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Research Paper

Single muscle fibre contractile properties differ between body-builders, power athletes and control subjects

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Experimental Physiology

New Findings

- **What is the central question of this study?**
  Do the contractile properties of single muscle fibres differ between body-builders, power athletes and control subjects?
- **What is the main finding and its importance?**
  Peak power normalized for muscle fibre volume in power athletes is higher than in control subjects. Compared with control subjects, maximal isometric tension (normalized for muscle fibre cross-sectional area) is lower in body-builders. Although this difference may be caused in part by an apparent negative effect of hypertrophy, these results indicate that the training history of power athletes may increase muscle fibre quality, whereas body-building may be detrimental.

We compared muscle fibre contractile properties of biopsies taken from the vastus lateralis of 12 body-builders (BBs; low- to moderate-intensity high-volume resistance training), six power athletes (PAs; high-intensity, low-volume combined with aerobic training) and 14 control subjects (Cs). Maximal isotonic contractions were performed in single muscle fibres, typed with SDS-PAGE. Fibre cross-sectional area was 67 and 88% ($P < 0.01$) larger in BBs than in PAs and Cs, respectively, with no significant difference in fibre cross-sectional area between PAs and Cs. Fibres of BBs and PAs developed a higher maximal isometric tension (32 and 50%, respectively, $P < 0.01$) than those of Cs. The specific tension of BB fibres was 62 and 41% lower than that of PA and C fibres ($P < 0.05$), respectively. Irrespective of fibre type, the peak power (PP) of PA fibres was 58% higher than that of BB fibres ($P < 0.05$), whereas BB fibres, despite considerable hypertrophy, had similar PP to the C fibres. This work suggests that high-intensity, low-volume resistance training with aerobic exercise improves PP, while low- to moderate-intensity high-volume resistance training does not affect PP and results in a reduction in specific tension. We postulate that the decrease in specific tension is caused by differences in myofibrillar density and/or post-translational modifications of contractile proteins.

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Introduction

The performance of a power athlete is largely determined by the following two traits: the maximal force- and power-generating capacity of the recruited muscles; and the ability to maintain force and power for a prolonged period of high-intensity efforts. Peak muscle power and force are largely dependent on muscle volume and physiological cross-sectional area of the muscle, respectively. A main determinant of maximal sustainable power is the mitochondrial density in the recruited muscle fibres. Ideally, an athlete seeks to maximize both muscle power and endurance. However, there exists an inverse relationship between the fibre cross-sectional area (FCSA) and its mitochondrial density. It is suggested that the FCSA at a given mitochondrial density is limited by the maximal extracellular oxygen tension (Van der Laarse *et al.* 1997; Wessel *et al.* 2010). There may thus be a limit to the amount of hypertrophy, beyond which it becomes disadvantageous for sustainable power (Degens, 2012).

Athletes who require both high power output and muscle endurance may, at least in theory, solve this conundrum by increasing peak power and maximal isometric force without a concomitant increase in fibre size. In other words, by increasing the peak power per unit muscle mass (specific power) and maximal force per unit cross-sectional area (specific tension; F_0). Such an improvement would not only have benefits for athletes participating in sports with weight restrictions or sports where a large body mass limits performance, but also for older people, in whom a large part of the muscle weakness is attributable to a loss of muscle fibre F_0 (Frontera *et al.* 2000; Degens *et al.* 2009; Narici & Maffulli, 2010). Is it possible to improve specific power and F_0 and, if so, what training programme will give the best result?

Using single skinned muscle fibre segments, it has been demonstrated that while F_0 of type II fibres was higher, F_0 of type I was lower in male body-builders compared with non-body-builders (D'Antona *et al.* 2006). Other studies reported, however, that F_0 of single muscle fibres was unaffected by resistance training (Widrick *et al.* 2002; Erskine *et al.* 2011). Such equivocal observations have also been reported for endurance training. In healthy young men, marathon training increased F_0 of type I and IIA fibres in the face of reducing FCSA, thereby maintaining maximal tension (Trappe *et al.* 2006), whereas others reported a decrease in F_0 of type I fibres after a 12 week aerobic exercise programme (Harber *et al.* 2012).

Aside from F_0, specific peak power is also determined by the maximal shortening velocity and the curvature of the force–velocity relationship, here expressed as a/F_0, where 'a' represents the heat constant in the Hill equation (Hill, 1938). A high a/F_0 signifies a low curvature, which results in a higher peak power for muscles or muscle fibres with identical F_0 and maximal shortening velocity. Maximal shortening velocity appears to be unaffected by resistance training (Widrick *et al.* 2002; Erskine *et al.* 2011) and, in the case of body-building, it may even be reduced (D'Antona *et al.* 2006). On the contrary, endurance training has been reported to increase maximal shortening velocity, particularly in type I fibres (Trappe *et al.* 2006). It is unclear via what mechanism maximal shortening velocity can be affected by different types of activity, but it may be related to post-translational modifications of the contractile proteins. Such post-translational modifications have been reported to contribute to the age-related slowing in the speed at which actin can be propelled by myosin in an *in vitro* motility assay (Li *et al.* 2015). To the best of our knowledge, training-induced changes in a/F_0 of single muscle fibre segments and the impact thereof on specific peak power have not been reported.

Whilst findings are equivocal, the overall impression emerges that F_0 and specific power can be altered by regular exercise. The discrepancies between studies may be the result of differences in the type of training and/or the duration of most conventional training studies that are too short to measure the long-term effects of training. Hence, the aim of the present study was to determine how different long-term resistance training programmes affect specific power and F_0. For this purpose, we analysed contractile properties of skinned single muscle fibre segments from body-builders (BBs), power athletes (PAs) and non-competitive control subjects (Cs).

The most important goal of BBs is to increase muscle size, which is achieved by performing low- to moderate-intensity and high-volume resistance training with aerobic training elements in the precompetition weight-cutting phase. For PAs instead, performance is determined by the combination of both peak force and peak power, and the ability to sustain and repeat these high-intensity efforts for extended periods during a competition. To achieve this, the exercise programme of PAs is characterized by high-intensity, low-volume resistance training with supplemental aerobic exercise. Given that BBs train for bulk and PAs for function, we hypothesize that fibres from PAs will have a higher specific power and F_0 than those from BBs. We expect an increase in specific power and F_0 in PAs and BBs (for BBs especially in type II fibres) compared with Cs.

Methods

Participants

Muscle biopsies were collected from the vastus lateralis of 12 male body-builders (BBs: 29.8 ± 4.8 years old; 177.8 ± 4.1 cm; and 91.7 ± 13.4 kg), six power athletes (PAs: 23.4 ± 3.9 years old; 185.0 ± 4.3 cm; 103.0 ± 7.3 kg) and 14 non-competitive control subjects (Cs: 24.0 ± 3.5 years old; 180.9 ± 5.3 cm; 77.9 ± 6.3 kg)

after they had given written informed consent. The study conformed with the latest revision of the *Declaration of Helsinki*. The vastus lateralis was chosen because it is one of the major knee extensors and therefore plays a key role in many activities of power athletes, body-builders and the control population. In addition, biopsies from the vastus lateralis can be obtained with minimal discomfort to the participant because of its superficial location. The PA group consisted of American football players, track and field athletes and weight lifters. The local ethical committees of the Lithuanian University of Health Sciences and the University of Primorska, Koper, Slovenia, approved obtaining BB, PA and C biopsies. Nine of the BBs also participated in the study by Seynnes *et al.* (2013) and three of them in the study by Salvadego *et al.* (2013). The PAs and Cs were involved in the study by Salvadego *et al.* (2013).

Training diaries of BBs showed moderate- to high-intensity and high-volume training regimens [four to five sets, eight to 15 repetitions, 60–80% of one-repetition maximum (1RM), three to five times a week; Seynnes *et al.* 2013], with no reported aerobic exercise. All body-builders had been in a between-competition phase for at least 6 months at the time of the biopsy. Training diaries of the PAs showed that their training consisted of high-intensity, low-volume resistance training, with additional aerobic exercise such as running and cycling for 127 ± 150 min per week. Exercise diaries of Cs revealed that they were physically active. On average, Cs performed endurance exercise in the form of running and cycling for 153 ± 133 min per week and other recreational sports activities for 102 ± 143 min per week. The Cs did not follow a training schedule or perform resistance training. In the previous studies (Salvadego *et al.* 2013; Seynnes *et al.* 2013), the volume of the quadriceps femoris was measured. In all PAs and Cs and in three BBs the volume was assessed with magnetic resonance imaging, while the quadriceps volume of the remaining BBs was measured using ultrasound. Body-builders had a quadriceps volume of 2852 ± 892 cm^3, PAs 3194 ± 349 cm^3 and Cs 2550 ± 630 cm^3; indicating that both PAs and BBs had developed significant hypertrophy.

Biopsy collection

Muscle biopsies were taken under local anaesthesia (1 ml of 2% lidocaine) with a conchotome. The biopsies were collected in relaxing solution, and after 24 h in glycerol–relaxing solution at 4°C they were sucrose treated and stored at −80°C (Frontera & Larsson, 1997; Degens *et al.* 2010). Before use, the muscle biopsy was desucrosed, stored in glycerol–relaxing solution at −20°C and used within 1 month of desucrosing (Degens *et al.* 2010).

Solutions

The solutions were as described previously (Frontera & Larsson, 1997; Degens *et al.* 2010). Briefly, relaxing solution contained 4.5 mM MgATP, 1 mM free Mg^{2+}, 10 mM imidazole, 2 mM EGTA and 100 mM KCl (pH 7.0). The glycerol–relaxing solution contained 50% glycerol (v/v). Trition–relaxing solution was made by adding 1% Triton X-100 (v/v) to the relaxing solution. The activating solution consisted of 5.3 mM MgATP, 1 mM free Mg^{2+}, 20 mM imidazole, 7 mM EGTA, 19.6 mM creatine phosphate and 64 mM KCl with a pH of 7.0 and a pCa of 4.5.

Preparation of the skinned single muscle fibre segment

The preparation of the muscle fibre and the experimental set-up have been described before (Larsson & Moss, 1993; Gilliver *et al.* 2009; Degens *et al.* 2010). Before analysis, (part of) the biopsy was permeabilized in 1% Triton X-100 in relaxing solution for 20 min. The biopsy was then moved into relaxing solution, where the single fibre segments were dissected and mounted onto the fibre test system (400; Aurora Scientific Inc., Aurora, Ontario, Canada) using nylon thread. The fibre was suspended between two insect pins connected to a force transducer (403A; Aurora Scientific Inc.) and a motor arm (312C; Aurora Scientific Inc.). The temperature of the relaxing and activating solutions was kept at 15°C and checked at regular intervals. In previous studies, we have seen that the optimal sarcomere length for human fibres is at 2.6 μm (S.F. Gilliver & H. Degens, unpublished data). Therefore, sarcomere length was set at 2.6 μm, as determined by a

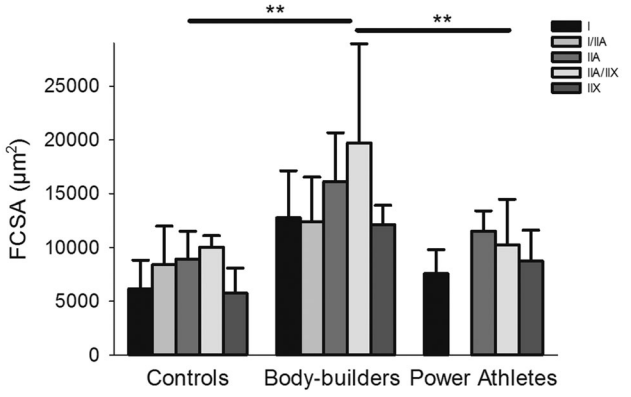

Figure 1. Fibre cross-sectional area (FCSA) of muscle fibres from the vastus lateralis of non-resistance-trained control subjects, body-builders and power athletes
Data are mean values + SEM. **Differences between indicated groups at $P < 0.01$.

Fourier transformation of the sarcomere pattern (900A; Aurora Scientific Inc.), by adjusting the length of the fibre. Fibre width was measured while the fibre was immersed in the relaxing solution, assuming a circular circumference. The FCSA, F_0 and specific power were not corrected for swelling. Fibre length was determined using a digital bore gauge (Gemred, Guangxi, China). After setting the sarcomere length and determination of fibre width and length, the fibre was transferred to activating solution.

Contractile properties

The protocol to assess force–velocity characteristics has been described previously (Gilliver *et al.* 2009; Degens *et al.* 2010). Briefly, muscle fibres were set at optimal length in relaxing solution and transferred to activating solution. After isometric muscle tension reached a plateau, the fibre was subjected to four sets of isotonic releases. After each set of isotonic releases, the fibre was restretched to optimal length. At the end of the four sets, the fibre was returned to relaxing solution. The total amount of shortening in each isotonic set of releases was <20% fibre length.

In a subset of fibres, the rate of force redevelopment (K_{TR}) was analysed. As previously described (Gilliver *et al.* 2009; Degens *et al.* 2010), maximally activated fibres were released to 20% optimal length and restretched after 15 ms, which forcibly uncouples myosin heads from actin. Force was then allowed to redevelop to maximal isometric tension. The K_{TR} was determined by fitting the force trace of this redevelopment.

SDS-PAGE

Myosin heavy chain (MHC) composition was determined via SDS-PAGE, essentially as described before (Larsson & Moss, 1993; Degens & Larsson, 2007). Briefly, SDS-PAGE was performed at 275 V for 27 h at 15°C (SE 600 vertical slab gel unit; Hoefer Scientific Instruments, Holliston, MA, USA). The total acrylamide concentration was 4 and 7% in the stacking and separating gel, respectively. The gel

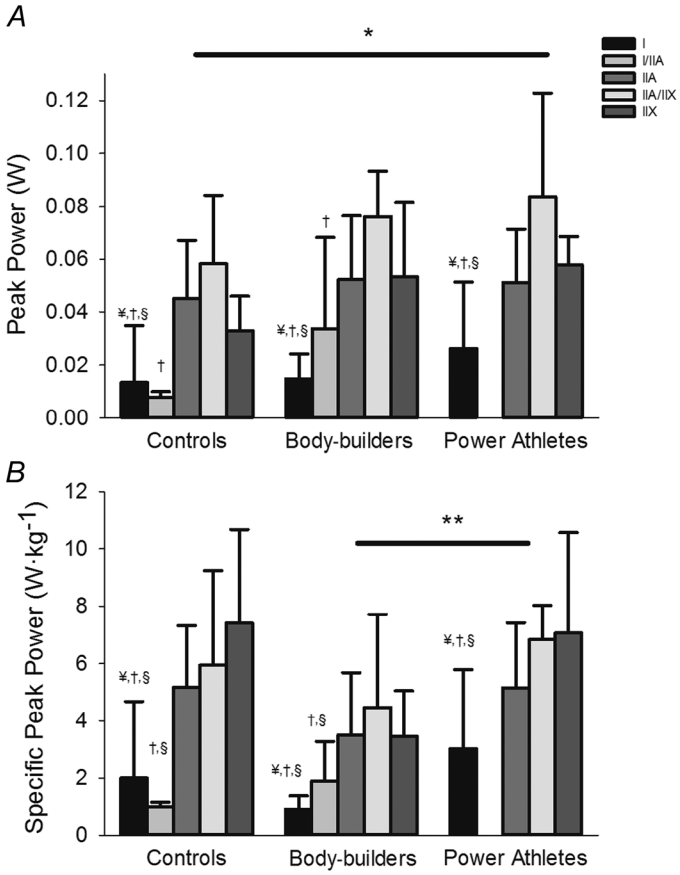

Figure 2. Peak power (in watts; *A*) and specific peak power (in watts per kilogram; *B*) of skinned muscle fibre segments from the vastus lateralis of non-resistance-trained control subjects, body-builders and power athletes
*Differences between groups at $P < 0.05$; **differences between indicated groups at $P < 0.01$; †different from type IIA at $P < 0.05$; §different from type IIA/IIX at $P < 0.05$; and ¥different from type IIX at $P < 0.05$.

matrix consisted of 35% glycerol. A 10-μm-thick section of a human soleus muscle biopsy dissolved in sample buffer was used as a marker for the three myosin heavy chain isoforms in human muscle (I, IIA and IIX). Gels were stained using a Silverstain Plus kit (Bio-Rad, Hemel Hempstead, UK).

Data analysis

Data analysis of the force–velocity relationship has been described before (Gilliver *et al.* 2009; Degens *et al.* 2010). The last 100 ms of each isotonic release was used to determine the velocity during the step. This resulted in 16 force–velocity data points, which were fitted to the Hill equation (Hill, 1938) using a non-linear least-squares regression. Maximal shortening velocity (V_{max}) in fibre lengths per second (FL s^{-1}) was extrapolated from this curve. The best-fit values for the Hill heat constants '*a*' and '*b*' (where '*b*' signifies '*a*' multiplied by the unloaded shortening velocity divided by the maximal isometric tension) were then used to calculate peak power using the following formulae (Gilliver *et al.* 2011):

$$M = \frac{\left(\sqrt{1+\left(\frac{F0}{a}\right)} - 1\right)}{\frac{P0}{a}}$$

$$\text{Peak power} = M^2 \times F_0 \times V_{max}$$

The K_{TR} was analysed by feeding the following formula to a non-linear least-squares regression:

$$F = F_{max}\left(1 - e^{-K_{TR}t}\right)$$

To determine the goodness of fit for the K_{TR} and the force–velocity curve, the fitted curves and the measured

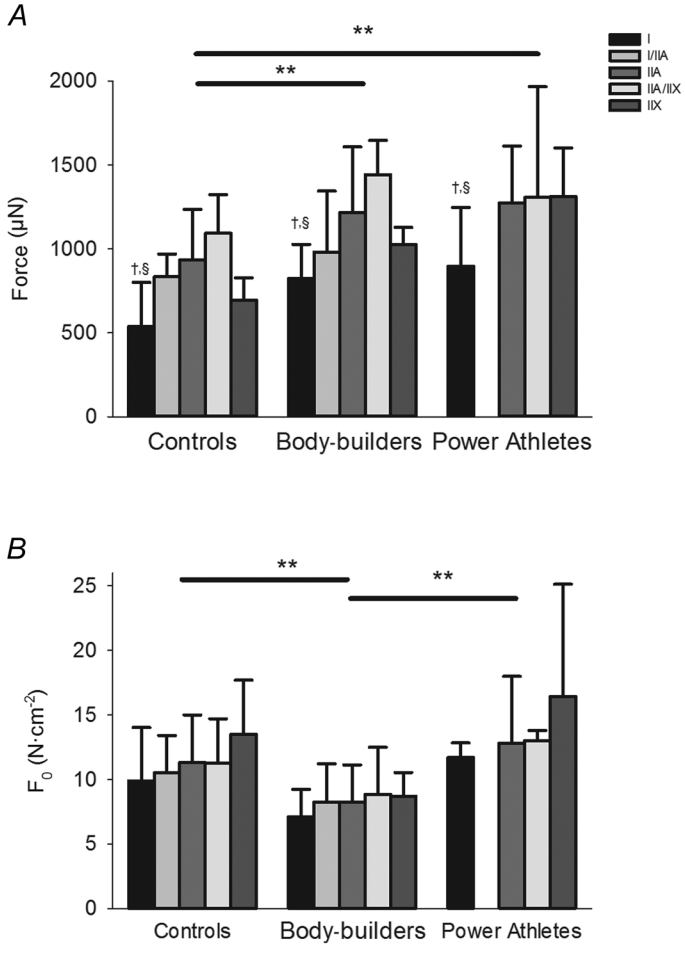

Figure 3. Maximal isometric tension (force in micronewtons; *A*) and specific tension (F_0 in newtons per square centimetre; *B*) of skinned vastus lateralis fibre segments of non-resistance-trained control subjects, body-builders and power athletes
Data are presented as mean values + SEM. **Differences between indicated groups at $P < 0.01$; †different from type IIA at $P < 0.05$; and §different from type IIA/IIX at $P < 0.05$.

force data were correlated using the Pearson correlation. For the force–velocity curve, data were accepted if $r^2 > 0.96$. The K_{TR} was analysed only if the data for force velocity met the criterion. For the general analyses of K_{TR}, an $r^2 > 0.90$ was used as an inclusion criterion. In addition to the criterion of goodness of fit, fibres were rejected if sarcomere length had changed by >0.1 μm or if maximal isometric tension was decreased by >10% after the four sets of isotonic releases. Only if a fibre was accepted was it dissolved in SDS sample buffer and stored at −80°C for later SDS-PAGE.

Statistical analysis

Data were analysed by averaging values of muscle fibres per type per person and feeding these into a linear mixed model [group (BB, PA or C)] × fibre type (I, IIA, IIA/IIX or IIX)]. Participant was included as a random factor. In the case of significant main effects, a *post hoc* analysis with Bonferroni correction was performed. Interactions are reported only when significant. Differences were considered significant at $P < 0.05$. We tested the significance of the difference between regression slopes with an *F*-test (Sokal & Rohlf, 2012). Reported figures and numbers are based on averages per fibre type per participant. Error bars show a single standard deviation.

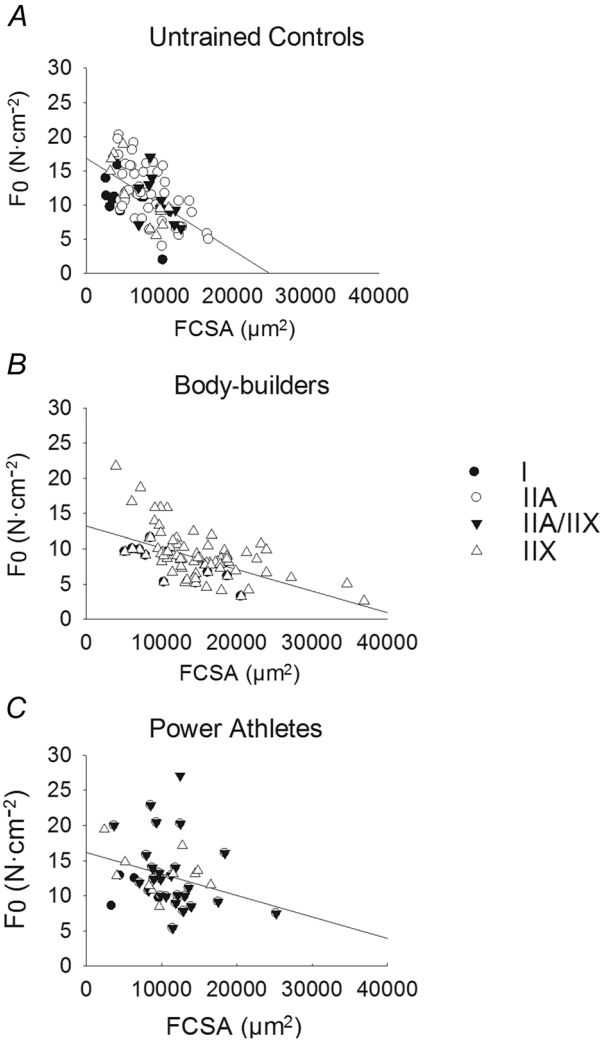

Figure 4. Specific tension of skinned vastus lateralis fibres is inversely related to muscle FCSA in non-resistance-trained control subjects (*A*), body-builders (*B*) and power athletes (*C*)

Results

In total, 14, five and 11 type I, four, zero and two type I/IIA, 43, 26 and 47 type IIA, 19, 12 and nine type IIA/IIX and six, two and 12 type IIX fibres were analysed from BBs, PAs and Cs, respectively. Measurements were performed in three separate batches.

As can be seen in Fig. 1, fibres from BBs were 67 and 88% ($P < 0.01$) larger than those of PAs and Cs, respectively, with no significant difference in FCSA between PAs and Cs. A significant main effect for FCSA was also found over fibre types, but *post hoc* analysis did not reveal the location of the differences between fibre types.

As shown in Fig. 2*A*, peak power in type I fibres was lower than that of IIA, IIAX and IIX fibres ($P < 0.01$). The peak power of I/IIA fibres was also lower than that of type IIA/IIX fibres ($P < 0.01$). Peak power was higher in PA fibres than those of Cs (58%; $P < 0.05$), whereas fibres of BBs tended to have a higher peak power than C fibres ($P = 0.07$).

Type I and I/IIA fibres had a lower specific power than IIX and IIA/IIX fibres ($P < 0.01$). The specific power of type I fibres was also lower than that of type IIA fibres ($P < 0.05$). Fibres from PAs had a 98% higher specific power than those of BBs ($P < 0.01$), whereas fibres from BBs tended to have a lower specific power than C fibres ($P = 0.08$).

As can be seen in Fig. 3, force generated by type I fibres was lower than that by IIA and IIA/IIX fibres ($P < 0.01$). Fibres of BBs and PAs produced a higher force than those of Cs (33 and 50%, respectively; $P < 0.01$), irrespective of fibre type. The F_0 did not differ between fibre types, but as can be seen in Fig. 3*B*, F_0 of fibres from PAs and Cs was higher than that of fibres from BBs (63%, $P < 0.01$; 41%, $P < 0.01$).

The observation that fibres of BBs have a lower F_0 and a larger FCSA than those of PAs and Cs gave rise to the hypothesis that excessive hypertrophy has a detrimental effect on F_0. To gain insight into this relationship, we plotted F_0 against FCSA. As can be seen in Fig. 4, there is an linear inverse relationship between FCSA and F_0 for all groups and fibre types, indicating that hypertrophy (or at least a large FCSA) is detrimental for F_0. There were no

significant differences between the slopes of the regression lines.

As can be seen in Fig. 5, type I and I/IIA fibres were slower than IIA, IIA/IIX and IIX fibres in terms of V_{max} (Fig. 5*A*; $P < 0.01$) and K_{TR} (Fig. 5*B*; $P < 0.01$). The K_{TR} of type I/IIA fibres was also lower than that of IIA and IIA/IIX fibres ($P < 0.01$). The value of a/F_0 did not differ significantly between fibre types. Values of V_{max}, a/F_0 and K_{TR} did not differ significantly between BBs, PAs and Cs. This indicates that it is unlikely that the lower F_0 in the BBs is a result of altered cross-bridge dynamics.

In order to look further into the possibility of altered cross-bridge kinetics, we studied the Huxley rate constants. The rate of force redevelopment is representative for $(f + g_1)$ in the Huxley model, where f represents the rate constant of attachment of cross-bridges and g_1 represents the rate constant of detachment of cross-bridges that exert a positive force (Huxley, 1957). The value V_{max}

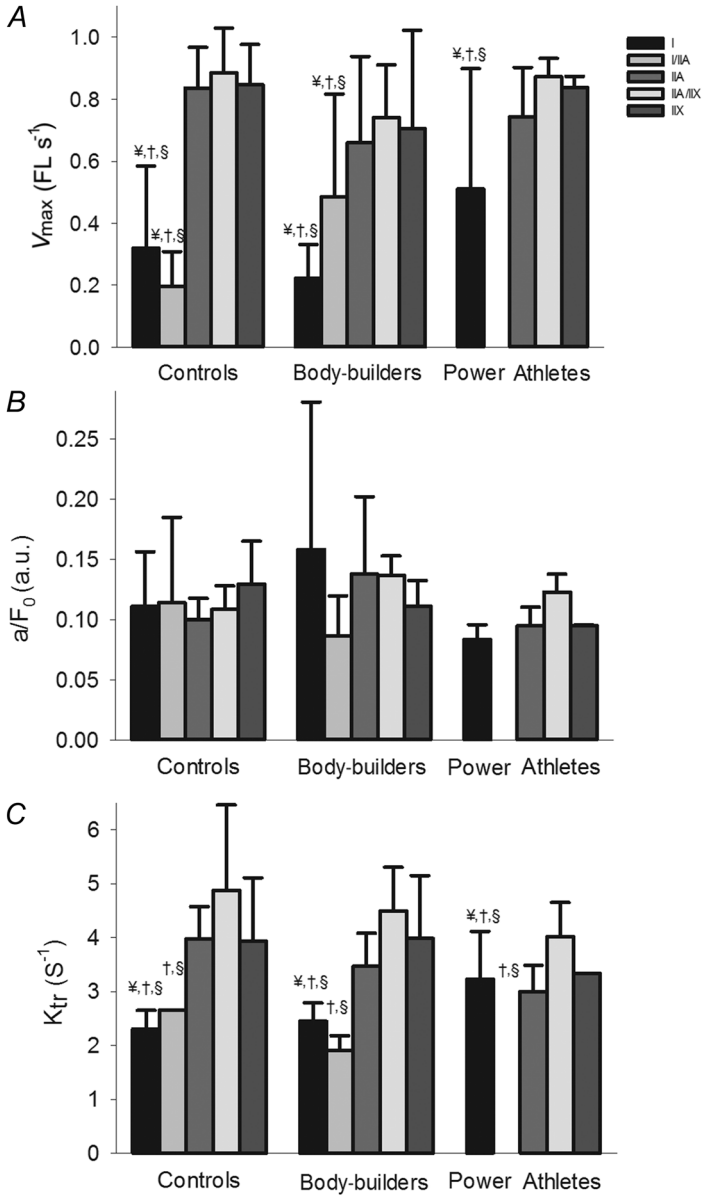

Figure 5. Maximal shortening velocity [V_{max} in fibre lengths (FL) per second; *A*], rate of force development (K_{TR} per second; *B*) and curvature of the force–velocity relationship (a/F_0 in arbitrary units; *C*) for control subjects, body-builders and power athletes according to fibre type
Data are mean values + SEM. †Different from type IIA at $P < 0.05$; §different from type IIA/IIX at $P < 0.05$; and ¥different from type IIX at $P < 0.05$.

is representative of g_2 in the Huxley model, where g_2 stands for the rate constant of detachment of cross-bridges exerting a negative force due to compression (Huxley, 1957). The intercept of a regression between K_{TR} and V_{max} may represent an approximation of g_1 (Gilliver *et al.* 2011). These regressions are shown in Fig. 6. The data for K_{TR} in this analysis had been subjected to stricter inclusion criteria (r^2 of 0.96 instead of 0.90) than the data for K_{TR} represented in Fig. 5. The intercepts (reflecting g_1) of Cs, BBs and PAs were 2.19 (95% confidence interval 1.51–2.86), 2.38 (95% confidence interval 1.24–3.53) and 2.34 (95% confidence interval 1.83–2.90). The intercept of each group falls within the confidence intervals of the other intercepts, indicating that g_1 does not differ significantly between the different groups. There were also no significant differences between the slopes of the regression lines.

In order to visualize the different contributions of V_{max}, F_0 and a/F_0 to power production, the force–velocity and force–power curves of three typical examples of IIA muscle fibres of each group are shown in Fig. 7. The values of V_{max} and a/F_0 are similar in all groups, whereas F_0 and specific power were greater in PAs than in BBs.

Discussion

We observed that muscle fibres of BBs and PAs generated a higher maximal isometric tension than those of non-resistance-trained men (Cs), indicating that years of resistance training has a positive effect on maximal tension. Peak power, however, was increased only in PAs and not in BBs, despite significant hypertrophy in BBs. Specific peak power (in watts per kilogram) was higher in PA than BB fibres, which can be explained largely by the higher fibre specific tension (maximal tension normalized by FCSA, F_0; in newtons per square centimetre) of PAs than BBs and Cs. The F_0 of BB fibres was also lower than that of C fibres. It has been reported before that regular physical activity may induce changes in specific tension (D'Antona *et al.* 2006, 2007; Degens *et al.* 2009; Erskine *et al.* 2011). While this may suggest that body-building is detrimental for specific tension, it may well be that hypertrophy itself is detrimental for specific tension regardless of exercise experience, because in all groups a similar inverse relationship between FCSA and F_0 was shown. Possible explanations are discussed below.

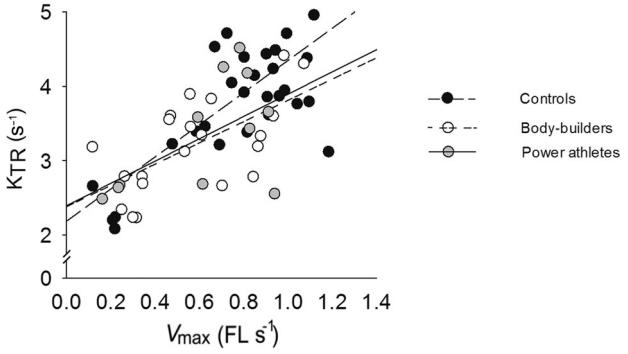

Figure 6. Rate of force redevelopment (K_{TR}) *versus* the maximal shortening velocity (V_{max}) of skinned vastus lateralis muscle fibre segments of non-resistance-trained control subjects, body-builders and power athletes
The intercepts of the regression lines represent g_1 (rate of detachment of cross-bridges exerting a positive force) in the Huxley model. In order to make a more precise estimation of g_1, a stricter inclusion criterion for rate of force redevelopment was used for this regression (see main text). In total, 44 fibres from 15 participants met the inclusion criteria. Separate fibres were regarded as separate observations.

Single skinned muscle fibre segments of BBs have been studied before (D'Antona *et al.* 2006). These authors reported, similar to the present study, that the F_0 of type I fibres of BBs was lower than those of Cs. In contrast to our observations, however, they reported for type II fibres a higher, rather than a lower F_0 in BBs than Cs (D'Antona *et al.* 2006). Part of this discrepancy may be related to the timing in sampling of the muscle biopsies, because BBs generally switch between a bulking phase, in which hypertrophy is maximized, and a calorie-deficient, weight-cutting phase. This phase switching may have a significant impact on specific tension, as illustrated in a case study where the loss of 3.9% of fat-free mass during the cutting phase was accompanied by a proportionally larger (13.8%) reduction in the one-repetition maximum squat (Rossow *et al.* 2013). Subjects in the present study had been in the between-competition phase for at least 6 months before the sampling of the biopsy, the period during which body-builders usually perform little aerobic exercise or cut down on caloric intake. Another possible factor may be differences in anabolic steroid use. In the study of (D'Antona *et al.* 2006), two of five BBs reported the use of anabolic steroids, whereas in the present study nine of 12 BBs admitted to using anabolic steroids. However, neither D'Antona *et al.* (2006) nor we found differences between contractile properties of steroid users and non-steroid users.

Altered F_0

The F_0 of BBs was lower than that of Cs and PAs, suggesting that their (excessive) hypertrophy has a detrimental effect on F_0. A negative trend between FCSA and F_0 has been reported before in single muscle fibre segments of untrained people (Gilliver *et al.* 2009) and frogs (Elzinga *et al.* 1989). In the present study, this negative trend is apparent across all groups, as can be seen in Fig. 4. It has been suggested that this may be related to accumulation of inorganic phosphate (P_i) in the larger fibres, owing to

longer diffusion times from the interior of the fibre to the surrounding incubation medium (Elzinga *et al.* 1989).

A relatively larger increase in FCSA than acquisition of new myonuclei in BBs would result in a larger myonuclear domain (MND). Indeed, it has been observed that the MND is increased after 90 days of progressive resistance training by healthy men (Kadi *et al.* 2004) and is larger in non-functional hypertrophy caused by myostatin knockout (Qaisar *et al.* 2012). Our preliminary data do suggest that the MND is indeed larger in BBs than Cs and smallest in PAs [BBs, 92.45 ± 42.48 pl ($n = 14$); Cs, 80.57 ± 32.09 pl ($n = 5$); PAs, 70.32 ± 32.08 pl ($n = 5$); MND was determined by 4′,6-diamidino-2-phenylindole staining of longitudinal single muscle fibres, n thus represents the number of single muscle fibres]. An enlarged MND may diminish the transcriptional capacity of the muscle cell, which may result in a slower replacement of proteins. This slower replacement would increase the chance of post-translational modifications that make the proteins work suboptimally. It remains to be seen whether the MND is also larger in BBs than in Cs and whether BBs have an elevated abundance of post-translationally modified myofibrillar proteins.

The potential accumulation of post-translationally modified actin or myosin heavy chains and local P_i accumulation and/or impaired local ATP supply should be reflected by impaired cross-bridge kinetics. In the cross-bridge model of Huxley, f_1 represents the rate constant of cross-bridge attachment, g_1 the rate constant of detachment of cross-bridges that exert a positive force, and g_2 the rate constant of detachment of cross-bridges exerting a negative force (Huxley, 1957). The percentage of attached cross-bridges is represented by $f_1/(f_1 + g_1)$ (Gilliver *et al.* 2011). However, a/F_0 $[(f_1 + g_1)/g_2]$, K_{TR} $(f_1 + g_1)$ and V_{max} (g_2) did not differ between groups. To estimate g_1, we plotted K_{TR} $(f_1 + g_1)$ against V_{max} (g_2), where the intercept may represent g_1 (Gilliver *et al.* 2011). We found that the intercept of the curve, g_1, was similar in the three groups. The absence of significant differences in K_{TR}, V_{max}, a/F_0 and g_1 between groups suggests that cross-bridge kinetics are similar and that the myofibrillar proteins therefore are not functioning differently in any group. Although it cannot be excluded entirely, the unaltered cross-bridge kinetics suggests that diffusion limitations and/or an increased abundance of post-translational myofibrillar protein modifications play only a minor role in the lower F_0 of BBs.

Alternatively, the observed differences in F_0 may be attributable to differences in myofibrillar density between fibres of the BBs, PAs and Cs. Changes in myofibrillar density, hence in the number of cross-bridges per unit cross-sectional area, have indeed been reported. Disuse and ageing have been associated with lower myosin concentrations (D'Antona *et al.* 2003), while weight training has been reported to increase the myosin concentration (Penman, 1970) and specific tension. The decrease in F_0 of fibres of BBs may therefore be attributable to a decrease in myofibrillar density, as also suggested by an increased cytoplasmic content of non-contractile elements (Schoenfeld, 2010).

Study limitations

Nine of 12 BBs admitted to recent use of anabolic steroids. Although one might expect that this could affect the contractile properties of skeletal muscle fibres, neither previous investigators (D'Antona *et al.* 2006) nor we found significant differences in contractile properties or FCSA between steroid users and non-users in our BBs. However, it has to be considered that it is difficult to obtain accurate information from athletes on steroid use, be they

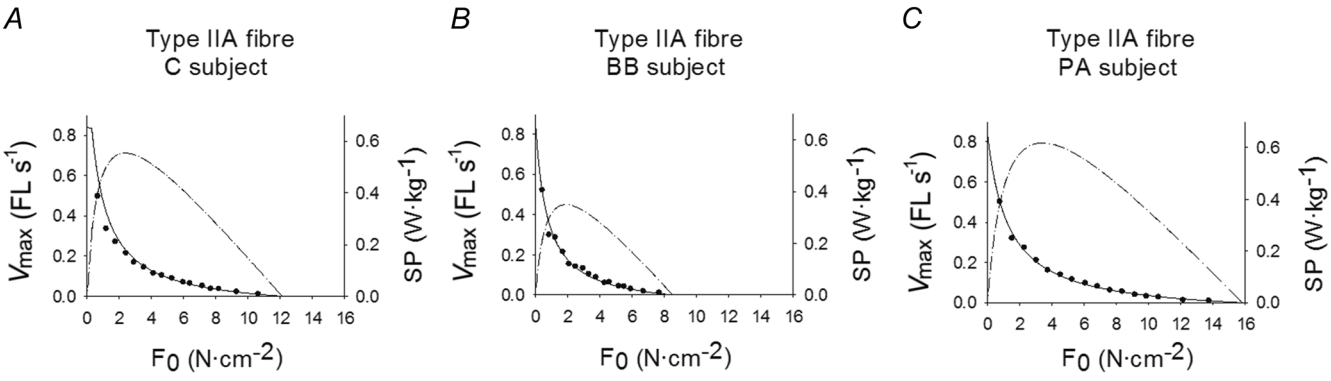

Figure 7. Typical examples of three type IIA muscle fibres of a body-builder, power athlete and non-resistance-trained control subject
Circles represent the measured force–velocity data. The continuous lines show the fitted force–velocity curves and the dashed lines show the force–power curves.

BBs, PAs or from other specialties. Finally, owing to the nature of single muscle fibre work, our data set contains a relatively small number of participants and fibres per participant, with large interindividual variations. As a result of this large variation, the statistical power may have been too low to reach statistical significance for all possible differences between groups. Nevertheless, the numbers of fibres and participants in our study are comparable to those in many other studies on single skinned muscle fibres (Frontera *et al.* 2000; Li *et al.* 2015) and were sufficient to reveal significant differences in fibre contractile properties between groups.

Perspective

This work shows that long-term resistance exercise, represented here by PAs and BBs, increases the force-generating capacity of muscle fibres. Only in PAs was this increase in force associated with a significant increase in the power-generating capacity of single muscle fibres, resulting primarily from an increase in FCSA. The power-generating capacity of BBs was, however, not higher than that of Cs, despite their significantly larger muscle fibres. This unexpected observation was explicable by a lower F_0 in BB compared with C fibres. The work therefore suggests that high-intensity, low-volume resistance training with aerobic exercise, as performed by PAs, is beneficial to peak power, whereas low- to moderate-intensity, high-volume training, as performed by BBs, does not affect peak power and is even detrimental to F_0. Given that Cs and PAs performed comparable amounts of aerobic exercise, it is likely that the effects shown in PAs can be attributed to the high-intensity, low-volume resistance training. We postulate that the decrease in specific tension is caused by differences in myofibrillar density and/or, possibly, post-translational modifications.

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Additional information

Competing interests

None declared.

Author contributions

J.P.M., R.T.J., J.R., O.R.S., S.K., M.B., A.S., R.P., B.S., M.V.N. and H.D. contributed to the conception and design of the work. J.P.M., R.T.J. and H.D. contributed to acquisition, analysis and interpretation of the data and drafting the manuscript and revising it critically.

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