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RESEARCH ARTICLE

Effects of load magnitude, muscle length and velocity during eccentric chronic loading on the longitudinal growth of the vastus lateralis muscle

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ABSTRACT

The present study investigated the longitudinal growth of the vastus lateralis muscle using four eccentric exercise protocols with different mechanical stimuli by modifying the load magnitude, lengthening velocity and muscle length at which the load was applied. Thirty-one participants voluntarily participated in this study in two experimental and one control group. The first experimental group (*N*=10) exercised the knee extensors of one leg at 65% (low load magnitude) of the maximum isometric voluntary contraction (MVC) and the second leg at 100% MVC (high load magnitude) with 90 deg s^{-1} angular velocity, from 25 to 100 deg knee angle. The second experimental group (*N*=10) exercised one leg at 100% MVC, 90 deg s^{-1}, from 25 to 65 deg knee angle (short muscle length). The other leg was exercised at 100% MVC, 240 deg s^{-1} angular velocity (high muscle lengthening velocity) from 25 to 100 deg. In the pre- and post-intervention measurements, we examined the fascicle length of the vastus lateralis at rest and the moment–angle relationship of the knee extensors. After 10 weeks of intervention, we found a significant increase (~14%) of vastus lateralis fascicle length compared with the control group, yet only in the leg that was exercised with high lengthening velocity. The findings provide evidence that not every eccentric loading causes an increase in fascicle length and that the lengthening velocity of the fascicles during the eccentric loading, particularly in the phase where the knee joint moment decreases (i.e. deactivation of the muscle), seems to be an important factor for longitudinal muscle growth.

KEY WORDS: Muscle size, Fascicle length, Fascicle kinetics, Ultrasonography

INTRODUCTION

Skeletal muscles follow three strategies to adapt in response to mechanical loading (Goldspink, 1985): (1) increase of physiological cross-sectional area (PCSA, i.e. radial growth), (2) increase of muscle fiber length (i.e. longitudinal growth) and (3) increase of specific muscle force (i.e. muscle force normalized to the PCSA). These three structural changes in the muscle phenotype directly affect the specific functional characteristics of muscle force generation, such as the force–length and force–velocity relationships (Goldspink, 1985). A functional consequence of an increased muscle fiber length is an increase in maximum shortening velocity and maximum mechanical power of the muscle. This means that the longitudinal muscle growth is, together with the radial muscle growth, an important component by which muscle power can be enhanced. However, although there are a lot of studies investigating the radial growth of muscles in humans (MacDougall et al., 1979; Sale et al., 1990; Reeves et al., 2004; Seynnes et al., 2007; Reeves et al., 2009; Franchi et al., 2014) and in other animals (Watt et al., 1982; Timson et al., 1985), there is little information about the longitudinal muscle growth in humans.

There is evidence that eccentric loading may affect the longitudinal muscle growth (Lynn and Morgan, 1994; Proske and Morgan, 2001; Butterfield and Herzog, 2006). Lynn and Morgan (Lynn and Morgan, 1994) were the first to report an increase in the amount of sarcomeres in series in the vastus intermedius muscle of rats after eccentric loading. They found a higher number of sarcomeres in series in vastus intermedius muscle in rats after one week of running on a decline (i.e. eccentric loading) compared with those running on an incline (i.e. concentric loading). More recent work from Butterfield et al. (Butterfield et al., 2005) supports the earlier findings from Lynn and Morgan (Lynn and Morgan, 1994) and summarizes that eccentric loading might be an important mechanical stimulus for the longitudinal muscle growth. One year later, Butterfield and Herzog (Butterfield and Herzog, 2006) directly investigated the effects of eccentric loading on the number of sarcomeres in series in the tibialis anterior muscle of rabbits. They demonstrated an increase in the number of sarcomeres in series after controlled eccentric loading and reported that the longitudinal muscle growth after eccentric loading depends on both the magnitude of the muscle fiber strain and the magnitude of the generated muscle force.

Although the underlying mechanism regarding the longitudinal muscle growth is not well known, it is believed that muscle damage in the Z-bands of the sarcomeres is one reason for the initiation of new sarcomeres in series (Lynn and Morgan, 1994; Proske and Morgan, 2001). Particularly, eccentric contractions, especially on the descending part of the force–length curve (i.e. long muscle length), have been identified to cause damage in the sarcomeres (Asmussen, 1956; Armstrong, 1984; Lieber and Fridén, 2002). It has been suggested that an increase in the number of sarcomeres in series could be a protective mechanism for further muscle damage after eccentric loading (Lynn and Morgan, 1994; Proske and Morgan, 2001). The functional consequence of the increased number of sarcomeres in series is a lower lengthening of each sarcomere by a given strain magnitude of the muscle fiber and, thus, a lower structural instability. Furthermore, independent of muscle damage after eccentric loading, it has been reported that the titin kinase domain can be directly activated by mechanical force to trigger signaling (Lange et al., 2005; Puchner et al., 2008). As titin is responsible for maintaining the structural organization of the sarcomere during tension (Granzier and Labeit, 2004) it is thought that, by application of extensional forces, the mechanical strain

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Received 23 November 2013; Accepted 5 May 2014

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List of abbreviations

EMG	electromyography
MVC	maximum voluntary isometric contraction
PCSA	physiological cross-sectional area

along the sarcomere is transmitted through titin kinase to the nucleus, initiating signaling processes that modulate protein synthesis (Lange et al., 2005; Tskhovrebova and Trinick, 2008). This means that the titin-based stretch sensing, and thus the role of titin as a mechanosensor in sarcomeres for initiating signaling, might be an additional mechanism for longitudinal fiber growth. Despite the difficulties with the identification of the underlying mechanism(s), there is evidence that the magnitude of the eccentric loading, the lengthening velocity of the fibers and the fiber length at which the eccentric load is applied affect the longitudinal growth of muscles (Butterfield and Herzog, 2006; Bryer and Koh, 2007). There is also evidence that the concentration of the satellite cells at fiber ends is an important factor affecting the longitudinal growth of muscle fibers and that the longitudinal muscle growth occurs at the terminal ends of the fibers (Allouh et al., 2008).

Recent studies have also reported longitudinal muscle growth in human muscles in response to eccentric loading (Duclay et al., 2009; Potier et al., 2009; Reeves et al., 2009). These studies used eccentric or combined (i.e. concentric and eccentric) contractions in their training protocols, but they did not isolate the possible independent mechanical stimuli that may affect the longitudinal growth of muscle (i.e. magnitude of the eccentric loading, the lengthening velocity of the fibers and the fiber length where the eccentric load is applied). Butterfield and Herzog (Butterfield and Herzog, 2006) have reported that both the magnitude of muscle fiber strain and magnitude of the generated muscle force during eccentric exercise might affect the longitudinal muscle growth. Furthermore, the titin-based stretch sensing and the role of titin as a mechanosensor in sarcomeres for initiating signaling (Lange et al., 2005; Tskhovrebova and Trinick, 2008) suggest an effect of lengthening velocity of the fibers for the longitudinal muscle growth. To our knowledge there are no studies in human that have investigated the longitudinal muscle growth by applying training interventions of controlled eccentric loading.

Therefore, the purpose of this study was to investigate the longitudinal growth of the vastus lateralis muscle using four different eccentric exercise protocols by modifying the load magnitude, lengthening velocity and the muscle length at which the load is applied (protocol 1, low load magnitude; protocol 2, high load magnitude; protocol 3, short muscle length; protocol 4, high lengthening velocity). Further, we investigated the vastus lateralis fascicle kinetics for the eccentric stimuli used in the four training protocols. We hypothesized that given a constant overall volume of loading, a higher magnitude of eccentric load, higher lengthening velocity and application of the eccentric load at a greater muscle length would introduce superior longitudinal growth of the muscle.

RESULTS

Fascicle kinetics during the eccentric protocols

All four eccentric training protocols demonstrated three characteristic phases regarding the fascicle kinetics of the vastus lateralis (Fig. 1). In the first phase, the muscle fascicles shortened while the knee joint moment increased. The shortening of the fascicle continued further after the initiation of the eccentric contraction (i.e. lengthening of the muscle tendon unit). In the first phase, the magnitude of shortening and the shortening velocity of the vastus lateralis fascicle did not differ significantly (P>0.05) between the four protocols (Table 1). In the second phase, the muscle fascicles were elongated while the knee joint moment increased further (Fig. 1). In this phase, the fascicles in protocols 3 and 4 showed a lower (P<0.05) elongation compared with the other two protocols, whereas the lengthening velocity of the fascicle did not differ between the four protocols (Table 1). The third phase was characterized by a further lengthening of the fascicles while the knee joint moment decreased. In this phase, the lengthening of the vastus lateralis fascicles did not differ significantly (P>0.05) between the four protocols; however, the lengthening velocity of the fascicle was significantly higher (P<0.05) in protocol 4 (Table 1). Furthermore, the lengthening and the lengthening velocity of the vastus lateralis fascicle were greater in the third phase compared with that in the second phase in all examined protocols (Table 1).

Fascicle length of the vastus lateralis at rest

The fascicle length of the vastus lateralis muscle in an inactive state ranged from ~12 cm at a knee joint angle of 25 deg to ~15 cm at a knee joint angle of 85 deg (Fig. 2). The fascicle length of the vastus lateralis did not differ between the examined groups in the pre measurements, indicating homogeneous groups regarding the vastus lateralis fascicle length. During the 10 weeks of eccentric exercises, the fascicle length of the vastus lateralis increased significantly (P<0.05) in exercise protocol 4 (i.e. high lengthening velocity). The increase in fascicle length was ~14% across the whole measured

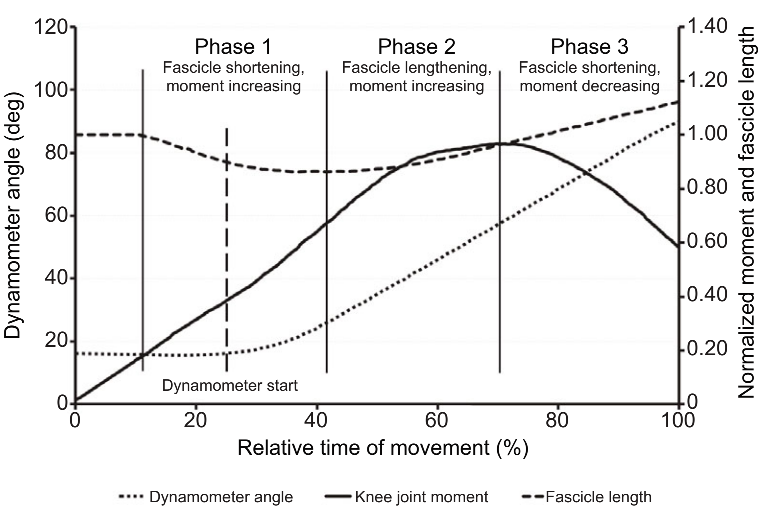

Fig. 1. Representative chart of the general fascicle behavior during eccentric contractions. Because the fascicle kinetics of the vastus lateralis in all for the four intervention protocols was similar, this figure is representative of the general fascicle behavior during eccentric contractions and shows the dynamometer angle (dotted line, primary axis), the normalized fascicle length (dashed line, secondary axis) and the normalized knee joint moment (solid line) as a function of the time of movement (from the inactive state until the end of the knee joint flexion). The beginning of the dynamometer and knee joint movement is indicated with ‘dynamometer start’. The vertical lines separate the three main phases of fascicle behavior during eccentric contractions. The first phase shows an increase in knee joint moment and a shortening of the muscle fascicle. In the second phase, a further increase in knee joint moment and an increase in fascicle length can be observed. The last phase shows a progressive increase in fascicle length with a decreasing moment.

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Table 1. Shortening, lengthening and the corresponding shortening and lengthening velocities of the vastus lateralis fascicle during the three phases of the investigated exercise protocols

	Phase 1	Phase 2	Phase 3
Protocol	Shortening (mm)	Lengthening (mm)	Lengthening (mm)
Protocol 1 (low load magnitude)	−11.48±6.46	13.82±6.94	23.76±12.57¶
Protocol 2 (high load magnitude)	−15.60±9.56	11.41±5.64	25.00±10.37¶
Protocol 3 (short muscle length)	−11.28±6.41	3.72±3.67*,‡	11.45±8.06¶
Protocol 4 (high lengthening velocity)	−10.61±5.33	3.20±3.12*,‡	23.04±14.12¶
Protocol	Shortening velocity (mm s^{-1})	Lengthening velocity (mm s^{-1})	Lengthening velocity (mm s^{-1})
Protocol 1 (low load magnitude)	−30.79±14.36	30.16±7.73	51.51±15.31¶
Protocol 2 (high load magnitude)	−47.72±30.84	26.98±11.08	52.69±18.54¶
Protocol 3 (short muscle length)	−44.73±21.88	17.27±10.80	54.14±21.31¶
Protocol 4 (high lengthening velocity)	−47.86±22.16	26.06±17.92	90.39±48.56*,‡,§,¶

Values are shown as Means ± s.d.; *statistically significant (P<0.05) differences to protocol 1; ‡statistically significant (P<0.05) differences to protocol 2; §statistically significant (P<0.05) differences to protocol 3; ¶statistically significant (P<0.05) differences between phase 2 and phase 3.

knee angle range. All other exercise protocols, including the control group, did not show any significant (P>0.05) differences in muscle fascicle length before and after the 10 week period (Fig. 2).

Moment–angle relationship

The knee extension moments of the involved participants were significantly (P<0.05) higher after the exercise intervention compared with the pre values in protocols 1, 2 and 4 in almost all measured knee angles (Fig. 3). In exercise protocol 3 (i.e. short muscle length) the maximal knee extension moments were significantly (P<0.05) higher only at a knee joint angle of 45 deg (Fig. 3). The control group did not show any significant changes in the knee extension moments after the 10 week period (Fig. 3). To investigate the effectiveness of the four training protocols on the muscle strength, we compared the ratio of the post to pre values of the maximum knee joint moment. The ratio of the post to pre values was higher (i.e. an increase in maximum resultant knee joint moment) in all exercise protocols compared with those of the control group (Fig. 4). This ratio was significantly greater (P<0.05) in protocol 2 compared with the ratios of protocols 1 and 3 and had a tendency to be greater when compared with that of protocol 4 (P=0.055).

Furthermore, we calculated the ratio of post to pre knee angle values, where the maximum knee extension moment was achieved as an indirect marker for a longitudinal muscle growth. A ratio of >1 indicated a shift in the maximum resultant moment towards a greater knee angle (i.e. longer muscle length). The post to pre ratios of the knee angle did not differ significantly (P>0.05) between the four exercise protocols and the control group, indicating the absence of a shift in the angle of maximum knee extension moment after 10 weeks of eccentric exercise (Fig. 5).

DISCUSSION

The muscle strength of the knee extensors improved after the 10 weeks of eccentric exercise in all training protocols; however, we found a significant increase of ~14% in the fascicle length of the vastus lateralis muscle only after the intervention with the high lengthening velocity. Therefore, our hypothesis has not been confirmed. These findings provide evidence that not every eccentric exercise loading causes an increase in fascicle length and that the lengthening velocity of the fascicles seems to be an important factor for longitudinal muscle growth. Furthermore, our results show that the fascicle kinetics of the vastus lateralis muscle during eccentric knee extension contractions is different to the elongation behavior expected from the knee joint angle motion. In the beginning of all contractions, the fascicles contract concentrically, despite a lengthening of the muscle-tendon unit (i.e. flexion in the knee joint), and the main lengthening of the fascicles occurs in the phase where the knee extension moment decreases. Furthermore, only in the last phase (i.e. lengthening of the fascicles while the knee joint moment decreased) did protocol 4 show a higher lengthening velocity of the muscle fascicles in comparison with the other protocols. The above fascicle behavior can be explained by the tendon compliance, which affects the fascicle kinetics in eccentric contractions. In conclusion, we can argue that muscle-tendon unit movement cannot predict fascicle kinetics during eccentric contractions in humans.

Although the underling mechanisms regarding the longitudinal growth of the muscle (i.e. increase of the number of sarcomeres in series) are not well known, it has been suggested that structural damage in the sarcomeres after eccentric muscle contractions is the major stimulus for the increase in fiber length (Lynn and Morgan, 1994; Proske and Morgan, 2001). Several studies report that eccentric muscle contractions with high lengthening velocity cause more severe muscle damage compared with eccentric contractions with low lengthening velocity (Chapman et al., 2008; Shepstone et al., 2005). Therefore, this type of loading (i.e. eccentric loading with high lengthening velocity) might be advantageous to induce longitudinal muscle growth and can partly explain our findings. Furthermore, we can argue that a high lengthening velocity of the fascicles might affect the interaction between titin and its interacting structures (i.e. Z-disc, M-line, I-band), affecting the titin-based stretch sensing and signaling in a lengthening-velocity-dependent manner. Accordingly, we found an increase of vastus lateralis fascicle length only in protocol 4. However, we did not find evidence for an intensity-dependent longitudinal muscle growth in relation to eccentric loading as stated in our hypothesis. In agreement with our results, Koh and Herzog (Koh and Herzog, 1998) found an increase of muscle mass in response to 12 weeks of eccentric exercise in rabbits. However, as they did not find an increase in the number of sarcomeres in series, as was the case in three out of four eccentric exercise protocols in the present study, it seems that not only the eccentric load per se but also the type of the fascicle kinetic affects longitudinal muscle growth.

Reports from the literature provide evidence that, during different kinds of sporting activity, the achieved maximal angular velocity at the knee joint is higher compared with the maximum angular velocity that we used in our experiment (i.e. 240 deg s^{-1}). For running and sprinting the reported maximal knee flexion angular velocities during the stance phase ranged from 500 to 600 deg s^{-1} (Albracht and Arampatzis, 2013; Bezodis et al., 2008) and for

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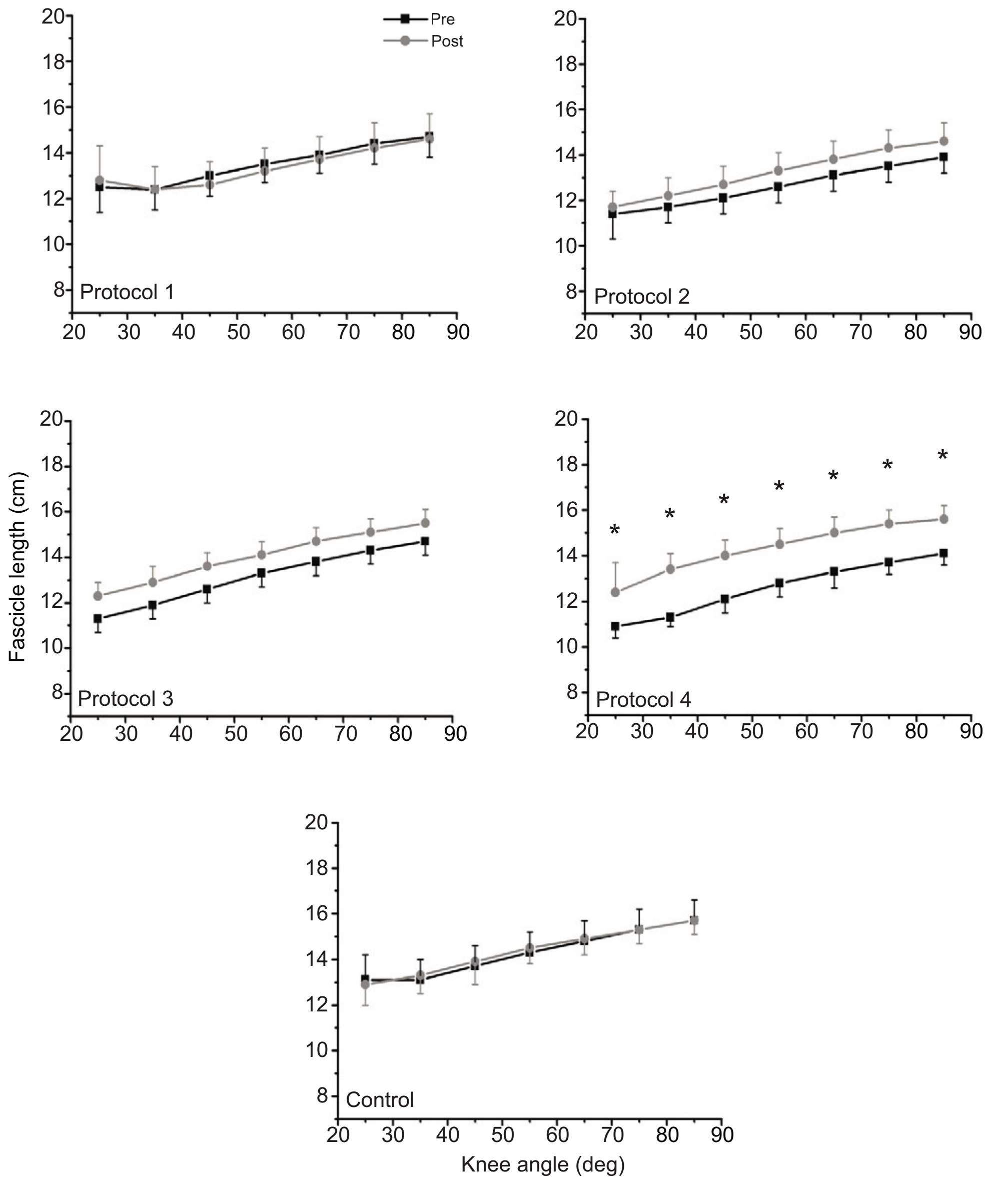

Fig. 2. Fascicle length of vastus lateralis muscle in relation to knee joint angle. Mean values and s.e.m. of the fascicle length of the vastus lateralis muscle as a function of knee angle before (pre) and after (post) the exercise intervention protocols at rest (protocol 1, low load magnitude protocol; protocol 2, high load magnitude protocol; protocol 3, short muscle length protocol; protocol 4, high lengthening velocity protocol; control, control group). *Statistically significant (P<0.05) differences between pre and post values.

counter movement and drop jumps from 280 to 540 deg s^{-1} (Bobbert et al., 1987a; Bobbert et al., 1987b). However, these are peak values lasting only for some milliseconds. The average angular velocity for these activities ranged from 190 to 230 deg s^{-1} for running (Arampatzis et al., 1999; Arampatzis et al., 2000), 70 deg s^{-1} to 225 deg s^{-1} for jumping (Arampatzis et al., 2001) and achieved values of ~320 deg s^{-1} for sprinting (Stafilidis and Arampatzis, 2007). In the present interventions, the training velocity was constant within the whole range of motion. Further, the participants in our experiments (i.e. protocols 2, 3 and 4) had to produce high forces (i.e. 100% MVC) during the whole eccentric contraction. Therefore, the maximal angular velocity used in our study can be considered as high with respect to the movement duration, magnitude of muscle loading and range of motion.

In the past few years, some studies (Blazevich et al., 2007; Duclay et al., 2009; Potier et al., 2009; Reeves et al., 2009) have reported longitudinal growth in human muscles from 7% to 34% in response to eccentric exercise. However, to our knowledge, there is only one study (Butterfield and Herzog, 2006) that investigated the fiber kinetics during eccentric contractions and this was on rabbit muscles. It was found that, in addition to the magnitude of the generated joint moment during the eccentric contraction, the lengthening of the muscle fiber and especially the lengthening during the deactivation of the muscle (i.e. decrease of joint moment) were the best predictors for the increase of the sarcomeres in series. In our training protocols 2, 3 and 4, the magnitude of the generated knee joint moment was equal (i.e. 100% MVC) and, therefore, cannot be the reason for the different findings regarding the fascicle length increase following protocol 4. Furthermore, the maximal strain magnitude of the vastus lateralis fascicle did not differ between the four protocols. However, in training protocol 4, the fascicle lengthening velocity of the vastus lateralis muscle in the phase where the knee joint moment decreased was significantly higher compared with that of the other three training protocols. In our experiments, we did not measure the electromyography (EMG) activity of knee extensors and therefore it could be argued that the decrease in knee extension moment in the last phase of motion (i.e. phase 3) is due to the force–length relationship curve (i.e. descending part) and not due to muscle deactivation. Fig. 6 shows the average normalized knee joint moment from 25 to 90 deg of

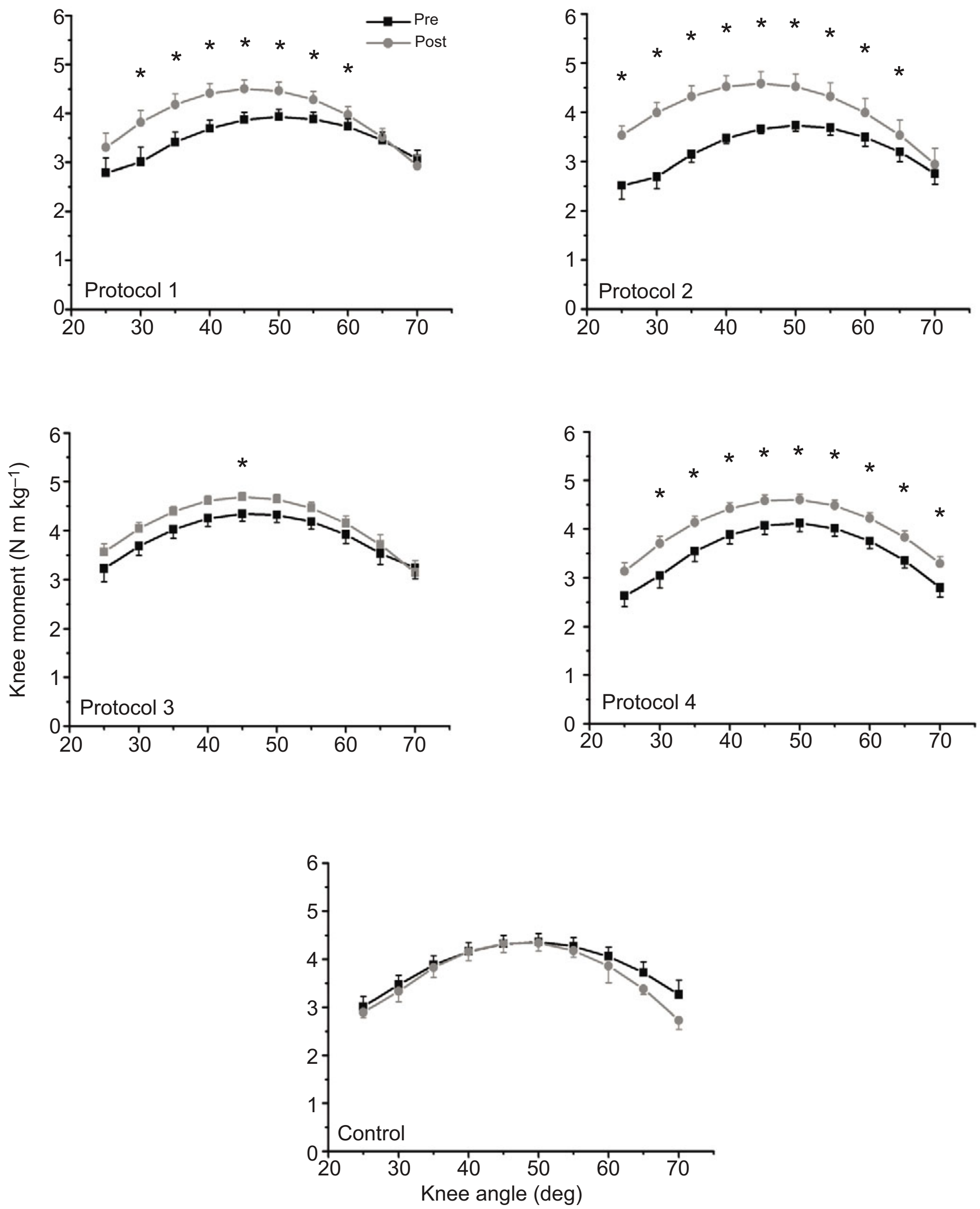

Fig. 3. Maximum resultant knee joint moment in relation to knee joint angle. Mean values and s.e.m. of the resultant knee joint moments as a function of the knee joint angle before (pre) and after (post) the exercise intervention protocols (protocol 1, low load magnitude protocol; protocol 2, high load magnitude protocol; protocol 3, short muscle length protocol; protocol 4, high lengthening velocity protocol; control, control group). *Statistically significant (P<0.05) differences between pre and post values.

knee joint angle for the maximal isometric contractions and for the isokinetic eccentric contraction we used in the protocols 2 (100% MVC, 90 deg s^{-1}, 75 deg range of movement) and 4 (100% MVC, 240 deg s^{-1}, 75 deg range of movement). It is clearly visible that in the protocol with the high angular velocity, the decrease in knee joint moment was higher than that expected due to the moment–angle relationship, indicating a deactivation of the knee extensor muscles at the end of movement in protocol 4. In contrast, in protocol 2, reduction of the knee joint moment was in line with that expected due to the moment–angle relationship (Fig. 6). The rapid lengthening of the muscle fibers in the descending part of the force–length relationship (i.e. long muscle fiber length) combined with a decrease in muscle force (i.e. deactivation of the muscle) may be an important trigger for muscle damage due to the instability of the sarcomeres and, thus, the homeostatic perturbation that facilitates longitudinal muscle growth (Butterfield and Herzog, 2005). Although we did not examine any biomarkers for muscle damage in our experiments and, therefore, there is no direct evidence regarding the amount of muscle damage between the investigated exercise protocols, we can argue that a high lengthening velocity of the fascicles combined with a decrease in muscle force is an important mechanical stimulus to trigger a homeostatic perturbation in muscles to induce longitudinal plastic changes.

Muscle strength was increased in all four interventions, providing evidence for the effectiveness of our training protocols. Furthermore, protocol 2 (i.e. 100% MVC, 90 deg s^{-1}) showed a tendency for a higher increase in muscle strength compared with protocol 4 (i.e. 100% MVC, 240 deg s^{-1}). These findings indicate a specificity of the lengthening velocity on the radial and longitudinal muscle growth. In protocol 2, we could not identify in phase 3 any decrease in knee joint moment due to the deactivation of the knee extensor muscles as in protocol 4 (Fig. 6), which might be a reason for the absence of longitudinal growth. Reeves et al. (Reeves et al., 2009) also report a specificity of the training stimulus for adding sarcomeres in series and in parallel by comparing conventional resistance training (i.e. concentric and eccentric contractions) to training with only eccentric contractions. However, Reeves et al. used, in both their interventions, a wide range of angular velocities

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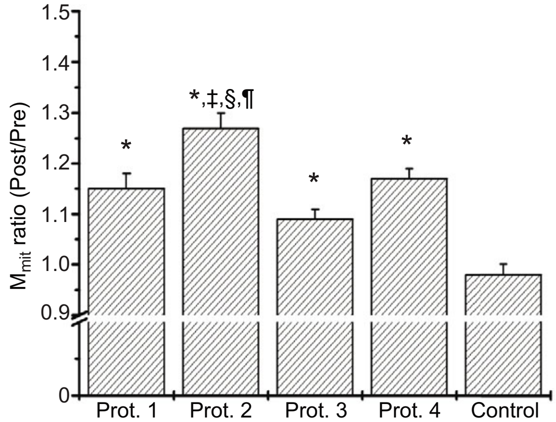

Fig. 4. Ratio of maximal resultant knee joint moment. Mean values and s.e.m. of the ratio (i.e. post to pre values) of maximal resultant knee joint moment in the different exercise protocols and the control group (protocol 1, low load magnitude protocol; protocol 2, high load magnitude protocol; protocol 3, short muscle length protocol; protocol 4, high lengthening velocity protocol; control, control group). *Statistically significant (P<0.05) difference to control group; ‡statistically significant (P<0.05) difference to protocol 1; §statistically significant (P<0.05) difference to protocol 3; ¶tendency towards a statistically significant (P=0.055) difference to protocol 4.

(i.e. 50 to 200 deg s^{-1}) and therefore it is not possible to differentiate the effect of angular velocity on the longitudinal growth.

We examined an additional functional parameter (i.e. moment–angle relationship), which is often used in the literature as an indicator for longitudinal muscle growth (Butterfield and Herzog, 2006; Proske and Morgan, 2001). The functional consequence of a longitudinal muscle adaptation is a shift of the maximum knee joint moment to longer muscle length (i.e. greater knee joint angle). However, in all investigated training protocols, we did not find a shift of the maximum knee joint moment. Even in the training protocol with the high lengthening velocity, where we measured an increase of ~14% in the vastus lateralis fascicle length, the maximum knee joint moment was achieved at the same knee joint angle in the pre and post conditions. These findings

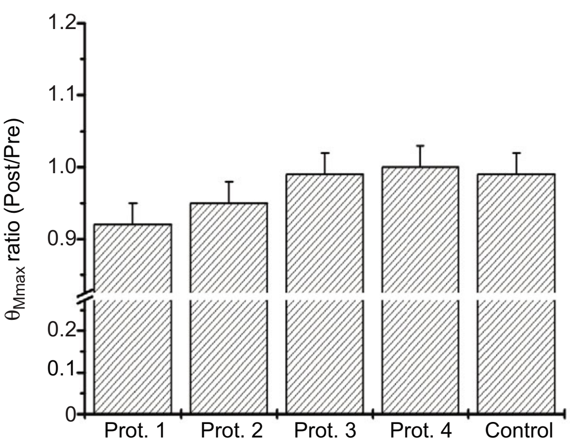

Fig. 5. Ratio of knee joint angle in which the maximum resultant knee moment was achieved. Mean values and standard errors of the ratio (i.e. post to pre values) of knee joint angle in which the maximum resultant knee joint moment was achieved in the different exercise protocols and the control group (protocol 1, low load magnitude protocol; protocol 2, high load magnitude protocol; protocol 3, short muscle length protocol; protocol 4, high lengthening velocity protocol; control, control group).

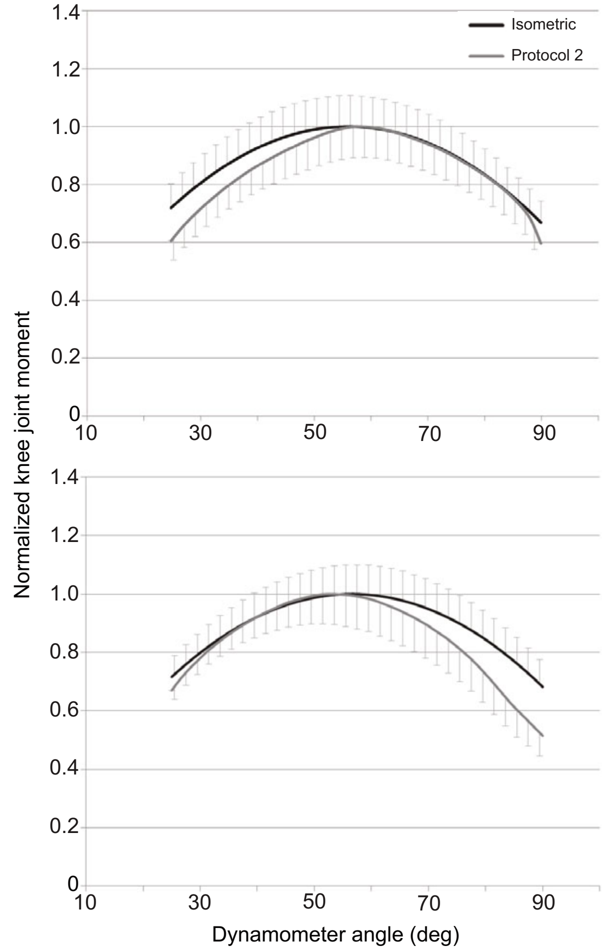

Fig. 6. Normalised knee joint moment over the range of movement. Mean values and s.e.m. of the average normalized knee joint moment from 25 to 90 deg of knee joint angle for the maximal isometric contractions (Isometric) and for the isokinetic eccentric contractions we used in the protocol 2 (high load magnitude protocol, top) and protocol 4 (high lengthening velocity protocol, below). The isometric values were measured at 30, 65 and 100 deg of the knee joint angle and then fitted with a second order polynomial to calculate the knee joint moment–angle relationship over the entire range of motion.

show how difficult it is to assess morphological changes in the fascicles from functional parameters, as there can be more than one possible adaptational change in the muscle-tendon unit that affects the moment–angle relationship of the knee extensors after an exercise intervention. Because the tendon is in series with the muscle, the magnitude of fascicle shortening may be affected by the elongation of the tendon (Narici and Maganaris, 2006). Recent studies show that resistance training increases the stiffness of the tendon (Arampatzis et al., 2007; Arampatzis et al., 2010; Reeves et al., 2004). Therefore, from a theoretical point of view, a shift of the maximum knee joint moment to a greater knee angle by an increase in fascicle length of the vastus lateralis could be compensated by an exercise-induced increase of the quadriceps tendon stiffness (i.e. shift of the maximum joint moment to a lower knee joint angle).

In summary, we conclude that not every type of eccentric exercise loading causes an increase in fascicle length. The lengthening velocity of the fascicles during the eccentric loading, and particularly in the phase where the knee joint moment decreases (i.e.

deactivation of the muscle), seems to be an important factor for longitudinal muscle growth.

MATERIALS AND METHODS

Experimental design

Fifty-three participants voluntarily participated in the present study and were randomly divided in two experimental and one control group. The participants provided written consent regarding their participation after being informed of all risks, discomforts and benefits of being involved in the study. The participants in the experimental groups eccentrically exercised the knee extensors muscles (especially the vastii) by using an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA) for 10 weeks, 3 days per week and five sets per training day. Thirty-one of the participants (10 for experimental group 1, 10 for experimental group 2, and 11 for the control group) successfully finished the designed intervention and performed all pre and post measurements.

The longitudinal growth of the vastus lateralis muscle was investigated using four different eccentric protocols by modifying the load magnitude, lengthening velocity and the muscle length where the eccentric load was applied. Therein, we modified the magnitude of the eccentric load, the lengthening velocity of the muscle and the muscle length where the eccentric load was applied. In protocol 1, the participants of the first experimental group (age, 24.9±4 years; body mass, 77.1±7.4 kg; height, 183.5±7.3 cm) exercised one leg (randomly assigned) at 65% of the maximum knee joint moment (i.e. low magnitude of eccentric load) that was examined during a knee extension MVC. The isometric values were measured at 30, 65 and 100 deg of the knee joint angle and then fitted with a second order polynomial to calculate the knee joint moment–angle relationship over the entire range of motion (i.e. 100% MVC). The second leg was exercised with protocol 2 at 100% of the MVC (i.e. high magnitude of eccentric load). The training in both exercise protocols was conducted at a knee angular velocity of 90 deg s^{-1} and a knee angle range of 25 to 100 deg (fully extended knee was defined as 0 deg).

The participants of the second experimental group (age, 29±3 years; body mass, 77.1±9.1 kg; height, 181.7±7.4 cm) followed protocol 3 and exercised one leg at 100% MVC by a knee angular velocity of 90 deg s^{-1} at a knee angle of 25 to 65 deg (i.e. in short muscle length). In this regard, the knee extensor muscles were trained in the ascending part of force–length relationship. The second leg of these participants was used for protocol 4 and exercised at a higher knee angular velocity (240 deg s^{-1}) at 100% MVC and at a knee angle of 25 to 100 deg (i.e. high lengthening velocity of the muscle). In all exercise protocols the hip angle was set to 85 deg to reduce the contribution of the bi-articular rectus femoris. The total eccentric load (integral of the knee joint moment over time) was equal in all four protocols. Therefore, in exercise protocol 1 (low load magnitude) the participants completed ten repetitions, in exercise protocol 2 (high load magnitude) six repetitions, in exercise protocol 3 (short muscle length) 12 repetitions and in exercise protocol 4 (high lengthening velocity) 16 repetitions per set, allowing a direct comparison of the results from all four protocols. The eccentric contraction driven by the isokinetic dynamometer started as the participants achieved 30% of their angle-specific MVC during the initial isometric contraction in the starting position. With this approach, we were able to start each movement with a proper pre-activation and muscle tension. During the whole training period, the participants received online feedback on the target and generated angle-specific moments and in this way it was possible to control the applied eccentric load in all exercise protocols. In our experiment, we controlled the magnitude of loading in relation to the maximal isometric contraction in the range of motion we used. This means that because of the higher force potential of the muscle during eccentric contraction, the activation level of the knee extensors during all our protocols was not maximal and in this way the participants were able to complete all repetitions at the target loading. At the beginning of every seventh training session during the intervention period, the MVCs of all participants were re-measured, and the relative values were updated. The participants of the control group (age, 28.6±4 years; body mass, 77±7.7 kg; height, 180±5.7 cm) did not participate in any specific training during the 10 week period.

Measurement of fascicle length at rest

The fascicle length of the vastus lateralis was measured before and after the intervention in inactive muscle as a function of the knee joint angle. For this purpose a linear array ultrasound probe (7.5 MHz, 10 cm wide, Esaote MyLab 60, Genova, Italy, recording frequency 43 Hz) was placed over the belly of the vastus lateralis muscle midway between trochanter major and epicondylus lateralis and fixed to the leg via elastic straps. The knee joint angle was measured by a motion analysis system (Vicon, Version 1.5.1, Oxford, UK, eight cameras, 250 Hz). The leg was passively rotated around the knee joint at 10 deg s^{-1} using the isokinetic dynamometer in a range of motion between 10 and 100 deg knee angle. After three preconditioning cycles, the ultrasound scans and the kinematic data were captured during the subsequent passive knee flexion. The fascicle length of the vastus lateralis for all participants was analyzed from a 20 to 90 deg knee angle by three independent observers. For the statistical analysis, the average values of the fascicle length from the three observers for seven knee joint angle intervals (i.e. 20–30, 30–40, 40–50, 50–60, 60–70, 70–80 and 80–90 deg knee joint angle) were used.

Measurement of moment–angle relationship

Functional parameters (indirect markers) were measured to further investigate the potential longitudinal growth of the muscles. A mechanical consequence of an increase of the number of sarcomeres in series in the quadriceps muscle would be a shift of the moment–angle relationship towards a greater knee angle (i.e. greater muscle length). This functional result has been observed previously in animal experiments (Butterfield and Herzog, 2006; Butterfield and Herzog, 2005) and is generally accepted as indirect evidence for longitudinal muscle growth. Therefore, the moment–angle relationship of the knee extensors of all participants was examined before and after the intervention. The participants performed 10 maximal isometric voluntary knee extension contractions in the range of 40 to 85 deg knee angle (measured at rest) every 5 deg in a random order [note that the knee joint angle differs from rest approximately 10 to 15 deg at the plateau of the MVC (Arampatzis et al., 2004)]. They were instructed to perform the MVCs with at least 3 min rest between trials. The hip angle was set to 85 deg to reduce the contribution of the bi-articular rectus femoris to the resultant knee moment (Herzog and ter Keurs, 1988; Herzog et al., 1990).

To account for the gravitational forces and the misalignment of the knee joint axis and the axis of the dynamometer during the maximal isometric contractions, kinematic data was recorded during the contractions to calculate the resultant knee joint moments (Arampatzis et al., 2004). Kinematic data was recorded using a Vicon motion capture system integrating eight cameras operating at 250 Hz. The antagonistic moment of the hamstrings during the MVC measurement was estimated by establishing a relationship between EMG amplitude and exerted moment for the hamstrings, whilst working as agonist (Baratta et al., 1988). In order to do that, the EMG activity of the hamstrings and the corresponding moment were measured in a relaxed condition and in two additional differing submaximal knee flexion contractions (Mademli et al., 2004). EMG activity was measured synchronously with the kinematic data at 1000 Hz. From the examined data of the MVCs (i.e. maximum knee joint moment as a function of measured knee joint angle), we calculated a second order polynomial and determined the knee joint moments from 25 to 70 deg knee angle in 5 deg intervals for each leg.

Measurement of fascicle kinetics associated with the applied eccentric stimuli

Butterfield and Herzog (Butterfield and Herzog, 2005; Butterfield and Herzog, 2006) demonstrated previously in the rabbit tibialis anterior muscle that the fiber kinetics during eccentric contractions are different to that of the whole muscle-tendon unit due to the tendon compliance. Therefore, in a second experiment, we investigated the vastus lateralis fascicle kinetics for the eccentric stimuli used in the four training protocols. Nineteen physically active persons were randomly assigned into two groups. The first group (N=10; age, 29.5±3.8 years; body mass, 79.7±22.7 kg; height, 178.3±11 cm) performed eccentric contractions of the knee extensors of one leg according to protocol 1 (i.e. low load magnitude) and on the second leg according to the protocol 2 (i.e. high load magnitude). The second group (*N*=9; age, 30.6±2.4 years; body mass, 75.3±9.6 kg; height, 175.6±8.2 cm) performed eccentric contractions with one leg on protocol 3 (i.e. short muscle length) and the second leg on protocol 4 (i.e. high lengthening velocity). For each participant, five eccentric contraction trials were analyzed. Lower limb kinematics, knee joint moments and fascicle length of the vastus lateralis were recorded during the eccentric contractions using the motion capture system (Vicon) and the ultrasound device (Esaote MyLab 60).

Statistics

The comparison of the knee joint moments and the fascicle length of the vastus lateralis muscle between the experimental and the control groups in the pre measurements were investigated using a one-way ANOVA. In order to determine the effects of the different eccentric protocols on the fascicle length of the vastus lateralis and on the knee joint moments, a two-factor ANOVA for repeated measurements (exercise protocol×pre-post condition) was applied. In case of a significant interaction effect, *post hoc* tests (Bonferroni) were conducted in order to determine the differences within the protocols. Finally, the comparison of the ratio of post to pre values and the fascicle behavior between the four eccentric protocols were examined using a one-way ANOVA. In all statistical tests, a significance level of α=0.05 was used.

Competing interests

The authors declare no competing financial interests.

Author contributions

A.S. designed and executed the experiments, interpreted the findings, and drafted and revised the article; R.M. executed the experiments, interpreted the findings, and drafted and revised the article; A.A. conceived and designed the experiments, interpreted the findings, and drafted and revised the article.

Funding

This research was supported by The Federal Institute of Sport Science (BISp), Germany.

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