Declines in skeletal muscle quality vs. size following two weeks of knee joint immobilization

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BACKGROUND. Disuse of a muscle group, which occurs during bedrest, spaceflight, and limb immobilization, results in atrophy. It is unclear, however, if the magnitude of decline in skeletal muscle quality is similar to that for muscle size. The purpose of this study was to examine the effects of two weeks of knee joint immobilization on vastus lateralis and rectus femoris echo intensity and cross-sectional area. **METHODS.** Thirteen females (mean ± SD age = 21 ± 2 years) underwent two weeks of left knee joint immobilization via ambulating on crutches and use of a brace. B-mode ultrasonography was utilized to obtain transverse plane images of the immobilized and control vastus lateralis and rectus femoris at pretest and following immobilization. Effect size statistics and two-way repeated measures analyses of variance were used to interpret the data. **RESULTS.** No meaningful changes were demonstrated for the control limb and the rectus femoris of the immobilized limb. Analyses showed a large increase in vastus lateralis echo intensity (i.e., decreased muscle quality) for the immobilized limb (p = .006, Cohen's d = .918). For vastus lateralis cross-sectional area, no time × limb interaction was observed (p = .103), but the effect size was moderate (d = .570). There was a significant association between the increase in vastus lateralis echo intensity and the decrease in cross-sectional area (r = -.649, p = .016). **CONCLUSION.** In female participants, two weeks of knee joint immobilization resulted in greater deterioration of muscle quality than muscle size. Echo intensity appears to be an attractive clinical tool for monitoring muscle quality during disuse.

DECLINES IN SKELETAL MUSCLE QUALITY VS. SIZE FOLLOWING TWO WEEKS OF KNEE JOINT IMMOBILIZATION

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Abstract

Background. Disuse of a muscle group, which occurs during bedrest, spaceflight, and limb immobilization, results in atrophy. It is unclear, however, if the magnitude of decline in skeletal muscle quality is similar to that for muscle size. The purpose of this study was to examine the effects of two weeks of knee joint immobilization on vastus lateralis and rectus femoris echo intensity and cross-sectional area.

Methods. Thirteen females (mean ± SD age = 21 ± 2 years) underwent two weeks of left knee joint immobilization via ambulating on crutches and use of a brace. B-mode ultrasonography was utilized to obtain transverse plane images of the immobilized and control vastus lateralis and rectus femoris at pretest and following immobilization. Effect size statistics and two-way repeated measures analyses of variance were used to interpret the data.

Results. No meaningful changes were demonstrated for the control limb and the rectus femoris of the immobilized limb. Analyses showed a large increase in vastus lateralis echo intensity (i.e., decreased muscle quality) for the immobilized limb ($p = .006$, Cohen's $d = .918$). For vastus lateralis cross-sectional area, no time × limb interaction was observed ($p = .103$), but the effect size was moderate ($d = .570$). There was a significant association between the increase in vastus lateralis echo intensity and the decrease in cross-sectional area ($r = -.649$, $p = .016$).

Conclusion. In female participants, two weeks of knee joint immobilization resulted in greater deterioration of muscle quality than muscle size. Echo intensity appears to be an attractive clinical tool for monitoring muscle quality during disuse.

Introduction

Muscle unloading or disuse, such as what occurs during bed rest and removal of weight bearing conditions, results in muscular atrophy (Berg et al. 1997; Tesch et al. 2004; Wall et al. 2014). These changes present several significant challenges for health care providers, particularly following surgical procedures and bedrest in older adults, as muscle weakness is a strong predictor of mortality (Newman et al. 2006). Responses to disuse vary by sex (Deschenes et al. 2009) and muscle group (Schulze et al. 2002; Tesch et al. 2004), with greater declines in females and the knee joint, respectively. In athletics, females have a roughly three times greater incidence of anterior cruciate ligament (ACL) tears during soccer and basketball versus males, with many of these injuries occurring during non-contact situations (Prodromos et al. 2007; Renstrom et al. 2008). Female athletes that play sports year-round have an ACL tear rate of approximately 5% (Renstrom et al. 2008). These issues are not limited to competitive athletes, however. ACL tears are common among any population that engages in physical activity and sports. It has been reported that the overall age- and sex-adjusted annual incidence of ACL tears among the general population was 68.6 per 100,000 person-years, with a substantial increase over time regardless of age (Sanders et al. 2016). Rehabilitation of the knee joint following surgical interventions has major social and economic consequences, with one study estimating a cost of > $2 billion for an annual incidence of 200,000 ACL reconstructions in the U.S (Mather et al. 2013). Following ACL reconstruction surgery, specifically with a concomitant meniscal

82 repair, patients are often placed under temporary bed rest to maintain graft integrity, as well as
83 reduce compressive forces and pain at the knee joint (Hiemstra et al. 2009). Following short-term
84 immobilization, patients are typically administered a knee brace to be worn for all weight
85 bearing activities.
86   Given the implications for long-term health, changes in muscle size (i.e., hypertrophy and
87 atrophy) following various interventions have historically garnered substantial interest from
88 researchers and clinicians. In recent years, however, investigators have begun to consider
89 changes in muscle quality via analysis of echo intensity (Lopez et al. 2017). The premise behind
90 its use is that the degree of darkness/brightness of a given region of interest characterizes tissue
91 composition, as darker images (lower values) represent skeletal muscle tissue and brighter
92 images (higher values) represent non-contractile elements. While echo intensity's precise
93 underpinnings have yet to be directly verified, anatomical investigations carried out in humans
94 and animals have demonstrated that it strongly reflects fibrous tissue content (Arts et al. 2012;
95 Pillen et al. 2009), as well as intramuscular adiposity (Nishihara et al. 2014; Reimers et al. 1993;
96 Young et al. 2015). Echo intensity also seems to be at least moderately correlated with
97 performance measures such as muscle strength (Mota et al. 2017), stair-climbing ability
98 (Kleinberg et al. 2016), and chair stand ability (Rech et al. 2014; Lopez et al. 2017), and these
99 associations have been reported from adolescents (Stock et al. 2017) through older adults
100 (Watanabe et al. 2013). Furthermore, echo intensity can be easily analyzed with B-mode
101 ultrasonography. Though its image quality still lags behind CT and MRI, ultrasonography's
102 portability, cost, and time efficiency make it a realistic option for clinicians and practitioners
103 with limited resources. While more research and normative data are needed, at this point,
104 ultrasonography-based echo intensity shows promise as a tool for tracking changes in muscle
105 quality.
106   Understanding the neuromuscular and morphological adaptations that occur during short-
107 term disuse will allow for the development of more effective and timely preventative
108 interventions. While there is evidence that resistance training may affect echo intensity in older
109 people (Radaelli et al. 2014), evidence of this relationship in young people is less clear.
110 Furthermore, as additional imaging analytical techniques become available, it is important to
111 understand which variables may be more or less sensitive to changes in muscle morphology,
112 particularly in clinical settings. While the atrophic effects of limb immobilization have been well
113 established (Hackney and Ploutz-Snyder 2012), the extent to which muscle quality deteriorates is
114 unclear. Thus, the purpose of this study was to examine the effects of two weeks of knee joint
115 immobilization on vastus lateralis and rectus femoris muscle quality and size in college-aged
116 females. Based on previous studies (Deschenes et al. 2017; Hackney and Ploutz-Snyder 2012),
117 we hypothesized that skeletal muscle size would show a small-to-moderate decrease following
118 immobilization. In contrast, we speculated that muscle quality would deteriorate to a greater
119 extent, which would be suggestive of rapid intramuscular fat and/or fibrous tissue infiltration
120 during disuse, rather than reduction in muscle size.
121

Materials & Methods

Experimental Approach to the Problem

This investigation utilized a within-participants design in which B-mode ultrasonography imaging was performed for the vastus lateralis and rectus femoris muscles of immobilized (left) and control (right) limbs. All participants were right leg dominant. Data was collected during baseline testing (PRE) and two weeks later (POST). All visits to the laboratory occurred at the same time of day (± one hour) throughout the course of the study. All imaging and image analyses were performed by the same investigators utilizing methods described in our previous publications (Burton and Stock 2018; Mota et al. 2017). Throughout the study, participants were asked to refrain from alcohol, keep their dietary habits consistent, and comply with the immobilization protocol. Participants kept a three-day food log, which was primarily meant to serve as a reminder for the need for consistency of health behaviors throughout the study. Each participant was assigned to a study investigator, who served as her direct point of contact and was always available.

Participants

Due to the novelty of this topic, the ability to predict the observed effect size was limited. As such, an *a priori* power analysis was not carried out as recommended by previous investigators (Beck 2013). It should be noted, however, that a recent systematic review found that lower limb immobilization studies have reported data for ≤ 13 participants, with ≤ 10 being typical (Campbell et al. 2019). Thus, the final sample size described below is ≥ what has previously been utilized in the relevant literature. This study's *post-hoc* power analyses for particularly relevant outcomes have been included in our Supplemental Files.

Twenty-six healthy, college-aged females originally enrolled in this study. Recruitment methods included the use of flyers, social media posts, marketing on laboratory and university websites, and presentations in group settings. Inclusion criteria included females between the ages of 18-35 years, a body mass index ≤ 30 kg/m², and willingness to comply with the study's demands. Major exclusion criteria included the following nine conditions: 1) personal or family history of blood clots; 2) neuromuscular or metabolic disease; 3) osteoarthritis; 4) previous surgery on the hip or knee joints; 5) use of an assistive walking device within the previous year; 6) myocardial infarction within the past year; 7) pregnancy; 8) use of contraceptives within the previous 90 days (Vinogradova et al. 2015); 9) musculoskeletal pain or discomfort in any of the major joints. Recent lower-body resistance or aerobic training participation was not considered exclusionary, trained and untrained individuals experience similar relative declines in lean mass following knee joint immobilization (Deschenes et al. 2017). Participants completed an in-house Pre-Testing Health Questionnaire and the Physical Activity Readiness Questionnaire + (PAR-Q +) in the laboratory to determine eligibility. All participants read, understood, and signed an informed consent form. The study protocol was approved by the University of Central Florida's Institutional Review Board (Study # BIO-17-13642). Participants were compensated $350 for completing the study.

161   Out of the 26 participants that enrolled, eleven withdrew. Reasons for withdrawing
162   included, but were not limited to, time constraints, changes in contraceptive use, difficulty
163   accomplishing daily activities and subsequent inability to comply with the study protocol, and
164   discomfort. Out of the fifteen participants that completed the study, two were removed from this
165   paper's statistical analysis, one because of difficulty with image analysis and another because of
166   accelerometer data demonstrating non-compliance (details described below). Therefore, 13
167   participants (mean ± SD age = 21 ± 2 years, mass = 61.6 ± 4.6 kg, height = 164.7 ± 6.1 cm) were
168   included in our final data set.

Immobilization Procedures

170   During the pretesting data collection session, participants were properly fit for the use of
171   a knee joint immobilization brace (T Scope® Premier Post-Op Knee Brace, Breg, Inc., Carlsbad,
172   CA, USA). Each brace was locked at a 90° angle, which allowed the left knee extensors to
173   remain relaxed and unloaded while preventing the toes from contacting the ground. A 90° knee
174   joint angle was a conservative approach to ensuring that the toes of the left foot did not come
175   into contact with the ground while ambulating on the crutches. Participants wore the brace at all
176   times, except during periods of sleep. Participants were also asked to keep their left leg in the
177   brace and covered with a plastic bag when showering. To ensure safety and comfort while
178   showering, each participant was offered a shower chair (Medline Shower Chair Bath Seat with
179   Padded Armrests and Back, Medline Industries, Inc., Northfield, IL, USA). Participants were
180   also provided and properly fit for axillary crutches (Cardinal Health Axillary Crutch, Adult,
181   Height 62-70 in, Adjustable, Cardinal Health, Inc., Dublin, OH, USA) using guidelines described
182   by Fairchild (2012). Training was provided on proper use of crutches in navigating the
183   community including curbs, stairs, and sidewalks (Figure 1). Participants were advised that they
184   would likely have trouble with activities of daily living such as transportation and cleaning, to
185   allow additional time to complete tasks, and to plan ahead in order to avoid unsafe conditions
186   (e.g., ensure a towel was near the shower and to sufficiently dry off). In addition to use of the
187   brace and crutches, participants were urged to refrain from weight bearing.

[FIGURE 1 ABOUT HERE]

189   Each participant wore a stocking (Rolyan Extra Soft Stockinette, 100% Cotton,
190   Performance Health, Warrenville, IL, USA) underneath their brace that spanned the length of the
191   left lower extremity from the proximal thigh to the ankle. This stocking was worn to improve
192   comfort while wearing the knee brace and to minimize the risk of adverse skin reactions. The
193   stocking could be taken off before bed but was worn during all daytime activities. To reduce the
194   risk of blood clots, participants were also given nighttime compression stockings (Medi-Pak
195   Anti-Embolism Stockings, McKesson, San Francisco, CA, USA) to be worn over the left leg
196   while they slept. Basic hygiene was discussed with the participants, and they were instructed to
197   notify the researchers immediately in the event that they noticed any irritation, redness, or
198   swelling; this was not reported by any of the participants.

199   Consistent with previous knee joint immobilization studies (Deschenes et al. 2008;
200   Deschenes et al. 2009), each participant performed light range of motion movements at the ankle

201   and knee while lying supine in bed. These activities were performed twice daily (morning and
202   evening) in an effort to minimize the risk of vascular pathology and muscular contractures. A
203   video containing instructions was provided to participants, as was as a handout with detailed
204   directions of performance of the exercises. The compressive stocking was worn during the
205   exercise. Finally, to further ensure compliance and safety, study investigators text messaged or
206   spoke with the participants via telephone daily.

207   **Ultrasonography Measurements and Analysis**

208   Ultrasonography images for the vastus lateralis and rectus femoris muscles of both limbs
209   were taken with a portable B-mode imaging device (GE Logiq e BT12, GE Healthcare,
210   Milwaukee, WI, USA) and a multi-frequency linear-array probe (12 L-RS, 5–13 MHz, 38.4-mm
211   field of view; GE Healthcare, Milwaukee, WI, USA). The panoramic function (LogiqView, GE
212   Healthcare, Milwaukee, WI, USA) was used to obtain images of the vastus lateralis and rectus
213   femoris in the transverse plane. Prior to ultrasound measurements, subjects laid in the supine
214   position for 5 minutes in order to allow the redistribution of fluids from their quadriceps muscles.
215   The images were taken at 50% of the length of the femur. A high-density foam pad was secured
216   around the thigh with an adjustable strap to ensure probe movement in the transverse plane.
217   Ultrasonography settings (Frequency: 10 MHz, Gain: 55dB, Dynamic Range: 72) were kept
218   consistent across participants. To ensure optimal image clarity, a standardized depth of 5.0 cm
219   was utilized. However, for three participants, a greater depth was necessary to adequately capture
220   each muscle belly. For these three participants, depths of 6.0, 6.0, and 7.0 cm were utilized.
221   Depth for each participant was kept consistent across trials. A generous amount of water-soluble
222   transmission gel (Aquasonic 100 ultrasonography transmission gel, Parker Laboratories, Inc.,
223   Fairfield, NJ, USA) was applied to the skin to allow immersion of the probe surface during
224   measurement to enhance acoustic coupling. Three images of each muscle were obtained, and
225   mean values were used for statistical analyses.

226   The ultrasonography images were digitized and examined with ImageJ software (version
227   1.46, National Institutes of Health, Bethesda, MD, USA) at the conclusion of the study. The
228   polygon function was used to outline the borders of the vastus lateralis and rectus femoris. After
229   scaling the image units from pixels to cm, muscle cross-sectional area (cm^2) was determined
230   with the polygon function. Echo intensity was also assessed by computer-aided gray-scale
231   analysis using the histogram function. The echo intensity values were determined as the
232   corresponding index of muscle quality ranging between 0 and 255 arbitrary units (AU).
233   Recently, Young et al. (2015) provided an equation that allows investigators to correct rectus
234   femoris echo intensity for the magnitude of subcutaneous thickness over the muscle (corrected
235   echo intensity = raw echo intensity + [subcutaneous fat thickness [cm] × 40.5278]). While
236   uncorrected rectus femoris echo intensity values have been reported herein, it should be noted
237   that correction for subcutaneous thickness would not have affected this study's overall
238   conclusions.

239   **Ultrasonography Test-Retest Reliability**

240   Test-retest reliability statistics were calculated for each of the dependent variables using
241 the equations described by Weir (2005). Specifically, we assessed the intraclass correlation
242 coefficient (ICC [model 2, k]), standard error of measurement (SEM [expressed both in absolute
243 terms and as a percentage of the mean]), and the minimal difference needed to be considered real
244 (MD). As presented in a recent publication (Burton and Stock 2018), our laboratory's test-retest
245 reliability statistics for rectus femoris echo intensity in college-aged females were: ICC = .928;
246 SEM = 4.21 AU (4.22%); MD = 11.68 AU. For rectus femoris cross-sectional area, these
247 statistics were as follows (Burton and Stock 2018): ICC = .982; SEM = 0.24 cm^2 (5.43%); MD =
248 0.67 cm^2. For the vastus lateralis, the echo intensity statistics were: ICC = .810; SEM = 3.31
249 (3.71%); MD = 9.17 AU. The test-retest reliability statistics for vastus lateralis cross-sectional
250 area were: ICC = .980; SEM = 0.52 (3.43%); MD = 1.45 cm^2.

Actigraphy

252   In order to assess compliance with the established protocol, participants were asked to
253 wear Actigraph GT9X accelerometers (ActiGraph Inc, Penscola, FL, USA) on both their right
254 and left legs, fastened securely around each ankle via Velcro straps. The accelerometers detect
255 normal human motion while filtering out high-frequency vibrations that would artificially
256 increase movement data. Participants were instructed to wear accelerometers around both ankles
257 for the entire study period, only removing the devices during showering. Two levels of
258 compliance were established for the current protocol: 1) overall wear-time compliance and 2)
259 compliance to the immobilization protocol. To meet wear-time compliance criteria, established
260 by Troiano (2007), participants were required to wear the device for a minimum of four days (10
261 hours per day) over a 7-day period. As participants were instructed to wear the device over a
262 two-week period, eight full days of data, including two weekend days, were required to meet
263 compliance criteria. To determine compliance to the immobilization protocol, criteria was
264 established based on a previous study by Cook et al. (2006), who examined differences in both
265 the number of steps and intensity of steps between legs in non-weight bearing participants on
266 crutches.

Statistical Analyses

268   All variables were first checked for normal distributions with Shapiro-Wilk tests. To
269 assess compliance with protocol, paired samples t-tests were conducted to determine differences
270 in activity intensity and steps between the immobilized and control limbs of each participant.
271 Two-way (time [pre, post] × limb [immobilized, control]) repeated measures analyses of
272 variance (ANOVAs) were used to examine mean differences for each muscle and dependent
273 variable. When appropriate, follow-up analyses included Bonferroni post-hoc comparisons and
274 paired samples *t*-tests. We also evaluated 95% confidence intervals for mean differences (CIs)
275 and effect sizes via Cohen's *d* statistics. Small, medium, and large Cohen's d corresponded to
276 values of 0.20, 0.50, and 0.80, respectively (Cohen 1988). All statistical procedures were carried
277 out with JASP software (version 0.8.3.1, University of Amsterdam, Amsterdam, The
278 Netherlands).

279

Results

281 Mean ± SD values for all ultrasonography variables have been displayed in Table 1.

282 **[TABLE 1 ABOUT HERE]**

Vastus Lateralis Echo Intensity

284 The results from the two-way repeated measures ANOVA indicated that there was a limb
285 × time interaction ($F = 9.14$, $p = .011$ [Figure 2]). Follow-up analyses revealed a significant
286 increase in echo intensity ($p = .006$, 95% CI = -5.60 – -1.16 AU) for the immobilized limb that
287 was considered large (mean increase = 3.40 AU, $d = .918$). In contrast, there was a small
288 decrease in echo intensity for the control limb that was not significant (mean decrease = 1.87
289 AU, $p = .189$, $d = .386$, 95% CI = -1.06 to 4.79 AU). Differences between limbs were
290 small/moderate but not significant at the pretest ($p = .064$, $d = .567$, 95% CI = -0.22 – 6.94 AU)
291 or posttest ($p = .169$, $d = .406$, 95% CI = -0.93 – 4.74 AU). For the immobilized limb, three
292 participants showed an increase in vastus lateralis echo intensity that exceeded the MD (9.17
293 AU). The mean increase in echo intensity for the immobilized limb (3.40 AU) did not exceed the
294 MD.

Vastus Lateralis Cross-Sectional Area

296 Despite a large effect size, the results from the two-way repeated measures ANOVA
297 indicated that there was no limb × time interaction ($F = 3.11$, $p = .103$ [Figure 3]) and no main
298 effects for limb ($F = 1.81$, $p = .204$) or time ($F = 2.75$, $p = .123$). Analysis of effect sizes and
299 95% CIs for mean differences revealed similar cross-sectional area values for the pretest and
300 posttest for the control limb ($d = .151$, 95% CI = -0.64 – 1.08 cm^2). However, a moderate effect
301 size was found for the immobilized limb ($d = .570$, 95% CI = -0.07 – 2.28 cm^2). For the
302 immobilized limb, five participants showed a decrease in vastus lateralis cross-sectional area that
303 exceeded the MD (1.40 cm^2). The mean decrease in cross-sectional area for the immobilized
304 limb (1.11 cm^2) did not exceed the MD.

Correlation between Changes in Vastus Lateralis Echo Intensity and Cross-Sectional Area

307 The correlation between change scores for vastus lateralis echo intensity and cross-
308 sectional area of the immobilized limb was statistically significant ($r = -.649$, $p = .016$ [Figure
309 4]).

310 **[FIGURES 2-4 ABOUT HERE]**

Rectus Femoris Echo Intensity

312 The results from the two-way repeated measures ANOVA indicated that there was no
313 limb × time interaction ($F = .308$, $p = .589$) and no main effects for limb ($F = .046$, $p = .833$) or
314 time ($F = .004$, $p = .953$ [Figure 2]). Analysis of effect sizes and 95% CIs for mean differences
315 revealed nearly identical echo intensity values for the pretest and posttest for the control ($d =$
316 .087, 95% CI = -3.34 – 4.47 AU) and immobilized ($d = .084$, 95% CI = -3.32 – 2.51 AU) limbs.
317 For the immobilized limb, none of the participants showed an increase in rectus femoris echo
318 intensity that exceeded the MD (11.68 AU). The mean increase in echo intensity for the
319 immobilized limb (0.41 AU) did not exceed the MD.

320 **Rectus Femoris Cross-Sectional Area**

321   The results from the two-way repeated measures ANOVA indicated that there was no
322 limb × time interaction ($F = .041$, $p = .794$) and no main effects for limb ($F = .067$, $p = .801$) or
323 time ($F = .450$, $p = .515$ [Figure 3]). Analysis of effect sizes and 95% CIs for mean differences
324 revealed similar cross-sectional area values for the pretest and posttest for the control ($d = .086$,
325 95% CI = -0.41 – 0.55 cm^2) and immobilized ($d = .309$, 95% CI = -0.21 – 0.65 cm^2) limbs. For
326 the immobilized limb, four participants showed a decrease in rectus femoris cross-sectional area
327 that exceeded the MD (0.67 cm^2). The mean decrease in cross-sectional area for the immobilized
328 limb (0.22 cm^2) did not exceed the MD.

329 **Correlation between Changes in Rectus Femoris Echo Intensity and Cross-**
330 **Sectional Area**

331   The correlation between the change in rectus femoris echo intensity and cross-sectional
332 area of the immobilized limb was not statistically significant ($r = .378$, $p = .202$ [Figure 4]).

333 **Compliance**

334   After removal of one participant because of difficulty with image analysis, the results
335 from the wear-time validation software indicated that all but one participant met the minimum
336 wear-time criteria (8 days of 10+ hours), with participants wearing the device for an average of
337 13.1 ± 1.3 days. Additionally, significant differences existed between minutes of vigorous
338 physical activity (mean difference of 70.2 minutes ± 52.8; $p = 0.002$) and total steps (mean
339 difference of 14,861 steps ± 19,535; $p = 0.04$) between the immobilized limb and the control
340 limb.

341

342 # Discussion

343   Echo intensity is becoming increasingly utilized by investigators to assess changes in
344 skeletal muscle composition (i.e., muscle quality) during various interventions. The major
345 finding of the present study was that two weeks of left knee joint immobilization resulted in a
346 significant increase in echo intensity for the vastus lateralis muscle, and this change was slightly
347 greater than that for the decrease in cross-sectional area. No changes were found for the rectus
348 femoris muscle, which is consistent with previous findings (Hackney and Ploutz-Snyder 2012)
349 and its continued role as a hip flexor throughout the two week intervention. Overall, these results
350 suggest that echo intensity, which appears to reflect intramuscular adiposity (Reimers et al. 1993;
351 Young et al. 2015) and fibrous tissue infiltration (Arts et al. 2012; Pillen et al. 2009), may be a
352 more sensitive measure of muscle morphology than cross-sectional area when studying limb
353 disuse. It is important to note, however, that the magnitude of these changes varied across
354 participants, as many of the pretest–posttest differences did not exceed our laboratory's MD
355 statistics.

356   The results of the present study supported the hypothesis that there would be greater
357 changes in echo intensity compared to cross-sectional area. Although our study design did not
358 include ultrasonography testing at multiple time points, these findings suggest that the
359 deterioration in muscle quality may occur early in the disuse period and – perhaps more

360   importantly – prior to the onset of notable atrophy. To our knowledge, our two week study is the
361   first to find potential indications of adipose tissue changes following as little as two week of
362   disuse. To our understanding, only two previous studies have investigated infiltration of
363   intramuscular adipose tissue following a period of disuse (Gorgey and Dudley 2007; Manini et
364   al. 2007). Manini et al. (2007) found a 14.5% increase in intermuscular adipose tissue in the
365   thigh musculature following a four-week period of unilateral lower limb suspension. Of
366   interesting note, this accumulation of intermuscular fat occurred prior to any significant changes
367   in subcutaneous fat content. The hypothesized physiological explanation for the increased
368   intermuscular fat content was due to a suppression of skeletal muscle lipase activity during
369   periods of inactivity. Similar findings were found in participants who had been diagnosed with
370   an acute, incomplete spinal cord injury (Gorgey and Dudley 2007). The disuse of the lower
371   extremities occurred in a more clinical manner, in which the participants were primarily
372   wheelchair dependent due to the nature of the injury. Substantial increases in intramuscular
373   adipose tissue were found in the thigh musculature six weeks after the injury. The largest and
374   most dramatic increase was seen during the initial six weeks, but there was continued infiltration
375   of intramuscular fat at three months follow up (Gorgey and Dudley 2007). While other studies
376   specifically addressing changes in muscle quality following disuse are limited, insight into this
377   topic may be gleamed from the aging literature, as aging and inactivity seem to manifest in
378   similar physiological adaptations (Bell et al. 2016). Specifically, high echo intensity values have
379   been associated with muscle weakness in the elderly population (Watanabe et al. 2013). Muscle
380   quality in older adults has also been shown to be a significant predictor of performance during
381   the 30-second chair stand test (Lopez et al. 2017; Rech et al. 2014), which has important
382   functional relevance. Given that periods of disuse are common in many medical scenarios, such
383   as ACL tears, our findings highlight the need for researchers and health care professionals to
384   focus their attention on deterioration of muscle quality, rather than just atrophy. A post-operative
385   ACL reconstruction patient, for whom weight bearing is contraindicated, may suffer a reduction
386   in muscle quality despite no noticeable change in muscle size. These results suggest that further
387   research is warranted to investigate early interventions to mitigate the effects of short-term
388   disuse on muscle quality.
389   The moderate decline in cross-sectional area observed in this study is in line with a
390   variety of previous studies. Following a comprehensive review of unilateral limb immobilization
391   studies, Hackney and Ploutz-Snyder (2012) determined that the vastus lateralis was significantly
392   more prone to disuse than the gastrocnemius and the rectus femoris. Based on the available
393   literature available at the time, it was estimated that the vastus lateralis atrophies at a rate of
394   .44% per day. Our design and results seem to be quite similar to the work of Wall et al. (2014),
395   who also studied a two-week duration. These authors reported significant atrophy of the vastus
396   lateralis after only five days of immobilization, demonstrating a 3.5% decrease. The decline in
397   cross-sectional area continued as the period of immobilization prolonged, reaching a 8.4%
398   decrease by 14 days. It should be noted that our focus was on healthy females, primarily due the
399   relevance to knee joint injuries in athletics (Prodromos et al. 2007; Renstrom et al. 2008).

400 Additional studies are needed to investigate if there are differences between sexes and other
401 muscle groups in the ability to preserve muscle quality versus size during a period of disuse.
402 Given that the decline in muscle size was lesser in magnitude than that observed for muscle
403 quality, it is evident that declines in muscle function during periods of immobility are
404 mechanistically related to a variety of physiological phenomena.
405   To the best of our knowledge, this is the first study to utilize ultrasonography to track
406 changes in echo intensity following limb immobilization. Previous immobilization studies have
407 primarily utilized MRI (Berg et al. 1997; Gorgey and Dudley 2007) and CT-scans (Dirks et al.
408 2014; Wall et al. 2014) to analyze decreases in cross-sectional area of the quadriceps femoris
409 muscles. While our results provide additional support for the use of ultrasonography-based echo
410 intensity as a time- and cost-effective means of studying muscle morphology, we believe that its
411 exact anatomical underpinnings require further investigation. On one hand, there is strong
412 evidence to support the notion that echo intensity is associated with intramuscular adiposity (Arts
413 et al. 2012; Young et al. 2015). A study by Young et al. (2015) conducted MRI and
414 ultrasonography imaging on 31 participants of varying ages for the rectus femoris, biceps
415 femoris, tibialis anterior, and medial gastrocnemius. They found that there were strong
416 correlations between the percentage of intramuscular fat as shown with MRI and echo intensity
417 as measured with ultrasonography, with the rectus femoris association being particularly high (r
418 = .91). On the other hand, the influence of fibrous tissue content should not be completely
419 discounted. Pillen et al. (2009) analyzed both echo intensity and muscle biopsies of golden
420 retrievers suffering from muscular dystrophy. The results of their study revealed that muscle
421 echo intensity was strongly correlated (r = .87) with the amount of intramuscular fibrous tissue.
422 In addition, the fact that echo intensity is altered by muscle tissue damage following eccentric
423 exercise (Nosaka and Sakamoto 2001) and potentially reflective of glycogen/hydration status
424 (Jenkins 2016) points to the need for more comprehensive studies in this area. Collectively,
425 while the term muscle quality is likely an appropriate term for ultrasonography-based echo
426 intensity, the precise explanation for the changes observed in the present study is worthy of
427 additional inquiry.
428   Like all studies, this investigation had several limitations that are worthy of brief
429 discussion. First, although our efforts to monitor and ensure compliance give us confidence in
430 these findings, it seems impossible to state with 100% certainty that our participants did not ever
431 weight bear. Future investigators wishing to perform similar studies in college-aged participants
432 should consider the use of a golf cart to provide transportation to and from classes.
433 Unfortunately, this option was not available when the present study began. Second, while we
434 were motivated to perform this study in young females, the external validity to other populations
435 is somewhat limited. Indeed, future studies are needed in young males as well as varying age
436 groups to determine if the enhanced sensitivity of echo intensity versus cross-sectional area is
437 consistent across sexes and age groups. As echo intensity appears to be lower in African
438 Americans (Melvin et al. 2014), racial and ethnic backgrounds of research participants should
439 also be studied. Third, as our study was designed to specifically examine limb immobilization,

440 these findings should not be extrapolated to other non-weight bearing conditions, and in
441 particular, bedrest. We believe that the ambulatory activities of the control limb should not be
442 discounted, particularly given the influence of cross education training (Carr et al. 2019) and the
443 central nervous system's role in maintaining muscle strength and mass (Clark et al. 2014).
444 Similarly, our implementation of a 90° joint angle at the knee to ensure that the participants' toes
445 did not come into contact with the ground during ambulating with crutches resulted in a unique
446 biomechanical condition, particularly for the biarticular muscles of the thigh. Finally, it is
447 interesting to note that although some group mean differences and moderate/large effect sizes
448 were demonstrated, only a small portion of the participants showed changes that exceeded the
449 MD. None of the mean differences exceeded the MD. This contrast was despite good-to-
450 excellent test-retest reliability statistics of well-trained and experienced investigators. These
451 results highlight the need to consider change scores on a participant-by-participant basis, as well
452 as the practical value of including multiple and comprehensive analytical approaches in research
453 (i.e., effect sizes, confidence intervals, p values, individual participant data, etc.).
454
Conclusions
456 We sought to examine the effects of two weeks of knee joint immobilization on vastus
457 lateralis and rectus femoris muscle quality and size. The findings of this study demonstrated that
458 muscle quality deteriorated to a greater extent than muscle size during two weeks of knee joint
459 immobilization in college-aged females. We believe that the lack of change for the rectus
460 femoris due to its role as a hip flexor provides further justification for this conclusion. Future
461 investigators may wish to verify these results in males and participants of various age and ethnic
462 groups. Based on these data, ultrasonography-based echo intensity appears to be a useful clinical
463 tool for tracking changes in skeletal muscle deterioration during extended periods of disuse.
464
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Two weeks of knee joint immoblization result in significant increases in vastus lateralis echo intensity.

Mean +/- SD echo intensity and cross-sectional area values for each muscle and testing session of the immoblized (A.) and control (B.) limbs. * = p < .05

A.			
Immobilized Limb			
Vastus lateralis cross-sectional area (cm^2)		Rectus femoris cross-sectional area (cm^2)	
PRE	POST	PRE	POST
16.71 ± 2.85	15.60 ± 2.38	6.15 ± 1.17	5.93 ± 0.86
Vastus lateralis echo intensity (AU)		Rectus femoris echo intensity (AU)	
PRE	POST	PRE	POST
82.77 ± 8.35	86.17 ± 9.54 *	82.85 ± 7.61	83.25 ± 10.30
B.			
Control Limb			
Vastus lateralis cross-sectional area (cm^2)		Rectus femoris cross-sectional area (cm^2)	
PRE	POST	PRE	POST
17.05 ± 3.09	16.84 ± 3.59	6.17 ± 1.44	5.95 ± 1.42
Vastus lateralis echo intensity (AU)		Rectus femoris echo intensity (AU)	
PRE	POST	PRE	POST
86.13 ± 10.47	84.26 ± 9.25	83.95 ± 10.90	83.38 ± 12.21

Figure 1

Use of crutches during knee joint immoblization

An example of a participant being instructed on proper use of crutches in navigating the community.

Figure 2

Changes in Muscle Quality During Limb Immobilization

Individual participant data showing changes in echo intensity for the vastus lateralis (A.) and rectus femoris (B.) of the immobilized limb. The thick, dotted lines correspond to the mean values. The mean +/- SD values are displayed for each muscle as well.
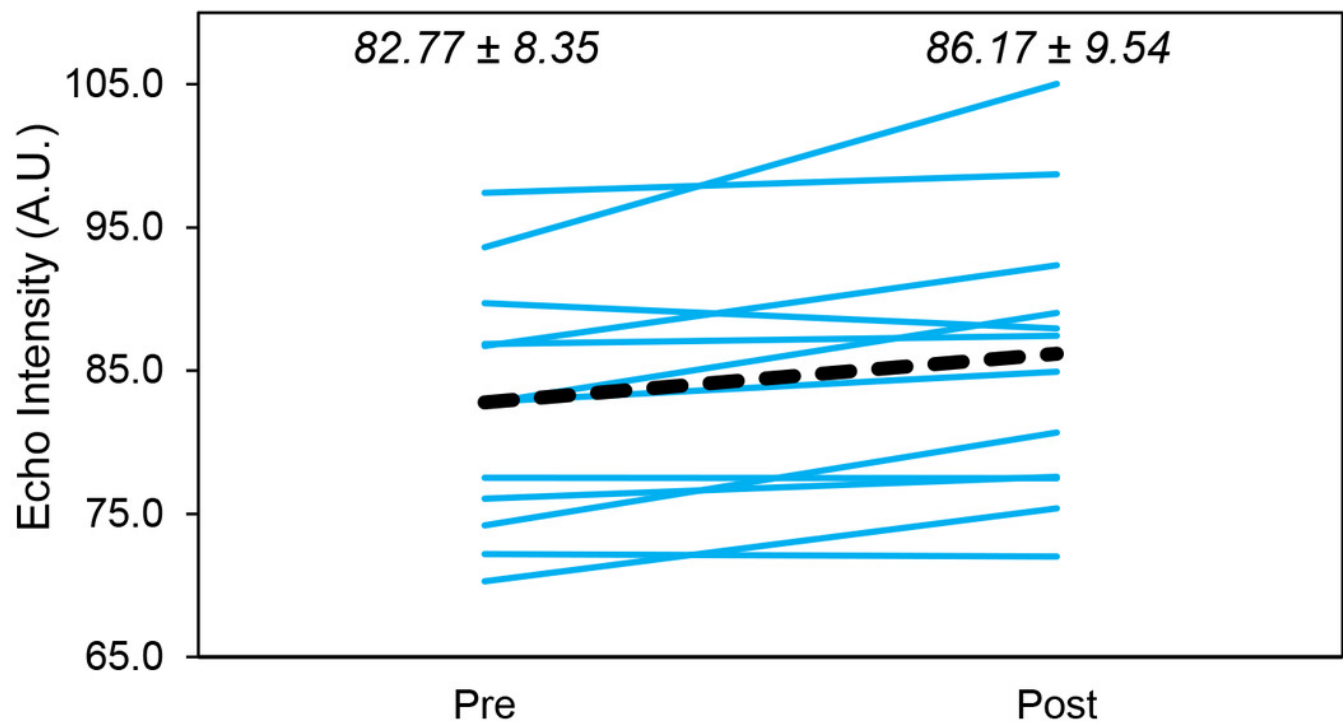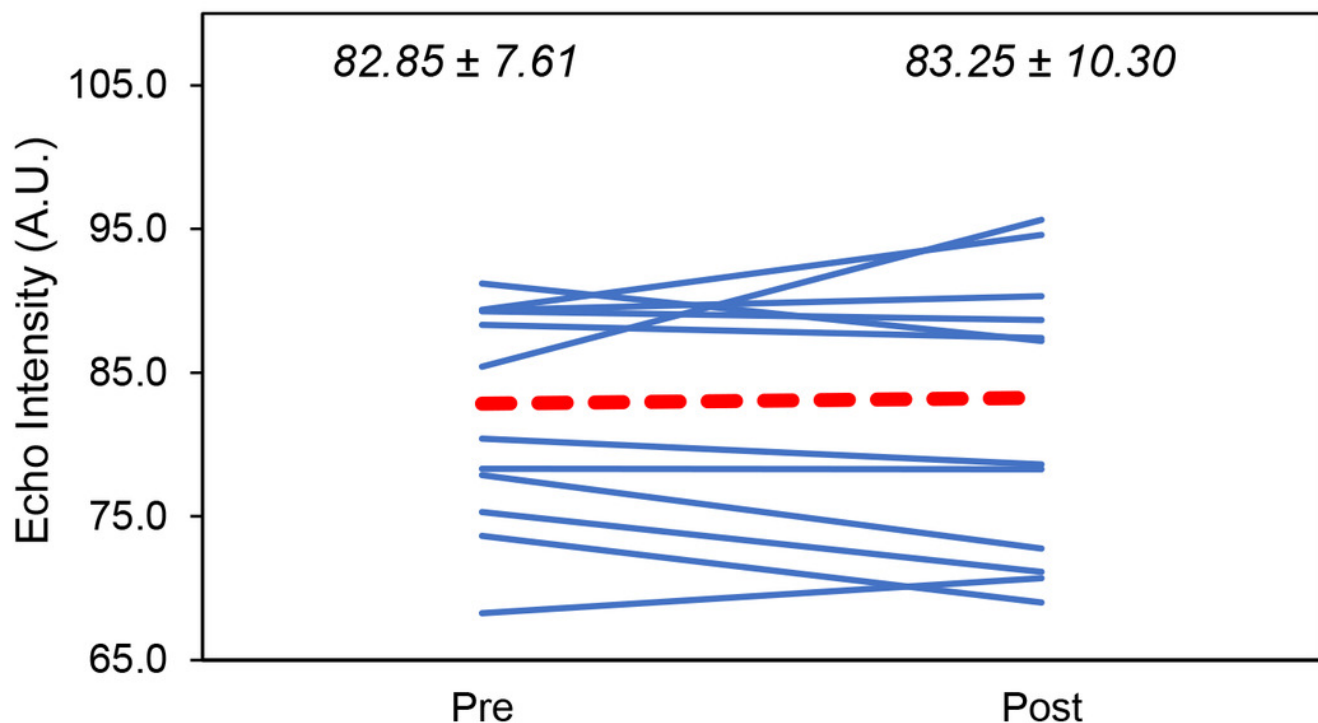
Figure 3

Changes in Muscle Size During Limb Immobilization

Individual participant data showing changes in cross-sectional area for the vastus lateralis (A.) and rectus femoris (B.) of the immobilized limb. The thick, dotted lines correspond to the mean values. The mean +/- SD values are displayed for each muscle as well.

A.

B.
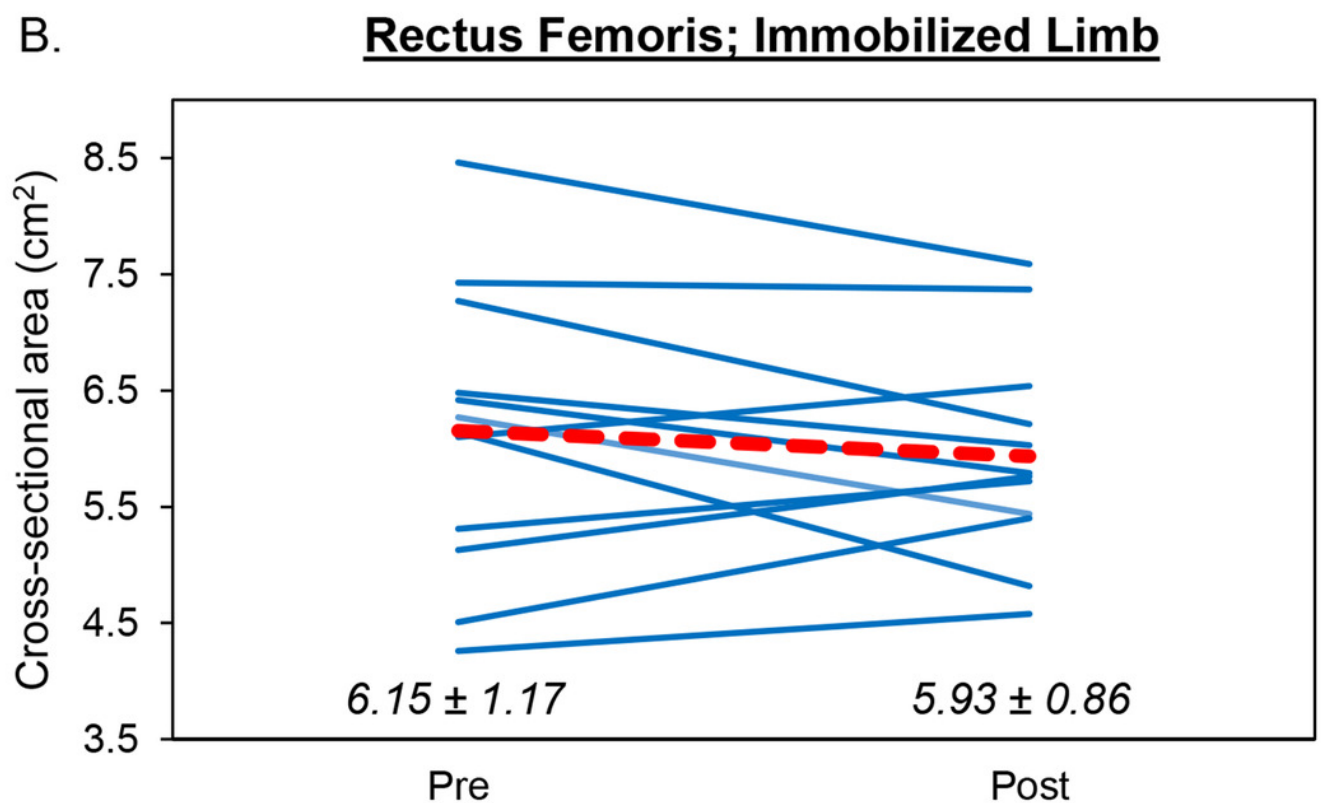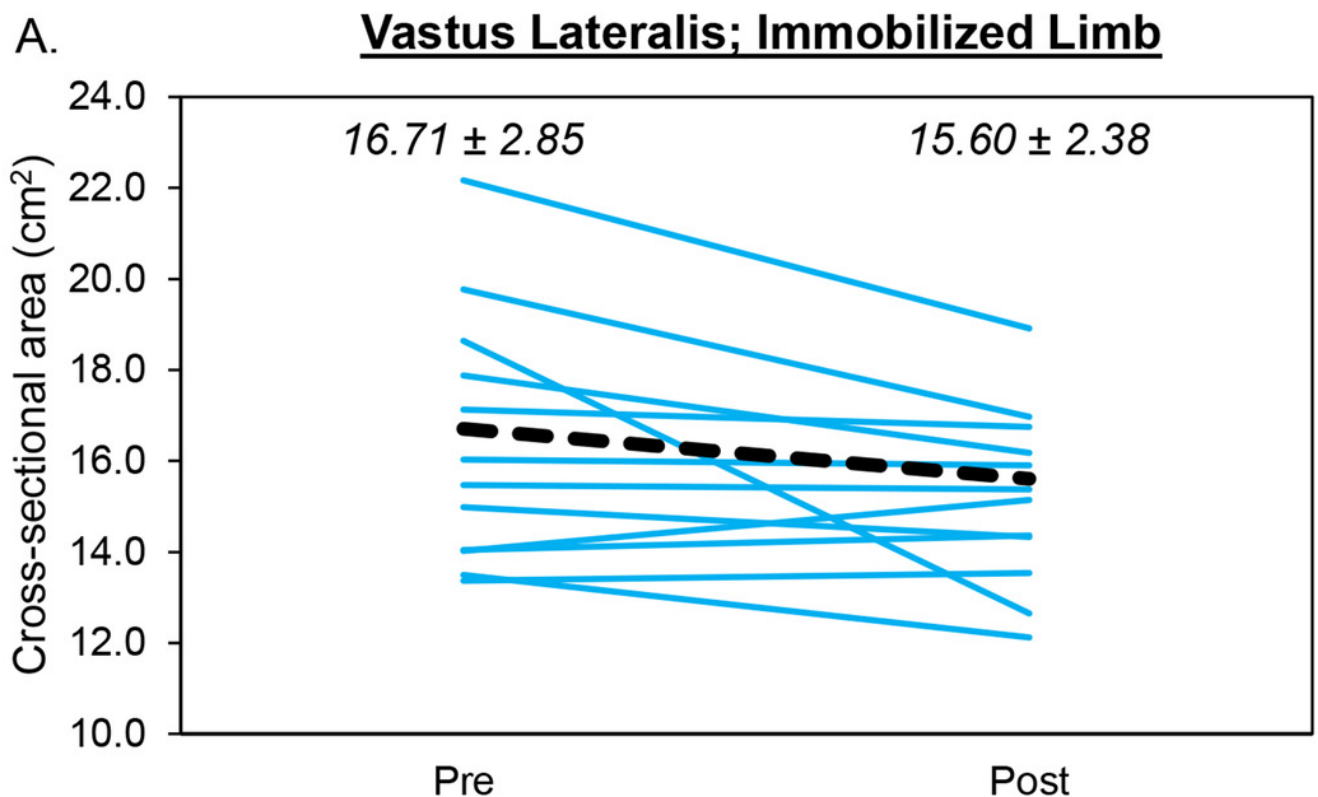
Figure 4

Significant Associations Between Changes in Muscle Quality and Size

Scatterplots displays the statistically significant correlations between changes in muscle cross-sectional area (CSA) and echo intensity (EI) for the vastus lateralis (A.) and rectus femoris (B.). It is important for the reader to keep in mind that changes in rectus femoris CSA and EI were small and likely not meaningful.

Vastus Lateralis

A.

$r = -.649, p = .016$

Rectus Femoris

B.

$r = .378, p = .202$