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Effect of fluid loss following hemodialysis on tibialis anterior muscle strength in people with end-stage renal disease

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A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

EFFECTS OF FLUID LOSS FOLLOWING HEMODIALYSIS ON TIBIALIS ANTERIOR MUSCLE STRENGTH IN PEOPLE WITH END-STAGE RENAL DISEASE

(Thesis format: Integrated Article)

by

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ABSTRACT

PURPOSE: The purpose of this study was to evaluate the effect of fluid loss following hemodialysis (HD) on tibialis anterior (TA) strength in participants with end-stage renal disease. Issues concerning measures of skeletal muscle hydration and efficacy of exercise as an anabolic intervention were also addressed.

METHODS: Data from published literature were combined in a meta-analysis to establish efficacy of exercise in participants on HD. Three clinical studies were undertaken using data acquired from healthy volunteers and participants on HD. Hydration of the calf muscles was estimated using bioelectrical impedance spectroscopy (BIS) [extracellular (ECF) and intracellular (ICF) fluid] and magnetic resonance imaging (MRI) [transverse relaxation time constants (T_2) and apparent diffusion coefficient (ADC)] acquired measures. Reliabilities and associations between the measures acquired using these two techniques were established using intraclass correlations and linear regression analyses. The maximal voluntary isometric contraction (MVIC) strength of TA was measured using a dynamometer.

RESULTS: A significant reduction ($p<0.05$) in MVIC strength by 1.54 Nm (95%CI: 0.05, 3.02), and ECF (T_2 shortened by 2.38ms; 95%CI: 1.04, 3.71) of TA, and calf ICF by 0.05 liters (95%CI: 0.01, 0.08) were observed between before and after HD measurements. In comparison with control group, participants on HD had significantly ($p<0.005$) lower mean MVIC by 9.76 Nm (95%CI: 3.64, 15.88) and 11.16 Nm (95%CI: 5.30, 17.01) and an expansion in ECF volumes of TA [T_2 of TA was significantly ($p<0.001$) prolonged by 9.07 ms (95%CI: 5.50, 12.64) and 6.83 ms (95%CI: 3.57, 10.09)], before and after HD respectively. Calf BIS acquired ECF was significantly associated with T_2 of TA ($\beta = 0.44$, $p=0.042$), medial gastrocnemius ($\beta = 0.47$, $p=0.027$) and ADC of lateral gastrocnemius ($\beta = 0.6$, $p= 0.003$) after HD only; hence these measures could not be used interchangeably as measures of TA interstitial fluid.

CONCLUSION: We observed reduction in strength (~6%) and volume (~ 15%) of TA following HD. Further research is required to evaluate the impact of myocellular lipids and muscle architecture on estimates of ECF and MVIC of TA for establishing absolute or relative effects of fluid loss on TA muscle volume and strength.

Keywords

End-stage kidney disease, Hemodialysis, Tibialis anterior, Strength, MRI, Transverse relaxation times, Extracellular fluid

CO-AUTHORSHIP STATEMENT

This thesis contains material from manuscripts in press (Chapters 2), published manuscript of chapter 3 and manuscripts from chapters 4 and 5 are in submission for publication. Anuradha Sawant designed the studies, recruited participants, collected and then analyzed the data for all the studies. On all papers Anuradha Sawant was the first author, Supervisors Tom Overend and Andrew House were co-authors of all manuscripts. Jayne Garland was co-author for manuscript on materials from Chapter 1. Tim Doherty was co-author for chapter 5. Collaborators of this project, Robert Bartha, Joe Gati, and Robert Lindsay were co-authors for chapters 3, 4 and 5; the manuscripts for which have either been submitted or are in the process of submission.

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List of Abbreviations

AAS – adjusted activity score

ACSA – anatomical cross-sectional area

ADC – apparent diffusion coefficient

BIS – bioelectrical impedance spectroscopy

BMI – body-mass index

cBIS – calf bioelectrical impedance spectroscopy

CI – confidence interval

CT – computerized tomography

CV – coefficient of variation

DXA – dual x-ray absorbtiometry

ECF – extracellular fluid

ESRD – end-stage renal disease

FFM – fat-free mass

HAP – human activity profile

HD – hemodialysis

ICF – intracellular fluid

ICC – intraclass correlations

LBM – lean body mass

LOA – limits of agreement

MAS – maximum activity score

MDC – minimal detectable change

METs –Metabolic equivalents

MRI – magnetic resonance imaging

MVIC - maximum voluntary isometric contraction

PD – peritoneal dialysis

ROI – region of interest

SEM – standard error of measurement

SMD – standardized mean difference

T_2 – transverse relaxation time constants

TA – tibialis anterior

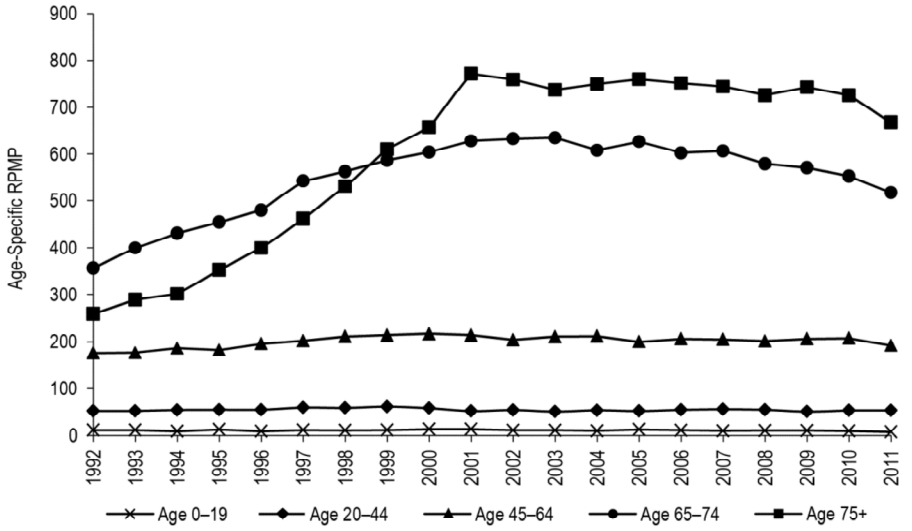
TW – total water

1 Chapter 1: Outline of Thesis and Purpose of Studies

1.1.0 Introduction

Chronic kidney disease occurs as a result of progressive failure of renal function over a period of months to years. Renal replacement therapies such as hemodialysis (HD) are required in its end stages, to prevent premature mortality.[1] In Canada; there were 5489 newly diagnosed people with end-stage renal disease (ESRD) on HD in 2011, double the number reported in 1992. Since 1999, the highest rate per million population of newly diagnosed ESRD was among those aged 75 and older (Figure 1.1).[2] This age group also had the largest rate increase over the reporting period, a trend that began in the 1980s and continued until 2001, when the incident rate per million population reached 771.8.

Figure 1:1: Incident End-stage Renal Disease Patients, Age-specific Rate per Million Population, Canada, 2002 -2011 (from CORR report published 2013).



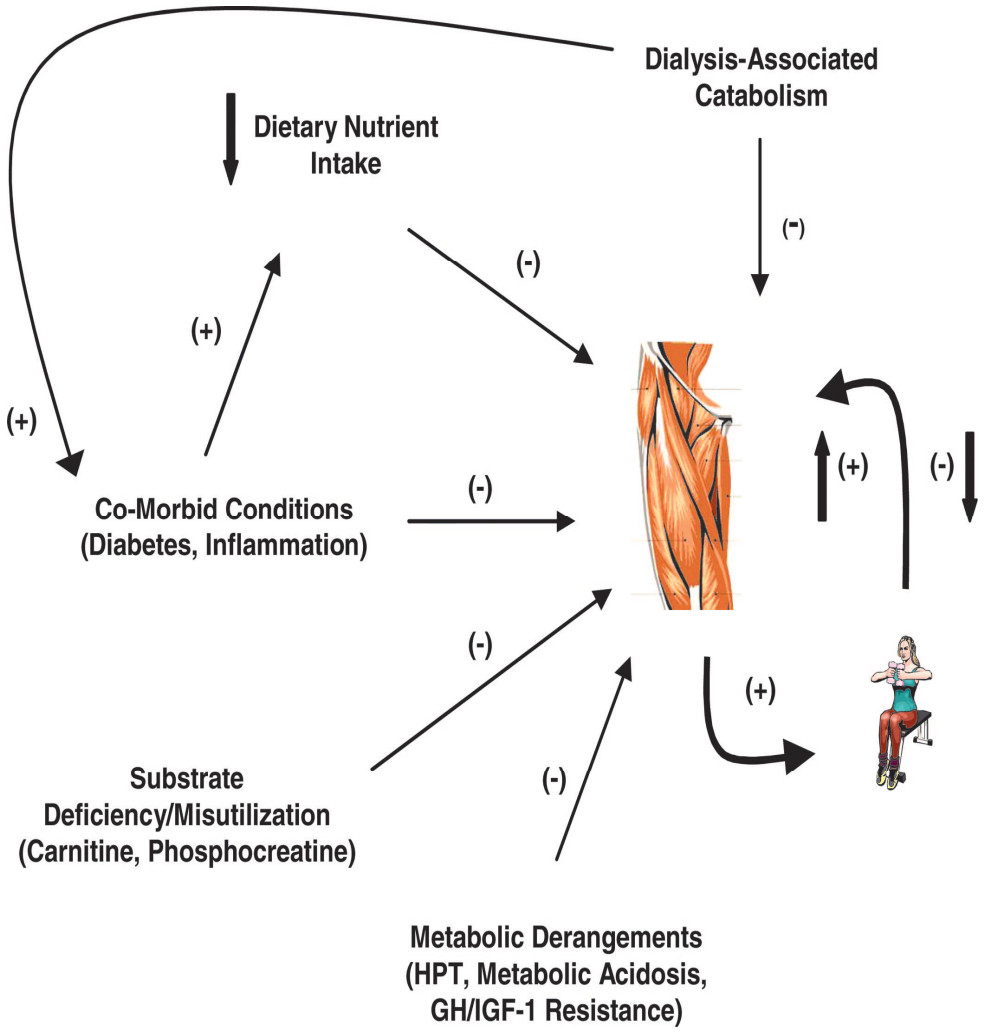
RRMP: rate per million populations

Reproduced from CORR Canadian Organ Replacement Register Annual Report: Treatment of End-Stage Organ Failure in Canada, 2002 to 2011[2]

End stage renal disease impacts multiple organ systems resulting in a constellation of symptoms including anemia, reduction in cardiac function, changes in skeletal muscle strength, and reduced aerobic capacity.[3] Several reviews have confirmed the presence of muscle weakness and reduced physical functional abilities in this population.[3 -6] A similar report of reductions in physical function in a Canadian ESRD/HD sample was reported by Overend et al;[7] mean scores of the physical function measures were in the 10th percentile of established normative values for community-dwelling residents between 65 and 85 years of age.[8] Such large declines in physical function (up to 50%) in population on renal replacement therapies, especially, in those receiving in-center HD are attributable to reduced physical activity associated with treatment time on HD, metabolic changes related to the disease and the catabolic effects of HD (Figure 1.2).[9,10]

Exercise interventions to address skeletal muscle weakness are important, as people with ESRD/HD have identified management of complications related to HD, including fatigue, as the second highest priority for health research.[11] Skeletal muscle force production is a complex phenomenon and is dependent on several factors such as morphological characteristics, neural mechanisms, muscle metabolism, size and architecture.[12] Knowledge of the underlying mechanisms underpins the rationale of safe exercise administration;[13] this may be particularly important in people with ESRD/HD who have documented evidence of muscle weakness and progressive deterioration of strength**.** For example, exercise administration in people with neurological disorders is targeted at improving neural plasticity[14] and strength[15] whereas for patients with myopathic disorders, exercise interventions require careful monitoring to avoid muscle damage potentially resulting from overuse or structural injury to the muscle.[16]

Figure 1:2: Mechanisms of Muscle Atrophy: Reproduced From Ikizler TA, Himmelfarb J: Muscle Wasting in Kidney Disease: Let's Get Physical. J Am Soc Nephrol 2006; 17: 2097-2098.[10]



1.2.0 Morphological Characteristics of the Muscles

Observations of progressive proximal muscle weakness in people with ESRD/HD by Floyd et al,[17] Lazaro and Kirshner,[18] and others were ascribed to myopathy (skeletal muscle damage). The findings of muscle damage observed on electron microscopy by Diesel et al[19] and

Kouidi et al[20] were similar to changes associated with muscle damage due to eccentric contraction in healthy controls.[21] These reports of muscle damage, analogous to changes associated with eccentric contractions in healthy participants, may support suggestions of progression of proximal muscle weakness.[17,18] Progression of proximal muscle weakness may result from ongoing muscle damage associated with the eccentric contractions of these muscles carrying out their postural stabilization role during antigravity activities such as sitting on a chair, standing, or walking in people with proximal myopathies.[22]

A literature review of the studies evaluating morphological features of the muscles using muscle biopsy, indicated myopathic (damage intrinsic to the muscle), neuropathic (loss of muscle mass and quality due to peripheral neuropathy) and mixed (neuropathic and myopathic) characteristics in skeletal muscle.[23] The common observations of type II fibre atrophy in studies using muscle biopsy suggests that the presence of weakness can be linked to sedentary lifestyle in people with ESRD/HD, since type II fibre atrophy is essentially the result of disuse. According to Henneman's size principle, type II motor units are the last to be recruited in voluntary actions.[24] The poor health status of people with ESRD/HD may thus restrict activities that require large force production (necessitating recruitment of type II motor units) further contributing to type II fibre atrophy.

1.3.0 Electrophysiological Properties

Factors related to ESRD/HD, such as sensorimotor neuropathy from uremia and defects of the neuromuscular junction, have also been considered as possible causes of muscle weakness in people with ESRD/HD.[25] A review of studies evaluating electrophysiological characteristics indicated prevalence of characteristics suggestive of myopathic, neuropathic, and mixed

(neuropathic and myopathic) features in the skeletal muscles investigated.[23] These findings support the suggestion of a possible effect of ESRD/HD on the different components of the muscle motor unit complex.[23]

1.4.0 Metabolic Characteristics of the Muscles

According to studies utilizing *in vivo* analysis of muscle metabolism using ^{31}P-nuclear magnetic resonance spectroscopy, muscle atrophy alone could not explain the changes in muscle metabolism during incremental submaximal exercise in people with ESRD/HD.[27] This phenomenon was described as "reduced effective muscle mass" (product of true muscle mass and metabolic efficiency of exercising muscle fibres) and suggested reduced intrinsic contractile efficiency.[26] Reports of increased resting cytosolic free phosphate (Pi) which is known to inhibit the force generation in skinned fibres[27] in a review by Kemp and Thompson[26] lends theoretical support to the notion of reduced effective muscle mass.

1.5.0 Anabolic Effect of Exercise Interventions: Current State of Evidence

The literature review of the characteristics of the muscles in people with ESRD/HD was inconclusive. Hence understanding the differential effects of exercises as anabolic interventions was important to elucidate exercise parameters that would yield maximal results. Dong and Ikizler[28] and Storer[29] recently reviewed anabolic interventions, including exercise, in people with ESRD/HD. These reviews indicate that the studies using aerobic or strength training or mixed (aerobic and strengthening) exercise as anabolic interventions were unable to consistently

demonstrate beneficial anabolic effects of exercise training. Two recent systematic reviews and meta-analyses of exercise training in ESRD/HD participants by Smart and Steele[30] and Heiwe et al[31] showed that exercise training is safe and imparts large improvements in peak oxygen consumption, sympathetico-adrenal activity, physical function and health-related quality of life. We did not find any study that comprehensively established the efficacy of exercise interventions by type in this population, nor could we find any study that evaluated the influence of variations in the extracellular fluid (ECF) during the interdialytic period on skeletal muscle size or strength.

1.6.0 Architecture of the Muscles

Anatomical cross-sectional area (ACSA), measured using magnetic resonance imaging (MRI) of anterior tibial[32] and calf[33] muscles confirmed the presence of muscle atrophy in ESRD/HD study participants who also showed muscle weakness. Contractile efficiency (force per unit ACSA) was similar to that of the control group based on the analysis of force per unit ACSA. Although ACSA has been considered a good predictor of muscle strength,[34] it does not account for the variation in muscle force production due to differences in the muscle architecture such as angle of muscle pennation. Muscle pennation angle influences muscle CSA and is a major determinant of the most fundamental mechanical properties of the muscle (force-length relationship).[34] Muscle CSA, taking into account the pennation angle, is referred to as physiological CSA. Thus the findings of intact contractile relationships by Johansen et al[32] and Kemp et al[33] may be incorrect as the muscle pennation angles were not considered in those studies.

1.7.0 Influence of Interdialytic Fluid Variations

Another factor complicating the accurate measure of size and strength relationships in people with ESRD/HD is hydration status. People on HD accrue ECF between their HD treatments. During this interdialytic period, fluid accumulates within superficial tissues, particularly in the lower extremities as the result of gravitational forces.[35] Kaysen et al[36] used bioelectrical impedance spectroscopy (BIS) to show that the intracellular fluid volume (ICF) does not vary significantly during interdialytic periods. According to Kushner et al[35] the ECF in people with ESRD/HD is ~107 ± 19% of control values and ICF is ~93 ± 18%. Other researchers have supported these results of Kushner et al using BIS.[37] Thus, extra body water accruing during the interdialytic period appears to accumulate mainly in the extracellular spaces. This suggests the possibility of a higher percentage of ECF volume in people on HD when compared to healthy control participants. This "excess ECF" may confound measurement of true muscle size. Assessment of contractile efficiency of the skeletal muscle (strength per unit muscle size) requires an accurate assessment of the size of the muscle contractile elements.[38] Hence, in order to establish contractile efficiency of the skeletal muscle in people on HD, it is necessary to correct the muscle size by taking into account the hydration status.

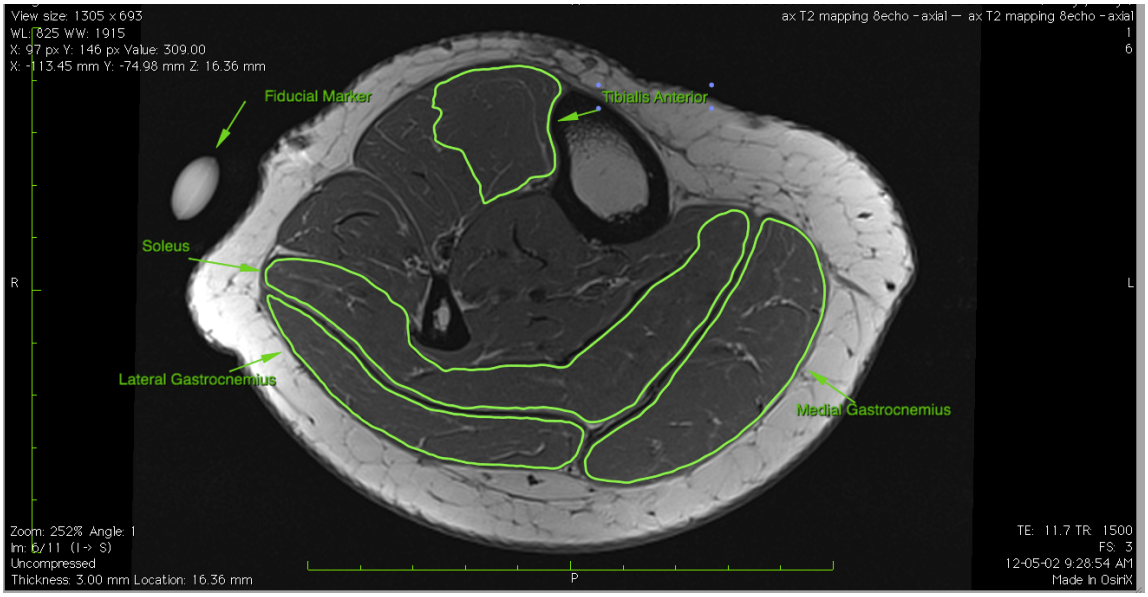
1.8.0 Measurement of Skeletal Muscle Hydration

Multi frequency BIS in one of the methods utilized to evaluate body composition in this population. It is important to establish fluid balance within the muscle environment. Euhydration or normal state of hydration of the skeletal muscles is an important factor for adequate force production and endurance. In athletes, dehydration of 1% to 2% of body weight begins to

compromise physiologic function and negatively influences performance.[39]About ¾ of ECF is within the interstitial spaces. Since BIS provides estimates of ECF and ICF within a body segment or whole body, it may be used for estimation of interstitial fluid/hydration of specific skeletal muscle; however this is yet to be established.

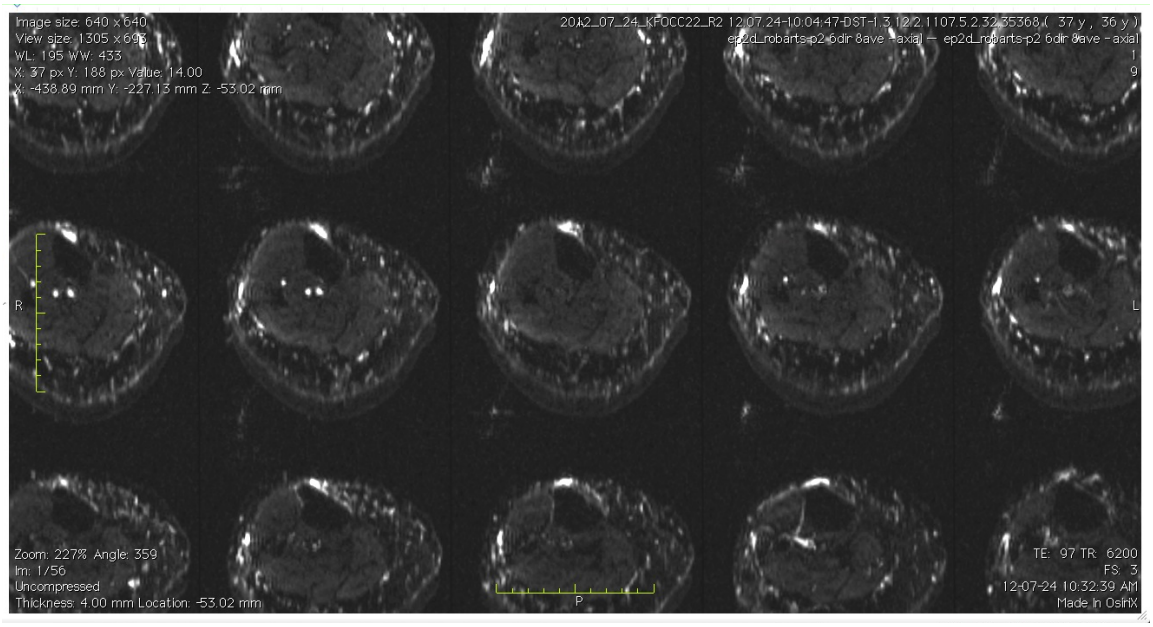
Magnetic resonance imaging-acquired images provide quantitative measures of skeletal muscle composition. It is presumed that ECF affects the transverse relaxation time contstants (T_2) (T_2 is a measure of how long transverse magnetization would last in a perfectly uniform external magnetic field) more than total tissue and ICF in resting skeletal muscle; hence, T_2 can be considered as an estimate of ECF.[40] However, a literature review did not indicate regular use of this technique for estimating ECF and hydration status of a skeletal muscle in people with ESRD/HD.[41]

Figure 1:3 T_2 image of the muscles used for generating Transverse Relaxation Time Constants



Apparent diffusion coefficient (ADC) is measured by a MRI-acquired diffusion-weighted imaging technique that is sensitive to the hydrodynamic environment of the tissue water. Apparent diffusion coefficient has been used to estimate water motion in the intra- and extracellular compartments related to small and random movements in the tissue.[41] These are probably related to the intra- and extracellular volumes, the size of the extracellular volume appears to be the most important contributing to the ADC values; e.g. ADC is increased in exercising muscles and is presumed to reflect increased water motion predominantly in extra-cellular volume.[43] This ADC technique has also not been widely used to estimate changes in the hydrodynamics of skeletal muscle.[43] This technique has been used to estimate brain water by Galons et al[44] in nephrectomised rats. Nygren and Kaijser[43] and other researchers[42] utilized this technique to estimate ECF in skeletal muscle in healthy people. However according to Yanagisawa et al[41] the ADC technique has not been widely used to estimate the changes in the hydrodynamics of the skeletal muscle. Thus, at the present time, it is unclear which of the two measures, T_2 or ADC, is superior for the estimation of ECF. Since both ADC and T_2 measurements can be acquired during one MRI session, we proposed to collect both and analyze as estimates of ECF.

Figure 1:4 Apparent Diffusion Coefficient Map of the Calf Muscles



1.9.0 Outline of the Thesis and Purpose of Studies

The studies reported in this thesis aimed at establishing the effect of fluid loss following hemodialysis on TA muscle strength in people with ESRD on HD, using the reliable surrogate measure of TA ECF. The studies included also addressed issues concerning measures of skeletal muscle hydration and efficacy of exercise as an anabolic intervention.

1.9.1 Study One:

Data extracted from published reports of exercise interventions evaluating its effect on muscle mass will be combined into a meta-analysis for determination of its anabolic effect in people with ESRD/HD.

1.9.2 Study Two:

Determine the reliability of skeletal muscle hydration measures, extracellular and intracellular fluid, and total water obtained using calf BIS, and MRI-acquired T_2 and ADC values of muscles of calf (tibialis anterior, lateral and medial gastrocnemius and soleus).

1.9.3 Study Three:

Determine associations between the BIS-acquired measures of calf hydration and MRI-acquired measures of single muscle hydration in people with ESRD/HD.

1.9.4 Study Four:

Determine the effect of whole-body fluid loss following hemodialysis on maximal voluntary isometric contraction strength and extracellular fluid volumes of tibialis anterior muscle in people with ESRD/HD compared to controls.

1.9.5 Summary:

This chapter will summarize the findings and any limitations of the above-mentioned studies. This chapter will also provide recommendations for future research to improve our understanding of the impact of this complex disorder on motor unit complex and improve outcomes of exercise interventions.

The experiments outlined above will enable us to establish the current state of evidence of exercise as an anabolic intervention in people with ESRD/HD; the measurement properties of the calf muscle hydration measures and determine which of these measures is the most reliable

and stable; association between the calf BIS and MRI-acquired measures of muscle hydration will establish if these measures can be employed interchangeably. These experiments will enable us to determine the effect of variations in interdialytic fluid on tibialis anterior muscle strength and provide directions for future research to improve the efficacy of exercise intervention for a measurable change in skeletal muscle mass and strength and hence survival and quality of life in people with ESRD/HD.

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2 Chapter 2: Anabolic Effect of Exercise Training in People with End-stage Renal Disease on Hemodialysis: A Systematic Review and Meta-analysis.

2.1.0 Introduction

Hemodialysis (HD) is considered to have more pronounced deleterious effects on skeletal muscle than peritoneal dialysis (PD).[1] One difference between these two dialysis techniques is fluctuation in toxin levels[2] which range from very low post-dialysis to very high pre-dialysis; people on HD typically dialyze three times per week whereas people on PD dialyze every four hours or daily.[2]

People with end-stage renal disease (ESRD) on HD encounter multiple catabolic processes such as loss of albumin and amino acids during dialysis, and metabolic derangements together with changes in skeletal muscle associated with conditions of muscle disuse.[3] These changes result in muscle atrophy (loss of lean muscle mass). Presence of neurogenic (muscle atrophy/loss associated with nerve disorder), myogenic (damage intrinsic to the muscle) and mixed (neurogenic and myogenic) changes intrinsic to the skeletal muscle in people with ESRD/HD[4] may further compromise the integrity of the motor-unit complex, and contribute to muscle atrophy.[5] As muscle wasting is a significant predictor of morbidity and mortality in this population,[6] preventing such wasting is clinically important to maintain or improve physical function, prevent falls and related hospital visits; a critical issue for healthcare costs.[7] Therefore, physical therapy interventions to counteract muscle atrophy and promote muscle anabolism have been strongly recommended in this population.[8]

Exercise may promote an anabolic milieu, increasing muscle mass, improving muscle strength, and ameliorating muscle catabolism.[9] Dong and Ikizler[10] and Storer[11] recently reviewed

anabolic interventions, including exercise in people with ESRD/HD. These reviews indicated that the studies using aerobic or strength training or mixed (aerobic and strengthening) exercise as anabolic interventions were unable to consistently demonstrate beneficial anabolic effects of exercise training. Two recent systematic reviews and meta-analyses of exercise training in hemodialysis patients by Smart and Steele[12] and Heiwe et al[13] showed that exercise training is safe and imparts large improvements in peak oxygen consumption, sympathetico-adrenal activity, physical function and health–related quality of life. Heiwe et al[14] reviewed outcomes evaluating muscle morphology and morphometrics. However, their results may not be relevant to us for two reasons. First, a study recruiting participants with chronic kidney disease not on HD[15] was included in cumulative analysis. Second, we have identified studies we deem to be relevant that were not considered relevant by Heiwe et al.[14] This may have impacted their results evaluating effect of exercise on mid-thigh muscle area. Thus, the primary objective of this systematic review was to evaluate the effectiveness of exercise training programs for increasing muscle mass in the adult population with ESRD/HD. The secondary objectives were to evaluate the influence of following parameters on the cumulative results; a) age; b) day of exercise intervention (dialysis day vs. off-dialysis day); c) mode of exercise (aerobic, strength-training or a combination); d) outcome measure used to reflect muscle mass, such as muscle cross-sectional area (CSA) or lean body mass, and e) measurement techniques such as dual energy x-ray absorptiometry (DXA), bio-electrical impedance spectroscopy (BIS), computed tomography (CT) and magnetic resonance imaging (MRI).

2.2.0 Methods

2.2.1 Study Design:

Our systematic review used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines throughout the literature search and reporting phases of development.[14]

2.2.2 Data Sources and Search Strategy:

Electronic databases CINAHL, Cochrane Library, EMBASE, PEDro, PubMed and SCOPUS from inception to the end of November 2012 were searched to identify relevant studies. The following terms were searched as "MeSH" terms and key words: kidney failure AND exercise AND muscle mass. These searches were limited to studies published in English and involving adult human subjects stable on HD. Reference sections of the retrieved articles were hand-searched for citations that were missed by the electronic searches.

2.2.3 Study Inclusion Criteria:

Inclusion criteria were as follows:

a) English language of publication;

b) Randomized studies;

c) Participants included in the studies had a diagnosis of ESRD requiring maintenance HD as renal replacement therapy;

d) Participants were ≥ 18 years of age, on HD for more than three months (indicating chronic exposure to HD intervention);

e) Received exercise training for a minimum duration of eight weeks either during dialysis sessions (intradialytic), or on non-dialysis days in a structured, supervised environment.

2.2.4 Study Exclusion Criteria:

Studies that evaluated skeletal muscle mass using biopsy or those that investigated the messenger RNA, a molecule of RNA that encodes a chemical "blueprint" for protein product, did not include measures of muscle mass, were not randomized clinical trials, cross-sectional or before-and-after design or cross-over design were excluded.

2.2.5 Outcome Measures:

Skeletal muscle is a heterogeneous structure; therefore 'muscle mass' has been measured in intervention and epidemiological studies at various levels such as cellular, molecular and tissue level.[15] Hence the primary outcome measures were validated surrogate measures of muscle mass; a) fat-free-mass (FFM) measured using multi-frequency BIS or DXA; b) lean body mass (LBM) measured using BIS or DXA; c) muscle CSA as measured by CT or MRI; d) muscle attenuation measured using Hounsfield unit values generated using CT scans.

2.2.6 Study Quality:

The internal validity of studies included in this review was assessed using statistical criteria of sample size and power required for establishing possible type I or type II error. We used the PEDro scale, a valid[16] and reliable[17] tool to evaluate study quality for RCTs.

2.2.7 Study Selection and Data Extraction:

Following the primary search, two authors (AS and TO) independently reviewed the titles, abstracts and full texts for assessing the study inclusion and exclusion criteria. Discussions, in case of discrepancy, were adequate for resolution. All articles were included by consensus.

Required data for calculations of effect sizes were extracted using a standardized form, from the included studies by one author and reviewed by the second author for accuracy. Data were coded using categorical numerical codes based on theoretical constructs including type of exercise (aerobic, resistance training or mixed, aerobic and strength training), outcome measure (muscle attenuation, lean limb mass, or LBM or muscle CSA) and measurement tool (CT, or MRI, or DXA, or BIS). Authors were contacted for further information where the published data were in a format other than that accepted by the meta-analysis software for analysis or if the data were not analyzed by intention-to-treat.

2.2.8 Statistical Analyses:

A Comprehensive Meta Analysis (Version 2.2.040; Biostat, Englewood, NJ, USA) software was used for computation of standardized mean differences (SMDs); expressed as

Hedges' g.[18] Hedges' g values and their 95% confidence interval (CI) were used to assess the influence of type of exercise, day of exercise intervention and the outcome measures used to measure muscle mass. Hedges' g values reported were calculated using the random effects model to account for methodological differences amongst studies. For the study by Dong et al,[19] data provided by the authors for participants completing the study were included in the analysis.

The statistical significance of the differences in the moderator variables was computed by Page's L statistic[20] with the use of IBM SPSS V. 20.0 statistical software to calculate the between-groups and total sum of squares (SS). The L statistic was then calculated using the formula $L = (N-1)\ r^2$ where N is the total number of effect sizes and r^2 is the product of $\frac{SS_{between}}{SS_{total}}$; the computed value was then compared against the χ^2 value for (k-1) degrees of freedom (df), where k is total number of effect sizes included in the analysis. The significance of the L statistic was evaluated using a χ^2 table. The presence of heterogeneity among the moderator variables was evaluated by the Q statistic using a random effects model. The studies were considered heterogeneous if the p value of the Q statistic was < 0.05. The SMDs (Hedges' g) were interpreted as small, medium and large if they were ≤ 0.2, ≤ 0.5 and ≤ 0.8, respectively.[21] Publication bias was assessed using a funnel plot of the standard error vs. the standard difference in the mean. A 95% CI around the point estimates of the effect sizes and the number of null studies required to change a statistically significant result to a non-significant finding (Fail Safe N) were used to assess robustness of the findings. The critical number of studies (Fail Safe N) was calculated using Hedges and Olkin's[22] formula $[K_0 = \frac{K\ (\text{mean } d - d_{trivial})}{d_{trivial}}]$, where K_0 = number of new studies needed to produce a trivial effect size, K = number of studies in the meta analysis,

mean d = the mean effect size from all studies, and d-trivial= is estimate of a trivial effect size, assumed at 0.05. The significance level for all statistical tests was set at $p < 0.05$.

2.3.0 Results

Five[6, 19,23-25] of the 469 citations retrieved following the primary search were included for analysis (Figure 2.1). A total of 10 SMDs were extracted, seven of which were analyzed to evaluate the efficacy of exercise training on muscle mass; i.e. one effect size per group receiving exercise intervention (Figure 2.2). Kopple et al[25] investigated the effect of strength, aerobic and mixed training in three separate groups of participants. Hence three SMDs extracted from this study were included in the analysis. Characteristics of studies included in the analysis of SMDs are presented in Table 2.1 and characteristics of participants included in these studies are presented in Table 2.2. Figure 2.3 indicates that there was no publication bias in the pool of studies included in this review. However, the absence of studies in the two-tailed areas indicates a lack of publications demonstrating large significant or non-significant effects of exercise.

2.3.1 Study Quality:

All studies reported protection against Type I error by convention; however, only one study[7] reported sample size calculations to protect against Type II error as well. Four[6, 19, 23, 24] of the five studies suggest adequate opportunity of inclusion in any one group by ensuring allocation concealment. None of the studies had blinded participants, therapists, or outcome assessors; however, use of objective quantitative measures such as MRI or CT reduces the risk of potential observer bias (Table 2.3).

2.3.2 Effect of Exercise on Muscle Mass:

The SMD (Hedges' g value) for exercise intervention on muscle mass under the random effects model was statistically significant [0.272, 95% CI (0.020, 0.525); p = 0.034] (Figure 2.2). Over 30 studies with null effects would be required to negate the significance of our findings (trivial difference = 0.05, Fail safe N = 31.08). Interestingly, the Q statistic assessing the heterogeneity of the studies was not statistically significant [Q (6df) = 1.864; p=0.932]. This lack of heterogeneity is likely due to the small number of studies in this review.

Figure 2:1: Flow Chart of Study Selection

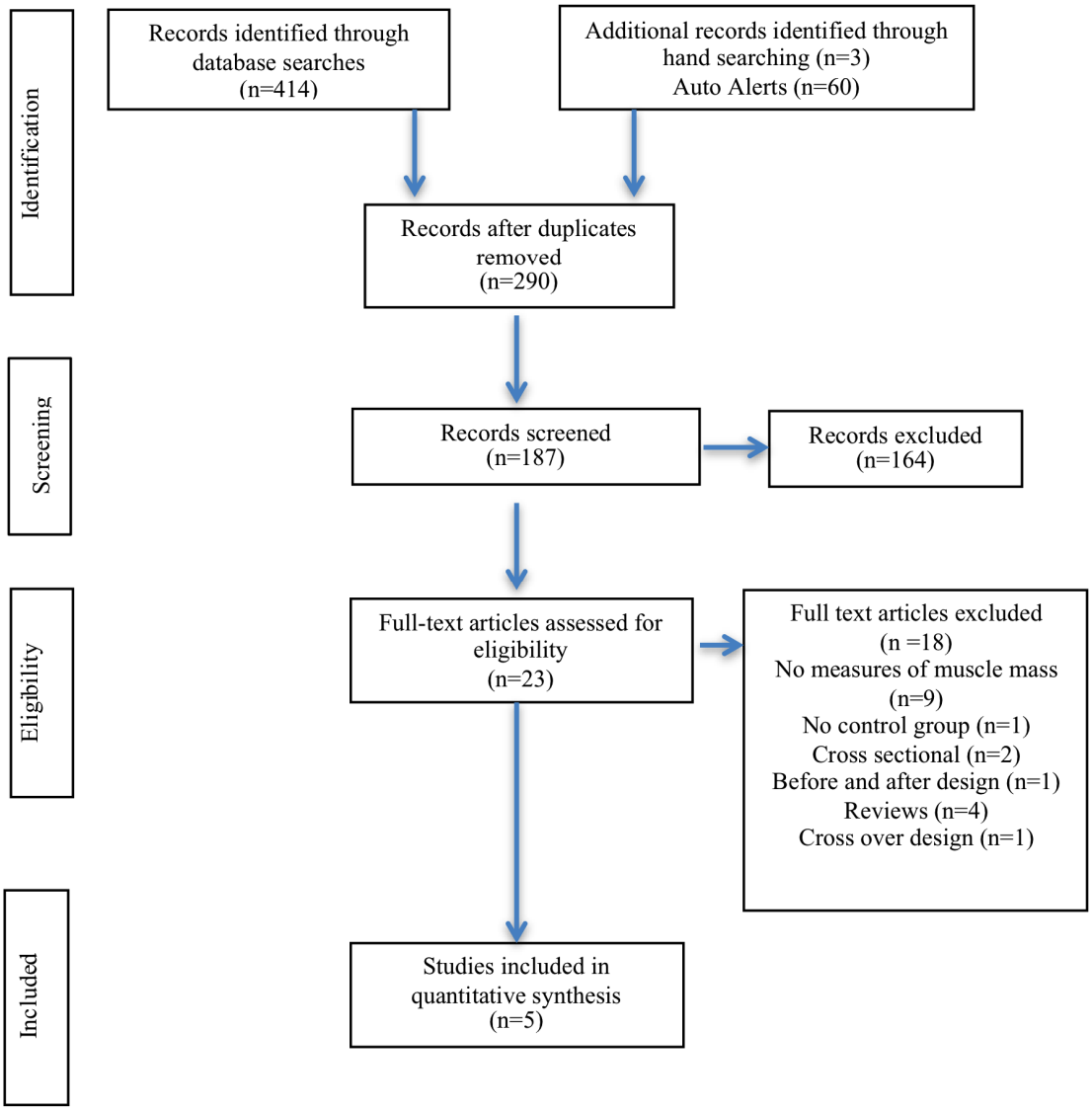
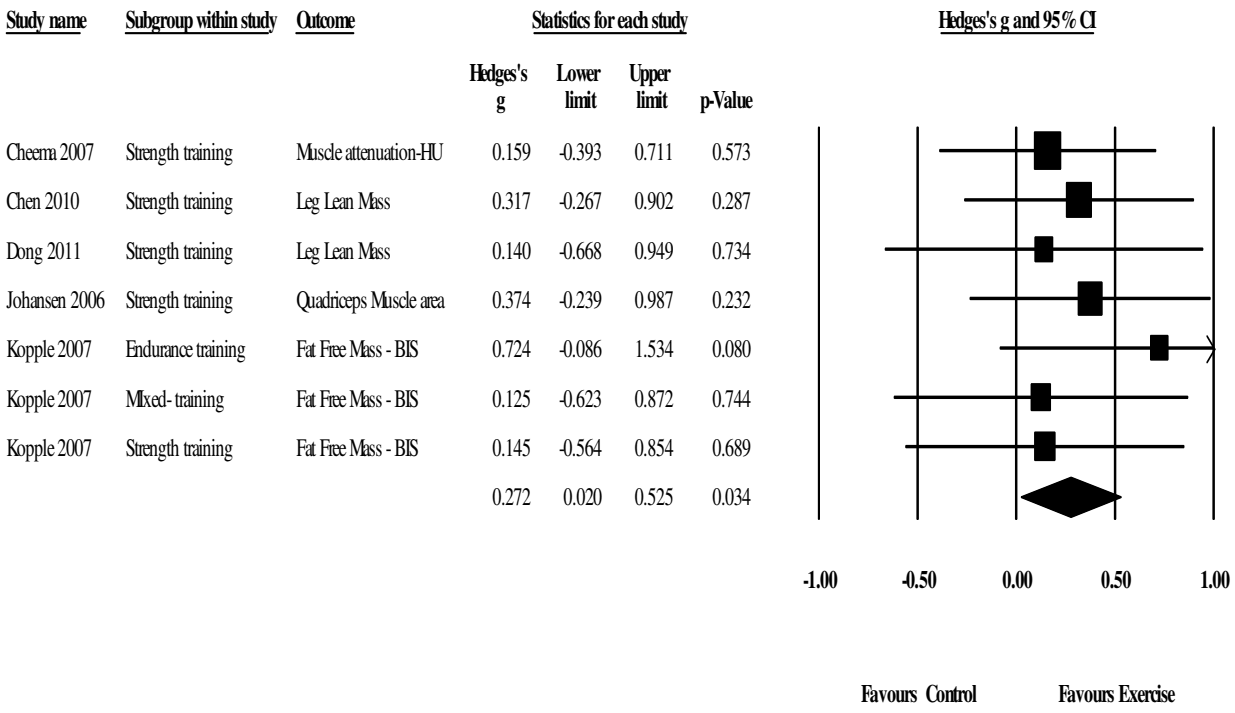


Figure 2:2: Effect of Exercises on Muscle Mass.



Heterogeneity
Q(df6):1.86; p:0.93; I^2:0

2.3.3 Effect of Type of Exercise Intervention:

The influence of type of exercise intervention [L (9df) = 4.05, p>0.05] on estimates of muscle mass following exercise was not statistically significant. Five SMDs were extracted from studies that provided a strength training intervention.[6, 19, 23-25] The effect of strength training on muscle mass was not statistically significant [Hedges' g = 0.238; 95% CI: (-0.046, 0.522); p =0.100]. Only one study[25] evaluated the effect of aerobic and mixed (aerobic and strength training). Both these effects were not statistically significant [aerobic training: Hedges' g =

0.0.724; 95% CI (-0.086, 1.534); p = 0.0.80 and mixed training: Hedges' g = 0.125; 95% CI (-0.623, 0.872); p = 0.744].

2.3.4 Effect of Differences in Outcome Measure:

The SMDs evaluating the influence of exercise (aerobic and/or strength training) on muscle mass using FFM [Hedges' g = 0.304, 95% CI (-0.130, 0.739); p=0.169], leg lean mass (LLM) [Hedges' g = 0.257, 95% CI (-0.217, 0.730); p=0.288], thigh/quadriceps muscle CSA [Hedges' g = 0.210, 95% CI (-0.2,0.62); p=0.316] or muscle attenuation (Hounsfield units) [Hedges' g = 0.159, 95%CI (-0.393, 0.711); p = 0.573] as outcome measures were not statistically significant [L (9df) = 2.059, p>0.05]. Kopple et al[25] reported estimates of muscle mass as FFM using DXA and BIS. For the analysis we used measures of FFM using BIS. Two studies[19, 24] provided estimates of muscle mass using LLM as an outcome measure. However, only one study[7]measured muscle attenuation using CT and one study[23] used MRI to measure quadriceps muscle CSA.

2.3.5 Effect of Differences in Outcome Tool:

The Hedges' g value for the effect sizes evaluating the influence of exercise (aerobic and/or strength training) on muscle mass using DXA [Hedges' g = 0.258, 95% CI (-0.064, 0.579); p=0.116], BIS [Hedges' g = 0.304, 95% CI (-0.130, 0.739); p=0.169], CT [Hedges' = 0.159, 95% CI (-0.393, 0.711); p = 0.573] or MRI [Hedges' g = 0.374, 95% CI (-0.239, 0.987); p=0.232] as outcome tools were not statistically significant [L (9df) = 1.418, p>0.05]. Three studies[19,24, 25] used DXA for estimating LLM or FFM and provided five SMDs. Only Kopple et

al[25] reported estimates of FFM using BIS providing three SMDs, one study used CT and one used MRI.[6, 23]

We were unable to evaluate the effect of age as none of the studies included in this review had categorically recruited participants over the age of 60 years; using 60 years as the critical age to form groups of "old" and "young," has been suggested elsewhere.[28] However the mean age of the participants recruited in two studies[7,26] was over 60 years and the mean age of the participants in three studies[21,25,27] was less than 60 years. An exploratory evaluation of the effect of exercise in the two studies[7,26] with "older" participants was smaller [Hedges' g = 0.234; 95%CI: -0.168, 0.635; p = 0.254] compared to the studies[21,25,27] in which the mean age of the participants was less than 60 years of age and approached significance [Hedges' g = 0.298; 95% CI: -0.027, 0.622; p = 0.072].

We were unable to evaluate the influence of day (on or off HD intervention day) of exercise intervention on muscle mass. All the included studies administered exercise intervention on dialysis days either just prior to or during dialysis.

Table 2-1: Characteristics of Individual Studies.

Study PEDro Score	Sample Size		Experimental Intervention	Control Intervention	Duration, day and frequency of intervention	Outcome tool	Results and p values	Hedge's g
	Exp	Con						
Cheema 2007 8	24	25	Strength training	No intervention	12 weeks; during dialysis 3 X week	CT	No change in thigh muscle CSA; p = 0.40	0.078
Chen 2010 6	22	22	Strength training	Stretching exercises with light resistance bands	24 weeks; during dialysis 2 X week	DXA	Leg lean mass changed: p = 0.0001	0.323
Dong 2011 5	10	12	Strength training and nutritional supplement	Nutritional supplement alone	6 months; Just prior to dialysis on dialysis day	DXA	Leg lean mass did not change significantly	0.146
Johansen 2006 7	20	20	Strength training	Placebo nandrolone	12 weeks	MRI	Change in quadriceps muscle area was significant; p = 0.02	0.362
Kopple 2007 4	ET - 10	14	Endurance training,	No exercises	21 weeks; ET- during first 60 mins of dialysis	DXA, BIS	Significant change in FFM between pre and post measurements in the ET, ST, EST or NT as measured with DXA ($p<0.05$)	BIS: 0.724; DXA: 0.527
	ST - 15		Strength training		ST- just prior to dialysis		Non significant when measured with BIA groups or three exercising groups combined	BIS: 0.144; DXA: 0.059
	EST- 12		Endurance and strength training		EST - as above; 3 X week			BIS: 0.124; DXA: 0.235

Abbreviations: BIA, Bio-electrical impedance analysis; Exp, experimental; Con, control; CT, Computerized Tomography; CSA, Cross-sectional area; DXA, Dual energy X-ray absorptiometry; ET, Endurance training; EST, Endurance and strength training; FFM, fat-free mass; MRI, Magnetic Resonance Imaging; NT, no training; ST, Strength training

Table 2-2: Characteristics of Participants Included in Individual Studies.

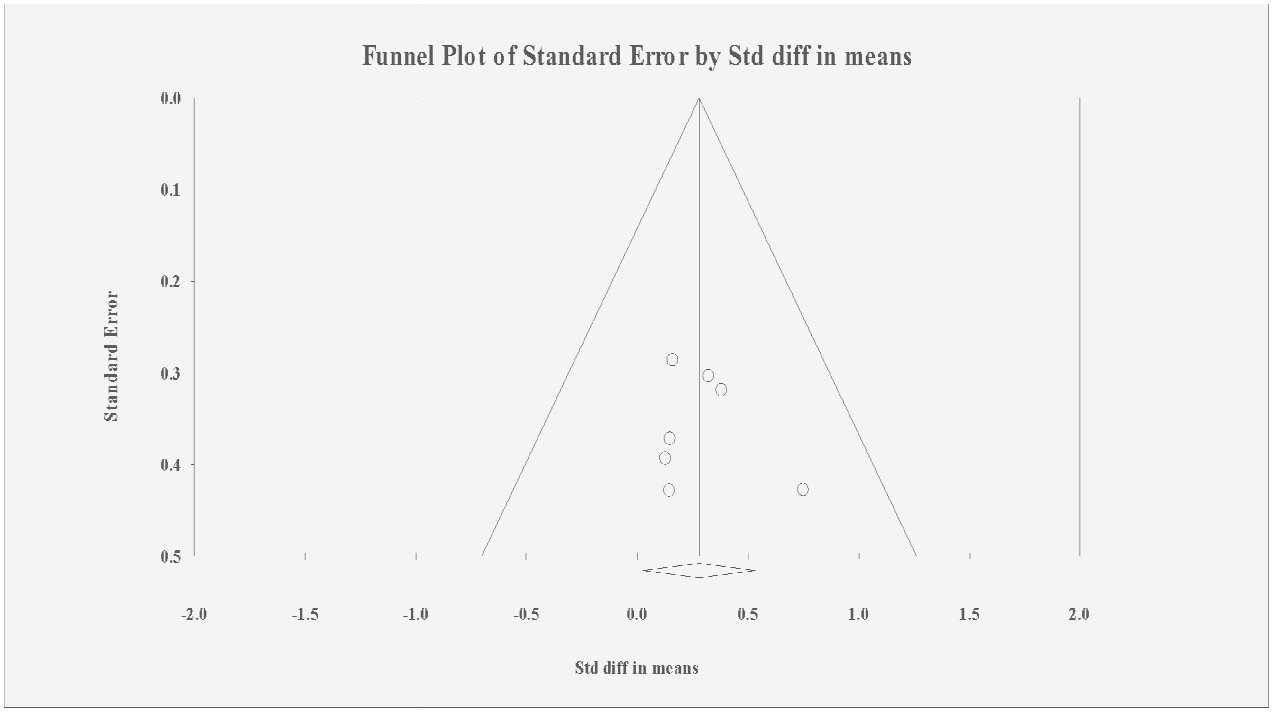
Study ID	Age of participants Mean (SD)	Number of co-morbidities: Mean (SD); Duration of dialysis; Mean (SD/range)	Inclusion Criteria
Cheema 2007	Exp: 60.0 (14.2) Con: 65.0 (15.3)	Exp: 4.9(1.6); 3.3 (0.3 - 16.7) yrs Con: 5.2 (2.0); 1.6 (0.6-10.3) yrs	Age - > 18 yrs; >3 mo on hemodialysis; without acute or chronic medical condition that could preclude PRT of data collection; be able to ambulate without assistive devices >50ms; Kt/V ≥ 1.2; stable during dialysis.
Chen 2010	Exp:71.1 (12.6) Con: 66.9 (13.4)	Exp: 7.3 (2.9); 1.54 (0.14) Con: 6.3 (2.4); 1.65 (0.38)	Age > 30 yrs.; Serum albumin <4.2 g/dl; on HD 3xwk and 80% compliance.
Dong 2011	Exp: 46.5 (12.1) Con: 40.2 (13.5)	Co-morbidities and duration of dialysis not indicated	Age > 18 yrs.; >3 mo on hemodialysis; Kt/V ≥ 1.2; on HD 3xwk using bio-compatible HD membrane.;
Johansen 2006	Exp: 54.4 (13.6) Con: 56.8 (13.8)	% of participants with DM, CAD, HTN, PAD and CVA in both groups has been reported. Total number of comorbidities - not reported Exp: 33.0 (3.5, 108) months Con: 25.5 (3, 156) months	Kt/V ≥ 1.2; on HD 3xwk; were free of HIV, malignancy and did not have infection in the past 3 mo;
Kopple 2007	1.ET - 45.9(4.1) 2.ST - 46.0(2.7) 3.EST-42.7(3.8)	Not indicated; Exp: 45.9 (4.1) months Con: 51.4 (21.0) months Exp: 46.0 (2.7) months Con: 51.4 (21.0) months Exp: 42.7 (3.8) months Con: 51.4 (21.0) months	Age between 25 and 65 years; Clinically stable HD; on HD 3xwk;

Abbreviations: CAD, Coronary Artery Disease; Con, control; CVA, cerebro-vascular accident; DM, Diabetes Mellitus; Exp, experimental; HTN, hypertension; PAD, peripheral arterial disease; SD: standard deviation.

Table 2-3: Risk of Bias: PEDro Scores of Included Papers.

		Cheema (2007)	Chen (2010)	Dong (2010)	Johansen (2006)	Kopple (2007)
Allocation	Random	Yes	Yes	Yes	Yes	Yes
	Concealed	Yes	Yes	Yes	Yes	No
Blinding	Subjects	No	No	No	No	No
	Therapists	No	No	No	No	No
	Assessors	Yes	No	No	No	No
Follow-up	Adequate	Yes	Yes	No	Yes	No
	Intention-to-treat	Yes	No	No	Yes	No
Baseline comparability		Yes	Yes	Yes	Yes	Yes
Between group comparison		Yes	Yes	Yes	Yes	Yes
Point estimates and variability		Yes	Yes	Yes	Yes	Yes

Figure 2:3 Funnel Plot for Publication Bias.



Diff: difference; Std: standard.

2.4.0 Discussion

The results of our systematic review and mathematical combination of the SMDs indicate a small, statistically significant positive effect of strength training on muscle mass in participants with ESRD/HD. This indicates that one in nine participants is likely to show increase in muscle mass following exercise intervention.[27]

All studies included in this review employed strength training interventions with only one study reporting results of aerobic and mixed exercise intervention in two separate groups of participants.[25] However, factors such as differences in the mean age of the participants recruited, level of physical activity of the participants, ratio of male and female participants included

within the study[28] or magnitude of effort[29] may have influenced the results of this systematic review. The participants in the studies included in this review performed strength training at various levels of intensity; participants in the studies by Chen et al[24] and Johansen et al[23] performed three sets of 10 repetitions of leg exercises at 60% of one and three repetition maximum (RM), respectively, whereas participants in the Cheema[6] study performed two sets of eight repetitions of 10 exercises targeting the major muscle groups of the upper and lower extremities at a rating of perceived exertion of "hard" to "very hard". Hence, the intensity of strength training adjusted to 60% of one or three RM may influence its effectiveness as an anabolic intervention. Interestingly, in the study by Dong et al,[19] the participants worked slightly harder (70% of one RM) but no increase in lean muscle mass was observed following 24 weeks of training. Whether differences in the intensity of exercise truly influence the effectiveness of exercise intervention for muscle anabolism requires further investigation.

Hakkinen et al[28] observed a difference in training effects by gender in men and women over the age of 70 years, a small (2%) non-significant increase in quadriceps muscle CSA in older men and a significant 6% increase in quadriceps muscle CSA in elderly women following six months of strength training. These results suggest larger gains in muscle mass in healthy older adult females than in males following similar strength training exercises**.** However, the training effects observed by Cheema et al[6] (adjusted mean difference of -0.4 in muscle attenuation; male to female ratio -17:7) and Johanesen et al[23] (mean change of 1.2% in muscle CSA; male to female ratio - 12:8) may have been influenced by the gender of the participants; the larger training effects observed in females may have been nullified by smaller training effects in the male participants. Since the studies included in this review did not report results by gender, the anabolic effect of exercise by gender remains inconclusive.

Reporting of participant characteristics requires further attention for adequate comparison of factors limiting outcomes of exercise training such as presence of peripheral neuropathies or physical activity levels of the participants. Since neuropathic and myopathic changes have been reported in the skeletal muscle of people with ESRD/HD,[4] unequal proportions of participants with neuropathies in the different groups may have influenced the outcomes.[30] However, the incidence of polyneuropathy in the participants was not reported by the studies in this review. Also, Cupisti et al[31] reported a positive relationship between mean daily metabolic equivalent of tasks (METs) and phase angle measured using bio-electrical impedance vector analysis; a proxy measure for muscle mass and hence strength.[32] It is intuitive to assume that level of physical activity may augment the effects of strength training in this population; particularly in people with ESRD/HD with physical activity levels 20%-50% lower than age- and gender-matched sedentary population controls.[33,34] However, as the level of physical activity was not reported in any of the studies included in this review, we were unable to confirm if it varied significantly to impact the outcomes.

The lack of significant difference in our analysis for type of exercise (aerobic or strength training) also should be interpreted with caution. Sakkas et al[35] were able to demonstrate increase in muscle fibre areas following six months of aerobic training in nine participants. However, further research in this area is required to confirm the differences in anabolic effect that result from aerobic compared to strengthening exercises.

Another factor requiring further investigation is the impact of accrued extracellular fluid between HD treatments. During the interdialytic period, fluid accumulates within intravascular spaces and tissues[36] and particularly in the lower extremities as the result of gravitational forces.[37] Kaysen et al[36] used BIS to show that the intracellular fluid volume (ICF) does not vary

significantly during interdialytic periods. According to Kushner et al,[38] the extracellular fluid (ECF) in people with ESRD/HD is ~107 ± 19% of control values and ICF is ~93 ± 18% of controls. Thus, extra body water accruing during the interdialytic periods appears to accumulate mainly in the extracellular spaces. This suggests the possibility of a higher percentage of extracellular fluid volume in people on dialysis when compared to controls. This "excess ECF" may thus confound the measurement of true muscle size. Further research is required to establish techniques for accurate estimates of ECF between dialysis treatments and its impact on measurements of muscle mass or size.

Several observational studies have shown increments in aerobic capacity following aerobic and/or strength training exercises on non-dialysis days.[39] Although we set out to evaluate if the exercise intervention day (dialysis or non-dialysis day) affects the anabolic effect of the exercises, we found no randomized clinical trial where training was carried out on non-dialysis days. Hence, our results can be interpreted for exercise administered during dialysis only.

One of the major limitations of this study is the fact that different estimates of skeletal muscle mass were used by the studies included. Johansen et al[23] showed quadriceps muscle area improvement following 12 weeks of progressive resistance training, as measured by MRI, but Cheema et al[6] were unable to demonstrate any increase in thigh muscle cross-sectional area using CT scans following a similar duration resistance training program. However, Cheema et al[6] did demonstrate an increase in the attenuation of the CT signal, suggesting an increase in lean muscle mass. These results from the study by Cheema et al[6] of muscle attenuation expressed in Hounsfield units, were included in our analysis. Muscle attenuation was included to reduce the bias of suggesting there was no improvement (as demonstrated by non-significant change in thigh-muscle CSA) associated with exercise intervention, when in fact there was an increase in

thigh muscle attenuation, reflective of possible muscle hypertrophy without "true" increase in size. Five effect sizes included in the analysis were calculated from the three studies [19,24, 25] using DXA to measure LBM or FFM and two effect sizes from the one study that used BIS to measure FFM.[25] Body composition analysis using DXA is considered to be at "molecular"[15] level and is dependent on several factors including the software utilized for analysis of the acquired images.[40] Measurement of body composition using BIS is considered to be at a "whole body"[15] level and is also dependent on several factors such as cellular resistance, amount of subcutaneous fats and equations used for calculations of the body composition.[40] While it is reasonable to question the wisdom and validity of combining the SMDs obtained for such heterogeneous outcomes, the authors felt that such an effort was warranted, at the very least to provide a crude estimate of the likely effect of exercise on skeletal muscle mass. Also this study emphasizes the need for further research to evaluate the anabolic effects of exercise using uniform, gold standard measurements of muscle mass for appropriate comparisons.

2.5.0 Conclusions

In conclusion, this study evaluated the anabolic effect of exercise training in people with ESRD/HD from five studies representing a total of 206 participants. Our results support use of exercise to promote anabolism; one in nine participants is likely to benefit from such an intervention.[28] These results indicate that exercises promote anabolic milieu increasing muscle mass, improving muscle strength and hence, reduce morbidity and mortality in this population. We did not find a study providing direct evidence to indicate a relationship between increments in muscle size and reduction of relative risk of all-cause mortality. However, participants with normal BMI/high muscle mass had a lower relative risk of all-cause mortality in participants on

hemodialysis,[42] or peritoneal dialysis[43] when compared to people with high BMI and lower muscle mass.

2.6.0 References

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3 Chapter 3: Reliability of Calf Bioelectrical Impedance Spectroscopy and Magnetic Resonance Imaging Acquired Skeletal Muscle Hydration Measures in Healthy People.

3.1.0 Introduction

Hemodialysis (HD) is considered to have more pronounced deleterious effects on skeletal muscle than peritoneal dialysis.[1] The consequences of HD on physical function and quality of life have been well documented.[2] In order to offset such effects, exercise interventions have been strongly recommended to improve muscle function and reduce the risk of falls.[3] Recent systematic reviews and meta-analyses of exercise training in participants diagnosed with end-stage renal disease (ESRD) undergoing HD support the use of resisted and/or aerobic exercise to promote limb muscle strengthening.[4] These benefits were small and inconsistent with only one in nine persons receiving the benefits of exercise interventions.[5]

Extra body water accruing during the interdialytic periods has been reported to accumulate mainly in the extracellular spaces.[6,7] Hemodialysis or ultra-filtration is aimed at achieving normal state of hydration.[8] However, factors such as weight gain associated with improved appetite and nutritional support,[9] may influence estimation of true “dry weight” or what the weight the person would be when all the extra fluid is removed from the body.[10] This may lead to dehydration or relative volume depletion.[11] Clinical symptoms such as hypotension and intradialytic cramps have been associated with volume depletion or dehydration.[12] Such HD related dehydration or volume depletion has not yet been linked to poor response to exercise interventions or fatigue in this population. Using single frequency bioelectrical impedance analysis, Jain and Lindsay[13] have demonstrated loss of leg and arm extracellular fluid (ECF) in participants receiving conventional HD (three times a week) that returned to baseline at 48 hours.

They suggested that the majority of fluid loss and refilling takes place in the extremities to maintain the central blood volume that consists of the blood volume in the cardiopulmonary circulation plus great vessels. Consequently, change in pre and post HD body mass cannot be used to establish hydration of a limb or single muscle. Hence, assessing hydration of the specific muscle may provide insight and understanding of the role of HD-related fluctuating hydration on skeletal muscle function in people with ESRD/HD; as in athletes, dehydration of 1% to 2% of total body weight begins to compromise an array of physiologic functions and negatively influences performance and force generation.[14]

Several methods have been used to estimate the hydration of the human body. Bioelectrical impedance spectroscopy (BIS) is a safe and inexpensive *in vivo* method for whole body or segmental composition assessment providing estimates of ECF and intracellular (ICF) fluid volume shifts.[15] While BIS can provide estimates of ECF and ICF for the whole body or a segment of the body, it does not provide such estimates for a specific muscle, which limits its utility to ascertain the adequacy of HD to maintain adequate hydration at a muscle level.

Magnetic resonance imaging (MRI) acquired images provide quantitative measures of skeletal muscle fibre type composition and transverse relaxation time constants (T_2).[16] Researchers have used T_2 (T_2 - a measure of transverse magnetization signal decay) to investigate the changes in ECF volume or extracellular space ratio following drug-induced myopathy,[17] neural damage,,[18,19] and exercise.[20] Magnetic resonance imaging also provides an apparent diffusion coefficient (ADC), measured by a diffusion-weighted imaging technique, which is sensitive to the hydrodynamic environment of the tissue fluid volume.[21]

Test-retest reliability of BIS has wide variability in the reliability coefficient for total water (TW), ECF and ICF.[22] Establishing such test-retest reliability of calf BIS (cBIS) measures and T_2 and ADC for accurate estimates of true differences in skeletal muscle hydration in healthy people with varying levels of physical activity, age, and body composition is essential to determine the most reliable measure of muscle hydration that can be applied in the population with ESRD/HD.[23] Thus the primary purpose of this study was to evaluate the measurement properties of cBIS measures and MRI-acquired T_2 and ADC. The secondary objectives were to 1) compare the relative variations of the cBIS and MRI-acquired measures of hydration, and 2) evaluate association between these two methods of acquiring estimates muscle hydration. Based on prior published reports on relationship between the ECF and T_2, we evaluated agreement between ratio of ECF:ICF and T_2 of the tibialis anterior (TA), lateral and medial gastrocnemius and soleus muscles within the same calf segment.

3.2.0 Methods

3.2.1 Sample Size:

A required sample of 32 participants was calculated as suggested by Donner and Eliasziw,[24] based on alpha = 0.05, statistical power of 80% and a correlation of greater than 0.9 for a single observer on two observations.

3.2.2 Inclusion Criteria:

Eligibility to participate in the study was determined as follows:

1. Participants were healthy adults 18 years of age or older.
2. Were able to understand instructions in English and provide informed consent.
3. Had no health condition, existence of a foreign body or medical device that would preclude them from having an MRI.

Participants not meeting all of the inclusion criteria were excluded from the study. Western University's Ethics Review Board for Human Subjects granted ethics approval for this study and all participants provided written informed consent prior to participation.

3.2.3 Related Data:

Levels of self-reported physical activity was quantified using the Human Activity Profile Questionnaire (HAP)[25] administered to participants using a question and answer or interview format. A Maximum Activity Score (MAS - HAP) (the highest –numbered activity the person reports still doing) and an Adjusted Activity Score (AAS-HAP) calculated by subtracting the number of activities marked as "stopped doing this activity" listed below the MAS -HAP were generated using the HAP questionnaire to quantify the physical activity of participants. The HAP consisted of 94 activities, ranked in ascending order of difficulty according to the energy requirements of the task. The presence of any comorbidity was determined using the Charlson Comorbidity Index[26] calculated using the automated calculator (Appendix 2).

3.2.4 Skeletal Muscles:

Tibialis anterior, a foot dorsiflexor muscle, was chosen to represent a muscle from the anterior compartment of the lower leg, and lateral and medial gastrocnemius and soleus were selected to represent muscles from the posterior compartment.[27]

3.2.5 Calf Bioelectrical Impedance Spectroscopy:

A multi-frequency device (XiTRON 4200, Xitron Technologies, San Diego, CA, USA 92126) was used for automatic continuous sequential measurements of calf with frequencies ranging from 5 kHz to 1 MHz. Measures of ECF, ICF, TW, and ECF:ICF ratios for the calf segment were calculated as estimates of hydration. Two measuring (E_{S1} and E_{S2}) and two injecting electrodes (E_{I1} and E_{I2}) were placed on the lateral side of the tested leg. The E_{SI} electrode was placed at maximum circumference of the calf; E_{S2} was placed 10 cms distal to E_{S1}. Injecting electrodes E_{I1} was placed 5 cms proximal to E_{S1} and E_{I2} was placed 5 cms distal to Es_2 (Appendix 3). A fiduciary marker (vitamin E capsule) was placed at the E_{S1} electrode site for identification of the first measuring electrode on MRI. Each measurement was repeated at least 10 times and the average value was used in subsequent computation of the calf hydration estimate for that test occasion. Calculations and curve fitting (Cole-Cole model) were done offline as described by Zhu et al.[28]

3.2.6 Magnetic Resonance Imaging:

All MRI-acquired data were collected on a 3.0 Tesla Tim Trio whole body imaging system (Siemens, Erlangen, Germany) using an 8-channel knee coil. A multi-echo spin-echo (8

echoes) volume {11 contiguous 3 mm transverse slices; 160 mm field of view (16X16); 384 x 384 matrix; TE (13.1 ms to 93.6 ms); TR = 1500 ms} and a diffusion weighted volume (b=400 s/mm^2; 6 directions; 22 contiguous 4 mm transverse slices; 160 mm field of view; 128 x 128 matrix; TE =61.6 ms; TR = 6200 ms; 8 averages) were used to measure the T_2 and ADC, respectively, of the TA, lateral and medial gastrocnemius and soleus muscles.

The muscles of interest were outlined, close to the fiducial marker visible on MRI, on the T_2 weighted images acquired for the purpose of calculating T_2 and T_2 maps for the muscle cross-sectional area (CSA) were generated in OsiriX using the "T2 Fit Map" plugin (OsiriX v 3.9.4, 32 bit, Pixmeo Sari). On this map, three areas or regions of interest (ROI) less than 0.22 cm^2 were selected taking care to avoid visible subcutaneous fat, septum, or neurovascular bundles (Figure: 3.1).[29] We sampled three areas of a muscle and values were averaged to obtain a representative T_2 for each muscle.

Figure 3:1: Example of Measuring Transverse Relaxation Times of Tibialis Anterior.

Calculations of ADC for each of the four muscles were completed using the CSA of muscle as outlined in the T_2 weighted image and imported into the ADC map generated using OsiriX plugin "ADC Map"(Appendix 4);[21] the software automatically calculated and displayed the ADC values.

3.2.7 Data Collection:

Data were collected on two different occasions at least one day apart preferably at the same time of day. Participants were asked to refrain from exercise and alcohol for 8 to 10 hours and not eat or drink anything for two hours prior to testing to control extraneous factors affecting hydration of the muscle/body for short durations.[22] Participants were positioned in supine lying on a standard hospital bed for 30 minutes prior to imaging to allow redistribution of water to the lower extremities.[30] Measurements of hydration using cBIS were collected while the participants were lying supine in preparation for MRI. All BIS and MRI-acquired measures of hydration were collected from the same calf segment.

3.2.8 Statistical Analysis:

Participant demographic data, including age, average body mass, body mass index, HAP scores,[25] and Charlson Comorbidity Index[26] were analyzed using descriptive statistics and reported as group data with means and standard deviations. The test-retest reliabilities for all measures of hydration (ECF, ICF, TW, ECF:ICF for the calf segment and T_2 and ADC measures for TA, lateral and medial gastrocnemius and soleus) were assessed using two-way random effects intra-class correlation coefficients ($ICC_{2,1}$).[31] An $ICC_{2,1} \geq 0.9$ was considered "excellent," and values ≥ 0.8 and < 0.9 were considered "good" and those below 0.8 were

considered to be of questionable clinical significance.[32] Minimal detectable change values at 95% confidence (MDC_{95}) were calculated using the formula: $MDC_{95} = SEM \times \sqrt{2} \times 1.96$ where SEM is the standard error of measurement calculated from the $ICC_{2,1}$ analysis of variance tables.[33] The MDC_{95} is an estimate of the amount by which 95% of participants' who remain unchanged will display random fluctuations. The variability in their change scores is expected to be within the bounds of MDC_{95} value.[33] The SEM estimates how repeated measures of a participant tend to be distributed around the true score.[34] For each measure, the difference between the measurements obtained on two occasions was plotted against the mean of those two measurements. The 95% limits of agreement (LOA) were estimated by the mean difference between the two occasions, $\bar{\boldsymbol{d}}$ and the standard deviation of that difference (s). Assuming a Gaussian distribution, 95% of the differences were calculated to lie between $\bar{\boldsymbol{d}}$ - 1.96s and $\bar{\boldsymbol{d}}$ +1.96s.[23] Coefficient of variation (CV) for each measure was computed using statistical software. Levene's test, used to evaluate the homogeneity of variances between the cBIS and MRI-acquired measures, was computed by using one-way analysis of variance on the absolute deviation of each score from the mean of its group.[35] The association between ECF:ICF and MRI-acquired T_2 and ADC of TA, lateral and medial gastrocnemius and soleus was evaluated using linear regression analysis.[36] A statistical software package (IBM SPSS v20.0) was used for all data analyses and Prism 4.0a for Macintosh (GraphPad Software Inc.) was used for plotting and analyses of differences-against-mean plots. Statistical significance was assumed for p-values < 0.05.

3.3.0 Results

3.3.1 Participants:

Thirty-two participants (16 men and 16 women) with mean age of 50.8 ± 14.0 years, meeting the inclusion criteria were recruited for this study. Table 3.1 summarizes the demographic characteristics of these participants.

Table 3-1: Characteristics of the Participants Included in Study (n=32).

Participant characteristic	Group mean (SD)
Weight (kg)	75.27 (13.24)
Height (cms)	168.1 (8.6)
BMI (kg/m^2)	26.3 (3.3)
Charlson Comorbidity Index	0.16 (0.72)
HAP-MAS	87.8 (5.3)
HAP-AAS	85.4 (8.3)

AAS: adjusted activity score; BMI: body mass index; HAP: human activity profile; SD: standard deviation.

Table 3-2: Lower Leg and Individual Skeletal Muscle Hydration Measures Using Bioelectrical Impedance Spectroscopy and Magnetic Resonance Imaging in Healthy Adults (n = 32).

Measure	Occasion 1 Mean (SD)	Occasion 2 Mean (SD)
BIS (Liters)		
ECF	0.15 (0.02)	0.15(0.02)
ICF	0.49 (0.15)	0.48 (0.15)
TW	0.64 (0.16)	0.62 (0.17)
ECF:ICF	0.31 (0.09)	0.31 (0.06)
T_2 (ms)		
Tibialis anterior	36.4 (1.4)	36.2 (1.6)
Medial gastrocnemius	38.7 (3.3)	38.8 (3.3)
Lateral gastrocnemius	40.2 (3.2)	40.3 (3.0)
Soleus	39.7 (2.4)	39.8 (2.4)
ADC (mm^2/s)		
Tibialis anterior	1.5 (0.1)	1.5 (0.1)
Medial gastrocnemius	1.6 (0.1)	1.6 (0.1)
Lateral gastrocnemius	1.6 (0.1)	1.6 (0.1)
Soleus	1.5 (0.1)	1.5 (0.1)

ADC: apparent diffusion coefficient; BIS: bioelectrical impedance spectroscopy; ECF: extracellular fluid; ICF: intracellular fluid; SD: standard deviation; T_2: transverse relaxation time constants; TW: total water.

3.3.2 Reliability of Measures of Muscle Hydration:

Results for the measures of hydration for occasion one and two are presented as group means and standard deviations in Table 3.2. Values for the $ICC_{2,1}$, the MDC_{95}, bias and the 95% LOA are presented for each measure of hydration in Tables 3.3 to 3.5. For the test-retest reliabilities of the cBIS measures (Table 3.3), ECF:ICF had the lowest reliability, while ICF and TW had the highest. For T_2, the average of three ROI yielded better test-retest reliability than estimates derived from a single ROI (Table 3.4). $ICC_{2,1}$ value for single measures of ADC of the leg muscles varied between 0.4 and 0.5 (Table 3.5). However, for average measures of ADC, $ICC_{2,1}$ ranged from 0.6 – 0.7.

Table 3-3: Test-retest Reliabilities of Calf Bioelectrical Impedance Spectroscopy Measures of Hydration in Healthy Adults (n =32).

Estimate of calf muscle hydration	$ICC_{2,1}$ (95% CI)	MDC_{95} (Liters)	Bias (SD)	95% LOA
ECF	0.8 (0.6, 0.9)*	-	-0.004 (0.1)	-0.03, 0.02
ICF	0.9 (0.8, 0.9)*	0.2	0.02 (0.05)	-0.08, 0.12
TW	0.9 (0.8, 0.9)*	0.2	0.02 (0.1)	-0.09, 0.12
ECF:ICF	0.6 (0.3, 0.8)*	0.1	-0.01 0.1)	-0.15, 0.12

*Indicates $p < 0.001$.

CI: confidence interval; ECF: extracellular fluid; ICC: intraclass correlation; ICF: intracellular fluid; LOA: limits of agreement; MDC: minimal detectable change; SD: standard deviation; TW: total water.

Table 3-4: Test-retest Reliabilities of Transverse Relaxation Time constants.

Muscle	Single measure (95% CI)	Average (2 measures) (95% CI)	Average (3 measures) (95% CI)	MDC_{95} (ms)	Bias (SD)	95% LOA
Tibialis Anterior	0.9 (0.8, 0.9)*	0.93 (0.9, 0.96)*	0.93 (0.9, 0.97)*	1.06	0.09 (0.56)	-0.99, 1.18
Lateral Gastrocnemius	0.98 (0.9, 0.98)*	0.97 (0.9, 0.99)*	0.99 (0.97, 0.99)*	1.29	-0.12 (0.55)	-1.19, 0.96
Medial Gastrocnemius	0.9 (0.9, 0.98)*	0.97 (0.9, 0.99)*	0.99 (0.97, 0.99)*	0.94	-0.05 (0.29)	-0.62, 0.52
Soleus	0.9 (0.8, 0.9)*	0.9 (0.9, 0.97)*	0.96 (0.92, 0.98)*	0.29	-0.03 (0.96)	-1.85, 1.90

* Indicates $p < 0.001$.

CI: confidence interval; ICC: intra-class correlation; LOA: limits of agreement; MDC: minimal detectable change; SD: standard deviation.

Table 3-5: Test-retest Reliability of Apparent Diffusion Coefficient Measures.

Muscle	$ICC_{2,1}$ (95% CI) Single Measure	$ICC_{2,1}$ (95% CI) Average Measures	MDC_{95} (mm^2/s)	Bias (SD)	95% LOA
Tibialis Anterior	0.4 (0.06, 0.67)*	0.6 (0.13, 0.79)*	0.96	0.03 (0.10)	-0.17, 0.23
Lateral Gastrocnemius	0.5 (0.1, 0.7)*	0.6 (0.2, 0.8)*	0.88	0.13 (0.74)	-1.31, 1.57
Medial Gastrocnemius	0.4 (0.1, 0.7)*	0.6 (0.2, 0.8)*	0.09	0.01 (0.078)	-0.15, 0.16
Soleus	0.5 (0.2, 0.7)*	0.7 (0.3, 0.8)*	0.13	0.01 (0.11)	-0.19, 0.22

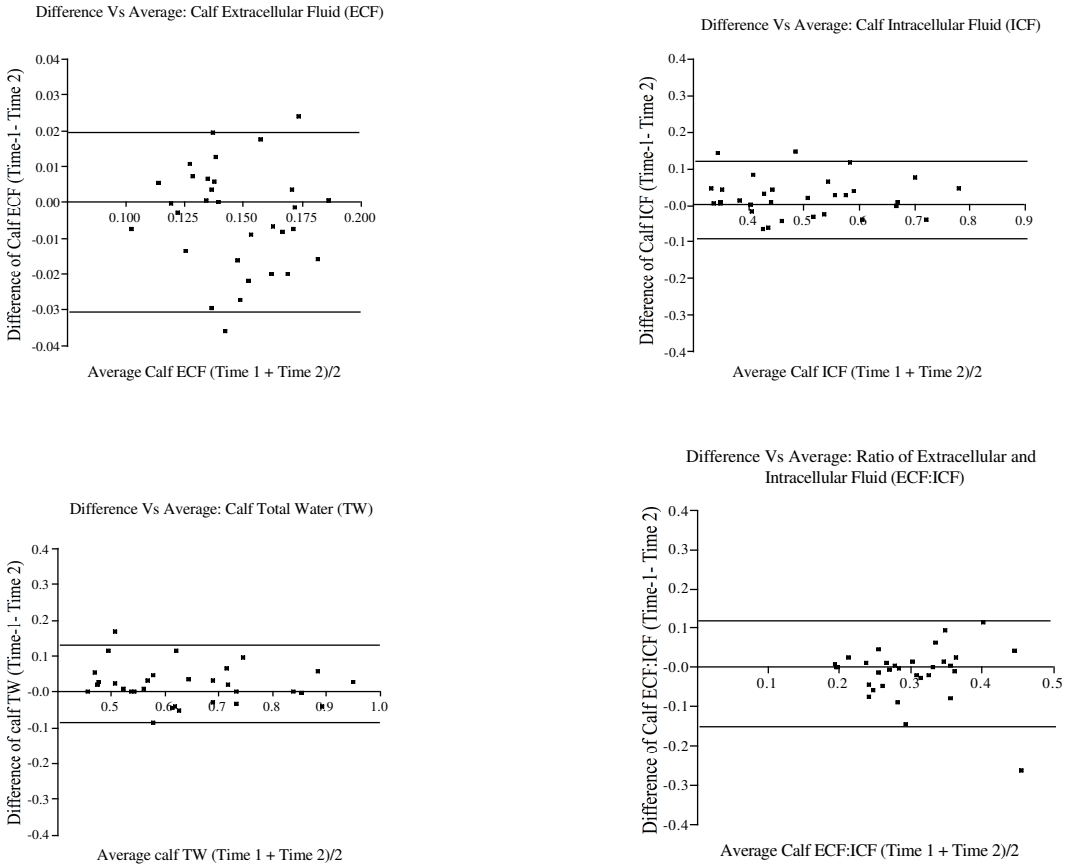
* Indicates $p < 0.05$

CI: confidence interval; ICC: intra-class correlation; LOA: limits of agreement; MDC: minimal detectable change; SD: standard deviation.

3.3.3 Limits of Agreement between Repeated Measures of Hydration:

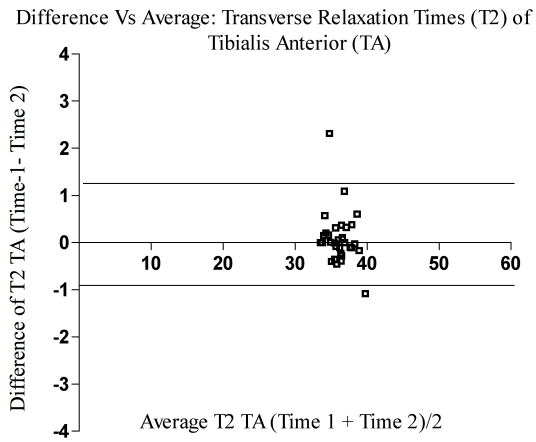
The difference-against-mean plots of ICF and TW indicate a larger positive bias at the lower end of the scale that is larger bias at smaller values of ICF and TW. The plot of the ECF:ICF ratio (Figure 3.2) indicates increasing bias at higher values of the ratio. The plots of T_2 (Figure 3.3) for all the muscles do not reveal any systematic bias; however, a single data point for T_2 of TA and three data points for soleus were observed to lie outside the 95% LOA. For the ADC, the plots (Figure 3.4) for all the muscles did not identify any systematic bias.

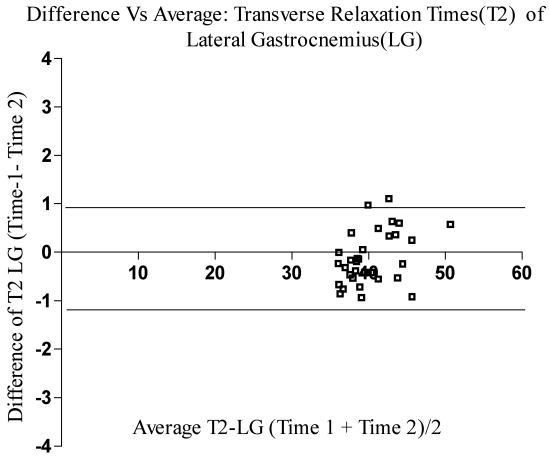
Figure 3:2: Plots of Difference vs. Average Calf Bioelectrical Impedance Spectroscopy Measures.

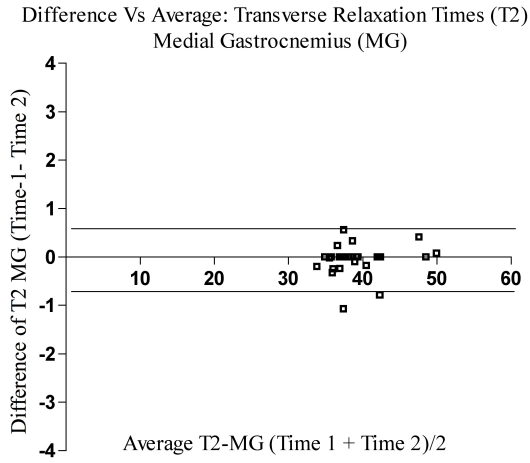


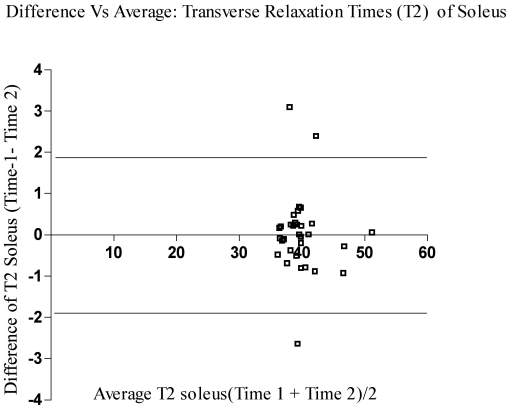
ECF: extracellular fluid; ICF: intracellular fluid, TW: total water.

Figure 3:3: Plots of Difference vs. Average of Transverse Relaxation Time Measures.



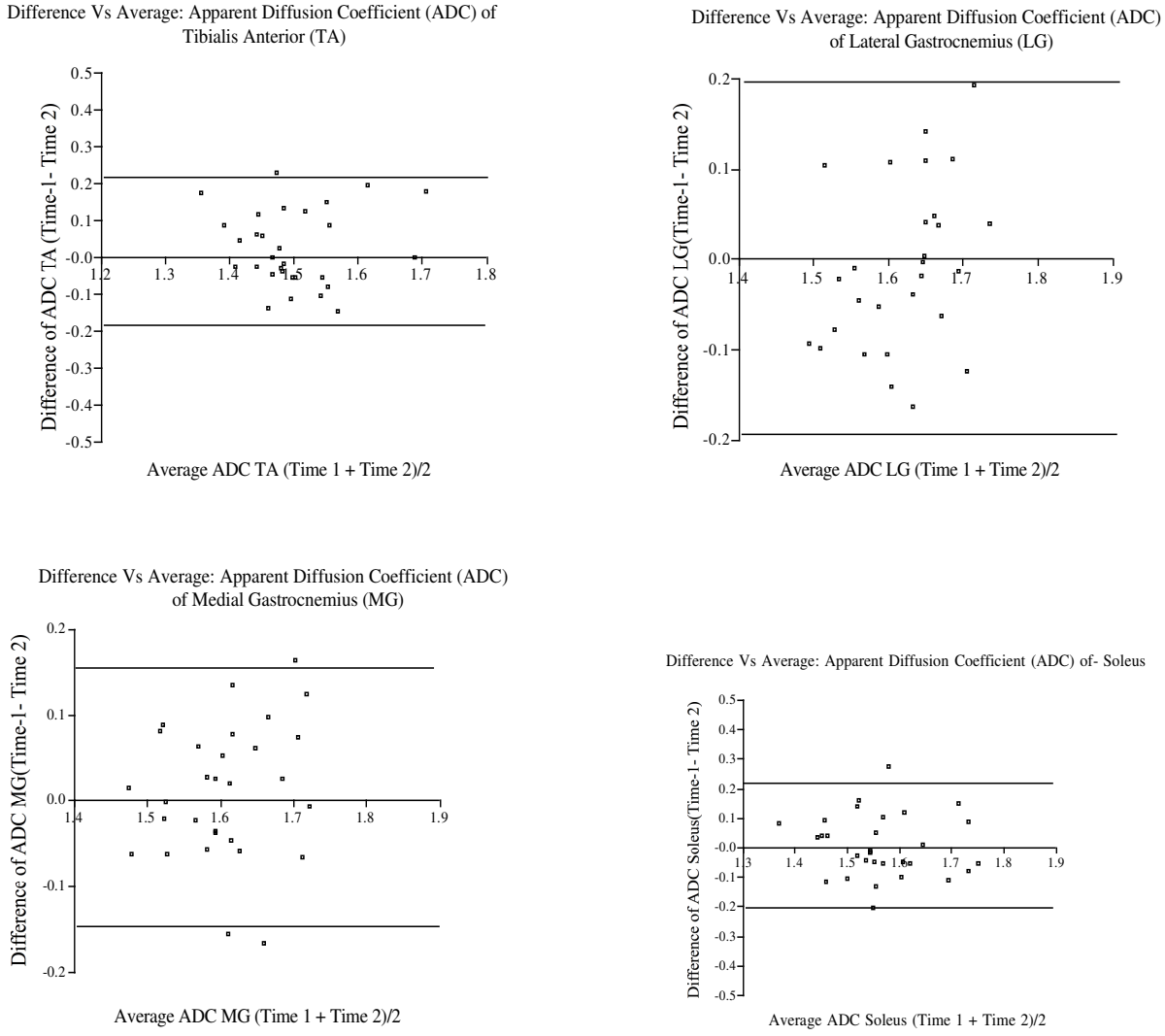






LG: lateral gastrocnemius; MG: medial gastrocnemius; TA: tibialis anterior; T_2: transverse relaxation times.

Figure 3:4: Plots of Difference vs. Average of Apparent Diffusion Coefficient Measures.



ADC: apparent diffusion coefficient; LG: lateral gastrocnemius; MG: medial gastrocnemius; TA: tibialis anterior.

3.3.4 Comparison of Variances in Calf BIS and MRI-acquired Measures of Hydration:

The CVs for each measure on occasion one and two are presented in Table 3.6. The Levene's test for equality of variances between the measures was significant ($F_{23,744} = 18.405$; $p<0.001$). Post hoc comparisons using Turkey's HSD indicated that the differences in mean variations between the MRI-acquired T_2 and ADC of the muscles investigated and ICF, TW and ratio of ECF:ICF were significant. However, these differences were not significant for T_2 of lateral and medial gastrocnemius and soleus, ADC of all the muscles and ECF.

Table 3-6: Coefficient of Variability of Calf BIS and MRI-acquired Measures of Hydration on Occasion One and Two.

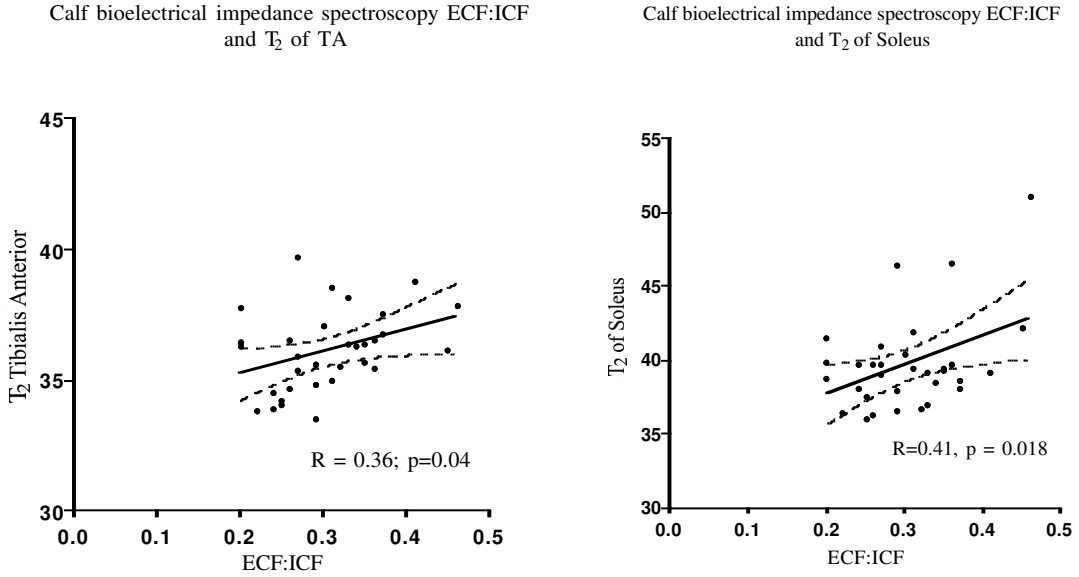
		T_2 -1	T_2 -2	ADC-1	ADC -2
Tibialis Anterior		4.2	4.5	6.5	6.1
Lateral Gastrocnemius		9.0	8.4	7.2	7.6
Medial Gastrocnemius		10.0	10.0	5.4	5.2
Soleus		8.1	8.2	6.5	7.2
		Occasion 1		Occasion 2	
cBIS	ECF	14.6		15.3	
	ICF	24.9		26.0	
	TW	20.7		22.6	
	Ratio (ECF:ICF)	24.9		25.6	

cBIS: calf bioelectrical impedance spectroscopy; ECF: extracellular fluid; ICF: intracellular fluid; T_2: transverse relaxation times; TW: total water.

3.3.5 Association between Calf BIS and MRI-acquired Measures of Hydration:

Ratio of ECF:ICF was significantly associated with T_2 of TA [$\beta = 0.4$, $p=0.041$; $b = 32.9$ (95%CI: 31.2, 36.1), $t_{(30)} = 20.38$ $p<0.05$] and soleus [$\beta = 0.4$, $p=0.018$; $b = 33.8$ (95%CI: 28.8, 36.1), $t_{(30)} = 13.75$, $p<0.05$]; Ratio of ECF:ICF explained a significant proportion of T_2 of TA [$R^2=0.13$, $F_{(1,30)} = 4.56$, $p < 0.05$] and soleus [$R^2=0.17$, $F_{(1,30)} =6.24$, $p <0.05$] (Figure 3.5). The agreement between ECF:ICF and T_2 of lateral gastrocnemius and medial gastrocnemius and ADC of all the muscles investigated were non-significant (Table 3.7).

Figure 3:5: Graph of ECF:ICF by T_2 of Tibialis Anterior and Soleus.



ECF: extracellular fluid; ICF: intracellular fluid; T_2: transverse relaxation times; TA: tibialis anterior.

Table 3-7: Results of Association between Calf BIS and MRI-acquired Measures.

		R		R^2		SEE		Constant (95% CI)		cBIS ECF:ICF (95%CI)	
		T_2	ADC	T_2	ADC	T_2	ADC	T_2	ADC	T_2	ADC
cBIS ECF:ICF	TA	0.36*	0.12	0.13*	0.01	1.45	0.08	33.6* (31.2,36.1)	1.5 (1.3, 1.6)	8.3* (0.4, 16.3)	0.1 (-0.3, 0.6)
	LG	0.29	0.08	0.08	0.01	3.39	0.1	35.7 (29.9, 41.4)	1.6 (1.4, 1.7)	15.0 (-3.4, 33.5)	0.1 (-0.4, 0.7)
	MG	0.28	0.15	0.08	0.02	3.8	0.07	33.9 (27.6, 40.4)	1.6 (1.4, 1.7)	17.2 (-19.1, 53.4)	0.16 (-0.2, 0.6)
	Soleus	0.41*	0.22	0.17*	0.05	3.1	0.09	33.8 (28.8, 38.8)	1.66 (1.51, 1.82)	19.8* (3.6, 35.9)	-0.3 (-0.81,0.21)

* indicates $p<0.05$

ADC: apparent diffusion coefficient; CI: confidence interval; ECF: extracellular fluid; LG: lateral gastrocnemius; MG: medial gastrocnemius; T_2: transverse relaxation times; TW: total water; SEE: standard error of estimate.

3.4.0 Discussion

To our knowledge this is the first study that has established test-retest reliability of the cBIS and MRI-acquired measures of skeletal muscle hydration, viz. T_2 and ADC. The results of this study indicate that T_2 time constants estimating hydration of muscles of the lower leg have excellent reliability and reproducibility when compared to cBIS and ADC. This study also established MDC_{95} values indicating random fluctuations in the measures of hydration in 95% of unchanged participants on repeated measurements. According to our results, an MDC_{95} of 0.96 ms between repeated measures of T_2 of lateral gastrocnemius indicates a minimum fluctuation of the measure one should expect to see in 95% of the participants whose measurements randomly fluctuate in repeated measurements.

Based on the characteristics of the participants recruited in this study, the results of this study can be generalized to a wide range of healthy and active population. The age of the participants in this study ranged from 22 to 81 years and the activity profile or the HAP scores ranged from 75 to 94. The activity levels of the population can impact the BIS measures[37] and hence we recruited active participants to reduce variability in cBIS measures.

Our findings of good to excellent reliability of the cBIS measures are in accordance with those reported in the literature for the repeated measurements of whole body and segmental BIS.[22] Several factors can impact the test-retest reliability of cBIS-acquired data. These include day-to-day variation in body hydration,[22] exercises,[20,38] participants' body type, and equations utilized to estimate ECF, ICF and TW. The reliability of the ECF:ICF may have been affected by the differences in body types of our participants (BMI 25.9± 2.8), and differences in offline

model fitting (range 0 to 2) affecting ECF volume calculations of these participants. These findings are supported by difference-against-the-mean plot that suggests that the errors in estimating ECF:ICF were influenced by the value of the ratio, i.e. the errors in measurements were larger at higher values of ECF:ICF ratio and vice versa. Hence contribution of the differences between measurements may not be associated with technical differences or operator error. Kyle et al[22] have reported similar findings in studies evaluating whole body composition as well.

Our findings of excellent $ICC_{2,1}$ for T_2 of all the muscles investigated indicate the measures ability to differentiate between the study participants. Factors such as exercise[20] and perhaps variations in placement of the limb in the MRI receiver coil influencing signal-to-noise ratio may impact MRI-acquired measures as well. In this study, about 4% of the measurements for T_2 of soleus lay outside the 95% limits of agreement; one was lower and two higher than the limits of agreement. Two of these participants' had performed light physical activity just prior to their scheduled data collection session and perhaps 30 minutes of supine positioning may not have been adequate to equilibrate the muscle ECF and ICF associated with effects of physical activity and led to the differences in the T_2 of soleus between the two occasions.[38] The increments in T_2 of the soleus on repeated measurements can be supported by findings of Ngygren et al[20] in participants who performed light exercises using a 1.5 Tesla magnet and four echoes. For the difference in repeated measures of T_2 of gastrocnemius muscle for the non-exercising leg they observed a change of 0.4% to 1.0% in the T_2. This change is slightly higher than the change we observed on repeated measurements of T_2 of the lateral and medial gastrocnemius (0.01% – 0.22% calculated based bias of 0.09 and mean of 40.19 ms for lateral gastrocnemius on bias of 0.004 and mean of 38.73 ms for medial gastrocnemius). These

differences between our results and those reported by Nygren et al[20] could be due to several factors such as differences in the mean age of the participants (mean age of participants in the study by Nygren et al[20] was 25 yrs), number of echoes utilized for measuring T_2 (Nygren et al[20] computed T_2 by fitting four echoes), sample size (Nygren el al[20] recruited six participants), and perhaps in variations in intra-muscular fat.

Our overall results indicate a smaller variation for the MRI-acquired muscle hydration measures, ranging from 4.2 to10%; whereas the same for the cBIS-acquired measures ranged from 14.6 to 25.6%. The variations in anatomical configurations of the muscles of the calf by age of the participants recruited in this study support our findings of minimum variability of T_2 for TA and largest variability in the ICF. Using segmental multi-frequency BIS Yamada et al[39] suggested that the expansion of ECF relative to ICF and the lean volume of the lower extremity masked actual muscle cell atrophy with aging. This supports our observations of larger relative within-subjects variations for the cBIS-acquired measures as we recruited participants aged from 22 to 81 years.

Transverse relaxation times of muscles investigated measured changes unique to each muscle; hence numerous studies on the dorsiflexors[40-42] reporting lack of significant difference in strength and contractile properties/kinetics between the young and old support our findings of smaller within-subject variability for TA. However, according to Vandervoort et al[43] age related changes in muscle strength and size were larger in the plantar flexor muscles. Hence the CV for T_2 of plantar flexor muscles (lateral and medical gastrocnemius and soleus) was larger than that for the T_2 time constants of TA values for these muscles.

Joseph et al[44] quantified reproducibility of T_2 of cartilage using coefficient of variation

(CV) ranged from 0.83 to 3.21. This is slightly higher than the results with CV for T_2 of TA (0.04) and medial gastrocnemius (0.09). These differences could be due to variations of the morphological characteristics and hydration of knee cartilage and skeletal muscle.

Skeletal muscle is a heterogeneous structure in its composition and architecture. Measurement of hydration for a limb segment such as calf using BIS can be considered to be at a "whole body" level, and MRI estimates of hydration at the "tissue system level".[45] A direct comparison of these two techniques, based on different assumptions and methods is challenging. Besides no study has yet determined a direct relationship between T_2 and wet/dry weight of the calf/shank muscles in healthy individuals. Our results of coefficient of regressions indicate that ECF:ICF ratio explained 13 and 17% variance observed in the T_2 of TA and soleus respectively. Several factors other than the interstitial fluid in TA may contribute to the variations in T_2 of TA and soleus. Generally T_2 time constants correlate most strongly with bulk water content of the tissue.[46] Tissues with high concentrations of aliphatic lipid protons have longer T_2. Hence MRI signals arising from bulk water content or total water and the degree of binding to the lipids may result in alterations of T_2. Since fats/lipids hold about 10-15% of total water,[47] the impact of possible increase to intra and extra-myocellular lipids and the amount of water bound within these lipids may have impacted our goodness-of-fit between T_2 of TA and soleus and ECF:ICF ratio; T_2 accounting for the water bound to lipids and ECF:ICF excluding this portion of water. We did not correct our data prior to analysis for the changes in T_2 associated with possible increase in intra and extra-myocellular lipids in this population. However we attempted to control for these changes by choosing small ROIs for analyses.

Factors other than the interstitial fluid in TA and soleus may contribute to the estimates ECF:ICF ratio such as plasma volume in the large vessels viz. the popliteal artery, anterior tibial

artery, saphenous veins, and intra and extracellular spaces embedded within the other muscles of the calf viz. lateral and medial gastrocnemius and peronei. The contribution of the interstitial space encompassed by TA and soleus to the ECF and ICF may perhaps also be related to the total space occupied by the muscle in relation to the total calf volume supporting our findings of total variance in T_2 of TA and soleus explained by ECF:ICF. Although ratio of ECF:ICF could significantly predict the T_2 of TA and soleus, the standard error of estimate was larger than the MDC_{95} value of T_2 of TA and soleus. Hence the predicted values for T_2 of TA and soleus using these equations may have larger error for estimating true value.

The T_2 of the muscles evaluated in this study ranged from 36.37 ms for TA and 40.13 ms for the soleus muscles. This reflects the differences in the fibre-type composition of these muscles. The heterogeneous muscle fibre composition and plasticity of skeletal muscles (ability to change the fibre type composition in response to environment/physical activity) can affect estimates of T_2 as MRI signals are strongly related to the histochemical composition of the tissue.[48] These findings of heterogeneous T_2 are in accordance to prior published reports investigating T_2 of various muscles. For example subscapularis muscle T_2 (31.5 ms) were slightly higher than anterior deltoid (29.5 ms); the same authors showed a change of 10 ms in the supraspinatus muscle T_2 following three sessions of exercise intervention (empty-can and full-can exercise) performed within one week.[49] Additionally, the diffusion rate of muscle cell membrane that takes place in the presence of magnetic local field in-homogeneities or variations in the magnetic field at different parts pf the sample that are constant in time, can impact the T_2.[16]

The poor reliability of ADC measures can be supported by the fact that body fluids are rarely stable. Study of volume of body fluid compartments, ICF and ECF concentration, using

isotope dilution methods requires several hours to equilibrate (3-5 hrs) the isotopes.[38] For practical reasons we allowed only 30 minutes for body fluids to equilibrate prior to acquiring MRI data and this may have perhaps affected the reliability of the ADC measures. However, as ADC can be influenced by changes in the osmolality, change in ADC may be more sensitive to the changes in microcirculation associated with activity or hydration status of the muscle and a difference greater than 0.1 to 0.12 mm/s^2 would indicate a true change on repeated measurements.[34] Following eccentric exercise interventions, Yanagisawa et al[21] were able to demonstrate significant change in ADC values of lateral gastrocnemius that reflected physiological changes affecting fluid movement associated with such exercises.

Our results can be applied to the studies utilizing similar methods of selecting smaller ROI and using OsiriX for data analysis with data collected as described in the methods section .[50] A review of literature indicates differences in the techniques of measurement of T_2. For example, Liu et al[51] measured T_2 of the rat TA, gastrocnemius and soleus using in-house software, and the whole muscle boundaries were outlined as regions of interest; Hatakenaka et al[17] selected ROIs between 20 to 30 mm^2 for each muscle and calculated T_2 by fitting the appropriate signal intensities to an equation. Hence, it would be inappropriate to compare our findings to results of their studies.

3.5.0 Conclusions

The findings of our study indicate that the MRI-acquired T_2 of the muscles of the lower leg have excellent test-retest reliability and are appropriate for studies utilizing repeated measurements. Besides the relative variability of this measure is smaller than the cBIS-acquired measures and hence can be utilized in studies recruiting participants of varying age. These results

can be applied to studies utilizing similar techniques for data collection and analysis. The ADC of the leg muscles can be used in studies with relatively large sample sizes, as ADC may be sensitive to minor variations in the circulation of the muscles associated with variations in body fluids and activity. Although cBIS-acquired measures have excellent test- retest reliability, whether these measures can be used to estimate interstitial fluid of a single muscle requires further investigations.

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4 Chapter 4: Association Between Measures of Skeletal Muscle Hydration Acquired Using Calf Bioelectrical Impedance Spectroscopy and Magnetic Resonance Imaging in People with End-stage Renal Disease on Hemodialysis.

4.1.0 Introduction

Falls commonly predict morbidity, mortality and perhaps need for institutional care.[1] People with end-stage renal disease (ESRD) on hemodialysis (HD) are known to have skeletal muscle weakness[2] and renal osteo-dystrophy,[3] predisposing them to increased risk for falls[4] and long bone fractures.[2] Hence, exercise training has been strongly recommended to maintain skeletal muscle function and prevent falls and related injuries.[5] However the uptake and adherence to exercise interventions is limited.[6] Besides the presumed benefits of exercise in this population have not been observed consistently.[7]

Expansion of extra cellular fluid (ECF) volume is one of the manifestations of ESRD, and HD is required to correct this, although it may lead to periods of relative volume contraction.[8] Clinical symptoms such as hypotension and intradialytic cramps have been associated with volume depletion or dehydration.[9] However such HD related dehydration or volume depletion has not yet been linked to poor response to exercise interventions or muscle weakness in this population. Using single frequency bioelectrical impedance analysis, Jain and Lindsay[10] have demonstrated loss of ECF in leg and arm segments of the whole body in participants receiving conventional HD (three times a week). Hence change in pre and post HD whole-body mass cannot be used to establish hydration of a limb segment or single muscle. The impact of this fluctuating hydration, pre and post HD, of the skeletal muscle in people receiving HD is yet to be elucidated. According to Cleary et al[11] a volume contracted individual who performs eccentric exercise/activities may exacerbate skeletal muscle damage, leading to

impairment of structural and contractile properties. Hence, assessing the HD-related changes in ECF volumes of skeletal muscle may provide insight and understanding of the effect of such fluctuating hydration on muscle function in people with ESRD/HD.

Since total body water turnover is complex, none of the measures of hydration based on laboratory techniques such as isotope dilution, neutron activation analysis, body mass change, and plasma volume change, have been established as "gold standard" measures to unquestionably represent accurate changes in total body water gain or loss.[12] Bioelectrical impedance spectroscopy (BIS) has been widely used to measure ECF and intracellular fluid (ICF) space within a limb segment such as calf, limb or whole body. This method of measuring whole-body ECF and ICF has been validated using deuterium and bromide dilution techniques. According to van Marken Lichtenbelt and colleagues a correlation of 0.95 ($p<0.05$) was observed between the dilution methods and BIS techniques for the ECF.[13] Researchers have utilized this method in people with ESRD/HD to evaluate the fluctuations in ECF and ICF before and after HD interventions.[14] However, whether BIS measures can be used to estimate hydration of a single muscle is yet to be established.

Magnetic resonance imaging (MRI) acquired transverse relaxation time constants (T_2 - a measure of transverse magnetization signal decay) or apparent diffusion coefficient (ADC) has been used as an estimate of changing interstitial fluids/ECF volumes of skeletal muscle. Hatakaneka et al[15] have indisputably demonstrated a relationship (Pearson $r = 0.813$, $p<0.05$) between extracellular space ratio and T_2 in an animal model (rabbits), using biopsy to microscopically measure extra and intracellular spaces for establishing these correlations. Hence T_2 can be considered as a valid estimate of the ratio of extracellular and total space within a

skeletal muscle; i.e. T_2 time constants will rise with an increase in extracellular space. Investigations using T_2 for estimating changes in ECF volume following neural damage,[16,17] and exercise[18] lend further support to use this measure as an estimate of ECF at the muscle/tissue level.

Similarly, MRI-acquired ADC has been used to evaluate fluid changes between the intra and extracellular compartments following denervation of hamstring muscle in rats[16] or following plantar-flexion exercise in humans.[18] The increase in ADC following denervation is considered to be due to an increase in ECF and shrinking of the intracellular space associated with atrophy. The increase in ADC following high intensity exercise is attributed to fluid movement between the intra and extracellular compartments.[18] Hence ADC can also be utilized to estimate ECF and ICF specific to a muscle; ADC increases as extracellular spaces increase (increase in muscle volume) and decreases as the extracellular spaces shrink. However, T_2 and ADC have not been commonly used as estimates of ECF in research.[19] Besides acquisition and analysis of MRI based methods are both expensive and resource intensive.

The primary objective of this paper was to evaluate association between calf ECF measured using BIS (cBIS) and MRI-acquired T_2 or ADC of TA to establish if cBIS ECF can be used to estimate interstitial fluid in TA muscle instead of resource and labor intensive MRI-acquired T_2/ADC. Secondary objectives were to evaluate association between 1) cBIS ECF and T_2 of medial and lateral gastrocnemius, and soleus muscles and 2) cBIS ECF and ADC of TA, medial and lateral gastrocnemius and soleus muscle to determine if cBIS ECF can be used to estimate interstitial fluid of these specific muscles.

To further investigate the association between the cBIS and MRI-acquired measures, we compared the equality of the pre and post HD coefficients of regression quantifying the mean rate of change in T_2/ADC of the muscles for each unit of cBIS ECF.

A priori hypotheses were grounded in theoretical assumptions based on distribution of type II fibres and the size/volume of the muscles. Previous reports have established presence of atrophy of type II fibres in people with ESRD/HD.[20] However; according to Johansen et al[21] the total muscle compartment for the TA was not significantly different in size from the control participants (people with no disease). As a result, the space occupied by these atrophic muscle fibres may expand the relative ECF volume of the muscle. Hence we hypothesized that T_2 of the TA muscle (~50% type II fibres)[22] will have a stronger relationship with cBIS ECF followed by T_2 of medial and/or lateral gastrocnemius (~43% type II fibres)[22] and the weakest association with the soleus muscle (20% type II fibres).[23]

As an alternative, we hypothesized that the muscle occupying maximum volume would also encompass the most interstitial fluid volume. Based on this assumption we hypothesized that the T_2 of soleus muscle will have the highest correlation with cBIS ECF followed by medial and lateral gastrocnemius and TA respectively.[24] However for ADC, as people on HD are known to have reduced muscle mass, reduced ratio of capillary to muscle fibre and perhaps degradation of cell wall due to substrate deficiency,[5] the relationship with cBIS ECF would be weak for all the muscles based on muscle fibre type distribution or muscle volume distribution hypotheses.

4.2.0 Methods

4.2.1 Participant Eligibility:

1. Participants were over the age of 18 years
2. Understood English and were able to provide informed consent
3. Were stable on HD treatment for at least 3 months
4. Had no documented evidence of any disease impacting the nervous system
5. Had no any condition that would preclude them from having an MRI or strength measurements.

Participants not meeting all of the above mentioned inclusion criteria were excluded from the study. Western University's Ethics Review Board approved the study and all participants provided written informed consent prior to participation.

4.2.2 Skeletal Muscles:

TA, lateral and medial gastrocnemius and soleus muscles were investigated. These muscles represented anterior and posterior compartment of the calf.[25]

4.2.3 Study Protocol:

Participants were positioned supine on a bed for 30 minutes to allow redistribution of water in the lower extremities prior to MRI.[26] Measurements of hydration using cBIS were collected while the participants were lying supine in preparation for MRI. All BIS and MRI-acquired measures of hydration were collected from the same calf segment.

4.2.4 Calf Extracellular Fluid:

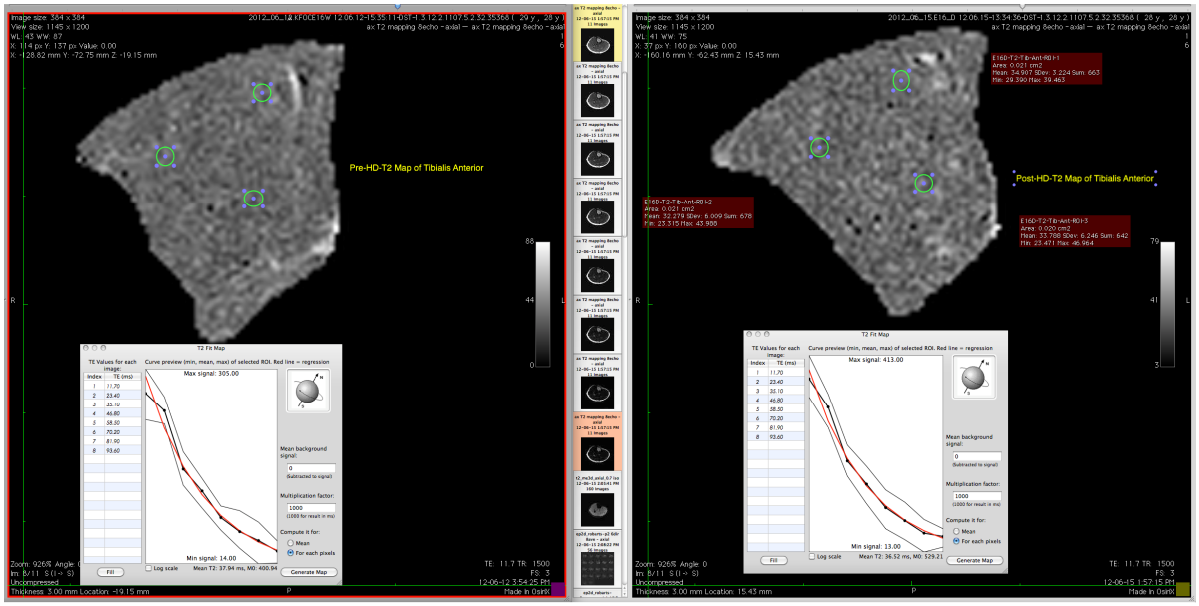
A multi-frequency BIS device (XiTRON 4200, Xitron Technologies, San Diego, CA, USA) was used for automatic sequential measurements of calf segment with frequencies ranging from 5 kHz to 1 MHz. Two measuring (E_{S1} and E_{S2}) and two injecting electrodes (E_{I1} and E_{I2}) were placed on the lateral side of the tested leg. First E_{SI} electrode was placed at maximum circumference of the calf; E_{S2} was placed 10 cms distal to E_{S1}. Injecting electrodes E_{I1} was placed 5 cms proximal to E_{S1} and E_{I2} was placed 5 cms distal to Es_2 (Appendix 3). A fiduciary marker (vitamin E capsule) was placed at the E_{S1} electrode site for identification of this first measuring electrode on MRI. Each measurement was repeated at least 10 times and the average value was used in subsequent computation of cBIS ECF. Calculations and curve fitting (Cole-Cole model) for data collected were done offline as described by Zhu et al.[27]

4.2.5 Transverse Relaxation Time Constants and Apparent Diffusion Coefficients:

Magnetic resonance imaging acquired data were collected on a 3.0 Tesla Tim Trio whole body imaging system (Siemens, Erlangen, Germany) using an 8-channel knee coil. A multi-echo spin-echo (8 echoes) volume {11 contiguous 3 mm transverse slices; 160 mm field of view; 384 x 384 matrix; TE (13.1 ms to 93.6 ms); TR = 1500 ms} and a diffusion weighted volume (b=400 mm/s^2); 6 directions; 22 contiguous 4 mm transverse slices; 160 mm field of view; 128 x 128 matrix; TE =61.6 ms; TR = 6200 ms; 8 averages) were used to measure the T_2 and ADC, respectively, of the TA, medial and lateral gastrocnemius and soleus muscles.

The muscles of interest were outlined close to the fiduciary marker visible on MRI, on the T_2 weighted images acquired for calculating the T_2. The T_2 maps for the muscle cross-sectional area were generated in OsiriX using the "T2 Fit Map" plugin. On this map three areas or regions of interest (ROIs) less than 0.22 cm^2 were selected taking care to avoid visible subcutaneous fats, septum, or neurovascular bundles (Figure 4.1). Averages of three small areas of the muscle were calculated to obtain appropriate representation of the T_2 for that muscle. Others have used a similar method of averaging T_2 of two or three small ROIs for the determination of T_2 of a muscle.[28]

Figure 4:1: Example of Measuring Transverse Relaxation Times of Tibialis Anterior.



Calculations of ADC for each of the four muscles were completed using the cross-sectional area of muscle as outlined in the T_2 image and was imported into the ADC map generated using OsiriX[19] plugin "ADC Map" (Appendix 4); the software automatically calculated and displayed the ADC values for the muscles.

4.2.6 Related Data:

Maximum activity score (MAS - HAP) (the highest –numbered activity the person reports still doing) and an Adjusted Activity Score (AAS-HAP) calculated by subtracting the number of activities marked as "stopped doing this activity" listed below the MAS -HAP were generated using the Human Activity Profile Questionnaire (HAP)[29] to quantify the physical activity of participants. The HAP consists of 94 activities, ranked in ascending order of difficulty according to the energy requirements of the task. The HAP has been validated against accelerometer values (an objective measure of physical activity) over a 7-day period in people with ESRD/HD ($r = 0.78$).[30] The presence of any comorbidity among the participants was quantified using the Charlson Comorbidity Index[31] (Appendix 2) calculated from the medical chart review of each participant.

4.2.7 Statistical Analysis:

Descriptive data for the participants such as age, average body mass, body mass index, Human Activity Profile[30] and Charlson Comorbidity[31] scores were calculated as means and standard deviations. The associations between the outcome measures of hydration were evaluated using simple linear regression analysis. The equality of the before and after HD regression coefficients was assessed as described by Paternoster et al[32] for N-4 degrees of freedom. The significance for the difference in equality of regression coefficients was determined only if the association between cBIS ECF and MRI measures was statistically significant before or after HD. A statistical software package (IBM SPSS v20.0) was used for all data analyses and Prism 4.0a for Macintosh (GraphPad Software Inc.) was used for plotting the associations between the cBIS and MRI-acquired measures. A p-value of <0.05 was required for

statistical significance.

4.3.0 Results

We recruited 22 participants (11 men and 11 women) on HD from Dialysis units affiliated with London Health Sciences Center. Table 4.1 summarizes the characteristics of the participants included in this study. Measures of hydration (cBIS ECF, T_2 and ADC) collected before and after HD are presented as means, and standard deviations in Table 4.2.

Table 4-1: Characteristics of Participants Included (n=22).

Subject characteristics	Group mean (SD)
Age (yrs.)	50.6 (15.7)
Weight (kg)	75.1 (26.3)
Height (cms)	167.3 (12.7)
BMI kg/m^2	26.7 (7.7)
Charlson Comorbidity Index	4.2 (2.4)
HAP-MAS	69.3 (16.5)
HAP-AAS	57.8 (16.7)
Number of Medications	11.4 (2.4)
Cause of ESRD	Glomerulonephritis - 6 Polycystic Kidney disease- 5 Hypertensive nephropathy-4 Other nephropathies -3 IgA nephropathy -1 Vasculitis-1 Rhabdomyolysis -1 ESRD-NYD-1

AAS: adjusted activity score; BMI: body mass index; ESRD: end-stage renal disease; HAP: human activity profile; NYD: not-yet-diagnosed; SD: standard deviation.

Table 4-2: Measures of Hydration before and after Hemodialysis.

Measure		Pre HD (SD)	Post HD (SD)
BIS-ECF (liters)		0.15 (0.05)	0.14 (0.05)
T_2 (ms)	Tibialis anterior	45.7 (6.8)	43.3 (6.2)
	Medial gastrocnemius	43.8 (6.7)	43.3 (6.8)
	Lateral gastrocnemius	47.7 (6.9)	45.4 (6.2)
	Soleus	45.5 (4.5)	42.8 (3.6)
ADC (mm^2/s)	Tibialis anterior	1.6 (0.1)	1.6 (0.1)
	Medial gastrocnemius	1.7 (0.2)	1.7 (0.1)
	Lateral gastrocnemius	1.8 (0.2)	1.7 (0.2)
	Soleus	1.6 (0.2)	1.6 (0.1)

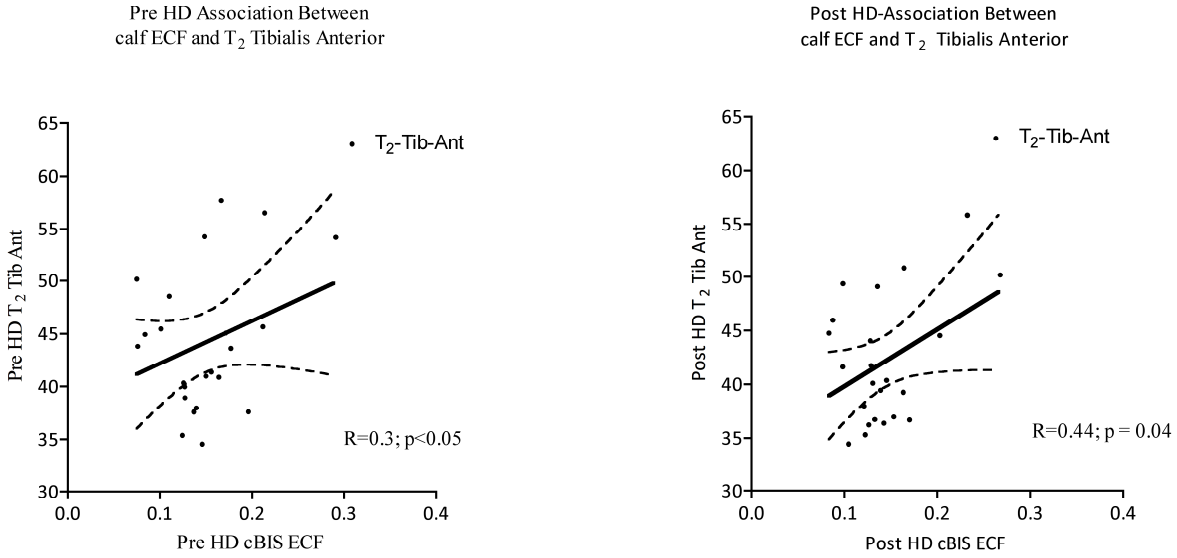
ADC: apparent diffusion coefficient; BIS: bioelectrical impedance spectroscopy; ECF: extracellular fluid; HD: hemodialysis; SD: standard deviation; T_2: transverse relaxation times.

The pair-wise comparisons for pre and post HD measures of cBIS ECF were not significant with a mean difference of 0.02 liters. However, the mean difference, pre and post HD, for the T_2 of TA, lateral and medial gastrocnemius and soleus ranged from 2.5 to 5.2 ms; $p<0.05$.

4.3.1 Association between Calf BIS ECF and T_2 and ADC of TA:

Following HD, cBIS ECF was significantly associated with T_2 of TA [$\beta = 0.44$, $p=0.042$; $b = 34.2$ (95%CI: 26.0, 42.4), $t_{(20)} = 8.7$, $p<0.05$]; cBIS ECF explained a significant proportion of variance in the T_2 of TA [$R^2=0.19$, $F_{(1,20)} = 4.72$, $p = 0.042$] (Figure 4.2). The associations between before-HD cBIS ECF and T_2; and cBIS ECF and ADC of TA on both occasions were non-significant (Table 4.3).

Figure 4:2: Pre and post HD Associations Between Calf ECF and T_2 of Tibialis Anterior.

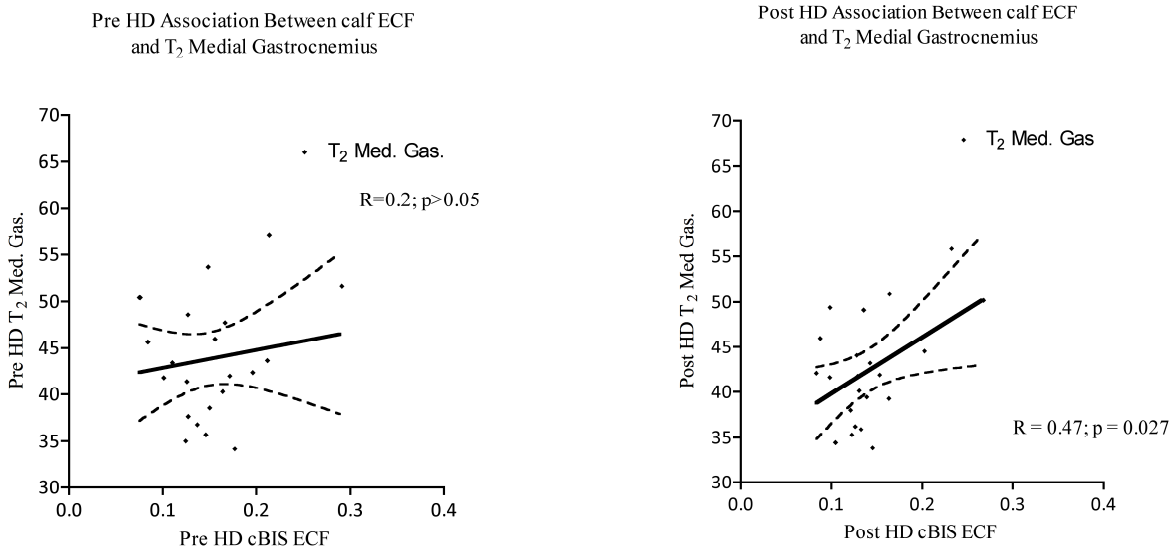


cBIS: calf bioelectrical impedance spectroscopy; ECF: extracellular fluid; HD: haemodilaysis; T_2: transverse relaxation times; Tib-Ant: tibialis anterior

4.3.2 Association between Calf BIS ECF and T_2 and ADC of Lateral and Medial Gastrocnemius, and Soleus:

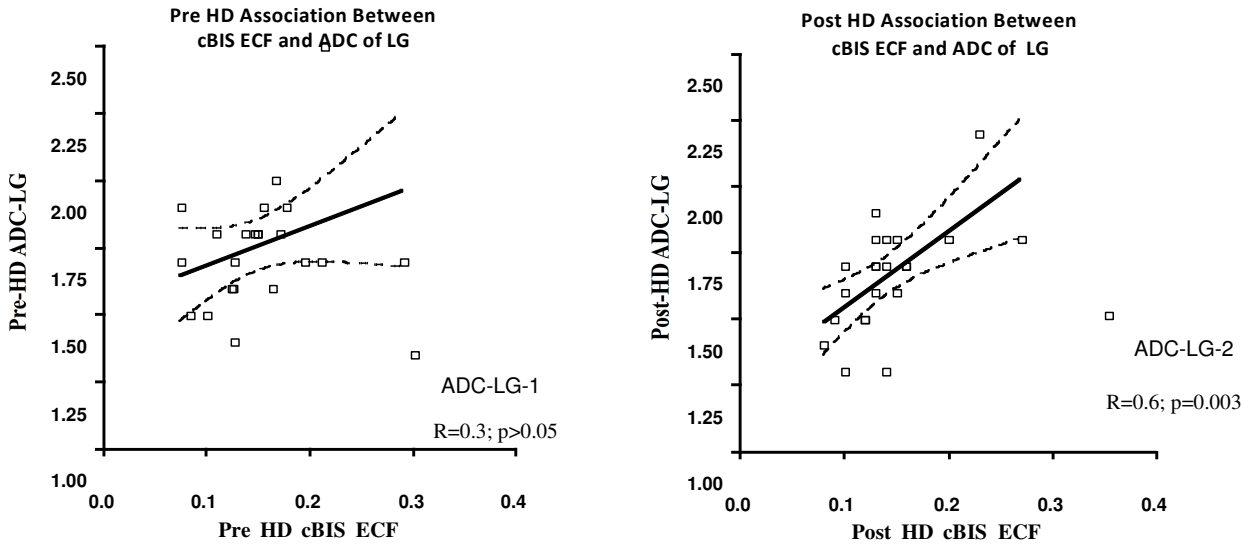
Following HD, cBIS ECF was significantly associated with T_2 of medial gastrocnemius [β= 0.47, p=0.027; b = 33.6 (95%CI: 25.5, 41.6), $t_{(20)}$ = 8.68, p<0.05] and ADC of lateral gastrocnemius [β= 0.6, p=0.003; b = 1.3 (95%CI: 1.1, 4.6), $t_{(20)}$ = 10.25, p<0.05]; also following HD, cBIS ECF explained a significant proportion of variance in the T_2 of medial gastrocnemius [R^2=0.22, $F_{(1,20)}$ =5.69, p =0.027] (Figure 4.3) and ADC of lateral gastrocnemius [R^2=0.36, $F_{(1,20)}$ = 11.34, p = 0.003] (Figures 4.4). The associations between the cBIS ECF and T_2/ADC were non-significant before HD. Following HD association between cBIS ECF and T_2 of lateral gastrocnemius and soleus and ADC of medial gastrocnemius and soleus muscles were non-significant as well.

Figure 4:3: Pre and Post HD Associations between Calf ECF and T_2 of Medial Gastrocnemius.



ECF: extracellular fluid; HD: haemodialysis; Med.: medial; Gas.: gastrocnemius.

Figure 4:4: Pre and post HD Associations between Calf BIS ECF and ADC of Lateral Gastrocnemius.



ADC: apparent diffusion coefficient; cBIS: calf bioelectrical impedance spectroscopy; ECF: extracellular fluid; HD: haemodilaysis; LG: lateral gastrocnemius

4.3.3 Effect of HD on Associations between Calf BIS ECF and MRI-acquired Measures:

The before and after HD coefficients of regression determining prediction of T_2 of TA ($t_{(18)}$= 2.175; $p<0.05$), medial gastrocnemius ($t_{(18)}$= 4.785; $p<0.05$) and ADC of lateral gastrocnemius ($t_{(18)}$= 5.386; $p<0.05$) by cBIS ECF were significantly different.

We explored the agreement between T_2 and ADC of all calf muscles investigated and cBIS ECF before and after HD. The pre (β=0.39; p = 0.57) and post HD (β=0.6; p = 0.094) exploration of the overall model to predict cBIS ECF by T_2 of all the muscles investigated was not significant. Similarly, the pre (β=0.59; p = 0.106) and post HD (β=0.62; p = 0.094) exploration of the overall model to predict cBIS ECF by ADC of all the muscles investigated was not significant.

Table 4-3: Results of Association between Calf BIS and MRI-acquired Measures of Hydration.

		R		R^2		SEE		Constant		Coefficient of regression	
		Pre HD	Post HD	Pre HD	Post HD	Pre HD	Post HD	Pre HD (95% CI)	Post HD (95% CI)	Pre HD (95% CI)	Post HD (95% CI)
cBIS-ECF	TA -T_2	0.29	0.44*	0.09	0.19	6.5	5.4	38.6 (29.3, 47.9)	34.2 (26.0, 42.3)	39.1 (-19.9, 98.1)	57.6 (2.3, 112.8)
	LG- T_2	0.02	0.3	0	0.09	7.6	5.7	47.3 (36.5, 58.0)	39.1 (30.5,47.6)	3.1 (-65.6, 71.7)	39.8 (-18.2, 97.7)
	MG -T_2	0.18	0.47*	0.03	0.22	6.3	5.3	40.3 (31.4, 49.2)	33.6 (25.5, 41.6)	22.8 (-34.1, 79.7)	62.4 (7.8, 116.9)
	Sol - T_2	0.18	0.09	0.03	0.01	4.2	3.4	43.2 (37.1, 49.2)	42.0 (36.9, 47.1)	15.0 (-23.3, 53.3)	6.4 (-28.0, 41.0)
	TA-ADC	0.06	0.29	0.003	0.09	0.15	0.13	1.6 (1.4, 1.8)	1.4 (1.2, 1.6)	0.2 (-1.1, 1.5)	0.9 (-0.4, 2.2)
cBIS-ECF	LG - ADC	0.32	0.6*	0.1	0.36	0.2	0.17	1.5 (1.2, 1.8)	1.3 (1.0, 1.5)	1.4 (-0.5, 3.3)	2.8 (1.1, 4.6)
	MG-ADC	0.37	0.37	0.14	0.14	0.15	0.12	1.5 (1.3, 1.8)	1.5 (1.3, 1.7)	1.2, (-0.2, 2.5)	1.1 (-0.2, 2.3)
	Sol-ADC	0.15	0.3	0.02	0.001	0.15	0.14	1.7 (1.5, 1.9)	1.6 (1.4, 1.8)	-0.4 (-1.8, 0.9)	-0.1 (-1.5, 1.3)

* Indicates $p<0.05$

ADC: apparent diffusion coefficient; cBIS: calf bioelectrical impedance spectroscopy; CI: confidence interval; ECF: extracellular fluid; HD- hemodialysis; LG: lateral gastrocnemius; MG: medial gastrocnemius; T_2: transverse relaxation times; TA: tibialis anterior; Sol- soleus; SEE: standard error of estimate.

4.4.0 Discussion

The main purpose of this study was to examine the association between the BIS and MRI-acquired measures of muscle hydration for the TA muscle. The results of the study indicated a significant association between T_2 of TA and cBIS ECF following HD only. For our secondary objectives, associations of T_2 of medial gastrocnemius and ADC of lateral gastrocnemius with cBIS ECF indicate post HD significance between these measures. Also the pre and post HD coefficients of regression were significantly different for the T_2 of TA and medial gastrocnemius and ADC of lateral gastrocnemius muscles. These results suggest that cBIS and MRI-acquired measures cannot be used interchangeably to estimate interstitial fluid volume of a single muscle in this population.

To our knowledge this is the first study that has explored the association between cBIS ECF and T_2 or ADC of the calf muscles. Our results show a smaller correlation between cBIS ECF volume and T_2 than that reported in the literature by Hatakenaka et al.[15] This could be due to several factors including the interval between the healthy and pathological state of the skeletal muscle (administration of steroids to induce myopathy for the study by Hatakenaka et al[15] and duration on HD in our participants). Hatakenaka et al[15] evaluated the T_2 relaxation time constants following six weeks of steroid administration. This can be described as early phase of fast progressive atrophy.[33] All of our participants had been on HD for a period greater than three months; a chronic phase where atrophic muscle fibres have been replaced with fibrous or lipid tissue.[33] Hence the contribution of the lipids replacing the atrophic muscles in participants on ESRD/HD to the T_2 of the muscles may have influenced its association with cBIS ECF.

Our findings suggest cBIS ECF explains ~ 20% (R^2=0.19) of the observed variation in T_2

of TA following HD. Factors related to skeletal muscle composition and structure that impact T_2 may explain our results of ~ 20% variation in T_2 of TA by cBIS ECF. Generally T_2 time constants correlate most strongly with bulk water content of the tissue.[34] Also tissues with high concentrations of lipid protons have longer T_2. Hence MRI signals arising from bulk water content or total water and the degree of binding will result in alterations of T_2. Using customized software for MRI images of the ankle muscles to differentiate between contractile and non-contractile areas of ankle muscles in participants on HD, Johansen et al[21] were able to show an increase in total non-contractile area. This non-contractile area could consist of lipid deposition. Since fats/lipids hold about 10-15% of total extracellular water,[35] the impact of possible increase in intra and extra-myocellular lipids and the amount of water bound within these lipids may have impacted our associations between cBIS ECF and T_2 of TA; T_2 accounting for the water bound to lipids and cBIS ECF excluding this portion of water. We did not correct our data prior to analysis for the changes in T_2 associated with possible increase in intra and extra-myocellular lipids in this population. However we attempted to control for these changes by choosing small ROIs for analyses.

Factors related to cBIS ECF as well may contribute to the variations in T_2 of TA explained by cBIS ECF. For the cBIS ECF this includes plasma in the large vessels viz. the popliteal artery, anterior tibial artery, saphenous veins, interstitial spaces embedded within the other muscles of the calf viz. lateral and medial gastrocnemius, soleus, and peronei. The variation in the cBIS ECF associated with TA may perhaps also be related to the total space occupied by the muscle in relation to the total calf volume and variations in T_2 of lateral and medial gastrocnemius and soleus may contribute to variations in cBIS ECF as observed in our exploratory model evaluating the association between T_2 or ADC of all muscles and cBIS ECF.

The results of this study exploring the association between cBIS ECF and T_2 of the calf muscles support our *a priori* hypothesis grounded in fibre type distribution. Tibialis anterior muscle composed of ~50% type II fibres[22] (the most commonly atrophied fibres in people with ESRD/HD with intact muscle volume) had the strongest association with cBIS ECF post HD. Similarly, medial gastrocnemius has about ~ 47% type II fibres and showed similar association with cBIS ECF.[22] The association with soleus with the least amount of type II fibres[23] was the weakest. A review of prior published reports[20] establishing presence of type II fibre atrophy lend further support to these observations of lack of association between cBIS ECF and soleus in participants with ESRD/HD.

Henriksen et al[36] observed an increase in the rat soleus interstitial fluid volume following suspension or space flight. The authors attributed this increase in the interstitial fluid volume to atrophy of the muscles due to suspension. In contrast, the same study observed an increase in the extensor digitorum longus muscle wet weight attributed to hypertrophy of this muscle. In our participants perhaps the soleus (an endurance muscle or muscle rich in type I fibres), had retained its strength or slowed atrophy by simple activities of daily living. Hence the association of cBIS ECF to soleus was poor following HD treatment ($\beta = 0.09$; $p>0.05$); also perhaps the relative increase in ECF volumes of the TA and medial gastrocnemius muscles associated with type II fibre atrophy in participants on HD was larger than the ECF volume of the soleus muscles affecting its association with cBIS ECF in this population.

The significant change in the coefficients of regression following HD for T_2 of TA and medial gastrocnemius and ADC of lateral gastrocnemius are in accordance to prior published reports of loss of fluid volumes from the calf segment associated with the HD treatments and

perhaps these muscles lost most fluid during the HD treatment. Kaysen et al[37] used whole body BIS to show that ICF volume does not vary significantly during interdialytic periods. According to Charra[38] this expansion of ECF appears to be largely corrected for the excess plasma volume following the HD sessions. During the few hours of HD treatment the plasma compartment is ultra-filtered down to its normalized volume. These findings suggest that the excess plasma volume may have confounded the associations between cBIS ECF prior to HD treatment and interstitial fluid measured using T_2. Measurement bias at higher values of ratio of ECF and ICF may also have contributed to poor associations prior to HD treatment.[39] These factors related to plasma volumes and measurement error of cBIS ECF support our findings of non-significant associations between the measures of individual skeletal muscle hydration obtained using MRI and BIS, before HD. Besides, skeletal muscle is a heterogeneous structure in its composition and architecture. Measurement of hydration of a limb segment such as calf using BIS is considered to be at a "whole body" level, whereas MRI estimates hydration at the "tissue system level".[40] A direct comparison between these two techniques, based on different assumptions and methods is challenging. No study has yet determined a direct relationship between T_2 and wet/dry weight of the calf/shank muscles in people with ESRD/HD.

The significant association between cBIS ECF and ADC of lateral gastrocnemius following HD only also confounds the use of cBIS ECF to confidently predict ADC values of a single muscle before and after HD. Besides the test-retest reliability of ADC is moderate for average measures and poor for single measures.[39] Hence, larger random fluctuations may contaminate the change in data collected on two occasions (minimal-detectable-change at 95% confidence).

The HAP-AAS scores (the best estimate of respondents' average level of energy expenditure in comparison with peers of same age) indicate that the participants included in this study were functioning at ~61% of the maximum possible activity level or at below-average fitness level.[29,41] The participants in this study had a comorbidity score greater than three (the mortality rate was zero for ESRD/HD cohort with a Charlson Comorbidity Index score of three).[42] The comorbidity scores correlate with the phase angle (suggesting an altered intra and extra cellular water distribution) of BIS and functional levels.[43] Hence these results can be applied to participants with HAP-AAS and comorbidity scores similar to participants in this study.

4.5.0 Conclusions

In conclusion this is the first study that has looked at the associations between the hydration measures of the calf muscles acquired using BIS and MRI. Although cBIS ECF can be used for estimation of ECF in the calf segment, T_2 of a calf muscle provides estimates of individual skeletal muscle hydration before and after HD treatment. Our findings of the overall model exploring correlations between cBIS ECF and T_2 or ADC of all the muscles require to be interpreted with caution due to relatively low numbers of test subjects and hence limited statistical power. The characteristics of the participants included in this study were comparable to those reported in literature and hence the results of this study can be applied to a broader population on HD except for those with neuromuscular disorders.

4.6.0 References

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5 Chapter 5: Effect of Fluid Loss Following Hemodialysis on Tibialis Anterior Muscle Strength in People with End-stage Renal Disease

5.1.0 Introduction

The incidence of chronic kidney disease is rising.[1] In its end stages, renal replacement therapies such as peritoneal dialysis, hemodialysis (HD) or kidney transplant are required.[2] People with end-stage renal disease (ESRD) on HD encounter multiple catabolic processes such as loss of albumin and amino acids during HD, and metabolic derangements together with changes in skeletal muscle associated with relative muscle disuse.[3] This results in muscle atrophy and loss of lean muscle mass. Presence of neurogenic (loss of muscle mass and quality due to peripheral neuropathy), myogenic (damage intrinsic to the muscle) and mixed (neurogenic and myogenic) changes in the skeletal muscles of people with ESRD/HD[4] may further compromise the integrity of the motor-unit complex, and contribute to muscle atrophy.[5] Since muscle wasting is the most significant predictor of morbidity and mortality in this population,[6] preventing muscle wasting is of clinical importance and a critical issue in terms of healthcare costs.[7] Exercise interventions have been strongly recommended in this population to increase muscle mass and strength. However, recent systematic reviews[8-10] indicate that the studies using aerobic, strength training or mixed (aerobic and strengthening exercises) were not able to consistently demonstrate the expected beneficial effects of exercise training in participants with ESRD/HD.[11] In order to establish consistent beneficial effects of exercise in people with ESRD/HD factors contributing to the lack of expected gains require further investigations. Because knowledge of underlying mechanisms underpins the rationale of safe exercise administration,[12] understanding these mechanisms may be particularly important in people with ESRD/HD with documented evidence

of muscle weakness, increased risk for falls and related injuries, progressive deterioration of strength and fluctuations in the extracellular fluid (ECF).

Expansion of ECF volume is one of the manifestations of ESRD and HD is required to correct the fluid and electrolyte imbalances.[13] Hence, assessing the changing ECF volumes of the specific muscles in people with ESRD/HD may provide insight into the influence of fluctuating hydration on its function. Thomas-Hawkins[14] has reported fluctuations up to 6% in daily physical function measured using a questionnaire (administered on different occasions in relation to the HD schedule within a seven-day period) associated with the interdialytic fluid variations. However, no previous studies have investigated the effect of fluid loss following HD on tibialis anterior (TA) muscle strength and volume or other muscle groups.

Bioelectrical impedance spectroscopy (BIS) has been widely used to measure changes in ECF volumes in people on HD;[15] BIS provides estimates of ECF or intracellular fluid (ICF) for a whole-body, limb or limb segment. Magnetic resonance imaging (MRI) acquired transverse relaxation time constants (T_2) and apparent diffusion coefficient (ADC) have been used to estimate changing ECF volume of a skeletal muscle in response to muscle atrophy or exercise.[16-20] Our earlier investigations have established that T_2 of all the calf muscles have excellent test-retest reliabilities ($ICC_{2,1}$ >0.9) while reliability of ADC of calf muscles was poor to moderate (0.4 – 0.7),[21] Besides, there was only a moderate association between MRI-acquired measure of T_2 for TA with BIS-acquired estimate of calf ECF following HD only; indicating that these measures could not be used interchangeably as measures of interstitial fluid/ECF of a single leg muscle.[22] Hence we proposed to estimate TA interstitial fluid volume using its T_2 time constants.

The purpose of this paper was to determine the effect of whole-body fluid loss following HD on maximal voluntary isometric contraction strength and ECF volumes, measured using T_2, of tibialis anterior muscle, in people with ESRD/HD compared to controls. The reductions in ankle dorsiflexor performance can impact activities of daily living and increase risk of falls in the elderly.[23] Hence we chose to investigate TA, an ankle dorsiflexor muscle.

5.2.0 Methods

5.2.1 Study Design:

This was a cross-sectional study. Data were collected on two occasions (1 to 10 days apart), in healthy participants and before and after HD in participants on HD (2 to 7 days apart). We avoided collecting experimental data for a minimum of five hours following HD to avoid confounding of the strength measures due to presence of immediate post-dialysis fatigue.[24]

5.2.2 Participants' Eligibility:

Participants with ESRD/HD were included in the study if they

1) Were over the age of 18 years,

2) Understood English and were able to provide informed consent,

3) Were stable on HD treatment for at least 3 months,

4) Had no documented evidence of any disease impacting the nervous system,

5) Had no condition that would preclude them from having an MRI or strength measurements.

Healthy volunteers were recruited as control participants if they had no documented evidence of any chronic condition and met criteria 1, 2, 4 and 5 mentioned above. Participants

not meeting all of the above mentioned inclusion criteria were excluded from the study. Western University's Ethics Review Board approved the study and all participants provided written informed consent prior to participation.

5.2.3 Study Protocol:

Participants were positioned supine on a bed for 30 minutes to allow redistribution of water in the lower extremities prior to MRI.[25] Measurements of hydration using cBIS were collected while the patients were lying supine in preparation for MRI. All BIS and MRI-acquired measures of hydration were collected from the same calf segment.

5.2.4 Calf Extracellular Fluid:

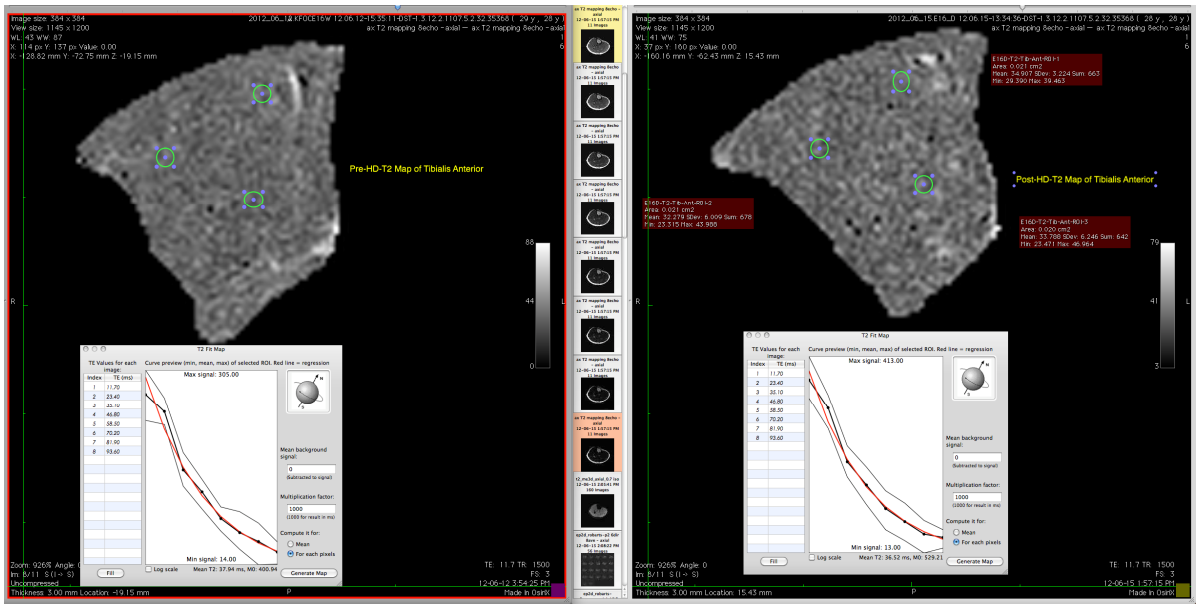
Multi-frequency BIS device (XiTRON 4200, Xitron Technologies, San Diego, CA, USA) was used for automatic sequential measurements of calf segment with frequencies ranging from 5 kHz to 1 MHz. Two measuring (E_{S1} and E_{S2}) and two injecting electrodes (E_{I1} and E_{I2}) were placed on the lateral side of the tested leg. First E_{SI} electrode was placed at maximum circumference of the calf; E_{S2} was placed 10 cms distal to E_{S1}. Injecting electrodes E_{I1} was placed 5 cms proximal to E_{S1} and E_{I2} was placed 5 cms distal to Es_2 (Appendix 3). A fiduciary marker (vitamin E capsule) was placed at the E_{S1} electrode site for identification of this first measuring electrode on MRI. Each measurement was repeated at least 10 times and the average value was used in subsequent computation of calf hydration estimate for that test occasion. Calculations and curve fitting for data collected were done offline (Cole-Cole Model) as described by Zhu et al.[26]

5.2.5 Transverse Relaxation Time Constants and Apparent Diffusion Coefficients:

Magnetic resonance imaging acquired data were collected on a 3.0 Tesla Tim Trio whole body imaging system (Siemens, Erlangen, Germany) using an 8-channel knee coil. A multi-echo spin-echo (8 echoes) volume {11 contiguous 3 mm transverse slices; 160 mm field of view; 384 x 384 matrix; TE (13.1 ms to 93.6 ms); TR = 1500 ms} and a diffusion weighted volume (b=400 mm/s^2); 6 directions; 22 contiguous 4 mm transverse slices; 160 mm field of view; 128 x 128 matrix; TE =61.6 ms; TR = 6200 ms; 8 averages) were used to measure the T_2 and ADC, respectively, of the TA muscle.

The muscles of interest were outlined on the T_2 weighted images acquired for calculating the T_2. The T_2 maps for the muscle cross-sectional area closest to the fiduciary marker were generated in OsiriX using the "T2 Fit Map" plugin. On this map three areas or regions of interest (ROIs) less than 0.22 cm^2 were selected taking care to avoid visible subcutaneous fats, septum, or neurovascular bundles (Figure 5.1). An average of three small areas of the muscle was calculated to obtain appropriate representation of the T_2 for that muscle. Others have used a similar method of averaging T_2 of two or three small ROIs for the determination of T_2 value for a single muscle.[27]

Figure 5:1: Example of Measuring Transverse Relaxation Times



Calculations of ADC for TA muscle were completed using the cross-sectional area of muscle as outlined in the T_2 image and was imported into the ADC map generated using OsiriX plugin "ADC Map" (Appendix 4);[20] the software automatically calculated and displayed the ADC values of TA muscle.

5.2.6 Measures of Muscle Strength:

We measured isometric strength from voluntary contractions of the ankle dorsiflexors (TA) of the dominant leg in our laboratory at ambient room temperatures between 22 and 25^0 C. All strength testing was performed on a Biodex multi joint dynamometer (System 3, Biodex Medical Systems, Shirley, NY). The torque sampled at 100 Hz was converted (analog-to-digital) with a 12-bit convertor (CED micro 1401 mk II, Cambridge Electronic Design Limited, Cambridge UK) and displayed in real-time on an online digital system using commercially

available software (Spike 2 ver. 5, Cambridge Electronic Design). The peak MVIC strength of TA was analysed offline.

Familiarization with the test procedure and strength testing (imaged limb) was conducted during the first visit to the laboratory. The available pain-free ankle range of motion was established for each participant prior to experimental procedure to determine the available range for the strength testing protocol. Participants were placed in a semi-reclined position with angles of 90 and 20° flexion at the hip and knee, respectively, and their leg was aligned parallel to the ground. Participants performed warm-up (one set of 10 repetitions of submaximal ankle plantar flexion and dorsiflexion) prior to data collection. Three repetitions of MVIC of dorsiflexors were performed at an ankle angle of 25° of plantar flexion. The peak MVIC strength of the best performance was selected to represent the strength measure of that participant. Participants were provided visual feedback and verbal cues to encourage best performance.

5.2.7 Related Data:

Maximum activity score (MAS-HAP) (the highest –numbered activity the person reports still doing) and an Adjusted Activity Score (AAS-HAP) calculated by subtracting the number of activities marked as "stopped doing this activity" listed below the MAS-HAP were generated using the Human Activity Profile Questionnaire (HAP)[28] to quantify the physical activity of participants. The HAP consists of 94 activities, ranked in ascending order of difficulty according to the energy requirements of the task and has been validated against accelerometer values (an objective measure of physical activity) in people with ESRD (r = 0.78).[29] The presence of any comorbidity among the participants was quantified using the Charlson Comorbidity Index[30] calculated from the medical chart review of each participant (Appendix 2).

5.2.8 Statistical Analysis:

Participants' demographics such as age, average body mass, body mass index, HAP scores[28] and Charlson Comorbidity Index[30] and were summarized using descriptive statistics. For the exploratory data analysis, box-whisker plots were used.[31] Effect of fluid loss following HD was evaluated using paired-samples t test and differences between the control and ESRD/HD groups were evaluated using independent samples t test. A statistical software package (IBM SPSS v20.0) was used for all data analyses and Prism 4.0a for Macintosh (GraphPad Software Inc.) was used for plotting the box-plots. A p-value of <0.05 was required for statistical significance.

5.3.0 Results

Eighteen healthy and 18 volunteers with ESRD/HD meeting the inclusion criteria were recruited. One participant in the ESRD/HD group dropped out due to inability to complete the MRI procedures. Data for the remaining 35 participants was analyzed. The participants' characteristics included in this study are summarized in Table 5.1. Means and standard deviations for measures of muscle hydration and TA strength collected on both occasions are provided in Table 5.2. We explored the variability in the T_2, cBIS ECF, cBIS ICF and MVIC strength of TA using boxplots (Figures 5.2 – 5.5).

Table 5-1: Characteristics of Participants.

	Control Mean (SD) n=18	ESRD/HD Mean (SD) n=17
Age (yrs)	56.7 (9.9)	51.6 (16.5)
Sex (M/F)	9/9	9/8
Weight (kgs)	74.01 (13.44)	75.27 (28.09)
Height (cms)	168.9 (9.4)	168.4 (13.4)
BMI	25.89 (3.42)	26.04 (7.84)
CCI	0.28 (0.96)	4.24 (2.54)
Medications	1 (1.33)	11.94 (5.18)
HAP-MAS	87.39 (5.89)	70.35 (16.25)
HAP-AAS	84.22 (9.99)	58.12 (16.86)
Dialysis Vintage		3 months to greater than 120 months
Diagnosis		Nephropathy-6 Glomerulonephritis -5 PCKD -3 ESRD NYD -1 Vasculitis -1 Rhabdomyolysis - 1

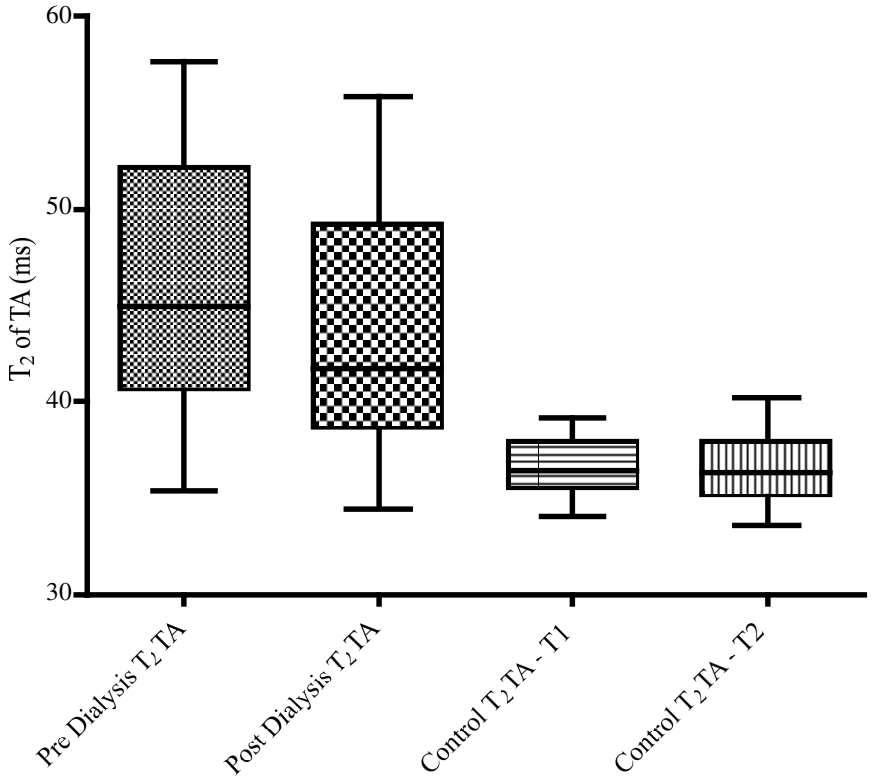
AAS: adjusted activity score; BMI: body-mass index; CCI: charlson comorbodity index; ESRD: end-stage renal disease; HAP: human activity profile score; HD: haemodialysis; NYD: not yet doagnosed; PCKD: polycystic kidney disease, SD: standard deviation.

Table 5-2: Tibialis Anterior Muscle Hydration and Strength Measures of the Control and Hemodialysis Participants.

	Control group (n=18)		ESRD/HD group (n=17)	
	Time 1 Mean (SD)	Time 2 Mean (SD)	Pre dialysis Mean (SD)	Post dialysis Mean (SD)
Muscle Hydration Measures - Calf BIS				
ECF (liters)	0.15 (0.02)	0.15 (0.02)	0.15 (0.05)	0.14 (0.05)
ICF (liters)	0.49 (0.15)	0.47 (0.14)	0.49 (0.17)	0.44 (0.16)
MRI-acquired Muscle Hydration Measures				
T_2 (ms)	36.59 (1.54)	36.45 (1.78)	45.66 (6.83)	43.29 (6.17)
ADC (mm^2/s)	1.51 (0.11)	1.50 (0.09)	1.63 (0.16)	1.55 (0.13)
Muscle Strength				
Ankle Dorsiflexors (Nm)	34.49 (10.18)	34.35 (9.73)	24.73 (6.98)	23.19 (6.98)

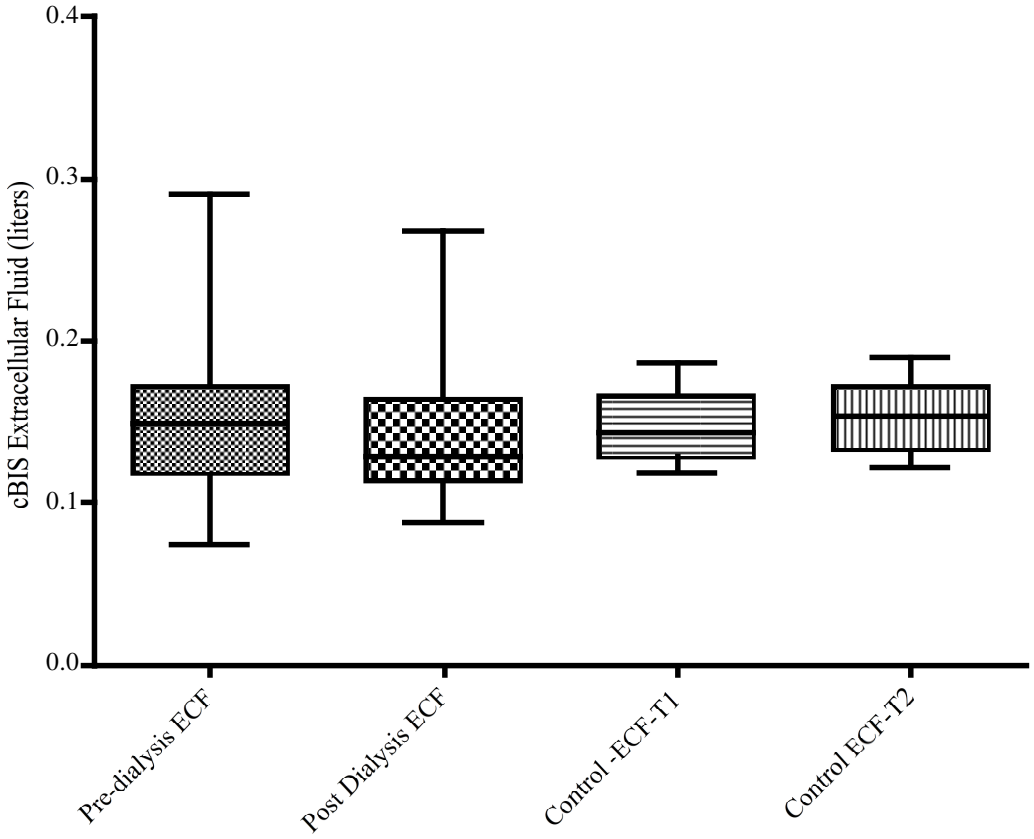
ADC: apparent diffusion coefficient; BIS: bioelectrical impedance spectroscopy; ECF: extracellular fluid; ICF: intracellular fluid; SD: standard deviation; T_2: transverse relaxation times.

Figure 5:2: Box-plots Transverse Relaxation Times: Hemodialysis (pre and post); Control (Time 1 and 2).



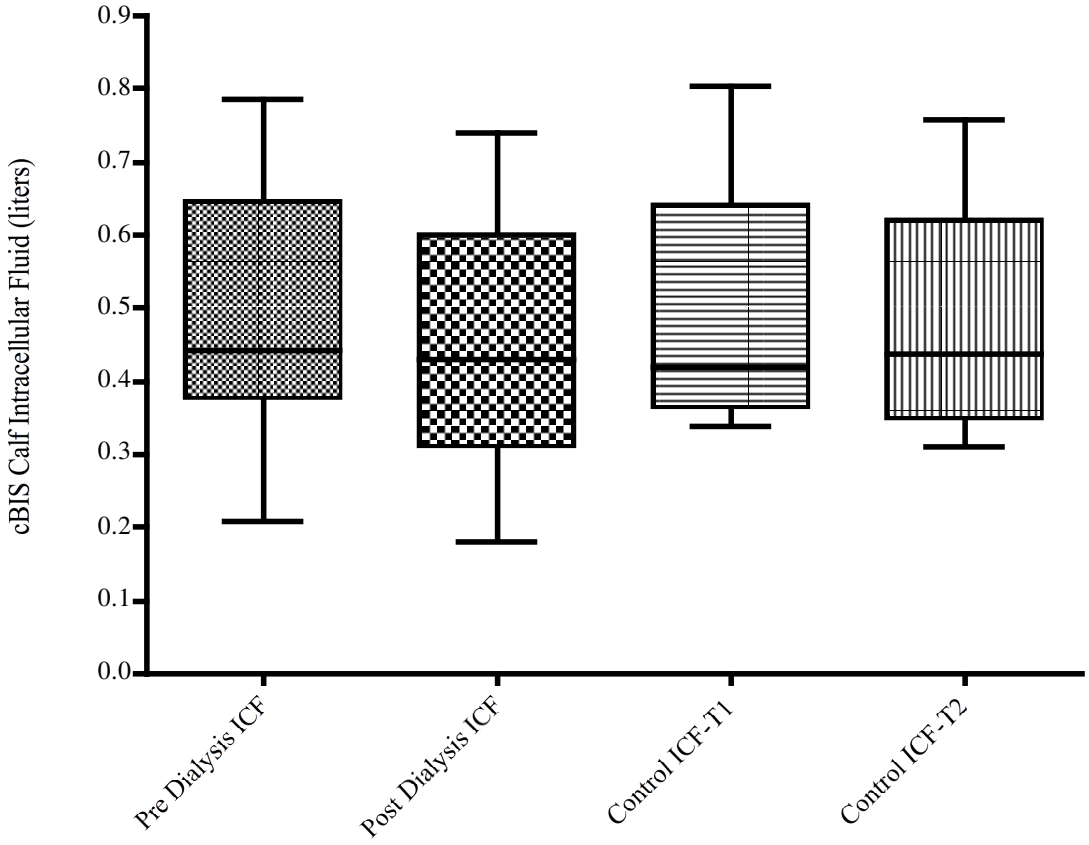
T1: Occasion one; T2: occasion two; T_2: transverse relaxation times; TA: tibialis anterior.

Figure 5:3: Box-plots Calf BIS Calf Extracellular Fluid: Hemodialysis (pre and post); Control (Time 1 and 2).



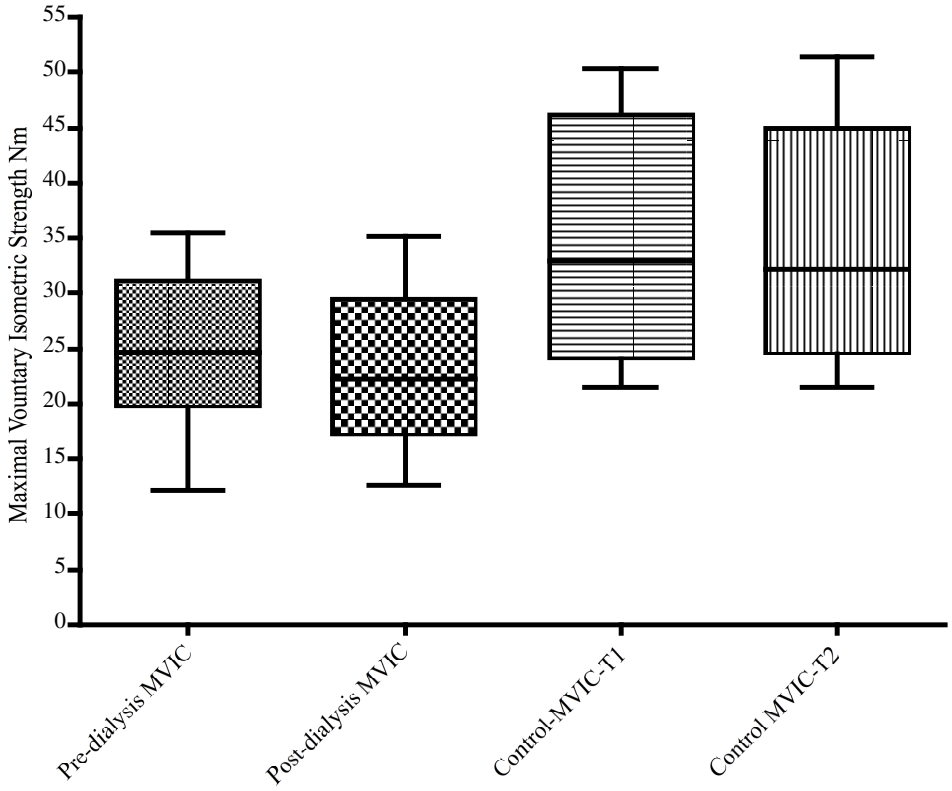
cBIS: calf bioelectrical impedance spectroscopy; ECF: extracellular fluid; T1: Occasion one; T2: occasion two.

Figure 5:4: Box-plots Calf BIS Calf Intracellular Fluid: Hemodialysis (pre and post); Control (Time 1 and 2).



cBIS: calf bioelectrical impedance spectroscopy; ICF: intracellular fluid; T1: Occasion one; T2: occasion two.

Figure 5:5: Box-plots Tibialis Anterior Muscle Strength on Both Occasions.



MVIC: maximum voluntary isometric contraction; T1: Occasion one; T2: occasion two.

5.3.1 Effect of Fluid Loss Following HD on MVIC of TA in Hemodialysis Participants:

The participants on HD were significantly weaker in their TA muscle strength as the peak MVIC strength of TA was lower by 1.54 Nm (95% CI: 0.05, 3.02; p = 0.043) following HD. However, seven of the 17 participants showed an increase in peak MVIC strength of TA

following HD session. The peak MVIC strength of TA was not significantly ($p>0.05$) different between the participants in the ESRD/HD group demonstrating a post-HD increment or decrement in strength before or after HD (Table 5.3).

Table 5-3: Measures of Hydration and Strength of Participants in Hemodialysis Group With or Without Increment in Strength Post Hemodialysis.

	Participants with increase in strength following HD (n=7)		Participants with decrease in strength following HD (n=10)		Pre HD Mean difference between the groups	Post HD Mean difference between the groups
	Pre HD (SD)	Post HD (SD)	Pre HD (SD)	Post HD (SD)	(95% CI)	(95% CI)
ECF (liters)	0.13 (0.05)	0.13 (0.05)	0.16 (0.5)	0.15 (0.05)	0.03 (-0.1,0.02)	0.02 (0.02, -0.07)
T_2 (ms)	46.6 (7.1)	45.4 (6.5)	45.0 (7.0)	41.8 (5.8)	1.5 (3.4, -5.8)	3.6 (3.1, -2.8)
ICF (liters)	0.48 (0.19)	0.44 (0.16)	0.51 (0.17)	0.45 (0.17)	-0.03 (-0.2, 0.16)	-0.11 (-0.19, 0.16)
MVIC strength (Nm)	22.3 (8.0)	23.3 (8.5)	26.4 (6.9)	23.1 (6.2)	-4.1 (-12.3, 3.6)	0.2 (-8.1, 8.5)

cBIS: calf bioelectrical impedance spectroscopy; CI: confidence interval; ECF: extracellular fluid; HD: hemodialysis; ICF: intracellular fluid; MVIC: maximum voluntary isometric contraction; T_2: transverse relaxation times.

5.3.2 Effect of Fluid Loss Following HD on ECF Volumes of TA in Hemodialysis Participants:

Following HD treatment the participants' on ESRD/HD showed a significant contraction of ECF volume of the TA muscle (measured using T_2) by 2.38 ms (95% CI: 1.04, 3.71; p = 0.002) and calf ICF volume (measured using BIS) by 0.05 liters (95% CI: 0.01, 0.08; p = 0.007). There was no significant difference (p>0.05) in the pre and post HD secondary measure of hydration, ADC.

For the subgroup of participants with observed increment or decrement of strength, the measures of hydration (T_2, cBIS ICF, and ADC) were not significantly different between the participants in the ESRD/HD group demonstrating a post-HD increment or decrement in strength.

5.3.3 Comparison of before and after HD MVIC of TA between Healthy and Hemodialysis Participants:

Although the participants in the control and ESRD/HD group were matched on height and body-mass index parameters, participants in the ESRD/HD group were significantly weaker in TA muscle strength than the control group as the peak MVIC strength for TA was lower in HD group by 9.76 Nm (95% CI: 3.64, 15.88; p = 0.003) and 11.16 Nm (95% CI: 5.30, 17.01; p<0.001), before and after HD respectively (Table 5.2).

5.3.4 Comparison of before and after HD ECF Volumes of TA between Healthy and Hemodialysis Participants:

The participants with ESRD/HD had significantly expanded ECF volumes as T_2 time constants were prolonged by 9.07ms (95% CI: 5.50, 12.64; p<0.001) and 6.83ms (95% CI: 3.57, 10.09; p<0.001) before and after HD respectively. Our secondary outcome measure of muscle hydration, ADC, showed a significant difference between the control and ESRD/HD group for the ADC of TA prior to HD by 0.11mm^2/s (95% CI: 0.02, 0.22; p = 0.02) but comparable to the control group following HD (mean difference: -0.05 (95% CI: -0.13, 0.03; p = 0.21).

5.4.0 Discussion

The results of this study show a small but significant decline in the peak MVIC strength of TA following fluid loss associated with HD session. Findings of shortened T_2 after HD suggests volume contraction of TA muscle for six to 12 hours following HD treatment, as we had refrained from collecting data immediate post HD to avoid confounding strength measures by HD related fatigue. Comparisons of MVIC and T_2, a measure of TA interstitial space, with the healthy participants confirms earlier reports of TA muscle weakness and persistent expansion of ECF consistent with reduced contractile fraction or muscle atrophy in participants with ESRD/HD. The reports of increase in non-contractile area of TA in people with ESRD/HD by Johansen et al[32] substantiate our results of persistent prolongation of T_2.

The earlier reports of limb fluid volume depletion by Jain and Lindsay[33] further support our results of volume contraction of TA muscle six to 12 hours following HD treatment. However this finding of volume contraction in our participants was also associated with a

decrement in the mean TA MVIC strength by 1.54 Nm or 6.23% of the pre HD mean MVIC strength. This pre HD mean MVIC strength of TA for the participants with ESRD/HD was ~71% of the mean MVIC of the control group on occasion one; comparable to the mean MVIC strength of TA in male participants aged ~ 78 years of age.[34] An increment in TA MVIC strength measured in Nm has been associated with a decrease in odds of loss-of-balance during sensory-organization test in healthy older adults;[35] although such relationship between quantitative estimates of MVIC strength of TA and loss of balance has not been established in people with ESRD/HD, our results of showing decrement of strength following HD can be considered to potentially increase the risk for falls in this population with ESRD/HD strengths. This is an important clinical association that requires further investigations, particularly in this population with documented increase in risk for falls.

Our results of differences in strength of the TA muscle following HD in participants with ESRD were similar to those reported by Saiki et al.[36] Saiki et al[36] explored the effect of a single HD treatment session in a sample of 10 participants. They measured quadriceps muscle and handgrip strength before and after HD. Quadriceps muscle strength improved in six, decreased in three and remained unchanged in one; while handgrip strength increased in five, decreased in three, and remained unchanged in one. These findings of changes in strength of the tested muscle groups following HD were unrelated to the serum concentrations of sodium, potassium, calcium, phosphorus, urea, nitrogen, creatinine, and changes in blood pressure or fluid balance. However these authors measured 'fluid balance' as change in pre and post HD body mass. A change in body mass is not a reliable measure of changes in hydration status as substantial loss of mass without an effective net negative fluid balance has been observed in people without kidney disease.[37] Besides, based on the reports of Jain and Lindsay[33] indicating limb fluid volume loss

following HD, a change in pre and post HD body-mass may not be adequate to establish hydration of a limb or single muscle. This confounds the findings of Saiki et al[35] regarding lack of relationship between muscle strength and fluid balance.

Schoffstall et al[38] showed a decrease in bench-press one repetition-maximum of experienced male power lifters by 5.6% associated with ~ 1.5% of loss of body mass related with acute dehydration. These results of loss of strength related to dehydration can be considered analogous to our results of volume depletion and loss of MVIC strength of TA muscle in participants with ESRD/HD. Our findings of decrements in strength four to 12 hours following HD treatment mean that the volume-contracted status may be prolonged. The muscle investigated in our study is of primary importance for ambulation. Since individuals must ambulate to function independently,[39] such basic activities performed in a volume- contracted individual could contribute to fatigue, and muscle damage.[40] Reports of decrement in quadriceps muscle area and AAS-HAP scores over a three-month period in a sample of 20 participants on conventional HD lends further support that perhaps adequacy of HD for maintaining appropriate hydration at muscle–level may be of importance to improve functional outcomes in people with ESRD/HD.[41] Since exercises have been shown to alter fluid dynamics,[19] intradialytic exercises may prevent muscular dehydration and hence improve outcomes in this population. This notion of intradialytic exercise as a measure to maintain adequate hydration can be supported by earlier findings of improved six-minute-walk test and HD efficacy associated with low intensity intradialytic exercise interventions.[42]

The primary outcome measure used to measure ECF volume of the TA muscle, viz, MRI-acquired T_2, indicated an expansion of ECF volume that persisted following HD and did not shrink to volumes comparable to healthy controls following HD treatment. These findings of

prolonged T_2 in participants with ESRD/HD can be interpreted as expansion of ECF volume in the TA muscle. Generally T_2 time constants correlate most strongly with bulk water content of the tissue.[43] Hence a mean difference of ~ 9 ms between the control and participants indicated an approximate increase of ~ 27% in TA muscle ECF expansion before HD and 18% following HD when compared to the control ECF volumes estimated using its T_2 time constants; this is consistent with the chronic state of expanded ECF that characterizes ESRD. Although no study has directly established the relationship between total water content and T_2 of skeletal muscle in this population we can draw these inferences from previous reports investigating muscle fluid contents in dystrophic mice,[44] muscle atrophy,[45] myocellular lipids[46] and intra/extra cellular fluid movements associated with exercise.[19] Dunn et al[44] observed a mean change of T_2 from 28.0 ms to 25.3 ms with change in mean ratio of dry and wet weight of calf muscles from 0.291 to 0.244. This supports our assumption of increased ECF content associated with the prolongation of T_2 in people with ESRD/HD.

These inferences regarding ECF volume expansion of the TA muscle requires further investigation to establish whether the prolongation of T_2 of TA reflects absolute or relative increase in ECF fluid volumes by the contractile fraction of this muscle. Psatha et al[45] observed a 17% decrease in TA volume following 43 days of cast immobilization of lower leg; an increase of T_2 from 27.0 ± 2.5ms to 29.6 ± 2.8 ms was also observed in the same muscle. This rise in T_2 can be associated with increase in ECF in relation to the ICF as reported earlier by Holl et al.[17] Schwenzer et al[46] showed a correlation of 0.75 (Spearman's rho) between T_2 of TA and percentage of fat content. Since we did not estimate the myocellular lipids in our participants the contribution of myocellular lipids in prolongation of T_2 merits consideration; we controlled for this by selecting smaller ROIs for estimating T_2. However a post HD mean-difference of 2.34 ms

in T_2 of TA in our participants indicates a reduction in muscle volume of ~ 15-17% that is more consistent with a rapid change in fluid content of the muscle. Interestingly, the ECF volume depletion measured using cBIS did not corroborate with that suggested by shortening of T_2 time constants following HD; as the difference in pre and post HD cBIS ECF volumes (mean difference between pre and post HD cBIS ECF: 0.001 liters; $t_{(16)} = 1.402$; $p=0.18$) was not significant. This questions the utility of cBIS as a measure of interstitial fluid volume and requires further investigation.

Muscle fat infiltration that often accompanies muscle atrophy may affect the quality of the muscle and lead to reductions in muscle function/strength.[47] In people with COPD intramuscular fatty infiltration was observed across thigh and calf muscles and was highly correlated with muscle function and functional performance.[48] We did not assess pre and post HD differences in myocellular lipids and their association with strength in our participants (Figure 5.8). This requires further investigation.

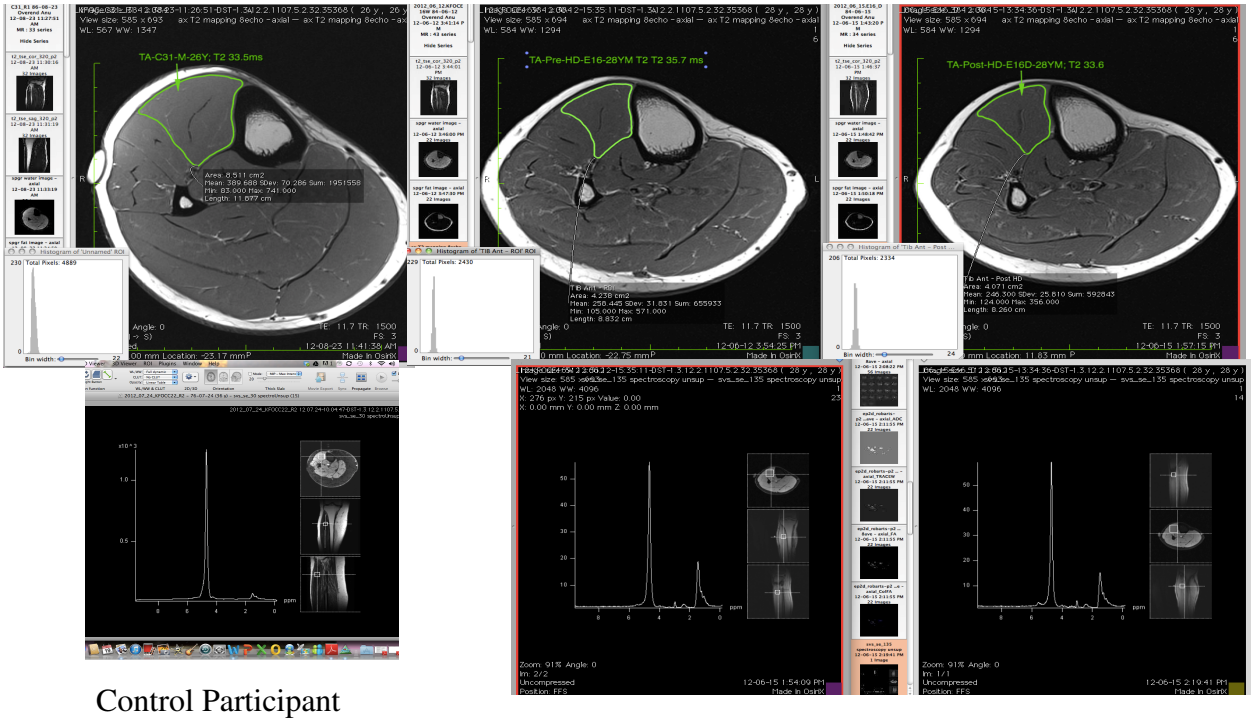
The AAS-HAP scores (the best estimate of respondents' average level of energy expenditure in comparison with peers of same age) indicate that the participants included in this study were functioning at ~62% of the maximum possible activity level or at below-average fitness level.[28,49] The participants in this study had a comorbidity score greater than three (the mortality rate was zero for ESRD/HD cohort with a Charlson Comorbidity Index score of three).[50] The comorbidity scores correlate with the phase angle (suggesting an altered intra and extra cellular water distribution) of BIS and functional levels.[51] Hence these results can be applied to participants with AAS-HAP and comorbidity scores similar to participants in this study.

A limitation of this study is its cross-sectional nature. Our study provides a snapshot of changes in the muscle hydration and strength related to a single HD intervention in participants with a wide range, from 3 months to > 120 months, of exposure to HD. Hence it is difficult to determine the longitudinal effect of HD on the skeletal muscle to understand the progression and/or onset of such changes in the muscles.

5.5.0 Conclusions

In conclusion, this is the first study that has examined the association between intradialytic fluid variations on TA muscle strength. This study used quantitative measures using unique techniques to evaluate hydration of individual muscle in this population for the first time. The effect of whole-body fluid loss following HD was associated with reductions in tibialis anterior strength and volume. Our findings of prolonged T_2 of the tibialis anterior indicated an expansion of relative ECF volume when compared to the control participants that persisted following HD. However, whether dehydration of the contractile fraction of the muscle, as indicated by loss of ECF of the tibialis anterior muscles following rapid fluid loss associated with HD treatment, impacts muscle strength requires further investigation.

Figure 5:6: T_2 Weighted Images and Corresponding Spectroscopy Data Showing Relationship between Water and Lipid Concentrations in Control and before and after HD in a Participant with ESRD.



HD: hemodialysis; T_2: transverse relaxation times.

5.6.0 References

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6 Chapter 6: Summary of Studies, Limitations, and Recommendations for Future Studies

6.1.0 Summary of Findings

To our knowledge this is the first study that has evaluated the effect of fluid loss following hemodialysis (HD) on extracellular fluid (ECF) or interstitial fluid of tibialis anterior (TA) muscle and its strength in participants with end-stage renal disease (ESRD). Using quantitative methods of ECF estimates at the tissue level, we investigated changes in the interstitial fluid of TA, before and after HD and their impact on TA muscle strength. The main results of this study showed that participants with ESRD/HD had reductions of strength six to 12 hours following fluid loss related to their HD. This was also accompanied by reductions in ECF volumes in the TA muscle. Based on prior published reports evaluating changes in magnetic resonance imaging (MRI) acquired transverse relaxation time constants (T_2) associated with muscle atrophy, the mean shortening of T_2 by 2.38ms following HD in the participants' with ESRD was analogous to a shrinkage of ~ 15 % of muscle volumes. Since a direct relationship between T_2 and wet/dry weight of TA muscle is yet to be established, factual shrinkages in TA muscle volume following HD require further investigation.

In comparison with the control participants, the TA muscle strength was significantly weaker and T_2 time constants were prolonged in participants with ESRD/HD before and after HD. This prolongation of T_2, before and after HD indicate persistent expansion of ECF volumes of the TA muscle in the ESRD/HD group, a characteristic finding of ESRD. However, these findings of post HD reductions in interstitial fluid of TA measured using T_2 differed from the findings of cBIS-acquired ECF volumes indicating no reductions in post HD calf ECF volumes.

Hence, matters related to adequacy of dialysis at the "muscle level" may require further investigations.

We observed a varying response to the fluid loss following HD to TA muscle strength; 10 of the 17 participants showed decreased strength while seven of the 17 participants showed increased strength following their HD session. The differences in the measures of hydration between these two subgroups did not reach statistical significance, however, the group demonstrating decreased strength following HD showed relatively greater loss of ECF measured using MRI-acquired T_2.

We established the reliability of cBIS-acquired calf ECF, intracellular fluid (ICF), ratio of ECF:ICF, total water (TW) and MRI-acquired T_2 and apparent diffusion coefficient (ADC) in healthy individuals using intraclass correlations ($ICC_{2,1}$). Our results indicated that $ICC_{2,1}$ for T_2 of all the calf muscles viz, TA, lateral and medial gastrocnemius and soleus had test-retest reliabilities of >0.9. An average of two or three small regions of interest representing the muscles T_2 provided best reliability. For the cBIS ICF and TW, the $ICC_{2,1}$ indicated "excellent"[1] test retest reliability, whereas the reliability of cBIS ECF was "good."[1] These findings indicate that the T_2 of the muscles of the lower leg with "excellent" test-retest reliability[1] are appropriate for studies utilizing repeated measurements. The relative variability of this measure is smaller than the cBIS-acquired measures and hence can be utilized in studies recruiting participants of varying age. These results can be applied to studies utilizing similar techniques for data collection and analysis. The ADC of the leg muscles can be used in studies with relatively large sample sizes, as ADC may be sensitive to minor variations in the circulation of the muscles associated with variations in body fluids and activity.

We explored the association between the cBIS and MRI-acquired measures to evaluate if these measures could be used interchangeably to estimate the hydration of individual calf muscles (TA, lateral and medial gastrocnemius or soleus) in people with ESRD/HD. Our results indicated a significant relationship between the T_2 of TA and medial gastrocnemius post HD but not prior to the treatment. These findings suggested that these measures could not be used interchangeably. Factors related to the measures (T_2 and cBIS ECF) and prior published reports related to the distribution of ECF in people with ESRD/HD before HD may account for the lack of agreement between these measures.

The results of our systematic review and mathematical combination of the standardized mean differences (SMDs) indicate a small, statistically significant positive effect of strength training on muscle mass in participants with ESRD/HD. The results indicated one in nine participants is likely to show an increase in muscle mass following exercise intervention. This review highlighted the paucity of research using standardized outcomes to evaluate the effect of exercise training as an anabolic intervention.

6.2.0 Limitations and Future Directions

Our study provides information on the effect of fluid loss related to a single exposure to HD treatment within the total exposure to HD interventions of a participant; our participants were on HD from three months to greater than 120 months. Whether the observed variability in strength measures following HD was associated with total duration of HD treatment exposure is unclear at this time. Johansen et al[2] observed a decline in the Human Activity Profile (Adjusted Activity Score)[3] score, an increase in fats and reductions in quadriceps muscle area at three months in participants with ESRD/HD who did not participate in a formal exercise program or receive nandrolone deconate (a synthetic testosterone that promotes anabolism). These changes

suggest possible longitudinal catabolic effects of HD. Whether these catabolic changes reflect variations of response in hydration and MVIC of TA following fluid loss related to HD is yet to be determined.

Our results of reductions in strength require further evaluation. Issues contributing to muscle atrophy and weakness such as myocellular lipids, muscle thickness and central activation ratio, of the TA muscle require further investigations. According to Visser et al[4] fat infiltration in the muscle was associated with poorer lower-extremity performance in well functioning older men and women. Similar results of reductions in muscle strength associated with fatty infiltration in participants with COPD have been reported.[5] We did not analyze the relationship between myocellular lipids and strength in our participants. In addition, lipids can hold up to 15% of total ECF;[6] we did not assess changes in lipid concentrations pre and post HD and its relationship with strength in our participants. Additional analysis of the data for lipid concentrations will provide information regarding the impact of myocellular lipids and strength for the participants in our study.

Previous studies have reported that muscle thickness is strongly correlated with muscle cross-sectional area in limb and trunk muscles in a large sample of healthy participants aging 20 to 95 years.[7] Muscle thickness is an established tool for quantifying sarcopenia/muscle atrophy and has been used to evaluate effect of exercise interventions in healthy participants.[8] Determining pre and post HD muscle thickness and pennation angles, strongly related to strength, may add further information regarding changes in muscle architecture on strength influenced by the dialysis related fluid loss.

In our final chapter reporting the effect of HD related fluid loss on MVIC strength of TA

muscle, we have proposed that exercise interventions may alter trans-cellular fluid movements. This may facilitate maintaining adequacy of hydration at the muscle level to improve outcomes in people with ESRD/HD. Such potential of intradialytic exercises should be further investigated. The research presented in this thesis is built on past studies into the muscle function of people with ESRD/HD. This thesis adds information regarding importance of adequacy of dialysis at the muscle level. We have proposed future experiments to improve outcomes and reduce health care costs associated with caring for population with this disease. The author of this thesis hopes that this research will be applied in clinical settings, impact policies and procedures, and guide future research.

6.3.0 References

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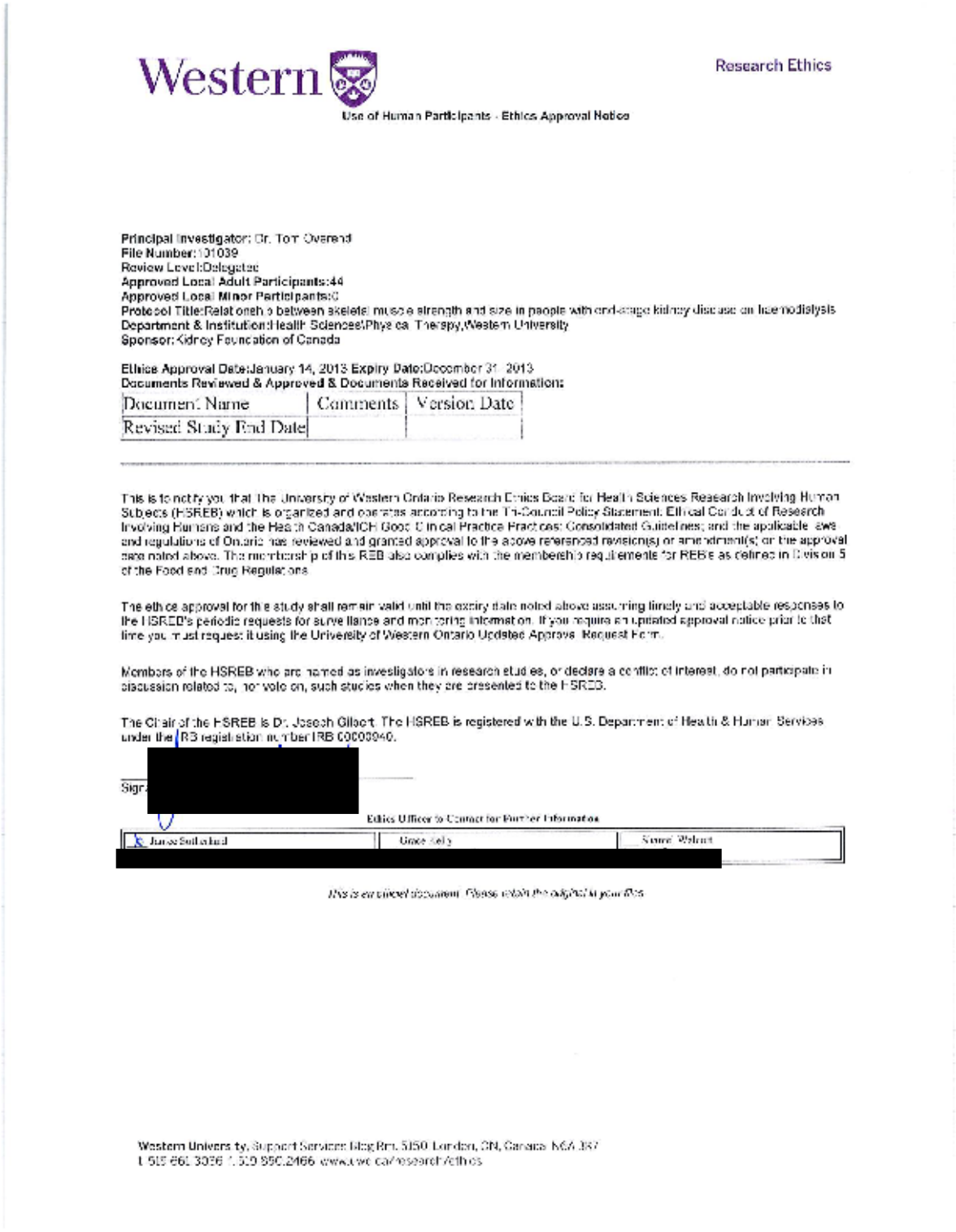
Appendix 1: Ethics Approval Form: Western University's Use of Human Participants



Western

Research Ethics

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Tom Overend
File Number: 101039
Review Level: Delegated
Approved Local Adult Participants: 44
Approved Local Minor Participants: 0
Protocol Title: Relationship between skeletal muscle strength and size in people with end-stage kidney disease on hemodialysis
Department & Institution: Health Sciences\Physical Therapy,Western University
Sponsor: Kidney Foundation of Canada

Ethics Approval Date: January 14, 2013 Expiry Date: December 31, 2013
Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Revised Study End Date		

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REBs as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for the study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request Form.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

The Chair of the HSREB is Dr. Joseph Gilbert. The HSREB is registered with the U.S. Department of Health & Human Services under the FWA registration number FWA 00000940.

[redacted]

Ethics Officer to Contact for Further Information

[redacted]	Grace Kelly	Susan Wilcox

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Western University, Support Services Bldg Rm. 5150 London, ON, Canada N6A 3K7
t. 519.661.3036 f. 519.850.2466 www.uwo.ca/research/ethics

Office of Research Ethics
The University of Western Ontario
Room 5150 Support Services Building, London, ON, Canada N6A 3K7
Telephone: (519) 661-3036 Fax: (519) 850-2466 [redacted]
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. T. Overend
Review Number: 17567 **Review Level:** Full Board
Review Date: December 14, 2010 **Approved Local # of Participants:** 44
Protocol Title: Relationship between skeletal muscle strength and size in people with end-stage kidney disease on haemodialysis
Department and Institution: Physical Therapy, University of Western Ontario
Sponsor: KIDNEY FOUNDATION
Ethics Approval Date: February 02, 2011 **Expiry Date:** June 30, 2013
Documents Reviewed and Approved: UWO protocol (including instruments noted in Section 8.1), Letter of Information and Consent Form - Patients dated 2010/12/21 and Control dated 2010/12/21, Poster Advertisement
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REBs as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

[redacted]

Chair of HSREB: Dr. Joseph Gilbert
FDA Reg. #: RB 00000940

Ethics Officer to Contact for Further Information

☐ Janice Sutherland	☑ Elizabeth Wentbolt	☐ Grace Kelly

[redacted]

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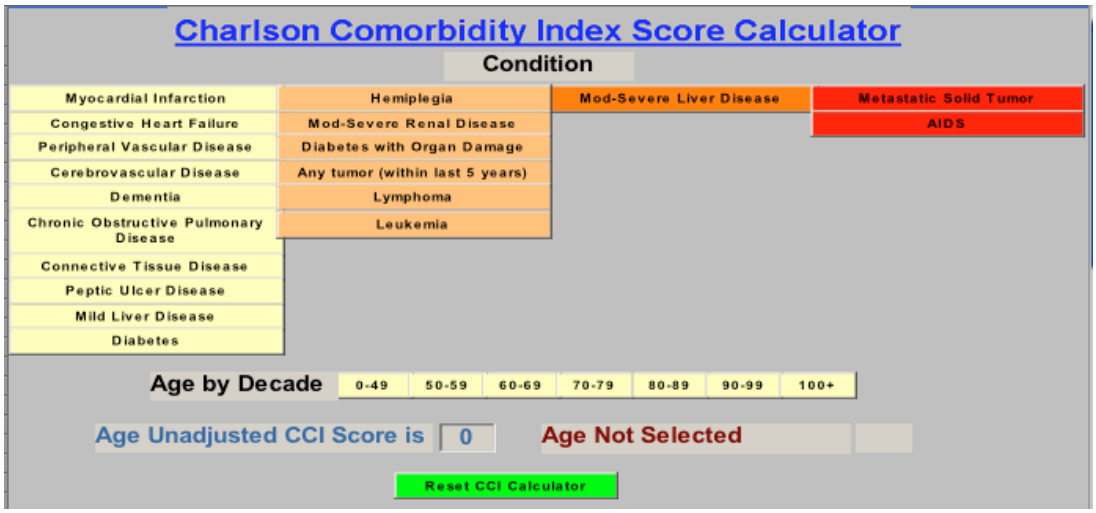
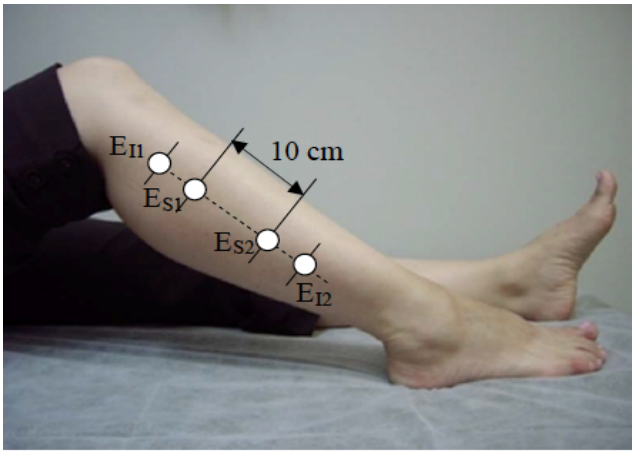
cc: ORE File

Appendix 2: On-line Charlson Comorbidity Index Calculator



Appendix 3: Electrode Placement for Measuring Calf Hydration Using Bioelectrical Impedance Spectroscopy



$E_{S1:}$ First measuring electrode placed at calf – maximum circumference

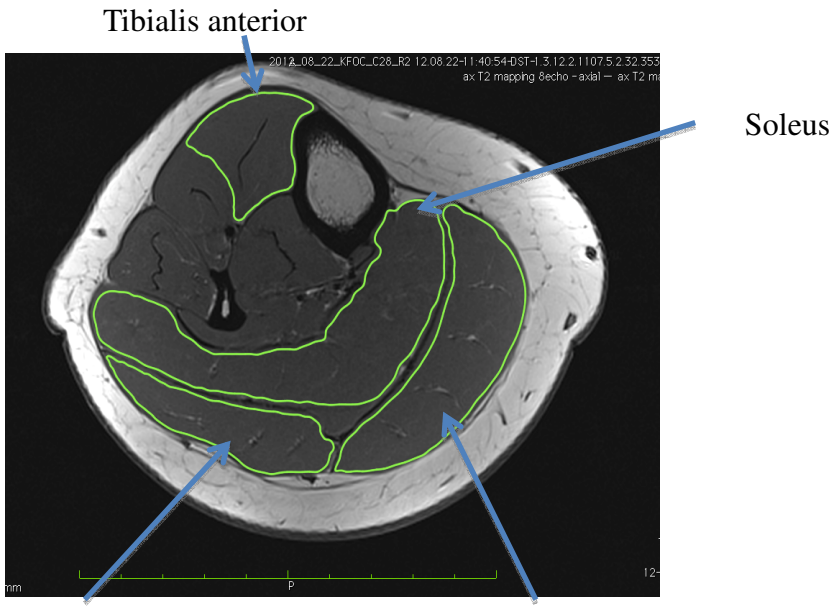
E_{S2}: Second measuring electrode 10 cms from the first electrode

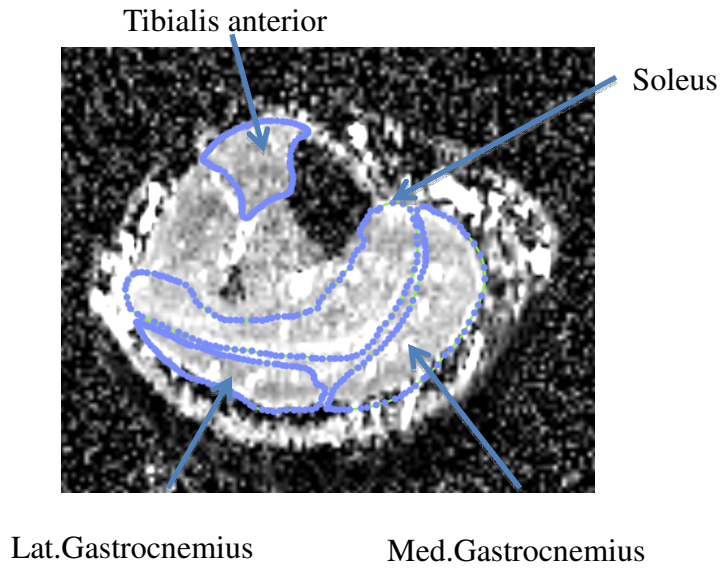
E_{I1}: Injection electrode -1

E_{I2}: Injection electrode – 2

Appendix 4: Example of Measuring Apparent Diffusion Coefficient

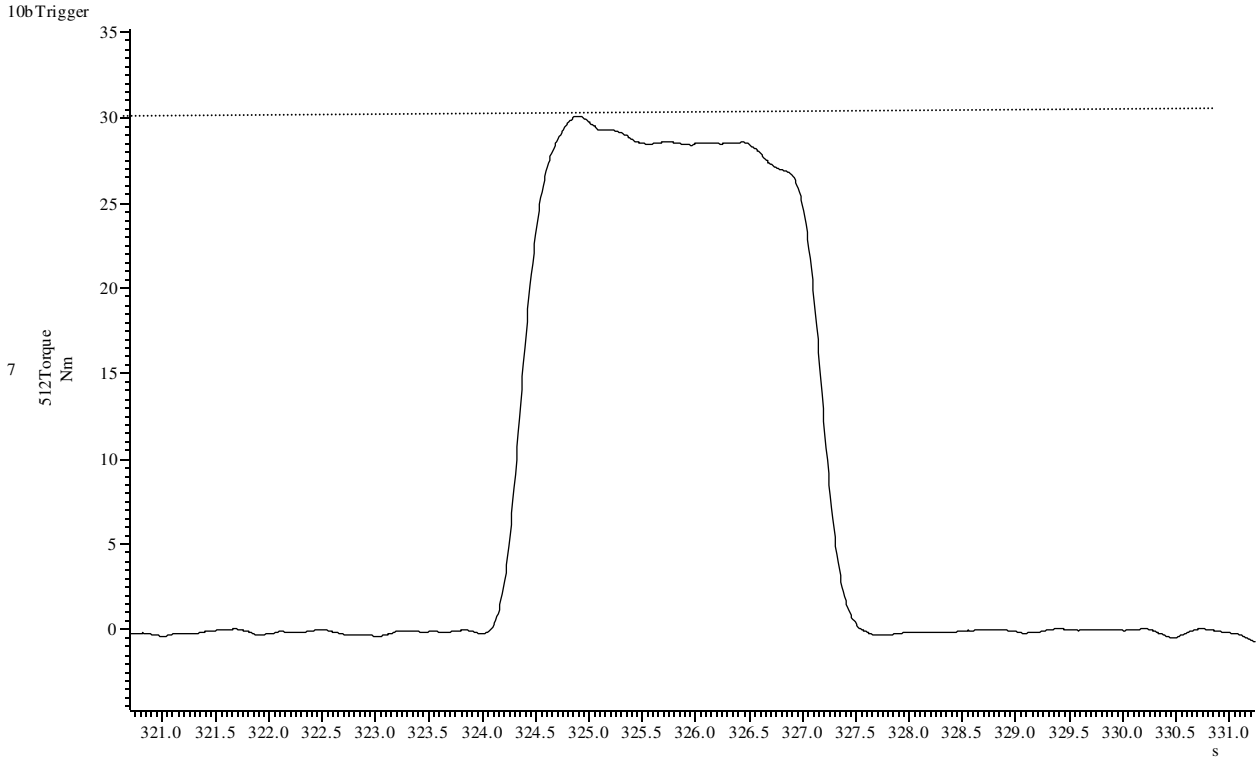
Image of skeletal muscle outlined on T2 weighted image



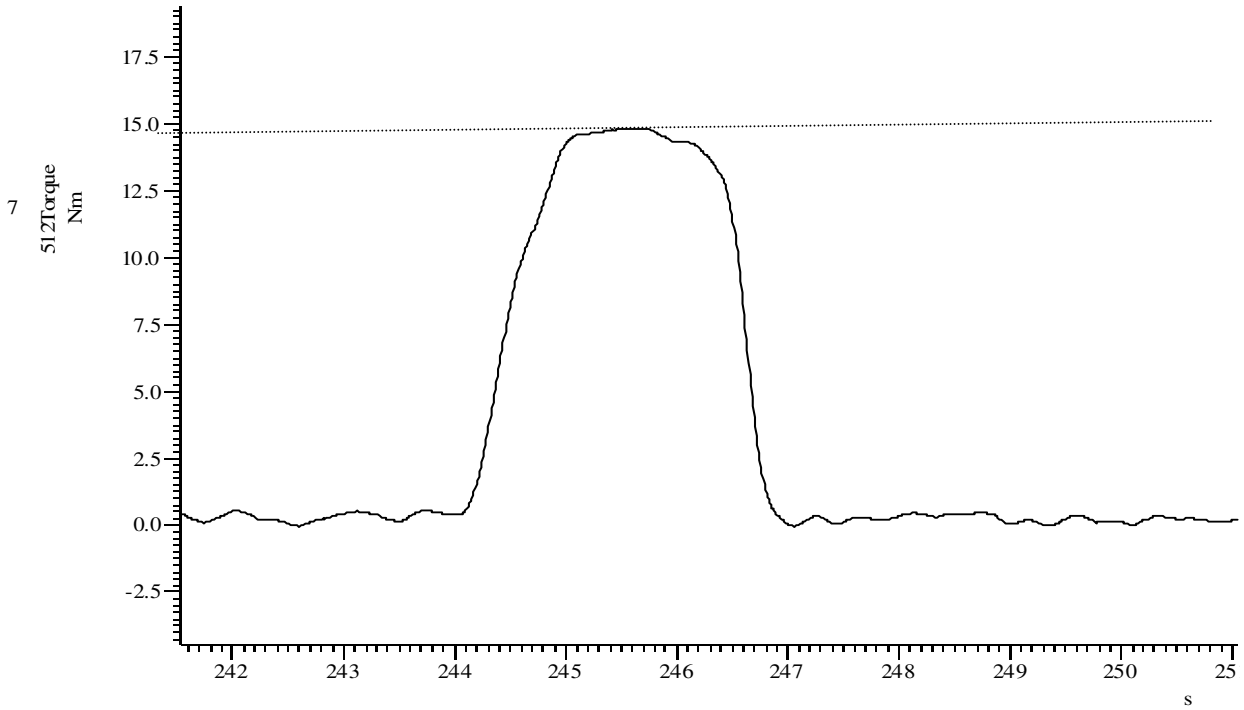


Lat: lateral; Med: medial.

Appendix 5: Peak MVIC – TA of a Control and Experimental Participant



Peak MVIC TA of a HD Participant



Appendix 6: Human Activity Profile Questionnaire

Dear HAP requestor,

We are sending you a copy of the HAP and some abbreviated information from our manual. You are granted permission to use the scale in your research studies. However, you are not given permission to re-transmit this scale to other people.

Thank you for your interest in the HAP.

Sincerely,

David Daughton, M.S.
Behavioral Researcher

DD/sm

HUMAN ACTIVITY PROFILE

Instructions

Please check each activity according to these directions:

Check Column 1 ("Still Doing This Activity") if:

You completed the activity unassisted the last time you had the need or opportunity to do so.

Check Column 2 ("Have Stopped Doing This Activity") if:

You have engaged in the activity in the past, but you probably would not perform the activity today even if the opportunity should arise.

Check Column 3 ("Never Did This Activity") if:

You have never engaged in the specific activity.

Human Activity Profile Test

By David M. Daughton and A. James Fix, Ph.D.

Name_____ Age____ Male____ Female____ Smoker_____ Non-Smoker____
(Optional)
Occupation_____ Married____ Single____ Separated/Divorced_____

Any chronic ailments? Yes____ No____ Highest school grade completed_____

	Still Doing This Activity	**Have Stopped Doing This Activity**	**Never Did This Activity**
1. Getting in and out of chairs or bed (without assistance)			
2. Listening to the radio			
3. Reading books, magazines or newspapers			
4. Writing (letters, notes)			
5. Working at a desk or table			
6. Standing (for more than one minute)			
7. Standing (for more than five minutes)			
8. Dressing or undressing (without assistance)			
9. Getting clothes from drawers or closets			
10. Getting in or out of a car (without assistance)			
11. Dining at a restaurant			
12. Playing cards/table games			
13. Taking a bath (no assistance needed)			
14. Putting on shoes, stockings or socks (no assistance needed)			
15. Attending a movie, play, church event or sports activity			
16. Walking 30 yards (27 meters)			
17. Walking 30 yards (non-stop)			

Human Activity Profile Test

	Still Doing This Activity	Have Stopped Doing This Activity	Never Did This Activity
18. Dressing/undressing (no rest or break needed)			
19.Using public transportation or driving a car (100 miles or less)			
20. Using public transportation or driving a car (99 miles or more)			
21. Cooking your own meals			
22. Washing or drying dishes			
23. Putting groceries on shelves			
24. Ironing or folding clothes			
25. Dusting/polishing furniture or polishing cars			
26. Showering			
27. Climbing six steps			
28. Climbing six steps (non-stop)			
29. Climbing nine steps			
30. Climbing 12 steps			
31. Walking ½ block on level ground			
32. Walking ½ block on level ground (non-stop)			
33. Making a bed (not changing sheets)			
34. Cleaning windows			
35. Kneeling, squatting to do light work			
36. Carrying a light load of groceries			
37. Climbing nine steps (non-stop)			

Human Activity Profile Test

	Still Doing This Activity	Have Stopped Doing This Activity	Never Did This Activity
38. Climbing 12 steps (non-stop)			
39. Walking ½ block uphill			
40. Walking ½ block uphill (non-stop)			
41. Shopping (by yourself)			
42. Washing clothes (by yourself)			
43. Walking one block on level ground			
44. Walking two blocks on level ground			
45. Walking one block on level ground (non-stop)			
46. Walking two blocks on level ground (non-stop)			
47. Scrubbing (floors, walls or cars)			
48. Making beds (changing sheets)			
49. Sweeping			
50. Sweeping (five minutes non-stop)			
51. Carrying a large suitcase or bowling (one line)			
52. Vacuuming carpets			
53. Vacuuming carpets (five minutes non-stop)			
54. Painting (interior/exterior)			
55. Walking six blocks on level ground			
56. Walking six blocks on level ground (non-stop)			
57. Carrying out the garbage			

Human Activity Profile Test

	Still Doing This Activity	Have Stopped Doing This Activity	Never Did This Activity
58. Carrying a heavy load of groceries			
59. Climbing 24 steps			
60. Climbing 36 steps			
61. Climbing 24 steps (non-stop)			
62. Climbing 36 steps (non-stop)			
63. Walking one mile			
64. Walking one mile (non-stop)			
65. Running 110 yards (100 meters) or playing softball/baseball			
66. Dancing (social)			
67. Doing calisthenics or aerobic dancing (5 minutes non-stop)			
68. Mowing the lawn (power mower, but not a riding mower)			
69. Walking two miles			
70. Walking two miles (non-stop)			
71. Climbing 50 steps			
72. Shoveling, digging or spading			
73. Shoveling, digging or spading (five minutes non-stop)			
74. Climbing 50 steps (non-stop)			
75. Walking three miles or golfing 18 holes without a riding cart			
76. Walking three miles (non-stop)			
77. Swimming 25 yards			

Human Activity Profile Test

	Still Doing This Activity	Have Stopped Doing This Activity	Never Did This Activity
78. Swimming 25 yards (non-stop)			
79. Bicycling one mile			
80. Bicycling two miles			
81. Bicycling one mile (non-stop)			
82. Bicycling two miles (non-stop)			
83. Running or jogging ¼ mile			
84. Running or jogging ½ mile			
85. Playing tennis or racquetball			
86. Playing basketball (game play)			
87. Running or jogging ¼ mile (non-stop)			
88. Running or jogging ½ mile (non-stop)			
89. Running or jogging one mile			
90. Running or jogging two miles			
91. Running or jogging three miles			
92. Running or jogging one mile in 12 minutes or less			
93. Running or jogging two miles in 20 minutes or less			
94. Running or jogging three miles in 30 minutes or less			

Scoring and Interpretation

Scores and Normative Information

The HAP produces a number of scores and classifications based on responses to the activity items. These scores, their definitions, method of calculation, and interpretation are outlined in Table 1 and described in detail below.

Several HAP scores have meaning only in comparison to an appropriate normative sample. Because studies have demonstrated age and gender effects, normative data are provided for different age groups for each gender. The normative sample for the HAP consisted of 477 individuals without significant medical problems. This sample ranged in age from 20 to 79.

Table 1
Outline of HAP Scores and Classifications

Scores and Classifications	Definition	Formula	Interpretation
Primary Scores Maximum Activity Score (MAS)	Highest oxygen-demanding activity that the respondent still performs	MAS = Highest item number answered *Still Doing*	Best estimate of respondent's highest level of energy expenditure, in comparison with peers of same age and gender
Adjusted Activity Score (AAS)	A measure of usual daily activities	AAS = MAS minus total number of *Stopped Doing* responses below MAS (i.e., with lower item numbers)	Best estimate of respondent's average level of energy expenditure, in comparison with peers of same age and gender

Development and Validation

Description of Research Samples

Several research samples were employed in the development of the HAP. These research samples were chosen to represent specific characteristics--age and physical health. Representing the extremes on the dimension of age were groups of healthy elderly adults and adolescents.

To analyze the effect of physical impairment on human daily activity, the HAP was administered to chronic lung disease patients, renal dialysis patients, and patients suffering chronic pain. To observe the effects of a critical health event that does not necessarily lead to permanent overall activity impairment, the HAP was given to a small group of patients with myocardial infarcts who were enrolled in a cardiac rehabilitation program.

To represent general overall normal adult health status, data were collected from several groups of essentially healthy adults. One group consisted of individuals who attended a local health fair, another group was comprised of students at two local colleges, and the remaining groups were selected because they represented occupations requiring widely differing skills. Employed city sewer workers, for example, were sought specifically because the occupation requires strenuous physical activity. Data were also collected from nurses and physician's assistant students, two groups with specific training in health issues and rehabilitation.

Table 12

Description of Research Samples

Sample	*N*	Age Range	MAS *M/SD*	AAS *M/SD*	Description of Sample
AD	96	9-18	90.1/3.2	87.0/5.4	Healthy adolescents living in a group home
IE	102	60-88	75.7/6.2	71.6/7.1	Elderly subjects living independently
HF	137	20-59	84.8/7.6	82.6/8.0	Health fair participants
CS	157	18-60	87.3/6.1	85.1/7.1	College students from two universities
TE	64	20-60	85.2/7.3	83.6/7.9	Teachers
NU	40	22-54	85.3/5.3	83.2/5.9	Nurses
PA	22	21-31	89.9/5.1	89.3/5.7	Physician's Assistant Students
SW	36	27-57	84.7/7.5	82.9/8.3	Municipal sewer workers
PAIN	83	16-65	63.3/13.1	51.6/16.2	Chronic pain patients treated in a pain center
COPD	30	37-77	58.8/13.2	48.7/14.2	Patients with chronic obstructive pulmonary disease
CARD	10	45-71	83.7/7.0	75.7/8.6	Myocardial infarct rehabilitation patients
RENAL	39	22-83	55.2/14.9	43.6/19.1	Renal dialysis patients
HEALTHY	654	9-88	84.8/7.8	82.2/8.9	Combined samples of AD, IE, HF, CS, TE, NU, PA, SW
IMPAIRED	162	16-83	61.8/14.8	50.7/17.6	Combined samples of PAIN, COPD, CARD, RENAL
NORM	477	20-79	85.3/7.0	83.2/7.8	Normative sample; subset of HEALTHY subjects between ages 20 and 79
YA	167	20-29	88.6/5.8	86.3/6.7	Young adult subset of HEALTHY subjects (ages 20-29)

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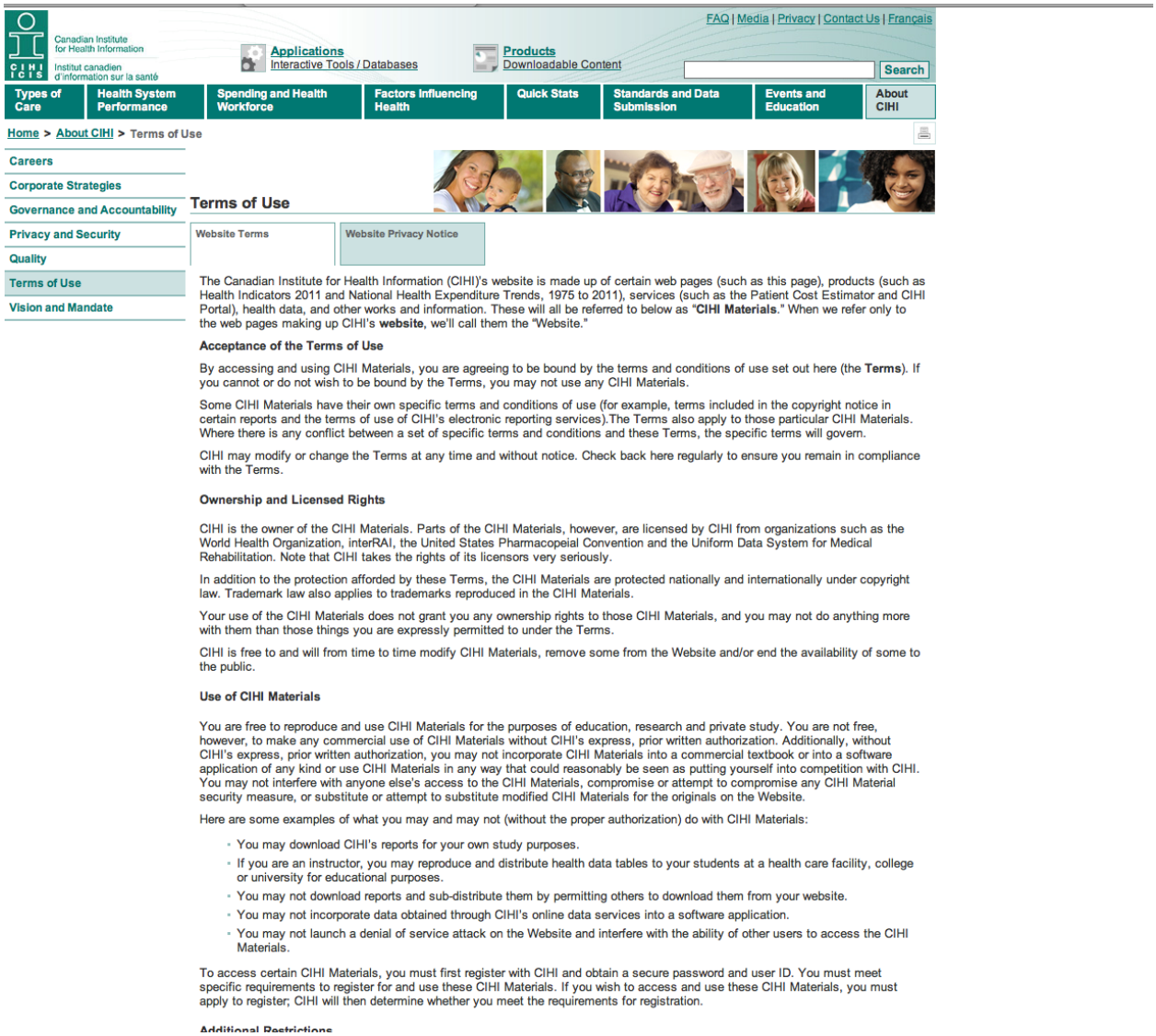

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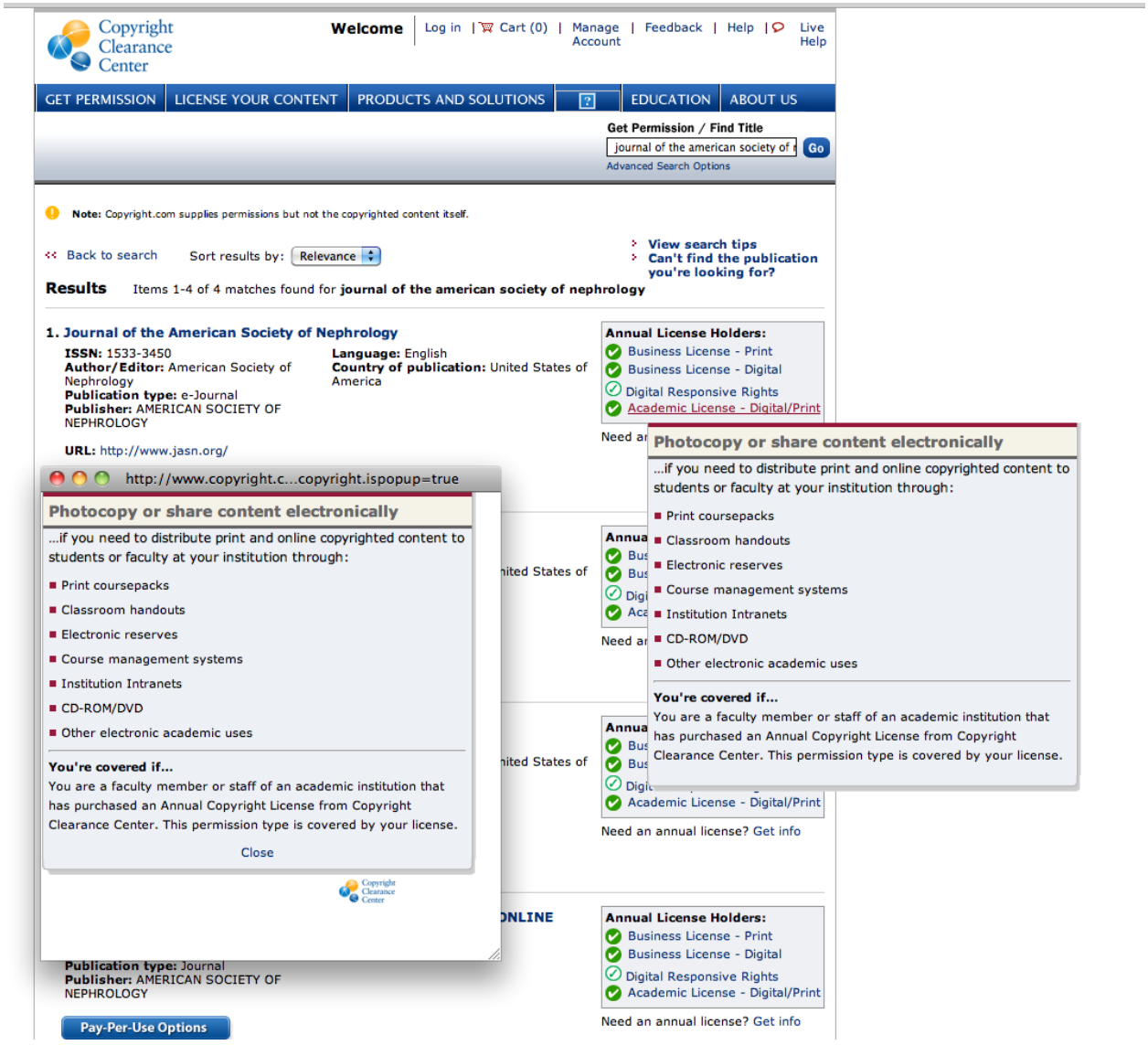

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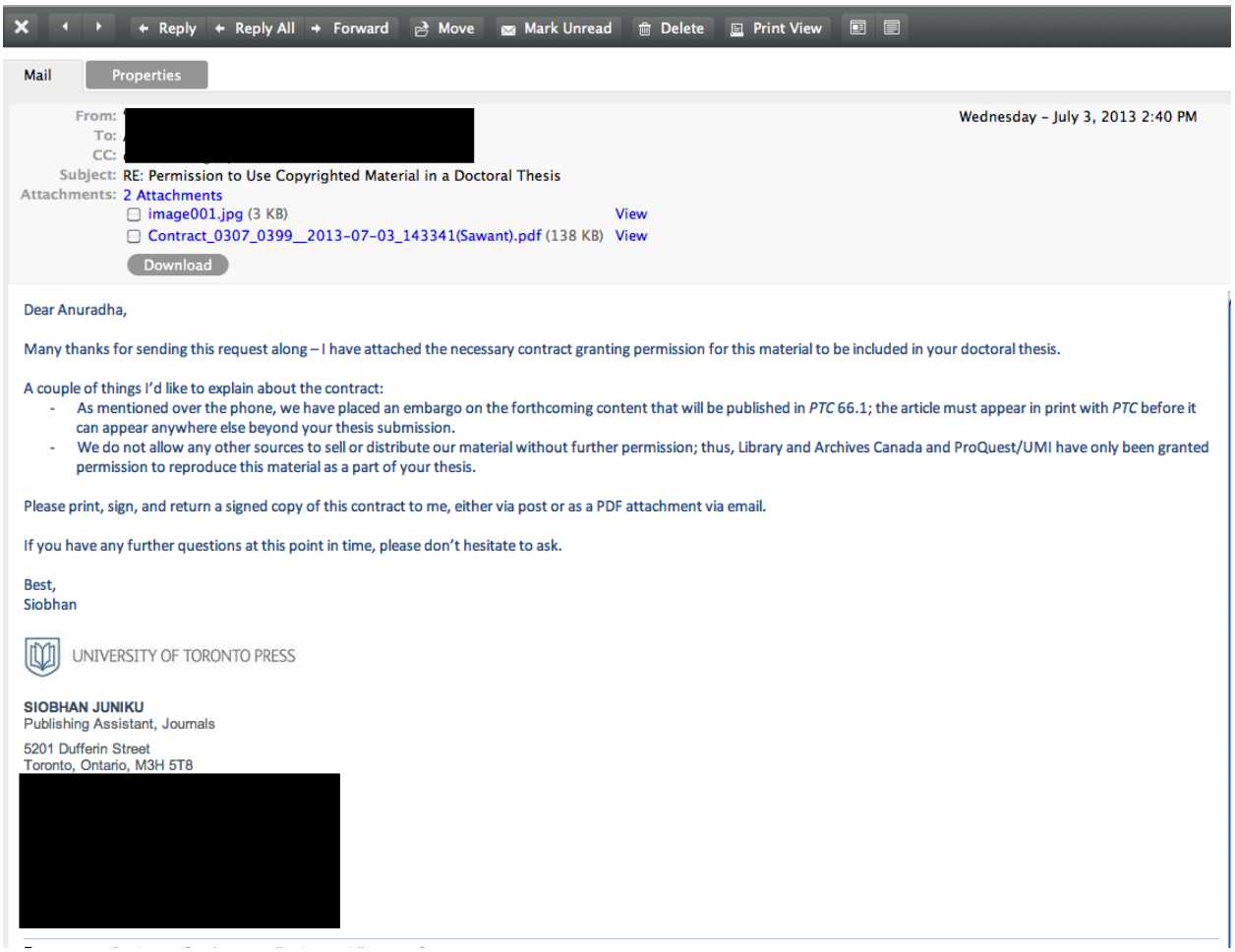

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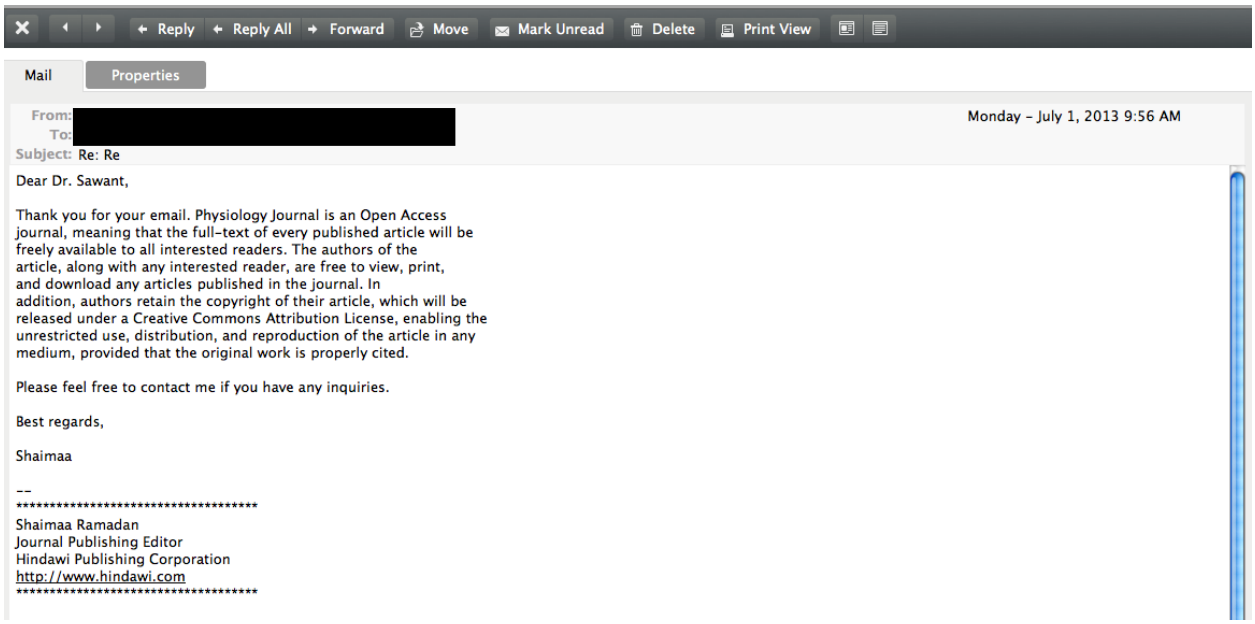

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Appendix 8: Curriculum Vitae

Anuradha Sawant

Education

- PhD candidacy at University of Western Ontario, London (Supervisor: Dr. T. Overend, since Sept 2007; expected date of completion Sept 2013)
- Doctor of Physical Therapy (DPT) A.T Still University, Arizona, USA (2006)
- Bachelor of Science [Physical therapy] Seth G.S. Medical College & K.E.M. Hospitals University of Bombay, India (1978)

Grants and Fellowships

- Allied Health Doctoral Fellowship: July 2010- June 2013
- Western University's Graduate Student Funding: September 2007 – August 2012
- Co-Investigator: Allied Health Research Grant. Kidney Foundation of Canada - 77,357.00 CAD

Academic Work Experience

- Course Manager/Rehabilitation 2008/3 2008/6 University of Western Ontario Physical Therapy
- Teaching Assistant 2007/3 2007/6 University of Western Ontario Physical Therapy

Journal Review Activities

Peer Reviewer Double Blind Physiotherapy Canada

University of Toronto Press

2012/10 2012/11

List of Publications

- **Sawant A,** House AA, Chesworth B et al: Reliability of calf bioelectrical impedance spectroscopy and magnetic resonance imaging acquired skeletal muscle hydration measure. Physiology Journal. In press
- **Sawant A,** House AA, Overend T. Effect of exercise training on skeletal muscle mass in people with end-stage renal disease on hemodialysis: A systematic review and meta-analysis. Physiotherapy Canada – In Press
- Busch AJ, Webber SC, Brachaniec M, Bidonde J, Bello-Haas VD, Danyliw AD, Overend TJ, Richards RS, **Sawant A**. Schachter C. Exercise therapy for fibromyalgia. Current Pain and Headache Reports 2011 October; 15(5): 358–367.
  Published online 2011 July 5. doi: 10.1007/s11916-011-0214-2
- **Sawant A,** Garland JS, House AA, Overend TJ. Morphological, electrophysiological and metabolic characteristics of skeletal muscle in people with end stage renal disease: A critical review. Physiotherapy Canada. 2011; 63:355–76.
- Overend T, Anderson C, **Sawant A,** Perryman B, Locking-Cusolito H. Relative and absolute reliability of physical function measures in people with end-stage renal disease. Physiotherapy Canada. 2010;62:122–128.
- **Sawant A.** Review of Exercise benefits for osteoarthritis of knee joint. Bahrain Physical Therapy Journal. 1999

Papers in Submission:

- **Sawant A,** House AA, Chesworth BM, et al. Association between calf bioelectrical impedance spectroscopy and magnetic resonance imaging acquired measures of skeletal muscle hydration in people with end-stage renal disease on hemodialysis. In submission.
- **Sawant A,** House AA, Doherty T, et al. Effect of fluid loss following hemodialysis on dorsiflexor muscle strength in people with end-stage renal disease on hemodialysis. In submission

Podium/Poster Presentations

- Effect of interdialytic fluid gain on muscle strength: A pilot project.

The Canadian Physiotherapy Association's National Congress -2013, Montreal, Quebec, Canada.

- Skeletal muscle hydration: Relationship between calf bioelectrical impedance spectroscopy and MRI-acquired measures in people with ESRD on haemodialysis.
  Canadian Society of Nephrologists- AGM – 2013, Montreal, Quebec, Canada.
- Reliability of skeletal muscle hydration measures: Relevance for utilization of these measures in people with ESKD on hemodialysis.
  15th International Conference on Dialysis Location Puerto Rico City Puerto Rico
- Falls in the Hemodialysis Population
  CANNT 2012 City Ottawa
- Best Volume: Best practice
  CANNT- 2013 City Ottawa
- The Effect of Exercise Interventions on Pain Intensity in Individuals with Fibromyalgia: A Systematic Review
  14th World Congress on pain Location Italy City Milan Main
- Exercise as an anabolic intervention in people on maintenance hemodialysis: A systematic review and meta-analysis
  Expanding Horizons Congress 2012, Canadian Physiotherapy Association, Saskatchewan
- Effect of interdialytic fluid gain of skeletal muscle strength and size
  Educational presentation, University of Toronto, Toronto
- Falls in Hemodialysis Population
  Patient Safety Summit, 2012 LHSC and St. Joseph's Quality & Patient Safety Summit
- Renal Program, London Health Sciences Centre
  Renal Rehab network Congress, Kingston
- Effect of exercise training on skeletal muscle mass in people with end-stage renal disease on hemodialysis
  Interaction 2012, Ontario Physiotherapy Association ,Toronto
  URL http://www.opa.on.ca/pdfs/Preliminary_Program_Final_2012.pdf
- Anabolic effect of exercise in older adults on maintenance Hemodialysis: A systematic review and meta analysis

Ideas to Action: Knowledge Translation at the point of Health Care, ARGC/FHS Research Symposium, Western University and St. Joseph's Health Care, London

- My Journey - Experiences from the field and the strategies used to reach the current stage in program
  Health & Rehabilitation Sciences Graduate Research Forum, Western University, London
- Skeletal Muscle Weakness in People on Hemodialysis: Review of Morphological, Electrophysiological and Metabolic Characteristics
  Falls and Mobility Network Meeting, Sunnybrook Health Sciences Centre, Toronto
- Weakness in people on hemodialysis: Review of morphological, electrophysiological and metabolic skeletal muscle characteristics
  Faculty of Health Sciences Research Day, Western University, London
- Weakness in people on hemodialysis: Review of morphological, electrophysiological and metabolic skeletal muscle characteristics
  Society of Graduate Studies Research Day , Western University, London
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  Society of Graduate Studies Research Day , Western University, London
- Absolute and relative reliability of physical function measures in people with end-stage kidney disease
  Faculty of Health Sciences Research Day, Western University, London

Book Reviews

- **Sawant A.** Book Review: Measurement of Range of Movement.3rd Ed Norkin CC & White DJ. Physiotherapy Canada. 2004.