25.0	0.91	17.37	0.99	40.9	39.9	19.2	59.1
Mean			80	3.80	0.90	23.3	0.92	17.87	0.96	34.5	36.4	29.1	65.5
SD			30.1	0.47	0.08	2.6	0.02	6.1	0.09	3.7	4.9	6.7	3.7
HS18	M	72	34	6.24	1.93	12.1	0.98	5.05	0.48	37.30	34.02	28.68	62.70
HS19	M	69	45	5.46	1.53	17.8	0.95	7.41	0.59	33.65	34.68	31.67	66.35
HS32	M	84	57	6.39	1.66	15.9	0.96	8.11	0.55	36.00	34.81	29.19	64.00
HS35	M	79	44	5.87	1.66	9.3	0.99	7.02	0.56	35.01	34.52	30.47	64.99
HS46	M	90	33	4.94	1.54	13.6	0.97	6.13	0.60	35.29	34.83	29.89	64.71
HS27	F	87	31	4.63	1.47	16.2	0.96	6.08	0.62	38.68	31.59	29.73	61.32
HS34	F	83	35	5.41	1.65	13.1	0.97	5.94	0.56	38.10	34.87	27.03	61.90
HS37	F	97	30	5.54	1.78	14.5	0.97	4.97	0.52	36.20	35.36	28.44	63.80
HS43	F	84	34	2.99	0.92	17.1	0.96	10.33	0.98	35.94	32.94	31.12	64.06
HS45	F	92	31	5.32	1.69	14.3	0.97	5.35	0.54	39.52	30.43	30.05	60.48
Mean			37	5.28	1.58	14.4	0.97	6.64	0.60	36.6	33.8	29.6	63.4
SD			8.6	0.97	0.27	2.5	0.01	1.7	0.14	1.8	1.6	1.4	1.8
			P=0.003*	P=0.001*	P<0.001*	P<0.001*	P<0.001*	P<0.001*	P=0.001*	P=0.182	P=0.035*	P=0.549	P=0.182