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Expression of Myosin Heavy Chain Isoforms in the Supraspinatus Muscle of Different Primate Species: Implications for the Study of the Adaptation of Primate Shoulder Muscles to Different Locomotor Modes

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Abstract The supraspinatus muscle is a key component of the soft tissues of the shoulder. In pronograde primates, its main function, in combination with the other rotator cuff muscles (subscapularis, infraspinatus, and teres minor), is to stabilize the glenohumeral joint, whereas in orthograde primates it functions together with the deltoid, to elevate the upper extremity in the scapular plane. To determine whether these functional differences are also reflected in the molecular biochemistry of the supraspinatus muscles involved in these different locomotor modes, we used real-time polymerase chain reaction (RT-PCR) to analyze the expression of the myosin heavy chain (MHC) isoforms in supraspinatus muscles from modern humans and 12 species of pronograde and orthograde primates. The MHC expression pattern in the supraspinatus muscle of pronograde primates was consistent with its function as a tonic and postural muscle, whereas the MHC expression pattern observed in the supraspinatus muscle of nonhuman orthograde primates was that of a muscle that emphasizes speed, strength, and less resistance to fatigue. These findings are

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consistent with the role of the supraspinatus in the posture and locomotor modes of these groups of nonhuman primates. The humans included in the study had an expression pattern similar to that of the nonhuman orthograde primates. In conclusion, molecular analysis of skeletal muscles via RT-PCR can contribute to a better understanding of the morphological and functional characteristics of the primate musculoskeletal system.

Keywords Myosin heavy chain · Primate locomotion · Real-time polymerase chain reaction · Supraspinatus

Introduction

The supraspinatus muscle, together with the subscapularis, infraspinatus, and teres minor muscles, forms part of the rotator cuff, which plays an important role in the movement and stabilization of the glenohumeral joint (Ashton and Oxnard 1963; Michener *et al.* 2003; Roberts 1974). The anatomical and functional characteristics of the supraspinatus vary among the different species of primates according to their posture and locomotor mode. Pronograde primates are characterized by a narrow thorax, a scapula located in the parasagittal plane, and a glenohumeral joint adapted for both arboreal and terrestrial quadrupedal locomotion. Orthograde primates, which include the family Hylobatidae (gibbons) and the species *Pongo pygmaeus* (orangutans), *Gorilla gorilla* (gorillas), *Pan troglodytes* (chimpanzees), *Pan paniscus* (bonobos), and *Homo sapiens* (modern humans), are characterized by a broad thorax, a more dorsally situated scapula, and a more mobile glenohumeral joint suitable for different locomotor modes—including brachiation, vertical climbing, suspensory posture and knuckle-walking—and in modern humans consistent with using the hand for complex manipulation (Aiello and Dean 1990; Schultz 1961).

In pronograde primates such as *Chlorocebus aethiops* (Larson and Stern 1989), *Papio anubis*, and *Macaca mulatta* (Larson and Stern 1992), the supraspinatus muscle is electromyographically active during the support phase of quadrupedal walking, acting as a stabilizer of the glenohumeral joint. Nonprimate quadrupeds, such as *Felis catus* (cat: English 1978) and *Didelphis virginiana* (Virginia opossum: Jenkins and Weijs 1979), have a similar pattern of activity. This function of stabilizing the glenohumeral joint is also important in orthograde primates using quadrupedal locomotion, such as gorillas and chimpanzees. Investigators have also recorded electromyographic activity in the supraspinatus in these primates during the support phase of knuckle-walking (Larson and Stern 1987; Tuttle and Basmajian 1978).

In addition to stabilizing the glenohumeral joint, the supraspinatus muscle works together with the deltoid muscle to elevate the upper extremity in the scapular plane (Alpert *et al*. 2000; Basmajian and de Luca 1985; Halder *et al.* 2001; Inman *et al.* 1944). This function is especially important in orthograde primates, in which the anatomical structure of the thorax and the shoulder allows the upper extremity to be elevated during brachiation, vertical climbing, and suspensory locomotion (Ashton and Oxnard 1964; Ciochon and Corruccini 1977; Inman *et al.* 1944; Oxnard 1963,

1967). The supraspinatus muscle of orthograde primates such as orangutans, gorillas, chimpanzees, and modern humans also shows electromyographic activity during the elevation of the hand (Inman *et al.* 1944; Larson and Stern 1986; Tuttle and Basmajian 1978).

The morphology of the supraspinatus muscle is adapted to the type of locomotion used by each species of primates. For example, the size, i.e., mass, of the supraspinatus relative to the total mass of deltoid, rotator cuff, and teres major muscles was larger in pronogrades than in hominoids (Inman *et al.* 1944). In addition, the difference in size between the supraspinatus fossa and the infraspinatus fossa is much greater in arboreal quadrupedal primates (*Miopithecus talapoin* and *Colobus* sp.) than in terrestrial or semiterrestrial quadrupedal primates (*Papio papio*, *Macaca* sp., *Chlorocebus aethiops*, and *Erythrocebus patas*: Roberts 1974). Similarly, the supraspinatus fossa is larger in relation to the infraspinatus fossa in orangutans than in arboreal quadrupedal primates, and it is larger still in chimpanzees and gorillas, both knuckle-walkers (Roberts 1974). Modern humans seem to be the exception among orthograde primates because their supraspinatus fossa is relatively small (Roberts 1974). The smaller supraspinatus fossa in terrestrial quadrupedal primates may be due to the shape of the humeral head, which is less spherical in terrestrial than in arboreal quadrupedal primates, with a resultant greater projection of the greater tubercle and greater leverage for the supraspinatus (Larson 1993). Different locomotor modes also lead to differences in the internal structure of the supraspinatus, e.g., angle of pinnation and muscle fiber length. In *Chlorocebus aethiops*, semiterrestrial quadrupeds, the internal structure of the supraspinatus is adapted for speed instead of strength, vs. *Cercopithecus ascanius*, arboreal quadrupeds, in which strength is a more important factor (Anapol and Gray 2003). In modern humans, the internal structure of the supraspinatus is designed for great strength but in small movements (Ward *et al.* 2006).

The skeletal muscles of adult mammals express 3 MHC isoforms—the slow MHC-I, the fast MHC-IIa, and the fastest MHC-IIx—in different proportions. A fourth isoform, MHC-IIb, is expressed only in skeletal muscles of very small mammals (Baldwin and Haddad 2001). The expression pattern of the MHC isoforms is directly related to the functional properties of the muscle fibers (Table I), such as contraction time, strength, and resistance to fatigue (Bottinelli and Reggiani 2000). The MHC-I isoform is expressed mainly in slow-oxidative, or type I, fibers; the MHC-IIa isoform in fast-oxidative, glycolytic, or type IIa, fibers; and the MHC-IIx isoform in fast glycolytic, or type IIx fibers. Type IIx fibers are more powerful, faster, and less resistant to fatigue than type IIa fibers, while type I fibers are the least powerful and fast, but the most fatigue-resistant of the 3 types (Botinelli *et al.* 1999; Pette and Staron 2000). In general, the slow postural muscles express mainly the slow MHC-I isoform, with a variable expression of the MHC-IIa isoform (Baldwin 1996; Fitts and Widrick 1996; Fitts *et al.* 1991; Rivero *et al.* 1999; Schiaffino and Reggiani 1996; Talmadge 2000). In contrast, the powerful, fast, but less fatigue-resistant muscles express all 3 MHC isoforms in variable proportions but with a generally higher expression of the MHC-II isoforms (Harridge *et al.* 1998; Larsson and Moss 1993).

Using ATPase staining, Singh *et al.* (2002) found that the supraspinatus of *Macaca mulatta* had a mean of 49% of type I fibers, 23% of type IIa fibers, and

Table I Main characteristics of the muscle fiber types of mammalian muscles

	Type I fibers	Type IIa fibers	Type IIx fibers
MHC isoform predominantly expressed	MHC-I	MHC-Iia	MHC-IIx
Metabolism	Slow oxidative	Fast oxidative	Fast glycolytic
Contraction velocity	Slow	Fast	Very fast
Resistance to fatigue	High	Intermediate	Low
Force production	Low	High	Very high
Mitochondrial density	High	High	Low
Oxidative capacity	High	High	Low
Glycolytic capacity	Low	High	High
Myoglobin content	High	High	Low
Capillary density	High	High	Low
Identification method	ATPase staining	ATPase staining	ATPase staining

MHC = myosin heavy chain

28% of type IIx fibers. Using the same technique, Schmidt and Schilling (2007) found that the percentage of type I fibers in the supraspinatus of *Saguinus oedipus* was greater than in the supraspinatus of *Saimiri sciureus* (55–70% vs. 45–60%, respectively), which the authors attributed to the greater stability of the glenohumeral joint in *Saguinus oedipus* and its greater mobility in *S. sciureus*. These authors also reported a heterogeneous distribution of the fiber types; the percentage of type I fibers increased from proximal to distal, and in the more proximal region, type I fibers were concentrated in the posterior muscle region near the scapular spine (Schmidt and Schilling 2007). Also using ATPase staining, Srinivasan *et al.* (2007) found that the supraspinatus of *Homo sapiens* had a mean of 50% of type I fibers, 21% of type IIa fibers, and 29% of type IIx fibers. Using immunohistochemistry with antibodies for specific MHC isoforms, Lovering and Russ (2008) found that the MHC-I isoform accounted for 54% of MHC expression in the modern human supraspinatus.

Although there is abundant information in the literature about the anatomy and the structure of the supraspinatus in different primate species, to date few studies have examined the molecular characteristics of this muscle, and the majority of them have been conducted in a small number of species. Most studies have analyzed the quantification and distribution of the different fiber types in the skeletal muscle (Schmidt and Schilling 2007; Singh *et al.* 2002), but none have examined the mRNA expression of the different MHC isoforms in the supraspinatus. Real-time quantitative polymerase chain reaction (RT-PCR) analysis of the MHC isoforms can provide new information on the molecular characteristics of the supraspinatus muscle in different primate species and their relation to different types of locomotion. RT-PCR has several advantages over both ATPase staining and immunohistochemistry. ATPase staining can be used only with muscles obtained from biopsies or immediately after death, because results can vary due to postmortem changes in pH. However, RT-PCR can be used with muscles from cryopreserved cadavers because any potential postmortem degradation of the mRNA can be accounted for by normalizing the values of each MHC

isoform with those of the endogenous gene 18 S, which remains intact for ≥8 d postmortem in the skeletal muscle (Bahar *et al.* 2007). Whereas immunohistochemistry does not distinguish between the MHC-IIa and the MHC-IIx isoforms, RT-PCR can identify all 3 MHC isoforms.

In the present study, we quantified the mRNA expression of the 3 MHC isoforms by RT-PCR in supraspinatus muscles from modern humans and 12 different species of primates. Our primary objective was to obtain molecular information on the functional characteristics of the supraspinatus muscle and to correlate our findings with adaptations of the supraspinatus to different locomotor modes. We hypothesized that a differential expression pattern of MHC isoforms would be related to the different functions of the supraspinatus in pronograde and orthograde primates.

Materials and Methods

Supraspinatus Muscle Samples

We obtained supraspinatus muscle samples from modern humans and nonhuman primates, cryopreserved within 24–48 h of death and not treated with any fixation method. The 24 modern human supraspinatus muscle samples included in the study were from cadavers from the Body Donation Service and dissection rooms of the University of Barcelona. The 12 male and 12 female cadavers, with a mean age of 74.8 yr (range, 44–97 yr), showed no macroscopic pathology. The 21 nonhuman primate supraspinatus muscle samples were obtained from 12 different species (Table II) from the Department of Anatomy and Radiology of the University of

Table II Means and standard deviations (SD) of the relative mass and mRNA expression of MHC isoforms in the supraspinatus muscle

Species	Sample	Locomotion	SUP/RC	% MHC-I	% MHC-II	% MHC-IIa	% MHC-IIx
Homo sapiens	24	B	0.16 (0.02)	36.86 (2.52)	63.14 (2.52)	33.38 (1.57)	29.77 (2.43)
Pan troglodytes	3	KW-AS	0.15 (0.01)	33.03 (2.07)	66.97 (2.07)	38.30 (2.42)	28.67 (1.98)
Gorilla gorilla	3	KW	0.20 (0.02)	36.77 (1.37)	63.23 (1.37)	35.69 (1.42)	27.54 (2.07)
Pongo pygmaeus	2	AS	0.18 (0.01)	37.64 (3.36)	62.36 (3.36)	39.43 (0.55)	22.94 (2.81)
Nomascus gabriellae	1	BR	0.17	33.59	66.41	35.79	30.62
Miopithecus talapoin	1	AQ	0.29	48.27	51.73	51.73	0
Macaca fascicularis	3	AQ	0.22 (0.01)	46.29 (4.01)	53.71 (4.01)	53.71 (4.01)	0
Colobus guereza	1	AQ	0.21	48.46	51.54	51.54	0
Lemur catta	2	STQ	0.25 (0.03)	53.46 (1.19)	46.54 (1.19)	46.54 (1.19)	0
Chlorocebus aethiops	1	STQ	0.26	49.51	50.49	50.49	0
Macaca silenus	2	STQ	0.26 (0.00)	48.15 (0.64)	51.85 (0.64)	51.85 (0.64)	0
Mandrillus sphinx	1	STQ	0.25	48.49	51.51	51.51	0
Erythrocebus patas	1	TQ	0.28	49.14	50.86	50.86	0

SUP = supraspinatus mass; RC = rotator cuff mass; B = biped; KW = knuckle-walker; AS = arm-swinger; BR = brachiator; AQ = arboreal quadruped; STQ = semiterrestrial quadruped; TQ = terrestrial quadruped

Valladolid. All of the focal subjects came from Spanish zoos and had died from causes unrelated to the present study. We included 9 orthograde primates: 1 male *Nomascus gabriellae* (gibbon), 2 female *Pongo pygmaeus*, 1 male and 2 female *Gorilla gorilla*, and 2 male and 1 female *Pan troglodytes*. We also included 12 pronograde primates: 2 female *Lemur catta*, 1 male *Miopithecus talapoin*, 1 male *Erythrocebus patas*, 1 female *Chlorocebus aethiops*, 2 male *Macaca silenus*, 3 male *Macaca fascicularis*, 1 male *Mandrillus sphinx*, and 1 female *Colobus guereza*. All the primates were adults, except the *Nomascus gabriellae*, which was 13 mo old.

In each specimen, the same investigator carefully dissected the subscapularis, supraspinatus, infraspinatus, and teres minor muscles (Figs. 1 and 2). He removed adipose tissue and fascia and recorded the weight of each of the muscles. He weighed each muscle 3 times at 5-min intervals and took the mean of the 3 measurements as the muscle's weight. He obtained the total weight of the rotator cuff (RC) for each individual by adding the weight of each of the muscles, and calculated the weight of the supraspinatus muscle (SUP) relative to the total RC (SUP/RC). Although the weight of the supraspinatus relative to total body weight may be a more appropriate parameter, we did not know the total body weight of the primates dissected because they were necropsied before our study was performed. We therefore selected the SUP/RC as a useful parameter to provide information on the degree of development of the supraspinatus in relation to the other stabilizers of the glenohumeral joint. After weighing the muscles, the investigator took 3 samples of 3–5 mm^3 from the central area of each of the supraspinatus muscles and froze them in saline solution for the molecular analyses.

In addition, to determine if MHC expression in the supraspinatus muscle is homogeneous or heterogeneous, we analyzed this expression in several regions of the supraspinatus of an orthograde primate (*Gorilla gorilla*) and a pronograde primate (*Macaca fascicularis*). We obtained samples from the anterior proximal, anterior central, anterior distal, posterior proximal, posterior central, and posterior distal regions of the supraspinatus. The proximal region of the supraspinatus corresponds to the region near the medial border of the scapula; the distal region is near the glenoid fossa; the anterior region is near the superior angle of the scapula;

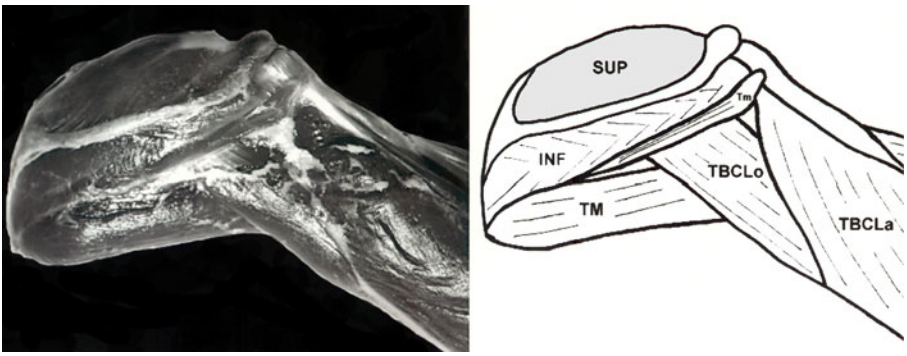

Fig. 1 Dissection and anatomical drawing of the dorsal region of the scapula in a pronograde primate (*Lemur catta*), showing the 3 posterior muscles of the rotator cuff and the teres major muscle. SUP = supraspinatus; INF = infraspinatus; Tm = teres minor; TM = teres major; TBCLo = triceps brachii caput longum; TBCLa = triceps brachii caput laterale.

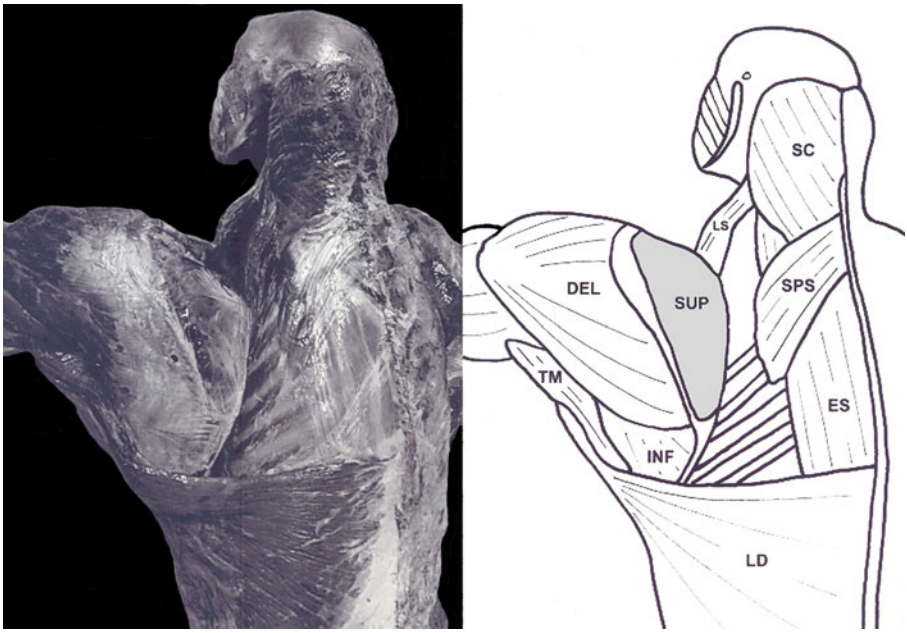

Fig. 2 Dissection and anatomical drawing of the dorsal region of the shoulder in an orthograde primate (*Pan troglodytes*) after the elimination of the trapezius, rhomboideus minor, and rhomboideus major muscles. SUP = supraspinatus; INF = infraspinatus; TM = teres major; DEL = deltoid; LD = latissimus dorsi; LS = levator scapulae; SC = splenius capitis; SPS = serratus posterior superior; ES = erector spinae.

the posterior region is near the scapular spine; and the central region is located between the proximal and the distal regions.

RNA Isolation and cDNA Synthesis

We extracted the RNA from the muscle samples using the commercial RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacturer's protocol. We used UV spectrophotometry to determine the concentration, purity, and amount of RNA and electrophoresis on 1% agarose gels to assess the integrity and quality of RNA. We used a NanoDrop 1000 Spectrophotometer to quantify RNA from the samples in duplicate.

We used the TaqMan Reverse Transcription Reagent Kit (Applied Biosystems, Foster City, CA) to synthesize cDNA. We performed reverse transcription using 330 ng of total RNA in 10 μl of TaqMan RT buffer, 22 ml of 25 mM magnesium chloride, 20 μl of dNTPs, 5 μl of random hexamers, 2 μl of RNAse inhibitor, 2.5 μl of 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 at 25°C, 48 min at 30°C, and 5 min at 95°C.

Gene Expression and Quantification by RT-PCR

Applied Biosystems supplied primers and probes. We labeled primers at the 5′ end with the reporter dye molecule FAM. We analyzed MYH-I (Hs00165276_m1),

MYH-IIa (Hs00430042_m1), MYH-IIx (Hs00428600_m1), and MYH-IIb (Hs00757977_m1) genes. We used an 18 S gene probe labeled at the 5′ end with the reporter dye molecule FAM (Hs99999901_s1) as a housekeeping gene.

We performed RT-PCR in a total volume of 20 μl in the ABI Prism 7700 Sequence Detection System (Applied Biosystems). We ran all samples for each gene in duplicate for 40 cycles using the following master mix and thermal cycler conditions: 10 μl of the TaqMan universal PCR master mix, 1 μl of the primers and probes, 2 μl of the cDNA, and 7 μl of the RNAse-free water for 2 min at 50°C, 10 min at 95°C, 15 s at 95°C, and 1 min at 60°C. We used genomic DNA as negative control in each run. We captured fluorescent emission data and quantified mRNA concentrations by using the critical threshold value and $2^{-\Delta\Delta Ct}$.

Finally, we calculated the expression of each MHC isoform (MHC-I, MHC-IIa, and MHC-IIx) relative to the total expression of all MHC isoforms and compared the expression of the 2 fast MHC-II isoforms taken together to the expression of the slow MHC-I isoform.

Statistical Analyses

We divided the modern humans and nonhuman primates into ≥1 of 7 subgroups (Table II) based on their most commonly used substrate and locomotor mode, according to Schmitt (2010). The bipeds included *Homo sapiens*; the knuckle-walkers included *Pan troglodytes* and *Gorilla gorilla*; the arm-swingers included *Pan troglodytes* and *Pongo pygmaeus*; the arboreal quadrupeds included *Miopithecus talapion*, *Macaca fascicularis*, and *Colobus guereza*; the semiterrestrial quadrupeds included *Lemur catta*, *Chlorocebus aethiops*, *Macaca silenus*, and *Mandrillus sphinx*; the brachiators included *Nomascus gabriellae*; and the terrestrial quadrupeds included *Erythrocebus patas*.

We used the Mann-Whitney test to compare orthograde vs. pronograde taxa and to compare the locomotor groups that contained ≥5 individuals each: bipeds (24 individuals), knuckle-walkers (6 individuals), arm-swingers (5 individuals), arboreal quadrupeds (5 individuals), and semiterrestrial quadrupeds (6 individuals). We set statistical significance at $p<0.05$. We used SPSS version 14.0 for all statistical analyses.

Results

Table II summarizes the results. The mean SUP/RC values are 0.16 in modern humans, 0.18 in nonhuman orthograde primates, and 0.25 in pronograde primates. We observed significant differences between modern humans and nonhuman orthograde primates ($p=0.018$, $U=49.5$, df=31), between modern humans and pronograde primates ($p<0.001$, $U=0$, df=34), and between nonhuman orthograde and pronograde primates ($p<0.001$, $U=5$, df=19). In the subgroups based on locomotor mode, the mean SUP/RC values are as follows: bipeds, 0.16; knuckle-walkers, 0.18; arm-swingers, 0.16; arboreal quadrupeds, 0.23; and semiterrestrial quadrupeds, 0.25. We observed significant differences in SUP/RC values between bipeds and arboreal quadrupeds ($p=0.001$, $U=0$, df=27), between bipeds and

semiterrestrial quadrupeds ($p<0.001$, $U=0$, df=28), between knuckle-walkers and arboreal quadrupeds ($p=0.045$, $U=4$, df=9), between knuckle-walkers and semiterrestrial quadrupeds ($p=0.006$, $U=1$, df=10), between arm-swingers and arboreal quadrupeds ($p=0.009$, $U=0$, df=8), and between arm-swingers and semiterrestrial quadrupeds ($p=0.006$, $U=0$, df=9). We observed no significant differences between bipeds and knuckle-walkers ($p=0.092$, $U=39.5$, df=28), between bipeds and arm-swingers ($p=0.371$, $U=44.5$, df=27), or between arboreal quadrupeds and semiterrestrial quadrupeds ($p=0.144$, $U=7$, df=9).

The expression pattern of the MHC isoforms is similar in the modern humans and the nonhuman orthograde primates, with higher expression levels of the MHC-II isoforms. The mean expression of the MHC-I isoform is 36.86% in modern humans and 35.36% in nonhuman orthograde primates ($p=0.157$, $U=73$, df=31). The mean expression of the 2 MHC-II isoforms taken together is 63.14% in modern humans and 64.64% in nonhuman orthograde primates ($p=0.157$, $U=73$, df=31). However, modern humans expressed a higher proportion of the MHC-IIx isoform (29.77% vs. 27.24%; $p=0.043$, $U=58$, df=31) and a lower proportion of the MHC-IIa isoform (33.38% vs. 37.4%; $p<0.001$, $U=10$, df=31). The pronograde primates have a higher proportion of the MHC-I isoform (48.83%) and a lower proportion of the MHC-II isoforms (51.17%) than either the modern humans ($p<0.001$, $U=0$, df=34) or the nonhuman orthograde primates ($p<0.001$, $U=0$, df=19). Intriguingly, none of the pronograde primates expressed MHC-IIx, though all the modern human and nonhuman orthograde primates expressed it. None of the modern humans or nonhuman primates expressed MHC-IIb.

The overall pattern of MHC expression is similar in the biped, knuckle-walker, and arm-swinger subgroups. There are no significant differences in the expression of MHC-I between bipeds and knuckle-walkers ($p=0.120$, $U=42$, df=28) or between bipeds and arm-swingers ($p=0.133$, $U=34$, df=27). There are no significant differences in the expression of MHC-IIx between bipeds and knuckle-walkers ($p=0.133$, $U=43$, df=28) or between bipeds and arm-swingers ($p=0.050$, $U=26$, df=27). However, the bipeds have a lower expression of MHC-IIa (33.38%) than the knuckle-walkers (36.99%) ($p=0.001$, $U=10$, df=28) and the arm-swingers (38.75%; $p=0.001$, $U=0$, df=27). In contrast, we observed significant differences in the expression of all 3 MHC isoforms when we compared the biped, knuckle-walker, and arm-swinger subgroups to the arboreal quadruped and the semiterrestrial quadruped subgroups. In the arboreal quadruped subgroup, the mean expression is 47.12% of MHC-I and 52.88% of MHC-IIa (arboreal quadrupeds vs. bipeds, $p=0.001$, $U=0$, df=27; arboreal quadrupeds vs. knuckle-walkers, $p=0.006$, U=0, df=7; arboreal quadrupeds vs. arm-swingers, $p=0.009$, $U=0$, df=8). In the semiterrestrial quadruped subgroup, the mean expression is 50.20% of MHC-I and 49.8% of MHC-IIa (semiterrestrial quadrupeds vs. bipeds, $p<0.001$, $U=0$, df=28; semiterrestrial quadrupeds vs. knuckle-walkers, $p=0.004$, $U=0$, df=10; semiterrestrial quadrupeds vs arm-swingers, $p=0.006$, $U=0$, df=7). We observed no significant differences in MHC isoform expression between the arboreal quadruped and the semiterrestrial quadruped subgroups ($p=0.100$, $U=6$, df=7).

There are no large variations in the MHC expression pattern according to the region of the supraspinatus muscle studied in 1 *Macaca fascicularis* and 1 *Gorilla gorilla* (Table III).

Table III mRNA expression of MHC isoforms in different regions of the supraspinatus muscle in 1 *Macaca fascicularis* and 1 *Gorilla gorilla*

Species	Region analyzed	% MHC-I	% MHC-IIa	% MHC-IIx
Macaca fascicularis	Anterior	46.8	53.2	0
	Posterior	46.32	53.68	0
	Proximal	46.64	53.36	0
	Central	46.77	53.23	0
	Distal	46.27	53.73	0
Gorilla gorilla	Anterior	33.43	36.15	30.42
	Posterior	34.17	36.13	29.7
	Proximal	34.26	36.2	29.54
	Central	34.23	36.12	29.65
	Distal	32.91	36.1	30.99

Discussion

In the present study, the mass of the supraspinatus muscle in relation to the total mass of the rotator cuff was larger in the pronograde than in modern humans and the nonhuman orthograde primates. These findings are in line with those of Inman *et al.* (1944), who compared the mass of the supraspinatus muscle with the combined mass of all the scapulohumeral muscles, including the rotator cuff, the deltoid, and the teres major muscles. The supraspinatus plays an important role in stabilizing the glenohumeral joint in the pronograde primates, preventing its collapse in retraction (Preuschoft *et al.* 2010). This role of the supraspinatus as an antigravity postural muscle may explain its larger proportional size in pronograde vs. orthograde primates because postural muscles, such as the human soleus or gluteus maximus, tend to be relatively large.

In contrast, in modern humans and nonhuman orthograde primates, the primary role of the supraspinatus muscle is the elevation of the upper extremity in the scapular plane, where it acts together with the deltoid (Inman *et al.* 1944; Larson and Stern 1986; Tuttle and Basmajian 1978). The relatively smaller size of the supraspinatus in modern humans and the nonhuman orthograde primates in comparison with the pronograde primates may be due to its role as an agonist of the deltoid muscle, which is especially large in hominoid primates (Aiello and Dean 1990; Inman *et al.* 1944). The different locomotor modes of modern humans and the nonhuman orthograde primates do not seem to be related to major changes in the relative size of the supraspinatus muscle because we found no significant differences in SUP/RC values among the biped, knuckle-walker, and arm-swinger subgroups. The SUP/RC values for the chimpanzees, orangutans, and the gibbon were similar to those for modern humans. However, the gorillas had a higher SUP/RC value. This proportionately larger supraspinatus may be related to the fact that the fundamental locomotor mode of adult gorillas is knuckle-walking (Fleagle 1999; Schmitt 2010), wherein the supraspinatus muscle is crucial to the stability of the glenohumeral joint (Larson and Stern 1987; Tuttle and Basmajian 1978). However, this larger supraspinatus was not evident in the chimpanzees, which combine knuckle-walking with other locomotor modes, including arm-swinging (Schmitt 2010). Further studies with a larger number of

chimpanzees and gorillas could help shed light on the potential effect of knuckle-walking on SUP/RC values.

The functional differences between the supraspinatus muscles of modern humans and nonhuman orthograde primates and pronograde primates are reflected in the differences in MHC expression patterns. Importantly, none of the pronograde primates expressed MHC-IIx in the supraspinatus muscle. MHC-IIx is the fastest and least resistant of the MHC isoforms and is not expressed in slow postural muscles (Baldwin 1996; Fitts and Widrick 1996; Fitts *et al.* 1991; Rivero *et al.* 1999; Schiaffino and Reggiani 1996; Talmadge 2000). Our findings thus provide molecular evidence for the importance of the supraspinatus as a postural muscle in pronograde primates. In relation to MHC-I isoform expression, our results confirm previous findings with ATPase staining in other pronograde primates, such as *Macaca mulatta* (Singh *et al.* 2002) and *Saimiri sciureus* (Schmidt and Schilling 2007). Specifically, Singh *et al.* (2002) found 44–58% of type I fibers in the supraspinatus muscle of *Macaca mulatta*, and Schmidt and Schilling (2007) found 45–60% of type I fibers in the supraspinatus of *Saimiri sciureus*. These percentages are along the lines of the 42.48–54.30% of MHC-I expression we observed in the 12 pronograde primates in the present study.

Schmidt and Schilling (2007) observed a heterogeneous distribution of the type I fibers in the supraspinatus of *Saguinus oedipus* and *Saimiri sciureus*, with a major percentage of these fibers in the distal region of the muscle and near the scapular spine. Intriguingly, however, we have observed a homogeneous expression of the MHC isoforms in the supraspinatus muscle of both *Macaca fascicularis* and *Gorilla gorilla*, leading us to speculate that the distribution of fiber types may be unrelated to the expression of MHC isoforms. Further analyses with a wider sample of primate species and individuals are warranted to shed light on this issue.

In contrast to that of the pronograde primates, the supraspinatus of all the modern humans and nonhuman orthograde primates expressed all 3 MHC isoforms, with a higher percentage of the 2 fast MHC-II isoforms than the slow MHC-I isoform. This expression pattern is typical of fast and powerful muscles with low resistance to fatigue (Harridge *et al.* 1996; Klitgaard *et al.* 1990), and our findings provide evidence for the elevatory function of the supraspinatus in pronograde primates. Although the nonhuman orthograde primates included in this study use various locomotor modes (brachiation, arm-swinging, knuckle-walking), we observed no large differences in the expression patterns of the MHC isoforms according to locomotor mode, but we were unable to make statistical comparisons between nonhuman orthograde primate locomotor groups because of the small sample size. Interestingly, we observed significant differences in the expression patterns of the MHC isoforms between knuckle-walking orthograde primates and the arboreal quadrupeds and semiterrestrial quadrupeds pronograde primates, indicating that the electromyographic activity in the chimpanzee and the gorilla supraspinatus (Larson and Stern, 1987; Tuttle and Basmajian, 1978) is not related to a pronograde-like MHC expression pattern.

The modern humans in the present study had an MHC expression pattern similar to that of the other orthograde primates, with expression of all 3 isoforms and a higher proportion of the 2 fast MHC-II isoforms. However, the modern humans expressed a higher percentage of the MHC-IIx isoform and a correspondingly lower

percentage of the MHC-IIa isoform. This higher expression of the fastest isoform may be due to the greater mobility and precision of the muscles of the upper extremity in modern humans, where the locomotor function has, to a large extent, been replaced by a manipulative function.

In conclusion, RT-PCR is a valuable technique for the molecular study of skeletal muscles and can complement information obtained via other techniques, such as electromyography, ATPase staining, and immunohistochemistry. We here observed molecular evidence of 2 different functional patterns in the supraspinatus muscle of primates, related to the anatomical pattern of modern humans and nonhuman orthograde vs. pronograde and to the locomotor modes of different species.

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