Single muscle fibre contractile properties differ between bodybuilders, power athletes and controls

JP Meijer[1,2], RT Jaspers[2], J Rittweger[3], OR Seynnes[4], S Kamandulis[5], M Brazaitis[5], A Skurvydas[5], R Pišot[6], B Šimunič[6], MV Narici[7], H Degens[1]

1; School of Healthcare Science, Manchester Metropolitan University (Manchester, United Kingdom), 2; Laboratory for Myology, MOVE Research Institute Amsterdam, Faculty of Human Movement Sciences, VU University Amsterdam (Amsterdam, The Netherlands), 3; Division Space Physiology, German Aerospace (Cologne, Germany), 4; Norwegian School of Sport Sciences (Oslo, Norway), 5; Sports Science and Innovation Institute, Lithuanian Sports University (Kaunas, Lithuania), 6; Institute of Kinesiology Research, University of Primorska, (Koper, Slovenia), 7; University of Nottingham (Nottingham, United Kingdom)

Running head: Muscle fibre force after resistance training

Key words: single skinned muscle fibre fragments, force characteristics, high-intensity low-volume resistance training, low- to moderate-intensity high-volume resistance training

Word count: 6255 **References:** 31

Address for correspondence:

Dr. ir. Hans Degens

School of Healthcare Science

Manchester Metropolitan University

John Dalton Building, Chester Street

Manchester, M1 5GD, United Kingdom

Phone: +44.161.247.5686

E-mail: h.degens@mmu.ac.uk

Abstract

We compared muscle fibre contractile properties of biopsies taken from *m. vastus lateralis* of 12 bodybuilders (BB; low- to moderate-intensity high-volume resistance training), 6 power athletes (PA; high-intensity low-volume combined with aerobic training) and 14 controls (C). Maximal isotonic contractions were performed in single muscle fibres, typed with SDS-PAGE. Fibre cross-sectional area (FCSA) was 67% and 88% ($P<0.01$) larger in BB than in PA and C, respectively, with no significant difference in FCSA between PA and C. Fibres of BB and PA developed a higher maximal isometric tension (32%, 50%, $P < 0.01$) than those of C. Specific tension (F_0) of BB fibres was 62% and 41% lower than that of PA and C fibres ($P < 0.05$), respectively. Irrespective of fibre type, peak power (P) of PA fibres was 58% higher than that of BB fibres ($P < 0.05$), while BB fibres –despite considerable hypertrophy- had similar PP as 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 F_0. We postulate that the decrease in specific tension is caused by differences in myofibrillar density and/or post-translational modifications of contractile proteins.

2

Introduction

The performance of a power athlete is largely determined by 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 that 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 muscle mass (specific power) and maximal force per 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 where 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). This raises the question whether it is possible to improve specific power and F_0 and if so, what training program 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 bodybuilders compared to non-bodybuilders (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 maximum tension (Trappe *et al.*, 2006), while others reported a decrease in F_0 of type I fibres after a 12-week aerobic exercise program (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 bodybuilding, it may even be reduced (D'Antona *et al.*, 2006). On the other hand, 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 *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 due to 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 this study was to determine how different long-term resistance training programs affect specific power and F_0. For this purpose, we analysed contractile properties of skinned single muscle fibre segments from bodybuilders (BB), power athletes (PA) and non-competitive controls (C).

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 pre-competition 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 program of PAs is characterized by high-intensity low-volume resistance training with supplemental aerobic exercise. Since 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 to C.

Methods

Participants

Muscle biopsies were collected from the *m. vastus lateralis* of 12 male bodybuilders (BB: 29.8 ± 4.8 y; 177.8 ± 4.1 cm; 91.7 ± 13.4 kg), 6 power athletes (PA: 23.4 ± 3.9 y; 185.0 ± 4.3 cm; 103.0 ± 7.3 kg) and 14 non-competitive controls (C: 24.0 ± 3.5 y; 180.9 ± 5.3 cm; 77.9 ± 6.3 kg) after giving written informed consent. The study was performed conform the Declaration of Helsinki. The *m. 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, bodybuilders and the control population. In addition, biopsies from the *m. vastus lateralis* can be obtained with minimal discomfort to the participant due to 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 participated also in the study by (Seynnes *et al.*, 2013) and 3 of them in the study by (Salvadego *et al.*, 2013). The PAs and Cs have been involved in the study by (Salvadego *et al.*, 2013). Training diaries of BBs showed moderate- to high-

intensity and high-volume training regimens (4-5 sets · 8-15 repetitions · 60-80% of 1-repetition maximum (1RM), 3 to 5 times a week) (Seynnes *et al.*, 2013), with no reported aerobic exercise. All bodybuilders 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 minutes 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 minutes per week and other recreational sports activities for 102±143 minutes per week. The C 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 *m. quadriceps femoris* was measured. In all PA, C and 3 BB volume was assessed with magnetic resonance imaging, while the quadriceps volume of the remaining BB was measured using ultrasound. BB had a quadriceps volume of 2852±892 cm^3, PA 3194±349 cm^3, and C 2550±630 cm^3; indicating that both PA and BB had developed significant hypertrophy.

Biopsy collection

Muscle biopsies were taken under local anaesthesia (1 mL 2% lidocaine) with a conchotome. The biopsies were collected in relax solution and after 24 hrs. in glycerol/relax solution at 4^0C, sucrose treated and stored at -80^0C (Frontera & Larsson, 1997; Degens *et al.*, 2010). Before use, the muscle biopsy was desucrosed, stored in glycerol/relax solution at -20^0C 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, relax solution contained 4.5 mM MgATP, 1 mM free Mg^{2+}, 10 mM imidazole, 2 mM EGTA, 100 mM KCl and pH

7.0. The glycerol/relax solution contained 50% glycerol (v/v). Trition/relax was made by adding 1% Triton X-100 (v/v) to the relax 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, 64 mM KCl with a pH of 7.0 and a pCa^{2+} 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 permeabilised in 1% triton-X100 in relax solution for 20 minutes. The biopsy was then moved into relax 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., Canada) and a motor arm (312C, Aurora Scientific Inc., Canada). Temperature of the relax and activating solutions was kept at 15°C and checked at regular intervals. In previous studies we have seen that the optimum sarcomere length for human fibres is at 2.6 μm (unpublished data). Therefore, sarcomere length was set at 2.6 μm., as determined by a Fourier transformation of the sarcomere pattern (900A, Aurora Scientific Inc., Canada), by adjusting the length of the fibre. Fibre width was measured while the fibre was immersed in the relax solution, assuming a circular circumference. FCSA, $F_{0,}$ and specific power were not corrected for swelling. Fibre length was determined using a digital bore gauge (Gemred, 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 optimum 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 re-stretched to optimum 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 less than 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% optimum length and restretched after 15 ms, which forcibly uncouples myosin heads from actin. Force was then allowed to redevelop to maximal isometric tension. K_{TR} was determined by fitting the force tracing 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 hours at 15°C (SE 600 vertical slab gel unit, Hoefer Scientific Instruments, U.S.A.). The total acrylamide concentration was 4 and 7% in the stacking and separating gel, respectively. The gel matrix consisted of 35% glycerol. A 10-μm 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, 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 constant 'a' and 'b' (where 'b' signifies 'a' multiplied with the unloaded shortening velocity divided by the maximal isometric tension) were then used to calculate peak power using the following formulas (Gilliver *et al.*, 2011):

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

$$Peak\ power = M^2 * F_0 * Vmax$$

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

$$F = F(max) * (1 - e^{-KTR*t})$$

To determine the goodness of fit for the K_{TR} and the force-velocity curve, the fitted curves and the actual force data were correlated using Pearson correlation. For the force-velocity curve, data were accepted if $R^2 > 0.96$. K_{TR} was only analysed 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 more than 0.1 μm or if maximal isometric tension was decreased by more than 10% after the four sets of isotonic releases. Only if a fibre was accepted, it was dissolved in sodium dodecyl sulphate (SDS) sample buffer and stored at -80°C for later SDS-polyacrylamide gel electrophoresis (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, C]) × fibre type [I, IIA, IIA/IIX, IIX]). Participant was included as a random factor. In case of significant main effects, a post-hoc analysis with Bonferroni correction was performed. Interactions are only reported 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 based on group averages based on averages per fibre type per participant. Error bars show a single standard deviation.

Results

In total 14, 5, and 11 type I; 4, 0, and 2 type I/IIA; 43, 26 and 47 type IIA; 19, 12, and 9 type IIA/IIX; and 6, 2, and 12 type IIX fibres were analysed of BBs, PAs, and Cs, respectively. Measurements were performed in three separate batches.

As can be seen in figure 1, BB fibres were 67% and 88% (P < 0.01) larger than those of PA and C, with no significant difference in FCSA between PA and C. 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 figure 2A, 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 controls (58%; P < 0.05), while fibres of BB 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). PA fibres had a 98% higher specific power than those of BB (P < 0.01), while fibres from BB tended to have a lower specific power than C fibres (P = 0.08).

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

The observation that fibres of BB have a lower F_0 and a larger FCSA than those of PA and C gave rise to the hypothesis that excessive hypertrophy has a detrimental effect on F_0. To gain insight in this relationship, we plotted F_0 against FCSA. As can be seen in figure 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 a no significant differences between the slopes of the regression lines.

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

To further look into the possibility of altered cross-bridge kinetics, we studied the Huxley rate constants. K_{TR} 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). V_{max} is representative for 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 figure 7. The data for K_{TR} in this analysis had been subject to stricter inclusion criteria (R^2 of 0.96 instead of 0.90) than the data for K_{TR} represented in figure 6. The intercepts (reflecting g_1) of C, BB, and PA were 2.19 (95%CI: 1.51 to 2.86), 2.38 (95%CI: 1.24 to 3.53) and 2.34 (95%CI: 1.83 to 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.

To visualize the different contributions of V_{max}, F_0 and a/F_0 on power production the force-velocity and power curves of three typical examples of IIA muscle fibres of each group are shown in figure 7. V_{max} and a/F_0 are similar in all groups, while F_0 and specific power were greater in PA than in BB.

Discussion

We observed that muscle fibres of bodybuilders (BB) and power athletes (PA) generated a higher maximal isometric tension (F) than those of non-resistance trained men (C), indicating that years of resistance training positively affects maximal tension. Peak power , however, was only increased in PA and not in BB, despite significant hypertrophy in bodybuilders. Specific peak power ($W \cdot kg^{-1}$) was higher in PA than BB fibres, which can be largely explained by the higher fibre specific tension (maximal tension normalized by FCSA, F_0; $N \cdot \mu m^{-2}$) of PA than BB and C. 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; D'Antona *et al.*, 2007; Degens *et al.*, 2009; Erskine *et al.*, 2011). While this may suggest that bodybuilding is detrimental for specific tension, it may well be that hypertrophy itself is detrimental for specific tension regardless of exercise experience, as in all groups a similar inverse relationship between FCSA and F_0 was shown. Possible explanations are discussed below

Single skinned muscle fibre segments of BB have been studied before (D'Antona *et al.*, 2006). These authors reported, similar to us, that the F_0 of type I fibres of BB was lower than those of C. In contrast to our observations, however, they reported for type II fibres a higher, rather than a lower F_0 in BB than C (D'Antona *et al.*, 2006). Part of this discrepancy may be related to the timing in sampling of the muscle biopsies, as BB generally switch between a bulking phase, in which hypertrophy is

12

maximized, and a caloric 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 bodybuilders 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), 2 out of 5 BB reported to use anabolic steroids, while in the present study 9 out of 12 BB 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

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 figure 4. It has been suggested that this may be related to accumulation of Pi in the larger fibres, due to longer diffusion times form 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 BB 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 knock-out (Qaisar *et al.*, 2012). Our preliminary data do suggest that the MND is indeed larger in BB than C and smallest in PA (BB: 92.45 ± 42.48 pL (*n*=14); C: 80.57 ± 32.09 pL (*n*=5); PA: 70.32 ± 32.08 pL (*n*=5); MND was determined by 4',6-diamidino-2-fenylindool (DAPI)

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 sub-optimally. It remains to be seen whether also in BB the

MND is larger than in C and whether BB have an elevated abundance of post-translationally modified

myofibrillar proteins.

The potential accumulation of post-translationally modified actin or myosin heavy chains and

local Pi 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, neither a/F_0 [*$(f_1+g_1)/g_2$*], K_{TR} [

f_1+g_1], or V_{max} [*g_2*] differed 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, *g1*,

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 BB.

Alternatively, the observed differences in F_0 may be attributable to differences in myofibrillar

density between fibres of the BB, PA and C. Changes in myofibrillar density, and hence 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 BB 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 out of 12 BB admitted to recently using 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 BB. However, it has to be considered that it is difficult to obtain accurate information from athletes on steroid use, be they BB, PA or from other specialties. Finally, due to the nature of single muscle fibre work, our data set contains a relatively small number of participants and fibres per participant, with large inter-individual variations. Because of this large variation the statistical power may have been too low to reach statistical significance for all possible differences between groups. Nevertheless, the number 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 was sufficient to reveal significant differences in fibre contractile properties between groups.

Perspective

This work shows that long-term resistance exercise, represented here by power athletes and body builders, increases the force generating capacity of muscle fibres. This increase in force was only in power athletes associated with a significant increase in the power generating capacity of single muscle fibres, resulting from primarily an increase in fibre cross-sectional area (FCSA). The power generating capacity of body builders was, however, not higher than that of control fibres, despite their significantly larger muscle fibres. This unexpected observation was explicable by a lower F_0 in BB compared to C

15

fibres. The work therefore suggests that high-intensity low-volume resistance training with aerobic exercise, as performed by power athletes, is beneficial to PP, while low- to moderate-intensity high-volume training, as performed by body builders does not affect PP and is even detrimental to F_0. Since C and PA performed comparable amounts of aerobic exercise, it is likely that the effects shown in PA 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.

Additional information

References

D'Antona G, Lanfranconi F, Pellegrino MA, Brocca L, Adami R, Rossi R, Moro G, Miotti D, Canepari M & Bottinelli R (2006). Skeletal muscle hypertrophy and structure and function of skeletal muscle fibres in male body builders. *The Journal of Physiology* **570,** 611-627.

D'Antona G, Pellegrino MA, Adami R, Rossi R, Carlizzi CN, Canepari M, Saltin B & Bottinelli R (2003). The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. *The Journal of Physiology* **552,** 499-511.

D'Antona G, Pellegrino M, Carlizzi C & Bottinelli R (2007). Deterioration of contractile properties of muscle fibres in elderly subjects is modulated by the level of physical activity. *European Journal of Applied Physiology* **100,** 603-611.

Degens H (2012). Determinants of skeletal muscle hypertrophy and the attenuated hypertrophic response at old age. *Journal of Sports Medicine & Doping Studies* **S1**.

Degens H, Bosutti A, Gilliver S, Slevin M, van Heijst A & Wüst R (2010). Changes in contractile properties of skinned single rat soleus and diaphragm fibres after chronic hypoxia. *Pflügers Archiv European Journal of Physiology* **460,** 863-873.

Degens H, Erskine RM & Morse CI (2009). Disproportionate changes in skeletal muscle strength and size with resistance training and ageing. *J Musculoskelet Neuronal Interact* **9,** 123-129.

Degens H & Larsson L (2007). Application of skinned single muscle fibres to determine myofilament function in ageing and disease. *J Musculoskelet Neuronal Interact* **7,** 56-61.

Elzinga G, Stienen GJ & Wilson MG (1989). Isometric force production before and after chemical skinning in isolated muscle fibres of the frog Rana temporaria. *The Journal of Physiology* **410,** 171-185.

Erskine RM, Jones DA, Maffulli N, Williams AG, Stewart CE & Degens H (2011). What causes in vivo muscle specific tension to increase following resistance training? *Experimental Physiology* **96,** 145-155.

Frontera WR & Larsson L (1997). Contractile studies of single human skeletal muscle fibers: A comparison of different muscles, permeabilization procedures, and storage techniques. *Muscle & Nerve* **20,** 948-952.

Frontera WR, Suh D, Krivickas LS, Hughes VA, Goldstein R & Roubenoff R (2000). Skeletal muscle fiber quality in older men and women. *American Journal of Physiology-Cell Physiology* **279,** C611-C618.

Gilliver S, Jones D, Rittweger J & Degens H (2011). Variation in the determinants of power of chemically skinned type I rat soleus muscle fibres. *Journal of Comparative Physiology. A: Sensory, Neural, and Behavioral Physiology* **197,** 311-319.

Gilliver SF, Degens H, Rittweger J, Sargeant AJ & Jones DA (2009). Variation in the determinants of power of chemically skinned human muscle fibres. *Experimental Physiology* **94,** 1070-1078.

Harber MP, Konopka AR, Undem MK, Hinkley JM, Minchev K, Kaminsky LA, Trappe TA & Trappe S (2012). Aerobic exercise training induces skeletal muscle hypertrophy and age-dependent adaptations in myofiber function in young and older men. *Journal of Applied Physiology* **113,** 1495-1504.

Hill AV (1938). The Heat of Shortening and the Dynamic Constants of Muscle. *Proceedings of the Royal Society of London. Series B - Biological Sciences* **126,** 136-195.

Huxley AF (1957). Muscle structure and theories of contraction. *Prog Biophys Biophys Chem* **7,** 255-318.

Kadi F, Schjerling P, Andersen LL, Charifi N, Madsen JL, Christensen LR & Andersen JL (2004). The effects of heavy resistance training and detraining on satellite cells in human skeletal muscles. *The Journal of Physiology* **558,** 1005-1012.

Larsson L & Moss RL (1993). Maximum velocity of shortening in relation to myosin isoform composition in single fibres from human skeletal muscles. *The Journal of Physiology* **472,** 595-614.

Li M, Ogilvie H, Ochala J, Artemenko K, Iwamoto H, Yagi N, Bergquist J & Larsson L (2015). Aberrant post-translational modifications compromise human myosin motor function in old age. *Aging Cell* **14,** 228-235.

Narici MV & Maffulli N (2010). Sarcopenia: characteristics, mechanisms and functional significance. *British Medical Bulletin* **95,** 139-159.

Penman KA (1970). Human Striated Muscle Ultrastructural Changes Accompanying Increased Strength without Hypertrophy. *Research Quarterly. American Association for Health, Physical Education and Recreation* **41,** 418-424.

Qaisar R, Renaud G, Morine K, Barton ER, Sweeney HL & Larsson L (2012). Is functional hypertrophy and specific force coupled with the addition of myonuclei at the single muscle fiber level? *The FASEB Journal* **26,** 1077-1085.

Rossow LM, Fukuda DH, Fahs CA, Loenneke JP & Stout JR (2013). Natural bodybuilding competition preparation and recovery: a 12-month case study. *Int J Sports Physiol Perform* **8,** 582-592.

Salvadego D, Domenis R, Lazzer S, Porcelli S, Rittweger J, Rizzo G, Mavelli I, Simunic B, Pisot R & Grassi B (2013). Skeletal muscle oxidative function in vivo and ex vivo in athletes with marked hypertrophy from resistance training. *J Appl Physiol (1985)* **114,** 1527-1535.

Schoenfeld BJ (2010). The Mechanisms of Muscle Hypertrophy and Their Application to Resistance Training. *The Journal of Strength & Conditioning Research* **24,** 2857-2872 2810.1519/JSC.2850b2013e3181e2840f2853.

Seynnes OR, Kamandulis S, Kairaitis R, Helland C, Campbell E-L, Brazaitis M, Skurvydas A & Narici MV (2013). Effect of androgenic-anabolic steroids and heavy strength training on patellar tendon morphological and mechanical properties. *Journal of Applied Physiology* **115,** 84-89.

Sokal RR & Rohlf FJ (2012). *Biometry: the principles and practice of statistics in biological research.* W. H. Freeman & Co., New York.

Trappe S, Harber M, Creer A, Gallagher P, Slivka D, Minchev K & Whitsett D (2006). Single muscle fiber adaptations with marathon training. *Journal of Applied Physiology* **101,** 721-727.

Van der Laarse W, Des Tombe A, Lee-de Groot M & Diegenbach P (1997). Size principle of striated muscle cells. *Netherlands journal of zoology* **48,** 213-223.

Wessel T, Haan A, Laarse WJ & Jaspers RT (2010). The muscle fiber type–fiber size paradox: hypertrophy or oxidative metabolism? *European Journal of Applied Physiology* **110,** 665-694.

Widrick JJ, Stelzer JE, Shoepe TC & Garner DP (2002). Functional properties of human muscle fibers after short-term resistance exercise training. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* **283,** R408-R416.

FIGURES
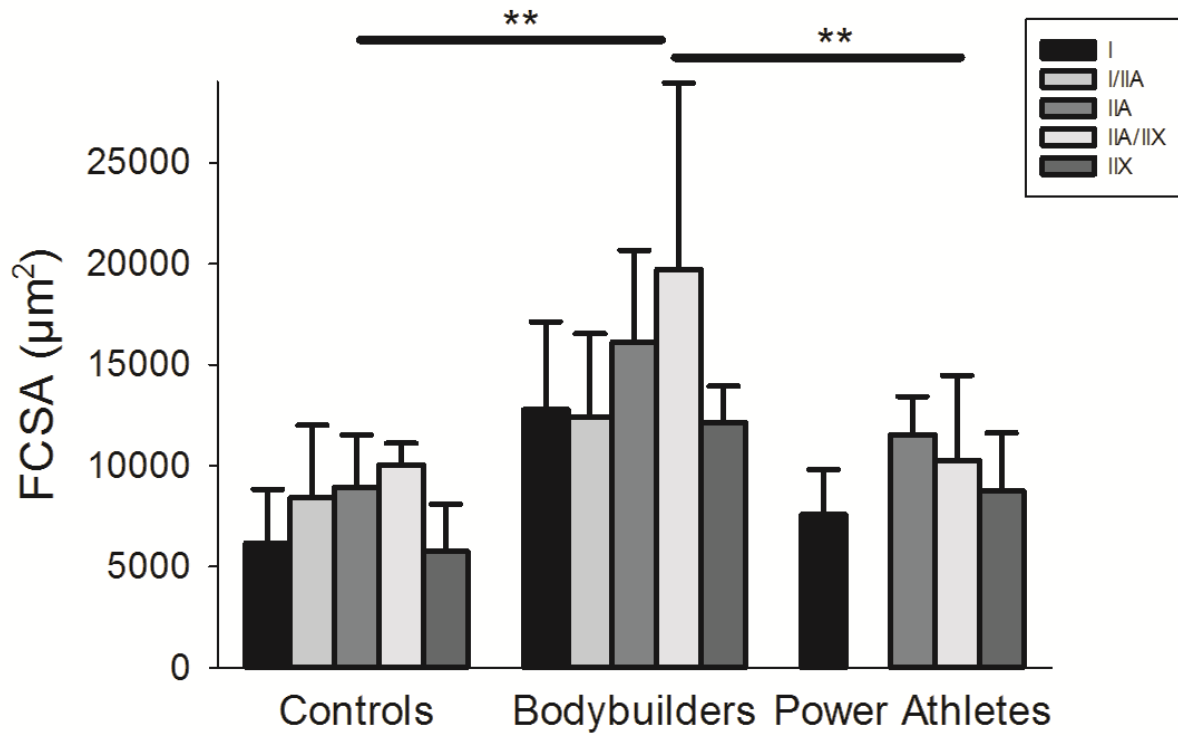
Figure 1. Fibre cross-sectional area (FCSA) of muscle fibres from the *m. vastus lateralis* of non-resistance trained controls (C), bodybuilders (BB) and power athletes (PA). Data are mean ± SEM. ** differences between indicated groups at *P* < 0.01.
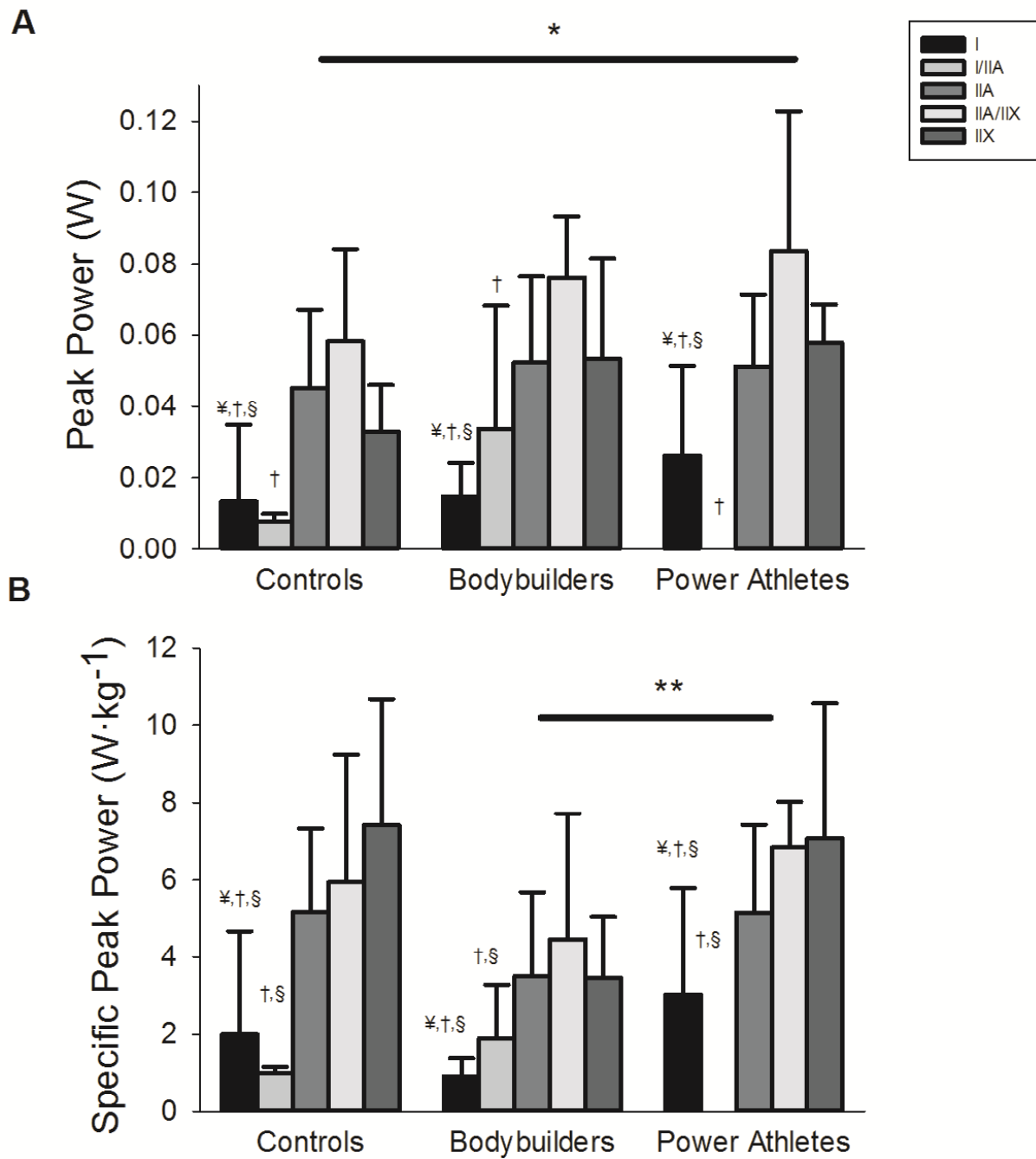
Figure 2. A) Peak power (PP in Watt) and **B**) specific peak power (Watt per kg) of skinned muscle fibre

segments from the *m. vastus lateralis* of non-resistance trained controls (C), bodybuilders (BB) and

power athletes (PA). * 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 $P < 0.05$; ¥ different from type IIX at $P < 0.05$.

A

B
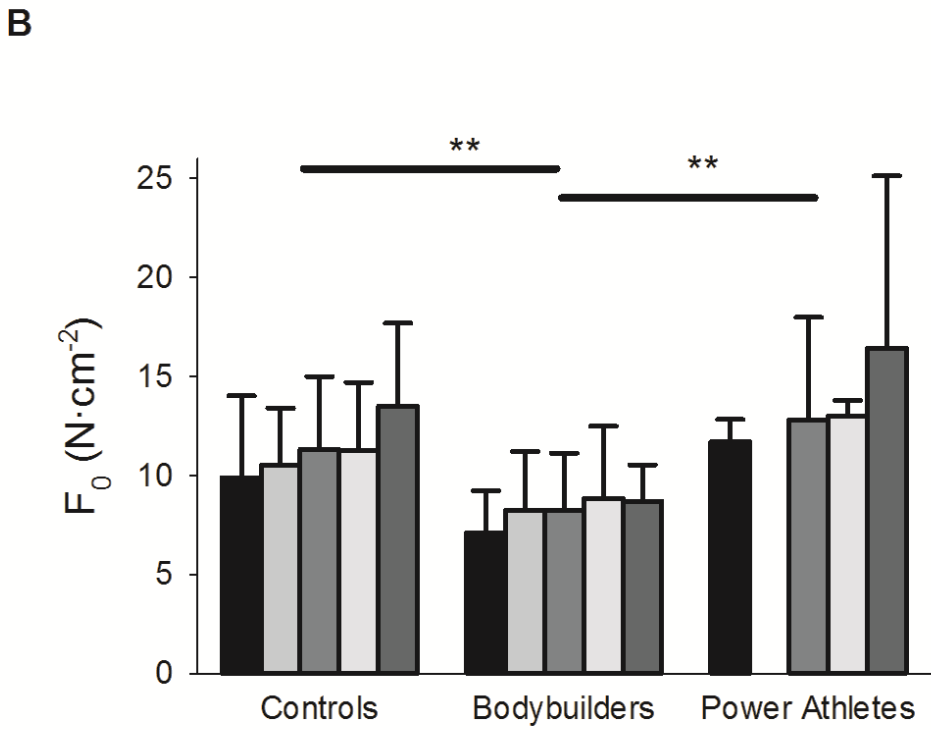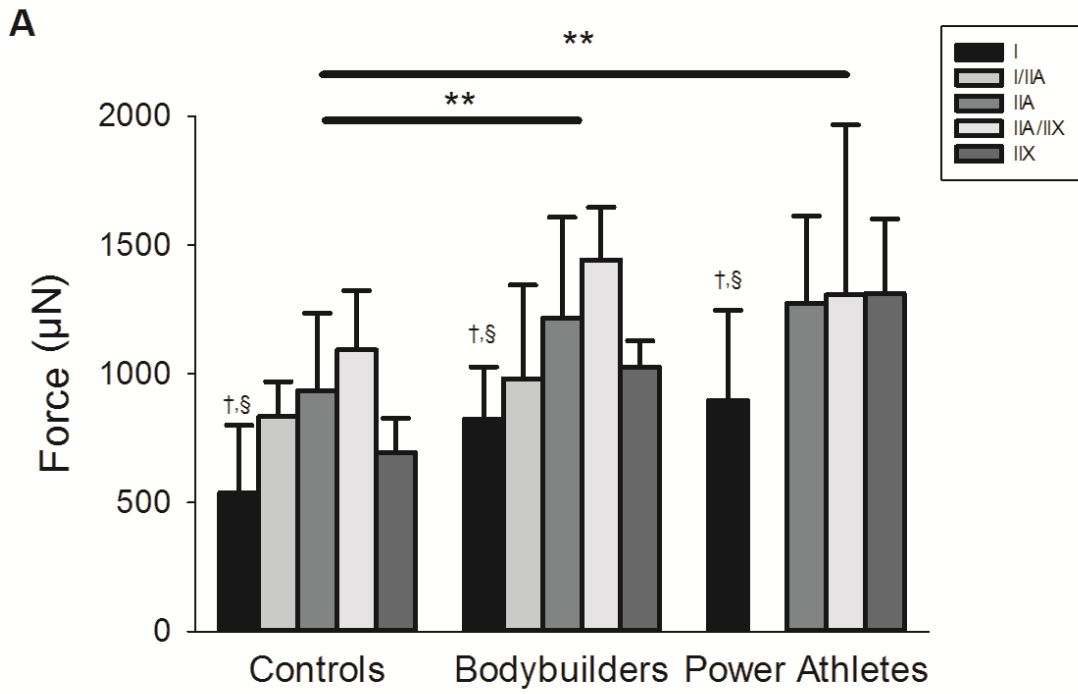
Figure 3. A) Maximal isometric tension (F in µN) and **B**) specific tension (F_0 in N·cm^{-2}) of skinned *m. vastus lateralis* fibre segments of non-resistance trained controls (C), bodybuilders (BB) and power athletes (PA). Data are presented as mean ± SEM. **differences between indicated groups at $P < 0.01$; † different from type IIA at $P < 0.05$; § different from type IIA/IIX at $P < 0.05$.

A Untrained Controls

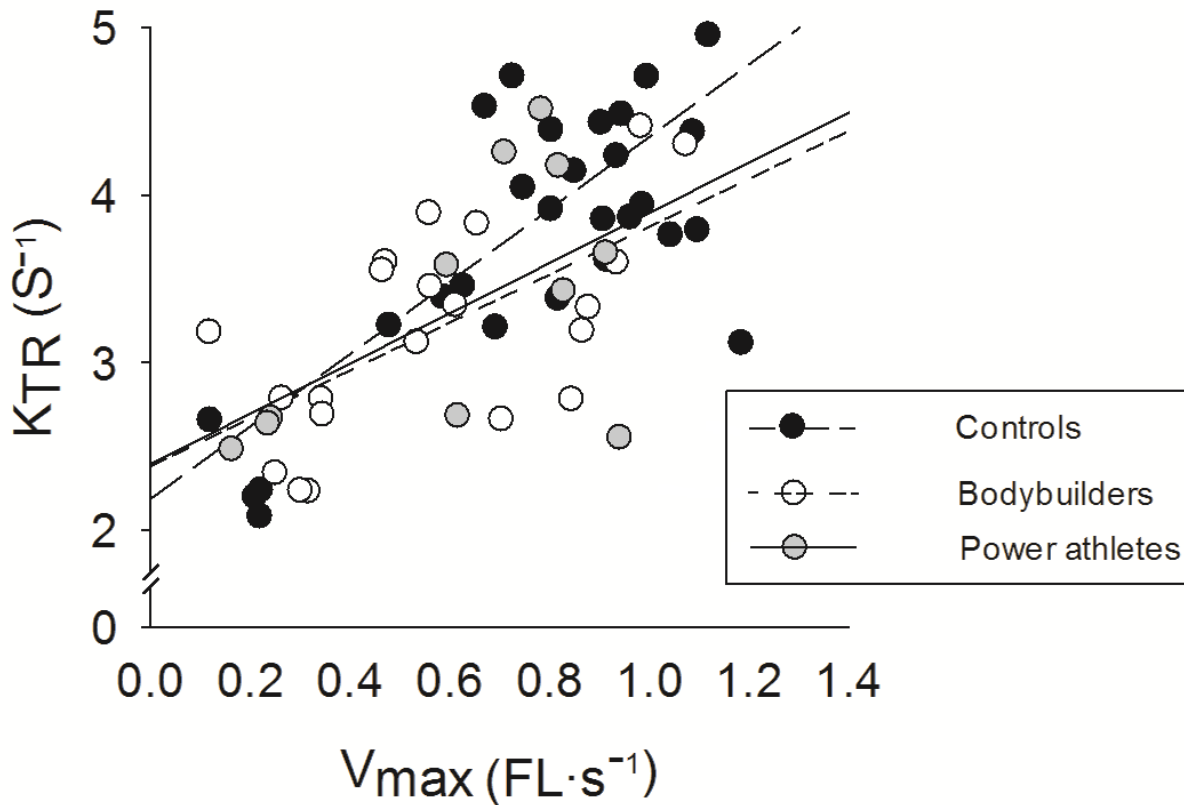**B** Bodybuilders
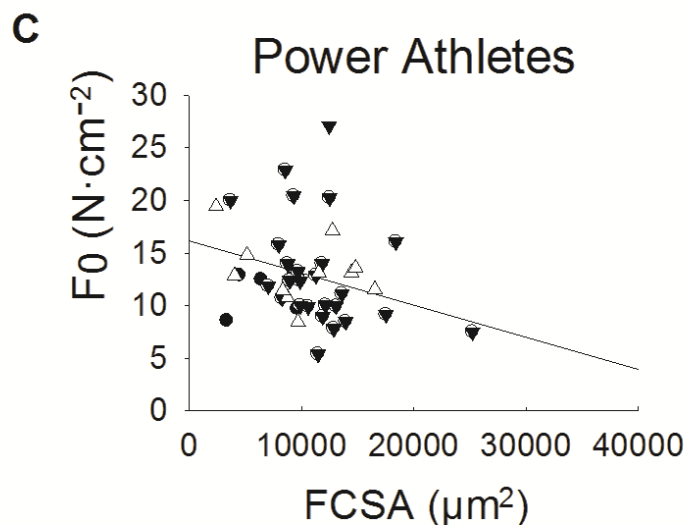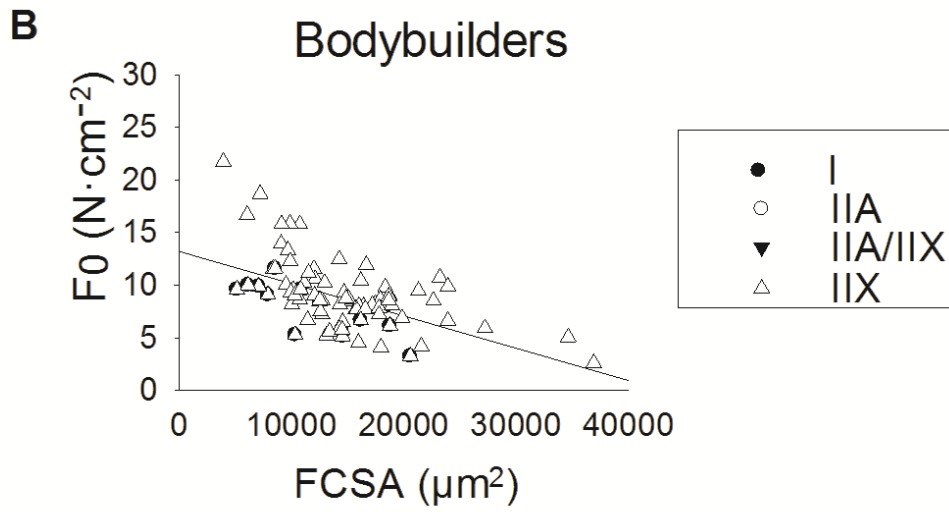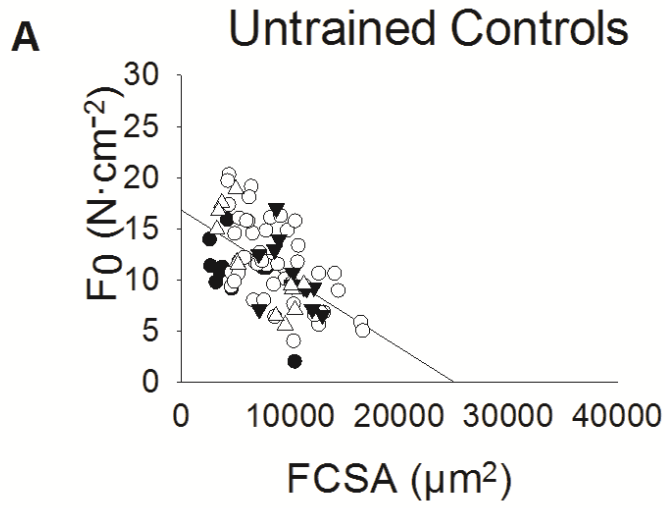
C Power Athletes

Figure 4. Specific tension of skinned *m. vastus lateralis* fibres is inversely related to muscle fibre cross-sectional area in non-resistance trained controls **(A)**, bodybuilders **(B)**, and power athletes **(C)**.
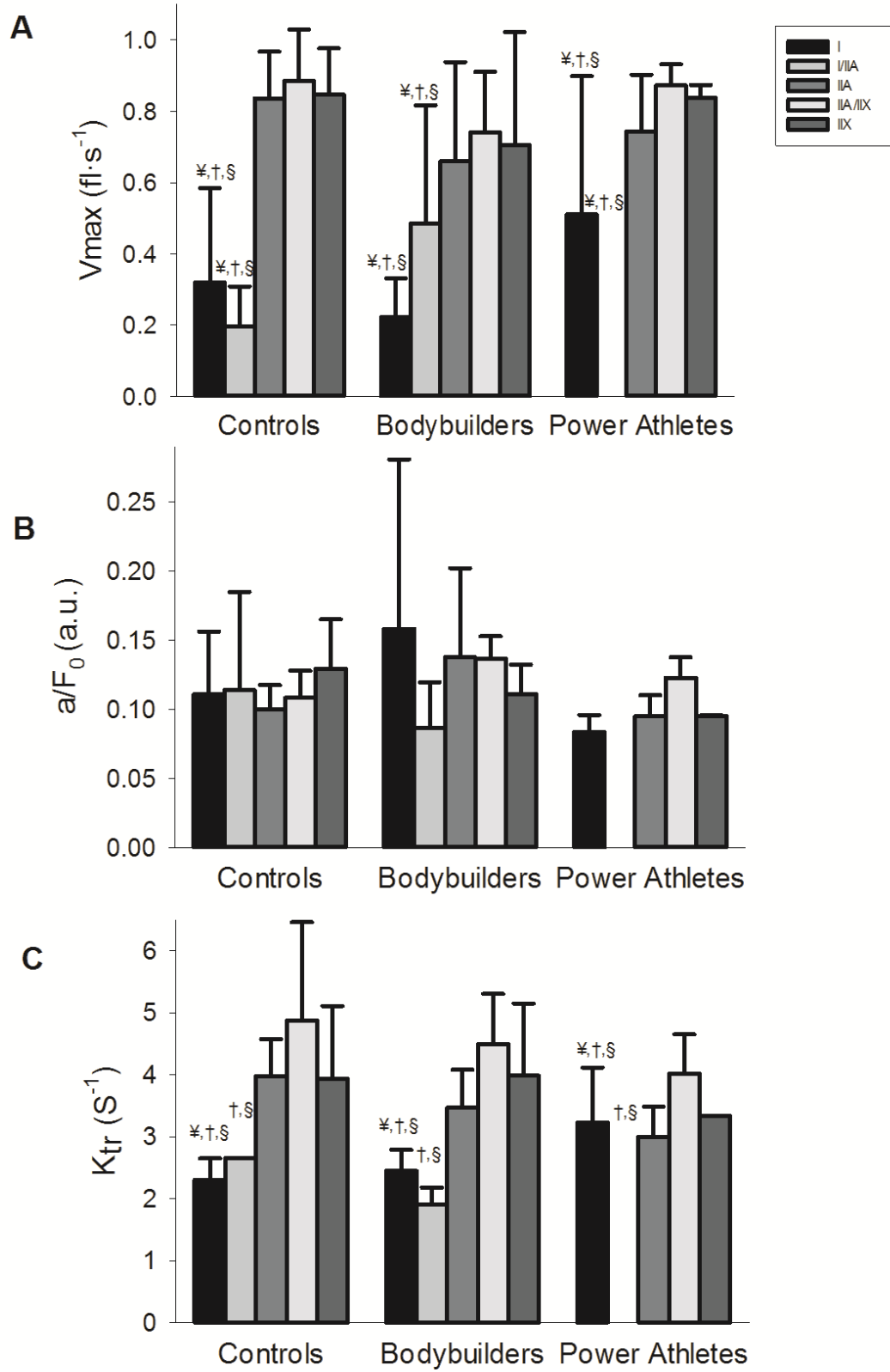
Figure 5. A) Maximal shortening velocity (V_{max} in fibre lengths per s), **B)** Rate of force development (K_{TR} in s^{-1}) and **C)** curvature of the force-velocity relationship (a/F_0 in arbitrary units) for controls, bodybuilders and power athletes per fibre type. Data are mean ± SEM. † different from type IIA at *P* < 0.05; § different from IIA/IIX at *P* < 0.05; ¥ different from IIX at *P* < 0.05.

Figure 6. Rate of force redevelopment (K_{TR}) versus the maximal shortening velocity (V_{max}) of skinned *m. vastus lateralis* muscle fibre segments of non-resistance trained controls, bodybuilders and power athletes. The intercepts of the regression lines, representing 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 criteria for rate of force redevelopment was used for this regression (see text). Total 44

fibres from in 15 participants met the inclusion criteria. Separate fibres were regarded as separate observations.
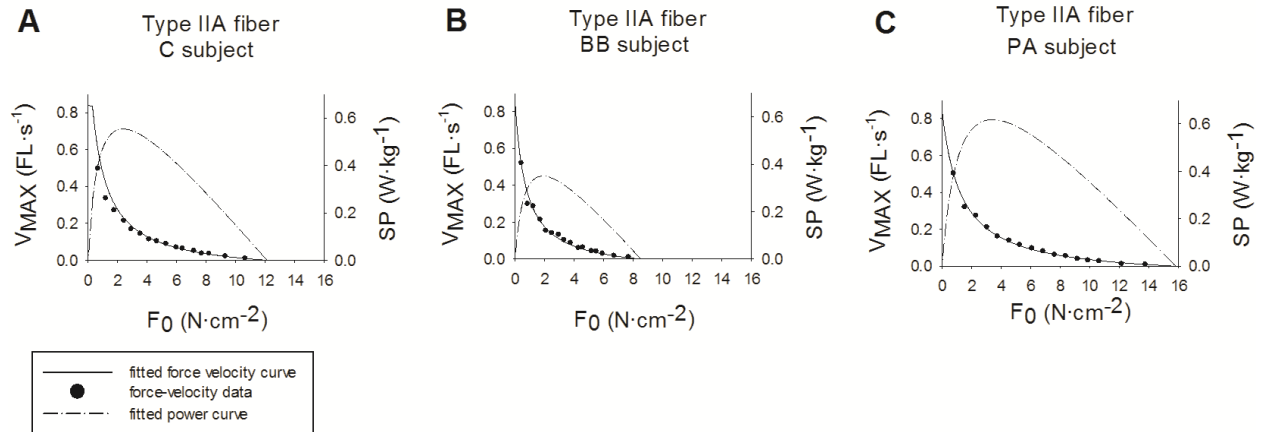
Figure 7. Typical examples of three IIA muscle fibres of a bodybuilder (BB), power athlete (PA) and non-resistance trained control (C). Circles represent the measured force-velocity data. The continuous line shows the fitted force-velocity curve and the dashed line shows force-power curve.