THIGH FAT AND PHYSICAL FUNCTION IN KNEE OSTEOARTHRITIS

THE RELATIONSHIP OF THIGH MUSCLE COMPOSITION AND FAT WITH MUSCLE POWER AND PHYSICAL FUNCTION IN WOMEN WITH KNEE OSTEOARTHRITIS

By

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Abstract

The aim of this study was to investigate the relationship between thigh intramuscular fat (intraMF) and intermuscular fat (IMF) with quadriceps and hamstrings power and physical performance in women (n=20) with clinical, radiographic knee osteoarthritis (OA). Secondarily, we investigated the correlation between thigh and calf fat volumes, and the agreement between 3.0T and 1.0T MRI for quantifying fat. The thigh and calf of the symptomatic leg were scanned using 3.0T MRI with the IDEAL sequence, and fat separated images were analyzed using semi-automated software to quantify intraMF, IMF and muscle. The calf was also scanned using 1.0T MRI with a Fast Spin Echo (FSE) sequence. Knee extensor and flexor isokinetic power was measured at 20% and 40% of individuals' maximum voluntary isometric contraction (MVIC) torque, and surface electromyography (EMG) measured activation. We found no relationship between quadriceps or hamstrings intraMF and knee extensor or flexor power, respectively. In addition, there were no relationships between intraMF and performance-based tests. There was a correlation between thigh and calf intraMF ($r=0.759$; $p=0.001$) and a trend toward a correlation in IMF ($r=0.436$; $p=0.055$). There was agreement and a correlation between calf intraMF ($r=0.779$; $p=0.001$) and IMF ($r=0.956$; $p=0.001$) using 3.0T and 1.0T MRI. There is disagreement about the relationship of intraMF and quadriceps strength, although studies have found that intraMF is related to decreased physical performance. The importance of calf fat subsets in physical performance of individuals with OA should be further investigated. Power analysis demonstrated a sample size (n=91) is recommended for future investigations of intraMF and power in OA.

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List of Abbreviations

AP: Anteroposterior
CSA: Cross-Sectional Area
EMG: Electromyography
FSE: Fast Spin Echo
FWR: Full-Wave Rectification
GPS: Global Pain Scale
ICC: Inter-class Correlation Coefficient
ICOAP: Intermittent and Constant OsteoArthritis Pain
IDEAL: Iterative Decomposition of water and fat with Echo Asymmetry and Least-squares estimation
IMF: Intermuscular Fat
IntraMF: Intramuscular Fat
JSN: Joint Space Narrowing
KAM: Knee Adduction Moment
K-L: Kellgren-Lawrence Grade
KOOS: Knee injury and Osteoarthritis Outcome Score
MCII: Major Clinically Important Improvement
MDC: Minimum Detectable Change
MRI: Magnetic Resonance Imaging
MVIC: Maximum Voluntary Isometric Contraction
mWORMS: Modified Whole-Organ Magnetic Resonance Imaging Score
NPRS: Numeric Pain Rating Scale
OA: Osteoarthritis
PASE: Physical Activity Scale for the Elderly
pMRI: Peripheral MRI
PURE: Phased array Uniformity Enhancement
Q/H: Quadriceps/Hamstrings ratio
RER: Rate of EMG Rise
RMS: Root-Mean-Square
SCAT: Subcutaneous Adipose Tissue
SCIC: Surface Coil Intensity Correction
sEMG: Surface Electromyography
TKA: Total Knee Arthroplasty
TUG: Timed Up-and-Go
WOMAC: Western Ontario and McMaster Osteoarthritis Index
6MWT: 6-Minute Walk Test
9SCT: 9 step Stair Climb Test
30s CST: 30 second Chair Stand Test
40m FPWT: 40 meter Fast-Paced Walk Test

Declaration of Academic Achievement

This thesis is the primary authorship of the Master of Science candidate, Michael Davison, who was responsible for the execution of the research study detailed within. Data presented in this thesis was collected during the Fall-Winter of 2013-2014. As primary author, his contributions include: study design, ethics approval, literature review, participant recruitment and consent, booking study appointments, data collection during participant visits, data analysis, and manuscript preparation. The research findings detailed within are intended for future publication in peer-reviewed scientific journals.

Supervisors and important contributors to this study include: Dr. Jonathan Adachi, the thesis supervisor who funded this study and oversaw the Master's student's progress; Dr. Karen Beattie, who provided expertise for the design of the study objectives and methodology, particularly involving MRI acquisition and analysis; Dr. Monica Maly, who provided resources and expertise for the biomechanical and electromyographical acquisition and analysis; and Drs. George Ioannidis and Peter Keir who provided guidance and expertise for the statistical and electromyographical analyses.

Other notable contributors include: Sandani Hapuhennedige, Bachelor of Science student, who helped establish the lower leg MRI SliceOmatic® analysis methods and assisted Michael Davison with participant data collection; and Amie Kron, Bachelor of Science student, who analyzed and scored participant questionnaires, assisted with participant recruitment, and helped establish a study database of participant data.

Chapter One: Muscular Strength and Physical Function in Osteoarthritis

Osteoarthritis (OA) is a degenerative joint disease that is among the most common musculoskeletal disorders globally, currently affecting over 4.4 million Canadians and projected to affect over 10 million Canadians in 30 years (1). Knee OA reduces quality of life by increasing pain, immobility and the inability to participate in activities of daily living (2). The onset and cause of knee OA are uncertain. Major risk factors have been identified which include ageing, obesity, family history of the disease, and severe joint trauma (3).

Weakness of the thigh muscles, particularly the quadriceps, is a risk factor for knee OA (4). Thigh muscle weakness in knee OA manifests as a decrease in the strength, or peak torque output, of the quadriceps or hamstrings. This deficit in strength in individuals with knee OA resembles the reduction in muscle mass, quality and function with aging known as sarcopenia (5). Alteration in neuromuscular activation patterns also contribute to the decline in muscle strength found in aging and knee OA (6). Decreased quadriceps strength is associated with increased pain, increased cartilage loss at the patellofemoral joint, and decreased physical function (7, 8).

The knee extensors, particularly the rectus femoris (RF), produces a moment to counter the external knee adduction moment (KAM) applied to the joint due to ground reaction forces during gait, thereby stabilizing the joint (9). Knee flexors, mainly the hamstrings, also contribute to a stabilizing moment. Indeed, co-contraction of the quadriceps and hamstrings allowed for larger KAM and reduced medial joint

compression during gait (10). Therefore, it is important to understand the factors that contribute to muscle weakness in aging, and particularly in the accelerated decline in thigh muscle strength exhibited in knee OA.

1.1 Quadriceps and Hamstrings Strength in OA

In individuals with knee OA, declines in muscle strength and function resemble an accelerated version of the sarcopenia seen in healthy aging. Evidence suggests that, compared to healthy aging, strength deficits are larger in the quadriceps than hamstrings, resulting in a lower quadriceps-to-hamstrings (Q/H) strength ratio in people with this disease (11). Quadriceps dysfunction in knee OA includes reduced maximal isometric, concentric and eccentric strength, an inability to control submaximal force, and reduced proprioception (12-14). Evidence suggests that hamstrings dysfunction is also related to knee OA. Tan et al. (1995) found that maximum peak torque deficits were exhibited in the quadriceps and hamstrings of patients with radiological and symptomatic knee OA compared to controls, and concluded that both muscle groups should be the focus of rehabilitation strengthening (15). Fisher and Pendergast (1997) found that men and women with knee OA had lower quadriceps and hamstrings endurance (force-time relationship), lower maximum isometric strength, and greater difficulty and pain in activities of daily living compared to controls (16). On the other hand, Aaboe et al. (2011) found that a relatively high hamstring strength was associated with a high KAM in obese patients with knee OA, and may be a factor in disease progression (17). A review by Alnahdi et al. (2012) concluded that in individuals with knee OA quadriceps and

hamstrings isometric strength were reduced by 10-56% and 4-35%, respectively (18). Therefore, it is important to understand the roles of both the quadriceps and hamstrings in knee OA.

There is uncertainty as to whether thigh muscle weakness is caused by knee OA, or precedes disease onset and is a factor in OA incidence and progression. Slemenda et al. (1998) found that knee extensor strength was 15-18% lower in women who later developed incident knee OA compared to age-matched controls, indicating that reduced knee extensor strength may be a risk factor for OA (19). Furthermore, in a longitudinal study of muscle strength and OA incidence, baseline quadriceps strength, but not Q/H strength ratio, protected against symptomatic knee OA at 30-month follow-up (20, 21). However, quadriceps strength did not protect against radiographic knee OA, based on the Kellgren-Lawrence (K-L) system of grading knee joint deformity including osteophytes, joint space narrowing (JSN) and bone sclerosis (20). Conversely, Sharma et al. (2003) found that greater quadriceps strength at baseline did not prevent knee OA progression, and in malaligned and lax knees, greater strength actually increased the likelihood of tibiofemoral OA progression (22).

The role of thigh muscle weakness in disease progression has also been investigated. Diraçoglu et al. (2009) found that isokinetic knee extensor and flexor strength was lower in individuals with knee OA compared to healthy controls, and that patients with stage II radiological OA had significantly lower isokinetic strength than those with stage I OA (23). However, in men and women with bilateral frequent knee pain, no cross-sectional difference was found between knees with medial JSN and

contralateral knees without JSN in quadriceps specific strength, or in 2-year changes in thigh muscle cross-sectional area (CSA) and strength. Thus, while thigh muscle weakness is clearly demonstrated in individuals with symptomatic knee OA, there is controversy concerning its role in radiological disease initiation and progression.

Quadriceps and hamstrings strength play an important role in self-reported and objective performance-based physical function in individuals with knee OA. Isometric peak knee extension torque was related to performance on several tests including the 5-minute walk test, 20-meter walk test, Timed Up-and-Go (TUG), and stair ascent/descent in men with knee OA (24). Maly et al. (2005) found that quadriceps isokinetic peak torque was related to performance of the 6-minute walk test and TUG and that hamstrings isokinetic peak torque was related to performance on a stair-climbing task, in individuals with radiographic knee OA (25). Quadriceps and hamstrings strength were also significantly related to high self-reported physical function using subscales of the Western Ontario McMaster Universities Osteoarthritis Index (WOMAC) and Short-Form Health Survey, in individuals with knee OA (26).

Recent findings suggest that muscle power (W or N*m/s) may better predict performance of physical function than maximum isometric torque. In assessing functional ability in individuals after total knee arthroplasty (TKA), normalized leg press muscle power, or the product of the torque and velocity, was a better predictor of physical performance, determined using a 10m fast walking test and 30s chair stand test, than normalized knee extensor strength (27). Similarly, Berger et al. (2012) found that knee extensor isotonic power at lower loads was a better predictor of physical function than

maximum voluntary isometric torque (28). Furthermore, high-velocity resistance training resulted in increased performance in the TUG and greater ultrasound echo intensity, a measure of muscle composition or contractile to adipose tissue content, of the gluteus maximus compared to low-velocity strength training, in individuals with hip OA (29). These findings suggest that both the velocity and magnitude of muscle force are important factors in muscle dysfunction in OA. Importantly, failure of quadriceps neuromuscular activation was an important mediator of the relationship between quadriceps weakness and low objective and self-reported physical function in individuals with knee OA (30). Thus, thigh muscle strength and power are important factors in the physical function of individuals with knee OA, but other factors including muscle mass and composition, as well as neuromuscular function of the thigh must also be considered.

1.2 Lean Muscle Mass

Lean muscle mass is a well-known factor contributing to muscle strength. Aging adults experience a loss of lean muscle mass and an associated decline in strength and function (3). On average, total muscle CSA declines by 40% between the ages of 20 and 80. Using ultrasound to determine CSA, Young et al. (1985) demonstrated that there is a 25% and 33% reduction in quadriceps size in healthy men and women, respectively, in their 70s compared to those in their 20s (31, 32). Using computed tomography (CT), Rice et al. (1989) found that men aged 65-90 not only had 28-36% smaller limb muscles, but also had 81% more non-muscle adipose and connective tissue comprising the plantar flexors compared to men aged 25-38 (33). Overend et al. (1992) found that while total

thigh CSA was not different in men aged 65-77 compared to men aged 19-34, the older men had significantly smaller (26.4%) quadriceps muscles (34). In a 12-year longitudinal study of the effect of aging on muscle mass, Frontera et al. (2000) found that the CSA of all thigh muscles declined by approximately 15% and that the knee extensors and flexors were significantly weaker in older men with a baseline age of 65.4±4.2 years compared to their younger counterparts (35). Associated with this decline in muscle mass is an increase in incident mobility limitations in the elderly (36, 37).

Knee OA has been associated with declines in thigh lean muscle mass beyond that typically seen with healthy aging. Using magnetic resonance imaging (MRI) of the thigh, Hart et al. (2012) found that individuals with patellofemoral joint OA had smaller vastus medialis (VM) and RF muscle volumes compared with controls (38). In individuals with structurally progressive knee OA, there was a greater decline in quadriceps CSA over 2 years than in individuals with non-progressive knee OA (39). Berry et al. (2008) found that increased VM CSA, but not vastus lateralis (VL) CSA, was associated with increased risk of patella cartilage defects and increased patella bone volume in healthy women (40). Conversely, evidence suggests that declines in thigh lean mass in individuals with knee OA are consistent with expected declines in healthy aging. Beattie et al. (2012) found that quadriceps muscle volume, measured using MRI, was not different between women with and without radiographic knee OA (41). Similarly, using CT scans of the thigh, Conroy et al. (2012) found no significant difference in quadriceps CSA in individuals with radiographic knee OA compared to those without radiographic knee OA (42). However, this sample had significantly lower muscle quality, or strength per unit lean mass, as

determined by decreased quadriceps specific torque. Thus, while decreased muscle strength is apparent in individuals with knee OA, reduced muscle mass may only be one of several factors which are important in this functional decline.

Lean muscle mass alone cannot explain age and OA-related declines in strength. Goodpaster et al. (2006) found that in older men the loss of leg strength is three times greater than the loss of leg muscle mass over a three year period, assessed using dual x-ray absorptiometry (43). Gür and Cakin (2003) found that quadriceps and hamstrings CSA explained only 24-61% or 38-51% of variation in concentric and eccentric peak torques, respectively (44). This indicates that while there is an age-related loss of muscle mass, there is also an age-related decline in the contractile quality of this muscle. Therefore, factors such as neuromuscular activation and muscle composition, which affect the quality of muscle, must be important in aging and OA-related strength deficits.

1.3 Neuromuscular Function

Changes in muscle activation and coordination patterns play a role in weakness and functional decline exhibited in aging individuals and those with OA. In women with tibiofemoral OA, quadriceps muscle weakness was found independent of knee pain or lean muscle mass, suggesting that weakness in OA may be the result of other factors, of which neuromuscular dysfunction could be one (19). This may have implications for disease initiation and progression, as inefficient muscle activation patterns may increase joint loading, impair motor control, and thus further cartilage damage.

Deficits in neuromuscular activation, including motor unit recruitment and rate coding or the rate of neuronal action potentials, are associated with healthy aging and sarcopenia. This decline in neuromuscular activation with aging includes a reduced maximal motor unit firing rate which is related to deficits in muscle torque production (45). Laroche et al. (2007) measured rate of EMG rise (RER), or the greatest rate of change in the integrated EMG signal during the period corresponding to the positive steep slope on the force versus time curve, and found that it was related to increased rate of torque production during visually cued, maximum voluntary isometric contraction (MVIC) knee extension in older women (46). Decline in quadriceps RER was also associated with decline in leg press power production at 70% of individuals' 1-repetition maximum over 3 years in older adults (47). This decline in RER may occur even when maximum sEMG amplitude is preserved, and is most apparent in older individuals with mobility impairments (48, 49). Thus, along with the peak amplitude, the rate of motor unit activation may be an important factor in age-related deficits in quadriceps strength.

In a comparative study of strength training in young and old men, an increase in peak amplitude of activation was the primary source of strength gains in older men across the entire 8 week program (50). The reduction in activation peak amplitude with age may be due to an inability to voluntarily activate the muscle. Stevens et al. (2003) found that healthy older adults have an 11% decrease in voluntary activation, defined using the central activation ratio which compares MVIC force production before and after the superimposition of an electrical current to an active muscle, compared to young adults (51). As such, neuromuscular activation appears to play an important role in age-related

strength deficits. With the accelerated decline in strength and function exhibited in knee OA, it may be that deficits in neuromuscular activation are also accelerated beyond those typically seen in the aged.

Numerous studies have investigated the relationship between weakness and neuromuscular activation in knee OA. Knee OA and joint trauma are associated with the inhibition of voluntary quadriceps activation, termed arthrogenic muscle inhibition (AMI). Petterson et al. (2008) found that decreased voluntary quadriceps activation, determined using burst superimposition, and lean muscle CSA both contribute to quadriceps MVIC strength deficits in individuals with OA (52). Importantly, although lean muscle CSA explained the most variance in strength in contralateral non-diseased limbs, voluntary activation explained the most variance in strength of muscles supporting joints with OA.

Conversely, Berger et al. (2012) concluded that variance in quadriceps MVIC torque and isotonic power were predominantly due to changes in muscle volume and not voluntary activation, measured using the interpolated twitch technique, in individuals with knee OA (28). Nonetheless, deficits in neuromuscular activation in individuals with knee OA are widely reported, and interventions which improve the strength of the diseased limb also improve its voluntary activation. Patients with knee OA who underwent TKA had a baseline deficit in voluntary activation, measured using twitch superimposition, of the quadriceps which was partially improved following surgery compared to healthy, age-matched controls (53). Similarly, patients with unilateral knee OA showed baseline deficits in quadriceps voluntary activation and MVIC strength,

which were partially improved following rehabilitation compared to their non-diseased limb (54). This inhibition of voluntary muscle activation in knee OA has implications for physical function and disease progression, particularly with respect to gait patterns.

Using EMG of the leg muscles, researchers have identified alterations in neuromuscular activation patterns in individuals with knee OA during gait. As stated earlier, external knee joint loading during gait occurs due to ground reaction forces. During gait, forces in the tibiofemoral joint are nearly three times an individual's body mass (55). The thigh musculature is partly responsible for counteracting this loading at the knee (56). Buchanan and Lloyd (1997) found that activation of the RF, the long head of the biceps femoris (BF) and the lateral gastrocnemius were important in producing these stabilizing external knee abduction forces (57). Alterations in knee OA include a preference for activation of the lateral quadriceps and hamstrings muscles during stance phase, and increased co-activation of the medial quadriceps and hamstrings during weight-bearing phase (58, 59). This increased co-activation, particularly of the hamstrings but also of the gastrocnemius, may be a means of mitigating the higher external KAM associated with the disease (60). Experimentally induced paralysis of the quadriceps increased the ground reaction loading rate during the heel strike phase of gait, supporting the notion that proper quadriceps function is essential for mitigating articular joint damage (61).

While these muscle-generated forces are believed to stabilize the joint, quadriceps and hamstrings co-activation may also subject the knee cartilage to additional joint contact forces (9). This relationship between co-activation and disease progression may

be due to strength imbalances between the quadriceps and hamstrings, as a sample of individuals with knee OA had greater weakness in the quadriceps than hamstrings (11). Aaboe et al. (2011) found that high MVIC peak torque of the hamstrings, but not the quadriceps, was associated with high peak KAM in obese individuals with knee OA (17). In malaligned or lax knees, baseline high quadriceps strength was associated with an increased likelihood of disease progression after 18 months (22). Thus, while adequate thigh muscle strength is essential for protecting the knee joint, the relative strength of the quadriceps and hamstrings, as well as body mass and knee alignment are important considerations that may modify the relationship between thigh strength and disease progression. The proper neuromuscular activation of these muscles is essential in generating joint stabilizing forces during gait. Thus, dysfunction of the thigh musculature which manifests as decreased activation and strength plays an important role in knee OA.

1.4 Pain

Lower limb pain plays a major role in OA-related disability and physical function. It is the most frequently reported symptom experienced by individuals with knee OA, and OA is hypothesized to be the primary cause of the high prevalence of regional joint pain in older adults (62). After initiation of the disease process, knee pain elicited during physical activity has a severe effect on quality of life and can lead to physical inactivity (63). Decreased physical activity is associated with loss of muscle strength and joint stability. Furthermore, physical exercise aimed at improving strength and stability also decreases knee pain (64). Dos Santos et al. (2014) found that high pain intensity,

measured using the WOMAC pain scale, was related to low self-reported physical function and the root-mean-square (RMS) slope of sEMG of the VM, in patients with knee OA (65). Knee pain and symptoms have also been specifically associated with isometric strength. Ruhdorfer et al. (2014) found that limbs with symptomatic OA had 11-13% and 7-16% reduced knee extensor and flexor isometric strength, respectively, compared to non-symptomatic limbs (12). A combination of quadriceps and hamstrings strengthening exercises over 12 weeks improved perceived knee pain and range of motion, and decreased functional performance limitations, providing further evidence of the association of muscle weakness and pain in older adults with knee OA (66).

Pain may also be a factor in the decreased neuromuscular activation found in knee OA. Park and Hopkins (2013) found that saline injection-induced pain in the infrapatellar fat pad of patients caused a 34% reduction in MVIC knee extension torque and a 5% reduction in quadriceps central activation ratio, compared to controls (67). A similar study of induced knee pain found that the onset of VM obliquus EMG was delayed relative to the VL, and normalized VL EMG amplitude was decreased in individuals experiencing knee pain compared to controls, while ascending and descending stairs (68). Thus, neuromuscular activation is a factor in the decreased knee extensor strength and function related to knee pain. This cycle of loss of strength and physical function, combined with worsening symptoms and physical inactivity must therefore be targeted to mitigate the process by which knee OA leads to disability.

Chapter Two: Thigh Muscle and Fat Composition in Osteoarthritis

2.1 Muscle Fiber Quantity and Quality

Various changes in the quality of muscle fibers, including contractility and fiber type composition, have been associated with lower limb strength declines in the aged. These changes suggest a reduced ability of aging muscle to produce high force and high velocity contractions and thus may have negative implications for physical function in older adults.

Numerous changes in muscle composition have been reported in the aged. There is an age-related decrease in muscle protein synthesis with no change in muscle protein degradation, particularly in an environment of high body fat (69). This suggests an age-related decline in muscle repair due to decreased 'recycling' of proteins, which may affect muscle fiber contractility. Furthermore, Larsson et al. (1983) found that the loss of lean muscle mass with age is characterized by a preferential decrease in Type II glycolytic muscle fibers (70). Type II fibers contract faster and produce greater tension than Type I fibers, so a preferential decline in these fast-twitch fibers may explain some of the strength and power losses seen with age. More recently, Houmard et al. (1998) contested this finding by showing that there were no changes in muscle fiber type composition in the VL or gastrocnemius in older compared to younger men (71). Similarly, Purves-Smith et al. (2014) suggest that in severely atrophied, aging muscle, fibers co-express myosin heavy chain isoforms making it difficult to properly quantify type I and II fibers (72).

Individual fibers undergo qualitative changes with age. The shortening velocities and specific tensions of Type I and IIa heavy myosin chain fibers were decreased in men ages 73-81 compared to men ages 25-31 (73). Frontera et al. (2008) found that while there was a reduction in lean muscle CSA in the anterior thigh, there was no change in fiber type distribution over 12 years in men with a baseline age of 71.1±5.4 years (74). Furthermore, there was an increase in Type IIa fiber diameter and a trend toward an increase in maximal force in both fiber types. They concluded that in atrophied muscle, the remaining fibers may attempt to compensate for age-related declines in lean mass and strength. However, this compensation is not enough to mitigate the declines in fiber quantity with age.

Differential atrophy between muscle fiber types has been investigated in older individuals with knee OA. Biopsy of VM specimens from 68% of individuals with knee OA undergoing TKA demonstrated a preferential decrease in Type II muscle fibers (75). The authors hypothesized that Type II fiber atrophy in patients with OA was due to pain-related immobilization of the limb. Similarly, 73.1% of women with knee OA demonstrated preferential atrophy of Type II fibers during biopsy of the VL, compared to only 6.3% of women who fractured without OA (76). However, Terracciano et al. (2013) found that muscle atrophy in individuals with OA was homogenous between fiber types and related to the duration of the disease (77). Thus, the precise changes in muscle fiber quality and quantity in knee OA remain controversial. Declines in the contractile quality of muscle fibers with age may be related to other age-related changes, such as increased muscle adiposity.

2.2 Muscle Adiposity

Fat mass is an important factor in muscle quality, or strength per unit lean mass, and physical function of older individuals. As individuals age, they experience a loss of lean mass with a concomitant increase in infiltrating adipose tissue in the muscle. In support of this, functional decline has been identified in obese individuals, despite these individuals having greater lean mass. Vilaça et al. (2014) found that elderly obese women had decreased absolute strength during a 1-repetition maximum of knee extension, and performance on the 6-minute walk test (6MWT), compared to healthy age-matched controls (78). Villareal et al. (2004) found decreased muscle quality despite greater lean mass in obese elderly individuals with frailty, compared to non-obese frail, and healthy elderly individuals (79). Furthermore, obesity had a significant interaction with muscle quality in its relationship to objective physical performance (80). Obesity may also have implications in the incidence of sarcopenia, as declines in muscle quality with age are exacerbated by the presence of obesity (81). Adipose tissue must therefore be partially responsible for this decrease in strength and muscle quality in obese frail individuals.

An increasing amount of literature is describing the phenomenon of 'sarcopenic obesity,' in which obesity and frailty work synergistically in the elderly to increase disability (82). The link may be due to adipokines such as tumor necrosis factor and interleukin-6, which are produced by adipose tissue and have a catabolic effect on lean muscle (83). Thus, a cycle of frailty and obesity may exist in the inactive elderly which leads to physical function limitations. A recurring issue in these investigations is the lack of a standard definition for sarcopenic obesity, and indeed for non-obese sarcopenia. Scott

et al. (2014) found that baseline "dynapenic obesity", or decreased muscle strength concurrent with obesity, but not sarcopenic obesity was associated with increased falls risk after 5 years, compared to non-dynapenic, non-obese elderly individuals (84). Among octogenarians, leg strength, arm strength, agility, walking speed and balance each predicted risk of sarcopenic obesity (85). Auyeung et al. (2013) found that the ratio of total body fat to lower limb muscle mass predicted 4-year incident physical limitations in men and women over 65 years of age. In men, this relationship only existed when the ratio was equal to or greater than 0.75, whereas for women this relationship existed across all values (86).

Concurrent with this change in body composition with age is a change in muscle composition, or the amounts of lean tissue and adipose tissue within a muscle. Using CT, Overend et al. (1992) found that elderly men had 59% and 127% greater non-muscle tissue in the quadriceps and hamstrings muscle, respectively, compared to young men (34). This non-muscle infiltrate within the quadriceps and hamstrings includes connective tissue and adipose tissue, and thus may provide a link between obesity and muscle quality. Obesity has also been linked to knee OA through a combination of biomechanical and physiological factors related to fat mass. Thus, there is interest in whether adipose tissue plays a role in the decreased quadriceps strength exhibited in knee OA.

As individuals with knee OA exhibit deficits in thigh muscle strength, fat mass may play a role in the concurrent loss of physical function associated with the disease. Emerging evidence suggests that the quadriceps weakness found in individuals with knee OA is partly due to increased fat mass. Slemenda et al. (1998) found that there was a

negative correlation between body mass and knee extensor strength in older women who developed knee OA (19). Particular fat subsets have been identified which may be responsible for this relationship between high fat mass and low strength in knee OA. Infiltration of adipose tissue within the fascia and surrounding muscle bellies, called intermuscular fat (IMF), has been implicated in OA-related physical function deficits. Individuals with radiographic knee OA have larger volumes of IMF in the thigh, greater total fat mass, larger thigh muscle CSA, and reduced muscle quality compared to individuals without radiographic knee OA (87). Furthermore, Maly et al. (2013) found that IMF, measured using T1-weighted MRI of the thigh, explained a small amount of variance in knee extensor strength and the repeated chair stand test in women with or at risk of knee OA (88). Beattie et al. (2012) found that changes over 2 years in quadriceps muscle and IMF volumes in the thigh, assessed using MRI, were similar between women with and without radiographic knee OA (41). While these results suggest that the relationship between IMF volume and knee OA may be small, another fat subset may play an essential role in the loss of physical function with aging and OA.

Emerging evidence suggests that intramuscular fat (intraMF), or adipose tissue infiltration within a muscle belly, plays an important role in strength deficits in the elderly, and may provide rationale for the marked physical function deficits observed in sarcopenic obese individuals. Segal et al. (2011) found a positive relationship between BMI and intraMF between groups categorized as <30 kg/m^2, 30-35 kg/m^2 and >35 kg/m^2, in both men and women with knee OA (89). Using histochemical analysis of a VL biopsy, intracellular lipid droplets were twice as abundant in the muscle fibers of obese

compared to non-obese individuals (90). A muscle's attenuation coefficient determined using CT scan provides an indirect measure of intraMF by estimating the density of the muscle in Hounsfield units, with lower attenuation indicating a higher proportion of infiltrating adipose tissue (91). Goodpaster et al. (2001) identified that intraMF and IMF in the thighs of obese patients could be reduced with weight loss, and that although these tissues make up a small proportion of total body fat, they are related to insulin resistance whereas subcutaneous adipose tissue (SCAT) is not (92). A relationship between intraMF and the specific force, or force per muscle CSA, has also been identified in the healthy elderly. Goodpaster et al. (2001) found in the Health ABC Study cohort that age was associated with decreased muscle attenuation, and that quadriceps and hamstrings specific force was highest in elderly individuals among the highest quartile for muscle attenuation (93). Therefore, thigh intraMF appears to be related to the loss of muscle quality and physical function with age, and may be a factor in the impaired quadriceps and hamstrings function exhibited in knee OA.

Thigh intraMF is related to the symptomatic and structural progression of knee OA. Kumar et al. (2014) found that individuals with radiographic knee OA had a greater quadriceps intraMF fraction compared to individuals without OA, and that intraMF fraction was associated with aging, worse K-L score, and cartilage and meniscus lesions (94). However, Segal et al. (2011) found that the sum of intraMF and IMF CSA and CT muscle attenuation did not differ between individuals with and without knee OA, and that peak quadriceps strength did not differ based on BMI group (89). Due to the limitation of measuring intraMF using indirect CT muscle attenuation, which includes non-adipose

connective tissue, intraMF has not been able to be accurately quantified to fully understand its relationship with muscle quality. Thus, it is necessary to further investigate the role of thigh intraMF and IMF in the muscle quality, strength and physical function of individuals with knee OA using noninvasive methods that can accurately quantify these fat subsets.

Chapter Three: Imaging of Fat and Muscle

3.1 Computed Tomography and Magnetic Resonance Imaging

Many studies focusing on muscle composition have used CT scanning to indirectly measure muscle attenuation, or the proportion of lean muscle to non-muscle tissue. However, the main drawback of CT is radiation exposure. Furthermore, other imaging modalities may provide a more direct measurement of fat tissue embedded in muscle. The use of MRI for quantifying fat also has limitations, including chemical-shift artifacts between water and fat-based tissue, which can distort images (95). The chemical-shift encoded, or three-point Dixon, technique in MRI has been developed to overcome this issue by creating separate water and fat images based on the differing resonant frequencies of these molecules. Images are then reconstructed to provide fat separation, and this technique is resistant to main field inhomogeneities.

The iterative decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL) method is a derivative of the Dixon technique which has been applied to whole-body MRI and produces reliable fat separated images (96). The IDEAL sequence has been successfully applied to investigate fatty infiltration into muscle. In patients with degenerative lumbar kyphosis, IDEAL MRI allowed for quantification of intraMF in the paraspinal muscles and revealed a reduction in muscle composition in these patients compared to healthy controls (97). This approach has also been used to quantify lingual intraMF deposits with a high degree of accuracy (98). Using the IDEAL sequence, Nardo et al. (2014) found that intraMF fraction of the rotator cuff

muscles was positively related to shoulder pain and range of motion (99). This evidence points not only to the uses of the IDEAL sequence in quantifying intraMF, but also the role of intraMF in physical function limitations. Recently, Kumar et al. (2014) applied the IDEAL method to determine that quadriceps intraMF fraction is related to structural and symptomatic progression in knee OA (94). Application of the IDEAL MRI method provides an accurate means to quantify thigh muscle intraMF, and may further the understanding between intraMF and muscle quality in individuals with knee OA.

3.2 Study Outline

The quadriceps and hamstrings strength deficits in older individuals with knee OA appear to be related to numerous variables, including the lean muscle mass, neuromuscular activation, and volumes of IMF and intraMF in the thigh. Due to the importance of quadriceps and hamstrings function in knee OA initiation and progression, and their roles in mobility, activities of daily living, and OA-related knee pain, it is important to understand the effect of fatty infiltration in these muscles on muscle power, as it relates to physical function.

3.2.1 Relationships of Muscle Power to IntraMF and IMF

For the first time, this study aims to determine the cross-sectional relationship between intraMF and IMF in the quadriceps and hamstrings and these muscles' peak power, while controlling for the neuromuscular activation in women with symptomatic, radiographic knee OA. We hypothesize that greater thigh intraMF, and IMF to a lesser

extent, will be related to decreased quadriceps and hamstrings muscle peak power. These findings will provide evidence of the role of these fat subsets in the quadriceps and hamstrings impairment seen in individuals with knee OA, independent of neuromuscular activation. As neuromuscular activation contributes to strength deficits in knee OA, controlling for this variable will enhance our understanding of the role of tissue composition on muscle function. By explaining the factors involved in OA-related strength deficits, better strategies can be devised to promote muscle strength in people with knee OA. Ultimately this work aims to prevent physical function limitations and disability in individuals with or at risk of knee OA.

3.2.2 Relationships of Calf and Thigh IntraMF and IMF

Secondarily, we aim to determine if there is a relationship between intraMF in the thigh and calf, as calf intraMF may also play an important role in the decline in physical function with age and in knee OA. We hypothesize that a strong, positive correlation exists between IMF and intraMF in the calf and thigh. As calf intraMF has not been investigated, and proper function of the gastrocnemius in particular is essential for gait and its role in OA progression, the relationship between thigh and calf intraMF may be of functional importance in these individuals. Our findings will provide an estimate of the relationship between IMF and intraMF in the upper and lower leg, and may direct future research in investigating the functional consequences of this adipose tissue in the leg.

3.2.3 Relationships of Fat Imaged using 3.0T MRI IDEAL and 1.0T MRI FSE

Lastly, measurement of intraMF volumes in the thigh and calf using an IDEAL sequence with whole-body 3.0T MRI will be compared with the measurement of calf intraMF volume using a FSE sequence with peripheral 1.0T MRI to compare the quality of analyses using these different scanners. As the IDEAL sequence is fat separated and provides high spatial resolution, allowing for greater contrast between adipose and muscle tissue, we hypothesize that while there will be a moderate correlation and agreement between intraMF and IMF measured using each scanner.

This research will advance our understanding of the role of fat in the strength and physical function in individuals with knee OA. A greater understanding of the contribution of all factors, including lean muscle mass, pain, neuromuscular activation, muscle composition and adiposity, to functional outcomes and disability in knee OA will provide the foundation for strategies to improve these individuals' physical function and quality of life.

Chapter Four: Study Methodology

4.1 Study Sample

This study includes a sample of women (n=20) between the ages of 56-76 with rheumatologist-diagnosed, radiographic and symptomatic knee OA consistent with the criteria set out by the American College of Rheumatology (Appendix A) (100). This age range was chosen due to the high prevalence of primary OA among women over 55 years of age. Women older than 76 years were excluded due to the strenuous physical activity required for the muscle power measurement (101). Exclusion criteria included contraindications to MRI (e.g., pacemaker); inability to sign informed consent; diagnosis with any other form of arthritis (e.g. rheumatoid, psoriatic); active non-arthritic knee disease (e.g. gout); conditions that might be exacerbated by the study (e.g. previous heart attack or stroke); neurological impairment of the lower limb; current or past knee surgery; use of a cane or walking aid for ambulation; injury to the hip, knee or ankle in the past three months; and pregnancy. Participants who met the inclusion criteria were asked to identify their most painful knee. If this painful knee also showed radiographic signs of OA then the leg was identified as the study leg for our analyses.

Patients attending a rheumatology clinic were invited to review the study purpose, methods, risks and benefits with a Master's student, Michael Davison, outside their circle of care. Interested participants were screened by the Master's student, Michael Davison, and signed informed consent upon enrollment (Appendix D). The study ethics and scientific rationale were reviewed and approved by the Hamilton Integrated Research

Ethics Board (HIREB) in accordance with their criteria. The imaging protocol and scientific rationale were also approved by the St. Joseph's Healthcare Brain-Body Institute's Imaging Research Centre (IRC). All data was collected using a Case Report Form (Appendix C). This is an exploratory study to investigate the relationship between intraMF and muscle power, using MRI with the IDEAL sequence for the first time in patients with knee OA. The data collected from this study will inform investigators for a future longitudinal study investigating changes in muscle power and intraMF volume in the thighs of a larger sample of women and men with knee OA.

4.2 Imaging Protocol

X-rays were obtained of the osteoarthritic knee using a fixed flexion standing radiograph in the anteroposterior (AP) view (102). Radiographs were used to diagnose radiological knee OA by trained medical radiologists in combination with the rheumatologists' clinical diagnosis.

We performed 3.0T MRI of the thigh and lower leg of participants in order to quantify SCAT, IMF, intraMF and muscle volumes. The St. Joseph's Healthcare IRC uses a Discovery MR750 3.0T scanner with software version 23.1 (General Electric Healthcare, Milwaukee WI). Specifications for the 3.0T MRI knee and torso coils can be found in Table 4.1. Prior to entering the MRI, participants filled out a questionnaire concerning their history of surgery, medical implants and other potential magnetic hazards such as tattoos and piercings (Appendix B). Participants were asked to remove any jewelry and accessories, and changed into a cotton hospital gown. Using the whole-

body 3.0T MRI, IDEAL images for the thigh and lower leg were collected separately. For the thigh scan, participants were positioned in the scanner with a torso array coil overlying the thigh region of interest. As such, the coil covered from their chest to approximately the knee joint space. Participants placed their arms either at their side or resting on top of the coil. Padding was placed under the knee to raise the thigh above the scanning bed to prevent anatomical distortion or flattening of the thigh. For some participants, placing additional padding under the lower back assisted with elevating the thigh and with comfort during the scans. The participants' feet were placed in an elevating block with two grooves to fit their heels, and in some cases padding was placed beneath the lower leg near the ankle for support. The scanning region of interest was distal from the 70% site on the line from the tibial spine/knee joint space to the lesser trochanter, and was labelled with a vitamin E tablet (Figure 4.1). The thigh MRI parameters were as follows: WATER:AX PD IDEAL, axial plane, 512x512 matrix, 75 slices, 280 mm display field of view, 3mm slice thickness, slice gap 0, TE=31.512msec, TR=2000msec, echo train length=6, bandwidth=195.3kHz, 1 NEX, and scan time 7.25 minutes. Calf and thigh 3.0T MRI were performed at the St. Joseph's Healthcare IRC in Hamilton, Ontario by a certified imaging technologist (MRT).

Table 4.1 Specifications of the 1.0T pMRI, as well as the torso and knee coil used with the Discovery MR750 3.0T MRI to image the thigh and calf, respectively.

OrthOne® 1.0T pMRI	**Torso Coil (Neocoil, WI)**	**Knee Coil (Invivo Corporation, FL)**
3 Receive/transmit channels	Receive only	Transmit/receive
Matrix size: 64-512 for transverse and up to 256 for z direction	32-channels, 16 anterior and 16 posterior	Single channel transmit, 8-channel receive
RF coils 80-180 mm diameter, FOV 40-160 mm	Anterior and posterior panel 50x50cm	Coil length 20cm, inside diameter 14cm

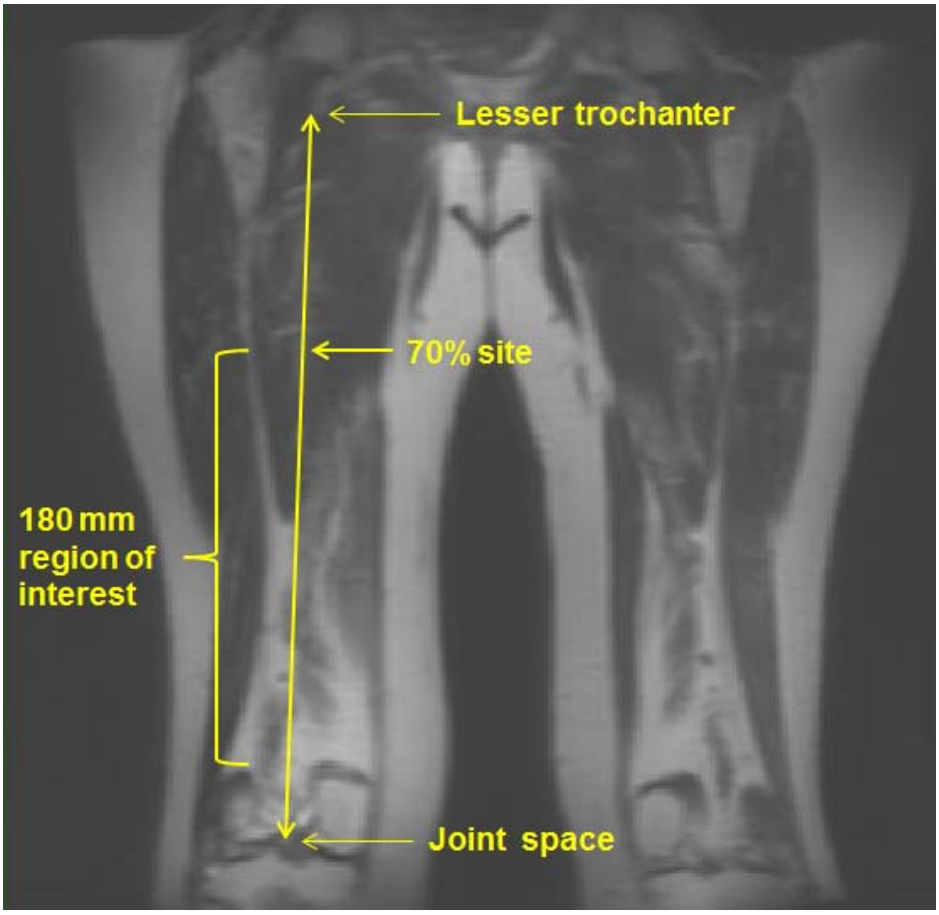

Figure 4.1. Whole-body 3.0T MRI coronal scout scan of the thigh, demonstrating the joint space to lesser trochanter measurement, the 70% proximal distance marker and the 180mm region of interest scanned and used to quantify muscle and fat subsets.

For the calf scan, the lower leg was positioned in a knee coil. The 66% site of the lower leg moving proximally on the line from the medial malleolus to the tibial spine was centered in the coil using a vitamin E tablet (Figure 4.2). As in the thigh protocol, the participants' feet were placed in an elevating block with two grooves to fit their heels.

Padding was placed under the ankle, between the knee coil and the foot block, for support and to elevate the calf off the bottom of the coil to prevent flattening. Similarly, padding was placed proximal to the coil near the thigh for elevation. Some participants required padding for lower back support. The lower leg MRI parameters were as follows: WATER:AX PD IDEAL, axial plane, 512x512 matrix, 52 slices, 160x110mm field of view, 3mm slice thickness, slice gap 0, TE=33.576msec, TR=2999msec, echo train length=6, bandwidth=195.312kHz, 4 NEX, scan time 6.00 minutes. Post-processing Surface Coil Intensity Correction (SCIC) was applied to thigh images acquired using the torso coil to correct image intensity homogeneity. Similarly, Phased array Uniformity Enhancement (PURE) was applied to lower leg images acquired using the knee coil for the same purpose.

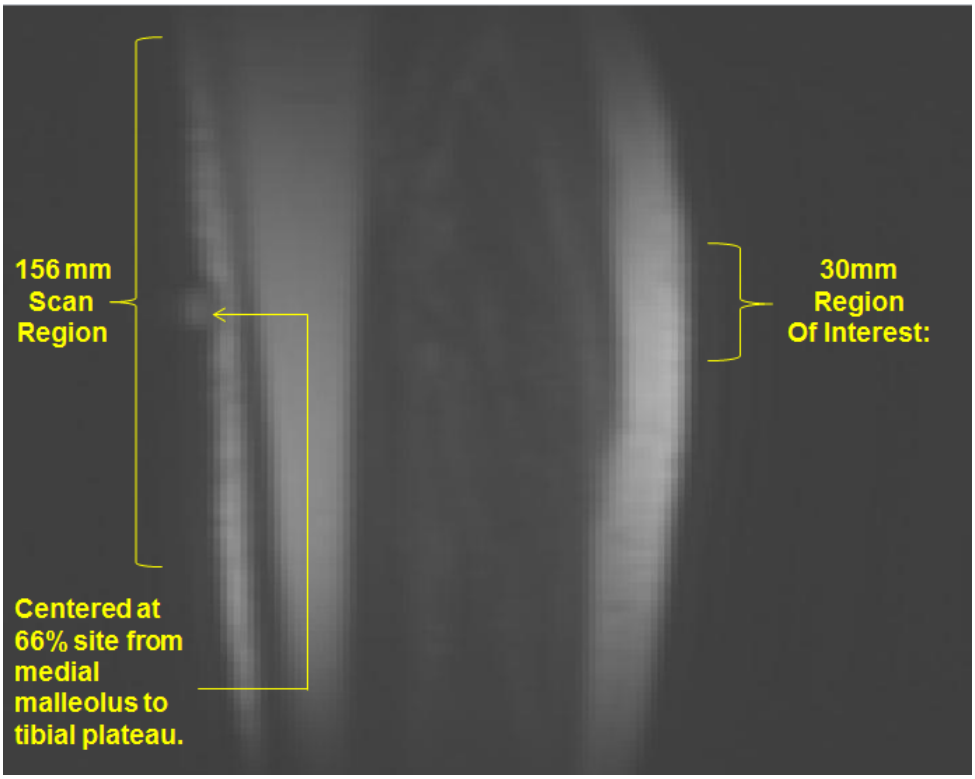

Figure 4.2. Whole-body 3.0T MRI coronal scout scan of the lower leg, demonstrating the 66% site marker from the medial malleolus to the tibial plateau, the 156 mm scan region and the 30mm region of interest used to quantify muscle and fat subsets.

On a separate visit, the lower leg was imaged using a 1.0T OrthOne® peripheral MRI (pMRI), now known as the ONI 1.0T MSK Extreme, with a T1-weighted FSE sequence, centered on the same region of interest as the 3.0T whole-body MRI IDEAL calf scan (GE Healthcare, ON; formerly from ONI Systems). Specifications for the OrthOne pMRI can be found in Table 1. Participants were positioned with their non-symptomatic leg raised and the symptomatic leg placed into a transmit-receive coil (160mm), such that the middle of the scanner is centered on the region of interest.

Padding was used to completely surround the lower leg, elevating the limb in the center of the coil and preventing it from touching the top or sides to prevent anatomical distortion (Figure 4.3). Secure padding also prevented movement artifacts during the scanning protocol. Localization was performed using axial and sagittal scout scans and centering the 66% site marker in the center of the coil (Figure 4.4). Images were obtained from the peripheral 1.0T MRI using a T1-weighted FSE sequence according to the following parameters: TE= 22.6 ms, TR= 1280ms, 24 slices, slice thickness = 3.0 mm, distance between the slides= 0, flip angle 90°, 50Hz band width, number of excitations = 2, field view of 160mm x 160mm, frequency= 512, phase= 512, echo train= 4, TI= 15, scan time = 11 minutes. These images were obtained by the Master's student, Michael Davison at the Centre for Appendicular MRI Studies (CAMRIS) in Hamilton, Ontario.

Figure 4.3. Setup for imaging the lower leg using a T1-weighted FSE sequence on the OrthOne®1.0T pMRI (General Electric Healthcare, Milwaukee MI).

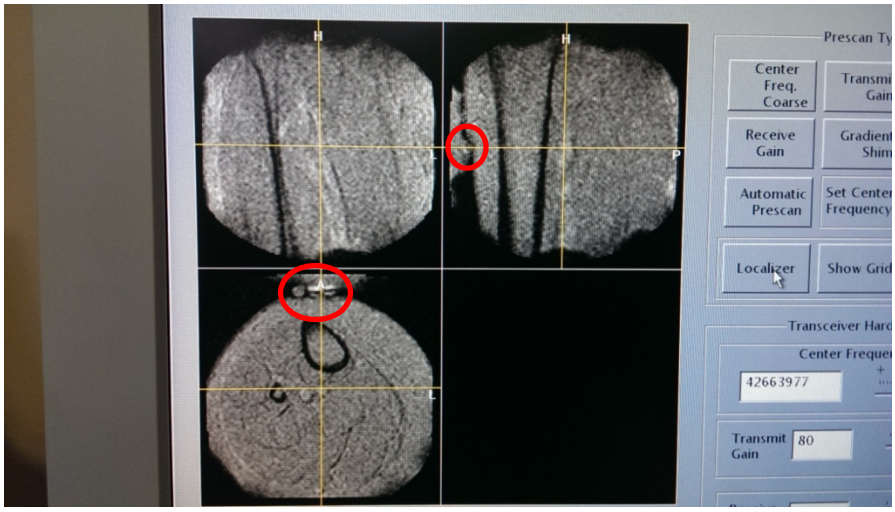

Figure 4.4. The sagittal (top right) and axial (bottom left) scout scans used for centering the lower leg region of interest in the OrthOne® 1.0T pMRI. Red circles delineate the series of three vitamin E tablets used for centering the region of interest.

4.3 Image Analysis

MRI scans were analyzed for fat and muscle volumes using the SliceOmatic 5.0 software (Tomovision, QC). For the lower leg, the 10 middle slices were selected out of the 52 or 24 slices acquired from 3.0T and 1.0T MRI, respectively. This number of MRI slices was shown to provide an accurate estimate of total extensors muscle volume in the lower leg (103). For the thigh, the 60 most proximal slices of 75 were analyzed for fat and muscle volumes, as this covers the regions of largest CSA for the quadriceps and hamstrings muscle groups.

The MRI slices were analyzed using a combination of the watershed and region growing algorithms in SliceOmatic. Fat compartments were measured sequentially, starting with the outer layer of SCAT, defined as fat outside the muscle fascia and below the skin, then proceeding with IMF, defined as muscle within the fascia and outside of muscle bellies, or between muscle and bone (also including veins, arteries and nerves surrounded by fat), and finally intraMF, defined as fat within the fascia and within a muscle belly. In the thigh, intraMF was compartmentalized and analyzed separately according to the muscle group: quadriceps including the VL, vastus intermedius, VM, and RF; hamstrings including the BF long and short head, semitendinosus (ST), and semimembranosus; and the medial compartment including the gracilis, adductors longus, adductor brevis, adductor magnus, and sartorius. Examples of a thigh 3.0T IDEAL fat separated axial slice before and after quantification of fat and muscle CSA using SliceOmatic can be seen in Figures 4.5 and 4.6, respectively.

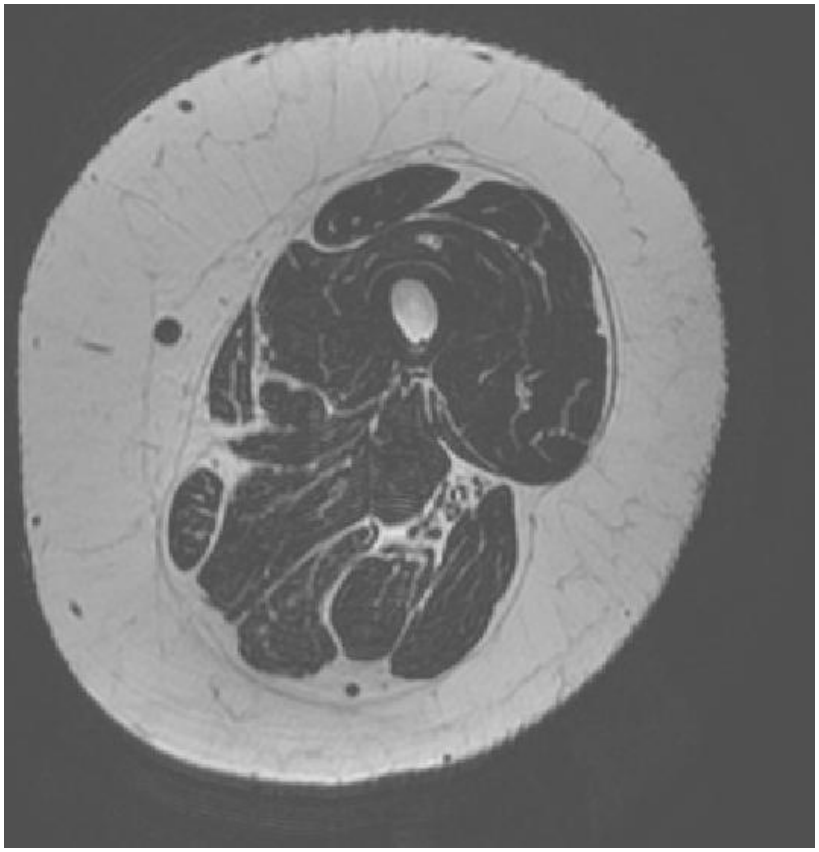

Figure 4.5. Fat separated axial image (3mm slice thickness) of the thigh acquired using the Discovery MR750 3.0T MRI with the IDEAL sequence (General Electric Healthcare, Milwaukee MI).

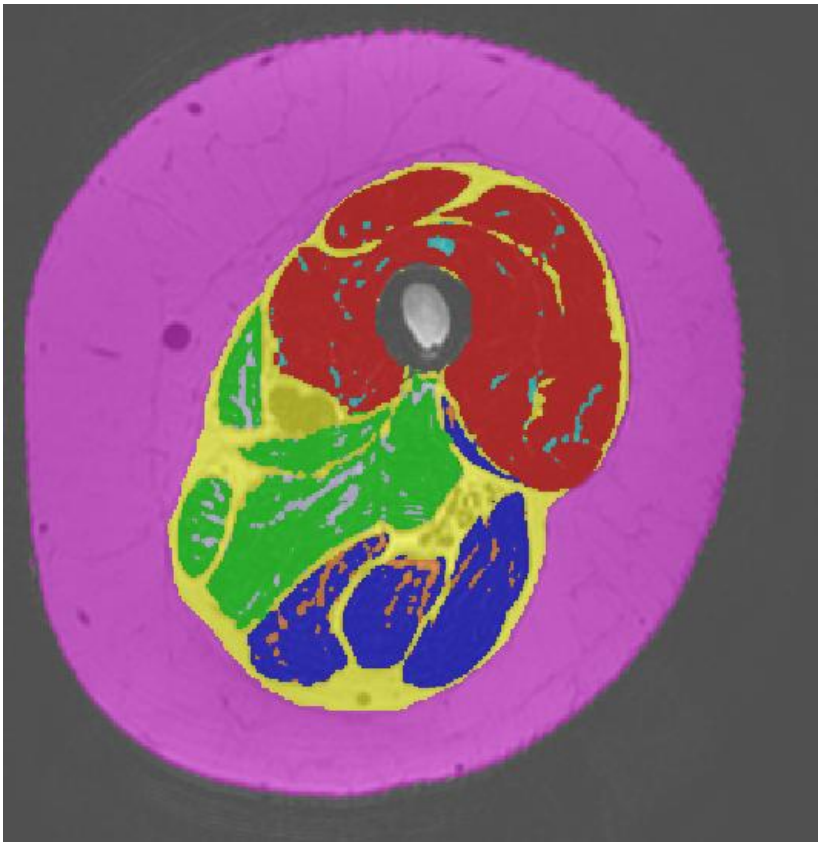

Figure 4.6. Axial image (3mm thickness) of the thigh acquired using the Discovery MR750 3.0T MRI with the IDEAL sequence and analyzed using SliceOmatic semi-automated segmentation (Tomovision, QC). Colour legend: pink (subcutaneous fat), yellow (intermuscular fat), red (quadriceps muscle), cyan (quadriceps intraMF), blue (hamstrings muscle), orange (hamstrings intraMF), green (intermediate compartment muscle), and grey (intermediate compartment intraMF).

In the lower leg, a similar sequence was used to quantify SCAT, IMF and intraMF using the same definitions. Muscle bellies and groups were separated to accurately quantify IMF and intraMF, including: the medial gastrocnemius, lateral gastrocnemius,

soleus, fibularis longus, extensor hallucis and digitorum, tibialis anterior, tibialis posterior, and flexor digitorum longus. Examples of a lower leg 3.0T IDEAL fat separated axial slice before and after quantification of fat and muscle CSA using SliceOmatic can be seen in Figures 4.7 and 4.8, respectively. In addition, examples of a lower leg 1.0T pMRI T1-weighted FSE sequence axial slice before and after quantification of fat and muscle CSA using SliceOmatic can be seen in Figures 4.9 and 4.10, respectively.

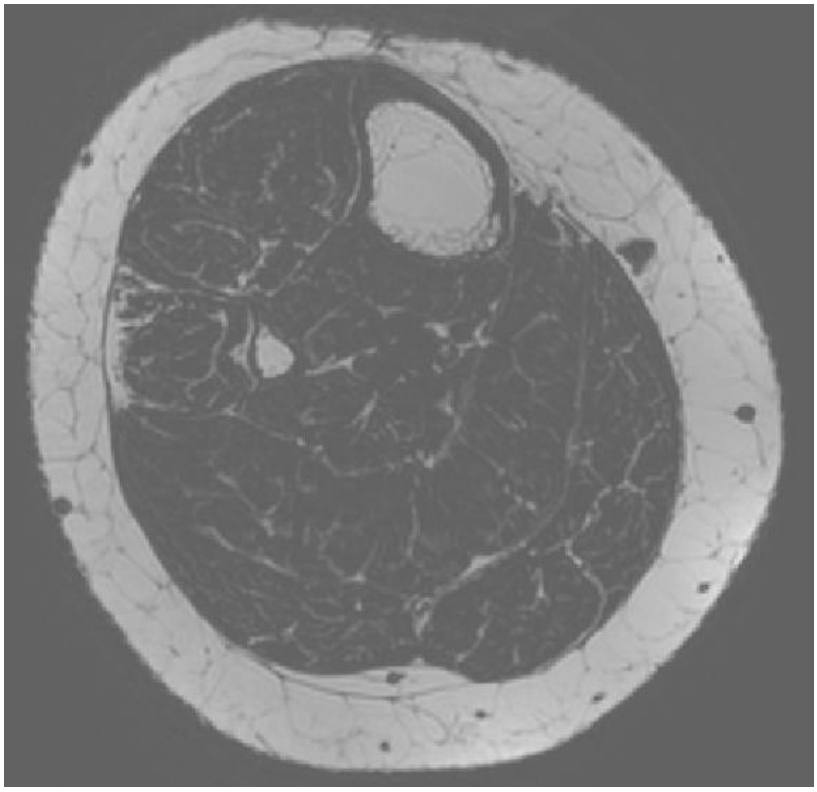

Figure 4.7. Fat separated axial image (3mm slice thickness) of the lower leg acquired using the Discovery MR750 3.0T MRI with the IDEAL sequence (General Electric Healthcare, Milwaukee MI).

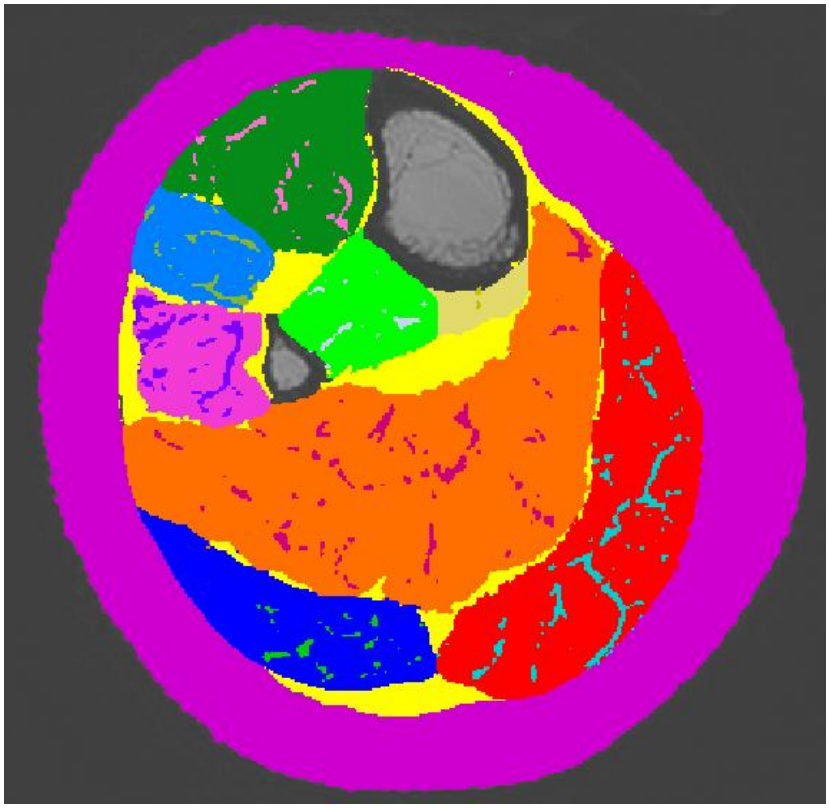

Figure 4.8. Fat separated axial image (3mm slice thickness) of the lower leg acquired using the Discovery MR750 3.0T MRI with the IDEAL sequence and analyzed using SliceOmatic semi-automated segmentation (Tomovision, QC). Colour legend: pink (SCAT), yellow (IMF), red (medial gastrocnemius muscle), cyan (medial gastrocnemius intraMF), blue (lateral gastrocnemius muscle), green (lateral gastrocnemius intraMF), orange (soleus muscle), magenta (soleus intraMF), light pink (peroneus muscle), dark purple (peroneus intraMF), light blue (extensor digitorum and hallucis muscle), olive green (extensor intraMF), dark green (tibialis anterior muscle), light pink 2 (tibialis anterior intraMF), light green (tibialis posterior muscle), grey (tibialis posterior intraMF), light brown (flexor digitorum longus muscle), tan (flexor digitorum longus intraMF).

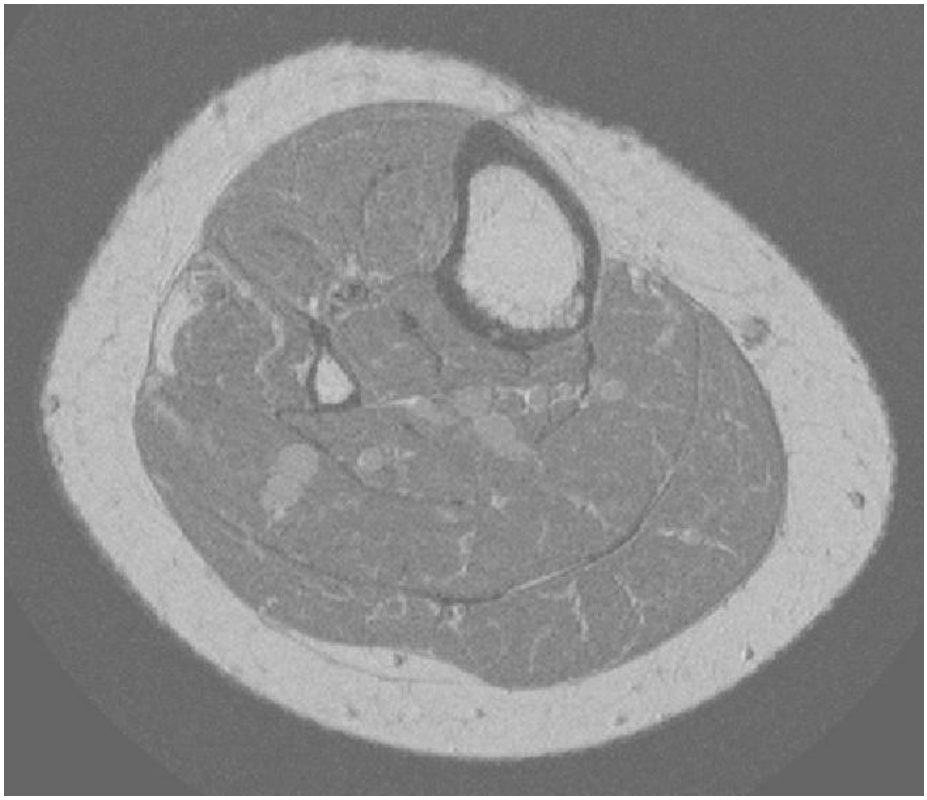

Figure 4.9. Axial image (3mm slice thickness) of the lower leg acquired using the OrthOne® 1.0T pMRI with a T1-weighted FSE sequence (General Electric Healthcare, Milwaukee MI).

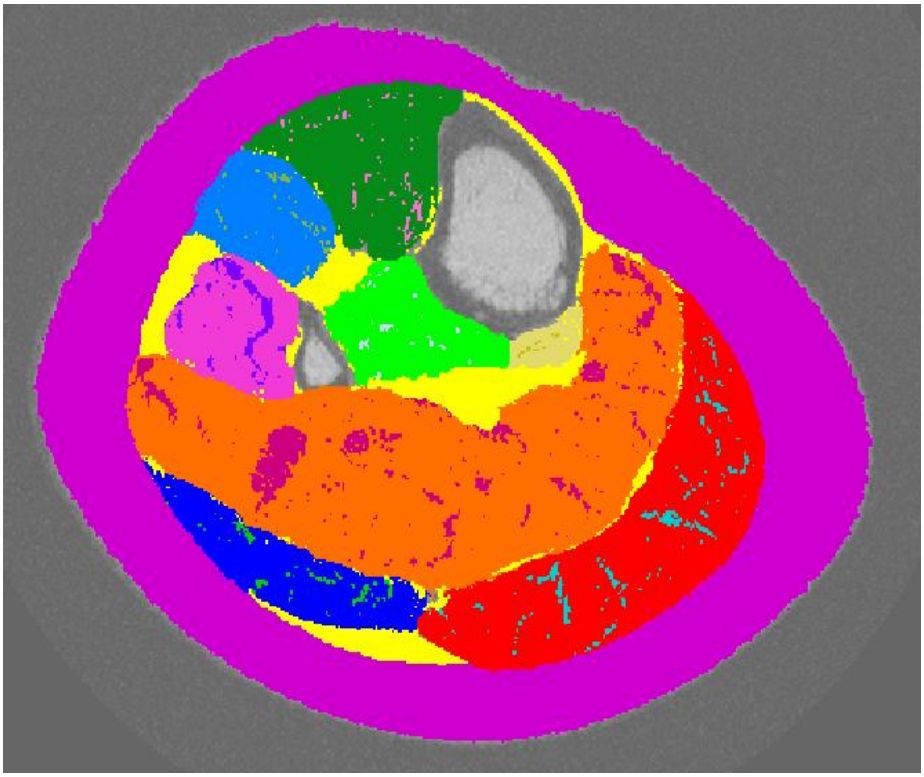

Figure 4.10. Axial image (3mm slice thickness) of the lower leg acquired using the OrthOne® 1.0T pMRI with a T1-weighted FSE sequence and analyzed using SliceOmatic semi-automated software (Tomovision, QC). For the colour legend, see Figure 8.

The region growing algorithm which used for the majority of IMF and intraMF volume measurements involves a user-designated threshold for separating water and fat, based on a histogram of pixel brightness set by the software. This analysis software has been validated through comparison with bioelectrical impedance analysis for the quantification of lean muscle (104). In addition, it is established as a reference standard for validation of other image analysis software to measure adipose tissue (105, 106). In

IDEAL fat separated images, the first slope of the region growing histogram represents low-intensity water tissue pixels while the high-intensity fat and fibrous tissue pixels are represented by the area beyond the first slope. As such, the upper limit of the region growing threshold was set to the maximum value, and the lower threshold was set to the base of the slope. This lower limit was adjusted depending on the signal intensity for each slice, and ranged between 740-1088. Demerath et al. (2007) used a similar method of a lower threshold range for quantifying visceral fat and SCAT (106).

For analysis of the 1.0T MRI FSE sequence images the histogram of pixel brightness exhibited two distinct peaks: the first representing the cortical bone, and the second representing water-based muscle tissue. Past the slope of the second peak the histogram represented the bright fat and fibrous tissue. As such, the upper limit was set at the maximum value, and the lower limit was set at the base of the second peak within the range of 34-40 on the histogram. After analyzing the entire catalogue of slices for each fat deposit, the software's color tag for the respective fat subset was locked so that analyses of other tissues would not overlap. When all the fat subsets had been analyzed and their color tags locked, the remainder of tissue to be measured within the fascia was entirely muscle, excluding cortical bone and marrow. Muscle volume was thus assessed by tagging this remaining tissue compartmentally for the quadriceps, hamstrings and medial compartment, as was done for intraMF. Using IDEAL fat separated images from the 3.0T MRI, cortical bone had to be manually excluded based on visual separation from muscle, as both tissues have the same dark field intensity. This challenge did not occur with 1.0T MRI T1-weighted images, in which cortical bone appears darker than muscle. An

identical analysis procedure was used for muscles in the lower leg. Finally, the program added the CSA for each slice and, accounting for slice thickness, calculated a volume (cm^3) for each tissue using the entire slice catalogue.

Several outcomes of interest were calculated based on the volume data determined using SliceOmatic. We were interested in representing intraMF volume as a percent of total muscle volume for each muscle group of interest. To do so, we used the calculation: % intraMF = intraMF /(intraMF+muscle)*100, separately for the quadriceps and hamstrings.

4.4 Physical Performance Tests

Participants were asked to perform the Osteoarthritis Research Society International (OARSI) recommended battery of five performance-based tests, which have been validated for use in older individuals with knee OA (107). This battery includes the 6 Minute Self-Paced Walk Test (6MWT), the Timed Up and Go (TUG), the 30 Second Chair Stand Test (30s CST), the 9-Step Stair Ascent and Descent Test (9SCT) and the 40 Meter Fast-Paced Walk Test (40m FPWT). Instructions for each test can be found online in the OARSI published guidelines at www.oarsi.org. A systematic review first identified the properties of all performance-based tests used in patients with hip or knee OA (108). These tests were assessed using the consensus-based standards for the selection of health status measurement instruments (COSMIN). For each physical performance measure, the COSMIN tool's 10 sections each scored one of the following: reliability, responsiveness, measurement error, internal consistency, interpretability, content validity, construct

validity (structural validity and hypothesis testing), cross-cultural validity, and criterion validity. Finally, a consensus was reached on the best individual tests to assess each of five clinically-relevant 'activity themes,' including sit-to-stand, walking short distances, walking long distances, stair negotiation, and ambulatory transitions.

This test battery was divided between two of the study visits to prevent physical fatigue and minimize the impact of pain. During the 1.0T MRI study visit, the TUG and 30s CST were performed due to limited space availability. During the biomechanics study visit, the 40m FPWT, 6MWT, and 9SCT were performed. The order in which the tests were performed was randomized for each visit to minimize any effect from physical fatigue or pain on declines in test performance. Participants were allowed to briefly rest between each test.

4.4.1 The 30s Chair Stand Test

The 30s CST has demonstrated good test-retest reliability with an intraclass correlation coefficient (ICC) of 0.95 at baseline assessment, and higher ICCs at 7-week (0.97) and 15-week (0.98) follow-up, in individuals awaiting knee or hip replacements (109). There was a significant difference between the first and second test results at baseline, indicating an effect from participant practice and familiarization. In addition, the 30s CST standard error of measurement (SEM) was 0.70 repetitions and the minimum detectable change (MDC) was 1.64 repetitions. Wright et al. (2011) found that the 30s CST showed greater responsiveness to change compared to other performance-based tests, including the 40m Self-Paced Walk Test and TUG (110). The 30s CST inter-rater

reliability ICC was 0.81, SEM was 1.27, test sensitivity was 66.7% and specificity was 67.9%. The major clinically important improvement (MCII) scores for the 30s CST were calculated using three variations of the anchor-based method and were 2.0, 2.1, or 2.6 repetitions. In the present study, participants started from the seated position on a 44cm high chair without armrests, stood up completely so their hips and knees were extended as fully as possible, then completely back down so that their bottom fully touched the seat. This was repeated for 30 seconds and the number of full stands was recorded. The chair was placed against a wall to prevent backward movement. The participant was asked to practice standing from sitting several times before beginning the test.

4.4.2 The 6-Minute Self-Paced Walk Test

The 6MWT has shown good responsiveness following TKA, with a preoperative to 1-month postoperative effect size of -0.49, and a 1-month to 12-month postoperative effect size of 1.36, as well as a significant mean improvement after physiotherapy in individuals with knee OA (111, 112). The 6MWT has a test-retest reliability coefficient of 0.94, the SEM was 26.29 m and the MDC_{90} was 61.34 m following total hip or knee arthroplasty (113). In the present study, participants were asked to walk continuously through a rectangular corridor, covering as much distance as possible in 6 minutes. Standardized encouragement was provided ("you're doing well, keep up the good work") every minute by the graduate student, Michael Davison, following behind the participant with a hodometer measuring distance to the nearest 10^{th} of a meter (114).

4.4.3 The 40-Meter Fast-Paced Walk Test

The 40m FPWT provides an assessment of walking quickly over short distances and changing direction during walking. The test has demonstrated good test-retest reliability with a coefficient of 0.91, a SEM of 1.73 s, and a MDC_{90} of 4.04 s in patients following total knee or hip replacement (113). The similar 40 meter Self-Paced Walk Test had an interrater reliability ICC of 0.95, a sensitivity of 66.7% and specificity of 85.5% in patients with hip OA (115). In the present study, a corridor was marked with two tape markers on the floor 10 meters apart and a pylon at 2 meters distance beyond each end of the 10 meter course. Participants were instructed to cover the 10 meters as quickly as possible, continue and loop around the pylon, then return by walking back over the 10 meters as quickly as possible, then repeat the same course without stopping. Each of the four passes on the 10 meter course (4*10=40 m) was measured to the nearest 100^{th} of a second and the average pace was calculated over the 40 meters.

4.4.4 The Timed Up-and-Go

The TUG is a multifaceted test of transitions during ambulation, involving a sit-to-stand movement, a test of walking short distances and of changing directions during walking. The TUG has shown good responsiveness following TKA, with a preoperative to 1-month postoperative effect size of -0.43, and a 1-month to 12-month postoperative effect size of 1.17, as well as a significant mean improvement after physiotherapy in individuals with knee OA (111, 112). The TUG has demonstrated a test-retest reliability coefficient of 0.75, a SEM of 1.07 s, and a MDC_{90} of 2.49 s (113). Furthermore, the TUG

has an interrater reliability ICC of 0.87, sensitivity of 55.6%, specificity of 78.2%, and a MCII of -0.8, -1.2 or -1.4 based on three calculation methods (110). In the present study, participants were asked to rise from a chair, walk 3 m (9 ft 10 inches), turn around, walk back to the chair, then sit down wearing regular footwear. The chair was against a wall so as not to slide backward. The participant started with their back against the 44cm high chair and arms on the armrests at 65cm height. A practice trial was performed. The time to perform the test once was measured to the nearest 10^{th} of a second.

4.4.5 The 9-Step Stair Climb Test

The 9SCT provides an assessment of the individual's ability to ascend and descend stairs, an activity which involves balance and body strength. The 9SCT has demonstrated a test-retest reliability coefficient of 0.90, a SEM of 2.35 s, and a MDC_{90} of 5.49 s in patients following total knee or hip arthroplasty (113). Furthermore, it demonstrated good responsiveness with a standardized response mean from preoperative to first postoperative visit of -1.74, and from first postoperative to second postoperative visit of 1.98. In the present study, participants were asked to ascend, turn around, and descend a flight of 9 steps as quickly as possible, using one armrail if necessary for their safety. The stairwell was cleared of all traffic and obstruction prior to commencing the test. The time to ascend and descend was measured separately to the nearest 100^{th} of a second.

4.5 Electromyography Acquisition

The surface EMG signals from quadriceps, hamstrings, and gastrocnemius muscles were acquired using the Myomonitor® IV System and bipolar electrodes, the specifications of which can be found in Table 4.2 (Delsys, MA). The receiver unit was attached with wire electrodes to 7 channels each acquiring a signal at 4000 Hz. The Delsys EMGWorks® 4.0 Acquisition software was used to obtain electromyographical signals (Delsys, MA). The participant was prepared for EMG surface-electrode placement on their most symptomatic leg. Alcohol cleansing and abrasion was used to help reduce skin impedance. Participants were also asked not to wear any skin cream or moisturizer on their legs that day. To identify muscle bellies for proper electrode placement, the participant was asked to lie on a plinth on either there front or back, and to extend or flex the knee while the Master's student applied gentle resistance to their lower leg in the direction opposite of contraction.

Preamplified bipolar surface electrodes with adhesive tape were placed on the muscle belly of the quadriceps, hamstrings, and calf muscles according to the European Union Surface ElectroMyoGraphy for the Non-Invasive Assessment of Muscles (SENIAM) guidelines found online at http://www.seniam.org/. In detail, electrodes were placed for the quadriceps at (1) the VL, 2/3 on the line from the anterior spina iliaca superior to the lateral side of the patella, (2) the VM, 80% of the distance on the line between the anterior spina iliaca superior and the joint space in front of the anterior border of the medial ligament, and (3) the RF, 50% of the distance on the line from the anterior spina iliaca superior to the superior part of the patella; for the hamstrings at (4)

the BF long and short heads, 50% of the distance on the line between the ischial tuberosity and the lateral epicondyle of the tibia, (5) the ST, 50% of the distance on the line between the ischial tuberosity and the medial epicondyle of the tibia; and for the calf at (6) the gastrocnemius lateralis, 1/3 of the distance on the line between the head of the fibula and (7) the gastrocnemius medialis on the most prominent bulge of the muscle (Figure 4.11). A ground electrode was placed on the bony prominence of the lateral malleolus (American Imex, CA). The EMG signal was measured for each of the isometric and isotonic knee extensors, knee flexors, and standing plantarflexion contractions.

Table 4.2. Specifications of the EMG system, electrodes and dynamometer system used for obtaining muscle power and activation data.

Myomonitor® IV EMG System	**DE-2.3 Differential EMG Sensor**	**Dermatrode® Reference Electrode**	**Biodex System 2 Pro Dynamometer**
8 channels	99.9% silver bipolar	Adhesive gel and perforated carbon	Speed 1-500 (con) or 1-300 (ecc) deg/sec
Signal input ±5 V	Bar dimensions 10mm length, 1mm diameter	Diameter 4cm	Torque 1-500 (con) or 1-300 (ecc) ft-lbs
Signal resolution 16 bits	Bar spacing 10mm	REF: DC-R02	Speed accuracy ±1 deg/sec
Sampling rate 64 kS/sec (aggregate)	REF: SP-E08		Torque accuracy ±1% of full scale

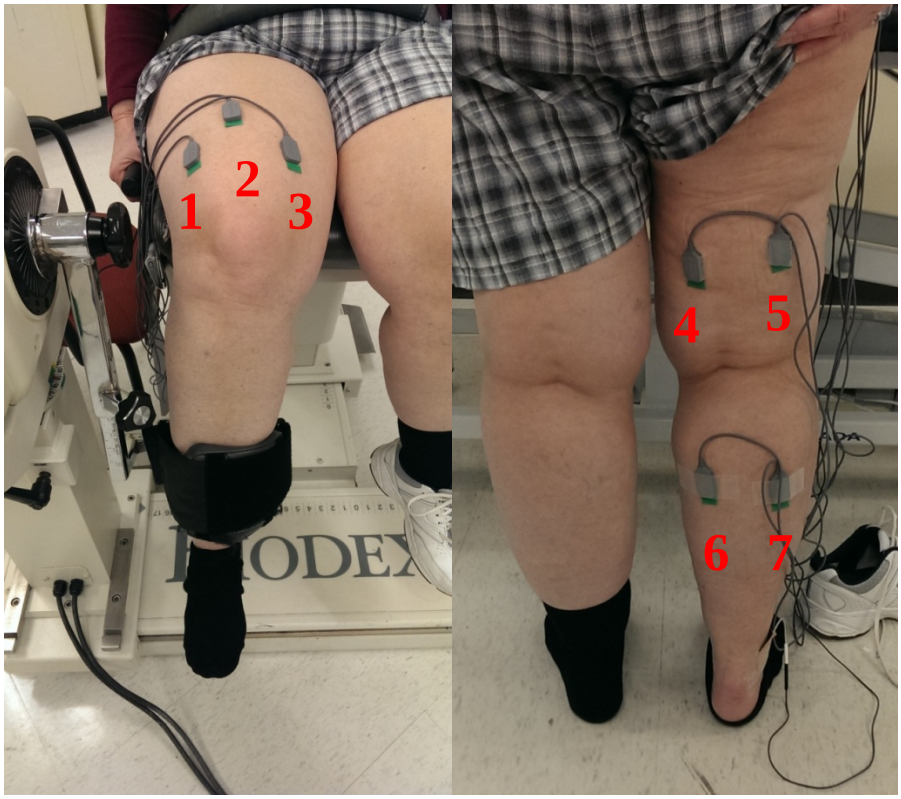

Figure 4.11. Myomonitor® IV (Delsys, MA) surface EMG setup with seven bipolar electrodes measuring neuromuscular activation of the (1) VL (2) RF (3) VM (4) ST (5) BF (6) medial gastrocnemius and (7) lateral gastrocnemius.

4.6 EMG Processing and Analysis

Electromyographical signals underwent post-processing using Delsys EMGWorks® 4.0 Analysis software (Delsys, MA). The same processing steps were used for the isometric and isotonic contraction trial data. Firstly, the data collected at 4000 Hz was resampled at 1000 Hz by using a moving average to calculate the mean of every four samples. Any trials with DC bias had the offset removed by subtracting the trial mean value from each data point. Next the data was filtered at 30 Hz high pass and 500 Hz low

pass cutoffs, which are commonly used in analyzing EMG signal's relationship to muscle force (116-118). A second order Butterworth bandpass filter with a high pass of 30 Hz and low pass of 500 Hz was selected. The data was Full Wave Rectified (FWR) using the software's simple math absolute value function.

Next the background physiologic noise was calculated and subtracted from each channel. The background noise was calculated using a quiet trial for each participant. This trial was collected before the isometric and isotonic contraction protocol, and the participant was asked to keep still while in a relaxed, seated position for ten seconds. The lowest average one second signal from the trial was calculated for each channel, and this value was subtracted from each data point from that channel in all trials. The RMS of the signal was calculated using a 300 millisecond window recommended by Farfan et al. (2010) for obtaining the most information from dynamic contractions (119). Lastly, signal normalization was performed on the isotonic contraction trials to represent the signal as a percent of maximum activation. Signal normalization is an essential step in EMG analysis, especially when comparisons are made between subjects, days, muscles, or studies (120). The maximum or 100% activation standard for normalization was selected for each of the seven channels or muscles by selecting the peak data point from either the MVIC or isotonic trial, whichever was highest. The reliability of MVIC as a representation of true maximal force has been disputed, as factors such as the muscle activated, the subject's training level and motivation can affect the outcome (121). However, the MVIC method was the most reliable for normalization when compared to the mean dynamic activity and peak dynamic activity signals (122). As such, we used the

method of obtaining the peak signal from either the MVIC or isotonic trial to avoid cases in which participants elicited a submaximal MVIC.

After post-processing of the data, the EMG outcomes of interest were calculated using MATLAB® 2010a Student (MathWorks, Natick MA). Briefly, the ten peak signals corresponding to each isotonic knee extension were identified. For the mean peak activation outcome, the five highest peaks of these ten were selected and averaged. Next, the minimum values preceding each peak were identified using MATLAB® and were verified with the raw data. The difference in time from the minimum to the corresponding maximum, or time to peak (TTP), was calculated for each peak. Our second outcome, the mean RER, was calculated by dividing the peak value by the TTP for each peak, or the slope of the EMG signal during isotonic contraction. The five highest slope activation values were averaged to determine the mean slope activation. Dos Santos et al. (2014) used a similar method to identify the RER of their EMG signal (65). A graph of the EMG normalized activation over time, and examples of activation peaks and RER for the BF during isotonic knee flexion at 20% MVIC are shown (Figure 4.12).

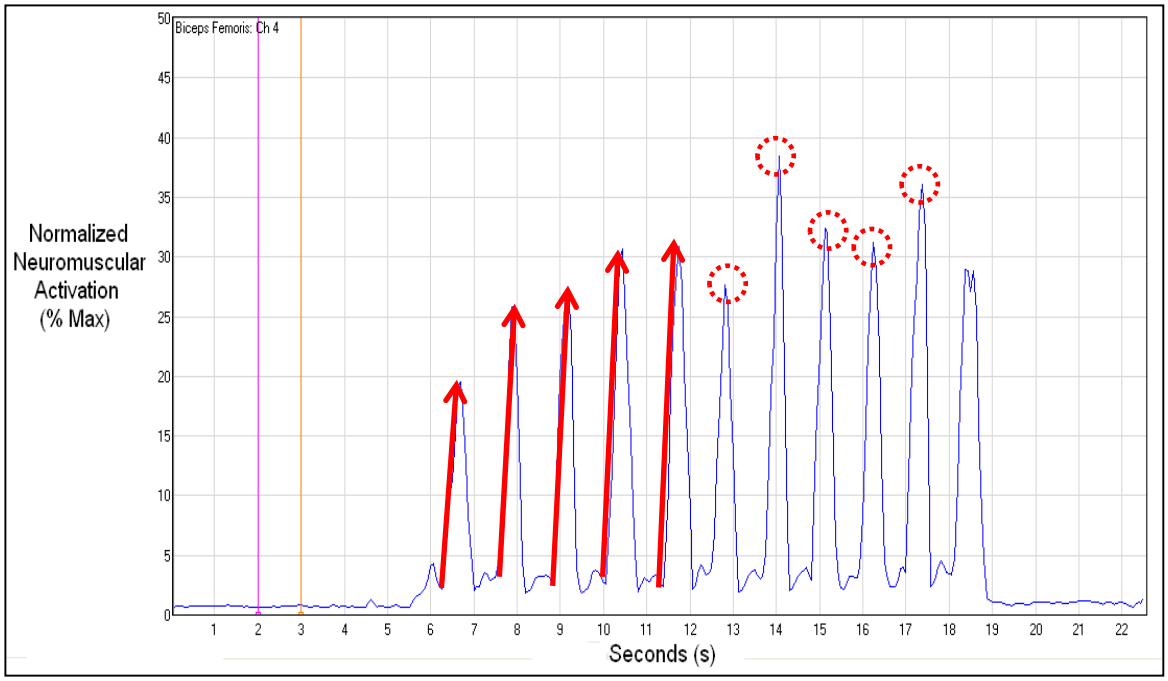

Figure 4.12. Normalized EMG of the BF during isotonic knee flexion at 20% MVIC, with examples of the peaks (red circles) and slopes (red arrows) used to calculate the mean peak amplitude and mean peak RER, respectively. Post-processing and graphing were performed with EMGWorks® 4.0 (Delsys, MA) and analysis performed using Matlab (MathWorks, Natick MA). Note: circles and arrows are used for illustrative purposes, and do not represent the true selected peaks or slopes.

4.7 Muscle Power

Following the physical performance tests and instrumentation with EMG, participants were asked to undergo a protocol of isometric and isotonic knee extensions and flexions. Torque and velocity were recorded using a Biodex System 2 dynamometer, the specifications of which can be found in Table 2 (Biodex, NY). Participants were led

by the Master's student through a protocol of MVIC and isotonic knee extension and flexion to assess muscle EMG and power.

Participants were asked to perform a measurement of MVIC knee extension and flexion with their symptomatic leg using the dynamometer. The participants were positioned for testing according to the Biodex Multi-Joint System Setup/Operation Manual. Specifically, after aligning the center of rotation of the knee with the axis of rotation of the dynamometer, participants were secured using waist, chest, and thigh straps to stabilize these regions (Figure 4.13). Their ankle was strapped to the movement arm which was oriented for either their right or left symptomatic leg. Participants were given the opportunity to perform twenty submaximal isokinetic knee extensions and flexions to familiarize themselves with the dynamometer. Participants performed five MVIC knee extensions at 60° flexion, and five MVIC knee flexions at 65° flexion (123). These angles produce the greatest force for each muscle group on the force-length curve (124, 125). Each contraction was held for five seconds, with a subsequent five second rest period. Verbal instructions for MVIC knee extension were as follows:

> *"For this test, push your leg outward, extending the knee as hard as possible. It is important that you give your maximum effort contracting your muscle, applying maximum force for 5 seconds. This will be followed by a 5s rest, and you will be prompted to repeat the maximum contraction for a total of 5 times. You may grip the handles for assistance. During this time, I will be yelling 'Push! Hard!' in order to encourage your maximum effort."*

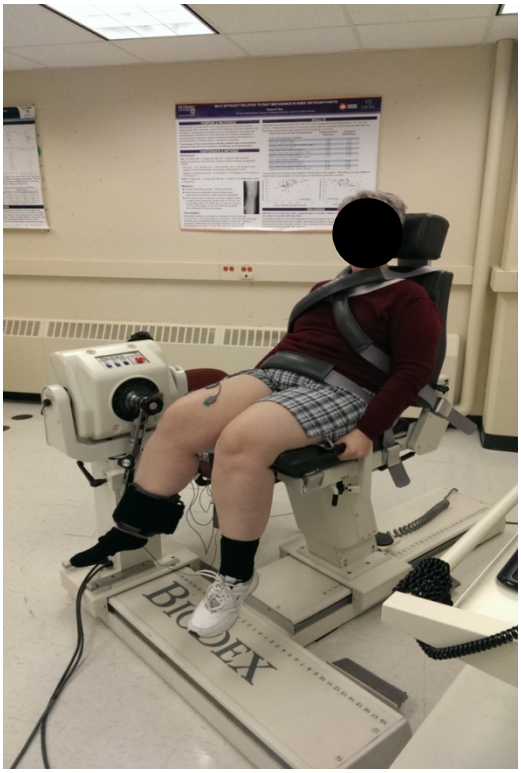

Figure 4.13. System 2 dynamometer setup for measuring knee extension and flexion MVIC and isokinetic torque in legs with symptomatic, radiographic knee OA (Biodex, NY).

Using the same instrumentation, the participant was then asked to perform consecutive isotonic knee extension and flexion. The isotonic extension began at 90° flexion and moved through a range of motion to just above 0° knee flexion (5°), and the isotonic flexion returned from this position to 90° flexion. The extension and flexion movements were repeated ten times consecutively. This series of ten extensions and flexions were performed at two isotonic resistances: 20% or 40% MVIC, with a one minute rest period between each set of ten. Power (W) has been shown to relate more

closely to physical function at lower isotonic resistances in individuals with knee OA (28). Verbal instructions for the isotonic contractions were as follows:

> *"For this test, you will move back-and-forth by pushing your leg outward and pulling it backward as fast as possible. You should push and pull your leg through its full range of motion as fast as possible. You will perform each movement 10 times, followed by a 60 second rest. You will then perform another 10 consecutive push/pull movements at a higher resistance, followed by another 60 seconds of rest, and a last set of 10 at an even higher resistance. During this time, I will be yelling 'Push! Pull!' to encourage you to continue. I will then tell you to stop."*

Following this, participants were removed from the dynamometer. Lastly, participants were asked to perform five MVIC standing, bilateral plantar flexions for activation of the gastrocnemius. They were instructed to move from neutral ankle position to full plantar flexion such that participants were balanced on the metatarsal heads and toes of both feet, holding the contraction for five seconds. A physiotherapy plinth, adjusted to each participant's height, was available for balance. Standing plantar flexion is the most effective exercise for eliciting maximal activation of the gastrocnemius (126).

Following these isometric and isotonic power tests, participants were asked to assess the intensity of the current pain in the knee of their leg for which power measurements are being obtained, using an 11-point Numeric Pain Rating Scale (NPRS).

4.8 Muscle Power Analysis

The change in torque (ft-lbs) and velocity (deg/s) over the consecutive isotonic contractions were measured by the dynamometer. Torque was converted to newton-meters and velocity was converted to radians/second. Finally, power (W or N*m/s) was calculated by multiplying the torque by the velocity for each data point. The mean peak power outcome was identified using MATLAB® 2010a Student (MathWorks, Natick MA). The onset of each contraction (extension or flexion) was determined by the minimum power, typically 0 W, located at a specified time interval for each participant, based on the speed of their contractions. Between each minimum, the peak power for the given contraction was identified as the highest single data point. Knee extension was identified as movements from the reference angle, corresponding to 90° flexion, to 0° flexion by the dynamometer; while flexion was identified as movement in the opposite direction. The knee extension or flexion mean peak power for each participant was calculated based on the five highest peak powers from the ten peaks corresponding to the particular contraction. The TTP (s) was calculated, similar to the protocol for EMG TTP, as the difference in time between the local minimum and maximum for each contraction.

4.9 Morphological Measurements and Questionnaires

The participant's height and body mass were measured, as well as their hip and waist circumference, used to determine the waist-to-hip ratio, according the World Health Organization protocol (127). Participant's height in centimeters and body mass in kilograms were measured. Participants were asked several health-related questions,

including whether they have type 1 or type 2 diabetes and the name, frequency and dose of any prescription medications the participant was taking. Participants were asked to complete four questionnaires between study visits, including: the Knee injury and Osteoarthritis Outcome Score (KOOS) questionnaire, the Intermittent and Constant Osteo-Arthritis Pain (ICOAP) measure, the Physical Activity Scale for the Elderly (PASE) questionnaire, and the Global Pain Scale (GPS) questionnaire.

4.9.1 The Knee Injury and Osteoarthritis Outcome Score

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire was developed for the elderly to assess OA-induced joint pain, stiffness and functional limitation. The Knee Injury and Osteoarthritis Outcome Score (KOOS) was created as an expansion of the WOMAC in order to evaluate both short-term and long-term symptoms and function in individuals with knee OA or injury (128). It contains 42 items in 5 separately scored domains: Pain, other Symptoms, Function in Activities of Daily Living (ADL), Function in Sport and Recreation (Sport/Rec), and knee-related Quality of Life (QOL). Due to the inclusion of questions from the WOMAC Osteoarthritis Index, WOMAC-specific subscale scores can be calculated from the KOOS questionnaire. The KOOS has been validated in a variety of populations, including previously meniscectomized individuals with and without OA (ages 38-76), and patients with OA who underwent total knee replacement (ages 43-86) (129, 130). It has demonstrated responsiveness, particularly in the QOL and Pain subscales, following total knee replacement, and the minimal perceptible clinical improvement (MPCI) has been

recommended at 8-10 points (131). The KOOS was included in the present study as a method of describing participants' knee-related symptoms and physical function (Appendix E).

4.9.2 The Measure of Intermittent and Constant Osteoarthritis Pain

Interviews with individuals with OA have identified that musculoskeletal pain can be intermittent and variable in type and intensity (132, 133). However, no previous pain assessment tools addressed these separate pain manifestations. To fill this gap, the ICOAP scale was developed as part of an Osteoarthritis Research Society International-Outcomes Measures in Rheumatology (OARSI-OMERACT) initiative (134). The ICOAP is a self-report questionnaire consisting of two subscales: 5 questions for constant pain, and 6 questions for intermittent pain. Each question is scored from 0 to 4 with 4 indicating more extreme or more frequent symptoms. Each subscale is summed (constant /20; intermittent /24) and transformed into a score out of 100. It has high retest reliability, high internal consistency and was validated through comparison with the WOMAC and KOOS pain subscales (135). The ICOAP has exhibited good responsiveness for changes in pain following physical therapy, a trial of duloxetine treatment, and following knee or hip replacement (136-138). The ICOAP scale was included in the present study as an alternative method of describing participants' knee pain from the KOOS Pain subscale (Appendix F).

4.9.3 The Physical Activity Scale for the Elderly

The PASE self-report measures physical activity in older adults and consists of 12 questions probing the frequency and duration of leisure activity, household activity, and work-related activity during the previous 7 days (139). Questions are assessed on either 4-point scales or with yes/no responses. Theoretically, a total score from 0 to 400 is calculated with a high score indicating high physical activity. The PASE has shown responsiveness following TKA with a significant improvement in physical activity (140). High PASE scores are associated with positive gait pace and chair stand outcomes in adults with knee OA, while high and very low PASE scores have been associated with increased OA progression using cartilage T2 measurements (141, 142). The PASE was included in the present study as a method of describing participants' self-reported physical activity (Appendix G).

4.9.4 The Global Pain Scale

The GPS self-report provides a measure of pain anywhere in the body as well as feelings, clinical outcomes and ability to perform daily activities, as they relate to pain. It comprises 20 questions each scored with a 11-point Likert scale, providing a total score out of 100 with a high score indicating extreme pain and its effect on daily living and quality of life. The GPS has demonstrated high criterion validity, high construct validity, reliability and a correlation with other pain scales in young adults with chronic pain (143). The GPS was included in the present study as a means of describing participants' total body pain (Appendix H).

4.10 Statistical Analyses

All statistical analyses were performed using SPSS Statistics version 22 (IBM, NY). Descriptive statistics were determined for the study sample of women with clinical, radiographic primary knee OA (n=20). Linear regression analysis was used to investigate the relationship between % intraMF muscle fraction of the quadriceps or hamstrings and isotonic knee extensor or flexor power (W), controlling for knee extensor or flexor neuromuscular activation. Isotonic muscle power as the dependent variable was investigated at 20% and 40% MVIC. For the neuromuscular activation covariate, we included the following: in the investigation of knee extensor power, we controlled for either the VL, VM or RF mean peak activation (%max) or mean RER (%max/s); and in the investigation of knee flexor power, we controlled for either the BF or ST mean peak activation or mean RER. In addition, linear regression analysis was used to investigate the relationship between intraMF volume (cm^3), as opposed to muscle fraction, of the quadriceps or hamstrings and isotonic knee extensor or flexor power at 20% and 40% MVIC, controlling for knee extensor or flexor neuromuscular activation. Linear regression analysis was also used to investigate the relationship between total thigh IMF volume (cm^3) and isotonic knee extensor or flexor power at 20% or 40% MVIC, controlling for knee extensor or flexor neuromuscular activation.

Linear regression analysis was also used to investigate the relationship between quadriceps or hamstrings % intraMF muscle fraction and physical performance outcomes. As the dependent variable, each of the five following tests were investigated: the TUG,

40m FPWT, 6MWT, 30s CST, and 9SCT. For these analyses we controlled for self-reported knee pain following physical activity using the NPRS.

We also used bivariate Pearson correlations to determine the linear relationships between thigh and calf intraMF or IMF volumes. Lastly, bivariate Pearson correlations were used to determine the relationships between calf intraMF or IMF assessed using the 3.0T whole-body MRI IDEAL method and the 1.0T peripheral MRI T1-weighted FSE method. In order to determine the agreement between these two MRI-based methods for quantifying fat subsets, we used two Bland Altman plots demonstrating the mean difference ±2SD between the two methods for quantifying IMF and intraMF, separately.

Lastly, we included calculations of the sample size required for a future study to investigate the relationship between quadriceps intraMF fraction and isotonic knee extensor power at 20% MVIC, based on the standard deviations found in this study, and provide a power calculation for the present study based on its sample size (n=20). These calculations were performed using the methods described by Dupont and Plummer (1998), including the use of their free software 'PS – Power and Sample Size Calculations' for simple linear regression, found at http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize (144).

Chapter Five: Results

5.1 Descriptive Statistics

Analyses were performed on a sample of women (n=20) with radiographic, symptomatic primary knee OA. Descriptive statistics were reported for this sample (Table 5.1). These women had a mean (±SD) age of 65.2±5.4 and mean BMI of 30.2±5.4 kg/m^2. Participants had a mean ICOAP total score of 22.6±14.6, a mean GPS total score of 21.3±14.8, and a mean PASE total score of 186.7±84.8. Participants rated their pain following the MVIC and isotonic knee extension and flexion protocol using a 11-point NPRS with a mean pain rating of 2.35±2.43. In addition, participants reported mean KOOS subscores as the following: Pain 64.4±12.8, Symptoms 62.3±16.3, Activities of Daily Living 65.9±21.0, Function in Sports and Recreation 40.5±29.2, and Quality of Life 53.3±18.5. In addition, the descriptive statistics for the thigh and calf variables of interest were reported (Table 5.2). The physical function outcomes including muscle power, performance tests and neuromuscular activation descriptive statistics were reported (Table 5.3). Lastly, descriptive statistics for the EMG covariates were reported (Table 5.4).

Table 5.1. Sample population descriptors for a sample of women (n=20) with radiographic, symptomatic knee OA.

Descriptive Statistic	Mean (±SD)	Minimum, Maximum
Age (years)	65.2±5.4	56, 76
Body Mass (kg)	77.7±12.3	57.6, 98.4
Height (m)	1.61±0.05	1.51, 1.67
Waist-to-Hip Ratio	0.903±0.067	0.802, 1.031
BMI (kg/m^2)	30.2±5.4	20.8, 40.3
ICOAP Total Score (/100)	22.6±14.6	0.0, 63.6
PASE Total Score (/400)	186.7±84.8	90.4, 360.5
GPS Total Score (/100)	21.3±14.8	4.5, 69.0
KOOS Pain Subscore*	64.4±12.8	41.7, 91.7
KOOS Symptoms Subscore*	62.2±15.9	21.4, 85.7
KOOS ADL Subscore*	65.9±21.0	23.5, 100.0
KOOS FSR Subscore*	40.5±29.2	0.0, 100.0
KOOS QoL Subscore*	53.3±18.5	0.0, 81.3
NPRS Pain Score	2.35±2.43	0.0, 8.0

Body Mass Index (BMI); Intermittent and Constant Osteoarthritis Pain (ICOAP); Physical Activity Scale for the Elderly (PASE); Global Pain Scale (GPS); Knee Injury and Osteoarthritis Outcome Score (KOOS); Numeric Pain Rating Scale (NPRS).

*Scores from KOOS are on a scale of 0-100, with low scores indicating worse symptoms/function.

Table 5.2. Descriptive statistics for 3.0T and 1.0T MRI intraMF and IMF.

Descriptive Statistic	Mean (±SD)	Minimum, Maximum
3.0T Thigh Quadriceps intraMF volume (cm^3)	35.82±9.94	17.05, 49.69
3.0T Thigh Hamstrings intraMF volume (cm^3)	42.00±13.37	20.95, 74.19
3.0T Thigh Quadriceps % intraMF	5.56±1.48	2.82, 7.76
3.0T Thigh Hamstrings % intraMF	9.82±2.46	5.42, 13.78
3.0T Thigh IMF volume (cm^3)	419.06±114.10	233.40, 689.90
3.0T Calf intraMF total volume (cm^3)	9.96±3.45	5.92, 19.21
3.0T Calf IMF volume (cm^3)	22.91±6.25	11.52, 35.62
1.0T Calf intraMF total volume (cm^3)	8.95±2.75	5.63, 16.35
1.0T Calf IMF volume (cm^3)	19.36±6.25	9.36, 31.97

Intramuscular fat (intraMF); intermuscular fat (IMF).

Table 5.3. Descriptive statistics for measures of physical function including muscle power, performance-based physical function and EMG neuromuscular activation.

Descriptive Statistic	Mean (±SD)	Minimum, Maximum
Power (W) Knee Extension 20% MVIC	252.54±89.99	69.63, 479.98
Power (W) Knee Flexion 20% MVIC	243.11±102.40	53.85, 524.41
Power (W) Knee Extension 40% MVIC	189.09±75.32	24.25, 327.87
Power (W) Knee Flexion 40% MVIC	169.61±65.16	29.05, 306.13
Timed Up-and-Go (s)	8.1±1.8	4.2, 11.1
6 Minute Self-Paced Walk Test (m)	497.3±81.7	305.0, 655.0
40 Meter Fast-Paced Walk Test (m/s)	1.62±0.34	1.06, 2.69
30 Second Chair Stand Test (repetitions)	11.7±5.1	6.0, 30.0
9 Step Stair Ascent/Descent Test (s)	11.0±5.8	5.8, 27.1

Maximum voluntary isometric contraction (MVIC).

Table 5.4. Descriptive statistics for EMG covariates from the knee extensors and flexors.

Descriptive Statistic	Mean (±SD)	Minimum, Maximum
VL Mean Peak Activation (%)	81.87±8.56	65.65, 96.50
VM Mean Peak Activation (%)	84.29±7.33	67.06, 94.10
RF Mean Peak Activation (%)	82.06±11.36	48.51, 94.08
BF Mean Peak Activation (%)	76.95±14.41	35.83, 94.70
ST Mean Peak Activation (%)	73.10±20.39	7.33, 92.45
VL Mean RER (%/sec)	139.01±36.51	51.71, 196.75
VM Mean RER (%/sec)	138.01±32.71	76.61, 201.69
RF Mean RER (%/sec)	138.51±32.99	90.04, 201.34
BF Mean RER (%/sec)	118.01±41.78	45.39, 183.23
ST Mean RER (%/sec)	122.97±49.75	10.83, 199.26

Rate of EMG Rise (RER); vastus lateralis (VL); vastus medialis (VM); rectus femoris (RF); biceps femoris (BF); and semitendinosus (ST).

5.2 Percent IntraMF Muscle Fraction and Peak Power

Linear regression analysis was used to investigate the cross-sectional relationships between quadriceps or hamstrings % intraMF muscle fraction and isotonic knee extensor or flexor power at 20% of MVIC, controlling for EMG mean peak activation and RER. Quadriceps % intraMF muscle was not significantly related to knee extensor power at 20% MVIC (B=5.433; p=0.714), controlling for VL mean RER (Table 5.5). Hamstrings

% intraMF was not significantly related to knee flexor power at 20% MVIC (B=-9.008; p=0.406), controlling for ST mean peak activation (Table 5.6).

The relationships between quadriceps or hamstrings % intraMF muscle fraction and knee extensor or flexor power at 40% MVIC, controlling for EMG activation, were also investigated. There were no significant relationships between quadriceps % intraMF and knee extensor power at 40% MVIC (B=-6.471; p=0.580) (Table 5.5) or hamstrings % intraMF and knee flexor power at 40% MVIC (B=-7.189; p=0.282) (Table 5.6), controlling for VM mean RER or ST mean peak activation, respectively.

Table 5.5. The relationships between quadriceps % intraMF muscle fraction and isotonic knee extensor power at 20% MVIC and 40% MVIC, controlling for neuromuscular activation of knee extensor muscles.

Dependent Variables	Neuromuscular Activation EMG Covariates	Quadriceps % intraMF B Coefficient (p-value)
Knee Extensor 20% MVIC Power (W)	VL Mean Peak Activation	4.351 (0.764)
	VM Mean Peak Activation	0.063 (0.997)
	RF Mean Peak Activation	2.444 (0.872)
	VL Mean RER	5.433 (0.714)
	VM Mean RER	-0.764 (0.956)
	RF Mean RER	-0.272 (0.986)
Knee Extensor 40% MVIC Power (W)	VL Mean Peak Activation	-4.162 (0.741)
	VM Mean Peak Activation	-6.079 (0.626)
	RF Mean Peak Activation	-4.054 (0.749)
	VL Mean RER	-1.657 (0.893)
	VM Mean RER	-6.471 (0.580)
	RF Mean RER	-5.759 (0.654)

Intramuscular fat (intraMF); maximum voluntary isometric contraction (MVIC); rate of EMG rise (RER); vastus lateralis (VL); vastus medialis (VM); rectus femoris (RF).

Table 5.6. The relationships between hamstrings % intraMF muscle fraction and isotonic knee flexor power at 20% MVIC and 40% MVIC, controlling neuromuscular activation of knee flexor muscles.

Dependent Variables	Neuromuscular Activation EMG Covariates	Hamstrings % intraMF B Coefficient (p-value)
Knee Flexor 20% MVIC Power (W)	BF Mean Peak Activation	-4.793 (0.645)
	ST Mean Peak Activation	-9.008 (0.406)
	BF Mean RER	-2.821 (0.775)
	ST Mean RER	-3.348 (0.744)
Knee Flexor 40% MVIC Power (W)	BF Mean Peak Activation	-3.082 (0.635)
	ST Mean Peak Activation	-7.189 (0.282)
	BF Mean RER	-2.592 (0.681)
	ST Mean RER	-3.422 (0.605)

Intramuscular fat (intraMF); maximum voluntary isometric contraction (MVIC); rate of EMG rise (RER); biceps femoris (BF); semitendinosus (ST).

5.3 IntraMF Volume and Peak Power

Linear regression analysis was used to investigate the cross-sectional relationships between quadriceps or hamstrings intraMF volume and isotonic knee extensor or flexor power at 20% of MVIC, controlling for EMG mean peak activation and mean RER. Quadriceps intraMF volume had a significant, positive relationship to isotonic knee extensor power at 20% MVIC (B=4.023; p=0.050), controlling for VL mean RER (Table

5.7). Hamstrings intraMF volume was not significantly related to isotonic knee flexor power at 20% MVIC (B=1.178; p=0.535), controlling for ST mean RER (Table 5.8).

The relationships between quadriceps or hamstrings intraMF volume and isotonic knee extensor or flexor power at 40% of MVIC, controlling for EMG activation, were also investigated. Quadriceps intraMF volume had a trend toward a positive relationship with knee extensor power at 40% MVIC (B=2.582; p=0.140), controlling for VL mean RER (Table 5.7). Hamstrings intraMF volume was not related to knee flexor power at 40% MVIC (B=-0.680; p=0.602), controlling for ST mean peak activation (Table 5.8).

Table 5.7. The relationships between quadriceps intraMF volume and isotonic knee extensor power, controlling for neuromuscular activation of knee extensor muscles.

Dependent Variables	Neuromuscular Activation EMG Covariates	Quadriceps intraMF (cm^3) B Coefficient (p-value)
Knee Extensor 20% MVIC Power (W)	VL Mean Peak Activation	3.085 (0.125)
	VM Mean Peak Activation	2.870 (0.180)
	RF Mean Peak Activation	4.016 (0.065)
	VL Mean RER	4.023 (0.050)*
	VM Mean RER	2.863 (0.154)
	RF Mean RER	3.057 (0.157)
Knee Extensor 40% MVIC Power (W)	VL Mean Peak Activation	1.796 (0.314)
	VM Mean Peak Activation	1.657 (0.364)
	RF Mean Peak Activation	2.551 (0.170)
	VL Mean RER	2.582 (0.140)
	VM Mean RER	1.643 (0.340)
	RF Mean RER	1.825 (0.318)

Intramuscular fat (intraMF); maximum voluntary isometric contraction (MVIC); rate of EMG rise (RER); vastus lateralis (VL); vastus medialis (VM); rectus femoris (RF).

*Statistically significant ($p<0.05$).

Table 5.8. The relationships between hamstrings intraMF volume and isotonic knee flexor power at 20% MVIC and 40% MVIC, controlling neuromuscular activation of knee flexor muscles.

Dependent Variables	Neuromuscular Activation EMG Covariates	Hamstrings intraMF (cm^3) B Coefficient (p-value)
Knee Flexor 20% MVIC Power (W)	BF Mean Peak Activation	0.713 (0.705)
	ST Mean Peak Activation	-0.082 (0.969)
	BF Mean RER	0.696 (0.695)
	ST Mean RER	1.178 (0.535)
Knee Flexor 40% MVIC Power (W)	BF Mean Peak Activation	-0.087 (0.528)
	ST Mean Peak Activation	-0.050 (0.722)
	BF Mean RER	-0.043 (0.750)
	ST Mean RER	-0.069 (0.621)

Intramuscular fat (intraMF); maximum voluntary isometric contraction (MVIC); rate of EMG rise (RER); biceps femoris (BF); semitendinosus (ST).

5.4 IMF and Peak Power

The relationships between thigh IMF volume and isotonic knee extensor or flexor power at 20% MVIC, controlling for mean peak activation and mean RER, were investigated. Thigh IMF was not significantly related to knee extensor power at 20% MVIC (B=-0.092; p=0.675), controlling for RF mean RER (Table 5.9). Thigh IMF was also not significantly related to knee flexor power at 20% MVIC (B=-0.105; p=0.634),

controlling for BF mean peak activation. The relationships between thigh IMF volume and knee extensor or flexor power at 40% MVIC, controlling for EMG activation, were also investigated. Thigh IMF was not significantly related to knee extensor power at 40% MVIC (B=0.099; p=0.529) or knee flexor power at 40% MVIC (B=-0.069; p=0.621), controlling for VM or ST mean RER, respectively (Table 5.9).

Table 5.9. The relationships between thigh IMF volume and isotonic knee extensor or flexor power at 20% or 40% MVIC, controlling for neuromuscular activation of extensor or flexor muscles.

Dependent Variables	Neuromuscular Activation EMG Covariate	Thigh IMF (cm^3) B Coefficient (p-value)
Knee Extensor 20% MVIC (W)	VL Mean Peak Activation	-0.025 (0.891)
	RF Mean RER	-0.092 (0.675)
Knee Extensor 40% MVIC (W)	RF Mean Peak Activation	0.054 (0.734)
	VM Mean RER	0.099 (0.529)
Knee Flexor 20% MVIC (W)	BF Mean Peak Activation	-0.105 (0.634)
	ST Mean RER	-0.086 (0.688)
Knee Flexor 40% MVIC (W)	BF Mean Peak Activation	-0.087 (0.528)
	ST Mean RER	-0.069 (0.621)

Intermuscular fat (IMF); maximum voluntary isometric contraction (MVIC); rate of EMG rise (RER); VL (VL); RF (RF); BF (BF); ST (ST).

5.5 Percent IntraMF Muscle Fraction and Physical Performance

The relationships between quadriceps or hamstrings % intraMF muscle fraction and a battery of five physical performance tests were also investigated, controlling for self-reported NPRS following physical activity. No significant relationships were found between quadriceps or hamstrings % intraMF and the 30s CST, the TUG, the 9SCT, the 40m FPWT, or the 6MWT) (Table 5.10).

Table 5.10. The relationships between quadriceps or hamstrings % intraMF and five objective physical performance test outcomes, controlling for NPRS.

Dependent Variables	Quadriceps % intraMF B Coefficient (P)	Hamstrings % intraMF B Coefficient (p-value)
30-s Chair Stand Test (reps)	-0.706 (0.378)	-0.399 (0.420)
Timed Up-and-Go (s)	0.142 (0.581)	0.161 (0.306)
Stair Ascent/Descent Test (s)	-0.467 (0.612)	0.391 (0.490)
40-m Fast-Paced Walk Test (m/s)	0.015 (0.788)	0.004 (0.913)
6-Min Self-Paced Walk Test (m)	2.241 (0.859)	-4.858 (0.560)

Intramuscular fat (intraMF).

5.6 Thigh and Calf IntraMF and IMF Assessed using 3.0T MRI IDEAL

Using linear bivariate correlation, we investigated the correlation between total thigh intraMF volume and total calf intraMF volume, both assessed using 3.0T MRI IDEAL sequences. Calf intraMF volume assessed using 3.0T MRI IDEAL was significantly, positively correlated with thigh intraMF volume assessed using 3.0T MRI IDEAL (r=0.759; p=0.001) (Figure 5.1). We also investigated the correlation between total thigh intraMF volume assessed using a 3.0T MRI IDEAL sequence and total calf intraMF volume assessed using a 1.0T MRI FSE sequence. Calf intraMF volume assessed using 1.0T MRI FSE was significantly, positively correlated with thigh intraMF assessed using 3.0T MRI IDEAL (r=0.688; 0.001) (Figure 5.2).

In addition, we investigated the correlation between total thigh IMF volume and total calf IMF volume, both assessed using 3.0T MRI IDEAL sequences. There was a trend toward a positive relationship between thigh IMF and calf IMF (r=0.436; p=0.055), each assessed using 3.0T MRI IDEAL sequences (Figure 5.3). We also investigated the correlation between total thigh IMF volume assessed using a 3.0T MRI IDEAL sequence and calf IMF volume assessed using a 1.0T MRI FSE sequence. There was a significant, positive correlation between thigh IMF volume assessed using 3.0T MRI IDEAL and calf IMF assessed using 1.0T MRI FSE (r=0.483; p=0.031) (Figure 5.4).

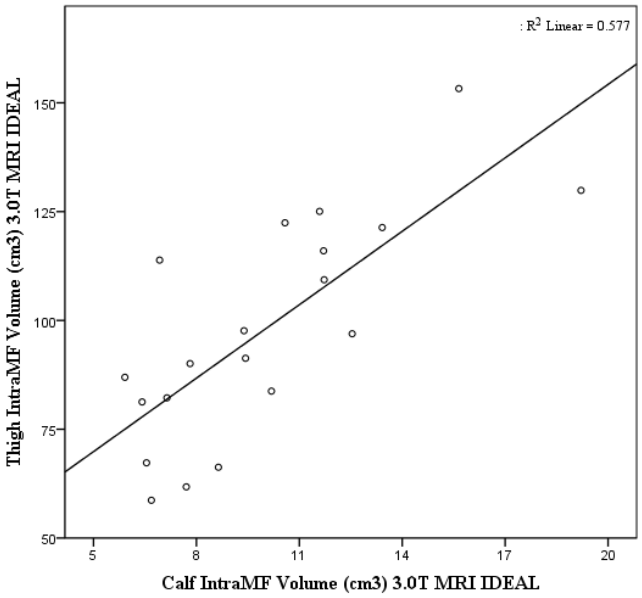

Figure 5.1. The linear correlation between calf and thigh intraMF volumes, each imaged using 3.0T MRI IDEAL sequences.

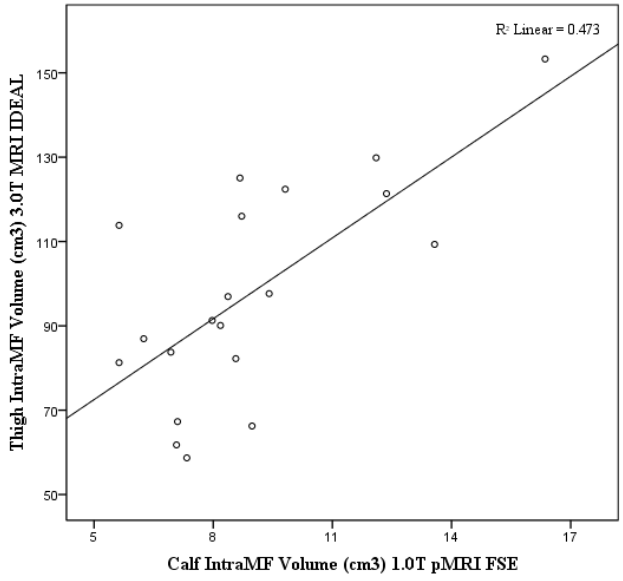

Figure 5.2. The relationship between calf intraMF volume imaged using a 1.0T MRI FSE sequence and thigh intraMF volume imaged using 3.0T MRI IDEAL sequence.

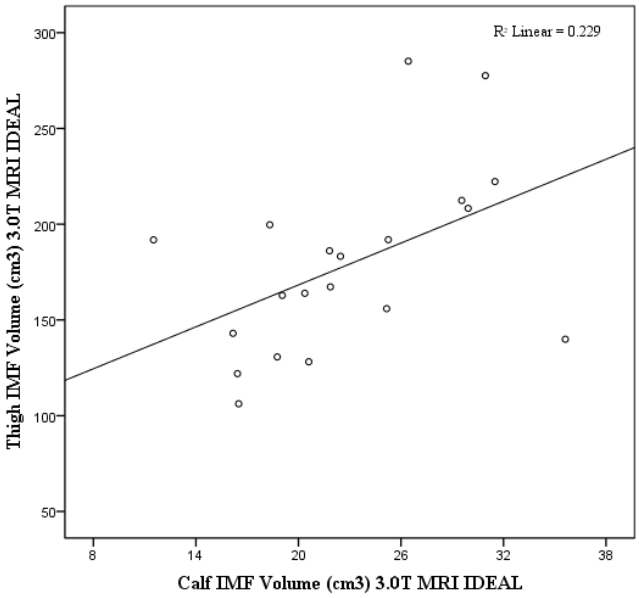

Figure 5.3. The linear relationship between calf and thigh IMF volumes, each imaged using 3.0T MRI IDEAL sequences.

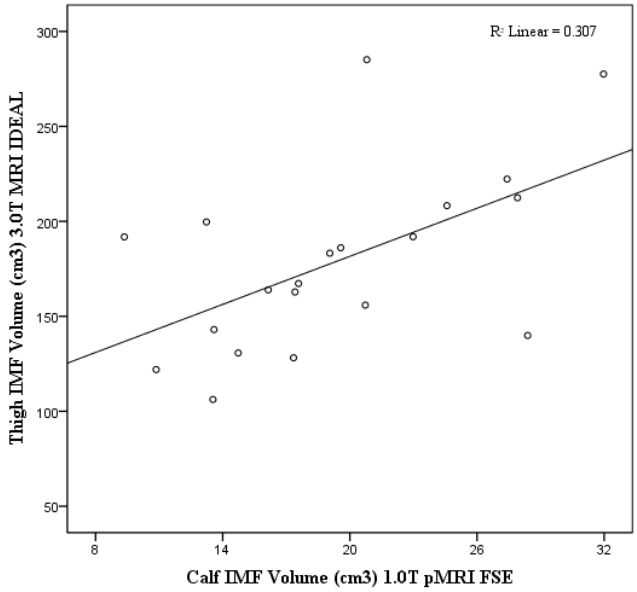

Figure 5.4. The relationship between calf IMF volume imaged using a 1.0T MRI FSE sequence and thigh IMF volume imaged using a 3.0T MRI IDEAL sequence.

5.7 Comparison of Fat Quantification using 3.0T MRI IDEAL and 1.0T MRI FSE

Using linear bivariate correlation, we investigated the correlation between total calf intraMF volume assessed using 3.0T MRI IDEAL sequence and the same fat subset assessed using 1.0T MRI FSE sequence. Calf intraMF assessed using 1.0T MRI FSE was significantly, positively correlated to calf intraMF assessed using 3.0T MRI IDEAL ($r=0.779$; $p=0.001$) (Figure 5.5). In addition, a Bland Altman plot of the mean calf intraMF values versus the difference in calf intraMF assessed using 3.0T MRI IDEAL or 1.0T MRI FSE sequences demonstrates good agreement, with all but one point falling within ±2 SDs of the mean difference (Figure 5.6).

Lastly, we investigated the correlation between calf IMF volume assessed using a 3.0T MRI IDEAL sequence and calf IMF assessed using a 1.0T MRI FSE sequence. There was a significant, positive correlation between calf IMF assessed using 3.0T MRI IDEAL and calf IMF assessed using 1.0T MRI FSE ($r=0.956$; $p=0.001$) (Figure 5.7). A Bland Altman plot of the mean calf IMF values versus the difference in calf IMF assessed using 3.0T MRI IDEAL or 1.0T MRI FSE sequences demonstrates good agreement, with all but one point falling within ±2 SDs of the mean difference (Figure 5.8).

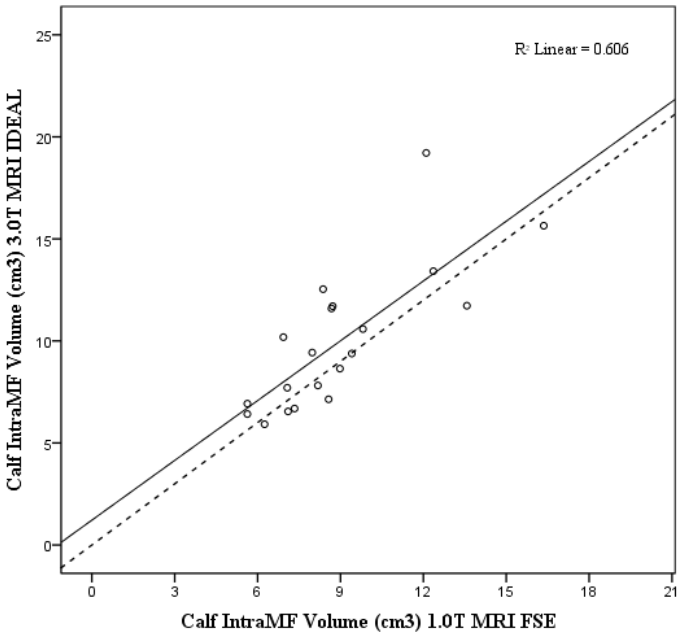

Figure 5.5. The linear relationship between calf intraMF volumes imaged using a 3.0T MRI IDEAL sequence or imaged using a 1.0T MRI FSE sequence. Solid line = linear correlation, dotted line = line of identity (y=x) illustrating scanner agreement.

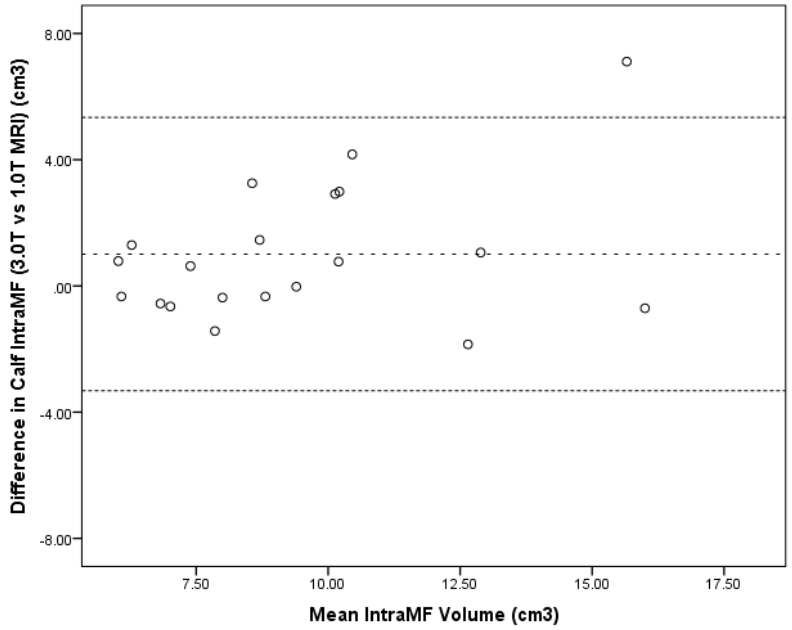

Figure 5.6. Bland Altman plot of the mean calf intraMF versus the difference in calf intraMF assessed using 3.0T IDEAL MRI and 1.0T FSE MRI.

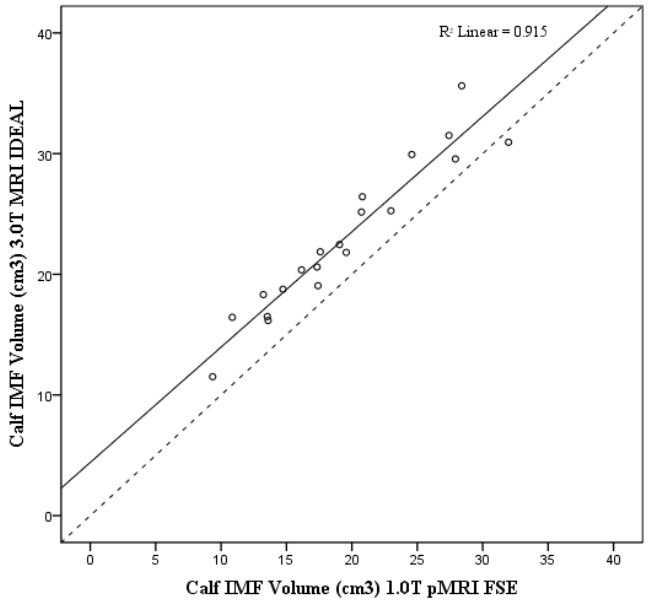

Figure 5.7. The linear relationship between calf IMF volumes imaged using a 3.0T MRI IDEAL sequence or using a 1.0T MRI FSE sequence. Solid line = linear correlation, dotted line = line of identity (y=x) illustrating scanner agreement.

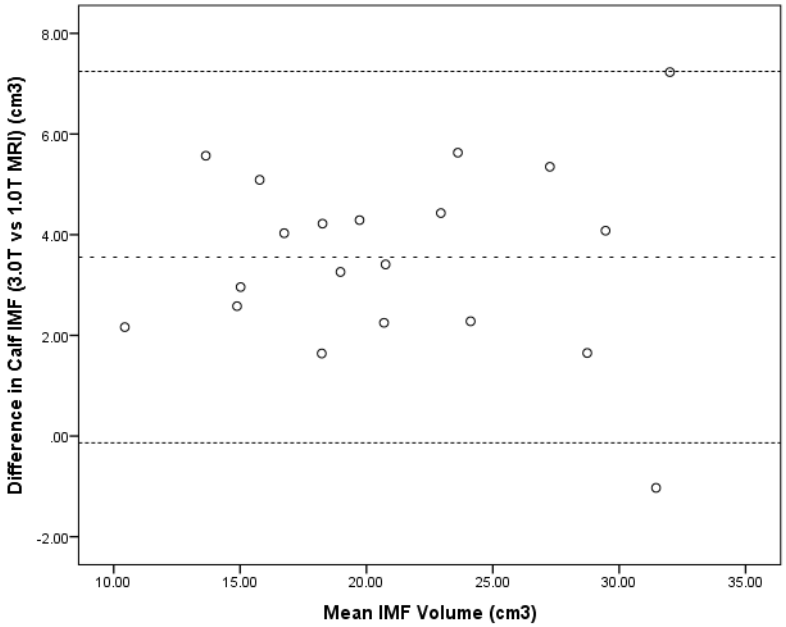

Figure 5.8. Bland Altman plot of the mean calf IMF versus the difference in calf IMF assessed using 3.0T IDEAL MRI and 1.0T FSE MRI.

5.8 Power and Sample Size Calculations

Based on the data from the present study, we sought to determine the power with which we can correctly reject the null hypothesis that the slope of the regression line between quadriceps intraMF fraction (X) and isotonic knee extensor power at 20% MVIC (Y) is equal to zero. Using the standard deviation of the X of ±1.48%, the standard deviation Y of ±89.99W, and the slope of our study's linear regression line of -1.5, we estimated the power $1-\beta$, where β is the false negative rate ($\alpha=0.05$). With a sample size of 20 and the observed regression slope of -1.5, the power of this study was estimated at 0.051. Thus, the probability of correctly rejecting the null hypothesis in the present study is low, particularly when compared to the recommendation of Cohen (1988) of a power of 0.80 (145). This agrees with our findings as we did not find a significant relationship between quadriceps intraMF fraction and knee extensor power.

Estimates of the relationship between intraMF and power were based on Taaffe et al. (2009), who found that a 7.7% decrease in muscle attenuation was associated with a 17% decrease in 1-repitition maximum strength. We assumed a strong negative relationship between CT muscle attenuation and MRI intraMF fraction, as well as a strong positive relationship between 1-repitition maximum torque and isokinetic power at 20% MVIC. Thus, it was estimated that a 1% increase in quadriceps intraMF fraction is associated with a 18W decrease in isokinetic knee extensor power (or a slope of -18 for the regression line) (2). These estimates utilize the percent changes found by Taaffe et al. (2009) in relation to the mean values of X and Y from the present study. In this case, the sample size required to correctly reject the null hypothesis would be n=91. Conversely,

the power of the present study (n=20) to correctly reject the null hypothesis when investigating the estimated regression slope of -18 was 0.225, below the recommendation of 0.80 set out by Cohen (1988) (145). As expected, a sample size of at least n=91 should be used to further investigate the relationship between quadriceps intraMF fraction and isokinetic knee extensor power at 20% MVIC.

Chapter Six: Discussion

This study characterized thigh intraMF and its relationship to knee extensor and flexor power in women with knee OA. In exploring these relationships, we accounted for the role of neuromuscular activation, recorded using EMG. Neuromuscular activation is an important factor in the relationship between fat, lean mass and power in women with knee OA. This study used the IDEAL MRI sequence to image the thighs of women with knee OA. Using semi-automated analysis software we were able to quantify fat within muscle bellies, or intraMF, separately from the lean muscle component of the quadriceps and hamstrings, while also quantifying IMF within the fascia and outside of muscle bellies. This study also investigated the relationship of intraMF with physical performance measures in women with knee OA. To our knowledge, this is the first study to investigate the relationship between intraMF and IMF in the thigh and calf, using IDEAL 3.0T MRI method with semi-automated analysis to separate muscle bellies in the calf, and thereby accurately measuring these fat subsets separate from lean muscle. This is also the first study to compare the 3.0T IDEAL method with a 1.0T FSE T1-weighted method of quantifying calf intraMF and IMF. Together this body of work has implications for future studies investigating intraMF, particularly relating to its role in the thigh muscles in individuals with knee OA.

6.1 Thigh IntraMF, Peak Power and Physical Function

A significant, positive relationship between quadriceps intraMF volume and knee extensor power at 20% MVIC, controlling for VL mean RER was found. The direction of this relationship is opposite to our initial hypothesis. However, no relationship existed between % intraMF muscle composition and knee extensor or flexor power in this sample of women with knee OA. Thus, factors other than quadriceps intraMF must be responsible for the positive relationship between quadriceps intraMF volume and knee extensor power. Obesity is related to both high lean muscle mass and high thigh muscle intraMF compared to non-obese individuals (78, 89). It is possible that women with greater quadriceps intraMF volume also have greater quadriceps lean muscle volume, particularly as the mean BMI (30.2 kg/m^2) of women in this study is categorized as class I obese. We did find a significant positive correlation between intraMF volume and lean muscle volume in the hamstrings, but not in the quadriceps (data not shown). While quadriceps lean muscle mass is an important factor in knee extensor strength, it does not explain the observed positive relationship between intraMF volume and knee extensor power in this study. Other studies that have found a negative relationship between quadriceps intraMF and knee extensor strength have represented intraMF as a fraction of total muscle or as a component of the muscle's total density, emphasizing the importance of controlling for lean muscle mass as a factor in the relationship between thigh intraMF and strength (93, 94).

Interestingly, studies have demonstrated that in older obese individuals there is a decrease in muscle quality, or the strength per unit CSA of muscle (78-81). Obesity

exacerbates the physical function deficits associated with aging in a phenomenon termed sarcopenic obesity (86). The IMF and intraMF fat subsets have been implicated in this decline in muscle quality and physical function in older individuals, and particularly individuals with knee OA (88, 93, 94). Goodpaster et al. (2001) found a positive relationship between quadriceps or hamstrings muscle attenuation and isokinetic specific force (93). However, the Pearson coefficient (r=0.26) was not strong for the correlation between mid-thigh muscle attenuation and quadriceps specific torque, and maximal torque was only slightly increased for women, but not for men, across muscle attenuation quartiles (93).

Several important differences between the Health ABC study population and the current sample may explain the observed differences in the relationship between intraMF and muscle strength. Women in the Health ABC cohort had a mean age of 73.4 years, higher than our sample mean age of 65.2 years. As such, the relationship between intraMF and muscle strength may be dependent on the individual's age as it relates to the extent of sarcopenia and loss of lean mass. Nearly half of the women in the Health ABC cohort were black, and it was shown that black women had a significantly lower mean attenuation value, or greater intraMF, compared to white women of the same age (93). Furthermore, our sample of women had a mean BMI of 30.2 kg/m^2 compared to women in the Health ABC cohort who had a mean BMI of 27.6 kg/m^2. Our high representation of obese women within a population of individuals with knee OA is representative of the strong association of these factors and knee OA (146). The relationship between intraMF and quadriceps specific force may be different in obese compared to non-obese

individuals. Lastly, the Health ABC study had a much larger sample size (n=2627) compared to the present study, and this additional power may have allowed it to more accurately investigate the relationship between intraMF and strength.

In a sample of obese and non-obese men and women ages 50-59, there was no relationship between quadriceps intraMF CSA and peak strength (89). Further, there was no difference in intraMF CSA between individuals with and without knee OA, when controlling for BMI (89). The current study supports these findings by showing that in women with knee OA, intraMF fraction was not related to knee extensor or flexor power. Segal et al. (2011) also found that 53% of quadriceps strength was accounted for by variation in lean muscle CSA. Thus, while the loss of lean mass with age does not account for all variation, it may be the single strongest factor affecting quadriceps strength in individuals with knee OA. In support of this, Toda et al. (2000) found that lean body mass of the lower extremities, but not the upper extremities or trunk, were reduced in women with knee OA compared to controls (147). A better understanding of the relationship between changes in body mass and muscle composition in individuals with knee OA is required, particularly in the context of sarcopenic obesity.

An important difference in the current study is the use of MRI for quantifying IMF and intraMF, as opposed to CT. Muscle attenuation determined using CT is related to muscle triglyceride content determined using muscle biopsy (r=-0.58) (91). However, CT scans of soft tissue have limited resolution, making it difficult to differentiate lipid and muscle tissue, and resulting in a portion of the surrounding IMF being included in the muscle attenuation value (91, 148). Conversely, MRI provides high tissue contrast

allowing for better assessment of tissue volume and architecture, and has been shown to be a more sensitive technique than CT or ultrasound in identifying skeletal muscle alterations associated with myotonic dystrophy (149). Using MRI also avoids the ionizing radiation associated with CT scans. Importantly, our study is one of the first to assess thigh intraMF using MRI spanning a large volume of the quadriceps and hamstrings muscle (18cm), whereas previous CT-based studies have used a single slice at the mid-thigh as representative of total thigh muscle attenuation (89, 93). By covering a greater volume of the thigh musculature, we provide a more accurate assessment of quadriceps and hamstrings muscle composition.

One recent study by Kumar et al. (2014) also quantified intraMF using IDEAL-based MRI, and investigated its relationship to muscle strength, physical function and disease severity in a large sample (n=96) of individuals with and without radiographic knee OA (94). They found that intraMF muscle fraction was related to worse KOOS Symptoms, Pain and ADL scores, as well as poor performance-based physical function based on the 6MWT and stair ascent/descent test. Furthermore, they found a correlation between intraMF and greater total cartilage and meniscus modified-whole-organ magnetic resonance imaging score (mWORMS) scores. It is important to note that they did not control for lean muscle CSA in the relationship between intraMF fraction and radiographic OA severity, suggesting the possibility that loss of lean mass may be responsible for the high intraMF fraction and worse mWORMS scores. In agreement with our study, they did not find a relationship between quadriceps intraMF fraction and quadriceps MVIC peak torque. However, we did not find an association between %

intraMF fraction and performance tests, including the 9-step SCT and 6MWT in women with knee OA, whereas they did. An important difference is that Kumar et al. investigated a smaller region of thigh muscle, spanning 2cm compared to the current study's 18cm region. Our sample of women had mean quadriceps and hamstrings intraMF fractions of 5.56% and 9.82%, respectively. These findings closely agree with those of Kumar et al. (2014) whose sample of men and women with knee OA had mean quadriceps and hamstrings intraMF fractions of approximately 6.5% and 11%, respectively. However, the larger sample size in the study by Kumar et al. may have been able to more accurately quantify the relationship between intraMF and physical function.

A unique aspect of the current study is that it investigated the relationship between intraMF and knee extensor and flexor isotonic power, the product of both contractile force and velocity, which is more closely related to physical function than muscle strength (28). As such, our finding that intraMF muscle fraction was not related to muscle power or physical performance suggests that this fat depot does not share a strong relationship with physical function in this sample of women with knee OA. Lang et al. (2010) investigated risk of hip fracture in older women and found that low midthigh muscle attenuation was related to increased risk of hip fracture even after adjusting for maximal knee extensor isokinetic strength, suggesting that intraMF is responsible for other functional impairments not encompassed by measures of strength (150). Visser et al. (2005) also found that older men and women in the lowest and highest quartile for muscle attenuation had incident mobility limitations hazard ratios of 1.91 and 1.68, respectively. Despite the fact that knee extensor and flexor power at low isotonic

resistance is more closely related to physical function than strength, we did not see a relationship between physical performance or muscle power with intraMF muscle fractions. Further investigation of the relationship between thigh intraMF and muscle power is needed to understand the underlying cause of functional limitations associated with this fat subset.

This study is also the first to investigate the relationship between thigh intraMF and isotonic power while controlling for neuromuscular activation. The decline in EMG peak amplitude and RER with age is well documented, making this an important factor in sarcopenia and dynapenia (46-49). Previous studies investigating the relationship between intraMF and strength in individuals with knee OA have not included neuromuscular activation in this relationship, despite the fact that it is an important factor in OA-related deficits in quadriceps strength (52). The RER in particular may reflect the fast-twitch or velocity related aspect of muscle power. Research has not yet investigated the possible role of intraMF in OA-related deficits in neuromuscular activation. This will be an important area of future research in understanding the relationship between quadriceps intraMF and physical function in individuals with knee OA.

6.2 Thigh IMF and Peak Power

Our study found that IMF volume in the thigh was not related to knee extensor or flexor power in women with knee OA. Thigh IMF has been described as a peripheral ectopic fat depot sharing vascularization with the thigh muscle it surrounds (151). Thigh IMF has been associated with decreased peak exercise oxygen consumption, and is a

factor explaining sex-related differences in physical performance in older adults (152, 153). Furthermore, high thigh IMF and low thigh lean mass independently predicted annual decline in gait-speed over four years in the Health ABC cohort of older adults (154).

Research has suggested that there is an optimal range of IMF volume for physical function. During a 3-month fall prevention program paired with either eccentric or traditional strength training, older adults that were stratified as high intermuscular adipose tissue, or those above the mean, had a reduction in these fat subsets with exercise; while individuals with low intermuscular adipose tissue had an increase in these fat subsets over the course of the exercise intervention. Their findings agree with those of Newman et al. (2003) who found that body fat mass had a quadratic relationship with lower leg specific force, in that high or low fat mass was associated with decreased specific force. Thus, being within a range encompassing 'optimal' amount of body fat, and thus IMF and intraMF, may be important for muscle quality and function. It is also important to note that the relatively small relationship between IMF and physical function may not have been accurately captured with the present study's sample size. However, another important difference with the present study is that these studies group intraMF and IMF into a single CSA measure, which they called intermuscular fat.

Differences between IMF, or fat within the fascia and between muscle bellies, and intraMF, or fat within the fascia and within muscle bellies, may exist in their relationships to physical function and strength. Maly et al. (2013) found that IMF, defined in the same manner as the present study, was weakly related to knee extensor strength and

performance on the repeated chair stands test (88). Beattie et al. (2012) found no difference in 2-year change in thigh IMF in individuals with and without radiographic knee OA (41). Although we hypothesized that thigh intraMF would be more closely related to muscle power than thigh IMF, neither intraMF nor IMF was related to knee extensor or flexor peak power in the present study.

However, studies have shown a relationship between these fat subsets. Goodpaster et al. (2001) found that IMF was related to BMI, and that CT muscle attenuation as a measure of intraMF was related to IMF volume (93). While their study found a negative relationship between muscle attenuation and quadriceps specific force, they did not find a relationship between thigh IMF and quadriceps specific force, similar to the present study. In the present study sample of women with knee OA we did not find a significant correlation between total thigh intraMF and IMF (data not shown). Kumar et al. (2014) also found no differences in thigh IMF volume in older adults with and without radiographic knee OA (94). Further investigation is needed of the difference between thigh intraMF and IMF and their relationship to physical function, and whether these fat subsets are independently related to physical function in older adults with knee OA.

6.3 Thigh and Lower Leg IntraMF and IMF

The current study found significant, positive relationships between the volumes of intraMF in the thigh and calf, as well as volumes of IMF. To our knowledge, we are the first study to demonstrate these relationships by separately quantifying intraMF and IMF using an IDEAL-based MRI method. Using this method, we were able to separately

identify muscle bellies in the lower leg to provide an accurate quantification of intraMF separate from IMF. There was a stronger relationship between intraMF compared to IMF between the thigh and calf. This finding indicates that decreases in the proportion of lean mass in muscle bellies, rather than fat accumulation between muscle bellies, is more uniform in the lower limb, and may suggest that intraMF accumulation is of more consequence than IMF to physical function in the lower limb of older adults. Our findings agree with those of Goodpaster et al. (2000) who found a positive correlation (r=0.60) between muscle attenuation in the mid-thigh and mid-calf similar to the correlation found in the present study. They also found that mid-thigh muscle attenuation was related to psoas and erector spinae muscle attenuation, suggesting that intraMF infiltration is related throughout the trunk and lower limbs. This may implicate intraMF in age-related functional declines in areas other than the lower limbs. To this effect, IDEAL-based MRI assessing intraMF infiltration of the subscapularis muscle found a relationship with increased shoulder pain and decreased range of motion (99).

Fat accumulation within the lower leg is of particular interest in individuals with knee OA due to the role of these muscles in gait and mobility. Using T1-weighted 3.0T MRI, Buford et al. (2012) found that intermuscular adipose tissue, defined as all fat within the fascia, in the tibiofibular and femoral regions were increased in older compared to younger adults, but not between high- and low-functioning older adults (155). In addition, they found that both femoral and tibiofibular intermuscular adipose tissue volumes were not related to scores on the short physical performance battery or usual gait speed in older adults. Their findings suggest that while older adults have high IMF and

intraMF mass and low lean muscle mass in both the calf and thigh, these alterations may not be of equal consequence to physical function in older adults. Nonetheless, proper activation of the lateral gastrocnemius is important in stabilizing the knee joint during gait, and alterations in lower leg strength due to fat accumulation may play a role in disease progression. At this point, the role of the lower leg muscles in knee OA is not well understood, and the accumulation of intraMF and IMF in the calf and their potential effects on calf muscle function warrant further investigation.

Due to the importance of intraMF and IMF in function in the elderly, as well as their associations with metabolic syndrome and insulin resistance, recent research has investigated interventions which can target these fat subsets. In a group of sedentary older men and women, Murphy et al. (2012) found that both exercise and calorie restriction interventions, based on the same energy deficit, caused decreases in bilateral thigh intermuscular fat volumes, defined as total fat within the epimysium fascia, although exercise caused a preferential reduction in this fat depot (156). Similarly, in a diverse population of individuals aged 18-87 years and with various disease states including individuals (n=17) who had undergone knee replacement surgery, a positive relationship was found between age and intermuscular adipose tissue (157). In the study, individuals older than 55 years (n=32) showed an 11% reduction in thigh intermuscular adipose tissue and a 7% increase in thigh lean tissue after 12-weeks of resistance training. Although the benefits of strengthening exercise on pain, range of motion and physical function in individuals with knee OA are well documented, the effect of this intervention on thigh IMF and intraMF is not well understood in individuals with knee OA. As

changes in quadriceps strength in older men are not strongly associated with increases in lean mass, it is possible that these interventions improve muscle composition through a reduction of fat deposits (50). This supports the notion that strengthening exercise is an ideal intervention for improving physical function in older adults with knee OA.

6.4 Assessments of Fat using 3.0T IDEAL MRI and 1.0T FSE pMRI

A significant, positive correlation existed between lower leg intraMF quantified using 1.0T FSE T1-weighted pMRI, and intraMF in the same region quantified using 3.0T whole-body MRI based on the IDEAL method of fat/water separation. Similarly, we found a significant, positive correlation between lower leg IMF quantified using the same imaging methods. In addition, using Bland Altman plots we demonstrated that these two methods show good agreement. These findings suggest that while 3.0T whole-body MRI based on the IDEAL method is widely regarded as optimal for the quantification of adipose tissue, 1.0T T1-weighted pMRI provides an accurate and more readily available substitute for the quantification of intraMF and IMF in the lower limb. Furthermore, due to the demonstrated significant correlations between these fat subsets quantified using 1.0T T1-weighted pMRI in the lower leg and 3.0T IDEAL MRI in the thigh, quantification of lower leg intraMF and IMF using pMRI may provide an accurate estimate of thigh intraMF and IMF.

Similar to our findings, Alabousi et al. (2011) found that there were no differences in mid-thigh and mid-calf SCAT assessed using 1.5T Spin Echo MRI, 3.0T T1-weighted spoiled gradient echo (SPGR) and 3.0T IDEAL-SPGR (158). However, they did find a

significant difference between IDEAL-SPGR and the other MRI methods for quantifying visceral adipose tissue in the abdomen, which they attributed to the ability of IDEAL to differentiate adipose tissue from blood vessels and bowel content. Because we included vessels and nerves in our quantification of IMF using both methods, this would explain the close agreement we observed between methods. However, based on the ability of IDEAL to produce images in which fat alone appears brightly, quantification of adipose tissue excluding these non-adipose structures would be more accurate with the IDEAL method had we approached segmentation with this method. Our inclusion of these non-adipose structures is based on a previously described method of segmentation using T1-weighted images which demonstrated a relationship between IMF, quadriceps strength and physical function in women with radiographic knee OA (41, 88). Alizai et al. (2012) found that a semi-quantitative method, the Goutallier system, for grading intraMF infiltration using T1-weighted MRI was strongly correlated with intraMF quantified using IDEAL-based water/fat separated images in the calf (159). However, they did find that the semi-quantitative method overestimated muscle fat infiltration.

The ability of MRI approaches based on fat-optimized spoiled gradient-echo sequences to accurately measure the distribution of muscular fat has been questioned. Schick et al. (2002) found that there were high inter-rater differences in lipid distribution in the lower leg, with relatively high lipid content in the soleus and peroneus muscles and low lipid content in the tibialis muscles due to variations in signal-to-noise ratios across scan regions (160). As the IDEAL approach for fat separation allows for high spatial resolution, it allows for reliable quantification of intraMF and IMF in localized regions.

Furthermore, the elimination of chemical shift artifacts due to fat suppression in the IDEAL method allowed for more accurate quantification of these fat subsets in the present study. The IDEAL sequence has been used to demonstrate that intraMF, but not IMF, in the calf muscles is increased in individuals with diabetes compared to controls (161). Therefore, while conventional T1-weighted MRI provides an accurate assessment of total intraMF and IMF in the lower leg in the present study, due to differences in spatial resolution, signal-to-noise ratio and chemical shift when compared to IDEAL sequence MRI, the localized measurement of fat subsets surrounding muscles and within specific muscle groups is best approached using the IDEAL method.

6.5 Study Strengths and Limitations

This is one of the first studies to separately quantify intraMF and IMF in the thighs and lower legs of women with clinical, radiographic knee OA. Another strength of the study was exploring the relationship between these thigh fat subsets and isotonic knee extensor and flexor power while controlling for EMG activation. The use of isotonic power as a dependent variable provides a more accurate representation of physical function than measures of strength in this sample of women with knee OA. Neuromuscular activation is an important factor in strength, particularly in individuals with knee OA, and future research investigating thigh muscle strength and power in knee OA should also control for this variable.

Our findings are strengthened by the diversity of this study's sample of women with symptomatic, radiographic knee OA. The age of women in this study ranged from 56

to 76, a span of years associated with significant declines in lean mass and strength (162). Knee pain assessed using the ICOAP scale demonstrated our participants symptoms ranged from no intermittent or constant pain to moderate (mean score 63.6 out of 100) knee pain. Similarly, KOOS Pain and Symptoms mean scores were within the midrange indicating moderate pain (mean score 64.4) and symptoms (mean score 62.3), with a wide distribution. The KOOS ADL and FSR scores showed the widest distribution with individuals demonstrating no limitations in ADL or FSR (score 100) and an individual demonstrating maximum limitation in FSR (score 0). Similarly, KOOS QoL had a mean score of 53.3, near the midrange.

Our sample had lower KOOS scores representing more severe symptoms and functional limitations than another sample of obese and overweight women with severe radiographic knee OA preparing for TKA (163). This may be due to the fact that our sample was initially selected based on the presence of symptomatic knee OA, followed by confirmation of radiographic knee OA. Conversely, this may be a factor of the proposed discordance between knee OA symptomatic and radiographic severity (12). Our sample mean PASE score agreed closely with the mean PASE of 182.8 found in individuals with radiographic or symptomatic knee OA in the Osteoarthritis Initiative (142).

Several strengths are associated with our MRI method of quantifying thigh and calf fat deposits. By quantifying fat and muscle within a region spanning 18cm of the thigh, we provide a highly accurate estimate of total tissue volumes in the thigh. Although smaller, the 3cm region of interest used for quantification of fat and muscle in the lower leg also encompasses a large volume of the calf muscles and provides a much stronger

estimate than a single-slice based approach for estimation of total calf tissue volumes. The use of the IDEAL sequence with 3.0T MRI allowed for high spatial resolution to separately identify muscle bellies in the thigh and lower leg, as well as fat within and outside of these muscles. This novel approach will allow for future research to non-invasively and accurately quantify the muscle composition of specific, deep muscle bellies.

The use of muscle peak power as a dependent variable was based on the finding that isotonic power, especially at low isotonic resistances, is more closely associated than MVIC peak torque with physical function (28). This may be attributed to the velocity- and time-dependent manner of muscle activation and contraction during functional tasks, such as gait, chair stands and stair climbing. Another strength of the study was the investigation of the relationship between intraMF or IMF and isotonic muscle power at moderate and low isotonic resistances (40% or 20% MVIC). However, it is possible that fat subsets are more strongly related to peak power at higher (50% or greater) isotonic resistances, or to isometric peak torque.

There are several limitations to the present study. Due to the small sample size (n=20) this study likely does not have enough power to quantify the relationship between intraMF and isotonic muscle power in women with knee OA. However, due to the accuracy of the imaging protocol we hypothesized that we would be able to accurately quantify these fat subsets and investigate their relationship to muscle power. As discussed in our results section statistical power analysis, a sample size of n=91 would be able to more accurately identify the relationship between quadriceps intraMF and knee extensor

power. In addition, due to limitations in study power we were unable to control for relevant covariates affecting thigh muscle power and physical function, such as the presence of knee pain, self-reported physical activity, radiographic disease severity, age, BMI, and lean muscle mass. We also do not provide K-L grades of radiographic disease severity, as our inclusion criteria of radiographic OA was based on clinical radiologist-assessed x-rays without scoring. In addition, our findings are limited to women with knee OA, as men were excluded in order to reduce sample variation.

Some limitations in the MRI-based quantification of fat and muscle in this study should be noted. Firstly, as the region-growing algorithm used with SliceOmatic software requires manual selection of a threshold separating muscle and fat tissue, threshold selection is subject to potential inter-rater variation, as well as variation in signal intensity within axial slices, between slices within the same subject, and between participant scans. However, we utilized the SCIC and PURE post-processing methods of correcting for signal intensity heterogeneity within each slice. Furthermore, in the present study a single observer was used to analyze all scans, eliminating inter-rater differences. The analysis of intraMF and IMF based on the region growing method described is time consuming, taking approximately 12 minutes for a highly trained observer to analyze all tissues on each slice. Secondly, imaging based assessments of fat and muscle, including the IDEAL sequence method used in this study, are sensitive to motion artifact causing distortions in signal intensity across the axial field of view. A small number of individuals in the present study exhibited slight motion artifact, although this artifact was exclusively limited to the posterior thigh. This motion was exclusively found in whole-body MRI of

the thigh and not of the lower leg. However, the scan times of approximately 6 to 7 minutes in this study are reasonably short.

6.6 Conclusions and Future Directions

We did not find a relationship between either quadriceps or hamstrings intraMF with isotonic knee extensor or flexor peak power, after controlling for EMG activation, in a sample of women with clinical, radiographic knee OA. Similarly, we did not find a relationship between thigh IMF and either isotonic knee extensor or flexor peak power, controlling for EMG activation. In addition, we found a strong correlation between intraMF in the thigh and lower leg, as well as a moderate correlation between IMF in the thigh and lower leg. We demonstrated that 1.0T FSE pMRI and IDEAL-based 3.0T whole-body MRI provide good agreement and strong correlations in the volume of lower leg intraMF and IMF.

Due to the importance of quadriceps and hamstrings strength, and potentially power in individuals with knee OA, further research is required into the role of these fat subsets in measures of physical function and strength. In particular, future research should aim to identify longitudinal changes in intraMF and IMF and their relationships to changes in power and physical function in the thighs of men and women with knee OA. Research should also investigate the relationship between these fat subsets and deficits in neuromuscular activation of the quadriceps and hamstrings of individuals with knee OA.

We present an MRI-based method using the IDEAL sequence to separately quantify intraMF and IMF volumes, as well as lean muscle volume in the thigh and lower

leg. This method has important implications for further understanding the role of muscle composition in several disease states, and particularly in the functional limitations of individuals with knee OA. Studies such as this that include diverse measures of physical function such as muscle power, neuromuscular activation and muscle composition will provide a clear understanding of the functional decline and disability associated with aging and knee OA.

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Appendix A: Participant Screening and Tracking

Inclusion Criteria (American College of Rheumatology Clinical Criteria):

Age between 60 and 75 years of age?	Yes	No
Knee pain on most days of the week?	Yes	No
Less than 30 minutes of morning stiffness?	Yes	No
Crepitus with active range of motion?	Yes	No
Bony enlargement?	Yes	No
Bony tenderness to palpation?	Yes	No
Signs of inflammation (warmth, swelling)?	Yes	No

Exclusion Criteria:

Any other forms of arthritis (rheumatoid, psoriatic)?	Yes	No
Knee surgery? Excluded: high tibial osteotomy, joint replacement, ligament repair Included: unrepaired lax ligament, arthroscopic debridement (or "clean up"), hyaluronic acid injections including "synvisc"	Yes	No
Do you use a cane or other helping aid to get around? Excluded: unable to ambulate 20' without an aid	Yes	No
Do you have an unstable heart condition or a history of stroke? Excluded: physician-advised restrictions to physical activity	Yes	No
Have you injured your hip, knee, or ankle in the past three months? If so, which leg?: _____	Yes	No
Are you currently receiving cancer treatment?	Yes	No
Are you/could you be pregnant?	Yes	No
Have you used/are you using oral steroids for >3 months?	Yes	No

Notes

Which knee will be studied (circle)? LEFT RIGHT

Identification:

Last Name		First Name	
Sex		Birthdate (MM/DD/YYYY)	
Phone Numbers	Home		
	Office		
	Mobile		
Email		Alternate Email	

***Please put an asterisk beside preferred mode of communication.**

Emergency Contact:

This person will be contacted in the unlikely event of an emergency

Name		
Relationship to You		
Phone Numbers	Home	
	Office	
	Mobile	
Email		

Appendix B: MRI Safety Screening Form

MRI system ownership:
IRC, St. Joseph's Hamilton
50 Charlton Avenue East, Hamilton, Ontario, Canada L8N 4A6

Date: _____/_____/_____ (d/m/y)

Study ID: _____

Year of birth: _____ Month of birth:_____

Weight: _____ (kg) Height: _____ (m)

Is there any possibility that you may be pregnant? **YES** □ **NO** □
Have you ever worked with metal (hobby/occupation)? **YES** □ **NO** □

Please check if you <u>have</u> any of the following:

Pacemaker, defibrillator, pace wires	Yes □	No □
Prosthetic heart valve	Yes □	No □
Electrodes, shunts, plates, aneurysm clips	Yes □	No □
Vascular access port or cathe er	Yes □	No □
Intravascular coils, filters or stents	Yes □	No □
Insulin pump or infusion pump	Yes □	No □
Cochlear, stapes or orbit/ear implants	Yes □	No □
Bone growth/fusion stimulator	Yes □	No □
Implanted neur stimulator	Yes □	No □
Metal or wire mesh implants	Yes □	No □
Artificial limb or joint	Yes □	No □
Metal rods, pins or plates in a joint or bone	Yes □	No □
Bullets or shrapnel in your body	Yes □	No □
Metal fragme ts in your eye(s)	Yes □	No □
Tattoos, tattooed makeup, body piercing	Yes □	No □
Any other implanted device	Yes □	No □

Please check if you <u>have ever had</u> any of the following:

Brain, ear, eye or head surgery	Yes □	No □
Vascular (vein) surgery	Yes □	No □
Bone or joint surgery	Yes □	No □

Before your MRI, please REMOVE shoes and ALL metal objects, including:

-hearing aid -barrettes/hair pins -safety pins/clips -jewelry/keys
-credit cards -pocket knife -coins/change -pens/pencils
-watch -cellular phone/pager -clothing/undergarments containing metal

Appendix C: Case Report Form

STUDY ID:_____ **DATE:_____/_____/_____**
DAY MONTH YEAR

Complete this form after patient has given informed consent.

1.0 Demographic Information

1.1 Age: _____years

1.2 Gender: _____

2.0 Anthropometric Data

2.1 Body Mass: _____ (kg) Height:_____(cm)

2.2 BMI: _____

2.3 Waist circumference:_____(cm) Hip circumference:_____(cm)

2.4 Waist-to-hip ratio:_____

3.0 Knee Osteoarthritis and Pain

3.1 Have you been diagnosed with knee osteoarthritis?

- ❑ Yes
- ❑ No

3.2 What, if any, prescribed medication is the participant currently taking?

Name	Frequency	Dose	Duration

4.0 Diabetes and Current Treatments

4.1 Does the participant have T1 or T2 Diabetes?

- ❑ Yes
- ❑ No

4.2 What treatment or medications are they receiving for their diabetes?

Name	Frequency	Dose	Duration

5.0 Physical Activity Tests

5.1 30 Second Chair Stands Test

The participant is asked to perform the 30 second Chair Stands Test. The participant is instructed to begin in a seated position, and stand from a sitting position as many times as possible within the 30 seconds. The participant will be allowed to practice standing from the sitting position once, then they will perform the timed test. The study coordinator will inform the participant when the 30 seconds are over.

Repetitions: _____Chair stands

5.2 Timed Up and Go (TUG) Test

The participant is asked to perform the Timed Up and Go Test. The participant is instructed to rise from sitting on a chair placed securely against a wall, walk 3m to a marker on the ground, turn, walk back to the chair, then sit down. The study coordinator times this test.

Time: _____seconds

5.3 6 Minute Self-Paced Walk Test

The participant is asked to perform the 6 Minute Walk Test in a rectangular corridor. The participant is instructed to cover as much distance as they can over 6 minutes, walking at a comfortable pace, and that they may stop or rest at any time if necessary.

Distance: _____meters

5.4 Stair Ascent and Descent Test

The participant is asked to perform the Timed Stair Ascent and Descent Test. The participant is instructed to ascend, turn around, and then descend 9 steps at a pace that is comfortable and safe. Both handrails are available for support, however, the participant is asked to use only one handrail. The ascent and descent are timed separately. This test is performed twice for increased measurement reliability.

Trial 1: Ascent Time:_____seconds Descent Time:_____seconds
Trial 2: Ascent Time:_____seconds Descent Time:_____seconds

5.5 40 Meter Fast-Paced Walk Test

The participant is asked to perform the 40 meter Self-Paced Walk Test. Participants are asked to walk as quickly but as safely as possible to a mark 10 m away, return, and repeat for a total distance of 40 m. Subjects are timed for this test and data is expressed as speed (m/s).

Speed: _____meters/second

6.0 Power and Electrical Potential Measurements

6.1 Peak Maximum Voluntary Isometric Contraction (Nm)

1. Knee Extension: _____

2. Knee Flexion: _____

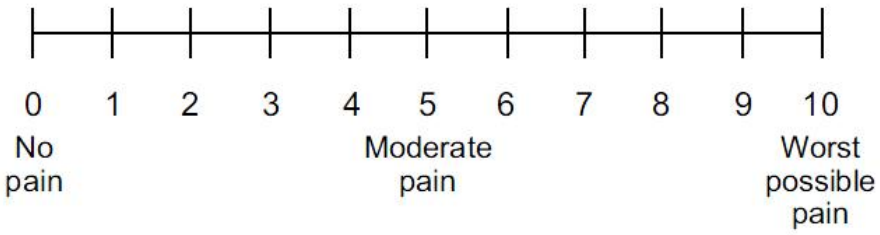
6.2 Peak Power Measurements (W)

	Extension	Flexion
1. 40% MVIC:	_____	_____
2. 20% MVIC:	_____	_____
3. 1 Nm:	_____	_____

7.0 Numeric Pain Rating Scaling Following Physical Activity

7.1 Please rate the intensity of the pain in your knee by circling the appropriate number on the scale below.

0-10 Numeric Pain Intensity Scale



Appendix D: Letter of Information and Consent

Thigh composition, Muscle Power and Activation in Women with Clinical Knee Osteoarthritis

Principal Investigator: Michael Davison
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Co-Investigators: Karen Beattie, Assistant Professor
Dr. Rick Adachi, Rheumatologist
Dr. Monica Maly, Assistant Professor
McMaster University

Introduction

You are being asked to take part in a study that investigates the association between fat and muscle volumes in your thigh and the strength of your leg. Before agreeing to participate, it is important that you read and understand the proposed study procedures. The information provided describes the purpose, procedures, benefits, discomforts, risks and precautions associated with this study. It also describes your right to refuse to participate or withdraw from the study at any time. To decide whether you wish to participate, you should understand enough about its risks and benefits to be able to make an informed decision. This is part of the informed consent process. Make sure all of your questions have been answered to your satisfaction before signing this document.

Background and Purpose

Knee osteoarthritis is a leading cause of mobility disability in North America and is most common in women above the age of 60. The factors that cause its onset and progression are poorly understood. A recently proposed factor in knee osteoarthritis is the strength and power of the thigh muscles. As the knee is supported and moved by the thigh muscles, it is believed that weaker muscles may increase risk and severity of osteoarthritis. The fat within the thigh muscles may reduce the ability of muscle to generate power. Until now, we have been unable to properly visualize and measure this fat using MRI.

The purpose of this study is to determine whether the amount of fat within your thigh muscles is related to the strength and power of your thigh muscles. We also aim to determine if there are similar fat contents in the thigh and calf muscles. To

participate in this study, you will need to be a woman between the ages of 60 and 75 years, with diagnosed knee osteoarthritis. We cannot include anyone who has metal anywhere in their body or anyone who has medical restrictions to physical activity, may be pregnant, or has a history of other forms of arthritis, injury or surgery in their legs. We expect that 20 people will participate.

Procedure

If you meet the above criteria, we welcome you to enroll. We will ask if we can contact you via telephone, or email over the course of the study to answer any questions that may arise, and book appointments for these visits. We will ask if we can retain contact information for a friend or family member in case we cannot contact you.

First, we will ask you to visit 25 Charlton Avenue East, Suite 501, across the road from St. Joseph's Hospital, for a visit with Dr. J. D. Adachi, rheumatologist. Here you will be asked questions and assessed by Dr. Adachi to determine the presence of knee osteoarthritis. You will also be directed to the parking level floor to the x-ray center for an x-ray of your symptomatic knee. X-rays are performed on a first-come first-serve basis, and you may return any other day to obtain the x-ray if need be. Parking is available behind the clinic off of Forest Ave, and you will be reimbursed for expenses.

Second, we will ask you to return to 25 Charlton Avenue East, this time to Suite 612 for a peripheral 1 Tesla MRI scan, and two physical performance tests. You will be asked to complete the following:

- A brief interview of your general health to ensure you do not have metal anywhere in your body.
- A timed chair sit, in which you will be asked to stand from a seated position as many times as possible in 30 seconds. You may set the pace and stop at any time.
- A Timed Up and Go test, in which you are asked to rise from sitting on a chair, walk three meters, turn around, return to the chair and sit down. You may set the pace and stop at any time.
- A scan of your calf of your leg with the most knee pain, in our peripheral MRI scanner to assess muscle and fat in your calf, and compare results with the previous whole-body scanner at the IRC.

If possible, we will ask you to book an appointment at the whole-body 3 Tesla scanner at St. Joseph's Imaging Research Centre (IRC) on the same day. If this is not possible, this second MRI appointment may be booked on a separate day. You will be directed from the 25 Charlton Ave. E. site across the road to St. Joseph's by Michael Davison. The IRC is in the Fontonebonne building F-126, at the south end of the hospital, and parking is

accessed off of James St. We ask you to visit this center only once, for no longer than 1 hour. Your parking costs will be covered. You will be asked to complete the following:

- A brief interview of your general health to ensure you do not have metal anywhere in your body.
- A scan of your thigh of your leg with the most knee pain, in our full-body magnetic resonance imaging (MRI) scanner to assess the muscle and fat in your thigh. This scan will take no longer than 8 minutes.
- A scan of your calf of your leg with the most knee pain, in our full-body MRI scanner to assess the muscle and fat in your calf. This scan will take no longer than 6 minutes.
- You will be provided with brief questionnaires about your general health, pain, physical abilities, and arthritis symptoms. You may complete these questionnaires at home, and return them at the third visit. These will take approximately 30 minutes to complete.

Third, we will ask you to visit the MacMobilize Laboratory, located at the 1400 Main Street entrance to McMaster University. We will ask you to visit this center once. This visit will take no longer than 1.5 hours. A designated parking spot is provided. At these visits, we will ask you to complete the following:

- Walk for 6 minutes in an indoor corridor. You may set the pace and stop at any time.
- Walk 40 meters in a corridor. You may set the pace and stop at any time.
- A timed ascent and descent of nine steps in a comfortable manner. You may set the pace and stop at any time.
- We will place surface skin electrodes on your thigh and calf using double-sided tape; these will measure the electrical currents controlling your leg muscles during movement. No electrical current is applied to your muscles.
- Maximally contract your thigh and calf muscles 3 times each. You will be set up in a chair with a leg attachment that will measure your muscle power.
- In the same chair, bend and straighten your leg as quickly and forcefully as possible 10 times consecutively. These movements will be repeated at different intensities, for a total of 3 sets.

Risks and Benefits

There is a minimal risk associated with participation in this study. As long as you have no metal in your body, this is no risk associated with the MRI. You will receive no radiation from this test. You may experience some muscle soreness around your knee, typical of the discomfort felt with physical activity. Any muscle soreness should settle after 24-48 hours. You may experience some skin irritation from the double-sided tape

used to attach electrodes to your skin. It is normal to have marks on your skin up to 24 hours after the test. Pain is not usually associated with the use of these surface electrodes. If you experience any serious discomfort following a study visit, please contact the Principal Investigator, **Dr. Karen Beattie at (905) 527-9100.**

There are minimal benefits to you. You will receive a $15 Tim Horton's gift card as a sign of our appreciation for your participation in the study. You will learn about your thigh strength, the muscle and fat composition of your legs, pain and physical activity. Your participation will help us to better understand how fat within the thigh and calf muscles affect each muscles' strength, and how this relates to knee osteoarthritis severity.

Confidentiality

All information obtained during the study will be held in strict confidence. You will be identified in the study by a code only. No names or identifying information will be used in any publication or presentation. No information identifying you will be available outside the investigators. The information we collect will be secured in a locked filing cabinet in ***Suite 612, 25 Charlton Ave. E.*** to which only the researchers will have direct access. This research space is also locked. Following completion of the study, the information we collect will be destroyed. Representatives of the McMaster University Health Sciences Research Board may require access to your study-related records or may follow up with you to monitor the conduct of the research.

Participation

Your participation in this study is voluntary. If you decide to participate, you can decide to stop at any time, even after signing the consent form or part-way through the study. If you drop out of the study your data will only be used with your explicit consent. You can withdraw from the study at any time, for any reason without any negative consequences. If you do not want to answer some of the questions, you do not have to, but you can still be in the study.

Questions

If you have any general questions, please call the principal investigator in charge of this study, Dr. Karen Beattie at (905) 527-9100. If you have any questions about your rights as a research participant or the conduct of the study, you may contact the Office of the Chair of the Hamilton Health Sciences/Faculty of Health Sciences Research Ethics Board at (905) 521-2100 ext. 42013. This person is not involved with the research project in any way and calling him will not affect your participation in this study. This letter is yours to keep for future reference.

Consent

I have read the Letter of Information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction. I will receive a *signed* copy of this form.

_____	_____	_____
Participant Name (please print)	Participant Signature	Date

I confirm that I have explained the nature and purpose of this study to the participant named above. I have answered all questions.

_____	_____	_____
Person Obtaining Consent	Signature	Date

_____	_____	_____
Principal Investigator	Signature	Date

Appendix E: KOOS Survey

STUDY ID:_____ Date: _____/_____/_____
Y M D

INSTRUCTIONS: This survey asks for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to perform your usual activities.
Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

Symptoms
These questions should be answered thinking of your knee symptoms during the last week.

S1. Do you have swelling in your knee?
Never Rarely Sometimes Often Always

S2. Do you feel grinding, hear clicking or any other type of noise when your knee moves?
Never Rarely Sometimes Often Always

S3. Does your knee catch or hang up when moving?
Never Rarely Sometimes Often Always

S4. Can you straighten your knee fully?
Always Often Sometimes Rarely Never

S5. Can you bend your knee fully?
Always Often Sometimes Rarely Never

Stiffness
The following questions concern the amount of joint stiffness you have experienced during the last week in your knee. Stiffness is a sensation of restriction or slowness in the ease with which you move your knee joint.

S6. How severe is your knee joint stiffness after first wakening in the morning?
None Mild Moderate Severe Extreme

S7. How severe is your knee stiffness after sitting, lying or resting later in the day?
None Mild Moderate Severe Extreme

Pain
P1. How often do you experience knee pain?
Never Monthly Weekly Daily Always

What amount of knee pain have you experienced the last week during the following activities?

P2. Twisting/pivoting on your knee
None Mild Moderate Severe Extreme

P3. Straightening knee fully
None Mild Moderate Severe Extreme

P4. Bending knee fully
None Mild Moderate Severe Extreme

P5. Walking on flat surface
None Mild Moderate Severe Extreme

P6. Going up or down stairs
None Mild Moderate Severe Extreme

P7. At night while in bed
None Mild Moderate Severe Extreme

P8. Sitting or lying
None Mild Moderate Severe Extreme

P9. Standing upright
None Mild Moderate Severe Extreme

Function, daily living

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A1. Descending stairs
None Mild Moderate Severe Extreme

A2. Ascending stairs
None Mild Moderate Severe Extreme

For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A3. Rising from sitting
None Mild Moderate Severe Extreme

A4. Standing
None Mild Moderate Severe Extreme

A5. Bending to floor/pick up an object
None Mild Moderate Severe Extreme

A6. Walking on flat surface
None Mild Moderate Severe Extreme

A7. Getting in/out of car
None Mild Moderate Severe Extreme

A8. Going shopping
None Mild Moderate Severe Extreme

A9. Putting on socks/stockings
None Mild Moderate Severe Extreme

A10. Rising from bed
None Mild Moderate Severe Extreme

A11. Taking off socks/stockings
None Mild Moderate Severe Extreme

A12. Lying in bed (turning over, maintaining knee position)
None Mild Moderate Severe Extreme

A13. Getting in/out of bath
None Mild Moderate Severe Extreme

A14. Sitting
None Mild Moderate Severe Extreme

A15. Getting on/off toilet
None Mild Moderate Severe Extreme

For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A16. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc)
None Mild Moderate Severe Extreme

A17. Light domestic duties (cooking, dusting, etc)

None Mild Moderate Severe Extreme

Function, sports and recreational activities

The following questions concern your physical function when being active on a higher level. The questions should be answered thinking of what degree of difficulty you have experienced during the last week due to your knee.

SP1. Squatting

None Mild Moderate Severe Extreme

SP2. Running

None Mild Moderate Severe Extreme

SP3. Jumping

None Mild Moderate Severe Extreme

SP4. Twisting/pivoting on your injured knee

None Mild Moderate Severe Extreme

SP5. Kneeling

None Mild Moderate Severe Extreme

Quality of Life

Q1. How often are you aware of your knee problem?

Never Monthly Weekly Daily Constantly

Q2. Have you modified your life style to avoid potentially damaging activities to your knee?

Not at all Mildly Moderately Severely Totally

Q3. How much are you troubled with lack of confidence in your knee?

Not at all Mildly Moderately Severely Extremely

Q4. In general, how much difficulty do you have with your knee?

None Mild Moderate Severe Extreme

Thank you very much for completing all the questions in this questionnaire.

Appendix F: A Measure of Intermittent and Constant Osteoarthritis Pain, ICOAP

People have told us that they experience different kinds of pain (including aching or discomfort) in their knee. To get a better sense of the different types of knee pain you may experience, we would like to ask you about any "constant pain" (pain you have all the time) separately from any pain that you may experience less often, that is, "pain that comes and goes". The following questions will ask you about the pain that you have experienced in your knee in the PAST WEEK. Please answer ALL questions.

A) CONSTANT PAIN

For each of the following questions, please select the response that best describes, on average, your constant knee pain in the PAST WEEK.

1. In the past week, how intense has your constant knee pain been?

0	1	2	3	4
Not at all	Mildly	Moderately	Severely	Extremely

2. In the past week, how much has your constant knee pain affected your sleep?

0	1	2	3	4
Not at all	Mildly	Moderately	Severely	Extremely

3. In the past week, how much has your constant knee pain affected your overall quality of life?

0	1	2	3	4
Not at all/	Mildly	Moderately	Severely	Extremely

4. In the past week, how frustrated or annoyed have you been by your constant knee pain?

0	1	2	3	4
Not at all	Mildly	Moderately	Severely	Extremely

5. In the past week, how upset or worried have you been by your constant knee pain?

0	1	2	3	4
Not at all	Mildly	Moderately	Severely	Extremely

B) PAIN THAT COMES AND GOES

For each of the following questions, please select the response that best describes your knee pain that comes and goes, on average, in the PAST WEEK.

6. In the past week, how intense has your most severe knee pain that comes and goes been?

0	1	2	3	4
Not at all	Mildly	Moderately	Severely	Extremely

7. In the past week, how frequently has this knee pain that comes and goes occurred?

0	1	2	3	4
Never	Rarely	Sometimes	Often	Very Often

8. In the past week, how much has your knee pain that comes and goes affected your sleep?

0	1	2	3	4
Not at all	Mildly	Moderately	Severely	Extremely

9. In the past week, how much has your knee pain that comes and goes affected your overall quality of life?

0	1	2	3	4
Not at all	Mildly	Moderately	Severely	Extremely

10. In the past week, how frustrated or annoyed have you been by your knee pain that comes and goes?

0	1	2	3	4
Not at all	Mildly	Moderately	Severely	Extremely

11. In the past week, how upset or worried have you been by your knee pain that comes and goes?

0	1	2	3	4
Not at all	Mildly	Moderately	Severely	Extremely

THANK YOU!

Appendix G: PASE Questionnaire

STUDY ID:_____ Date: _____/_____/_____
Y M D

Please complete this questionnaire by either circling the correct response or filling in the blank. Answer all items as accurately as possible. All information is strictly confidential.

LEISURE TIME ACTIVITY

Over the past 7 days, how often did you participate in sitting activities such as reading, watching TV or doing handcrafts?

[0.] NEVER → GO TO Q.#2 | [1.] SELDOM (1-2 DAYS) | [2.] SOMETIMES (3-4 DAYS) | [3.] OFTEN (5-7 DAYS)

1a. What were these activities?

_____

1b. On average, how many hours per day did you engage in these sitting activities?

[1.] LESS THAN 1 HOUR [2.] 1 BUT LESS THAN 2 HOURS

[3.] 2-4 HOURS [4.] MORE THAN 4 HOURS

Over the past 7 days, how often did you take a walk outside your home or yard for any reason? For example, for fun or exercise, walking to work, walking the dog, etc.?

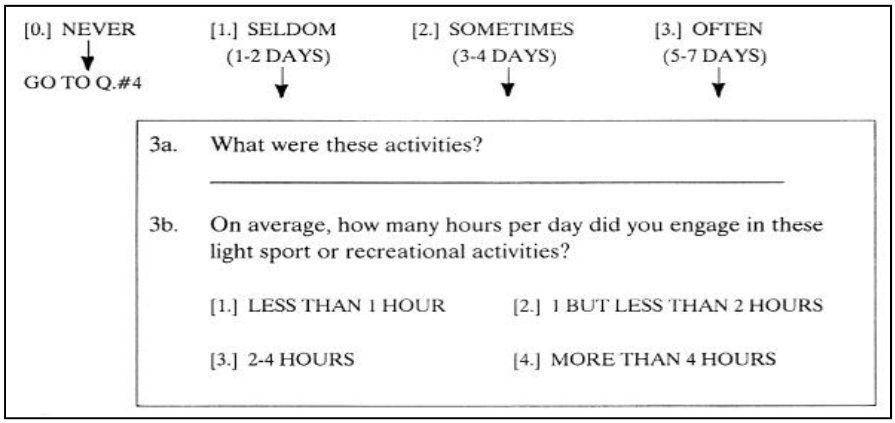
[0.] NEVER → GO TO Q.#3 | [1.] SELDOM (1-2 DAYS) | [2.] SOMETIMES (3-4 DAYS) | [3.] OFTEN (5-7 DAYS)

2a. On average, how many hours per day did you spend walking?

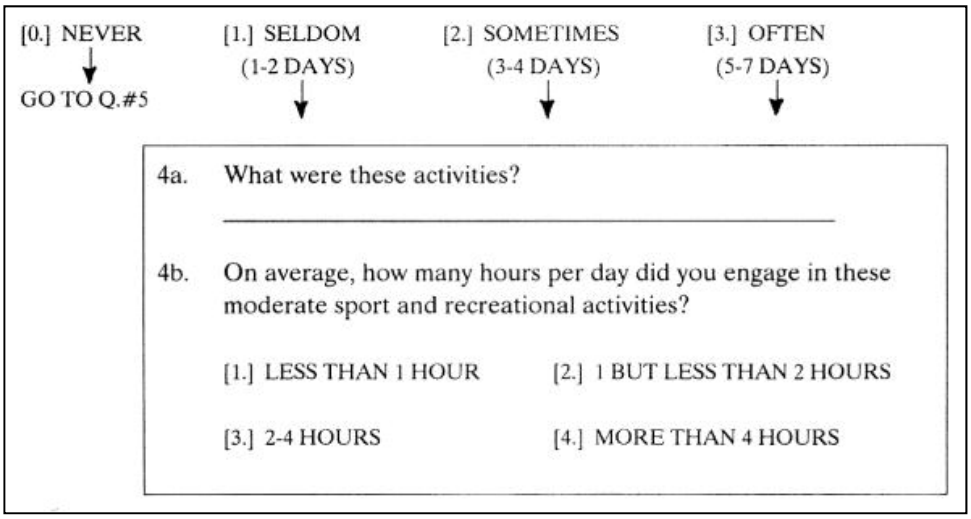
[1.] LESS THAN 1 HOUR [2.] 1 BUT LESS THAN 2 HOURS

[3.] 2-4 HOURS [4.] MORE THAN 4 HOURS

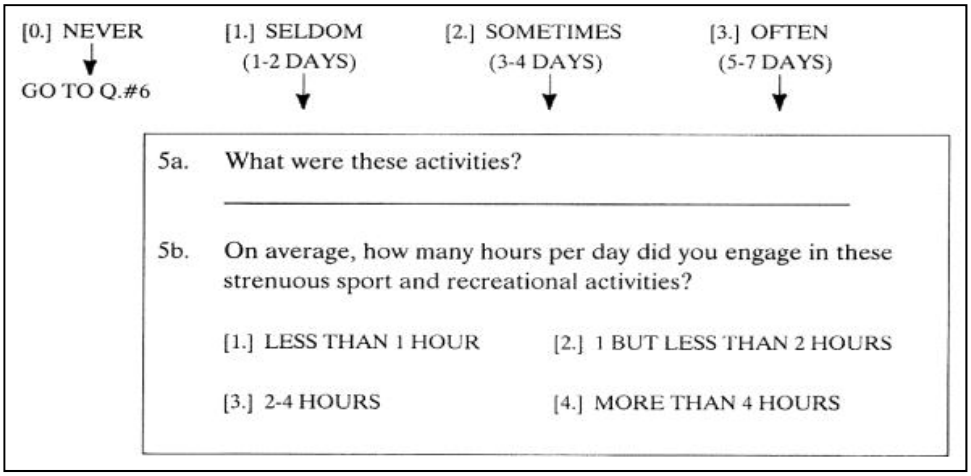
Over the past 7 days, how often did you engage in light sport or recreational activities such as bowling, golf with a cart, shuffleboard, fishing from a boat or pier or other similar activities?



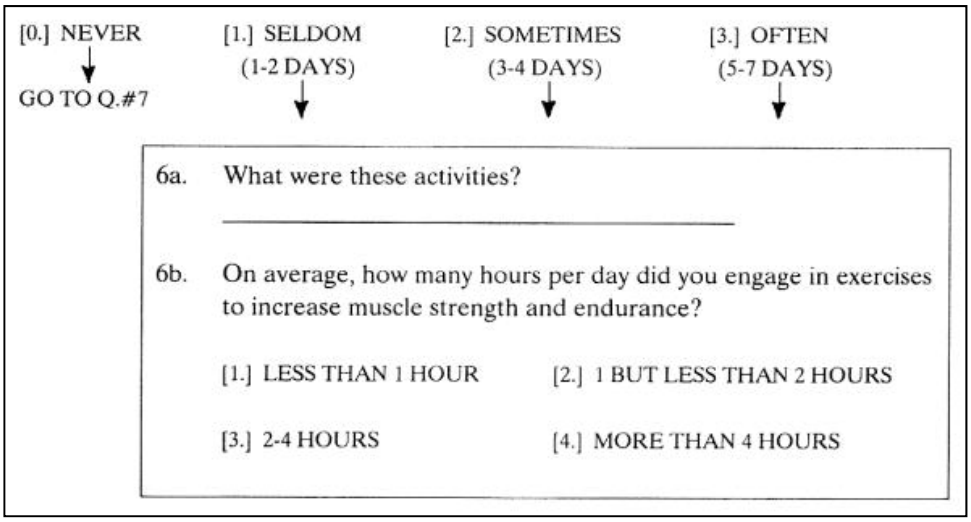
Over the past 7 days, how often did you engage in moderate sport and recreational activities such as doubles tennis, ballroom dancing, hunting, ice skating, golf without a cart, softball or other similar activities?



Over the past 7 days, how often did you engage in strenuous sport and recreational activities such as jogging, swimming, cycling, singles tennis, aerobic dance, skiing (downhill or cross-country) or other similar activities?



Over the past 7 days, how often did you do any exercises specifically to increase muscle strength and endurance, such as lifting weights or pushups, etc.?



During the past 7 days, have you done any light housework, such as dusting or washing dishes?

- ❑ Yes
- ❑ No

During the past 7 days, have you done any heavy housework or chores, such as vacuuming, scrubbing floors, washing windows, or carrying wood?

- ❑ Yes
- ❑ No

During the past 7 days, did you engage in any of the following activities? Please answer YES or NO for each item.

		NO	YES
a.	Home repairs like painting, wallpapering, electrical work, etc.	1	2
b.	Lawn work or yard care, including snow or leaf removal, wood chopping, etc.	1	2
c.	Outdoor gardening	1	2
d.	Caring for an other person, such as children, dependent spouse, or an other adult	1	2

During the past 7 days, did you work for pay or as a volunteer?

10a. How many hours per week did you work for pay and/or as a volunteer?

_____ HOURS

10b. Which of the following categories best describes the amount of physical activity required on your job and/or volunteer work?

[1] Mainly sitting with slight arm movements. [**Examples:** office worker, watchmaker, seated assembly line worker, bus driver, etc.]

[2] Sitting or standing with some walking. [**Examples:** cashier, general office worker, light tool and machinery worker.]

[3] Walking, with some handling of materials generally weighing less than 50 pounds. [**Examples:** mailman, waiter/waitress, construction worker, heavy tool and machinery worker.]

[4] Walking and heavy manual work often requiring handling of materials weighing over 50 pounds. [**Examples:** lumberjack, stone mason, farm or general laborer.]

Appendix H: Global Pain Scale

Instructions: For each question, please indicate your level of pain by circling a number from 0 to 10.

YOUR PAIN:

My **current** pain is .....**No pain:** 0 1 2 3 4 5 6 7 8 9 10

During the *past week,*
the **best** my pain has been is.....**No pain:** 0 1 2 3 4 5 6 7 8 9 10

During the *past week*,
the **worst** my pain has been is...**No pain:** 0 1 2 3 4 5 6 7 8 9 10

During the *past week*,
my **average** pain has been.....**No pain:** 0 1 2 3 4 5 6 7 8 9 10

During the *past 3 months*,
my **average** pain has been.....**No pain:** 0 1 2 3 4 5 6 7 8 9 10

YOUR FEELINGS: During the past week I have felt:

Afraid.....**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

Depressed .....**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

Tired .....**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

Anxious .....**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

Stressed.....**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

YOUR CLINICAL OUTCOMES During the past week:

I had trouble sleeping .....**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

I had trouble
feeling comfortable ……..**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

I was less independent…..**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

I was unable to work
(or perform normal tasks)..**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

I needed to take
more medication…….. **Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

YOUR ACTIVITIES: During the past week I was **NOT** able to:

Go to the store ....**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

Do chores in my home.....**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

Enjoy my friends and family .....**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

Exercise (including Walking)…….**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

Participate in my favorite hobbies...**Strongly Disagree:** 0 1 2 3 4 5 6 7 8 9 10

Scoring: Add up the total score and divide by 2. Each subset is worth 25 points. The maximum total score is 100.