



# The influence of muscle length on gastrocnemius and vastus lateralis muscle oxygen saturation and endurance

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## ABSTRACT

Increasing muscle length (passive stretch) has been shown to reduce muscle oxygen levels by increasing intramuscular pressure.

**Purpose:** To measure the effect of passive stretch on muscle-specific endurance and oxygen saturation in the vastus lateralis and medial gastrocnemius muscle groups.

**Methods:** Muscle Endurance (EI), Muscle blood flow (MBF), and Muscle oxygen saturation (MVO<sub>2</sub>) were measured on the vastus lateralis and medial gastrocnemius muscles in a passive stretched (lengthened) and relaxed (shortened) positions in 10 healthy individuals (21 ± 1 yrs.). Muscle endurance was measured with tri-axial accelerometer. Muscle oxygen saturation and blood flow were measured using a continuous wavelength Near Infrared Spectroscopy (NIRS).

**Results:** Muscle at stretched position showed a lower endurance index in the gastrocnemius (51 ± 9.6% versus 77 ± 9.1%,  $p = 0.008$ ) and vastus lateralis (54 ± 8.9% versus 75 ± 9.6%,  $p < 0.001$ ). The time to half recovery of oxygen levels during reactive hyperemia was slower in the stretched positions for the gastrocnemius (11.4 ± 1.0 s versus 8.2 ± 1.1 s,  $p < 0.001$ ) and the vastus lateralis (9.8 ± 1.9 s versus 6.3 ± 0.7 s,  $p < 0.001$ ). However, oxygen saturation during the endurance tests were not different between stretched and relaxed conditions in both muscle ( $p > 0.05$  for all comparisons).

**Conclusions:** Studies of muscle endurance need to control for muscle length as changes in muscle length can influence muscle endurance.

## 1. Introduction

Muscle fatigue has been defined as a muscle producing less than the expected force of contraction due to metabolic changes associated with continued muscle activation (Edwards, 1983). Muscle fatigue occurs with the buildup of metabolic byproducts such as hydrogen ion and phosphate during muscle activation (Wilson et al., 1988). Muscle fatigue has also been studied using a number of different experimental protocols (Al-Mulla et al., 2011; Allen et al., 2002; Enoka and Stuart, 1992; Grassi et al., 2015; McCully et al., 2002). Recently, an endurance protocol has been developed to study muscle fatigue using electrical stimulation and muscle twitches (Willingham and McCully, 2017a). This approach uses low-frequency surface neuromuscular electrical stimulation (NMES) to produce twitch contractions and a triaxial accelerometer to measure the acceleration of muscle movement (aMMG). With the development of muscle fatigue, the acceleration declines, and the rate of acceleration at the end of the stimulation is used to calculate an endurance index (Bossie et al., 2017; McCully et al., 2018;

Willingham et al., 2018).

Muscle endurance is dependent on muscle blood flow (Cole and Brown, 2000). Studies on animal muscle have shown evidence that blood flow decreases with muscle length (Poole and Mathieu-Costello, 1997). Muscle contractions can reduce blood flow due to increases in intramuscular pressure and shear closing off intramuscular blood vessels (Gray and Staub, 1967; Poole et al., 1997b). Previous studies have shown that muscle stretch can alter oxygen levels in skeletal muscle as measured by near-infrared spectroscopy (McCully, 2010a; Miura et al., 2004); these studies were performed on human gastrocnemius muscles, and the stretch was thought to reduce oxygen levels by increasing intramuscular pressure related to changes in muscle fiber pennation angle. It is not clear if the effect of muscle stretch also influences other muscles, such as the vastus lateral muscle, as these two muscles have different pennation angles at rest. It is also not clear if the magnitude of the change in oxygen levels and blood flow with stretch are sufficient to influence muscle function, such as muscle endurance. Previous studies have shown that decreased muscle length reduces muscle fatigue, by

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reducing the energy cost of muscle contractions (Fitch and McComas, 1985). However, we were not able to find studies that increased muscle length and measured muscle endurance or fatigability.

The purpose of this study is to evaluate the impact of passive stretch on muscle endurance, muscle blood flow, and muscle oxygen levels. The hypothesis being tested was that increased muscle length (passive stretch) would reduce muscle blood flow and thus reduce muscle endurance. This study also evaluated changes in muscle lengths in two different muscle groups commonly used in studies of human muscle fatigue: the gastrocnemius and vastus lateralis muscles.

## 2. Methods

This study involved ten healthy participants (5 male, 5 Female); who had no injuries to their lower limbs in the past five years. Study was approved by the Institutional Review Board at the University of Georgia. All subjects signed the informed consent form before being tested. The gastrocnemius and vastus lateralis muscles were tested in this study. All testing was performed on the same day in the following order: left Gastrocnemius lengthened, right Gastrocnemius shortened, right Vastus Lateralis lengthened, and left Vastus Lateralis shortened.

### 2.1. Gastrocnemius muscle testing arrangement

The participants were positioned supine on a padded table with their foot placed in custom-built leg pedal (Ryan et al., 2013). For the stretched position (lengthened), the ankle joint was set to 90 degrees. For the shortened position, the ankle was plantarflexed to 110 degrees.

### 2.2. Vastus lateralis testing arrangement

The participants sat upright with their leg strapped in an isokinetic ergometer (Biodex) machine. For the stretched position (lengthened), the knee joint was set to 90 degrees. For the shortened position, knee joint was set to 180 degrees.

### 2.3. Testing procedure

Continuous wavelength NIRS device (Portamon, Artinis, Ltd) was placed on the belly of the muscle. Stimulation electrodes (2 × 4 cm) were positioned proximal and distal to the NIRS device. Electrical twitch stimulation was performed using a Theratouch electrical stimulator (Richmar). Stimulation current was set to tolerable levels that also resulted in strong visual contractions (Willingham and McCully, 2017b). A blood pressure cuff attached to a commercial air compressor was wrapped around the leg proximal to the NIRS device (Hokanson) to occlude blood flow to calibrate the NIRS signals. A wireless tri-axial accelerometer (WAX3, Axivity, UK) was placed adjacent to the NIRS device and used to measure the acceleration of muscle twitch contractions (Willingham and McCully, 2017a).

### 2.4. Testing protocol

Baseline NIRS data was collected at 10 Hz for 5 min; then an endurance test protocol was performed (Willingham and McCully, 2017a). The muscle was stimulated at a frequency of 2 Hz, 4 Hz, and 6 Hz at three minutes each with 10 s rest between each frequency. Muscle acceleration data was collected at 400 Hz during the electrical stimulation periods. Following the endurance test, the cuff was inflated for 5 min ischemia to lower the muscle oxygen level close to 0%. The cuff was then rapidly deflated; this was done to measure the rate at which blood flow back to the muscle. The range of NIRS signals between the lowest point at the end of five minutes of ischemia and the highest point during reactive hyperemia was collected. The rate of recovery of the NIRS signal during the reactive hyperemia was used as a measure of blood flow (Willingham et al., 2016).

## 2.5. Data analysis

Data are reported as means with standard deviations. NIRS signals were analyzed from the farthest separation distance (4.5 cm) to increase the signal contribution from skeletal muscle. NIRS data obtained from the Portamon device were exported to Excel for analysis. NIRS data are presented as the difference signal O<sub>2</sub>Hb, although the deoxygenated (HHb) and Tissue oxygen index (TSI) signals were also analyzed. Statistical conclusions were the same for all three signals unless indicated. Oxygen saturation values were imported to Microsoft Excel, and the 3rd channel from the NIRS device was analyzed. The percent saturation values were taken at the end of each interval of the endurance test, and time points were measured consistently between channels. The accelerometer measured the surface oscillations resulting from the muscle twitches via wireless Bluetooth transmission. Muscle twitch acceleration was quantified as the resultant acceleration from all three axes (X, Y, and Z). Raw acceleration data was analyzed using custom-written routines in MATLAB R2014b (MathWorks Inc., USA). Peak to peak (p-p) analysis was employed to determine the magnitude of acceleration measured during each contraction. The endurance index was calculated as the percentage each of movement at the end of the fatigue test compared to the initial peak value ( $EEI = a_e/a_p \times 100$ ) (Willingham and McCully, 2017a). The blood flow values were determined by analyzing the post-5-minute cuff ischemia recovery period; the time to half recovery was analyzed using a custom method (Willingham et al., 2016).

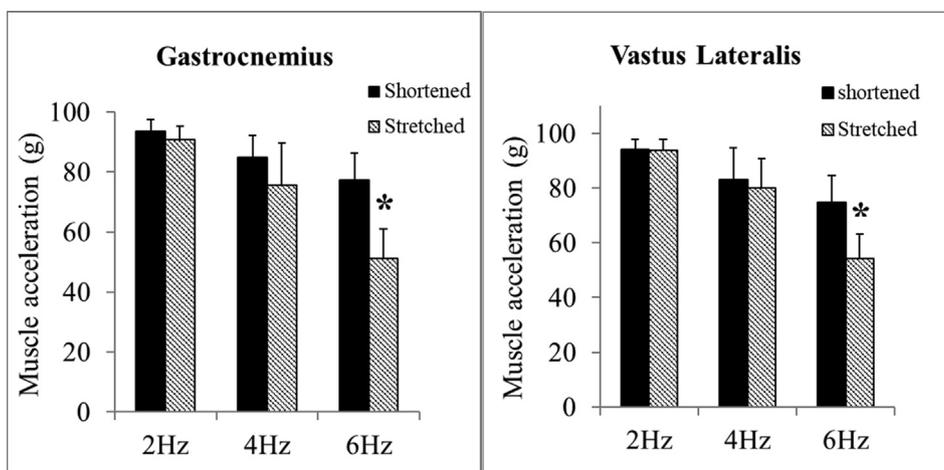
Statistical analysis was done with SPSS IBM version 24. Two way ANOVA was used to find the difference in muscle endurance value of the Quadriceps and vastus Lateralis muscle in the shortened and stretch condition. Two-way ANOVA was also used to find the difference in blood flow between both muscles in the shortened and stretch position. ANOVA was used to find the difference in the oxygen saturation between shortened muscle and lengthened muscle position for both muscles. Significance was accepted at 0.05 alpha level.

## 3. Results

Two way ANOVA shows a there was a significant difference between endurance index in the shortened and lengthened muscle position of the gastrocnemius ( $77\% \pm 9.1$ ,  $51\% \pm 9.6$ ) and in the vastus lateralis ( $75\% \pm 9.6$ ,  $54\% \pm 8.9$ ) respectively ( $F(1,18) = 85.2$ ),  $p < 0.01$ ), the lengthened position of the muscle showed a lower endurance value compared to the shortened position in both muscle (Fig. 1). There was no interaction effect of the leg ( $F(1,18) = 1.08$ ),  $p = 0.312$ ). There was no significant difference in the endurance value between the quadriceps and the vastus lateralis muscle ( $F(1,18) = 0.007$ ),  $p = 0.933$ . Fig. 2 shows a significant difference in blood flow (time to half recovery of oxygen level during reactive hyperemia) between the shortened and lengthened muscle position of the gastrocnemius ( $8.2 \pm 1.0$ ,  $11.4 \pm 1.1$ ,  $p < 0.001$ ) and in the vastus lateralis ( $6.3 \pm 0.7$ ,  $9.8 \pm 1.9$ : SD,  $p < 0.001$ ). There was also an interaction effect of the muscle ( $F(2,72) = 8.86$ ,  $p = 0.001$ ), but there was no interaction effect of the different muscle position ( $F(2,72) = 0.44$ ,  $p = 0.59$ ). Fig. 3 shows there was no significant difference in oxygen saturation during the endurance tests between the stretched and shortened conditions for the quadriceps ( $76.8 \pm 6.8\%$ ,  $82.2 \pm 11.9\%$ ) or the gastrocnemius ( $75.0 \pm 11.6\%$ ,  $80.3 \pm 5.5\%$ ) ( $p = 0.304$ ).

## 4. Discussion

The major finding in our study is that passive stretch reduced muscle endurance in both vastus lateralis and medial gastrocnemius muscles.



**Fig. 1.** The mean and standard deviation of the Endurance Index; muscle acceleration (g) of the two muscle (Gastrocnemius and Vastus lateralis) using 2 Hz, 4 Hz and 6 Hz of electrical stimulation at both muscle positions (Lengthened and Shortened). \* shows significance difference. Significance was accepted at 0.05 alpha level.

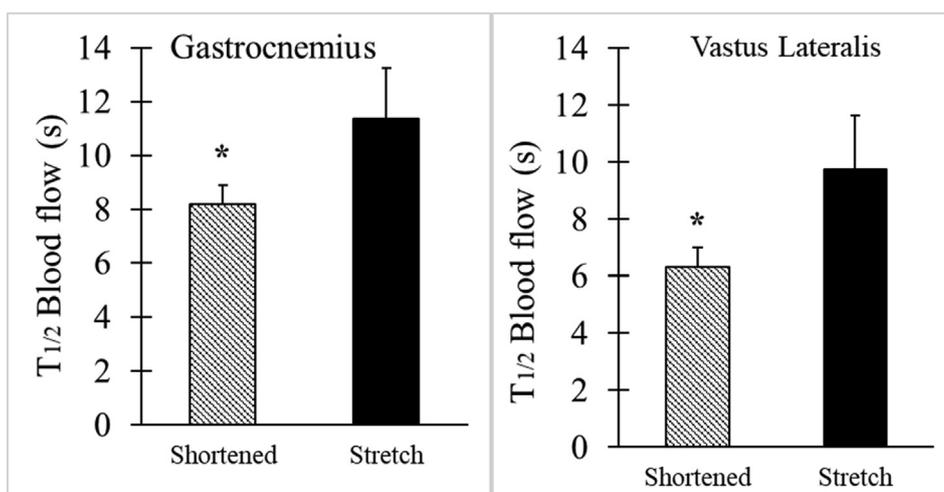
#### 4.1. Muscle blood flow and passive muscle stretch

This study found that passive stretching decreased muscle blood flow as measured by the rate of recovery of oxygen saturation during reactive hyperemia. The reductions in reactive hyperemia with stretching were similar to that seen with leg elevation (Willingham et al., 2016), which is another model of flow restriction. Evidence for decreased blood flow would support the hypothesis that stretching reduced muscle endurance by reducing oxygen delivery. We did not find that flow restriction lowered oxygen saturation levels during the endurance test. Previous studies have reported that passive stretching did lower oxygen levels in the medial gastrocnemius muscle (McCully, 2010a). The reduction in length of the sarcomere of the muscle has been reported to reduce the diameter of the capillaries in the capillary bed attached to the muscle fiber (Mathieu-Costello, 1987; Poole et al., 1997a) which can result to reduction in blood flow and muscle oxygen exchange (Welsh and Segal, 1996; Supinski et al., 1986). Kingdig and Poole, in their study on ‘sarcomere length-induced alteration of haemodynamics in rats spinotrapezius’, reported that red blood cell flux and red blood cell velocity reduced as muscle sarcomere increased during a passive stretch of the rats spinotrapezius muscle (Kingdig and Poole, 2001), although was not dependent on the vasodilatory state of the muscle and there was no change in hematocrit. The lack of change of oxygen saturation values in our study might be explained by the

reduced contraction levels which would then reduce oxygen consumption. Thus it is hard to know if the unchanged levels of oxygen in the stretched muscle was because blood flow was not reduced, or because fatigue caused by reduced blood flow reduced oxygen consumption to match the delivery of oxygen.

#### 4.2. Muscle endurance with muscle stretch in two different muscles

Previous studies have found that blood flow restriction increased muscle fatigue (Broxterman et al., 2015; Farup et al., 2015). The reduction of muscle endurance with stretching in our study is consistent with the increase in fatigue seen in studies where blood flow was restricted. Stretching has been shown to decrease in blood flow by closing blood vessels by either changing the vascular architecture or by increasing intramuscular pressure (Gray and Staub, 1967; Miura et al., 2004; Poole et al., 1997b). Previous studies have shown that stretch of the gastrocnemius muscle influences muscle oxygen levels (McCully, 2010a). In our study we found that stretching had similar reductions in muscle endurance in the medial gastrocnemius and vastus lateralis muscles. Oxygen saturation was not significantly different in both muscle during the reduced muscle endurance at 6 Hz; this could be explained because muscle fatigue reduces muscle activation such that oxygen levels may rise as the muscle fatigues.



**Fig. 2.** Mean and standard deviation of the blood flow (measured as time (s) to reach half of the blood flow recovery after transient ischemia) for the Vastus Lateralis and the gastrocnemius at the two muscle positions (Lengthened and Shortened). \* shows significance difference. Significance was accepted at 0.05 alpha level.

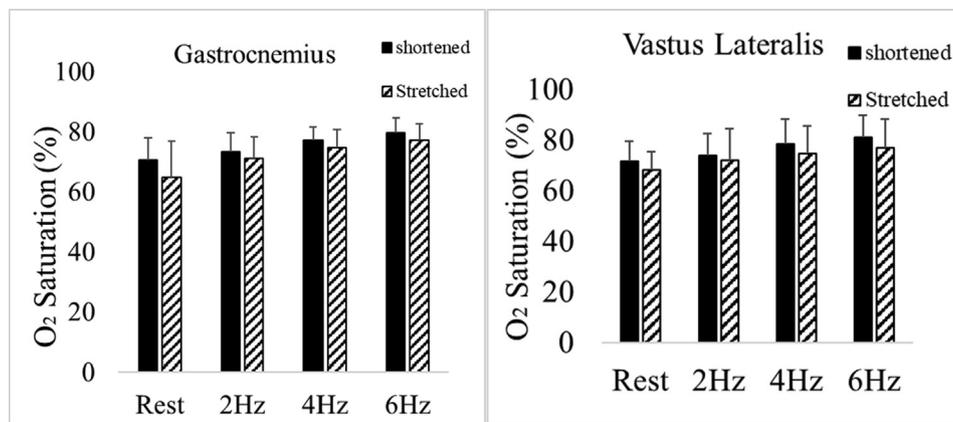


Fig. 3. Mean and standard deviation of the oxygen saturation (%) at rest, 2 Hz, 4 Hz and 6 Hz for the Gastrocnemius and Vastus Lateralis at the muscle positions (Lengthened and Shortened).

#### 4.3. Potential study limitations

One limitation of this study was the difficulty of comparing the magnitude of the effect of stretch between the two muscles, the vastus lateralis and the medial gastrocnemius. These two muscles have very different pennation angles, and previous studies have suggested that pennation angle is important in the effect of stretch (Miura et al., 2004). Both muscles were influenced by stretch in a similar fashion; but we were not able to accurately compare the amount of change in muscle length between the two preparations. The changes in joint angle were not the same between the two muscle groups, and the effect of change in joint angle was not expected to be the same between the two muscles. However, the changes in muscle length used in this study for both muscles were within the ranges expected during muscle testing and exercise. Another potential limitation to the study was the use of sub-maximal levels of stimulation. Since the intensity of electrical stimulation was dependent on the individual, it is possible that oxygen levels in the muscle could have varied during the stimulation based on the levels of stimulation. Previous studies found oxygen levels to decrease with passive stretch, and lack of proper muscle activation could be the cause of this (McCully, 2010b). Another limitation of the study is the lack of randomizing the order of testing of the different muscle position. In our previous studies we have not found an order effect of performing repeated muscle endurance tests (Willingham and McCully, 2017a), so we feel it is unlikely that the order of testing the relaxed and stretched positions influenced our results.

In conclusion, this study found that both the vastus lateralis and medial gastrocnemius muscles had endurance index values that were sensitive to muscle length. Longer muscle lengths resulted in reduced muscle endurance. Because longer muscle lengths were associated with reduced rate of muscle hyperemia, the reduced muscle endurance with longer lengths could be due to reduced muscle blood flow. This study shows that contracting muscles in a stretch position are more due to fatigue as a result of reduced blood flow. Muscle length should be taken into consideration when measuring muscle endurance using the electrical twitch, and muscle blood flow when using the Near Infrared Spectroscopy (NIRS).

#### Declaration of Competing Interest

Prof. Kevin McCully is the President of Infrared Rx, Inc., a company that designed NIRS data analysis software. No company software was used in this study. No other conflicts are reported.

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