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Physiology

# What drives activation-dependent shifts in the force–length curve?

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Skeletal muscles are rarely recruited maximally during movement. However, much of our understanding of muscle properties is based on studies using maximal activation. The effect of activation level on skeletal muscle properties remains poorly understood. Muscle optimum length increases with decreased activation; however, the mechanism responsible is unclear. Here, we attempted to determine whether length-dependent calcium effects, or the effect of absolute force underpin this shift. Fixed-end contractions were performed in frog plantaris muscles at a range of lengths using maximal tetanic (high force, high calcium), submaximal tetanic (low force, high calcium) and twitch (low force, low calcium) stimulation conditions. Peak force and optimum length were determined in each condition. Optimum length increased with decreasing peak force, irrespective of stimulation condition. Assuming calcium concentration varied as predicted, this suggests that absolute force, rather than calcium concentration, underpins the effect of activation level on optimum length. We suggest that the effect of absolute force is due to the varying effect of the internal mechanics of the muscle at different activation levels. These findings have implications for our understanding of *in vivo* muscle function and suggest that mechanical interactions within muscle may be important determinants of force at lower levels of activation.

## 1. Introduction

Skeletal muscle provides the mechanical output for all movement. To meet the varied demands of locomotion, muscle activation level, defined here as the force produced by the muscle relative to its maximum force output, is varied both by changing the activation levels of individual muscle fibres and by activating different numbers of fibres. However, the fundamental properties of muscle, such as the force–velocity and force–length relationships, are generally determined in maximally activated muscle [1,2]. These properties are often used to interpret [3] and make predictions [4] about *in vivo* muscle performance. However, the basic properties of muscle are not consistent across activation levels [5,6]. Hence, using the properties of maximally activated muscle to understand the performance of submaximally activated muscle has limitations [7]. A better understanding of the effects of activation level on muscle performance is important in advancing our understanding of *in vivo* muscle function.

The length at which a muscle can produce maximum isometric force, its optimum length, varies with activation level. Optimum length increases with decreasing activation level [5,8]. The shift towards longer optimum lengths at low activation levels has been attributed largely to a length-dependence of calcium sensitivity [8]. This is supported by the reduced calcium threshold for force generation at longer lengths [9] and the increase in optimum length with decreased stimulation frequency [5] and low calcium concentrations [10]. However, the length-dependence of calcium sensitivity does not necessarily explain the effect of activation level on optimum length. The effect of calcium concentration is often confounded by the effect of force. When calcium is low, force is also low. Hence, the shift in optimum length with activation level

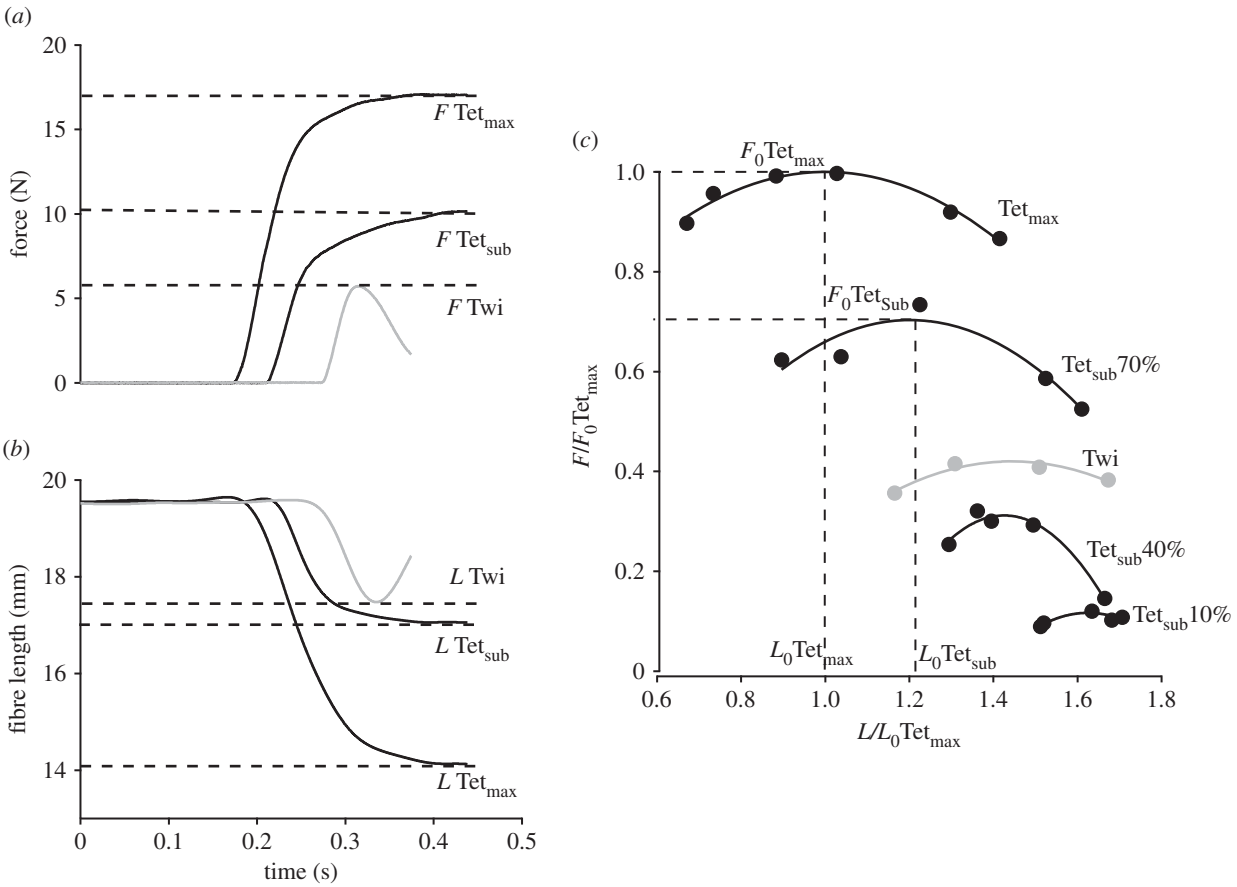


**Figure 1.** Sample force (*a*) and fibre length traces (*b*) during $Tet_{max}$, $Tet_{sub}$ and Twi contractions showing the variation in force and length profiles of contractions in the different stimulation condition and denoting the points at which force and fibre length were measured. Force and length were normalized to peak force and optimum length in the $Tet_{max}$ condition and plotted for each of the stimulation conditions (*c*). A third-order polynomial fitted to the data in each stimulation condition and the location of $F_0$ and $L_0$ in the $Tet_{max}$ and a $Tet_{sub}$ condition are shown. A representative force–length curve for each $Tet_{sub}$ group (approx. 70%, 40% and 10% of force in the $Tet_{max}$ condition) is also shown.

could be due to differences in absolute force, rather than changes in calcium sensitivity. The aim of this study was to further our understanding of the mechanisms underlying the effect of activation level on optimum length by determining whether such an effect is predominately owing to intracellular calcium concentration ($[Ca^{2+}]_i$) or absolute force.

## 2. Material and methods

The optimum fibre length of the bullfrog plantaris muscle was determined *in vitro* during fixed-end contractions under maximal tetanic ($Tet_{max}$), a range of submaximal tetanic ($Tet_{sub}$) and twitch (Twi) conditions. These conditions aimed to separate the effect of $[Ca^{2+}]_i$ and absolute force. In the $Tet_{max}$ condition, a high-frequency train of supramaximal pulses was applied to the nerve innervating plantaris. All muscle fibres will have been fully activated; $[Ca^{2+}]_i$ and muscle force will have been high. In the twitch condition, a single supramaximal pulse was applied. All muscle fibres will have been briefly activated; $[Ca^{2+}]_i$ and muscle force will have been low. In the submaximal tetanic condition, a high-frequency train of submaximal pulses was applied. Theory predicts that this will have maximally activated a subset of muscle fibres [11]. Hence, we assume that $[Ca^{2+}]_i$ will have been high in the active motor units [12], but muscle force will have been low. If the effect of activation level on optimum length was due to $[Ca^{2+}]_i$, optimum length would have been the same in the $Tet_{max}$ and $Tet_{sub}$ contractions. However, if it was a result of absolute force, optimum length would have declined with decreasing force, irrespective of stimulation condition, and been the same in force-matched Twi and $Tet_{sub}$ contractions.

The plantaris muscle and sciatic nerve were isolated ($n = 10$), sonomicrometry transducers were implanted along the length of a fascicle and the muscle was secured in a stereotaxic frame and connected to an ergometer. A stimulating electrode was placed around the sciatic nerve. Muscle length, fibre length and muscle force were measured in response to activation. Fixed-end contractions were elicited at a range of lengths using $Tet_{max}$, $Tet_{sub}$ and Twi stimulation conditions (table 1). Maximum active force ($F$) and final fibre length ($L$) were determined during each contraction (figure 1*a*,*b*). Force was plotted against length for each stimulation condition. A third-order polynomial was fitted to the data; peak active force and optimum length were determined. For each muscle, force and length were normalized to peak active force and optimal length in the $Tet_{max}$ condition and normalized peak active force ($F_0$) and optimum length ($L_0$) calculated (figure 1*c*). $Tet_{sub}$ contractions were grouped according to force (approx. 10%, 40% and 70% of peak force in the $Tet_{max}$ condition) and Kruskal–Wallis and pairwise Wilcoxon tests used to determine the effect of stimulation condition on $L_0$. A power analysis was used to determine the minimum effect size that could have been detected between $Tet_{sub}$40% and Twi conditions. A mixed-effects model was used to examine whether there was an effect of peak active force on optimum length accounting for effects of stimulation condition and individual.

## 3. Results

Isometric force production varied with stimulation condition (figures 1*a*,*c* and 2), but was well matched between the $Tet_{sub}$40% and Twi conditions (figure 2). This allowed for

**Table 1.** Parameters for the various stimulation conditions.

| | $Tet_{max}$ | $Tet_{sub}$ | twitch |
|---|---|---|---|
| pulse duration (ms) | 0.2 | 0.2 | 0.2 |
| train duration (ms) | 400 | 400 | n.a. |
| pulse frequency (Hz) | 100 | 100 | n.a. |
| pulse amplitude (V) | 3–5 | 0.3–0.9 | 3–5 |

comparison of optimum lengths in different stimulation conditions, without the confounding effect of muscle force. Optimum length varied significantly with stimulation condition ($p < 0.001$; figures 1*c* and 2). However, despite the overall effect of stimulation condition on optimum length, there was no difference in the optimum length in $Tet_{sub}$40% and Twi conditions ($p = 0.43$). Power analysis demonstrated that we would have been able to detect, with a power of 0.9, a difference of 0.05 in optimum length between conditions. Hence, we can be confident that there is no physiologically relevant difference in $L_0$ between Twi and $Tet_{sub}$40% conditions. The mixed-effects model showed that there was a significant effect of peak force on optimum length that was independent of stimulation condition ($p < 0.001$).

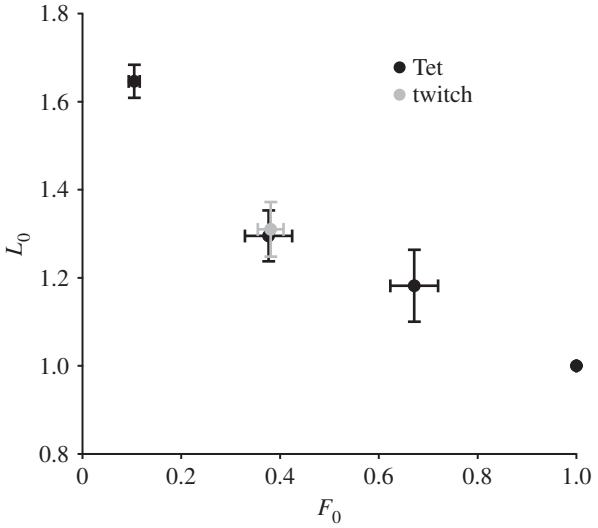


**Figure 2.** The relationship between normalized force and optimum length for all stimulation conditions. $n = 12, 5, 5, 3, 6$ for $Tet_{max}$, $Tet_{sub}$ (70%, 40%, 10%) and Twi conditions, respectively. Optimal length increases significantly with decreased force, but does not vary between force-matched Twi and $Tet_{sub}$ conditions. Data are means ± s.e.m.

## 4. Discussion

Optimum length increased with decreased activation level ($p < 0.001$; figures 1*c* and 2), as has previously been demonstrated [5,8]. This variation in optimum length with activation level suggests that there is an activation level-dependent factor that interacts with the actin–myosin effects [2] to determine optimum length at submaximal activation levels. There was no significant difference in optimum length between $Tet_{sub}$40% and Twi conditions, and there is an effect of peak force on optimum length independent of stimulation condition (figure 2). Assuming high intracellular calcium concentrations in $Tet_{max}$ and $Tet_{sub}$ conditions and low intracellular calcium concentration in the Twi condition, these findings imply that absolute force has a greater effect on optimum length than intracellular calcium concentration. While it may be a little simplistic to assume that intracellular calcium concentration is exactly the same in active fibres in the $Tet_{max}$ and $Tet_{sub}$ conditions, it is hard to envision a way in which the high-frequency stimulation used in the $Tet_{sub}$ condition would not result in a higher intracellular calcium concentration than the single pulse used in the Twi condition (table 1) [12]. Hence, we conclude that there is an effect of absolute force, rather than just a length-dependence of calcium sensitivity, underpinning the increase in optimum length with decreasing activation level.

We propose that the effect of absolute force on optimum length is a result of the varying effects of internal mechanics, the interaction between force generating cross-bridges and the physical properties of the muscle, at different activation levels. In this case, we suggest that the changing interaction between cross-bridges and the force transmission system with activation level may be responsible for the effect of absolute force on optimum length. The force output of a muscle depends on both the intrinsic force production capacity of the contractile apparatus and the effectiveness of the force transmission system within muscle. The force transmission system of muscle is structurally complex, consisting of longitudinal pathways along myofilaments as well as lateral pathways across connective tissue elements [13]. Some amount of internal work is likely needed to stretch and reorient the passive elements to allow for effective force transmission. This internal work will be relatively constant across activation levels. However, fewer cross-bridges are active at lower activation levels, so the constant requirement for internal work is likely to have a relatively greater effect. This internal work is, however, likely to vary with muscle length. Stretching the muscle to longer lengths may lower intramuscular compliance and decrease the internal work needed for effective force transmission. Hence, at low activation levels, where internal work may be more important, increasing muscle length beyond optimal actin–myosin overlap may ultimately result in greater force output owing to the reduction in internal work requirements. Experimental manipulations aimed at elucidating the details of such a mechanism will deepen our understanding of how mechanical interactions within a muscle constrain force production.

We provide indirect evidence demonstrating that the observed increase in optimum length with decreased activation level is likely to be due, at least in part, to the effect of different levels of absolute force. We suggest that the increase in optimum lengths at low activation levels may be due varying effects of internal mechanics at different activation levels: specifically, the requirement for internal work in force transmission pathways. Both the effect of activation level and the underlying mechanism have implications for our understanding of *in vivo* muscle function. It is generally considered that muscle should operate around optimum length *in vivo*, so as to maximize force [14]. However, the operating lengths of muscles *in vivo* will be complicated by the fact that optimum length depends on activation level and the state of force transmission pathways. If muscles always operate at the length at which maximal force can be

produced, then operating lengths would shift to longer lengths at lower activation levels. Future work linking the operating length of muscles with force–length curves characterized under submaximal activation conditions will greatly advance our understanding of *in vivo* muscle function.

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