Eur J Appl Physiol (2014) 114:2437–2446
DOI 10.1007/s00421-014-2967-x

ORIGINAL ARTICLE

Effects of isometric training on the knee extensor moment–angle relationship and vastus lateralis muscle architecture

Luis M. Alegre · Asunción Ferri-Morales ·
Raúl Rodriguez-Casares · Xavier Aguado

Received: 9 January 2014 / Accepted: 26 July 2014 / Published online: 7 August 2014

Abstract
Purpose To analyse the muscle adaptations induced by two protocols of isometric training performed at different muscle lengths.
Methods Twenty-eight subjects were divided into three groups: one (K90) performed isometric training of the knee extensors at long muscle lengths (90° of knee flexion) for 8 weeks, and the second group (K50) at short muscle lengths (50°). The subjects of the third group acted as controls. Isokinetic dynamometry was utilized to analyse the net moment–angle relationship and vastus lateralis muscle thickness at three different locations, and pennation angles and fascicle length at 50 % of thigh length were measured at rest with ultrasonography.
Results Only subjects from K90 group showed significant increases in isokinetic strength (23.5 %, $P < 0.001$), while K50 group showed no increases in isokinetic strength: (10 %, $P > 0.05$). There was a shift in the angle of peak torque of the K90 group to longer muscle lengths (+14.6 %, $P = 0.002$) with greater increases in isokinetic strength, while the K50 angle shifted to shorter muscle lengths (−7.3 %, $P = 0.039$). Both training groups showed significant increases in muscle thickness, (K90 9–14 % vs. K50 5–9 %) but only K90 significantly increased their pennation angles (11.7 %, $P = 0.038$). Fascicle lengths remained unchanged.
Conclusions Isometric training at specific knee angles led to significant shifts of peak torque in the direction of the training muscle lengths. The greater strength gains and the architectural changes with training at long muscle lengths probably come from a combination of different factors, such as the different mechanical stresses placed upon the muscle–tendon complex.

Keywords Fascicle length · Pennation angle · Quadriceps femoris · Length–tension relationship · Hypertrophy

Abbreviations

ANOVA	Analysis of variance
BF	Biceps femoris long head
CV	Coefficient of variation
ES	Effect size
EMG	Electromyography
KE	Knee extensors
MT	Muscle thickness
RMS	Root mean square
ROM	Range of movement
VL	Vastus lateralis

Introduction

Long-term physical activity leads to changes in the moment–angle relationship of the human skeletal muscles (Brughelli et al. 2010a; Herzog et al. 1991; Ullrich and Brueggemann 2008). Furthermore, the literature has shown that this relationship can also be modified through both dynamic (Blazevich et al. 2007; Brughelli et al.

Communicated by Olivier Seynnes.

L. M. Alegre (✉) · A. Ferri-Morales · R. Rodriguez-Casares ·
X. Aguado
Human and Sports Biomechanics Research Group, Faculty of Sports Sciences, University of Castilla-La Mancha, Avda. Carlos III s/n, 45071 Toledo, Spain
e-mail: luis.alegre@uclm.es

A. Ferri-Morales
Department of Physical Therapy, University of Castilla-La Mancha, Toledo, Spain

2010b; McMahon et al. 2013) and isometric (Kubo et al. 2006; Ullrich et al. 2009; Noorkoiv et al. 2014) resistance training. This relationship can be assessed in vivo by analysing the moment–angle relationship of isokinetic or isometric tests performed at different joint angles (Brockett et al. 2004; Brughelli and Cronin 2007; Ullrich et al. 2009). Shifts in the moment–angle relationship towards longer muscle lengths have been related to lower injury risk in the knee flexor and extensor muscles, because the sarcomeres of the muscle are operating near the plateau of their length–tension curves during a greater part of the joint range of motion (ROM) (Brockett et al. 2004; Brughelli and Cronin 2007). The ability to change the length at which a muscle can develop its maximum force is also important for functional performance, given that, for example cyclists compared to other athletes from sports with different force profiles throughout their usual joint ROMs, also show specific moment–angle relations in the involved muscles (Brughelli et al. 2010a; Herzog et al. 1991; Savelberg and Meijer 2003; Ullrich and Brueggemann 2008).

Muscle architecture, i.e., the geometrical arrangement of muscle fibres, strongly determines muscle function, especially length–tension and force–velocity relationships (Blazevich 2006). Therefore, it is not surprising that some studies have found associations between muscle architecture parameters, specifically fascicle length, and the moment–angle relationship (Blazevich et al. 2007), given that one of the proposed mechanisms for the shift is the addition of more sarcomeres in series (Lynn and Morgan 1994). Furthermore, other factors involved in strength, like muscle activation, fibre type and the mechanical behaviour of the muscle–tendon complex, may have prevented from finding stronger relationships. However, only a few works have focused on the effects of resistance training on the moment–angle relationship together with muscle size and architecture measurements (Blazevich et al. 2007; Brughelli et al. 2010a; Kubo et al. 2006; Noorkoiv et al. 2014).

Studies in literature that have analysed changes in fascicle length with dynamic resistance training have shown increases, especially when lengthening contractions have been used as training exercise (Reeves et al. 2009). However, the information available on the training joint angle on the muscle architecture of the knee extensors is limited. Isometric resistance training can be an effective alternative to dynamic training, because of its lower risk–benefit ratio (Burgess et al. 2007) and the limited equipment that requires. Therefore, it is not surprising that this contraction mode is utilized in rehabilitation and physical therapy contexts and even in sports performance (Ullrich et al. 2009). For example, specific angle isometric strength training programs can be applied in clinical populations where pain or impairments in some parts of the ROM are present. To our knowledge, few studies have compared isometric strength training at long and short knee extensor muscle length (Kubo et al. 2006; Lindh 1979; Weir et al. 1995; Noorkoiv et al. 2014), and the information provided about the changes in muscle geometry is limited. Besides, most of the studies had only focused on the effects over the isometric strength, without testing the dynamic muscle function. Dynamic testing of the moment–angle relationship allows the measurement of the strength of a given muscle group through the whole ROM, and gives a more applicable analysis of the muscle function than isometric testing (Bowers et al. 2004; Brughelli et al. 2010a).

Therefore, the aim of the present study was to analyse the adaptations in muscle function produced by two similar protocols of isometric training: one performed at long muscle lengths and another one at short muscle lengths. It was hypothesized that there would be shifts of the angle of peak torque towards the specific training angles, and that these shifts would be related to specific changes in fascicle length.

Methods

Experimental design

All measurements were performed before and after 8 weeks of isometric strength training and the moment–angle relationship of the knee extensor muscles (KE) was also tested at week 5. The training groups completed 8 weeks of unilateral isometric knee extension training, with three sessions per week. Testing included isokinetic strength, electromyographic (EMG) activity of the thigh muscles and muscle architecture of the vastus lateralis muscle (VL).

Participants

Twenty nine healthy young men and women, all of them university students without previous experience in resistance training, volunteered for the study. They were randomly divided into three similar groups. Subjects of the K90 group (*n* = 10, 8 men and 2 women, age, 19.3 ± 1.5 years; body mass, 65.6 ± 7.5 kg; and height, 1.75 ± 0.07 m) performed isometric strength training of the KEs at long muscle lengths for 8 weeks (60–80 % of maximal voluntary contraction, 90° knee angle, 0° = full extension), and participants of the K50 group (*n* = 9, 6 men and 3 women; age 18.8 ± 1.7 years; body mass 67.9 ± 10.4 kg; and height 1.72 ± 0.08 m) performed the same training protocol at short muscle lengths (50° knee angle). The subjects of the third group acted as controls (*n* = 10, 8 men and 2 women), and continued their daily physical activity, that did not involve resistance training of the lower limb in

Table 1 Training protocol for the experimental groups

	Intensity (%MVC)	Contraction time (s)	Reps	Sets	Days/weeks	Volume/week (s)	Rest between repetitions (s)	Rest between sets (s)
Weeks 1–2	60	5	6	3	2	180	10	60
Weeks 3–4	70	5	7	3	3	315	10	60
Week 5	80	5	5	4	2	200	15	60
Weeks 6–8	80	5	6	4	3	360	15	60

MVC maximal voluntary contraction

any case. During the study, one man dropped out from the control group for personal reasons unrelated to the study, therefore, only 7 men and 2 women were included in the control group for the analysis ($n = 9$; age 20.9 ± 5.4 years; body mass 71.4 ± 6.9 kg; and height 1.75 ± 0.06 m). The participants were informed about the design, potential risks of the study and discomforts related to the measurements, and all of them signed a written informed consent. The study was approved by the local Ethics Committee.

Training program

Details of the training protocol are shown in Table 1. It consisted of 2–3 sessions per week, separated by at least 1 day each. The subjects had to perform, depending on the training week, 3–4 sets of 5–7 reps of 5-s unilateral isometric knee extensions, with 10 or 15-s rest between repetitions and 60-s between sets. Training was performed on both legs, in a Biodex System 3 Pro isokinetic dynamometer (Biodex Medical, Shirley, NY, USA). The K90 group trained at 90° of knee flexion during the whole training period, while the K50 trained at 50° of knee flexion. The participants had 1 s to achieve the target torque, and had to maintain the torque with real-time feedback during all the repetitions. All the participants performed a 5-min cycling warm up at 60–75 W min^{-1}, and several submaximal isometric repetitions before the training sets. Stretching was not allowed before or after the sessions.

Isokinetic strength testing

All testing procedures were performed on the right leg of all participants. The participants were familiarized with all the strength testing protocols on a separate session before the actual testing days. Moment–angle relationship of the KE was assessed in the Biodex dynamometer. The subjects were seated with a 85° hip angle (supine position = 0°) and their trunk and thigh were fixed to the dynamometer chair with velcro straps. A leg cuff, fixed 1 cm above the medial malleolus, was used to securely connect the dynamometer's lever arm to the lower leg. The participants were instructed to grip the side handles to help stabilize the trunk. The centre of the knee joint was carefully aligned to the axis of rotation of the dynamometer. The individual positioning for each subject of the dynamometer setup was similar throughout the whole study. All the subjects performed two warm up sets of progressively greater intensity and three sets of six maximal concentric repetitions for their right leg at an angular velocity of 60° s^{-1}, with interset rests of 2 min. Knee joint ROM was set to ~100°, from 110° to 10°. The torque, angle and angular velocity signals from the Biodex along with the EMG signals (see below) were recorded online at 1,500 Hz in a laptop with the MyoResearch XP software v. 1.06 for offline analysis. Torque signal was offline gravity corrected according to Aagaard et al. (1995). From the six repetitions, the first and last contractions were excluded from the data analysis. The four remaining repetitions were averaged for the determination of the angle of peak torque by fitting a six-order polynomial curve in a custom-made Excel 2003 (Microsoft, USA) spreadsheet. Peak torque and angle of peak torque were determined from the fitted curve. The set with the highest peak isokinetic torque was selected for further analysis. This procedure has been modified from one previously utilized in the literature (Brughelli et al. 2010a). Test–retest coefficients of variation (CV), computed from the Control group measurements, were 5.0 and 3.4 % for the peak isokinetic torque and optimum angle, respectively.

Surface EMG recordings

EMG activity of VL, vastus medialis, rectus femoris and biceps femoris long head (BF) muscles was recorded using a wireless EMG recording system (Telemyo 2400TG2, Noraxon USA Inc., USA) during the isokinetic strength tests. The dynamometer and EMG signals were synchronously sampled at a 1,500 Hz, with a 12 bit analogue-to-digital conversion card (Telemyo 2400T v2, Noraxon, USA). Raw EMG signals were amplified and filtered with a band-pass filter between 10 and 500 Hz (common mode rejection ratio >100 dB, input impedance >100 MΩ and gain = 500), and were stored with commercially available software (MyoResearch XP v 1.06, Noraxon, USA). The EMG recordings were obtained by a bipolar electrode configuration (Blue-Sensor N-00-S, AMBU, Ballerup, Denmark) with a 2 cm inter-electrode distance and the electrodes placed according to SENIAM recommendations (Hermens et al. 2000). Before applying the electrodes, the skin was carefully shaved, abraded, and cleaned with alcohol. The electrode location and anatomical landmarks were marked onto an acetate sheet to ensure identical placement throughout the study. A common ground electrode was placed according to manufacturer's instructions.

For the EMG signals during the strength tests, a symmetric moving root-mean-square (RMS) filter with a time constant of 200 ms was applied to smooth the EMG data. The two repetitions with the highest peak torque were divided into data windows of 10° each, from 50° to 100° of knee flexion. Then, the EMG RMS values were normalized to the average RMS value recorded during the 65–75° data window of the ROM in the repetition with the highest torque to ensure comparisons with the same point in the ROM (i.e., similar muscle length) at an intermediate ROM window between the training angles, and look for shifts in the angle–EMG relationship. Finally, KE normalized RMS EMG amplitude was calculated as the average RMS EMG of VL, vastus medialis and rectus femoris (KE_{RMS100}, KE_{RMS90}, KE_{RMS80}, KE_{RMS70} and KE_{RMS60}). Biceps femoris long head EMG was analysed following the same criteria. No offline digital filtering was applied to the EMG values, according to manufacturer's recommendations. The same protocol was repeated for comparison in the post-training measurements.

Measurement of muscle architecture

Before the strength measurements, muscle thickness, pennation angles and fascicle length of the right VL muscle were measured in vivo by B-mode ultrasonography (MySono 201, Medison, South Korea) with a 5-cm, 7.5-MHz linear array probe, which was coated with water-soluble transmission gel to provide acoustic contact without depressing the dermal surface. Before measurements, the subjects were laid on a couch for 15 min to allow osmotic fluids to shift. During measurements, the subjects were laid supine with the knees fully extended and muscles relaxed. The images for the measurements of muscle thickness were recorded at 25, 50 and at 75 % of the distance between greater trochanter and lateral condyle of the femur (MT25, MT50 and MT75, respectively), with the ultrasound probe placed in the transversal plane and perpendicular to the skin. The distance between the subcutaneous adipose tissue–muscle interface and intermuscular interface at mid belly was defined as muscle thickness. To assess pennation angle and fascicle length at the mid-thigh location, the probe was placed in sagittal position at the mid belly and the angles between the echoes of the deep aponeurosis of the muscles and the echoes from interspaces among the fascicles were measured. For the fascicle length measurements, the portion of the fascicles visible in the screen were measured, and the nonvisible portions were estimated by linear extrapolation (Csapo et al. 2011). This has been recently reported as a valid method of fascicle length estimation (Ando et al. 2014).

To increase the reliability of repeated measures, the location of the probe was recorded onto acetate paper and pre-training and post-training images were compared during the measurements to ensure that the location was the same based on identifiable markings viewed in the muscle fascicles or adipose tissue. The ultrasound images were recorded and subsequently analysed by custom-made software. The examiner analysed two images from each location and averaged the measurements for the three variables. Test–retest CVs were 2.9, 1.9 and 3.6 % for muscle thickness (MT25, MT50 and MT75, respectively), 3.6 % for the pennation angle and 2.7 % for fascicle length.

Statistical analysis

Statistical analysis was performed with SPSS Version 19.0 (SPSS Inc., IBM, USA). The data are presented as mean ± SD. One-way ANOVA was used to evaluate whether there were any differences between groups for any of the variables at baseline. Two-way, repeated measures ANOVA (group × time) was used to evaluate the effects of isometric training on isokinetic strength and optimum angle in the training groups (three time points), muscle architecture and EMG variables (two time points). Post hoc tests with Bonferroni corrections were used to further analyse significant main interactions. Greenhouse–Geisser adjustment was applied on occasions when the assumption of sphericity was violated (Mauchly's test of sphericity, $P < 0.05$). Pearson's correlation coefficients were assessed to analyse relationships between the changes in the measured variables. The results were considered significant at $P < 0.05$. The mean effect sizes (ES) were also calculated to examine and compare the practical significance of the changes among the experimental groups. Based on Cohen (1988), who suggested ES of 0.2, 0.5, and 0.8 to represent small, moderate, and large effects, respectively, practical relevance was defined as an ES >0.8. A priori power analysis based on an ES of 0.35 on the isokinetic strength tests (isokinetic torque and optimum angle) revealed that nine participants were needed per group to reach a statistical power of 0.95.

Results

Isokinetic strength and moment–angle relationship

Outcomes from the isokinetic test are shown in Fig. 1. ANOVA showed significant interactions in absolute peak torque and optimum angle: $P = 0.014$ and $P < 0.001$. Only subjects from K90 group showed significant increases in isokinetic strength at week 5 and post-training: pre-training, 190 ± 36 N m, week 5, 212 ± 44 N m and post-training, 233 ± 42 N m, $P = 0.046$ and $P < 0.001$, respectively, with an $\mathrm{ES}_{\text{pre–post training}} = -1.1$ (23.5 ± 11.4 %). K50 group showed no significant changes in isokinetic strength: pre-training, 195 ± 61 N m, week 5, 195 ± 53 N m and post-training, 211 ± 60 N m, ns, with an $\mathrm{ES}_{\text{pre–post training}} = -0.26$ (10 ± 12.4 %). Optimum angle shifted to longer muscle lengths in the K90 group, from 77.5 ± 7.9° at pre-training to 86.3 ± 11.3° at week 5 and to 88.5 ± 8.0° at post-training ($P = 0.010$ and $P = 0.002$, pre-training vs. week 5 and post-training, respectively, $\mathrm{ES}_{\text{pre–post training}} = -1.38$; 14.6 ± 9.3 %). The K50 group followed the opposite trend, with a shift towards shorter muscle lengths that was only significant at post-training: from 70.4 ± 6.1° at pre-training to 70.8 ± 10.1° at week 5 and to 65.1 ± 5.6° at post-training ($P = 1.000$ and $P = 0.039$, pre-training vs. week 5 and post-training, respectively, with an $\mathrm{ES}_{\text{pre–post training}} = -0.91$; −7.3 ± 7.1 %). Subjects in the control group showed no changes in any of the dependent variables.

No significant shifts or increases in EMG values were found in the isokinetic strength tests of the K90 group. However, K50 group showed significant increases and large effect sizes at the knee angles of 70–60° ($P = 0.036$, $\mathrm{ES}_{\text{pre–post training}} = -1.00$) and moderate effect sizes at 60–50° ($P = 0.205$, $\mathrm{ES}_{\text{pre–post training}} = -0.77$) (Fig. 2). The control group showed no significant changes in any of the EMG values of the isokinetic test.

Muscle architecture

Pre- and post-training data on muscle architecture are shown in Table 2. VL muscle thickness at 25 and 50 % of thigh length significantly increased in both the training groups. MT25 (K90 9.3 ± 3.5 %; K50 6.1 ± 7.4 %). MT50 (K90 13.5 ± 9.7 %; K50 5.2 ± 6.5 %). However, VL muscle thickness at 75 % of thigh length only increased significantly in the group that trained at long muscle lengths, although the changes in K50 group were almost significant, with a moderate effect size: MT75 (K90 9.0 ± 7.1 %; K50 9.0 ± 11.1 %). ANOVA revealed a significant time × group interaction in MT50 ($P = 0.024$). Vastus lateralis pennation angles only significantly increased in the group that trained at long muscle lengths (K90 11.7 ± 14.7 %, $P = 0.038$; K50 7.3 ± 10.2 %, $P = 0.076$). No significant changes were found in any of the muscle architecture variables of the control group. Fascicle length did not significantly change in any of the groups. K90 4.2 ± 12.7 %; K50 −0.3 ± 12.5 %; and control 2.0 ± 4.7 %.

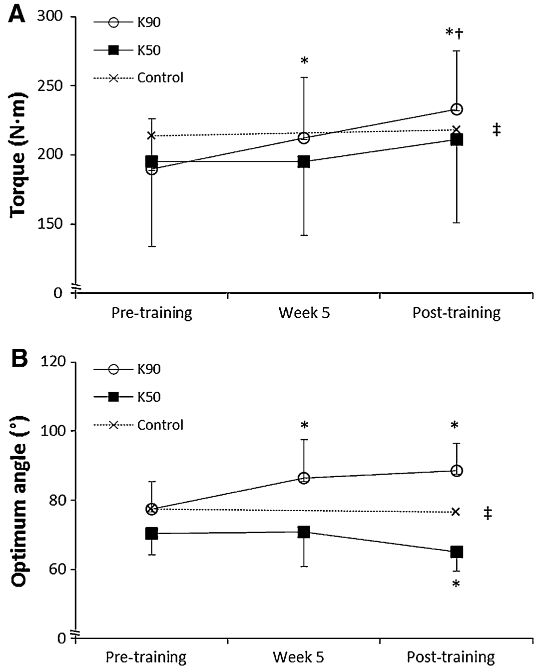

Fig. 1 Peak torque at 60° s^{-1} (**a**) and optimum angle (**b**) of the three groups throughout the 8-week period. To improve readability, error bars of the control group are not shown. *Asterisk* significantly different from pre-test, $P < 0.05$, *dagger symbol* significantly different from mid-test, $P < 0.05$; *double dagger symbol* significant interaction between K90 and K50

No significant relationships were found between the changes in muscle architecture and changes in either isokinetic strength or moment–angle relationship.

Discussion

The main finding of the present study is that isometric training performed at long muscle lengths led to greater changes in isokinetic strength, muscle size and architecture than one performed at short muscle lengths. Both the training groups showed significant shifts towards their training muscle lengths in their moment–angle relationship.

The mechanisms underlying greater changes with training at longer muscle lengths could be influenced by a number of factors. First, isometric contractions at stretched fascicle positions could have produced greater muscle damage, and hence, greater muscle adaptations, when compared with the contractions performed at muscle shortened positions, similar to other reports based on acute and chronic protocols (Philippou et al. 2004; Hunter and Faulkner 1997; McMahon et al. 2013). Second, although we did not measure quadriceps moment arm, the literature has consistently reported smaller quadriceps moment arms at more flexed knee angles (Pal et al. 2007; Reeves et al.

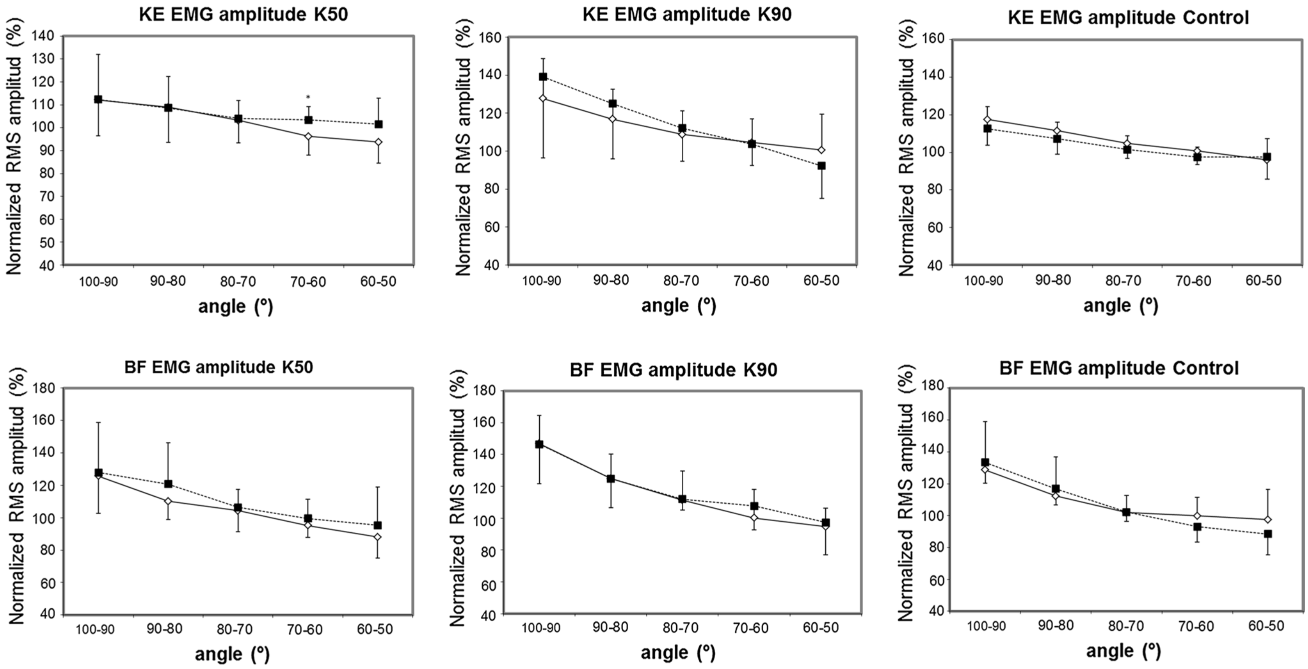

Fig. 2 EMG measurements during the isokinetic tests at pre- (*open circles*) and post-testing (*closed squares*). EMG RMS values were normalized to the average RMS values recorded during the 65–75° data window in the repetition with the highest torque. *KE* knee extensors, *BF* biceps femoris, *asterisk* significantly different from pre-test, $P < 0.05$

Table 2 Comparison of the variables from the muscle architecture measurements among the three groups throughout the 8-week period

Group	Pre-test	Post-test	ES pre–post
K90 ($n = 10$)			
25 % VL MT (cm)	2.17 ± 0.63	2.38 ± 0.73*	−0.31
50 % VL MT (cm)	2.27 ± 0.45	2.56 ± 0.45*	−0.65
75 % VL MT (cm)	2.04 ± 0.34	2.22 ± 0.39*	−0.50
Pennation angle (°)	15.1 ± 3.6	16.7 ± 3.5*	−0.45
Fascicle length (cm)	8.91 ± 1.93	9.23 ± 2.07	−0.16
K50 ($n = 9$)			
25 % VL MT (cm)	2.39 ± 0.55	2.51 ± 0.51*	−0.24
50 % VL MT (cm)	2.44 ± 0.50	2.55 ± 0.44*	−0.23
75 % VL MT (cm)	1.84 ± 0.31	1.98 ± 0.25	−0.51
Pennation angle (°)	15.5 ± 2.3	16.5 ± 2.0	−0.47
Fascicle length (cm)	8.30 ± 1.73	8.21 ± 1.64	0.05
Control ($n = 9$)			
25 % VL MT (cm)	2.39 ± 0.36	2.45 ± 0.35	−0.17
50 % VL MT (cm)	2.70 ± 0.37	2.76 ± 0.35	−0.15
75 % VL MT (cm)	1.79 ± 0.37	1.83 ± 0.37	−0.13
Pennation angle (°)	16.3 ± 1.7	16.1 ± 1.4	0.17
Fascicle length (cm)	9.81 ± 1.90	10.01 ± 1.97	−0.13

VL MT vastus lateralis muscle thickness

* Significantly different from pre-test, $P < 0.05$

2004b), leading to greater mechanical stress at the fascicle level to achieve the same torque output. Moreover, as the 90° knee training angle of the K90 group was probably closer to their optimum knee angle than that at the K50 (Kubo et al. 2006), they were able to generate greater absolute torque during the whole training program. The shorter quadriceps moment arm at long muscle lengths has been reported to be compensated by a number of factors, such as additional recruited motor units and higher discharge rates in the already activated motor units (Altenburg et al. 2009), increased afferent signal from the ligament mechanoreceptors (Johansson et al. 1991) and also increased sensitivity of the myofilaments for intracellular Ca^{2+} concentration (Balnave and Allen 1996). Therefore, the combination of greater muscle damage, fascicle stress, optimal working range and neural factors may have caused greater training response in the K90 group. The strength changes found in the present study seem to be mainly related to increases in whole muscle mass and also possibly related to preferential type II muscle fibre hypertrophy (Aagaard et al. 2001). To the best of our knowledge, no study has evaluated the differences in fibre type transition between resistance training at short and long muscle lengths. We speculate that the magnitude and direction of this specific adaptation would have been similar in both groups, meaning that all the subjects might had reached the peak torque more rapidly after training, that is, at more flexed knee angles (longer muscle lengths). However, despite this potential shift of the moment–angle curves toward longer muscle lengths in both training groups, we think that the effect of fibre of fibre type on this potential shift was small because of the limited overall contribution to the total strength changes from this parameter over a 10-week period.

Changes in the moment–angle relationship

It is worthy to note that both groups showed a significant shift in their optimum angle towards their training muscle lengths, although again, the change was greater in the group that trained at long muscle lengths. The changes in the EMG activity did not appear to be a key factor in the strength increases observed in the group that trained at long muscle lengths, given that, similar to other studies (Blazevich et al. 2009; Ullrich et al. 2009; Noorkoiv et al. 2014), we did not find any significant shift or increases in the agonist EMG amplitude. However, the group that trained at short muscle lengths showed significant increases in the EMG RMS of the KEs measured during the isokinetic test (Fig. 2). The greatest increases were found in the ROM intervals nearest to the training angle, i.e. 70–60° and 60–50° (ES of −1.21 and −1.22, respectively), and are quite similar to the increases found by Kubo et al. (2006) and Noorkoiv et al. (2014) which is, to our knowledge, the only studies that have analysed changes in the EMG activity comparing isometric training at short and long muscle lengths. As pointed out by others, neural activation could be different between isometric contractions performed at long and short muscle lengths (Babault et al. 2003; Noorkoiv et al. 2014), and this could be related to the shift in optimum angle toward short muscle lengths in the K50 group. The changes in dynamic strength observed in the present study (23 and 10 %, K90 and K50 group, respectively, Fig. 1) were fairly similar to those reported by McMahon et al. (2013) after 8 weeks of ROM restricted resistance training at long and short muscle lengths (26 and 7 %, respectively). However, they tried to control the influence of quadriceps moment arm over the muscle–tendon complex by adjusting the intensity, thus, the group that trained at long muscle lengths used a 32 % lower training intensity (55 vs. 80 % of one repetition maximum). The similar strength increases with the lower relative loads utilized by McMahon et al. could be related to differences in testing protocols and in the tensions of the working muscles during the training workouts due to the force–velocity relation. Their training included isometric contractions (50 % of the time under tension) and testing was also isometric, while we used isokinetic testing in order to analyse the whole ROM and the optimum angle. Therefore, the strength gains reported in the present study could be slightly underestimated, compared to McMahon's.

In the present study, like Noorkoiv et al. (2014), there were no significant relationships between the shifts in the moment–angle relationship and changes in fascicle length. Although the subjects of Noorkoiv et al., who also trained isometrically at short and long muscle lengths, showed significant increases in fascicle length after a 6-week training program, the tendency was similar in both of their training groups. Therefore, from our findings and the aforementioned study, it seems that the addition of sarcomeres in series is not the key factor of the shifts in the moment–angle relationship, at least for the quadriceps muscles.

Changes in muscle architecture

Surprisingly, unlike others (McMahon et al. 2013; Noorkoiv et al. 2014) no increases in fascicle length were found in the K90 group (4.2 %, ns, Table 2), despite the participants isometrically training during the whole training period with the quadriceps muscles at long lengths. The increases in the VL pennation angle (K90 11.7 %, $P < 0.05$; K50 7.3 %, $P = 0.07$) parallel to the increases found in muscle thickness (K90 9–13.5 %, $P < 0.05$; K50 5.2–9 %, $P < 0.05$) have been previously taken as indirect evidence of the addition of sarcomeres in parallel (Reeves et al. 2004a), although others have associated increases in physiological cross sectional area of pennate muscles like the VL to right shifts of the muscle optimum length, produced by increased muscle length (Heslinga et al. 1995; Huijing 1998; Swatland 1980). The results described by Heslinga et al. seem to be an exception among the usual findings from atrophy and hypertrophy studies. Besides, they were found in rat gastrocnemius medialis muscle that in humans is more pennated than the quadriceps muscles (especially the VL) (Blazevich et al. 2006). Thus, from our point of view, the possible functional implications of the increase in muscle length from a greater physiological cross sectional area would have been limited. On the other hand, evidence on human skeletal muscles (Erskine et al. 2010), with a model much closer to ours (that is, changes from resistance training in human quadriceps muscles) showed increases in physiological cross sectional area without changes in fascicle length and no shifts in the angle of peak torque, which seems to be in line with our results. In fact, the K50 group also showed increases (not significant, but with moderate effect sizes) in their pennation angles, but with left shifts in their optimum angle. If their likely change in physiological cross sectional area would have increased muscle length this left-shift would have been neglected. Therefore, the findings of Heslinga et al. in atrophied rats could have limited application to adult human models of hypertrophy, at least for the quadriceps muscles.

From previous findings (McMahon et al. 2013; Boakes et al. 2007), muscle excursion, that is, the change in muscle–tendon complex length, especially in lengthened positions, has been postulated as the main factor responsible for the change in fascicle length and metabolic stress has been reported to be the responsible mechanism for the changes in pennation angles (Reeves et al. 2009). The group that trained at long muscle lengths probably underwent greater metabolic stress, because for the same contraction time, oxygen consumption and, therefore fatigue (de Ruiter et al. 2005) would have been greater than those in the group that trained at short muscle lengths (Philippou et al. 2004). Blazevich et al. (2007) reported no differences in fascicle lengthening after an only-concentric or only-eccentric strength training period. They claimed muscle excursion as the main factor responsible for fascicle lengthening. The results of the present investigation may support these combined findings, given that although the group that trained at long muscle lengths showed slightly greater increases in their VL pennation angles and fascicle lengths, the lack of difference in muscle excursion during the training workouts seemed not adequate to bring about significant changes in fascicle length.

The group that trained at short muscle lengths showed more hypertrophy in the distal part of the VL, while the K90 group showed the greatest increases in muscle thickness in the mid-thigh (Table 2). This differential hypertrophy has been recently reported (Noorkoiv et al. 2014), and was related to shifts in the moment–angle relationship. We failed to find relationships between the changes in muscle thickness and the shifts in the optimum angle. Differences in the optimum angle calculations and in the imaging techniques (muscle thickness vs. cross sectional area from magnetic resonance imaging) could have prevented the finding of these relationships. A possible explanation could be the differential activation and mechanical loading of quadriceps components depending on the knee angle (Folland and Williams 2007), inhomogeneity of the VL architecture (Blazevich et al. 2006) and differences in myofascial force transmission within the KE muscles depending on the knee angle (Huijing and Jaspers 2005). Any of these three factors together could be the cause of the shifts reported in the present study.

The present study is not free from limitations. Fascicle angle and lengths were only measured at a single location of one muscle among the four that form the quadriceps complex. With this approach, we could have missed different changes among the quadriceps components or even in the VL itself. However, literature has consistently reported findings in the same single measurement site, and thus we understand that our results are still comparable and meaningful. Even more, changes in fascicle length in the slack muscle might not completely reflect actual changes in serial sarcomere number, but changes in resting muscle compliance or tendon stiffness. Our analysis, compared to extended field of view ultrasound imaging could have slightly underestimated the changes in fascicle length (Noorkoiv et al. 2010).

Said this, we still do believe that with conventional ultrasonography, the measurement of fascicle length at full knee extension minimizes the nonvisible part of the fascicles. We used, among the indirect methods to assess fascicle length, the one with the lowest error of estimation (Ando et al. 2014).

Conclusions

To sum up, the present study showed greater strength and changes in muscle size and structure with isometric training performed at long muscle lengths. Isometric training at specific muscle lengths led to significant changes of the angle of peak torque in the direction of the training knee angles. These findings probably come from a combination of different factors, such as the mechanical configuration of the knee joint through its functional ROM, the different metabolic responses of muscles contracting at long or short muscle lengths, and the different mechanical stresses placed upon the muscle–tendon complex depending on its length–tension relationship.

The findings of this study might be important for the design of strength training interventions for sports performance and rehabilitation. More specifically, populations that need to produce force at specific muscle lengths should include resistance training at specific joint angles into their training programs. These results provide a valuable basis for further studies into the mechanisms of the muscular responses produced by isometric training at specific muscle lengths.

Acknowledgments This study was supported by research funds from the regional government of Castilla-La Mancha, Spain (project PII1I09-0192-6593).

Conflict of interest No conflicts of interest are declared by the authors.

References

Aagaard P, Simonsen EB, Trolle M, Bangsbo J, Klausen K (1995) Isokinetic hamstring/quadriceps strength ratio: influence from joint angular velocity, gravity correction and contraction mode. Acta Physiol Scand 154(4):421–427

Aagaard P, Andersen JL, Dyhre-Poulsen P, Leffers AM, Wagner A, Magnusson SP, Halkjaer-Kristensen J, Simonsen EB (2001) A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. J Physiol 534(Pt. 2):613–623

Altenburg TM, de Haan A, Verdijk PW, van Mechelen W, de Ruiter CJ (2009) Vastus lateralis single motor unit EMG at the same absolute torque production at different knee angles. J Appl Physiol 107(1):80–89. doi:10.1152/japplphysiol.90322.2008

Ando R, Taniguchi K, Saito A, Fujimiya M, Katayose M, Akima H (2014) Validity of fascicle length estimation in the vastus lateralis and vastus intermedius using ultrasonography. J Electromyogr Kinesiol 24(2):214–220. doi:10.1016/j.jelekin.2014.01.003

Babault N, Pousson M, Michaut A, Van Hoecke J (2003) Effect of quadriceps femoris muscle length on neural activation during isometric and concentric contractions. J Appl Physiol 94(3):983–990. doi:10.1152/japplphysiol.00717.2002

Balnave CD, Allen DG (1996) The effect of muscle length on intracellular calcium and force in single fibres from mouse skeletal muscle. J Physiol 492(Pt 3):705–713

Blazevich AJ (2006) Effects of physical training and detraining, immobilisation, growth and aging on human fascicle geometry. Sports Med 36(12):1003–1017 (pii:36122)

Blazevich AJ, Gill ND, Zhou S (2006) Intra- and intermuscular variation in human quadriceps femoris architecture assessed in vivo. J Anat 209(3):289–310. doi:10.1111/j.1469-7580.2006.00619.x

Blazevich AJ, Cannavan D, Coleman DR, Horne S (2007) Influence of concentric and eccentric resistance training on architectural adaptation in human quadriceps muscles. J Appl Physiol 103(5):1565–1575. doi:10.1152/japplphysiol.00578.2007

Blazevich AJ, Cannavan D, Horne S, Coleman DR, Aagaard P (2009) Changes in muscle force-length properties affect the early rise of force in vivo. Muscle Nerve 39(4):512–520. doi:10.1002/mus.21259

Boakes JL, Foran J, Ward SR, Lieber RL (2007) Muscle adaptation by serial sarcomere addition 1 year after femoral lengthening. Clin Orthop Relat Res 456:250–253. doi:10.1097/01.blo.0000246563.58091.af

Bowers EJ, Morgan DL, Proske U (2004) Damage to the human quadriceps muscle from eccentric exercise and the training effect. J Sports Sci 22(11–12):1005–1014

Brockett CL, Morgan DL, Proske U (2004) Predicting hamstring strain injury in elite athletes. Med Sci Sports Exerc 36(3):379–387 (pii:00005768-200403000-00005)

Brughelli M, Cronin J (2007) Altering the length-tension relationship with eccentric exercise: implications for performance and injury. Sports Med 37(9):807–826 (pii:3794)

Brughelli M, Cronin J, Nosaka K (2010a) Muscle architecture and optimum angle of the knee flexors and extensors: a comparison between cyclists and Australian Rules football players. J Strength Cond Res 24(3):717–721. doi:10.1519/JSC.0b013e318197009a

Brughelli M, Mendiguchia J, Nosaka K, Idoate F, Arcos AL, Cronin J (2010b) Effects of eccentric exercise on optimum length of the knee flexors and extensors during the preseason in professional soccer players. Phys Ther Sport 11(2):50–55. doi:10.1016/j.ptsp.2009.12.002

Burgess KE, Connick MJ, Graham-Smith P, Pearson SJ (2007) Plyometric vs. isometric training influences on tendon properties and muscle output. J Strength Cond Res 21(3):986–989. doi:10.1519/R-20235.1

Cohen J (1988) Statistical power analysis for the behavioral sciences, 2nd edn. Erlbaum Associates, Hillsdale

Csapo R, Alegre LM, Baron R (2011) Time kinetics of acute changes in muscle architecture in response to resistance exercise. J Sci Med Sport 14(3):270–274. doi:10.1016/j.jsams.2011.02.003

de Ruiter CJ, de Boer MD, Spanjaard M, de Haan A (2005) Knee angle-dependent oxygen consumption during isometric contractions of the knee extensors determined with near-infrared spectroscopy. J Appl Physiol 99(2):579–586. doi:10.1152/japplphysiol.01420.2004

Erskine RM, Jones DA, Williams AG, Stewart CE, Degens H (2010) Resistance training increases in vivo quadriceps femoris muscle specific tension in young men. Acta Physiol (Oxf) 199(1):83–89. doi:10.1111/j.1748-1716.2010.02085.x

Folland JP, Williams AG (2007) The adaptations to strength training: morphological and neurological contributions to increased strength. Sports Med 37(2):145–168 (pii:3724)

Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G (2000) Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kinesiol 10(5):361–374 (pii:S1050-6411(00)00027-4)

Herzog W, Guimaraes AC, Anton MG, Carter-Erdman KA (1991) Moment-length relations of rectus femoris muscles of speed skaters/cyclists and runners. Med Sci Sports Exerc 23(11):1289–1296

Heslinga JW, te Kronnie G, Huijing PA (1995) Growth and immobilization effects on sarcomeres: a comparison between gastrocnemius and soleus muscles of the adult rat. Eur J Appl Physiol Occup Physiol 70(1):49–57

Huijing PA (1998) Muscle, the motor of movement: properties in function, experiment and modelling. J Electromyogr Kinesiol 8(2):61–77

Huijing PA, Jaspers RT (2005) Adaptation of muscle size and myofascial force transmission: a review and some new experimental results. Scand J Med Sci Sports 15(6):349–380. doi:10.1111/j.1600-0838.2005.00457.x

Hunter KD, Faulkner JA (1997) Pliometric contraction-induced injury of mouse skeletal muscle: effect of initial length. J Appl Physiol 82(1):278–283

Johansson H, Sjölander P, Sojka P (1991) A sensory role for the cruciate ligaments. Clin Orthop Relat Res 268:161–178

Kubo K, Ohgo K, Takeishi R, Yoshinaga K, Tsunoda N, Kanehisa H, Fukunaga T (2006) Effects of isometric training at different knee angles on the muscle-tendon complex in vivo. Scand J Med Sci Sports 16(3):159–167. doi:10.1111/j.1600-0838.2005.00450.x

Lindh M (1979) Increase of muscle strength from isometric quadriceps exercises at different knee angles. Scand J Rehabil Med 11(1):33–36

Lynn R, Morgan DL (1994) Decline running produces more sarcomeres in rat vastus intermedius muscle fibers than does incline running. J Appl Physiol 77(3):1439–1444

McMahon G, Morse CI, Burden A, Winwood K, Onambele GL (2013) Muscular adaptations and insulin-like growth factor-I (IGF-I) responses to resistance training are stretch-mediated. Muscle Nerve. doi:10.1002/mus.23884

Noorkoiv M, Stavnsbo A, Aagaard P, Blazevich AJ (2010) In vivo assessment of muscle fascicle length by extended field-of-view ultrasonography. J Appl Physiol (1985) 109(6):1974–1979. doi:10.1152/japplphysiol.00657.2010

Noorkoiv M, Nosaka K, Blazevich AJ (2014) Neuromuscular adaptations associated with knee joint angle-specific force change. Med Sci Sports Exerc. doi:10.1249/MSS.0000000000000269

Pal S, Langenderfer JE, Stowe JQ, Laz PJ, Petrella AJ, Rullkoetter PJ (2007) Probabilistic modeling of knee muscle moment arms: effects of methods, origin-insertion, and kinematic variability. Ann Biomed Eng 35(9):1632–1642. doi:10.1007/s10439-007-9334-6

Philippou A, Bogdanis GC, Nevill AM, Maridaki M (2004) Changes in the angle-force curve of human elbow flexors following eccentric and isometric exercise. Eur J Appl Physiol 93(1–2):237–244. doi:10.1007/s00421-004-1209-z

Reeves ND, Narici MV, Maganaris CN (2004a) Effect of resistance training on skeletal muscle-specific force in elderly humans. J Appl Physiol 96(3):885–892. doi:10.1152/japplphysiol.00688.2003

Reeves ND, Narici MV, Maganaris CN (2004b) In vivo human muscle structure and function: adaptations to resistance training in old age. Exp Physiol 89(6):675–689. doi:10.1113/expphysiol.2004.027797

Reeves ND, Maganaris CN, Longo S, Narici MV (2009) Differential adaptations to eccentric versus conventional resistance training in older humans. Exp Physiol 94(7):825–833. doi:10.1113/expphysiol.2009.046599

Savelberg HH, Meijer K (2003) Contribution of mono- and biarticular muscles to extending knee joint moments in runners and cyclists. J Appl Physiol 94(6):2241–2248. doi:10.1152/japplphysiol.01001.2002

Swatland HJ (1980) Analysis of growth in a complex muscle (*M. supracoracoideus*, *Anas platyrhynchos*). Growth 44(2):139–146

Ullrich B, Brueggemann GP (2008) Moment–knee angle relation in well trained athletes. Int J Sports Med 29(8):639–645. doi:10.1055/s-2007-989322

Ullrich B, Kleinoder H, Bruggemann GP (2009) Moment–angle relations after specific exercise. Int J Sports Med 30(4):293–301. doi:10.1055/s-0028-1104589

Weir JP, Housh TJ, Weir LL, Johnson GO (1995) Effects of unilateral isometric strength training on joint angle specificity and cross-training. Eur J Appl Physiol Occup Physiol 70(4):337–343