INFLAMMATION AND PHYSICAL FRAILTY IN WOMEN WITH KNEE OSTEOARTHRITIS

SARAH KARAMPATOS

INFLAMMATION AND PHYISCAL FRAILTY IN WOMEN WITH KNEE OSTEOARTHRITIS

By SARAH KARAMPATOS BASc

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AUTHOR: Sarah Karampatos, BASc (Guelph Humber University)

SUPERVISOR: Dr. Monica Maly PhD

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LAY ABSTRACT

Arthritis is a chronic disease that has a debilitating effect on the lives of more than 4.6 million Canadians. In 2015, the cumulative economic burden of osteoarthritis was 195.2 billion dollars and is expected to increase significantly in the next two years. Knee osteoarthritis is the most common form of arthritis in older adults. Knee osteoarthritis is associated with increased pain, decreased physical function and decreased quality of life (QOL). In vulnerable older adults increased exhaustion, decreased physical function and muscle loss can increase the risk of developing frailty. Frail older adults are at higher risk of adverse health outcomes such as falls, hospitalization and death. Previous research has shown that older adults with knee OA are at higher risk of developing frailty however, it is not understood what underlying mechanisms increase this risk. This thesis provides fundamental information aimed at understanding potential mechanisms associated with knee osteoarthritis and frailty in women. Our study found that despite their relatively young age, nearly half of the women with knee OA are pre-frail. This data shows that inflammatory cytokines in particular, tumor necrosis factor alpha is related to symptomatic knee osteoarthritis severity in particular, self-reported pain. Overall, early detection of frailty is important when managing this condition. These data suggest that chronic knee pain associated with OA may be a useful trigger for early assessments of frailty in women.

ABSTRACT

Background:

Knee osteoarthritis (OA) is the most common form of arthritis in older adults. Knee OA is associated with limitations in physical function. Functional limitations are also associated with another geriatric condition, frailty. Frailty is characterized by reduced strength, endurance and physiological function.

Purpose:

The primary purpose of this study is to determine if there is a difference in radiographic or symptomatic knee OA severity between non-frail and pre-frail women with knee OA. Secondary objectives include: a) the relationship between radiographic and symptomatic OA severity with serum inflammatory cytokines, and b) if there is a difference in inflammatory cytokines between non-frail and pre-frail women with knee OA.

Methods:

We included 21 community-dwelling women with knee OA. Frailty was assessed using the Fried Frailty Phenotype. Knee OA severity was characterized by the Kellgren and Lawrence (KL) score and the Knee Injury and Osteoarthritis Outcome Questionnaire (KOOS). Inflammatory cytokines included serum interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis alpha (TNF α) and C reactive protein (CRP).

Results:

Data from 20 participants (66.1 [9.6] years, BMI 29.7 [4.9] kg/m^2, non-frail=55%; pre-frail=45%) were analyzed. Radiographic severity was not different between frailty

groups (p= 0.11). There was no difference in symptomatic knee OA severity, measured using the KOOS subscales, between frailty groups (p>0.17). Radiographic OA severity and inflammatory markers were not associated (p>0.30). There was a negative relationship between TNF α and self-reported pain (r=0.26), no relationships between inflammatory cytokines with any other KOOS sub-scales. Lastly, there was no difference in any inflammatory cytokines between non-frail and pre-frail groups.

Conclusion:

Despite the relatively young age, nearly 50% of our participants were pre-frail. Pre-frailty was unrelated to the severity of the knee OA, or inflammatory cytokines. TNF α may be involved in the experience of pain in these women. While it appears women with knee OA frequently demonstrate pre-frail status, more work is necessary to examine the link between these diseases.

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LIST OF ABBREVIATIONS

Abbreviation	**Definition**
(6MW)	6 Minute Walk
(ADL)	Activities of Daily Living
(BMI)	Body Mass Index
(CI)	Confidence Interval
(CRP)	C-Reactive Protein
(CES-D)	Centre for Epidemiologic Studies Depression Scale
(ELISA)	Enzyme-linked Immunosorbent Assay
(HR)	Hazard Ratio
(IADL)	Instrumental Activities of Daily Living
(ICOAP)	Intermittent and Constant Osteoarthritis Pain Questionnaire
(IGF-I)	Insulin-like growth factor I
(IL-1)	Interleukin-1
(IL-4)	Interleukin-4
(IL-6)	Interleukin-6
(IL-10)	Interleukin-10
(IL-13)	Interleukin-13
(IQR)	Interquartile range
(KL)	Kellgren and Lawrence
(KOOS)	Knee Osteoarthritis Outcome Scale
(ICC)	Intraclass Correlation
(MOST)	Multicenter Osteoarthritis Study

(OARSI)	Osteoarthritis Research Society International
(OR)	Odds Ratio
(OA)	Osteoarthritis
(OAI)	Osteoarthritis Initiative
(PR)	Prevalence Ratios
(QOL)	Quality of Life
(RR)	Relative Risk
(SD)	Standard Deviation
(SF-36)	Short Form Health Survey 36
(SOF)	Study of Osteoporotic Fractures
(TNF α)	Tumor Necrosis Factor Alpha
(TUG)	Timed Up and Go
(VAS)	Visual Analog Scale
(WBC)	White Blood Cell
(WOMAC)	The Western Ontario and McMaster Universities Arthritis Index

DECLARATION OF ACADEMIC ACHIEVEMENT

This thesis is the primary work of Master of Science candidate, Sarah Karampatos. Sarah was part of a team responsible for the planning and execution of the following research study, the data included is baseline data of a larger randomized controlled trial. Data enclosed in this thesis was collected during the 2015-2016 academic year. As the primary author, Sarah's contributions include: literature review, ethics applications, participant recruitment and consent, data collection, data analysis, and preparation of manuscripts. The second chapter of this thesis is intended for future publication.

Important contributors to this thesis include: Dr. Monica Maly, the thesis supervisor who funded this study, developed the study design and interpretation of the results, and edited all submitted documents; Dr. Dawn Bowdish, who provided resources, guidance, and help for overall inflammatory cytokine analysis, interpretation, and analysis. Dessi Loukov for her assistance on analysis of inflammatory cytokines; Dr. Alexandra Papaioannou who provided expertise in aging and frailty; and Alexander Kuntz, Jaclyn Chopp-Hurley, Elora Brenneman and Emily Wiebenga who helped with ethics applications, participant recruitment and consent and data collection.

CHAPTER 1
INTRODUCTION

Osteoarthritis

Osteoarthritis (OA) is considered to be the most prevalent chronic joint disease in the world. In fact, half the world's population over the age of 65 years suffer from OA (Castell, van der Pas et al. 2015). OA is characterized by progressive degenerative changes in articular cartilage within synovial joints, hypertrophy of the bone, subchondral bone deterioration, and thickening of the joint capsule (Woolf and Pfleger 2003). OA commonly affects the hands and weight bearing joints including the hips and knees (Canada 2011). Unfortunately, individuals with OA often experience joint pain, joint tenderness, restriction of movement and local inflammation however, the cause of OA is unknown (Woolf and Pfleger 2003).

Burden of Osteoarthritis

OA has a great impact on function and mobility and can cause limitations in daily activity. Although OA commonly places great burden on the individual, this disease can also place a large burden on the loved ones of the person living with OA (Canada 2011). Day-to-day tasks often require assistance including personal care activities, shopping, preparing meals and cooking, household chores or moving around at home (Badley, Rothman et al. 1998). These tasks often become increasingly difficult due to decreased range of motion, increased joint pain and limitation of movement (Badley, Rothman et al. 1998). Individuals with OA have higher rates of comorbidities such as cardiovascular

disease, cancer and dementia and therefore, are at risk increased risk of mortality (Nüesch, Dieppe et al. 2011).

Osteoarthritis is costly not only to the individual but also to society. The economic burden of knee OA results in both direct and indirect costs. Direct costs include medical expenses such as hospitalization, diagnosis, outpatient treatments, and medication (Canada 2011). Joint replacement has been identified as a potential intervention strategy for managing the burden of arthritis. It is thought to be a cost-effective treatment for OA as it alleviates pain and improves physical function. It is thought that total joint replacement would have a cumulative savings of $17 billion dollars for the Canadian government over the next 30 years (Canada 2011). The Arthritis Alliance of Canada estimates that OA currently accounts for $10 billion in direct health care costs (Canada 2011). Indirect costs include inability to work and informal care by family members (Canada 2011). Individuals with OA have both activity and workplace limitations (Gignac, Cao et al. 2008). They often face both the inability to work or live independently (Gignac, Cao et al. 2008). The Arthritis Alliance of Canada estimates that OA currently accounts for $17 billion in indirect costs and these costs are expected to rise due to decreased physical activity, increased rates of obesity and increased life expectancy (Canada 2011).

Knee Osteoarthritis

Prevalence of Knee Osteoarthritis

Osteoarthritis most commonly affects the knee due to the weight-bearing nature of the joint. Symptomatic knee OA affects 13% of women and 10% of men aged 60 years and older (Zhang and Jordan 2010). Higher risks of knee OA are associated with individuals who are obese (Felson, Anderson et al. 1988). Previous meta-analysis showed that those who were obese or overweight had 2.96 times higher risk of the incidence of knee OA compared to individuals of normal weight (95% confidence interval [CI] 2.56-3.43) (Blagojevic, Jinks et al. 2010). The prevalence of knee OA is higher in women compared with men and they also generally have increased severity of knee OA compared to men (Srikanth, Fryer et al. 2005). Women tend to have more risk factors for knee OA including; higher rates of obesity most often measured using body mass index (BMI), poor knee alignment and decreased muscle strength (Neogi and Zhang 2013). Knee malalignment, in particular varus alignment is one of the strongest predictors of progressive knee OA (Sharma, Song et al. 2010). The effect of knee malalignment is primarily biomechanical. Often joint loads put on the knee are altered and therefore, forces about the knee are changed (Cerejo, Dunlop et al. 2002, Sharma, Song et al. 2010). Malalignment and progression of disease is a vicious cycle (Sharma, Song et al. 2010). As knee adduction moment increases, there is increased knee malalignment and in turn increased risk of knee OA (Sharma, Song et al. 2010).

Pathology of Knee Osteoarthritis

Knee OA is degenerative joint disease that affects the entire joint. Often there is damage to the articular cartilage and formation of osteophytes. Previous research has suggested that decreased joint function is a result of loss of articular cartilage (Buckwalter, Mankin et al. 2005). Articular cartilage has two functions. It distributes stress through deformation and provides a smooth and to provide a frictionless surface to facilitate joint motion (Creamer and Hochberg 1997). Decreased chondrocytes due to aging can cause degeneration of articular cartilage (Buckwalter, Mankin et al. 2005). Degeneration of articular cartilage leads to joint pain and decrease physical function (Buckwalter, Mankin et al. 2005).

Other tissues such as muscle play an important role in early stages of OA (Li, Wei et al. 2013). Sarcopenia is a syndrome characterized by progressive and general loss of muscle mass and strength (Cruz-Jentoft, Baeyens et al. 2010). The number of determinants assesses severity of sarcopenia. Pre-sarcopenia is characterized by low muscle mass with no impact on muscle strength or low physical performance (Cruz-Jentoft, Baeyens et al. 2010). Sarcopenia is characterized by low muscle mass and low muscle strength or low physical performance (Cruz-Jentoft, Baeyens et al. 2010). Severe sarcopenia is characterized by low muscle mass, low muscle strength and low physical performance (Cruz-Jentoft, Baeyens et al. 2010). Patients affected by knee OA are more likely to develop sarcopenia (Lee, Kim et al. 2012). Previous studies have shown that quadriceps muscle weakness is associated with increased risk of knee OA (Slemenda, Heilman et al.

1998, Brandt, Heilman et al. 1999). Severity of sarcopenia will determine the amount of physical disability placed on the individual.

Methods of Measuring Knee Osteoarthritis

There are multiple methods of diagnosing knee OA. There are currently 3 different definitions of knee OA: clinical, radiographic, and laboratory diagnostic criteria. Clinical methods of diagnosing knee OA including patient history, physical examination and diagnostic criteria are commonly used to diagnose knee OA (Altman, Asch et al. 1986). Radiographic methods are used to aid the diagnosis of knee OA if pain persists (Felson 2006). Laboratory testing, specifically blood tests are rarely used for the diagnosis of knee OA however, are more commonly used to exclude a diagnosis of rheumatoid arthritis (Felson 2006). A variety of diagnosis methods can cause a problem when diagnosing knee OA. There are two methods of measuring knee OA; radiographic and symptomatic measurements. Previous literature shows that there is a weak correlation between both radiographic and symptomatic measurements (Hannan, Felson et al. 2000, Muraki, Oka et al. 2009, Neogi, Felson et al. 2009).

Radiographic Measures

Knee OA is commonly diagnosed through radiography. Once a clinical examination has been completed, radiographic images are used to confirm diagnosis (Altman and Gold 2007). There are multiple methods used to assess the severity of joint disease, including the Kellgren and Lawrence (KL) and the Osteoarthritis Research International grading

scales (Bauer, Hunter et al. 2006). The most commonly used radiographic grading system is the KL grading system (Table 1-1) (Emrani, Katz et al. 2008). The KL grading scale has previously received criticism as it places a large emphasis on osteophyte formation with relatively less emphasis on joint space narrowing (Altman and Gold 2007). The first two grades of the KL grading system are defined by presence of osteophytes. This becomes a problem, as a patient with joint space narrowing but no osteophyte formation would not be characterized as having knee osteoarthritis according to the KL grading system. Therefore, the KL grading system assumes that osteophyte formation occurs before joint space narrowing.

The Osteoarthritis Research International (OARSI) grade is a new grading system developed in 2004 (Altman and Gold 2007). Similar to the KL, the OARSI grading system takes into account both joint space narrowing and osteophytes however, both are weighted equally (Altman and Gold 2007). Unlike the KL grading system, the OARSI grading system is compartment specific (Altman and Gold 2007). Table 1-2 describes the criteria of the OARSI grading system. Although knee OA is commonly diagnosed through radiographs, the signs of knee OA do not always correlate with radiographic measurements (Heidari 2011).

Symptomatic Measures

Symptoms of OA of the knee may include; pain, stiffness, swelling, crepitus and decreased mobility. Physical performance tools such as the Timed Up and Go (TUG) and

the 6-Minute Walk (6MW) are often used to measure mobility. Measurements frequently used to assess symptoms of pain include self-report questionnaires such as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) and the Knee Osteoarthritis Outcome Scale (KOOS). Patients with radiographic signs of knee OA (joint space narrowing and osteophyte formation) are often asymptomatic (Li, Wei et al. 2013). The most common symptom of knee OA is pain, but the correlation with the radiographic severity of knee OA is controversial (Hannan, Felson et al. 2000, Muraki, Oka et al. 2009, Neogi, Felson et al. 2009). Knee pain severity is an important determinant of disability (McAlindon, Cooper et al. 1993).

Functional Consequences of Knee Osteoarthritis

Pain and other symptoms of OA may have a profound effect on QOL. It is painful and debilitating disease that causes functional limitation and disability (Guccione, Felson et al. 1994, Mallen, Peat et al. 2007). Recent research has highlighted the clinical importance of activity-related pain (Wideman, Finan et al. 2014). Knee OA is generally linked with pain during weight-bearing activities, which results in trouble walking and climbing stairs (Guccione, Felson et al. 1990, Guccione, Felson et al. 1994, Felson, Lawrence et al. 2000, Wideman, Finan et al. 2014). Knee OA is associated with increased number of comorbidities however; obesity is arguably one of the largest health risks. Obese individuals are at risk for multiple health conditions such as hypertension, type 2 diabetes, coronary heart disease, stroke, cancer and low QOL (Pi-Sunyer 1991).

Frailty

Frailty is a medical syndrome with multiple causes and contributors that is characterized by decreased strength, endurance and decreased physiological function that increases an individual's vulnerability for developing increased dependency or death (Morley, von Haehling et al. 2014). This geriatric syndrome is complex and heterogeneous as it increases an individual's vulnerability and homeostasis affecting multiple systems (Buckinx, Rolland et al. 2015). The worldwide prevalence of frailty ranges between 6.9% to 42.6%, prevalence is dependent upon the method used to identify frailty. (Ottenbacher, Ostir et al. 2005, Cesari, Leeuwenburgh et al. 2006, Ávila-Funes, Helmer et al. 2008). The incidence of frailty increases with age and being female.

Pre-frailty is an intermediate stage between non-frail and frail (Fairhall, Kurrle et al. 2015). Pre-frail individuals have more than twice the risk of becoming frail compared to non-frail individuals (Fried, Tangen et al. 2001). Individuals that can be classified as pre-frail respond better to an intervention compared to those that are frail (Fried, Tangen et al. 2001). Currently there are five clinical randomized controlled trails registered on clinicaltraials.gov that are targeted toward improving frailty status of pre-frail individuals. Although there has been a consensus on the consequences associated with frailty, there has been no consensus on a gold standard for measuring frailty.

Methods of Identifying and Measuring Frailty

Identification of frailty is important, as frailty is a dynamic, treatable condition (Morley, von Haehling et al. 2014). Measuring the level of frailty can be problematic as there are numerous functional tests, questionnaires and indexes used to categorize frailty (Hubbard, O'Mahony et al. 2009, De Vries, Staal et al. 2011). One method of measuring frailty is, The Fried Frailty Phenotype is one of the most commonly used tools used to assess physical frailty in older adults.

Fried Frailty Phenotype

A "phenotype", that is a combination of five pre-defined physical frailty criteria used to identify individuals who are pre-frail or frail. However, it does not provide any insight into the underlying cause of the patient's condition (Cesari, Gambassi et al. 2014). The Fried Frailty Phenotype can be used by both clinicians and researchers as it does not need an assessment completed by a physician (Cesari, Gambassi et al. 2014). The Fried Frailty Phenotype is a combination of both subjective and objective measurements (Fried, Tangen et al. 2001). Frailty is identified by the presence of 3 or more of the following; self-reported weight loss, self-reported exhaustion, low activity, muscle weakness and decreased gait speed (Fried, Tangen et al. 2001). Table 1-3 describes the Fried Frailty Phenotype criteria used to define frailty.

The Fried Frailty Phenotype has been tested for predictive validity of mortality (Fried, Tangen et al. 2001, Fugate Woods, LaCroix et al. 2005, Rockwood, Andrew et al. 2007,

Kulminski, Ukraintseva et al. 2008). Previous research has shown that baseline frailty independently predicted risk of death (hazard ratio (HR)=1.71, 95% confidence interval (CI)=1.48-1.97) (Fugate Woods, LaCroix et al. 2005) and falls (Fried, Tangen et al. 2001, Kiely, Cupples et al. 2009). Previously, frail individuals have been identified using the Study of Osteoporotic Fractures (SOF) Frailty Phenotype. The SOF Frailty Phenotype has predicted risk of hospitalizations (hazard ratio (HR)(frail)=2.2, 95% confidence interval (CI)=1.2-4.0 (OR=1.95, 95% CI=1.72-2.22) and emergency department visits (Fried, Tangen et al. 2001). Concurrent validity including; ADL and IADL (Fugate Woods, LaCroix et al. 2005) ADL disability was related to frail individuals (odds ratio (OR)=3.15, 95% CI=2.47-4.02). Convergent validity comparing the Fried Frailty Phenotype to Frailty Index score, these measures correlated moderately well with each other (R=0.65) (Rockwood, Andrew et al. 2007).

A recent systematic review found that the prevalence of pre-frailty, assessed by the Fried Frailty Phenotype, in community dwelling older adults aged 65 years or older was 38-53% and the prevalence of frailty was 4-17% (Collard, Boter et al. 2012).

Impact of Frailty

Previous research has shown that frail individuals have poorer health and higher rates of comorbidity (Buckinx, Rolland et al. 2015). Compared to non-frail counterparts, frail older adults require greater usage of community resources (Buckinx, Rolland et al. 2015) and experience an increased risk of adverse health outcomes such as increased dependency, institutionalization, falls, fractures, hospitalization and mortality (Boyd, Xue

et al. 2005, Fugate Woods, LaCroix et al. 2005, Cawthon, Marshall et al. 2007, Ensrud, Ewing et al. 2007, Ávila-Funes, Helmer et al. 2008, Bouillon, Kivimaki et al. 2013). Frailty is a dynamic state; although it is possible to improve, most individuals become worse over time (Buckinx, Rolland et al. 2015). Once an individual is pre-frail, they are more likely to progress to becoming frail (Ahmed, Mandel et al. 2007). However, frailty is a treatable condition therefore, it is important to diagnose and manage frailty at an early state (Morley, von Haehling et al. 2014). Common and effective treatments of frailty include a combination of exercise, protein supplementation, vitamin D supplementation and reduced polypharmacy (Morley, Vellas et al. 2013).

Relationship between Knee Osteoarthritis and Frailty

Knee OA and frailty also share common risk factors including age, female sex and obesity (Castell, van der Pas et al. 2015, Misra, Felson et al. 2015). OA of the knee increases in prevalence with age in particular over the age of 50 (Altman, Asch et al. 1986). The exact mechanism of knee OA is unknown (Felson 2006). Age related changes to articular cartilage may contribute to the development of OA (Li, Wei et al. 2013). Knee OA is more common in women than men (Felson 2006). Higher rates of obesity, poor knee alignment and decreased muscle strength are risk factors more often seen in women compared to men (Neogi and Zhang 2013). Obesity is associated with a high prevalence of knee OA (Garstang and Stitik 2006). Obesity can contribute to higher mechanical loads on the joint. These increased abnormal loads are attributed to obesity, joint instability, or trauma. Muscle forces are a main determinant of how loads are

distributed across a joint surface. Decreasing the muscle forces acting on a joint will ultimately alter loading conditions (Sowers and Karvonen-Gutierrez 2010). Age, female sex and obesity are also risk factors for frailty.

Frailty is a common medical syndrome that is seen in the elderly specifically over 80 years of age (Ahmed, Mandel et al. 2007). However, it is suggested that individuals over the age of 70 years with weight loss is suggested to be assessed for frailty (Morley, Vellas et al. 2013). The incidence of frailty increases with age (Ahmed, Mandel et al. 2007). Frailty is more prevalent in women than men (Morley, Vellas et al. 2013). However, unlike weight gain seen in individuals with knee OA, weight loss is commonly seen in frail individuals (Morley, Vellas et al. 2013).

The co-existence of these conditions may have several consequences. For example, it is possible that individuals who meet the frailty criteria and have knee OA may be at a greater risk for vulnerability and progressing down the frailty cascade (Castell, van der Pas et al. 2015, Misra, Felson et al. 2015). Table 1-4 summarizes recent work has begun studying the associations between frailty and knee OA. Both studies assessed physical frailty in individuals over the age of 60 years with knee OA. Although it is understood that frailty is more prevalent in individuals with radiographic or symptomatic knee OA than those without, the underlying mechanisms are not understood (Castell, van der Pas et al. 2015, Misra, Felson et al. 2015). One potential mechanism promoting the co-existence of frailty among older adults with knee OA is through systemic inflammation.

Chronic inflammation appears to be related to knee OA and frailty but more research is necessary to fully understand whether a common pathophysiology exists (Fulop, Larbi et al. 2010). Previous literature has shown that systemic inflammation is associated with predicting radiographic changes, pain and physical function in individuals with knee OA (Table 1-5). Systemic inflammation has also been shown to be associated with frailty, assessed by the Fried Frailty Phenotype or a Frailty Index (Table 1-6).

Inflammatory Cytokines

Most recent theories on aging focus on an immune response and takes into account inflammatory cytokines response to age-related disease and infection.

Interleukin-6

Interleukin-6 (IL-6) is produced by immune cells, vascular and endothelial cells, adipocytes, and skeletal muscles (Singh and Newman 2011). This cytokine is involved in the regulation of immune response, acute phase response and inflammation (Minciullo, Catalano et al. 2015). It is known to have both pro-inflammatory and anti-inflammatory properties (DeRijk, Michelson et al. 1997, Xing, Gauldie et al. 1998, Yao, Li et al. 2011). IL-6 expression is low and serum levels are nearly undetected in the absence of inflammation (Minciullo, Catalano et al. 2015). Increased serum levels of IL-6 is a characteristic of aging (Minciullo, Catalano et al. 2015). The pro-inflammatory functions of this cytokine have a negative influence on skeletal muscle and lead to weakness and fatigue (Leng, Chaves et al. 2002). It has also shown to have increased circulating levels

in older adults (Yao, Li et al. 2011). Age-related increases of IL-6 are implicated in atherosclerosis, sarcopenia and frailty they are also associated with functional decline and decreased strength and mortality (Reuben, Cheh et al. 2002, Visser, Pahor et al. 2002, Yao, Li et al. 2011). Frail older adults have shown to have higher serum IL-6 levels (Ferrucci, Penninx et al. 2002, Leng, Chaves et al. 2002, Leng, Xue et al. 2007). The frail, community dwelling older adults had significantly higher serum IL-6 levels than the non-frail subjects (4.4 +/-2.9 versus 2.8 +/- 1.6 pg/mL) (Leng, Chaves et al. 2002). In a longitudinal study, Ferrucci and colleagues showed that increased levels of IL-6 at baseline predicted higher risk for the development of physical disability and decreased strength after a follow-up period of 3.5 years in older community-dwelling women (Ferrucci, Penninx et al. 2002).

There has been increasing evidence that has shown that serum levels of IL-6 are associated with knee cartilage loss (Stannus, Jones et al. 2010) and risk of progression (Livshits, Zhai et al. 2009) in older adults. At baseline, those in the highest quartile of IL-6 had an increased prevalence of medial tibiofemoral joint space narrowing (OARSI grade ≥ 1) with an odds radio 1.42, $p<0.05$ (Stannus, Jones et al. 2010). Longitudinally, baseline IL-6 predicted loss of both medial and lateral tibial cartilage volume (β:-1.19% and -1.35% per annum per quartile, $p< 0.05$ and $p<0.01$, respectively (Stannus, Jones et al. 2010).

Tumor Necrosis Factor α

Tumor Necrosis Factor α (TNF α) is a key regulator of the inflammatory response that is produced by macrophages, lymphoid cells, mast cells, vascular endothelial cells, cardiac monocytes, adipocytes, fibroblasts and neuronal tissue. Increased levels of TNF α TNF α contributes to IL-6 production (Yao, Li et al. 2011) and is associated with decreased muscle strength and poor physical function (De Martinis, Franceschi et al. 2006).

Research looking at the relationship between TNF α and frailty is inconsistent. A study of 386 institutionalized older adults (mean 81.5 years SD 49 years) found no association between frailty and TNF α. The median of TNF α was 1.20pg/ml (range 0.13-28.2pg/ml) (Lai, Chang et al. 2014). However, Hubbard and colleagues found that a frailty index correlated significantly with log-transformed TNF α ($r=0.379$, $P<0.01$) in 110 adults, suggesting this cytokine may play a role in the severity of frailty (Hubbard, O'Mahony et al. 2009).

This cytokine likely has a role in knee OA also. However, the inconsistent relationship seen in TNF α and frailty is also seen in TNF α and knee OA. A study including 172 older adults (mean 63 years, range 52-78 years) with radiographic knee OA showed that TNF α is associated with changes in medial cartilage volume and total knee pain over time. A change in TNF α was negatively associated with change in medial cartilage volume (β:-1.27% per annum per quartile, $p<0.05$) (Stannus, Jones et al. 2010). A 2-year longitudinal study completed by Botha-Scheepers and colleagues that included 104

sibling pairs (median baseline age 60.1 years) found that patients in the highest quartile of TNF α production had a six-fold increase of joint space narrowing progression (RR 6.1, 95% CI 1.4 to 9.8) (Botha-Scheepers, Watt et al. 2008). Overall, this study found the production of the inflammatory cytokine TNF α is associated with radiological progression of knee over a follow-up period of 2 years (Botha-Scheepers, Watt et al. 2008). However, in the Chingford Study, a prospective population-based study of 1,003 women aged 45-65 years, TNF α was not associated with radiographic knee OA (Livshits, Zhai et al. 2009).

Interleukin-10

Interleukin-10 (IL-10) is arguably the most powerful anti-inflammatory cytokine (Minciullo, Catalano et al. 2015). It is produced by both innate and adaptive immune cells and inhibits the release of proinflammatory cytokines (Mitchell and Cotran 2003, Lio and Caruso 2006) to help keep the inflammatory response short and to resolve inflammation after infection (Lio, Scola et al. 2002). Elderly individuals have higher levels of inflammation and have trouble down-regulating the anti-inflammatory response; therefore, the anti-inflammatory response is no longer acute but chronic (Lio, Scola et al. 2002). IL-10 has been recognized for the ability to inhibit the activation and function of T cells, monocytes and macrophages (Moore, de Waal Malefyt et al. 2001). High IL-10 found in serum has been associated with successful aging (van den Biggelaar, de Craen et al. 2004).

Little research has been conducted in the relationship of IL-10 and frailty in older adults. Previous research has been focused mostly on mouse models. Walston and colleagues found that these nice did not produce the inflammatory cytokine IL-10 and therefore, express elevated levels of proinflammatory cytokines. A frail mouse model was genetically manipulated in order to mimic a frail human (Walston, Fedarko et al. 2008). They showed that IL-$10^{tm/tm}$ mice exhibited inflammation (increased serum IL-6 levels) and muscle weakness at an earlier age than wild-type controls (Walston, Fedarko et al. 2008). These finds are consist with several human frailty characteristics (Walston, Fedarko et al. 2008).

IL-10 production has been shown to be associated with radiological progression of knee OA. Botha-Scheepers and colleagues presented that patients in the highest quartile of IL-10 production had a fourfold increased risk of joint space narrowing (adjusted RR 4.3, 95% confidence interval [CI] 1.7 to 6.3), in comparison to patients in the lowest quartile (Botha-Scheepers, Watt et al. 2008) .

C Reactive Protein

C Reactive Protein (CRP) is a nonspecific protein that responds in the acute phase of inflammation to most forms of infection and tissue damage (Pepys and Hirschfield 2003). CRP is produced by the liver and is a sensitive marker of inflammation and tissue damage (Pepys and Hirschfield 2003). CRP is known to increase with elevated IL-6 (Singh and Newman 2011). Elevated levels of CRP are associated with cardiovascular disease (Hage

and Szalai 2007), poor physical performance and muscle weakness (Cesari, Penninx et al. 2004).

The Cardiovascular Health Study (Walston, McBurnie et al. 2002) showed a significant association between elevated CRP levels with frailty. Compared to non-frail (2.7±4.0 mg/L), frail participants had higher levels of CRP (5.5±9.8 mg/L) (Walston, McBurnie et al. 2002).

Some studies have reported that serum CRP levels were significantly higher in individuals with OA (Stürmer, Brenner et al. 2004, Pearle, Scanzello et al. 2007, Stannus, Jones et al. 2013). For example, Spector and colleagues showed that levels of CRP were higher in women with radiographic knee OA (median 2.4 mg/liter, interquartile range [IQR] 1.0-5.1), compared to women without knee OA (median 0.7 mg/liter, IQR 0.3-1.8) ($p<0.001$) (Spector, Hart et al. 1997). Meanwhile, others showed no association between CRP and OA after adjusting for covariates such as body mass (Davis, Karl et al. 2007, Vlad, Neogi et al. 2011).

White Blood Cell

White blood cells (WBC) are an important cellular component of the inflammation process (Leng, Xue et al. 2007). An acute increase in total WBC counts is recognized as a clinical indicator of systemic inflammation (Yao, Li et al. 2011). White blood cell counts are associated with frailty and mortality in older adults (Leng, Xue et al. 2007).

A significant association between WBC count and pre-frailty and frailty has been seen in previous research. Women with total WBC counts in the top tertile were significantly more likely to be pre-frail (OR= 2.43, 95% confidence interval (95% CI)=1.21-4.89) or frail (OR=4.25, 95% CI 1.89-9.58) than those with WBC counts in the bottom tertile, after adjusting for potential cofounders (Leng, Xue et al. 2007). There however, have been no associations shown between WBC and knee OA.

Inflammatory Cytokines and Aging

Aging is a dynamic and active process and it is associated with complex continuous remodeling of the immune system, which can decrease immune competence (Sergio 2008). The immune system requires a balance of pro and anti-inflammatory activity. The aging immune system is characterized by a low-grade, chronic, systemic inflammatory state, sometimes referred to as "Inflamm-aging" (Franceschi, Bonafè et al. 2000, Yao, Li et al. 2011). Many age related diseases such as obesity, depression, cardiovascular disease and cancer share an inflammatory pathogenesis (Minciullo, Catalano et al. 2015). The activation of inflammatory pathophysiology seems to be involved in the pathophysiology of frailty and sarcopenia (Minciullo, Catalano et al. 2015). It is initiated by increased circulating levels of pro-inflammatory cytokines, predominantly interleukin-1, interleukin-6, tumor necrosis factor α (Sergio 2008).

Long lived centenarians have been shown to cope with inflammation through an anti-inflammatory response known as "anti-inflammaging" (Minciullo, Catalano et al. 2015). Anti-inflammaging is marked by the decline of immunological functions and occurs with

the increased levels of proinflammatory cytokines (Sergio 2008). Anti-inflammatory cytokines elevated in older adults include interleukin-4, IL-6, interleukin-13, IL-10 (Sergio 2008). We can view frailty as a consequence of a poor balance between pro- and anti-inflammatory functions, affecting multiple systems and tissues (Sergio 2008). Frail individuals have the inability to repair molecular damage or adapt to the damage that has occurred (Minciullo, Catalano et al. 2015). The presence of elevated inflammatory cytokines is associated with increased morbidity and mortality in older adults (Roubenoff, Parise et al. 2003, De Martinis, Franceschi et al. 2006).

Increased levels of pro-inflammatory cytokines have negative impacts on muscle mass and strength in older adults (Sergio 2008). Sarcopenia is the loss of muscle mass, strength and function (Roubenoff 2007). It is often associated with age and a common characteristic of frailty (Roubenoff 2007). Increased levels of inflammation damages skeletal muscle in humans through a mechanism of apoptosis (Anker, Ponikowski et al. 1999, Chung, Cesari et al. 2009). Apoptosis is the normal death of cells that is controlled by the body (Anker, Ponikowski et al. 1999). Specifically, it has shown that TNF α is one of the major signals for prompting apoptosis of muscle cells (Phillips and Leeuwenburgh 2005). Along with TNF α, IL-6 has also been associated with reduced muscle strength (Chung, Cesari et al. 2009) and functional performance (Ferrucci, Penninx et al. 2002) in older adults.

Inflammatory Cytokines and Knee Osteoarthritis

Recent studies have suggested that local inflammation plays a large role in the pathogenesis of OA (Jin, Beguerie et al. 2015). The development and the progression of knee OA is mediated between the joint cartilage and surrounding tissues (Rainbow, Ren et al. 2012). Proinflammatory cytokines, such as IL-6 and TNF α, are produced by the synovium and chondrocytes, a major cell type in cartilage tissue (Rainbow, Ren et al. 2012). These cytokines contribute to tissue break down by disrupting the balance of catabolic and anabolic changes of chondrocytes (Rainbow, Ren et al. 2012, Jin, Beguerie et al. 2015). However, IL-10 inhibits the apoptosis of chondrocytes, promoting anti-inflammatory cytokine production (John, Müller et al. 2007). While local inflammation is important in knee OA, multiple studies have suggested that local inflammation may be detected systemically (Otterness, Weiner et al. 2001, Lavigne, Benderdour et al. 2004, Filková, Lišková et al. 2009).

In the synovial joint, the surface of articular cartilage is exposed to synovial fluid within the joint cavity (Flandry and Hommel 2011). Therefore pro-inflammatory factors circulating in the synovial fluid will influence the articular cartilage. The joint cavity is lined with two types of cells, synoviocytes and macrophages (IWANAGA, Shikichi et al. 2000). Synoviocytes are synovial intimal cells that are thought to be responsible for the production of synovial fluid components, absorption from the joint cavity and blood and synovial exchanges (IWANAGA, Shikichi et al. 2000). Macrophages are a type of WBC that engulf foreign substances they are important components of the innate immune

system (Mosser and Edwards 2008). They initiate a defense mechanism by recruiting other immune cells such as lymphocytes (Mosser and Edwards 2008). Synovitis is a common feature of knee OA and occurs when the synovium becomes inflamed and is marked by an increase in proinflammatory cytokines (Revell, Mayston et al. 1988, Rainbow, Ren et al. 2012). Several key changes in the synovium occur with knee OA however, these changes are more pronounced in late stages of knee OA. Some of the changes in the synovium include thickening of the lining layer, increased vascularity and inflammatory cell infiltration (Wenham and Conaghan 2010). In both early and late stage OA the synovium is infiltrated with activated B cells and T lymphocytes and proinflammatory mediators (Wenham and Conaghan 2010). Anti-inflammatory cytokines have also been found in the synovial fluid of patients with OA (Martel-Pelletier, Alaaeddine et al. 1999). Anti-inflammatory cytokines decrease TNF α in-vitro (Alaaeddine, Di Battista et al. 1999).

Joint pain, swelling and joint stiffness is common in knee OA which eventually causes breakdown of cartilage (Stannus, Jones et al. 2010). Previous studies have shown that elevated levels of CRP are associated with a decrease in cartilage volume and disease progression (Spector, Hart et al. 1997, Kobayashi, Squires et al. 2005).

Unfortunately, the literature documenting a role for inflammatory cytokines in both frailty and knee OA is inconsistent. The following inflammatory cytokines and cells have been implicated in aging, frailty, knee OA, and muscle function in older adults.

Gaps in the Literature

It is possible that, among older adults, the presence of knee OA is associated with an increased risk of developing frailty. Nonetheless, there exist gaps in knowledge, which need to be addressed to understand the underlying mechanisms associated with frailty and knee OA. Some of these gaps include:

1) Does choosing pre-frail "young" women also demonstrate an elevated presence of inflammatory cytokines?

   Most frailty research that has been previously published has focused on choosing older adults over the age of 70 years. Younger women may be more likely to be pre-frail or non-frail compared to older adults. Recently, it has been suggested that at the pre-frail stage, frailty may be able to be reversed (Ahmed, Mandel et al. 2007). Early intervention with pre-frail young women will potentially improve QOL, increase independence and reduce health care costs.

2) Is there a relationship between the severity of frailty and presence of inflammatory cytokines in individuals with knee OA?

   The relationship between the severity of frailty and presence of inflammatory cytokines remains unstudied. Filling this gap is necessary to better understand the potential mechanisms affecting both frailty and knee OA. Identifying potential

mechanisms would provide the possibility to develop novel strategies to identify, prevent and manage both conditions.

3) Does the severity of frailty relate with pain and physical function in older women with knee OA?

   The severity of frailty relates with pain in older adults with knee OA. Frailty was more prevalent among individuals with symptomatic knee OA (5.88% vs 2.79%; PR 1.92 [1.35, 2.74]) compared to those without, respectively. However, the relationship of frailty with pain and physical function has not been shown in "younger" women with knee OA.

Objectives of this Thesis

This project provides an opportunity to determine an association between knee OA, frailty and inflammatory cytokines in women living in the community.

The primary purpose of this study is to determine if there is a difference in radiographic or symptomatic knee OA severity between non-frail, pre-frail and frail women with knee OA. Secondary objectives include the following:

a) Determine the relationship between each of radiographic and symptomatic OA severity and the presence of inflammatory cytokines in serum.

b) Determine if there a difference in inflammatory cytokines between non-frail, pre-frail and frail women with knee OA.

Our primary hypothesis is that both radiographic and symptomatic severity will be greater in participants categorized as pre-frail/frail compared to those that are non-frail. For our secondary hypotheses, we anticipated that:

a) Both radiographic and symptomatic knee OA severity would share a positive relationship with the level of pro-inflammatory cytokines in serum (and negative relationship with anti-inflammatory cytokines).
b) Elevated levels of serum cytokines would be present in pre-frail/frail participants relative to non-frail participants.

This thesis advances knee OA and pre-frailty research as it could promote the use of a diagnosis of knee OA among women as a time point to "catch" and potentially stop or reverse pre-frailty. By identifying and treating pre-frailty early, we are able to determine a conservative intervention with low risk of potential harm. This thesis also helps to fill the gap in understanding if systemic inflammation is a shared underlying mechanism affecting both frailty and knee OA. Ultimately, advancing the early identification of frailty as comorbidity in knee OA, and examining systemic inflammation as a potential underlying cause of both conditions has great potential to improve QOL in younger adults without the complications of potential comorbidities.

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Tables

Table 1-1. Kellgren and Lawrence Radiographic Knee Osteoarthritis Grading System.

Grade	Description
Grade 1	Doubtful narrowing of joint space and possible osteophytic lipping
Grade 2	Definite osteophytes and possible narrowing of joint space
Grade 3	Moderate multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends
Grade 4	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends
	(Kellegren and Lawrence 1957)

Table 1-2. OARSI criteria of Radiographic Knee Osteoarthritis (tibiofemoral) Grading System.

Description	Scoring
Marginal osteophytes	
Medial femoral condyle	(0-3+)
Medial tibial plateau	(0-3+)
Lateral femoral condyle	(0-3+)
Lateral tibial plateau	(0-3+)
Joint space narrowing	
Medial compartment	(0-3+)
Lateral compartment	(0-3+)
Other	
Medial tibial attrition	(absent/present)
Medial tibial sclerosis	(absent/present)
Lateral femoral sclerosis	(absent/present)
	(Altman and Gold 2007)

Table 1-3. Fried Frailty Phenotype Scoring Criteria.

			Score _____/5
Frailty Domain	Item	0 point	1 point
Weight Loss	Have you lost more than 10lbs unintentionally?	No	Yes
Exhaustion	(a) I felt that everything I did was an effort; (b) I could not get going How often in the last week did you feel this way?	"Rarely or none of the time (1 day)" "Some or a little of the time (2 days)"	"Moderate amount of the time (3–4 days)" "Most of the time (5+days)"
Physical Activity	Minnesota Physical Activity Questionnaire	Men ≥ 383 kcal Women ≥ 270 kcal	Men < 383 kcal Women < 270 kcal
Grip Strength	Calculated using a JAMAR dynamometer and taking the average of 3 trials	Men BMI ≤24 ≤29 kg BMI 24.1–26 ≤30 kg BMI 26.1–28 ≤30 kg BMI >28 ≤32 kg Women BMI ≤ 23 ≤17 kg BMI 23.1–26 ≤17.3 kg BMI 26.1–29 ≤18 kg BMI >29 ≤21 kg	
Gait Speed	15 Foot Walk	Men Height ≤ 173 cm ≥ 7 sec Height >173 cm ≥ 6 sec Women Height ≤ 159 cm ≥ 7 sec Height > 159 cm ≥ 6 sec	
Details on scoring the Fried Frailty Phenotype can be found in Fried LP et al. (Fried, Tangen et al. 2001) Weight loss: self-report measure Exhaustion: self-report measure The Minnesota Physical Activity Questionnaire: a weighted score of kilocalories expended per week Grip strength: is categorized by gender and body mass index Gait speed: is categorized by sex and height			

Table 1-4. Participant characteristics and approach to identifying knee OA and frailty in community dwelling older adults.

	Misra D et al (2014) n=3,707(Misra, Felson et al. 2015)	Castell MV et al (2015) n= 2,455 (Castell, van der Pas et al. 2015)
Participant Characteristics		
Age	≥ 60 years old	65-85 years
Patients (country of development)	Community dwelling older adults participating in the Multicenter Osteoarthritis Study (MOST) and Osteoarthritis Initiative (OAI) Study	Community dwelling older adults living in Germany, United Kingdom, Netherlands, Italy, Spain and Sweden
Inclusion Criteria	MOST Study: Individuals at risk for knee OA OAI Study: Individuals that have early risk factors for knee OA	Random samples from population based cohorts were included
Knee OA Identification		
Method of Knee OA Identification	Identified both radiographically and symptomatically. Radiographic knee OA (ROA) was diagnosed with a Kellgren and Lawrence grade ≥2 in at least one knee. Symptomatic knee OA was defined as ROA in at least one knee plus pain in the same knee, severity of ROA based upon the highest Kellgren and Lawrence grade and number of knees with ROA	Clinical diagnosis: history and physical examination Pain: Western Ontario and McMaster Universities OA Index pain sub score, plus any three of: age 50+, morning stiffness lasting <30 minutes and no palpable warmth of synovium in both knees
Frailty Identification		
Method of Measuring Frailty	Study of Osteoporotic Fracture Index	Fried Frailty Phenotype

Table 1-5. Population studies of older adults with knee OA and possible consequences of elevated serum inflammatory cytokines.

Predictor of Radiographic Knee OA and/or Radiographic Changes			
Author, year of publication, journal number of participants	Objective	Inflammatory Cytokines Measured	Conclusions
Botha-Scheepers S et al. (2008) Ann Rheum Dis, n=191 (Botha-Scheepers, Watt et al. 2008)	Investigated whether innate differences in the inflammatory response regarding cytokine production associated with radiological progression of knee OA	TNF α and IL-10	Patients in the highest quartile of TNF α production had a 6 fold increased risk of tibiofemoral joint space narrowing progression (RR 6.1, 95% CI 1.4 to 9.8). Patients in the highest quartile of IL-10 production also had a 4 fold increased risk of tibiofemoral joint space narrowing progression (RR 4.3, 95% CI 1.7 to 6.2) (Botha-Scheepers, Watt et al. 2008).
Livshits G et al. (2009) Arthritis and Rheumatism, n=908 (Livshits, Zhai et al. 2009)	Determine whether circulating levels of IL-6, TNF α and CRP can serve as useful markers of radiographic knee OA in a normal human population	IL-6, TNF α and CRP	During a 15-year follow up, the prevalence of radiographic knee OA (KL ≥ 2) increased from 14.7% to 48.7% ($p<0.0001$) (Livshits, Zhai et al. 2009). Circulating levels of IL-6 and CRP were consistently higher in subjects diagnosed with having radiographic knee OA (Livshits, Zhai et al. 2009). However, TNF α did not have an association with radiographic knee OA (Livshits, Zhai et al. 2009).
Sharif M et al. (2000) Ann Rheum Dis, n=90 (Sharif, Shepstone et al. 2000)	To determine if CRP can predict radiological progression of knee OA	CRP	Serum CRP during a 3 year follow up was predictive of progression (OR 1.2 95% CI 1.01, 3.28) (Sharif, Shepstone et al. 2000)
Sowers MF et al. (2002) Osteoarthritis and	Evaluation of CRP as a potential biomarker of	CRP	Higher CRP concentrations were associated with both prevalent and incident of radiographic knee

Cartilage, n=2078 (Sowers, Jannausch et al. 2002)	prevalent and incident of knee OA		OA ($p<0.0001$ and $p<0.0001$, respectively) (Sowers, Jannausch et al. 2002). For each KL score increase from 0 to 3, there was a significantly higher mean CRP level (Sowers, Jannausch et al. 2002). Higher levels of CRP were associated with radiographic knee OA.
Spector TD et al. (1997) Arthritis and Rheumatism, n=875 (Spector, Hart et al. 1997)	Examine the role of low-grade inflammation etiology and progression of early OA of the knee	CRP	Levels of CRP were higher in 105 women with radiographic knee OA (KL ≥ 2) (median 2.4 mg/L, interquartile range [IQR] 1.0-5.1), compared to women without radiographic knee OA (median 2.6 mg/L, IQR 0.3-1.8) (Spector, Hart et al. 1997). CRP was associated with radiographic knee OA.
Stannus O et al. (2010) Osteoarthritis and Cartilage, n=172 (Stannus, Jones et al. 2010)	Determine the associations between serum levels of IL-6 and TNF α, knee radiographic AA and cartilage loss over 2.9 years in older adults	IL-6 and TNF α	IL-6 and TNF α were associated with increased prevalence of medial tibiofemoral joint space narrowing (OARSI ≥ 1) [odds ratio (OR): 1.42 and 1.47 per quartile, respectively, $p<0.05$] (Stannus, Jones et al. 2010). Change in IL-6 predicted loss of both medial and lateral tibial cartilage volume (-1.18% and -1.06% per annum per quartile, $p<0.05$) and change in TNF α was also negatively associated with change in medial cartilage volume (-1.27% per annum per quartile, $p<0.05$) (Stannus, Jones et al. 2010). Both IL-6 and TNF α were associated with medial tibiofemoral joint space narrowing and loss or medial and lateral tibial cartilage volume.
Pain			
Author, year of publication, number of participants	Objective	Inflammatory Cytokines Measured	Conclusions
Aguiar GC et al. (2015). Rheumatology	The aim of the present study was to determine the effects	IL-6 and TNF α	A decrease in the perception of pain was evident on visual analog scale (VAS) ($p < 0.001$) and pain

International, n=22 (Aguiar, Do Nascimento et al. 2015)	of an exercise therapy protocol on inflammatory markers, perception of pain, and physical performance of individuals with knee OA.		subscale of the WOMAC ($p < 0.001$) (Aguiar, Do Nascimento et al. 2015). There was a decrease in serum levels of IL-6 ($p < 0.001$) with exercise (Aguiar, Do Nascimento et al. 2015). However, changes in the levels of the TNF α were not statistically significant. Physical function subscale score and the WOMAC global score improved significantly ($p < 0.001$) (Aguiar, Do Nascimento et al. 2015). This study suggests that this exercise therapy protocol could be a strategy for reducing IL-6 levels, managing pain, and improving function in individuals with OA of the knee.
Imamura M et al. (2015). International Journal of Inflammation, n=101 (Imamura, Ezquerro et al. 2015)	The objective of this study was to investigate the relationship between IL-6, TNF α and IL-10 in patients with painful knee osteoarthritis, functional capacity and pressure pain threshold values	IL-6, TNF α and IL-10	This study showed that serum levels of IL-6 and IL-10 were higher in knee OA than in healthy controls. IL-6 ($p =0.031$) and IL-10 ($p= 0.030$) levels were significantly elevated in patients (Imamura, Ezquerro et al. 2015). Both IL-6 and IL-10 correlated positively with visual analog scale (VAS) and WOMAC scores for rigidity (Imamura, Ezquerro et al. 2015). Only IL-6 correlated positively with WOMAC scores for pain.
Shimura et al. (2013). Osteoarthritis and Cartilage, n=160 (Shimura, Kurosawa et al. 2013)	Factors associated with pain in knee OA varied according to disease progression.	IL-6	Serum IL-6 is associated with pain severity, visual analog scale (VAS) in early stage knee OA (Shimura, Kurosawa et al. 2013). Serum IL-6 is associated with pain severity (Japanese knee OA measure) in early stage (Shimura, Kurosawa et al. 2013).
Stannus OP et al. (2013). Annals of Rheumatic Diseases, n=149 (Stannus, Jones et al.	To determine the association between inflammatory markers and change in knee pain over 5 years	CRP, TNF α and IL-6	CRP was positively associated with change in total knee pain ($\beta=0.33$ per mg/l, $p=0.032$). Change in TNF α was positively associated with change in total knee pain ($\beta=0.66$ per mg/l, $p=0.020$). Baseline

2013)			TNF α and IL-6 was associated with change in pain with standing (β=0.06 per ml/pg, p=0.033); (β=0.16 per ml/pg, p=0.035) respectively (Stannus, Jones et al. 2013). Both CRP and TNF α were associated with change in total knee pain.
Physical function & muscle strength			
Author, year of publication, number of participants	Objective	Inflammatory Cytokines Measured	Conclusions
Aguiar GC et al. (2015). Rheumatology International, n=22 (Aguiar, Do Nascimento et al. 2015)	The aim of the present study was to determine the effects of an exercise therapy protocol on inflammatory markers, perception of pain, and physical performance of individuals with knee OA.	IL-6 and TNF α	A decrease in the perception of pain was evident on visual analog scale (VAS) ($p < 0.001$) and pain subscale of the WOMAC ($p < 0.001$) (Aguiar, Do Nascimento et al. 2015). There was a decrease in serum levels of IL-6 ($p < 0.001$) with exercise (Aguiar, Do Nascimento et al. 2015). However, changes in the levels of the TNF α were not statistically significant. Physical function subscale score and the WOMAC global score improved significantly ($p < 0.001$) (Aguiar, Do Nascimento et al. 2015). This study suggests that this exercise therapy protocol could be a strategy for reducing IL-6 levels, managing pain, and improving function in individuals with OA of the knee.
Cohen HJ et al. (1997) Journal of Gerontology Medical Sciences, n=4,164 (Cohen, Pieper et al. 1997)	Determine the relationship between the level of IL-6 with age, sex, race, self-reported medical conditions and functional status	IL-6	IL-6 levels were higher with age (p=0.001) even in adults older than 70 years (Cohen, Pieper et al. 1997). There was a positive correlation between IL-6 and functional disability (p=0.0001), as well as self-rated health and IL-6 (Cohen, Pieper et al. 1997). IL-6 is associated with functional disability in older adults.
Penninx BW et al.	To investigate whether	IL-6, CRP	Higher serum levels of IL-6 tended to be associated

(2004). The journal of Rheumatology, n=274 (Penninx, Abbas et al. 2004)	serum concentrations of various inflammatory markers are associated with physical function and disease severity among older obese adults with knee OA		with slower walking speed, but no significant associations were observed for CRP (Penninx, Abbas et al. 2004).
Samut G et al. (2015) Modern Rheumatology, n=42 (Samut, Dinçer et al. 2015)	Investigate the effects of isokinetic and aerobic exercise training programs on serum pro-inflammatory cytokine levels, pain and functional activity in patients with knee OA	TNF α, IL-6, CRP	There was no statistically significant decrease in TNF α and IL-6 levels in both intervention groups (isokinetic and aerobic) (Samut, Dinçer et al. 2015). Serum CRP levels strongly tended to decrease in both intervention groups, but they did not reach a statistically significant level (p=0.072) (Samut, Dinçer et al. 2015).
Sanchez-Ramierz DC et al. (2014) Arthritis Research & Therapy, n=186 (Sanchez-Ramirez, van der Leeden et al. 2014)	Examine the associations of evaluated serum CRP with change in muscle strength in patients with established knee OA	CRP	Patients with elevated CRP values at both baseline and 2-year follow-up exhibited a lower increase in knee muscle strength for a period of 2 years (β = −0.22; P = 0.01) compared with the group with non-elevated levels at both times of assessment (Sanchez-Ramirez, van der Leeden et al. 2014).
Santos MLAS et al. (2011) Archives of Gerontology and Geriatrics, n=80 (Santos, Gomes et al. 2011)	Verify the correlation between IL-6 plasma levels and muscular function of the knee joint and functionality in elderly women with knee OA.	IL-6	IL-6 was inversely correlated to the muscle resistance and muscle balance with the hamstring muscle. No significant correlation between IL-6, muscle strength and physical function was found (Santos, Gomes et al. 2011).

Table 1-6. Population studies of frailty in older adults and the possible consequences of elevated serum inflammatory cytokines.

Population studies of frailty in older adults and the possible consequences of elevated serum inflammatory cytokines				
Frailty & Inflammatory Cytokines				
Author, year of publication, journal number of participants	Objective	Frailty measurement used	Inflammatory Cytokines Measured	Conclusions
Arts MHL et al. (2015) JAGS, n=366 (Arts, Collard et al. 2015)	To determine whether physical frailty is associated with low-grade inflammation in older adults with depression	Fried Frailty Phenotype	IL-6 and CRP	The physical frailty phenotype was not associated with inflammatory markers. Of the individual criteria, hand grip strength was associated with CRP (β=-0.21, p=0.002), and IL-6 (β=-0.25, p<0.001) (Arts, Collard et al. 2015).
Collerton J et al. (2012). Mechanisms of Ageing and Development, n=845 (Collerton, Martin-Ruiz et al. 2012)	Relationship between frailty and biomarkers	Fried Frailty Phenotype	CRP and WBC	A greater risk of frailty was associated with high levels of CRP and neutrophils (odds ratios (95% CI) 1.82 (1.19-2.80), p=0.006 and 1.65 (1.06-2.56), p=0.027 respectively (Collerton, Martin-Ruiz et al. 2012).
Darvin K et al. (2014) J Gerontol A Biol Sci Med Sci, n=65 (Darvin, Randolph et al. 2013)	The purpose of the study was to confirm if plasma levels of IL-6 are increased with frailty in community dwelling older adults	Fried Frailty Phenotype	IL-6	Higher levels of IL-6 (p=0.0035) are associated with frailty status (Darvin, Randolph et al. 2013).
Hubbard et al. (2009). Journal of cellular and molecular medicine, n=110 (Hubbard, O'Mahony et al. 2009)	Investigate inflammation in older patients across different frailty criteria	Fried Frailty Phenotype and Frailty Index	TNF α, IL-6, CRP, WBC	The frailty index is correlated significantly with log-transformed TNF α (r=0.379, p<0.01), IL-6 (r=0.369, p<0.01), CRP (r=0.221, p<0.05) and albumin (r=-0.545, p<0.01) (Hubbard, O'Mahony et al. 2009).

Lai HY et al. (2014) Maturitas, n=386 (Lai, Chang et al. 2014)	Determine whether higher serum levels of IL-6, TNF α and CRP were associated with frailty in the older institutionalized men.	Fried Frailty Phenotype	IL-6, TNF α and CRP	Higher IL-6 levels were positively associated with the frailty. After adjusting for covariates, Il-6 showed significant trend across frailty categories (p=0.03 [95% CI 1.4-5.24]). No significant association was seen between TNF α, CRP and frailty (Lai, Chang et al. 2014).
Leng S et al. (2002). JAGS, n=30 (Leng, Chaves et al. 2002)	Test physiological parameters as potential correlates of frailty	Fried Frailty Phenotype	IL-6	Frail subjects had significantly higher serum IL-6 levels and significantly lower haemoglobin and hematocrit than non-frail subjects (4.4±2.9 vs 2.8±1.6 pg/mL, 12.1±1.1 vs 13.9±1.0g/dL, and 35.8% ±3.1 vs 40.6% ±2.8, respectively) (Leng, Chaves et al. 2002). No significant difference observed in white blood cell and platelet counts between the frail and non-frail groups (Leng, Chaves et al. 2002).
Leng SX et al. (2007) JAGS, n=558 (Leng, Xue et al. 2007)	Evaluate the relationships between WBC count and IL-6 and prevalent frailty	Fried Frailty Phenotype	WBC, IL-6	When women with total WBC counts in the top tertile were significantly more likely to be pre-frail (OR=2.43, 95% confidence interval (95% CI)=1.21-4.89) or frail (OR=4.25, 95%CI 1.89-9.58) than those with WBC in the bottom tertile (Leng, Xue et al. 2007). Women with IL-6 levels in the top tertile were significantly more likely to be pre-frail (OR 2.28, 95% CI 1.13-4.60) or frail (OR=3.98, 95% CI=1.76-9.00) than those with IL-6 levels in the bottom tertile (Leng, Xue et al. 2007).
Puts MTE et al. (2005). Clinical	Examine the association of serum concentrations	Frailty Index	IL-6, CRP	Moderately elevated serum concentrations of CRP (3.0-10.0μg/ml) predicted frailty, with

Endocrinology, n=242 (Puts, Visser et al. 2005)	of IL-6 and CRP with prevalent and incident frailty			an OR of 1.77 (95% CI 1.15-2.68) vs low serum concentrations of CRP when adjusting for sex and age. There was no significant association between IL-6 and incident of frailty when adjusting for cofounders (Puts, Visser et al. 2005).
Walston et al. (2002). JAMA, n=4735 (Walston, McBurnie et al. 2002)	To establish the biological correlates of frailty in the presence and absence of concurrent cardiovascular disease and diabetes mellitus	Fried Frailty Phenotype	CRP	Frail vs non-frail participants mean CRP 5.5±9.8, 2.7±4.0 mg/L, respectively (Walston, McBurnie et al. 2002). There is a significant association between inflammatory markers and frailty both in the presence and absence of cardiovascular disease and diabetes.

CHAPTER 2
ASSESSING INFLAMMATORY CYTOKINES & FRAILTY IN KNEE OSTEOARTHRITIS

Systemic Inflammation and Physical Frailty in Women with Knee Osteoarthritis

Authors: Sarah Karampatos[1], Dessi Loukov[2], Jaclyn N. Chopp-Hurley[1], Alexander B. Kuntz[3] , Elora C. Brenneman[3], Dawn M. Bowdish[2], Alexandra Papaioannou[4,5,6], Monica R. Maly[1,3]

1. School of Rehabilitation Science, McMaster University, 1400 Main St W, Hamilton, ON L8S 1C7
2. Department of Pathology and Molecular Medicine, McMaster University, 1280 Main St W, Hamilton, ON L8S 4L8
3. Department of Kinesiology, McMaster University, 1280 Main St W, Hamilton, ON L8S 4L8
4. Department of Medicine, McMaster University, 1280 Main St W, Hamilton, ON L8S 4L8
5. Hamilton Health Sciences, 88 Maplewood Ave, Hamilton, ON L8M 1W9
6. Geriatric Education and Research in Aging Sciences, 88 Maplewood Ave, Hamilton, ON L8M 1W9

Introduction

Osteoarthritis (OA) is the most prevalent chronic joint disease in the world (Woolf and Pfleger 2003). Half the population over the age of 65 years suffer from OA (Castell, van der Pas et al. 2015), with prevalence and severity higher in women than men (5). Individuals with OA often experience joint pain, joint tenderness and inflammation which can increase pain and functional limitations (Woolf and Pfleger 2003). In Canada, it is estimated that OA currently accounts for $10 billion in direct health care costs (Canada 2011). Osteoarthritis most commonly affects the knee due to the weight-bearing nature of the joint.

Knee OA is the most common form of arthritis in older adults (Misra, Felson et al. 2015). There are multiple methods used to measure knee OA severity, including both radiographic and symptomatic approaches. Individuals with radiographic changes may have no symptoms; and individuals with symptoms may show no radiographic joint changes. Accordingly, there is a weak correlation between radiographic and symptomatic severity in this condition (Hannan, Felson et al. 2000, Muraki, Oka et al. 2009). Knee OA is characterized by damage to articular cartilage and formation of osteophytes (Buckwalter, Mankin et al. 2005). Damage to the articular cartilage may lead to abnormal joint loading which can increase pain and cause mobility limitations (Bhatia, Bejarano et al. 2013). Knee pain is often the most prominent and disabling symptom of knee OA (Stannus, Jones et al. 2013). The primary goals of knee OA management are to reduce pain and improve physical function. Functional limitation is also strongly associated with another condition that affects older adults, frailty.

Frailty is a multi-faceted medical syndrome where individuals are at increased risk of adverse health outcomes, including hospitalization, institutionalization and mortality (Fugate Woods, LaCroix et al. 2005, Cawthon, Marshall et al. 2007, Ensrud, Ewing et al. 2007, Ávila-Funes, Helmer et al. 2008, Bouillon, Kivimaki et al. 2013). The prevalence of frailty ranges from 6.9% to 42.6% depending on the definition of frailty measurement used (Ottenbacher, Ostir et al. 2005, Cesari, Leeuwenburgh et al. 2006, Ávila-Funes, Helmer et al. 2008). A recent study by Aarts and colleagues showed that there is heterogeneity within the frail population (Aarts, Patel et al. 2015). Although there has been a consensus on the consequences of frailty, there has been no consensus on a gold standard for measuring frailty. There are multiple physical function tests, questionnaires and indexes used to categorize frailty (Hubbard, O'Mahony et al. 2009, De Vries, Staal et al. 2011) However, a very common definition of frailty is a decrease in strength, endurance and physiological function that increases an individual's vulnerability to adverse health outcomes (Morley, von Haehling et al. 2014). Pre-frailty occurs at an earliest stage of the frailty spectrum and may be a better an ideal target for intervention (Castell, van der Pas et al. 2015). Frailty is a treatable, dynamic condition (Morley, von Haehling et al. 2014). Treatments for frailty include exercise, protein-calorie supplementation, vitamin D supplementation and decrease polypharmacy. It is likely that treating pre-frailty is the most effective way to halt and reverse this age-related condition.

Knee OA may be a useful early marker of risk for frailty (Castell, van der Pas et al. 2015, Misra, Felson et al. 2015). A cross-sectional analysis showed that frailty was more

prevalent among patients with radiographic knee OA (4.39% vs. 2.77%) and symptomatic knee OA (5.88% vs. 2.79%) compared to those without radiographic and symptomatic knee OA, respectively (Misra, Felson et al. 2015). It is likely that knee OA and frailty share common risk factors, such as obesity, female sex, decreased strength and physical function (Felson, Anderson et al. 1988, Blaum, Xue et al. 2005). Nonetheless, the potential mechanisms through which knee OA and frailty are associated are not understood. While knee OA and frailty both affect aging adults, little work has explored the co-existence of these conditions. Systemic inflammation may be the linking mechanism between knee OA and frailty.

Local inflammation plays a role in the pathogenesis of osteoarthritis (Rainbow, Ren et al. 2012). Proinflammatory cytokines including interleukin-6 (IL-6), tumor necrosis factor α (TNF α) and C reactive protein (CRP) were higher in individuals with knee OA (Spector, Hart et al. 1997, Botha-Scheepers, Watt et al. 2008, Livshits, Zhai et al. 2009, Stannus, Jones et al. 2013, Sanchez-Ramirez, van der Leeden et al. 2014, Aguiar, Do Nascimento et al. 2015). For example, IL-6 was associated with radiographic knee OA (Livshits, Zhai et al. 2009), joint space narrowing (Stannus, Jones et al. 2010) and pain (Aguiar, Do Nascimento et al. 2015). In a 3-year cohort study of 172 randomly selected subjects (mean 63 years, range 52-78), change in IL-6 was associated with greater loss of medial and lateral tibial cartilage volume (β: -1.18% and -1.06 per annum per quartile, both $p<0.05$) (Stannus, Jones et al. 2010). Elevated TNF α was associated with joint space narrowing (Botha-Scheepers, Watt et al. 2008, Stannus, Jones et al. 2013), radiographic knee OA severity (Livshits, Zhai et al. 2009) and pain intensity (Aguiar, Do Nascimento

et al. 2015). Increased change in TNF α was also negatively associated with change in medial cartilage volume (β: -1.27% per annum per quartile, $p<0.05$) over 3 years (Stannus, Jones et al. 2010). Elevated serum levels of C reactive protein was positively associated with radiographic knee OA severity (Spector, Hart et al. 1997, Sowers, Jannausch et al. 2002, Livshits, Zhai et al. 2009), pain intensity (Stannus, Jones et al. 2010) and a decrease in knee muscle strength (Sanchez-Ramirez, van der Leeden et al. 2014). Serum CRP levels were higher in 105 women with radiographic knee OA (KL ≥ 2) (median 2.4 mg/L, interquartile range [IQR] 1.0-5.1), compared to women without radiographic knee OA (median 2.6 mg/L, IQR 0.3-1.8) (Spector, Hart et al. 1997). Finally, elevated serum levels of interleukin-10 (IL-10) may also be associated with joint space narrowing (Botha-Scheepers, Watt et al. 2008). These findings suggest that inflammatory cytokines may play a role in knee OA initiation; and likely play a role in the progression of this disease.

Inflammation is not isolated to knee OA. In fact, the aging immune system is characterized by low-grade, chronic, systemic inflammatory state (Franceschi, Bonafè et al. 2000). The association between inflammation and frailty has been previously shown (Walston, McBurnie et al. 2002, Puts, Visser et al. 2005, Leng, Xue et al. 2007, Hubbard, O'Mahony et al. 2009, Collerton, Martin-Ruiz et al. 2012, Darvin, Randolph et al. 2013, Lai, Chang et al. 2014, Arts, Collard et al. 2015). It is initiated by increased circulating levels of pro-inflammatory cytokines such as IL-6, TNF α and CRP (Sergio 2008). In a cross-sectional study of 110 patients aged over 75 years (mean 83.9 years), a frailty index correlated with log-transformed TNF α ($r=0.38$, $p<0.01$), IL-6 ($r=0.37$, $p<0.01$) and CRP

($r=0.22$, $p<0.05$) (Hubbard, O'Mahony et al. 2009). Meanwhile, IL-10 is considered to be one of the most dominant anti-inflammatory cytokines (Minciullo, Catalano et al. 2015). IL-10 blocks immune responses and inhibits the production of inflammatory cytokines (Minciullo, Catalano et al. 2015). However, most of this research has focused on mouse models, with little in older, frail adults. What is known in humans is that IL-10 is associated a decreased risk of death of cardiovascular event (van den Biggelaar, de Craen et al. 2004).

The relationship between the severity of frailty and presence of inflammatory cytokines remains unknown. This project provides a chance to investigate the relationship between the severity of frailty and presence of inflammatory cytokines, which currently remains unstudied. Filling this gap is necessary to better understand the potential mechanisms affecting both frailty and knee OA. Identifying potential mechanisms would provide the possibility to develop novel strategies to identify, prevent and manage both conditions.

The primary purpose of this study is to determine if there is a difference in radiographic or symptomatic knee OA severity between non-frail, pre-frail and frail women with knee OA. Secondary objectives include the following:

a) Determine the relationship between each of radiographic and symptomatic knee OA severity and the presence of inflammatory cytokines in serum.

b) Determine if there is a difference in inflammatory cytokines between non-frail, pre-frail and frail women with knee OA.

Our primary hypothesis is that both radiographic and symptomatic severity will be greater in participants categorized as pre-frail/frail compared to those that are non-frail. For our secondary hypotheses, we anticipated that

a) Both radiographic and symptomatic knee OA severity would share a positive relationship with the level of pro-inflammatory cytokines in serum (and negative relationship with anti-inflammatory cytokines).
b) Elevated levels of serum cytokines would be present in pre-frail/frail participants relative to non-frail participants.

Methodology

This study was a secondary analysis of baseline data collected in a subsample of participants who enrolled in a larger randomized controlled trial (clinical trial number NCT02370667). Participants were asked provide written, informed consent to obtain additional data necessary for this analysis (specifically venous blood samples).

Participants

Community-dwelling women (over the age of 50) diagnosed with mild to moderate symptomatic knee osteoarthritis were recruited. This age group is commonly affected by knee OA (Oliveria, Felson et al. 1995). Participates were recruited through newspaper and community newsletter advertisements, a yoga studio newsletter, a rheumatologist office, and postering in the community.

Inclusion/Exclusion Criteria

Participants were included if they were women ≥ 50 years and answered yes to 3 or more of the following items that are used to confirm symptomatic knee OA: (a) Have knee pain in most days of the week (b) Have fewer than 30 minutes of morning stiffness (c) Have crepitus with active range of motion (d) Have a bony enlargement (e) Have bony tenderness with palpation (f) Have signs of inflammation and/or have been diagnosed with radiographic osteoarthritis. Also, if participants consented, standing radiographs of the most symptomatic knee were taken at the beginning of study to ensure that participants had radiographic disease.

Participants were excluded if they had any other forms of arthritis (e.g., rheumatoid, psoriatic), active non-arthritic knee disease (e.g., bursitis), patellofemoral symptoms, knee surgery (e.g., high tibial osteotomy, joint replacement, ligament repair), history of an osteoporotic fracture, planned surgery in the next 6 months, lower extremity trauma in the last 3 months, an unstable heart condition or neurological conditions (e.g., stroke), recent or current exposure to radiation, or other health conditions that might be exacerbated by the protocol. All participants had written informed consent before beginning the study. Ethics approval was received through the Hamilton Integrated Research Ethics Board.

Measurements

Frailty Measurement: Fried Frailty Phenotype

The Fried Frailty phenotype (Fried, Tangen et al. 2001) is one of the most commonly used frailty assessment tools that was developed to be used by both clinicians and researchers. The Fried Frailty phenotype is an assessment of physical frailty that is composed of a combination of five self-reported items and objective performance items: self-reported weight loss, self-reported exhaustion, self-reported physical activity, grip strength and walking speed. Fried Frailty Phenotype scoring can be found in Table 2-1. Each item is scored between 0 (non-frail) and 1 (frail). The tool has a maximum score of 5; higher scores indicate a greater degree of frailty. Individuals who have a total score of 0 are considered to be non-frail, individuals who are scored 1-2 are considered to be pre-frail and individuals who are scored 3 or greater are considered to be frail (Fried, Tangen et al. 2001).

The tool has been tested for predictive validity for mortality. Baseline frailty independently predicted risk of death (hazard ratio (HR)=1.71, 95% confidence interval (CI)=1.48-1.97) (Fugate Woods, LaCroix et al. 2005) and falls (Fried, Tangen et al. 2001, Kiely, Cupples et al. 2009). Convergent validity, when comparing commonly used tools used to assess frailty (frailty index compared to the Fried Frailty Phenotype), there was moderate correlation between measures (R=0.65) (Rockwood, Andrew et al. 2007).

Radiographic Knee Osteoarthritis Severity: Kellgren and Lawrence (KL)

The KL grading score is the most widely used to identify radiographic OA (Kellegren and Lawrence 1957, Braun and Gold 2012). The KL is considered the "gold standard" diagnostic test for knee OA (Bauer, Hunter et al. 2006). Participant KL grades were determined for each knee from a frontal plane, fixed-flexion, standing radiograph (Kothari, Guermazi et al. 2004) by one experienced radiologist. The grading scores OA range between 0 (normal) to 4 (severe OA) (Kellegren and Lawrence 1957). A score of ≥ 2 is often used as a cutoff to classify OA (Schiphof, Boers et al. 2008). Nonetheless, the KL grading has received criticism because it places a large emphasis on osteophyte formation with relatively less emphasis on joint space narrowing (Altman and Gold 2007). The first two grades of the KL grading system are defined by presence of osteophytes (Altman and Gold 2007). This becomes a problem, as a patient with joint space narrowing but no osteophyte formation is not characterized as having osteoarthritis according to the KL grading system (Altman and Gold 2007).

Symptomatic Knee Osteoarthritis Severity: The Knee Injury and Osteoarthritis Outcome Score (KOOS)

The KOOS is a self-report questionnaire used for both research and clinical purposes (Collins, Misra et al. 2011). The 42-item questionnaire contains five domains including pain, symptoms, activities of daily living (ADL), and sport/recreation activities and knee related QOL (Collins, Misra et al. 2011). Scores are transformed to a scale of 0-100. It is important to note that a transformed score of 0 indicates extreme problems and a transformed score of 100 indicates no problems (Collins, Misra et al. 2011). The tool has shown high construct validity when compared with SF-36 (physical function vs.

activities of daily living, r = 0.57, physical function vs. sport and recreation function, r = 0.47, bodily pain vs. pain, r = 0.46) (Ware Jr, Snow et al. 1993, Roos, Roos et al. 1998) and high test-retest reproducibility (ICC >0.75) (Roos, Roos et al. 1998, Collins, Misra et al. 2011).

Inflammatory Markers from Serum

Venous blood samples were taken from all participants in the morning. Fasting was not required. The blood samples were centrifuged at 1.5 x *g* for 10 minutes at 25°C and serum was stored at -140°C until processed 4 months later. Serum cytokines IL-6, IL-10, TNF α, except CRP, were measured using Milliplex MAP 60K cytokine panel as per manufacturer's protocol (EMD Millipore). CRP was analyzed by an in-house ELISA. In short, the wells were coated with capture antibody (1ug/mL, ab8279, Abcam) overnight, blocked with blocking buffer (PBS with 10% FBS) for 2h. Samples were diluted 1/500 and added to the wells, incubated for 1h before being washed with washing buffer (PBS with 0.05% Tween 20). Plate was incubated for 1h with HRP-conjugated detection antibody (1ug/mL, abcam24462, Abcam) diluted in blocking buffer for 1h before being washed. Plates were developed with TMB as per manufacturer's protocol. In all cases, the serum values were measured in duplicate and an average score was reported.

Descriptors

Performance Outcome Measurement: Six Minute Walk Test (6MW)

The 6MW is a mobility performance measure commonly used in a range of patients, including those with lower extremity OA (Davis, Ettinger et al. 1991). The 6MW

measures the distance that individuals can walk in 6 minutes on an indoor surface (Davis, Ettinger et al. 1991, Bennell, Dobson et al. 2011). Individuals were instructed to walk as quickly as possible without running or jogging and were allowed to use walking aids. Shorter distances are associated with poorer mobility (Bennell, Dobson et al. 2011). The 6MW produces valid (Hamilton and Haennel 2000, Eileen Collins PhD, Susan O'Connell MHA et al. 2008) and reliable (Kennedy, Stratford et al. 2005) data to assess the sub-maximal level of mobility performance in older adults.

Depressive Symptoms: The Centre for Epidemiologic Studies-Depression Scale (CES-D)

The CES-D is a measure of depressive symptoms. It is a 20-item questionnaire that asks individuals to rate how often over the past week they have experienced symptoms of depression, poor appetite and feeling lonely (Lewinsohn, Seeley et al. 1997). Scores range from 0 to 60 with higher scores indicating greater depressive symptoms (Lewinsohn, Seeley et al. 1997). The CES-D also provides a cut-off score of 16 or greater that identifies individuals at risk for clinical depression. Data from the CES-D demonstrate good sensitivity in the general population (Radloff 1977, Lewinsohn, Seeley et al. 1997).

Anthropometrics

Anthropometric measurements collected include height, mass, body mass index. Height and mass were measured barefoot using a standard stadiometer. Body mass index was calculated as body mass (kilograms)/height (meters)^2.

Statistical Analysis

Normality of all data was assessed using the Shapiro-Wilk test and histograms. Raw data for the inflammatory cytokines were not normally distributed. These data were log-transformed (Figure 2-1). From the whole sample, the Fried Frailty Phenotype score categorized participants into non-frail (score 0) and pre-frail (score 1) or frail (score 2 or greater).

The primary purpose of the study was to determine if radiographic and symptomatic severity of knee OA was different between participants that were non-frail versus pre-frail/frail. If independent variable data were normally distributed, a t-test was used; however if the independent variable data were not normally distributed a Mann-Whitney U test was used.

For the secondary objectives, first, a linear regression was performed to determine if there was a relationship between radiographic knee OA (KL score) and inflammatory cytokines. A separate regression was run for each cytokine (IL-6, IL-10, TNF α, CRP). Regression was also used to evaluate the relationship between symptomatic knee OA severity (KOOS subscales) and inflammatory cytokines. A separate regression was run individually for each cytokine.

Second, mean values for the serum level of inflammatory cytokines were compared between the non-frail and pre-frail/frail categories. A test of normality showed that IL-6, IL-10, CRP and TNF α were not normally distributed. A variance test showed that IL-6,

IL-10 and CRP had equal variance and TNF α had unequal variance. Therefore, a Mann-Whitney U Rank Sum test used to establish where there was a difference between each inflammatory cytokine between pre-frail/non-frail women. All analysis was performed using STATA version 13.1. A two-sided p value of 0.05 was considered to be significant.

Results

Twenty-one women participated in this study. Data from one was excluded due to elevated inflammatory cytokines and neutrophils indicating illness. Data from 20 women (70.3 [8.8] years, BMI 31.2 [4.0] kg/m^2) were used for analyses. Of these, 11 women were considered to be non-frail and 9 women were considered to be pre-frail. Table 2-2 presents the frequency distribution for each item of the Fried Frailty Phenotype. Grip strength and self-reported exhaustion were the items most likely to identify pre-frailty; while no participants in this sample reported physical activity or demonstrated walking speeds indicative of frailty. Table 2-3 represents the characteristics of the whole study sample and each of the non-frail and pre-frail groups separately. It is important to note that participants that were pre-frail had larger BMI, higher CES-D depression score, decreased 6MW score and lower grip strength compared to those that are non-frail.

In this cross-sectional analysis, there was no a difference in radiographic knee OA severity between pre-frail individuals compared to those who are non-frail (p= 0.11). There was no difference in symptomatic knee OA severity in pre-frail participants compared to non-frail participants for any domains of the KOOS scale (Table 2-4).

Radiographic knee OA severity was not associated with log-transformed values for IL-6 (r=0.004, p=0.42), IL-10 (r=0.06, p=0.35), TNF α (r=0.07, p=0.30) and CRP (r=0.001, 0.99). Log-transformed TNF α was significantly associated with self-reported pain assessed by KOOS (r=0.26, p=0.02). However, there was no relationship between self-reported symptoms, ADL, sport and QOL assessed by KOOS with log-transformed IL-6, log-transformed IL-10 (Table 2-5).

There was no difference in pre-frail individuals with inflammatory cytokines compared to those who are non-frail (Table 2-6).

Discussion

In this sample, women with knee OA, though relatively young, showed signs of pre-frailty. Although it is not surprising, none of the women with knee OA were considered to be frail. Identifying risk of frailty in younger women with knee OA may be useful to initiate early intervention.

Inflammation and Knee Osteoarthritis

We confirmed the importance of inflammatory cytokines and self-reported pain in the younger old women with symptomatic knee OA. Specifically we found a negative relationship between pain and log-transformed TNF α. TNF α is a key regulator of an inflammatory response, which can promote cell death. Previous research has shown that higher levels of TNF α have been associated with change in total knee pain (β=0.66 ml/pg, p=0.02). The cause of joint pain in knee OA is not well understood. It has also been shown that individuals may show no radiographic signs of knee OA however,

experience a lot of pain and reduced physical function (Li, Wei et al. 2013). Similar to findings in the current study, self-reported pain assessed by the Western Ontario and McMaster Universities Arthritis Index (WOMAC) was associated with increased levels of TNF α (Roos, Roos et al. 1998). The KOOS pain subscale is identical to the WOMAC pain subscale. However, we did not find any cross-sectional relationships of other inflammatory cytokines with KOOS subscales.

It is likely that the pathogenesis of knee OA is the immune system (Goldring and Otero 2011). Evidence for the role of TNF α in knee OA is debated and associations between TNF α and knee OA are inconsistent. In our study we found a relationship between KOOS pain (symptomatic knee OA) and TNF α. This relationship may be important as previous literature has shown that TNF α can induce the production of other cytokines, and inhibit the synthesis of proteoglycans and type II collagen and therefore, be important in cartilage matrix degradation and bone resorption in OA (Goldring and Goldring 2004). Therefore, TNF α may be an important primary inflammatory cytokine in knee OA. Conversely, Penninx et al (Penninx, Abbas et al. 2004) reported in 274 patients with knee OA participating in an exercise and nutrition intervention study serum TNF α was not associated with WOMAC knee pain, radiographic scores. These results suggest that TNF α plays a role in self-report pain however, future studies should look at the longitudinal relationships between TNF α and self-report pain. Nonetheless, a major limitation of inflammatory markers is the difficulty to discriminate if inflammatory status is due to aging, knee OA, frailty or a combination of all co-morbidities.

We found no relationship between radiographic and symptomatic OA severity and IL-6. Previous studies have shown inconsistent relationships between IL-6 and knee OA. IL-6 is produced by T cells, B cells, monocytes and fibroblasts and can stimulate osteoclast stimulation, which leads to cartilage destruction (Park and Pillinger 2007). Penninx et al (Penninx, Abbas et al. 2004) reported that serum IL-6 was not associated with WOMAC pain, stiffness and function and radiographic knee OA in baseline assessments in 274 patients with knee OA participating in an exercise and nutrition intervention study. These results are consistent with our study. We found that there was no relationship between radiographic or symptomatic knee OA and IL-6 and there was no difference in IL-6 between non-frail and pre-frail women with knee OA. However, Livshits et al. (Livshits, Zhai et al. 2009) reported that serum IL-6 predicted the incidence of radiographic knee OA assessed by the KL scoring system in a prospective population-based study of healthy, middle aged British women.

Our study found no relationship between radiographic and symptomatic OA severity and CRP. C reactive protein is a sensitive, acute phase protein that responds to inflammation and infection (Pepys and Hirschfield 2003). In a systematic review, Jin et al (Jin, Beguerie et al. 2015) reported that the pooled odds ratio showed no significant predictive value of CRP level in the progression of OA (OR=0.99, 95% CI 0.81 to 1.21, p=0.93) (Sharif, Shepstone et al. 2000, Mazières, Garnero et al. 2006, Engström, de Verdier et al. 2009). However, Stannus et al (Stannus, Jones et al. 2013) found that baseline high sensitivity CRP was positively associated with change in total knee pain (β=0.33 per mg/l, p=0.03) in 149 randomly selected subjects. The reason for inconsistencies between

radiographic and symptomatic knee OA and inflammatory cytokines are unclear but may be due to sample size, multiple methods of assessing radiographic knee OA for example KL scoring/ OARSI scoring or inaccurate self-report of symptomatic knee OA symptoms. As a whole, though, it appears that the impact of inflammatory cytokines on knee OA severity may not be large.

Knee Osteoarthritis and Frailty

While previous cross-sectional and longitudinal work in epidemiological studies show that frailty is more prevalent among people with knee OA, the current findings showed that the severity of knee OA, either radiographically or symptomatically, was not different between women who were non-frail versus women who were pre-frail. Recent cross-sectional and longitudinal analyses show that frailty occurs more commonly among people with knee OA than those without knee OA (Castell, van der Pas et al. 2015, Misra, Felson et al. 2015). In the Multicenter Osteoarthritis Study (n=3,026; aged 50-79 years) and Osteoarthritis Initiative (n=4,796; aged 45-79 years), cross-sectionally frailty was more prevalent among patients with radiographic knee OA (4.39% vs. 2.77%; PR 1.60 [1.07, 2.39]) and symptomatic knee OA (5.88% vs. 2.79%; PR 1.92 [1.35, 2.74]) compared to those without (Misra, Felson et al. 2015). Longitudinally, risk of developing frailty was greater among those with radiographic knee OA (4.73% vs. 2.50% PR 1.45 [0.91, 2.30]) and symptomatic knee OA (6.30% vs. 2.83%; RR 1.66 [1.11, 2.48]) (Misra, Felson et al. 2015). It is likely that the small sample size in the current study limited the ability to identify a difference in knee OA severity between frailty groups. Further, women that participated in the study were younger than previous frailty studies. It is

likely that the presence of the disease is sufficient to promote frailty, independent of the level of severity experienced by the individual with this disease. Nonetheless, a larger sample may be useful to confirm these results.

Inflammation and Frailty

We found no difference in TNF α between non-frail and pre-frail women with knee OA; although, previous evidence for TNF α is mixed. Increased levels of TNF α is associated with decreased muscle strength and poor physical function (De Martinis, Franceschi et al. 2006). Lai et al (Lai, Chang et al. 2014) found that higher serum levels of TNF α was not associated with frailty in 386 older institutionalized men. However, Hubbard et al (Hubbard, O'Mahony et al. 2009) found that the frailty index was related to log-transformed TNF α ($r=0.38$, $p<0.01$) in 110 patients.

Unfortunately, we did not find a difference in IL-6 between non-frail and pre-frail women with knee OA. A cohort study the physical frailty phenotype was not associated with IL-6 serum levels in frail patients compared to non-frail patients (0.56 ± 10.8 vs. 0.46 ± 5.64 pg/L). However, Leng et al reported that in a population-based case-control study 11 frail subjects have significantly higher serum IL-6 levels than the 19 non-frail subjects (4.4 ± 2.9 vs. 2.8 ± 1.6 pg/mL). The inconsistency in the literature suggests that the impact of these systemic cytokines may not be large in women with knee OA.

Similarly, our study did not find a difference in CRP between non-frail and pre-frail women with knee OA. Previous studies have shown that CRP serum levels increase in

response to acute or chronic illness (Ershler and Keller 2000). C reactive protein may increase inflammation (Walston, McBurnie et al. 2002). Lai et al (Lai, Chang et al. 2014) found that there was no relationship between CRP and frailty in 386 older institutionalized men. However, Walston et al (Walston, McBurnie et al. 2002) found that levels of serum CRP was greater in frail compared to non-frail patients (5.5 ± 9.8 vs. 2.7 ± 4.0 mg/L) in 4735 community dwelling older adults 65 years and older. In the current study, it is likely due to the small sample size that we did not see a difference in inflammatory cytokines between frailty groups. Also, our sample did not include any frail women with knee OA; perhaps with a greater diversity in frailty severity, we would be able to see a difference between inflammatory cytokines. We also looked at frailty in younger older adults; many frailty studies have assessed frailty in adults over the age of 70 years. With this in mind, perhaps future studies should focus on assessing inflammatory cytokines in older adults.

Characteristics of the Pre-frail Participants

It is important to note that while our key measure were not different between the non-frail and pre-frail women in this sample, significant differences were noted between the groups in depressive symptoms and walking performance. This finding provides some confidence that the distinction between non-frail and pre-frail groups in this sample were meaningful. The mean score for the depressive symptoms reached the threshold for clinical depression in the pre-frail group. This finding may point to a role for depressive symptoms in the pathology underlying the development of frailty among women with symptomatic knee OA. Further, in this sample, women in the pre-frail group also

demonstrated significantly worse walking performance than those who were non-frail. Impaired walking performance is a common consequence of knee OA. It is possible that this walking impairment, and the likely physical inactivity that results, could increase the likelihood for the development of frailty. It is also interesting to note that while the pre-frail showed slower walking speeds, this reduction in speed was not sufficient to identify an element of frailty on the Fried Frailty Phenotype. This element of the tool may not be sensitive to pre-frailty perhaps due to strict criteria; or due to the length of the walk required to complete the Fried. Further research is necessary to explore whether depressive symptoms, and/or walking impairment, are important in the pathway from chronic joint disease to frailty among older women.

Strengths and Limitations

Our study also had several strengths. Firstly, we used a validated measurement of frailty. The Fried Frailty Phenotype is a validated measurement of frailty that can be used both clinically and in research. Secondly, the assessment of knee OA included both radiographic and symptomatic scoring. Radiographs are commonly used to diagnose knee OA; combinations of symptomatic (pain) and radiographic (KL) scores are often used to characterize knee OA (Li, Wei et al. 2013). Previous studies have shown that patients with radiographic signs of knee OA (joint space narrowing and osteophyte formation) are often asymptomatic (Li, Wei et al. 2013).

We acknowledge that our study has limitations. Firstly, the sample size is small. It is possible that a greater number of study participants may have improved power to note group differences and relationships between variables. However, given the

inconsistencies in the literature, increasing sample size may not show a relationship between frailty and inflammatory cytokines in women with knee OA. Secondly, individuals that participated in the study were also enrolled in a larger randomized controlled trail of exercise; thus the sample is likely biased towards individuals who are willing to engage in a regular exercise program. Thirdly, the participants were younger women and therefore, may not be able to be generalized to older adults with knee OA or older adults without knee OA. Lastly, inflammatory cytokine samples taken in this study were obtained from systemic serum therefore; do not directly reflect knee tissue measures only.

Conclusion

In this cross-sectional study, we found that nearly half of the women with knee OA showed signs of pre-frailty despite a relatively young age. There was a significant negative relationship between symptomatic pain severity and TNF α; however, no difference found with any other inflammatory cytokines. There was also no difference found in inflammatory cytokines between non-frail and pre-frail women with knee OA. There was no difference in radiographic or symptomatic knee OA severity between non-frail and pre-frail women with knee OA. There was no relationship between radiographic knee OA and inflammatory cytokines. We recognize that firm conclusions cannot be drawn from this data given the small number of participants. Nevertheless, these preliminary results provide further rationale for further studies looking for potential mechanisms between knee OA and frailty. Future work should aim at increasing sample size in older adults, rather than middle-aged adults in order to perhaps see a relationship between knee OA and frailty.

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Tables

Table 2-1. The Fried Frailty Phenotype Scoring.

			Score _____/5
Frailty Domain	Item	0 point	1 point
Weight Loss	Have you lost more than 10lbs unintentionally?	No	Yes
Exhaustion	(a) I felt that everything I did was an effort; (b) I could not get going How often in the last week did you feel this way?	"Rarely or none of the time (1 day)" "Some or a little of the time (2 days)"	"Moderate amount of the time (3–4 days)" "Most of the time (5+days)"
Physical Activity	Minnesota Physical Activity Questionnaire	Men ≥ 383 kcal Women ≥ 270 kcal	Men < 383 kcal Women < 270 kcal
Grip Strength	Calculated using a JAMAR dynamometer and taking the average of 3 trials	Men BMI ≤24 ≤29 kg BMI 24.1–26 ≤30 kg BMI 26.1–28 ≤30 kg BMI >28 ≤32 kg Women BMI ≤ 23 ≤17 kg BMI 23.1–26 ≤17.3 kg BMI 26.1–29 ≤18 kg BMI >29 ≤21 kg	
Gait Speed	15 Foot Walk	Men Height ≤ 173 cm ≥ 7 sec Height >173 cm ≥ 6 sec Women Height ≤ 159 cm ≥ 7 sec Height > 159 cm ≥ 6 sec	
Details on scoring the Fried Frailty Phenotype can be found in Fried LP et al. (Fried, Tangen et al. 2001) Weight loss: self-report measure Exhaustion: self-report measure The Minnesota Physical Activity Questionnaire: a weighted score of kilocalories expended per week Grip strength: is categorized by gender and body mass index Gait speed: is categorized by sex and height			

Table 2-2. Frequency distribution for each item of the Fried Frailty Phenotype.

Frequency distribution for each item of the Fried Frailty Phenotype (n=20)		
	Non-frail (n,%)	Pre-Frail (n,%)
Self-reported weight loss	19, 95%	1, 5%
Self-reported exhaustion	17, 85%	3, 15%
Self-reported physical activity	20, 100%	0, 0%
Grip strength	15, 75%	5, 25%
Walking speed	20, 100%	0, 0%

Table 2-3. Characteristics of Study Sample According to Frailty Status.

Characteristic	Total, n=20	Non-frail, n=11	Pre-Frail, n=9
Sociodemographic			
Age, years, mean ± SD	66.1 ± 9.6	62.6 ± 9.2	70.3 ± 8.8
Use of medication, mean ± SD			
Medications, number, *mean ± SD*	1.5 ± 1.6	1.5 ± 1.8	1.4 ± 1.4
Lifestyle Characteristics			
Body mass index, kg/m^2, mean ± SD	29.7 ± 4.9	28.5 ± 5.5	31.2 ± 4.0
Depression			
CES-D Depression Scale sum score, mean ± SD	10.1 ± 6.3	6.5 ± 3.4	14.4 ± 6.3
Frailty			
Handgrip strength, kg, mean ± SD	23.2 ± 6.9	26.5 ± 4.8	19 ± 6.9
Gait speed, seconds, mean ± SD[a]	3.8 ± 0.7	3.5 ± 0.6	4.1 ± 0.7
Mobility Performance Measures			
Chair stand, total number, mean ± SD	10.0 ± 2.8	10.0 ± 2.8	8.8 ± 2.4
Six Minute Walk Distance, meters, mean ± SD	460.7 ± 64.3	493.9 ± 47.3	420.1 ± 60.6
Pain Measurements			
KOOS pain, total score, mean ± SD	54.8 ± 15.4	59.2 ± 8.9	49.4 ± 20.1
KOOS symptoms, total score, mean ± SD	50.7 ± 15.0	48.1 ± 13.7	53.9 ± 16.7
Inflammatory Cytokines			
Log transformed Interleukin-6, mean ± SD	0.6 ± 0.5	0.6 ± 0.5	0.5 ± 0.5
Log transformed Interleukin-10, mean ± SD	1.4 ± 1.6	1.3 ± 1.4	1.6 ± 1.8
Log transformed Tumor Necrosis Factor α, mean ± SD	2.1 ± 0.3	2.0 ± 0.4	2.2 ± 0.2
Log transformed C Reactive Protein, mean ± SD	3.4 ± 0.9	3.2 ± 0.9	3.6 ± 0.8
White Blood Cells			
Neutrophils, mL, mean ± SD	4805000 ± 3688314	4830000 ± 3948291	4755556 ± 3842236
Monocytes, mL, mean ± SD	290475 ± 184877.5	315900 ± 184412.2	244277.8 ± 190912.6
T cells, mL, mean ± SD	1745304 ±1257294	1702447 ±1053940	1631289 ± 1491071
B cells, mL, mean ± SD	182464 ± 126822.4	218841 ± 119434.4	136430 ± 133395.3

a. gait speed, seconds (n=19), 10 participants = non-frail, 9 participants = pre-frail due to operation error

Abbreviations: Standard Deviation, SD; Center for Epidemiologic Studies Depression Scale, CES-D; Knee Injury and Osteoarthritis Outcome Score, KOOS

Table 2-4. Differences between symptomatic knee OA severity in pre-frail vs. non-frail women.

Differences between symptomatic knee OA severity in pre-frail vs. non-frail women (n=20)			
	Non-frail (n=11) Mean ± SD	Pre-Frail (n=9) Mean ± SD	P value
Pain [a]	41.64 ± 17.31	32.22 ± 18.05	0.25
Symptoms [a]	48.09 ± 13.67	53.89 ± 16.71	0.42
ADL [a]	69.73 ± 15.01	54.33 ±18.39	0.61
	Non-frail (n=11) Median, IQR (Range)	Pre-Frail (n=9) Median, IQR (Range)	z, prob >z
Sport [b]	50, 55 (15-70)	25, 60 (15-75)	1.386, 0.166
QOL [b]	31, 43 (13-56)	25, 56 (19-75)	0.231, 0.817

[a] Data was normally distributed and therefore, differences were assessed using a t-test
[b] Data was not normally distributed and therefore, differences were assessed using a Mann-Whitney U test
Abbreviations: Standard Deviation, SD; Interquartile Range, IQR; Activities of Daily Living, ADL; Quality of Life, QOL.

Table 2-5. Relationship between symptomatic knee OA severity and inflammatory cytokines.

Relationship between symptomatic knee OA severity and inflammatory cytokines in women assessed using logistic regression (n=20)												
	Log-transformed IL-6			Log-transformed IL-10			Log-transformed TNF α			Log-transformed CRP		
	β	p value	R^2	β	p value	R^2	β	P value	R^2	β	p value	R^2
Pain	-0.006	0.457	0.311	-0.019	0.450	0.032	**-0.010**	**(0.022)**	**0.260**	-0.001	(0.998)	0.001
Symptoms	-0.002	0.759	0.004	-0.029	0.237	0.077	0.002	(0.583)	0.017	0.023	(0.103)	0.158
ADL	-0.001	0. 887	0.001	0.008	0.721	0.007	-0.005	(0.165)	0.104	0.002	(0.906)	0.001
Sport	0.003	0.692	0.009	-0.009	0.680	0.010	-0.001	(0.835)	0.003	0.008	(0.529)	0.025
QOL	-0.011	0.148	0.112	-0.010	0.681	0.010	-0.007	(0.087)	0.154	0.023	(0.062)	0.202
Abbreviations: Interleukin-6, IL-6; Interleukin-10, IL-10; Tumor Necrosis Factor α, TNF α; C Reactive Protein, CRP; Activities of Daily Living, ADL; Quality of Life, QOL *Note:* Symptomatic knee OA was measured using the Knee Injury and Osteoarthritis Outcome Score; normalized scores of 100 indicate no symptoms and normalized score of 0 indicate extreme symptoms												

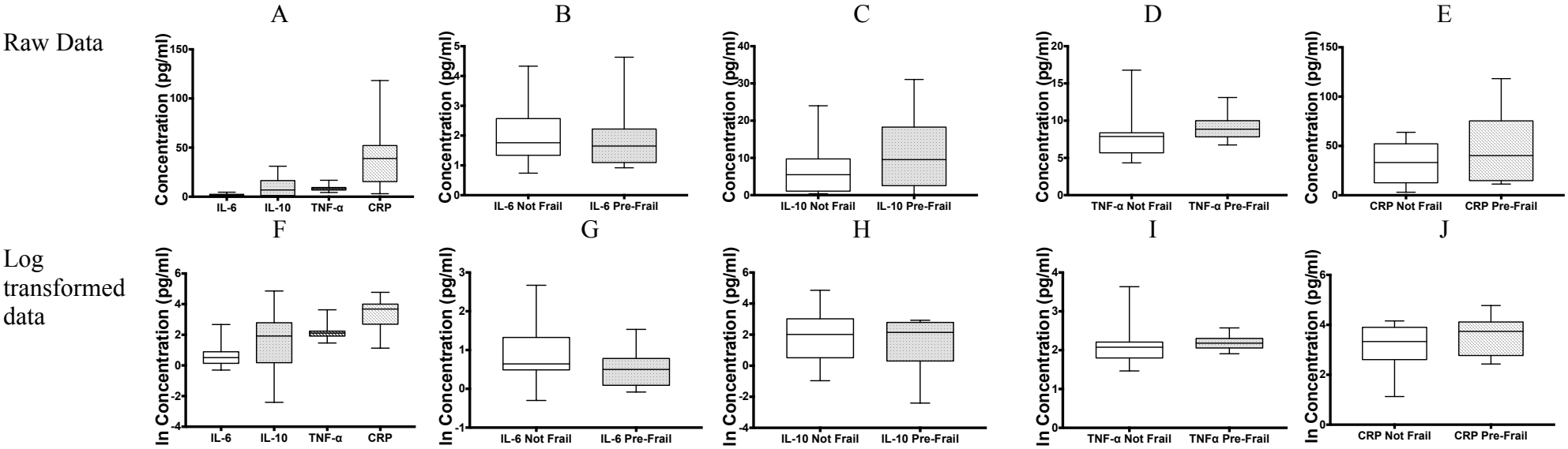
Table 2-6. Differences between inflammatory cytokines in pre-frail vs. non-frail women.

Differences between inflammatory cytokines in pre-frail vs. non-frail women assessed using the Mann-Whitney U test (n=20)			
	Non-frail (n=11) Median, IQR (Range)	Pre-Frail (n=9) Median, IQR (Range)	z, prob >z
Log-transformed IL-6	0.62, 0.84 (0.49-1.32)	0.50, 0.50 (-0.08- 1.53)	0.49, 0.62
Log-transformed IL-10	1.81, 2.20 (0.77-4.86)	2.25, 1.46 (-2.41, 3.44)	-0.80, 0.43
Log-transformed TNF	2.21, 0.41 (1.47-3.63)	2.18, 0.18 (1.91-2.57)	-1.86, 0.06
Log-transformed CRP	3.33, 1.29 (1.13-4.16)	3.74, 1.34 (2.43-4.11)	-0.77, 0.44
Abbreviations: Interleukin-6, IL-6; Interleukin-10, IL-10; Tumor Necrosis Factor α, TNF α; C Reactive Protein, CRP			

Figures

Figure 2-1. Raw Data and log-transformed Inflammatory Cytokines

Raw Data and log-transformed Inflammatory Cytokines (interleukin-6, interluekin-10, tumor necrosis factor α and C reactive protein) between Pre-Frail and Non-frail women with knee osteoarthritis categorized by the Fried Frailty Phenotype



CHAPTER 3
DISCUSSION

Overview

Nearly half of the women with knee OA who participated in this study showed signs of pre-frailty, despite a relatively young age. These findings suggest that it may be useful to consider screening these women for pre-frailty to initiate interventions that could slow, halt, or reverse the process of frailty early. We also found that there was a significant negative relationship between pain severity and TNF α. It is likely that TNF α plays a role in the pain experience of women with symptomatic knee OA.

Why is this study important?

In this study that incorporated markers of frailty, we chose to look at relatively younger older adults (66.1 years ± 9.6 years; mean age ± SD). These younger older adults have fewer chronic conditions compared to older adults. The Canadian Institute for Health Information found that 74% of adults 65 years of age or older reported having at least one of eleven chronic health conditions including; arthritis, asthma, cancer, chronic pain, depression, diabetes, emphysema or chronic obstructive pulmonary disease, heart disease, high blood pressure, a mood disorder other than depression and stroke (Information January 2011). By comparison, this percentage is substantially lower in adults aged 45-65 years and older, at 48% (Information January 2011). These statistics clearly show that adults between 45-65 years of age are healthier than their older counterparts. Nevertheless, data from the current study suggest that pre-frailty was common in women with knee OA in this younger age group.

Pre-frail individuals are more likely than non-frail persons to develop the full frailty syndrome (Fried, Tangen et al. 2001). Pre-frail individuals are at increased risk of falls, institutionalization and mortality however, their risk is not as high as in frail elderly persons (Fried, Tangen et al. 2001). It may be easier to manage or even reduce these risks at the pre-frail stage frailty (Ahmed, Mandel et al. 2007); however it was unclear if pre-frailty could be detected in a relatively younger age group. We decided to explore pre-frailty in women with painful knees starting at age 45 to directly address this gap. Women with symptomatic knee OA may be an ideal target for early intervention to minimize frailty and its consequences.

Previous frailty literature has consistently advocated that frailty screening should be implemented in persons 70 years and older (Morley, Vellas et al. 2013). There are effective treatments available and simple screening tests that have been developed to produce more beneficial than harmful outcomes for frail individuals (Theou and Rockwood 2012). Effective treatments include a combination of exercise (aerobic and resistance), caloric and protein support, vitamin D supplementation and reduction of polypharmacy (Morley, Vellas et al. 2013). For example Theou and colleagues completed a systematic review showing that multicomponent training 45 to 60 minutes of exercise 3 times a week that lasted at least 5 months improved function in frail older adults (Theou, Stathokostas et al. 2011). Exercise in frail individuals increases walking speed, chair stands, stair climbing, balance, and reduces depression and fear of falling (Morley, Vellas et al. 2013). Data from the current study suggest that, among women with chronic knee pain, it is likely useful to approach earlier identification of pre-frailty, to provide an opportunity to implement these effective treatment strategies. It would be interesting

to see whether exercise, nutrition and pharmaceutical support offered in relatively young older women could prevent the onset of frailty.

Knee osteoarthritis (OA) and frailty are two conditions that are associated with functional limitation and disability and are commonly seen in geriatric patients. Two previous studies conducted cross-sectional and longitudinal explorations of frailty among men and women 60 years and older with knee OA (Misra, Felson et al. 2015). Both were large epidemiological studies designed to study risk factors in knee OA: The Multicenter Osteoarthritis Study (MOST) (n=3,026) (Misra, Felson et al. 2015) and the Osteoarthritis Initiative (OI) (n=4,796) (Misra, Felson et al. 2015). In a cross-sectional analysis Mirsa and colleagues found that frailty was more prevalent among patients with radiographic knee OA (4.39% vs. 2.77%; prevalence ratio 1.60 [1.07, 2.39]) and symptomatic knee OA (5.88% vs. 2.79%; prevalence ratio 1.92 [1.35, 2.74]) compared to those without any evidence of knee OA (Misra, Felson et al. 2015). In a longitudinal analysis, risk of developing frailty (over a 60 months for MOST study and 48 months for OAI time period) was greater among individuals with radiographic knee OA (4.73% vs 2.50%; relative risk 1.45 [0.91, 2.30]) and symptomatic knee OA (6.30% vs 2.83%; relative risk 1.66 [1.11, 2.48]) than those without any knee OA, respectively (Misra, Felson et al. 2015). Frailty was assessed using the Study of Osteoporotic Fracture (SOF) (Ensrud's) index, a modified version of the Fried Frailty Phenotype. Presence of ≥2 out of three of the following criteria: (i) weight loss >5% between baseline and the subsequent follow-up visit; (ii) inability to rise from chair five times without using support (hereafter referred to as difficulty with chair stands); and (iii) poor energy derived from the SF12 questionnaire response of "little at a time" or "none at a time" to the question "in the past 4 weeks, did you have a lot of energy?".

The current study expands on the findings from the MOST and OAI cohorts. In our study, the mean age was similar in both our study and the combined MOST and OAI cohorts. We selected different measurements in the current study compared to the MOST and OAI studies of frailty. In our study, symptom severity was measured using the pain subscale of the Knee Injury and Osteoarthritis Outcomes Score (KOOS); whereas the MOST and OAI cohorts used radiographic knee OA in at least one knee plus pain in the same knee. The underlying mechanisms that could be tying the relationship between frailty and knee OA is unclear. It has been hypothesized that the underlying mechanism may be the presence of inflammatory cytokines. Filling this gap is important to better understand the potential role that inflammation has in both frailty and knee OA. Identifying potential mechanisms could provide the possibility to develop novel strategies to identify, prevent and manage both conditions. However our study did not include men. Women were chosen as they have a higher prevalence of both knee OA (Srikanth, Fryer et al. 2005) and frailty (Morley, Vellas et al. 2013) compared to men. Women tend to have more risk factors for knee OA including a higher prevalence of obesity, poor knee alignment and decreased muscle strength (Neogi and Zhang 2013).

Inflammatory Cytokines

To our knowledge, this is the first study to examine a potential mechanism linking knee OA and frailty. This information is essential to advancing early detection; inflammatory cytokines might be an early marker of frailty in women with knee OA. Systemic inflammation has been associated with frailty (Leng, Xue et al. 2007, Li, Manwani et al. 2014) and knee OA (Botha-Scheepers, Watt et al. 2008, Livshits, Zhai et al. 2009, Aguiar, Do Nascimento et al. 2015) but in

the current study, inflammatory cytokines appeared unrelated to the severity of knee OA from either the radiographic or symptomatic severity. Unfortunately, there were large variances in cytokines in the present sample; this emphasizes the importance of increasing sample size when measuring inflammatory cytokines. Previous studies have shown that elevated inflammatory cytokines, specifically IL-6 (Livshits, Zhai et al. 2009), TNF α (Botha-Scheepers, Watt et al. 2008) and CRP (Stannus, Jones et al. 2013), are present in knee OA; however, there have also been studies that have showed no relationship between inflammatory cytokines and knee OA (Santos, Gomes et al. 2011, Samut, Dinçer et al. 2015). There are also inconsistencies with inflammatory cytokines and frailty. Previous studies have shown that increase in inflammatory cytokines with frailty specifically IL-6 (Darvin, Randolph et al. 2013), TNF α (Hubbard, O'Mahony et al. 2009) and CRP (Collerton, Martin-Ruiz et al. 2012); however, a previous study showed no relationship between inflammatory cytokines and frailty (Arts, Collard et al. 2015). These inconsistencies may reflect the challenges of measuring systemic inflammation when exploring specific health conditions. This general inflammatory state may represent frailty as frailty can be considered to be heterogeneous. However, not understanding where the inflammation is coming from does not allow us to create a targeted intervention. Also, there may be other influences of systemic inflammation such as illness. The first line of defense essential for common bacterial infections is the increase of macrophages, neutrophils, chemokines and cytokines throughout the body. When measuring systemic inflammation we are unable to measure the direct source of inflammation. For example, in our study one participant demonstrated extremely high levels of serum inflammatory cytokines and neutrophils, which was likely attributed to an upper respiratory infection (reported on a descriptive questionnaire).

Therefore, systemic inflammation reflects a wide variety of influences, ranging from OA, aging and frailty, to upper respiratory infections.

In our study, systemic inflammation was measured using the enzyme-linked immunoassay (ELISA) (Milliplex map kit, EMD Millipore Corporation). The Milliplex map kit, EMD Millipore Corporation is considered to be one of the most sensitive map kits available for research purposes (Breen, Reynolds et al. 2011). However, results vary based on the kits chosen; therefore, it may only be compared with other studies that have also used to same map kits to measure inflammatory cytokines therefore, making it less generalizable.

Lastly, due to feasibility, serum collection was somewhat inconsistent between participants. This study was secondary analysis of baseline data of a randomized controlled, cross-sectional study. Participants were randomized into three groups; yoga, traditional exercise and meditation. In some cases, difficulty attaining a blood sample during the baseline data collection meant that we obtained blood samples before, during and after exercise. Prescribing 300 minutes/week of moderate-to-vigorous aerobic exercise did not improve inflammatory cytokines in women (Friedenreich, O'Reilly et al. 2015).

Frailty

Since multiple frailty measurements have been developed based on different models of frailty, there is no gold standard measurement of frailty. The physical phenotype of frailty, the Fried Frailty Phenotype, is a biological syndrome that results from cumulative decline of multiple physiological systems (Buckinx, Rolland et al. 2015). However, the Fried Frailty Phenotype

does not provide information about the underlying causes of the condition (Cesari, Gambassi et al. 2014). For this study, the Fried Frailty Phenotype was chosen because it focuses on physical frailty. The women that participated in the current study were younger than previous frailty studies that recruited individuals 70 years and older (Ferrucci, Guralnik et al. 2004). As well, that with having knee OA, they would have physical limitations that may in turn cause them to show physical frailty indicators.

Frailty is described as a state of increased vulnerability. The prevalence of frailty depends on the operational definition used to define the geriatric condition (Rodríguez-Mañas, Féart et al. 2013). Frailty has been used interchangeably with multimorbidity and disability. Previous research has shown that frailty and multimorbidity are independent risk factors for disability (Rodríguez-Mañas, Féart et al. 2013). Heterogeneity within the frail population should not be ignored (Aarts, Patel et al. 2015). Overall, heterogeneity makes it difficult to understand if frailty is caused by disease burden, disability or if it is related to vulnerability (Aarts, Patel et al. 2015).

Knee Osteoarthritis

Multiple measurements are currently used to measure knee OA including radiographic and symptomatic diagnostic criteria. Unfortunately, previous literature shows a weak correlation between both measurements (Hannan, Felson et al. 2000, Muraki, Oka et al. 2009, Neogi, Felson et al. 2009).

Radiographic Knee Osteoarthritis Severity

Radiographic knee OA severity is commonly measured using the Kellgren and Lawrence (KL) grading score (Kellegren and Lawrence 1957, Braun and Gold 2012). The KL grading score is

considered to be the gold standard diagnostic test for knee OA (Bauer, Hunter et al. 2006). However, the KL grading score has been criticized. A major criticism is the emphasis placed on osteophytes and the lack of emphasis placed on joint space narrowing in the diagnosis knee OA (Kijowski, Blankenbaker et al. 2006). The first two grades of the KL scale are defined exclusively by the presence of osteophytes therefore, patients with joint space narrowing but no osteophyte formation cannot be diagnosed with knee OA according to the KL grading system (Kijowski, Blankenbaker et al. 2006).

Symptomatic Knee Osteoarthritis Severity

Symptoms of OA of the knee may include pain, stiffness, swelling, crepitus and decreased mobility. Measurements often used to assess symptoms of pain include self-report questionnaires such as the Knee Osteoarthritis Outcome Scale (KOOS). However, previous literature has shown that there is a weak correlation between radiographic and symptomatic measurements of knee OA (Hannan, Felson et al. 2000, Muraki, Oka et al. 2009, Neogi, Felson et al. 2009). It can be argued that symptomatic knee OA measures such as pain are more important than radiographic measures. Individuals may have severe pain and no osteophyte formation or joint space narrowing. Ultimately it is pain, not signs of joint destruction, which creates a burden on the individual during the daily activity, and on healthcare utilization. It can also be argued that individuals with pain are those that benefit most from an intervention.

Our study has confirmed the importance of inflammatory cytokines, specifically TNF α and self-reported pain in the younger old women with symptomatic knee OA. TNF α is a key regulator of an inflammatory response including cell death promotion. Higher levels of TNF α have been

associated with change in total knee pain (β=0.66 ml/pg, p=0.02). However, the cause of joint pain in knee OA is not well understood. It has also been shown that individuals may not present any radiographic signs of knee OA however, experience a lot of pain and reduced physical function (Li, Wei et al. 2013). Unfortunately, cross-sectional relationships of other inflammatory cytokines with KOOS subscales were not found.

New Insights

Although we did not find significant findings between frailty and knee OA, frailty and inflammatory cytokines and inflammatory cytokines and knee OA, we did find significant differences between groups when looking at depression and walking speed measured by the 6 minute walk test. These data suggest that depression and walking speed may be two measurements that can be used to screen for frailty in younger older women with knee OA. Therefore, diagnosis of both frailty and depression early and understanding the risks of both syndromes can help clinicians care for patients more confidently and decrease risk of adverse health outcomes. Health promotion provided to older adults suffering from depressive symptoms may help reduce risk of depression or frailty, respectively (Markle-Reid, Weir et al. 2006). Future studies should also explore the relationship between social vulnerability and depression. Social vulnerability may perhaps lead to depression or loss of mobility due to social isolation.

Implications of Findings

The results of this study are important, as we have learned that despite a relatively young age, women with knee OA showed signs of pre-frailty. This could promote the use of a diagnosis of

knee OA among women as a time point to "catch" and potentially stop or reverse pre-frailty. By identifying and treating pre-frailty early, we are able to implement a conservative intervention with low risk of potential harm, such as exercise and nutritional support. This study helps to fill the gap in understanding if systemic inflammation is a shared underlying mechanism affecting both frailty and knee OA. Ultimately, advancing the early identification of frailty as comorbidity in knee OA, and examining systemic inflammation as a potential underlying cause of both conditions has great potential to improve QOL in younger adults without the complications of potential comorbidities.

Limitations

We acknowledge that our study has limitations. Firstly, the sample size is small. It is possible that a greater number of study participants may have improved power to see differences and relationships between groups. Secondly, women who participated in the study were also enrolled in a larger randomized controlled trail of exercise thus the sample is likely biased toward individuals who are willing and able to engage in a regular exercise program and may not be generalizable to a broader population. Thirdly, the participants were younger women and therefore, may not be able to be generalized to older adults with knee OA or older adults without knee OA. Lastly, inflammatory cytokine samples taken in this study were obtained from systemic serum therefore; do not directly reflect knee tissue measures only.

Future Directions

Future studies should use a larger sample to explore the relationships between; (1) depressive symptoms, (2) walking impairment, and (3) inflammatory cytokines with frailty among older women with knee OA.

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APPENDIX

Consort Diagram

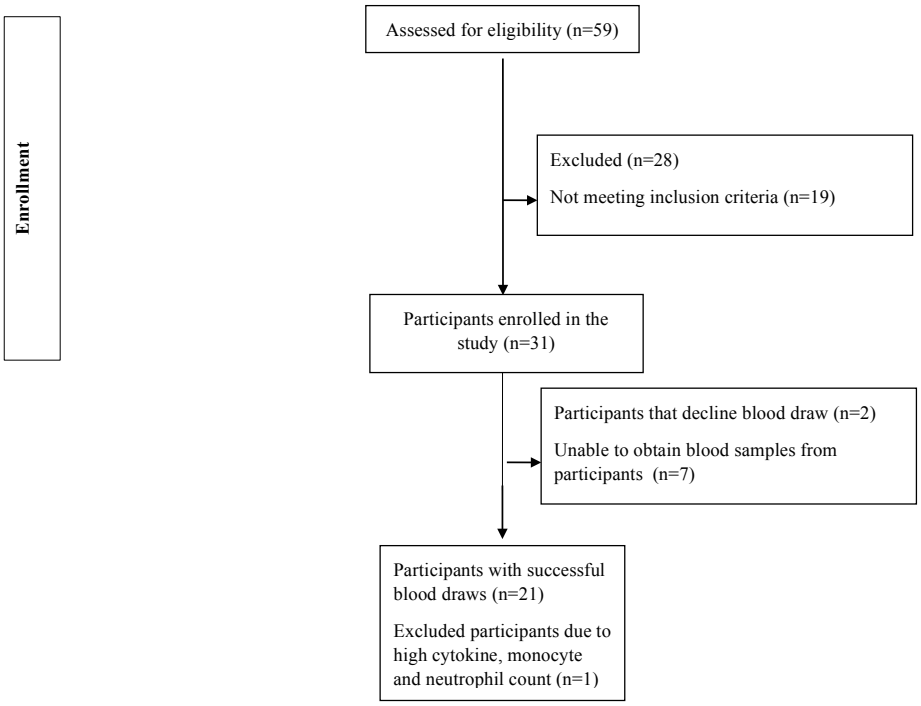

Figure 1. CONSORT (Consolidated Standards of Reporting Trials) diagram of participant flow throughout recruitment and blood serum data collection.

Fried Frailty Scale

Please place a check mark beside the best answer for you.

1. **Weight Loss**
   "In the last year, have you lost more than 10 pounds unintentionally?"

   - ☐ Yes; why _____
   - ☐ No

2. **Exhaustion**
   How often in the last week did you feel this way? "I have felt that everything I did was an effort and I could not get going"

   - ☐ 0 = Rarely or none of the time (<1 day)
   - ☐ 1 = Some of little of the time (1-2 days)
   - ☐ 2 = Moderate amount of the time (3-4 days) (Frail)
   - ☐ 3 = Most of the time (Frail)

3. Physical Activity
Do you perform the following activities?

ACTIVITY	Did you perform this activity? (check mark) NO	YES	Number of Times per Week	Duration of Time on Each Occasion hrs	min
Walking	☐	☐			
Home Chores	☐	☐			
Mowing the lawn	☐	☐			
Raking	☐	☐			
Gardening	☐	☐			
Hiking	☐	☐			
Jogging	☐	☐			
Biking	☐	☐			
Exercise cycling	☐	☐			
Dancing	☐	☐			
Aerobics	☐	☐			
Bowling	☐	☐			
Golf	☐	☐			
Singles Tennis	☐	☐			
Doubles Tennis	☐	☐			
Racquetball	☐	☐			
Calisthenics (e.g., push-ups, pull-ups, Jumping jacks)	☐	☐			
Swimming	☐	☐			

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Please pass the questionnaire back to the research assistant; they will take you through the next two assessments.

4. Grip Strength

Right Hand	Left Hand
kg	kg
kg	kg
kg	kg
Average:	Average:

5. Gait Speed (15 foot walk)

Time: _____sec

Infections Questionnaire

The following questions will ask you about cold or flu symptoms, vaccination history and contact with others in the PAST 2 WEEKS. Please answer ALL questions.

Have you had any cold or flu symptoms? ☐ No ☐ Yes
If yes, please list your symptoms below:
1.
2.
3.
4.
5.

If yes, please answer question below

For these cold and flu symptoms listed above have you
☐ Not received medical attention, self-care ☐ Visited a doctor
☐ Been hospitalized ≤1 day ☐ Been hospitalized >1 day

Select vaccination history
Have you ever been vaccinated against Influenza?
(e.g. Annual Influenza vaccine)

☐ Yes ☐ No ☐ Unsure

Have you ever been vaccinated against *Streptococcus pneumoniae*?
(e.g. pneumococcal polysaccharide vaccine, 23-valent polysaccharide vaccine)

☐ Yes ☐ No ☐ Unsure

Contact with others
Do you live with others? ☐ No ☐ Yes

Do you have contact with children? ☐ No ☐ Yes
If **yes,** list ages of children: _____

Do you have caregiving responsibilities? ☐ No ☐ Yes

The following questions are to be completed by an investigator. Please hand this questionnaire back to investigator. Thank you for completing this questionnaire.

Knee Injury & Osteoarthritis Outcome Score

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.

A Measure of Intermittent and Constant Osteoarthritis Pain, ICOAP: Knee Version

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 an "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/ No constant knee pain	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/ No constant knee pain	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/ No constant knee pain	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/ No constant knee pain	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/ No constant knee pain	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/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

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

0	1	2	3	4
Not at all/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

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/ No knee pain that comes and goes	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/ No knee pain that comes and goes	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/ No knee pain that comes and goes	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/ No knee pain that comes and goes	Mildly	Moderately	Severely	Extremely

THANK YOU!

Six Minute Walk Test

Instructions

"The object of this test is to walk as far as possible for 6 minutes. You will walk in a circle in this hallway for 6 minutes. Six minutes is a long time to walk, so you will be exerting yourself. You will probably get out of breath or become exhausted. You are permitted to slow down, to stop, and to rest as necessary. You may lean against the wall while resting, but resume walking as soon as you are able."

"Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but don't run or jog. I am also going to record your time at the 40m mark. Start now, or whenever you are ready."

5 minute remaining: "You are doing well. You have 5 minutes to go."

4 minutes remaining: "Keep up the good work. You have 4 minutes to go."

3 minutes remaining: "You are doing well. You are halfway done."

2 minutes remaining: "Keep up the good work. You have only 2 minutes left."

1 minute remaining: "You are doing well. You have only 1 minute to go."

15 seconds remaining: "In a moment I'm going to tell you to stop. When I do, just stop right where you are."

**If the patient stops walking during the test and needs a rest, do not stop the timer.

Scoring

Time in SECONDS covered in **15m**:	
Time in SECONDS covered in **40m**:	
Distance in METERS covered in **6 minutes**:	
BORG score upon completing:	

Timed Up and Go Test

Purpose: A "transition" test of ambulatory activity. A test incorporating multiple activity themes including a test of sit-to-stand activity, a test of walking short distances and a test of changing direction during walking, and the transitions between the activities. Also a test of strength, agility and dynamic balance.

Description: Time (seconds) taken to rise from a chair, walk 3 m, turn, walk back to the chair, then sit down at a regular pace wearing footwear and using a walking aid if required.

Equipment:

- A stopwatch
- Standard chair with arm rests: seat height approximately 44 cm and arm rest height approximately 65 cm.
- Tape or other marker on the floor 3 m away from the chair.

Instructions to Participant:

"For this test, do the best you can and walk at your regular pace.

1. *Start by sitting in the chair with you back resting on the back rest and your hands on the arm rest.*
2. *On start, stand up, walk to the mark, turn around, return and sit back into the chair with your back resting on the back of the chair.*
3. *Walk at your regular pace.*
4. *Get ready and START".*

Scoring:

2 trials are performed and the fastest trial is recorded to the nearest 10^{th} of a second.

Fastest trial time: | |

The Center for Epidemiologic Studies Depression Scale

Instructions for questions: Below is a list of the ways you might have felt or behaved. Please tell me how often you felt this way during the past week.

During the past week:	Rarely or none of the time (less than 1 day)	Some or a little of the time (1 – 2 days)	Occasionally or a moderate amount of time (3 – 4 days)	Most or all of the time (5 – 7 days)
1. I was bothered by things that usually don't bother me	0	1	2	3
2. I did not feel like eating; my appetite was poor	0	1	2	3
3. I felt that I could not shake off the blues even with help from my family or friends	0	1	2	3
4. I felt that I was just as good as other people	0	1	2	3
5. I had trouble keeping my mind on what I was doing	0	1	2	3
During the past week:	Rarely or none of the time (less than 1 day)	Some or a little of the time (1 – 2 days)	Occasionally or a moderate amount of time (3 – 4 days)	Most or all of the time (5 – 7 days)
6. I felt depressed	0	1	2	3

7. I felt that everything I did was an effort	0	1	2	3
8. I felt hopeful about the future	0	1	2	3
9. I thought my life had been a failure	0	1	2	3
10. I felt fearful	0	1	2	3
11. My sleep was restless	0	1	2	3
12. I was happy	0	1	2	3
13. I talked less than usual	0	1	2	3
14. I felt lonely	0	1	2	3
15. People were unfriendly	0	1	2	3
16. I had crying spells	0	1	2	3
17. I felt sad	0	1	2	3
18. I felt that people dislike me	0	1	2	3
19. I could not get “going”	0	1	2	3

Consent Form

Letter of Information and Consent

Clinical and Tissue Outcomes of a Biomechanical Exercise Program for Knee Osteoarthritis

Principal Investigator: Dr. Monica Maly (PT, PhD)
Associate Professor, Rehabilitation Sciences
McMaster University
(905) 525-9140 x 27823

Co-investigators: Dr. Jonathan Adachi (MD, FRCPC)
Dr. Dawn Bowdish (PhD)
Dr. Mike Noseworthy (PhD)
Prof. Paul Stratford (PT, MSc)
McMaster University

Student Investigators: Alexander Kuntz, MSc Candidate, Kinesiology
Sarah Karampatos, MSc Candidate, Rehab. Sciences
McMaster University
(905) 525-9140 x 20748

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Introduction

You are invited to participate in a randomized controlled trial that will compare 12 weeks of specialized exercise designed for an arthritic knee versus traditional routine exercise and no exercise on knee joint and muscle health, mobility and strength, and pain.

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. In deciding whether you wish to participate, you should understand enough about the 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

Osteoarthritis, the most common type of arthritis in Canada, affects one in ten Canadians. This condition is associated with pain, problems with mobility, and additional health complications including – but not limited to – chronic inactivity, heart disease, diabetes, and depression. Keeping individuals with knee osteoarthritis physically active is critically important. Exercise is effective at reducing pain while improving physical function. However, some exercises may overload the knee resulting in a worsening of knee pain and health of the knee joint. Yoga exercises focus on static postures that improve muscle strength and joint flexibility. Using sophisticated technology that allows us to calculate the loads inside the knee, we have designed a yoga exercise class that is ideal for people with knee osteoarthritis. **The purpose of the study is to investigate whether these yoga exercises improve muscle and joint health, mobility, and pain in comparison to traditional exercise and no exercise.**

To participate in this study, you need to be a woman that is 50 years of age or older, with knee osteoarthritis. We cannot include anyone who has the following:

- Medical restrictions to physical activity
- Fractures from osteoporosis
- Other forms of arthritis (e.g. psoriatic, rheumatoid)
- Active non-arthritic knee disease (e.g. gout, patellofemoral symptoms)
- Current use of intra-articular injections or knee surgery
- History of stroke or heart disease
- Allergy to medical tape
- Use of aids such as a cane
- Inability to safely climb 2 flights of stairs
- Leg injury in the last 3 months
- Hip or ankle problems
- Radiation (e.g. cancer treatment)
- Pregnancy
- Metal anywhere in the body. Examples of metal in the body include a pacemaker; heart valve; blood vessel shunt/stent; ear implant; metal or wire mesh implants; artificial joints; metal rods, pins or plates; bullets or shrapnel; tattoos involving metal in the ink; or body piercings.

Finally, to ensure that your knee has osteoarthritis instead of another joint condition that could be worsened by our exercise program, we ask that you complete two x-rays of your knee. The x-rays will be arranged and paid for by us. The x-rays will be completed at 25 Charlton Avenue East (across the street from St. Joseph's Hospital). This visit should be only 30 minutes in length. It is possible that after the x-rays, we discover you are not eligible to participate.

Procedure

If you meet the above criteria and are interested in participating, we welcome you to join our study! You will be asked to provide us with your phone number or email address so that we can contact you to answer any questions that may arise and to book an initial appointment. Also, we will ask you to provide us with the name and contact information for your family physician, and permission to contact your family physician if a health concern arises during your participation in the study.

Your involvement in the study will consist of attending supervised classes **at least 3 times weekly, for 12 weeks**, in your assigned study arm (yoga exercise program, traditional exercise program, and no exercise program). Your assigned study arm will be chosen randomly for you. Unfortunately we cannot let you choose which study arm you will participate in. If you are assigned to receive no exercise, you will be offered 3 months of a free membership to receive exercise if you complete the study.

We will ask you to attend 2 visits (one before and one after the 12 week intervention) at the MacMobilize lab at McMaster University. We may also ask you to attend 2 visits (one before and one after the 12 week intervention) at the Centre for Appendicular Magnetic Resonance Imaging (CAMRIS) at St. Joseph's Hospital, downtown Hamilton. There will be no cost for you to participate in the study. Parking at McMaster University, St. Joseph's Hospital, and at your instructed classes will be provided free of charge.

Measurements at the Start of the Study

- *MacMobilize Laboratory at McMaster University*
  1. Complete this form
  2. Body size measurements
  3. Resting heart rate and blood pressure
  4. Analyses of walking and yoga poses using laboratory equipment
  5. Mobility performance measurements
     a. 6-minute walk
     b. Stair climb
     c. 30-second chair stand
  6. Strength assessment
  7. Questionnaires
  8. Blood sample and nasal swab

  This visit will take approximately 2.5 hours to complete.

- *CAMRIS at St. Joseph's Hospital, downtown Hamilton*
  1. Questionnaire and MRI eligibility form
  2. MRI image of the thigh to examine muscle and fat content
  3. Second MRI scan of the knee joint to examine cartilage health

  This visit will take approximately 1 hour to complete.

Study Arms

Study Arm 1: New Exercise Program – De La Sol Yoga at Dundas and Hamilton Streets, Waterdown *(http://www.delasolyoga.com)*

- 12-weeks of yoga exercise classes. Each class is 1 hour.
- Weekly attendance at 3 of 4 available classes offered each week.
- Classes include a warm-up, series of standing postures with a focus on lower extremity strength and flexibility, cool-down.
- Commercial yoga studio with a certified instructor. Yoga mats and other equipment are available for your use. The studio also has bathroom/change room facilities including showers.

Study Arm 2: Traditional Exercise Program – Physical Activity Centre of Excellence (PACE), McMaster University, Hamilton

- 12-weeks of exercise classes. Each class is 1 hour.
- Weekly attendance at 3 of 4 available time slots offered each week.
- Supervised exercise including a warm-up, cardiovascular activities (i.e., walking or bicycling), balance activities, and strength training.
- All classes will be taught by a Registered Kinesiologist. All equipment are available for your use. The PACE also has bathroom/change room facilities including showers.

No Exercise Program – De La Sol Yoga at York & Locke Streets, Hamilton (http://www.delasolyoga.com)

- 12-weeks of meditation classes. Each class is 1 hour.
- Weekly attendance at 3 of 4 available classes offered each week.
- Classes include body awareness, relaxation, deep breathing and meditation. These classes will not include physical exercise.
- All classes will be taught by a certified yoga instructor will a specialization in meditation.

Measurements at the End of the Study

- *MacMobilize Laboratory at McMaster University*
  1. Body size measurements
  2. Resting heart rate and blood pressure
  3. Analyses of walking and yoga poses using laboratory equipment
  4. Mobility performance measurements
     a. 6-minute walk
     b. Stair climb
     c. 30-second chair stand
  5. Strength assessment
  6. Questionnaires
  7. Blood samples and nasal swab

  This visit will take approximately 2.5 hours to complete.

- *CAMRIS at St. Joseph's Hospital, downtown Hamilton*
  1. Questionnaire and MRI eligibility form
  2. MRI image of the thigh to examine muscle
  3. Second MRI scan of the knee joint to examine cartilage health
  4. Stipend

  This visit will take approximately 1 hour to complete.

You must complete these measurements at the end of the study to be eligible to receive the $50 stipend.

Total time commitment if you choose to participate in the study

Activity	Time per session	Number of sessions	Total time
X-ray visit	0.5 h	1	0.5 h
Measurement visit	2.5 h	2	5 h

MRI visit	1 h	2	2 h
Classes	1 h	36	36 h
Total:			**43.5 hours**

Risks & Benefits
There are risks associated with exercise in this study, including the following: fatigue, soreness, heart complications in those with poor heart health, increased risk of fracture especially in those with osteoporosis, and worsening of knee pain in those with patellofemoral syndrome. With regards to the potential muscle soreness, we will teach you gentle stretches and how to use an ice pack to reduce the soreness. There is a risk to that, in unusual cases, you may experience a skin allergy to medical tape used to keep the skin markers in place. Finally, exposure to x-rays can increase your risk of health problems like cancer. However the exposure to x-ray involved in this study is lower than x-rays at the dentist. If you have concerns about this x-ray, please be sure to ask questions. If you experience any serious discomfort following a yoga class or lab visit, please contact Dr. Monica Maly at (905) 525-9140 x 27823.

Risks associated with blood draw are minimal and may include bruising or pain around the stick site. Very rarely, infection may occur on the skin or the vein, or introduce infection into the blood stream.

You may gain the physical and/or mental benefits associated with the three groups in this study. Your participation will help improve our understanding of how exercise, a non-invasive treatment option, affects osteoarthritis of the knee.

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 investigation. The information we collect will be secured in a locked filing cabinet in the MacMobilize Laboratory at McMaster University to which only the researchers will have direct access. This research space is also locked. Blood samples and nasal swabs processed at the McMaster Immunology Research Centre will remain anonymous. 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. You will receive a $50 stipend upon completion of the study. There will be draws following some yoga classes to win Tim Horton's gift certificates and rewards for best attendance during the 12 weeks. Those randomized into the no exercise group who complete the study will be eligible for three free months of exercise offered at the Physical Activity Center of Excellence at McMaster University.

Questions
If you have any general questions, please call the MacMobilize Research Laboratory at (905) 525-9140 x 20748. If you have any questions about your rights as a research participant or the conduct of the study, you may contact the Dr. Monica Maly, the principal investigator at (905) 525-9140 x 27823. This letter is yours to keep for future reference.

Clinical and Tissue Outcomes of a Biomechanical Exercise Program for Knee Osteoarthritis

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