RESEARCH ARTICLE

Anatomical and molecular analyses of the deltoid muscle in chimpanzees (*Pan troglodytes*) and modern humans (*Homo sapiens*): Similarities and differences due to the uses of the upper extremity

Mónica Gómez[1] | Aroa Casado[1,2] | Marina de Diego[1] | Juan Francisco Pastor[3] | Josep Maria Potau[1,2]

[1]Department of Surgery and Surgical Specializations, Unit of Human Anatomy and Embryology, University of Barcelona, Barcelona, Spain

[2]Institut d'Arqueologia de la Universitat de Barcelona (IAUB), Faculty of Geography and History, University of Barcelona, Barcelona, Spain

[3]Department of Anatomy and Radiology, University of Valladolid, Valladolid, Spain

Correspondence
Josep Maria Potau, Unit of Human Anatomy and Embryology University of Barcelona, C/ Casanova 143, 08036 Barcelona Spain.
Email: jpotau@ub.edu

Abstract

In the deltoid muscles of *Pan troglodytes* and *Homo sapiens*, we have analyzed the muscle architecture and the expression of the myosin heavy chain (MHC) isoforms. Our aim was to identify differences between the two species that could be related to their different uses of the upper limb. The deltoid muscle of six adult *Pan troglodytes* and six adult *Homo sapiens* were dissected. The muscle fascicle length (MFL) and the physiological cross-sectional area (PCSA) of each muscle were calculated in absolute and normalized values. The expression pattern of the MHC-I, MHC-IIa and MHC-IIx isoforms was analyzed in the same muscles by real-time polymerase chain reaction. Only the acromial deltoid (AD) presented significant architectural differences between the two species, with higher MFL values in humans and higher PCSA values in chimpanzees. No significant differences in the expression pattern of the MHC isoforms were identified. The higher PCSA values in the AD of *Pan troglodytes* indicate a greater capacity of force generation in chimpanzees than in humans, which may be related to a greater use of the upper limb in locomotion, specifically in arboreal locomotion like vertical climbing. The functional differences between chimpanzees and humans in the deltoid muscle are more related to muscle architecture than to a differential expression of MHC isoforms.

KEYWORDS
chimpanzee, deltoid muscle, muscle architecture, myosin heavy chain

1 | INTRODUCTION

The deltoid is the main shoulder muscle and covers the glenohumeral joint anteriorly, laterally and posteriorly (Klepps et al., 2004). It consists of three portions: the anterior portion or clavicular deltoid (CD); the middle portion or acromial deltoid (AD); and the posterior portion or spinal deltoid (SD) (Figure 1). The CD originates at the lateral third of the anterior border of the clavicle and at the anterior border of the acromion; the AD originates at the lateral border of the acromion; and the SD originates at the inferior border of the spine of
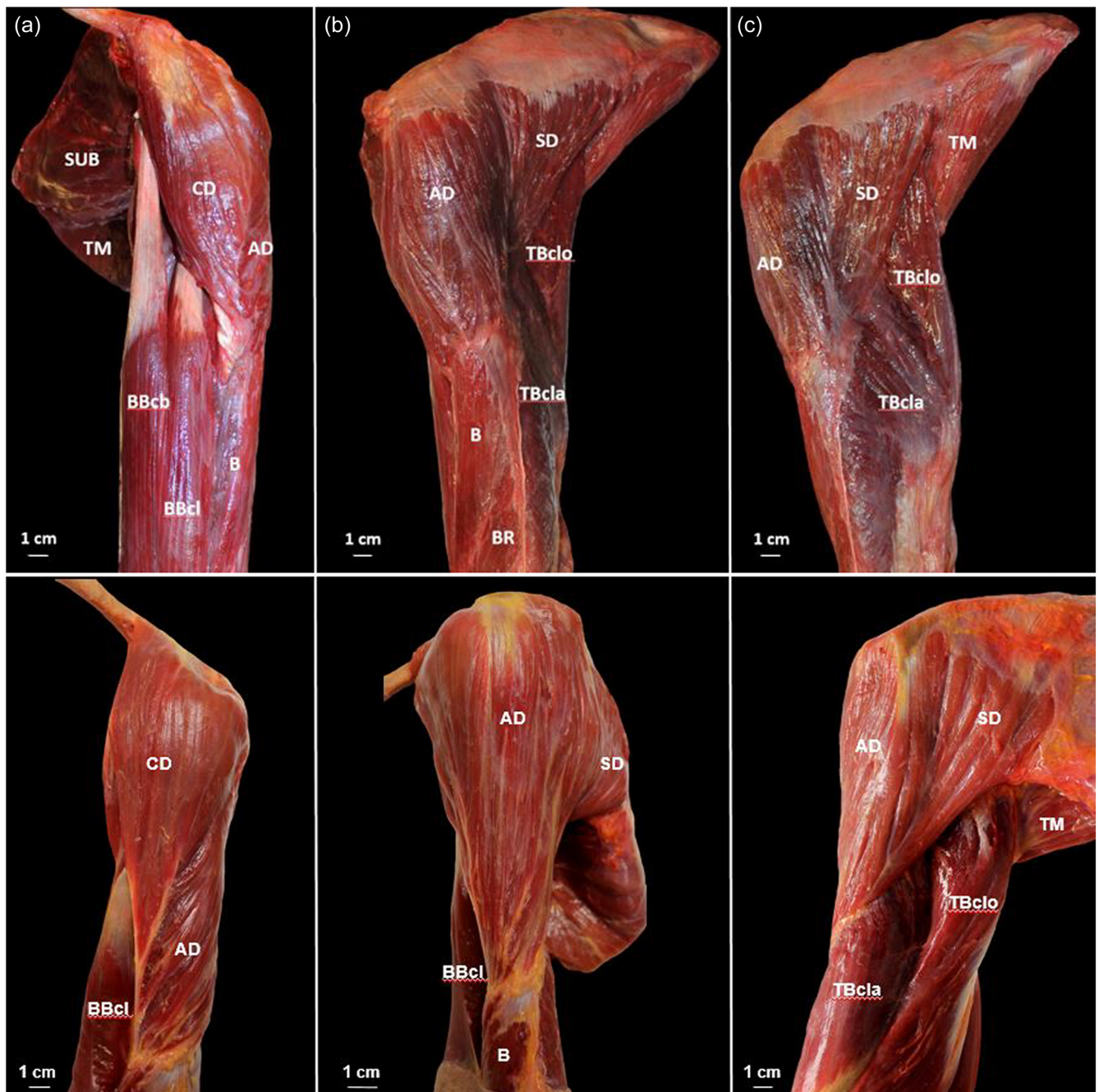

FIGURE 1 Dissection of the deltoid muscle in a specimen of *Pan troglodytes* (upper panel) and *Homo sapiens* (lower panel). (a) anterior view; (b) lateral view; (c) posterior view. SUB = *M. Subscapularis*; T = *M. Teres major*; BBcl = *M. Biceps brachii caput longum*; BBcb = *M. Biceps brachii caput breve*; B = *M. Brachialis*; BR = *M. Brachioradialis*; TBcl = *M. Triceps brachii caput longum*; TBcl = *M. Triceps brachii caput laterale*. AD, acromial deltoid; CD, clavicular deltoid; SD, spinal deltoid

the scapula (Leijnse et al., 2008; Rispoli et al., 2009; Sakoma et al., 2011; Williams & Warwick, 1980). The three portions converge at the deltoid tuberosity, located in the middle region of the lateral surface of the humeral diaphysis (Gómez et al., 2020; Klepps et al., 2004). Functional studies in humans indicate that the deltoid is jointly involved in the elevation of the upper limb in the scapular plane (Inman et al., 1944; Klepps et al., 2004; Michilsens et al., 2010; Perry et al., 1989; Reddy et al., 2000; Rosso et al., 2014) and also ensures

the stability of the glenohumeral joint (Ackland et al., 2011; Kido et al., 2003; Ovesen & Nielsen, 1986; Rosso et al., 2014; Wysiadecki et al., 2014). Electromyographic studies have shown that the CD acts as a flexor and medial rotator of the glenohumeral joint, the AD acts as an abductor of the glenohumeral joint, and the SD acts as an extensor and lateral rotator of the glenohumeral joint (Basmajian & de Luca, 1985; Gorelick & Brown, 2007; Gray & Standring, 2005; Levangie & Norkin, 2001).

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The elevating and stabilizing functions of the deltoid are shared by humans and other primates (Dunham et al., 2016; Kikuchi et al., 2012; Michilsens et al., 2009; Zihlman & Underwood, 2019), especially those in the Hominoidea superfamily, which includes the primates most closely related to humans: the Hylobatidae family (gibbons and siamangs) and the Hominidae family (orangutans, gorillas, chimpanzees, bonobos and humans) (Almécija et al., 2021). All these primates share a shoulder morphology that facilitates postures with the upper limb elevated (Larson, 1998; Myatt et al., 2012; Thompson et al., 2018; Ward, 2007), thus enabling different forms of arboreal locomotion, including brachiation and vertical climbing (Thompson et al., 2018). The functional importance of the deltoid in these forms of locomotion is reflected in the fact that the deltoid represents approximately 40% of the scapulohumeral muscles in hominoid primates but only approximately 20% in nonhominoid primates (Ashton & Oxnard, 1963; Inman et al., 1944).

Along with bonobos (*Pan paniscus*), chimpanzees (*Pan troglodytes*) are the hominoid primates most directly related phylogenetically to humans (Cagan et al., 2016; Prado-Martinez et al., 2013; Rieux et al., 2014). Anatomically and functionally, they are also one of the most studied species of hominoid primates. *Pan troglodytes*, *Homo sapiens* and other hominoid primates share anatomical characteristics of the upper limb (Aiello & Dean, 1990; Fleagle, 1999; Myatt et al., 2012; Oishi et al., 2009), but chimpanzees have a mixed-type upper limb (McLean and Dickerson, 2020) that allows them to combine quadrupedal terrestrial locomotion like knuckle-walking, arboreal locomotion like vertical climbing and brachiation, and non-locomotor behavior (Cartmill & Smith, 2009). This nonlocomotor behavior includes several manipulative tasks, such as picking up food and objects, building nests, using tools, and communicating in signs (Goodall, 1986; Hernandez-Aguilar et al., 2007; Marzke et al., 2015). Chimpanzees can combine different types of terrestrial locomotion with different types of arboreal locomotion although up to 90% of their movements are made on the ground (Doran, 1992). Knuckle-walking accounts for up to 90% of their terrestrial locomotion (Hunt, 1992), while vertical climbing accounts for up to 50% of their arboreal locomotion, followed by quadrupedal walking in trees (up to 31%) (Hunt, 1992). In contrast, the upper limb of humans is not normally used for locomotion but can participate in a wide range of manual activities that are preferably performed with the hand located below the shoulder (Cartmill & Smith, 2009; Lewis et al., 2001; Veeger & Van der Helm, 2007). The greater participation of the upper limb in chimpanzee locomotion is reflected anatomically in the greater relative mass of their upper limb, which represents 16% of their body mass, compared to only 9% in *Homo sapiens* (Thorpe et al., 1999; Zihlman, 1992).

The deltoid plays a fundamental role in both terrestrial and arboreal locomotion in chimpanzees. During knuckle-walking, the CD and AD are active in the second half of the support phase to control the degree of retraction of the upper limb. The AD is also active in the swing phase to abduct the outer upper limb when moving forward, while the SD is active in the swing phase to produce a lateral rotation of the inner upper limb at the glenohumeral joint (Larson &

Stern, 1987; Tuttle & Basmajian, 1978a). During the swing phase of vertical climbing and brachiation, the CD and AD raise the upper limb in the scapular plane, while the SD is active during the support phase of vertical climbing and brachiation to act as a propellant (Larson & Stern, 1986; Tuttle & Basmajian, 1978b). The different portions of the deltoid also participate in movements associated with manipulative tasks. The CD and the AD show light or moderate electromyographical activity during movements of the upper extremity where the abduction of the glenohumeral joint is less than 30°, while the SD is not usually active during these movements (Larson & Stern, 1986).

In the present study, we have analyzed the anatomical and functional characteristics of the deltoid in *Pan troglodytes* and *Homo sapiens*. Our main objective was to find significant differences between these two closely related species that could be related to their different uses of the upper limb—more aimed at manipulative functions in humans (McLean and Dickerson, 2020) and at terrestrial and arboreal locomotion in chimpanzees (Cartmill & Smith, 2009). We have studied the functional anatomy of the deltoid by analyzing its muscle mass (MM), its muscle fascicle length (MFL), and its physiological cross-sectional area (PCSA) (Michilsens et al., 2009). MM is proportional to muscle volume and is related to the capacity of a muscle for power generation (Payne et al., 2006; Zajac, 1992). MFL is related to the number of sarcomeres arranged in series and is proportional to the speed of shortening of a muscle (Lieber & Friden, 2000; Thorpe et al., 1999). The PCSA is related to the number of sarcomeres arranged in parallel and provides information on the ability of a muscle to generate force (Jones et al., 2004; Kikuchi, 2010; Nigg & Herzog, 2007).

In addition, we have studied the functional characteristics of the deltoid by analyzing the expression patterns of myosin heavy chain (MHC) isoforms with real-time polymerase chain reaction (RT-PCR) (Bottinelli & Reggiani, 2000). The main MHC isoforms expressed in skeletal muscles of adult mammals are MHC-I, MHC-IIa, and MHC-IIx (Sciote & Morris, 2000). The MHC-I isoform is characteristic of type I muscle fibers, which are mainly present in tonic and postural muscles, which have a low contraction speed, limited force generation, and high fatigue resistance (Kohn et al., 2011; Schiaffino & Reggiani, 2011). In contrast, the MHC-IIa and MHC-IIx isoforms are mostly expressed in type IIa and IIx muscle fibers, respectively, which are mostly located in fast-twitch phasic muscles, with great force generation capacity but low fatigue resistance (Kohn et al., 2011; Schiaffino & Reggiani, 2011). The MHC-IIx isoform is characterized by the highest contraction speed, greatest force generation capacity, and lowest resistance to fatigue of all three isoforms, while the MHC-IIa isoform is associated with intermediate contraction speed, force generation capacity and resistance to fatigue (Bottinelli et al., 1999).

We hypothesized that the three portions of the deltoid in *Pan troglodytes* would present architectural and molecular parameters related to a greater capacity for force generation as an adaptation to knuckle-walking, brachiation, and vertical climbing, while the three portions of the deltoid in *Homo sapiens* would present parameters related to a greater contraction speed that would facilitate the

manipulative functions of the upper limb (McLean and Dickerson, 2020). Since we had access to a relatively large number of chimpanzee specimens, a rare opportunity in comparative anatomy studies of hominoid primates, and we were able to carry out the architectural and molecular analyses in the same specimens, we believe that our findings on the functional anatomy of the deltoid will enhance our knowledge both of human adaptations to manipulation and chimpanzee adaptations to locomotion.

2 | METHODS

2.1 | Muscle samples

The upper limbs of six *Pan troglodytes* (two males and four females) and six *Homo sapiens* were included in the study. The chimpanzee specimens came from adults from different Spanish zoos, all of which have both natural and artificial supports that allow chimpanzees to use different types of locomotion, including knuckle-walking, vertical climbing and brachiation. All the chimpanzees included in this study had died from causes unrelated to this study and were dissected at the Anatomical Museum of the University of Valladolid. The human specimens came from males, with a mean age of 85 years (range: 81–91 years) and came from the Body Donation Service of the School of Medicine of the University of Barcelona. They were dissected at the Unit of Human Anatomy and Embryology of the University of Barcelona. All the individuals included in the study had been cryopreserved at −18°C without chemical fixation at 24–48 h postmortem. None of the dissected individuals had traumatic or degenerative injuries that could have affected the bones, joints or muscles of the upper limb.

All the specimens were dissected by the same researcher (JMP), who identified all the muscles of the upper limb and recorded information about their anatomical characteristics for use in future studies. For the present study, the deltoid muscle was identified, as much connective tissue and fat as possible were removed, and the muscle was isolated and weighed with a precision digital scale (model Sartorius PT610 and resolution of 0.1 g) to calculate its MM in grams. The muscle was then photographed with a Canon Eos-50 digital camera to identify the architecture of its muscle fascicles. Finally, samples of 0.5 cm^3 were taken from the central region of each of the three portions of the deltoid and were preserved in saline solution at −18°C for the molecular analysis.

2.2 | Muscle architecture

The photographs of the deltoid showed an arrangement of bipennate muscle fascicles in the CD and the SD and multipennate fascicles in the AD, both in humans and chimpanzees (Figure 2). Each of the photographic images was analyzed with ImageJ software (Rueden et al., 2017) to calculate the MFL in centimeters and the pennation angle (θ). These two values were obtained in three different regions of each pennation and the mean of the three values was calculated. We then calculated the mean value of the MFL and θ in each portion of the deltoid according to the number of pennations (two each in the CD and SD, six in the AD) (Figure 2). Once we had identified the MM, the mean MFL and the mean θ, we calculated the PCSA of each portion of the deltoid using the formula PCSA = (MM x cos θ)/(ρ x MFL), where ρ = muscle density (1.06 g/cm^3) (Kikuchi et al., 2012).

Because the deltoid of chimpanzees is larger than that of humans, we normalized the absolute MFL (aMFL) and absolute PCSA (aPCSA) values to compare the two species. The MFL values were normalized relative to MM$^{1/3}$ and the PCSA values were normalized relative to MM$^{2/3}$ (Michilsens et al., 2009). The mean aMFL, nMFL, aPCSA and nPCSA values of each of the three portions of the deltoid were also calculated.

Finally, the MM of the deltoid was calculated relative to the total MM of all the muscles that act on the glenohumeral joint (pectoralis major, subscapularis, supraspinatus, infraspinatus, teres minor, teres major, deltoid, coracobrachialis, biceps brachii, triceps brachii and dorsoepitrochlearis). The latissimus dorsi muscle could not be included in this analysis because this muscle was not available from any of the dissected specimens.

2.3 | Expression of the MHC isoforms

RNA from the muscle samples was extracted using the commercial RNeasy mini kit (Qiagen). We used a NanoDrop 1000

FIGURE 2 The architectural pattern of the deltoid muscle in (a) a human; (b) a chimpanzee; and (c) a schematic drawing. AD, acromial deltoid; CD, clavicular deltoid; SD, spinal deltoid

Spectrophotometer to determine the concentration, purity and amount of RNA and TaqMan Reverse Transcription Reagent Kit (Applied Biosystems) to synthesize cDNA. We performed reverse transcription 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 25°C, 48 min 30°C and 5 min 95°C.

Applied Biosystems supplied primers and probes. Primers were labeled at the 5′ end with the reporter dye molecule FAM. MYH-I (Hs00165276_m1), MYH-IIa (Hs00430042_m1), and MYH-IIx (Hs00428600_m1) genes were analyzed. To avoid any possible effects of postmortem messenger RNA (mRNA) degradation, the mRNA values for each of the MHC isoforms were normalized using the reference gene ACTB (Bustin et al., 2009). The mRNA of ACTB is detectable for more than 22 days postmortem in skeletal muscle fibers preserved at 4°C (Bahar et al., 2007), and it is one of the reference genes that is least affected by muscular degeneration (Yüzbaşıoğlu et al., 2010).

We performed RT-PCR in a total volume of 20 µl in the ABI Prism 7700 Sequence Detection System (Applied Biosystems) using the following master mix 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. We ran all samples for each gene in duplicate using the following thermal cycler conditions: 2 min 50°C, 10 min 95°C and ×40 (15 s 95°C, 1 min 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 percentage of expression of each MHC isoform relative to the total expression of all MHC isoforms (%MHC-I,%MHC-IIa, and %MHC-IIx).

2.4 | Statistical analyses

Sample normality was tested using the Shapiro–Wilk test. Variables with a normal distribution were compared with the parametric t test and those without a normal distribution with the non-parametric Mann–Whitney U test. We compared the following variables between humans and chimpanzees: the absolute MM of the deltoid; the MM of the deltoid relative to that of all the muscles acting on the glenohumeral joint; the MM of each of the portions of the deltoid relative to the other portions; the parameters of muscle architecture (MM, aMFL, nMFL, aPCSA, nPCSA) both overall and in each of the different portions of the deltoid; and the expression patterns of the MHC isoforms both overall and in each of the different portions of the deltoid. Our aim was to identify significant differences between humans and chimpanzees that could be related to the different functions of the deltoid in the two species. All statistical analyses were performed with SPSS Statistics 25 and significance was set at $p \le 0.05$.

3 | RESULTS

The muscle architecture of the deltoid presented the same structural pattern in humans and chimpanzees. Both the CD and SD had a bipennate pattern, consisting of two pennations in each portion, while the AD had a multipennate pattern, with six pennations grouped into three bipennations (Figure 2). The main quantitative results obtained in the study of muscle architecture are summarized in Table 1. Chimpanzees had a higher MM than humans (319.5 ± 144.5 g vs. 192.7 ± 75.3 g), although this difference was not significant ($p = 0.078$). However, the proportion of the deltoid MM relative to the total MM of the muscles that act on the glenohumeral joint was significantly higher in humans than in chimpanzees (21.7 ± 2.0% vs. 14.6 ± 1.1%; $p < 0.001$).

In chimpanzees, the CD represented 21.3% of the total deltoid MM, the AD 58.5%, and the SD 20.2%, while in humans, the CD represented 17.5% of the total deltoid MM, the AD 53%, and the SD 29.1% (Table 1). These differences were only significant in the SD ($p = 0.029$) but not in the CD ($p = 0.093$) or AD ($p = 0.144$). Significant differences were observed between chimpanzees and humans in the MFL and PCSA values for the AD. Both aMFL and nMFL values were lower in chimpanzees (aMFL: 3.1 ± 0.5 cm vs. 4.5 ± 0.7 cm; $p = 0.003$; nMFL: 0.57 ± 0.11 vs. 0.98 ± 0.14; $p < 0.001$) (Figure 3). Both aPCSA and nPCSA values were higher in chimpanzees (aPCSA: 53.3 ± 26.2 cm² vs. 20.9 ± 9.0 cm²; $p = 0.016$; nPCSA: 1.60 ± 0.34 vs. 0.94 ± 0.13; $p = 0.001$) (Figure 4). Neither the aMFL, nMFL, aPCSA, nor nPCSA values for the CD and SD showed significant differences between chimpanzees and humans (Table 1, Figures 3 and 4).

In chimpanzees, the CD and AD showed significant differences in aMFL ($p < 0.001$), nMFL ($p = 0.004$), aPCSA ($p = 0.004$), and nPCSA ($p = 0.004$). The AD and SD also showed significant differences in aMFL ($p < 0.001$), nMFL ($p = 0.004$), aPCSA ($p = 0.004$), and nPCSA ($p = 0.004$). The AD had higher PCSA values, while the CD and SD had higher MFL values (Table 1). No significant differences were observed between the CD and SD in aMFL ($p = 0.248$), nMFL ($p = 0.109$), aPCSA ($p = 0.631$), or nPCSA ($p = 0.150$). A similar pattern was observed in humans. Significant differences were observed between the CD and AD in aMFL ($p < 0.001$), nMFL ($p = 0.004$), aPCSA ($p = 0.004$), and nPCSA ($p = 0.004$). Significant differences were also found between the AD and SD in aMFL ($p < 0.001$), nMFL ($p = 0.004$), aPCSA ($p = 0.004$), and nPCSA ($p = 0.004$). The AD had higher PCSA values, while the CD and SD had higher MFL values (Table 1). However, when comparing the CD with the SD, the only significant differences were observed in aMFL values ($p = 0.010$), with the SD presenting higher values, while there were no significant differences in the values for nMFL ($p = 0.873$), aPCSA ($p = 0.200$), or nPCSA ($p = 0.522$).

The expression patterns of the MHC isoforms are summarized in Table 2. In both chimpanzees and humans, the CD, AD, and SD showed an expression pattern characteristic of phasic muscles, with the MHC-I isoform accounting for less than 50% and the MHC-IIx isoform for more than 20% of the total expression. There were no significant differences between chimpanzees and humans in the

TABLE 1 Results of the analysis of the muscle architecture of the deltoid in chimpanzees and humans

Sample	Sex	AGE (years)	D MM	%CD	%AD	%SD	CD aMFL	CD nMFL	CD aPCSA	CD nPCSA	AD aMFL	AD nMFL	AD aPCSA	AD nPCSA	SD aMFL	SD nMFL	SD aPCSA	SD nPCSA
HS01	M	91	139.2	13.5	44.5	42.0	6.5	2.4	2.6	0.37	3.4	0.86	15.8	1.01	10.7	2.77	4.7	0.31
HS02	M	91	187.2	25.9	48.6	25.5	8.3	2.3	5.3	0.40	4.6	1.02	18.1	0.89	9.1	2.52	4.5	0.34
HS03	M	81	337.9	18.9	58.9	22.2	7.7	1.9	7.2	0.45	4.7	0.81	38.8	1.14	10.5	2.48	6.3	0.35
HS04	M	85	157.9	17.1	52.0	30.9	7.8	2.6	3.1	0.34	5.2	1.19	14.5	0.77	8.9	2.42	5.0	0.37
HS05	M	81	136.5	17.1	57.2	25.6	7.0	2.4	3.0	0.37	4.0	0.94	17.9	0.98	7.5	2.30	4.2	0.39
HS06	M	81	197.7	12.5	59.3	28.2	7.6	2.6	3.0	0.35	5.3	1.08	20.3	0.85	8.6	2.25	5.6	0.38
Mean			**192.7**	**17.5**	**53.4**	**29.1**	**7.5**	**2.4**	**4.0**	**0.38**	**4.5**	**0.98**	**20.9**	**0.94**	**9.2**	**2.46**	**5.0**	**0.36**
SD			**75.3**	**4.8**	**6.1**	**7.0**	**0.6**	**0.3**	**1.8**	**0.04**	**0.7**	**0.14**	**9.0**	**0.13**	**1.2**	**0.18**	**0.8**	**0.03**
PT01	M	A	494.7	20.7	55.4	23.9	6.6	1.4	13.9	0.63	3.5	0.54	68.9	1.63	10.8	2.20	9.5	0.40
PT02	F	A	500.2	19.0	60.2	20.9	10.6	2.3	7.7	0.37	2.7	0.40	98.2	2.19	12.6	2.68	7.0	0.31
PT03	F	A	293.2	23.2	57.1	19.7	12.6	3.1	4.4	0.26	2.8	0.51	51.5	1.70	9.6	2.48	5.2	0.35
PT04	M	A	233.6	21.9	51.2	27.0	8.3	2.2	5.4	0.39	3.5	0.71	30.1	1.24	10.6	2.66	5.2	0.33
PT05	F	A	156.1	21.7	62.5	15.8	6.3	2.0	4.4	0.42	2.6	0.56	33.0	1.56	8.0	2.74	2.7	0.32
PT06	F	A	239.3	21.1	64.8	14.0	8.8	2.4	4.9	0.36	3.7	0.68	37.9	1.31	10.2	3.15	3.0	0.29
Mean			**319.5**	**21.3**	**58.5**	**20.2**	**8.9**	**2.2**	**6.8**	**0.41**	**3.1**	**0.57**	**53.3**	**1.60**	**10.3**	**2.65**	**5.4**	**0.33**
SD			**144.6**	**1.4**	**5.0**	**4.8**	**2.4**	**0.6**	**3.7**	**0.12**	**0.5**	**0.11**	**26.2**	**0.34**	**1.5**	**0.31**	**2.6**	**0.04**
			$p = 0.078$	$p = 0.093$	$p = 0.144$	$p = 0.029$[a]	$p = 0.262$	$p = 0.569$	$p = 0.078$	$p = 0.631$	$p = 0.003$[a]	$p < 0.000$[a]	$p = 0.016$[a]	$p = 0.001$[a]	$p = 0.206$	$p = 0.215$	$p = 0.731$	$p = 0.179$

Note: Bold values indicate means, standard deviations and p values.

Abbreviations: A, adult; AD, acromial deltoid; aMFL, absolute muscle fascicle length; aPCSA, absolute physiological cross-sectional area; CD, clavicular deltoid; D, deltoid; F, female; HS, Homo sapiens; M, male; MM, muscle mass; nMFL, normalized muscle fascicle length; nPCSA, normalized physiological cross-sectional area; PT, Pan troglodytes; SD, standard deviation; SD, spinal deltoid.
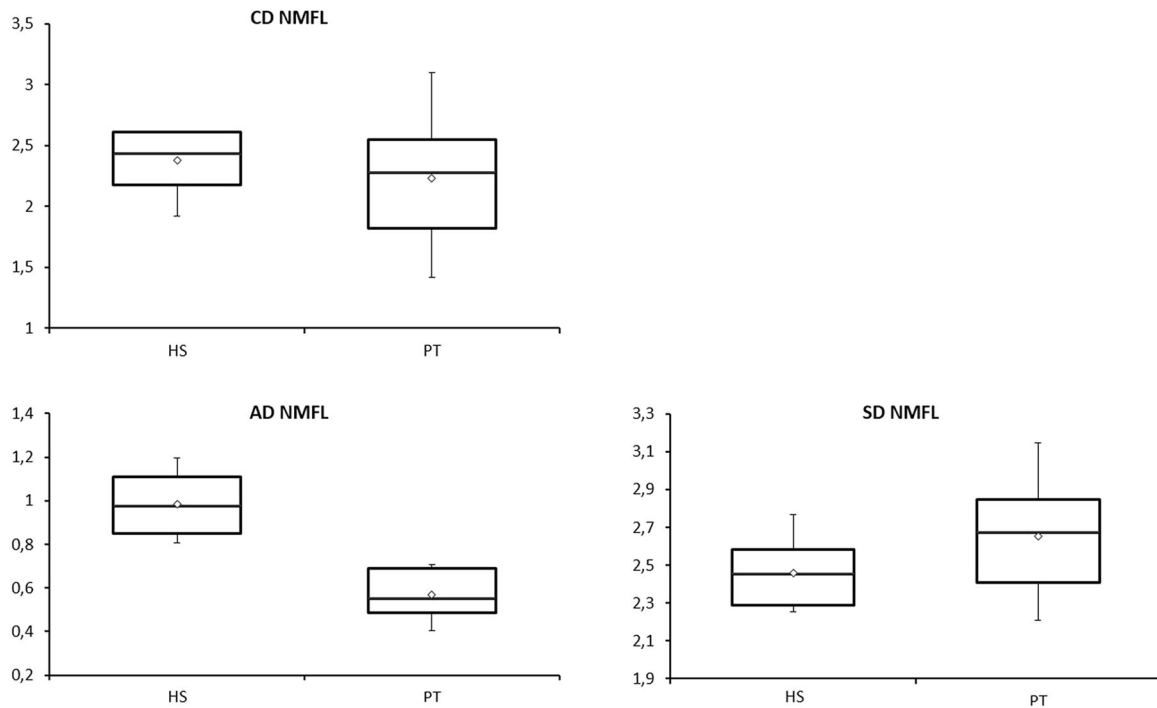
[a]Statistical significance.

FIGURE 3 Boxplot of the differences between humans and chimpanzees in the normalized muscle fascicle length of the three portions of the deltoid muscle. AD, acromial deltoid; CD, clavicular deltoid; HS, *Homo sapiens*; NMFL, normalized muscle fascicle length; PT, *Pan troglodytes*; SD, spinal deltoid
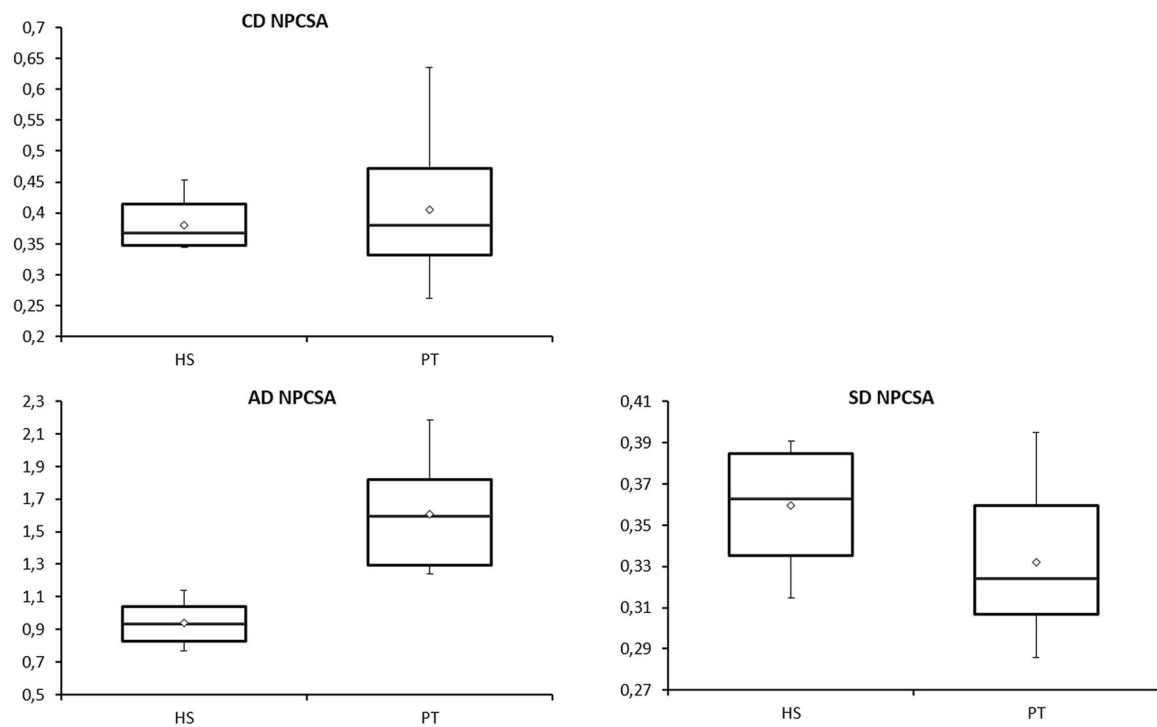

FIGURE 4 Boxplot of the differences between humans and chimpanzees in the normalized physiological cross-sectional area of the three portions of the deltoid muscle. AD, acromial deltoid; CD, clavicular deltoid; HS, *Homo sapiens*; NPCSA, normalized physiological cross-sectional area; PT, *Pan troglodytes*; SD, spinal deltoid

TABLE 2 Results of the analysis of the patterns of expression of MHC isoforms in the deltoid muscle of chimpanzees and humans

Sample	Sex	Age (years)	CD %MHC-I	CD % MHC-IIa	CD % MHC-IIx	AD %MHC-I	AD % MHC-IIa	AD % MHC-IIx	SD % MHC-I	SD % MHC-IIa	SD % MHC-IIx
HS01	M	91	39.1	28.5	32.4	37.8	36.0	26.2	42.8	29.1	28.1
HS02	M	91	35.2	35.4	29.4	43.2	38.5	18.3	35.6	38.1	26.3
HS03	M	81	32.7	32.4	34.8	41.0	27.8	31.2	29.1	34.9	36.0
HS04	M	85	34.6	37.5	27.9	37.8	39.9	22.2	36.4	38.5	25.1
HS05	M	81	37.8	45.5	16.7	36.4	51.4	12.1	35.6	37.8	26.6
HS06	M	81	34.3	39.7	26.0	34.5	36.2	29.3	33.0	44.4	22.6
Mean			**35.6**	**36.5**	**27.9**	**38.5**	**38.3**	**23.2**	**35.4**	**37.1**	**27.4**
SD			**2.4**	**5.9**	**6.3**	**3.1**	**7.7**	**7.2**	**4.5**	**5.0**	**4.6**
PT01	M	A	39.6	38.1	22.4	41.9	33.0	25.1	42.9	30.4	26.8
PT02	F	A	33.3	34.9	31.8	36.0	35.6	28.4	33.5	36.7	29.8
PT03	F	A	27.0	35.7	37.4	29.3	30.7	40.0	32.9	39.6	27.5
PT04	M	A	31.6	40.5	27.9	30.1	45.4	24.5	28.9	42.6	28.5
PT05	F	A	28.9	44.7	26.3	29.4	45.3	25.3	23.0	46.6	30.5
PT06	F	A	39.4	36.0	24.6	38.1	39.5	22.4	40.2	30.2	29.6
Mean			**33.3**	**38.3**	**28.4**	**34.2**	**38.2**	**27.6**	**33.6**	**37.7**	**28.8**
SD			**5.3**	**3.8**	**5.4**	**5.3**	**6.2**	**6.4**	**7.3**	**6.6**	**1.4**
			p= 0.346	**p= 0.544**	**p= 0.877**	**p= 0.118**	**p= 0.986**	**p= 0.289**	**p= 0.605**	**p= 0.879**	**p= 0.510**

Note: Bold values indicate means, standard deviations and *p* values.

Abbreviations: A, adult; AD, acromial deltoid; CD, clavicular deltoid; F, female; HS, Homo sapiens; M, male; MHC, myosin heavy chain; PT, Pan troglodytes; SD, standard deviation; SD, spinal deltoid.

percentages of expression of the MHC-I, MHC-IIa, and MHC-IIx isoforms in the CD, AD or SD (Table 2). When the three portions of the deltoid were compared with each other, no significant differences in the expression patterns of the MHC isoforms were observed in either chimpanzees or humans.

4 | DISCUSSION

The anatomical structure of the deltoid as a whole is similar in chimpanzees and humans despite the different uses they give to the upper limb (Cartmill & Smith, 2009; Lewis et al., 2001; McLean and Dickerson, 2020; Veeger & Van der Helm, 2007). In fact, all primates of the Hominoidea superfamily have certain anatomical characteristics of the deltoid in common, including its high degree of development, the almost total fusion of its different portions, and its relatively distal insertion into the humeral diaphysis (Ashton & Oxnard, 1963; Dunham et al., 2016). In the present study, the absolute MM of the deltoid was greater in chimpanzees than in humans, which could be related to their greater use of the upper limb for arboreal locomotion (Dunham et al., 2016; Myatt et al., 2012). Although the difference was not significant, the lower overall body mass of the chimpanzees would account for the larger relative MM of

the deltoid in chimpanzees than in humans. Moreover, the three portions of the deltoid had a similar relative MM in the two species, with the only significant differences occurring in the SD, which had a lower relative MM in chimpanzees (Table 1). This could be related to the functional importance of the CD and AD in arboreal locomotion, especially vertical climbing (Larson & Stern, 1986; Tuttle & Basmajian, 1978b). In contrast, when comparing the MM of the deltoid relative to that of the set of muscles that act on the glenohumeral joint, the humans had significantly higher values. This could be related to the important function of the deltoid in stabilizing the glenohumeral joint in humans (Rosso et al., 2014; Wysiadecki et al., 2014) since its MM creates pressure that may increase joint stability and thus decrease the upper translation of the humeral head when the upper limb is raised (Ackland et al., 2011; Kido et al., 2003; Ovesen & Nielsen, 1986). Nonetheless, the absolute and relative differences in the MM between humans and chimpanzees must be interpreted with caution when drawing conclusions about the function of the deltoid since these differences may well be partly the result of the older age of the human specimens. Humans suffer a loss of MM with age, a condition known as sarcopenia (Kirkendall & Garrett, 1998). This loss occurs at a rate of approximately 1%–2% annually after the age of 50 years (Rolland et al., 2008), and elderly individuals can have as much as 25%–35% less MM than younger ones (Lexell, 1995). The human

specimens included in the present study came from individuals donated to the Body Donation Service of our university, most of whom were elderly, and we can suggest that future studies should include specimens from younger individuals if possible.

There were also no major differences in the architecture of the deltoid muscle as a whole between chimpanzees and humans. All the individuals analyzed had a similar architectural pattern that coincides with that described by Leijnse et al. (2008) in the human deltoid, in which the CD and SD have a downward-pointing bipennate structure, while the AD presents a multipennate structure formed by three downward-pointing bipennations (Figure 2). In both humans and chimpanzees, the bipennate structure of the CD and SD was accompanied by a longer MFL, suggesting the need for forces to be exerted over a wide range of motion (Isler, 2005; Myatt et al., 2012), while the multipennate structure of the AD prioritizes the generation of force over fatigue resistance (Gorelick & Brown, 2007; Lieber & Friden, 2000). In both chimpanzees and humans, we observed significant differences in nMFL and nPCSA values between the different portions of the deltoid. The AD showed significantly higher nPCSA values, indicating a greater capacity of force generation in this portion (Kikuchi et al., 2012; Michilsens et al., 2009; Nigg & Herzog, 2007; Thorpe et al., 1999; Zajac, 1992), which has a fundamental role in the abduction and stabilization of the glenohumeral joint (Basmajian & de Luca, 1985; Gorelick & Brown, 2007; Klepps et al., 2004; Rosso et al., 2014). In contrast, the CD and SD had significantly higher nMFL values, indicating a higher contraction speed in these portions (Isler, 2005; Michilsens et al., 2009; Thorpe et al., 1999), which are mainly involved in the flexion, extension and rotation of the glenohumeral joint (Basmajian & de Luca, 1985; Gorelick & Brown, 2007; Zihlman & Underwood, 2019). The CD and SD had a bipennate structure in both chimpanzees and humans, which could account for the lack of significant differences in their nMFL and nPCSA values.

The functional differences observed between the two species in the CD and SD did not translate into significant anatomical or structural differences. In chimpanzees, both the CD and SD participate in different phases of vertical climbing and brachiation (Larson & Stern, 1986; Tuttle & Basmajian, 1978b) and in different phases of knuckle-walking (Larson & Stern, 1987; Tuttle & Basmajian, 1978a). In addition, the CD participates in movements of the upper extremity during manipulative tasks and other movements where there is only a small degree of abduction of the glenohumeral joint (Larson & Stern, 1986). In humans, the CD and SD are electromyographically active in movements of flexion-extension and medial lateral rotation, which are associated with manipulative tasks where there is less than a 90° abduction of the glenohumeral joint (Basmajian & de Luca, 1985; Gorelick & Brown, 2007; Klepps et al., 2004; Reddy et al., 2000). Despite these different uses of the CD and SD—for both manipulative and locomotor behavior in chimpanzees and for mainly manipulative tasks in humans—we did not observe significant differences in either of the functional parameters between the two species (Table 1). This may be due to the fact that the CD and SD are active in movements that do not require a high degree of

abduction of the glenohumeral joint, which are more closely associated with the AD (Larson & Stern, 1986). The greater participation of the AD in the locomotor behavior of chimpanzees (Larson & Stern, 1986; Larson & Stern, 1987; Tuttle & Basmajian, 1978a; 1978b) and the greater relative MM of the upper extremity in chimpanzees (McLean and Dickerson, 2020) can explain both the lack of differences between humans and chimpanzees in the functional parameters of the CD and SD as well as the differences between the two species in the AD.

For example, we observed significant differences between chimpanzees and humans in the architecture of the AD. In humans, the AD showed significantly higher aMFL and nMFL values (Figure 3), indicating the capacity for greater speed and wider excursions in humans than in chimpanzees (Kikuchi, 2010; Lieber & Friden, 2000; Michilsens et al., 2009; Thorpe et al., 1999; Zajac, 1992). These characteristics can be related in humans to the high speed and precision required for the manipulative tasks where the AD actively participates, such as using pulleys in the sagittal and the scapular planes, donning and doffing a button-up shirt, using a dowel, and other movements that require a lateral rotation of the glenohumeral joint with less than a 90° abduction (Burke et al., 2016; Escamilla et al., 2009). In chimpanzees, the AD had significantly higher aPCSA and nPCSA values (Figure 4), indicating a greater capacity of force generation in chimpanzees than in humans (Kikuchi, 2010; Michilsens et al., 2009; Thorpe et al., 1999; Zajac, 1992). This greater capacity could be related to the important role of the AD in different phases of brachiation and vertical climbing (Kikuchi et al., 2012; Larson & Stern, 1986; MacLean & Dickerson, 2020; Michilsens et al., 2009; Tuttle & Basmajian, 1978b) and in knuckle-walking (Larson & Stern, 1987; Tuttle & Basmajian, 1978a). In addition, the greater capacity of force generation in the AD of chimpanzees may be related to its participation in the abduction of the glenohumeral joint during manipulative tasks (Larson & Stern, 1986), where chimpanzees need to generate a greater force than humans because 16% of their body mass is in the upper extremity, compared to only 9% in humans (McLean and Dickerson, 2020).

In addition to the similarity between chimpanzees and humans in the muscle architecture of the deltoid, the patterns of MHC isoform expression were also similar in the two species (Table 2). In both chimpanzees and humans, the CD, AD and SD all had an expression pattern characteristic of phasic muscles, with a higher percentage of expression of MHC-II isoforms (Bottinelli & Reggiani, 2000). The expression patterns that we observed at the mRNA level by RT-PCR are similar to those that had previously been reported at the protein level by ATPase staining (Srinivasan et al., 2007), although protein expression identified slightly higher percentages of type I muscle fibers, which express the MHC-I isoform (Scott et al., 2001). This difference may be due to the presence of hybrid fibers expressing more than one MHC isoform, which may have affected the quantification of muscle fiber types by ATPase staining (Pette & Staron, 2000). In contrast, RT-PCR directly quantifies the mRNA transcripts of the MHC isoforms without taking into account the type of muscle fiber where they are expressed, thus providing more

realistic values of the percentages of expression of the different isoforms. Unfortunately, to the best of our knowledge, no studies have analyzed the expression patterns of MHC isoforms at the protein level in the deltoid of chimpanzees, precluding the comparison of our mRNA results with other protein-based studies. However, several studies in other muscles and in other species indicate that the expression of MHC isoforms is controlled at the transcription level, suggesting that there is a good correlation between the mRNA and protein levels (Cox & Buckingham, 1992; Eizema et al., 2005; Short et al., 2005; Zurmanova & Soukup, 2013). The expression pattern we have observed in both chimpanzees and humans indicates that the deltoid behaves as a fast, strong muscle with relatively low resistance to fatigue (Schiaffino & Reggiani, 2011). Unexpectedly, however, the greater participation of the upper limb in locomotor behavior in chimpanzees did not translate into significant differences in the expression patterns of MHC isoforms in any of the three portions of the deltoid (Table 2). The greater development of the upper limb muscles in chimpanzees (McLean and Dickerson, 2020; Myatt et al., 2012; Thorpe et al., 1999; Zihlman, 1992) would be expected to accompany an increase in type I muscle fibers, which mostly express the MHC-I isoform (Scott et al., 2001), as occurs in the deltoid of human athletes involved in activities like weight lifting (Mandroukas et al., 2010; Tesch & Larsson, 1982). However, our analysis did not identify any significant differences in the expression patterns of MHC isoforms between humans and chimpanzees. Interestingly, the AD showed a significantly higher nMFL in humans than in chimpanzees, which suggests that the human AD is structurally prepared for high contraction speed. However, the human AD also showed a high percentage of expression of the MHC-I isoform, which is associated with low contraction speed. This finding could be related to the older age of our human specimens, since a higher expression of the MHC-I isoform has been reported in the upper extremities of elderly individuals (Wu et al., 2020).

In conclusion, the different functional uses of the upper limb in chimpanzees and humans (McLean and Dickerson, 2020) moderately affect the anatomical characteristics of the deltoid but have no impact on the expression patterns of MHC isoforms. Of the differences proposed in our initial hypothesis, only those regarding the muscle architecture of the AD have been borne out by our study, with significantly higher nMFL values in humans, an adaptation to manipulative activities, and significantly higher nPCSA values in chimpanzees, an adaptation to both their locomotor behavior, especially vertical climbing and brachiation (Thorpe et al., 1999) and to manipulative activities, given the higher percentage of body mass located in their upper extremities

(McLean and Dickerson, 2020). However, we found no significant differences in muscle architecture between humans and chimpanzees when the deltoid was assessed as a whole. The large absolute and relative size of the deltoid in humans is a typical feature of hominoid primates (Ashton & Oxnard, 1963; Dunham et al., 2016; Inman et al., 1944), and its great force generation capacity can be related to its important function as a stabilizer of the glenohumeral joint in humans (Lee & An, 2002; Rosso et al., 2014; Yanagawa et al., 2008).

This stabilizing function is especially important in humans due to the relatively smaller MM of the rotator cuff, the main stabilizing structure of the glenohumeral joint (Potau et al., 2009).

Our study has several limitations, including the small sample size. Although six adult specimens of *Pan troglodytes* is a relatively high number in studies on comparative soft tissue anatomy in hominoid primates, it would be interesting to expand this sample to see if our results are confirmed. It would also be informative to repeat the study with other species of hominoid primates to see how the deltoid behaves in primates using other types of locomotion. Another limitation of the study is the use of human specimens obtained from the Body Donation Service, which tend to come from elderly individuals. This may have affected our RT-PCR results, since in older individuals, a higher expression of the MHC-I isoform relative to the MHC-II isoforms has been reported in other muscles, such as the vastus lateralis (Short et al., 2005; Toth et al., 2005; Wu et al., 2020). Finally, we analyzed the expression of the MHC isoforms in muscle samples obtained from an intermediate part of the deltoid—equidistant from the most superficial and the deepest fascicles. In this way, we were able to obtain a value representative of the overall expression of the isoforms. Nevertheless, as this expression can vary between the superficial and deep parts of the muscle (Schmidt & Schilling, 2007), we can suggest that future studies should analyze this potential variation in the deltoid of humans and chimpanzees and compare findings with those of the present study. In spite of these limitations, to the best of our knowledge, this is the first study to compare muscle architecture, functional parameters and MHC isoform expression in the deltoid of chimpanzees and humans. Importantly, we were able to perform all our analyses in the same specimens, which added to the reliability of our findings and has provided new information about the adaptation of the deltoid to manipulative activities in humans and to different types of locomotion in chimpanzees.

AUTHOR CONTRIBUTIONS

Mónica Gómez: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing-original draft preparation. **Aroa Casado:** data curation, formal analysis, funding acquisition, investigation, methodology, validation, visualization. **Marina de Diego:** data curation, formal analysis, investigation,

methodology, validation, visualization. **Juan Francisco Pastor:** data curation, formal analysis, investigation, methodology, resources, supervision, validation, visualization. **Josep Maria Potau:** conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, supervision, validation, visualization, writing-original draft preparation.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICS STATEMENT

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 and to the principles of the American Society of Primatologists for the ethical treatment of nonhuman primates.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/ajp.23390

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