EFFECTS OF ACUTE SUPINE REST AND HYDRATION STATUS ON MID-THIGH MUSCLE SIZE AND QUALITY AS MEASURED BY ULTRASONOGRAPHY

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ABSTRACT

Nicholas William Shea: Effects of Acute Supine rest and Hydration Status on Mid-Thigh Muscle Size and Quality as Measured by Ultrasonography
(Under the direction of Eric D. Ryan)

Ultrasonography (US) has emerged as a method of assessing muscle size and quality. These measurements may be confounded by fluid shifts resulting from a change in position. The purpose of this study was to investigate the time course of fluid shifts and its influence on muscle size and quality measurements. Thirty-five males were recruited for the study. Ultrasound images and segmental bioelectrical spectroscopy assessments of the right thigh occurred after transition to supine and sequentially every five minutes, to 30 minutes. Subcutaneous fat and corrected echo intensity (EI) for the young and older men showed a significant age × time interaction ($P<0.05$). Changes in muscle size were noted after 20 minutes, whereas changes in corrected EI were seen after 10 minutes only in the older men. Our findings suggest that muscle size and quality US measurements should be taken within the first 10 minutes of supine rest.

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LIST OF ABBREVIATIONS

ATFSM	Appendicular adipose tissue-free skeletal muscle
BIA	Bioelectrical impedance
BIS	Bioelectrical spectroscopy
CSA	Cross sectional area
CT	Computerized tomography
ECW	Extracellular water
EI	Echo intensity
IAT	Intramusclular adipose tissue
ICC	Inter-correlation coefficient
ICW	Intracellular water
LDM	Low Density Muscle
MRI	Magnetic resonance imaging
NDM	Normal density muscle
Re	Extracellular water resistance
Ri	Intracellular water resistance
SFAT	Subcutaneous fat tissue
S-BIS	Segmental bio-electrical spectroscopy

SEM	Standard error of measurement
TBW	Total Body Water
TW	Total Water
US	Ultrasonography

CHAPTER I

INTRODUCTION

Assessing muscle size and quality *in vivo* is commonly used to determine the effectiveness of various exercise and nutritional interventions[1-3] and the influence of various clinical conditions on muscle function.[4,5] For example, previous studies have demonstrated that resistance training,[6,7] aging,[8,9] unweighting,[10] and disuse,[11] result in significant changes in muscle size. Sarcopenia, the age associated decrease in muscle mass, has been estimated to cost $18.5 billion annually in healthcare expenses.[12] More recently, muscle quality, (i.e. a measure of lean tissue composition and density), has also been shown to influence muscle function independent of muscle size.[13,14] For example, Goodpaster and colleagues[15] found that initially well-functioning older adults lost three times as much strength than muscle mass over three years, thus underscoring the importance muscle quality has in maintaining strength. Another finding from these authors[15] showed that the maintenance or even increases in lean mass of older individuals did not necessarily prevent decreases in strength; therefore, it may be assumed that muscle quality is a critical variable in determining strength in older adults.

Historically, computerized tomography[16] and magnetic resonance imaging (MRI) have been used to measure muscle size and quality.[17,18] The high contrast observed between tissues of different molecular properties make MRI an attractive method for determining muscle size and quality[19] while CT assessment speed may make it a desirable method for assessing similar variables.[14] In a recent study, Mitsiopoulos and colleagues[20] compared MRI and CT in

measuring human cadavers for appendicular adipose tissue-free skeletal muscle, interstitial adipose tissue, and subcutaneous fat (SFAT) tissue. These authors[20] concluded that both MRI and CT are reference methods for appendicular and whole body skeletal muscle assessments.[20]

Although accurate, CT and MRI assessments require expensive equipment, can expose participants to ionizing radiation,[16] and may not be readily available.[2,21] Ultrasonography (US) has recently emerged as a method of assessing muscle size and quality due to its portability, patient safety, and is low cost.[2,21,22] Ultrasound operates by emitting and receiving sound waves that are reflected from tissues and processes the received waves to digital images.[23] Initial US technology limited researchers to a narrow field of view;[19] however, more recent advancements in panoramic US imaging have enabled a larger field of view.[1] Many recent studies have demonstrated that panoramic US imaging provides reliable measures of both muscle size and quality, even when measuring highly curved surfaces.[21,24] Furthermore, a recent study by Athtiainen and colleagues[1] demonstrated that panoramic US cross sectional area (CSA) values showed good repeatability with an intraclass correlation coefficient of 0.905 and a standard error of measurement of 0.87 cm^2 in the vastus lateralis (VL) muscle. In the same study, VL changes were tracked over 21 weeks of resistance training. Ultrasound showed a high degree of validity when compared with MRI in tracking changes in VL CSA (ICC of 0.929, SEM of 0.94 cm^2).[1] More recent studies[22,25,26] have focused on using US imaging as a method to examine muscle quality from echo intensity (EI). Echo intensity can be used as an index of muscle quality obtained through quantitative gray-scale analysis. Increases in intramuscular fat and connective tissue result in higher echogenicity and subsequently whiter images,[27] which are suggested to represent decreased muscle quality.[25] The accuracy of US EI values in assessing muscle quality, has improved with the establishment of calibration equations developed by Young and

colleagues[22] which correct for SFAT thickness. Young et al.[22] found strong correlations (r = 0.91 for rectus femoris, r = 0.80 for biceps femoris and tibialis anterior) between MRI percent fat and SFAT corrected EI values. A study by Akima and colleagues[26] compared US with MRI in detecting intramyocellular lipids (IMCL) and extramyocellular lipids (EMCL). The authors found US EI values were related to EMCL (r = 0.658) but not IMCL (r = 0.341) content in the biceps femoris; suggesting that increased EI is due to the adipose tissue located outside the muscle cells.[26]

Fluid shifts have been shown to occur in thigh muscle CSA and SFAT[28] after 15 minutes[29] and 20 minutes[28] of supine rest. Bioelectrical impedance (BIA) was used to confirm fluid losses in the thigh and lower leg after one-hour of bed rest with decreases of 2.1% and 9.2% respectively.[28] Muscle quality has also been reported to be affected by changes in position as Berg and colleagues[28] reported an increase in radiological density in the lower leg of 2.9% after one hour of bed rest; however, there was no change in radiological density after 20 minutes of supine rest. Cerniglia and colleagues[29] reported a 1.6% decrease in thigh specific normal density muscle (NDM) measured by CT from 5 to 15 minutes of supine rest. In addition, recent studies[30,31] have suggested that aging results in a relative expansion of extracellular water (ECW) to intracellular water (ICW) ratio using segmental bioelectrical spectroscopy (S-BIS). Thus, it is possible that the time course in fluid shifts may be different in older adults.

To our knowledge, no previous studies had determined if the fluid shifts that occur from the standing to supine position influences US-derived muscle size and quality. The purpose of this study was to determine the posture induced muscle size and quality changes in both young and older populations that may occur from fluid shift changes.

SIGNIFICANCE

The size and quality of quadriceps muscles are important as they are independent predictors of strength[15] and functional performance,[32] and decrease with aging.[15] The significance of this study was in the functional properties and measurements of the quadriceps and more specifically the VL as a muscle pivotal for walking[33] and activities of daily living.[34-36] As fluid shifts have shown to alter assessments of muscle size and quality after a change in posture, it was important to establish a time frame to assess these measures using ultrasonography.

RESEARCH QUESTIONS

1. At what time point do measurements of muscle size and quality decrease due to fluids shifts occurring after a change from standing to supine position?
2. Does aging influence the time course of changes in muscle size and quality when compared to the younger population?

INDEPENDENT VARIABLES

1. Time
2. Age

DEPENDENT VARIABLES

1. VL muscle CSA
2. VL muscle EI
3. Thigh ICW
4. Thigh ECW

LIMITATIONS

1. Participant recruitment took place in the local community and university; therefore, recruitment was not completely random.

DELIMITATIONS

1. Participants were healthy and not highly active.

CHAPTER II

REVIEW OF LITERATURE

MUSCLE CROSS-SECTIONAL AREA

Skeletal muscle size is often reported as the anatomical cross-sectional area (CSA) of a given muscle at a specific landmark (i.e. half the distance of the limb). Muscle CSA is considered an important measure of function and performance and numerous studies have examined the influence of various interventions and conditions on CSA.[7,9,37] Changes in muscle size can be influenced by a number of factors, some of which include: training,[6] aging,[8] and disuse.[11] For example, Hakkinen and colleagues[6] reported that leg extensor muscles CSA increased in both middle-aged and elderly subjects (men age 40 yrs 4.9 ± 2.5% and women age 40 yrs 9.7 ± 2.5%) after the combination of a six-month heavy resistance and explosive exercise training program. The influence of age on muscle CSA has been well documented as previous authors[9,37] have reported 25-35% reductions in muscle CSA in older men and women when compared to younger control groups. Prolonged disuse has been shown to play a significant role in the reduction of muscle CSA by Gibson and colleagues[38] who reported a decrease in the calculated size of thigh muscle of 8.3 ± 3.1% between uninjured and injured/immobilized legs. Furthermore, LeBlanc and colleagues[16] showed that the muscle size of quadriceps and hamstrings measured by MRI decreased by 16-18% after 17 weeks of bedrest.

MUSCLE QUALITY

Muscle quality has traditionally been examined as the ratio of strength per unit of muscle size.[39] Many previous studies have demonstrated that in older adults, the rate of strength

loss (dynapenia) occurs much faster than the rate of reduction in muscle size (sarcopenia),[15] which may suggest that the quality of skeletal muscle is impaired. Older and obese populations have been proposed to have decreased muscle quality.[40-42] The decreases in muscle quality associated with aging and obese populations may be due to lower physical fitness and the infiltration of non-contractile tissue (i.e. adipose and connective tissue) into skeletal muscle tissue.[17,43-46] In a study performed by Ryan and colleagues[41] the investigators found positive correlations between age and mid-thigh low density lean tissue ($r = 0.58$ $P < 0.001$) and percent body fat and mid-thigh low density lean tissue ($r = 0.53$ $P < 0.001$).

Muscle quality is thought to be a meaningful indicator of overall muscle function, independent of other variables such as muscle size, especially in older adults.[15] Visser and colleagues[46] found that individuals with more skeletal muscle fat and fibrous tissue infiltration showed decreases in lower extremity performance as measured by walking and standup-sit down tests. Decreases in strength have also been shown to be related to decreased muscle quality. In a classic study performed by Goodpaster and colleagues,[15] the investigators found that the decline in muscle strength occurred three times faster than the decline in muscle mass over the course of three years and that gains in body weight and lean body mass did not necessarily contribute to increased strength.

MEASURING MUSCLE SIZE AND QUALITY

Historically, CT[16] and MRI have been traditionally used to assess muscle size and quality. An early study performed by Engstrom and colleagues[47] found MRI measured muscle size within 7.5% of cadaver estimates, while CT tended to overestimate muscle size by 10-20%. The authors of this study concluded that MRI was highly reliable, although subjective interpretations of muscle boundaries are possible. The reliability of CT and MRI has also been

supported by Mitsiopoulos and colleagues[20] in measuring adipose tissue free skeletal muscle (ATFSM) in a cadaver study and reported a coefficient of variation of ~2% in both assessment techniques. Mitsiopoulos and colleagues[20] concluded that both MRI and CT are capable of detecting small differences or changes in soft tissue composition. The reliability of MRI in detection of muscle CSA has further been supported by Beneke and colleagues[48] who found a test-retest correlation of $r = 0.993$ while the coefficient of variation ranged from 0.69% - 5.74%. The authors[48] concluded that MRI was appropriate to quantify individual muscles independent of their size, location and neighboring tissue. Furthermore, Mitsiopoulos and colleagues[20] found both MRI and CT measured ATFSM and cadaver values adipose tissue values to have a correlation of $r = 0.99$. Mitsiopoulos and colleagues[20] concluded that CT and MRI are valid methods for estimating appendicular ATFSM, IAT, and SAT in vivo. Sensitivity of MRI in detecting changes in muscle size has been supported by Hudelmaier and colleagues[49] who observed changes in individual muscle morphology after an endurance training or strength training intervention. The strength training intervention group experienced an increase in muscle volume in the extensors (+3.1%), flexors (3.5%), and adductors (3.9%), while the endurance training group experienced increases in muscle size of the extensors (+3.7%) and the sartorius (5.1%). Hudelmaier and colleagues[49] concluded that MRI can be effectively used to monitor location-specific effects of exercise intervention on muscle size.

Although accurate and reliable, MRI and CT technology requires expensive equipment, exposes patients to ionizing radiation and may be limited in accessibility.[2,21] Ultrasound (US) scanning may be an attractive alternative to MRI and CT to assess muscle size and quality due to its portability, patient safety, and lower financial obligation.[2,21,22] Historically US muscle size and quality assessments were limited to the muscle tissue accessible in the field-of-view for a

single scan. However, panoramic US imaging has the ability to overcome this shortfall by providing a simultaneous assessment of muscle size and quality in a relatively short acquisition time even among curved surfaces.[21]

Ultrasonography has been reported to be a reliable instrument in assessing muscle size. The test-retest reliability of measuring muscle CSA has been demonstrated by Rosenberg and colleagues[21] who reported an ICC value of 0.914 with SEM values of 5.8% on the medial gastrocnemius. Similar findings were reported by Lima et al.[50] who reported an ICC value of 0.877 and a coefficient of variation value of 8.9% when analyzing the rectus femoris muscle. When compared to gold standard imaging techniques, US has been strongly correlated with MRI measured CSA. For example, there was a strong relationship ($r = 0.90$) between US and MRI measured CSA of the adductor muscles[51] A disparity of 10 ± 4% has been reported between US and MRI and may be attributed to the processing algorithms of panoramic US.[52] The stitching of multiple US images may omit minute sections of individual images, resulting in a smaller final image[53] or from the exclusion of fasciae when analyzing US CSA images;[2] whereas in MRI and CT fasciae is not visible and may be included in determining muscle size. Other shortcomings of US may lie in the skill of the operator and that US imaging may not be applicable in all body regions.[54]

A recent study by Young et al.[22] demonstrated that SFAT corrected grayscale values from US images may provide an index of intramuscular fat. Young and colleagues[22] observed correlations between EI and MRI derived estimates of percent intramuscular fat in the rectus femoris ($r = 0.91$) and in the biceps femoris ($r = 0.80$). Furthermore, when comparing MRI and US estimates of muscle quality, Akima and colleagues[26] found strong correlations between EI and MRI derived estimates of extramyocellular lipids in the vastus lateralis ($r = 0.485$ and $r =$

0.506, respectively) and biceps femoris (r = 0.648 and r = 0.591, respectively); however, correlations between EI and MRI derived estimates of intramyocellular lipids of the vastus lateralis were not significant (r = 0.060). These authors determined that US derived EI values could be used as an index of intramuscular adipose tissue and that increases in EI are primarily due to extramyocellular lipids.[26] Strong test-retest reliability of US assessment of muscle quality has been found across multiple studies reporting ICC values of 0.720-0.917; while simultaneously reporting standard error of measurement values of 3.6%-4.1%[21,24] and coefficient of variation value of 5.2%.[55]

TIME COURSE FOR FLUID SHIFTS

Fluid content of skeletal muscle and adipose tissue is a significant component to tissue density and consequently muscle size and quality. Therefore, fluid shifts resulting from changes in posture have the potential to alter assessments of muscle size and composition.[29] Fluid shifts occurring in various regions of the body are generally dictated by posture due to the action of gravitational force.[28] A biphasic fluid shift has been shown to occur following the transition from a standing to supine position.[28,56] An initial rapid phase occurs as venous blood is being redistributed towards the heart from the lower limbs.[56] The second phase of the shift involves fluid moving from interstitial and intracellular spaces to venous capillaries due to decreases in fluid pressure on the venous side of capillary bed.[28,57]

Fluid shifts have been well documented in the literature. The argument for local extracellular fluid loss has been reported under conditions including weightlessness,[58] head-down tilt,[59] unweighting[10] and bed rest.[60,61] Hargens and colleagues[59] found that interstitial fluid pressure of the lower leg drops significantly during a five-degree head down body tilt procedure over the course of eight hours. This drop in pressure may be a driving force for fluid shift from

intracellular to extracellular fluid compartments. The reasons for fluid shift may be explained in large part by hydrostatic pressure. Hydrostatic pressure (HP) is an important factor for the circulatory system's efforts to maintain consistent cardiovascular pressure.[29,62] Hydrostatic pressure changes with postural changes and may also drive daily fluid shifts in the caudal region[63] resulting in the swelling of the foot and ankle. For example, Berg and colleagues[28] found a decrease in calf and thigh CSA after two hours of supine bed rest measured via CT. Simultaneously, limb water, as measured by BIA, was also reported to decrease, occurring twice as much in the calf vs. the thigh. This difference between calf and thigh may be due to greater hydrostatic pressures in the lower leg in the erect position.[28] Gibson and colleagues studied the fluid shifts from the transition from supine to standing position and found an ECW expansion after 30 minutes of standing.[64] The findings of this study are in agreement with Zhu and colleagues where the ECW expansion was attributed to redistribution of fluids held in the torso during supine towards the ECW of the limbs.[65] Nixon and colleagues[58] documented a significant fluid shift from the legs to upper body, using a five-degree head down body tilt for 24 hours. In a study performed by Hargens and colleagues,[59] the authors reported a local dehydration effect, over the course of eight hours, on the muscle and SFAT tissue of the lower leg measured by water displacement. Hargens and colleagues[59] concluded that tissue dehydration is greater in the early stages of head-down bed rest. Additionally, fluid shifts have been reported to decrease up to six percent after 30 minutes of five-degree head down bed rest, and a 10-19% from initial leg volume after 24 hours using five-degree head down bed rest.[58] As a part of the second phase of fluid shift, transcapillary fluid reabsorption can occur from muscle tissue ICW to the ECW compartment, and finally to local capillaries due to changes in hydrostatic pressure that result from moving from a standing to a supine position.[66] Decreases in ECW pressure, within muscle

and SFAT tissues of the lower leg, support the transcapillary fluid shifts occurring from ICW to ECW and eventually to capillaries.[59] Hargens and colleagues[59] have postulated that drops in capillary blood pressure in tissue below the heart is most likely the causative factor for extravascular fluid loss in simulated or actual weightlessness.

Larger fluid movements have been reported to occur nearly twice as much in the lower leg than the thigh.[56] Berg and colleagues[28] found a smaller movements of fluid (-1.6%) in the initial rapid phase of twenty minutes to occur in the thigh. This difference in fluid flux has been hypothesized to occur from a greater hydrostatic column and intra-capillary pressure in the erect position.[28] A decrease in HP could result in a reduction of capillary pressure, causing ICW to move out of the muscle cells towards the extracellular space and eventually into plasma inside the capillaries. Both fat and muscle tissue are subject to variation via fluid shifts. With water composing 75% of skeletal muscle[67] the loss of ICW can cause muscle cells to shrink and therefore decrease CSA measurements. Fat tissue, with its relatively low water content, has surprisingly been reported to have an equal or greater fluid flux than muscle. This was demonstrated by Berg and associates[28] who reported a four percent decrease in both thigh and calf fat CSA after two hours of bed rest; which was similar to the approximate four percent fluid efflux from muscle tissue. Furthermore, Cerniglia and colleagues[29] found a significant decrease in CSA of normal density muscle (NDM) after 15 minutes of supine rest but no decrease in low density muscle (LDM) CSA. This may be attributed to the anhydrous nature of LDM and a longer amount of time needed for fluid to efflux from such tissue.[68] Therefore, higher amounts of intramuscular fat and connective tissue may increase the time needed for fluid to move from ICW to ECW.

This may underlie the importance of collecting an US scan quickly after the transition from standing to supine in lean populations as they hold more water in ICW and thus have greater potential for fluid efflux. To the current investigator's knowledge the time course of fluid shifts measured by US resulting from changes in posture was previously undetermined.

AGE RELATED DIFFERENCE IN EXTRACELLULAR WATER AND INTRACELLULAR WATER

Bioelectrial impedance spectroscopy (BIS) has been reported to distinguish the differences between ICW and ECW.[69] Bioelectrical impedance spectroscopy uses a spectrum of frequencies typically ranging from 5 kHz to 100 kHz. In assessing ECW, it is hypothesized that the lower impedance frequencies (i.e. 5kHz) are better at detecting ECW, while higher frequencies (i.e. 100 kHz) are better at detecting TBW.[70,71] When the resistance to current data is modeled in a Cole-Cole plot (resistance vs. reactance over the frequency spectrum), the resistance of ICW (Ri) and ECW (Re) can be determined, and specific volumes of each can then be calculated.[69] Because BIS operates on the cellular level, where the body is divided into extracellular fluid, extracellular solids, and body cell mass, it is able to differentiate fluctuations in ECW and ICW.[69] Bioelectrical impedance spectroscopy can also be used to determine total body water and total water volumes of specific limbs, if the limb is cylindrically shaped.[69] Segmental Bioelectrical spectroscopy has been demonstrated to be a valid tool in in monitoring fluid changes in segmental limbs during position changes having strong correlations with MRI muscle volumes of the calf ($r = 0.93$) and arm ($r = 0.96$);[65,69,72] while the thigh measured by the ImpediMed SFB7 BIS has been supported to be relatively reliable with a relative error percentage range of 3.63% at 5 kHz to 26.63% at 50kHz.[73]

Muscle size is comprised of both ECW and ICW compartments.[30,74] An age-related increase in the whole body ECW: ICW ratio has been reported using BIS, chemical dilution, whole-body counting, or neutron activation analysis.[30,75-77] Yamada and colleagues[30] observed a relative expansion of ECW in the lower leg in older adults when compared to the younger populations, when using S-BIS estimations. These authors concluded expansions of ECW may confound the measurement of muscle size. However, the retention and increases of ECW may occur concurrently with muscle atrophy and/or declines in body composition, as higher ECW:ICW ratios have been reported in the limbs of quadriplegic and obese participants when compared to healthy individuals.[69] This change in ECW:ICW distribution can be attributed to the composition of skeletal muscle and adipose cells. Adipose tissue has a ECW:ICW ratio of 3.5:1 grams compared to skeletal muscle which has an ECW:ICW ratio of 0.42:1 grams.[77-79] Therefore, the infiltration of adipose tissue into skeletal muscle may explain the increase ECW:ICW ratio of skeletal muscle in older and diseased populations.[77,80] Furthermore, Chamney and colleagues[74] found that normal hydrated adipose tissue was reported to have a much lower water content but higher ECW:ICW ratio compared to normal hydrated lean tissue, using chemical dilution techniques. When normal hydrated lean tissue dominates body weight, whole body ICW tends to be in higher proportion than whole body ECW. The opposite has been shown to hold true; that is when normal hydrated adipose tissue dominates body weight lower values in whole body ICW:ECW are observed meaning expansions of the ECW may indicated increased body fatness and/or muscle atrophy.[74,81]

HYDRATION STATUS AND INFLUENCE OF FLUID SHIFTS

Hypo- and hyperhydration can seldom occur without concurrent changes in ECW, ICW, and electrolyte content.[82] Hypo- and hyperhydration refer to body fluid deficit and fluid excess,

respectively.[83] Changes in electrolyte content can confound BIS measurements for estimating fluid changes.[82] Euhydration is more typically reflected by higher ECW volume; while ICW is more reflective of body cell mass which may increase due to increases in intracellular dry matter associated with resistance training[84] and decrease due to aging, malnutrition, and tissue wasting diseases.[85,86] Certain hydration states or methods of hydration can manipulate the ECW:ICW ratio such as, hypertonic hypohydration or hypernatriema which results in increases in plasma osmolality, sodium, and chloride, urine osmolality, and specific gravity of urine.[69] During hypertonic hypohydration, the relatively greater water loss compared to solute can result in a redistribution of fluid from ICW to ECW.[87] In a study by O'Brien and colleagues,[88] estimates of ICW and ECW decreased due to isotonic hypohydration, where both water and solutes are lost, were underestimated by BIS when compared to isotope dilution technique. Mixed data has been reported in studies using BIS to estimate hyperhydration through water or ionic rehydration beverage ingestion at a euhydrated state. Gomez et al.[89] reported initial (3-5 min) decreases in ICW and ECW estimates after ingestion of three percent body weight in either water, hypotonic fluid, or isotonic fluid; however, after 90 minutes post fluid consumption only water and hypotonic fluid groups showed increased estimates of ICW and ECW. These initial decreases in estimated water values have been attributed to posture induced (standing to supine) redistribution of body fluids from the limbs to the trunk region; therefore, emphasizing the importance of time needed for BIA to detect changes in ICW:ECW after ingestion of fluids.[90] The studies listed above suggest that BIA and BIS are better at assessing hydration in a "steady state"[91] and that neither method is sufficiently accurate in estimating TBW in fluctuating conditions.[82] Therefore, it is of critical importance to control for hydration and hydration methods when estimating body composition or TBW.[82]

CHAPTER III

METHODOLOGY

PARTICIPANTS

Thirty-eight male participants were enrolled in the study while thirty-five participants participated in the study; including 23 young participants (demographics listed in table 1) and 12 older adults (demographics listed in table 1). This age range was chosen because older adults are defined as 65 years of age or older[92] and the strength and performance relationship is altered at age 75.[35,93] Participants were recruited via the local university and surrounding communities (i.e. Chapel Hill, Carrboro, etc.). Participants were excluded if they had sustained a neuromuscular or metabolic disease, if they had a current or recent (previous three months) lower extremity joint or muscle problem, had gained or lost 20 pounds in previous two months, or exercised over three hours per week. Interested participants were screened on the phone to ensure potential participants fit inclusion and exclusion criteria before visiting the lab for testing. Using data from our lab and data reported by Berg et al.[28] to detect between and within group differences in muscle quality [effect size (ES): 0.366][94,95] and muscle size (ES: 0.22), we needed a sample of 14-32 participants respectively, to provide 80% power at a 5% significance level.[96]

EXPERIMENTAL DESIGN

At the start of testing session participants were asked to refrain from vigorous exercise for 48 hours and arrive to the laboratory following an eight hour fast.[66] Upon arrival,

Participants were asked to read and sign an informed consent form stating the risks and benefits of the experimental procedures and completed a health history and exercise status questionnaire. During the testing sessions participants had their body stature and mass to ensure participants meet the BMI category, followed by a measurement of specific gravity of urine for hydration assessment. Seven measurements of thigh TW, ECW, ICW and ultrasound derived CSA and EI measurements were taken initially after lying down and sequentially every five minutes during a 30-minute supine rest period. Data collection took place during week day mornings, typically starting between 8-9AM. Therefore, participants had limited standing, walking and physical activity prior to supine muscle size and quality measurements.

SPECIFIC GRAVITY OF URINE

To account for hydration status, urine specific gravity was measured directly after the completion of paperwork. Urine specific gravity was measured using a clinical handheld refractometer (Model 1.33Ade Advanced Optics, Oregon city, OR).[66] A few drops of urine were placed on the stage of the refractometer and held under a light source which passed through urine for measurement of specific gravity.[97]

ULTRASONOGRAPHY

The VL CSA and EI were assessed in the supine position with the right knee supported at 50 degrees of flexion. Ultrasound measurements were taken immediately after the transition to supine position and sequentially every five minutes thereafter, for thirty minutes for a total of seven measurements. Measures of CSA and EI were assessed using a portable brightness mode (B-mode) US imaging device (LOGIQ e 6, General Electric Company, Milwaukee, WI, USA) and a multi-frequency linear-array probe (12L-RS; 5-13 MHz; 38.4 mm FOV) (General Electric

Company, Milwaukee, WI, USA). The musculoskeletal mode with standardized settings for gain (56 dB), depth (6 cm), and frequency (10 MHz) were used to optimize image quality and the settings remained consistent across all subjects. All ultrasound measures were assessed with the panoramic (extended field of view) function.

A single scan of the VL was conducted perpendicular to the longitudinal axis of the thigh at half the distance between the greater trochanter and the lateral epicondyle of the femur.[98] Transmission gel was applied to the skin on the thigh to enhance acoustic coupling.[98] A custom foam pad was applied to the thigh to ensure movement of the probe remained consistent in the transverse plane.[21] Panoramic US scans were performed starting from the lateral aspect of the thigh moving medially, across the entire VL. Care was taken to ensure steady speed of US probe and consistent application of mild pressure to the skin without muscle compression.

IMAGE ANALYSIS

Image-J software (version 1.46r, National Institutes of Health, USA) was used to analyze all VL US images. Images were first scaled from pixels to centimeters using the straight-line function and then the polygon function was used to assess muscle CSA and quality by outlining the region of interest that included as much as the muscle as possible without the surrounding fascia. Muscle quality was determined from the mean EI values using computer aided gray-scale analysis ranging from 0-255 arbitrary units (A.U.; black = 0, white = 225).[21] Echo intensity values were corrected for SFAT thickness by using the equation: (40.5278 x SFAT average) + raw EI = corrected EI, outlined by Young and colleagues.[22] Subcutaneous fat was measured using the straight line function and drawing a line from directly beneath skin to superficial muscle aponeurosis at three different locations of the muscle including the most medial border,

the 50% distance point, and the most lateral aspect of the VL. The average of these three measurements was taken to calculate SFAT as described by Ryan and colleagues.[99]

REGIONAL FLUID COMPARTMENTS

A multi-frequency BIS (SFB7, ImpediMed, Queensland, Australia) was used to determine TW per the procedures[100] recommended by the manufacturer. The BIS device uses a four-electrode system where the first pair of electrodes emits a constant current while the second pair receives voltage fluctuations dependent on body resistance.[101] Emitting (red and black) electrodes were placed 10cm distally to the anterior superior iliac spine and 10cm proximally from the tibial tuberosity, respectively. Sensing electrodes (yellow and blue) were placed five cm distal from the red electrode and five cm proximal from black electrode, measured center to center of electrodes.[70] All measurements and electrode placements were performed prior to the transition from standing to supine position.

Bioelectrical spectroscopy began immediately after transition to supine position and every five minutes, up to 30 minutes after the transition to supine in order to detect any fluid shifts that may have occurred with the change in position (Figure 1). Regional measurements of ECW and ICW were calculated using ImpediMed multi-frequency software to derive whole-body resistance of intracellular water and whole-body resistance of extracellular water values. Extracellular resistance, intracellular resistance and thigh length values were imputed into proprietary ImpediMed equations used for calculating regional ECW and ICW. The ImpediMed segmental fluid compartment equations that were used: $\text{Volume}_{ECW} = \rho ECW^{2/3}/3(4\pi)^{1/3} * L * (C_1^2 + C_2^2 + C_1 + C_2) * (L/C_1 * C_2 * R_E)^{2/3}$ and Volume of ICW was calculated using the following two equations: $\rho TW = \rho ICW - (\rho ICW - \rho ECW) * (R_I/R_E + R_I)^{2/3}$ and $\text{Volume}_{ICW} = V_{ECW} * \{(\rho TW * (R_E+R_I)/\rho ECW * R_I)^{2/3} - 1\}$ and $TW_{Volume} = V_{ECW} + V_{ICW}$, where L is the length

between the center blue and yellow (lead) electrodes, R_E is the resistance to extracellular water, C_1 is the circumference of the thigh at the blue electrode while C_2 is the circumference of the thigh at the yellow electrode, R_I is the resistance to intracellular water, ρICW and ρECW are male and female dependent value constants: ρICW = 191.63 (male), 185.35 (female) and ρECW = 49.77 (male), 47.91 (female), C_1 is the circumference of the blue lead electrode and C_2 is the circumference of yellow lead electrode.[65,69,102]

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS version 21.0 (SPSS, In., Chicago, IL, USA). All descriptive data are presented as mean ± SD. Potential differences in demographic and hydration status were examined using an independent samples t-test. Multiple 2 x 7 mixed factorial analysis of variance (ANOVA) was used to analyze the changes in muscle CSA, muscle EI, ECW and ICW that may have resulted from a change in position across seven time points (0 min, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min) and between the two age groups. An alpha level was established a priori $P<0.05$. If significance is found, a Tukey HSD post-hoc test was implemented as post-hoc analysis between significant data points to determine between data differences.

CHAPTER IV

RESULTS

All demographic and USG values are presented in Table 1. There was a significant difference in age (P<0.001) and BMI (P=0.04). However, no differences were observed for stature (P=0.540), weight (P=0.490), or USG (P=0.265).

VASTUS LATERALIS CROSS-SECTIONAL AREA

For VL CSA, there was no significant interaction (P=0.130); however, there was a main effect for time (P=0.006) and young compared to old (P<0.001). Muscle CSA was greater for the young (25.3±3.6 cm^2) vs older men (17.2±3.1cm^2). (P<0.001) and CSA was greater at 20 minutes when compared to 30 minutes (P=0.049).

SUBCUTANEOUS FAT

For SFAT, there was a significant interaction (P=0.007). SFAT was significantly greater in the younger man (0.66±0.29 cm) when compared to the older men at 25 (0.51±0.21 cm) (P=0.026) and 30 (P=0.046) minutes. In the older men, SFAT was greater at 15 minutes than 30 minutes (P=0.026), however there was no change in SFAT across time (P=0.283) for the young men.

UNCORRECTED ECHO INTENSITY

For the VL EI, there was no significant interaction (P=0.082). However, there was a main effect for time (P<0.001) and age (P<0.001). The raw EI values were greater at 5 minutes than

25 and 30 minutes (P=0.004-0.003) and 10 minutes was greater than 15, 20, 25, 30 minutes (P≤0.045-0.001). The young raw EI values (53.7±4.9 a.u.) were smaller than the older men (66.5±8.7 a.u.) (P<0.001).

CORRECTED ECHO INTENSITY

For corrected EI there was a significant interaction (P<0.001). Corrected EI was greater in the older men (90.8±15.1 a.u.) compared to the younger men (80.95±12.4 a.u.) at minute 10 (P<0.046). The younger men did not see a change in corrected EI over time (P=0.743); while corrected EI was greater at 10 minutes than 25 (P=0.008) and 30 (P<0.001) minutes in the older men.

THIGH ICW:TW

For the thigh ICW:TW ratio, there was no significant interaction (P=0.604), however, there was a main effect for age (P<0.001) and time (P<0.001). The thigh ICW:TW ratio was greater in the young men when compared to the older men (P<0.001). The thigh ICW:TW ratio at minute 0 was less than 10, 15, 20, 25, and 30 minutes (P<0.001), the 10 minute measurement was less than 20, 25, and 30 minutes (P<0.001), the 20 minute measurement was less than 25 and 30 minutes (P≤0.046-0.001), and the 25 minute measurement was less than 30 minutes (P=0.001).

THIGH ECW:TW

For the thigh ECW:TW ratio, there was no significant interaction (P=0.529), however, there was a main effect for age (P<0.001) and time (P<0.001). The thigh ECW:TW ratio was less in young men when compared to the older men (P<0.001). The thigh ECW:TW ratio at 0 minute was greater than 10, 15, 20, 25 and 30 minutes (P<0.001), the 10 minute measurement

was greater than 20, 25 and 30 minutes (P<0.001), the 20 minute measurement was greater than 30 minutes (P=0.004) and the 25 minute measurement was greater than 30 minutes (P<0.001).

CORRELATIONS AMONG VARIABLES

Given the majority of changes for the aforementioned variables occur between 10 and 30 minutes, follow-up analyses examined the relationship between the changes in each respective variable using Pearson's correlation coefficients. The change in CSA was not related to the change in ICW:TW (r=0.245; P=0.156) or ECW:TW (r=-0.088; P=0.614). The change in raw EI was not related to the change in ICW:TW (r=0.260; P=0.131) or ECW:TW (r=-0.130; P=0.457). The change in corrected EI was not related to the change in ECW:TW (r=0.004; P=0.984), but was related to the change in ICW:TW (r=0.356; P=0.036). Furthermore, the change in corrected EI was related to the change in raw EI (r=0.544; P<0.001) and the change in SFAT (r=0.799; P<0.001). Hydration status as measured by USG was not related to the change in raw EI (r=0.042; P=0.816), the change in corrected EI (r=0.006; P=0.972), the change in SFAT (r=0.036; P=0.844), nor the change in CSA (r=-0.103; P=0.569).

CHAPTER V

DISCUSSION

The primary findings of the current study indicated that both young and older men experienced a similar time dependent decrease in muscle CSA induced by a supine rest (Figure 3). The VL CSA remained stable for the first 20 minutes of rest and then decreased at 30 minutes. The corrected EI values demonstrated an age-specific response with the young men experiencing no change over the course of 30 minutes, whereas the older group experienced similar values that peaked at 10 minutes of supine rest and decreased 4.6-5.0% at 25 and 30 minutes (Figure 2). These changes may be influenced by similar responses in changes in SFAT (Figure 2).

The current study found that muscle CSA in the young men was 31% greater than the older men (Figure 3). Our data is in agreement with many previous studies[14,38,46,102,103] demonstrating sarcopenia of the quadriceps muscles. Our decreases in thigh CSA measurements were apparent at minute 30, with CSA at 30 minutes being less then CSA at 20 minutes (Figure 3). This data is in contrast with previous data; where decreases in thigh muscle CSA became apparent at 10, 15, and 20 minutes of supine rest.[28,29,95] The discrepancy between studies may reside in the physical activity differences in subjects between studies (Table 1), where our subjects were limited to 3 hours of exercise per week and previous studies recruited physically active subjects.[28] More specifically, our subjects engaged in a relatively low amount of physical activity. A low amount of exercise or physical activity may show a lower ICW:ECW ratio,

especially in the young groups, due to less myofibrillar protein content and metabolic enzymes.[105-107] Therefore, expansions of ECW may come from a lower ICW to ECW fluid efflux capacity.[29,68,77] Interestingly, in the current study both young and older groups had similar responses in muscle CSA to the time course of supine rest; which has been suggested to be related to decreases in hydrostatic pressure in the capillary bed, resulting in a central venous fluid redistribution.[28,56,108,109] However, it is unlikely that posture induced changes to ECW and ICW resulted to decreases to CSA (Figure 3 and Figure 4).

The current study reported an increase in corrected EI from the young to the older group, indicative of a decrease in muscle quality. The difference in corrected EI is similar to many previous studies that have reported increases in raw EI in older adults.[22,110-112] The current study utilized corrected EI as increasing subcutaneous fat amounts have been shown to reduce raw EI values due to the attenuation of ultrasound waves.[22] Nevertheless, the data is in agreement with previous literature showing increased corrected EI with age.[113] The age-related difference in corrected EI was ironically found in contrast with SFAT; where the younger men reported a 29% greater depth of SFAT than the older group (Figure 1) despite a slightly greater BMI (Table 1).[22] The discrepancy between BMI and SFAT depth between age groups may be indicative of an age associated decrease to muscle density and/or an inability to store adipose tissue in the lower body.[112,114-118] More specifically, older adults have shown a decrease in ability to store adipose tissue as SFAT in the lower body; where SFAT adipocytes may have a reduced ability to uptake circulating free fatty acids. The result of SFAT dysregulation may manifest itself with increased visceral fat and decreased muscle quality, while body weight may remain the same or even decrease.

Corrected EI remained relatively stable over the course of 30 minutes for the younger group; while the older group experienced changes over the initial 20 minutes with a peak occurring at 10 minutes of supine rest, which was 9.2-7.5% greater than the 25 and 30-minute time points, respectfully (Figure 2). Interestingly, the responses for raw EI values were similar between groups, with raw EI values peaking at 5 and 10 minutes and subsequent decreases of 4.3% to 30 minutes (Figure 3). These improvements in muscle quality measurements may be clinically significant, as previous studies have reported raw EI SEM values of 3.6%.[21] However, our data showed no change in SFAT across time for the young men, but a significant decrease in SFAT from 10 minutes to 25 and 30 minutes in the older men. Thus, it is possible that the significant changes to corrected EI observed in the older men group may be related to the changes in SFAT (r=0.799; Figure 5). The younger group's lack of posture induced change to corrected EI is in contrast to previous literature which demonstrated a 1.4-1.6% improvement in measurements of muscle quality.[28] For example, both Berg et al.[29] and Arroyo et al.[94] found improvements in muscle quality after 20 and 10 minutes, respectively. The current study's discrepancy in muscle quality measurements between age groups may be related to the changes to SFAT between age groups (Figure 2 and Figure 5). Furthermore, previous studies did not account for SFAT, which may have contributed to different time course changes to muscle quality observed between studies.[22,95] With decreased muscle quality, as seen with older adults, may come an expanded ECW:ICW ratio and therefore faster venous return.[46,57,107]

The change in raw EI (r=0.042), corrected EI (r=0.006), SFAT (r=0.036) and muscle CSA (r=-0.103) was unrelated to hydration status. One explanation for the lack of influence is the relatively tight control of water in muscle homeostasis.[68] For example, skeletal muscle holds the majority of its fluid content in the ICW and decreases in the ECW are the first to occur with

decreases in hydration.[119] Given that most of our subjects were relatively hydrated (Table 1) with most USG's < 1.030 and recommendations for hydration remaining below 1.030[120] combined with the "steady state" pre testing conditions of our participants; the lack of change across 30 minutes could be expected.[100] More specifically, fluid shifts have been shown to occur from ICW to ECW when changes to plasma sodium concentrations are induced, which can occur during exercise and water deprivation;[87,121] whereas the current study did not induce a hydration or dehydration stimulus that could provoke changes to muscle size and/or quality.

In summary, this study is in agreement with previous literature[9,15] in that measurements of muscle size were larger in the young men versus the older men. Muscle size responded similarly between groups to a change in posture, in that decreases in size were observed after 20 minutes of supine rest. Similar physical activity levels between subject groups may have played a role in the lack of fluid efflux observable via muscle size measurements. Corrected EI was higher in the older men (i.e. poorer muscle quality) compared to the young men, in agreement with previous literature,[122] despite having lower SFAT measurements. It is noteworthy that differences in corrected EI occurring after 10 minutes were seen in the older group but not in the young group. These changes across time in the older group may be due to the greater amount of adipose tissue in the muscle, which may contribute to changes in ECW. However, it is also possible that SFAT directly influenced the responses in corrected EI values across time between groups. Therefore, the timeframe of data acquisition for muscle quality may be different between young and older men, in that the older men may be limited to a 5-9 minute timeframe while measurements in young men are less affected by time. More research is needed to investigate how an age-related change in adipose tissue storage may influence measurements of muscle size and quality. Furthermore, based off the findings of this research and previous research,[28,29,95] it

is advised to take measurements of muscle size and quality within 5-9 minutes of supine rest to avoid changes in muscle CSA, raw EI, corrected EI, and SFAT, regardless of age. Due to time constraints, ease of assessment, and consistency of assessments, a 5-9 minute window of time for US assessments may be both applicable and reliable for a data collection setting.[123]

TABLES

Table 1. Mean ± standard deviation (SD) values for demographics and specific gravity of urine.

	Younger Men	Older Men
Age (years)*	21.35±2.53	69.8±83
Mass (kg)	70.92±7.64	72.9±8.33
Stature (cm)	177.59±5.87	176±6.77
BMI*	22.39±1.3	23.4±1.34
USG	1.031±0.037	1.018±0.011

* $P<0.05$, significant difference between the younger and older men

FIGURE LEGENDS

Figure 1. An example of thigh specific BIS. Placement of injecting electrodes (red and black) were determined from 10cm distal from ASIS and 10cm proximal from the tibial tuberosity. Sensing electrodes (yellow and blue) were placed 5cm from injecting electrodes measured from center to center of electrode.

Figure 2. Subcutaneous fat (A) and corrected echo intensity (B) for the young and older men across time. # Indicates a significant age × time interaction ($P<0.05$). * Indicates significant difference between age groups ($P<0.001$). + Indicates 15 min > 30 min ($P < 0.026$) in the older group. ++ Indicates 10 min > 25 and 30 min (P =0.008-0.001) in the older group.

Figure 3. Marginal means for raw echo intensity (A) across time. * Indicates main effect for time (5 min > 25 and 30 min; P=0.030 – 0.004). β Indicates main effect for time (10 min > 15, 20, 25, and 30 min; $P\leq$0.045 – 0.001). Marginal means for vastus lateralis cross sectional area across time (B). + indicates main effect for time (20 min > 30 min; P=0.049).

Figure 4. Marginal means for thigh ICW:TW ratios (A) and ECW:TW ratios (B) across time. *Indicates 0 min < 10, 20, 25, 30 minutes; 10 < 20, 25, 30; 20 < 25, 20; 25 < 30 ($P<0.046$). ** Indicates 0 min > 10, 15, 20, 25, 30; 10 min > 20, 25, 30 min, 20 min > 30 min, 25 min > 30 min; $P<0.001$).

Figure 5. Changes in young and older groups combined from 10 min to 30 min to corrected EI plotted against raw EI (A) (P<0.001) and SFAT (B) (P<0.001).

Figure 6. Changes in young and older groups combined from 10 to 30 min to ICW:TW (A) ($p<0.036$) and ECW:TW (B) ($p<0.983$).

Figure 1

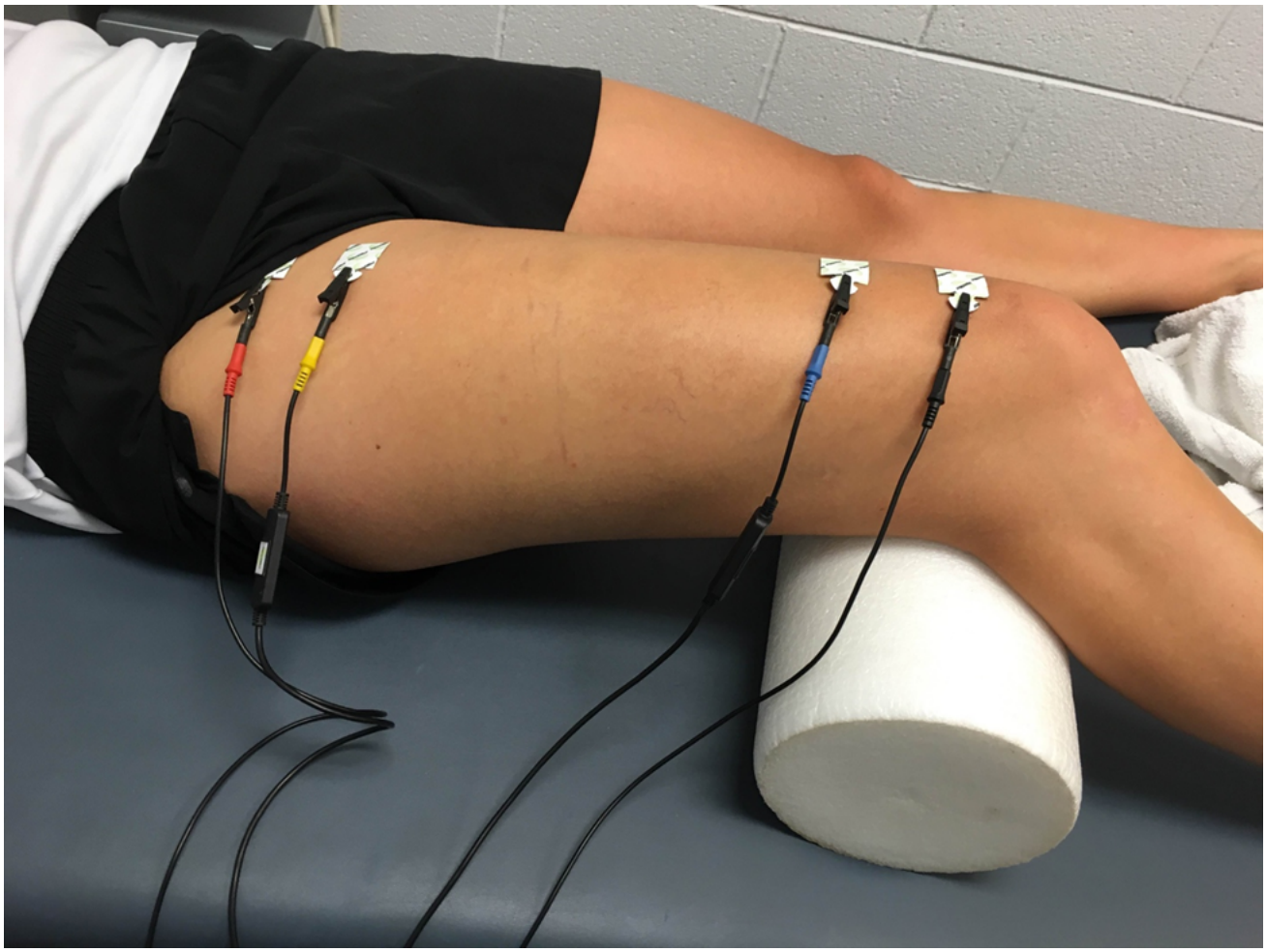


Figure 2

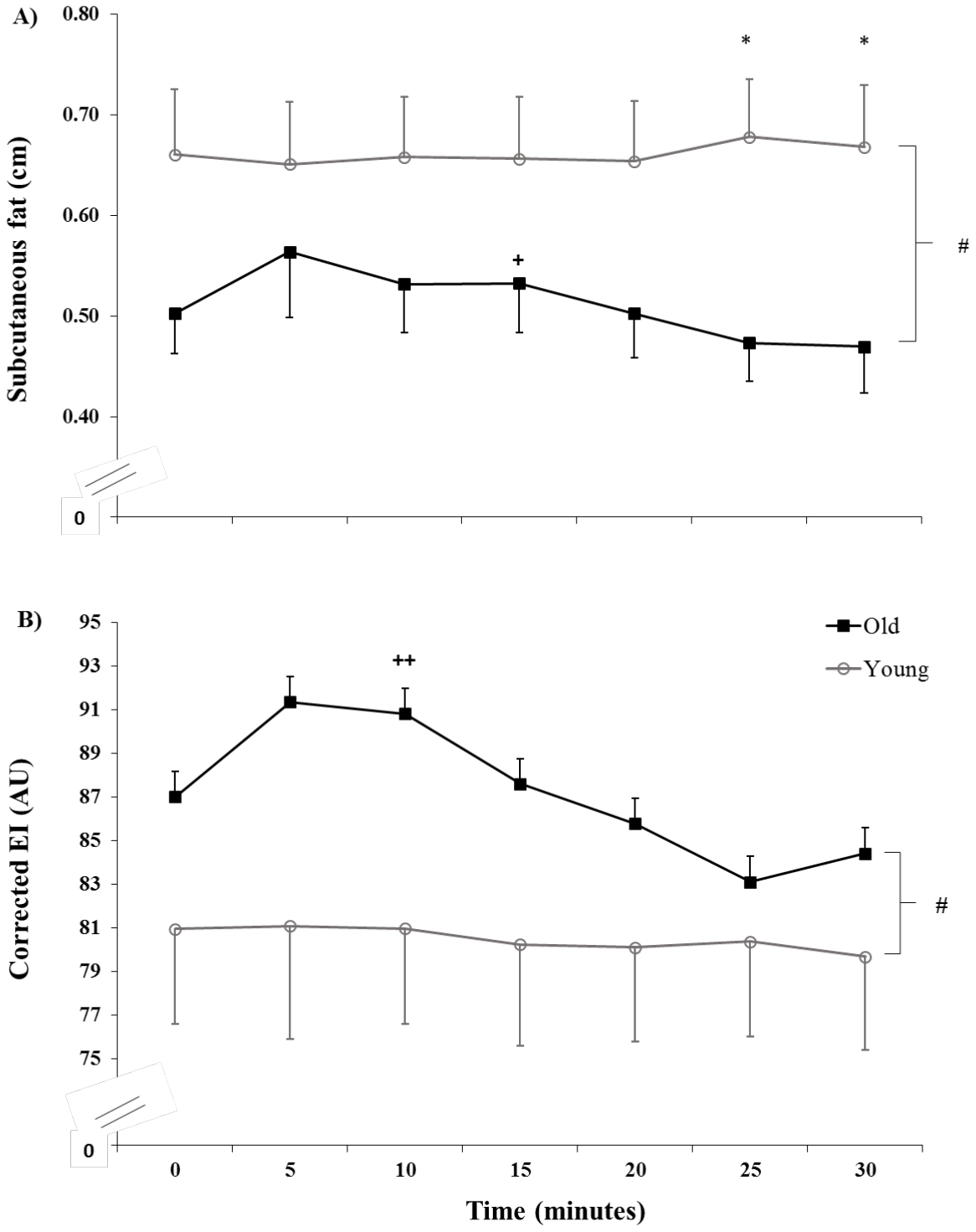

A)
Subcutaneous fat (cm)
0.80
0.70
0.60
0.50
0.40
0
*
*
+
#
B)
Corrected EI (AU)
95
93
91
89
87
85
83
81
79
77
75
0
++
#
Old
Young
0
5
10
15
20
25
30
Time (minutes)

Figure 3

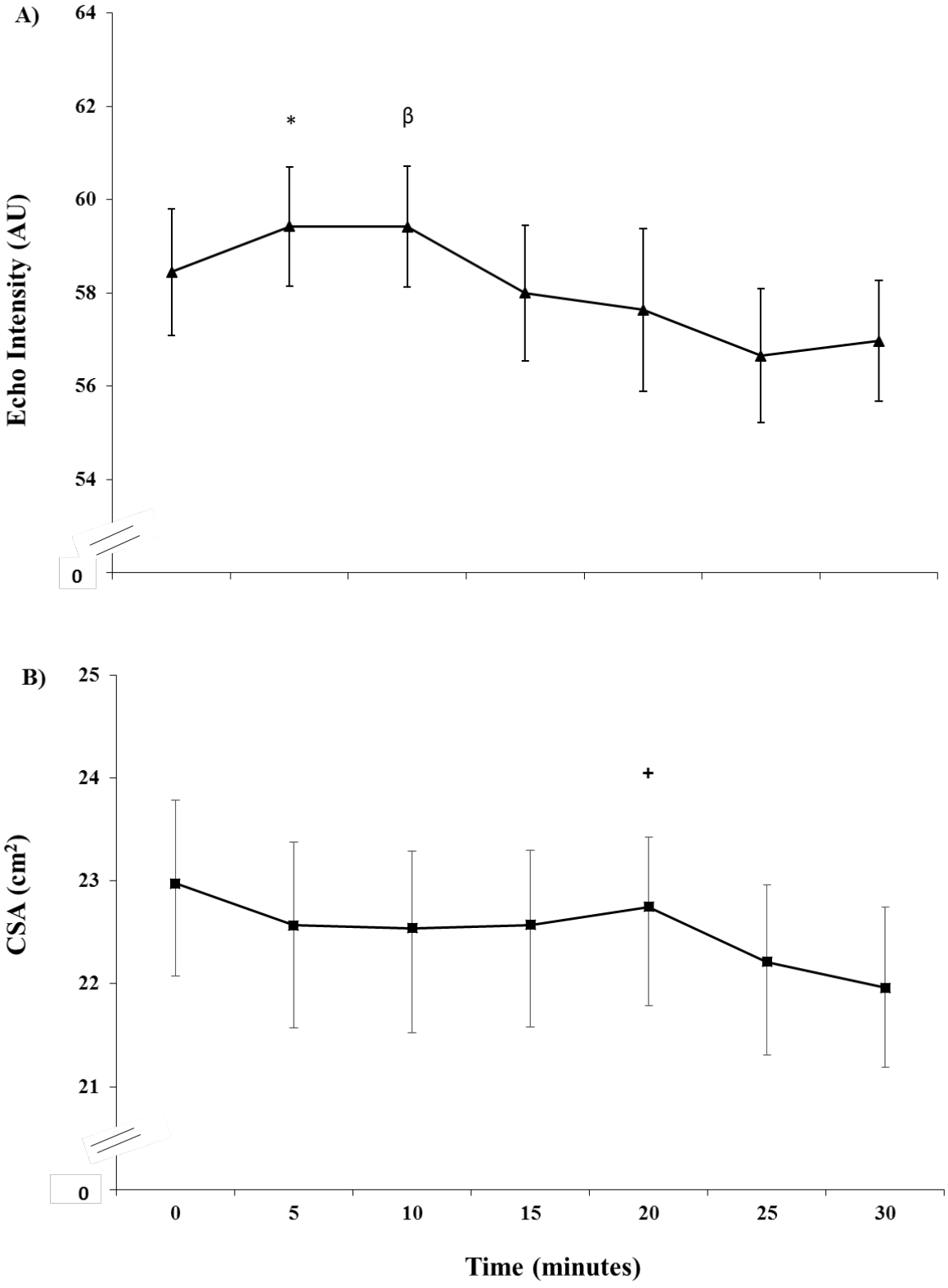

A)
64
62
60
58
56
54
0
Echo Intensity (AU)
*
β
B)
25
24
23
22
21
0
CSA (cm²)
+
0
5
10
15
20
25
30
Time (minutes)

Figure 4

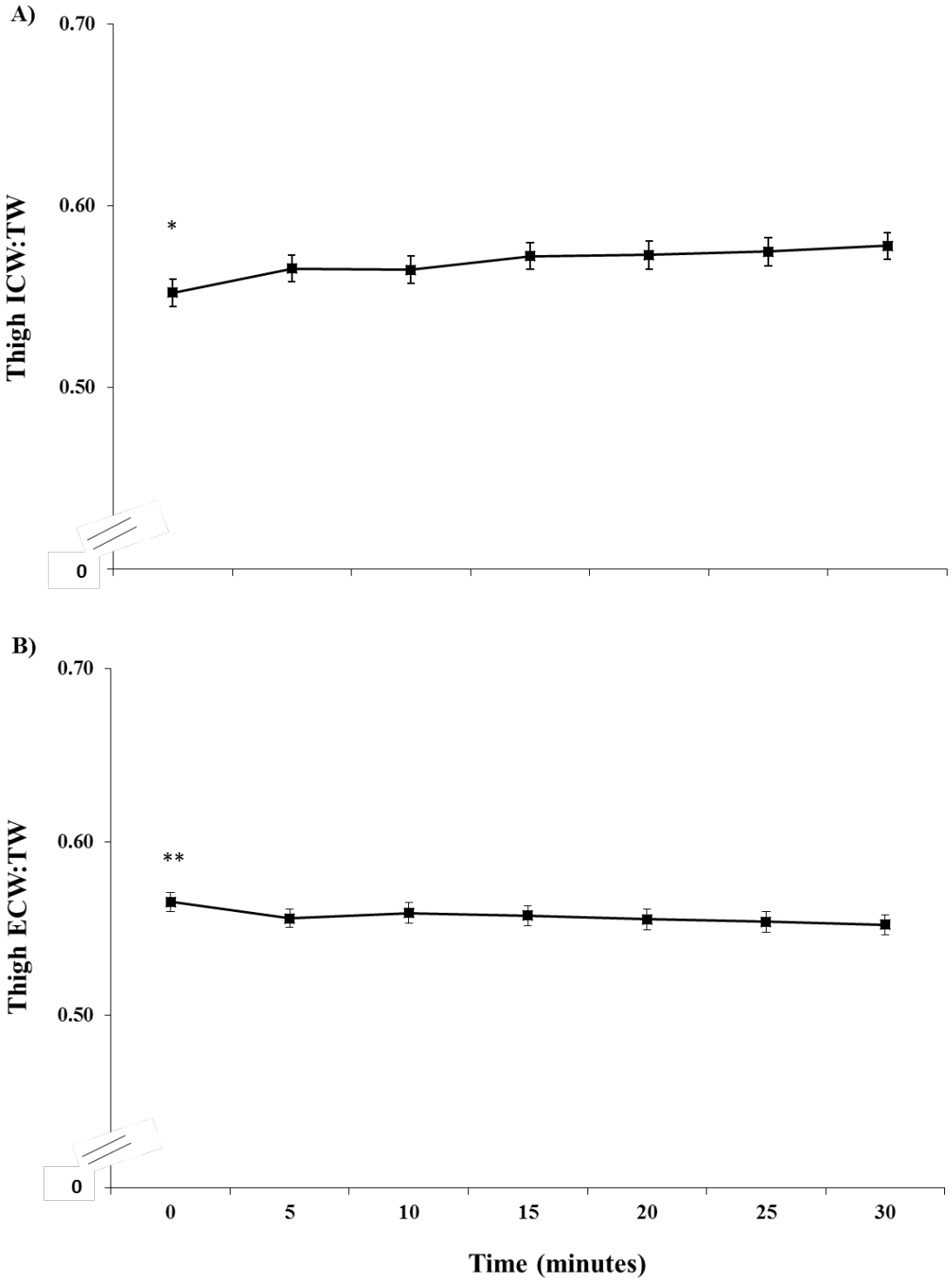

A)
0.70
0.60
0.50
0
Thigh ICW:TW
*
B)
0.70
0.60
0.50
0
Thigh ECW:TW
**
0
5
10
15
20
25
30
Time (minutes)

Figure 5

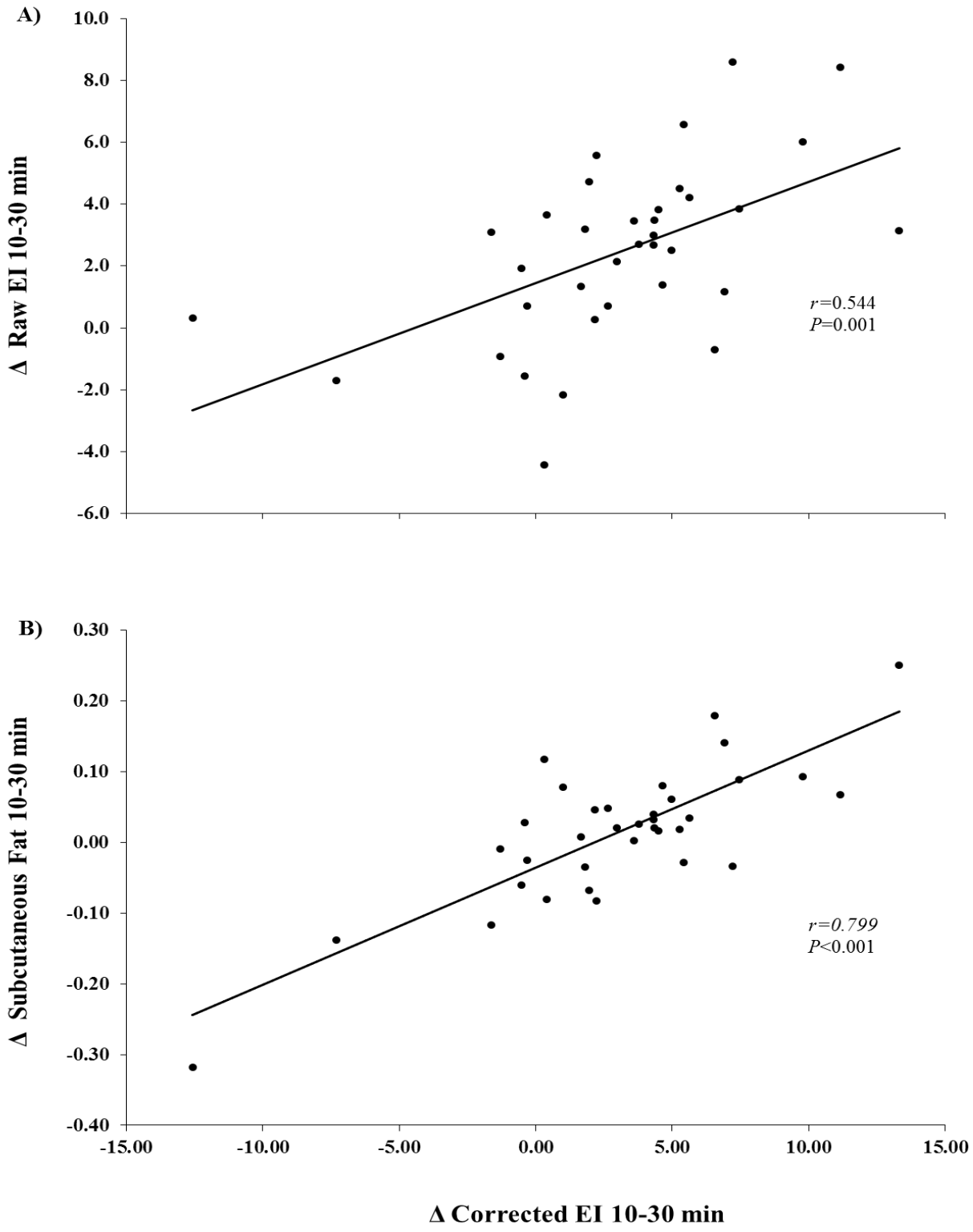
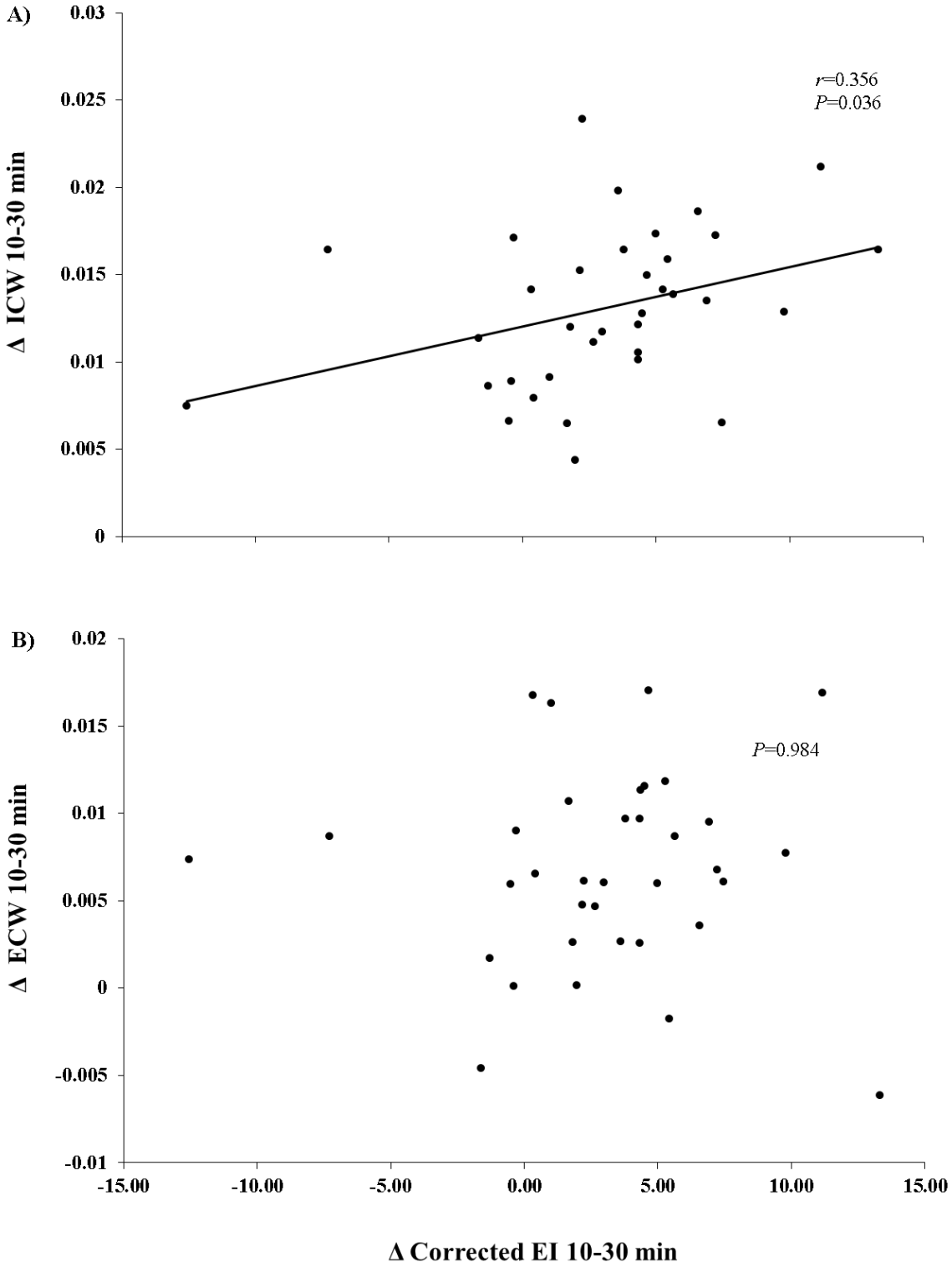

A)
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
Δ Raw EI 10-30 min
r=0.544
P=0.001
B)
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
-0.40
Δ Subcutaneous Fat 10-30 min
r=0.799
P<0.001
-15.00
-10.00
-5.00
0.00
5.00
10.00
15.00
Δ Corrected EI 10-30 min

Figure 6


A)
0.03
0.025
0.02
0.015
0.01
0.005
0
Δ ICW 10-30 min
r=0.356
P=0.036
B)
0.02
0.015
0.01
0.005
0
-0.005
-0.01
Δ ECW 10-30 min
P=0.984
-15.00
-10.00
-5.00
0.00
5.00
10.00
15.00
Δ Corrected EI 10-30 min

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