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RESEARCH ARTICLE

Muscle contractile properties as an explanation of the higher mean power output in marmosets than humans during jumping

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ABSTRACT

The muscle mass-specific mean power output ($P_{MMS,mean}$) during push-off in jumping in marmosets (*Callithrix jacchus*) is more than twice that in humans. In the present study it was tested whether this is attributable to differences in muscle contractile properties. In biopsies of marmoset m. vastus lateralis (VL) and m. gastrocnemius medialis (GM) (*N*=4), fibre-type distribution was assessed using fluorescent immunohistochemistry. In single fibres from four marmoset and nine human VL biopsies, the force–velocity characteristics were determined. Marmoset VL contained almost exclusively fast muscle fibres (>99.0%), of which 63% were type IIB and 37% were hybrid fibres, fibres containing multiple myosin heavy chains. GM contained 9% type I fibres, 44% type IIB and 47% hybrid muscle fibres. The proportions of fast muscle fibres in marmoset VL and GM were substantially larger than those reported in the corresponding human muscles. The curvature of the force–velocity relationships of marmoset type IIB and hybrid fibres was substantially flatter than that of human type I, IIA, IIX and hybrid fibres, resulting in substantially higher muscle fibre mass-specific peak power ($P_{FMS,peak}$). Muscle mass-specific peak power output ($P_{MMS,peak}$) values of marmoset whole VL and GM, estimated from their fibre-type distributions and force–velocity characteristics, were more than twice the estimates for the corresponding human muscles. As the relative difference in estimated $P_{MMS,peak}$ between marmosets and humans is similar to that of $P_{MMS,mean}$ during push-off in jumping, it is likely that the difference in *in vivo* mechanical output between humans and marmosets is attributable to differences in muscle contractile properties.

KEY WORDS: Primate, Comparative physiology, Muscle mass-specific power, Force–velocity relationship, Immunohistochemistry, Muscle fibre-type distribution, Myosin heavy chain, Single muscle fibre, Skinned muscle fibre

INTRODUCTION

In the animal kingdom there are large differences among species in performance during vertical jumping (Marsh and John-Alder, 1994; Aerts, 1998; Harris and Steudel, 2002; Legreneur et al., 2010; Bobbert et al., 2014). For comparison among different species, the performance of jumping is best defined as body mass-specific mean power output ($P_{BMS,mean}$) or leg muscle mass-specific mean power output ($P_{MMS,mean}$) during push-off. Recently, *in vivo* mean power output during the push-off in vertical jumping of the common marmoset, *Callithrix jacchus* (Linnaeus 1758), was shown to be 52 W kg^{-1} $P_{BMS,mean}$ and 430 W kg^{-1} $P_{MMS,mean}$ (Bobbert et al., 2014). These values are twice those reported for humans (*Homo sapiens* L.; 27 W kg^{-1} $P_{BMS,mean}$ and 181 W kg^{-1} $P_{BMS,mean}$, respectively; Bobbert et al., 2014).

The higher power-generating capacity of marmosets may be explained by a larger proportion of fast muscle fibres and/or differences in force–velocity (*F–V*) characteristics of the muscle fibres. In general, the major leg muscles consist of higher percentages of fast-type fibres in smaller primates, such as rhesus monkey (*Macaca mulatta*), green vervet monkey (*Chlorocebus aethiops sabaeus*) and bushbaby (*Galago senegalensis*), than in larger primates such as humans (Ariano et al., 1973; Green et al., 1981; Petter and Jouffroy, 1993; Fitts et al., 1998; Jouffroy et al., 1999; Feng et al., 2012). Moreover, based on reports on muscle fibre-type distribution, in small primates one of the major leg muscles, m. vastus lateralis (VL), seems to consist of relatively large proportions of fast glycolytic fibre types [fibres expressing IIB and IIX, myosin heavy chain (MHC) not distinguished] (Ariano et al., 1973; Petter and Jouffroy, 1993; Feng et al., 2012). However, little is known regarding the muscle fibre *F–V* characteristics within small primates. Tracing differences in *in vivo* mechanical output to differences in muscle contractile properties it is not sufficient to reveal muscle fibre-type distribution – one also needs information about muscle fibre *F–V* characteristics. Some reports on *F–V* characteristics of skinned muscle fibre segments from primates suggest that the maximal shortening velocity (V_{max}) of type IIA fibres is higher in small primate species (e.g. green vervet monkey and rhesus monkey) than in humans, whilst specific tension (P_0) seems to be similar (Larsson and Moss, 1993; Bottinelli et al., 1996; Fitts et al., 1998; Gilliver et al., 2009; Choi et al., 2012). However, to the best of our knowledge, there are no data available on the *F–V* characteristics of fast glycolytic muscle fibres (type IIB and IIX) of the smallest primates.

The aim of this study was to investigate whether the 2-fold difference in *in vivo* mean power output (both $P_{BMS,mean}$ and $P_{MMS,mean}$) between marmosets and humans is attributable to differences in muscle contractile properties. First, fibre-type distributions were assessed within marmoset m. vastus lateralis (VL) and m. gastrocnemius medialis (GM). Subsequently, *F–V* characteristics of marmoset and human single muscle fibre segments were determined. From these data, estimates of muscle mass-specific peak power ($P_{MMS,peak}$) were calculated for marmoset VL and GM and compared with those for humans.

RESULTS

Fibre-type distribution of marmoset VL and GM

Immunohistochemical staining of cross-sections of marmoset VL biopsies showed that a very small number of muscle fibres

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List of symbols and abbreviations

a/P_0	curvature of Hill's relationship
FCSA	fibre cross-sectional area
FL	fibre length
F–V	force–velocity
GM	m. gastrocnemius medialis
MHC	myosin heavy chain
$P_{BMS,mean}$	body mass-specific mean power
$P_{FMS,peak}$	fibre mass-specific peak power
$P_{MMS,mean}$	muscle mass-specific mean power
$P_{MMS,peak}$	muscle mass-specific peak power
P_0	specific tension
P–V	power–velocity
VL	m. vastus lateralis
V_{max}	maximal shortening velocity

expressed type I MHC (0.2±0.05% in the distal region of VL and 1.1±0.53% in the proximal region). In VL, 68.9±5.1% (distal) and 56.5±7.50% (proximal) of the fibres were pure type IIB fibres, while the remaining fibres were hybrid fibres, expressing multiple MHCs.

In GM, the proportion of type I fibres (8.6±1.9% in the distal region and 9.7±5.3% in the proximal region) was also very small. In GM, 48.0±4.5% (distal) and 40.6±12.37% (proximal) of the muscle fibres expressed type IIB MHC exclusively. The proportions of the different fibre types are summarized in Fig. 1 and Table 1. No main effects for muscle and region and no interaction effects were found.

Marmoset optimal sarcomere length

To measure *F–V* characteristics around optimal sarcomere length, we determined the optimal sarcomere length by measuring the length–force characteristics of five muscle fibre segments. Force deterioration due to transferring the fibre from relaxing to activating solution was on average 2.5±0.2% per activation. Mean optimal sarcomere length derived from polynomial fits was 2.59±0.06 μm.

F–V characteristics of marmoset and human muscle fibres

Using electrophoresis, 77 of all 82 marmoset muscle fibres were identified as type IIB fibres. As immunohistochemical staining showed that type IIB MHC is the only isoform expressed in muscle fibres singularly and hybrid fibres always contain type IIA and IIX MHC, we considered marmoset muscle fibre segments expressing one MHC band as pure type IIB muscle fibres (see Fig. 2 for three typical examples in combination with a rat soleus homogenate). Of the 52 human fibres analysed, six consisted of type I MHC, 27 consisted of only type IIA MHC, nine consisted of only type IIX MHC and 10 contained multiple MHCs.

F–V characteristics for each fibre type and species are displayed in Table 2. As none of the marmoset muscle fibres were similar in type to the tested human muscle fibres, it was not possible to use a mixed model analysis with fibre type and species. Therefore, to test for differences in *F–V* characteristics between marmoset type IIB and hybrid fibres, a Student's *t*-test was used. To test for differences in *F–V* characteristics between different human muscle fibre types, a one-way ANOVA was used. Subsequently, Student's *t*-tests were performed to test for differences between different human and marmoset fibre types. Within marmoset fibres, the muscle fibre cross-sectional area (FCSA) was significantly larger in marmoset type IIB fibres than in marmoset hybrid fibres. ANOVA in human muscle fibres indicated only a significant main effect for V_{max}, with type I muscle fibres being slower compared with all other human fibre types (type IIA, IIX and hybrid). Subsequent testing indicated that marmoset type IIB fibres had a significantly higher muscle fibre mass-specific peak power output ($P_{FMS,peak}$) than all human fibre types. Marmoset hybrid fibres also had a higher $P_{FMS,peak}$ than human type I, IIA and hybrid fibres. This difference in $P_{FMS,peak}$ was mainly caused by a significantly higher curvature of Hill's relationship (a/P_0) in marmoset fibres (both type IIB and hybrid) compared with that in all human fibre types (I, IIA, IIX and hybrid). For type IIB marmoset muscle fibres, a/P_0 was 3-fold higher than that for human fibres. V_{max} of marmoset type IIB and hybrid muscle fibres was significantly higher than that of human type I fibres and significantly lower in marmoset type IIB than in human type IIA muscle fibres. FCSA of marmoset type IIB fibres was significantly smaller than that of human type IIA, IIX and hybrid fibres. FCSA of marmoset hybrid fibres was significantly smaller than that of all human fibre types. P_0, calculated as force divided by FCSA, did not differ among different fibre types (of both marmoset and human). However, the question remains to what extent estimates of $P_{MMS,peak}$ of whole VL and GM of marmosets and humans differ.

Total VL- and GM-specific velocity–force and velocity–power estimates: marmoset versus human

To estimate $P_{MMS,peak}$ of VL and GM, *F–V* and *P–V* curves of both muscles were estimated. The calculation took the average *F–V* characteristics of each fibre type and the fibre-type distribution into account. Human VL consists of 46.3% type I, 44.3% type IIA and 8.9% type IIX fibres, while human GM consists of 49.4% type I, 42.8% type IIA and 6.6% type IIX fibres (Green et al., 1981). Unfortunately, we were not able to measure *F–V* characteristics of type I muscle fibre segments derived from marmoset muscles. Instead, we used reported values for *F–V* parameters of human type I muscle fibres as conservative estimates for marmoset type I muscle fibres. Our calculations yielded whole-muscle $P_{MMS,peak}$ of marmoset VL and GM of more than twice the corresponding

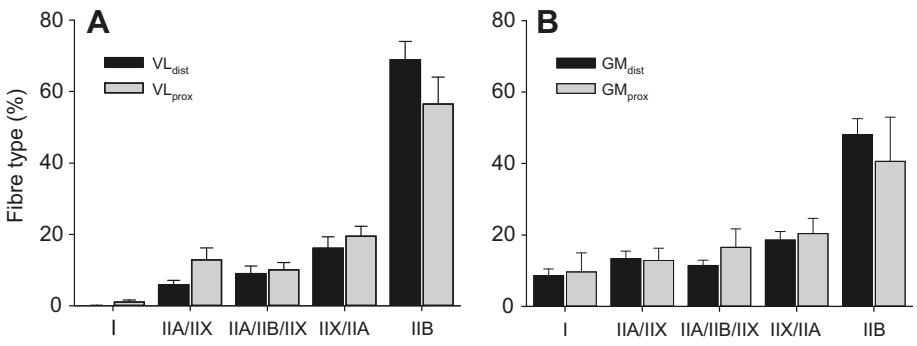

Fig. 1. Muscle fibre-type distribution in marmoset leg muscles. (A,B) Fibre-type distribution in distal and proximal regions of m. vastus lateralis (VL_{dist} and VL_{prox}, respectively; A) and m. gastrocnemius medialis (GM_{dist} and GM_{prox}, respectively; B). Data are means+s.e.m., *N*=4.

The Journal of Experimental Biology (2015) 218, 2166-2173 doi:10.1242/jeb.117655

Table 1. Fibre-type distribution, fibre mass-specific peak power and estimated whole-body muscle mass-specific peak power in marmoset and human m. vastus lateralis (VL) and m. gastrocnemius medialis (GM)

	Human				Marmoset		
	Type I	Type IIA	Type IIX	Hybrid	Type I	Type IIB	Hybrid
VL fibre-type distribution (%)[a]	46.3±3.9	44.3±3.2	8.9±2.3		0.6±0.3	62.7±6.3	36.7±7.4
GM fibre-type distribution (%)[a]	49.4±2.8	42.8±3.5	6.6±2.6		9.1±3.6	44.3±8.4	46.6±9.4
$P_{FMS,peak}$ (W kg^{-1})	2.53±1.36	5.45±0.61	6.51±1.25	5.15±0.75		9.17±0.35	8.90±1.86
VL $P_{MMS,peak}$ (W kg^{-1})	3.7				8.7		
GM $P_{MMS,peak}$ (W kg^{-1})	3.5				8.0		

Data are means±s.e.m.

$P_{FMS,peak}$ (fibre mass-specific peak power) was measured *in vitro* at 15°C; $P_{MMS,peak}$ (muscle mass-specific peak power) was estimated at 15°C.

[a]Human values for fibre-type distribution are taken from Green et al. (1981).

human values: 8.7 W kg^{-1} for marmoset VL versus 3.7 W kg^{-1} for human VL, and 8.0 W kg^{-1} for marmoset GM versus 3.5 W kg^{-1} for human GM (Table 1, Fig. 3).

DISCUSSION

The aim of this study was to investigate whether the 2-fold difference in *in vivo* power output (both $P_{BMS,mean}$ and $P_{MMS,mean}$) between marmosets and humans is attributable to differences in muscle contractile properties. We showed that marmoset VL consists mainly of fast-type muscle fibres (>99.0%), of which the majority express pure type IIB MHC (63%). Almost all other muscle fibres express multiple fast MHC isoforms in different proportions. *F–V* characteristics differed among fibre types. Significantly higher $P_{FMS,peak}$ values were found in marmoset type IIB and hybrid fibres than in human type I, IIA, IIX and hybrid fibres, mainly because of a significantly higher a/P_0 in marmoset.

$P_{MMS,peak}$ of whole VL and GM were estimated by combining our measured *F–V* characteristics of skinned single fibre segments of marmosets and humans with fibre-type distribution in GM and VL of the marmoset and reported fibre-type distributions in humans, respectively. The estimates for marmoset VL and GM $P_{MMS,peak}$ were 2.4 and 2.3 times those for human VL and GM $P_{MMS,peak}$. These differences are similar to differences reported for mean power output (both $P_{BMS,mean}$ and $P_{MMS,mean}$) delivered during push-off of a vertical jump. In marmoset, $P_{BMS,mean}$ was 1.9 times and $P_{MMS,mean}$ 2.4 times that of human (Bobbert et al., 2014). These results indicate that during a maximal vertical jump the higher *in vivo* mean power output (both $P_{BMS,mean}$ and $P_{MMS,mean}$) delivered by marmosets compared with that by humans is mainly caused by a larger proportion of fast muscle fibres in the main leg extensor muscles of the marmoset.

Some methodological limitations need to be considered regarding the accuracy of estimates of whole-muscle $P_{MMS,peak}$ based on the present data and those of the cited studies. First, biopsies for assessment of *F–V* characteristics of marmosets were taken from the middle part of the muscles and biopsies for assessment of fibre-type distribution from the proximal and distal parts. Theoretically, it is possible that muscle fibre types were not homogeneously distributed throughout the muscles. However, muscle fibre-type distribution in the analysed sections was highly uniform and no difference was shown between proximal and distal biopsies. It is therefore unlikely that our estimates are biased as a result of measurement errors caused by the location of biopsies. Second, biopsies of marmosets were taken post mortem, whereas those of humans were sampled *in vivo*. However, as marmoset biopsies were taken within 15 min post mortem, it seems highly unlikely that the results would have been affected by differences in harvesting conditions. Moreover, if this had influenced the results, the estimated $P_{MMS,peak}$ of marmosets would be underestimated rather than overestimated. Third, current estimates for whole-muscle $P_{MMS,peak}$ reported are still far from values reported for *in vivo* $P_{BMS,mean}$ and $P_{MMS,mean}$. This difference is caused by the fact that reported $P_{FMS,peak}$ for different fibre types is determined at 15°C whilst *in vivo* muscles operate at about 37°C. Making a fair translation from skinned single fibre segment force characteristics to whole-muscle force characteristics *in vivo* requires a comparative study of the temperature dependence of single muscle fibres. For such a translational step between *ex vivo* and *in vivo*, measurements with *in situ* whole muscles seem indicated. Another factor affecting the estimate of whole-muscle power output is that dissection and treatment of muscle tissue for determining muscle fibre *F–V* characteristics involves permeabilization of muscle fibres and, whilst suspended in relaxing solution, swelling of the fibre (Degens and Larsson, 2007). Both the skinning and the swelling of the muscle fibre may decrease the measured $P_{FMS,peak}$ (Moss, 1979; Elzinga et al., 1989; Degens and Larsson, 2007). However, these factors will not affect the conclusions of the current study as a direct comparison between human and marmoset fibre *F–V* characteristics was made using the same method and set-up. Because the protocol, the solutions used and temperature have a large effect on *F–V* characteristics, we refrain from comparing our results with those obtained in other studies.

Taking all limitations into account, it seems safe to say that estimated whole-muscle $P_{MMS,peak}$ of VL and GM, calculated on the basis of muscle fibre-type distributions and fibre *F–V* characteristics, are twice as high in marmosets as in humans. These differences are mainly attributable to differences in the MHC expression between the two species.

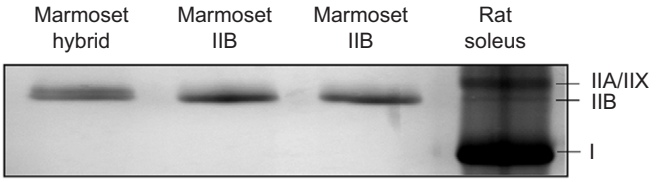

Fig. 2. Myosin heavy chain (MHC) composition of three marmoset single muscle fibres and a rat soleus homogenate. Three single muscle fibre segments were analysed using SDS-PAGE. A hybrid fibre is shown in the left lane and pure type IIB fibres are shown in the middle two lanes. The right lane contains a rat m. soleus homogenate as reference.

Marmosets have fast muscles and a unique expression of type IIB MHC

To the best of our knowledge, the present study is the first to distinguish between fast-type MHC isoforms expressed in

The Journal of Experimental Biology (2015) 218, 2166-2173 doi:10.1242/jeb.117655

Table 2. Contractile properties of human type I, IIA, IIX and hybrid fibres and marmoset type IIB and hybrid fibres

Species	*N*	FCSA (μm^2)[a]	P_0 (N cm^{-2})	a/P_0 [b]	V_{max} (FL s^{-1})[c]
Human type I	6	5100±893*	12.24±2.30	0.10±0.02*	0.33±0.10*
Human type IIA	27	7867±501*	12.26±0.91	0.09±0.00*	0.86±0.03*
Human type IIX	9	6429±821*	12.94±1.78	0.12±0.02*	0.80±0.10*
Human hybrid	10	7659±688*	11.37±0.79	0.10±0.01*	0.80±0.06*
Marmoset type IIB	77	4271±157*	10.90±0.31	0.33±0.02*	0.80±0.02*
Marmoset hybrid	5	2561±586*	12.45±1.42	0.24±0.03*	0.77±0.12*

Data (means±s.e.m.) are shown for fibre cross-sectional area (FCSA), specific tension (P_0), curvature of Hill's relationship (a/P_0) and maximal shortening velocity (V_{max}). *N*, number of fibres.

Significant differences between fibre types are indicated with an asterisk (*P<0.05).

[a]Significant differences for FCSA were found between: human type I and marmoset hybrid, human type IIA and marmoset type IIB, human type IIA and marmoset hybrid, human type IIX and marmoset type IIB, human type IIX and marmoset hybrid, human hybrid and marmoset type IIB, human hybrid and marmoset hybrid, and marmoset type IIB and marmoset hybrid.

[b]Significant differences for a/P_0 were found between: marmoset type IIB fibres and all human fibre types (I, IIA, IIX and hybrid), and marmoset hybrid and all human fibre types (I, IIA, IIX and hybrid).

[c]Significant differences for V_{max} were found between: human type I fibres and all other fibre types, and human type IIA and marmoset type IIB.

non-human primate skeletal muscle. Our study shows that skeletal muscles of the small marmoset primate express MHC IIB, like small rodents such as mouse and rat. Comparison of our findings on marmoset muscles with those reported in the literature for VL and GM of other primates reveals some similarities and differences. Overall, small primates have relatively more fast fibres than large primates (Ariano et al., 1973; Petter and Jouffroy, 1993; Fitts et al., 1998; Jouffroy et al., 1999; Myatt et al., 2011; Feng et al., 2012) (Table 3). Myatt et al. (2011) reported fibre-type distributions in one orangutan (GM, *Pongo abelii*) and two chimpanzees (GM, *Pan troglodytes*). Orangutan GM had a muscle fibre-type distribution similar to that of humans, and chimpanzee GM had more fast-type muscles fibres. In rhesus macaque (*Macaca mulatta*), both VL and GM consist mainly of fast muscle fibres (Fitts et al., 1998; Jouffroy et al., 1999). In green vervet monkeys (*Chlorocebus aethiops sabaeus*), VL consists of an even higher percentage of fast fibres (Feng et al., 2012). In the bushbaby (*Galago senegalensis*), VL and GM mainly consist of fast glycolytic muscle fibres (Ariano et al., 1973), similar to the values reported in the present study for marmoset muscle. The smallest primate of which the muscle fibre-type distribution has been studied is the grey mouse lemur (*Microcebus murinus*), of which the VL contained only fast muscle fibres (Petter and Jouffroy, 1993). Taken together, our findings on muscle fibre-type distributions in marmoset seem to be in line with values reported in the literature for other primates. In smaller primate species, VL and GM consist of relatively more fast muscle fibres than in larger primate species. However, whether other primates also express type IIB MHC remains to be determined.

An intriguing question is why type IIB MHC is abundantly expressed in leg muscles of marmosets but not in adult human leg muscles (Smerdu et al., 1994; Ennion et al., 1995; Pereira Sant'Ana et al., 1997; Wu et al., 2000; Horton et al., 2001). Type IIB MHC mRNA expression in human skeletal muscle occurs only at very low levels; IIB MHC mRNA expression is upregulated only in cases of severe muscle degeneration/regeneration, but protein levels remain undetectable (Harrison et al., 2011). The lack of IIB MHC expression in human skeletal muscle is due to specific, key promoter sequences of the human gene which differ from those of the mouse IIB MHC gene (Harrison et al., 2011). These sequences prevent binding of transcription factors (i.e. serum response factor and myocyte enhancer factor 2), which enhance transcription of IIB MHC. In addition, post-transcriptional control also may play a role (Harrison et al., 2011). It has been suggested that the *F–V* characteristics of IIB muscle fibres may be incompatible with the biomechanical constraints of larger muscles (Pereira Sant'Ana et al., 1997; Allen et al., 2001). If this applies to the order of primates, it remains to be determined which other primate species express MHC IIB like the marmoset and which do not. Comparison of myosin IIB expression levels and sequence differences in the MHC promoter will allow determination of the extent to which the ability to express MHC IIB relates to primate body size. Such insight will improve our understanding of evolutionary development of the musculo-skeletal system.

Muscle power-generating capacity: marmoset versus other (primate) species

Marmoset *in vivo* mean power output (both $P_{BMS,mean}$ and $P_{MMS,mean}$) is about twice as high as that of human. Other primates seem to have the ability to generate an even higher $P_{BMS,mean}$ or $P_{MMS,mean}$ during push-off in a vertical jump. For instance, for the

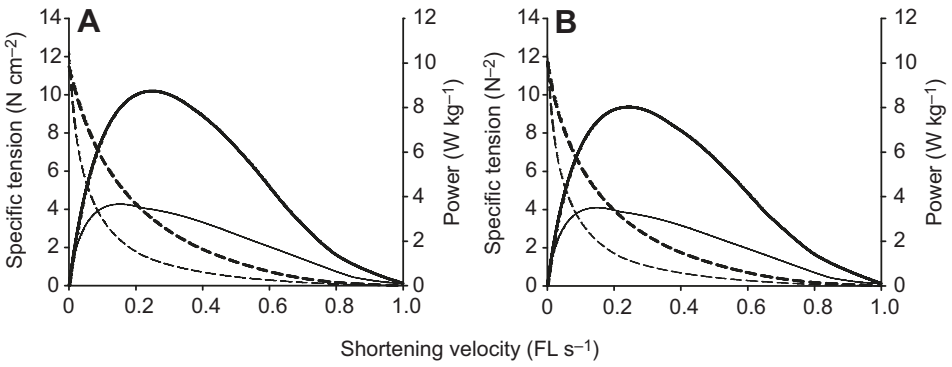

Fig. 3. Force–velocity and power–velocity curves of marmoset and human muscles. Muscle mass-specific peak power ($P_{MMS,peak}$) was derived from the whole-muscle *F–V* relationship, which was estimated by adding at each velocity the force output of the relative fractions of different fibre types. (A,B) *F–V* (dashed) and *P–V* (solid) curve of marmoset (thick) and human (thin) VL (A) and GM (B). FL, fibre length.

Table 3. Literature overview of fibre-type distribution in different (primate) species

Species	Author	Mass (kg)	Muscle	Type I	Type II				Method
					A	B	X	Hybrid	
Human (*Homo sapiens*)	Green et al., 1981	79.1	VL	46.3	44.3		8.9		ATPase
			GM	49.4	42.8		6.6		
Chimpanzee (*Pan troglodytes*)[a]	Myatt et al., 2011	56/62	GM	14/16	83/82				MAb
Orangutan (*Pongo abelii*)	Myatt et al., 2011	42	GM	47	51				MAb
Rhesus macaque *(Macaca mulatta)*	Fitts et al., 1998	9.4	GM	23	24		49	4	MAb
	Jouffroy et al., 1999	5–6	VL	15	85				MAb
			GM	22	78				
Green vervet monkey (*Chlorocebus aethiops sabaeus*)	Choi et al., 2012	5.6	VL		79			21	SDS-PAGE
	Feng et al., 2012	5.6	VL	6	78	16			ATPase
Marmoset (*Callithrix jacchus*)	Present study	0.34	VL Dist	0.1		68.9		31.0	MAb
			VL Prox	1.1		56.5		42.4	
			GM Dist	8.6		48.0		43.4	
			GM Prox	9.7		40.6		49.7	
Bushbaby (*Galago senegalensis*)	Ariano et al., 1973	0.25–0.31[b]	VL		13	87			ATPase
			GM	15	29	56			
Grey mouse lemur (*Microcebus murinus*)	Petter and Jouffroy, 1993		VL		47.3	52.7			ATPase
			GM	21.8	37.8	40.3			

MAb, monoclonal antibodies; Dist, distal; Prox, proximal.
[a]Chimpanzee data are for females/males.
[b]Values for mass are not reported by Ariano et al. (1973) so values displayed are from Aerts (1998).

bushbaby, power values of 171 W kg^{-1} $P_{BMS,mean}$ and 683 W kg^{-1} $P_{MMS,mean}$ have been calculated from data in the literature (for references, see Bobbert et al., 2014). These values are 6.3-fold and 3.7-fold those reported for humans, respectively, and 3.3- and 1.6-fold those shown here for the marmoset. Again, the question arises of whether this difference in *in vivo* power-generating capacity is related to differences in fibre-type distribution and/or muscle fibre *F–V* characteristics. As reported above, muscle fibre-type distribution is likely to be fairly similar in marmosets and bushbabies (Ariano et al., 1973). However, to the best of our knowledge, as yet no data are available on the power-generating capacity of bushbaby muscle fibres. $P_{FMS,peak}$ of different species needs to be compared within the same experimental set-up using the same solutions in order to make definitive statements about possible differences in power-generating capacity. In this context it is interesting to note, as we did before (Bobbert et al., 2014), that the reported $P_{MMS,mean}$ delivered by marmosets and bushbabies during a vertical jump is similar to or even higher than $P_{MMS,peak}$ measured for supramaximally stimulated *in situ* rat m. gastrocnemius medialis (i.e. 530 W kg^{-1} muscle mass) at 35°C (Furrer et al., 2013).

In a recent forward simulation study, it was shown that isometric downscaling of a human musculoskeletal model to the size of a marmoset leads to a reduction in jump height from about 40 cm to about 10 cm, because the *F–V* relationship limits power and work production (Bobbert et al., 2014). The higher percentage of fast muscle fibres in smaller primates and the higher power output is one of the factors that helps us understand why real marmosets, despite their small size, actually jump higher than humans (Bobbert et al., 2014).

Further research is warranted to uncover mechanisms underlying the differences between *in situ* and *in vitro* muscle power measurements on the one hand, and an animal's jumping performance *in vivo* on the other hand. As a first next step, development of a realistic musculoskeletal model of the marmoset seems indicated.

Conclusions

The results of this study show that the 2-fold difference observed in *in vivo* mean power output (both $P_{BMS,mean}$ and $P_{MMS,mean}$) between marmosets and humans is attributable to differences in muscle fibre contractile properties. This is explained by marmosets having a higher percentage of fast muscle fibres. Surprisingly, marmosets seem to be unique amongst primates in that they have a high percentage of type IIB fibres in leg muscles. To the best of our knowledge, type IIB MHC expression in leg muscles fibres has not been reported yet for any other primate species.

MATERIALS AND METHODS

Marmoset muscle biopsies

Muscle biopsies were obtained within 15 min post mortem from four adult common marmosets (*C. jacchus*, three males and one female, age 3.5± 0.5 years, mass 0.343±0.035 kg).

Animals were housed in pairs in spacious cages enriched with climbing attributes. Intensive veterinary supervision was present during the study. The facility was under controlled conditions of humidity (>60%), temperature (22–26°C) and lighting (12 h light:12 h dark cycles). The marmosets were fed daily with pellet chow for New World monkeys (Special Diet Services, Witham, Essex, UK), enriched with peanuts, biscuits, fruit, vegetables and an occasional mealworm. Water was available *ad libitum*. Animals were enrolled in another project at the Biomedical Primate Research Centre (BPRC) regarding the effects of Parkinson's disease. According to the Dutch law on animal experimentation, the study was approved by the Ethical Review Committee of the BPRC. For the project regarding effects of Parkinson's disease, the animals had received behavioural training and their behaviour had been monitored weekly. Animals were killed in an early stage of the induction of Parkinson's disease and were considered as lightly affected or not affected by the disease, as judged from behavioral measurements. Furthermore, post mortem, no sign of neurodegeneration was observed by immunohistochemical staining of the target brain tissue.

For measurement of muscle fibre *F–V* characteristics, a small piece (2×2×5 mm) of muscle tissue was cut from the middle of VL. For immunohistochemical analysis, biopsies (2×2×5 mm) from VL and GM were taken from both the distal and proximal parts of each muscle.

Human muscle biopsies

Human muscle biopsies were collected from the VL of nine human subjects (age 24.3±3.9 years) who were enrolled in a previous study (Salvadego et al., 2013). All subjects had given their informed consent. The local ethical committees of the University of Primorska, Koper, Slovenia, approved obtaining the human biopsies.

The Journal of Experimental Biology (2015) 218, 2166-2173 doi:10.1242/jeb.117655

Determination of marmoset fibre-type distribution

Immunohistochemistry

Muscle biopsies were frozen and stored in liquid nitrogen until use. Sections (10 µm) were cut using a cryostat at –20°C, air dried and stored at −80°C until use. Fibre-type distribution was determined by immunohistochemical staining of MHCs. Monoclonal antibodies were used against MHC I (BA-D5, 1:1000), MHC IIA (SC-71, 1:1000), MHC IIB (F30, 1:1000) and MHC IIX (6H1, 1:200) (Developmental Studies Hybridoma Bank, The University of Iowa) (Miller et al., 1985; Schiaffino et al., 1989; Lucas et al., 2000). Secondary antibodies against mouse IgG2b (Alexa Fluor 488, 1:200), IgG1 (Alexa Fluor 488, 1:200) and IgM (Alexa Fluor 647, 1:200) (Invitrogen, USA) were used. Sections were blocked with goat serum (Invitrogen, USA) in phosphate-buffered saline (PBS) for 30 min at 25°C. Subsequently, sections were incubated with primary antibody for 60 min followed by incubation with secondary antibody incubation for 60 min, both at 25°C. Each incubation was followed by three 3 min washes in PBS with Tween (PBST) (Sigma-Aldrich, USA). Cell membranes were visualized by incubation of the sections with wheat germ agglutinin (WGA, 1:50) (Invitrogen, USA) for 20 min at 25°C followed by washing 2 times with PBST for 3 min and once with PBS for 3 min. Slides were mounted with Vectashield HardSet containing 4′,6-diamidino-2-phenylindole (DAPI). Images were captured using a CCD camera (PCO, Sensicam, Kelheim, Germany) with a 20× objective connected to a fluorescence microscope (Axiovert 200M, Zeiss, Göttingen, Germany) with image-processing software (Slidebook 4.1, Intelligent Image Innovations, Denver, CO, USA). Typical examples of staining of different MHC isoforms are displayed in Fig. 4.

Data analysis

Per biopsy, MHC expression was assessed in 500 fibres. Fibres were subdivided into type I and type IIB fibres and the following hybrid forms: mainly type IIA with IIX, mainly type IIB with IIA and IIX, and mainly type IIX with IIA.

F–V characteristics of human and marmoset skinned single muscle fibre segments

Solutions

Solutions were as described previously (Larsson and Moss, 1993; Degens and Larsson, 2007; Degens et al., 2010). Relaxing solution contained (mmol l^{-1}): MgATP 4.5, free Mg^{2+} 1, imidazole 10, EGTA 2 and KCl 100; pH was adjusted to 7.0 with KOH. Activating solution (pCa 4.5) contained, in addition to Ca^{2+} (mmol l^{-1}): MgATP 5.3, free Mg^{2+} 1, imidazole 20, EGTA 7, creatine phosphate 19.6 and KCl 64; pH 7.0.

Preparation of muscle biopsies

Muscle biopsies were cut into small bundles and immersed for 24 h in a 50% glycerol, 50% relaxing solution at 4°C. For prolonged storage, muscle fibre bundles were treated in relaxing solution with increasing sucrose concentration and were stored in 2 mol l^{-1} sucrose at −80°C until further analysis (Frontera and Larsson, 1997). Prior to analysis, biopsies were desucrosed and stored in glycerol/relaxing solution at −20°C and used within 1 month.

Preparation of skinned single muscle fibre segments

Single muscle fibre segments were prepared as described previously (Larsson and Moss, 1993; Gilliver et al., 2011). In summary, the muscle fibre bundles were placed for 20 min in relaxing solution containing 1% Triton X-100 to permeabilize the membranes and sarcoplasmic reticulum. Single fibre segments were extracted from the bundles in the relaxing solution and mounted in a permeabilized fibre test system (400, Aurora Scientific Inc., Aurora, ON, Canada). Each fibre segment was then attached with nylon thread to fine insect pins mounted on a force transducer (403A, Aurora Scientific Inc.) and motor arm (312C, Aurora Scientific Inc.). The force transducer and motor arm were mounted over a moveable plate containing a set of wells, with a glass base. The plate was cooled to 15°C and the set-up (plate, force transducer and motor) was mounted on an inverted microscope (Olympus IX71, Tokyo, Japan). The average sarcomere length

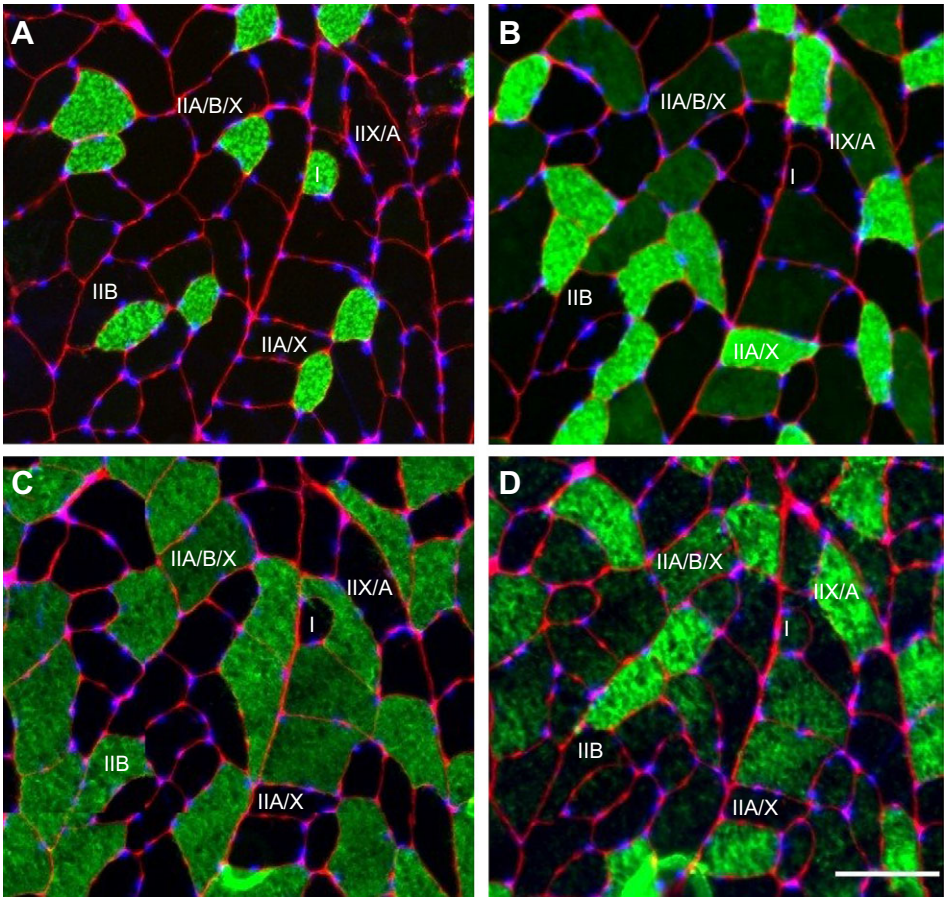

Fig. 4. Immunofluorescence images of marmoset GM. Serial sections were stained (green) with monoclonal antibodies against MHC I (A), MHC IIA (B), MHC IIB (C) and MHC IIX (D). Nuclei were stained with DAPI (blue) and cell membranes were stained with wheat germ agglutinin (WGA, red). Scale bar indicates 100 µm.

The Journal of Experimental Biology (2015) 218, 2166-2173 doi:10.1242/jeb.117655

was determined using a Fourier transformation of the sarcomere striation pattern (900A, Aurora Scientific Inc.). Fibre segment length was adjusted to optimum sarcomere length.

Prior to the *F–V* measurements, the diameter of the muscle fibre segment was assessed at three locations along its length while being submerged in relaxing solution. Fibre width was measured in liquid, assuming a circular circumference. Fibre diameter measured in air and liquid are closely correlated (R^2≈0.90) (Degens and Larsson, 2007). In order to be able to compare data of this study with those from others that measured fibre CSA while the fibre was suspended in air, values for FCSA, P_0 and P_{peak} were multiplied by 1.25 (Degens and Larsson, 2007). No correction was made for swelling of the fibre during skinning. Fibre segment length was measured to the nearest 0.01 mm.

Determination of marmoset optimal sarcomere length

For marmoset muscle fibre segments, optimum sarcomere length had to be determined. Optimum sarcomere length was determined in five fibre segments by repeatedly moving the segments from relaxing to activating solution at randomly determined sarcomere lengths around 2.6 µm. To correct for force decrements due to switching between relaxing and activating solution, a least squares linear regression line was fitted to the force data over activations. The slope of this line shows the average decrement in force after a transfer of the muscle fibre from relaxing to activating solution. Using this slope, force data were corrected for this force decrement.

Determination of contractile properties

To determine contractile properties of muscle fibre segments, a fibre was transferred from relaxing solution into activating solution (pCa^{2+} 4.5 mmol l^{-1}) and, once an isometric force plateau was reached, subjected to four sequences of four isotonic shortening steps, as described elsewhere (Bottinelli et al., 1996) (Fig. 5A,B). During each sequence, the lever arm moved at a speed sufficient to maintain muscle fibre force at a predetermined percentage of its isometric force. Each sequence consisted of four force steps (each 150 ms long). Force levels ranged from 0.90 to 0.05 of maximal isometric force. Following each sequence, fibre segments were rapidly re-stretched to their original lengths while still in activating solution. This rapid re-stretch was shown to help maintain sarcomere integrity and maintain stability of the muscle fibre segments during long series of contractions (Brenner, 1983). During isotonic shortening protocols, maximal shortening of fibre segments was less than 20% of the initial length.

Determination of MHC composition of skinned single muscle fibre segments

After determination of muscle fibre contractile characteristics, fibre segments were stored in sodium dodecyl sulphate (SDS) sample buffer and kept at −20°C if MHC typing was performed within a week or at −80°C if this occurred more than a week after the contractile measurements. All chemicals were obtained from Sigma-Aldrich (The Netherlands) unless stated otherwise. Before analysis, samples were denatured at 100°C for 2 min. MHCs were separated using SDS-PAGE as previously described (Larsson and Moss, 1993; Degens and Larsson, 2007; Degens et al., 2010). Briefly, samples were separated for 27 h at 275 V on a 7% polyacrylamide gel containing 35% glycerol. Gels were stained using a Silverstain Plus kit (Bio-Rad, Hemel Hempstead, UK). MHC isoforms were identified based on migration distances of the proteins. Human fibres were subdivided into pure type I, pure IIA, pure IIX and hybrid fibres. Marmoset fibres were subdivided in pure type IIB fibres and hybrid fibres. Single bands were considered to identify pure type IIB fibres while multiple bands identified hybrid fibres.

Data analysis

Force and length data were analysed as described previously (Gilliver et al., 2009). Force was averaged over the last 50–120 ms. Shortening velocity was calculated by fitting a least squares linear regression line to the same last 50–120 ms of the length trace. The force and velocity data points were used to fit a hyperbolic Hill equation (Hill, 1938) using a non-linear least-squares regression (Fig. 5C). This fit yielded Hill constants *a* and *b*, which together with specific tension (P_0 in N cm^{-2}), were used to estimate maximal unloaded shortening velocity of muscle fibre segments [V_{max} in fibre lengths (FL) s^{-1}]. The fraction (M) of maximal force (P_0) or shortening velocity (V_{max}) at which muscle fibre peak power ($P_{FMS,peak}$) was generated was derived from Hill's equation as:

$$M = (\sqrt{1 + G - 1})/G,$$

where G is P_0/a or V_{max}/b (Woledge et al., 1985). $P_{FMS,peak}$ was calculated as $M^2 \times P_0 \times V_{max}$.

Data were rejected if during the isotonic protocol the isometric force decreased by more than 10% and/or sarcomere length at rest changed by more than 0.1 µm and/or if R^2 for V_{max} was <0.96 when fitting Hill's equation to the data.

Human and marmoset total VL and GM peak power estimates

Using relative proportions of muscle fibre types in VL and GM and their contractile characteristics for both humans and marmosets, $P_{MMS,peak}$ (in W kg^{-1} muscle) was estimated for whole VL and GM. For fibre-type distributions of human VL and GM, we used data reported in the literature (Green et al., 1981). $P_{MMS,peak}$ was derived from the whole-muscle *F–V* relationship, which was estimated by adding at each velocity the force output of the relative fractions of different fibre types.

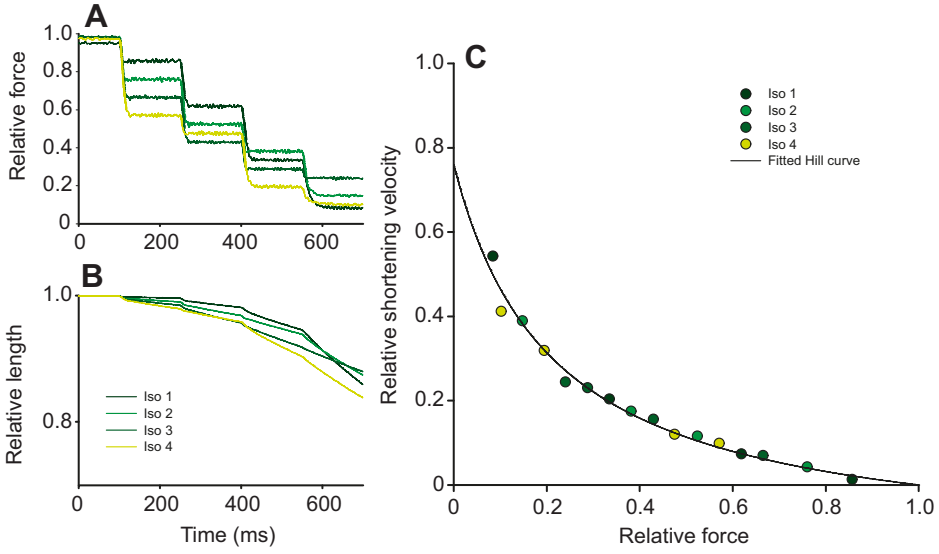

Fig. 5. Typical examples of muscle fibre segment *F–V* measurements. (A) Muscle fibre relative force during the four isotonic shortening sequences. (B) Muscle fibre relative length during the four isotonic shortening sequences. (C) Using the average force (over the last 50–120 ms) and shortening velocity (incline of fibre fragment length in the same period) of each of the 16 isotonic force levels, a Hill curve was fitted.

Statistics

To test whether the muscle fibre-type distribution differed between muscles and regions, data were submitted to a 2×2 mixed model design ANOVA with between-subjects factor muscle (VL and GM) and within-subjects factor regions (distal and proximal).

ANOVA and Student's *t*-tests were performed to test whether *F–V* outcome variables were significantly different between muscle fibre types.

All data were analysed using SPSS statistics. Values are presented as means±s.e.m. Differences were considered significant at $P<0.05$.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

R.L.C.P. conceived, designed and executed the experiments, analysed and interpreted the results, and drafted and revised the article. H.D. conceived and designed the experiments, analysed and interpreted the results, and drafted and revised the article. J.P.M. executed some of the experiments and interpreted results. G.M.J.d.W. designed and performed the assays for protein analyses and interpreted results. I.H.C.H.M.P. conceived and designed the experiments. M.F.B. conceived and designed the experiments, analysed and interpreted the results, and drafted and revised the article. R.T.J. conceived and designed the experiments, analysed and interpreted the results, and drafted and revised the article.

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