VASTUS LATERALIS MAXIMUM FORCE-GENERATING POTENTIAL OCCURS AT OPTIMAL FASCICLE LENGTH REGARDLESS OF ACTIVATION LEVEL

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Abstract:

Purpose: Despite the fact that everyday movements are hardly ever performed with muscles contracting maximally, our understanding of the force-length relationship is mostly based on in vitro studies using maximal activation. In this study, the in vivo submaximal and maximal force-length relationships of vastus-lateralis were investigated. Force-length relationships were obtained based on maximal and submaximal levels of force and, also, on EMG activation.

Methods: Nine subjects performed isometric knee extensor contractions at ten knee angles (80° to 170°). Knee extensor torque, and vastus-lateralis EMG and fascicle lengths were acquired simultaneously. Fascicle lengths and knee angles at peak force occurrence were compared across maximal and submaximal conditions.

Results: The submaximal force-fascicle length relationships depend crucially on the approach used: in the force-based approach, peak forces are constrained to occur at the same MTU length and, because of series elasticity, occur at longer fascicle lengths for decreasing force levels. In contrast, in the activation-based approach, peak force occurrence is not constrained to a given muscle length for submaximal contractions and occurs at similar fascicle lengths but shorter MTU lengths (more extended knee angles) as force decreases.

Conclusions: Our results support the hypothesis that vastus-lateralis fascicle length for maximal force production is about constant for maximal and submaximal levels of activation, presumably taking advantage of optimal myofilament overlap at that fascicle length. This result implies that optimal vastus-lateralis lengths occur at different knee angles for different levels of activation, which is in stark contrast to findings in the literature in which submaximal force-fascicle length relationships were based on force rather than activation.

Keywords: force-length relationship; fascicle; series elasticity; muscle properties; calcium sensitivity; muscle adaptation, electromyography, ultrasound imaging, in vivo muscle function

Abbreviations:

ANOVA Analysis of variance

EMG Electromyography

MTU Muscle tendon unit

MVC Maximal voluntary contraction

RMS Root mean square

SEE Series elastic elements

VL Vastus lateralis

The force generating potential of skeletal muscles depends on their length. This mechanical property obtained for isometric, steady-state contractions is known as the force-length relationship. The force-length relationship can be observed across all structural levels of muscle and is thought to reflect the extent of overlap between the myosin and actin filaments within sarcomeres: the optimal length for maximum force production is the one at which muscles are working with maximum overlap between filaments (Ramsey and Street 1940; Gordon et al. 1966).

Despite the fact that everyday movements are hardly ever performed with muscles contracting maximally, our understanding of the force-length relationship is mostly based on studies using maximal activation. The few studies using submaximal levels of activation have shown that the maximum force generating potential of isolated muscles or single fibres is shifted towards longer lengths with decreasing levels of activation/force (Rack and Westbury 1969; Vaz et al. 2012). This shift of the optimal muscle/fibre length has been associated with a length-dependent increase in calcium sensitivity with increasing muscle/fibre lengths (Martyn and Gordon 1988; Rassier et al. 1999) and also, more recently, with changes in the internal mechanics and force transmission that occurs at the different levels of absolute muscle force (Holt and Azizi 2014).

Although there are no major limitations in obtaining the maximal and submaximal force-length relationships in skeletal muscles in-vitro, the relevance and applicability of such measurements for in-vivo muscle properties remains uncertain. Results obtained from artificial electrical activation of isolated muscle fibers may not well represent the fibre behaviour in an intact muscle with its complex architectural arrangement of fibres and their interaction with essentially “elastic” aponeuroses and tendons. While research on isolated muscle fibres and myofibrils under controlled conditions continues to define the molecular and cellular properties of force generation, investigations of whole muscle tendon mechanics in-vivo are essential for expanding our understanding of muscle performance and muscle function in everyday life (Ichinose et al. 1997; Finni et al. 2003; Fontana et al. 2014; Lai et al. 2015).

With the advance of ultrasound imaging techniques, it has become possible to measure in vivo fascicle lengths and their orientation with respect to the aponeuroses with good accuracy and reliability (Kwah et al. 2013). Ichinose et al. (1997) used ultrasound muscle imaging to measure the maximal and submaximal fascicle force-length relationships of human vastus lateralis (VL) in-vivo. They determined submaximal force-length curves based on the percentage of force relative to the force measured during maximal voluntary contractions (MVCs) at a given muscle length. They observed a shift in the force-length relationship towards longer fascicle lengths with decreasing levels of force. This result has been interpreted as evidence that the force generating potential of the muscle and, consequently, its optimal length in-vivo is shifted to longer fascicle/fiber lengths (Austin et al. 2010).

However, it is important to realize that Ichinose's curves were obtained based on the percentage of maximum force at each joint angle. For example, they found that for MVCs, the peak force for the VL occurred at a knee joint angle of 70° of flexion. When deriving their force-length curves for the submaximal contractions based on force (80%, 50%, 30% and 10% of the maximal force), naturally the peak force at the submaximal levels also occurred at 70° of knee flexion. Therefore, peak force, by definition of the experimental protocol, always occurred at the same muscle tendon unit (MTU) length. Since with decreasing force, series elastic elements (SEE) are less

stretched than for the maximal contractions, fibre length at optimal length (always 70° of knee flexion in their case), had to be increasing with decreasing force levels. Therefore, the observed shift in “optimal” fascicle length was predictable, and the experimental design constrained the result to be what they found. Consequently, interpreting their results as evidence that a muscle’s maximum force generating potential in-vivo is shifted to longer fascicle lengths for submaximal contractions may be misleading, as it contains the unstated assumption that force and activation are proportionally related. However, submaximal force and submaximal activation may not be related in a proportional manner, and therefore, it is perceivable that muscles may take advantage of the optimal overlap between actin and myosin filaments even during submaximal contractions, and thus a muscle’s maximal force potential may occur at lengths close to the optimal fascicle length for all levels of activation.

To our knowledge, there are no data on in vivo, submaximal fascicle force-length relationships that are based on submaximal activation rather than submaximal force. If force and activation at all muscle lengths are perfectly proportional, the force-based and activation-based submaximal force-length relationships would be identical. However, there is good evidence that force and activation (or its in-vivo human proxy – electromyographic activity, EMG) are not linearly related (Guimaraes et al. 1995; Pincivero and Coelho 2000). Therefore, the aim of this study was to determine the submaximal force-length relationship for human VL muscle at the fascicle and MTU level. The relationship was obtained using the traditional force-based approach (e.g., Austin et al. 2010; Ichinose et al. 1997) and the novel EMG-based approach.

We hypothesized that (i) force and activation at a given muscle length are not proportional and, therefore, the submaximal force-length curves based on submaximal activation differ from those based on submaximal force. More specifically, (ii) we expected that peak forces at submaximal levels of activation occur close to the optimal fascicle length, and thus, that fascicle length at peak force occurrence was about constant, while those based on submaximal force are (predictably) associated with increasing fascicle length with decreasing forces (Ichinose et al. 1997; Austin et al. 2010).

METHODS

Nine subjects (7 male and 2 female) took part in this study. All subjects gave written, informed consent to participate in this research, and all procedures were approved by the University of Calgary Conjoint Health Research Ethics Board. The subjects were recreational athletes participating in sports such as running, swimming or soccer. Mean $\pm$ SD age, height and weight were 27 ± 3 years, 1.76 ± 0.13 m and 69.0 ± 15.1 kg respectively.

Knee extensor torques and knee joint angles were measured on a Biodex Multi-Joint System II isokinetic dynamometer (Biodex Medical Systems, Inc., Shirley, New York, USA). Subjects were seated with the back supported and the hip joint flexed at 80°. Straps across the shoulders, waist and thigh were used to stabilize subjects. The dynamometer was positioned so that the knee joint rotational axis was aligned with the axis of the dynamometer arm. Active (submaximal) isokinetic knee extensions were performed to verify that both axes remained aligned throughout the entire knee range of motion. In case of noticeable misalignment, subjects were re-positioned until alignment throughout the entire active range was achieved. Knee angles were analyzed in the sagittal plane, and the

end of the active range of motion (full extension) was defined as 180 degrees on the Biodex machine. The tibia was strapped to the dynamometer arm 3 cm proximal to the lateral malleolus of the tested limb.

Surface EMG was recorded from VL using bipolar electrodes (Norotrode 20™, inter electrode spacing 22 mm). The skin was shaved and cleaned with alcohol before placing the electrodes approximately two-thirds along a line from the anterior superior iliac spine to the proximal end of the patella. A ground electrode was placed on the tibial tuberosity. Skin impedance was checked and pronounced acceptable if it was less than 5 kΩ (if it was greater than 5 kΩ, the electrodes were removed and skin preparation continued). EMG, torque and joint position were recorded at 1000 Hz using the Windaq data acquisition system (Dataq Instruments, Akron, OH, USA).

Longitudinal plane images of the VL muscle were obtained at 37-49 Hz using a 12.5-MHz linear array ultrasound probe (50mm, Philips Envisor, Philips Healthcare, Eindhoven, the Netherlands). The ultrasound probe was attached to the skin on the lateral mid-thigh region using a custom-built holder, and was supported by the examiner during testing. Probe orientation and location were adjusted until the best image of VL fascicles was obtained and the superficial aponeurosis direction was approximately parallel to the deep aponeurosis. An external function generator (B-K Precision 3010, Dynascan Corp., Chicago, IL, USA) was used for synchronization of all measured signals.

By using the described setup, knee joint angle, knee extensor torque, VL EMG activity, and VL mid-length ultrasound images were obtained. The patellar tendon moment arm was calculated based on the regression equation defined by Herzog and Read (1993).

VL forces were calculated from the knee extensor torque as: VL force = $[TQ \cdot K / \text{moment arm}]$, where TQ is the knee extension torque and K (0.34) is the proportion of force associated with VL based on the physiological cross-sectional area of VL relative to the entire knee extensor group (34%; (Akima et al. 1995)).

From the VL ultrasound images, the fascicle angle and the distance between the deep and superficial aponeuroses were measured using freely available software (MicroDicom version 0.7.8). Fascicle length at each frame of interest was calculated using standard trigonometry ($Fl = h / (\sin \theta)$), where h is the distance between aponeuroses and θ is the fascicle angle (the angle formed between the fascicle and the aponeuroses). In frames where the aponeuroses were not perfectly parallel, a line starting at the deep attachment point of the fascicle of interest was drawn parallel to the superficial aponeurosis. In those cases, h was defined as the distance between the added straight line and the superficial aponeurosis, and θ was defined as the angle between the fascicle and the superficial aponeurosis. Fascicles were assumed to be straight lines and an error of 2-7% in fascicle length determination has been ascribed to this approach (Finni et al. 2003; Ishikawa et al. 2003; Kwah et al. 2013; Earp et al. 2014; Ando et al. 2014).

After being fully informed about the experimental protocol, subjects took part in a standardized warm up, which consisted of 10 submaximal reciprocal concentric knee extension and flexion repetitions. For data collection, subjects performed two maximum isometric knee extensor contractions at ten different knee angles (80° to 170°) in a randomized order and the higher value of the two measurements was used for analysis. Subjects were asked to build up to their maximal isometric torque over a period of about 5 seconds and then hold the maximal torque for approximately another 2 seconds. Verbal encouragement and visual feedback of the knee extensor torque were

provided to subjects during all contractions. To minimize fatigue, a rest period of at least 2 min was strictly enforced and was extended upon a subject's request.

Knee angle, torque, EMG and fascicle length were exported to Microsoft Office Excel (Microsoft Corp. Redmond, WA, USA) and analyzed using Matlab (Math-Works, Natick, MA). Root mean square values (RMS) of the EMG signal were calculated (100 ms window) throughout the entire 5s-ramp contraction and were then smoothed (recursive moving average) and normalized by the maximal RMS value reached at each joint angle. For the activation-based force-length relationship, fascicle lengths and VL forces were determined for each 10% increase in EMG activation level from 0-100% activation.

For the force-based force-length relationship, fascicle lengths and VL forces were determined for forces ranging from 0-100% of the maximal force using 10% increments. Fascicle length was normalized to optimal fascicle length (the length at which peak VL force at MVC was observed), while VL force was normalized to the maximum VL force. Figure 1 contains original recordings of EMG activity, normalized torque (top) and fascicle length (bottom) for one subject at 100 degrees of knee joint angle.

Fig. 1 Original recordings of EMG, normalized torque (top) and fascicle length (bottom) for one subject at 100 degrees of knee joint angle. Note that percentages of maximum force (left Y-axis) do not coincide with percentages of maximum activation (identified with the filled red circles in the force signal) and are, therefore, related to different fascicle lengths (graph at the bottom). The differences between gray and blue arrows (percentages of activation and force respectively) indicate the shift in fascicle length for this contraction. Changes in the force length relationship between the two approaches result from a combination of small shifts in the force x EMG relationship that occurs at each angle

Statistical analyses were carried out using SPSS 20.0, and a normal distribution of variables was confirmed with the Shapiro-Wilk normality test. Means and standard deviations were calculated for fascicle lengths and joint angles at peak VL force for the maximal and submaximal force-length relationships obtained with the force-based and the activation-based approach. Two one-factor repeated measures analyses of variance were carried out to test for significant effects of activation/force levels on fascicle length and on joint angle for maximal force production (optimal). A level of significance of $\alpha = 0.05$ was used for all statistical tests. Eta-squared values are shown for non-statistically significant effects.

RESULTS

The VL force for maximum activation varied, on average, from 418 N at a knee joint angle of 170° to 1588 N at 100° . The mean optimal fascicle length across subjects was 9.2 ± 1.2 cm. The activation-based VL fascicle force-length relationships differed from the force-based relationships in significant ways. This finding can be directly attributed to the non-proportional relationship between VL force and the corresponding EMG (Figure 2). Typically, the ratio FORCE/EMG was greater than 1, particularly for activation levels between 20-70% of maximum. The FORCE/EMG relationship also deviated from a linear proportionality, as indicated by the best-fitting 2nd order polynomial approximation, which had an excellent coefficient of determination exceeding 0.91.

Fig. 2 Relationship between VL Force and VL Activation at a variety of muscle tendon unit lengths (different colors indicate mean values at different muscle tendon unit lengths). Instead of a perfectly linear relationship (dotted line), a slightly curvilinear relationship was observed. Values are mean normalized values. EMG and Force were normalized to the maximum values at a given muscle length

The length dependence of submaximal force production based on the percentages of maximum force (Figure 3) differed significantly from the results based on percentages of maximum EMG activation (Figure 4). In the force-based approach, by definition, peak forces always occurred at the same joint angle (100° , bold dashed line in Figure 3) and thus the same MTU length. At submaximal levels of force, SEE were less stretched than for maximal contractions, and consequently, peak force occurred at longer fascicle lengths (indicated by bold “x” for every force level in Figure 3) since the MTU length was the same. At a submaximal force level of 10%, the average fascicle length at peak force was 23% longer than the optimal fascicle length at maximum force ($p < 0.001$).

Conversely, using the new approach introduced here (Figure 4), in which fascicle lengths and force were based on the amount of activation (EMG), rather than a percentage of force, peak forces were not constrained to occur at the same joint angle/MTU length. In fact, using EMG as the measure of submaximal activation, peak forces at submaximal contractions occurred at similar fascicle lengths (indicated by “x” in Figure 4; $p = 0.222$, Eta squared = 0.165) but shorter MTU lengths ($p < 0.001$) compared to those obtained for the maximal contractions. Muscle-tendon unit lengths decreased with decreasing levels of activation and thus the knee joint angles of maximal force occurrence increased (i.e. they became more extended) from about 100° for maximal activation to about 135° for the 10% submaximal activation level (bold dashed lines in Figure 4).

Fig. 3 Length dependence of submaximal force production based on percentages of maximal force. Colored lines represent the mean values for fascicle lengths at maximal and submaximal force production (0-100%), while the dashed lines correspond to the different knee angles analyzed (specified in the MVC curve). Best fitting, 3rd order polynomial approximations were made for each level of force. Note that for decreasing levels of force, by definition, peak forces occur at the same MTU length (and thus the same joint angle – i.e. 100° indicated by the bold dashed line) but longer fascicle lengths (indicated by the “x” symbols)

Fig. 4 Length dependence of submaximal force production based on percentages of maximal activation. Colored lines represent the mean values for the force generating potential per fascicle length at the different levels of activation, while the dashed lines are the different knee angles. Best fitting, 3rd order polynomial approximations were made for each level of activation. Note that for decreasing levels of activation, peak forces (indicated by the black “x” symbol) occur at similar fascicles lengths but shorter MTU lengths and thus increasing knee angles (from about 100° for the maximal contractions to about 135° for the 10% of maximal activation – indicated by the bold dashed lines)

In Table 1, the means and standard deviations for fascicle lengths and joint angles at peak VL force for the submaximal force-length relationships based on percentages of maximum force and percentages of maximum activation are presented.

Table 1 Mean (SD) fascicle lengths and joint angles at peak VL force for submaximal force-length relationships based on the percentage of maximum force (force-based) and the percentage of maximum EMG activation (activation-based). Fascicle lengths were normalized by the optimal fascicle length (that is the fascicle length at which peak VL force was observed for maximal effort contractions, mean = 9.2 cm)

OPTIMAL		SUBMAXIMAL LEVELS									
		10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Fascicle length (%)	Force-based[†]	123 (11)	120 (9)	117 (7)	115 (6)	111 (5)	109 (5)	107 (4)	105 (3)	103 (1)	100
	Activation-based	107 (16)	98 (12)	106 (11)	109 (9)	107 (15)	106 (11)	102 (11)	101 (9)	99 (3)	101 (1)
	Activation-based (best fit)*	109	109	112	111	109	109	108	106	107	105
Joint angle (degrees)	Force-based	103 (10)									
	Activation-based[†]	130 (12)	122 (8)	115 (10)	110 (8)	107 (9)	103 (9)	101 (7)	103 (9)	100 (9)	99 (15)
	Activation-based (best fit)*	131	120	111	110	108	105	103	102	100	100

* Best fit values based on 3rd order polynomial approximations.

[†]Statistically significant effect based on repeated measures ANOVA ($p < 0.05$)

DISCUSSION

The purpose of this study was to investigate the maximal and submaximal force-length relationships of VL muscle across a physiological range of *in vivo* lengths. Force-length relationships were obtained based on submaximal levels of force and based on submaximal levels of EMG activity. Our results confirm that increasing activation is not associated with a proportional increase in force for isometric contractions of VL. Therefore, the predicted force generating potential of VL fascicles at a given length depends crucially on the way the force potential is calculated: (i) based on submaximal levels of force, or (ii) based on submaximal levels of EMG activity.

Based on force, fascicle lengths of greatest predicted force potential (optimal fascicle length) increase with decreasing force. In contrast, based on EMG activity, optimal fascicle lengths remain approximately constant. This latter result supports the hypothesis that optimal fascicle length remains the same, independent of the level of activation. Decreasing levels of activation are associated with continuously smaller forces and increasingly less stretch of SEE. Therefore, maximal forces occurred at increasingly smaller MTU lengths with decreasing levels of activation.

Muscle function at the plateau region of the force-length relationship is usually regarded as favourable as it allows fascicles to work at a length where actin and myosin overlap optimally and thus isometric force potential is maximal (Gordon et al. 1966). For example, Lutz and Rome (1994) found that the mechanical properties of the semimembranosus muscle of the frog were perfectly suited for maximizing power output during jumping, in part because the muscle was allowed to work on the plateau region of the typical (maximal) force-length relationship. Herzog et al. (1991) found that the maximal force-length relationship of human rectus femoris muscle differed between cyclists and runners such that fibres worked on the plateau of the force-length relationship for the cyclists and runners, despite the very different demands and different hip/knee joint angles required for these two activities..

Although the plateau region of the force-length relationship is regarded as optimal for force production, many muscles are found to work on the ascending limb of the force-length relationship when maximally activated (Kulig et al. 1984; Kawakami et al. 1998). This is the case for the quadriceps muscle, which, within the primary range of knee joint motion (100 to 180°), produces more force as the muscle gets longer and very rarely performs work at the optimal fascicle length for maximal activation. It is possible, however, that the knee extensors, as well as other muscles that work on the ascending limb, are actually adapted to work at the optimal fascicle length for submaximal contractions. Our findings suggest that the ascending limb, plateau and descending limbs of the force-length relationship change relative to joint angles, depending on the level of muscle activation. Muscles that work on the ascending limb of the force length relationship for maximal contractions can potentially work closer to the plateau during submaximal daily activities. Less force in submaximal contractions leads to less elongation of SEE and therefore to longer fascicles for a given MTU length.

Optimal length in the submaximal context may be defined as the length at which the activation – or corresponding energy cost (Hug et al. 2004) – required to produce a specific force is minimum, or equivalently, where the force is greatest for a given amount of activation or energy expenditure. Therefore, analyses of the force

generating potential of muscles at specific lengths (the force-length relationship) should be specific to the required activation level. Given the non-proportional relationship between force and EMG in many muscles (Moritani and deVries 1978; Metral and Cassar 1981; Lawrence and De Luca 1983; Guimaraes et al. 1995; Pincivero and Coelho 2000), the optimal length determined for submaximal levels of force (e.g. Ichinose et al. 1997) does not necessarily represent the length at which the muscle force generating potential is maximal.

In the current study, the maximal and submaximal force generating potential of VL across a physiological fascicle length range was determined based on force and based on EMG activity level. The force-based approach has been used previously, but needs careful scrutiny. For example, Austin et al (2010) used the force-based approach of Ichinose et al. (1997) to determine the submaximal force-length relationships of VL corresponding to cycling at submaximal power outputs of 100 and 250 Watts. The comparisons were made by determining the percent effort for the submaximal cycling conditions relative to the maximal effort, and then interpolating the data of the sub-maximal force-length relationships obtained by Ichinose et al. (1997).

When superposing the fascicle length-time trajectories for submaximal and maximal cycling to the corresponding maximal and submaximal fascicle force-length relationships, Austin et al. (2010) found that fascicle excursions for cycling enclosed the plateau region for the maximal and all submaximal activation conditions; that is, the increase in fascicle lengths for submaximal cycling corresponded to the increase in optimal length of the corresponding submaximal VL force-length relationship. They interpreted this result as saying that, independent of the cycling effort, VL was working in the region of optimal length. However, the optimal fascicle lengths in the force-based force-length relationship (Ichinose et al. 1997) are constrained to occur at a constant MTU length, and thus, the results do not necessarily imply that muscle fascicles for submaximal contractions during cycling correspond to the true plateau region of the submaximal VL force-length relationships.

In our (activation/EMG-based) approach, the VL's maximum force potential occurred at similar fascicle lengths, independent of the level of EMG activity, thus presumably taking advantage of the optimal overlap of myofilaments and the maximal force that can be produced at these lengths (Gordon et al. 1966). The quotient FORCE/EMG is maximal when the muscle is contracting at lengths close to the plateau region of the force-length relationship, regardless of the level of activation (Figure 4). This result implies that any adaptation of a muscle's force-length relationship to a certain daily task is specific to the level of activation required. The argument made by Austin et al. (2010) that VL fascicles are used optimally for cycling independent of the cycling effort may not hold, as fascicle lengths increase with decreasing efforts of cycling and our findings point to an optimal fascicle length that remains about constant. If VL fascicles for a certain athlete were found to work in an optimized manner when cycling at maximal effort/activation, then, for submaximal cycling, fascicles would likely be contracting at lengths longer than the optimal fascicle length and would reach optimal length at a later stage in the pedaling cycle when the knee is more extended and VL is shorter. For the same task (same kinematics), submaximal activation, and the associated decrease in force, would lead to less stretched SEE and consequently to a fascicle trajectory that would enclose lengths longer than those found for the maximal condition, shifting the muscle to work on the (non-optimal) descending limb of the force-length relationship. A possible strategy to compensate for this shift might be achieved by shortening the MTU during the submaximal activation. Although we are not aware of studies on the effect of seat

height on fascicle excursion during cycling, increasing seat heights would lead to shorter VL MTU lengths, and, could possibly cancel increases in fascicle lengths with decreasing levels of activation.

While Ichinose's and Austin's force-based approach predicts that VL optimum force generating potential occurs at a constant MTU length across all submaximal levels, the alternative, activation-based approach presented here, suggests that the optimal MTU length is shifted to shorter lengths for submaximal contractions. The optimal MTU length – and, therefore, joint angle – for a given activation level is the one that, following the force induced elongation of SEE, allows fascicles to function at a length in which myofilament interaction is maximum. Our findings of the importance of the series elasticity's role in optimal fascicle length for force production are supported by previous research that evaluated dynamic contractions *in vivo*. For example, Kawakami et al. (2002) observed that the decrease in the VL MTU length (or increase in the knee extension angle) at which peak knee extensor torques occurred with increasing angular speeds of isokinetic knee extension corresponded well with the load-elongation properties of the SEE (which were measured separately, using isometric contractions). Based on this observation, the authors suggested – but did not measure – that fascicle lengths were similar at peak torque occurrence independent of the angular speed of knee extension, and therefore, that the shift in peak torque towards more extended knee positions with increasing knee extension speeds (shorter MTU lengths) was fully accounted for by the SEE recoil. More recently, Fontana et al. (2014) measured VL fascicle lengths throughout isokinetic knee extension contractions at eight different angular speeds and found that fascicle lengths at the instant of maximum force production were similar across the different speed conditions. These results seem to be in agreement with our present findings that optimal fascicle lengths remain approximately constant while optimal MTU becomes shorter for the submaximal force-length relationships.

Muscle “activation” has been defined in different ways in the skeletal muscle literature. For example, it has been defined by the relative frequency of stimulation of a muscle or fibre (Rack and Westbury 1969; Vaz et al. 2012; Holt and Azizi 2014); by the intracellular levels of free Ca^{2+} (Martyn and Gordon 1988); or by the EMG activity of voluntary muscle contraction (Pincivero and Coelho 2000). In experiments with *in vitro* and *in situ*, electrically stimulated or calcium activated preparations, the force-length relationship is found to be shifted towards longer lengths for decreasing levels of activation (Rack and Westbury 1969; Close 1972; Stephenson and Wendt 1984; Martyn and Gordon 1988; Morgan et al. 2000; Vaz et al. 2012; Holt and Azizi 2014). The reason for this shift is not fully understood, and although an increase in the length-dependent calcium sensitivity of muscles has been the most commonly proposed mechanism (Martyn and Gordon 1988; Rassier et al. 1999), there is no consensus in the literature. Holt and Azizi (2014) described a shift equal to 70% of the optimal length (at maximal activation) for contractions at 10% of the maximum tetanic force. They suggested that the observed shift in optimal length was caused by the different internal muscle mechanical responses at the different levels of force, rather than a change in calcium sensitivity. While a shift in optimal length seems to be a consistent finding among *in vitro* and *in situ* studies, it has never been tested systematically whether such a shift also occurs in muscles *in vivo* when activated by voluntary contraction, or if this result is an artifact of *in vitro* preparations and/or artificial electrical stimulation. In either case, shifts of 70% in optimal length, as observed by Holt and Azizi (2014), correspond to fibre lengths that are beyond actin-myosin filament overlap (Herzog et al. 1990), which seems unreasonable, and thus needs to be

considered with caution. Furthermore, it is known that the physiological operating range for most muscles appears to be considerably smaller than the total theoretical excursion available based on myofilament overlap (Cutts 1988), thus again, an increase in fascicle length of 70% beyond optimal needs careful evaluation.

The reason why we did not observe a shift in optimal fascicle lengths in-vivo during voluntary contractions needs further investigation. In electrically stimulated preparations, submaximal contractions are often produced by reducing the stimulation frequency to levels as low as single twitches or 3-5 Hz (e. g. Rack and Westbury 1969; Close 1972; Vaz et al. 2012). Maximal stimulation frequencies might be as high as 100 or 200 Hz. During human voluntary contractions, decreasing levels of activation are accompanied by decreasing frequencies of stimulation (but not substantially below about 10 Hz (Basmajian and De Luca 1985)), and simultaneously by a decrease in the number of activated motor units, while maximal stimulation frequencies probably never exceed about 50-60 Hz for a given motor unit (Bigland-Ritchie et al. 1992; Adam and De Luca 2005; Pucci et al. 2006). Therefore, we would expect that during voluntary contractions, the frequency range is much smaller than that typically used in electrically stimulated muscle and fibre preparations, and thus, variations in the free calcium are much smaller in vivo than those produced in situ and in vitro, thus reducing, possibly eliminating the length dependent calcium sensitivity for the physiological conditions encountered in involuntary human skeletal muscle contractions. Similarly, in calcium activated skinned fibre or myofibril preparations, calcium sensitivity is often evaluated over a range from [pCa] of 8 to [pCa] 3.5 or 4.0 with the sensitivity occurring over a relatively small range of calcium concentrations. It is quite possible that during voluntary contractions calcium concentrations in fibres stimulated at their lowest frequencies (8-12 Hz (Basmajian and De Luca 1985)) are higher than the critical values where calcium sensitivity is exhibited in these in vitro preparations. Therefore, it seems possible that the increased calcium sensitivity observed in artificially activated preparations may not play a significant role, or may be offset by other factors, during voluntary contractions.

Our results support the hypothesis that the maximal force potential of voluntarily activated muscles may occur at lengths close to the optimal fascicle length for all levels of activation, therefore causing optimal MTU lengths to vary according to SEE elongation/recoil. For VL, the observed shift of the MTU optimal length towards shorter lengths with decreasing levels of activation seems to fit well with the functional role of VL in everyday movements. VL is a one-joint knee extensor muscle that is frequently involved in closed kinetic chain movements in which small forces are required at short MTU lengths and high forces at long MTU lengths. For example, when standing still with the knees fully extended and the VL in its shortest possible position, just small knee extensor forces are required to maintain a stable standing position. However, when flexing the knees and moving into a squatting position, for example when lowering into a chair, the VL MTU becomes longer and, as the torque caused by the subject's weight around the knee joint increases, the force produced by VL must increase too. Similar conditions arise during the descending phase of a counter-movement jump. It is possible that, by combining increasing MTU lengths with increasing levels of force (as in squatting), and decreasing MTU lengths with decreasing levels of force (as in standing), the resulting fascicle excursion may be minimal leaving the fascicles at a nearly constant length, possibly working right at the plateau of the force-length relationship throughout the

movement, thus producing force in an optimized manner with SEE elongation matching MTU elongation and leaving fascicle lengths approximately constant.

The following assumptions were made in this study and need to be kept in mind when interpreting the results. First, VL forces were assumed to be proportional to the whole quadriceps force for all knee angles and activation levels analyzed and were calculated using moment arm values obtained by a regression model that does not account for individual moment arm length variations. VL surface EMG activity was assumed to represent the activation level of VL muscle fibers. The effect of muscle movement under the electrodes was minimized by calculating angle-specific percentage values of activation. EMG RMS values were not calculated for steady state contractions but during contractions in which force was slowly but continuously increasing. With a familiarization period and the visual feedback, all subjects were capable of increasing force in a fairly linear manner and no big deviations from linearity were observed during data collection. Electromechanical delay was not considered in the data analysis but we believe that, for this quasi-static contractions (slow increase in force over a 5s period), it would probably not play a big role in the EMG and force coupling. In addition, measurements of fascicle lengths were made based on two-dimensional ultrasound image analysis, even though muscle contraction leads to three-dimensional muscle deformations. Fascicles were assumed to be straight lines, an assumption that has been found to cause errors in absolute fascicle lengths of 2-7% (Finni et al. 2003; Ishikawa et al. 2003). Since some of the assumptions lead to systematic errors in fascicle length and VL force determination, the shapes of the normalized force-length relationships are likely not affected by these assumptions, or if they are, then only to a small degree. It is known that dynamometer testing is associated with compliance, and the Biodex system is no exception. Therefore, we would expect that with increasing force/activation, knee angles might become slightly more extended, and thus VL MTU lengths slightly shorter than at rest. Therefore, the observed fascicle lengths, although measured directly with ultrasound, might be associated with small changes in MTU lengths across the different activation/force levels of the “isometric” contractions. These small changes in MTU lengths would affect the force- and activation-based approaches to a similar degree, and therefore, would likely not be the source for the differences observed between the two approaches. Since changes in MTU lengths would probably be accommodated to a great degree by corresponding changes in fascicle lengths, the relationship between MTU and fascicle lengths would not be expected to differ much. A repeated measures design was also used to eliminate some of the systematic errors that might have occurred by assuming, for example, subject independent moment arms. Finally, one person was responsible for fascicle tracking and all data collection was conducted by the same people for consistency across all subjects.

CONCLUSIONS

To our knowledge, this is the first study in which in-vivo, submaximal force-length relationships for different levels of activation were analyzed and in which the force generating potential of VL under controlled submaximal contractions was explored. Force and activation across muscle lengths are not necessarily proportional and, therefore, the submaximal force-length curves based on activation differ from the ones based on force that have

been reported in the literature. Most importantly, the activation-based force-length relationships result in nearly constant optimal fascicle lengths and decreasing MTU lengths with decreasing levels of activation. In contrast, the force-based force-length relationships predict increasing optimal fascicle lengths and constant MTU length (by definition) for decreasing levels of force. The results of this study suggest that muscles may take advantage of the optimal overlap between actin and myosin filaments in fibers, and thus have the maximal force potential at lengths close to the optimal fascicle length regardless of the activation level.

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Ethical approval: “All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.”

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