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Ultrasound Does Not Detect Acute Changes in Glycogen in Vastus Lateralis of Man

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Abstract

Purpose: To examine the validity of ultrasound (via cloud based software that measures pixilation intensity according to a scale of 0-100) to non-invasively assess muscle glycogen in human skeletal muscle.

Methods: In Study 1, 14 professional male rugby league players competed in an 80-minute competitive rugby league game. In Study 2 (in a randomized repeated measures design), 16 recreationally active males completed an exhaustive cycling protocol to deplete muscle glycogen followed by 36 hours of HIGH or LOW carbohydrate intake (8 v 3 $g.kg^{-1}$ body mass). In both studies, muscle biopsies and ultrasound scans were obtained from the vastus lateralis (at 50% of the muscle length) before and after match play in Study 1 and at 36 h after glycogen depletion in Study 2. **Results:** Despite match play reducing ($P< 0.01$) muscle glycogen concentration (Pre-game: 443 ± 65; Post-game: 271 ± 94 $mmol.kg^{-1}$ dw, respectively) in Study 1, there were no significant changes ($P=0.4$) in ultrasound scores (Pre-game: 47 ± 6, Post-game: 49 ± 7). In Study 2, muscle glycogen concentration was significantly different ($P < 0.01$) between HIGH (531 ± 129 $mmol.kg^{-1}$ dw) and LOW (252 ± 64 $mmol.kg^{-1}$ dw) yet there was no difference ($P = 0.9$) in corresponding ultrasound scores (HIGH: 56 ± 7, LOW: 54 ± 6). In both studies, no significant correlations ($P>0.05$) were present between changes in muscle glycogen concentration and changes in ultrasound scores. **Conclusion:** Data demonstrate that ultrasound (as based on measures of pixilation intensity) is not valid to measure muscle glycogen status within the physiological range (i.e. 200-500 $mmol.kg^{-1}$ dw) that is applicable to exercise-induced muscle glycogen utilization and post-exercise muscle glycogen re-synthesis.

Key Words: carbohydrate, vastus lateralis, glycogen depletion

Introduction

It is well established that high carbohydrate (CHO) availability improves both endurance and high-intensity intermittent exercise performance (1). As such, the practice of CHO loading to super-compensate muscle glycogen stores in the day(s) immediately prior to competition remains the cornerstone of sport nutrition practices (2). The practice of ensuring high CHO availability before and during exercise emerged from the seminal studies of Bergstrom and colleagues (3-6) demonstrating that high muscle glycogen availability (as achieved via consumption of a high CHO diet) improved exercise capacity. The emergence of the muscle biopsy technique therefore provided the platform for the following decades of research examining the regulation of glycogen storage and metabolism during exercise. Nonetheless, the technique of removing muscle tissue has associated risks due to potentially endangering neurovascular structures, thus limiting its use to muscles with low vascularity such as the vastus lateralis. Complications such as local skin infections, arterial bleed, ecchymosis or hematoma, prolonged pain (i.e. > three days) and a small area of local numbness distal to the biopsy site have also been reported (7). Whilst the use of the microbiopsy technique is considered less invasive and painful than traditional Bergstrom needles (8) the invasive nature of muscle biopsy sampling may still induce complications that limit its application to elite athletic populations.

The recent emergence of an ultrasound (US) technique to non-invasively assess muscle glycogen (9, 10) therefore holds much promise for those practitioners working at the coalface of elite sport. This technique is now available through commercially available cloud based software (9, 10) and is based on the premise that glycogen storage can be assessed through its association with water. In this way, glycogen depletion within the muscle (and hence loss of intracellular water) can manifest as changes in pixilation intensity upon US imaging. In the initial study in

this area, Hill and Milan (9) observed significant correlations between changes in pixel intensity scores (a score ranging from 0-100) of rectus femoris ultrasound scans and changes in muscle glycogen concentration (as directly assessed from biopsies obtained from the rectus femoris) before and after 90-minutes of laboratory based steady-state cycling exercise performed at an intensity below lactate threshold. Despite similar correlations and associations between glycogen utilisation within the vastus lateralis muscle during cycling (a laboratory-based 75-km time trial equating to 2-3 hours of exercise) and the ultrasound scoring system (10), there have been no further assessments of validity of this scoring system in either laboratory or field-based settings. Indeed, it is important to assess the validity of such software to assess glycogen utilization in both laboratory-based and field-based high-intensity activity, given the potential differences in mechanical loads and fluid shifts that are induced by the contrasting contractile stimuli. In an attempt to eliminate the potential confounding effects of acute changes in fluid shifts associated with differing exercise stimuli, an alternative approach to evaluate the validity of ultrasound is to also assess its ability to detect changes in muscle glycogen re-synthesis across a time-scale in which acute changes in fluid shifts have been restored to pre-exercise values. If this approach proves valid, then the ultrasound-based technique is potentially assessing "true" fluctuations in muscle glycogen as opposed to artefacts associated with acute changes in intracellular and extracellular fluid changes.

Accordingly, we aimed to assess the validity of ultrasound-based software to non-invasively assess muscle glycogen in the vastus lateralis muscle of man. To this end, we conducted two studies to assess the validity of ultrasound to detect muscle glycogen within the physiological range (i.e. 200-500 $mmol.kg^{-1}$ dw) that is applicable to exercise-induced muscle glycogen utilization (Study 1) and post-exercise muscle glycogen re-synthesis (Study 2).

Methods

Study 1

Subjects. Fourteen professional rugby league (RL) players (mean ± SD, age 18.2 ± 0.8 years, body mass 88.4 ± 12.4kg, height 180 ± 8.1cm) from a Super League rugby club academy in the United Kingdom provided written informed consent to participate in this study. All players competed on the same team and completed a calendared 80-min competitive RL match against a team from the same league. None of the subjects had a history of neurological disease or skeletal muscle abnormality and none were under pharmacological intervention during the course of the study. The study was approved by the Research Ethics Committee of Liverpool John Moores University.

Study Design. Muscle biopsy samples (vastus lateralis, VL) and ultrasound scans were obtained at a site corresponding to 50% of the length of the VL muscle at approximately 60 minutes prior to kick off and within 40 minutes upon completion of match play. During the time between the pre- and post-match muscle biopsy, players were permitted to consume water ad libitum though no form CHO (i.e. fluids, gels or solids) was consumed. To locate 50% of the VL, anatomical locations were identified through utilizing the ultrasound probe to identify distal and proximal locations of the VL alongside the medial and lateral portions of the muscle belly. This study was completed in February 2015.

Ultrasound scans. Prior to each biopsy sample, an ultrasound measurement was taken in a supine position at ~50% of the length, and 50% of the width, of the VL using a 12 MHz linear

transducer and a standard diagnostic high resolution Terason ultrasound machine (Terason t300, Burlington, USA). Ultrasound gel was applied to the marked site and the probe was placed on the site in the sagittal plane. The muscle was relaxed during imaging and minimal pressure was applied to the probe to ensure it produced an image with an superficial aponeurosis (connective tissue fascia immediately beneath the layer of subcutaneous fat) parallel with the flat skin surface. Using standardized (pre-determined) brightness, contrast and image depth settings, an image was taken of the muscle and uploaded to commercially available cloud based software (MuscleSound, LCC, Denver, CO). The software automatically examined the pixel intensity of a region of interest, identified as the area beneath the superficial aponeurosis, and provided an intensity ("glycogen") score (on a scale from 0-100) within seconds (muscles with high glycogen concentrations apparently display darker pixel intensities, with muscle containing lower glycogen concentrations displaying brighter pixel intensities). The decision to scan at 50% of the length and 50% of the width of the VL muscle was based on a preliminary investigation whereby 100 subjects (n=78 males: age, 24.5 ± 6.3 years; body mass, 82.1 ± 8.3 kg; height, 183.5 ± 12.3 cm; n=22 females: age, 20.3 ± 2.1 years; body mass, 65.4 ± 7.4 kg; height, 141.9 ± 7.1 cm) completed a test-retest reliability study, where two scans were obtained from the same limb in the resting state (60 minutes apart during which the subjects remained supine). In this initial reliability study, we obtained scans at locations corresponding to 25, 50 and 75% of the VL and rectus femoris muscle as well as 50% of the vastus medialis (see Table 1). Data demonstrate that 50% of the VL muscle was the anatomical site that produced the most reproducible ultrasound scores, as collectively evidenced from a relatively low coefficient of variation, high intra-class correlation (with narrow 95% confidence intervals) and close ratio limits of agreement (11). Estimates of reliability were obtained in December 2014.

Muscle Biopsies. Muscle samples were obtained from the same limb that was scanned (from the lateral portion of the vastus lateralis at 50% of the muscle length) using a Bard Montopy Disposable Core Biopsy Instrument (12 gauge x 10cm length, Bard Biopsy Systems, Tempe, AZ, USA). Samples (~20 mg) were obtained under local anesthesia (~2-2.5 ml of 0.5% Marcaine), were subsequently blotted on filter paper upon collection and then frozen in liquid nitrogen for later analysis. Pre- and post-game muscle biopsies and ultrasound scans were obtained from the same limb and from separate location points (approximately ~1-2 cm proximal) and the ultrasound scan was always performed prior to the biopsy. All muscle samples were immediately snap frozen in liquid nitrogen and stored at -80^{O}C for later analysis. Muscle glycogen concentration was determined through acid hydrolysis. Approximately 3-5 mg of freeze-dried sample was powdered, dissected free of all visible non-muscle tissue and subsequently hydrolyzed by incubation in 500 µl of 1M HCI for 3-4 h at 100^{o}C. After cooling to room temperature, samples were neutralized by the addition of 250 µl 0.12 mol 1^{-1} Tris/2.1 mol 1^{-1} KOH saturated with KC1. Following centrifugation, 150 µl of the supernatant was analyzed in duplicate for glucose concentration according to hexokinase method using a commercially available kit (GLUC-HK, Randox Laboratories, Antrim, UK). Glycogen concentration was expressed as mmol kg $^{-1}$ dry weight (dw) and intra-assay coefficients of variation were <5%.

Statistical analysis. Statistical analysis was conducted using the Statistical Package for Social Sciences software program (SPSS; version 17, IBM, Armonk, NY). A student's paired t-test was performed to assess changes in muscle glycogen concentration and ultrasound scores as a result of match play. Pearson correlations were performed between the change in muscle glycogen

concentration and change in ultrasound score. All data in text, figures, and tables are presented as means ± SD with *P* values <0.05 indicating statistical significance.

Study 2

Subjects. Sixteen recreationally active males provided written informed consent to participate in this study (VO_{2max}: 49.9 ± 7.5 ml.kg^{-1} min^{-1}; age, 21 ± 1.8 years; body mass, 84 ± 8.3 kg; height, 176 ± 8.4 cm). None of the subjects had a history of neurological disease or skeletal muscle abnormality and none were under pharmacological intervention during the course of the study. The study was approved by the Research Ethics Committee of Liverpool John Moores University.

Study Design. In a randomized, repeated measures, cross over design and after an initial assessment of maximal oxygen uptake, subjects completed two 36 h experimental protocols at 7-9 day intervals in conditions of high (HIGH) or low (LOW) carbohydrate (CHO) availability. Subjects reported to the laboratory on the evening of Day 1 in order to perform a glycogen-depleting bout of intermittent exhaustive cycling. At the cessation of exercise, subjects were provided with either high or low CHO foods (88 g CHO v 2 g CHO, respectively) to be consumed immediately. During the LOW trial, subjects also returned to the laboratory on the following morning to perform an additional 45 min bout of steady state cycling in an attempt to induce further muscle glycogen depletion. Upon cessation of exercise, subjects then consumed a standardized diet of low CHO (LOW: 2g.kg^{-1} CHO, 3.5 g.kg^{-1} Protein, 4 g.kg^{-1} Fat and 6 L fluid) for the next 24 h whereas subjects in the HIGH trial did not perform any morning exercise but consumed an isocaloric high CHO diet (HIGH: 8g.kg^{-1} CHO, 2g.kg^{-1} Protein, 0.6g.kg^{-1} Fat and 6

L fluid). In both trials, subjects returned to the laboratory on the morning of Day 3 (0800 h) in a fasted state to undergo both ultrasound scanning and collection of a muscle biopsy (from the vastus lateralis). In this way, each subject completed an exercise-dietary intervention that induced a state of high or low muscle glycogen in a fully rested and hydrated state. Muscle biopsy samples and ultrasound scores were obtained and analysed, as described previously in Study 1. All subjects completed their second trial within 7-9 days of completing their first. This study was completed in February-August 2015. An overview of the experimental design is presented in Figure 1.

Assessment of Peak Oxygen Uptake and Peak Power Output. Peak oxygen uptake and peak power output (PPO) were determined using an incremental cycle test to exhaustion on a self-regulated cycle ergometer (Lode Excalibur, Netherlands). The test commenced with a 5 min warm up at 100 W at a cadence corresponding to 70 revs min^{-1}. Following the warm up phase, power output was increased by 30 W every 120 s until volitional exhaustion. Breath-by-breath measurements were collected throughout the exercise protocol using a CPX Ultima series online gas analysis system (Medgraphics, MN, US). Peak power output was defined as the highest power output that could be maintained for 60 s. Saddle and handle bar position was recorded and replicated for the exercise trials and matched to that of the preliminary testing.

Glycogen Depletion Protocol. On the evening of Day 1, subjects arrived at the laboratory at 1700 h and underwent an ultrasound scan at 50 % of the VL alongside measurement of body mass and urine osmolality. After a 5 min warm up at 100 W, subjects cycled for 2 min at 90% PPO, followed immediately by a 2-min recovery period at 50% PPO. They repeated this work to

rest ratio until they could no longer complete 2 min cycling at 90% PPO, determined as an inability to maintain a cadence of 60 revs min. At this point, exercise intensity was lowered to 80% PPO and when participants could no longer cycle for two minutes at 60% PPO the exercise protocol was terminated (12). The intermittent nature of the exercise protocol was designed in order to deplete both type 1 and 2 muscle fibres of glycogen during the exercise. Heart rate and RPE were recorded at the end of each two-minute exercise and recovery phase. The total exercise duration and time spent exercising at each specific power output (i.e. repetitions completed) were recorded and replicated for the second trial. Water intake was consumed ad libitum during the first trial with the pattern of intake replicated in the subsequent trial. Before and after completion of the evening depletion exercise protocol, ultrasound scans were also obtained as described in Study 1.

Morning Glycogen Depletion Protocol. In the LOW condition, subjects returned to the laboratory on the following morning at 0800 h in a fasted state. Subjects commenced a bout of steady state cycling at 60% PPO for 45 minutes with heart rate and RPE being measured every five minutes. Ultrasound scans were also performed before and after this exercise protocol as described in Study 1.

Muscle Water Content. Muscle samples were weighed prior to entry into the dry freezer and on exit from the freeze drier after a 48 h period. Muscle water content was calculated using the following equation (wet weight – dry weight)/(dry weight/100) from Costill et al (13).

Statistical Analysis. Statistical analysis was conducted using the Statistical Package for Social Sciences software program (SPSS; version 17, IBM, Armonk, NY). Differences between muscle glycogen concentration and ultrasound score between the HIGH and LOW trials were analysed using a student's t-test for paired samples. Additionally, differences between physiological responses to exercise during the glycogen depletion protocol were analysed using two-way repeated measures General Linear Model, where the within factor was time (Pre-exercise vs post-exercise) and condition (HIGH vs. LOW). Pearson correlations examined relationships between the change in muscle glycogen concentration and change in ultrasound score. All data in text, figures, and tables are presented as means ± SD with *P* values <0.05 indicating statistical significance.

Results

Study 1

Changes in muscle glycogen concentration have been published previously (14) and are presented here as pooled subject data. Despite significant differences in muscle glycogen concentration ($P < 0.001$) between pre- and post-game (443 ± 65 v 271 ± 94 mmol.kg^{-1} dw, respectively), there was no corresponding decrease (P=0.4) in the ultrasound score (47 ± 6 v 49 ± 7, see Figure 2A). Additionally, no significant correlation was evident ($R^2 = 0.03$, $P = 0.55$) between the change in muscle glycogen concentration and change in ultrasound score as a result of match play (see Figure 2B).

Study 2

Physiological Responses and Changes in Muscle Ultrasound Score in Response to Evening and Morning Glycogen Depletion Protocols. Subjects cycled for 102 ± 8 min during the evening interval cycling protocol completed on Day 1. There was no significant differences (P = 0.7) in average heart rate between the HIGH (168 ± 8 $b.min^{-1}$) and LOW (170 ± 10 $b.min^{-1}$) trials. Similarly, RPE was also not significantly different (P = 0.9) between trials (HIGH: 14.8 ± 1.3; LOW: 15 ± 0.5). The glycogen depletion protocol induced a significant decrease (P < 0.001) in ultrasound score in both the HIGH (Pre: 67 ± 7; Post: 51 ± 10) and LOW trial (Pre: 58 ± 11; Post 48 ± 9) trials (Figure 3A), though no difference (P = 0.42) was observed between conditions. When subjects completed the LOW trial, they returned to the laboratory on the following morning to complete 45 minutes of exercise at 60% PPO of in an attempt to further induce muscle glycogen depletion. Additionally, this exercise protocol also induced a significant decrease (P < 0.001) in ultrasound score (Pre: 52 ± 7; Post: 45 ± 8) (Figure 3B).

Comparison of Resting Muscle Glycogen Concentration and Ultrasound Score. Following completion of glycogen depletion protocols, subjects then consumed the standardized dietary protocols of high or low CHO intake in an attempt to induce HIGH and LOW muscle glycogen concentrations. Subjects then returned to the laboratory on the morning of Day 3 to have a resting muscle biopsy sampled from the vastus lateralis. A comparison of muscle glycogen concentration, muscle water content and ultrasound scores are shown in Figure 4 A-C, respectively. Despite significant differences (P < 0.001) in muscle glycogen concentration between HIGH (531 ± 129 $mmol.kg^{-1}$ dw) and LOW (252 ± 64 $mmol.kg^{-1}$ dw), there was no significant difference in muscle water content (HIGH: 265 ± 50 mL/100 g dry mass, LOW: 250

± 50 mL/100 g dry mass; P = 0.3) or ultrasound score (HIGH: 56 ± 7, LOW: 54 ± 6; P = 0.9). There was also no significant difference (all P<0.05) in wet weight or dry weight of the muscle samples for each condition (HIGH wet weight: 24.3 ± 3.3 mg, LOW wet weight: 25.7 ± 4.0 mg; HIGH dry weight: 6.9 ± 1.7 mg, LOW dry weight 7.8 ± 2.2 mg). Additionally, there was no significant correlation between the changes in ultrasound score and muscle glycogen concentration (R^2 = 0.16, P = 0.11) or muscle water content (R^2 = 0.03, P = 0.55) (Figure 5A and B, respectively).

Discussion

We aimed to assess the validity of ultrasound to non-invasively assess muscle glycogen in the vastus lateralis muscle of man. Although the competitive rugby league match reduced muscle glycogen concentration in the vastus lateralis muscle by 40-50% (as evidenced through biochemical assessment of biopsies), we observed no significant reductions in ultrasound pixel intensity score (Study 1). In Study 2, we also observed that ultrasound scores did not differ between scans obtained after 36 h of a high or low CHO intake (as consumed after glycogen depleting exercise) despite differences in absolute muscle glycogen concentration. When taken together, our data demonstrate that ultrasound (as based on measures of pixilation intensity) is not valid to assess muscle glycogen status within the physiological range (i.e. 200-500 $mmol.kg^{-1}$ dw) that is applicable to exercise-induced muscle glycogen utilization and post-exercise muscle glycogen re-synthesis.

Previous studies assessing the validity of ultrasound in determining glycogen usage have utilised cycling based exercise protocols and have observed significant correlations between the changes

in biochemical determination of muscle glycogen concentration and associated ultrasound-based scores (9, 10). In contrast, our data demonstrate no significant changes in ultrasound scores as a result of rugby league (RL) match play. It is difficult to explain the discrepancies between studies given that the absolute glycogen utilisation observed here (i.e. 200-250 $mmol.kg^{-1}$ dw) is also comparable to that studied previously where mean absolute utilisation of 150 (9) and 300 $mmol.kg^{-1}$ dw (10) were observed. On this basis, it is possible that differences in the nature of the contractile stimuli and absolute force production between the intermittent exercise intensities associated with RL match play versus steady-state cycling activities may subsequently affect the image obtained via ultrasound. The ultrasound scoring system methodology is based upon the measurement of water content associated with glycogen in the muscle. For example, when glycogen content is high, the ultrasound image is hypoechoic (dark) and with glycogen depletion and water loss, the image is hyperchoic (brighter). During prolonged cycling based models, in which the intensity is typically controlled and below lactate threshold (i.e. low force production), it is indeed possible that the muscle loses considerable fluid (15,16), which is detectable in the post-exercise recovery period, an effect that may be especially apparent in those situations in which plasma volume has been considerably reduced due to dehydration. In contrast, it is possible that the loading pattern of RL match play induces different responses in terms of fluid shifts. Support for this hypothesis is provided by the observation that in response to the high mechanical loads occurring with resistance exercise, muscle actually increases cross-sectional area due to movement in fluid from the vascular space into active muscle (17). Such observations, coupled with the environmental temperature ($8^{O}C$, $43^{O}F$) in which Study 1 was completed, may explain why we observed no decrease in pixilation intensity (ultrasound score) in the vastus lateralis muscle. Unfortunately, neither we nor previous researchers (9, 10)

controlled for or assessed any parameter of hydration status, total body water content or muscle volume before and after the chosen exercise protocol.

To eliminate the potential confounding effects of acute changes in fluid shifts associated with differing exercise stimuli, in Study 2 we assessed the validity of the ultrasound scoring system to assess muscle glycogen status during experimental conditions where acute changes in fluid shifts are likely restored to pre-exercise values. To this end, we utilised an experimental protocol consisting of a cycling based glycogen depleting exercise protocol followed by 36 h of a high or low CHO diet (that was fluid matched) in order to achieve high and low muscle glycogen concentrations. In agreement with the previous validation studies using cycling based protocols (9, 10), we also observed that acute cycling based exercise induced significant declines in ultrasound scores from the vastus lateralis muscle, as evident in both the evening glycogen depleting protocol (see Figure 3A) and morning steady state exercise protocol (see Figure 3B). Whilst we acknowledge the limitation of not obtaining muscle biopsies before and after these protocols, it is possible that we may have also observed positive correlations between changes in muscle glycogen concentration and ultrasound scores. On the basis of glycogen depleting protocols previously completed in our laboratory (12, 19), it is likely that subjects' post depletion muscle glycogen concentration would be <150 $mmol.kg^{-1}$ dw. Nonetheless, when considering that we observed no changes in ultrasound scores as a result of RL match play (Study 1), these data provide further support for the hypothesis that variations in the mechanical load and alterations in fluid shifts between exercise protocols can significantly affect the ultrasound image pixel intensity and ultrasound score obtained.

Having completed the glycogen depleting protocol, the subjects in Study 2 then ingested a high or low CHO diet for 36 h in order to induce distinct differences in muscle glycogen concentration. Accordingly, on the morning of day 3, subjects presented with muscle glycogen concentrations of 531 ± 129 mmol.kg^{-1} dw and 252 ± 64 mmol.kg^{-1} dw when completing the HIGH and LOW trials, respectively. On the basis of the subjects' training status (e.g. VO_{2max} of 49.9 ± 7.5 ml.kg^{-1} min^{-1}) and absolute CHO intake consumed (e.g. 8 g.kg^{-1}), these data agree well with the normative values of resting glycogen concentration presented in a recent meta-analysis (18). In relation to muscle water content, we observed no differences between the HIGH: (265 ± 58 mL/100 g dry mass) or LOW trials (250 ± 50 mL/100 g dry mass). Given that muscle water content has previously been shown to play a minor role in modulating the magnitude of muscle glycogen utilisation during cycling (15) and post-exercise muscle glycogen re-synthesis (20), such data further question the rationale that changes in tissue water content can be used to infer subtle but physiologically relevant changes in muscle glycogen concentration. In this way, our data clearly demonstrate that in rested conditions where muscle is matched for water content but contains marked differences in absolute glycogen concentration (>250 mmol.kg^{-1} dw), the ultrasound scoring system does not provide a valid assessment of muscle glycogen status.

In summary, our data demonstrate that the ultrasound software used to analyze pixel intensity is not valid to assess muscle glycogen status within the physiological range (i.e. 200-500 mmol.kg^{-1} dw) that is applicable to exercise-induced muscle glycogen utilization and post-exercise muscle glycogen re-synthesis. Such a conclusion is evidenced from observations arising from field sport activity and laboratory based cycling protocols, respectively. Despite reasonable test-retest

reproducibility (especially at 50% of the length of the vastus lateralis muscle), we therefore conclude that ultrasound is not a valid measurement tool to non-invasively assess muscle glycogen status of human skeletal muscle.

Acknowledgments

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Conflict of Interest

The authors declare no conflicts of interest. The results of the present study do not constitute endorsement by ACSM. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation

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TABLE 1 – Estimates of Ultrasound score test-retest reliability within and between muscles. CV, ICC (95% CI) and Ratio Limits of agreement (*/÷) for 25, 50 and 75% of the vastus lateralis (VL) and vastus medialis (VM) muscles and 50% of the rectus femoris (RF) muscle. N = 100.

FIGURE 1 – Overview of the experimental design for Study 2.

FIGURE 2 – (A) Mean and individual Ultrasound score (0-100) pre- and post- an 80-minute Rugby League game. (B) correlation between changes in Ultrasound score (0-100) and muscle glycogen concentration (mmol.kg^{-1} dw). Data obtained from Study 1.

FIGURE 3 – (A) Mean and individual Ultrasound score (0-100) pre- and post- the evening glycogen depletion protocol for both the HIGH and LOW trials. (B) Mean and individual Ultrasound score (0-100) pre- and post- the morning steady state protocol (representative from the LOW trial only). * denotes significant difference between pre- and post-exercise, $P<0.05$. Data obtained from Study 2.

FIGURE 4 – (A) Mean and individual muscle glycogen concentration (mmol.kg^{-1} dw), (B) Mean and individual muscle water content (mL/100 g dry mass) and (C) Mean and individual Ultrasound score (0-100) in the HIGH and LOW trials. * significant difference between HIGH and LOW trials, $P<0.05$. Data obtained from Study 2.

FIGURE 5 – (A) Correlation between changes in muscle glycogen concentration (mmol.kg^{-1} dw) and Ultrasound score (0-100) and (B) changes in muscle water content (mL/100 g dry mass) and change in Ultrasound score (0-100). Data obtained from Study 2.

Figure 1

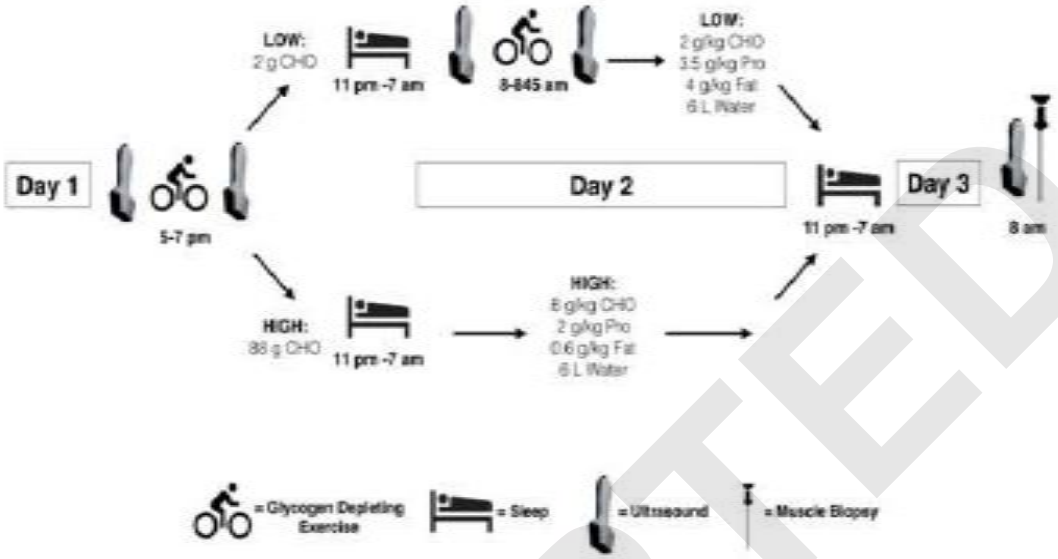

Figure 2

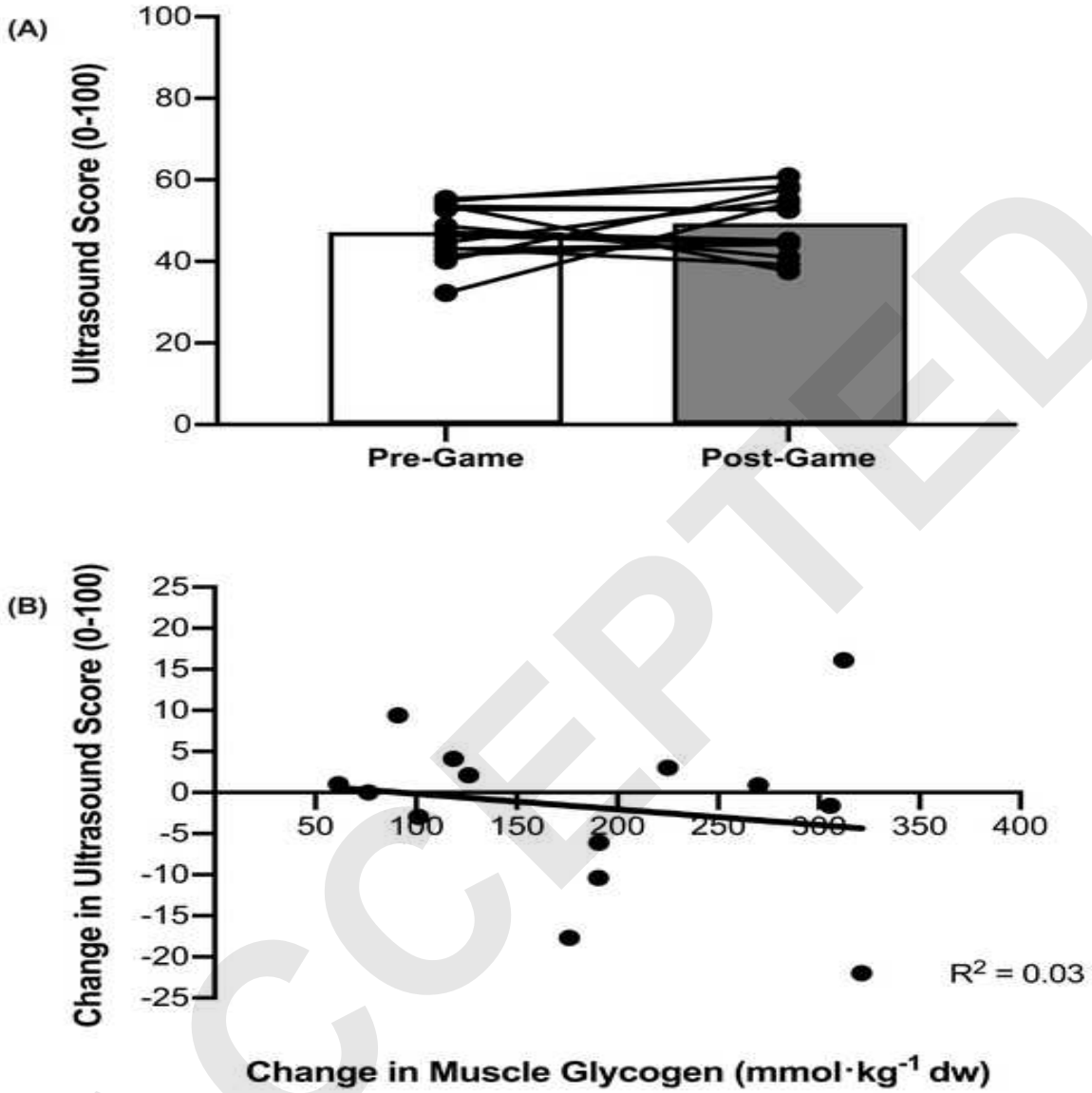

Figure 3

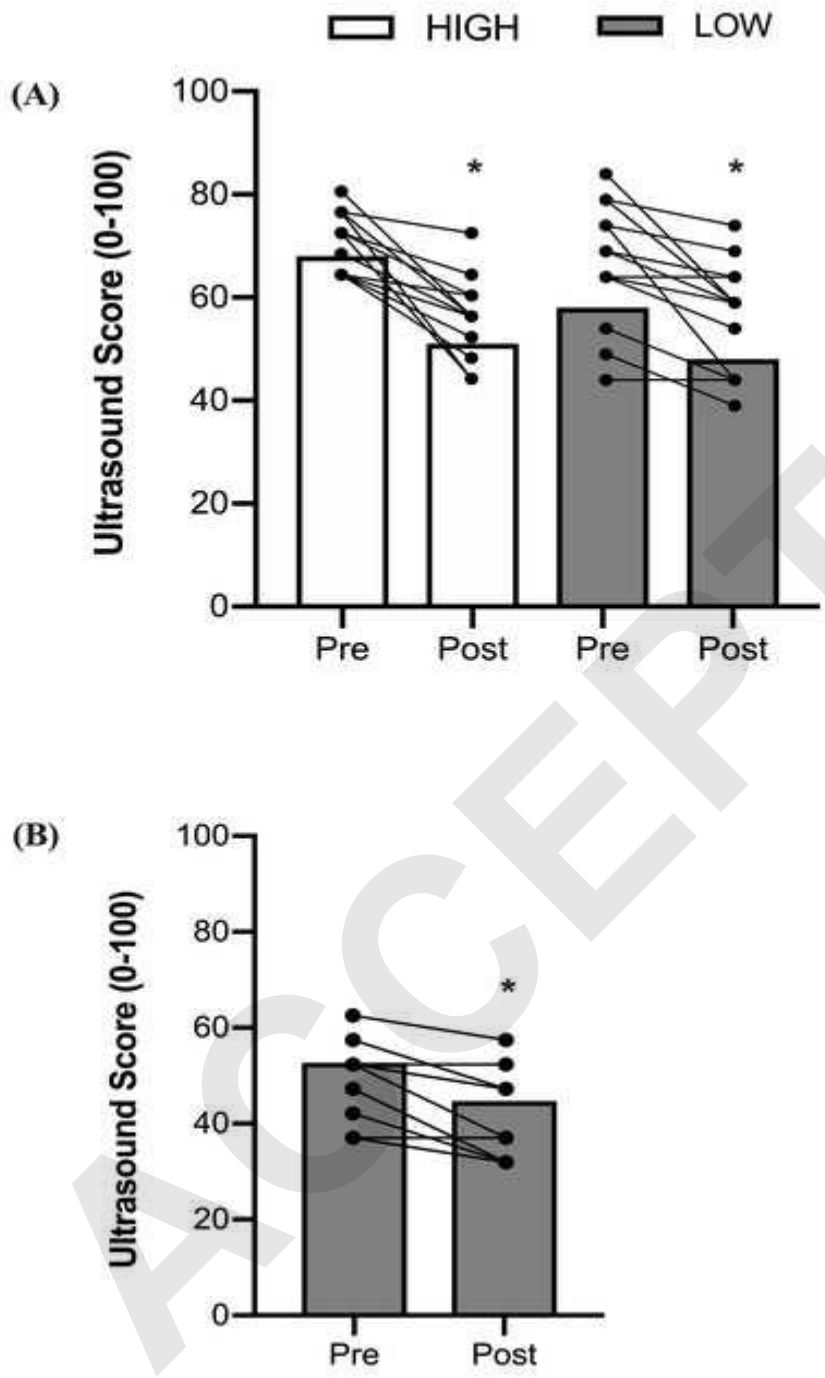

Figure 4

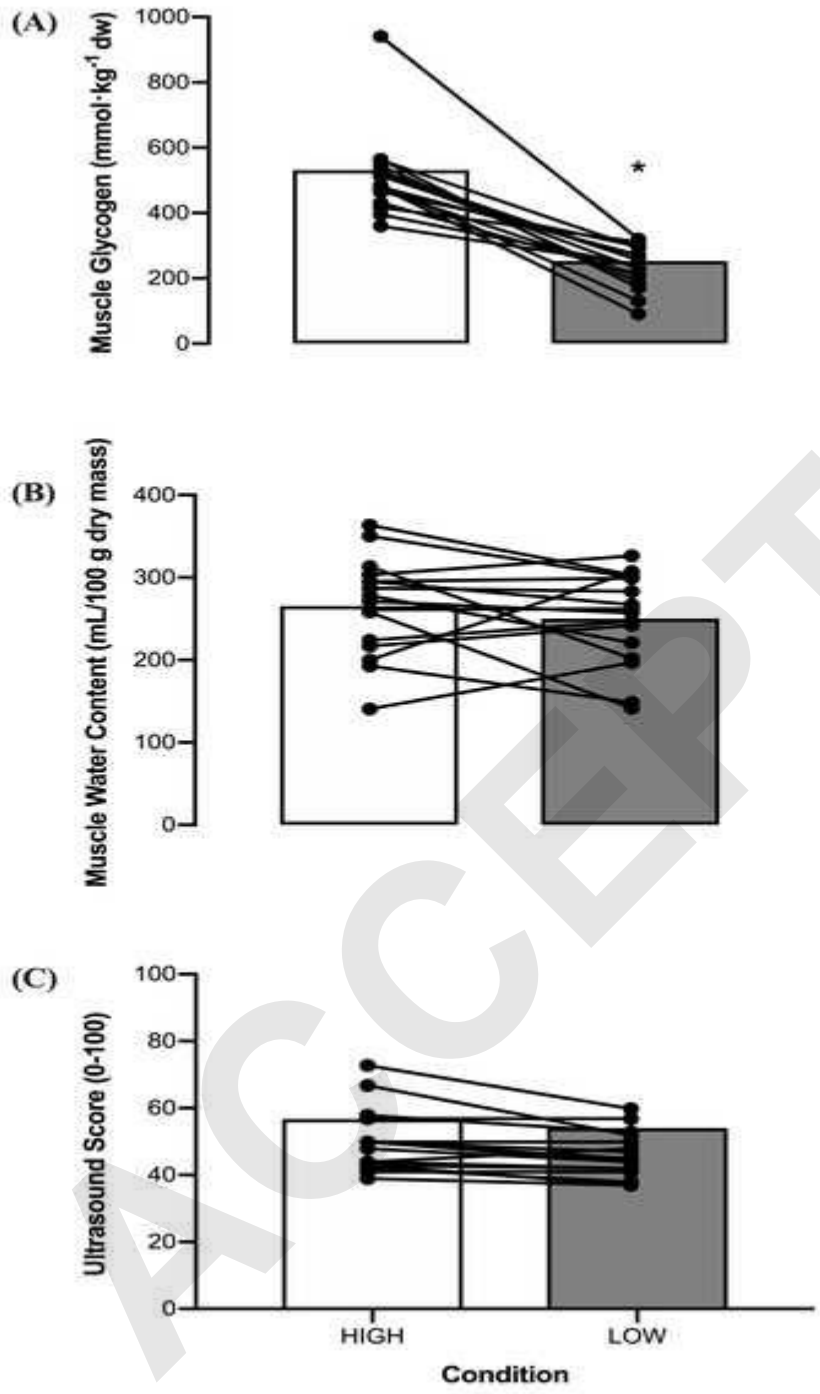

Figure 5

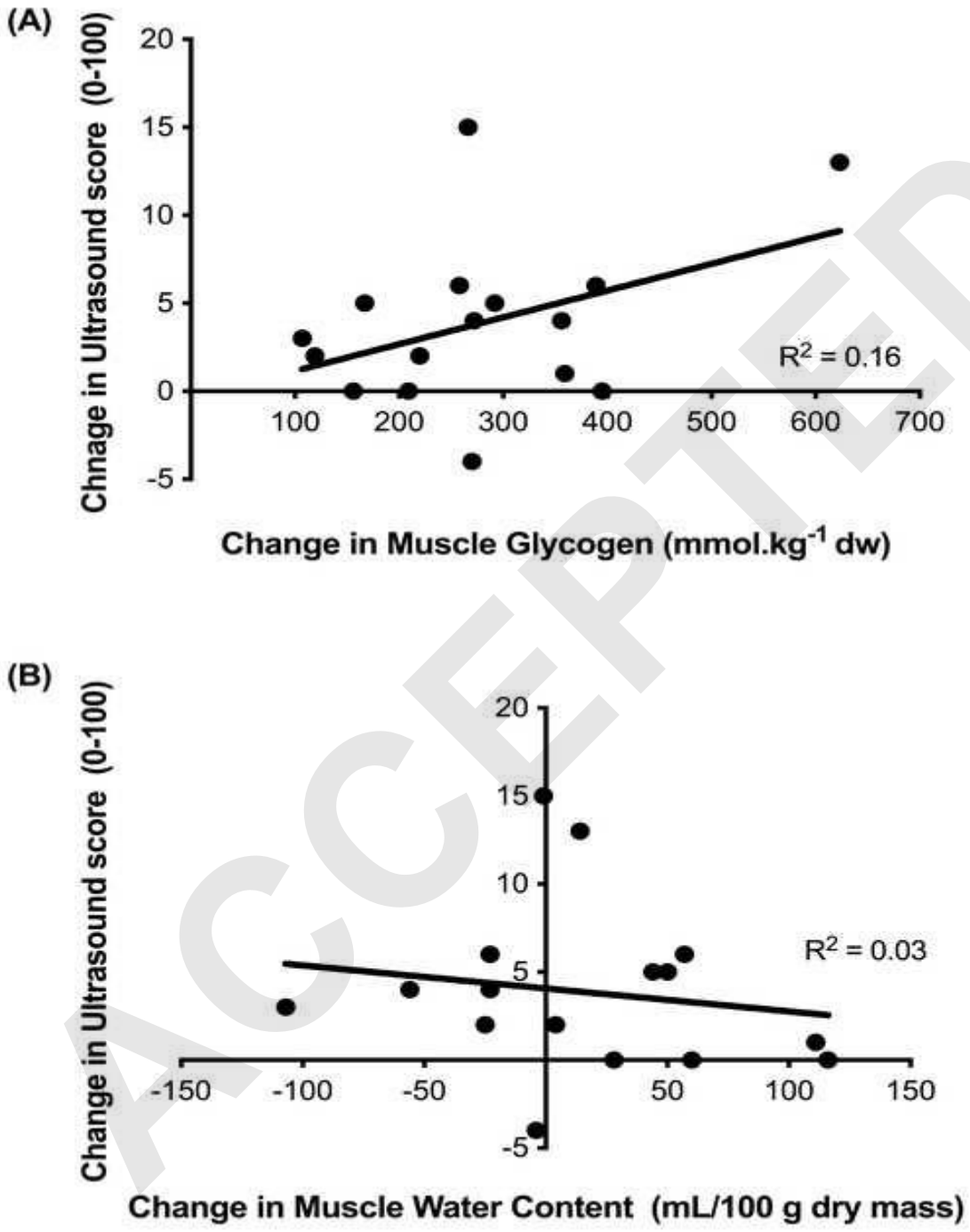

TABLE 1 – Estimates of Ultrasound score test-retest reliability within and between muscles. CV, ICC (95% CI) and Ratio Limits of agreement (*/÷) for 25, 50 and 75% of the vastus lateralis (VL) and vastus medialis (VM) muscles and 50% of the rectus femoris (RF) muscle. N = 100

Muscle and Length	Trial 1 Mean (±SD)	Trial 2 Mean (± SD)	CV	ICC (95% CI)	Ratio Limits of Agreement (*/÷)
VL 25%	62.6 (± 11.2)	63.8 (± 12.4)	7.3	0.85 (0.78 – 0.89)	1.02 (1.23)
VL 50%	64.5 (± 10.7)	66.2 (± 10.8)	5.8	0.89 (0.83 – 0.92)	1.02 (1.17)
VL 75%	65.9 (± 12.5)	67.8 (± 12.4)	6.3	0.89 (0.85 – 0.93)	1.03 (1.21)
VL Average	***64.4 (± 10.2)***	***65.9 (± 11.1)***	***4.5***	***0.94 (0.91 – 0.96)***	***1.02 (1.12)***
RF 25%	59.2 (± 11.6)	60.5 (± 10.8)	7.6	0.83 (0.76 – 0.89)	1.02 (1.25)
RF 50%	59.8 (± 12.1)	62.1 (± 11.5)	8.8	0.81 (0.73 -0.87)	1.04 (1.3)
RF 75%	64.5 (± 12.6)	64.9 (± 12.1)	9.0	0.77 (0.67 – 0.85)	1 (1.3)
RF Average	***60.7 (± 11.2)***	***62.1 (± 10.4)***	***6.5***	***0.87 (0.81 – 0.91)***	***1.03 (1.21)***
VM 50%	***62.1 (± 14.5)***	***60.9 (± 15.0)***	***11.1***	***0.79 (0.69 – 0.86)***	***0.97 (1.46)***