Validation of the MuscleSound® Ultrasound Device for Quantifying Change in Skeletal Muscle Glycogen Content

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Background MuscleSound® utilizes portable, diagnostic high-frequency ultrasound technology and cloud-based software to non-invasively measure change in muscle glycogen content. This methodology is based upon measurement of the water content associated with glycogen in the muscle. When a muscle is full of glycogen, the ultrasound image is hypoechoic (dark), and with glycogen and water loss, the image is hyperchoic (brighter). The MuscleSound® software quantifies change in muscle glycogen content using image processing and analysis through segmentation of the region of interest and measurement of the mean signal intensities.

Purpose Validation of the MuscleSound® device using direct measurement of glycogen content from skeletal muscle biopsies has not yet been conducted. The purpose of this study was to compare estimation of change in muscle glycogen content from the MuscleSound® device with direct quantification from pre- and post-exercise muscle biopsies taken from cyclists participating in a 75-km cycling time trial.

Methods Well-trained cyclists (N=20, age 38.4±6.0 y, 351±57.6 $watts_{max}$) participated in a 75-km cycling time trial on their own bicycles on CompuTrainer Pro Model 8001 trainers (RacerMate, Seattle, WA). Heart rate and rating of perceived exertion (RPE) were recorded every 30 minutes, and workload was continuously monitored using the CompuTrainer MultiRider software system (version 3.0). Oxygen consumption and ventilation were measured using the Cosmed Quark CPET metabolic cart (Rome, Italy) after 16 km and 55 km cycling. Subjects were allowed to ingest water ad libitum during the 75-km cycling time trial. Blood samples were taken pre- and post-exercise and analyzed for cortisol, myoglobin, glucose, and lactate. Muscle biopsy samples and MuscleSound® measurements were taken pre- and post-exercise. Specific locations on the vastus lateralis and rectus femoris were marked followed by three MuscleSound® measurements at each site conducted by a trained technician using a 12 MHz linear transducer and a standard diagnostic high resolution GE LOGIQ-e ultrasound machine (GE Healthcare, Milwaukee, WI). MuscleSound® images were pre-processed to isolate the muscle fibers under analysis (center crop within muscle section 25 mm from the top of the muscle), with the mean pixel intensity of the muscle averaged from the three scans and scaled (0 to 100 scale) to create the glycogen score. Pre- and post-exercise muscle biopsy samples were acquired at the vastus lateralis location (2 cm apart) using the percutaneous needle biopsy procedure modified to include suction. Muscle glycogen quantification was determined by a coupled enzyme assay which produces a colorimetric (570 nm)/fluorometric (λ_{ex} = 535/ λ_{ex} = 587 nm) product, proportional to the glycogen present (Sigma-Aldrich, St. Louis, MO; glycogen assay kit MAK016).

Results The 20 cyclists completed the 75-km cycling time trial in 168±26.0 minutes. Table 1 indicates that oxygen consumption averaged 69.6±10.3 % VO_{2max} at a power output of 193±57.8 watts (54.2±9.6 % $watts_{max}$) and heart rate of 160±11.5 bpm (89.4±5.9 % maximal heart rate). Serum cortisol increased 165%, serum myoglobin 654%, and blood lactate increased 1.05 mmol/L. These measures support that the cyclists exercised at high intensity which requires high utilization of muscle glycogen.

Table 1 Performance Variable (average, 75 km cycling time trial)	**Mean±SD**
VO_2 ($ml \cdot kg^{-1} min^{-1}$)	33.2±6.4
VO_2 (%VO_{2max})	69.6±10.3
Watts	193±57.8
% $Watts_{max}$	54.2±9.6
HR (beats/min)	160±11.5
%HR_{max}	89.4±5.9
Ventilation (L/min)	74.0±16.7
RPE	14.7±1.6
Blood values	
Serum cortisol (μg/dl)	
Pre-Exercise	10.7±4.3
Post-Exercise	28.4±10.5
Serum myoglobin (ng/mL)	
Pre-Exercise	32.1±14.7
Post-Exercise	242±216
Blood lactate (mmol/L)	
Pre-Exercise	0.97±0.3
Post-Exercise	2.02±1.0
Blood glucose (mg/dl)	
Pre-Exercise	70.0±14.1
Post-Exercise	76.6±13.5
Body Weight (lb)	
Pre-Exercise	184.8±15.5
Post-Exercise	181.2±16.0

Muscle glycogen decreased 77.2±17.4% (Figure 1), with an absolute change of 71.4±23.1 mmol glycogen per kilogram of muscle. The absolute change in muscle glycogen varied substantially between subjects (32 to 110 mmol/kg), as shown in Figure 2.

Figure 1

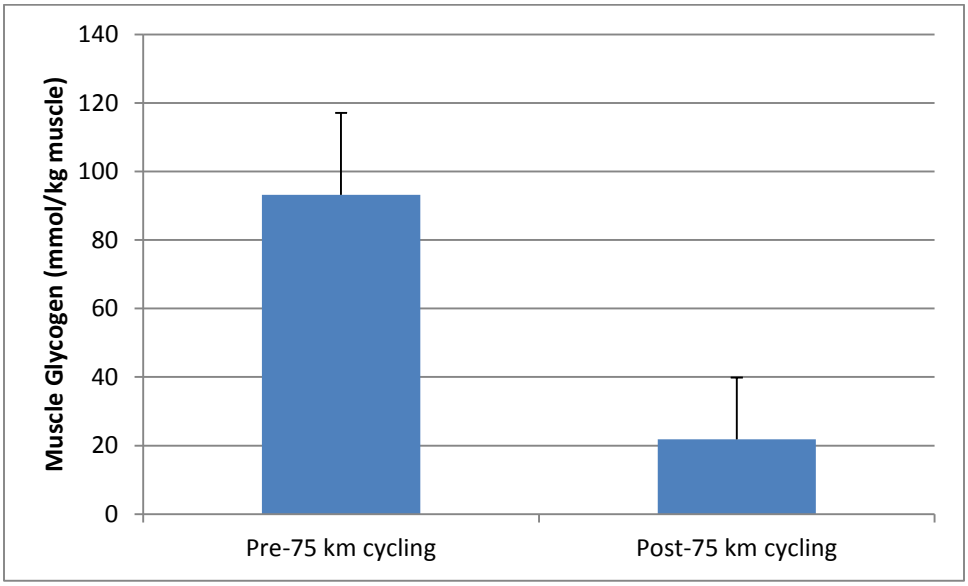
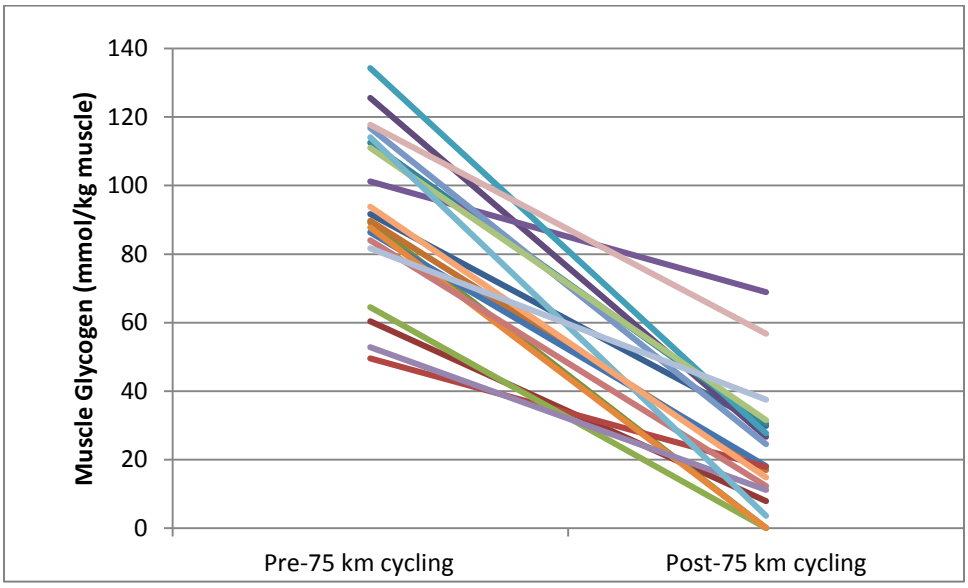


Figure 2



The MuscleSound® change score at the vastus lateralis site correlated highly with change in vastus lateralis muscle glycogen content (R=0.92, P<0.001) (Figure 3). The MuscleSound® change score at the rectus femoris site also correlated highly with change in vastus lateralis muscle glycogen content (R=0.87, P<0.001) (Figure 4). Correlations of vastus lateralis and rectus femoris MuscleSound® scores with vastus lateralis glycogen content measurements ranged from R=0.88 to 0.92 for pre-exercise and post-exercise time points. Figure 5 summarizes correlations for vastus lateralis MuscleSound® scores and muscle glycogen content for pre-exercise (A) and post-exercise (B) time points.

Figure 3

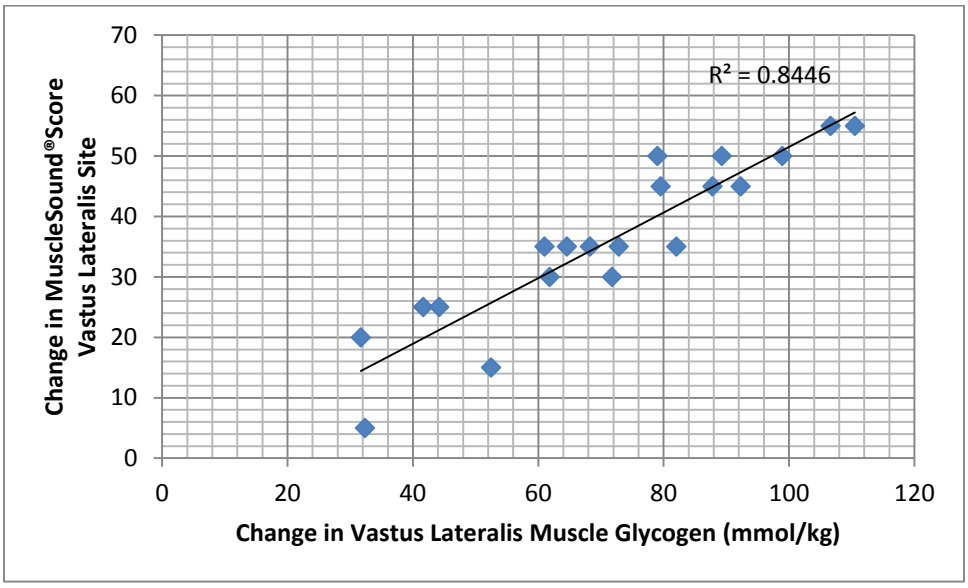


Figure 4

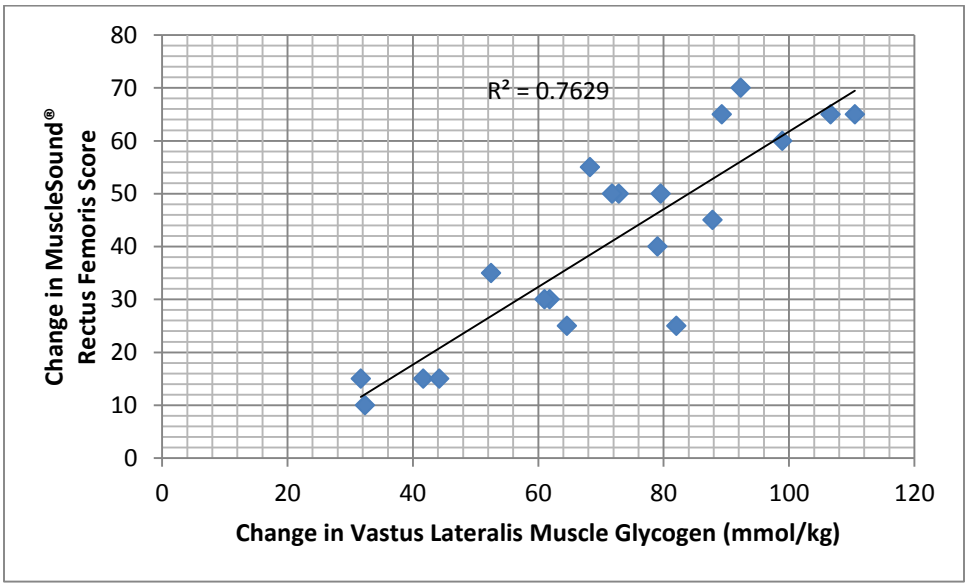
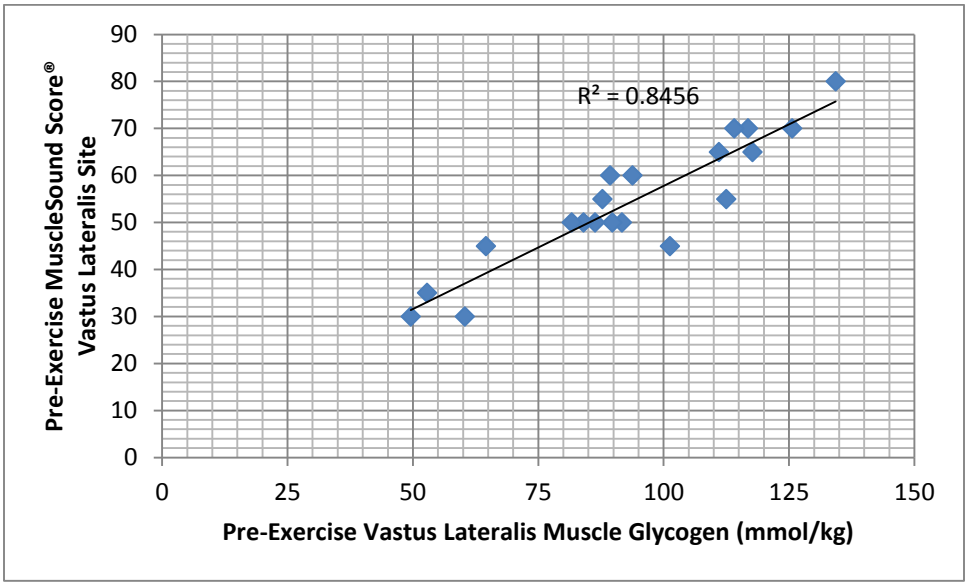
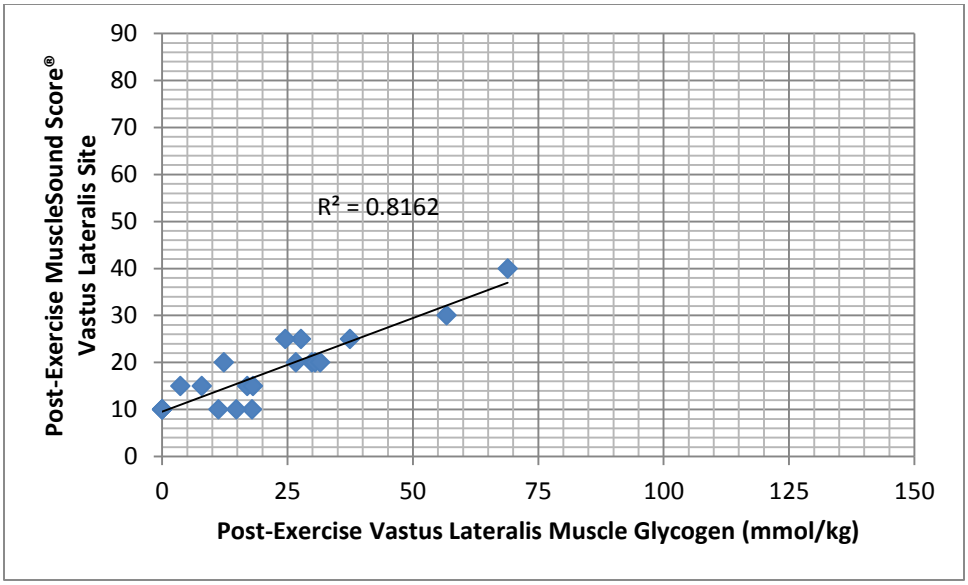


Figure 5A&B





Conclusions Twenty well-trained male cyclists engaged in a 75-km cycling time trial and experienced an average decrease of approximately three-fourths of glycogen content in the vastus lateralis, as determined with pre- and post-exercise skeletal muscle biopsies. The absolute decrease in muscle glycogen content varied widely between subjects (32 to 110 mmol/kg). MuscleSound® change scores acquired from an average of three ultrasound scans at the vastus lateralis and rectus femoris sites correlated significantly with change in vastus lateralis muscle glycogen content (R=0.92 and 0.87, respectively, P<0.001). Pre- and post-exercise MuscleSound® scores were highly correlated with muscle glycogen measurements. These data support the use of the MuscleSound® system in accurately estimating quadriceps muscle glycogen content and exercise-induced decreases in muscle glycogen content.