

# Measuring Muscle and Fat with Peripheral Quantitative Computed Tomography:

Precision, Annual Changes, Monitoring Intervals, and  
Associations with Fall Status in Older Adults

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*A Thesis Submitted to the College of Graduate Studies and Research in Partial*

*Fulfillment of the Requirements for the Doctoral Degree*

*in the College of Kinesiology, University of Saskatchewan*

*Saskatoon, Saskatchewan, Canada*

**By: Andrew William Frank-Wilson**

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# Abstract

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**Objectives:** The overall aim of this thesis was to investigate the precision error, annual changes, and monitoring time intervals of muscle and fat outcomes measured by peripheral quantitative computed tomography (pQCT), as well as explore the strength of their associations with fall status in older adults.

**Methods:** Participants aged >60 years old (N=190) were recruited from the Saskatoon Cohort of the Canadian Multicentre Osteoporosis Study (CaMOs). The precision error (Root Mean Squared Co-efficient of Variation,  $CV\%_{RMS}$ ) of soft-tissue outcomes from previously reported pQCT image analysis protocols (n=6) were calculated and compared using repeat forearm and lower leg scans collected from a random sub-sample of women (n=35). Prospective scans were collected with 1 and/or 2 years of follow-up (n=97) to estimate annual changes and monitoring time intervals for pQCT-derived muscle and fat outcomes in women. Imaging data and responses from a retrospective fall status questionnaire were analyzed to investigate the associations of muscle density, functional mobility, and health-related factors to fall status for both men and women (n=183).

**Results:** Precision errors of muscle and fat outcomes ranged from 0.7 to 6.4% in older women, however not all protocols were equally precise. Muscle cross-sectional area decreased by 0.8 to 1.2% per year, with greater losses in the lower limb. Biological changes in muscle area and density may be detected with 80 and 95% certainty within monitoring time intervals of 4 to 9 years. The odds of having reported a fall increased by 17% for every unit decrease in muscle density (mean 70.2, SD 2.6mg/cm<sup>3</sup>) after adjusting for age, sex,

body mass index, general health status, diabetes, the number of comorbidities, and functional mobility.

**Discussion:** This dissertation demonstrated the potential for pQCT to study changes in muscle and fat outcomes in older adults. Both muscle area and density can be precisely measured. Observed annual changes in soft-tissue outcomes were small in older adults; highlighting the importance of precise measurements to detect changes beyond measurement error. Together with the estimated monitoring time intervals, these findings can assist the planning of prospective investigations of musculoskeletal health in aging. Furthermore, based on the observed independent association between muscle density and fall status, monitoring muscle density may further complement the study of musculoskeletal health and fall risk in community-dwelling older adults.



# Preface & Contributions of Authors

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Sections of this thesis have been published or submitted for publication in refereed journals as multi-authored papers. In this section, the roles of each author are defined.

Published/Submitted Papers:

1. Frank-Wilson AW, Johnston JD, Olszynski WP, Kontulainen SA. Measurement of muscle and fat in postmenopausal women: precision of previously reported pQCT imaging methods. *Bone*. 2015;75:49-54.

*Author's contribution: AFW and SK had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conception and Design of the study: AFW, SK, JJ, WO. Data acquisition: AFW, SK, WO. Data analysis: AFW, SK. Interpretation: AFW, SK, JJ. Drafting: AFW, SK. Critical revision and final approval: All authors. This research is discussed in Chapter 4 of this thesis.*

2. Frank-Wilson AW, Johnston JD, Davison KS, Olszynski WP, Kontulainen SA. Monitoring pQCT-derived muscle and fat outcomes in older women. *Journal of Cachexia, Sarcopenia and Muscle*. Submitted 2015 July 27.

*Author's contribution: AFW and SK had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conception and Design of the study: AFW, SK, JJ, WO. Data acquisition: AFW, SK, WO. Data analysis: AFW, JJ, SK. Interpretation: AFW, SK, JJ, KD. Drafting:*

*AFW, SK. Critical revision and final approval: All authors. This research is discussed in Chapter 5 of this thesis.*

3. Frank-Wilson AW, Farthing JP, Chilibeck PD, Arnold CM, Davison KS, Olszynski WP, Kontulainen SA. Lower Leg Muscle Density is Independently Associated With Fall Status in Community-Dwelling Older Adults. *Osteoporosis International*, Submitted 2015 August 22.

*Author's contribution: AFW and SK had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Conception and Design of the study: AFW, SK, JF, PC, CA, WO. Data acquisition: AFW, SK, WO. Data analysis: AFW, SK. Interpretation: AFW, SK, JF, PC, CA, KD. Drafting: AFW, SK. Critical revision and final approval: All authors. This research is discussed in Chapter 6 of this thesis*

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The time and energy invested in me by Drs. Jon Farthing, Phil Chilibeck, and J.D. Johnston are also noteworthy; their frequent encouragement, thoughtful recommendation, availability for discussion, and enthusiastic work ethic are appreciated and admired. I also value the augmentation of my research through the clinical perspectives of Drs. Cathy Arnold, Soo Kim, and Saskatoon CaMOs co-directors Wojciech Olszynski and Shawn Davison.

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# Dedication

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For my loving and supportive wife, Alannah, and our perceptive and playful dog Ellammenno; both of whom have helped me de-stress throughout my studies and bring me great joy.

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# Glossary of Terms

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aBMD – areal Bone Mineral Density ( $\text{g}/\text{cm}^2$ )

AFW - Andrew Frank-Wilson

AUC – Area Under Curve

BMD – (volumetric) Bone Mineral Density ( $\text{mg}/\text{cm}^3$ )

BMI – Body Mass Index ( $\text{kg}/\text{m}^2$ )

CaMOs – Canadian Multicentre Osteoporosis Study

CI – Confidence Interval

CRT\_A - Cortical Area ( $\text{mm}^2$ )

CRT\_DEN - Cortical Density ( $\text{mg}/\text{cm}^3$ )

CRTSUB\_A - Cortical and Subcortical Area ( $\text{mm}^2$ )

CRTSUB\_DEN - Cortical and Subcortical Density ( $\text{mg}/\text{cm}^3$ )

CT – Computed Tomography

CV%<sub>RMS</sub> - Root Mean Squared Coefficient of Variation

DICOM - Digital Imaging and Communications in Medicine (Image Format)

dSAT – Deep Subcutaneous Adipose Tissue

DXA – Dual energy X-ray Absorptiometry

EMCL – Extra-myocellular Lipid

EWGSOP - European Working Group for Sarcopenia in Older People

FNIH - Foundation of the National Institutes of Health

Health ABC – Health, Aging, & Body Composition Study

H<sup>1</sup> - Proton

HU – Hounsfield Unit

IL6 – Interleukin-6

IMAT – Inter-muscular Adipose Tissue

IMF – Intramuscular Fat

IMCL – Intra-myocellular Lipid

InCHIANTI - Invecchiare in Chianti (“Aging in the Chianti Area”) Study

IntraFatA - IMAT Area (cm<sup>2</sup>)  
ISCD – International Society for Clinical Densitometry  
IWG - International Working Group on Sarcopenia  
LSC – Least Significant Change (%)  
MRI – Magnetic Resonance Imaging  
MRS – Magnetic Resonance Spectroscopy  
MTI – Monitoring Time Interval (years)  
MuA - Muscle Area (cm<sup>2</sup>)  
MuD - Muscle Density (mg/cm<sup>3</sup>)  
OR – Odds Ratio  
pQCT – Peripheral Quantitative Computed Tomography  
ProFaNE - Prevention of Falls Network Europe  
QCT – Quantitative Computed Tomography  
ROC Curve – Receiver Operator Characteristic Curve  
SAT – Subcutaneous Adipose Tissue  
SD – Standard Deviation  
SD<sub>RMS</sub> – Root Mean Squared Standard Deviation  
SF-36 - Short Form 36 Health Status Questionnaire  
SubCutFatA - SAT Area (cm<sup>2</sup>)  
TNF $\alpha$  – Tumour Necrosis Factor Alpha  
TOT\_A - Total Area (mm<sup>2</sup>)  
TOT\_CNT - Total Content (mg/mm)  
TRAB\_A - Trabecular Area (mm<sup>2</sup>)  
TUG – Timed Up and Go test

# 1 Introduction

---

The proportion of Canadians above age 60 is expected to increase 30% by 2031, almost exclusively from a doubling of those beyond age 70 [1]. This historically unprecedented demographic shift is not exclusive to high-income countries. Global projections estimate that the population of adults older than 60 will reach 2 billion by the middle of this century [2]. Thus, the 21st century will be defined by an older global society and factors that impact aging human health. The aging process is characterized by a diminished capacity to regulate the internal environment of the body [3]. No one is immune from the effects of aging, even the most fit masters athletes demonstrate physical decline [4]. Brooks et al. [3] observed that while mean life expectancy increased over the last century, the maximum lifespan has not changed. Thus, much focus has shifted towards identifying modifiable factors that can delay the onset of chronic disease and disability to enhance “successful aging”; the physical, psychological, and social success with which adults age [5]. While age affects every physiological system in the body, changes in body composition and neuromuscular function are of great interest. Age-related redistributions of fat, declines in muscle mass and physical function translate into reduced functional capacity to perform activities of daily living, which eventually lead to heightened risk of injury and a loss of independence [6].

Declines in muscle mass and strength among older adults have undergone tremendous study since Irwin Rosenberg coined the term “sarcopenia” to describe this geriatric phenomenon [7,8]. Initial operational definitions exclusively focused their attention on measuring muscle mass using Dual energy X-ray Absorptiometry (DXA) [9]. As this realm of health research matured, the importance of also measuring fat [10,11], strength [12], and physical function gained traction [13-15]. Although there are several variations in how sarcopenia is defined [7], many of these definitions are prospectively associated with increased likelihood of mobility impairments [16,17], falls [18-20], and fracture risk [21,22].

Furthermore, evidence suggests a connection between muscle adiposity and physical function [23-25]. Both heightened adiposity and reduced physical function have been identified as major components of the risk of disability development in older adults [26,27]. Adiposity refers to the presence of adipose tissue, which exists throughout the body in various regions and quantities. Of these regions, the adiposity of muscle or “myosteatorsis” is of particular interest due to reported increases with age, regardless of changes in body weight and the quantity of subcutaneous adipose [28]; as well as associations with hospitalization [29], and hip fracture incidence [30,31].

Sarcopenia and muscle adiposity are also of special interest to the field of Kinesiology. Resistance exercise has long been a recommended treatment for adults with low muscle mass [32]. Accumulating evidence in exercise physiology suggests that cardiovascular and resistance exercise may be promising countermeasures for reducing muscle adiposity in older adults. Cardiovascular exercise has been demonstrated to be effective for the reduction of non-myogenic satellite cells, as well the adiposity of older rat gastrocnemii [33], and there is recent evidence of a similar adiposity reduction in older humans [34]. Furthermore RCTs of aerobic, strength, flexibility and balance exercise regimens maintained muscular strength, improved physical function, and prevented or reversed decreases in muscle tissue attenuation (a surrogate of adiposity) in the elderly [35,36]. Progressive resistance training has also demonstrated positive effects in a small group of elderly men and women; significant concomitant changes were observed in thigh muscle attenuation and muscle strength with detraining, as well as increases with retraining [37], suggesting a connection between muscular fitness and adiposity. Observational data also supports the existence of a relationship between muscle function and adiposity, and the specificity of adipose depot location. Thigh muscle attenuation, a computed tomography (CT) measure of

muscle adiposity predicted isokinetic quadriceps muscle torque, independent of age, race and the size of inter-muscular and subcutaneous fat depots [23]. Persons in the lowest quartile of muscle attenuation were reported to be 50 – 80% more likely to develop mobility impairments, independent of baseline muscle size, strength, and total body fat mass [24]. Although the pathophysiology of changes in body composition and neuromuscular function observed with age are not fully understood [32], medical imaging provides a non-invasive avenue for their study in vivo [38].

Research concerning soft tissues and the age related changes that occur in human muscular and/or functional performance is particularly intriguing [39]. There are several imaging methods available to study muscle and adipose tissue depots in vivo [38,40]. Among these tools, pQCT is a desirable technology for assessing limb muscle area, density, and subcutaneous adipose tissue (SAT) area (Figure 1). When compared to alternative radiographic tools, such as DXA and CT, pQCT exposes participants to substantially lower effective doses of radiation [41,42]. Originally designed for measures of trabecular bone density [43], pQCT is also becoming a popular research tool for the measurement of soft tissues in aging research [27,44-56]. Particularly interesting are the findings of the InChianti study, which demonstrated pQCT-derived muscle density's association with motoneuron axonal degeneration [46], frailty [45], and incident disability [27]. Significant differences were recently reported in pQCT-derived lower leg muscle density when comparing female fallers to non-fallers [53], and wrist fracture patients with healthy controls [54]. Falls are a major public health concern for the elderly, responsible for over 90% of hip fractures, and 65% of hospitalizations [57,58]. Given the significance of falls, and the overlapping evidence suggesting a potential connection to muscle adiposity [26,29-31,53], further investigation

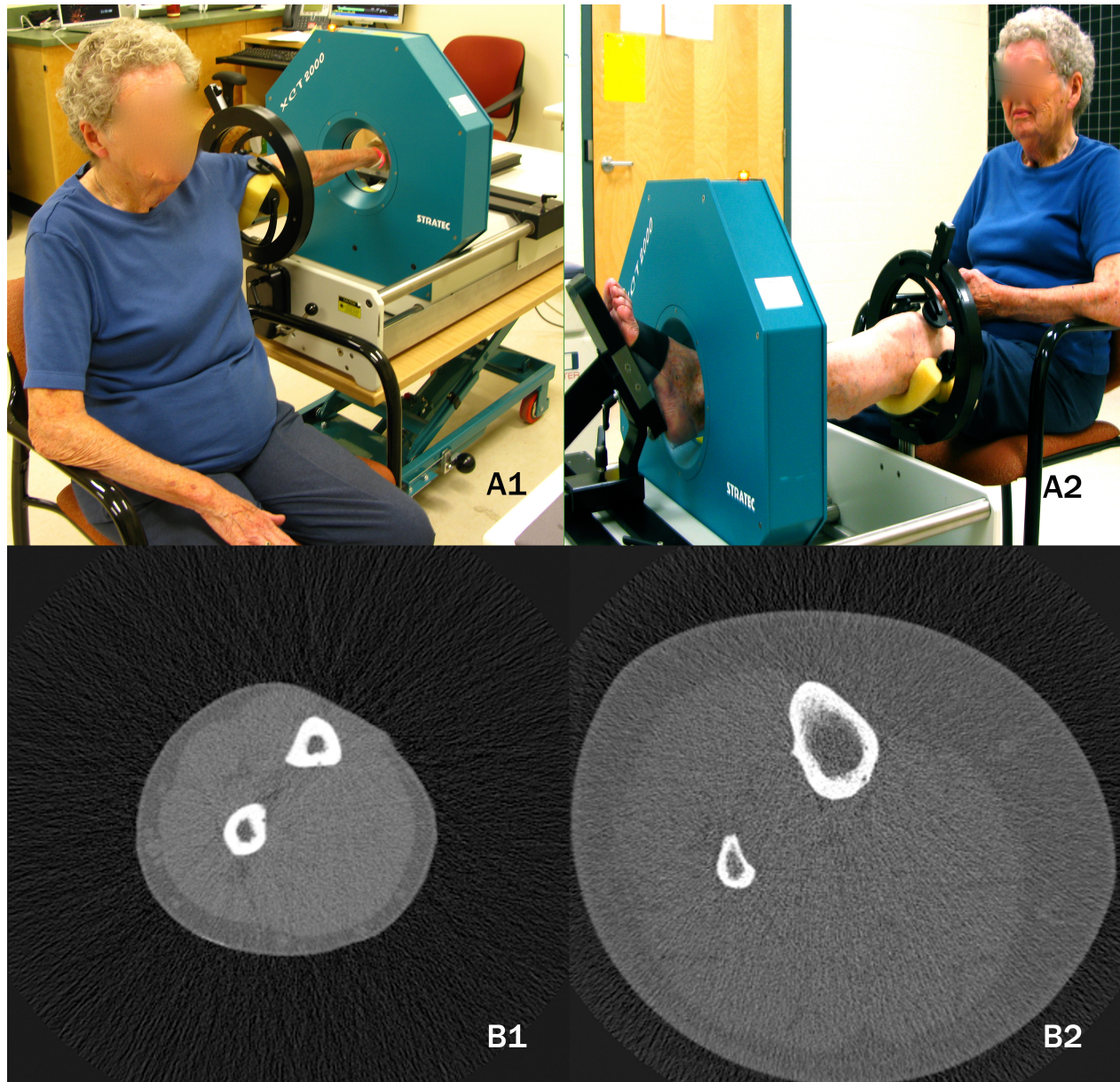


Figure 1: Positioning a participant for pQCT scanning of the forearm (A1) and lower leg (A2). Scans provide quantitative axial images of fat, muscle, and bone tissues at both the forearm (B1) and lower leg (B2) allowing for the study of how the size and composition of these tissues change and relate to human health.

of the association between pQCT-derived lower leg muscle density and fall status is warranted. However, there are multiple image analysis protocols reported (Table 1) and no consensus on how to best analyze pQCT scans for the derivation of soft-tissue outcomes, including muscle density. This makes it difficult to compare results between studies, as



values are dependent on how the images are segmented into fat, muscle and bone.

Furthermore, there is a lack of precision error data (derived using International Society for Clinical Densitometry (ISCD) guidelines) for pQCT-derived soft-tissue outcomes, as well as estimates of the longitudinal sensitivity of these outcomes in aging adults. The magnitude of biological change necessary to exceed the precision error of the instrument, as well as the length of time to observe these changes are important data for the efficient design of prospective studies.

Therefore the aim of this dissertation was to investigate the precision of pQCT image analysis protocols for muscle and fat outcomes in older adults, estimate the annual changes, and monitoring time intervals [59] for these outcomes, and explore their associations with fall status in a sample of community-dwelling older adults. To achieve this aim, three sub-studies were conducted utilizing data collected from the Saskatoon cohort of the CaMOs. Study one utilized a subset of repeat scans collected from older women to compare previously reported image analysis protocols for muscle and fat outcomes. Study two pooled imaging data collected one and two years apart to estimate annual changes and monitoring time intervals for muscle and fat outcomes in older adult women. Finally, study three explored the associations between muscle, fat, health related factors and functional mobility to fall status.

**Table 1: Summary of Reported pQCT Muscle and Fat Image Analysis Protocols**

Ref [#]	First Author	Year	Age Range or Mean (SD)	Software	Contour Modes	Density Ranges		ROI	Filters	Precision
						Fat	Muscle			
[44]	Cesari	2004	74.8 (6.8)	BonAllyse	N/A		15 to 179			
[60]	Bechtold	2005	15.3 (2.5)	Stratec XCT		<20	20 to 79			
[45]	Cesari	2006	74.8 (6.8)	BonAllyse	N/A		15 to 179			
[46]	Lauretani	2006	21-96	BonAllyse	N/A		15 to 179			
[61]	Fricke	2008	54.4 (4.1)	Stratec XCT		<20	20 to 59			
[62]	Fricke	2008	5-19	Stratec XCT		<20	20 to 59			Yes
[63]	Fricke	2008	5-19	Stratec XCT		<20	20 to 59			Yes
[64]	Frotzler	2008	41.9 (7.5)	Stratec XCT						
[47]	Miljkovic-Gacic	2008	40-91	Stratec XCT						
[48]	Miljkovic-Gacic	2008	18-103	Stratec XCT						Yes
[65]	Schweizer	2008	7.3 (2.7)	Stratec XCT		1 to 29	30 to 69			Yes
[66]	Cesari	2009	74.5 (7)	BonAllyse	N/A		15 to 179			
[67]	Coupaud	2009	40 (C.S.)	Stratec XCT		<36	36 to 279			
[68]	Coupaud	2009	35-41.2	Stratec XCT		<36	36 to 279			
[69]	Dubner	2009	5-22	Stratec XCT						Yes
[70]	Ducher	2009	8.4 (0.4)	Stratec XCT	CM3/PM1	-39 to 39	40 to 279			Yes
[71]	Ducher	2009	7-10	Stratec XCT		-39 to 39	40 to 279			Yes
[72]	Eser	2009	18-44	Stratec XCT	CM3/PM1	-39 to 39	40 to 279			
[73]	Fricke	2009	5-19	Stratec XCT		<20	20 to 59			Yes
[74]	Fricke	2009	5-19	Stratec XCT		<20	20 to 59			Yes
[75]	Frotzler	2009	38.6 (8.1)	Stratec XCT						
[76]	Martin	2009	4-12	Stratec XCT		<30	30 to 69			Yes
[77]	Sergi	2009	24-57							Yes
[78]	Sherk	2009	18-30	Stratec XCT	CM3/PM1	-100 to 39	40 to 710		F03F05	Yes
[79]	Wetzsteon	2009	5-21	Stratec XCT						Yes
[80]	Wetzsteon	2009	9-12	Stratec XCT						Yes

[81]	Bechtold	2010	12.2 (2.7)	Stratec XCT		<30	30 to 59			
[82]	Bechtold	2010	6-21	Stratec XCT						Yes
[83]	Eser	2010	20-90	Stratec XCT		-39 to 39	40 to 279			
[84]	Leonard	2010	5-35	Stratec XCT		<40	40 to 710			Yes
[49]	MacIntyre	2010	52-87	Stratec XCT	CM3/PM1	<40	40 to 279			
[85]	Sherk	2010	18-64	Stratec XCT					F03F06U 01	
[86]	Swinford	2010	26.6 (8.7)	Stratec XCT	CM3/PM2	-100 to 39	40 to 710	Box	7x7	ISCD
[50]	Farr	2011	10.7 (1.1)	Stratec XCT	BDI	-100 to 39	40 to 149	Box	BDI	Yes
[87]	Micklesfield	2011	13 (0.2)	Stratec XCT	CM3	<40	40 to 179			
[88]	Sherk	2011	24-49	Stratec XCT		-100 to 39	40 to 710		Multiple	Yes
[89]	Sukumar	2011	25-71	Stratec XCT	CM1/PM1					
[90]	Szabo	2011	42 (16)	Stratec XCT		-100 to 39	40 to 710	Matrix		Yes
[91]	Talla	2011	68 (11)	Stratec XCT		-39 to 39	40 to 279			Yes
[92]	Wetzsteon	2011	5-35	Stratec XCT						Yes
[93]	Butner	2012	38.6 (4.7)	Stratec XCT	CM3/PM1	<40	40 to 279	Box	F03F05 F05/F07	ISCD
[51]	Farr	2012	10.7 (1.1)	Stratec XCT	BDI	-100 to 39	40 to 149	Box	BDI	ISCD
[94]	Loenneke	2012	22 (3)	Stratec XCT					F03F06U 01	Yes
[95]	Mostoufi-Moab	2012	5.1-25.5	Stratec XCT						Yes
[96]	Putzker	2012	19.7 (0.5)	Stratec XCT						
[97]	Sayers	2012	15.5 (0.3)	Stratec XCT					F03F05 F05/F07	Yes
[98]	Van-Caenegem	2012	38 (8)	Stratec XCT		<60	60 to 279			
[52]	Baker	2013	21-78	Stratec XCT	BDI	-100 to 39	40 to 149	Box	BDI	Yes
[99]	Deere	2013	17.8 (0.4)	Stratec XCT					F03F05 F05/F07	
[100]	Farr	2013	10.7 (1.1)	Stratec XCT	BDI	-100 to 39	40 to 149	Box	BDI	Yes
[101]	Laing	2013	18-19	Stratec XCT	CM3/PM1		34 to 279	Manual		Yes
[102]	Loenneke	2013	23 (3)	Stratec XCT						Yes
[103]	Mueller	2013	43.8 (5.5)	Stratec XCT			35 to 710			Yes

[104]	Rantalainen	2013	23 (5)	BoneJ	N/A	-40 to 40	41 to 199	Matrix	7x7 median	Yes
[105]	Rittweger	2013	31 (6)	Stratec XCT	N/A	N/A	N/A	Manual	None	Yes
[106]	Vandewalle	2013	10-19	Stratec XCT		<40	40 to 279			
[107]	Veilleux	2013	6-60	Stratec XCT			40 to 279			
[108]	Blew	2014	9-13	Stratec XCT	BDI	-100 to 39	40 to 149		BDI	Yes
[109]	Stagi	2014	23.1 (6.2)	Stratec XCT						

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Abbreviations: SD = Standard Deviation; ROI = Region of Interest; BDI = utilized Bone Diagnostics Incorporated protocols; ISCD = derived using International Society for Clinical Densitometry guidelines; CM1/3 = Stratec XCT Contour Mode 1/3; PM1/2 = Stratec XCT Peel Mode 1/2; C.S. = Case Study.

## 2 Review of Literature

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To establish a conceptual framework for investigating the precision and annual changes of pQCT-derived muscle and fat outcomes as well as their relationship with fall status, a discussion of the following topics is necessary: In vivo imaging of muscle and fat, sarcopenia, myosteatosis, neuromuscular aging, and falls in older adults. The aim of this chapter is to summarize these relevant topics and concepts to provide the framework upon which the purpose of this dissertation was established.

## **2.1 *Imaging Muscle & Fat***

Longitudinal research has demonstrated that after age 45, adults lose approximately 12 to 17% of thigh muscle strength per decade, a change that is partly related to the loss of muscle mass [110]. Objective, imaging based measurements of muscle and adipose tissues are central to the study of sarcopenia and aging because they do not vary with arthritic pain or patient motivation. Furthermore, precise imaging outcomes are sensitive to subtle changes in soft-tissues that can help us better understand pathology, even before these changes manifest in clinical outcomes [111,112]. Although DXA measures of appendicular lean mass are commonly used to quantify muscle as a primary technique among definitions of sarcopenia, there is no expert consensus on methodology for routine assessment of skeletal muscle [113]. Furthermore, the need for data obtained from more sophisticated imaging techniques that are able to discern secular changes in soft-tissues has been a recent subject of much methodological discussion in muscle aging and sarcopenia research [38,40,112,114]. Although there are promising advanced MRI methods under development to provide in vivo measures of mitochondrial energetics [38,115], muscle creatine [38], and muscle fibre orientation [38,116], they are largely out of the scope of this dissertation. To reflect the evidence discussed in this literature review, this section will focus primarily on methodological aspects of the precision and monitoring of muscle and fat outcomes, as well

as soft-tissue measures derived from DXA, clinical CT, anatomical and proton spectroscopic MRI outcomes, and pQCT methods.

### 2.1.1 *Precision and Monitoring of Imaging Outcomes*

Understanding the precision error of a measurement tool is necessary to know if apparent changes in an outcome are real, or simply a reflection of noise due to analytical and biological variation [117]. Furthermore, a technique's longitudinal sensitivity (ability to monitor changes) is limited by its precision error [59]. Longitudinal evaluation seeks to determine three things: 1) Meaningful and clinically relevant changes; 2) The time interval that allows accurate assessment of treatment response or disease progression; and 3) The technique best suited to detect changes quickly and accurately [59].

Precision errors calculated with less than 27 degrees of freedom, or the use of arithmetic means (compared to root mean squared averages) may underestimate the true imprecision by up to 41% and 25% respectively [118]. An accurate (defined as 90% certainty of being within 30% of the actual error value) assessment of the precision error of an imaging technique requires the calculation of the root of the mean squared differences from measurement pairs with at least 27 degrees of freedom (i.e. 27 participants measured twice) [118]. These recommendations have since been adopted by the ISCD [41,119], which now hosts a convenient web-based precision calculation tool, and recommends repeat measures from at least 30 individuals [120]. Precision errors may be expressed as absolute values (root mean square standard deviation;  $SD_{RMS}$ ) or as a percentage of the sample mean  $CV\%_{RMS}$  [118]. See Appendix D for a worked example of the calculation of these values.

To characterize longitudinal sensitivity, the responsiveness of the outcome to change needs to also be taken into account [59]. Precision error, together with median annual changes, can be used to estimate monitoring time intervals (MTIs) between measurement

occasions. MTIs provide a time estimate (in years) to reliably (with 80% and 95% certainty) detect changes in disease progression or treatment effect. The calculation of these intervals allow for follow-up measures to be efficiently performed within the optimal window for capturing true biological change in imaging outcomes, and minimize both the radiation exposure to participants as well as the costs associated with repeated scanning in prospective studies [59]. To calculate these estimates of longitudinal sensitivity, we must first derive Least Significant Change (LSC) values which are described as the “criterion for the smallest change between two measurement results that can be considered statistically significant with 80 or 95% confidence” [59]. A change less than an 80% LSC is not considered clinically relevant, and a change less than the 95% LSC is not statistically proven [59]. The LSCs are calculated similarly by multiplying the precision error by  $\sqrt{2}$  times the critical Z-value for the desired level of confidence (1.28 and 1.96) [59,117]. To obtain MTIs the LSC values are divided by an outcome’s median response (per unit of time) [59].

Because precision error can vary with the unique characteristics of each participant, it should be specific to the population under study [118]. Technique precision derived from a young, healthy convenience sample may not be appropriate when assessing values derived from an aged or diseased population. Furthermore, precision values should be determined from measures collected on separate days but close enough together to negate possible biological changes. Same-day image collection has been known to underestimate precision errors for muscle and adipose tissue area due to enhanced technician recall when repositioning participants, and/or failure to capture between-day fluctuations in the precision error of the scanner [86]. Sample heterogeneity can also affect longitudinal estimates of an outcomes median response, therefore MTIs for populations with different characteristics, i.e. healthy normal, diabetic, etc., should be calculated separately [59].



### 2.1.2 *Fat and Adipose Tissue Outcomes*

In the study of body composition and metabolism, “fat” and “adipose tissue” are distinct [121]. At the molecular level fat is usually lipid in the form of triglycerides, and although adipose tissue contains ~80% fat, it also has water, protein and mineral components, and secretes adipokines that interact with surrounding tissues [121]. The terminology used to describe various adipose-tissue outcomes varies with the technique utilized, as well as the field of study. Komolka et al. [122] provides an excellent overview of the terminology used for various types of adipose tissue and fat in human, rodent, and livestock literature and provides a visual example of each (Figure 2). In the human limb the following depots are studied using direct and surrogate imaging measures: SAT, inter-muscular adipose tissue (IMAT), intramuscular fat (IMF); consisting of adipocytes within muscle, extramyocellular lipids (EMCL) and intramyocellular lipids (IMCL). DXA measures separate fat mass from lean mass for the total body, which can also be analyzed into sub-regions for estimates of central fat mass, and limb fat and lean mass, but cannot provide information about intra-tissue fat (IMAT, EMCL, IMCL) [40,112,114]. Both SAT and IMAT areas can be estimated using MRI and CT techniques [123]. While only MRI proton ( $H^1$ )-spectroscopy can directly measure IMCL and EMCL in vivo, CT muscle attenuation serves as a surrogate measure of IMCL and EMCL not accounted for by measures of IMAT [124,125]. CT Tools such as pQCT are calibrated differently and report tissue properties in density values rather than attenuation. Outcomes reported in this dissertation include commonly reported pQCT-derived soft-tissue measures: muscle, SAT, and IMAT cross-sectional areas ( $cm^2$ ), as well as muscle density ( $mg/cm^3$ ).

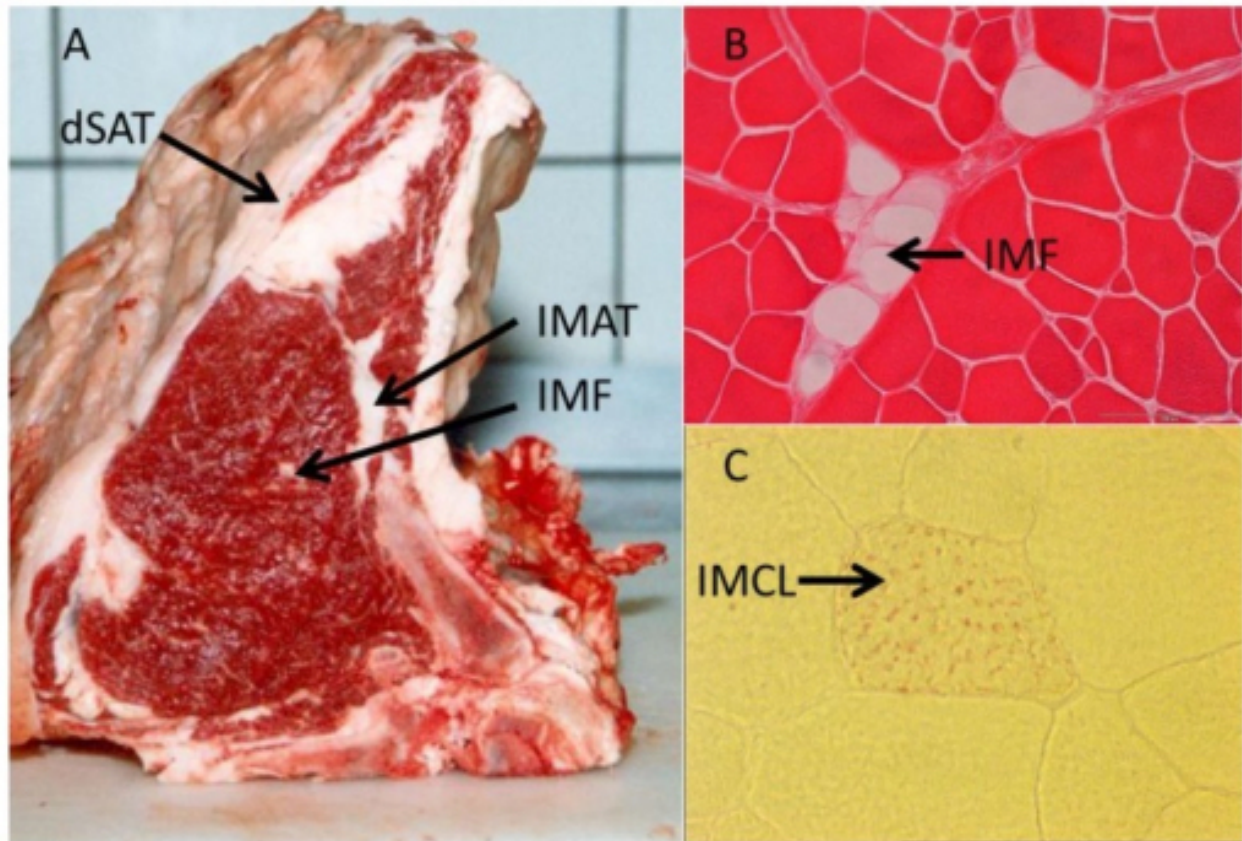


Figure 2: Illustration of different muscle-related adipose tissue depots. (A) Deep subcutaneous adipose tissue (dSAT) covering *M. serratus dorsalis*, inter-muscular adipose tissue (IMAT) between *M. intercostalis interni* and *M. longissimus dorsi*, and intramuscular fat (IMF) within *M. longissimus dorsi* in cattle. (B) Cellular structure of IMF in *M. longissimus dorsi* (cattle, Eosin stained). (C) Intramyocellular lipids (IMCL, red dots, Oil-red O stained) in a muscle cell (*M. longissimus dorsi*, mouse). Reprinted with permission from Komolka et al. [122] and The Journal of Genomics.

Muscle area is related to contraction torques [126], SAT and IMAT area quantify the adipose in subcutaneous and inter-muscular depots, and muscle density provides an analogue of muscle attenuation, estimating both adipose [55] and fat [127]. These outcomes reflect the unique limitations of the pQCT scanner. Unlike CT (Figure 3) and MRI, pQCT does not have a high enough contrast to separate the individual muscles of the forearm and lower leg (Figures 4 & 5), both muscle area and density are measures that quantify all soft tissues (including muscle, IMAT, EMCL, IMCL) between the inner edge of the SAT and the outer edge of the bones.

### 2.1.3 *Dual energy X-ray Absorptiometry*

DXA-derived lean and fat mass ( $\text{g}/\text{cm}^2$ ) parameters are some of the most commonly utilized measures in modern body composition research. The DXA scanner uses a projection imaging technique with two different energy beams to differentiate soft tissues from bone [128]. Thus, the tissue densities are not true volumetric densities, but are areal bone mineral densities (aBMD), with an area based unit " $\text{g}/\text{cm}^2$ " calculated by dividing the bone mineral content by bone area [41,128]. DXA can also separate mineral free soft tissue (lean mass) from fat mass, and appendicular lean mass has become a central component of the operational definitions of sarcopenia [7]. Be that as it may, the accuracy of DXA lean and fat mass measures are not well established, and have been shown to vary inconsistently with body type [129,130]. In obese adults, DXA underestimates fat mass when compared with computed tomography, and this bias has been shown to increase in heavier participants [130].

The advantages of DXA are the ability to quantify whole body fat and lean mass, and the comparatively low radiation doses associated with the technique. Limitations include an inability to provide tissue-specific lean and fat mass, (e.g., subcutaneous from visceral or muscle adipose), as well as the increased errors in lean and fat mass estimates that can occur if a patient is excessively under or overweight [128,131]. Furthermore, it may prove impossible to properly position a participant for a scan if they are kyphotic and cannot lay flat on the scanner table [128]. Newer scanner models have demonstrated better precision ( $\text{CV}\%_{\text{RMS}}$  of 1.2 to 1.6%) for determining the lean mass arm and leg sub-regions in younger and mixed-age, healthy samples [132,133]; however, poorer appendicular lean mass precision ( $\text{CV}\%_{\text{RMS}}$  of 2.7 to 10.9%) has been reported for older scanner models and aging cohorts [134].

#### 2.1.4 *Magnetic Resonance Imaging & Spectroscopy*

Magnetic resonance techniques are among the cutting edge of body composition imaging. Although new techniques are being developed that will provide muscle health and function data beyond anatomical tissue sizes and volumes [38], the principal use of magnetic resonance imaging (MRI) in musculoskeletal research has been to characterize the quantity and distribution of fat and muscle tissue [38,135]. Unlike DXA and CT methods, MRI does not utilize ionizing radiation, which allows for the measurement of muscle and fat properties throughout the entire body and across the human life span (from fetus to cadaver). Whole body 3D composition scans are able to monitor changes in the various depots of fat, allowing for the study of the redistribution of fat and its pathophysiological implications [136]. MRI scans can also provide a quantitative assessment of myocellular lipid via magnetic resonance spectroscopy (MRS) analyses with the use a radio-frequency coil tuned to H<sup>1</sup> [135]. In addition to quantifying IMCL, this technique can also identify triglycerides existing within and outside of the muscle fibres (EMCL) [125]. Conventional MRI often only measures the cross-sectional areas or volumes of large stores of inter-muscular adipose tissue [38,135]. The ability of MRI to measure muscle and adipose cross-sectional areas has been validated with cadaveric specimens with a precision (CV%) of ~2% [123], and muscle lipid content via H<sup>1</sup>-spectroscopy has been cross-validated with CT attenuation [125]. Substantial cost, limited availability, and poor standardization of imaging protocols are the primary limitations of this technique.

#### 2.1.5 *Computed Tomography*

Similar to MRI, computed tomographic imaging is also capable of providing 3D images of the soft-tissues of the body. CT can provide estimates of muscle size as well subcutaneous, visceral, inter-muscular fat depots [123]. Furthermore, muscle attenuation (Figure 3)

provides an imaging-based analogue of IMCL and EMCL content [124,125]. The ability of CT to assess muscle size and adiposity has also been cross validated with both MRI and magnetic resonance spectroscopy [123,125]. Goodpaster, Kelley, et al. [124] demonstrated that muscle attenuation values have a near perfect linear association with lipid content in vitro. Furthermore in vivo muscle attenuation values were negatively associated with muscle biopsy lipid and triglyceride content [124,125].

The methodological variability (CV%) of muscle attenuation was found to be less than 1% for both the mid-calf and mid-thigh [124], but this CV% was calculated from repeat measures of only 6 volunteers and likely underestimates the true precision error of these CT measurements. The Health, Aging and Body Composition (Health ABC) study has generated a wealth of data demonstrating the value of CT muscle imaging in aging research [29,30,137-142]. Muscle attenuation and adipose tissue area are associated with a number of different functional [23,24,26], clinical [30,31] and public health [29], outcomes in older adults.

Although clinical CT scanners allow for imaging in any part of the body, the technology has limited feasibility for large-scale use. Clinical scanners are expensive, and while the radiation dose associated with their use varies with the scan protocol, it is among the highest of all radiological imaging techniques [143]. For these reasons, a smaller-scale CT cousin “pQCT” has also become a popular tool in musculoskeletal health research.

#### **2.1.6**      *Peripheral Quantitative Computed Tomography*

Peripheral Quantitative Computed Tomography technically refers to any QCT scan of the periphery of the body. Despite that, a set of devices dedicated to this function have usurped this terminology in the literature [41]. The acronym “pQCT” most often refers to a class of small step-and-scan QCT tools [143,144]. These scanners collect axial tomographic

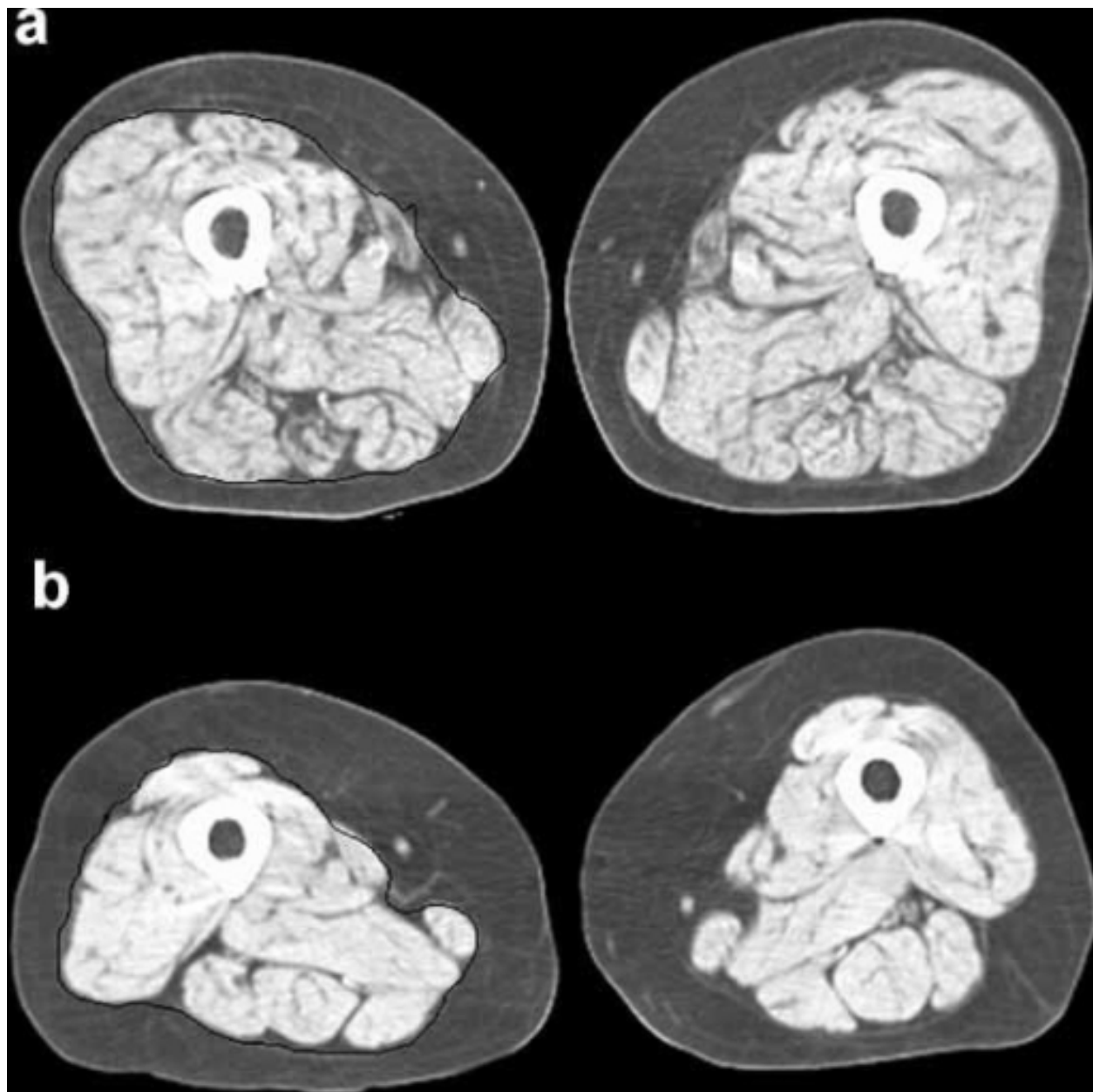


Figure 3 : Representative CT images of the midthigh showing in black the outline of the region of interest encompassing the thigh muscle bundle used for area and attenuation measurements. (a) Axial image showing extensive fatty infiltration of the muscle and having a thigh muscle lean tissue attenuation coefficient of 26 HU. (b) Axial image with a thigh muscle lean tissue attenuation coefficient of 38.6 HU. Reprinted with creative commons licensing from Lang et al. [30].

slices with a thickness of 2.1 to 2.5mm, through 15-30 translations of 12 degrees around a limb [144,145]. Scanners are factory calibrated to the European Forearm Phantom, which consisted of a water equivalent soft tissue simulating material [42,146,147]. Thus, contrary

to CT attenuation values (HU), pQCT quantifies all tissues in terms of volumetric bone mineral density (BMD,  $\text{mg}/\text{cm}^3$ ), measured in milligrams of hydroxyapatite per cubic centimeter and calibrated with fat equal to  $0 \text{ mg}/\text{cm}^3$  and a resin based water-equivalent material as  $60 \text{ mg}/\text{cm}^3$  [143,146,147]. Relative to clinical CT devices, pQCT scanners are more compact, cost-effective, and expose participants to substantially lower effective doses of radiation [143]. With the exception of Single Photon Absorptiometry, pQCT emits the lowest effective dose of all densitometric techniques [42,143]. Similar to a full body CT scanner, pQCT is also able to image fat, muscle, and bone to determine tissue content, density, and area. However, pQCT is limited to scanning the arms and legs, and does not provide enough contrast to distinguish individual muscles. Thus, pQCT derived lower leg and arm muscle density and cross sectional area provide global measures of appendicular muscle. Furthermore, scanner resolution restricts the ability to measure thin structures due to partial volume effects [148].

Along with relatively low radiation doses, pQCT provides the advantages of being able to separate appendicular SAT from muscle, as well as provide precise measures of muscle size, density and SAT area. The precision error ( $\text{CV}_{\text{RMS}}\%$ ) of these measurements range from 0.7 to 6.5% in the forearms and lower legs of older women [149]. Measures of pQCT-derived muscle and SAT area have been demonstrated to be similar to those obtained using MRI; although, pQCT image analysis protocols and filtering methods can influence the agreement between these two techniques [88]. Research in lemmings demonstrated pQCT-derived liver attenuation values accounted for 96% of the variance in chemically extracted liver lipid content [127]. In a cross-validation study, MRI-derived IMAT accounted for 50% of the variance in pQCT-derived muscle density [55], the remaining unexplained variance may be accounted for by other muscle tissue properties, and stores of EMCL, and IMCL that exist



below the resolution of the MRI scanner. Measures of pQCT-derived IMAT are not well described in the published literature [48,93]. Some evidence suggests large precision errors for pQCT-derived IMAT [93], yet there currently is no context (i.e., annual change data) within which these precision errors can be evaluated. Fat and muscle methods are an area of development for pQCT, and methodological heterogeneity currently limits the comparison and pooling of results. Various threshold values, software suites, and image filters (Table 1) have been applied to derive muscle and fat measures [45,52,53,88,93,104,149-151], but there is no consensus on how to best define these tissues and few methodological comparisons exist [149,151].

## **2.2 Sarcopenia**

The term sarcopenia is derived from the Greek sarx, meaning “flesh”, and penia “loss”. The term was originally coined in 1988 by Irwin Rosenberg who noted that lean body mass underwent the most dramatic and significant decline with age, negatively affecting metabolism, ambulation, mobility, and independence [152]. A decade later, Baumgartner et al. [9] analyzed data from the New Mexico Elder Health Survey and the Rosetta Study to establish the first operational definition of sarcopenia. Sarcopenia was defined as a DXA-derived appendicular skeletal muscle mass index (appendicular muscle mass/height<sup>2</sup>) less than two standard deviations below the mean value for young adults from the Rosetta Study [9]. Recognizing both the physiological importance of fat mass in muscle loss [10] and that progressive strength loss was outpacing declines in lean mass [153,154], alternative definitions have since been proposed. A “sarcopenic obesity” phenotype was defined [155,156], to account for the synergistically greater functional decline experienced by persons with both sarcopenia and obesity [32]. Be that as it may, there are still difficulties with how we define sarcopenia and sarcopenic obesity. When eight different definitions of



sarcopenic obesity were compared using data from the National Health and Nutrition Examination Survey, the variability among them produced up to a 26-fold difference in prevalence [156]. Although no medical consensus currently exists for sarcopenia or sarcopenic obesity, the most recent criteria for sarcopenia adjusts lean mass and grip strength values for body mass index (BMI) to account for the effect of obesity [15]. Despite the variability in how sarcopenia is defined, nearly two decades of research has been produced to help us better understand the mechanisms and relationships of age-related declines in muscle mass and function [32].

### *2.2.1 Moving Towards a Consensus Definition for Sarcopenia*

Recent efforts have focused their attention on arriving at a consensus for an operational definition of sarcopenia [157,158]. In the past five years, six consensus statements have been produced with different criteria for the definition of sarcopenia [13-15,159-161]. While there is variation among the consensus statements, all of them acknowledge that a definition of sarcopenia should include both low muscle mass (defined 3 different ways) and a variety of cut-points for poor function measured as gait speed, grip strength, or 6 min walk [7]. Among these 6 definitions, the Foundation of National Institutes of Health (FNIH) definition is unique as it is the first data-driven criteria for sarcopenia and does not rely on a distribution of lean body mass values from young adults [157]. The FNIH analysis defined sarcopenia for men and women as hand grip strength below 26 and 16kg, in the presence of a DXA-derived appendicular lean mass over BMI ratio of <0.789 and <0.512 [15,157]. A 3-year likelihood of incident mobility impairment was predicted by both FNIH criteria for low grip strength (OR 2.31 [95% CI 1.34 to 3.99]; OR 1.99 [95% CI 1.23 to 3.21]) and low appendicular muscle mass adjusted for BMI (OR 1.58 [95% CI 1.12 to 2.25]; OR 1.81 [95% CI 1.14 to 2.87]) for men and women respectively [16]

### 2.2.2 *Epidemiology*

Data on the prevalence of sarcopenia are dependent on the definition used, and its appropriateness for the population described. Estimates based on the European Working Group on Sarcopenia in Older People (EWGSOP) criteria range between 5 and 8% in England, 22 and 23% in Japan, and 33% of nursing home residents [7]. The International Working Group on Sarcopenia (IWG) criteria demonstrated a lower prevalence than the EWGSOP when compared in a population-based cohort of elderly Taiwanese [162]. The FNIH definition was found to be more restrictive than EWGSOP and the IWG, with only 1.3% of men and 2.3% of women being defined as having sarcopenia [163].

A recent British analysis compared the EWGSOP and FNIH sarcopenia criteria's ability to determine slowness and difficulties walking on the Timed Up and Go (TUG) test. Low lean mass determined using the FNIH criteria was associated with higher odds of mobility impairment, yet EWGSOP lean mass was not [17]. Using the EWGSOP criteria, a cohort of sarcopenic Italians had a 3-fold increased risk of falls over a period of 2 years [19]. Similarly, sarcopenia was shown to increase the odds of being a faller in a cohort of elderly Japanese men and women [20]. Sarcopenia has also been demonstrated to have a relationship with fall-induced injuries. Sarcopenia predicts incident fractures [21], and improves fracture risk prediction when combined with the Fracture Risk Assessment tool [22] in elderly Chinese men.

### 2.2.3 *Mechanisms & Therapies*

Sarcopenia has multiple contributing factors closely tied to age-related changes in physiologic function. Identified factors include: mitochondrial dysfunction, neuronal degeneration, weight loss, malabsorption, declines in vascular function, low testosterone, growth hormone, insulin growth factor 1, and vitamin D, increased cortisol, and pro-

inflammatory cytokines such as interleukin-1/6, and tumor necrosis factor alpha, and decreased physical activity [7,32,164]. Sarcopenia accelerates with diabetes and insulin resistance, due to decreased protein synthesis and increased protein degradation [32].

Resistance training has proven to be an effective treatment to stimulate protein synthesis, increase muscle mass and strength [32,154,161]. The safety and effectiveness for resistance exercise has been demonstrated, even in old and frail individuals living in nursing home care [165]. Aerobic exercise may also benefit aging skeletal muscle by improving insulin sensitivity via the stimulation of GLUT-4 mediated glucose transportation [32,166]. Nutritional interventions, particularly leucine-enriched amino acid supplementation, have demonstrated potential [7], but larger RCTs are needed to confirm these findings [32]. Anabolic hormone therapies have not proven successful and can carry safety risks [7,166]. Clinical trials are currently underway for antibodies that modulate myostatin, and ghrelin agonists that could increase food intake and release growth hormone [7,157]. Nevertheless, the lack of a medical consensus on a definition has created a barrier for investment and clinical development of therapies for sarcopenia. The U.S. Food and Drug Administration requires proposed therapies to treat diseases or conditions recognized by the medical community [158].

#### 2.2.4 *Challenges*

More research is needed comparing definitions of sarcopenia for the medical community to definitively determine which criteria has the best predictive ability and arrive at a medical consensus [7,157]. The timely and correct identification of sarcopenia or persons who are at risk of becoming sarcopenic may play an important role in the success of therapeutic interventions. There is a need for earlier detection and intervention in adults exhibiting trends in muscle loss. Although DXA-derived appendicular lean mass measurements are a

primary component of the majority of sarcopenia definitions, the utility of these DXA outcomes for monitoring changes across serial measures of appendicular lean mass is not well described. There is also a growing recognition that tools that can better evaluate and differentiate more specific body composition features (e.g., muscle area, muscle density/attenuation, SAT, IMAT) are necessary for the research and development of therapies targeting muscle and physical performance [40,112,114].

### **2.3 Myosteatorsis**

Adipose tissue undergoes changes in distribution and function with age. The increased infiltration of muscle tissue with adipose and fat has been referred to as “myosteatorsis” [37,167]. There are several mechanisms that may contribute to myosteatorsis. Metabolic hypotheses speculate that increases in ectopic fat storage in both visceral and muscular sites are a reflection of the diminished ability of subcutaneous fat depots to expand and regulate fatty acids in the blood stream [168,169]. This is characterized by a decline in subcutaneous fat storage (a depot which is dependent on adipocyte proliferation) and a shift towards visceral and ectopic adipose tissue storage (hypertrophy dependent adipocytes) in muscle and other organs [169]. Over time, these changes have deleterious effects on metabolic homeostasis, and are also often observed earlier in life in obese and diabetic populations [170]. Although multiple factors contribute to this phenomenon, the progression of fat redistribution in old age is believed to begin with increased expression of pro-inflammatory cytokines (tumor necrosis factor- $\alpha$  [TNF $\alpha$ ] and Interleukin-6 [IL6]) and the emergence of senescent pre-adipocytes; two factors which reduce the differentiation of active pre-adipocytes into lipid-storing adipocytes [171]. Supporting this relationship, Beasley et al. noted that inter-muscular adipose tissue of the thigh was significantly related to

inflammation in older adults, and a trend towards lower pro-inflammatory marker concentrations was also observed with increased subcutaneous fat [139].

Data also suggest that myosteatosis may have origins in the aging of muscle tissue. Prospective results from the Health Aging and Body Composition Study indicated five year increases in inter-muscular adipose tissue area regardless of changes in body weight or subcutaneous adipose, suggesting myosteatosis is a consistent characteristic of aging muscle [28]. Altered mitochondrial activity and oxidative stress are suspected to contribute to increased myosteatosis in the elderly [164,172]. Johannsen et al. reported that muscle fibre ATP and O<sub>2</sub> consumption did not differ between young and elderly groups; however, the elderly demonstrated a trend towards greater oxidative stress, reduced mitochondrial respiratory efficiency, and greater muscle adiposity [172]. Both myocytes and neurons contain large quantities of mitochondria and are susceptible to oxidative damage.

The increased failure of motoneurons to reinnervate muscle fibres undergoing remodeling [173] may also promote the replacement of muscle with adipose. Muscle fibres orphaned by the neuromuscular regeneration process either atrophy or are reinnervated by neighbouring motor units [174]. Muscle fibre atrophy can signal the fibro/adipocyte progenitors that assist in muscle fibre regeneration to differentiate into fibroblasts and adipocytes [175]. There is cellular evidence which suggests that muscle precursor cells increase their expression of adipocytic phenotypes with age [176-179]. Data from the InCHIANTI study linked  $\alpha$ -motoneuron degeneration to muscle adiposity; after adjusting for confounding variables, older men and women who had peroneal nerve compound muscle action potentials below the clinically relevant threshold of 4mV were between 1.9 and 2.4 times greater odds of having a lower leg muscle density more than an SD lower than young adult values [46].

Beyond the loss of contractile fibres with age, the chronic and gradual accumulation of adipocytes [139,180] and the pathophysiological effects of their pro-inflammatory cytokines [181-183] may precede declines in muscular function in aging [182]. Pro-inflammatory cytokines can promote peripheral insulin resistance [183], muscle catabolism [142,181], decrease the production of myofilament proteins [181,182], increase oxidative stress, and reduce contractility and strength [142,181]. A heightened inflammatory state lowers the anabolic response to resistance exercise, making it more difficult for older adults to maintain and build muscle [181,182]. This was recently demonstrated in a study of older adult fallers. Participants with higher baseline levels of muscle adipose demonstrated blunted muscle quality improvement from 3-months of training in resistance, endurance and balance exercises [184].

Although the mechanisms of myosteatorosis are still being elucidated, cross-sectional evidence has linked muscle adiposity with muscle strength [23,49], six meter walk and chair stand performance [25,185], ascending and descending stairs and the TUG test [185], diabetes [47,48], insulin resistance [167], obesity [39], frailty [45], falls [53], wrist and hip fracture [30,31,54]. Prospective evidence links muscle adiposity with physical function and mobility limitations [24,141,186], incident non-spine fractures [187,188], hospitalization [29], disability [26,27], as well as all-cause and cardiovascular mortality [66,189]. Lastly, randomized controlled trials in older adults have demonstrated that imaging-based measures of muscle adiposity can be decreased with structured exercise [35,36] and resistance training [37]. These effects coincided with improved strength [37], and were independently related to improved physical function [36]; suggesting measures of muscle adiposity have potential as a relevant marker for healthy and successful aging.

## **2.4 Neuromuscular Aging**

Muscle strength has three major components: neural-motor, contractile, and elastic; all of which combine to maximize dynamic muscle strength [190]. The neural coordination of the contractile and elastic properties of muscle is necessary to achieve efficiency in human movement and maximize force production [191]. The integrated control of human movement is guided by the central nervous system (CNS). Reflex inhibition can influence the excitability of the CNS, which can affect net joint torque [192]. This is particularly an issue for older adults with chronic, painful conditions. For example, the arthritic swelling of joint capsules are believed to put pressure on the Golgi tendon organs and afferent pain receptors in synovial membranes, leading to arthrogenous muscle inhibition [192,193]. Aches and pains can also cause neural inhibition of contraction [3,190,193]. Studies of the role of motor unit recruitment in maximal voluntary contraction torque with age are equivocal and depend on the muscle group [193-196]. Several studies of the knee extensors have demonstrated no differences between young and old adults as well as significant impairments in central activation with age [195]. A recent longitudinal analysis of 16 healthy older adults found that a reduction in neuromuscular activation was significantly associated with declines in muscle power [196]. This healthy older cohort was also contrasted with a group of older mobility limited adults who demonstrated no change in neuromuscular activation despite losses in power, contraction velocity, and muscle area [197]. Interestingly, both healthy and mobility impaired groups demonstrated a significant relationship between declines in contraction velocity and increases in IMAT [197].

Changes in the peripheral nervous system and muscle tissues are also considered to be a culprit of age-induced deficits in strength and power. Alpha-motoneurons are the efferent nerves that originate in the brainstem or spinal cord and innervate muscle fibres to

facilitate the propagation of action potentials to the muscles [191]. Action potentials terminating at the end of a motoneuron set in motion a cascade of electrochemical signaling within the muscle fibre that results in contraction. An alpha-motoneuron and the fibres it innervates are known as “motor units”. With age, a pattern of motor unit remodeling becomes apparent [3,164,174,191]. In young healthy muscle, a regenerative cycle of denervation, motoneuron axon terminal sprouting and re-innervation occurs without any apparent difficulty. Cross-sectional research indicates that into the seventh decade of life the normal turnover of synaptic junctions changes and an increase in the loss of alpha-motoneurons occurs [192,198]. As a result of muscle fibre denervation, neighbouring motoneuron axons sprout new terminals in an attempt to assimilate orphaned fibres into their motor unit [164]. Myoplasticity allows these orphaned fibres to adapt their contractile properties to more closely match those of the adopting motor unit [174]. With age, a pattern emerges where a larger number of fibres exist in each motor unit, and the location of these innervated fibres, which were previously intermixed throughout the muscle, are now clustered closer together. As a consequence of the amalgamation of muscle fibres into fewer motor units, force steadiness and fine motor control is also reduced [199]. Among the fibre types, fast twitch muscles appear to naturally undergo more neuromuscular junction remodeling cycles than slower phenotypes [200]. There also appears to be a preferential loss of fast twitch motor units [164]. This is either due to a greater susceptibility of fast twitch motoneurons to atrophy, or a reduced ability to sprout new axon terminals and re-innervate their type II fibres; giving slow twitch motoneurons the upper hand in the remodeling process [164].

The exact mechanisms of denervation are not completely understood. Early cadaveric studies highlighted the reduced number and size of alpha-motoneurons in the ventral root of



the spinal cord leading to the initial hypothesis that declines in muscle mass and strength may be driven by alpha-motoneuron atrophy [201]. Current evidence suggests that motoneuron death may not account for all age related deficits. Edstrom, Altun, et al. [173] note that lost muscle fibres often appear halted part way through their typical regenerative process. Due to their inability to replicate throughout the lifespan, neurons depend on maintenance proteins for structural integrity and plasticity. The accumulation of oxidative damage over time can impair the proteins that facilitate neuronal plasticity, compromising a motoneuron's ability to sprout new axon terminals and re-innervate muscle fibres [173].

Oxidative damage to the mitochondrial DNA, and oxidative stress-induced inefficiencies in cellular respiration are also hypothesized to be factors in the accumulation of muscle lipid [167,172], and inter-muscular fat observed with age. The production of incomplete, or inactive enzymes are also observed, and may be a symptom of dysfunctional protein production due to age-related genetic damage [3]. This is particularly common to the mitochondria, which are more often exposed to reactive nitrogen & oxygen species generated through respiration [202]. In muscle, decreases in glycolytic enzymes, and mitochondrial mass impact the production of ATP across all fibre types [3].

Changes in the elastic properties and composition of muscle also play a role in muscle performance with age. In the aging lower leg, increased tendon compliance impairs the ability of the musculature to rapidly transmit force onto the skeleton [203], and may reduce the efficiency of force production [204]. Evidence suggests that muscle power declines at a greater rate than strength, with a reported annual loss of 3.5% (compared to 1-2% isometric strength) between the ages of 65 and 89 [205]. The age-related leftward shift in the force-velocity curve of muscle contraction reduces both peak power, and the optimal velocity for power production [192]. Thus, deficits in power are more pronounced as the velocity

requirements of a task increase [206]. The serious implications of changes in muscle power are especially pronounced in tasks which require high velocities of movement, such as preventing a fall and recovering one's balance from a trip [207-209]. As such, muscle power has a profound impact on mobility, functional status, and fall risk in older adults. Deficits in whole muscle power exist between old and young groups when normalized to whole muscle cross sectional area [210]. Decreased muscle attenuation can account for differences in strength not attributed to muscle cross-sectional area in older adults [23]. This is suggestive of a role of muscular adipose in the specific force of whole muscle in older adults; yet there is little research directly investigating this possibility. An MRI study by Hilton, Tuttle, et al. [211] demonstrated a significant correlation between IMAT volume and dorsiflexor and plantar flexor isokinetic strength and power. Functional comparisons have noted differences in the amount of inter-muscular adipose tissues and muscle densities between healthy older subjects and older adults with mobility deficits [25,211,212], as well as fallers [53] and fracture patients [30,31,54,187,188].

## **2.5 Falls**

In Canada approximately 30% of community-dwelling elders ( $\geq 65$  y of age [WHO 1984]) fall each year, and 12% experience multiple falls [213]. Studies in other industrialized populations have reported the prevalence of falling to be 28 - 35% of persons over 65, with an increase to 32 - 42% of persons 75 and older [214]. Women are more likely to experience a fall, and even well-functioning older persons are not immune to fall events [215-217]. The experience of a fall can be a cruel event; with consequences that can include serious injury, hospitalization, fear, a loss of independence, and fatality [218]. Almost one quarter of fall events result in serious injury, half of fallers report developing a fear of falling, and a quarter of fallers restrict their activities (shopping, household chores, physical activity) due to this

fear [219,220]. Falls are the leading cause of non-fatal emergency room visits [58], and the primary cause of hip fractures [57]. In America, falls are estimated to account for 0.53 million osteoporotic fractures annually [216]. A systematic review of falls and their associated costs in the western world estimated the societal burden of falls to account for 1.5% of all health care costs, with the average cost of a single fall ranging from \$1,059 to \$10,913 USD [221]. These costs can reach as high as \$42,840 in severe fall-related injuries [221,222]. There is concern that these costs will rise at a greater rate than the growth of the elderly population. Age-adjusted data suggests that the rate of fall-induced injury in older persons is increasing [223], with an annual increase of 1.3% for men and 0.7% for women over the last 27 years [224]. While the case has been made that falls are prevalent and costly (for both the victims and society), how fall data are ascertained is fundamental in our understanding of these events.

### *2.5.1 Definition and Ascertainment of Falls*

How a fall event is defined and interpreted can influence the occurrence of falling reported by older adults. Many studies have defined falls differently, some with broad definitions and others with definitions intrinsically or environmentally narrowed to exclude falls that occur due to specific events (syncope, violence, car accidents, sports, etc.) [214]. In 2005 Hauer et al. noted that there were approximately 40 different variations of the definition of a fall in the literature; few of which could be combined [225]. The Prevention of Falls Network Europe (ProFaNE) sought to address this problem by publishing a fall definition consensus [226]. According to the ProFaNE consensus statement, a fall is broadly defined as “any event where any part of your body unexpectedly contacted the ground or another lower surface”. Utilization of this consensus definition is important for the assembly of comparable fall data, which can be pooled to further enhance fall research and health policy [225]. Many fall

studies have acquired fall data retrospectively [215,225,227,228], primarily for the convenience of collecting fall data in a very short span of time. Prospective methodologies are more resource intensive and may include daily record keeping of falls on a falls calendar, with weekly or monthly telephone follow-ups [225]. While the reliability of a retrospective recall in an elderly population is often a concern, data from a prospective fall monitoring study demonstrated that only 13% of elderly men and women failed to recall a fall event in the previous 12 months [229], whereas short-term recall was worse (32% and 26%) at 3 and 6 months respectively. It should be noted that the effect of participation in this prospective study was not controlled for, and these recall percentages likely reflect an overestimation of recall ability.

### *2.5.2 Fall Risk Factors & Muscle Weakness*

Risk factors are variables that may increase the likelihood of experiencing a harmful event [230]. The identification and monitoring of these factors is an important aspect of fall prevention research [217]. Fall risk factors include muscle weakness, a previous history of falls, gait deficits, balance deficits, use of an assistive device, visual deficits, arthritis, impaired activities of daily living, depression, cognitive impairment, vitamin D deficiency, polypharmacy, and age [218,231,232]. Due to the multifactorial nature of falls it is both unlikely and unreasonable to assume that any single physical performance test or biomarker will be shown to have excellent predictive accuracy [233]. There presently is no gold standard screening test for the prediction of falls in community-dwelling older adults [234]. The TUG test (measured as the time required to rise, walk three meters, turn and return to a seated position in a chair), was previously recommended for use as the primary falls risk screening tool in the joint statement from the American Geriatrics Society and the British Geriatrics Society (2001) [232]. Meta-analyses have recently demonstrated the TUG to have

poor univariate predictive ability for falls in high-functioning, healthy older people [235,236], and it was not recommended as the primary screening tool in the 2011 statement update [231].

A meta-analysis reported by the American Geriatrics Society and British Geriatrics Society provides insight into the relative importance of fall risk factors [218,232]. Muscle weakness was the single most important univariate risk factor; elderly persons with muscle weakness were 4.4 times more likely to fall than their fit peers [218,232]. History of falls, gait and balance deficits produced a mean relative risks of 3.0, 2.9 and 2.9, with other risk factors ranging between 2.6 and 1.7 [218,232]. Weakness, gait, and balance appear to be inter-related [207,237]. Lower limb muscle weakness is apparent in persons who display poor balance, abnormal gait, and reduced mobility [237,238]. A more rigorous meta-analysis of only prospectively ascertained falls and muscle weakness in community-dwelling adults reported a combined OR of 1.66 [95% CI 1.20 to 2.29] for the association between lower extremity weakness and falls [239].

### 2.5.3 *Fall Prevention*

Almost one in five falls (17%) are believed to be caused by muscle weakness or gait and balance disorders, which are second only to environmental hazards (31%) as the primary cause of falls [218]. Previously deemed ineffective [240], recent evidence now supports the efficacy of home safety assessments and modifications, particularly when delivered by an occupational therapist [241]. Carefully managed exercise programs, which target muscle weakness, are also an efficacious avenue to reduce the overall risk of falls. Several group and individual exercise interventions have reduced falls and fall risk [242] and improved lower body and postural strength, balance, flexibility and endurance in the community-dwelling elderly [241]. Furthermore, a meta-analysis of 59 randomized controlled trials

determined that multi-component group and home-based exercise interventions were effective in reducing the number of fallers, and the rate of falling [241]. This is also reflected in the current clinical guidelines for falls prevention, which recommend that all multifactorial interventions for community-dwelling older people have an exercise component that includes balance, gait, and strength training [231].

## **2.6 Summary of Literature Review**

The study of factors that influence human health in aging is of paramount importance as the world undergoes an unprecedented demographic shift towards an aged society. Changes in body composition and neuromuscular health are known to influence the risk of deleterious outcomes in old age. Among these changes, sarcopenia, “the loss of muscle mass and function”, and myosteatosis “the increased infiltration of muscle with adipose” are two phenomenon that share common etiology, and causal relationships with mobility limitations, fractures, and falls. Furthermore, muscle mass and adiposity appear to be modifiable targets for exercise interventions in older adults. Improved muscle mass and adipose profiles predict improvements in the physical function of older adults. The in vivo study of anatomical changes in muscle mass and adiposity is partially facilitated by biomedical imaging tools. Among these tools clinical CT and MRI offer high-contrast axial images of soft-tissue compartments; however, these tools often have limited availability and, in the case of CT, have a high effective radiation dose. Commonly utilized DXA imaging provides low-dose estimates of total body and appendicular measures of bone, lean and fat mass. Unlike axial CT and MRI, DXA cannot measure adipose infiltrating muscle for an assessment of muscle adiposity. Peripheral quantitative computed tomography scanners fill a musculoskeletal imaging niche between clinical CT, MRI and DXA. Although limited to the extremities, pQCT scanners capture axial images and can quantify muscle size & adiposity, as well as SAT in

three dimensions with a low radiation dose comparable to DXA scans. There are a variety of protocols for the derivation of soft-tissue outcomes from pQCT scans, yet no data currently exists comparing the precision error of these methodological variations in older adults. Furthermore, the longitudinal sensitivity of pQCT to changes in the soft-tissues of older adults has also not been assessed. Determining the most precise image analysis protocols for soft-tissue outcomes will facilitate more efficient longitudinal research and optimize the estimated observation time required before changes in these tissues can be detected. Together this data can extend the utility of pQCT for the study of changes in muscle and fat in older adults. Finally, preliminary evidence suggests that low pQCT-derived leg muscle density may be a biomarker for fall risk; a clinically relevant event that can precipitate serious injury, chronic disability, and loss of independence. The strength of the association between muscle density as a bivariate and multivariate predictor of fall status has not yet been described. Methodological and exploratory analyses may be able to resolve some of the uncertainty that exists around pQCT-derived soft-tissue outcomes, and extend the capabilities of this tool for measuring and monitoring muscle and adipose tissues as indicators of healthy aging.

# 3 Objectives & Hypotheses

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### **3.1 Study One: Comparing the Precision of Reported Protocols**

The objective of study one was to survey the literature and compare the precision of previously reported image analysis protocols for quantifying muscle area and density, as well as IMAT and SAT area in the commonly imaged lower leg and forearm using repeated pQCT images from older, community-dwelling postmenopausal women. The hypothesis was that the precision errors would differ across reported pQCT soft-tissue image analysis protocols.

### **3.2 Study Two: Estimating Annual Changes and Longitudinal Sensitivity**

The objectives of study two were to assess: 1) the annual changes in pQCT-derived muscle area and density as well as IMAT and SAT at both the forearm and lower leg; and 2) estimate MTIs for each of the aforementioned muscle and adipose tissue outcomes in older community-dwelling women. The hypothesis was that annual changes would be observed in pQCT-derived soft-tissue outcomes when monitored over time.

### **3.3 Study Three: The Association Between Muscle Density and Falls**

The primary objective of this study was to explore the relationships of muscle density, functional mobility, and health-related factors to fall status. The primary hypothesis was that muscle density would be independently associated with fall status after controlling for age, sex, BMI, general health status, diabetes, the number of comorbidities, and functional mobility. The secondary objective was to determine the independent and combined relationship of muscle density and functional mobility to fall status after adjusting for health-related factors. The secondary hypothesis was that models which include both muscle density and TUG test time will have a better fit with the data than models that include them separately.

## **4 Study One: Measurement of Muscle and Fat in Postmenopausal Women: Precision of Previously Reported pQCT Imaging Methods**

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## 4.1 Synopsis

This chapter outlines the methodological details of reported pQCT image analysis protocols utilized to derive muscle and fat outcomes in the literature. This chapter also presents a comparison of the precision errors inherent to variations in these protocols when analyzing a common set of repeat images collected from a random sub-sample of older adult women.

*This chapter has been published as an original investigation article in a peer-reviewed journal [149]. With the exception of some minor wording and/or format changes that were necessary for the conversion to graduate thesis format, it is presented in its published form. The introduction section below may repeat key aspects of the literature review directly pertinent to the purpose of the study.*

## 4.2 Introduction

For nearly 20 years, pQCT has been used to precisely measure and study volumetric density and distribution of bone mineral tissue [41,243]. In more recent years, pQCT has also been used to quantify muscle area [126,244], muscle density [45,46,49-51,93,105,245,246], subcutaneous adipose tissue (SAT) area [70,72,131], and inter-muscular adipose tissue (IMAT) area [48,93]; all valuable measures for the study of musculoskeletal health. These measures are important given increased interest in the study of soft-tissues as a factor in bone development and health [50,52,100], as well as for studying the development of diabetes [47,93,246], sarcopenia [46,114], falls [53], and frailty [45].

Although pQCT is proving to be a useful tool for measurement of muscle and fat, there is limited information regarding precision (i.e., repeatability) of muscle and fat measures in older adults. To date, precision errors for pQCT-derived muscle area, density, IMAT and SAT area have been reported for children (ages 7-12y) [50,70], young (26.6, SD 8.7y) [86] and premenopausal women (38.6, SD 4.7y) [93]. Prior to a recent muscle area and density paper

by Wong et al. [151], only lower leg muscle area and SAT precision values had been reported for postmenopausal women [90]. Both lower leg IMAT and forearm soft-tissue data have yet to be reported for older adults—a population with unique soft-tissue morphometry and a clinically relevant risk of muscle loss [114,164] and fat infiltration [28,51].

In addition to limited muscle and fat precision information, previous studies reporting precision errors used different image analysis protocols. Employed protocols have included manufacturer recommendations (Stratec Medizintechnik GmbH, Pforzheim, German) [247,248], customized manufacturer's protocols [52,105,249,250], or third-party software (e.g. BonAlyse, BoneJ) [45,46,251,252], each with specific segmentation approaches capable of influencing precision (e.g., contour detection algorithms, noise reduction filters, grayscale intensity thresholds). It is currently unknown which previously reported analysis protocols are most appropriate for precisely characterizing muscle area, density, and fat area in older adults. This is important because the most precise analysis techniques are required for detection of small effect sizes in soft-tissue outcomes. For example, an annual increase of 6.5% in CT-derived IMAT was reported in weight-stable older adult women [28]. According to the 95% LSC criterion [59], a precision error less than 2.3% ( $6.5\%/2.77$ ) would be necessary for detecting a true change or difference with 95% confidence. A precision error greater than 2.3% would imply that, due to analysis imprecision, observed (statistically significant) changes or differences may not truly exist. As such, identification of the most appropriate analysis protocols is of paramount importance for the measurement of soft tissues with pQCT.

Using repeated pQCT images from older, community-dwelling postmenopausal women, the objective of this study was to compare the precision of previously reported image

analysis protocols for quantifying muscle area and density, as well as IMAT and SAT area in both the commonly imaged lower leg and forearm.

## **4.3 Methods**

### **4.3.1 Participants**

Female participants aged 60 years and above were recruited from the Saskatoon cohort of the CaMOs; a longitudinal study of the associated factors and burden of osteoporotic fractures in a random, population-based sample of Canadian community-dwelling men and women. The CaMOs sample was assembled in 1995-1996, and consists of Canadians living within a 50km radius of each study centre. Households within each geographic area were contacted using a random list of telephone numbers, and one member (>25 y of age, non-institutionalized) was recruited from each household [253]. A randomly selected sub-sample of thirty-five women volunteered for this pQCT precision study (mean age: 73.7, SD 7.2) years, height: 159.8 (5.6) cm, weight: 71.9 (12.5) kg, BMI: 28.1 (4.7) kg/m<sup>2</sup>, SF-36 General Health Status score: 80.7 (14.7)). None of the aforementioned characteristics of this sub-sample were statistically different ( $P>0.05$ ) from their respondent CaMOs peers (N=115).

### **4.3.2 Data Acquisition**

One investigator (AFW) performed repeated pQCT imaging of the forearm and lower legs scans an average of 9.7 (3.6) days apart. The non-dominant (self-reported) forearm and ipsilateral lower leg were scanned using a Stratec XCT 2000 pQCT scanner (Stratec Medizintechnik GmbH, Pforzheim, Germany). A scout scan was performed over both the wrist and ankle joint. The scanner reference line was positioned at the most proximal aspect of the distal tibia endplate and the medial tip of the distal radius endplate. Images were acquired at 66 % of the tibia length and 65 % of the radius length proximal to the reference line as previously reported [254]. All images were collected at a scan speed of 20mm/s with

a voxel size of 400 $\mu$ m x 400 $\mu$ m x 2.4mm. Stratec XCT scanners are factory calibrated against the European Forearm Phantom for a single energy. As such, each scanner measures fat, water/lean tissue, and cortical bone with hydroxyapatite equivalent volumetric densities of 0 mg/cm<sup>3</sup>, 60 mg/cm<sup>3</sup>, and 1200 mg/cm<sup>3</sup>, respectively [146]. Image quality was visually assessed following data acquisition. The scan was rejected and repeated if the cortical shell was irregular due to movement artifacts. Using this criterion, one participant's lower leg was rescanned to achieve an acceptable image. The University of Saskatchewan Biomedical Research Ethics Board reviewed and approved this research. All participants provided written informed consent.

#### 4.3.3 *Image Analysis Selection*

A search of the literature was conducted using Web of Science™ 5.13.2 (Thompson Reuters, Philadelphia, PA, USA) for journal articles published as recent as March 2014 containing the topic terms: (peripheral quantitative computed tomography OR pQCT) AND (muscle OR fat OR adipos\*) NOT (mice OR rats OR rabbits OR porcine). The resulting 317 abstracts were searched for articles that used pQCT to study muscle or fat in humans, which were then retrieved for a review of their methodology. We focused this analysis on unrestricted software resources: Stratec XCT (supplied with every scanner), and the freely available BoneJ, a software plugin for the open source ImageJ image analysis software [150,252,255]. These software resources were used in 93% of the retrieved abstracts. Six (I-VI) protocols were identified that were reported with reproducible details for application. These protocols were not exclusive, but they were described in sufficient detail to be replicated and applied to a set of 35 repeat scans in women aged 60-90 y. Five protocols relied on Stratec XCT (manufacturer's software) and one used the pQCT Density Distribution tool in BoneJ:

- I) Modified Stratec recommendation A – Muscle Smooth Filter 3 (with added -40 fat threshold) [49,70,71,93,247]
- II) Stratec recommendation A – Muscle Smooth Filter 2 [247]
- III) Stratec recommendation B - Muscle Smooth Filter 2 (with manual bone regions of interest (ROI)) [53,126,248,250,256-258]
- IV) Bone Diagnostic Incorporated [50-52,100,249,259]
- V) Manual Segmentation [105]
- VI) BoneJ [104,251,252,255]

As automated contour detection algorithms do not always succeed in correctly identifying tissue boundaries, all image analyses were visually inspected for errors using the CALCBD/CORTBD functions in Stratec XCT and the visual result saved by BoneJ.

#### 4.3.4 *Stratec XCT Protocols*

The details of the placement of ROIs, contour modes, thresholds, filters employed, and outcomes produced for each of the analysis protocols are summarized in Table 2. A variety of median filters with NxN mask sizes of 3x3, 5x5, and 7x7 are combined by Stratec XCT to reduce noise and aid contour detection. According to the Stratec XCT manuals, Contour Mode 1 acts by removing all image voxels below a set density threshold, whereas Contour Mode 3 is an iterative algorithm that searches for a gradient of difference between voxels to define the existence of an edge. Each set of voxels are proofed for this gradient step until returning to the starting voxel [147,248]. While the ROIs, thresholds, contour detection algorithms and filters vary across the Stratec protocols (I-V), they all calculate soft-tissue values in a similar fashion by identifying tissue boundaries and then subtracting area and content values to isolate the tissue (muscle, fat or bone) of interest. The derivation of

**Table 2: Summary of Image Analysis Settings**

Protocol	Outcomes	Region of Interest	Voxels (mg/cm <sup>3</sup> )	Algorithm(s)	Median Filters
<b>XCT I</b>	Subcutaneous Fat	Entire Image Matrix	-40 to 39	Contour Mode 3	3x3, 5x5, 5x5
	Muscle	Entire Image Matrix	40 to 279	Contour Mode 3	3x3, 5x5, 5x5
	Bone	Entire Image Matrix	≥280	Contour Mode 1	3x3, 5x5, 5x5
<b>XCT II</b>	Muscle	Entire Image Matrix	40 to 279	Contour Mode 3	3x3, 5x5
	Bone	Entire Image Matrix	≥280	Contour Mode 1	3x3, 5x5
<b>XCT III</b>	Muscle	Entire Image Matrix	40 to 279	Contour Mode 1	3x3, 5x5
	Bone	Manual Trace T/R	≥280	Contour Mode 1	None
	Bone	Manual trace F/U	≥280	Contour Mode 1	None
<b>XCT IV</b>	Subcutaneous Fat	Entire Image Matrix	-100 to 39	Contour Modes: 3, 31, 1 Peel Modes: 2, 2, 2	3x3, 5x5, 5x5
	Muscle	Entire Image Matrix	40 to 149	Contour Mode 31 Peel Mode 2 Separation Mode 4	3x3, 5x5, 5x5
	IMAT	Entire Image Matrix	39 to -100	Contour Mode 3 Peel Mode 2 Separation Mode 4	None
	Bone	Entire Image Matrix	>710	Separation Mode 4	3x3, 5x5, 5x5
<b>XCT V</b>	Muscle	Manual trace	N/A	N/A	None
	Bone	Manual Trace T/R	N/A	N/A	None
	Bone	Manual trace F/U	N/A	N/A	None
<b>BoneJ VI</b>	Subcutaneous Fat	Entire Image Matrix	-40 to 40	Gradient Free Boundary Tracking	7x7
	Muscle	Entire Image Matrix	41 to 139	Gradient Free Boundary Tracking	7x7
	IMAT	Entire Image Matrix	40 to -40	Gradient Fee Boundary Tracking	7x7

T/R = Tibia/Radius; F/U =Fibula/ T/R = Tibia/Radius; F/U =Fibula/Ulna; Manual Trace indicates that the region of interest was traced around the tissue by a technician prior to analysis, this is in contrast to protocols that use the entire image matrix and rely exclusively on a threshold-driven analysis to segment tissues.



muscle and fat area and density requires post-analysis calculation by the user. The exact calculations used for each protocol are provided in Appendix C. Muscle area (Figure 4: light gray area including internal dark gray spots) and content (used to define density) were derived by the area and content of all tissues greater than the muscle threshold (40 mg/cm<sup>3</sup>), less tissues higher than the bone threshold (Figure 4: white/red areas). To determine muscle density (mg/cm<sup>3</sup>), muscle content (mg/cm) was divided by muscle area (cm<sup>2</sup>) (note that muscle area and density measures include IMAT). IMAT area was calculated as the area of tissue within the muscle area below the muscle threshold (Figure 4: dark gray spots). SAT area (Figure 4: dark gray border) was determined by the total limb area, minus the area of all tissues deeper than the SAT-muscle boundary.

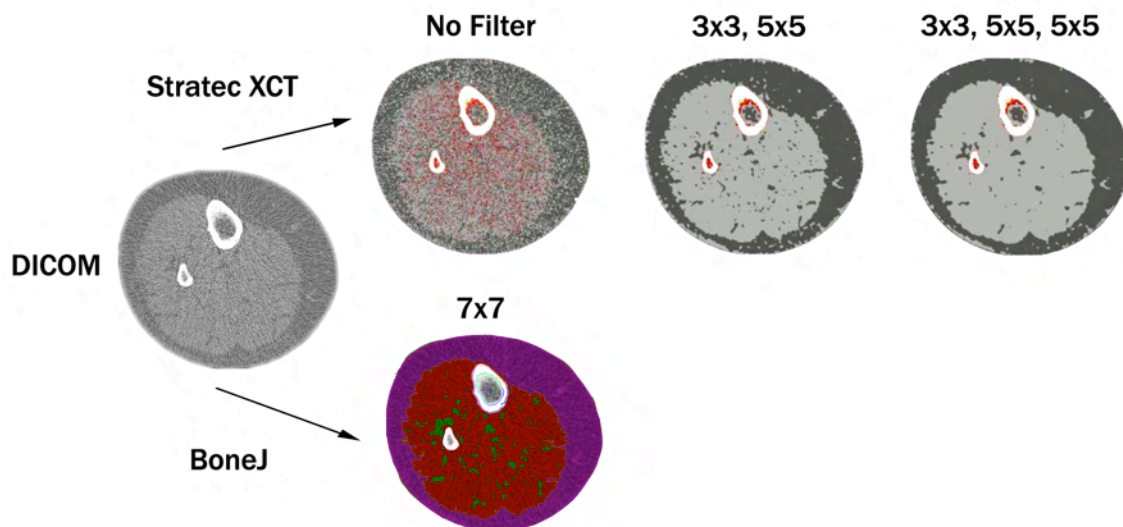


Figure 4: An unprocessed DICOM image of a pQCT lower leg scan (left) after a Stratec analysis (top) and subsequent filtering; BoneJ analysis (bottom). The portioned BoneJ image illustrates muscle area (red + green), inter-muscular adipose tissue (IMAT) (green) and subcutaneous adipose tissue (SAT) (purple).

#### 4.3.5 BoneJ Protocols

The source code for the pQCT density distribution plugin for BoneJ (Version 1.3.11) is freely available online [252,255]. BoneJ's soft-tissue analysis uses a 7x7 median filter to reduce

noise. The image is binarized according to the tissue thresholds selected. Starting in the top left corner of the image matrix, a gradient-free boundary tracking algorithm [260] searches for and traces the edge of the tissues of interest until it returns to its origin. For muscle (41 to 139mg/cm<sup>3</sup>), the traced object with the largest area is retained along with any smaller objects that are greater than 1% of total limb size (Figure 4: crimson area). These muscle objects are then eroded by one pixel. For SAT (-40 to 40mg/cm<sup>3</sup>), the traced object with the largest area is retained and then eroded by three pixels to remove the skin (Figure 4: purple area). IMAT was identified by searching within the muscle objects for pixels in the fat density range (-40 to 40mg/cm<sup>3</sup>) (Figure 4: green area). Muscle area and density, as well as IMAT and SAT area, were all provided automatically in the BoneJ analysis output.

#### 4.3.6 *Statistical Methods*

Mean and standard deviations of the repeat measurement averages are reported for each outcome. Precision error was calculated as absolute values (SD<sub>RMS</sub>) and as a percentage of the sample mean (CV%<sub>RMS</sub>) [118]. The 95% LSC, a “criterion for the smallest change in measurement results that can be considered statistically significant with 95% confidence”, calculated by multiplying CV%<sub>RMS</sub> by 2.77 [59].

Individual log-transformed CV% from repeat measures were utilized to compare the precision of image analysis protocols for each outcome. The data were checked for normality and outliers using skewness and kurtosis Z-scores as well as histograms and boxplots. Failed analyses were excluded from the precision error determination and comparison. All statistical comparisons were performed using shareware statistical software (R, version 3.0.2, Foundation for Statistical Computing, Vienna, Austria, [www.R-Project.org](http://www.R-Project.org)). Only two protocols (IV, VI) calculated IMAT area; therefore, they were compared using either paired t-tests or a Wilcoxon Signed-Rank test if normality was violated. Outcomes produced by three

or more analysis protocols were compared using multilevel linear models and post-hoc Tukey contrasts (R-Packages “nlme” [261] and “multcomp” [262]). Both omnibus chi-squared and post-hoc *P*-values are reported.

## 4.4 Results

### 4.4.1 Image Analysis Failures

While attempting to measure muscle and fat, protocols I and II accounted for all failures (3-9% and 6-14%, respectively) (Tables 3 and 4).

### 4.4.2 Precision Error Comparison

In the lower leg (Table 3), there were no significant precision differences for muscle area across the six protocols ( $\chi^2(4) = 5.82, P=0.325$ ). Statistically significant differences existed in the precision of muscle density across the six protocols ( $\chi^2(5) = 64.86, P<0.0001$ ). Post-hoc analysis revealed that protocol I ( $CV\%_{RMS} = 3.2\%$ ) was less precise ( $P<0.001$ ) than all other protocols ( $CV\%_{RMS} = 0.7$  to  $1.9\%$ ) and protocol III ( $CV\%_{RMS} = 1.9\%$ ) was less precise than both protocol IV ( $CV\%_{RMS} = 0.7\%, P<0.01$ ) and VI ( $CV\%_{RMS} = 0.7\%, P<0.05$ ). For IMAT area, a paired t-test revealed that the precision error ( $CV\%_{RMS} = 3.3\%$ ) of protocol IV was lower ( $P<0.001$ ) than protocol VI ( $CV\%_{RMS} = 28.0\%$ ). Three protocols reported SAT area (I, IV, VI), with precision differences observed across the protocols ( $\chi^2(2) = 9.04, P<0.011$ ). Post hoc analysis revealed that protocol IV ( $CV\%_{RMS} = 3.1\%$ ) was significantly less precise ( $P<0.01$ ) than protocol VI ( $CV\%_{RMS} = 2.4\%$ ).

In the forearm (Table 4), precision of muscle area differed significantly across the six protocols ( $\chi^2(4) = 37.55, P<0.0001$ ). Post-hoc analysis revealed that protocol IV ( $CV\%_{RMS} = 5.3\%$ ) was significantly less precise ( $P<0.01$ ) than all other protocols ( $CV\%_{RMS} = 2.1$  to  $3.2\%$ ). The precision of muscle density differed across the six protocols ( $\chi^2(5) = 29.33, P<0.0001$ ). Post-hoc analysis revealed that protocol I ( $CV\%_{RMS} = 3.2\%$ ) was significantly less precise

( $P < 0.001$ ) than protocols III, IV, V, and VI ( $CV\%_{RMS} = 1.4$  to  $1.6\%$ ). For IMAT area, a Wilcoxon Signed-Rank Test revealed that the precision error of protocol IV ( $CV\%_{RMS} = 7.0\%$ ) was significantly lower ( $P < 0.001$ ) than VI ( $CV\%_{RMS} = 42.2\%$ ). The precision of SAT area differed significantly across protocols I, IV, VI ( $\chi^2(2) = 6.92$ ,  $P < 0.0315$ ). Post-hoc analysis revealed that protocol IV ( $CV\%_{RMS} = 6.5\%$ ) was significantly less precise ( $P < 0.05$ ) than protocol VI ( $CV\%_{RMS} = 5.2\%$ ). All other differences among analysis protocols did not reach statistical significance.

**Table 3: Lower Leg Precision**

Protocol	N-Pairs	Mean	SD	CV% <sub>RMS</sub>	SD <sub>RMS</sub>	95% LSC (CV%*2.77)	Contrasts P<0.05
<b>Muscle Area (cm<sup>2</sup>)</b>							
I	32	58.3	8.5	3.3	1.9	9.2	N.S.
II	30	57.5	8.1	3.7	2.1	10.3	N.S.
III	35	62.9	10.6	3.5	2.2	9.8	N.S.
IV	35	56.1	8.3	2.6	1.5	7.2	N.S.
V	35	52.3	7.8	3.3	1.8	9.3	N.S.
VI	35	59.4	8.7	2.5	1.5	6.9	N.S.
<b>Muscle Density (mg/cm<sup>3</sup>)</b>							
I	32	70.7	3.4	3.2	2.3	8.9	II, III, IV, V, VI
II	30	71.0	2.9	1.2	0.9	3.4	I
III	35	67.6	3.4	1.9	1.3	5.3	I, IV, VI
IV	35	71.6	2.4	0.7	0.5	2.0	I, III
V	35	70.2	3.1	0.8	0.6	2.3	I
VI	35	70.0	2.8	0.7	0.5	1.8	I, III
<b>IMAT Area (cm<sup>2</sup>)</b>							
IV	35	16.4	2.7	3.3	0.5	9.2	VI
VI	35	1.4	1.1	28.0	0.4	77.5	IV
<b>SAT Area (cm<sup>2</sup>)</b>							
I	32	34.5	11.9	3.2	1.1	8.9	N.S.
IV	35	31.0	11.3	3.1	1.0	8.6	VI
VI	35	33.4	11.5	2.4	0.8	6.6	IV

SD = Standard Deviation; CV%<sub>RMS</sub> = Root-Mean-Squared Coefficient of Variation; SD<sub>RMS</sub> = Root-Mean-Squared Standard Deviation; LSC = Least Significant Change; P<0.05 = Tukey Contrasts; Least Significant Difference; IMAT = Inter-muscular Adipose Tissue; SAT = Sub-cutaneous Adipose Tissue; N.S. = Not Significant.

**Table 4: Forearm Precision**

Protocol	N-Pairs	Mean	SD	CV% <sub>RMS</sub>	SD <sub>RMS</sub>	95% LSC (CV%*2.77)	Contrasts P<0.05
<b>Muscle Area (cm<sup>2</sup>)</b>							
I	34	24.0	4.1	3.2	0.8	8.7	IV
II	33	24.0	4.0	2.7	0.6	7.5	IV
III	35	25.6	4.4	2.1	0.5	5.9	IV
IV	35	21.9	4.1	5.3	1.2	14.6	I, II, III, V, VI
V	35	21.3	3.5	2.9	0.6	8.0	IV
VI	35	24.4	4.1	2.7	0.7	7.6	IV
<b>Muscle Density (mg/cm<sup>3</sup>)</b>							
I	34	74.2	2.6	3.2	2.4	9.0	III, IV, V, VI
II	33	74.5	2.2	1.9	1.4	5.3	N.S.
III	35	72.0	2.6	1.4	1.0	3.9	I
IV	35	76.1	1.6	1.4	1.1	3.8	I
V	35	73.5	2.2	1.6	1.2	4.5	I
VI	35	72.3	1.9	1.5	1.1	4.1	I
<b>IMAT Area (cm<sup>2</sup>)</b>							
IV	35	4.9	0.9	7.0	0.3	19.5	VI
VI	35	1.0	0.5	42.2	0.4	116.8	IV
<b>SAT Area (cm<sup>2</sup>)</b>							
I	34	14.4	6.3	6.4	0.9	17.6	N.S.
IV	35	12.1	6.0	6.5	0.8	18.0	VI
VI	35	14.1	6.2	5.2	0.7	14.4	IV

SD = Standard Deviation; CV%<sub>RMS</sub> = Root-Mean-Squared Coefficient of Variation; SD<sub>RMS</sub> = Root-Mean-Squared Standard Deviation; LSC = Least Significant Change; P<0.05 = Tukey Contrasts; IMAT = Inter-muscular Adipose Tissue; SAT = Sub-cutaneous Adipose Tissue; N.S. = Not Significant.

#### 4.5 Discussion

To date, a variety of image analysis protocols have been used to derive soft-tissue outcomes from pQCT images of the lower leg and forearm. This study is the first to report and directly compare the precision error values of previously reported image analysis methods to derive soft-tissue outcomes for both the lower leg and forearm. This study found that most protocols produced similar precision error values (apart from IMAT area) with subtle differences among the various outcomes.

In the lower leg and forearm, most protocols (with the exception of IV in the forearm) provided comparable CV%<sub>RMS</sub> precision errors ranging between 2.1 and 3.7% for muscle

area. These values are higher than those previously reported for children and college students ( $CV\%_{RMS}$  1.4%) [51,70,86]. It may be that the discrepancy between these data and that of younger individuals is reflective of age-related morphological differences in musculature. One of these differences may be the amount of adipose within the muscle tissue, which pQCT-derived muscle area does not characterize.

To estimate the relative amount of muscle adipose, pQCT-derived muscle density is a hydroxyapatite-calibrated analogue of X-ray attenuation, which is validated to estimate muscle lipids and triglycerides [124,125]. Muscle density combines all soft tissues between the inner edge of the subcutaneous fat and the outer edge of the bones as a composite index of IMAT and myocellular adipose [52]. Most protocols provided similar muscle density precision errors within a small range (0.7 to 1.9%) in the lower leg and forearm. Protocols IV, V and VI demonstrated precision values below 1% in the lower leg; the latter agreeing with that reported for children (0.9%) using protocol IV [51]. Both protocols IV and VI were also an improvement ( $P < 0.05$ ) over protocol III (1.9%). In both limbs the largest error ( $P < 0.001$ ) occurred with protocol I (3.2%). This contrasts with other protocol I results for the lower legs of middle aged (0.8%) [93], and older women (1.8%) [151] as well as the forearms of children (1.2%) [70]. This discrepancy, as well as the occurrence of failed analyses [151], suggests that investigators need to be cautious when using protocol I for deriving muscle density from pQCT scans in postmenopausal women.

Another metric for muscle adiposity is inter-muscular adipose tissue (IMAT) area. A few pQCT studies have reported IMAT area or IMAT as a percentage of total muscle area [48,93]. Measuring the IMAT depot is challenging due to its small size relative to the pQCT voxels. The two protocols used to determine lower leg and forearm IMAT (IV and VI) used different image processing methods to help quantify IMAT area. Protocol IV quantifies the area of voxels

below 40mg/cm<sup>3</sup> in an unfiltered muscle image, whereas protocol VI does this after pre-processing the muscle with a 7x7 median filter. Precision error for the two protocols ranged between 3.3 and 42.2%. Protocol IV was much more precise (3.3% and 7.0%) in the lower leg and forearm ( $P < 0.001$ ), yet mean IMAT values were twelve and five times larger (respectively). Protocol VI results were similar to lower leg and forearm IMAT precision errors of 15.1% and 40.4% reported using a different filtering protocol in a sample of premenopausal women [93]. These results and the limited literature available [48,93] suggest that IMAT analysis protocols need further development and validation, with muscle density used in lieu of IMAT to quantify inter-muscular adipose tissue content.

Several recent papers report using pQCT to quantify SAT area in the lower leg [52,70,72,83,86,91,104]; however, only three studies reported precision estimates for this outcome [70,86,90]. Lower leg and forearm precision ranged from 2.4 to 6.4% for SAT area. These lower leg results were comparable to children (3.2%) [70] and college students (2.7%) [86]. However, forearm SAT precision error was nearly twice that of the lower leg and the reported error (2.5%) for children [70]. This may be a reflection of both the increased risk of minor movement in the upper limb [70] which may be greater in older individuals [151] as well as differences in data acquisition protocols (e.g. time between repeated scans, differences in voxel size).

Although observed differences in the precision errors across the pQCT protocols were small, a precision error of a mere 1% can affect the 95% LSC by a factor of 2.77. These small differences are important for quantifying modest musculoskeletal changes. For example, exercise interventions in older adults have reported between-group differences in muscle density (5.4%) [37] and IMAT (18%) [35] changes over 24 and 52 weeks respectively. Given the 95% LSC values below 5.4%, lower leg and forearm images analyzed using any method,

apart from method I, could reliably quantify changes in muscle density. Furthermore, with a 95% LSC below 18%, method IV would be needed to reliably quantify a similar change in IMAT at the lower leg.

This study has specific strengths related to sample population, sample size and scanning methodology. First, we have provided the first statistical comparison of precision errors across several pQCT analysis protocols for soft tissue outcomes clinically relevant for postmenopausal women [45,46,53]. Contrasted with results from younger participants [51,70,86], these data suggest that precision errors may be higher for forearm muscle area and SAT area in postmenopausal women. Other pQCT-derived soft-tissue precision studies have only reported lower leg precision values [86,90,151]. Second, this study met conservative recommendations (minimum of 27 degrees of freedom) for a precision error estimate within 30% of the mean error with an upper 90% confidence interval [118]. Failure to meet these recommendations can result in underestimation of precision errors [118]. In cases where the results included all 35 participants, precision error estimates can be trusted with a narrow 95% confidence interval [118]. Third, we collected images a mean of 9.7 days apart to avoid underestimating the precision error of soft tissues [86]. This is important because same day image collection can underestimate muscle and fat area precision errors due to enhanced technician recall when repositioning participants, or failure to capture between-day fluctuations in the precision error of the scanner [86].

This study has specific limitations related to age-specificity and accuracy. First, these results are only applicable to older postmenopausal women (mean age: 73.7, SD 7.2y). Second, the validity of pQCT measures of muscle and fat cross sectional area have been demonstrated against MRI [88]. QCT measures of muscle density and IMAT have been validated [124,125]; but these measures have not been directly validated with pQCT.



Comparison of pQCT-derived soft-tissue outcomes with MRI and MRS is a logical next step for the most precise image analysis protocols identified by this analysis.

The results indicate that pQCT-derived muscle area, density, and SAT area can be determined in older adults with a  $CV\%_{RMS}$  ranging between 2.1 to 3.7%, 0.7 to 1.9%, and 2.4 to 6.4% respectively. Precision for IMAT area varied considerably from 3 to 42%. While precision results were similar across most protocols, we have observed some subtle differences in methodology that can be used to aid both prospective and intervention studies of pQCT-derived soft-tissue outcomes in older adults.

## **5 Study Two: Monitoring pQCT-derived Muscle and Fat Outcomes in Older Women**

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## 5.1 Synopsis

This chapter describes estimates of the annual changes in muscle and fat outcomes measured using pQCT, using data collected over 1 and 2 year intervals in older adult women. Precision error and annual change estimates were utilized to estimate prospective monitoring time intervals for pQCT-derived muscle and fat outcomes.

## 5.2 Introduction

The loss of muscle mass and function with age (termed “sarcopenia”) has been a vigorous area of aging research for over 20 years [8]. Sarcopenia increases the risk of physical disability, reduced quality of life, and can lead to death [13]. The etiology of sarcopenia is complex, and is primarily characterized by chronic loss of muscle tissue and function in older adults [166]. The ectopic accumulation of adipose tissue within muscle is thought to play a role in the chronic catabolic state observed with aging [164,184,263], and can be quantified *in vivo* with the use of clinical imaging techniques such as MRI and CT [164]. Clinical CT and MRI offer high-contrast images of soft-tissue compartments; but these tools often have limited availability and, in the case of CT, may have a high effective radiation dose [40]. Peripheral quantitative computed tomography scanners fill a musculoskeletal imaging niche between clinical CT and MRI [40]. Images of the upper and lower extremities can be acquired using pQCT scanners to quantify muscle size and adiposity [55,127], as well as subcutaneous adipose tissue (SAT) area [143]. Depot-specific adipose data are important because aging, sarcopenic obesity, and cachexia are each associated with different adipose redistribution patterns [113,166,169].

In addition to providing insight into the composition of appendicular skeletal muscle, pQCT also has the capability to assess these measures with a high degree of precision [55,149,254]—a requisite for efficient longitudinal and intervention studies targeting muscle

and adipose tissues. Importantly, measurement precision, together with median annual changes, can be used to estimate MTIs between pQCT measurement occasions. MTIs provide a time estimate (in years) with 80% and 95% certainty, respectively, to detect progressive changes in pQCT-derived muscle and fat outcomes. MTIs with 80% certainty would be useful when the clinical benefits of the early detection of a trend in soft-tissue changes outweigh the need for statistical significance. These estimates allow follow-up measures to be performed within the optimal window for capturing clinically relevant and true biological changes, as well as minimizing participant radiation exposure and costs associated with repeated scanning in prospective studies. The ability of pQCT to detect changes in muscle or adiposity (in terms of both annual changes and MTIs) has not yet been reported in older adults. The purpose of this two-year prospective study was to: 1) assess the annual changes in pQCT-derived muscle area and density as well as IMAT and SAT at both the forearm and lower leg; and 2) estimate MTIs for each of the aforementioned muscle and adipose tissue outcomes in a cohort of older community-dwelling women.

## **5.3 Methods**

### **5.3.1 Participants**

Female participants aged >60 years old were recruited from the Saskatoon cohort of the CaMOs. The CaMOs sample is described in section 4.1.3.1. All 336 eligible female Saskatoon CaMOs participants were mailed an invitation to participate in this study. Baseline measures for 147 participants that accepted the invitation have been previously described [53]. After one year, 115 participants returned for follow-up measurements. After two years, 75 participants returned. To be eligible for this study, CaMOs participants had to have a lower leg circumference of less than 44cm (to fit the aperture of the scanner gantry) and be still for the scanning duration (approximately 4 minutes). Furthermore, valid forearm and/or

lower leg scans were required from at least two of the three annual imaging time points (T1, T2, or T3) for inclusion. Participants with diabetes, neuromuscular disease (Parkinson's disease, multiple sclerosis, or other), eating disorders, immobilization (defined as confinement to a bed, wheelchair or cast for longer than one month) or a diagnosis or treatment for cancer during in the past six years were excluded from this analysis. All scanned participants were assigned a number and then sub-sampled using a random number generator for repeat pQCT measurements to determine the precision of muscle and adipose outcomes. A total of 35 women underwent repeat pQCT imaging of their non-dominant forearm and lower leg within an average 9.7 (SD 3.6) days, as previously reported [149]. The University of Saskatchewan Biomedical Research Ethics Board reviewed and approved this research in accordance with the 2010 Canadian Tri-Council Policy Statement II: Ethical Conduct for Research Involving Humans. All participants provided written, informed consent.

### 5.3.2 *Descriptive Measures - Anthropometrics*

To characterize the sample, participant age, height (cm), weight (kg), and BMI (kg/m<sup>2</sup>) were assessed. Height was measured using a wall-mounted stadiometer (Holtain Ltd., Crosswell, Wales, UK) accurate to  $\pm 1$  mm and weight (in slacks and a t-shirt) from a calibrated scale (Toledo Ltd., Columbus OH, USA) accurate to  $\pm 0.1$  kg.

### 5.3.3 *Descriptive Measures - Physical Activity & Strength*

To assess whether physical activity and strength declined following baseline measures, participants completed self-reported minutes of physical activity and SF-36 Health Status questionnaire data. As well, participants performed the TUG [264] and isometric handgrip strength tests.

Two trained research assistants conducted the TUG test. Precision error ( $CV\%_{RMS}$ ) calculated from repeat measurements of 35 women was 5.4% in this cohort. The protocol involved rising from a chair, walking three meters, turning around, and returning to their seat in a seated position. Each participant was asked to walk at their usual walking pace. A stopwatch measured the time between the moment the research assistant said the word “go” to the moment the participant’s body resumed contact with the backrest of the chair. Participants were able to make use of the chair arms, as well as any walking aids they would normally use in their daily lives. Each participant was granted a practice trial to ensure they understood the test protocol, followed by three timed trials with short rest intervals. The fastest completion time was recorded.

For the grip strength measure, participants underwent maximal isometric grip strength testing on their non-dominant hand using a JAMAR Hydraulic Hand Dynamometer (Lafayette Instrument Co. Lafayette IN, USA). Each participant was seated, with their shoulders adducted, elbows flexed at 90 degrees and forearms in neutral according to the American Society of Hand Therapists recommendations [265]. Three, 3-second long maximal attempts occurred with a half minute break provided between attempts. The highest score, accurate to  $\pm 1$  kg, was recorded with a precision error ( $CV_{RMS}\%$ ) of 11%.

#### 5.3.4 *pQCT Image Acquisition*

Scans were acquired on the non-dominant (self-reported) lower leg and forearm using an XCT 2000 pQCT (Stratec Medizintechnik GmbH, Pforzheim, Germany). The XCT 2000 is calibrated to provide hydroxyapatite mineral equivalent tissue densities, such that adipose tissue has a density of  $0 \text{ mg/cm}^3$  and water  $60 \text{ mg/cm}^3$  [146]. All images were collected at a scan speed of 20mm/s with a voxel size of  $400\mu\text{m} \times 400\mu\text{m} \times 2.4\text{mm}$ . For both the forearm and lower leg, a scout scan was conducted to set a reference line at the medial aspect of the distal

radial epiphysis and the distal tibia plafond, respectively. A single image was collected at the site corresponding to 65% of total radius length or 66% of the total tibia length proximal from the reference line. These sites are the two most commonly pQCT-imaged locations for muscle and are the approximate anatomical location of the greatest limb girths [266,267]. The imaging technician visually assessed image quality using a subjective criterion; if motion artifacts interrupted cortical bone, the scan was not used and acquisition was repeated. All scans were acquired by the same technician (AFW).

### 5.3.5 *Image Analysis*

All scans were analyzed using BoneJ Version 1.3.11; a plugin for the freely-available image processing software ImageJ 1.48q (National Institutes of Health, Bethesda MD, USA) [104,255]. This image analysis protocol was selected for its relatively high precision of soft-tissue outcomes as compared to other previously published pQCT image analysis techniques [149]. We used an upper threshold of  $139\text{mg}/\text{cm}^3$  for soft-tissue to minimize the occurrence of rare tissue segmentation errors in older adult cohorts. The specifics of this image analysis technique and the parameters used are described elsewhere [149]. The automated image analysis software calculated muscle area and density as well as intramuscular adipose tissue (IMAT) and SAT areas for each scan.

Automated contour detection algorithms did not always succeed in correctly identifying soft tissue boundaries; therefore, each analyzed image was visually inspected for errors using the BoneJ visual result output. All images were graded by two blinded technicians (AFW, CB) for severity of motion artifacts according to the visual inspection rating scale (1 to 5) developed by Blew et al. [108].

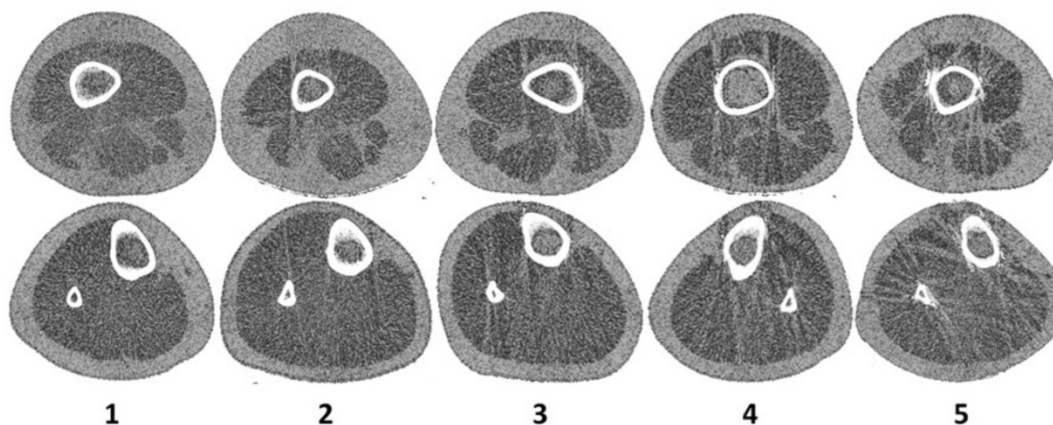


Figure 5: Visual inspection rating scale for femur (upper row) and tibia (lower row). Each score reflects the level of movement: 1 none, very minimal; 2 minimal; 3 moderate; 4 severe; 5 extreme. Reprinted with permission from Springer [108].

When two technicians' ratings did not agree, a third blinded reviewer (SK) was consulted and the median value was reported. All scans rated  $>3$  were excluded. To further minimize the effect of image quality on prospective soft-tissue changes, baseline and follow-up images that differed by more than one grade were also excluded.

### 5.3.6 *Statistical Methods*

For all participants, the longest available time between measures was utilized to determine annual change. This approach was taken to maximize both the observation time and sample size to provide accurate estimates of change [254]. Annual percentage change values were calculated by subtracting (adjusted) 1-year follow-up values from baseline values, and represented as a percentage change from baseline. To assess which outcomes actually changed, baseline and (adjusted) follow-up measures were statistically compared using IBM SPSS Statistics Version 22 (IBM, Armonk NY, USA). Skewness and kurtosis Z-scores as well as histograms and boxplots were used to check the data for normality ( $Z < 1.96$ ) and outliers ( $Z > 2.58$ ). Non-normal data was log-transformed, and re-checked for normality. Normally



distributed outcomes and transformed outcomes were compared using paired t-tests. Where log-transformation failed, the original data were compared using a Wilcoxon Signed-Rank test.

MTIs were defined as the ratio of measurement precision errors (specifically 80 and 95% LSCs) to median annual percent changes. These two intervals represent the longitudinal sensitivity of pQCT measures, and estimate the time needed for 50% of the population to demonstrate age-related changes exceeding the instrument and operator's measurement error [59]. For this study, LSCs from the related precision study of 35 women [149] were used to calculate MTIs. In this previous study, LSC was calculated based upon root-mean-squared coefficient of variation ( $CV\%_{RMS}$ ) precision errors and an adjusted Z-score for the selected level of statistical confidence ( $Z = 1.8$  for a two-tailed confidence of 80%; or  $Z = 2.77$  for a two-tailed 95% confidence) via the equation  $LSC = Z\text{-score} \times CV\%_{RMS}$  [59,149].

#### **5.4 Results**

Baseline and follow-up data for a total of 108 and 120 valid forearm and lower leg scans were collected (Figure 6). Of those with forearm scans, 22 were ineligible due to health conditions and 9 were excluded due to motion artifacts. Of those with lower leg scans, 24 were ineligible due to health conditions and 4 were excluded due to motion artifacts. Data collected from a total of 97 participants were analyzed, for an overall average of 1.7 years (SD 0.6) of observation.

Annual changes in height (-0.3 cm,  $P < 0.001$ ), and self-reported physical activity (-3min/day,  $P < 0.01$ ) occurred over this time but they were not correlated with changes in muscle area, muscle density, SAT or IMAT area. Weight, BMI, TUG test time, grip strength, and SF-36 Health Status Score did not demonstrate an annual change (Table 5). The median annual change in forearm muscle area was -0.81% per year ( $P < 0.001$ ) (Table 2). In the

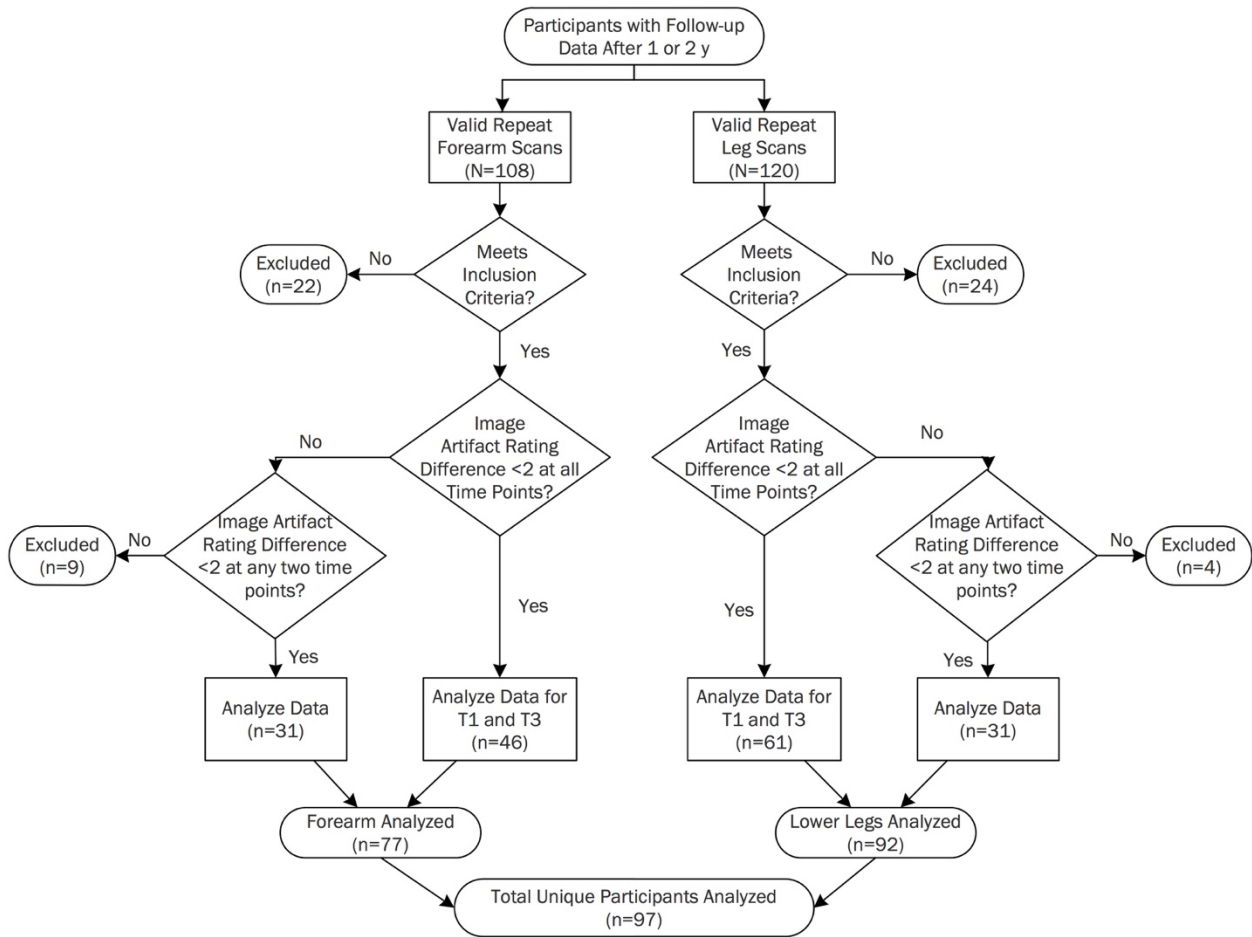


Figure 6: Flow chart of the image analysis process. Participants were excluded if they indicated the presence of diabetes, neuromuscular disease (Parkinson’s disease, multiple sclerosis, or other), eating disorders, immobilization >1 month or cancer diagnosis or treatment within the previous 6 years. Image motion artifacts were rated on a scale of 1 (none) to 5 (extreme) according to the subjective criteria provided by Blew et al. [108]. Scans rated 4 (severe) or higher were not considered valid. Scans collected at two of the three time points (T1, T2, or T3) were analyzed for annual changes in soft-tissue outcomes if they were rated similarly or within <2 scores.

lower leg, muscle area change was -1.23% per year ( $P=0.004$ ). No annual change was observed in muscle density, IMAT or SAT at either site (Table 6).

The MTI results estimate that clinically relevant and significant reductions in forearm muscle area could be detected in half of the older female adult population after 6 and 9 years of follow-up respectively (Table 5). In the lower leg, a decline in muscle area and

**Table 5: Baseline and Annual Follow-Up Participant Characteristics**

	Baseline		Follow-Up*		P-Value
	Mean	SD	Mean	SD	
N=97					
Time Between Measurements (y)	1.7	0.6			
Age (y)	74.5	7.8			
Height (cm)	158.8	6.1	158.5	6.2	0.001
Weight (Kg)	69.8	12.7	69.3	12.9	0.060
Body Mass Index (Kg/m <sup>2</sup> )	27.7	4.7	27.5	4.7	0.266
Physical Activity (min/day)**	20	24	17	19	0.011
SF36 Health Status Score (Max=100)**	74	17	74	17	0.862
TUG test (s)**	9.6	2.6	9.6	2.4	0.715
Grip Strength (kg)	17.1	4.8	17.3	4.6	0.583

Abbreviations: SD = Standard Deviation; TUG = Timed Up and Go.

P-Values are from Paired T-tests unless indicated otherwise.

\*Values Adjusted to 1-yr of follow-up time.

\*\*Wilcoxon Signed Rank Test.

density could be detected within 4 and 6 years using pQCT (Table 6). MTIs for forearm muscle density, IMAT and SAT measures ranged between 18 and 143 years (Table 6).

## 5.5 Discussion

This is the first study to estimate annual changes and MTIs for pQCT-derived muscle and adiposity measures in community-dwelling older women. Robust estimates of the years of observation required to observe real, biological changes in muscle area and density using pQCT will assist with the planning of aging cohort studies [35]. We observed annual declines in pQCT-derived muscle area by 0.8 to 1.2% in older women for estimated follow-up intervals of 4 to 9 Years. Using these rate-of-change values, we estimate that 6 to 9 and 4 to 6 years of age-related atrophy in forearm and lower leg muscle area are needed before a clinically relevant or biological difference could be detected in half the population. Statistically significant annual changes were not observed for muscle density, IMAT or SAT area.

**Table 6: Estimated Annual Changes & Monitoring Time Intervals**

Outcome	N	Baseline		Follow-up*		P-Value	Median Annual Change (%)	Confidence Level			
		Mean	SD	Mean	SD			80%		95%	
								LSC (%)	MTI (y)	LSC (%)	MTI (y)
<b>65% Forearm</b>											
Muscle Area (cm <sup>2</sup> )	77	24.9	3.1	24.7	3.3	0.001	-0.81	4.9	6	7.6	9
Muscle Density (mg/cm <sup>3</sup> )**	77	72.4	2.1	72.2	2.1	0.370	-0.04	2.7	66	4.1	92
IMAT (cm <sup>2</sup> )	77	0.9	0.5	0.9	0.5	0.751	-4.15	76	18	117	28
SAT (cm <sup>2</sup> )	77	14	7.6	13.9	7.6	0.296	-0.10	9.4	94	14.4	143
<b>66% Lower Leg</b>											
Muscle Area (cm <sup>2</sup> )	92	60.6	9.1	59.6	9.0	0.001	-1.23	4.5	4	6.9	6
Muscle Density (mg/cm <sup>3</sup> )	92	70.3	3.0	70.0	3.1	0.271	-0.28	1.3	5	1.8	6
IMAT (cm <sup>2</sup> )	92	1.4	1.4	1.4	1.2	0.839	-1.61	50	31	78	48
SAT (cm <sup>2</sup> )	92	32.8	12.6	32.4	12	0.223	-0.24	4.3	18	6.6	27

Abbreviations: SD = Standard Deviation; LSC = Least Significant Change; MTI = Monitoring Time Interval; IMAT = Inter-muscular Adipose Tissue; SAT = Subcutaneous Adipose Tissue.

P-Values are from Paired T-Tests, unless indicated otherwise.

\* Values adjusted to 1-yr of follow-up time.

\*\*P-Values from a Wilcoxon Signed Rank Test.

To date, the majority of prospective muscle aging research has relied on DXA-derived lean mass [268-270] and clinical CT based measures of muscle area, SAT, and attenuation/density [28]. DXA-derived analogs of appendicular muscle size, specifically arm and leg lean mass are the most commonly cited. Changes in leg lean tissue mass have been reported as -0.15%/year ( $P<0.01$ ) in healthy American women observed over 2 years [268], -0.40 to -0.65%/year ( $P<0.001$ ) in Japanese women observed over 6 years [269], and -0.79%/year ( $P<0.001$ ) in Italian women observed over 5 years [270]. Similar to the muscle area results from this study, annual changes in arm lean mass were smaller than those reported for the leg [268-270]. The precision error ( $CV\%_{RMS}$ ) of these outcomes was reported to be 10.9 and 2.7% for arm and leg lean mass, respectively [134]. Using these  $CV\%_{RMS}$  errors with previously-mentioned mean change values in older adult women [268-270], we can estimate DXA-derived leg lean mass MTIs to range between 9 and 50 years, and are likely even longer for arm lean mass (due to greater precision error). Similarly, 5 years of prospective clinical CT data in healthy American women aged 70-79 years of age demonstrated a -0.64%/year decline in thigh muscle area [28]. With a reported precision error of approximately less than 5% [25], we can estimate a substantially longer MTI of up to 21 years for CT measures of the thigh muscle area. These results suggest that pQCT-derived muscle area may be a more useful metric than CT when assessing prospective biological changes in appendicular muscle size with age.

In this sample, muscle area changed concurrently with a decline in self-reported physical activity, but without changes in muscle density, IMAT, SAT area, weight ( $P=0.058$ ), BMI, health status, or functional test performance. Functional test performances are known to vary with comorbidities such as arthritis and obesity [112]. Handgrip and TUG performances did not change despite a decline in forearm and lower leg muscle size. These

results support the notion that precise imaging-based measures may allow for the detection of changes in muscle, adipose and bone before changes in physical function become clinically evident [112]. Tools that can better evaluate and differentiate more specific body composition features (e.g., muscle area, muscle density/attenuation, IMAT) are necessary for the development of therapies targeting muscle and physical performance [112].

Recently, Murphy et al. [271] reported that older adults can transition between normal and pre-sarcopenic states; yet once sarcopenic they were unlikely to transition back to a more normal state. This emphasizes the need for early identification of persons at risk of adverse changes in muscle size and composition for preventive intervention. Clinical researchers can utilize MTI estimates to plan appropriate follow-up intervals when monitoring soft-tissue outcomes in older women. Conditions such as sarcopenic obesity and cachexia display secular trends in weight, muscle, and adipose loss which can obscure relationships between general body composition measures (BMI, total body fat percentage) and functional outcomes [13,113]. Clinical researchers can make use of LSC values to inform decision making when observing changes in body composition in response to conditions such as HIV-associated lipodystrophy, cachexia, or the effects of treatments like bariatric surgery. Use of liberal (80% confidence) versus conservative (95% confidence) estimates will depend on the context, and relative importance of modifying clinical intervention before a statistical change is observed [59].

There were no changes in SAT or IMAT in the forearm or lower leg. Static SAT values, concurrent with changes in muscle composition have also been reported at the mid-thigh in weight-stable 70-79 year old women [28]. Delmonico et al. [28], reported a 6% increase in the adipose infiltration of muscle tissue, regardless of changes in weight, muscle size or SAT. However, contrary to the clinical CT results of Delmonico et al. [28], we did not observe

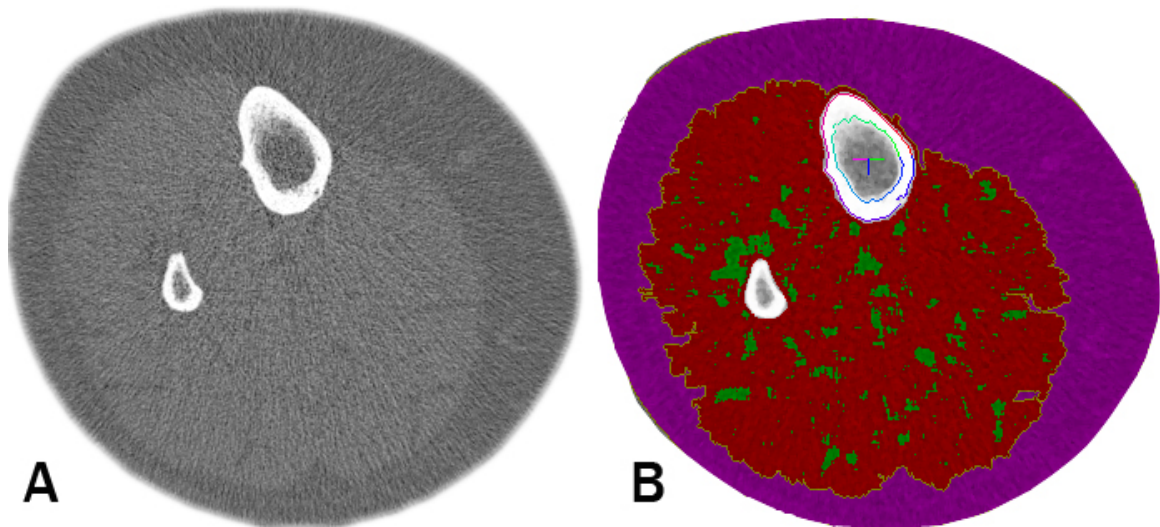


Figure 7: Typical lower leg grayscale image collected using a Stratec XCT2000 peripheral quantitative computed tomography scanner (A). The lower leg image is filtered and segmented by BoneJ (B) into subcutaneous adipose tissue (SAT; purple), muscle area (red + green), and inter-muscular adipose tissue (IMAT; green).

changes in pQCT-derived IMAT. This discrepancy may be due to the different muscles imaged (the thigh has a larger IMAT depot than the lower leg) and both the inconsistency and imprecision of pQCT-derived IMAT [93,149]. The 0.4 mm in-plane pixel size and contrast of pQCT imaging makes measuring small IMAT areas challenging (Figure 7). We also analyzed images using another IMAT technique (Bone Diagnostic Inc., Fort Atkinson WI, USA) that does not utilize image filters [149,249]; despite that, these alternative measures did not change the results (data not shown). Thus, current pQCT image analysis techniques do not appear capable of reliably isolating IMAT from surrounding muscle tissue and require further development. The establishment of open-source pQCT image analysis software [251,255] increases the potential for improvement in pQCT-derived IMAT. These results support the suggestion that pQCT researchers should consider reporting muscle density [93,149], rather than IMAT [55], as a surrogate measure of muscle adiposity [127].

Muscle density combines all soft tissues between the inner edge of the SAT and the outer edge of the bones as a precise [149] composite index of muscle adiposity [52]. Scanner attenuation or hydroxyapatite-calibrated tissue density is a validated, inversely related measure of tissue adipose content [55,124,125,127]. In this cohort, annual changes in muscle density were not significant, yet the high precision of pQCT-derived lower leg muscle density allows for estimated MTIs of 5 and 6 years. Therefore pQCT-derived lower leg muscle area and density may provide valuable insight into changes in muscle size and quality within the same 4 to 6 year time interval. Median annual percentage change values for forearm muscle density did not yield useful MTIs. The data here do not offer a concrete explanation for this upper and lower limb discrepancy. However, these findings may be linked to observations of greater skeletal muscle loss in the lower body [113] and previously demonstrated differences between lower and upper body neuromuscular properties and performance with age [272-274].

This study has several strengths related to sample, scanning and analysis methodology. First, we prospectively measured a sub-sample of a well-described population-based Canadian cohort [253]. The TUG test and grip strength results of this sub-sample are characteristic of normative reference values for normal, unimpaired older women [264,275]. Furthermore, in an attempt to isolate natural age-related changes in muscle we have excluded women with chronic conditions (i.e., immobilization, eating disorders, neuromuscular diseases, diabetes, cancer) that are known to accelerate muscle loss. Second, the precision error estimates for measures included in this study exceed conservative recommendations (minimum of 27 degrees of freedom) for robust  $CV\%_{RMS}$  values [149]. These precision error estimates were determined with 35 degrees of freedom, in randomly selected participants sampled from this same cohort and therefore can be



trusted within a narrow 95% confidence interval [118]. Furthermore, the repeat images utilized were collected a mean of 9.7 days apart to avoid underestimating the precision error of soft tissues [86]. This is important as same-day image collection can underestimate muscle and adipose tissue area precision errors due to enhanced technician recall when repositioning participants, or failure to capture between-day fluctuations in the precision error of the scanner [86]. Third, we have carefully minimized the risk of erroneous soft-tissue changes from image artifacts. This was achieved through the application of a standardized motion rating scale [108] by up to three independent observers. This conservative quality assurance process excluded low quality images and did not analyze prospective images that differed by more than one motion rating increment. Finally, we utilized data from two-year observation periods when both the participation rate and image quality allowed us to do so. This provided a more robust estimate of annual changes than a reliance on shorter observation periods [59].

This study has specific limitations related to age-specificity and accuracy. First, these results are only applicable to older women and the cohort they were sampled from was based on an urban population that is predominantly Caucasian. Furthermore, this voluntary sub-project ran between the scheduled Year 10 and Year 16 follow-up periods of the CaMOs study. Thus, it is not possible to nest this sub-sample within previously collected CaMOs data for a direct comparison of the characteristics of non-respondent CaMOs peers. As such, selection bias for healthier participants cannot be ruled out. Therefore, this analysis may underestimate the magnitude of soft-tissue changes and subsequently overestimated MTIs. Thus, these findings likely provide conservative estimates to assist the design of interventions and prospective studies in community dwelling older women. Furthermore, unrecorded behavior modifying comorbidities (i.e. arthritis, peripheral artery disease, etc.)

may have influenced these soft-tissue change estimates. Lastly, beyond excluding participants with self-reported eating disorders, the nutritional status of participants was not monitored during this period of observation. This is important because nutritional deficits are considered to be a secondary cause of sarcopenia [166], a potential confounder for this data. However, the use of median annual change values to calculate MTIs minimizes the effect of outliers and acute behavioural variability in these estimates; better reflecting the naturally occurring annualized changes in the cohort.

In conclusion, researchers can reasonably expect annual age-related changes of -0.8 to -1.2%/year in pQCT-derived forearm and lower leg muscle area. Accounting for measurement precision error, researchers can reasonably expect to detect declines in pQCT forearm muscle area within 4 to 9 years and lower leg muscle area and density within 4 to 6 years in community-dwelling Caucasian women >60 years.

## **6 Study Three: Lower Leg Muscle Density is Independently Associated with Fall Status in Older Adults**

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## **6.1 Synopsis**

This chapter describes an analysis of the associations of pQCT derived muscle density, health outcomes, and functional mobility to fall status. Muscle density is associated with fall status, independent of biologically relevant covariates and functional mobility, which suggests that it may serve as an important biomarker for fall risk and musculoskeletal health in older adults.

## **6.2 Introduction**

Falls are the leading cause of non-fatal emergency room visits in the US [58], and the primary cause of hip fractures in adults greater than 65 years old [57]. Approximately one third of older adults report a fall each year, and 20% of falls require medical attention [213,241]. Older, community-dwelling adults who fall cost health care systems an average (US) \$3,476; in cases where surgery and/or hospitalization are required, costs can rise to approximately \$26,483 per individual [222]. The need for research on fall etiology is accentuated by age-adjusted data suggesting the rate of fall-induced injury is increasing [224].

Falls are multi-factorial events caused by a combination of demographic, environmental, behavioral, and sensorimotor factors [241]. Among these, the strength and power of lower leg muscles are major contributing factors for falls, postural stability, and balance recovery in older adults [239,276,277]. Aging muscle undergoes changes in composition, defined by a decline in lean mass and an increase in adipose and non-contractile tissue [164]. Increased quantities of muscle adipose may contribute to the catabolic state observed in muscle with age owing to the secretion of pro-inflammatory cytokines which stimulate muscle catabolism [181,182,184]. The relative amount of intra- and inter-muscular adipose can be quantified using imaging techniques such as MRI and CT.

Similar to CT attenuation values [124] pQCT-derived density measures are inversely related to lipid content, and approximately 50% of the variance in muscle density is explained by inter- and intra-muscular adipose tissues [55]. In older individuals, muscle adiposity has been linked to reduced lower extremity performance as well as muscle torque [23,24]. Two large prospective studies on aging, the Health Aging and Body Composition and the Invecchiare in Chianti Studies both revealed that adiposity (percent body fat, muscle density) and physical performance (walking speed, chair stand ability) but not lean mass, muscle size, or strength were risk factors for disability in healthy, older adults [26,27]. There is a growing body of evidence that suggests muscle adiposity and associated functional deficits manifest in clinically significant fall-related outcomes. Older persons with low muscle attenuation (which reflects higher adiposity) are 50 - 80% more likely to develop mobility limitations [24], and are at a greater risk of fracture [30,31,187,188], disability [26,27] and hospitalization [29].

Recent results from our lab found pQCT-derived muscle density to be significantly lower in the legs of well-functioning older women who reported one or more falls in the past year when compared to controls matched for age, BMI, and general health status [53]. Furthermore, no significant difference on the TUG test was observed between fallers and non-fallers, despite this functional mobility test consisting of both walking speed and chair rise components [53,264]. The association between lower leg muscle density (a surrogate of fat infiltration) and falls has not been previously described in the literature. Furthermore, the role of functional mobility in the association between muscle density and falls is not known. Therefore, the primary objective of this study was to explore the relationships of muscle density, functional mobility, and health-related factors to fall status. The primary hypothesis was that muscle density would be associated with fall status after adjusting for age, sex,

BMI, general health status, diabetes, a number of pertinent comorbidities, and functional mobility. The secondary objective was to determine the independent and combined relationship of muscle density and functional mobility to fall status after adjusting for health-related factors. The secondary hypothesis was that models which include both muscle density and TUG test time will have a better fit with the data than models that include these factors separately.

## **6.3 Methods**

### **6.3.1 Participants**

Participants aged  $\geq 60$  years were recruited from the Saskatoon cohort of CaMOs as described previously. All 501 eligible CaMOs participants were mailed an invitation to participate in this study. Eligibility criteria, recruitment and measurements for 147 female participants have been previously described in detail [53]. For these analyses, an additional 43 males were recruited a year later using the same methodology. The University of Saskatchewan Biomedical Research Ethics Board reviewed and approved this research (BIOREB 10-83), and all participants provided written informed consent.

### **6.3.2 Descriptive Measures**

To characterize the sample, fall status was determined by a response to the retrospective fall recall question: “have you fallen in the previous 12-months?”. Defined according to the Prevention of Falls Network Europe Consensus as “an unexpected event in which the participants come to rest on the ground, floor, or lower level” [226]. Retrospective fall recall results demonstrated good agreement (Kappa = 0.77), when repeated an average of 9.7 (3.6) days apart in a random sub-sample of 35 female participants.

Participant age (y), height (cm), weight (kg), BMI ( $\text{kg}/\text{m}^2$ ), hand grip strength (Kg) and SF-36 Health Status questionnaire data were recorded. Height was measured using a wall-

mounted stadiometer (Holtain Ltd., Crosswell, Wales, UK) accurate to  $\pm 1$ mm and weight (in slacks and a t-shirt) from a calibrated scale (Toledo Ltd., Columbus OH, USA) accurate to  $\pm 0.1$  kg. Body mass index ( $\text{kg}/\text{m}^2$ ) was derived from height and weight measures. Isometric grip strength measures of the non-dominant hand were collected using a JAMAR Hydraulic Hand Dynamometer (Lafayette Instrument Co. Lafayette IN, USA) according to American Society of Hand Therapists recommendations. The highest score from three, 3-second long maximal attempts accurate to  $\pm 1$  kilogram was recorded with a precision error ( $\text{CV}_{\text{RMS}}\%$ ) of 11%. Confounding medical conditions for muscular health including diabetes, neuromuscular diseases (NMD; Parkinson's, Multiple Sclerosis, or other), osteoarthritis, eating disorders, and immobilization or a diagnosis of cancer in the past six years were recorded.

### 6.3.3 *Timed Up and Go Test*

Two trained research assistants conducted the TUG test. The precision error ( $\text{CV}\%_{\text{RMS}}$ ) calculated from repeat measurements of 35 women in this cohort was 5.4%. The timed protocol involved rising from a chair, walking 3 meters, turning around, and returning to their seat, at their usual walking pace [264]. Participants were able to make use of the chair arms, as well as any walking aids they would normally use in their daily lives. Each participant was granted a practice trial to ensure they understood the test protocol, followed by three timed trials with short rest intervals. The fastest completion time was recorded.

### 6.3.4 *pQCT Image Acquisition*

Scans were acquired on the non-dominant lower leg using an XCT 2000 pQCT (Stratec Medizintechnik GmbH, Pforzheim, Germany). The XCT 2000 is calibrated to provide hydroxyapatite mineral equivalent tissue densities, such that fat tissue has a density of 0  $\text{mg}/\text{cm}^3$  and water 60  $\text{mg}/\text{cm}^3$ . All images were collected at a scan speed of 20mm/s using

a voxel size of 400 $\mu$ m x 400 $\mu$ m x 2.4mm. A single image was collected at the site corresponding to 66% of the total tibia length proximal from a reference line at the medial aspect of the distal tibia plafond. This is the most common location for lower leg muscle scans, and the anatomical location of the greatest limb girth.

### 6.3.5 *Image Analysis*

All scans were analyzed using BoneJ Version 1.3.11; a plugin for the freely-available image processing software ImageJ 1.48q (National Institutes of Health, Bethesda MD, USA). We used density thresholds 40-139mg/cm<sup>3</sup> to define muscle tissue. Technical aspects of the image analysis protocol have previously been described in detail [149]. The precision error (CV%<sub>RMS</sub>) calculated from repeated measurements of 35 women in this cohort was 2.5% for muscle area and 0.7% for muscle density [149].

All images were graded by the consensus of up to three blinded technicians for the severity (1 to 5) of motion artifacts according to the visual inspection rating scale developed by Blew et al. [108]. Scans rated >3 were excluded.

### 6.3.6 *Statistical Methods*

Faller and non-faller descriptive results and statistical comparisons were calculated for all variables. Categorical proportions were compared using Fisher's Exact test. Non-normal data was log-transformed, and re-checked for normality. Normally-distributed outcomes and transformed outcomes were compared using independent t-tests. Where log-transformation failed, the original data were compared using the Wilcoxon Rank-Sum test. Logistic regressions were reported for fallers (1) and non-fallers (0). To test the associations of muscle density and functional mobility with fall status, forced entry multivariable logistic regression models were generated controlling for biologically relevant covariates: age, sex, BMI, general health status, diabetes and a number of pertinent comorbidities. Three models



were generated to test the predictive ability of 1) muscle density, 2) TUG test time and 3) both muscle density and TUG. Likelihood ratio tests determined if the model fit improved between independent (Models 1 & 2) and combined (Model 3) variables. We report descriptive means, SDs, relative proportions (%) and counts, *P*-values, the area under the curve (AUC) for each multivariable model, as well as odds ratios (OR) and 95% confidence intervals [95% C.I.] for each predictor. Statistical significance was set at  $P < 0.05$ . All statistical analyses were performed with IBM SPSS Statistics, Version 22 (IBM, Armonk NY, USA).

#### **6.4 Results**

A total of 190 older adults were recruited. A flowchart (Figure 8) details the participants included and excluded at each step of the analysis. A detailed description of the 183 eligible adults is provided in Table 7.

Bivariate logistic regression analyses revealed significant odds ratios for several factors (Table 8). For every unit higher in general health status score, the odds of being a faller (defined as having reported one or more falls in the past year) was reduced by 3% (OR 0.97 [95% C.I. 0.95 to 0.99]). For every second increase in TUG test result, the odds of being a faller increased by 16% (OR 1.16 [95% C.I. 1.03 to 1.30]). Similarly, for every  $\text{mg}/\text{cm}^3$  increase in muscle density, the odds of being a faller decreased by 15% (OR 0.85 [95% C.I. 0.75 to 0.95]). Being diabetic increased the odds of being a faller by 329% (OR 3.29 [95% C.I. 1.12 to 9.64]).

Multivariable models controlled for biologically relevant confounders (age, sex, BMI, general health status, diabetes, number of comorbidities) to compare the ability of muscle density and TUG test to independently predict fall status (Table 9). All three models provided

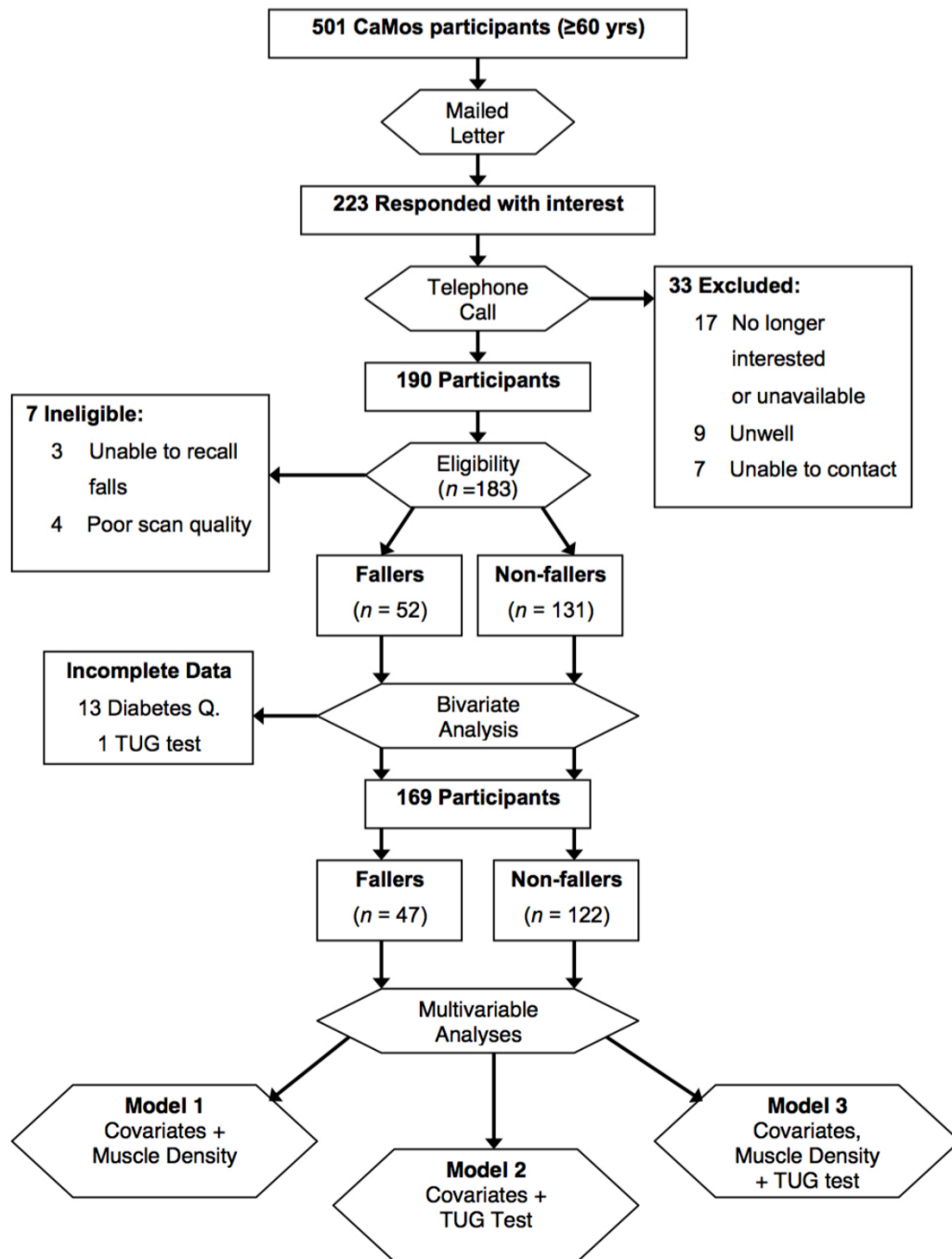


Figure 8: Participant flow-chart detailing recruitment, bivariate, and multivariable analyses for models 1-3. Diabetes Q. = Questionnaire; 13 participants did not indicate a response and were excluded, along with 1 TUG test refusal.

**Table 7: Descriptive Statistics**

	Faller (n=52)	Non-Faller (n=131)	P-Value
Sex (male/female)	15/37	27/104	0.246 <sup>a</sup>
Age (y)	74.3 (8.6)	74.6 (7.6)	0.806 <sup>b</sup>
Height (cm)	161.9 (8.7)	162.0 (8.4)	0.867 <sup>c</sup>
Weight (kg)	75.8 (14.9)	71.7 (13.2)	0.095 <sup>b</sup>
BMI (kg/m <sup>2</sup> )	28.8 (5.1)	27.3 (4.3)	<b>0.041<sup>b</sup></b>
General Health Status (0 - 100)	64.6 (19.5)	73.0 (15.2)	<b>0.022<sup>c</sup></b>
TUG (s)	10.7 (3.1)	9.5 (2.4)	<b>0.044<sup>c</sup></b>
Grip Strength (kg)	19.7 (8.1)	20.4 (9.5)	0.584 <sup>c</sup>
Lower leg muscle			
Density (mg/cm <sup>3</sup> )	69.2 (3.5)	70.5 (2.3)	<b>0.045<sup>c</sup></b>
Area (cm <sup>2</sup> )	64.1 (11.2)	62.4 (11.2)	0.382 <sup>b</sup>
Comorbidities (count)			
Diabetes	15% (8)	5% (7)	<b>0.034<sup>a</sup></b>
NMD	4% (2)	10% (12)	0.356 <sup>a</sup>
Osteoarthritis	48% (25)	45% (59)	0.744 <sup>a</sup>
Eating disorders	2% (1)	0 (0)	0.284 <sup>a</sup>
*Immobilization >1month	6% (3)	2% (2)	0.096 <sup>a</sup>
*Cancer	10% (5)	6% (8)	0.354 <sup>a</sup>
Number of Comorbidities			0.307 <sup>a</sup>
0	33% (17)	47% (61)	
1	52% (27)	41% (54)	
2	14% (7)	10.7% (14)	
3	2% (1)	2% (2)	

Abbreviations: BMI = Body Mass Index; TUG = Timed Up and Go; NMD = Neuromuscular Diseases. P<0.05 are **bolded**. P-Values from <sup>a</sup> Fisher's Exact Test; <sup>b</sup> Independent T-Test; <sup>c</sup> Wilcoxon Rank-Sum Test

\*Diagnoses/occurrences within the previous 6 years

acceptable discrimination with significant AUC values between 0.74 and 0.76, and significant ( $P<0.05$ ) improvement over the covariates (Table 9 & Figure 9). In Model 1, higher muscle density (mean 70.2, SD 2.6mg/cm<sup>3</sup>) reduced the odds of being a faller by 19% (OR 0.81 [95% C.I. 0.67 to 0.97]). In Model 2, each second of TUG test time (mean 9.8, SD 2.6s) independently increased the odds of being a faller by 17% (OR 1.17 [95% C.I. 1.01 to 1.37]).

**Table 8: Bivariate Associations with Fall Status**

	Odds Ratio (e <sup>β</sup> )	95% C.I.	
		Lower	Upper
Sex (female=0)	1.56	0.75	3.25
Age (y)	1.00	0.96	1.04
Height (cm)	1.00	0.96	1.04
Weight (kg)	1.02	1.00	1.05
BMI (kg/m <sup>2</sup> )	1.08	1.00	1.16
General Health Status (0-100)	<b>0.97</b>	<b>0.95</b>	<b>0.99</b>
TUG (s)	<b>1.16</b>	<b>1.03</b>	<b>1.30</b>
Grip Strength (Kg)	0.99	0.96	1.03
Lower leg muscle			
Density (mg/cm <sup>3</sup> )	<b>0.85</b>	<b>0.75</b>	<b>0.95</b>
Area (cm <sup>2</sup> )	1.01	0.99	1.04
Comorbidities (reference=0)			
Diabetes	<b>3.29</b>	<b>1.12</b>	<b>9.64</b>
NMD	0.40	0.09	1.84
Osteoarthritis	1.13	0.59	2.15
*Immobilization >1month	4.98	0.79	31.49
*Cancer	1.70	0.53	5.48
Number of Comorbidities (None=0)			
1	1.79	0.88	3.65
2	1.79	0.63	5.15
3	1.79	0.15	21.00

Bivariate relationship between fallers (1) and non-fallers (0). There were not enough participants with eating disorders to generate a bivariate odds ratio. **Bolded values** indicate odds ratios with 95% C.I.s that do not include 1.00. Abbreviations: C.I.=Confidence Interval; BMI = Body Mass Index; TUG = Timed Up and Go; NMD = Neuromuscular Diseases.

\*In previous 6 years

In Model 3 age independently reduced the odds of being a faller by 8% (OR 0.93 [95% C.I. 0.87 to 0.99]). Model 3 included both muscle density and TUG test time as predictors, yet only muscle density independently reduced the odds of being a faller by 17% (OR 0.83 [95% C.I. 0.69 to 0.99]). Furthermore, when comparing Model 2 and Model 3, the addition of muscle density to a model that included TUG improved ( $X^2_1=4.46$ ,  $P=0.03$ ) the overall fit with

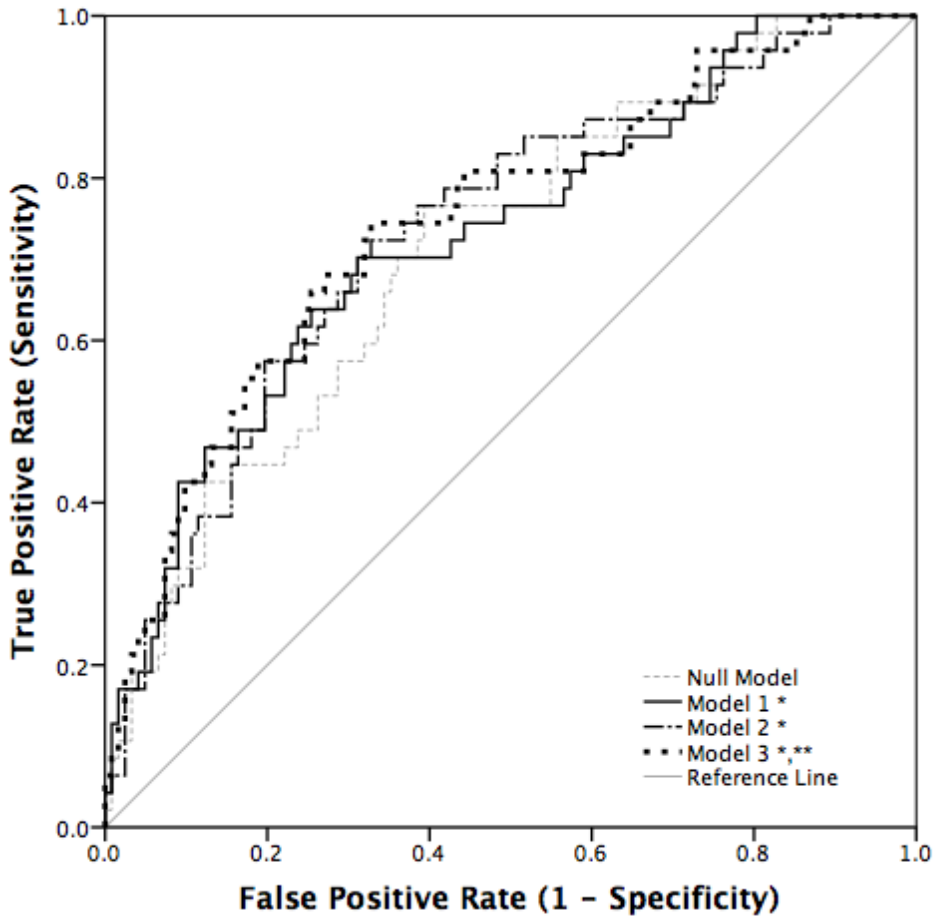


Figure 9: Receiver operating characteristic plot for multivariate models discriminating fallers (1) from non-fallers (0). Model values are the area under the curve. Null Model: age, sex, BMI, health status, diabetes, comorbidities; Model 1: Null Model + muscle density (MD); Model 2: Null Model + TUG test; Model 3: Null Model + TUG test and muscle density. Likelihood Ratio Test Significant ( $P < 0.05$ ) improvement from: \* Null Model; \*\*Model 2.

the data compared to a model with just TUG test time. The addition of TUG test time (Model 3) did not improve ( $\chi^2_1 = 2.81, P = 0.09$ ) overall fit when compared muscle density only (Model 1).

**Table 9: Multivariable Associations**

	Model AUC <sup>a</sup>	95% C.I.		Odds Ratio (e <sup>β</sup> )	(e <sup>β</sup> ) 95% C.I.	
		Lower	Upper		Lower	Upper
<b>Null Model</b>	0.71	0.63	0.80			
Age (y)				0.98	0.93	1.02
Sex (female=0)				1.91	0.84	4.36
BMI (kg/m <sup>2</sup> )				1.07	0.99	1.16
General Health Status (0-100)				<b>0.97</b>	<b>0.95</b>	<b>0.99</b>
Diabetes (no=0)				3.11	0.86	11.26
Number of Comorbidities (none=0)						
	1			1.59	0.67	3.77
	2			1.05	0.26	4.29
	3			0.91	0.06	14.96
<b>Model 1 *</b>	0.73	0.64	0.81			
Age (y)				0.94	0.88	1.00
Sex (female=0)				1.74	0.75	4.06
BMI (kg/m <sup>2</sup> )				1.05	0.97	1.14
General Health Status (0-100)				0.98	0.96	1.00
Diabetes (no=0)				2.62	0.70	9.86
Number of Comorbidities (none=0)						
	1			1.63	0.68	3.92
	2			1.11	0.26	4.73
	3			1.63	0.10	27.03
Lower Leg Muscle Density (mg/cm <sup>3</sup> )				<b>0.81</b>	<b>0.67</b>	<b>0.97</b>
<b>Model 2 *</b>	0.73	0.65	0.82			
Age (y)				0.96	0.91	1.01
Sex (female=0)				2.01	0.87	4.68
BMI (kg/m <sup>2</sup> )				1.06	0.98	1.15
General Health Status (0-100)				0.98	0.96	1.00
Diabetes (no=0)				3.03	0.81	11.28
Number of Comorbidities (none=0)						
	1			1.73	0.72	4.13
	2			0.93	0.22	3.99
	3			1.23	0.07	20.67
TUG test (s)				<b>1.17</b>	<b>1.01</b>	<b>1.37</b>
<b>Model 3 *, **</b>	0.75	0.66	0.83			
Age (y)				<b>0.93</b>	<b>0.87</b>	<b>0.99</b>
Sex (female=0)				1.85	0.78	4.37
BMI (kg/m <sup>2</sup> )				1.04	0.96	1.13
General Health Status (0-100)				0.98	0.96	1.01
Diabetes (no=0)				2.63	0.68	10.13
Number of Comorbidities (none=0)						
	1			1.75	0.72	4.25
	2			0.98	0.22	4.41
	3			1.99	0.12	33.73
Lower Leg Muscle Density (mg/cm <sup>3</sup> )				<b>0.83</b>	<b>0.69</b>	<b>0.99</b>
TUG test (s)				1.14	0.98	1.34

Multivariable models discriminate fallers (1), from non-fallers (0). **Bolded values** indicate odds ratios with 95% C.I.s that do not include 1.00. Comorbidities included the presence or recent history of diabetes, NMD, osteoarthritis, eating disorders, immobilization, or cancer. Abbreviations: AUC=Area Under Curve; L.R.T.=Likelihood Ratio Test; C.I.=Confidence Interval

<sup>a</sup>Range 0.5-1.0. Degree of discrimination: 0.7-0.8 acceptable, 0.8-0.9 excellent, 0.9-1.0 outstanding.

Likelihood Ratio Test Significant ( $P < 0.05$ ) improvement from: \* Null Model; \*\* Model 2.

## 6.5 Discussion

This is the first study to explore the relationship between muscle adiposity and fall status in older adults. The results indicate that every  $\text{mg}/\text{cm}^3$  increase in muscle density decreases the odds of being a faller by 17%, independent of age, BMI, health status, diabetes, the number of comorbidities and functional mobility. Muscle density was recently identified as a potential fall risk factor when comparing female fallers and controls matched for age, BMI and health status [53]. These results build on evidence from two clinical CT studies supporting a negative relationship between imaging-based measures of adiposity and fall-induced fractures [30,31]. Adjusting for similar covariates, Lang et al. reported an increase in hip fracture odds of 40% per SD decrease in thigh muscle attenuation independent of age, sex, BMI, muscle size, strength, physical function, and hip bone mineral density [30]. Given that less than 10% of falls result in a fracture [241], these results advance the hypothesis that muscle density is relevant to the most common mechanism of serious injury in older adults [57,58,241].

Prospective studies have established muscle adiposity as a risk factor for incident disability [26,27], reduced gait [141], mobility [24], and fractures [30,187,188]. Thus, measures of muscle adiposity can serve as adjunct biomarkers of metabolic and musculoskeletal health. The clinical relevance of imaging-based measures of muscle adiposity among other fall factors [231] such as vitamin D status, fall history, balance, visual deficits, arthritis, depression, and cognition requires further study. These results indicate pQCT-derived muscle density to have a modest multivariate association with fall status even after accounting for variability in functional mobility. The excellent precision ( $\text{CV}\%_{\text{RMS}}=0.7\%$ ) of lower leg muscle density measures in older adults [149] may facilitate the longitudinal sensitivity and clinical utility of this outcome for monitoring changes in muscle composition.

Several physical activity and exercise studies suggest muscle adiposity to be an important and modifiable biometric target in older adults [35-37,184]. Taaffe et al. reported a 5.5% increase in thigh muscle density with 12-weeks of resistance training in older community-dwelling men and women [37]. In the context of the results, a lower leg muscle density increase of half that magnitude would reduce the odds of being a faller by more than 30%. A recent randomized controlled trial demonstrated the specificity of 12-months of physical activity to reduce inter-muscular adipose, independent of total fat mass in sedentary older adults [36]. Most importantly, those physical-activity driven reductions in muscular adipose were independently associated with improved physical performance [36]. These data further emphasize the importance of muscle adiposity as a biomarker in aging musculoskeletal health.

Early identification of persons with greater muscle adiposity may improve their chances of a positive response to exercise; a recommended component for all multifactorial fall interventions [231]. Myosteatosis, “the accumulation of adipocytes within muscle” [167] and their secretion of pro-inflammatory cytokines exert pathophysiological effects which precede declines in muscular function with aging [182]. Pro-inflammatory cytokines promote peripheral insulin resistance, muscle protein catabolism [142,181], and decreased myofilament protein synthesis [181,182], increase oxidative stress, and reduce contractility and strength [142,181]. A heightened inflammatory state lowers the anabolic response to resistance exercise, making it more difficult for older adults to maintain and build muscle [181,182]. Older adult fallers with higher baseline levels of muscle adipose recently demonstrated blunted improvements in specific torque from three months of training with resistance, endurance and balance exercises [184].



The association between lower muscle density and fall status may also be a manifestation of the aging neuromuscular system. The degeneration of  $\alpha$ -motoneuron axons inhibits the reinnervation of regenerating muscle fibres [173] and is related to muscle density [46]. Denervated “orphaned” fibres have two possible fates: 1) atrophy; signaling the differentiation of fibroblasts and adipocytes [175] or 2) reinnervation by a neighbouring motor-unit; resulting in the co-expression of contractile phenotypes [174], slowing contractile velocity, and reducing force and power. Thus, motoneuron degeneration reduces muscle density [46], and the power available for high-velocity fall-avoidance tasks such as recovering balance from a trip.

The secondary objective of this investigation was to explore the independent and combined associations between muscle density and functional mobility to fall status. The TUG test is a composite measure of functional mobility [264], used to predict health decline and activities of daily living disabilities in community-dwelling older adults [278,279]. We demonstrated that the time to complete the TUG test was associated with fall status (OR 1.17, [95% C.I. 1.01 to 1.37]), independent of age, sex, BMI, health status, diabetes, and the number of comorbidities. These results agree with prospective findings in a sample of community-dwelling Britons [280] who similarly demonstrated multivariate association between TUG and fall status (OR 1.09 [95% C.I. 1.00 to 1.19]) after adjustment for age, sex, number of comorbidities and falls in the previous year. The present analysis demonstrated that the overall model fit improved with the addition of muscle density ( $P=0.03$ ), but TUG was no longer an independent predictor of fall status (OR 1.14 [95% C.I. 0.98 to 1.34]). The proximity of the confidence interval to unity, and the relatively larger precision error ( $CV\%_{RMS} = 5.4\%$ ) of TUG measures, suggests that a larger sample may demonstrate an independent effect of functional mobility. Incident disability data indicates that the effect size for

performance on TUG-like functional mobility tasks may be smaller than measures of muscle adiposity in relatively healthy older adult cohorts [26]. Given that mean faller and non-faller TUG times were faster than many of the recommended fall risk cut-points for community-dwelling older adults [281], it is also possible that a combined effect may predict fall risk in a sample with greater functional limitations and fall risk.

This study has several strengths related to sample and analysis methodology. First, we have collected data from a sub-sample of a well-described randomly selected population-based Canadian cohort [53,253]. Approximately 28% of the sample classified as fallers, similar to the estimated 29% of Canadian community-dwelling elders who experience a fall on an annual basis [213]. The TUG test results of this sub-sample are characteristic of normative reference values for normal, unimpaired older adults of this age [264]. Second, the multivariable analysis controls for several established confounding factors: age [28], sex [215], BMI [124], general health [53], and diabetes [137,167]. This study also has limitations related to the specificity of the sample and retrospective study design. First, these results were obtained from an urban sample of predominantly Caucasian older men and women. Furthermore, this voluntary sub-project ran between scheduled CaMOs study follow-up periods. Thus it is not possible to directly compare participant characteristics with their non-respondent peers. Selection bias for healthier participants is likely given that age independently reduced the odds of being a faller in some models. Furthermore, the retrospective design did not allow us to determine whether the differences observed between fallers and non-fallers preceded or followed a fall event [239]. Lower muscle densities could also be a reflection of an injurious fall event and/or subsequent muscle deconditioning due to a fear of falling. The use of retrospective fall recall can minimize the magnitude of the observed effects through misclassification. A 23% underestimation of falls

has been reported for 12-month retrospective recall methods and approximately 6% of non-fallers misclassify themselves in retrospective recall [282]. We provided the option of “unsure” on the falls questionnaire to mitigate the potential for misclassification.

Nevertheless, the presence of non-differential misclassification, or the previously reported misclassification rates would reduce effect sizes, lending further support to the results. The robustness of these results will have to be tested through further investigation of muscle adiposity using prospective fall monitoring methods.

This investigation provides further insight into the etiology of falls, by exploring the association between muscle density and fall status. The results expand upon the lower muscle density observed in female fallers [53], and supports indirect evidence of muscle adiposity as a risk factor for fall-related health outcomes such as frailty [45], fracture [30,31,187,188], hospitalization [29], and incident disability [26,27]. This research provides the impetus for the prospective study of muscle adiposity and fall risk. Muscle density is independently associated with fall status in a relatively unimpaired healthy older cohort, and the magnitude of this association is maintained after adjustment for biologically relevant covariates, as well as a measure of functional mobility. Thus, pQCT-derived muscle density may provide a physiological biomarker to further complement the assessment of musculoskeletal health and fall risk in well-functioning community-dwelling older adults.

# 7 General Discussion

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## 7.1 Summary

The overall aim of this thesis was to investigate the precision, annual changes, and longitudinal sensitivity of pQCT-derived muscle and fat outcomes, as well as explore the strength of their association with fall status in a cohort of community-dwelling older adults. The purpose of study one was to compare the precision of variations in image analysis protocols used to derive soft-tissue outcomes in older adults. This was achieved by first surveying the pQCT literature for studies that analyzed both muscle cross-sectional area and SAT, IMAT or muscle density. Protocols that were similar were sorted and given an identifier, and then each unique method was applied to a data set of repeat scans from 35 female CaMOs participants. The results of study one rejected the null hypothesis. Significant differences were detected among the precision errors for soft-tissue outcomes derived from each of the unique image analysis protocols. Precision errors appeared higher in the forearm than the lower limb, and varied considerably with the outcome. Muscle density provided precision error rates below 1%, whereas the precision error for IMAT was over 42%. Subtle differences existed in the precision of each of the methods applied. Protocols using the manufacturer's recommendations appeared to experience more errors segmenting muscle from fat and bone when compared to third-party protocols and software. The precision error results corroborated with previously reported IMAT precision errors as high as 40% in middle-aged women [93]. This was the first study to conduct a comparison of previously reported image analysis protocols. Furthermore, only two other studies [90,151] reported pQCT-derived soft-tissue precision data for older adults, and only did so for some of the outcomes reported in this thesis. The results of study one provided the evidence necessary to select the most precise image analysis protocols for the prospective analysis of soft-tissue outcomes. This is important because the precision error is multiplied by a factor of 2.77

when assessing whether or not an observed change exceeds the error rate of a measurement tool with 95% confidence; and annual age-related changes in both DXA and CT measures of muscle mass and area have previously proven to be less than 1% [28,268-270].

Study two expanded on the results of study one, and focused on determining the annual changes and longitudinal sensitivity of pQCT-derived soft tissue outcomes by collecting and analyzing scans over one and two years. It was hypothesized that significant annual changes would be observed in pQCT soft-tissue outcomes. Statistically significant annual changes of -0.8 and -1.2% per year were observed in the muscle areas of the forearm and lower leg respectively. These findings were of a similar magnitude to declines reported in CT-derived muscle area and DXA-derived lean tissue mass for Italian and Japanese women, and showed a similar trend of greater changes in lower body muscles [268-270]. Significant annual changes were not detected for muscle density, SAT or IMAT, despite reports of changes in these soft-tissue parameters with aging [28,113,169,197]. Clinical CT studies that have demonstrated significant differences in IMAT tracked participants for 3 to 5 years [28,197], which may partially explain the failure to detect an effect in pQCT-derived IMAT and muscle density in study two. Using both the precision data for soft-tissues and the annual changes observed, study two estimated MTIs wherein researchers could be either 80 or 95% certain that measured changes in muscle and fat tissues exceeded the limitations of both machine and operator error. These follow-up intervals can assist the planning of longitudinal studies that observe soft-tissue changes with pQCT. The more liberal MTI is an estimate wherein a clinical researcher could be 80% certain that observed changes are real biological effects and not an anomaly; whereas the more common 95% MTI provides an estimate of when observed changes would be statistically certain in 50% of the population [59]. Liberal

MTIs could be useful when monitoring clinically relevant trends that could influence the decision on whether or not a treatment or intervention should begin or be discontinued; situations where the benefits of pre-emptive action outweigh those of scientific certainty [59]. Simply having musculoskeletal imaging measures conducted can motivate people to modify their behaviour for better health [112]. The aim of estimating MTIs is to establish the most appropriate follow-up, so as to reduce measurement risk and cost burdens on both participants, clinics and/or laboratories [59]. The results of study two suggest that decreases in forearm and lower leg muscle area may be detected in 6 to 9 and 4 to 6 years respectively. Furthermore decreases in lower leg muscle density might also be detectable within 5 years. These estimates are likely conservative given the good physical function profile and potential for selection bias in this sample. Furthermore, in an attempt to generate estimates that characterize healthy aging; chronic conditions that can accelerate changes in muscle and fat outcomes were excluded from this analysis. Sub-populations with diabetes, neuromuscular disease, malnutrition, prolonged bed-rest or cancer will likely exhibit different (and likely shorter) MTIs. Although there are many MTIs reported for DXA and pQCT-derived bone outcomes [41,254], this thesis provides the first estimates generated for pQCT imaging based measures of muscle and fat outcomes in relatively healthy older adults.

Lastly, study three involved an investigation of the bivariate and multivariable associations between fall status, pQCT-derived muscle density, and functional mobility. Study three correctly hypothesized that muscle density would be independently associated with fall risk after adjusting for biologically relevant covariates and functional mobility. The results demonstrated that for every unit increase in pQCT-derived muscle density (mean 70.2, SD 2.6mg/cm<sup>3</sup>), the odds of having reported a fall dropped by 17% after adjusting for biologically relevant factors, and functional mobility performance. These results are similar to

odds ratios reported by Lang et al. [30,31] who observed a 40% decrease in other odds of hip fracture per SD increase in thigh muscle attenuation. Although every fall does not result in a hip fracture, falls are the primary mechanism by which older adults experience non-violent hip fractures [57]. This investigation provides further insight into the etiology of falls, by exploring the strength of the association between muscle density and fall status. Research suggesting a relationship between muscle power, strength, and muscle adiposity [23,211] as well as the importance of lower body muscle strength in fall risk [239], may explain the mechanism that determines the association observed in study three. These results expand upon earlier research that reported lower muscle density in female fallers [53], and supports indirect evidence of muscle adiposity as a risk factor for fall-related health outcomes such as frailty [45], hip fracture [30,31], hospitalization [29], and incident disability [26,27]. The secondary hypothesis of study three was that models that included both muscle density and TUG would better fit the data than models that included them separately. This hypothesis was true when comparing the combined fit with a model that only included TUG and covariates, but was rejected when comparing the combined fit with muscle density. Put simply, muscle density improved the prediction of fall status after accounting for the variance explained by functional mobility, but improvements were not observed when adding functional mobility to models that already contained muscle density. Although the relative improvement in model fit is small, this result supports the prospective investigation of the predictive power and multivariate associations of muscle density and functional testing to falls in older adults.



## **7.2 Strengths and Limitations**

This research has several strengths and limitations. Among its strengths, the data were collected from a sample of relatively high-functioning community-dwelling older adult men and women. This sample is clinically relevant, because it can help us understand and prevent outcomes that may lead to a loss of independence, such as falling and fall-induced injuries. The use of common functional tests, such as the TUG and isometric hand grip strength, allowed for a comparison of this sample with age-appropriate reference values for healthy older adults [264,275]. This improved the ability to describe the participants in a functional context, and may also facilitate the pooling of data with other researchers. Lastly, the pQCT image analysis protocol utilized for this thesis was selected based on a comparison of the precision of all available options. As such, these results present estimates for pQCT derived muscle area, density, and SAT area in older adult women using the most precise protocol available.

There are several limitations of this thesis related to study design. These studies were part of a local subproject of a much larger national, multi-centre study. CaMOs participants volunteered to be involved in this research between their regular 5-year follow-up intervals for the CaMOs Study. As such, maintaining low participant burden was a significant design consideration that limited the number and complexity of outcomes measured with each participant's appointment. Study two could have benefitted from the collection of behavior-modifying comorbidity (i.e., arthritis, peripheral artery disease) and nutrition data when interpreting changes in soft-tissue outcomes over time. CaMOs questionnaires for self-reported physical activity and simple functional tests such as handgrip strength and the TUG were utilized to measure behavior and physical function over time. The inclusion of a more detailed health condition questionnaire, objective measures of physical activity, and direct

measures muscle function may have yielded a more complete picture of the observed soft-tissue changes. In study three, falls were ascertained via retrospective questionnaires to avoid participant fatigue and confusion, but are best recorded prospectively on calendars, diaries, or via regular telephone follow-ups. More detailed, prospective falls data would have facilitated analyses beyond the baseline measures, and investigations of recurrent fallers (who are more likely to have functional deficits). Lastly, because the baseline year (2010) for this sub-project did not coincide with CaMOs follow-up intervals, it is not possible to directly compare the participants in this thesis project with their non-responding cohort peers. Had this sub-project been nested, it would have been interesting to compare the groups on 16 years of prospective health data to describe and assess the degree of health/survivor bias in the sub-sample.

### **7.3 Conclusions**

In conclusion, this thesis demonstrated that pQCT imaging can provide precise measurements of muscle and fat outcomes in older adults. This is important for the study of muscular health and aging because the annual rate of change in these tissues can be quite low in older adults. Thesis data suggest a relative loss of muscle cross-sectional area of 0.8 to 1.2% per year, with greater losses in the lower limb. Assuming that muscle tissues are not negatively impacted by other health conditions, biological changes can be detected with 80 and 95% certainty within 4 to 9 years follow-up time. This data can assist with the planning of prospective research investigating the complex interactions between muscle, fat, and human health in aging. Lastly, this thesis demonstrated the strength of the association between one of these soft-tissue outcomes (muscle density) and falls, suggesting a 17% increase in odds of reporting a fall for every unit decrease in muscle density (mean 70.2, SD 2.6mg/cm<sup>3</sup>).

## **7.4 Future Directions**

The clinical importance of the association between fall status and muscle density will need to be studied prospectively, and could be an interesting area of discovery. This thesis provides the methodological and effect-size data necessary to design a prospective investigation of this phenomenon using pQCT imaging. Currently, nutrition and physical exercise interventions are known to maintain [35] or improve muscle mass and physical function [7,32,165,166] and reduce muscle adiposity [36,37], increase insulin sensitivity [32,283], and reduce the number of falls and fall risk [241]. Very few studies provide a direct analysis of muscle adiposity and muscle torque [23,211] or power [211], even though there is considerable health outcome data (incident disability, reduced gait, mobility) indirectly suggesting a possible connection [24,26,27,141]. Future research that analyzes direct measures of human behaviour (i.e., multiphasic measures of activity), muscle function (i.e., muscle power) with anatomical (i.e., diffusion tensor imaging of muscle structure, measures of muscle size and adiposity), and physiological properties (i.e., mitochondrial energetics, inflammatory markers) will strengthen our understanding, and compliment research connecting changes in this organ with age.

As we continue to study the etiology and prevention of sarcopenia and myosteatosis, tools that can provide further insight into the physiology of aging muscle and fat are needed [38,40,113,114]. More effective interventions will be possible through an enhanced understanding of the antecedents, natural history, and magnitude of changes in muscle loss and adipose infiltration observed with age [28,167]. Some of the data collected for this thesis was shared with a cross-disciplinary team of CaMOs Study investigators who have established a network of pQCT scanners in cities across Canada, and have been pooling imaging data with the goal of investigating the associations and development of muscle

atrophy and adiposity, frailty, and fractures in aging Canadians. Furthermore, the Canadian Health Measures Survey recently acquired two pQCT scanners to collect musculoskeletal health data on Canadians as part of Statistics Canada's nationwide health survey. The relative affordability and ability of pQCT to provide precise, tissue-specific estimates of adipose content and muscle size make it a compelling tool that could compliment MRI in the next generation of muscle, fat, and bone health research. There is growing musculoskeletal research interest in the interactions between muscle, fat and bone [284]. Muscle and bone tissues have long been known to interact biomechanically [285,286]. Recent studies of the coordination of shared paracrine and endocrine signals in the development, aging, and injury of these tissues has spurred new research directions in muscle, bone and fat cross-talk, as well as integrated approaches to developing treatments for sarcopenia and osteoporosis [284].

Pharmacological interventions are now turning their focus to the manipulation of myostatin levels and the neuromuscular junction [166] to reverse the effects of aging on muscle [32]. Relevant to these targets, pQCT has been an effective tool for the study of appendicular muscle size and adiposity with respect to muscle torques [126], in relation to motoneuron atrophy [46], as well as spinal cord injury [287] diabetes [48,93,288], frailty [45], development of disability [27], fall risk [53], and fractures [54]. By no means a new tool, pQCT may still have an important role to play in our understanding of metabolic and neuromuscular health in aging.

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# Appendix A

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*Copies of Human Biomedical Research Ethics Approval*



# Certificate of Approval

PRINCIPAL INVESTIGATOR Saija Kontulainen	DEPARTMENT Kinesiology	Bio # 10-83
---	---------------------------	----------------

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT  
College of Kinesiology  
87 Campus Drive  
Saskatoon SK S7N 5B2

STUDENT RESEARCHERS  
Juliegh Clark, Andrew Frank, Megan Labas

SPONSORING AGENCIES  
UNIVERSITY OF SASKATCHEWAN

TITLE: Do pQCT Derived Muscle Cross Sectional Area and Muscle Density Differ Between Multiple Fallers and Non-Multiple Fallers?

ORIGINAL REVIEW DATE 06-May-2010	APPROVED ON 17-May-2010	APPROVAL OF Researcher's summary (10-May-2010) (15-Mar-2010) Research Participant Information and Consent Form(15-May-2010) Appendix B Fall, Medication History, Limb Dominance and Activity Questionnaire Appendix C RAND SF-36 Questionnaire Instrument Letter to Participant (1) Letter to Participant (2) Short questionnaire confirmation letter	EXPIRY DATE 16-May-2011
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Delegated Review:  Full Board Meeting:

### CERTIFICATION

The study is acceptable on scientific and ethical grounds. The Bio-REB considered the requirements of section 29 under the Health Information Protection Act (HIPA) and is satisfied that this study meets the privacy considerations outlined therein. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research study, and for ensuring that the authorized research is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved protocol or consent process.

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Gordon McKay, Ph.D., Vice-Chair  
University of Saskatchewan  
Biomedical Research Ethics Board

Please send all correspondence to:

Research Ethics Office  
University of Saskatchewan  
Box 5000 RPO University  
1607 - 110 Gymnasium Place  
Saskatoon, SK Canada S7N 4J8



# Certificate of Approval

PRINCIPAL INVESTIGATOR Saija Kontulainen	DEPARTMENT Kinesiology	Bio # 12-209
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INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT  
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 103 39 23rd St E                      87 Campus Drive

Saskatoon SK S7K 0H6                      Saskatoon SK S7N 5B2

SUB-INVESTIGATOR(S)  
Wojciech P. Olszynski, Jonathan Adachi

STUDENT RESEARCHER(S)  
Andrew Frank, Andy Kin-On Wong

FUNDER(S)  
CANADIAN INSTITUTES OF HEALTH RESEARCH (CIHR)

TITLE  
Protocol : Canadian Multicentre Osteoporosis Bone Quality Study (CaMos-BQS)

ORIGINAL REVIEW DATE 10-May-2012	APPROVED ON 01-Jun-2012	APPROVAL OF Application for Biomedical Research Ethics Review (Rec'd 14-May-2012) Participant Information and Consent Form for women 60-75 years (Version 1.0, dated 01-May-2012) Participant Information and Consent Form for women 76-85 years (Version 1.0, dated 11-May-2012) Participant Information and Consent Form Pilot (Version 1.0, dated 11-May-2012) Fall, Medication History, Limb Dominance and Activity Questionnaire (Appendix B) RAND SF-36 Questionnaire Instrument (Appendix C) Miniature Mental Status Examination (Appendix D) Demographic and Medical History Questionnaire (Appendix E) Confirmation Letter for Participant Participant Invitation Letter to CaMos Participants Email Invitation for participant recruitment Poster	EXPIRY DATE 31-May-2013
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Delegated Review:  Full Board Meeting:

**CERTIFICATION**

The study is acceptable on scientific and ethical grounds. The Bio-REB considered the requirements of section 29 under the Health Information Protection Act (HIPA) and is satisfied that this study meets the privacy considerations outlined therein. The principal investigator has the responsibility for any other administrative or regulatory approvals that may pertain to this research study, and for ensuring that the authorized research is carried out according to governing law. This approval is valid for the specified period provided there is no change to the approved protocol or consent process.

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PRINCIPAL INVESTIGATOR  
Saija Kontulainen

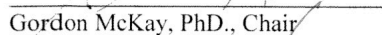
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In respect to clinical trials, the University of Saskatchewan Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Part 4 of the Natural Health Products Regulations and Division 5 of the Food and Drug Regulations and carries out its functions in a manner consistent with Good Clinical Practices. Members of the Bio-REB who are named as investigators, do not participate in the discussion related to, nor vote on such studies when presented to the Bio-REB. This approval and the views of this REB have been documented in writing. The University of Saskatchewan Biomedical Research Ethics Board has been approved by the Minister of Health, Province of Saskatchewan, to serve as a Research Ethics Board (REB) for research projects involving human subjects under section 29 of The Health Information Protection Act (HIPA).

  
Gordon McKay, PhD., Chair  
University of Saskatchewan  
Biomedical Research Ethics Board

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# Appendix B

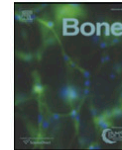
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*Measurement of Muscle and Fat in Postmenopausal Women: Precision of Previously Reported pQCT Imaging Methods. As Published in the Journal Bone.*



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## Technical Note

## Measurement of muscle and fat in postmenopausal women: precision of previously reported pQCT imaging methods

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## ABSTRACT

Peripheral quantitative computed tomography (pQCT) imaging has been used to quantify muscle area and density as well as intermuscular adipose tissue (IMAT) and subcutaneous adipose tissue (SAT) area in the lower and upper limb. Numerous protocols have been reported to derive these soft-tissue outcomes, but their precision has not been assessed in community-dwelling postmenopausal women. The objective of this study was to compare the precision of previously reported analysis protocols for quantifying muscle area and density, as well as IMAT and SAT area in postmenopausal women.

Six image analysis protocols using two available software suites (Stratec XCT, BoneJ) were identified from the pQCT literature. Analysis protocols were applied to a sample of 35 older female adults (mean age 73.7; SD 7.2 years), randomly selected from a population based-cohort and scanned twice within an average of 9.7 (SD 3.6) days. Relative precision was calculated as absolute values and as a percentage of the sample mean (root mean square coefficient of variation; CV<sub>RMS</sub>). Soft-tissue outcomes across protocols were compared on their log-transformed coefficients of variation using multilevel linear models and Tukey contrasts.

For most protocols, CV<sub>RMS</sub> for muscle area, density, and SAT area ranged between 2.1 and 3.7%, 0.7 and 1.9%, and 2.4 and 6.4%, respectively. Precision for IMAT area varied considerably, from 3 to 42%. Consideration of these study results will aid in the selection of appropriate image analysis protocols for pQCT-derived soft-tissue outcomes in postmenopausal women.

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## Introduction

For nearly 20 years, peripheral quantitative computed tomography (pQCT) has been used to precisely measure and study volumetric density and distribution of bone mineral tissue [1,2]. In more recent years, pQCT has also been used to quantify muscle area [3,4], muscle density [5–13], subcutaneous adipose tissue (SAT) area [14–16], and intermuscular adipose tissue (IMAT) area [10,17]; all valuable measures for the study of musculoskeletal health. These measures are important given increased interest in the study of soft-tissues as a factor in bone development and health [9,18,19], as well as for studying the development of diabetes [10,13,20], sarcopenia [7,21], falls [22], and frailty [5].

Although pQCT is proving to be a useful tool for measurement of muscle and fat, there is limited information regarding precision (i.e., repeatability) of muscle and fat measures in older adults. To date, precision errors for pQCT-derived muscle area, density, IMAT and SAT area have been reported for children (ages 7–12 y) [9,15], young

(26.6, SD 8.7 y) [23] and premenopausal women (38.6, SD 4.7 y) [10]. Prior to a recent muscle area and density paper by Wong et al. [24], only lower leg muscle area and SAT precision values had been reported for postmenopausal women [25]. Both lower leg IMAT and forearm soft-tissue data have yet to be reported for older adults—a population with unique soft-tissue morphometry and a clinically relevant risk of muscle loss [21,26] and fat infiltration [6,27].

In addition to limited muscle and fat precision information, previous studies reporting precision errors used different image analysis protocols. Employed protocols have included manufacturer recommendations (Stratec Medizintechnik GmbH, Pforzheim, Germany) [28,29], customized manufacturer's protocols [12,19,30,31], or third-party software (eg. Bonalyse, BoneJ) [5,7,32,33], each with specific segmentation approaches capable of influencing precision (e.g., contour detection algorithms, noise reduction filters, grayscale intensity thresholds). It is currently unknown which previously reported analysis protocols are most appropriate for precisely characterizing muscle area, density, and fat area in older adults. This is important because the most precise analysis techniques are required for detection of small effect sizes in soft-tissue outcomes. For example, an annual increase of 6.5% in CT-derived IMAT was reported in weight-stable older adult women [27].

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# Appendix C

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*Calculations for Muscle and Fat Outcomes from Study One*

The detailed steps of the Stratec XCT and BoneJ analysis protocols for muscle density and area, as well as SAT and IMAT area are provided below.

#### A) *Stratec XCT*

Stratec XCT was intended for bone outcomes and therefore the output will always be stated in bone terms (i.e. cortical area, content, and density); however, the values are only representative of the tissue corresponding to the thresholds employed. Modifying the analysis thresholds to reflect soft tissues (i.e. -40 or 40mg/cm<sup>3</sup>) is a common feature of most XCT soft tissue analyses.

#### Filtering

Stratec XCT median filters F03, F05, U01 and U04 are used. Every voxel within a filter's density range is modified to the median value of the NxN mask. Filter F03 is a 3x3 mask that acts on voxels in the density range of -500 to 500mg/cm<sup>3</sup>, F05 a 5x5 mask with a range of -500 to 300mg/cm<sup>3</sup>, and U01 a 7x7 mask with a range of -300 to 3000mg/cm<sup>3</sup>. The U04 filter is a custom-made 3x3 median filter with a range of 120 to 2000mg/cm<sup>3</sup> and is available from Bone Diagnostic Inc. [249].

**Appendix Table 1: Stratec XCT Image Analysis Loop Settings**

Method	Outcomes	Analysis Step #	ROI	Threshold	Inner Threshold	Contour Mode	Peel Mode	Cortical Threshold	Inner Cortical Threshold	Separation Mode	Filters 2
XCT I	Muscle Area, Density, SAT Area	1	Entire Matrix	-40		3	1				F03F05F05
		2	Entire Matrix	40		3	1				F03F05F05
		3	Entire Matrix	280		1	2				F03F05F05
XCT II	Muscle Area, Density	1	Entire Matrix	40		3	1				F03F05
		2	Entire Matrix	280		1	2				F03F05
XCT III	Muscle Area, Density	1	Entire Matrix	40		1	1				F03F05
		2	Manual Trace T/R	280		1	2				
		3	Manual trace F/U	280		1	2				
XCT IV	Muscle Area, Density, SAT Area, IMAT	1	Entire Matrix	-100	40	3	2	149	40	4	F03F05F05
		2	Entire Matrix	40	40	31	2	710	40	4	F03F05F05
		3	Entire Matrix	-100	40	3	2	710	-100	4	
		4	Entire Matrix	40	40	1	2	-100	2000	4	U01U01U01U04
XCT V	Muscle Density	1	Manual trace Muscle	-100		1	2				
		2	Manual Trace T/R	-100		1	2				
		3	Manual trace F/U	-100		1	2				

ROI = Region of Interest; T/R = Tibia/Radius; F/U =Fibula/Ulna; Manual Trace indicates that the ROI was traced around the tissue by a technician prior to analysis, this is in contrast to methods that use the entire image matrix and rely exclusively on a threshold-driven analysis to segment tissues.

**Calculations:**

Abbreviations refer directly from Stratec's XCT output, but reflect tissues corresponding to the thresholds applied in Appendix Table 1:

TOT\_A = Total Area (mm<sup>2</sup>)

TOT\_CNT = Total Content (mg/mm)

CRTSUB\_A = Cortical and Subcortical Area (mm<sup>2</sup>)

CRTSUB\_DEN = Cortical and Subcortical Density (mg/cm<sup>3</sup>)

CRT\_A = Cortical Area (mm<sup>2</sup>)

CRT\_DEN = Cortical Density (mg/cm<sup>3</sup>)

TRAB\_A = Trabecular Area (mm<sup>2</sup>)

For all calculations "#1-4" denote the analysis step # listed in Appendix Table 1.



## Method XCT I

### SAT Area

**#1 TOT\_A** is skin, SAT, muscle, IMAT, bone and marrow area (mm<sup>2</sup>)

**#2 TOT\_A** is muscle, IMAT, bone and marrow area (mm<sup>2</sup>)

Equation:

$$(\mathbf{\#1\ TOT\_A} - \mathbf{\#2\ TOT\_A}) * 0.01\text{cm}^2/\text{mm}^2 = \mathbf{SAT\ (cm}^2\mathbf{)}$$

### Muscle Area

**#2 TOT\_A** is muscle, IMAT, bone and marrow area (mm<sup>2</sup>)

**#3 TOT\_A** is bone and marrow area (mm<sup>2</sup>)

Equation:

$$(\mathbf{\#2\ TOT\_A} - \mathbf{\#3\ TOT\_A}) * 0.01\text{cm}^2/\text{mm}^2 = \mathbf{Muscle\ Area\ (cm}^2\mathbf{)}$$

### Muscle Density

**#2 TOT\_CNT** is muscle, IMAT, bone and marrow total content (mg/mm)

**#3 TOT\_CNT** is bone and marrow total content (mg/mm)

Equation:

$$(\mathbf{\#2\ TOT\_CNT} - \mathbf{\#3\ TOT\_CNT}) * 0.1\text{cm}/\text{mm} = \mathbf{Muscle\ Content\ (mg/cm)}$$

$$\mathbf{Muscle\ Content\ (mg/cm) / Muscle\ Area\ (cm}^2\mathbf{) = Muscle\ Density\ (mg/cm}^3\mathbf{)}$$

## Method XCT II

### Muscle Area

**#1 TOT\_A** is muscle, IMAT, bone and marrow area (mm<sup>2</sup>)

**#2 TOT\_A** is bone and marrow area (mm<sup>2</sup>)

Equation:

$$(\mathbf{\#1\ TOT\_A} - \mathbf{\#2\ TOT\_A}) * 0.01\text{cm}^2/\text{mm}^2 = \mathbf{Muscle\ Area\ (cm}^2\mathbf{)}$$

### Muscle Density

**#1 TOT\_CNT** is muscle, IMAT, bone and marrow content (mg/mm)

**#2 TOT\_CNT** is bone and marrow total content (mg/mm)

Equation:

$$(\mathbf{\#1\ TOT\_CNT} - \mathbf{\#2\ TOT\_CNT}) * 0.1\text{cm}/\text{mm} = \mathbf{Muscle\ Content\ (mg/cm)}$$

$$\mathbf{Muscle\ Content\ (mg/cm)} / \mathbf{Muscle\ Area\ (cm}^2\mathbf{)} = \mathbf{Muscle\ Density\ (mg/cm}^3\mathbf{)}$$

## Method XCT III

### Muscle Area

**#1 TOT\_A** is muscle, IMAT, bone and marrow area (mm<sup>2</sup>)

**#2 TOT\_A** is tibia or radius bone and marrow area (mm<sup>2</sup>)

**#3 TOT\_A** is fibula or ulna bone and marrow area (mm<sup>2</sup>)

Equation:

$$(\mathbf{\#1\ TOT\_A} - (\mathbf{\#2\ TOT\_A} + \mathbf{\#3\ TOT\_A})) * 0.01\text{cm}^2/\text{mm}^2 = \mathbf{Muscle\ Area\ (cm}^2\mathbf{)}$$

### *Muscle Density*

**#1 TOT\_CNT** is the total content of tissues that are not SAT (mg/mm)

**#2 TOT\_CNT** is the tibia or radius total content (mg/mm)

**#3 TOT\_CNT** is the fibula or ulna total content (mg/mm)

Equation:

**(#1 TOT\_CNT - (#2 TOT\_CNT + #3 TOT\_CNT)) \* 0.1cm/mm = Muscle Content (mg/cm)**

**Muscle Content (mg/cm) / Muscle Area (cm<sup>2</sup>) = Muscle Density (mg/cm<sup>3</sup>)**

### **Method XCT IV**

Method IV attempts to correct values that may be distorted by the presence of positive and/or negative movement artifacts. Positive movement artifacts are distortions of the voxels that cause the density to be higher than they should be, and negative lower. If the artifact exceeds a tissue threshold, it can impact the soft-tissue area results. Furthermore skin is removed from SAT area.

### *SAT Area*

**#1 TRAB\_A** is the skin, SAT, marrow and negative movement artifact area (mm<sup>2</sup>)

**#2 TRAB\_A** is the marrow and negative movement artifact area (mm<sup>2</sup>)

**#4 CRT\_A** is the skin area (mm<sup>2</sup>)

**#3 CRT\_A** is the bone and marrow area (mm<sup>2</sup>)

**#2 CRT\_A** is the bone area (mm<sup>2</sup>)

Equation:

**(#1 TRAB\_A - #2 TRAB\_A - #4 CRT\_A - (#2 TRAB\_A - (#3 CRT\_A - #2 CRT\_A)))**

**\*0.01cm<sup>2</sup>/mm<sup>2</sup> = SAT (cm<sup>2</sup>)**

### Muscle Area

#2 CRTSUB\_A is the muscle, IMAT and bone area (mm<sup>2</sup>)

#2 CRT\_A is the bone area (mm<sup>2</sup>)

#2 TRAB\_A is the marrow and negative movement artifact area (mm<sup>2</sup>)

#3 CRT\_A is the bone and marrow area (mm<sup>2</sup>)

Equation:

$$(\#2 \text{ CRTSUB\_A} - \#2 \text{ CRT\_A} + (\#2 \text{ TRAB\_A} - (\#3 \text{ CRT\_A} - \#2 \text{ CRT\_A})))$$

$$*0.01\text{cm}^2/\text{mm}^2 = \text{Muscle Area (cm}^2\text{)}$$

### Muscle Density

#2 CRTSUB\_DEN is the muscle, IMAT, and bone density (mg/cm<sup>3</sup>)

#2 CRTSUB\_A is the muscle, IMAT and bone area (mm<sup>2</sup>)

#1 CRT\_A is the bone and positive movement artifact area (mm<sup>2</sup>)

#1 CRT\_DEN is the bone and positive movement artifact density (mg/cm<sup>3</sup>)

Equation:

$$((\#2 \text{ CRTSUB\_DEN} * (\#2 \text{ CRTSUB\_A} / \#1 \text{ CRT\_A})) - \#1 \text{ CRT\_DEN}) / ((\#2 \text{ CRTSUB\_A} -$$

$$\#1 \text{ CRT\_A}) / \#1 \text{ CRT\_A} = \text{Muscle Density (mg/cm}^3\text{)}$$

### IMAT Area

#3 TRAB\_A is the SAT, IMAT, marrow, negative movement area (mm<sup>2</sup>)

#1 TRAB\_A is the skin, SAT, marrow, negative movement area (mm<sup>2</sup>)

#4 CRT\_A is the skin area (mm<sup>2</sup>)

Equation:

$$(\#3 \text{ TRAB\_A} - \#1 \text{ TRAB\_A} + \#4 \text{ CRT\_A}) * 0.01\text{cm}^2/\text{mm}^2 = \text{IMAT Area (cm}^2\text{)}$$

## Method XCT V

### Muscle Area

**#1 TOT\_A** is the manually traced muscle, IMAT, bone and marrow area (mm<sup>2</sup>)

**#2 TOT\_A** is the manually traced tibia or radius bone and marrow area (mm<sup>2</sup>)

**#3 TOT\_A** is the manually traced fibula or ulna bone and marrow area (mm<sup>2</sup>)

Equation:

$$(\#1 \text{ TOT\_A} - (\#2 \text{ TOT\_A} + \#3 \text{ TOT\_A})) * 0.01 \text{cm}^2/\text{mm}^2 = \text{Muscle Area (cm}^2\text{)}$$

### Muscle Density

**#1 TOT\_CNT** is the manually traced muscle, IMAT, bone and marrow area content (mg/mm)

**#2 TOT\_CNT** is the manually traced tibia or radius bone and marrow content (mg/mm)

**#3 TOT\_CNT** is the manually traced fibula or ulna bone and marrow content (mg/mm)

Equation:

$$(\#1 \text{ TOT\_CNT} - (\#2 \text{ TOT\_CNT} + \#3 \text{ TOT\_CNT})) * 0.1 \text{cm}/\text{mm} = \text{Muscle Content (mg/cm)}$$

$$\text{Muscle Content (mg/cm)} / \text{Muscle Area (cm}^2\text{)} = \text{Muscle Density (mg/cm}^3\text{)}$$

## **B) BoneJ (Method VI)**

The source code [255] and description of the BoneJ outcomes can be obtained online [252]. No additional calculations necessary. BoneJ abbreviations are defined below.

**SubCutFatA [cm<sup>2</sup>] = SAT Area**

**MuA [cm<sup>2</sup>] = Muscle Area**

**MuD [mg/cm<sup>3</sup>] = Muscle Density**

**IntraFatA [cm<sup>2</sup>] = IMAT Area**

BoneJ can analyze a batch of images with the appropriate macro, reducing the analysis time. We have appended the text, and instructions for use of the batch macro from our analysis on the following page.

### **BoneJ Image Batch Analysis Macro**

Copy the code on the following page into the ImageJ Macro Text Editor Found in:

Plugins > New> Macros >

- 1) Make sure the text in the macro editor matches the text below.
- 2) Replace the file directory and save directory paths with the paths to folders on your computer. (Of note, the example file path is for a Macintosh OS, Windows users will need to use the appropriate file path for their system; i.e. C:\filefolder\)
- 3) Hit Save

To Run:

Plugins> Macros> Select saved macro.txt file

```

macro "Batch Soft Tissue Analysis"{
    setBatchMode(true);

    sourceDir = "/Users/imagefolder/"; //Replace this path with
the path to the folder containing Stratec \.M01 or 02, 03, etc."
image files on your computer

    visualDir = "/Users/imageexport/"; //Replace this path with
the path to where you want to save the visualization images on
your computer

    parameterString = " air_threshold=-40.0 fat=40.0
muscle_threshold=40.0 marrow_threshold=80
soft_tissue_threshold=140 rotation_threshold=169.000
area=550.0000 bmd=690.0000 roi_selection=Bigger
soft_tissue_roi_selection=Bigger
rotation_selection=According_to_Imax/Imin
analyse_density_distribution analyse_soft_tissues
suppress_result_image set_distribution_results_rotation_manually
manual_rotation_[+-_180_deg]=90.0000
save_visual_result_image_on_disk image_save_path="+visualDir;

    files = getFileList(sourceDir);
    for (i = 0; i<files.length;++i){
        showProgress(i+1, files.length);
        run("Stratec pQCT", "select="+sourceDir+files[i]);
        run("Distribution Analysis", parameterString);
        close();
    }
    setBatchMode(false);
}

```

# Appendix D

---

*Example: Calculating of Relative ( $CV\%_{RMS}$ ) and Absolute ( $SD_{RMS}$ ) Precision Error*



1		A	B	C	D	E
2	Participant 1	Measure 1	Measure 2	Mavg	$\Delta$ mean	$\Delta$ mean <sup>2</sup>
3	Participant 2	25.5	26.1	25.81	-0.63	0.39
4	Participant 3	30.2	31.9	31.07	-1.70	2.89
5	Etc.,	18.8	20.8	19.80	-2.01	4.03
6		...	...	...	...	...
7			Average	25.56	<b>SD<sub>RMS</sub></b>	1.10
					<b>CV%<sub>RMS</sub></b>	4.32

Gluer  
Equation #  
[118]

C2-5 AVERAGE(A2:B2)

C6 AVERAGE(C2:C5)

D2-5 (A2-B2)

E2-5 D2<sup>2</sup>

4b

E6 (SD<sub>RMS</sub>) SQRT(SUM(E2:E5)/(2\*COUNT(E2:E5)))

5

E7 (CV%<sub>RMS</sub>) (E6/C6)\*100

# Appendix E

---

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## Figure 2: Komolka et al. [122]

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---

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MS

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Steffen

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Best regards,

---

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Figure 3: Lang et al. [30]



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Figure 5: Blew et al. [108]

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