Validation of Musculoskeletal Ultrasound to Assess Muscle Glycogen Content. A Novel Approach

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ABSTRACT: Glycogen storage is essential for exercise performance. The capacity to assess muscle glycogen levels should confer an important advantage for performance. However, skeletal muscle glycogen assessment has only been available and validated through muscle biopsy. We have developed a new methodology using high frequency ultrasound to assess skeletal muscle glycogen content in rapid, portable and non-invasive way using MuscleSound® technology. PURPOSE. To validate the utilization of high frequency musculoskeletal ultrasound for muscle glycogen assessment and correlate it with histochemical glycogen quantification through muscle biopsy. METHODS. Twenty two male competitive cyclists (Cat 1-4) (183.7 ± 4.9 cm, 76.8 ± 7.8 kg) performed a steady state test on a cyclergometer for 90 minutes at a moderate-high exercise intensity eliciting a carbohydrate oxidation (CHOox) of 2-3 g·min^{-1} and a blood lactate concentration ($[La^-]_b$) of 2-3mM. pre- and post-exercise glycogen content from rectus femoris muscle was measured using histochemical analysis through muscle biopsy and through high frequency ultrasound scans using MuscleSound® technology to measure glycogen content. RESULTS. Correlations between muscle biopsy glycogen histochemical quantification (mmol·kg^{-1}) and high-frequency ultrasound methodology through MuscleSound® technology were r=0.93, p<0.0001 pre-exercise and r=0.94, p<0.0001 post-exercise. The correlation between muscle biopsy glycogen quantification and high-frequency ultrasound methodology for the change in glycogen from pre- and post-exercise was r=0.81, p<0.0001. CONCLUSIONS. These results show that the use of high-frequency ultrasound through MuscleSound® technology is a reliable way to measure skeletal muscle glycogen in a fast and non-invasive way.

BACKGROUND: Carbohydrates and proper glycogen stores are a key element in athletic performance. Glycogen assessment has only been possible through muscle biopsy technique and to a limited extent, nuclear magnetic resonance spectroscopy (NMRS). These methodologies are either invasive and aggressive or very expensive, therefore, making them impractical for athletes to monitor performance and nutrition. We propose a novel methodology to assess muscle glycogen through high frequency musculoskeletal ultrasound which is fast and non-invasive. When a muscle is full of glycogen, the ultrasound image is generally hypoechoic (dark) due to water associated with glycogen. As a molecule of glycogen leaves the skeletal muscle it takes 3-4 molecules of water with it. The depleted glycogen stores result in a hyperechoic muscle image (bright). The qualitative picture is then processed pixel by pixel to give a quantitative value of the skeletal muscle glycogen content.

PURPOSE: The purpose of this study is to validate the methodology we have developed through high-frequency ultrasound to assess skeletal muscle glycogen with the traditional muscle biopsy methodology, which has long been the gold standard. Through this validation we propose a new, more practical and non-invasive methodology to measure muscle glycogen content through high-frequency ultrasound.

METHODS: Twenty two (N=22) male competitive cyclists (Professional and Amateurs, Categories 1-4, 183.7 ± 4.9 cm, 76.8 ± 7.8 kg) performed a steady state test on a cyclergometer for 90 minutes at a moderate-high exercise intensity eliciting a carbohydrate oxidation (CHOox) of 2-3 $g{\cdot}min^{-1}$ and a blood lactate concentration ($[La^-]_b$) of 2-3mM. pre-and post-exercise glycogen content from rectus femoris muscle was measured using histochemical analysis through muscle biopsy and through high frequency ultrasound scans using MuscleSound® technology to measure glycogen content. Muscle biopsy samples and high frequency ultrasound scans were obtained from rectus femoris muscle pre-and post-exercise. An ultrasound-guided muscle micro-biopsy technique was developed *ad hoc* in order to access rectus femoris without compromising major vascular structures. Muscle scans were performed with a 12 MHz linear transducer and a standard diagnostic high resolution ultrasound machine, GE LOGIQe (GE Healthcare, Milwaukee, WI). Software developed by MuscleSound® processed images to isolate the muscle fibers under analysis (center crop within muscle section 25 mm from the top of the muscle, using feature extraction to subtract the skin, fat, connective tissue and blood vessels), with the mean pixel intensity of the muscle averaged from the eight scans (both long and short axis) then scaled (0 to 100 scale) to create the glycogen score.

RESULTS Correlations between rectus femoris biopsy glycogen histochemical quantification ($mmol{\cdot}kg^{-1}$) and high-frequency ultrasound methodology through MuscleSound® technology were $r=0.93$, $p<0.0001$ pre-exercise and $r=0.94$, $p<0.0001$ post-exercise (Fig-1 and Fig-2).

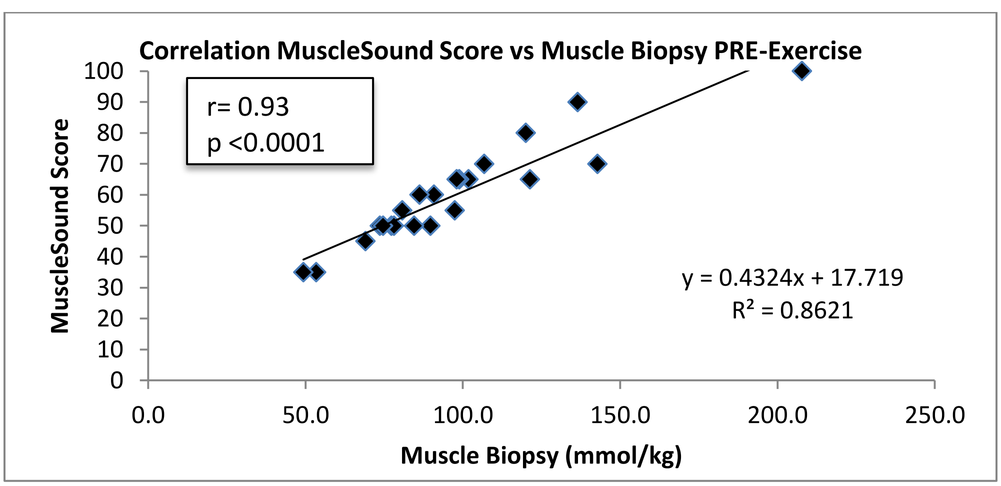

Fig-1

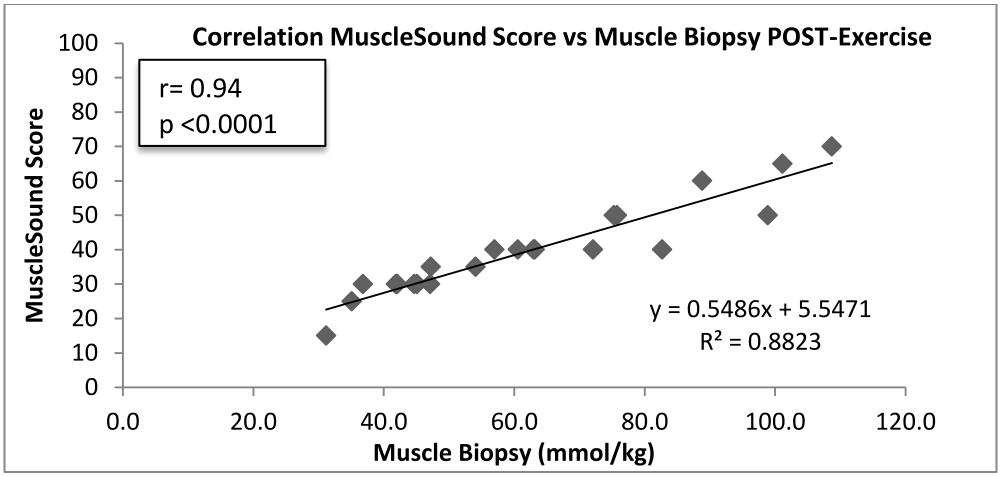

Fig-2

The correlation between muscle biopsy glycogen quantification and high-frequency ultrasound methodology for the change in glycogen from pre- and post-exercise was r=0.81, p<0.0001 (Fig-3)

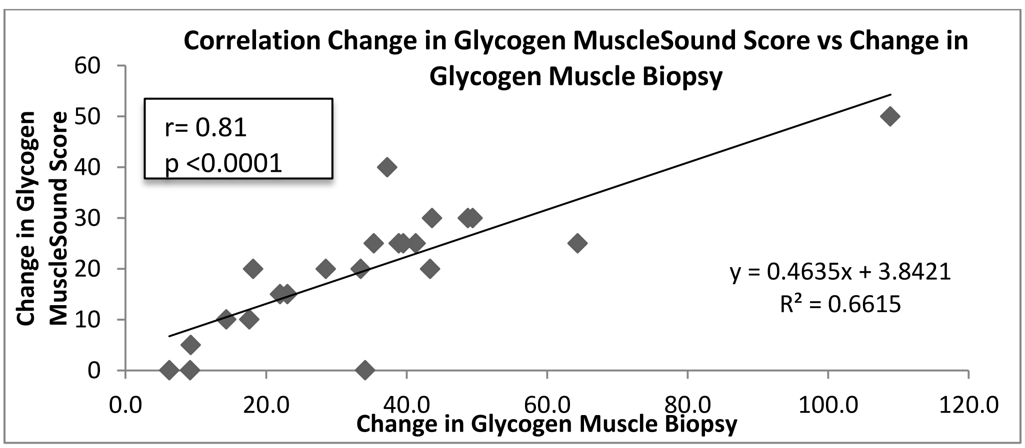

Fig-3

The absolute change in muscle glycogen varied substantially between subjects (109 to 6 mmol/kg), (Fig-4). The absolute change in the MuscleSound score also varied substantially between subjects (50 to 0), (Fig-5), but followed the same individual trends as did the muscle glycogen content as evidenced by the correlation in change r= 0.81.

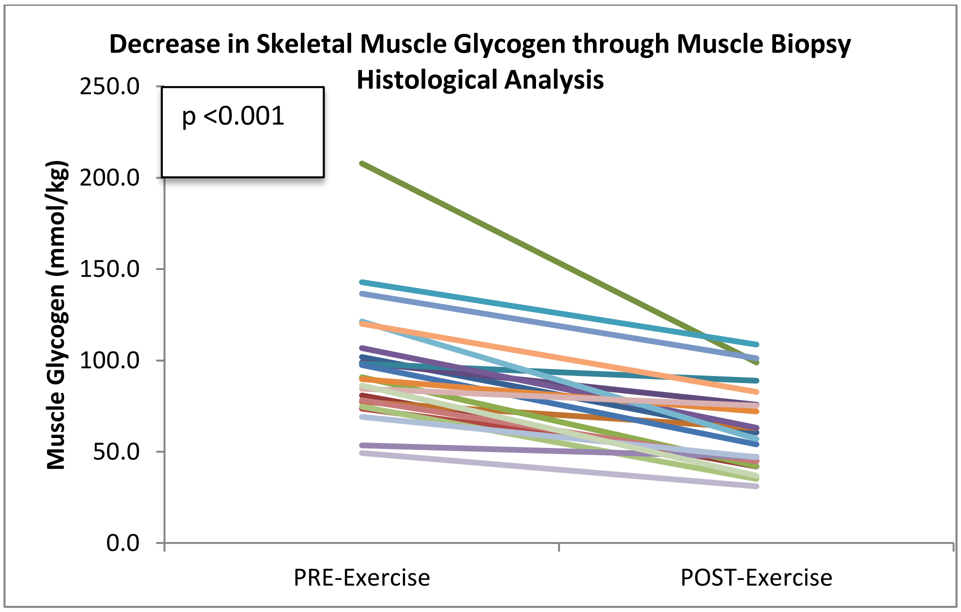

Fig-4

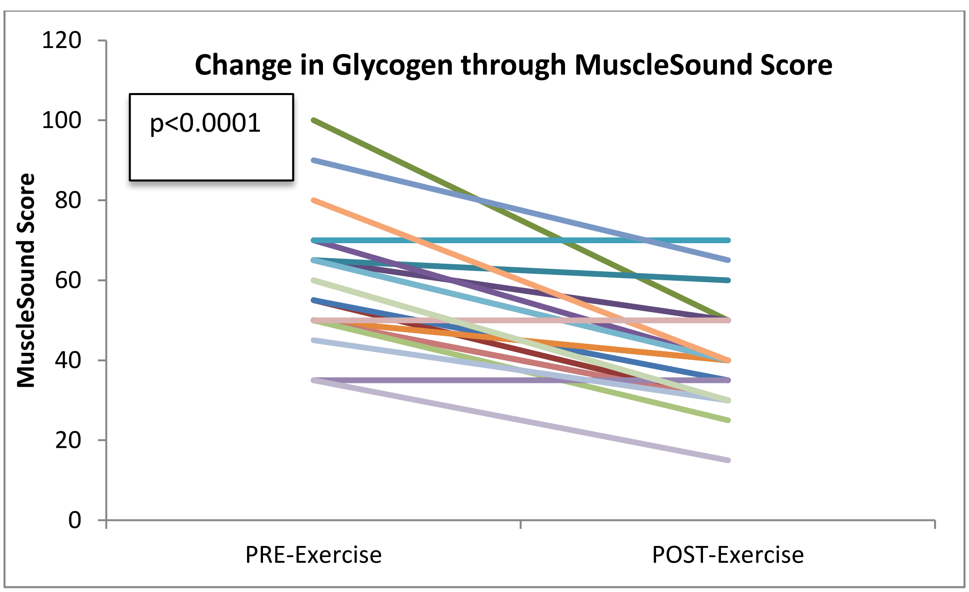

Fig-5

Correlations between rectus femoris muscle and vastus lateralis muscle through MuscleSound® technology were r=0.93, p<0.0001 pre-exercise and r=0.91, p<0.0001 post-exercise (Fig 6 and Fig 7)

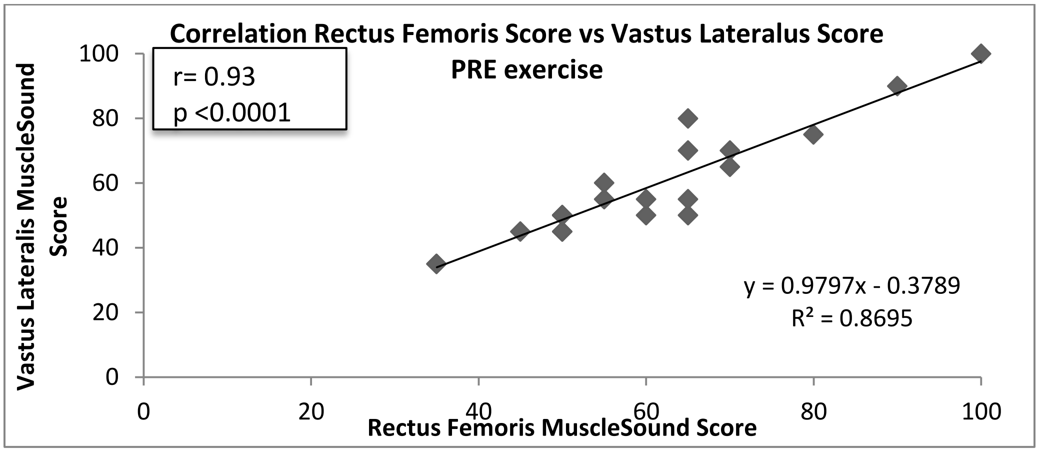

Fig-6

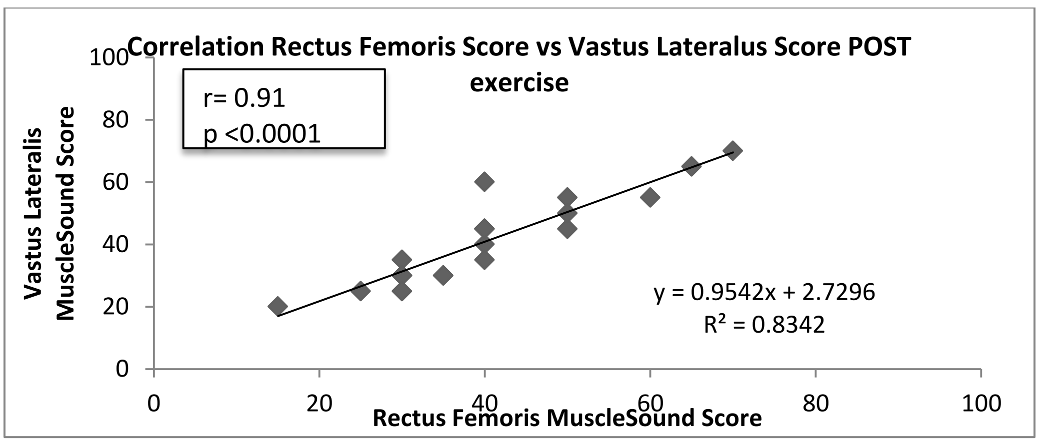

Fig-7

Finally, the correlation between rectus femoris and vastus lateralis muscles for the change in glycogen from pre- and post-exercise through MuscleSound® technology was r=0.76, p<0.0001 (Fig-8).

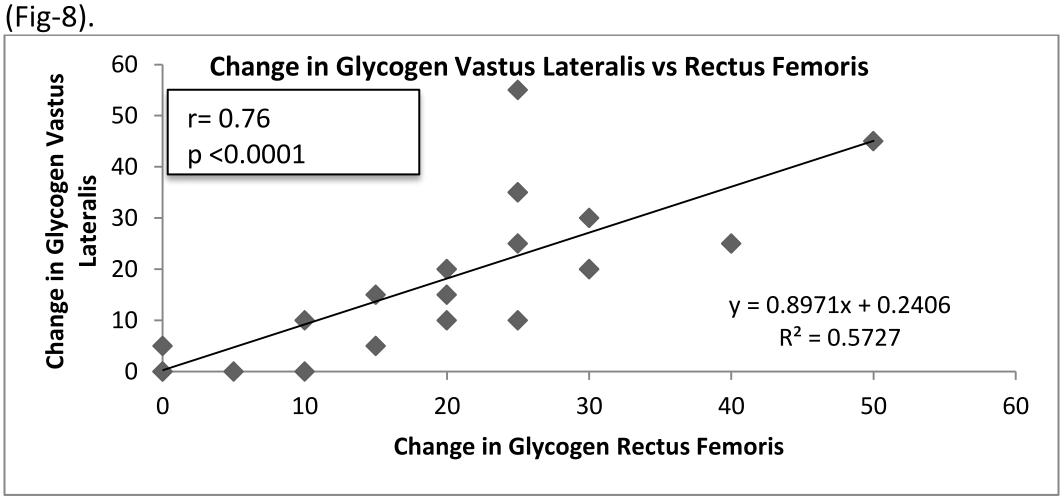

Fig-8

CONCLUSIONS: Pre- and post-exercise ultrasound scans using MuscleSound® technology were highly correlated with histochemical glycogen assessment through muscle biopsy. Changes in glycogen content from pre- and post-exercise were also highly correlated between MuscleSound® technology and muscle biopsy histochemical analysis. These results show that the use of high-frequency ultrasound through MuscleSound® technology is an accurate and reliable method to measure skeletal muscle glycogen in a practical, rapid and non-invasive way.

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