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

Rogier L.C. Plas[1,2], Hans Degens[2], J.P. (Peter) Meijer[1,2], G.M.J. de Wit[1], Ingrid H.C.H.M. Philippens[3], Maarten F. Bobbert [1], Richard T. Jaspers[1]

[1]MOVE Research Institute Amsterdam, Faculty of Human Movement Sciences, VU University Amsterdam, Van der Boechorstraat 9, NL-1081 BT Amsterdam, The Netherlands.

[2]School of Healthcare Science, Cognitive Motor Function Research Group, School of Healthcare Science, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, United Kingdom.

[3]Department of Immunobiology, Division Neuropathology, Biomedical Primate Research Centre, Rijswijk, The Netherlands

Contact information:

Richard T. Jaspers
MOVE Research Institute Amsterdam
Faculty of Human Movement Sciences
VU University Amsterdam
Van der Boechorstraat 9
1081 BT Amsterdam
THE NETHERLANDS
Phone: +31 20 59 88463
E-mail: r.t.jaspers@vu.nl

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Abstract

The muscle mass specific mean power output ($P_{MMS,mean}$) during push-off in jumping is in marmosets (*Callithrix jacchus*) 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) fiber type distribution was assessed using fluorescent immunohistochemistry. In single fibers from four marmoset and nine human VL biopsies the force-velocity characteristics were determined.

Marmoset VL contained almost exclusively fast muscle fibers (>99.0%), of which 63% were type IIB and 37% hybrid fibers, fibers containing multiple myosin heavy chains. GM contained 9% type I fibers, 44% type IIB and 47% hybrid muscle fibers. The proportions of fast muscle fibers 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 fibers was substantially flatter than that of human type I, IIA, IIX and hybrid fibers resulting in substantially higher muscle fiber mass specific peak powers ($P_{FMS, peak}$). Muscle mass specific peak power output ($P_{MMS,peak}$) of marmoset whole VL and GM, estimated from their fiber type distributions and force-velocity characteristics were more than twice the estimates for the corresponding human muscles.

Since the relative difference in estimated $P_{MMS,peak}$ between marmoset and human is similar to that $P_{MMS,mean}$ 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, human, comparative physiology, muscle mass specific power, force-velocity relationship, immunohistochemistry, marmoset, muscle fiber type distribution, myosin heavy chain, single muscle fiber, skinned muscle fiber

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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*) 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*; 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 fibers and/or differences in force-velocity (F-V) characteristics of the muscle fibers. In general, the major leg muscles consist of higher percentages of fast type fibers 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 fiber 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 fiber types (fibers 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 fiber F-V characteristics within small primates. To trace differences in *in vivo* mechanical output to differences in muscle contractile properties it is not sufficient to know muscle fiber type distribution, one also needs information about muscle fiber F-V characteristics. Some reports on F-V characteristics of skinned muscle fiber segments from primates suggest that the maximal shortening velocity (V_{max}) of type IIA fibers 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 is no data available on the F-V characteristics of fast glycolytic muscle fibers (type IIB and IIX) of the smallest primates.

The aim of this study was to investigate whether the two-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, fiber 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 fiber 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

Fiber type distribution of marmoset VL and GM

Immunohistochemical staining of cross-sections of marmoset VL biopsies showed that a very small number of muscle fibers 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 fibers were pure type IIB fibers, while the remaining fibers were hybrid fibers, expressing multiple MHCs.

In GM, the proportion of type I fibers (8.6±1.9% in distal region and 9.7±5.3% in proximal region) was also very small. In GM, 48.0±4.5% (distal) and 40.6±12.37% (proximal) of the muscle fibers expressed type IIB MHC exclusively. The proportions of the different fiber 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 fiber segments. Force deterioration due to transferring the fiber from relax 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 fibers

Using electrophoresis, 77 of all 82 marmoset muscle fibers were identified as type IIB fibers. As immunohistochemical staining showed that type IIB MHC is the only isoform expressed in muscle fibers singularly and hybrid fibers always contain type IIA and IIX MHC, we considered marmoset muscle fiber segments expressing one MHC band as pure type IIB muscle fibers (see figure 2 for three typical examples in combination with a rat soleus homogenate). Of the 52 human fibers, 6 consisted of type I MHC, 27 of only type IIA MHC, 9 of only type IIX MHC and 10 contained multiple MHC's.

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

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

estimates for marmoset type I muscle fibers. Our calculations yielded whole muscle $P_{MMS,peak}$ of marmoset VL and GM of more than twice the corresponding values of human: 8.7 W kg^{-1} for marmoset VL vs. 3.7 W kg^{-1} for human VL, and 8.0 W kg^{-1} for marmoset GM vs. 3.5 W kg^{-1} for human GM (Table 1 and Fig. 3).

Discussion

The aim of this study was to investigate whether the two-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 fibers (>99.0%), of which the majority express pure type IIB MHC (63%). Almost all other muscle fibers express multiple fast myosin heavy chain isoforms in different proportions. F-V characteristics differed among fiber types. Significantly higher $P_{FMS,peak}$ were found in marmoset type IIB and hybrid fibers than in human type I, IIA, IIX and hybrid fibers, mainly because of 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 fiber segments of marmosets and humans with fiber type distribution in GM and VL of the marmoset and reported fiber 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 the push off of a vertical jump. $P_{BMS,mean}$ in marmoset was 1.9 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 than humans is mainly caused by a larger proportion of fast muscle fibers 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 fiber type distribution from the proximal and distal parts. Theoretically, it is possible that muscle fiber types were not homogeneously distributed throughout the muscles. However, muscle fiber type distribution in the analyzed sections was highly uniform and no difference was shown between proximal and distal biopsies. It is therefore unlikely that our estimates are biased due to measurement errors caused by the location of biopsies. Second, biopsies of marmosets were taken post mortem, whereas

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those of humans were sampled *in vivo*. However, since marmoset biopsies were taken within 15 minutes post mortem it seems highly unlikely that the results 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 $P_{MMS,peak}$ for different fiber types is determined at 15°C whilst *in vivo* muscles operate at about 37°C. Making a fair translation from skinned single fiber segment force characteristics to whole muscle force characteristics *in vivo* requires a comparative study into the temperature dependence of single muscle fibers. 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 fiber F-V characteristics involves permeabilization of muscle fibers and, whilst suspended in relaxing solution, swelling of the fiber (Degens and Larsson, 2007). Both the skinning and the swelling of the muscle fiber 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 since a direct comparison between human and marmoset fiber F-V characteristics has been made with one and the same method and set-up. Because the protocol, solutions used as well as 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 fiber type distributions and fiber F-V characteristics, are twice as high in marmosets as in humans. These differences are mainly attributable to differences in the MHC expression between both species.

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

To the best of our knowledge the present study is the first that distinguishes between fast type MHC isoforms expressed in 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 fibers 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. (Myatt et al., 2011) reported fiber type distributions in one orangutan

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(GM, [*Pongo abelii*]) and two chimpanzees (GM, [*Pan troglodytes*]). Orangutan GM had a muscle fiber type distribution similar as those of humans and chimpanzee GM had more fast type muscles fibers. In rhesus macaque (*Macaca mulatta*), both VL and GM consist mainly of fast muscle fibers (Fitts et al., 1998; Jouffroy et al., 1999). In Green vervet monkeys (*Chlorocebus aethiops sabaeus*), VL consists of an even higher percentage of fast fibers (Feng et al., 2012). In the bushbaby (*Galago senegalensis*), VL and GM mainly consist of fast glycolytic muscle fibers (Ariano et al., 1973), similar to the values reported in the present study for marmoset muscle. The smallest primate of which the muscle fiber type distribution has been studied is the Gray mouse lemur (*Microcebus murinus*), of which the VL contained only fast muscle fibers (Petter and Jouffroy, 1993). Taken together, our findings on muscle fiber type distributions in marmoset seem to be in line with values reported in literature for other primates. In smaller primate species, VL and GM consist of relatively more fast muscle fibers 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; 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 quantity; IIB MHC mRNA expression is upregulated only in case 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 fibers may be incompatible with the biomechanical constraints of larger muscles (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 do 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.

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Muscle power generating capacity: marmoset vs 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 bushbaby, powers 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 whether this difference in *in vivo* power generating capacity is related to differences in fiber type distribution and/or muscle fiber F-V characteristics. As reported above, muscle fiber type distribution is likely to be fairly similar in marmoset and bushbaby (Ariano et al., 1973). However, to the best of our knowledge, as yet no data is available on power generating capacity of bushbaby muscle fibers. $P_{FMS,peak}$ 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, like we did before (Bobbert et al., 2014), that the reported $P_{MMS,mean}$ delivered by marmosets and bushbabies during a vertical jump is similar or even higher than $P_{MMS,peak}$ measured for supramaximally stimulated in situ rat m. gastrocnemius medialis (i.e. 530 W per kg muscle mass) at 35 °C (Furrer et al., 2013).

In a recent forward simulation study it has been 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 force-velocity relationship limits power and work production (Bobbert et al., 2014). The higher percentage of fast muscle fibers in smaller primates and the higher power output is one of the factors that help 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 two-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 fiber contractile properties. The latter is explained by marmosets having a higher percentage of fast muscle fibers. Surprisingly, marmosets seem to be unique primates

in that they have a high percentage of type IIB fibers in leg muscles. To the best of our knowledge, type IIB MHC expression in leg muscles fibers has not been reported yet for any other primate species.

Materials and Methods

Marmoset muscle biopsies

Muscle biopsies were obtained from four adult Common Marmosets (*Callithrix jacchus*, three males and one female, age 3.5±0.5 years, mass 0.343±0.035 kg) within 30 minutes after sacrifice.

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 hour light/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 the animals had received behavioral training and their behavior had been monitored weekly. Animals were sacrificed in an early stage of the induction of Parkinson's disease and were considered as lightly or not affected by Parkinson, as judged from behavioral measurements. Furthermore, post mortem no sign of neurodegeneration was observed by immunohistochemical staining of the target brain tissue.

For measurements of muscle fiber F-V characteristics, a small piece (2x2x5 mm) of muscle tissue was cut from the middle of *m. vastus lateralis*. For immunohistochemical analysis, biopsies (2x2x5 mm) from *m. vastus lateralis* and *m. gastrocnemius medialis* were taken from both the distal and proximal parts of each muscle.

Human muscle biopsies

Human muscle biopsies were collected from the *m. vastus lateralis* of 9 human subjects (age 24.3±3.9 y) who were enrolled in a previous study (Salvadego et al., 2013). All subjects had

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given their informed consent. The local ethical committees of the University of Primorska, Koper, Slovenia, approved obtaining the human biopsies.

Determination of marmoset fiber 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. Fiber type distribution was determined by immunohistochemical staining of myosin heavy chains (MHCs). Monoclonal antibodies were used against myosin heavy chain I (MHCI (BA-D5, 1:1000), MHCIIA (SC-71, 1:1000), MHCIIB (F30, 1:1000) and MHCIIX (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 minutes at 25 °C. Subsequently, sections were incubated with primary antibody for 60 minutes followed by incubation with secondary antibody incubation for 60 minutes, both at 25°C. Each incubation was followed by three 3-minute washes in phosphate buffered saline with tween (PBST) (Sigma-Aldrich, USA). Cell membranes were visualized by incubation of the sections with Wheat Germ Agglutinin (1:50) (Invitrogen, USA) for 20 minutes at 25°C and then washed 2 times with PBST for 3 minutes and once with PBS for 3 minutes. Slides were mounted with Vectashield HardSet containing 4',6-diamidino-2-phenylindole (DAPI). Images were captured using a CCD camera (PCO; Sensicam, Kelheim, Germany) at 20x objective connected to a fluorescent microscope (Axiovert 200M; Zeiss, Göttingen, Germany) with image processing software (Slidebook 4.1; Intelligent Image Innovations, Denver, Colorado). Typical examples of stainings of different MHC isoforms are displayed in Fig. 4.

Data analysis

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

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F-V characteristics of human and marmoset skinned single muscle fiber segments

Solutions

Solutions were as described previously (Larsson and Moss, 1993; Degens and Larsson, 2007; Degens et al., 2010). Relaxing solution contained (mM): 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+} (mM): 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 24h in a 50% relaxing, and 50% glycerol solution (glycerol/relax) at 4°C. For prolonged storage, muscle fiber bundles were treated in relax solution with increasing sucrose concentration and were stored in 2 M sucrose at -80°C until further analysis (Frontera and Larsson, 1997). Prior to analysis, biopsies were desucrosed and stored in glycerol/relax solution at -20°C and used within 1 month.

Preparation of skinned single muscle fiber segments

Single muscle fiber segments were prepared as described previously (Larsson and Moss, 1993; Gilliver et al., 2011). In summary, the muscle fiber bundles were placed for 20 minutes in relaxing solution containing 1% Triton X-100 to permeabilize the membranes and sarcoplasmic reticulum. Single fiber segments were extracted from the bundles in the relaxing solution and mounted in a permeabilized fiber test system (400, Aurora Scientific Inc., Aurora, Ontario, Canada). Each fiber segment was then attached with nylon thread to fine insect pins mounted onto a force transducer (403A, Aurora Scientific Inc., Aurora, Ontario, Canada) and motor arm (312C, Aurora Scientific Inc., Aurora, Ontario, Canada). 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 was determined using a Fourier transformation of the sarcomere striation pattern (900A, Aurora Scientific Inc., Aurora, Ontario, Canada). Fiber segment length was adjusted to optimum sarcomere length.

Prior to the F-V measurements, the diameter of the muscle fiber segment was assessed at three locations along its length while being submerged in relaxing solution. Fiber width was measured in liquid assuming a circular circumference. Fiber diameter measured in air and liquid are closely correlated ($R^2 \approx 0.90$) (Degens and Larsson, 2007). In order to be able to compare data of this study with those from others who measured fiber CSA while the fiber 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 fiber during skinning. Fiber segment length was measured to the closest 0.01 mm.

Determination of marmoset optimal sarcomere length

For marmoset muscle fiber segments, optimum sarcomere length had to be determined. Optimum sarcomere length was determined in five fiber segment 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 fiber 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 fiber segments, a fiber was transferred from relaxing solution into activating solution (pCa^{2+} 4.5 mM) 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 fiber 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, fiber 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 fiber segments during long series of contractions (Brenner, 1983). During isotonic shortening protocols, maximal shortening of fiber segments was less than 20% of the initial length.

Determination of myosin heavy chain composition of skinned single muscle fiber segments

After determination of muscle fiber contractile characteristics, fiber 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 minutes. MHCs were separated using SDS-PAGE as previously described (Larsson and Moss, 1993; Degens and Larsson, 2007;

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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 fibers were subdivided in pure type I, pure IIA, pure IIX and hybrid fibers. Marmoset fibers were subdivided in pure type IIB fibers and hybrid fibers. Single bands were considered to identify pure type IIB and fibers while multiple bands identified hybrid fibers.

Data analysis

Force and length data were analyzed 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 fiber segments (V_{max} in fiber-lengths (FL) s^{-1}). The fraction (M) of maximal force (P_0) or shortening velocity (V_{max}) at which muscle fiber 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 $M^2 \times P_0 \times V_{max}$.

Data was 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 fiber types in VL and GM and their contractile characteristics for both human and marmoset, $P_{MMS,peak}$ (in W/kg muscle) was estimated for whole VL and GM. For fiber 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 velocity-force (V-F) relation, which was estimated by adding at each velocity the force output of the relative fractions of different fiber types.

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Statistics

To test whether the muscle fiber type distribution differed between muscles and regions, data was submitted to a 2x2 mixed model design ANOVA with between subjects factor muscle (VL and GM) and within subjects factor regions (distal and proximal).

Analysis of variance (ANOVA) and Student's t-tests were performed to test whether F-V outcome variables were significantly different between muscle fiber types.

All data were analyzed using SPSS statistics. Values are presented as mean±standard error of the mean (s.e.m.). Differences were considered significant at $p<0.05$.

List of symbols and abbreviations

a/P_0	Curvature of Hill's relationship
FCSA	Fiber Cross Sectional Area
FL	Fiber Length
F-V	Force-Velocity
GM	*m. gastrocnemius medialis*
MHC	Myosin Heavy Chain
$P_{BMS,average}$	Body mass specific average power
$P_{FMS,peak}$	Fiber mass specific peak power
$P_{MMS,average}$	Muscle mass specific average power
$P_{MMS,peak}$	Muscle mass specific peak power
P_0	Specific tension
s.e.m.	standard error of the mean
V-F	Velocity-Force
V-P	Velocity-Power
VL	*m. vastus lateralis*
V_{max}	Maximal shortening velocity

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Competing interests

The authors declare to have no competing financial interests.

Author contributions

R.L.C.P. conceived, designed and executed the experiments, analyzed and interpreted the results, and drafted and revised the article.

H.D. conceived and designed the experiments, analyzed and interpreted the results, and drafted and revised the article.

J.P.M. executed part of the experiments, interpreted results.

G.M.J.d.W designed and performed the assays for protein analyses, interpreted results.

I.H.C.H.M.P. conceived and designed the experiments.

M.F.B. conceived and designed the experiments, analyzed and interpreted the results, and drafted and revised the article.

R.T.J. conceived and designed the experiments, analyzed and interpreted the results, and drafted and revised the article.

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Figures

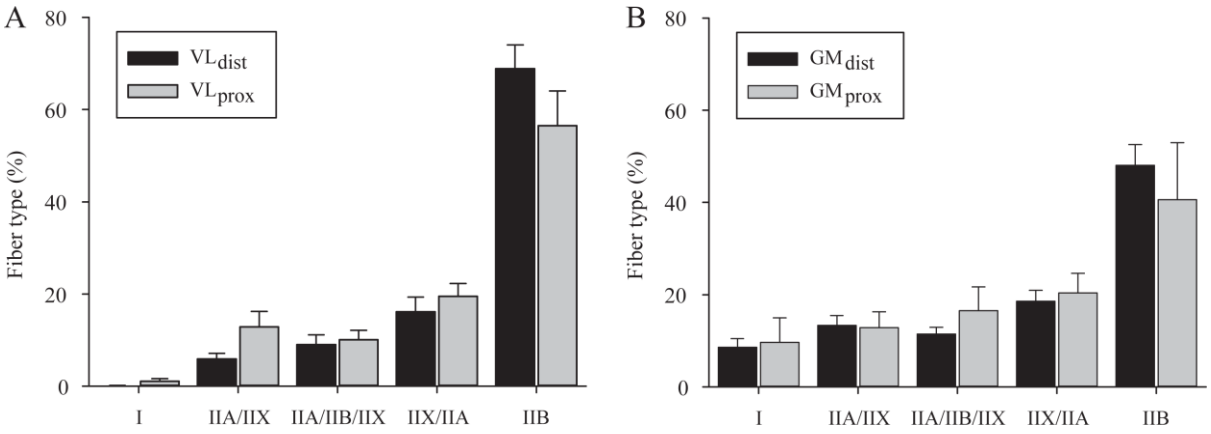

***Fig. 1* – Muscle fiber type distribution in Marmoset leg muscles (*N*=4).** (A) Fiber type distribution in distal and proximal regions of VL (VL_{dist} and VL_{prox}, respectively). (B) Fiber type distribution in distal and proximal regions of GM (GM_{dist} and GM_{prox}, repectively). Data are mean ± s.e.m..

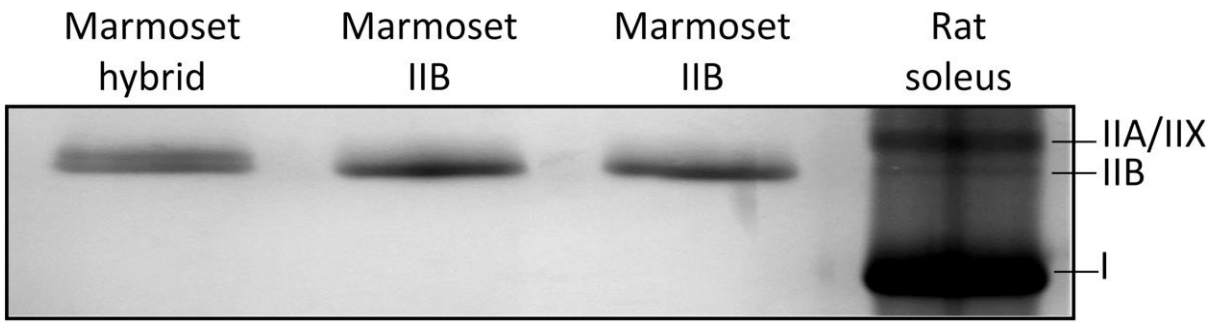

***Fig. 2* – SDS-PAGE of three marmoset single muscle fibers and a rat soleus homogenate.** Example of myosin heavy chain (MHC) composition in three single muscle fiber segment analysed using SDS-PAGE. The left fiber segment is a hybrid fiber and the middle two sements are pure type IIB fibers. The right lane contains a rat m. soleus homogenate as reference.

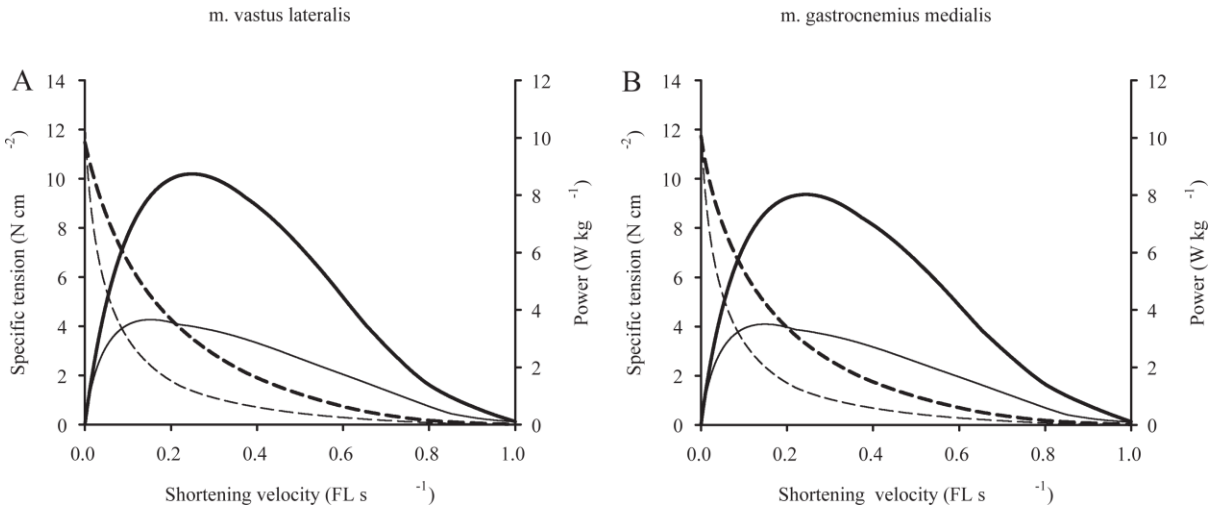

***Fig. 3* – Velocity-Force (V-F) and Velocity-Power (V-P) curve of marmoset and human.** $P_{MMS,peak}$ was derived from the whole muscle V-F relation, which was estimated by adding at each velocity the force output of the relative fractions of different fiber types. (A) V-F (dashed) and V-P (solid) curve of marmoset (thick) and human (thin) GM. (B) V-F (dashed) and V-P (solid) curve of marmoset (thick) and human (thin) VL.

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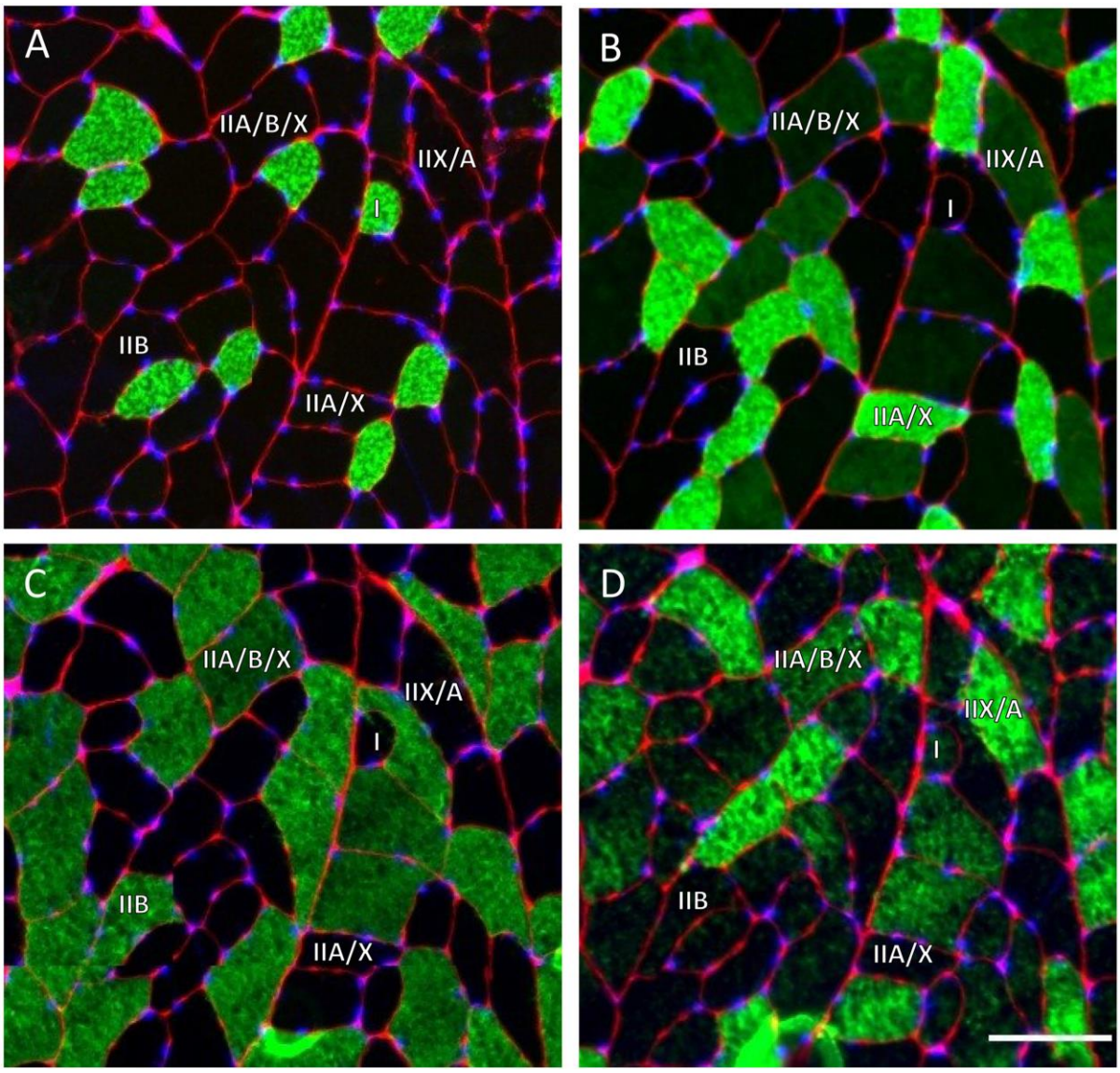

***Fig. 4* – Immunofluorescent images of marmoset *m. gastrocnemius medialis*.** Serial sections were stained (green) with monoclonal antibodies against MHC I (A), MHC IIA (B), IIB (C) and IIX (D). Nuclei were stained with DAPI (blue) and cell membranes were stained with WGA (red). Scale-bar indicates 100 µm.

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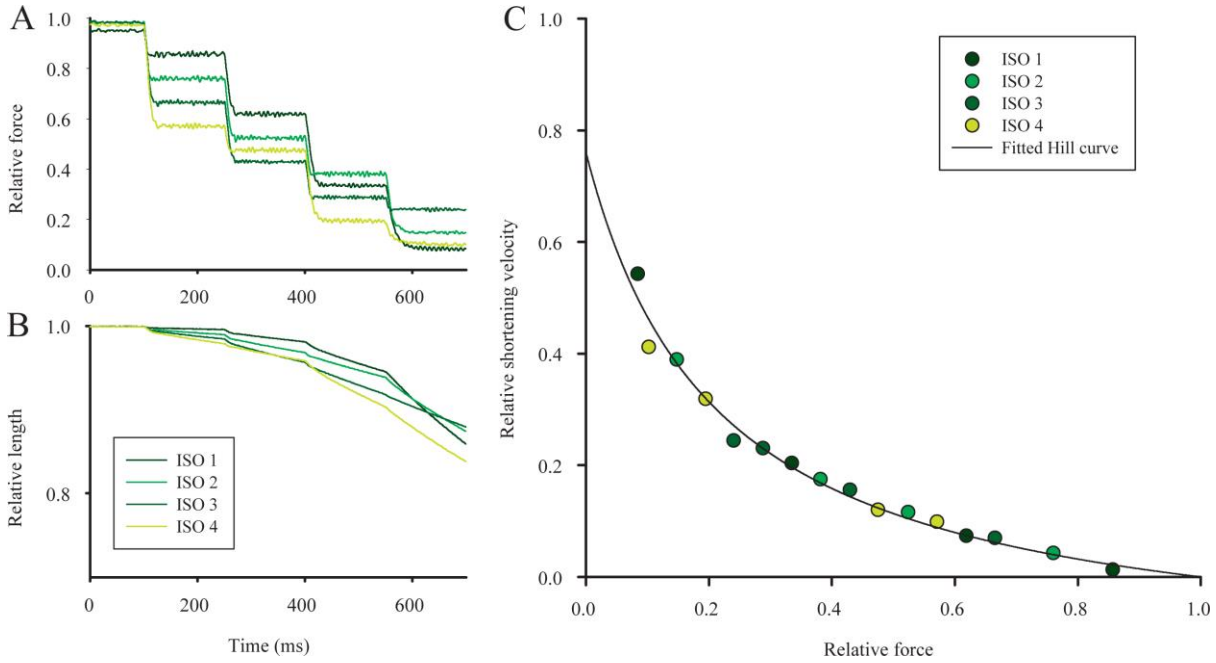

***Fig. 5* – Typical examples of muscle fiber segment F-V measurements.**

(A) Muscle fiber relative force during the four isotonic shortening seqeunces. (B) Muscle fiber relative length during the four isotonic shortening sequences. (C) Using the average force (over last 50-120 ms) and shortening velocity (incline of fiber fragment length in the same last 50-120 ms) of each of the 16 isotonic force levels, a Hill curve was fitted.

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Tables

Table 1 **– Fiber type distribution, fiber mass-specific peak power and estimated whole body muscle mass-specific peak power in marmoset and human vastus lateralis (VL) and gastrocnemius (GM).**

		Human				Marmoset		
VL fibre type distibution (%)[a]		**Type I**	**Type IIA**	**Type IIX**		**Type I**	**Type IIB**	**Hybrid**
		46.3	44.3	8.9		0.6	62.7	36.7
	s.e.m.	3.9	3.2	2.3		0.3	6.3	7.4
GM fibre type distibution (%)[a]		**Type I**	**Type IIA**	**Type IIX**		**Type I**	**Type IIB**	**Hybrid**
		49.4	42.8	6.6		9.1	44.3	46.6
	s.e.m.	2.8	3.5	2.6		3.6	8.4	9.4
$P_{FMS,peak}$ *in vitro* at 15°C (W kg^{-1})		**Type I**	**Type IIA**	**Type IIX**	**Hybrid**		**Type IIB**	**Hybrid**
		2.53	5.45	6.51	5.15		9.17	8.90
	s.e.m.	1.36	0.61	1.25	0.75		0.35	1.86
VL $P_{MMS,peak}$ estimates at 15°C (W kg^{-1})				3.7			8.7	
GM $P_{MMS,peak}$ estimates at 15°C (W kg^{-1})				3.5			8.0	

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

Table 2 **– Contractile properties of Human type I, IIA, IIX and hybrid fibers and Marmoset type IIB and Hybrid fibers (Mean and Standard Error of the Mean (s.e.m.)).**

Species	Number of Fibers (n)	FCSA (µm2):		P_0 (N/cm2)	a/P_0		V_{max} (FL/s)	
Human type I	**6**	**5100**	***,a**	**12.24**	**0.10**	***,b**	**0.33**	***,c**
s.e.m.		893		2.30	0.02		0.10	
Human type IIA	**27**	**7867**	***,a**	**12.26**	**0.09**	***,b**	**0.86**	***,c**
s.e.m.		501		0.91	0.00		0.03	
Human type IIX	**9**	**6429**	***,a**	**12.94**	**0.12**	***,b**	**0.80**	***,c**
s.e.m.		821		1.78	0.02		0.10	
Human Hybrid	**10**	**7659**	***,a**	**11.37**	**0.10**	***,b**	**0.80**	***,c**
s.e.m.		688		0.79	0.01		0.06	
Marmoset type IIB	**77**	**4271**	***,a**	**10.90**	**0.33**	***,b**	**0.80**	***,c**
s.e.m.		157		0.31	0.02		0.02	
Marmoset Hybrid	**5**	**2561**	***,a**	**12.45**	**0.24**	***,b**	**0.77**	***,c**
s.e.m.		586		1.42	0.03		0.12	

Data is shown for fiber cross-sectional area (FCSA), specific tension (P_0), curvature of Hill's relationship (a/P_0), maximal shortening velocity (V_{max}) and peak power per kg of muscle (P_{Peak}). Significant differences between fiber types are indicated with an asterisk (* P < 0.05). FL/s, fiber lengths per second, W/kg Watts per kilogram.

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

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

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

Table 3 **– Literature overview of fiber type distribution in different (primate) species.**

(Primate) 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		ATP-ase
			GM	49.4	42.8		6.6		
Chimpanzee (female/male) *(Pan troglodytes)*	(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
Rhesus Macaque *(Macaca Mulatta)*	(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
Green Vervet Monkey *(Chlorocebus aethiops sabaeus)*	(Feng et al., 2012)	5.6	VL	6	78	16			ATP-ase
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[a]	VL		13	87			ATP-ase
			GM	15	29	56			
Gray Mouse Lemur *(Microcebus murinus)*	(Petter and Jouffroy, 1993)		VL		47.3	52.7			ATP-ase
			GM	21.8	37.8	40.3			

MAb, Monoclonal Anti-bodies.

[a] Values for mass are not reported by (Ariano et al., 1973), values displayed are from (Aerts, 1998)

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