**Relationships between maximal strength, muscle size, myosin heavy chain isoform composition and post-activation potentiation**

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Title Page

Relationships between maximal strength, muscle size, myosin heavy chain isoform composition and post-activation potentiation

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Abstract

The purpose of this study was to examine the relationships between maximal voluntary post-activation potentiation (PAP) and maximal knee extensor torque, quadriceps cross-sectional area (CSA) and volume, and type II myosin heavy chain (MHC) isoform percentage in the human skeletal muscle. Thirteen resistance-trained men completed a test protocol consisting of two isokinetic knee extensions at 180°·s⁻¹ performed before and 1, 4, 7 and 10 min after the completion of 4 maximal knee extensions at 60°·s⁻¹ (i.e. a conditioning activity). Magnetic resonance imaging and muscle microbiopsy procedures were completed on separate days to assess quadriceps CSA and volume, and MHC isoform content. Maximal voluntary PAP response was assessed as the ratio of the highest knee extensor torques measured before and after the conditioning activity. There were large to very large correlations between maximal voluntary PAP response and maximal knee extensor torque (r=0.62), quadriceps CSA (r=0.68) and volume (r=0.63). Nonetheless, these correlations were not statistically significant after adjusting for the influence of type II MHC percentage using partial correlation analysis. By contrast, the strongest correlation was observed for type II MHC percentage (r=0.77), and this correlation remained significant after adjusting for the other variables. Maximal voluntary PAP response is strongly correlated with maximal knee extensor torque, quadriceps CSA and volume, but mostly clearly associated with the type II myosin isoform percentage in the human skeletal muscle.

Keywords: post-activation potentiation, conditioning activity, fibre type, myosin isoform, muscle torque, muscle size
Résumé

Le but de cette étude était d’examiner les relations entre la potentialisation musculaire (PAP) volontaire et la moment de force maximal des extenseurs du genou, l’aire de section transverse (CSA) et volume du quadriceps, et le pourcentage de chaînes lourdes de myosine (MHC) de type II dans le muscle squelettique humain. Treize sportifs ont complété un protocole test comprenant deux extensions iso-cinétiques du genou à 180°·s⁻¹ réalisées avant et 1, 4, 7 and 10 min après quatre extensions maximales du genou à 60°·s⁻¹ (stimulus conditionnant). Une Imagerie Résonance Magnétique et micro-biopsie musculaire ont été complétés pour mesurer la CSA et le volume du quadriceps, et les isoformes de MHC. La magnitude maximale de PAP volontaire a été calculé comme le ratio du moment de force maximal des extenseurs du genou mesuré avant et après le stimulus conditionnant.

Il existent de fortes à très fortes corrélations entre la magnitude maximale de PAP volontaire et le moment de force maximal des extenseurs du genou (r=0.62), CSA (r=0.68) et volume (r=0.63) du quadriceps. Néanmoins, ces corrélations ne sont plus statistiquement significatives après la prise en compte de l’influence du pourcentage de MHC de type II à l’aide d’une analyse partielle de corrélation. En revanche, la plus forte corrélation est observée pour le pourcentage de MHC de type II (r=0.77), qui reste significative après la prise en compte des autres variables. La magnitude maximale de PAP volontaire est fortement corrélée avec le moment de force maximal des extenseurs du genou, la CSA et volume du quadriceps mais plus fortement corrélée avec le pourcentage de MHC de type II dans le muscle squelettique humain.

Mots-clés: potentialisation musculaire, stimulus conditionnant, typologie musculaire, isoformes de myosine, moment de force, volume musculaire
**Introduction**

Post-activation potentiation, or PAP, is the phenomenon in which a preceding contraction (conditioning activity; CA) elicits an acute improvement in muscular performance during a subsequent test contraction (Tillin and Bishop 2009). Voluntary PAP can be defined as the improvement in muscular performance during a voluntary contraction in response to a CA (Seitz, et al. 2015). PAP has been ubiquitously reported in studies using electrical or voluntary contractions as CA and/or test contraction (the reader is directed to the reviews by Hodgson et al., 2005 and Tillin & Bishop, 2009). However, an important and consistent finding in these studies is a high inter-individual variability in the voluntary PAP response. Overall, increases (Wilson, et al. 2013), no change (Gossen and Sale 2000; Gourgoulis, et al. 2003) or decreases (Duthie, et al. 2002; Chiu, et al. 2003) in muscular performance after the CA have been reported, which is indicative of a responder versus non-responder phenomenon. Thus, despite the practical benefits of improving voluntary muscular performance with the use of CAs, some individuals do not seem able to utilize the strategy effectively.

Studies have shown that the ability to exhibit voluntary PAP is influenced by the volume and intensity of the CA and by the rest period between the CA and the subsequent muscular performance (Wilson et al. 2013). Moreover, several characteristics that potentially differ between responders and non-responders might also explain the difference in voluntary PAP susceptibility. First, PAP may be more clearly elicited in individuals with a high proportion of type II muscle fibres, as indicated by the strong relationship between type II muscle fibres proportion and PAP magnitude (Hamada, et al. 2000; Hamada, et al. 2003). This makes sense from the perspective that PAP has been shown to occur through an increase in myosin regulatory light chain (RLC) phosphorylation in response to a CA, and that this process is most notable in fibres with a greater proportion of the type II myosin heavy chain (MHC) isoform in animals (Klug, et al. 1982; Manning and Stull 1982; Moore and Stull
However, the relationship between type II MHC isoform and PAP has not always been observed in human skeletal muscle (Stuart, et al. 1988), and that between type II MHC isoform and voluntary PAP remains to be determined.

There is also a large body of literature indicating that stronger individuals may be able to express higher level of voluntary potentiation (Chiu et al. 2003; Ruben, et al. 2010; Seitz, et al. 2014a; Seitz, et al. 2014b). For example, Seitz et al. (2014b) found a significant correlation (r=0.67) between back squat strength and the magnitude of potentiation during a sprint task. Similarly, Ruben et al. (2010) reported a greater PAP effect during a horizontal jump task in stronger individuals in comparison to their weaker counterparts. Stronger individuals are often shown to have a higher percentage of type II muscle fibres (Thorstensson, et al. 1976; Maughan, et al. 1983b; Aagaard and Andersen 1998) and therefore would be likely to exhibit greater increases in myosin RLC phosphorylation in response to the CA (Houston, et al. 1985) or respond more to increases in the ability to recruit type II muscle fibres, resulting in a greater voluntary PAP response. It could also be argued that a greater muscle cross-sectional area (CSA) (Maughan, et al. 1983a) or volume (Fukunaga, et al. 2001) would be a characteristic of stronger individuals, whereby any increase in tissue-specific force elicited by voluntary PAP will be amplified in these individuals. However, although muscle size might in some way be a causative factor influencing voluntary PAP, the possibility exists that its relationship with voluntary PAP is also explicable by its relationship with fibre (motor unit) type. Nonetheless, to our knowledge no attempt has been made to quantify the relationships between voluntary PAP and muscle strength, muscle size, percentage of type II MHC isoform in the human skeletal muscle.

Given the above, the purpose of the present study was to examine the relationship between maximal voluntary PAP magnitude and maximal voluntary knee extensor torque, quadriceps CSA and volume and type II MHC isoform percentage in the human skeletal
muscle. It was hypothesized that participants displaying a greater voluntary PAP magnitude would also display a greater maximal voluntary knee extensor torque, quadriceps CSA and volume and type II MHC isoform percentage, and that type II MHC isoform would be the strongest correlate.

Materials and Methods

Participants

Thirteen resistance-trained men (mean ± SD: age, 24.1 ± 3.0 y; height, 1.85 ± 0.11 m; body mass, 86.1 ± 10.1 kg) volunteered for the study. They were recruited on the basis that they had been involved in a lower body resistance-training program for muscular strength and/or power for at least one year. Each participant signed an informed consent form, and the procedures of the investigation were approved by the Edith Cowan University Human Research Ethics Committee and were in agreement with the Declaration of Helsinki.

Study design and overview

The participants visited the laboratory on four separate occasions (familiarisation, experimental, magnetic resonance imaging (MRI) and muscle microbiopsy sessions), each separated by 5 to 7 days. The participants were familiarized with an isokinetic knee extension task during the first visit. During the experimental session, the participants completed a procedure consisting of a task-specific warm-up procedure (described below) and a test protocol that was performed before and 1, 4, 7 and 10 min after completing a knee extension CA. During the task-specific warm-up procedure, test protocol and CA, the participants were seated on an isokinetic dynamometer (Biodex System 3 Pro, Biodex Medical System, Shirley, New York) with their dominant (strongest) thigh strapped to the chair and the ankle fixed to the dynamometer’s lever arm. The lateral femoral epicondyle was aligned to the axis of
rotation of the dynamometer, and the knee and hip joints were flexed at 90° and 85°, respectively. The participants were asked to move through a range of motion that was set from 90° to 0° (0° = full knee extension), with a repetition completed when the lever arm was stopped at the mechanical stop position of the Biodex. The participants were asked to relax their leg before extending their knee so the knee angle was at 90° before initiating a knee extension. The leg was passively returned to the starting position after the shortening contraction. The participants were unaware of the study hypotheses as well as task-specific warm-up and test protocol scores.

*Task-specific warm-up procedure*

Based on previous work, a task-specific warm-up procedure was used to ensure that any increases in voluntary knee extensor torque after completing a CA could be specifically attributed to acute changes in response to the CA rather than warm-up or familiarisation effects (Seitz et al. 2015). The participants performed two isokinetic knee extensions at 180°·s⁻¹ at 20, 40, 60 and 80% of their perceived maximal force at 45 s intervals through the 90° range of motion. The knee extensions were then performed at 100% of maximum ‘as fast and hard as possible’ every minute until peak torque production in three consecutive contractions differed by less than 2% (Seitz et al. 2015). A 90-s rest period was then imposed prior starting the test protocol.

*Test protocol and conditioning activity*

A test protocol requiring the performance of 2 isokinetic knee extensions at 180°·s⁻¹ (separated by 15 s) was completed 90 s before (pre-CA) and 1, 4, 7 and 10 min after (post-CA) a CA involving 4 isokinetic knee extensions at 60°·s⁻¹. The knee extensions at 60°·s⁻¹ were separated by a 10-s rest period. The test protocol and CA were chosen based on previous
studies that used these specific velocities (Miyamoto, et al. 2011; Seitz et al. 2015). The knee extensions resulting in the highest voluntary torques captured during pre- and post-CA testing were selected for further analysis. Voluntary PAP was calculated as:

\[
\% \text{Voluntary PAP} = \left( \frac{\tau_{\text{vol,post-CA}} - \tau_{\text{vol,pre-CA}}}{\tau_{\text{vol,pre-CA}}} \right) \times 100
\]

Where \( \tau_{\text{vol,post-CA}} \) is the highest voluntary torque measured at any time point during the test protocol after the CA (post-CA) and \( \tau_{\text{vol,pre-CA}} \) is the highest voluntary torque measured at any time point during the test protocol before the CA (pre-CA).

During the test protocols and CA, the participants received verbal encouragement to extend their knee ‘as fast and as hard as possible’ throughout the 90° range of motion.

**Magnetic resonance imaging**

A 1.5 Tesla MRI scanner (Magnetom Essenza, Siemens Medical Solutions, Erlangen, Germany) was used to measure the CSA and volume of the quadriceps muscle group of the dominant leg whilst participants lay supine. A proton density turbo spin-echo axial (cross-sectional) slice sequence (field of view = 256 × 256 mm², TR = 3720 ms, TE = 25 ms, base resolution = 384 × 384 pixels, voxel size 0.7×0.5×5.0 mm) was utilized to acquire multiple 5-mm thick serial sections contiguously from the anterior superior iliac spine to the tibial tuberosity. Vastus lateralis, vastus medialis, rectus femoris and vastus intermedius total CSA and volume were determined by manually tracing MRI slices from the proximal point of the greater trochanter to the distal region of the femoral lateral condyle using open-source DICOM imaging software (OsiriX MD v.1.4, OsiriX foundation, Geneva). Care was taken to exclude adipose tissue incursions from each slice. When clear delineation of the vasti muscles was not possible because of a lack of observable intermuscular septum, a line was
traced from the end of the observable septum to a landmark on the muscle's perimeter where the septum had intersected in distal slices (Blazevich, et al. 2007). An MRI scan obtained from one individual and depicting thigh muscle cross-section is shown in Figure 1. In order to calculate quadriceps CSA, the area of each muscle in each slice was computed automatically by summing the given tissues’ pixels and multiplying by the pixel surface area. Muscle volume was obtained by multiplying muscle tissue area by slice thickness (Lee, et al. 2000). The participants were required to abstain from any exercise including experimental sessions for at least 72 h prior the MRI procedure.

**Insert Figure 1 about here**

*Muscle microbiopsy procedure*

Muscle biopsy samples were taken from the vastus lateralis of the dominant leg by percutaneous needle microbiopsy. After careful preparation of the skin by shaving, lightly abrading and cleaning with alcohol in order to minimize the risk of infection, a eutectic mixture of local anaesthetics cream (EMLA, Astra Pharmaceuticals, Sydney, Australia) was applied to the biopsy area and subsequently covered with a plastic wrap for 30 min. The plastic wrap and cream were then removed, the skin was sterilized with povidone iodine (Betadine solution, Faulding Consume, Virginia, Qld, Australia), and then punctured at a 2-cm depth with a 13 gauge insertion cannula at 50% of the distance from the greater trochanter to the lateral epicondyle of the femur in the middle of the muscle belly. A 14-gauge triggered microbiopsy needle (Bard Biopsy Systems, Covington, GA, USA) was then inserted into the cannula and a muscle sample was taken. The triggered microbiopsy needle was then removed while the 13 gauge insertion cannula remained in place, the tissue was extracted and immediately frozen in liquid nitrogen, and two samples were further obtained from the same
site. A total of ~30 mg of tissue was obtained and stored at -80°C for further analyses. This microbiopsy procedure was validated by a study reporting a similar MHC isoform distribution in vastus lateralis using the microbiopsy technique and the traditional, more invasive, Bergström technique (Hayot, et al. 2005). The participants were required to abstain from any exercises including experimental sessions for at least 72 h prior the microbiopsy procedure.

* Determination of myosin heavy chain isoform distribution

A 4-gel vertical electrophoresis system (Mini-PROTEAN, Tetra Cell for mini precast gel, 165-8004, Bio-Rad Laboratories, Hercules, CA) was used to separate myosin isoform.

* Samples.* Frozen samples were cut into slices using a scalpel and placed in 40 µl of refrigerated homogenisation buffer (250 mM sucrose, 100 mM KCl, 5 mM EDTA and 20 mM Tris, pH 6.8). A micropestle was used to homogenize samples. The protein content of each sample was determined using a spectrophotometer with a wavelength of 595 nm using Bradford reagent (Bio-Rad Laboratories; Hercules, CA) in order to standardize the amount of protein loaded per well (14.5 cm width, 8.3 cm height and 1 mm thickness; Bio-Rad Laboratories, Hercules, CA).

* Stacking and separating gel.* The stacking gel contained 33.6% dH₂O, 14% Tris-HCl 0.5 (pH 6.7), 13.3% acrylamide:Bis (50:1), 30% glycerol, 4% 100mM EDTA (pH 8.0) and 4% SDS. The separating gel was produced by mixing 15% dH₂O, 13.3% Tris-HCl 1.5 (pH 8.8), 26.7% acrylamide:Bis (50:1), 30% glycerol, 10% glycine and 4% SDS. In order to initiate polymerization, TEMED and ammonium persulfate were added to both the separating and stacking gels to a final concentration of 0.1 and 1%, respectively.
Running buffers. The lower running buffer consisted of 100 mM Tris, 150 mM glycine and 0.1% SDS. The upper running buffer contained five times the concentration of the lower running buffer. Lower and upper running buffers were cooled to between 4 and 5°C in a refrigerator before use.

Electrophoretic runs. The gel unit was cooled to between 4 and 5°C in a refrigerator for the duration of the electrophoretic runs (14 h at 140 V, constant voltage).

Staining and densitometry. All gels were stained with Coomassie Blue stain solution for 30 min and destained three times (30 min each) with 70% dH2O, 20% methanol and 10% acetic acid. Gels were then scanned with a computer scanner and the densitometric profile was calculated using ImageJ analysis software for Macintosh (v.1.48, National Institutes of Health, Bethesda, Maryland). The determination of the densitometric profile proved to be reliable with coefficient of variation and intra-class correlation coefficient being 1.9 and 0.89, respectively.

Statistical analyses

Pearson’s correlation coefficients were computed to determine the relationships between voluntary PAP magnitude and maximal voluntary knee extensor torque measured at 60°·s\(^{-1}\) during the CA, quadriceps CSA and volume and type II MHC isoform percentage. Initially, the strength of relationships was quantified by calculating coefficients of determination (R\(^2\)) of linear regressions. The strength of relationships was then quantified by means of polynomial fits and by calculating R\(^2\) using the method of least squares. The order of the polynomial was determined in a stepwise fashion. Starting with an order of one, R\(^2\) was ascertained. The order of the polynomial was then increased until the R\(^2\) value did not
increase by more than 2% if another order was added (Waugh, et al. 2012). $R^2$ values of linear and non-linear regressions were then compared. Because the difference in $R^2$ values between the linear and non-linear regressions was negligible for all the relationships, only linear models were plotted. Where significant correlations were observed between independent variables, partial correlation analyses were used to determine the relationship between voluntary PAP and maximal voluntary knee extensor torque, quadriceps CSA and volume and/or type II MHC isoform percentage while controlling for a third, independent variable. In addition to the correlation analysis, we used a Bayesian variable selection procedure to estimate the posterior probability of all possible models (Kruschke 2014). For the three variables there are 23 possible models and all models were given equal prior probability (i.e. they were all equally credible). The inclusion of each variable was dictated by a random sample from a Bernoulli distribution, taking values 0 or 1. The probability of including a variable in the model was 0.5, so each model had a prior probability of 0.125 (i.e. $0.5^3$). We used a non-committal broad prior distribution of the standardised regression coefficients (gamma distribution with mode of 1 and SD of 10).

The strength of relationships was assessed using the following criteria (Cohen 1988): trivial ($r < 0.1$), small ($0.1 \leq r < 0.29$), moderate ($0.3 \leq r < 0.49$), large ($0.5 \leq r < 0.69$), very large ($0.7 \leq r < 0.89$) and nearly perfect ($r \geq 0.9$). The magnitude of the effect size (ES) was considered trivial ($<0.20$), small ($0.20 \leq ES < 0.50$), medium ($0.50 \leq ES < 0.80$), large ($0.80 \leq ES < 1.30$) or very large ($> 1.30$). For all statistical analyses, the level of significance was set at $p \leq 0.05$. One-way repeated measures ANOVAs were used to compare the torque produced during the last three knee extensions of the task-specific warm-up and that produced during the pre-CA knee extensions to determine whether the task-specific warm-up was complete. All statistical analyses were conducted using Stata 12 (Stata Corp., College Station, TX, USA) for Macintosh.
Results

Task-specific warm-up procedure

No differences were observed between the knee extension torque produced during each of the last three knee extensions of the warm-up (e.g. performed at 100% of maximum) and the knee extension during pre-CA testing (p=0.87; Figure 2). The lack of statistical difference in torque production between these contractions indicates that maximal muscle contractile capacity was achieved before undertaking the CA and the task-specific warm-up was complete since no further improvement in torque production could be elicited by further practice using a 45-s rest interval.

Insert Figure 2 about here

Voluntary PAP magnitude

The highest voluntary knee extensor torque captured during post-CA testing (i.e. maximal voluntary PAP response) was significantly higher (+7.2 ± 4.6%; p<0.001) than the voluntary knee extensor torque captured during pre-CA testing. The magnitude of change (ES=0.57) was considered medium.

Relationships between maximal voluntary PAP and muscular variables

The muscular characteristics of the participants are described in Table 1. There were large to very large correlations between maximal voluntary PAP and muscular variables including maximal voluntary knee extensor torque at 60°·s⁻¹, quadriceps CSA and volume and type II MHC isoform percentage (Table 2; Column A and Figure 3). Therefore, a greater voluntary PAP magnitude was observed in participants who could produce higher knee extensor torque, had larger quadriceps CSA and volume, and had a greater percentage of the
fast-MHC isoform. An electrophoretic separation of the various MHC isoforms of a high- and low-PAP responders is shown in Figure 4.

The relationships between maximal voluntary PAP and maximal voluntary knee extensor torque at $60^\circ \cdot \text{s}^{-1}$, quadriceps CSA and volume were not statistically significant after adjusting for the influence of type II MHC isoform percentage using partial correlation analysis (Table 2; Column B). By contrast, the correlation between maximal voluntary PAP and type II MHC percentage remained significant after adjusting for the other variables (Table 2; Column C).

In relation to the Bayesian variable selection procedure, the most probable model included only ‘type II MHC isoform percentage’ and had a posterior probability of ~50%. The regression coefficient for type II MHC isoform percentage had median 0.38 and 95% HDI limits of 0.15 – 0.52. The next most probable model contained ‘type II MHC isoform percentage’ and ‘maximum torque’, with a posterior probability of about 12% (24% of best model). Median regression coefficient of marginal posterior distributions were 0.30 (0.03-0.52; 95% HDI) for type II MHC isoform percentage and 0.05 (-0.03-0.16; 95% HDI) for maximum torque, barely excluding zero from the 95% HDI. The model with ‘type II MHC isoform percentage’, ‘maximum torque’ and ‘quadriceps CSA’ had a probability of only 5% (10% of best model). 95% HDIs of all marginal posterior distribution regression coefficients.
included zero in the model. Consequently, we selected the most probable model, which has regression equation: PAP (%) = -14 + 0.38×type II MHC isoform percentage.

Discussion

The relationships between maximal voluntary PAP response and muscular variables including maximal voluntary knee extensor torque at 60°·s⁻¹, quadriceps CSA and volume and type II MHC isoform percentage were examined in the human skeletal muscle. Maximal voluntary PAP was strongly correlated with maximal voluntary knee extensor torque at 60°·s⁻¹, and quadriceps CSA and volume. However, maximal voluntary PAP was most strongly associated with the type II MHC isoform percentage and this correlation remained significant even after accounting for the influence of muscle strength and size (r=0.52-0.66) and was the single inclusion in the Bayesian most probable model.

The results of the present study are in line with previous research demonstrating that the performance of an isokinetic CA can contribute to increased voluntary torque production (i.e. voluntary PAP) (Babault, et al. 2008; Chaouachi, et al. 2011; Fukutani, et al. 2013). Because the participants in the present study completed a task-specific warm-up in which maximal contractile capacity was achieved before performing the pre-CA (baseline) testing, our data demonstrate that increases in voluntary knee extensor torque production following a CA most likely result from acute physiological changes in response to the CA rather than being either a warm-up or familiarization effect. This finding is in agreement with a previous study reporting the presence of voluntary PAP after maximal voluntary contractile capacity was achieved following the performance of a complete task-specific warm-up (Seitz et al. 2015).

The large correlation (r=0.62; p=0.027) between maximal voluntary PAP response and maximal voluntary muscle torque production is also in accordance with previous studies
reporting a notable relationship between muscle strength and voluntary PAP (Chiu et al. 2003; Jo, et al. 2010; Ruben et al. 2010; Seitz et al. 2014a; Seitz et al. 2014b). This might be explained by the fact that maximal voluntary knee extensor torque production at 60°·s\(^{-1}\) was significantly, although only moderately, correlated (r=0.55; p=0.05) with type II MHC isoform percentage, which indicates a link between muscular strength and fibre type (Thorstensson et al. 1976; Aagaard and Andersen 1998; Maughan and Shirreffs 2010). Therefore, stronger individuals, who tend to have a greater percentage of the type II MHC isoform will also exhibit a greater voluntary PAP response since PAP is most notable in fibres with a greater proportion of the type II MHC isoform (Klug et al. 1982; Manning and Stull 1982; Moore and Stull 1984), although this has not always been observed in human skeletal muscle (Stuart et al. 1988). Additionally, the correlation between maximal voluntary PAP and maximal voluntary knee extensor torque production at 60°·s\(^{-1}\) might also be explained by the fact that individuals producing higher torque levels exhibited a greater quadriceps size (CSA and volume) than their weaker counterparts. Indeed, quadriceps CSA and volume were significantly correlated with maximal voluntary PAP. Therefore, it could be argued that any increase in tissue-specific force elicited by voluntary PAP might have been amplified in the strongest individuals. Future research should attempt to specifically determine whether an increase in tissue-specific force elicited by voluntary PAP is amplified in individuals with larger muscles.

An important finding of the present study was that the relationships between maximal voluntary PAP response and maximal voluntary knee extensor torque production at 60°·s\(^{-1}\), quadriceps CSA and volume were not statistically significant after adjusting for the influence of type II MHC isoform percentage. By contrast, the correlation between maximal voluntary PAP response and type II MHC percentage remained significant after adjusting for the other variables (Table 2; Column C). Furthermore, we used a Bayesian variable selection procedure.
to estimate the posterior probability of all possible models. The most promising model was found to contain only ‘type II MHC isoform percentage’, and the addition of other variables relating to knee extension torque (strength) and muscle size did not improve model probability. Based on this analysis, the most probable model indicated that a 10% increase in type II MHC isoform percentage was associated with a 3.8% increase maximal voluntary PAP response. Importantly, the data from this Bayesian analysis were consistent with the outcome of the partial correlation analysis, and indicated a correlative link between type II MHC isoform percentage and maximal voluntary PAP in the human skeletal muscle. Cumulatively, these findings suggest that maximal voluntary PAP was most clearly associated with the type II MHC isoform in the human skeletal muscle, which is in agreement with studies showing that individuals with a greater percentage of type II twitch fibers express higher levels of PAP (Hamada et al. 2000; Hamada et al. 2003). However, these studies utilized relatively small sample sizes (i.e. 8 participants) and thus may have been prone to type I error, however our results using a larger (n=13) sample are consistent with their findings.

The strong correlation between maximal voluntary PAP and type II MHC isoform may be explained by the fact that myosin RLC phosphorylation, one proposed mechanism responsible for PAP, has been shown to be greater in type II twitch fibres (Klug et al. 1982; Manning and Stull 1982; Moore and Stull 1984). Phosphorylation of myosin RLCs through the activation of MLC kinase is thought to potentiate subsequent contraction by increasing the sensitivity of actin-myosin to Ca\(^{2+}\) released by the sarcoplasmic reticulum (Palmer and Moore 1989; Grange, et al. 1993; Vandenboom, et al. 1995) and thus increasing the likelihood of myosin cross-bridge interaction with actin (Levine, et al. 1996). The result is an increase in the number and rate of myosin cross-bridges binding to the actin filament, resulting in an increase in muscle tension (Barany, et al. 1980; Manning and Stull 1982; Metzger, et al.
Individuals with a higher percentage of type II twitch fibers may be most likely to exhibit greater myosin RLC phosphorylation because of the higher content of MLC kinase in these fibers (Moore and Stull 1984). An alternative explanation is that voluntary PAP may be accompanied by an increase in central drive, in line with evidence of increases in H-reflex (Gullich and Schmidtbleicher 1996; Trimble and Harp 1998; Folland, et al. 2008) and EMG (Hough, et al. 2009) amplitudes, which may theoretically increase the contribution of larger motor units to muscular contraction (Hodgson, et al. 2005). Therefore, individuals with a higher percentage of type II muscle fibres might benefit more from an improved ability to recruit this type of fibres, resulting in a greater voluntary PAP response, although explicit testing of this hypothesis is required in future research.

In conclusion, the present results show that maximal voluntary PAP is strongly correlated with maximal voluntary knee extensor torque production (i.e. muscular strength), quadriceps CSA and volume (i.e. muscle size) and the percentage of type II MHC isoform (i.e. fibre type) in the human skeletal muscle. However, the findings that (i) the strongest correlation with maximal voluntary PAP response was observed with the type II MHC isoform percentage, and (ii) this correlation remained significant after accounting for other variables using partial correlations analysis, and (iii) type II MHC isoform percentage was the single inclusion in the Bayesian most probable model, suggest that maximal voluntary PAP response is most clearly associated with the type II myosin isoform percentage in human skeletal muscle. Therefore, this finding fills a gap in the literature since to date the relationship between voluntary PAP and type II MHC isoform percentage in the human skeletal muscle was unclear (Stuart et al. 1988). In addition, the present results are of interest from a practical standpoint as they suggest that training interventions leading to an increase in type II MHC isoform content may allow for greater voluntary PAP magnitudes to be achieved.
Moreover, the association between type II MHC isoform and maximal voluntary PAP response also provides insights regarding the potential influence of peripheral mechanisms on voluntary PAP. Future research should use more direct techniques to assess potential changes in peripheral function, particularly in the excitation-contraction coupling process, following a PAP-inducing CA. A secondary finding was that the maximal voluntary PAP response was also correlated with maximal voluntary knee extensor torque production, and quadriceps muscle size. Nonetheless, these associations were no longer significant after accounting for the possible influence of type II MHC percentage. Therefore, these factors likely play a lesser, or associative, role in the maximal voluntary PAP response.

Conflict of interest

The authors declare that there are no conflicts of interest.

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References


Table 1. Voluntary PAP magnitude, maximal KE torque, muscle size and MHC isoform characteristics of the participants

<table>
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<tr>
<td>Voluntary PAP (%)</td>
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<tr>
<td>Maximal KE Torque at 60°·s⁻¹ (Nm)</td>
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<td>Quadriceps CSA (cm²)</td>
<td>83.3 ± 9.36</td>
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<tr>
<td>Quadriceps volume (cm³)</td>
<td>2653.9 ± 171.5</td>
</tr>
<tr>
<td>Type II MHC isoform (%)</td>
<td>56.3 ± 9.2</td>
</tr>
</tbody>
</table>

PAP = post-activation potentiation; KE = knee extensor; CSA = cross-sectional area; MHC = myosin heavy chain. SD = standard deviation.
Table 2. Pearson’s and partial correlations between maximal KE torque, muscle size, type II MHC isoform and voluntary PAP magnitude.

<table>
<thead>
<tr>
<th></th>
<th>(A)</th>
<th>(B)</th>
<th>(C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>Maximal KE Torque at 60°·s⁻¹ (Nm)</td>
<td>0.62</td>
<td>0.037</td>
<td>0.38</td>
</tr>
<tr>
<td>Quadriceps CSA (cm²)</td>
<td>0.68</td>
<td>0.010</td>
<td>0.17</td>
</tr>
<tr>
<td>Quadriceps volume (cm³)</td>
<td>0.63</td>
<td>0.020</td>
<td>0.23</td>
</tr>
<tr>
<td>Type II MHC isoform (%)</td>
<td>0.77</td>
<td>0.002</td>
<td>-</td>
</tr>
</tbody>
</table>

(Column A = Correlations between voluntary PAP and various muscular variables. Column B = Partial correlations between voluntary PAP and various muscular variables adjusted for type II MHC isoform %. Column C = Partial correlations between voluntary PAP and type II MHC isoform (%), adjusted for the various muscular variables).

PAP = post-activation potentiation; KE = knee extensor; CSA = cross-sectional area; MHC = myosin heavy chain.
**Figure 1.** Magnetic resonance imaging scan depicting thigh muscle cross-section obtained in one individual. Perimeters of the four vastii muscles are shown: VI = vastus intermedius; VL = vastus lateralis; VM = vastus medialis; RF = rectus femoris.

**Figure 2.** Voluntary knee extensor torque produced during the last three knee extensions of the warm-up (i.e. warm-up 1, warm-up 2 and warm-up 3) and the pre-CA knee extension. The lack of statistical difference in knee extensor torque production among these contractions indicated that maximal voluntary contractile capacity was achieved before undertaking the conditioning activity (i.e. the task-specific warm-up was complete). CA = conditioning activity.

**Figure 3.** Relationship between voluntary PAP magnitude and type II MHC isoform percentage in vastus lateralis. PAP = post-activation potentiation; MHC = myosin heavy chain.

**Figure 4.** Electrophoretic separation of the various myosin heavy chain (MHC) isoforms in vastus lateralis. (A) Electrophoretic separation from a high-PAP responder (maximum PAP response= 14.92 %). (B) Electrophoretic separation from a low-PAP responder (maximum PAP response= 1.22 %). PAP = post-activation potentiation.
Voluntary Knee Extensor Torque at 180°·s⁻¹ (N.m)

- Warm-up 1
- Warm-up 2
- Warm-up 3
- Pre-CA
Voluntary PAP response (%) vs. Type II MHC isoform (%)

- $r = 0.774$
- $p = 0.002$
- $r^2 = 0.599$