



## **The effect of aerobic exercise on quadriceps echo intensity and cross-sectional area**

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### **Abstract**

The two primary measurements involving Ultrasound Imaging used to assess changes in muscle following exercise are Echo Intensity (EI), which is the gray scale of the image and cross-sectional area (CSA). Due to limited literature, it is unclear how and to what degree muscles composition changes following aerobic exercise.

**Purpose:** This study evaluated the change in CSA and EI following aerobic cycling exercise.

**Methods:** Sixteen males (age:  $24 \pm 7$ , height  $184 \pm 6$  cm, mass  $80 \pm 7$  kg) voluntarily cycled for 60 minutes, Pre and post EI and CSA of the Rectus Femoris (RF) and Vastus Lateralis (VL) were measured using US.

**Results:** There was a statistically significant ( $P < 0.01$ ) increase in EI in the RF 34.30 to 38.0 A.U. and the VL 27.74 to 32.11 A.U., but there was no significant difference in CSA.

**Conclusions:** Aerobic exercise does influence EI but not CSA during aerobic exercise.

**Keywords:** cycling, muscle swelling, muscle quality, ultrasound imaging

### **1. Introduction**

The ability of Ultrasound (US) to measure the effect of exercise on muscle is a relatively new concept. Previous forms of measuring the level of intensity or effect of exercise were either subjective (rate of perceived exertion scores) or invasive (blood draws or muscle biopsies). The recent advancement in US technology has made it easier and affordable to analyze muscle, thus US is potentially a noninvasive option to evaluate the effect of exercise on muscle. This would allow for researchers and practitioners to measure both acute and long-term changes following exercise. Muscle size is an example of such a measure. It has been compared to CT scans <sup>[1]</sup> and has shown to be both reliable and a valid measurement for measuring Cross-Sectional Area (CSA) in muscle. US images have been able to measure transient muscle hypertrophy (muscle swelling) <sup>[2, 3]</sup> and long term adaptations in muscle size <sup>[4]</sup>. Echo Intensity (EI) is another application of US that uses a grey scale analysis with 0 being a pure black pixel and 255 being a pure white pixel <sup>[5]</sup>. It is generally accepted that the darker the pixels of the muscle (low echogenicity) the better the muscle quality, as low frequency beams penetrate further into soft tissue rather than reflect connective and adipose tissue (high echogenicity) <sup>[6]</sup>. EI values have been correlated with fibrous tissue <sup>[5, 7]</sup>, intramuscular adiposity <sup>[8, 9]</sup>, glycogen stores <sup>[10, 11]</sup> and an increase in water content <sup>[12]</sup>.

US images have recently been used to measure changes in muscle following acute bouts of exercise. Stock et al. <sup>[2]</sup> showed that muscle thickness increased after a concentric workout but did not alter EI. Yu et al. <sup>[13]</sup> observed an increase in muscle thickness following eccentric bouts of exercise.

Muddle et al. <sup>[14]</sup> also showed that isometric contraction at varying intensities increased both muscle EI and CSA. Elevated EI is commonly reported as a marker of muscle damage <sup>[15]</sup> as it has shown to increase with both concentric <sup>[16]</sup> and eccentric <sup>[17]</sup> exercise as well as plyometric training <sup>[18]</sup>. The majority of the research evaluating EI as a marker of muscle damage is a result of resistance exercises, yet EI has not shown to change with endurance resistance type exercise <sup>[17]</sup>. Even less research has evaluated EI and CSA following aerobic exercise.

In endurance activities such as cycling or running, there are only two manuscripts that have evaluated EI and endurance activity <sup>[10, 11]</sup>. Hill et al. <sup>[10]</sup> demonstrated that EI increased with 90 minutes of moderate to high intensity cycling which also strongly correlated to glycogen depletion in muscle. Nieman et al. <sup>[11]</sup> also showed the relationship between EI and glycogen content as the changes in EI were highly correlated with changes in muscle glycogen content after a 75 km cycling time trial. Recent research <sup>[19]</sup> conducted in professional rugby players in an 80 minute match resulted in no significant changes to EI, but did show changes in muscle glycogen depletion. It is of note that all three of these studies used a commercial analysis tool to evaluate EI that is unable to report muscle CSA. The majority of US research <sup>[2, 14, 15]</sup> is done via open source software. There is no study to date evaluating aerobic exercise on muscle EI and CSA simultaneously. If US images are going to be used to quantify acute changes in muscle following exercise, it is important to know if it is sensitive enough to measure changes in muscle following aerobic exercise. Therefore, the purpose of this study was to determine if CSA and EI change following aerobic cycling exercise.

## 2. Materials and Methods

### 2.1 Participants

Sixteen males (Mean  $\pm$  SD: age:  $24 \pm 7$ , height:  $184 \pm 6$  cm, mass:  $80 \pm 7$  kg) volunteered for this study. Participants were self-reported aerobically trained (12 or more hours per week) and were familiar with long bouts of training on a bike. Prior to any testing, participants read and signed an informed consent and a health history questionnaire. All participants were free of any neurological disease or musculoskeletal injuries. This study was approved by the Institutional Review Board for protection of human participants. A priori power analysis was conducted to evaluate an adequate sample size of 13 for this study design.

### 2.2 Protocol

Sixteen males (Mean  $\pm$  SD: age:  $24 \pm 7$ , height  $184 \pm 6$  cm, mass  $80 \pm 7$  kg) volunteered for this study. Participants were self-reported aerobically trained (12 or more hours per week) and were familiar with long bouts of training on a bike. Prior to any testing participants read and signed an informed consent and a health history questionnaire. All participants were free of any neurological disease or musculoskeletal injuries. This study was approved by the Institutional Review Board for protection of human participants.

Each participant visited the lab on two separate occasions separated by at least 48 hours. Pilot data showed no significant difference in CSA or EI 48 hours post exercise ( $P$  0.18-0.22). Average time between visits was 5 days. Day one consisted of a ramped protocol on a Lode cycle ergometer (Lode Excalibur Sport B.V. Groningen, Netherlands) starting at 50 watts and increase 25 watts per minute until volitional fatigue was met. Max wattage was recorded and used for initial starting intensity for the following exercise day. Prior to and following each exercise session each participant had an ultrasound assessment performed. All testing was performed with the same equipment and protocol.

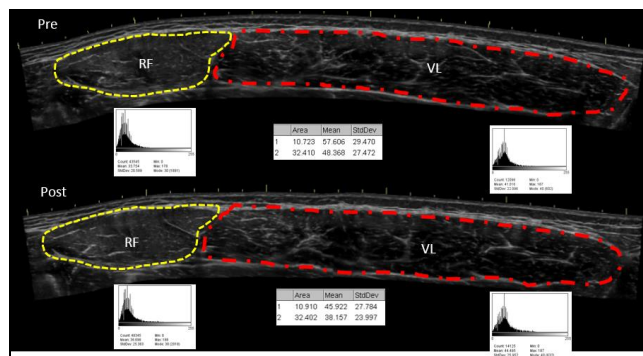
Participant performed 60 minutes session that consisted of continuous cycling starting out at 75% of max workload the wattage was then adjusted as participants fatigued so that they could continue the exercise session.

### 2.3 Ultrasound Assessment

US images were taken with a portable B-mode imaging device (GE Logiq e BT12, GE Healthcare, Milwaukee, WI, USA) and a multi-frequency linear-array probe (12 L-RS, 5–13 MHz, 38.4-mm field of view, GE Healthcare, Milwaukee, WI, USA). The panoramic function was used to obtain images of the right Rectus Femoris (RF) and Vastus Lateralis (VL) in the transverse plane. The images were taken at 1/2 of the distance between the anterior superior iliac spine and the superior border of the patella. A high-density foam pad was secured around the right thigh with an adjustable Velcro strap to ensure probe movement in the transverse plane. US settings (Frequency: 10 MHz, Gain: 45 dB, Dynamic Range: 72) were kept consistent across participants. To ensure optimal image clarity, scanning depth was individualized for each participant between 3.5–6.0 cm. A generous amount of water-soluble transmission gel (Aquasonic 100 ultrasound transmission gel, Parker Laboratories, Inc., Fairfield, NJ, USA) was applied to the skin such that it immersed the probe surface during testing

to enhance acoustic coupling. Consistent with the work of Young *et al.*, [8], three images were taken for each participant and the mean values have been reported herein. Skin was marked so that following the exercise protocol the foam pad could easily be place back on the leg and measurements were taken at the exact same spot.

The US images were digitized and examined with ImageJ Software (version 1.46, National Institutes of Health, Bethesda, MD, USA). The polygon function was used to outline the border of the RF and VL, and then both the EI and CSA were measured and assessed by a computer-aided grey-scale analysis using the histogram function. The EI values were determined as the corresponding index of muscle quality ranging between 0 and 255 A.U. (black = zero, white = 255). An example of the US measurements is displayed in Figure 1.



**Fig 1:** An example Rectus Femoris (RF: Yellow) and Vastus Lateralis (VL: Red) images and their associated analyses. The lines correspond to the border of the muscle. The corresponding grey-scale histogram has been provided under each muscle and the area measurements are in the middle.

### 2.4 Statistical Analysis

A paired sample t-test was used to compare pre vs post measurements. Effects sizes were calculated using Cohen's *d*. All statistical analyses were performed using Statistical Package for Social Science (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY).

## 3. Results

For EI there was significant ( $p < 0.001$ ) increase in EI for both the VL and RF values. However, there was no change in CSA for either muscle (Table 1).

**Table 1:** Cross-Section Area (CSA) and Echo Intensity (EI)

		Pre	Post	<i>d</i>
Rectus Femoris	CSA (cm <sup>2</sup> )	13.5 $\pm$ 3.9	13.7 $\pm$ 3.7	0.05
	EI (AU)	34.3 $\pm$ 6.4	38.0 $\pm$ 8.3*	0.49
Vastus Lateralis	CSA (cm <sup>2</sup> )	31.1 $\pm$ 4.8	30.4 $\pm$ 5.8	0.13
	EI (AU)	27.7 $\pm$ 5.8	32.1 $\pm$ 7.2*	0.67
Values are means $\pm$ standard deviations.				
* $p < 0.05$ , significant difference				
<i>d</i> = Cohens d for effect size				

## 4. Discussion

This is the first study to evaluate whether EI and CSA are affected by aerobic exercise. The result of this study demonstrated that EI increases with aerobic cycling exercise,

but has no effect on CSA.

It has been speculated that changes in EI may be a result of the depletion of glycogen<sup>[10]</sup>, however recent research<sup>[19]</sup> has shown that EI has no relation to glycogen stores. It is further speculated that changes in EI could be heat and water related<sup>[14]</sup>. Changes in EI have also been attributed to muscle swelling<sup>[15]</sup>. Muscle Swelling is associated with an increase in plasma enzyme levels<sup>[20]</sup>, edema<sup>[3]</sup> and buildup of metabolites as a result of exercise<sup>[21]</sup>. Our results did not find an increase in CSA to correspond to the increase in EI, which leads to speculation that EI may not be a result of muscle swelling in aerobic exercise. The effect size for EI values resulted in a moderate to strong effect size ( $d=0.49-0.67$ ), clearly indicating that EI is changing. These results clearly indicated that EI does increase following aerobic exercise.

This is a novel finding, as research has shown that not all exercise types illicit the same acute EI and CSA responses; as concentric<sup>[22]</sup> and eccentric<sup>[16]</sup> workouts increase CSA, but not EI, while isometrics<sup>[14]</sup> illicit increase in both EI and CSA immediately following exercise. Our results demonstrate that EI may be acutely affected by cycling exercise whereas resistance training affects CSA. Further investigation is needed to determine the mechanisms that lead to increases in EI and CSA.

A limitation to this current study is EI was not tracked over time to observe recovery with similar strategies employed by Radaelli et al.<sup>[16]</sup>. Another limitation consisted of reducing wattage on the bike so the participants could finish the exercise session as seen in Table 1. The goal was 75% for 60 minutes, but the participants' averaged 63%. This could be seen as a weakness, but also a strength as the participants were going at their maximal aerobic ability for the whole duration.

## 5. Conclusion

In conclusion, aerobic exercise increases EI, but not CSA. The results demonstrate that not all exercise (i.e. resistance vs aerobic) affects muscle the same. There is no conclusive evidence to the mechanisms that lead to changes in EI and CSA following different types and intensities of exercise. Whatever the mechanisms that lead to changes in EI and CSA from one type of exercise might not be the same as another, as demonstrated by this study. Therefore, to use US imaging as a blanket tool for analyzing muscle damage is probably outside the scope of US imaging.

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