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The Effects of Dietary Manipulations on Ultrasound Assessment of Muscle Size and Quality: A Pilot Study

Original Research

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Abstract

Introduction: This pilot study examined the effects of carbohydrate loading (CHO) and oral creatine monohydrate loading (Cr) on ultrasound measurements of the lower limbs. **Methods:** Twelve recreationally-active males (25.5 ± 6.2 y, 81.5 ± 9.6 kg, 180.9 ± 8.8 cm) completed baseline (BL) bioelectrical impedance analysis (BIA) and muscle ultrasound imaging of the rectus femoris (RF) and the vastus lateralis (VL). Following baseline measurements, participants completed one day of CHO loading (10g CHO/kg), and five days of Cr loading (20g/day). Following each treatment, participants reported to the lab after an overnight fast for BIA and ultrasound testing in which muscle thickness (MT), cross-sectional area (CSA) and echo intensity (EI) were assessed on the RF and VL. A repeated measures analyses of variance were used for each variable to assess differences between dietary conditions. **Results:** Significant main effects ($p<0.05$) were observed for RF and VL MT, RF CSA. RF MT increased from BL-Cr ($p<0.00$, +6.85%) and CHO-Cr ($p=0.002$, +4.59%). VL MT increased from BL-Cr ($p=0.008$, +6.46%) and CHO-Cr ($p=0.006$, +3.71%). RF CSA increased between CHO-Cr ($p=0.034$, +3.58%). No significant differences were seen for EI. **Conclusions:** These data show that acute dietary manipulations may influence muscular ultrasound measurements of MT and CSA.

Key Words: Body Composition, carbohydrate loading, creatine loading

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Introduction

Ultrasonography is a reliable, accurate, and cost-effective method of measuring muscle thickness (MT), cross-sectional area (CSA), and echo-intensity (EI) of the quadriceps [1-4]. These measurements are often utilized for tracking changes in muscle size and quality following resistance training protocols, nutritional interventions, and as a predictor of health and safety of older adults [5-7]. Furthermore, quantitative measures of MT and CSA acquired from ultrasound images strongly correlate with those obtained from magnetic resonance imaging (MRI) and computerized tomography (CT) [2-4]. Currently, researchers and practitioners implement standardized protocols to increase the reliability and precision of musculoskeletal imaging [1,4-6]. While these standardized protocols are important to reduce variability and error, there may be other factors which affect the reliability of ultrasound derived measures of muscle size and quality.

Recently, there has been an increased interest in examining the effects of acute dietary manipulations on measures of body composition utilizing dual x-ray absorptiometry (DXA) and bioelectrical impedance assessment (BIA) [8-10]. Glycogen and creatine are both associated with changes in hydration status and fluid retention in various body compartments [8,11,12]. Using standardized carbohydrate (CHO) or creatine loading protocols it is apparent that acute dietary strategies can artificially increase estimates of lean total mass and reduce estimated fat mass [13,14]. While these are important revelations for researchers and clinicians, little is known about the effects of acute dietary alterations on other forms of body composition assessment.

As ultrasound assessment is commonly used to measure change across various training and dietary interventions, it is important to understand how acute dietary changes affect the reliability of ultrasound measures of muscle size and quality. Furthermore, while dietary changes may affect measures of body composition, currently, there are few studies which can contribute to developing dietary guidelines prior to skeletal muscle ultrasonography. Therefore, the purpose of this pilot investigation was to determine if CHO and creatine loading altered muscle size and quality of the RF and VL.

Methods

Participants

Twelve recreationally-active men (25.5±6.2y, 81.5±9.6kg, 180.9±8.8cm) were recruited to participate in this investigation. This study was approved by the University's Institutional Review Board and each subject completed a written informed consent, a medical history, and activity form prior to enrollment into the study. Exclusion criteria included the use of medication or other dietary supplements, creatine supplementation in the past 30 days, or suffering from any medical, muscular, or metabolic contraindications.

Protocol

All participants visited the laboratory three times during the study for baseline (BL), post- carbohydrate (CHO) loading, and post-creatine (Cr) loading testing. Prior to the baseline visit, participants completed a 24- hour dietary recall to determine each participants average daily carbohydrate intake [9]. For each visit, participants reported to the laboratory following a 12- hour fast and subsequently completed body composition testing and muscle ultrasound measurements [9,10]. Participants were asked to refrain from consuming alcohol, caffeine, and nicotine while also refraining from vigorous physical activity for 24-hours prior to testing days which was verbally confirmed at each visit.

Dietary Analysis

Participants were asked to complete a 24-hour dietary recall of all food, beverages and condiments consumed at the BL visit and during the 1-day CHO loading protocol. The BL recall was utilized to determine the appropriate amount of supplemental CHO needed during the CHO loading protocol and to confirm that all participants adhered to the CHO loading threshold ($10g \cdot day^{-1} \cdot kg^{-1}$). All dietary analysis was completed using the MyFitnessPal application (Under Armour Inc., Baltimore, MA) which contains a large, detailed US-branded food database.

Bioelectrical Impedance

Body mass, total body water (TBW), intracellular water (ICW), extracellular water (ECW), body fat percentage (BF%), and skeletal muscle mass were measured during each visit using multi-frequency bioelectrical impedance analysis (BIA) using the InBody® 570 (Biospace, Inc., Seoul, Korea). Body composition from BIA is obtained from the measures of resistance and reactance when an electrical current travels throughout the body and is a valid measurement tool for determining TBW [15]. Prior to each assessment the participants' hands and feet were thoroughly cleansed with InBody® provided tissues. Age, height, and gender are manually entered after weight is determined by a scale positioned within the device. The participant is then instructed from the software to stand fully erect, arms extended while not touching the sides of the body, and to refrain from moving or talking until the assessment was completed.

Muscle Ultrasonography

Non-invasive measurements of MT, CSA and EI of the rectus femoris (RF) and vastus lateralis (VL) were collected using B-mode ultrasound imaging with a 12 MHz linear probe (General Electric LOGIQ P5, Wauwatosa, WI) coated in a water based conduction gel on the participant's right side. Participants laid in the supine position for approximately ten minutes before images of the rectus femoris were taken at the 50% distance between the anterior inferior iliac spine (AIIS) and the proximal border of the patella [4,5]. Images of the VL were taken at the 50% distance between the greater trochanter of the femur and the lateral border of the patella [5]. Each image was taken using the same standardized ultrasound settings in order to ensure echo intensity consistency (frequency:12 MHz, gain:54 dB, dynamic range:72, and depth:5 cm) [5]. Prior to the investigation, intraclass correlation coefficients (ICC3,k), standard error of measurements (SEM), and minimal differences (MD) for the ultrasound technician were calculated for the RF MT ($ICC_{3,k}$=0.99, $SEM_{3,k}$=0.07, MD=0.19 cm),VL MT ($ICC_{3,k}$=0.99, $SEM_{3,k}$=0.01, MD=0.03 cm), RF CSA ($ICC_{3,k}$=0.99, $SEM_{3,k}$=0.42, MD=1.17 cm), VL CSA ($ICC_{3,k}$=0.99, $SEM_{3,k}$=0.30, MD=0.84 cm), RF EI ($ICC_{3,k}$=0.97, $SEM_{3,k}$=0.90, MD=2.47 cm), and VL EI ($ICC_{3,k}$=0.98, $SEM_{3,k}$=1.17, MD=3.19 cm) from analysis of 10 healthy individuals separated by 24 hours.

Images were analyzed using ImageJ software (version 1.45s; National Institutes of Health, Bethesda, MD, USA). The software was calibrated based off of a known distance of 1 cm on each of the ultrasound images [5]. Muscle thickness was measured from the inferior border of the superficial aponeurosis and the superior border of the deep aponeurosis whereas CSA was calculated using the polygon function by tracing around the lean mass without including any bone or fascia. [1]. Echo intensity was calculated from the same traced location using the standard histogram function which is a quantification of the grayscale of individual pixels based off of a 0 to 256 scale. For each measurement, three images were analyzed and the mean of the three images was calculated and recorded.

Carbohydrate Loading Protocol

Following the BL visit, participants completed a 1-day CHO loading protocol ($10 g \cdot day^{-1} \cdot kg^{-1}$) which has been shown to effectively maximize muscle glycogen stores in 24 hours [16]. Based on each participant's BL dietary recall, participants were provided prepackaged high-glycemic index (GI) CHO drinks (Gatorade, Gatorade Co., Chicago, IL) along with additional individually packaged high CHO snacks to assist them in reaching their individual CHO goal [9,16]. During the CHO loading phase, the participants were instructed to track their diet using the same dietary tracking software utilized during BL testing [10,16].

Creatine Loading Protocol

The day following the CHO loading assessment, participants began a five-day Cr loading protocol. It has been demonstrated that glycogen levels return to baseline five days after carbohydrate loading [17,18]. Thus it was assumed that by the Cr loading assessment visit (6 days following CHO loading), muscle glycogen would have returned to baseline with participants following their normal diet. Five days of Cr loading has previously been shown to significantly increase intramuscular Cr concentrations in young healthy adults [19]. Over the course of the five days participants consumed four doses of 5 g of creatine monohydrate (Optimum Nutrition, Aurora, Illinois) spaced out throughout the day [8,14]. Each individual dose (5g) was weighed and provided to the participant in separate bags to be mixed with 10oz of water for ingestion [13,14]. Over the course of the Cr loading protocol participants were instructed to consume a diet similar to their normal diet recorded during the BL dietary recall.

Statistical Analysis

Analysis of anthropometric, body composition, and muscle ultrasound data was accomplished using a repeated measures analysis of variance (RMANOVA). Prior to the RMANOVA, all data were assessed for normal distribution, homogeneity of variance and sphericity. In the event of a significant F- ratio, LSD post-hoc tests were performed for pairwise comparisons. In addition, effect sizes (η^2_p; partial eta squared) for all dependent variables were assessed. Effect sizes were interpreted as small (0.01–0.059), medium (0.06–0.139), or large (>0.14) as previously recommended [20]. An alpha level was set at $p \leq 0.05$, and all analyses were performed using SPSS version 24.0 (SPSS, Inc., Chicago, IL).

Results

All 12 participants completed each loading protocol and complied with the supplementation protocols. All participants met their CHO loading goal of $10 g \cdot kg^{-1} day^{-1}$ with an average carbohydrate intake of $10.9 g \cdot kg^{-1} day^{-1}$ for the one-day loading protocol.

Body Composition and Body Water Changes

Changes in body composition and body water are presented in Table 1. There was a main effect for time for body mass (F=7.053, p=0.004, η^2=0.391). Significant increases in body mass were observed in CHO (+2.1kg; p=0.006) and Cr (+2.8kg; p=0.004) compared to BL measurements. A main effect for time was observed for skeletal muscle mass (F=19.042, p<0.000, η^2=0.634). Pairwise comparisons showed a significant increase in skeletal muscle mass for CHO (0.86kg; p=0.012) compared to BL. Additionally, skeletal muscle mass for Cr was significantly greater than BL (2.37 kg; p<0.000) and CHO (1.51; p=0.010). There was a main effect for time for % BF (F=4.396, p=0.025, η^2=0.286). Pairwise comparisons showed a significant decrease in % BF in Cr (-0.58%; p=0.003) compared to BL.

There was a main effect for time for ICW (F=18.319, p<0.000, η^2=0.6.25). Significant increases in ICW were observed between BL and CHO (+0.64L; p=0.017), BL and Cr (+1.78L; p<0.000), and CHO and Cr (+1.14L; p=0.011). There was a main effect for time for ECW (F=6.283, p=0.007, η^2=0.364). Pairwise comparisons showed a significant increase between BL and CHO (+0.81L; p=0.006) and BL and Cr (+0.67L; p=0.011). There was a main effect for time for TBW (F=11.595, p<0.000, η^2=0.513). Pairwise comparisons showed a significant increase between BL and CHO (+1.44L; p=0.006) and BL and Cr (+2.45L; p<0.000).

Table 1. Body composition and body water changes across treatments

Variable	Baseline	CHO Loading	Cr Loading
Body Mass (kg)	81.5 ± 9.6	82.4 ± 9.9*	82.7 ± 9.6*
Skeletal Muscle Mass (kg)	41.1 ± 4.5	41.5 ± 4.6*	42.2 ± 4.4*#
Body Fat%	12.0 ± 4.8	12.0 ± 5.0	11.4 ± 4.8*
Total Body Water (L)	115.1 ± 12.2	116.6 ± 12.6*	117.6 ± 12.2*
Intracellular Water (L)	72.7 ± 7.6	73.4 ± 7.8*	74.5 ± 7.5*#
Extracellular Water (L)	42.4 ± 4.6	43.2 ± 4.8*	43.1 ± 4.7*

All data represented as mean ± SD. CHO=Carbohydrate CR=Creatine Monohydrate
*significantly different from Baseline. p<0.05
#significantly greater than CHO Loading. p<0.05

Muscle Ultrasound Measures

Changes in muscle ultrasound measures are presented in Table 2. There was a main effect for time for RF MT (F=16.793, p<0.000, η^2=0.604). Pairwise comparisons showed a significant increase between BL and Cr (+0.14 cm and +6.85%; p<0.000) and CHO and Cr (+0.10cm and +4.59%; p=0.002). There was a main effect for time for VL MT (F=8.541, p=0.002, η^2=0.437). Pairwise comparisons showed a significant increase between BL and Cr (+0.11cm and +6.46%; p=0.008) and CHO and Cr (+0.10cm and +3.71%; p=0.006). There was a main effect for time for RF CSA (F=4.102, p=0.031, η^2=0.272). Significant increases in RF CSA were observed between CHO and Cr (+0.45cm^2 and 3.58%; p=0.034).

Table 2. Changes in muscle ultrasound measures across treatments

Variable	Baseline	CHO Loading	Cr Loading
RF Muscle Thickness (cm)	2.09 ± 0.39	2.13 ± 0.38	2.23 ± 0.36*#
VL Muscle Thickness (cm)	1.66 ± 0.35	1.70 ± 0.35	1.76 ± 0.38*#
RF Cross-Sectional Area (cm^2)	12.46 ± 3.07	12.47 ± 2.92	12.92 ± 2.99#
VL Cross-Sectional Area (cm^2)	31.58 ± 7.08	31.52 ± 6.99	32.22 ± 7.55
RF Echo Intensity (AU)	21.16 ± 2.88	20.66 ± 3.32	20.46 ± 3.16
VL Echo Intensity (AU)	22.73 ± 2.96	22.83 ± 3.27	22.82 ± 3.15

All data represented as mean ± SD. CHO=carbohydrate, CR=Creatine Monohydrate, RF= Rectus Femoris, VL=Vastus Lateralis, AU= Arbitrary Units
*significantly greater than Baseline. p<0.05
#significantly greater than CHO Loading. p<0.05

Discussion

The purpose of this study was to investigate the effects of carbohydrate and creatine loading on ultrasound measures of muscle thickness, cross-sectional area, and echo intensity. The results indicated that five days of creatine loading significantly increased MT of both the RF and VL, while only a trend toward increased MT was found following CHO loading in the RF and VL. Creatine loading significantly increased RF CSA, while no other changes in CSA were seen in RF or VL for either treatment. Additionally, echo intensity was not significantly altered by carbohydrate or creatine loading in either of the muscles examined.

Participants experienced significant increases in MT of the RF and VL after five-days of creatine monohydrate loading. Following creatine loading, a 2.8kg increase in BW, 2.5L (2.13%) increase in TBW, and a 1.8L (2.5%) increase in ICW was observed. This coincided with a 6.5% increase in VL MT and a 6.9% increase in RF MT. This is in concert with previous reports of body fluid retention following creatine loading as Volek *et al.* [12] documented increases in body weight (0.75kg) and body water (0.4kg) following a 7-day 0.3 $g \cdot day^{-1} \cdot kg^{-1}$ creatine loading protocol. Bone *et al.* [8] found significant increases in TBW (1.3%) and ICW (1.4%) following a 5-day 20g·day creatine loading protocol using BIA. The overall increases in MT documented in our study can be compared specifically to the segmental changes associated with creatine loading in Bone *et al.* [8] where they saw a significant increase in leg mass of 1.1% and non-significant increases in the lean leg mass of 1.4%. Therefore, acute creatine loading seems to effect MT which is likely driven by increased ICW.

McBride and colleagues[21] originally demonstrated in an animal model that for each gram of glycogen stored in liver muscle, roughly 2.7g of water was additionally deposited. Furthermore, CHO depletion followed by a high CHO diet resulted in a 2.4kg increase in body weight (2.2L TBW) as roughly 3-4g of water was stored per gram of glycogen [11]. In the present study, body mass significantly increased following CHO loading by 2.1kg and TBW increased significantly by 1.4L. However, our findings did not present increases in muscle CSA or MT following 1-day of CHO loading. Nevertheless, a 2.66% increase in VL MT is similar to previous work documenting significant VL CSA increases of 3.5% using MRI following a 4-day CHO loading protocol[22]. Additionally, significant increases in body mass, lean body mass, and appendicular body mass using DXA scans following a 3-day high carbohydrate diet have been reported[17]. Thus while we only observed a trend for increased MT, it is possible that systemic changes in muscle composition contributed to increased BIA predicted skeletal muscle mass which is similar to other reports utilizing various techniques [22,23].

This pilot investigation had limitations that may have affected the interpretation of our results. While previous work has demonstrated that muscle glycogen levels return to baseline 72-96 hours following CHO loading [17,18], one may suggest that the significant increases in MT and CSA may have been partially driven by residual increased glycogen stores. A previous study found that 3-day CHO loading (~9.1 $g \cdot day^{-1} \cdot kg^{-1}$) significantly increased muscle glycogen following loading, however, muscle glycogen did not remain significantly elevated past 72 hours [17]. Furthermore, it has been demonstrated that in absence of an exercise and dietary CHO depletion phase, muscle glycogen is only significantly elevated for 96 hours [18]. Therefore, while it is reasonable to conclude any potential carry over effects from the CHO loading protocol were negligible, follow-up investigations should utilize a longer washout period before creatine loading to confirm our findings. As CHO and Cr loading strategies are commonly implemented simultaneously in athletes, future work examining washout periods for CHO, Cr, and CHO + Cr are needed to determine baseline muscle size and quality.

This pilot study provides evidence that creatine loading may alter the results of ultrasound assessments while acute CHO loading has no effect on muscle size and quality. In light of these results, new parameters prior to ultrasound testing may be needed to allow for more accurate and reliable results during training or dietary interventions. However, a further clinical trials with a larger sample size and longer washout periods are needed to provide additional support to our findings.

Media-Friendly Summary

Muscle ultrasound is a very common diagnostic tool used to measure acute and long-term changes in muscle size during resistance training and nutrition interventions. Current research has demonstrated that manipulating acute dietary strategies can influence estimates of body composition, but no study has examined the effects of dietary alterations on ultrasound measures of muscle size. Twelve recreationally active male participants enrolled in the study. Following baseline measurements of muscle size and body composition, the participants completed one day of CHO loading (10 $g \cdot day^{-1} \cdot kg^{-1}$) and a five day creatine loading protocol ($20g \cdot day^{-1}$). Significant increases in muscle thickness were found following creatine loading while no other significant changes in ultrasound measures were observed. Further research on the effect of dietary manipulations on ultrasound measures of muscle size is warranted.

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Disclosures
The authors have no conflicts of interest.

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