Knee Osteoarthritis: A Clinical Trial Examining Therapeutic Effects of High-Rosmarinic Acid Spearmint Tea and Investigations into Relationships of Pain with Modifiable Lifestyle Factors and Biomarkers of Joint Metabolism and Inflammation

by

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A Thesis
Presented to
The University of Guelph

In partial fulfillment of requirements for the degree of Doctor of Philosophy In Human Health and Nutritional Sciences + Toxicology

Guelph, Ontario, Canada

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Abstract

Knee Osteoarthritis: A Clinical Trial Examining Therapeutic Effects of High-Rosmarinic Acid Spearmint Tea and Investigations Into Relationships of Pain with Modifiable Lifestyle Factors and Biomarkers of Joint Metabolism and Inflammation

A. Erin Connelly
University of Guelph, 2015

Advisors: Dr.Alison M. Duncan & Dr.Amanda J. Wright

Osteoarthritis (OA) is a chronic, progressive disease involving the degeneration of cartilage and joint tissue, resulting in pain and disability. This thesis investigated OA knee pain and physical function, namely associations with lifestyle factors and biomarkers as well as a novel therapeutic product to manage symptoms of knee OA. In a survey of healthy adults with knee OA (n=197), OA characteristics, health history and information about smoking history, alcohol consumption, height, body weight, medication and supplement intake, and exercise habits were collected. The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) was used to assess pain. OA medication use and higher body mass index (BMI) category were associated with higher pain. Supplement use and meeting physical activity guidelines (150 min/week) were associated with lower pain. Next, associations between OA symptoms and biomarkers of joint metabolism and inflammation (n=54) were analyzed using Spearman correlation coefficients, controlling for age, gender and BMI. Serum hyaluronic acid was significantly associated with better performance in the 6-minute walk test (6MWT) and stair climb test (SCT). Serum matrix mellatoproteinase-3 was significantly associated with higher pain. Interleukin-6 was significantly associated with higher pain, stiffness and worse performance in the SCT. Finally, in a randomized, double-blind clinical trial, participants (n=46) consumed tea brewed from a high-rosmarinic acid (rosA) spearmint or a commercially available spearmint twice daily for 16 weeks. Pain significantly decreased within the high-rosA group, but not the control group and scores for stiffness and physical disability significantly decreased within both groups. Increased quality of life score on the bodily pain index in the short form (SF)-36 questionnaire was observed within the high-rosA group only. In the 6MWT, only the high-rosA group increased distance walked at Week 16, but the increase was not statistically significant. There were no changes in the SCT within either group. Overall, this thesis found associations between OA symptoms and biomarkers of joint metabolism and inflammation and
characterized the relationship between OA pain and certain lifestyle factors. Also, for the first time, a decrease in pain with consumption of a high-rosA acid spearmint tea was observed, rationalizing further consideration in the management of knee OA pain.
Dedication

This thesis is dedicated to my grandmother, Elizabeth Connelly. Your love of knowledge and education is inspiring and has served as one of the greatest influences in my life. You are a model of strength, courage, and resolve.
Acknowledgements

I am forever grateful for everyone who has supported, challenged, and helped me throughout this process. I generally gravitate to strong female role models, and I really hit the jackpot with these three; Dr. Alison Duncan, Dr. Amanda Wright, and Dr. Amy Tucker. Alison and Amanda, you are both such well-rounded, hard working professors. You successfully balance family, research, teaching, students, business, and I have learned so much from watching you in action. You both want the best for your students and work hard for that to happen. Alison, it is hard to comprehend how anyone can accomplish as much as you do in a day/week/year and I am grateful for all you have done for me, including passing on an appreciation for statistics. Amanda, thank you for all of your patience with me. You always challenge me to be the best version of myself, as a scientist and as a person, and I am indebted to you for your persistence. Thank you both for providing me with this wonderful opportunity and guiding me through this process with kindness and support. Amy, thank you so much for being a mentor and a friend. I don’t know how other people get through doctoral degrees without an ‘Amy’. You think about science in such a complete, creative, and thoughtful way. I have learned so much from you and had so many revelations in your office. Thank you for your patience, kindness, and guidance.

Dr. Laima Kott, thank you for your invaluable contributions to this thesis and for serving on my advisory committee. Dr. Lindsay Robinson and Dr. Monica Maly, thank you for your important roles in my examination. Thank you to both Dr. Bill Bettger and Dr. Jim Kirkland for serving on my advisory and comprehensive exam committees. Bill, you have been an important role model and I have learned so much for you. Jim, I am grateful for our time together and it has also been a pleasure being your teaching assistant for the last 4 years and I will miss it.

I have had the opportunity to work with many different types of people, all of whom were important to this process, and my growth, and I value those experiences. Natasha, you were a life saver during a stressful time, I am so grateful that we got to work together and I know that you are going to do great things. Thank you to all my past and present research collaborators and lab/HNRU friends.
HHNS is truly a remarkable place. I have met and been inspired by so many wonderful people in this department. Brilliant, hard-working, fun, athletic, charitable, weird, and overall amazingly well-rounded individuals walk the halls and amaze me with their seemingly effortless awesomeness. I have made so many wonderful friendships in this department and they have made me a better person, scientist, and intramural athlete (excluding softball), and they are dear to me. Jessica Ralston, your friendship, support and kindness has meant the world to me. You have taught me so many things and I admire your intelligence, creativity and insane work ethic. Kaitlin Roke, I look up to you in so many ways, you are so smart, so kind, and so caring. Tara McDonald and Emily McIntosh, you are incredible scientists and humans and I am grateful for your friendship. Thank you to my wonderful partner and friend, Andrew. You have been a major part of this process and always know exactly what I need. Thank you for making me laugh, being my sounding board, a shoulder to cry on, and a kick in the pants (when needed). Thank you for your patience, love, and encouragement.

I need to thank my tiny, furry friend, Domi, who has been by my side and who always brings a smile to my face. I am lucky to have a large, warm supporting family who have been so proud and excited for me during this process. Mom and Dad, you have shown me what life is like when you are passionate about your job, work hard, and have big goals. Thank you for your unconditional support, trust, and love. You are my biggest cheerleaders, source of strength, and best friends.
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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>6MWT</td>
<td>6-minute walk test</td>
</tr>
<tr>
<td>AAOS</td>
<td>American Academy of Orthopaedic Surgeons</td>
</tr>
<tr>
<td>ACL</td>
<td>Anterior cruciate ligament</td>
</tr>
<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
</tr>
<tr>
<td>ADAMTS</td>
<td>A disintegrin and metalloproteinase with thrombospondin motifs</td>
</tr>
<tr>
<td>AGEs</td>
<td>Advanced glycation end products</td>
</tr>
<tr>
<td>ASU</td>
<td>Avocado/soybean unsaponifiables</td>
</tr>
<tr>
<td>AIMS</td>
<td>Arthritis Impact Measurement scale</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>BMLs</td>
<td>Bone marrow lesions</td>
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<tr>
<td>BW</td>
<td>Body weight</td>
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<tr>
<td>CGRP</td>
<td>Calcitonin gene related peptide</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CIA</td>
<td>Collagen-induced arthritis</td>
</tr>
<tr>
<td>COMP</td>
<td>Cartilage oligomeric matrix protein</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CVD</td>
<td>Cardiovascular diseases</td>
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<tr>
<td>ECM</td>
<td>Extra cellular matrix</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>ES</td>
<td>Effect size</td>
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<tr>
<td>EULAR</td>
<td>European League Against Rheumatism</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Association</td>
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<tr>
<td>GAG</td>
<td>Glycosaminoglycan</td>
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<tr>
<td>GI</td>
<td>Gastrointestinal</td>
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<td>GSH</td>
<td>Glutathione</td>
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<tr>
<td>HA</td>
<td>Hyaluronic acid</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>HO-1</td>
<td>Heme oxygenase-1</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormone replacement therapy</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IKK-b</td>
<td>Inhibitor of nuclear factor kappa-B kinase subunit beta</td>
</tr>
<tr>
<td>JSN</td>
<td>Joint space narrowing</td>
</tr>
<tr>
<td>KL</td>
<td>Kellgren-Lawrence</td>
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<tr>
<td>KOOS</td>
<td>Knee injury and OA outcome score</td>
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<tr>
<td>KOS-ADL</td>
<td>Knee outcomes survey activities of daily living scale</td>
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<tr>
<td>LIF</td>
<td>Leukemia inhibitory factor</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<tr>
<td>MMP</td>
<td>Matrixmetalloproteinase</td>
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<td>MPQ</td>
<td>McGill pain questionnaire</td>
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<tr>
<td>MS</td>
<td>Millisecond</td>
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<tr>
<td>MSK</td>
<td>Musculoskeletal</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NHP</td>
<td>Natural health product</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NOAEL</td>
<td>No observed adverse effect level</td>
</tr>
<tr>
<td>NRS</td>
<td>Numerical rating scale</td>
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<tr>
<td>NSAIDs</td>
<td>Nonsteroidal anti-inflammatory drugs</td>
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<tr>
<td>NF-kB</td>
<td>Nuclear factor-kB</td>
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<tr>
<td>Nrf2</td>
<td>Nuclear factor-like 2</td>
</tr>
<tr>
<td>OA</td>
<td>Osteoarthritis</td>
</tr>
<tr>
<td>OARSI</td>
<td>Osteoarthritis Research Society International</td>
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<tr>
<td>OMERACT</td>
<td>Outcome measures in rheumatology</td>
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<tr>
<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PASE</td>
<td>Physical Activity Scale for the Elderly</td>
</tr>
<tr>
<td>PGE$_2$</td>
<td>Prostaglandin E$_2$</td>
</tr>
<tr>
<td>PIIANP</td>
<td>Procollagen type II N-terminal propeptide</td>
</tr>
<tr>
<td>QoL</td>
<td>Quality of life</td>
</tr>
<tr>
<td>RA</td>
<td>Rheumatoid arthritis</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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</tr>
<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RosA</td>
<td>Rosmarinic acid</td>
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<tr>
<td>SCT</td>
<td>Stair-climb test</td>
</tr>
<tr>
<td>SMD</td>
<td>Standard mean difference</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide dismutase</td>
</tr>
<tr>
<td>TBARS</td>
<td>Thiobarbituric acid reactive substances</td>
</tr>
<tr>
<td>TEAC</td>
<td>Trolox equivalent antioxidant capacity</td>
</tr>
<tr>
<td>TNFa</td>
<td>Tumor necrosis factor-alpha</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analog scale</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WOMAC</td>
<td>Western Ontario and McMaster Universities Osteoarthritis Index</td>
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Chapter 1: Introduction and Review of the Literature

1.1 Introduction

Osteoarthritis (OA) is a progressive disease characterized by degeneration of articular cartilage and alteration of joint tissues, resulting in pain, stiffness and disability (Martel-Pelletier and Pelletier 2010). It is the most common form of arthritis and a leading cause of pain and disability in older adults (Loser et al 2012). The knees, hips and hands are most commonly affected, with knee OA having the greatest impact on disability (Jordan et al 2004b). The pain in OA is the most significant clinical issue and impacts function, mobility and quality of life (Hawker 2012). There is no cure for OA or method of halting joint tissue destruction. Treating OA pain is complex as pain is a multifactorial biopsychosocial phenomenon that is not completely understood. Additionally, pain and other symptoms of OA are not strongly correlated to radiographic evidence of the disease (Bijlsma et al 2011). This complicates research trying to evaluate the efficacy of different therapies. Current analgesic treatments are not effective and the overall lack of successful treatment options remains a large and critical gap in the management of OA. Many of the limitations in discovering effective treatments for OA stem from an incomplete understanding of OA etiology, a poor ability to measure and define the disease or assess disease progression, and response to new treatment options (Felson 2014). In general, this thesis investigates the impact of lifestyle factors, including a bioactive-enriched spearmint tea, on the management of knee OA and relationships between potential biomarkers and symptoms of OA. Investigating novel diet-derived therapeutic products is an important area of research that holds potential for managing symptoms of OA. The literature review provides an overview of OA, including its pathology and risk factors, as well as methods for measuring OA and current management strategies.
1.2 Review of the Literature

1.2.1 Osteoarthritis

1.2.1.i Definition of Osteoarthritis

OA is a disease of the synovial joints where degeneration of joint tissue results in pain, stiffness, and impaired physical function (Lane et al 2011; Litwic et al 2013). Pain is the most prominent and disabling symptom of OA, resulting in reduced participation in activities and negative effects on mood, sleep, and overall quality of life (Dieppe and Lohmander 2005; Lane et al 2011; Shimura et al 2013). OA can arise in any synovial joint in the body, but is most common in the hands, hips, knees and spine (Dieppe and Lohmander 2005). Knee OA results in the most severe disability and the knee is the joint most extensively studied in OA research (Dieppe and Lohmander et al 2005; Hunter and Felson 2006; Hayashi et al 2014). Although there is no standard definition for OA, it can be defined structurally or symptomatically (Nelson et al 2012; Litwic et al 2013). Radiographic OA is used in research settings and is based on structural characteristics of the joint, including joint space narrowing (JSN), osteophytes, bone cysts, and sclerosis visualized on radiographs (Felson et al 1997). Symptomatic OA is used in clinical and research settings and is based on radiographic evidence, as well as assessment of pain, stiffness, and cracking of joints (Altman et al 1986).

1.2.1.i.a Clinical Diagnosis

Diagnosis of OA is based on an overall clinical impression of age, history, location of joint abnormalities, and sometimes, radiographic evidence (Wu et al 2005). The American College of Rheumatology developed standards for diagnosis of knee OA in 1986 that are still used today (Table 1.1) (Altman et al 1986; Litwic et al 2013).
Table 1.1 Standards developed by the American College of Rheumatology for the diagnosis of knee OA based on history and type of examination.

<table>
<thead>
<tr>
<th>Must Have</th>
<th>History and Physical Examination</th>
<th>History, Physical Examination and Radiographic Findings</th>
<th>History, Physical Examination and Clinical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knee pain</td>
<td>Knee pain, and osteophytes</td>
<td>Knee pain</td>
<td></td>
</tr>
<tr>
<td>And at least 3 of:</td>
<td>And at least 1 of:</td>
<td>And at least 5 of:</td>
<td></td>
</tr>
<tr>
<td>- Over 50 years of age</td>
<td>- Over 50 years of age</td>
<td>- Over 50 years of age</td>
<td></td>
</tr>
<tr>
<td>- Less than 30 minutes of morning stiffness</td>
<td>- Less than 30 minutes of morning stiffness</td>
<td>- Less than 30 minutes of morning stiffness</td>
<td></td>
</tr>
<tr>
<td>- Crepitus on motion</td>
<td>- Crepitus on motion</td>
<td>- Crepitus on motion</td>
<td></td>
</tr>
<tr>
<td>- Bony tenderness</td>
<td>- Bony tenderness</td>
<td>- Bony tenderness</td>
<td></td>
</tr>
<tr>
<td>- Bony enlargement</td>
<td>- Bony enlargement</td>
<td>- Bony enlargement</td>
<td></td>
</tr>
<tr>
<td>- No palpable warmth of synovial membrane</td>
<td>- No palpable warmth of synovial membrane</td>
<td>- No palpable warmth of synovial membrane</td>
<td></td>
</tr>
<tr>
<td>- ESR &lt; 40 mm/hour</td>
<td>- RF &lt; 1:40</td>
<td>- Synovial fluid signs of OA</td>
<td></td>
</tr>
</tbody>
</table>

*Adapted from Altman et al 1986. ESR = erythrocyte sedimentation rate; RF = rheumatoid factor.

1.2.1.ii. Prevalence of OA

In Canada, OA affects approximately 4.4 million people (Bombardier et al 2012). Worldwide, OA is the most common joint disorder (Storheim and Zwart 2014), and the World Health Organization (WHO) estimates that 10% of the world’s population have clinical problems attributed to OA (Woolf and Pfleger 2003). Of the total OA burden, knee OA is estimated to account for 83% (Vos et al 2013; Murray et al 2013). Due to increased longevity, reduced physical activity levels, and increasing prevalence of obesity, the burden of OA is increasing (Bombardier et al 2012; Hunter et al 2014). It has been predicted that by 2040, more than 10 million Canadians will be diagnosed with OA (Bombardier et al 2012).

1.2.1.iii. Societal and Individual Costs

OA is a costly disease with Canada, United States of America, United Kingdom, France, and Australia spending approximately, 1 to 2.5% of the gross national product on care for arthritis (Hunter et al 2014). In 2010 in Canada, direct and indirect health care costs of OA were estimated to be $10.2 billion and $17.3 billion, respectively (Bombardier et al 2012). Direct costs
include drugs, side effects of treatment, non-pharmacological treatments, visits to health professionals, home adaptations, tests, hospitalizations, surgical procedures and use of community services (Figure 1) (Gupta et al 2005; Bombardier et al 2012; Chen et al 2012). Indirect costs are incurred based on absence from work, productivity losses at work, leaving the work force, caregiver time off work, and premature mortality (Gupta et al 2005; Bombardier et al 2012; Chen et al 2012).

The burden of OA must also be evaluated in terms of its impact on quality of life and other intangible costs, as there are significant physical and psychological effects on the individual (Figure 1.1) (Chen et al 2012; Hunter et al 2014). Pain, suffering, decreased quality of life, depression and anxiety, and reduced participation in activities are common in adults with OA (Chen et al 2012; Hunter et al 2014). Adults with OA report more pain, worse quality of life, greater number of hospitalizations and reduced productivity than those without (daCosta DiBonaventura et al 2012). The total economic burden of OA in Canada is expected to rise to over $1,455 billion in 2040 (based on 2010 values) (Bombardier et al 2012).

Figure 1.1 The burden of OA is affected by direct, indirect and intangible costs. (Modified from Hunter et al 2014).
1.2.2 Pathology of OA

OA is a heterogeneous disease, involving complex and interacting mechanical, biological, biochemical, molecular, and enzymatic feedback loops with cartilage degeneration as the common, final event (Martel Pelletier and Pelletier 2010; Umlauf et al 2010). Despite this degeneration, OA is an active process and a network of mechanisms reacting to stress or injury on the joint (Umlauf et al 2010; Loeser et al 2012). All joint features are affected in OA (Figure 1.2) (Hunter 2011). Structural changes include cartilage fibrillation, degeneration of articular cartilage, thickening of subchondral bone, osteophyte formation, synovial inflammation, degeneration of ligaments and meniscus, hypertrophy of joint capsule, cellular and molecular changes in nerves, as well as changes to periarticular muscle, bursa, fat pads (Figure 1.2) (Goldring and Goldring 2006; Martel Pelletier and Pelletier 2010; Loeser et al 2012). The loss of cartilage and modifications to bone and synovial membrane contribute to an unfavourable biomechanical environment which increases stress on the joint and furthers the progression of cartilage degradation (Goldring and Goldring 2006; Heijink et al 2012).

Figure 1.2 Cross-sectional picture of healthy knee joint on the left and characteristic changes to those structures in OA on the right. Taken from Hunter 2011.
1.2.2.i. Tissues involved

In OA, there is degeneration and alteration of the cartilage, bone, synovial membrane, menisci, and ligaments, which contributes to an unfavourable biomechanical and biochemical environment (Hunter 2011).

1.2.2.i.a Cartilage

Articular cartilage covers the ends of bones in synovial joints to provide a smooth surface with low friction for efficient gliding, load bearing, impact absorption, and to sustain shear forces during movement (Martel-Pelletier et al 2008; Struglics and Hansson 2012). It can accomplish these functions because of the structure and composition of the extra cellular matrix (ECM), which is composed of collagen, proteoglycans, and water (Martel Pelletier and Pelletier 2010). A fibrillar collagen network, composed of type II collagen linked together by cartilage oligomeric matrix protein (COMP) and chondroadherinin, is arranged in triple helices and gives cartilage its stiffness and tensile strength (Martel-Pelletier et al 2008; Sofat 2009; Goldring and Goldring 2010). The other main component of cartilage is the proteoglycan aggrecan. This is a large aggregating polymeric structure with a large protein core and 3 globular domains (Sofat 2009). Glycosaminoglycan (GAG) side chains on aggrecan contain a large number of negatively charged groups, which attract and retain water to form a gel that gives cartilages its compressive ability (Sofat 2009). There are no blood vessels, lymphatic vessels, or nerves in cartilage and chondrocytes are the only cell type present (Martel Pelletier and Pelletier 2010). As such, chondrocytes are responsible for the synthesis, breakdown, and maintenance of the ECM and respond to mechanical forces, osmotic stresses, growth factors, cytokines, and other inflammatory mediators (Sofat 2009; Goldring and Goldring 2010; Loeser et al 2012; Struglics and Hansson 2012).

The dynamic equilibrium between synthesis and degradation of ECM maintained by chondrocytes is disrupted in OA, with degradation being favoured (Lee et al 2013a). Catabolic activity increases as a response to cartilage loss, and cell proliferation and enhanced production of matrix proteins occurs (Goldring and Marcu 2009; Goldring and Goldring 2010; Martel Pelletier and Pelletier 2010). Despite the repair attempt, proteoglycans are degraded, exposing type II collagen fibrils which are then accessible for degradation (Loeser et al 2012).
Chondrocytes produce and release matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) enzymes, responsible for cleaving collagen fibrils and aggregans, respectively (Anderson and Loeser 2010). In a cyclical manner, cleaved parts of ECM can act on chondrocytes or diffuse to the synovial membrane to initiate kinase signaling, producing more enzymes and inflammatory mediators (Figure 1.3) (Goldring 2012; Loeser et al 2012). Although the exact roles of these mediators are unknown, interleukin 1-beta (IL-1b), tumor necrosis factor-alpha (TNFa), interleukin 6 (IL-6), leukemia inhibitory factor (LIF), interleukin 17 (IL-17), interleukin 18 (IL-18), nitric oxide (NO), prostaglandin E2 (PGE2), and leukotriens are increased in OA and involved in the disease pathogenesis (Martel-Pelletier et al 2008; Umlauf et al 2010). It is not known if inflammation in OA occurs as an initiating event or secondary to cartilage breakdown (Conde et al 2011). With the loss of cartilage and increased inflammatory and oxidative stress, apoptosis of chondrocytes occurs (Lee et al 2013a). Chondrocytes synthesize new aggregan and collagen in a repair attempt, but the new aggregan is similar to juvenile proteoglycans and the collagen produced is type X and neither of these can maintain the same mechanical properties as healthy adult cartilage (Figure 1.3) (Lohmander et al 1999; Martel-Pelletier et al 2008). This abnormal composition contributes to an adverse biomechanical environment, further stimulating degradation (Loeser et al 2012). The changes to the cartilage are not directly responsible for OA pain as cartilage is aneural and not capable of generating pain, although the other joint structures are richly innervated (Lee et al 2013a).
Figure 1.3 Complex cyclic processes in cartilage, bone, and synovial membrane in knee OA contribute to the disease pathogenesis. Chondrocytes produce matrix degrading enzymes (MMP and ADAMTS) and inflammatory mediators that can act on the chondrocytes or enter the synovial membrane to cause inflammation. Chondrocytes produce type X collagen in a repair attempt, but it is not suitable for adult ECM. In the bone, osteophytes and bone marrow lesions lead to an adverse biomechanical environment and are potentially painful. Adapted from Lee et al (2013a).

1.2.2.i.b. Bone

Bone alterations are recognized as important in the pathogenesis of OA, although less well understood than changes in cartilage (Dieppe and Lohmander 2005). In OA, the structure and properties of subchondral bone are altered with increases in subchondral plate thickness, modification of the architecture of the trabecular bone, formation of new bone at joint margins, and changes in the vascularity (Figure 1.3) (Dieppe and Lohmander 2005; Goldring and Goldring 2010; Martel Pelletier and Pelletier 2010). Bone marrow lesions (BMLs) are also
common and consist of bone marrow necrosis, trabecular abnormalities, bone marrow fibrosis, and edema (Figure 1.3) (Raynauld et al 2008; Ding et al 2010). These are hypothesized to result from excessive repetitive loading or an acute inflammatory response (Raynauld et al 2008; Loeser et al 2012). There is evidence that subchondral bone changes precede cartilage degradation (Goldring and Goldring 2010; Martel Pelletier and Pelletier 2010). Changes in bone could promote abnormal cartilage metabolism through reduced structural support, impaired nutrient supply, and provision of catabolic factors (Ding et al 2010; Goldring and Goldring 2010; Martel Pelletier and Pelletier 2010). Increased bone stiffness could also make the cartilage less effective at accommodating mechanical loads (Goldring and Goldring 2010). As bone is richly innervated, it may be a potential source of pain in OA (Cotofana et al 2013). Indeed, several studies have correlated bone abnormalities observed by magnetic resonance imaging (MRI) with knee pain in OA (Link et al 2003; Kornaat et al 2006; Cotofana et al 2013).

1.2.2.i.c. Synovial Membrane

The synovial membrane is a thin sheet of connective tissue that covers all structures in the joint except the cartilage (Martel Pelletier and Pelletier 2010). It is well vascularized and innervated and contains cells that phagocytize debris and secrete hyaluronic acid (HA) and lubricin to facilitate gliding during movement (Martel Pelletier and Pelletier 2010; Loeser et al 2012).

Inflammation of the synovial membrane as well as synovial hypertrophy and hyperplasia occur in OA (Martel Pelletier and Pelletier 2010). Products of cartilage degradation diffuse into the synovial membrane where they are phagocytosed by macrophages, initiating the release of cytokines, reactive oxygen species (ROS) and synovial inflammation (Figure 1.3) (Martel Pelletier and Pelletier 2010). These cytokines are then released into the synovial fluid where they can diffuse into the cartilage to act on chondrocytes, continuing the cycle of degradation and inflammation (Martel Pelletier and Pelletier 2010). Synovial cells are also capable of producing MMPs and ADAMTS in response to inflammatory signaling (Bondeson et al 2010). ROS produced in synovial inflammation are also believed to play a role in OA by breaking down ECM, up regulating matrix degrading enzymes, and inhibiting anabolic signals (Goldring and Berenbaum 2004; Im et al 2008). As the synovial membrane is richly innervated, synovial inflammation is potentially an important source of pain in OA and significant relationships
between synovitis and OA symptoms has been reported in several studies (Benito et al 2005; Loeuille et al 2005; Martel Pelletier and Pelletier 2010; Sowers et al 2011; Ballegaard et al 2014).

1.2.2.i.d. Menisci and Ligaments

The knee menisci are two pads of fibrocartilage that lie between the femur and tibia and are attached to the joint capsule (Englund et al 2012). The menisci are an integral part of the biomechanical system of the knee joint and work to stabilize the joint, resist tension, compression, and shear stress, and absorb shock during dynamic movement (Martel Pelletier and Pelletier 2010; Englund et al 2012). There are 3 ligaments in the knee. These consist of collagenous fibers arranged as parallel bundles that provide anteroposterior and rotational stability of the knee, prevent hyperextension, and provide proprioceptive information (Vincent et al 2012; Witt and Vilensky 2014). Pathological changes in the menisci and ligaments are common in adults with knee OA, even in those without previous injury (Ding et al 2010; Loeser et al 2012). These pathologic changes include matrix disruption, fibrillation, cell clusters, calcification, and cell death, which can alter knee kinematics and strain areas of cartilage that are not accustomed to those loads, promoting cartilage degradation (Loeser et al 2012). In addition, increases in vascular penetration and innervation have been reported in OA menisci, suggesting a potentially important source of pain in OA (Ashraf et al 2011).

1.2.2.ii Pain Pathology

It is not well understood how the process of joint degeneration in OA causes pain (Witt and Vilensky 2012; Heijink et al 2012). The structural origins of pain in OA also are unclear, but pain fibers innervate the synovial membrane, ligaments, menisci, and subchondral bone (Witt and Vilensky 2012). The central mechanisms of interpreting pain signals are also not well understood, but there is an emotional component of chronic pain (McDougall 2006; Lee et al 2013a). Depression and anxiety are common co-morbidities in OA and have been associated with the chronic pain experienced (Hawker et al 2011). Pain is multifactorial and in OA, believed to arise from nociceptive and neuropathic mechanisms (Mease et al 2011). Nociceptive pain occurs with local tissue damage, where mechanical or chemical stimulation of nociceptors causes the transmission of a pain signal from the joint to the dorsal root ganglion in the spinal
cord and then up the spinothalamic tract to cortical centers for processing (Lee et al 2013a). When a tissue is damaged, chemicals are released that cause physiological pain and which can also sensitize nociceptors (Mease et al 2011; McDougall and Lindon 2012). Neuropathic pain is generated from damage to the nerves (Hochman et al 2010) and has been detected in individuals with OA using questionnaires that identify the type and quality of pain sensations (i.e. burning, numbness, etc.) (Hochman et al 2010; Ohtori et al 2012). In OA and other chronic pain conditions, ongoing activation of pain fibers produces continuous neurotransmitter release into the spinal cord, causing increased neuronal activity and alterations in peripheral and central pain processing (McDougall 2006; Mease et al 2011). Table 1.2 shows and defines a number of these alterations in pain processing that occur in OA.

**Table 1.2 Alterations in pain processing that occur in OA.**

<table>
<thead>
<tr>
<th>Alteration in Pain Processing</th>
<th>Explanation/Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allodyina</td>
<td>Innocuous stimuli is perceived and transmitted as noxious stimuli (i.e. pain while walking).</td>
<td>Witt and Vilensky 2012</td>
</tr>
<tr>
<td>Hyperalgeisa</td>
<td>Heightened pain intensity to a noxious insult (i.e. increased sensitivity to painful sensations).</td>
<td>McDougall 2006</td>
</tr>
<tr>
<td>Peripheral and central sentization</td>
<td>Decrease in the activation threshold of a neuron, from chronic nociceptor stimulation.</td>
<td>Hochman et al 2010</td>
</tr>
<tr>
<td>Enlargement of receptor field</td>
<td>Increase in the area that a nociceptor innervates. Can occur beside injured or inflamed tissue.</td>
<td>Mease et al 2011</td>
</tr>
<tr>
<td>Spontaneous firing</td>
<td>Nociceptors send signals in the absence of mechanical stimulation (i.e. pain at rest).</td>
<td>McDougall 2006</td>
</tr>
<tr>
<td>Altered cortical processing</td>
<td>Individual variability in central processing of nociceptive stimuli. Has been hypothesized to explain the lack of association between structural damage and pain in OA.</td>
<td>Lee et al 2013</td>
</tr>
</tbody>
</table>

The same inflammatory mediators involved in joint destruction play a role in the altered pain processing in OA (Omoigui 2007; Lee et al 2013a). During inflammatory conditions, the number of cytokine receptors on neurons is increased (Witt and Vilensky 2012). Cytokines can directly or indirectly stimulate neurons and lower the firing threshold to initiate the alterations described in Table 1.2 (Konttinen et al 2012). Inflammatory mediators can also activate silent nociceptors in a joint. These are pain fibers that are not active unless the body is under stress (Witt and Vilensky 2012). Neuropeptides are chemical mediators released from the terminals of joint afferents that can cause neurogenic inflammation (McDougall 2006). The neuropeptides,
substance P, calcitonin-gene-related-peptide, and neurokinin-1, are believed to play a role in OA pain (Konttinen et al 2012; Witt and Vilensky 2012). It has also been suggested that neurogenic inflammation may contribute to joint damage by promoting and amplifying the inflammatory response (Kidd et al 2003).

1.2.3 Risk factors for OA

Several large prospective cohort studies have provided a wealth of information on risk factors for the development of OA. The main non-modifiable risk factors associated with incidence of OA are age and female sex, while family history and developmental conditions that affect bone growth or joint shape have also been identified (Felson et al 2000; Blagojevic et al 2010). Modifiable risk factors for OA include body mass index (BMI), joint injury, physical activity and diet. Table 1.3 presents odds ratios (OR) calculated from a meta-analysis examining risk factors for the onset of knee OA (Blagojevic et al 2010).

Table 1.3 Odds Ratio for developing knee OA for different risk factors (Adapted from Blagojevic et al 2010)

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>OR (95% CI)</th>
<th>Studies Included</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Not calculated</td>
<td>15</td>
<td>OR wasn’t calculated due to differing classification of age groups. Age was a risk in all studies.</td>
</tr>
<tr>
<td>Female Gender</td>
<td>1.84 (1.32-2.55)</td>
<td>8</td>
<td>Gender was often used an as adjustment factor and gender specific effects were not often reported.</td>
</tr>
<tr>
<td>BMI</td>
<td>2.18 (1.86-2.55)</td>
<td>23</td>
<td>OR for overweight versus normal.</td>
</tr>
<tr>
<td></td>
<td>2.63 (2.28-3.05)</td>
<td>17</td>
<td>OR for obese versus normal.</td>
</tr>
<tr>
<td>Knee Injury</td>
<td>3.86 (2.61–5.70)</td>
<td>16</td>
<td>OR wasn’t calculated due to varied definitions of physical activity. Higher quality studies showed a general increased risk with intense exercise.</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>Not calculated</td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

*BMI = body mass index; OR = odds ratio
1.2.3.i. Non-Modifiable Risk Factors

1.2.3.i.a. Age

Age is the primary risk factor for OA and the incidence of OA increases with age (Anderson and Loeser 2010; Litwic et al 2013). However, everyone who ages does not get OA (Anderson and Loeser 2010). It is generally thought that normal changes with aging increase the susceptibility to OA, but additional risk factors are required for the development of symptomatic or clinical OA (Figure 1.4) (Anderson and Loeser 2010). Radiographic damage in a knee also increases with age, even in the absence of disease, demonstrating that mild joint degradation may occur and accumulate with aging (Ding et al 2010; Anderson and Loeser 2010). It is nearly impossible to distinguish if these radiographic changes are a part of normal aging or preclinical OA that has not yet developed. Aging also decreases the ability of the joint to respond to stressors and maintain homeostasis in the cartilage, as observed by the fact that older adults develop OA more rapidly than younger adults following similar injuries (Roos et al 1995; Anderson and Loeser 2010).

Cellular changes that occur with aging can also increase the susceptibility to OA (Figure 1.4). As there is little to no cell division or cell death in adult cartilage, chondrocytes can accumulate age-related changes over time (Heijink et al 2012). The accumulation of advanced glycation end products (AGEs) can increase collagen cross-linking, making cartilage more brittle (Heijink et al 2012). There is also evidence that aged chondrocytes show a senescence-associated secretory phenotype that includes increased production of cytokines, chemokines and MMPs that may contribute to matrix degradation (Heijink et al 2012; Loeser et al 2012).
Aging alone does not cause OA. In aging, changes that affect joint function and tissues increase susceptibility to OA. For the development of symptomatic OA, additional risk factors or OA factors, need to be present. (Modified from Anderson and Loeser 2010).

1.2.3.i.b. Female Sex

The prevalence of OA is similar in both sexes until the age of 50, after which the prevalence in women increases significantly (Felson et al 1997; Srikanth et al 2005; Maleki-Fischbach and Jordan 2010). Not only are women diagnosed with OA more often, they usually have more severe pain and disability than men (Keefe et al 2000; Perrot et al 2009; Sims et al 2009; Elbaz et al 2011; Tonelli et al 2011; Glass et al 2014).

Pre-clinical studies have shown that a complex and incompletely understood relationship exists between cartilage metabolism and circulating gonadal steroids (Reginster et al 2003). Estrogen receptors are present on human and animal chondrocytes and estrogen has been found to have chondroprotective roles via its effect on growth factors, cytokines, MMPs, and ROS (Rosner et al 1982; Tanko et al 2008). A 10-year prospective cohort study found that lower serum estradiol
and urinary estrogen metabolite levels were associated with increased risk of knee OA (Sowers et al 2006). Because of this evidence, the effect of hormone replacement therapy (HRT) on the development and progression OA has been thoroughly examined. Several cross-sectional (Hannan et al 1990; Samanta et al 1993; Nevitt et al 1996; Spector et al 1997) and longitudinal (Zhang et al 1998; Hart et al 1999) studies have found that HRT use is associated with a decreased risk of knee OA, although other studies have not found an association (Sowers et al 1996; Erb et al 2000; Maheu et al 2000; Nevitt et al 2001). Some have even demonstrated an increased risk (Oliveria et al 1996; Sandmark et al 1999). Although it is possible that changes in estrogen levels can account for the increased risk of OA in women, it is a complicated relationship that has yet to be fully elucidated.

Several studies have found that men have larger cartilage volume and thickness, and a larger subchondral bone/cartilage area than women after controlling for BMI (Otterness and Eckstein 2007; Maleki-Fischbach and Jordan 2010). This relationship has also been found in children. In a study with children aged 9-18 years (n=92), boys had greater cartilage thickness and volume than girls, after accounting for body weight, bone size, and physical activity (Jones et al 2000). There was a significant association between increased cartilage volume and vigorous activity for more than 20 minutes, so it was suggested that increased physical activity in young boys may account for cartilage differences (Jones et al 2000). These gender differences in cartilage may play a role in the initiation of knee OA, but more research in this area is required.

Psychosomatic influences may also mediate the greater proportion of women diagnosed with OA, as radiographic OA is common, but a diagnosis of OA relies on pain as well. Some studies have shown that women more commonly employ emotion-focused pain coping strategies and pain catastrophizing than men when dealing with pain or stressful events (Keefe et al 2000; France et al 2004). Emotion-focused pain coping and pain catastrophizing have been associated with higher pain in OA (Keefe et al 2000). However, when Keefe et al (2000) controlled for pain catastrophizing, gender differences in OA pain were eliminated. Increased risk of OA for females is well established, and although hypotheses exist to explain this, the reasons are still unclear.
1.2.3.ii. Modifiable Risk Factors

1.2.3.ii.a. Obesity

Obesity is a well-established risk factor for the development OA (Cooper et al. 2000; Hunter 2009; Elbaz et al. 2011; Goulston et al. 2011; Lee et al. 2013b; Riddle and Stratford 2013). A prospective cohort study revealed that losing weight (≥ 2 BMI units) was associated with a 50% decrease in risk of OA (Felson et al. 1992). Obesity can result in excessive mechanical demand and increased loading and forces on the knee joint, which can directly damage articular cartilage (Messier et al. 1996; Heijink et al. 2012). This has been demonstrated in animal studies where abnormal loads due to obesity result in alterations in the composition, structure, metabolism and mechanical properties of cartilage (Griffin et al. 2009; Guilak 2011). In addition, slower walking with short and wide steps, greater stance duration and other altered gait and joint biomechanics in obese adults may contribute to OA risk; however, the specific role of such changes in terms of OA is not fully understood (Guilak 2011; Runhaar et al. 2011). Obese individuals are also at a higher risk for hand OA, indicating that the relationship between obesity and OA is not just mechanical (Katz et al. 2010). Adipose tissue is a source of local and systemic inflammation and a number of adipokines associated with obesity (IL-6, IL-1, TNFa) have also been shown to influence cartilage biology (Katz et al. 2010; Issa and Griffin 2012). In addition, hypertension, hypercholesterolemia, and high blood glucose are associated with knee OA, independent of BMI, indicating that a common set of metabolic factors are involved in those diseases (Hart et al. 1995; Sowers et al. 2009, Issa and Griffin 2012). Obesity is a primary risk factor for knee OA, with strong evidence for the role of biomechanical and metabolic factors contributing to disease initiation and progression.

1.2.3.ii.b. Knee Injury

Knee injury is a strong risk factor for the development of knee OA due to direct damage to the tissues, disruption of biomechanics, and a large inflammatory response (Roos et al. 1995; Blagojevic et al. 2010; Litwic et al. 2013). It has been reported that 50-60% of patients with injuries to ligaments or menisci are diagnosed with OA 12-15 years later and that this relationship is not altered by surgical intervention (Roos et al. 1995; Jarvholm et al. 2005; Blagojevic et al. 2010). Anterior cruciate ligament (ACL) injury plus traumatic meniscus damage seems to be the strongest predictor of early joint cartilage changes and OA (Neuman et al. 2011;
Englund et al 2012). Indeed, in animal models ACL transection and damage to the meniscus is used to induce rapid and extensive OA-like damage to the joint (Goldring 2012).

Knee cartilage adapts to cyclic loading and thickens in areas of greatest loading, with the thickest cartilage found at the lateral facet of the tibia and thinnest cartilage on the medial facet (Vincent et al 2012). With knee injury, the meniscus or ligament loses its function and loading during weight bearing activities can occur in areas of cartilage that are not adapted (Vincent et al 2012). Loading in non-adapted regions has been shown to lead to cartilage fibrillation, loss of proteoglycans, increased surface friction, increased sheer stress, trabecular bone changes, subchondral bone lesions, joint malalignment, up regulation of catabolic factors, and cartilage degradation (McDougall 2006; Englund et al 2012; Vincent et al 2012).

The large immune and inflammatory response that occurs with injury could initiate a cycle of inflammation and degradation in the knee joint (McDougall 2006; Lotz 2010). Immediately following an injury, inflammatory mediators are released into the joint from nerves, synoviocytes, and vascular endothelium to orchestrate healing responses (McDougall 2006). The trauma itself and inflammatory mediators cause cell necrosis, collagen rupture, GAG loss, and rupture of blood vessels in the joint capsule, synovial membrane, menisci, or subchondral bone that leads to intraarticular bleeding (Lotz 2010). Intraarticular bleeding promotes ECM loss and leads to synovial cell hypertrophy and synovitis (Hooiveld et al 2003; Lotz 2010). Shortly thereafter, there is an activation of viable cells to respond to the trauma, which also includes the generation of ROS, matrix degrading enzymes, and more inflammatory mediators (Marks and Donaldson 2005; Lotz 2010). In joint injury, there is also an increase of nociceptors and activation of silent nociceptors (McDougall and Linton 2012). Injured regions in healed joints display truncated and varicose nerves and have been reported to be full of inflammatory neuropeptides including, substance P and calcitonin gene related peptide (CGRP), which have been shown to increase pain responses and contribute to the inflammation (McDougall and Linton 2012).
1.2.3.ii.c Physical Activity

The role of physical activity as a risk factor for knee OA is controversial (Ding et al 2010; Barbour et al 2014). There are studies showing protective (Hart et al 1999), damaging (Felson et al 1997; Cheng et al 2000; Cooper et al 2000), and no (Felson et al 2007; Barbour et al 2014) effects, as well as increasing risk with different levels of physical activity (McAlindon et al 1999; Lin et al 2013). Importantly, the lack of a standard definition of physical activity or method of measuring levels makes quantifying risk difficult. Examining the risk of OA from sporting participation is also complicated because of the different types of sports, duration of participation, and frequent occurrence of joint injuries (Kujala et al 1995). Repetitive, excessive forces on the knee joint from intense exercise or sport participation may compromise structural integrity of the cartilage and promote degradation (Lin et al 2013). The risk of OA development does seem to be moderately increased with sporting participation, which could be mediated through associated joint injury (Bennell et al 2011b). However, joint loading and movement is needed for bone and joint health, and therefore walking or other low impact activities may be protective for knee OA (Felson et al 2007; Barbour et al 2014). In older adults without knee OA or previous knee injuries, vigorous activity was associated with increased cartilage volume and regular walking was associated with reduced risk of BMLs (Racunica et al 2007). The risk of knee OA with physical activity is most likely only present with high levels or risky types of exercise and is different between different ages, types of activities and genetics.

1.2.3.ii.d. Diet

The role of diet as a risk factor for OA is also controversial. Low intake of vitamins has been examined as potential risk for the initiation of OA. Vitamin D has been most extensively studied, as it has biological functions in cartilage, bone and muscle (McAlindon et al 1996b; Tetlow and Woolley 2001; Ding et al 2009). However, cross-sectional studies have not found associations between serum levels of 25(OH)D and radiographic knee OA (Al-Jarallah et al 2011; Muraki et al 2011). Furthermore, in 3 large, prospective cohort studies, baseline intake of vitamin D and serum levels of 25(OH)D were not related to incident knee OA, 6.5 to 22 years later (McAlindon et al 1996b; Bergink et al 2009; Konstari et al 2012). The role of ROS in the pathogenesis of OA has led to the hypothesis that high intake of antioxidant micronutrients could help to protect against OA by mediating oxidative damage (Jordan et al 2004a). An inverse association between
fruit intake and BMLs was found in healthy adults, suggesting that fruit constituents, including antioxidants, could have a role in protecting against knee structural damage (Wang et al 2007). Along with its antioxidant actions, vitamin C has a role in collagen synthesis, which may increase its relevance to OA. Vitamin E is a potent chain-breaking antioxidant and protector against lipid peroxidation (Jordan et al 2004a; Jeon et al 2013). However, baseline intake of vitamin C and E was not associated with incidence of OA 6.5 years later (McAlindon et al 1996a). The role of diet as a risk factor for the initiation of OA remains controversial and the complex interplay of bioactive components in whole foods and other lifestyle factors associated with diet quality complicate analysis.

1.2.4 Measuring OA and the Progression of OA

Measuring OA and its progression is a significant challenge in OA research and clinical practice. In the research setting, the disease can be defined radiographically but symptoms are necessary for medical diagnosis. Unfortunately, there is a weak correlation between radiographic and symptomatic evidence in OA (Dieppe and Lohmander 2005). Importantly, this limits the ability to measure response to a novel treatment. In 1996, the Outcome Measures in Rheumatology (OMERACT) group recommended that all clinical trials include the assessment of pain, physical function, patient global assessment and for studies longer than 1 year, imaging (Altman et al 1996). Quality of life, physician global assessment, inflammation, biological markers, time to surgery, and analgesic consumption were also included as potential endpoints (Altman et al 1996).

1.2.4.i. Imaging

1.2.4.i.a Radiography/X-rays

Radiography is the main method in epidemiology and research to define and measure OA (Petersson 1996; Lawrence et al 1998). Radiographs can visualize the space between bones, osteophytes, subchondral cysts, and bone sclerosis (Guermazi et al 2011). Cartilage cannot be visualized, so as an indirect measure, the space between the joint and JSN is used (Hayes et al 2005). The Kellgren-Lawrence (KL) scale from 0-4 is used to define severity of OA, based on JSN and the presence of osteophytes (Ding et al 2010). However, in some studies, KL scores are
based on JSN alone and a separate osteophyte score is given, making comparisons between studies challenging (Altman et al 2007; Neogi et al 2009). Various positions can be used when taking a knee x-ray, but in research, the gold standard is the standing (i.e. weight bearing) antero-posterior with knees fully extended (Altman et al 1996). However, only the medial and lateral compartments can be viewed and the patello-femoral joint is missed (Hayes et al 2005; Bedson and Croft 2008). It has also been postulated that improvements in joint pain during a clinical trial may alter the ability of the patient to extend the knee, changing joint position and altering JSN, without changing actual cartilage structure (Mazzuca et al 2002). Another limitation of radiographs is that they are not sensitive to change. It has been shown that when radiographic OA is detected, at least 10% of knee cartilage has already been lost (Jones et al 2004).

Radiographs do not correlate well with joint symptoms and, as such, cannot be used alone to evaluate OA patients (Bedson and Croft 2008). A number of cross-sectional studies have found weak associations between radiographic OA and pain (Hart et al 1999; Hannan et al 2000; Barker et al 2004; Bedson and Croft et al 2008). Structural joint damage most likely predisposes the joint to pain, but the severity of the joint damage bears little relation to the severity of the pain experienced (Dieppe and Lohmander 2005). However, it has been argued that accounting for factors that affect pain leads to strong associations between KL score and pain. Neogi et al (2009) controlled for psychosocial and genetic factors by comparing knees within a participant and found a strong correlation between pain and KL score. Similarly, Pereira et al (2013) observed a strong correlation when controlling for depressive symptoms. Despite limitations, radiography still remains the main method to define OA in research settings.

**1.2.4.i.b. MRI**

MRI is a powerful, non-invasive imaging tool that overcomes many of the limitations of the radiograph as all joint structures are visualized. Additionally, joint abnormalities can be detected before changes can be visualized on a radiograph allowing for the tracking of early joint abnormalities to OA (Spector and Cooper 1993; Colwell et al 2001; Amin et al 2005; Hayes et al 2005; Ding et al 2007). Different MRI techniques allow for examination of many types joint abnormalities and biochemical properties of cartilage (Hayes et al 2005) (Table 1.4). However, interpreting MRI images require fully trained and experienced readers to be able to distinguish between signal abnormalities from pathological changes (Guermazi et al 2011; Hayashi et al
The relevance of MRI outcomes to symptomatic knee OA is not clear. In systematic reviews of MRI outcomes and knee pain, just over half of the studies demonstrated statistically significant associations (Yusuf et al 2010; Hunter et al 2013). BMLs and synovitis/effusion have the strongest evidence for association with pain (Yusuf et al 2011). However, joint lesions on MRI are common in individuals with no knee pain and KL scores of 0, and it is unknown if these are pre-OA lesions or innocuous tissue changes that occur with aging (Hayes et al 2005; Hunter et al 2013). Standardization of MRI outcome measurements and defined parameters for knee OA is required in this area (Hayes et al 2005; Guermazi et al 2011).

Table 1.4 Joint structures and their endpoint and outcomes visualized with MRI

<table>
<thead>
<tr>
<th>Joint Structure Abnormality</th>
<th>Endpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cartilage defects</td>
<td>Graded and based on location, size, severity</td>
</tr>
<tr>
<td>Cartilage volume or surface area</td>
<td>Measured in millimeters</td>
</tr>
<tr>
<td>Bone marrow lesions</td>
<td>Presence of, size, site, severity</td>
</tr>
<tr>
<td>Osteophytes</td>
<td>Length</td>
</tr>
<tr>
<td>Meniscal or ligament tears</td>
<td>Graded on severity</td>
</tr>
<tr>
<td>Joint effusion</td>
<td>Presence of fluid, small-large</td>
</tr>
<tr>
<td>Synovial cysts</td>
<td>Presence of, small-large</td>
</tr>
<tr>
<td>Synovitis</td>
<td>Presence of, mild to marked</td>
</tr>
</tbody>
</table>


Although MRI is more sensitive and can visualize cartilage, radiography is widely available, has better defined outcome measures, takes less time and has a much lower cost (Guermazi et al 2011). Finding strong correlations between pain and either imaging method will be difficult until potential cofounders that affect pain are understood and accounted for.

1.2.4.ii. Clinical Symptoms

Symptoms of OA can vary greatly among patients and include joint pain and stiffness, swelling, decreased function, and cracking noise with joint movement (Dieppe and Lohmander 2005; Alshami 2014). Pain is the main concern for those with OA, what drives health care use, and plays a large role in deciding treatment options (Hawker et al 2011; Bombardier et al 2012). There are countless ways to measure clinical symptoms of OA and many different methods are used in the literature. In addition, quality of life, sleep quality, mood, pain coping strategies, participation in valued experiences, and others are recognized as contributing to the full OA experience and are being measured in research settings (Hawker et al 2011; Lane et al 2011).
1.2.4.ii.a. Pain

Pain is a subjective experience with a neurophysiologic basis (Hunter et al 2009). It is not well understood but it is complex and influenced by a multitude of factors (Neogi et al 2009). Measures of pain can include the severity, intensity, frequency, in activity, and impact on mobility, mood, sleep, and quality of life (Alshami 2014). In OA research, pain can be measured with a simple presence or absence of pain question, pain rating scale, or questionnaire (Neogi et al 2009; Allen et al 2010). Multiple methods are used in the literature, which can make comparisons between studies difficult. Table 1.5 presents pain assessment methods used in OA research. There are a number of multidimensional pain questionnaires available, which are proposed to provide a more comprehensive assessment of pain and overall response to a treatment in OA (Dworkin et al 2011). Dworkin et al (2011) examined responsiveness of various outcome measures in OA clinical trials and despite heterogeneity, found non-significant differences in favour of using the WOMAC pain scale. Overall, there is no pain measure recommended for use above others. Clinicians and researchers should choose a questionnaire specific to their purpose (Hawker et al 2011).
Table 1.5 Different Methods of Assessing Pain in Individuals with Knee OA

<table>
<thead>
<tr>
<th>Pain Assessment</th>
<th>Intended Population</th>
<th>Number and Type of Questions</th>
<th>Outcome</th>
<th>Examples of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>One-dimensional Pain Assessments</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain in the last # of days, weeks, or months</td>
<td>General</td>
<td>Yes/No</td>
<td>Pain or not</td>
<td>Neogi et al 2009</td>
</tr>
<tr>
<td>Visual analog scale (100mm) or numerical rating scale</td>
<td>General</td>
<td>1</td>
<td>Score (0-100)</td>
<td>Glass et al 2014, Allen et al 2010, Riecke et al 2010, Tonelli et al 2011</td>
</tr>
<tr>
<td><strong>Multidimensional Pain Questionnaires</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SF-36 - Bodily Pain Subscale</td>
<td>General</td>
<td>2 Likert questions about pain intensity as a subscale from the medical outcomes SF-36</td>
<td>Score (0-100), higher score = lower pain</td>
<td>Tonelli et al 2011; Terwee et al 2006</td>
</tr>
<tr>
<td>McGill Pain Questionnaire</td>
<td>Chronic pain conditions</td>
<td>4 subscales; 78 pain descriptors are categorized into subclasses</td>
<td>A score is assigned to each pain descriptor</td>
<td>Creamer et al 2000; Hughes and Carr 2002</td>
</tr>
<tr>
<td>Arthritis Impact Measurement Scale – pain subscale</td>
<td>Arthritis</td>
<td>5 questions (Likert scale)</td>
<td>Score (0-25), usually scales are combined</td>
<td>France et al 2004, Keefe et al 2000</td>
</tr>
<tr>
<td>Lequesne Algofunctional Index</td>
<td>OA</td>
<td>3 subscales with 10 questions</td>
<td>Score (0-24)</td>
<td>Oben et al 2009; Case et al 2003</td>
</tr>
</tbody>
</table>

VAS = Visual analog scale; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index.

1.2.4.ii.b. Physical Function

Physical function can be defined as the ability to move around and perform daily activities (Terwee et al 2006; Bennell et al 2011a; Dobson et al 2013). There is no gold standard of assessment and debate exists about the use of self-reported or performance-based measures for assessing physical function (Terwee et al 2006). Self-reporting is easier, less time consuming and
not influenced by observer bias but it can be influenced by culture, language, and education level and may not reflect actual ability of the participant (Dobson et al 2013). Performance-based measures examine functioning in an artificial situation, can be influenced by motivation to participate, and may not reflect how the person functions in their own environment (Terwee et al 2006). Performance based measurements examine what a patient can do in a specific timed/distance test, while the self-report is what the patient thinks they can do, and it has been suggested that pain plays a larger role in that aspect (Terwee et al 2006). Table 1.6 presents different assessment methods of physical function used in OA research. Assessment of physical function is complex and overall self-reported and performance-based outcome measures capture different, but complementary, aspects of physical function and should both be included when assessing physical function (Dobson et al 2013; Stratford and Kennedy 2006). An international, multidisciplinary expert advisory group reviewed performance-based measures and recommended that the 30-s chair-stand test, 40-m fast-paced walk test, a stair-climb test, timed up-and-go test and 6-min walk test be used for assessment of physical function in hip and knee OA (Dobson et al 2013). There is no consensus on which self-reported questionnaire of physical function is superior, as it depends on the population being studied and the purpose of the assessment.

Table 1.6 Performance-Based and Self-Reported Physical Function Assessments

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Measures</th>
<th>Outcome Variable</th>
<th>Examples of Use</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Performance Based</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chair stand test</td>
<td>Assesses ability to rise and then sit back down in a chair, as well as lower body strength and power</td>
<td>Count (#/repetitions performed in 30 seconds) with greater values indicating better performance</td>
<td>McAlindon et al 2013</td>
</tr>
<tr>
<td>Self-paced walk test</td>
<td>Assesses time taken to walk short distances (&lt;50 meters)</td>
<td>Time (seconds) with lower values indicating better performance</td>
<td>McAlindon et al 2013</td>
</tr>
<tr>
<td>Stair-climb test</td>
<td>Assesses ability to ascend and descend a flight of stairs, as well as strength, power, and balance</td>
<td>Time (seconds) with lower values indicating better performance</td>
<td>Rejeski et al. 1995, Messier et al 2004, Miller et al 2006</td>
</tr>
<tr>
<td>Timed up and go</td>
<td>Assesses ability to rise from a stool or chair</td>
<td>Time (seconds) with lower values indicating better performance</td>
<td>Oliveira et al</td>
</tr>
<tr>
<td>Test</td>
<td>Description</td>
<td>Score</td>
<td>Reference(s)</td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td>-------</td>
<td>--------------</td>
</tr>
<tr>
<td>6-minute walk test</td>
<td>Assesses endurance and ability to walk longer distances</td>
<td>Distance (meters) with a greater distance indicating better performance</td>
<td>Rejeski et al. 1995, Messier et al 2004, Miller et al 2006</td>
</tr>
<tr>
<td><strong>Self-Reported</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee Outcome Survey-Activities of Daily Living Scale (KOS-ADL)</td>
<td>Examines symptoms and functional limitations in daily living caused by knee pathologies with 17 questions and 2 Likert subscales</td>
<td>Score (0-100) greater score means no knee related symptoms or functional limitations</td>
<td>Collins et al 2011</td>
</tr>
<tr>
<td>Arthritis Impact Measurement scale (AIMS)</td>
<td>Measures different aspects of the impact of arthritis, including physical function. 28 questions in 6 domains on a 5-point Likert scale</td>
<td>Score (0 – 10)</td>
<td>Tonelli et al 2011</td>
</tr>
<tr>
<td>Knee injury and Osteoarthritis Outcome Score (KOOS)</td>
<td>Evaluate knee problems in young-middle aged people with OA following a knee injury with 42 Likert questions</td>
<td>Score (0-100)</td>
<td>Tonelli et al 2011, Riecke et al 2010</td>
</tr>
<tr>
<td>WOMAC Physical Function Subscale</td>
<td>17 questions designed for adults with hip or knee OA</td>
<td>Score (0-68, Likert or 0-1700, VAS)</td>
<td>Messier et al 2004, Miller et al 2006; Wluka et al 2002; McAlindon et al 2013</td>
</tr>
</tbody>
</table>

VAS = Visual analog scale; WOMAC = Western Ontario and McMaster Universities Osteoarthritis Index.

### 1.2.4.iii. Biomarkers

A biomarker is a measurable characteristic evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to therapeutic interventions (van Spill et al 2010). Molecules that are released during metabolism of joint tissues could serve as biomarkers of the OA process. The ‘BIPED’ framework, proposed by Bauer et al (2006) is
widely used and classifies the roles of biomarkers. A biomarker can belong in more than one of the categories.

- **Burden of disease** biomarkers measure the extent or severity of OA by correlating levels to extent of severity of OA.
- **Investigative** biomarkers capture markers that do not meet criteria for other categories.
- **Prognosis** biomarkers indicate new or worsening OA by examining association between marker and onset or progression of OA.
- **Efficacy** biomarkers indicate a response to a treatment.
- **Diagnostic** biomarkers are used to differentiate someone with OA from someone without.

Currently, there are no biomarkers accepted for use in any of the BIPED categories (Felson 2014). In 2011, the Osteoarthritis Research Society International (OARSI) and the United States’ Food and Drug Association (FDA) recommended further investigation of 16 urine and serum biomarkers that reflect different tissues and processes involved in OA (Table 1.7). No biomarkers were considered appropriate to serve as the primary outcome measure in clinical trials, although the continued study of biomarkers was encouraged. Also, as no single biomarker can represent all the complex biological changes occurring in the joint, it was recommended that a panel of biomarkers should be measured in research settings. As presented in Table 1.7, the selected biomarkers represent the main tissues affected in OA (i.e. cartilage, bone, synovial membrane) as well as different pathological processes (i.e. collagen degradation and synthesis).
Table 1.7 OARSI recommended biomarkers for continued investigation

<table>
<thead>
<tr>
<th>Biomarker*</th>
<th>Pathological Process</th>
<th>Proposed BIPED Classification#</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urinary Biomarkers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX-II</td>
<td>Type II collagen degradation</td>
<td>B, P, E, D</td>
</tr>
<tr>
<td><strong>Urinary or Serum Biomarkers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NTX-I</td>
<td>Bone resorption</td>
<td>P, E</td>
</tr>
<tr>
<td>CTX-I</td>
<td>Bone resorption</td>
<td>B, P, D</td>
</tr>
<tr>
<td>C1, 2C</td>
<td>Type I and II collagen degradation</td>
<td>D</td>
</tr>
<tr>
<td>C2C</td>
<td>Type II collagen degradation</td>
<td>E, D</td>
</tr>
<tr>
<td>Coll2-1</td>
<td>Type II collagen degradation</td>
<td>D, B</td>
</tr>
<tr>
<td><strong>Serum Biomarkers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COMP</td>
<td>Cartilage degradation</td>
<td>B, P, D</td>
</tr>
<tr>
<td>HA</td>
<td>Synovitis</td>
<td>B, P, E, D</td>
</tr>
<tr>
<td>PIIANP</td>
<td>Type II collagen synthesis</td>
<td>B, P, E, D</td>
</tr>
<tr>
<td>CPII</td>
<td>Type II collagen synthesis</td>
<td>D</td>
</tr>
<tr>
<td>CS-846</td>
<td>Aggrecan synthesis/turnover</td>
<td>P</td>
</tr>
<tr>
<td>MMP-3</td>
<td>Enzyme involved in tissue breakdown</td>
<td>E</td>
</tr>
</tbody>
</table>

Modified from Kraus et al 2011

#B = burden of disease; I = investigative; P = prognosis; E = efficacy; D = diagnostic
*C1, 2C= collagenase-generated neoepitope of types I and II collagens; C2C = collagenase-generated neoepitope of type II collagen; COMP = cartilage oligomeric matrix protein; CPII = type II collagen carboxy-propeptide; CTX-I = carboxy-telopeptide of type I collagen; CS-846 = aggrecan chondroitin sulfate 846 epitope; CTX-II = carboxy-telopeptide of type II collagen; HA = hyaluronan; NTX-I = N-telopeptide of type I collagen; MMP-3 = metalloproteinases-3.

Despite the promise of biomarkers, there are limitations. Many of these molecules arise from multiple body tissues and serum levels may not be representative of degradation in the target joint. In addition, measuring biomarkers from synovial joint fluid is difficult as collection is challenging and dilution can be uncontrolled (Martel-Pelletier et al 2008). Serum and urine concentrations of biomarkers can also be affected by synovial clearance as well as metabolism, degradation, and clearance from the circulation, which is not currently understood (Martel-Pelletier et al 2008). Age, gender, ethnicity, BMI, physical activity, diurnal variation, and other physiological processes or diseases may also affect biomarker levels complicating their utility (Martel-Pelletier et al 2008). However, biomarkers of OA deepen the understanding of disease progression and their inclusion in research will hopefully aid in the discovery of new treatments.
1.2.5. Non-Surgical Treatment and Management of OA

There is no cure or disease modifying therapy for OA. Therefore treatment goals include pain management and maintenance or improvement of physical function (Hochberg et al 2012; McAlindon et al 2014). International, evidence-based management guidelines recommend a combination of non-pharmacological and pharmacological options (Hunter 2009). In general, management of knee OA should follow a sequential, pyramidal approach where effective and less risky options are used by all individuals with knee OA and only where these options fail, more risky options are utilized (Dieppe and Lohmander 2005; Hunter 2009), as displayed in Figure 1.5. Invasive procedures like intraarticular injections of corticosteroids or hyaluranon are reserved for individuals who do not respond to other treatment options (Dieppe and Lohmander 2005). Total joint replacement is an effective treatment for end-stage OA, but only suitable for those who have exhausted available conservative interventions (Choong and Dowsey 2011; Dowsey et al 2014).
Management of knee OA should follow a sequential, pyramidal approach where options at the bottom of the pyramid are suitable for all with OA, simple, non-surgical options are suitable for some, advanced non-surgical options are suitable for few with OA and surgery is the last, least desirable options. Modified from Dieppe and Lohmander 2005.

1.2.5.i. Management Recommendations for All Individuals with Knee OA

OA guidelines are consistent in stating that education and self-management, weight loss (if overweight/obese), and exercise should be included as core management practices for knee OA (Hochberg et al 2012; AAOS, 2013; Fernandes et al 2013; McAlindon et al 2014).

1.2.5.i.a. Education and Self-Management

Patient-education programs are a method for achieving self-management. This describes an individual’s ability to manage the symptoms, treatment, physical, and psychological consequences of a chronic condition (Du et al 2011). Although education is widely recognized as an important aspect of disease management and included in all management guidelines, evidence from the literature is not overwhelmingly positive. In a meta-analysis of 14 randomized controlled trials (RCTs) examining effects of self-management programs in OA patients, a significant effect size (ES) for pain relief of 0.06 (95% confidence interval (CI) = 0.02 to 0.10) was found (Chodosh et al 2005). In 2013, a meta-analysis with 29 RCTs comparing self-management programs to attention control, usual care, information alone, or another intervention
concluded that low to moderate quality evidence exists for beneficial effects of self-management programs in persons with OA (Kroon et al 2014). There seems to be a small benefit of self-management and education for pain in adults with OA. Even with small effects, education should be incorporated into OA management plans, as there is no risk of adverse effects.

1.2.5.i.b. Weight Loss/Weight Maintenance

Although weight loss is included in all the guidelines, weight loss as a treatment for knee OA has not been extensively examined in the literature. A meta-analysis examined 4 weight-loss RCTs in knee OA patients and found that 5% weight reduction was associated with insignificant improvements pain (ES = 0.20, 95% CI = 0 to 0.39), but significant improvements in physical function (ES = 0.23, 95% CI = 0.04 to 0.42) (Christensen et al 2007). Miller et al (2006) presented similar findings in older, obese adults with knee OA where greater weight loss following a 6-months intensive weight loss program (dietary changes, nutrition and physical activity education, and supervised exercise 3 days/week) resulted in better physical function scores, 6MWT distances, and faster SCT times, when compared to a weight stable group. In an 18-week RCT examining weight loss from diet, exercise, diet plus exercise, or a healthy control group in overweight or obese adults with knee OA, only the diet plus exercise group had significant reductions in pain and improvements in physical function (Messier et al 2004). The diet-only intervention group lost more weight than other groups, but no significant differences in pain or physical function between diet only and healthy lifestyle group were present (Messier et al 2004). Alternatively, in obese patients with knee OA, a calorie-restricted intensive weight loss RCT resulted in reductions in pain and physical function scores with no difference between very-low energy diet (415 kcal/day) and low-energy diet (810 kcal/day) groups, given their respective diet for 8 weeks, followed by 1,200 kcal/day for 8 weeks in both groups (Riecke et al 2010). However, in a longer study in obese adults with knee OA, an intensive low energy diet group lost significantly more weight than a minimal attention control group, but there were no significant differences in pain at 1 year (Bliddal et al 2011). As described, education programs, exercise, and diet interventions are used in weight loss studies, which makes it difficult to decipher the effect of each intervention alone. To examine weight loss alone and eliminate the effect of attention and education that comes in an intervention study, Riddle and Stratford (2013) examined longitudinal data from 2 large cohort studies following adults with knee OA for 30-
months or 3 years. A significant dose-response relationship was found between body weight changes and changes in pain and physical function scores for weight loss. Overall, weight loss for those who are overweight represents an important method to controlling pain and improving function in patients with knee OA.

1.2.5.i.c. Exercise

Although weight loss is often an outcome of exercise interventions trials, there are also benefits of exercise without weight loss. A number of systematic reviews and meta-analyses have been published in this area examining aerobic and strengthening exercises for the treatment of knee OA (Table 1.8). All meta-analyses concluded that exercise has a small to moderate positive effect on pain and physical function in adults with knee OA. Heterogeneity between populations (age, gender, BMI, disease duration) and exercise type, duration, frequency, and intensity existed in all meta-analyses and limited the strength of conclusions. Examining exercise ‘dose’ is difficult as it is a factor of frequency and intensity, and the duration of intervention varies widely between studies. In addition, blinding is not possible in exercise studies and most have been less than 6 months, so conclusions cannot be made about long-term benefits. There was some evidence of greater effects with one-type exercise programs over mixed (Jamtvedt et al 2008; Juhl et al 2014); however, no differences between types of exercise (i.e. walking vs strengthening) was found. The fact that a specific type of exercise is not beneficial over others can be seen as a positive, as OA patients can choose an exercise program that they prefer, which may benefit adherence, which is known to be an important factor in maintaining positive effects of exercise (Roddy et al 2005). Exercise should be an important part of an OA management program as improvements are observed with a variety of exercises.
**Table 1.8** Results from Systematic Reviews and Meta-Analysis Examining Exercise Interventions in Knee OA

<table>
<thead>
<tr>
<th>Reference (Number of studies included)</th>
<th>Exercises Included</th>
<th>Results ES or SMD* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roddy et al 2005 (13)</td>
<td>Aerobic walking and quadriceps strengthening</td>
<td>Aerobic Walking</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain: ES = 0.52 (0.34 to 0.70)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF: ES = 0.46 (0.25 to 0.67)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Quadriceps Strengthening</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain: ES = 0.32 (0.23 to 0.42)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF: ES = 0.32 (0.23 to 0.41)</td>
</tr>
<tr>
<td>Devos-Comby et al 2006 (16)</td>
<td>Strength, walking, aerobic, balance, and flexibility</td>
<td>PF: ES = 0.29 (0.23 to 0.36)</td>
</tr>
<tr>
<td>Fransen and McConnell 2008 (32)</td>
<td>Strength, aerobic, walking, balance, &amp; tai chi</td>
<td>Pain: SMD = 0.40 (0.30 to 0.50)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF: SMD = 0.37 (0.25 to 0.49)</td>
</tr>
<tr>
<td>Jansen et al 2011 (12)</td>
<td>Strength training and mixed exercise (strength,</td>
<td>Strength training:</td>
</tr>
<tr>
<td></td>
<td>aerobic, and range of motion exercises)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain: ES = 0.38 (0.23 to 0.54)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF: ES = 0.41 (0.17 to 0.66)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mixed exercise:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain: ES = 0.34 (0.19 to 0.49)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF: ES = 0.25 (0.03 to 0.48)</td>
</tr>
<tr>
<td>Juhl et al 2014 (48)</td>
<td>Aerobic, resistance, performance, mixed</td>
<td>Aerobic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain: SMD = 0.67 (0.39 to 0.94)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF: SMD = 0.56 (0.24 to 0.87)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Resistance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain: SMD = 0.62 (0.45 to 0.79)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF: SMD = 0.60 (0.37 to 0.83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Performance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pain: SMD = 0.48 (0.11 to 0.85)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PF: SMD = 0.56 (0.14 to 0.98)</td>
</tr>
<tr>
<td>Tanaka et al 2014 (17)</td>
<td>Strength and aerobic</td>
<td>Pain: SMD = 0.57 (0.40 to 0.74)</td>
</tr>
</tbody>
</table>

*Physical function is self-reported.

CI = confidence interval; ES = effect size; PF = physical function; SMD = standard mean difference.

### 1.2.5.ii. Management Recommendations for some with Knee OA

The guidelines do not completely agree on their recommendations for pharmaceuticals, biomechanical interventions and aids, and dietary constituents and natural health products (NHPs). Dieppe and Lohmander (2005) suggest that these interventions should be used in a supervised fashion in those where safer methods have failed (Figure 1.5).
1.2.5.ii.a. Pharmaceuticals

Despite management guidelines, medication is the primary intervention used by OA patients. Medication can promote function by alleviating symptoms, but also can reduce function by causing drug-related morbidity and mortality (Ross et al 2001). Results from RCTs have revealed that nonsteroidal anti-inflammatory drugs (NSAIDS) are more effective at relieving pain in OA than acetaminophen but are also associated with a greater risk of adverse effects. Therefore, acetaminophen remains the first line pharmaceutical option for pain management (McAlindon et al 2014). Despite the widespread use of the drug and agreement between guidelines, there is not much data to support acetaminophen for pain relief in OA. As presented in Table 1.9, three meta-analyses have found small effects of acetaminophen over placebo. However, Bannuru and McAlindon (2010) reported an increase risk for gastrointestinal (GI) adverse effects with acetaminophen consumption. When examining epidemiological data, Garcia Rodriguez et al (2001) reported that users of acetaminophen at doses greater than 2 g had a greater risk of upper GI complications (relative risk = 3.7, 95% CI = 2.6 to 5.1). Topical NSAIDs should be used before oral NSAIDs. A meta-analysis in 2004 found that topical NSAIDS were effective at relieving pain only up to 2 weeks (Lin et 2004); however, later meta-analyses found pain relief for topical NSAIDS in RCTs longer than 4 weeks (Biswal et al 2006; Towheed 2006). There is a lower risk of GI adverse effects from topical NSAIDS when compared to oral NSAIDS (Lin et al 2004; Chou et al 2006), but increased risk of local adverse effects, like burning, rash and itch (Towheed 2006; Chou et al 2006). Non-selective NSAIDS and oral COX-2 inhibitors are widely used to manage OA pain and have been shown to be effective over placebo and acetaminophen in meta-analyses (Table 1.9). Despite these superior effects, all studies, as well as management guidelines, highlight the increased risk for serious gastrointestinal ulcers with bleeding, perforation, or obstruction, renal dysfunction, cardiovascular events, and the risk of death (Zhang et al 2004; Towheed 2006; Chou et al 2006; Argooff and Gloth 2012; Hochberg et al 2012; McAlindon et al 2014). Generally, opioids are only recommended in situations where there are serious or absolute contraindications to NSAIDS (Adebajo 2012). A systematic review revealed moderate pain relieving effect of codeine,
oxycodone, and morphine over placebo but patients were 4 times as likely to withdraw from studies because of adverse events (Nuesch et al 2009; McAlindon et al 2014).

Despite the widespread use of pharmaceuticals for pain relief in OA, the evidence from meta-analyses reveals mild to moderate benefits and risk of adverse events, especially in older, chronic users (Zhang et al 2004; Craig et al 2014). Only OA patients at low-risk for adverse events should use pharmaceuticals, and short-term use and conservative dosing should be followed as well as individual management strategies based on patient history, co-morbidities and risk for adverse events (Hochberg et al 2012; McAlindon et al 2014).

**Table 1.9** Results from Systematic Reviews and Meta-Analysis Examining Pain Relieving Effects of Pharmaceutical Products in Adults with Hip or Knee OA

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of RCTs included</th>
<th>ES or SMD (95% CI) for pain relief</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acetaminophen vs. Placebo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhang et al 2004</td>
<td>2</td>
<td>ES = 0.21 (0.02 to 0.41)</td>
</tr>
<tr>
<td>Towheed et al 2006</td>
<td>7</td>
<td>SMD = 0.13 (0.04 to 0.22)</td>
</tr>
<tr>
<td>Bannuru and McAlindon 2010</td>
<td>10</td>
<td>ES = 0.18 (0.11 to 0.25)</td>
</tr>
<tr>
<td><strong>Topical NSAIDs vs. Placebo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lin et 2004</td>
<td>13</td>
<td>ES = 0.40 (0.15 to 0.65)*</td>
</tr>
<tr>
<td>Biswal et al 2006</td>
<td>4</td>
<td>ES = 0.28 (0.14 to 0.42)</td>
</tr>
<tr>
<td>Towheed 2006</td>
<td>4</td>
<td>SMD = 0.33 (0.18 to 0.48)</td>
</tr>
<tr>
<td><strong>Oral NSAIDs vs. Placebo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bjordal et al 2004 (All oral NSAIDs)</td>
<td>23</td>
<td>ES = 0.32 (0.24 to 0.39)</td>
</tr>
<tr>
<td>Lee et al 2005 (Only COX-2 inhibitors)</td>
<td>15</td>
<td>ES = 0.44 (0.33 to 0.55)</td>
</tr>
<tr>
<td><strong>Oral NSAIDs vs. Acetaminophen</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zhang et al 2004</td>
<td>8</td>
<td>ES = 0.20 (0.10 to 0.30)</td>
</tr>
<tr>
<td>Wegman et al 2004</td>
<td>5</td>
<td>SMD = 0.33 (0.15 to 0.51)</td>
</tr>
<tr>
<td>Towheed 2006</td>
<td>10</td>
<td>SMD = 0.25 (0.17 to 0.33)</td>
</tr>
<tr>
<td>Verkleij et al 2011</td>
<td>15</td>
<td>ES = 0.29 (0.22 to 0.35)</td>
</tr>
<tr>
<td><strong>Opioids vs Placebo</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuesch et al 2009</td>
<td>22</td>
<td>SMD = 0.28 (0.20 to 0.35)</td>
</tr>
</tbody>
</table>

* This result was at 2 weeks; no significant difference was present at 4 weeks vs. placebo
CI = confidence intervals; COX-2 = cyclooxygenase 2; ES = effect size; NSAIDs = nonsteroidal anti-inflammatory drugs; RCTs = randomized controlled trials; SMD = standard mean difference.

1.2.5.ii.b. Biomechanical Interventions and Aids

Biomechanical interventions for knee OA include knee braces, knee sleeves, taping, foot orthoses and aids include canes, crutches and walkers. There is not much agreement between the

34
guidelines in this area. However, it is generally agreed upon that none of these are sufficient as stand-alone treatment. Meta-analysis and systematic reviews are limited by poor quality of trials and heterogeneity of interventions. There are multiple types of braces for knee OA, from a simple sleeve to an unloader brace that aims to change the distribution of the force on the knee (Page et al 2011). Insoles have been advocated as easy ways to decrease loading on a compartment of the knee and decrease pain (Page et al 2011). Two systematic reviews examined braces and insoles together and concluded that there is evidence of small beneficial effects on pain and function in adults with knee OA (Brouwer et al 2005; Raja and Dewan 2011). It was noted that long-term adherence is an important factors to consider and that longer high-quality trials are required. Alternatively, Wang et al (2012) examined insoles and shoes alone in a meta-analysis and did not find any effects on function in adults with knee OA. Taping the knee at the patella aims to realign the patella to reduce stress and unload painful soft tissues (Page et al 2011). A meta-analysis of 2 RCTs for taping did not suggest any beneficial effects on pain, function, or gait but pooled analysis was not possible due to differences in reporting (Wang et al 2012). In general, appropriate footwear, walking aids and assistive technologies to reduce pain and increase participation in activities for adults with knee OA is recommended and should be based on individual situations (Fernandes et al 2013).

1.2.5.ii.c. Natural Health Products/Supplements

With the lack of strong pain relieving effects of pharmaceuticals and the challenge of chronic pain in OA, there is a great opportunity for NHPs, oral supplement products, and dietary constituents in the management of OA (Elder et al 2012). There is a demand for these products, as arthritis is among the top 6 conditions for which NHPs and supplements are used (Lapane et al 2012). Evidence of the demand and potential for these products includes the fact that The Arthritis Society provides free educational guides including “Nutrition and Arthritis” and “Complementary and Alternative Therapies”.

A number NHPs and supplements have claimed to modify the course of OA and to control symptoms, but there is no consensus in the guidelines on the efficacy of these products. A large number and variety of products have been examined. The most commonly studied are glucosamine, chondroitin, capsaicin, avocado/soybean unsaponifiables (ASU), and rosehip...
Glucosamine and chondroitin are constituents of GAGs in the ECM of articular cartilage and have been used as therapeutic agents for OA since the 1980’s (McCarty 1994; Lee et al 2010). However, most OA management guidelines recommend against the use of glucosamine and chondroitin, based on small or non-significant ES in systematic reviews (Reichenbach et al 2007; Lee et al 2010; Wandel et al 2010), inconsistent results between industry and independent trials (Clegg et al 2006), and heterogeneity among studies (Vlad et al 2007; Wandel et al 2010). The use of glucosamine continues to rise despite a lack of evidence of its effectiveness (Block et al 2010). Capsaicin is a compound present in chili peppers that, when applied topically, causes acute pain and sensitivity by stimulating pain fibers and release of substance P in the skin (Mason et al 2004). Repeated applications desensitize pain fibers and, possibly, deplete substance P (Mason et al 2004). Meta-analyses in adults with OA and other chronic pain conditions have found moderate pain relieving effects of capsaicin over placebo (Zhang and Li Wan Po 1994; Mason et al 2004; de Silva et al 2011). However, capsaicin is associated with transient rash, itching, and burning, when compared to placebo. ASU mixtures are made up of unsaponifiable fractions of avocado and soybean oil and are suggested to have anti-inflammatory effects (Christensen et al 2008b). A systematic review and meta-analysis of short-term trials in adults with hip or knee OA found small benefit for ASU over placebo with an ES for pain relief of 0.39 (95% CI = 0.01 to 0.76) (Christensen et al 2008b). However, a recent meta-analysis revealed ASU supplementation up to 12 months resulted in pain 8 points lower than placebo (95% CI = 1 to 16) (Cameron and Chrubasik 2014). Powder made from the seeds and husks of the fruit of the wild-briar hedgerow rose have shown anti-inflammatory actions in in vitro studies (Christensen et al 2008a). A meta-analysis of 3 RCTs showed a benefit of rosehip powder over placebo with an ES of 0.37 (95% CI = 0.13 to 0.60) (Christensen et al 2008a). Data from rosehip studies is promising, but its efficacy and safety require confirmation in larger and longer RCTs. Chinese herbal mixture SKI306X is a purified extract from a mixture of 3 oriental herbal medicines (Clematis mandshurica, Trichosanthes kirilowiiia, and Prunella Vulagris) (Jung et al 2001). Pooling results from 2 studies demonstrated that treatment for 4 weeks resulted in pain reduction of 17.36 (95% CI = 12.15 to 22.57) compared to placebo (Cameron and Chrubasik 2014). A proprietary extract from Boswellia serata, 5-Loxin®, has shown anti-inflammatory actions in vitro and when results from 2 clinical trials carried out by the same authors were pooled, results revealed that treatment with...
5-Loxin® for 90 days resulted in a significant decrease in pain of 16.94 (95% CI = 11.50 to 22.39) compared to placebo (Cameron and Chrubasik 2014). For the remainder of the products described in Table 1.10, no meta-analyses were available due to low number or quality of studies, heterogeneity between trials, and different preparation of extracts (Cameron and Chrubasik 2014). For almost all products listed the proposed mechanism for beneficial effects in OA stems from in vitro anti-inflammatory and anti-oxidant mechanisms.

**Table 1.10** Selected references from commonly studied NHP and supplement products for the treatment of OA

<table>
<thead>
<tr>
<th>Product</th>
<th>Meta-Analysis Results</th>
<th>Proposed Mechanism</th>
<th>Comment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucosamine</td>
<td>Inconclusive</td>
<td>Anti-inflammatory and suggested cartilage sparing</td>
<td>Inconsistent results between industry and independent trials and significant heterogeneity between trials</td>
<td>Lee et al 2010; Vld et al 2007; Wandel et al 2010</td>
</tr>
<tr>
<td>Chondroitin</td>
<td>Inconclusive</td>
<td>Anti-inflammatory and suggested cartilage sparing</td>
<td>Inconsistent results between industry and independent trials and significant heterogeneity between trials</td>
<td>Lee et al 2010; Reichenbach et al 2007; Wandel et al 2010</td>
</tr>
<tr>
<td>Capsaicin (topical)</td>
<td>Moderate pain relieving effects over placebo</td>
<td>Desensitization of pain fibers</td>
<td>Associated with transient rash, itching, and burning</td>
<td>Zhang and Li Wan Po 1994; Mason et al 2004; de Silva et al 2011</td>
</tr>
<tr>
<td>Avocado/soybean unsaponifiables</td>
<td>ES for pain relief = 0.39 (95% CI = 0.01 to 0.76)</td>
<td>Anti-inflammatory, anti-oxidant, anabolic and anti-catabolic in human chondrocytes</td>
<td>Minimum of 3 months supplementation suggested</td>
<td>Christensen et al 2008b; Cameron and Chrubasik 2014; Cameron et al 2009; Henrotin et al 2010</td>
</tr>
<tr>
<td>Rosehip powder</td>
<td>ES for pain relief = 0.37 (95% CI = 0.13 to 0.60)</td>
<td>Anti-inflammatory, anti-oxidant</td>
<td>Longer trials for confirmation of efficacy and safety required</td>
<td>Christensen et al 2008a; Cameron et al 2009</td>
</tr>
<tr>
<td>Chinese herbal mixture</td>
<td>Pain relief 17.36 (95%)</td>
<td>Anti-inflammatory</td>
<td>Modest improvements</td>
<td>Cameron and Chrubasik 2014</td>
</tr>
<tr>
<td><strong>Extract</strong></td>
<td>**CI = 12.15 to 22.57)</td>
<td><strong>Pain relief observed with 4 to 6 weeks supplementation</strong></td>
<td>** Longer trials for confirmation of efficacy and safety**</td>
<td><strong>Cameron and Chrubasik 2014</strong></td>
</tr>
<tr>
<td>------------------</td>
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<td>---------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td><strong>Boswellia serrata extract (5-Loxin®)</strong></td>
<td>Anti-inflammatory, analgesic</td>
<td>CI = 11.50 to 22.39</td>
<td>Modest improvements in pain, up to 12 weeks</td>
<td>Debbi et al 2011</td>
</tr>
<tr>
<td><strong>Methylsulfonylmethane</strong></td>
<td>Not available</td>
<td>Anti-inflammatory</td>
<td>Similar pain reducing effects as NSAIDs in short and long term trials</td>
<td>De Silva et al 2011</td>
</tr>
<tr>
<td><strong>S-adenosylmethionine</strong></td>
<td>Not available</td>
<td>Anti-inflammatory, anabolic actions in human chondrocytes</td>
<td>Evidence of pain relief from poorly designed studies with various types of extracts</td>
<td>Cameron et al 2009</td>
</tr>
<tr>
<td><strong>Ginger extract (Zingiber officinale)</strong></td>
<td>Not available</td>
<td>Anti-inflammatory, anti-oxidant</td>
<td>Inconclusive results from different products of the roots</td>
<td>Cameron and Chrubasik 2014; Cameron et al 2009</td>
</tr>
<tr>
<td><strong>Devils claw (Harpagphytum procumbens)</strong></td>
<td>Not available</td>
<td>Anti-inflammatory, anti-oxidant</td>
<td>Modest evidence for improvements in pain</td>
<td>Cameron and Chrubasik 2014; Farid et al 2007</td>
</tr>
<tr>
<td><strong>Pycnogenol® pine bark extract</strong></td>
<td>Not available</td>
<td>Anti-inflammatory, anti-oxidant</td>
<td>Inconclusive results from 2 studies</td>
<td>De Silva et al 2011; Cameron and Chrubasik 2014</td>
</tr>
<tr>
<td><strong>Willow bark (Salix daphnoides)</strong></td>
<td>Not available</td>
<td>Anti-inflammatory</td>
<td>Modest pain relieving effects observed after 8 week supplementation</td>
<td>Farid et al 2010</td>
</tr>
<tr>
<td><strong>Purple passion fruit peel extract</strong></td>
<td>Not available</td>
<td>Anti-inflammatory, anti-oxidant</td>
<td>Modest pain relieving effects after 8 week supplementation</td>
<td>Henriotin et al 2010; Nakagawa et al 2014</td>
</tr>
<tr>
<td><strong>Curcumin</strong></td>
<td>Not available</td>
<td>Anti-inflammatory, anti-oxidant, analgesic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CI = confidence interval; ES = effect size; NSAIDS = non-steroidal anti-inflammatory drugs
1.2.5.ii.d. Dietary Constituents

Supplementation with vitamin E and D has also been investigated for the treatment of knee OA. However, no significant differences from placebo was observed in pain, function or structural joint changes between supplementation with 500 IU vitamin E/day for 6 months or 2 years in RCTs in adults with knee OA (Brand et al 2001; Wluka et al 2002). Similarly, supplementation of 2,000 IU of vitamin D/day for 2 years did not result significant differences from placebo in terms of pain, function or structural joint changes (McAlindon et al 2013). Another RCT enrolled patients with knee OA and vitamin D insufficiency (defined as serum 25(OH)D < 50 nmol/L) and demonstrated small, but significant improvements in pain and function over placebo in patients randomized to 60,000 IU vitamin D/day for 10 days followed by 60,000 IU vitamin D/month for 12 months (Sanghi et al 2013). The authors concluded that the changes bordered on being of no difference. There is not strong evidence to suggest that a specific vitamin will be able to treat the structural or symptomatic features of knee OA. However, a balanced diet, resulting in an adequate dietary intake of vitamins may slow progression of knee OA and protect against worsening (Cao et al 2013).

1.2.5.ii.d.i. Rosmarinic Acid

With the significant role of inflammation and oxidative stress in joint tissue breakdown and alterations in pain processing in OA, a product that can counter these actions holds potential to control pain. An example of such a potential compound is rosmarinic acid (rosA). This is a polyphenolic carboxylic acid that is an ester of caffeic acid and 3,4-dihydrophenyllactic acid (Figure 1.6) (Peterson and Simmonds, 2003). RosA is a naturally occurring compound consisting of 2 phenolic rings, both of them containing 2 ortho-dihydroxy groups (Furtado et al 2008). A carbonyl group, an unsaturated double bond and a carboxylic acid group are located between the two rings (Figure 1.6).
**Sources of RosA**

RosA was first isolated from rosemary in 1958 by two Italian chemists, Scarpati and Oriente (Peterson and Simmonds, 2003). RosA is found in 39 plant families and it has been isolated from many species of the *Lamiaceae* family, which comprise the majority of oil-rich culinary and medicinal herbs (Petersen and Simmonds, 2003; Petersen 2013). These plants include basil, rosemary, sage, savory, marjoram, oregano, thyme, lavender, perilla, self-heal, hyssop, lemon balm, peppermint, fennel, painted nettle, comfrey, thyme, rosemary, oregano, peppermint, and spearmint (Youn et al 2003; Ranjbar et al 2006; Pearson et al 2010). It has been suggested that certain species of this family (i.e. oregano, sage, thyme, spearmint, peppermint) have antioxidant constituents in amounts significant enough to make a contribution to the dietary intake of phenolic antioxidants (Nurmi et al 2006). RosA is also present in the roots of the medicinal plant saliva miltiorrhiza, also known as Dashen (Liu et al 2010).

**Toxicity of RosA**

Safety of a dry-spearmint extract containing 15.4% rosA was assessed in a 90-day study in Sprague-Dawley rats given up to 1,948 mg dry spearmint extract/kg bw/day, by gavage
(equivalent to 300 mg rosA/kg bw/day or 21 g rosA/day for a 70 kg adult) (Lasrado et al 2015). There were no adverse effects on body weight, food consumption, neurological parameters, hematology, clinical chemistry, gross pathology, and histopathology and so, the no-observed adverse effect level (NOAEL) in rats can be assumed to be 1,948 mg/kg bw/day. In 19 healthy adults consuming perilla frutescens extracts containing 50 or 200 mg rosA for 21 days, no adverse events were reported and there were no abnormalities detected in complete blood cell counts, hepatic and renal function tests, total protein and proteinograms, electrolytes, lipids, uric acid, and concentrations of creatine phosphokinase (Takano et al 2003).

Pharmacokinetics of RosA

In animal and human studies, rosA has been shown to be rapidly absorbed, metabolized, conjugated, and excreted in the urine following oral consumption. Metabolites of rosA include methylated rosA, caffeic acid, ferulic acid, and coumaric acid (Baba et al 2003; 2005). In male Sprague-Dawley rats, rosA, methylated rosA and coumaric acid were detected at peak levels 0.5, 1, and 8 hours, respectively, after rosA administration (Baba et al 2003). In the urine, rosA, and rosA metabolites methylated rosA, caffeic acid, ferulic acid, and coumaric acid were detected, with 83% being excreted 8 to 18 hours after administration. In the plasma and urine, the majority of components were conjugated.

Similar results were reported in 6 healthy men following consumption of perilla extract containing 200 mg rosA (Baba et al 2005). In plasma, rosA and it’s conjugates reached peak levels of 1.15 ± 0.28 umol/L at 0.5 hours after administration, with ferulic acid and methylated rosA also being detected. In the urine, rosA, methylated rosA, caffeic acid, ferulic acid and a trace of coumaric acid was detected, with the majority being present in conjugated forms. The proportion of rosA and its metabolites excreted in the urine after oral administration to humans was 6.3% of the total dose with 75% of these appearing 6 hours after consumption (Baba et al 2005).
**Biological Activities**

In the last 5 years, research exploring the biological activities of rosA has greatly expanded, resulting in several recent extensive reviews (Bulgakov et al 2012; Khojasteh et al 2014; Kim et al 2015). Additionally, rosA has been examined for its potential therapeutic effects in a large number of diseases (Appendix 1). RosA has demonstrated antimicrobial, anti-tumor, anti-viral, anti-allergenic, antioxidant, and anti-inflammatory actions (Youn et al 2003; Petersen and Simmonds 2003; Bulgakov et al 2012; Khojasteh et al 2014; Kim et al 2015).

**Proposed Mechanisms**

The mechanisms by which rosA can exert such a range of biological activities is not understood (Bulgakov et al 2012) although numerous possibilities have been proposed. A key mechanism that has been shown in a variety of cell lines and conditions is the inhibition of the activation and/or translocation of the pro-inflammatory transcription factor, nuclear factor-kB (NF-kB) with rosA treatment (Bulgakov et al 2012; Domitrovic et al 2012; Kim et al 2015; Hwang et al 2014; Fallarini et al. 2009; Lee et al 2006; Kim et al 2008; Moon et al 2010). In one study, rosA decreased the inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-b) downstream signaling pathway and NF-kB activation in TNFa-induced human dermal fibroblasts (Lee et al 2006). In general, treatment of rosA to chemically induced cells results in the inhibition of ROS generation (Moon et al 2010; Vostalova et al 2010; Kim et al. 2005; Zdarilova et al 2009; Lee et al 2008) as well as the inhibition of production of pro-inflammatory cytokines like IL-6, IL-1B, TNFa, and PGE$_2$ (Vostalova et al 2010; Chu et al. 2012; Hwang et al 2014; Zdarilova et al 2009). Furthermore, rosA has been demonstrated to increase intracellular anti-oxidant defense systems, including enhanced levels and/or activation of nuclear factor-like 2 (Nrf2) (Domitrovic et al 2012; Hwang et al 2014), heme oxygenase 1 (HO-1) (Domitrovic et al 2012; Hwang et al 2014; Lee et al 2008), glutathione (GSH) (Kim et al. 2005; Zdarilova et al 2009; Fallarini et al 2009), and superoxide dismutase SOD (Kim et al 2005; Chu et al 2012). Perez-Fons et al (2010) reported that the antioxidant abilities of rosA are from its ability to stabilize membranes [as measured in the thiobarbituric acid reactive substances].
(TBARS) assay] and restrict free radical movement [as measured in trolox equivalent antioxidant capacity (TEAC) assay].

In vitro studies have reported opposing effects of rosA on apoptosis in different cell lines. In human leukemia U937 cells, rosA treatment sensitized TNFa-induced apoptosis through activation of caspases, and suppression of NF-kB and ROS (Moon et al 2010). However, Kim et al (2005), Domitrovic et al (2012), Vostalova et al (2010) all found that rosA treatment resulted in an inhibition of chemically induced apoptosis in H9c2 cardiac muscle cells, hepatic cells, and human keratinocyte cells, via inhibition of caspase-3 activation. Clearly, the diverse biological activities of rosA demonstrate that this compound has potential to benefit to human health. Further investigations to elucidate the detailed molecular pathways underlying the biological functions are needed.

Animal Models

In animal models, several effects have been demonstrated that suggest rosA may be beneficial in the treatment of OA, specifically anti-nociceptive, anti-inflammatory effects, and anti-arthritic effects. In male and female Swiss mice, oral administration of 3 mg/kg rosA isolated from lemon balm inhibited glutamate induced pain (Guginski et al 2009). Similarly, oral administration of 150 mg/kg rosA extracted from thunbergia laurifolia demonstrated significant anti-nociceptive effects in laboratory tests for behavioral responses to thermal and chemical induced noxious insults in male ICR mice (Boonyarikpunchai et al 2014). In addition, rosA decreased paw edema 3-6 hours following carrageenan injection, demonstrating an anti-inflammatory effect. Finally, in male Wistar rats with streptozotocin induced diabetic neuropathy, oral administration of 10 and 30 mg/kg rosA for 8 weeks resulted in anti-nociceptive effects in behavioural laboratory tests (Hasanein and Zaheri 2014). Male DBA/1 mice were intraperitoneally injected with collagen for 21 days to create a rheumatoid arthritis state and then given 0 or 50 mg/kg rosA for 15 days from Day 21 post injection (Youn et al 2003). Animals treated with rosA had significantly decreased clinical manifestation of collagen-induced arthritis (CIA), as indicated by significant decreases in mean arthritis index and number of affected paws. In addition, histopathological examination of each hindpaw revealed that animals treated with rosA had normal joint composition when
compared to the inflamed joints of CIA mice. Finally, cyclooxygenase-2 (COX-2) levels were significantly decreased in the joint tissue of mice treated with rosA.

Anti-inflammatory and cartilage sparing effects of a high-rosA acid spearmint plant have also been demonstrated in animal models. Porcine cartilage explants were exposed up to 400 µg/mL of high-rosA spearmint, up to 10 mg/mL control spearmint, or 0.64 µg/mL of rosA and all explants were exposed to either 0 or 3 µg/mL of lipopolysaccharide (LPS) to induce inflammation (Pearson et al 2010). A strong inhibition of LPS-induced PGE₂ and NO production occurred in the presence of high-rosA spearmint, but not control spearmint or rosA, highlighting the potential for complex interactions between bioactives that may impact efficacy. In addition, high-rosA spearmint and rosA significantly inhibited to LPS-induced increased in GAG levels, whereas control spearmint had no effect on GAG levels.

High-rosA spearmint was also examined in 8 healthy, mature standardbread horses free of articular inflammation who were fed either 0 or 54 mg/kg of high-rosA spearmint mixed into hay and sweet feed for 24 days (Pearson et al 2012). Following intra-articular injection of LPS in all horses, PGE₂ and GAG synovial levels were significantly increased in control horses. LPS injection did not increase PGE₂ or GAG synovial levels in horses consuming high-rosA spearmint. LPS injection induced a small but insignificant increase in NO in all horses; however, there were no significant differences in NO levels between groups at any point.

**Humans**

There have been very few investigations with rosA in humans; although it has been examined for its effects on mild allergic rhinitis and nasal polyps. In a randomized, placebo-controlled study, patients with mild allergic rhinitis were given either perilla frutescens extracts containing 0 mg rosA (n=10), 50 mg rosA (n=9), or 200 mg rosA (n=10) for 21-days (Takano et al 2003). There were no differences in symptom scores between groups. RosA consumption did decrease levels of polymorphonuclear leukocytes or neutrophils in nasal lavage fluid on day 3, but the changes were not statistically significant on day 21. High-rosA spearmint tea was examined for its effects on nasal polyps, a chronic inflammatory disease of the nasal mucosa (Goronfolah et al 2009).
a double-blind, placebo-controlled, crossover trial, healthy adults with history of nasal polyp symptoms during the previous 12 months were randomized to consume 1 cup of high-rosA spearmint tea or 1 cup regular spearmint tea for 4 weeks each, with a 4-week washout between treatments. Twenty-two subjects completed the study and no significant differences between the treatments in terms of nasal stuffiness or peak nasal inspiratory flow, sleep, sense of smell or patient’s symptoms overall.

1.2.6 Summary

OA is a chronic, progressive disease resulting in degeneration of joint tissues, leading to significant pain, stiffness, and impaired physical function. The pathology of OA is not completely understood, but involves mechanical, biological, biochemical, molecular, and enzymatic feedback loops with inflammatory mediators playing large role in disease pathology and alterations in pain processing. The inability to completely characterize and measure OA remains a significant challenge in OA research that limits understanding of the disease and assessment of response to novel treatments. Biomarkers of joint metabolism hold promise, but require further investigation and validation. Effective treatment strategies in OA remain an important and critical area for research. Many management strategies exist, but none are particularly effective and the highly utilized pharmaceuticals have a strong risk for adverse effects. Anti-inflammatory and antioxidant dietary constituents and NHPs hold a lot of promise for the management of knee OA and large, high-quality investigations should continue. Overall, investigations that deepen our understanding of OA pathology and pain will enhance the ability to manage and treat this disease and better and faster methods of measuring OA will aid the ability to assess new treatments.
Chapter 2: Aims of Thesis

As reviewed above, there is no cure for knee OA and although there are many treatment options, many are ineffective and people with OA live with considerable pain and disability. The aim of this thesis is to examine relationships between pain and lifestyle factors and symptoms of knee OA and biomarkers and to investigate the effects of a novel therapeutic product in adults with knee OA.

Study 1 investigates the association between lifestyle factors and pain in individuals with knee OA. The specific objectives are to characterize OA disease characteristics and lifestyle factors at different levels of pain in adults with knee OA and to investigate the relationship between those factors and OA pain. It is hypothesized that higher pain scores would be associated with higher BMI, bilateral knee OA, increased disease duration, pain medication use, no supplement use, no alternative therapies use, poor self-reported health, increased number of co-morbidities, low intake of fruits and/or vegetables, and not meeting physical activity guidelines.

Study 2 examines biomarkers of joint metabolism and inflammation and their association with pain and physical function in adults with knee OA. The specific objective is to relate a panel of serum biomarkers of cartilage metabolism, synovial membrane, and inflammation to OA clinical characteristics (pain, stiffness, physical function scores, 6-minute walk test performance, stair climb task performance) in adults with knee OA. It is hypothesized that biomarkers of synovial membrane and inflammation will be associated with pain.

Finally, study 3 evaluates a novel treatment for the management of pain and physical function in adults with knee OA. The specific objective is to examine the effects of daily consumption of tea brewed from a high-rosA spearmint plant on measures of pain, stiffness, disability, quality of life, and physical function in adults with OA of the knee. It is hypothesized that participants consuming the high-rosA spearmint tea will have improved measures of pain, stiffness, disability, quality of life, and physical function after 4 months.
Chapter 3: Modifiable lifestyle factors are associated with lower pain in adults with knee osteoarthritis

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In press, accepted by \textit{Pain and Research Management} on Thursday April 17\textsuperscript{th}, 2015.
3.1 Abstract

With no cure or effective treatments for osteoarthritis (OA), the need to identify modifiable factors to decrease pain and increase physical function is well recognized. The objective of this study is to examine factors that characterize OA patients at different levels of pain and to investigate the relationships between those factors and pain. Details of OA characteristics and lifestyle factors were collected from interviews with healthy adults with knee OA (n=197). The Western Ontario and McMaster Universities Osteoarthritis Index was used to assess pain. Factors were summarized across 3 pain score categories and Chi-square and Kruskal-Wallis tests examined differences. Multiple linear regression using a stepwise selection procedure examined associations between lifestyle factors and pain. Multiple linear regression indicated that pain was significantly higher with the use of OA medications and higher BMI category and significantly lower with use of supplements and meeting physical activity guidelines (≥ 150 min/week). Stiffness and physical function scores, bilateral knee OA, BMI category, and OA medication use were significantly higher across pain categories, whereas self-reported health, servings of fruit, supplement use, and meeting physical activity guidelines significantly lower. No significant differences across pain categories were found for sex, age, number of diseases, duration of OA, ever smoked, alcoholic drinks/week, over-the-counter pain medication use, OA supplement use, physical therapy use, servings of vegetables, or minutes walked/week. Healthy weight maintenance, exercise for at least 150 min/week and correct use of medications and supplements represent important modifiable factors related to reduced knee OA pain.

3.2 Introduction

Knee osteoarthritis (OA) is the most common cause of knee pain and lower limb disability in older adults (Jordan et al 2009). Approximately 4.4 million Canadians have OA and, due to increased longevity, reduced physical activity, and increased obesity, this number is expected to increase to 10 million within 30 years (Bombardier et al 2012). Pain is the most important OA symptom among sufferers and contributes to disability, fatigue and decreased quality of life (Briggs et al 1999; Hawker et al 2011). Pain is also what drives healthcare use in OA, with direct and indirect healthcare costs of 27 billion dollars in Canada in 2010 (Bombardier et al 2012). In general, pain is poorly understood and, in OA specifically, much of its variability cannot be
explained by markers of disease severity (Bedson and Croft 2008; Felson 2014; van Spill et al 2010). Many modifiable factors, including psychological and physical health, social support, coping behaviors, and self-efficacy, have been found to influence OA pain (Hawker 2012).

With no cure available, management of OA focuses on analgesia and maintenance of physical function (Hunter 2009). International evidence-based guidelines suggest that OA management begin with non-pharmacological options, including weight loss or maintenance, moderate exercise and physical activity, physical therapies and assistive devices, followed by pharmacological interventions, starting with over-the-counter (OTC) pain medications like acetaminophen (Fernandes et al 2013; Hochberg et al 2012; McAlindon et al 2014). However, non-pharmacological approaches are infrequently recommended by clinicians and used by patients (Dhawan et al 2014; Hunter 2009). Individuals with OA also tend to use pain medications incorrectly and many live in pain and seek to manage it by limiting activities and movement (Merkle and McDonald 2009; Sale et al 2006). With the lack of successful treatment options, expected rise in OA cases, and the increasing burden on the healthcare system, there is significant need to revisit OA management strategies and to focus on modifiable lifestyle changes to decrease pain and increase or maintain physical function (Hunter 2009; Turkiewic et al 2014). Modifiable factors that could affect pain in OA include oral management techniques (e.g. OTC pain medications, supplements), use of physical therapies (e.g. physiotherapy, chiropractor, etc), assistive devices (e.g. walking aids, braces, etc), home treatment methods (e.g. heat, ice) as well as physical activity and diet.

The most important modifiable risk factor for knee OA is body weight. Excess body weight is the strongest and most consistent risk factor for the onset and progression of knee OA (Blagojevic et al 2010; Elbaz et al 2011; Hunter 2009; Riddle and Stratford 2013). The excessive loads to the knee, inflammation, and associated inactivity in overweight and obese individuals contributes to knee OA pathogenesis and pain (Hunter 2009; Lee et al 2013b).

With the exception of diet-induced weight loss, the literature examining the effect of diet on knee OA is mixed. Epidemiological studies have shown an increased risk of disease progression with low intake of antioxidant micronutrients (i.e. vitamin C, vitamin E, beta-carotene), while most
intervention studies report no significant effects of these micronutrients on the progression of knee OA (Brand et al. 2001; McAlindon et al. 1996a, 1996b, 1997, 2013; Wluka et al. 2002). It is possible that antioxidant vitamins only exert an influence on knee OA when they are in their original food matrix; however, the association of fruit and vegetable intake with OA has not been investigated.

The important role of exercise and physical activity in the development and management of knee OA is recognized, but it is complex and not well understood. OA clinical guidelines also recognize regular physical activity as pivotal in OA management (Hunter 2009; McAlindon et al. 2014). Modest volumes of low impact exercise can strengthen the muscular support around the joints, help to avoid obesity, increase mood and psychological health, and can help to preserve function and delay disability (Cheng et al. 2000; Hawker et al. 2011; Hawker 2012). Exercise intervention studies consistently show that exercise decreases pain and improves function in adults with OA (Baker et al. 2001; Ettinger et al. 1997; Messier et al. 2004; Roddy et al. 2005). However, no association between physical activity level and knee pain was found in a large prospective cohort study (Mansournia et al. 2012).

Although OA pain is poorly understood, it is the leading complaint by persons with OA and is influenced by many factors. The objective of this study was to examine factors that characterize OA patients at different levels of pain and to investigate the relationships between those factors and OA pain.

3.3 Methods

This study is based on cross-sectional data collected as part of a screening interview for a nutrition intervention clinical trial (n=157) (Connelly et al. 2014) with additional interviews (n=40) completed to increase the sample size for a total of 200 participants, based on previous cross-sectional analyses (Cruz-Almedia et al. 2013; Goncalves et al. 2011; Terwee et al. 2006; Tonelli et al. 2011; Blamely et al. 2009).

For the intervention and additional interviews, males and females aged ≥18 years with self-reported physician diagnosed knee OA were recruited from the Guelph area using posters and
newspaper advertisements. Exclusion criteria included other systemic or rheumatic arthritis, concomitant inflammatory processes, upcoming knee replacement surgery, smoking, clinically significant, uncontrolled cardiovascular, hepatic, or renal disorder, and any serious medical condition within 6 months such as heart attack, stroke, cancer, or diabetes. The study was conducted at the Human Nutraceutical Research Unit at the University of Guelph, approved by the University of Guelph Human Research Ethics Board (REB#13OC002), and all participants provided written consent.

Study Procedures

Trained study coordinators conducted the interviews, which included a questionnaire developed for the study with health history and lifestyle questions about OA diagnosis, general health, smoking history, alcohol consumption, body weight, height, medication and supplement intake, co-morbidities, exercise habits, and use of physical therapies. Participants were asked how many minutes per week they participated in weight training/resistance exercise, aerobic exercise, aquatic exercise or other types of exercise (e.g. walking, sport participation, mixed fitness classes, etc). Body mass index (BMI) was calculated from self-reported height and body weight and then categorized into a BMI category. Participants were asked to bring all medications and supplements to the interview, which were grouped into medications (any prescription and OTC), medications for OA (prescription and OTC taken specifically for OA pain), prescription medications for OA, and over the counter pain (OTC) medications. Supplements were grouped as OA specific and non-OA specific. Participants were considered to use physical therapy if they currently visited a practitioner. The time spent performing different types of exercises was summed to a total number of minutes of exercise per week, and the time spent walking was summed to a total number of walking minutes per week. A participant was considered meeting physical activity guidelines when total number of minutes of exercise per week met or exceeded 150 minutes.

The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) has 3 subscales to evaluate pain, stiffness and physical function associated with OA (Bellamy et al 1988). The 100 mm visual analog scale (VAS) version of the questionnaire was completed in the
presence of a study coordinator and scored according to the WOMAC® Osteoarthritis Index User Guide IX (Bellamy et al 2011). If a participant had OA in both knees, they were instructed to choose the more painful knee as the signal joint. Higher scores indicate greater pain and stiffness, and worse physical function.

A 24-hour dietary recall was conducted by a trained study coordinator and involved leading the participant through a series of questions regarding all food and beverage intake in the previous 24 hours. Detailed questions were asked during the recall to ensure specific amounts, preparation methods, brand names, and complete information about foods and beverages were recorded. All were entered into ESHA Food Processor software version 9.12 (Salem, OR). Foods were classified into one of the four main food groups (i.e. fruits and vegetables, grain products, milk and alternatives, and meat and alternatives) according to the current Canadian Food Guide to Healthy Eating (Health Canada 2014). Fruit and vegetable servings were also calculated and analyzed separately. Foods not included in these groups were classified as “other”. Based on previous nutrition research, mixed meals were broken down into their component foods appropriate to the serving sizes, juice was classified as a fruit or vegetable depending on content (e.g. orange versus carrot) and potatoes and french fries were classified as vegetables (Attorp et al 2014).

Statistics

All analyses were performed using the Statistical Analysis System (version 9.3; Cary, NC, USA) with \( p < 0.05 \) considered statistically significant. Continuous variables are presented as mean ± standard deviation. These data were not normally distributed, as determined by the Shapiro-Wilk test. Dichotomous and categorical variables are presented as N (%).

To examine health history and lifestyle factors at different levels of pain, WOMAC pain scores were grouped into 4 categories, mild (0 - 125), moderate (126 – 250), severe (251 – 375), and extreme (376 – 500). Although there is no standardized method of dividing WOMAC scores, categories were devised similar to previous work by Blamely et al (2009) and Kim et al (2013). Only 3 participants reported a pain score over 375 (378, 398 and 442), and were included in the
severe category, leaving 3 pain groups (mild, moderate, and severe). Health history and lifestyle factors were summarized for each pain category and differences were examined with Chi-square tests for dichotomous and categorical variables and Kruskal-Wallis tests for continuous variables. Linear regression analysis using continuous WOMAC scores was conducted using a stepwise multiple regression was performed to determine the best predictors of pain, setting probability to enter the model at $p<0.15$. Examination of residual plots revealed that the data was homoscedastic and the relationship was linear and the distribution of the residuals was normal. Variance inflation factors were between 1 and 2 for all values, revealing that multicollinearity was not an issue.

3.4 Results

Participant Characteristics

Participant characteristics are presented in Table 3.1. There were 197 adults (69% female) with a mean age of 63 years. Mean disease duration was 98 months and 59% of individuals had OA in both knees. Fifty-one percent of participants reported “good” health and the mean number of diseases or conditions, including OA, was 3. The most commonly reported co-morbidities were cardiovascular diseases (CVD), depression, hypertension, high cholesterol, hyperthyroidism, and cancer (data not shown). Eighty-one percent of the participants reported using at least one medication and 69% used at least one medication for OA. A high percentage of participants (88%) took supplements, although only 37% took an OA-specific supplement. The most commonly consumed supplements were vitamin D, multi-vitamin and minerals, calcium, omega-3/fish oil, glucosamine, and vitamin C. The most commonly reported OA-specific supplement taken was a glucosamine product (glucosamine or a combination of glucosamine and chondroitin or methylsulfonylmethane). Only 33% of participants reported current use of physical therapy, with physiotherapy, acupuncture and chiropractic being the most common. Forty-eight percent of participants met the physical activity guidelines of 150 minutes of exercise/week (36), with walking being the most frequently reported activity. Walking minutes were highly variable with a mean of 58 and median of 40 minutes/week. Overall average WOMAC scores revealed a mild to moderate level of pain, stiffness, and physical function in the study participants.
Table 3.1 Summary of Participant Characteristics (N = 197)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Females: N (%)</td>
<td>136 (69%)</td>
</tr>
<tr>
<td>Age (years): Mean ± SD</td>
<td>63 ± 10.9</td>
</tr>
<tr>
<td>Disease duration (months): Mean ± SD</td>
<td>98.1 ± 95.6</td>
</tr>
<tr>
<td>Bilateral OA: N (%)</td>
<td>117 (59%)</td>
</tr>
<tr>
<td>Number of diseases: Mean ± SD</td>
<td>3.0 ± 1.5</td>
</tr>
<tr>
<td><strong>Self-Reported Health:</strong> N (%)</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>6 (3%)</td>
</tr>
<tr>
<td>Good</td>
<td>100 (51%)</td>
</tr>
<tr>
<td>Very Good</td>
<td>79 (40%)</td>
</tr>
<tr>
<td>Excellent</td>
<td>12 (6%)</td>
</tr>
<tr>
<td>Ever smoked: N (%)</td>
<td>94 (48%)</td>
</tr>
<tr>
<td>Number of alcoholic drinks/week: Mean ± SD</td>
<td>3.4 ± 4.5</td>
</tr>
<tr>
<td><strong>Medication and Supplement Use:</strong> N (%)</td>
<td></td>
</tr>
<tr>
<td>Medications</td>
<td>159 (81%)</td>
</tr>
<tr>
<td>Medications for OA</td>
<td>136 (69%)</td>
</tr>
<tr>
<td>Prescription Medications for OA</td>
<td>53 (27%)</td>
</tr>
<tr>
<td>OTC Pain Medication for OA</td>
<td>116 (59%)</td>
</tr>
<tr>
<td>Supplements</td>
<td>173 (88%)</td>
</tr>
<tr>
<td>Supplements for OA</td>
<td>73 (37%)</td>
</tr>
<tr>
<td>Participates in Physical Therapy: N (%)</td>
<td>65 (33%)</td>
</tr>
<tr>
<td>Met Physical Activity guidelines: N (%)</td>
<td>95 (48%)</td>
</tr>
<tr>
<td>Walking minutes: Mean ± SD</td>
<td>58.7 ± 71.6</td>
</tr>
<tr>
<td><strong>WOMAC Subscale Scores:</strong> Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Pain score (max 500)</td>
<td>159.1 ± 92.9</td>
</tr>
<tr>
<td>Stiffness score (max 200)</td>
<td>100.6 ± 50.1</td>
</tr>
<tr>
<td>Physical function score (max 1700)</td>
<td>578.6 ± 347.8</td>
</tr>
</tbody>
</table>

OA = Osteoarthritis; SD = Standard deviation.

Factors by Pain Categories

Table 3.2 shows the health history and lifestyle factors for participants across the 3 pain categories. There were no significant differences across the pain categories in terms of distributions of sex, age, number of diseases, duration of OA, ever smoked, number of alcoholic drinks/week, use of OTC pain medication, use of OA supplements, use of physical therapy, servings of vegetables, or minutes walked/week.
Table 3.2 Health history and lifestyle factors across mild, moderate and severe WOMAC pain categories

<table>
<thead>
<tr>
<th></th>
<th>Mild Pain (n=83)</th>
<th>Moderate Pain (n=79)</th>
<th>Severe Pain (n=35)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>64.9 ± 11.8</td>
<td>63.0 ± 9.7</td>
<td>60.2 ± 10.8</td>
<td>0.14</td>
</tr>
<tr>
<td>OA duration (months):</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>90.1 ± 83.9</td>
<td>103.3 ±106.0</td>
<td>105.2 ± 98.3</td>
<td>0.86</td>
</tr>
<tr>
<td># Diseases:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td>2.9 ± 1.4</td>
<td>3.0 ± 1.6</td>
<td>3.2 ± 1.7</td>
<td>0.79</td>
</tr>
<tr>
<td>WOMAC Scores:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiffness score</td>
<td>68.3 ± 45.1</td>
<td>117.6 ± 39.9</td>
<td>139.3 ± 33.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Disability score</td>
<td>304.4 ± 208.0</td>
<td>689.2 ± 249.0</td>
<td>977.8 ± 267.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>57 (69%)</td>
<td>53 (67%)</td>
<td>26 (74%)</td>
<td>0.74</td>
</tr>
<tr>
<td>Males</td>
<td>26 (31%)</td>
<td>26 (33%)</td>
<td>9 (26%)</td>
<td></td>
</tr>
<tr>
<td>Unilateral or Bilateral OA:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>44 (53%)</td>
<td>27 (34%)</td>
<td>9 (26%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Bilateral</td>
<td>39 (47%)</td>
<td>52 (66%)</td>
<td>26 (74%)</td>
<td></td>
</tr>
<tr>
<td>BMI Category:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal weight</td>
<td>25 (31%)</td>
<td>12 (16%)</td>
<td>5 (14%)</td>
<td>0.008</td>
</tr>
<tr>
<td>Underweight</td>
<td>1 (1%)</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Overweight</td>
<td>34 (41%)</td>
<td>29 (38%)</td>
<td>8 (23%)</td>
<td></td>
</tr>
<tr>
<td>Obese</td>
<td>22 (27%)</td>
<td>36 (46%)</td>
<td>22 (63%)</td>
<td></td>
</tr>
<tr>
<td>Self-Reported Health:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>0</td>
<td>1 (1%)</td>
<td>5 (14%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Good</td>
<td>40 (48%)</td>
<td>46 (58%)</td>
<td>14 (40%)</td>
<td></td>
</tr>
<tr>
<td>Very good</td>
<td>37 (45%)</td>
<td>27 (34%)</td>
<td>15 (43%)</td>
<td></td>
</tr>
<tr>
<td>Excellent</td>
<td>6 (7%)</td>
<td>5 (6%)</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>Smoking History &amp; Alcohol Consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever smoke:</td>
<td>36 (44%)</td>
<td>37 (47%)</td>
<td>21 (60%)</td>
<td>0.25</td>
</tr>
<tr>
<td># Alcoholic drinks/week:</td>
<td>3.1 ± 3.8</td>
<td>3.8 ± 5.3</td>
<td>3.3 ± 3.9</td>
<td>0.86</td>
</tr>
<tr>
<td>Medication, Supplement, and Alternative Therapy Use:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OA medications</td>
<td>44 (53%)</td>
<td>59 (75%)</td>
<td>33 (94%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>OA prescriptions</td>
<td>14 (17%)</td>
<td>25 (32%)</td>
<td>15 (43%)</td>
<td>0.009</td>
</tr>
<tr>
<td>OTC pain</td>
<td>43 (52%)</td>
<td>49 (62%)</td>
<td>25 (71%)</td>
<td>0.12</td>
</tr>
<tr>
<td>Supplements</td>
<td>78 (94%)</td>
<td>69 (87%)</td>
<td>26 (74%)</td>
<td>0.01</td>
</tr>
<tr>
<td>OA supplements</td>
<td>37 (45%)</td>
<td>22 (28%)</td>
<td>14 (40%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Physical therapy</td>
<td>28 (34%)</td>
<td>23 (29%)</td>
<td>14 (40%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Serving of Fruit and Vegetables:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits</td>
<td>2.1 ± 1.5</td>
<td>1.6 ± 1.4</td>
<td>1.5 ± 1.4</td>
<td>0.03</td>
</tr>
<tr>
<td>Vegetables</td>
<td>2.5 ± 1.3</td>
<td>2.7 ± 1.4</td>
<td>2.4 ± 1.4</td>
<td>0.49</td>
</tr>
<tr>
<td>------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------</td>
<td>------</td>
</tr>
</tbody>
</table>

**Physical Activity**

<table>
<thead>
<tr>
<th>Meet guidelines:</th>
<th>N (%)</th>
<th>53 (64%)</th>
<th>34 (43%)</th>
<th>8 (23%)</th>
<th>0.0001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Walking minutes:</td>
<td>mean ± SD</td>
<td>67.1 ± 78.0</td>
<td>59.6 ± 67.4</td>
<td>38.6 ± 61.7</td>
</tr>
</tbody>
</table>

BMI = Body mass index; OA = Osteoarthritis; OTC = Over-the-counter; SD = Standard deviation; WOMAC = Western Ontario and McMaster Universities Arthritis Index

*Underweight: BMI <18.5 mg/kg², Normal weight: BMI = 18.5 – 24.9 mg/kg², Overweight BMI = 25.0 – 29.9 mg/kg², and Obese BMI > 30.0 mg/kg² (Health Canada 2012).


WOMAC stiffness and physical function scores were different across the pain categories and increased with increasing WOMAC pain score (p<0.0001).

Having OA in only one knee was most common in the mild pain category (53%) (p=0.008) and this proportion decreased across pain categories. Having OA in both knees was more common in the moderate (66%) and severe pain (74%) categories than the mild pain category (47%) (p=0.008).

The proportion of participants in each BMI category differed across pain categories (p=0.008). In the mild pain category, the greatest percent of participants were in the normal weight (31%) and overweight (41%) categories, and the percent of normal weight participants was lower in the moderate (16%) and the severe (14%) pain category. The percent of participants in the obese category was higher across increasing pain levels (Figure 3.1A).

Self-reported health categories were also different among pain categories, with fewer participants reporting poor health in the mild (0%) and moderate (1%) versus severe (14%) pain categories and more participants reporting very good health in the mild (45%) versus moderate (34%) pain categories (p=0.001) (Figure 3.1B).

Servings of fruit were highest in the mild pain category (2.1), which was significantly higher than the moderate (1.6) and severe (1.5) pain categories (p=0.03).
The proportion of participants who used a medication for OA increased across pain categories ($p<0.001$) (Figure 3.1C), with a similar pattern observed for prescription medications ($p=0.009$) (Figure 3.1D). The opposite trend was observed with supplements, with participants in the mild pain category reporting the highest proportion of supplement use and decreasing supplement use across pain categories ($p=0.003$) (Figure 3.1E).

Participating in exercise for at least 150 min/week was most common in the mild pain category (64%), and this percentage was lower as pain category increased ($p=0.0001$) (Figure 3.1F).
Figure 3.1 Chi-square analysis revealed significant differences in the percent of participants in the mild pain (0-125), moderate pain (126-250), and severe pain (251-500) categories in terms of BMI category [normal weight (N), overweight (OW), or obese (OB)] (A), self-reported health (B), use of medications for OA (C), use of prescription medications for OA (D), use of supplements (E), and met physical activity guidelines (F). BMI = body mass index.

Regression Analysis

Multiple linear regression using a stepwise selection procedure revealed that BMI category, use of supplements, use of OA medications, and meeting physical activity guidelines was the best combination of factors to predict pain score, with the model accounting for 28.4% (p<0.0001) (Table 3.3). The model revealed that pain score increased with use of OA medications and increasing BMI category and decreased with use of supplements and meeting physical activity
guidelines. Factors that were not included in the model included age, sex, disease duration, ever smoke, alcohol/week, number of diseases, use of OA supplements, use of OA prescriptions, use of OTC pain, use of physical therapy, fruit consumption, vegetable consumption, and walking minutes/week.

Table 3.3 Stepwise multiple regression examining factors related to pain score in adults with knee osteoarthritis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Unstandardized beta</th>
<th>Standard Error</th>
<th>R² of model (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Use of OA medications</td>
<td>60.1</td>
<td>13.1</td>
<td>14.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Meet Physical Activity Guidelines</td>
<td>-33.7</td>
<td>12.4</td>
<td>20.0</td>
<td>0.007</td>
</tr>
<tr>
<td>BMI Category</td>
<td>19.7</td>
<td>7.6</td>
<td>24.0</td>
<td>0.02</td>
</tr>
<tr>
<td>Use of Supplements</td>
<td>-35.3</td>
<td>17.8</td>
<td>25.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Self-Reported Health</td>
<td>-16.4</td>
<td>9.1</td>
<td>26.4</td>
<td>0.07</td>
</tr>
<tr>
<td>Use of Medications</td>
<td>26.4</td>
<td>15.0</td>
<td>27.5</td>
<td>0.08</td>
</tr>
<tr>
<td>Bilateral Knee OA</td>
<td>11.8</td>
<td>7.7</td>
<td>28.4</td>
<td>0.13</td>
</tr>
</tbody>
</table>

BMI = Body mass index; OA = Osteoarthritis.
* p<0.15 was used as cutoff for inclusion in the model

3.5 Discussion

This study examined modifiable lifestyle factors and their associations with OA pain in a sample of adults with knee OA. Sixty-nine percent of participants in the present study were women, with a mean age of 63 years and the majority of BMIs in the overweight and obese categories. This gender distribution, age and overweight status is reflective of Canadian OA patients (Public Health Agency of Canada, 2010). The majority of participants had mild (WOMAC pain score 0-125; n=83) or moderate (WOMAC pain score 125-250; n=79), pain, providing an opportunity to characterize modifiable lifestyle factors in adults with relatively low OA knee pain. A number of the lifestyle factors (stiffness score, physical function score, bilateral knee OA, BMI category, OA medication use, self-reported health, servings of fruit, supplement use, and meeting physical activity guidelines) were significantly different among pain categories. Additionally, linear regression analysis revealed that BMI, meeting physical activity guidelines, OA medication use, and supplement use accounted for 28.4% of the variance in pain score. The unexplained variability is potentially attributable to structural, psychological, and socioeconomic factors not measured in this study.
Use of medications was reported by 81% of participants, which may reflect that participants also reported an average of 3 diseases or conditions. Use of OA medications was a significant predictor for higher pain score, and although not included in the regression model, the use of any medication and OA prescription significantly increased as pain category increased. Use of OA medications has previously been associated with higher levels of pain (Fisher et al 2012; Lapane et al 2012), which could indicate that only individuals with high pain levels take medications, current OA pain medications are not effective at relieving pain, or that adults with OA are not using medications correctly. It has been found that individuals with OA use pain medications at lower doses and less frequently than recommended (Davis et al 2002; Ross et al 2001; Sale et al 2006). Pharmaceutical pain management is often seen by OA sufferers as undesirable and a last resort, due to concerns about side effects, addiction and tolerance, and general dislike for taking pills (Ross et al 2001; Sale et al 2006). Furthermore, patient knowledge and beliefs about self-management with medication is poor (Davis et al 2002; Hill and Bird 2007). OA patients may benefit from education on the correct use of OA medications.

Use of supplements was significant in the regression model for pain and 94% of individuals in the mild pain category reported using a supplement. Overall, supplement use was high in this study at 88%, which may have been influenced by the fact that screening data from a nutrition intervention study was used and interviews were conducted in a nutrition research unit. Estimates of supplement use in adults with OA vary from 34 to 90% based on geography, population and the definition of supplement (Lapane et al 2012). The most commonly consumed supplements in this study were vitamin D, multi-vitamin, calcium, omega-3/fish oil, glucosamine product, and vitamin C. As discussed previously, research into the effects of vitamin intake on OA is mixed. It has been suggested that in individuals with vitamin deficiencies, supplements may be beneficial in preventing OA progression (Wang et al 2004). In addition, higher dietary intakes of vitamin C and beta-carotene were associated with lower OA pain in adults with knee OA (McAlindon et al 1996a). The same trends of lower OA pain were observed with serum levels of vitamin D (Muraki et al 2011) and with supplementation with vitamin E (Bhattacharya et al 2012). Vitamin D may reduce inflammatory pain through regulation of cytokines and macrophage activity, although more studies are warranted (Cao et al 2013). Intervention studies with omega-3 rich oils
in adults with OA have demonstrated significantly reduced pain (Deutsch 2007; Zawadzi et al 2013), ostensibly based on anti-inflammatory mechanisms. Although not investigated in the present study, it is possible that, individuals with higher pain may have taken more medications to manage the pain and been less likely to take supplements for fear of interactions or general dislike of taking multiple pills (Davis et al 2002; Ross et al 2001).

Increasing BMI category was a significant predictor of higher pain score in this study. The association of BMI with pain is well established (Elbaz et al 2011; Felson et al 1997; Goulston et al 2011) and reducing body weight has been related to dose-dependent decreases in pain and improvements in physical function (Blagojevic et al 2010).

Not meeting physical activity guidelines was a significant predictor of higher pain score and, as pain category increased, the percent of participants meeting physical activity guidelines decreased. Similarly, walking min/week decreased as pain category increased, although this was highly variable and not statistically significant (p=0.09). In exercise intervention studies, pain decreases with increased activity levels (Baker et al 2001; Ettinger et al 1997; Messier et al 2004; Roddy et al 2005); however, the monitored exercise regimes used in interventions are not replicated in everyday life. Indeed, physical activity levels quantified with a the Physical Activity Scale for the Elderly (PASE) questionnaire did not correlate to WOMAC pain scores in a large, prospective cohort study in adults with or at risk for knee OA (Mansournia et al 2012). Participants in the present study were active, with 48% meeting the Canadian physical activity guidelines of ≥150 min/week. Previous reports in adults with OA are highly variable, ranging from 10% (Dunlop et al 2011), to 30-40% (Farr et al 2008; Fontaine et al 2004; Holden et al 2014) and as high as 55.9% (Barbour et al 2014). Different assessment techniques could explain this variation, including accelerometers (Dunlop et al 2011), questionnaires (Felson et al 1997; Fontaine et al 2004), and self-reporting of activities (Felson et al 2007; Holden et al 2014), as well as different parameters, including distance walked/week (Felson et al 2007), years of sporting participating (Cooper et al 2000), categories of questionnaire scores (Felson et al 2007; Holden et al 2014), and percent of participants meeting physical activity guidelines (Barbour et al 2014; Dunlop et al 2011). Exploring the role of exercise and physical activity levels in OA management would benefit from standardized methods and outcome measures. It is also possible
that individuals with severe pain in this study did not feel that they could exercise. However, there are reports of individuals with knee OA walking at a high cadence or velocity with low pain levels (Messier et al 2004; White et al 2013). Further, after total knee replacement, improvements in pain are reported, but are not associated with increases in physical activity (Harding et al 2014), supporting the argument that pain is not the limiting factor to exercise in knee OA. Similar to the general population, time, effort, scheduling, and commitment have been reported as barriers to exercise in persons with OA (Ross et al 2001; White et al 2013). Exercise is a key modifiable factor to manage pain in OA as well as to decrease risk of other chronic diseases, which were common in this study (83%). Interestingly, all-cause mortality is higher in adults with OA versus without OA and the increased risk for death is predicted by walking disability, highlighting the importance of physical activity and exercise in this population (Nuesch et al 2011).

In this study, physical function score significantly increased across pain category, indicating worse physical functioning with higher pain. The strong correlation commonly observed between the pain and physical function scales on the WOMAC is suggested by Terwee et al (2006) to indicate that self-reported physical function assessments innately measure both pain and exertion, in addition to functioning. In the SF-36 questionnaire, pain questions are placed after the function questions, and there is lower correlation observed between scales when compared to the WOMAC where the pain questions are asked before the physical function questions (Terwee et al 2006). Similarly, correlations between self-reported physical function and performance-based physical function tests were strengthened when patient rated pain and exertion of the performance-based test was combined into a composite score (Stratford and Kennedy 2006). In addition, adults with painful knee OA report keeping still and limiting movement as a way to manage pain. This lack of activity could contribute to de-conditioning and reduced functioning (Davis et al 2002; Sale et al 2006). Another factor that significantly increased across pain categories was the percentage of adults with bilateral knee OA. This has previously been associated with higher pain scores, which may reflect the presence of additional tissue degradation and inflammation (Adegoke et al 2012; Schiphof et al 2013).
Daily fruit servings reported by participants were significantly higher in the mild versus moderate and severe pain categories. Of note, regardless of pain category, the number of daily fruit servings (i.e. 1.5 – 2.6) was below recommended levels. Fruits are high in antioxidants and polyphenols, and fruit consumption has been associated with lower musculoskeletal pain in adults (Høstmark et al 2014), and berries, tart cherries, blueberries, and concord grapes, specifically, have been found to have anti-nociceptive effects in rats (Jensen et al 2011). Healthy eating patterns are also associated with better self-reported health, more time in exercise, and less depressive symptoms, which have all been associated with lower pain in OA (Lee et al 2013b). Although the 24-hour diet recall isn’t the most robust method of dietary assessment available, increasing fruit consumption and improving diet quality are important modifiable factors that warrant continued investigation for the potential to reduce pain in adults with knee OA.

In this study, self-reported health status was significantly different across pain categories, even though most participants reported good or very good health. Self-reported health has been shown to be reliable in OA populations (McAlindon et al 1999), although associations with pain have not always been consistent. Reichmann et al (2009) found that lower income, greater number of co-morbidities, and greater functional limitations, but not pain, were associated with self-reported health status in adults with knee OA. In contrast, Allen et al (2010) found that lower self-reported health was associated with higher pain scores in adults with hip or knee OA. In this study, 91% of participants reported “good” or “very good” health, which could be related to the fact that 82% of participants were in the mild or moderate pain categories.

Several factors were not significantly different among pain categories or significant in the regression analysis. For example, age showed a non-significant protective directional trend with increasing age being associated with decreased pain, consistent with previous OA research (Schiphof et al 2013). Surprisingly, sex was not a significant factor in the regression model and there was no difference in pain categories. Women frequently report higher pain than men in OA (Elbaz et al 2011; Glass et al 2014; Tonelli et al 2011), but no differences in pain have been reported between the sexes in some studies (Debi et al 2009; Dillon et al 2006). There were more females in the severe (74%) compared to the mild and moderate (~68%) categories and more males in the mild and moderate (~32%) than the severe (26%) categories. There were also no
significant differences in duration of OA across pain category. Although Rosemann et al (2007) reported that duration of OA was a predictor of pain, several studies have shown that OA can remain stable for years (Cooper et al 2000). Number of diseases reported did not differ across pain category, consistent with a previous report (Leite et al 2011). However, the relationship between co-morbidities in OA and pain is mixed and examination into specific diseases may reveal that different co-morbidities impact OA pain in different ways. For example, strong links between depression and pain has been found several studies in adults with OA (Hawker et al 2011; Rosemann et al 2007). OA supplement use was not significantly related to pain. Glucosamine products were almost exclusively used in this category, and evidence for its effect on pain is limited due to high variation among studies (McAlindon et al 2014; Vlad et al 2007). Physical therapy was only used by 33% of adults in this study and its use did not different across the pain categories. A systematic review and meta-analysis of physical therapies for OA concluded that only a few physical therapy interventions were effective (Wang et al 2012), but it is possible that effects are not seen in intervention studies due to the fact that a true placebo group doesn’t exist for any of the physical therapies.

Limitations of this cross-sectional study include that causality of the factors studied cannot be concluded and that the data was self-reported. In addition, a number of factors that have been shown to affect pain were not measured in this study, including psychological variables, self-efficacy, education level, socioeconomic data, and fatigue (Blamely et al 2009; Cleveland et al 2013; Cruz-Almedia et al 2013; Hawker et al 2011). Selection bias towards adults with less severe OA is also a possibility, as participants were required to be mobile enough to travel to the research centre for interviews. However, this gave the opportunity to characterize individuals with lower pain, as some studies investigating factors of OA pain examine patients waiting for knee replacement, thereby representing a much different population and more extreme level of pain than is representative of many people with knee OA (Cooper et al 2000). Lastly, BMI calculations for this study were based on self-reported heights and weights, which has also been used in previous OA research (Allen et al 2010; Fontaine et al 2004; Goulston et al 2011; Thiem et al 2013). Given the potential for inaccuracies, BMI categories were used.
3.6 Conclusion

Several modifiable factors were associated with OA pain including BMI, meeting physical activity guidelines, OA medication use, and supplement use. Having a BMI in the healthy range is associated with lower pain levels, as well as exercising for at least 150 minutes/week. Exercise plus a diet high in fruits and vegetables is important in weight maintenance, and increasing fruit consumption could be important in this study population who are consuming low levels. Supplement use was associated with lower pain and use of OA medication was associated with higher pain. Patients should be educated on proper use of medications to manage pain in OA. Research efforts and clinical practice should continue to focus on modifiable lifestyle changes to decrease pain in OA.

3.7 Acknowledgments

We thank all the study participants as well as Natasha Sheikh, Svitlana Yurchenko, Kate Faughnan and Bonnie Huang for their assistance in data collection.
3.8 Bridge to Chapter 4

In Study 1, several modifiable lifestyle factors were associated with OA pain, identifying potential targets for management of OA. However, there are still no completely satisfactory treatments and many adults with OA live in daily pain. A barrier to the development of new therapies for OA is the low sensitivity of change in radiographs which necessitates long-term trials with a large number of patients (Kraus et al 2011). MRI has overcome many of the limitations of the radiograph, but is not standardized for OA outcomes, and as such, biomarkers represent an exciting opportunity for measurement of OA. Study 2 examines serum biomarkers of OA and their association with pain and physical function scores, as well as performance based measures of physical function, the stair-climb test and 6-minute walk test. Study 1 examined what modifiable lifestyle factors were associated with OA pain, and now Study 2 will examine how serum biomarkers of joint metabolism and inflammation are associated with pain and other symptoms of OA.
Chapter 4: Serum biomarkers of joint metabolism and inflammation in relation to clinical symptoms and physical function in adults with knee osteoarthritis

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Submitted to *Arthritis* on April 18\(^{th}\), 2015, with minor revisions for thesis.
4.1 Abstract

The relationship between osteoarthritis (OA) symptoms and biomarkers is relatively unexplored. In adults with knee OA, the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) assessed pain, stiffness, and physical function, and the 6-minute walk test (6MWT) and stair climb test (SCT) assessed physical performance. Serum cartilage oligomeric matrix protein (COMP), type-IIA collagen N-propeptide (PIIANP), hyaluronic acid (HA), matrix metalloproteinase 3 (MMP-3), nitric oxide (NO), and interleukin 6 (IL-6) were measured by enzyme-linked immunoassay. C-reactive protein (CRP) was measured by immunoturbidimetric assay, and interleukin 18 (IL-18) and leukemia inhibitory factor (LIF) were measured by multiplex immunoassay. Spearman’s coefficient examined correlations between biomarkers and clinical variables, controlling for age, sex and body mass index (BMI). In participants (n=54; mean age 60 ± 11.9 years, BMI 32.6 ± 7.5 kg/m²) HA was associated with better physical performance (6MWT: R=0.33, p=0.02 and SCT: R= - 0.38, p=0.006), MMP-3 with higher pain score (R=0.30, p=0.03) and IL-6 with higher pain (R=0.31, p=0.03) and stiffness score (R=0.30, p=0.03) and worse physical performance (SCT: R=0.32, p=0.02). COMP, PIIANP, IL-18, CRP, and NO were not associated with any variables and LIF was not detected. This study reports significant associations between biomarkers and clinical characteristics in OA.

(Research supported by the Ontario Ministry of Agriculture, Food and Rural Affairs, project #200121).

4.2 Introduction

Osteoarthritis (OA) is a major cause of disability and the number one cause of joint pain in older adults (Dieppe and Lohmander 2005). The primary pathology in OA is joint matrix destruction caused by an impaired balance between cartilage matrix synthesis and degradation, mechanical stress, inflammation, and other unknown factors, leading to cartilage loss, new bone formation at joint margins, subchondral bone changes, and synovial inflammation (Dean et al 1989; Dieppe and Lohmander 2005). However, understanding of the mechanisms involved is incomplete due to the disease’s slowly progressive nature, complex cartilage biochemistry, and the intricate role of inflammation (Abramson 2008). Current treatments are ineffective and, despite the use of pharmaceuticals, most individuals with OA suffer significant pain and impaired physical
functioning (Messier et al 2013; Sofat et al 2011). Specifically, pain is the primary concern for persons with OA, as it significantly affects mood, fatigue and quality of life and the symptom that drives healthcare use (Hawker et al 2011). Investigations into OA mechanisms and disease-modifying treatments are significantly hindered by uncertainties related to OA assessment. Radiographs only allow for an estimation of the space between joints and large bony changes (Bijlsma et al 2011). While magnetic resonance imaging (MRI) allows for a more specific examination of cartilage, it is time-consuming, costly and requires complex interpretation and improved standardization (Bijlsma et al 2011).

Biomarkers of joint metabolism have the potential to contribute to earlier OA diagnosis and to provide a more rapid and complete picture of therapeutic responses to investigational treatments (Felson 2014). However, no single biomarker can represent all of the complex biological changes occurring in the joint during OA (Kraus et al 2011). To this end, in 2011, the Osteoarthritis Research Society International and the United States’ Food and Drug Association recommended further investigation of 16 urine and serum biomarkers that reflect different tissues and processes involved in OA. This included the serum markers cartilage oligometric matrix protein (COMP) as a measure of cartilage degradation, procollagen type II N-terminal propeptide (PIIANP) as a measure of type II collagen synthesis, hyaluronic acid (HA) as an indication of synovial membrane metabolism, and matrix metalloproteinase 3 (MMP-3) an enzyme involved in joint tissue degradation (Kraus et al 2011). Since inflammatory pathways are known to be up-regulated in OA, inflammatory cytokines also have relevance as OA biomarkers (Dieppe and Lohmander 2005). C-reactive protein (CRP) is a marker of general inflammation that has been investigated for its role in OA pathogenesis, with conflicting evidence (Jin et al 2013). Interleukin (IL)-6 is a pro-inflammatory cytokine that plays a role in cartilage destruction and has been shown to stimulate synovial proliferation, osteoclast activation, and MMP production (Park et al 2007). IL-18 is well known for its role in rheumatoid arthritis, where it is involved in induction of cartilage degradation through production of MMPs (Futani et al 2002). However, serum IL-18 has not been examined in relation to OA clinical characteristics. Leukemia inhibitory factor (LIF) plays a role in the regulation of bone metabolism and has been shown to degrade proteoglycans ex vivo (Futani et al 2002). It has been detected in the synovial fluid of OA patients and correlated to degree of degraded cartilage tissue (Jiang et al 2014; Westacott and Sharif 1996).
OA biomarker research has primarily sought to relate biomarker levels to joint structural characteristics determined from x-rays and/or MRI (van Spill et al 2010). For example, a meta-analysis showed that serum COMP was consistently elevated in knee OA patients and was predictive of radiographic progression over time (Hoch et al 2011). Similarly, PIIANP and MMP-3 have been shown to predict radiographic progression in adults with knee OA (Garnero et al 2002; Lohmander et al 2005). HA has also been positively associated with presence, severity, and number of knees with radiographic OA (Elliott et al 2005). Despite this progress, relatively less is known about how OA biomarkers correlate with clinical characteristics of the disease (Bijlsma et al 2011). The objective of this study was to relate a panel of serum biomarkers of cartilage metabolism, synovial membrane, and inflammation to OA clinical characteristics (pain, stiffness, physical function scores, 6-minute walk test performance, stair climb task performance) in participants with mild to moderately painful knee OA. There is a critical need to better understand how joint and inflammatory biomarkers relate to OA pain and physical function (Hawker et al 2011; Ishijima et al 2011; van Spill et al 2010).

4.3 Methods

Study design

A cross-sectional study using baseline data from adults with knee OA who participated in an intervention study (Connelly et al 2014) was conducted at the Human Nutraceutical Research Unit at the University of Guelph. The study was approved by the University of Guelph Human Research Ethics Board (REB#11JA040) and all participants provided informed consent.

Participants

Male and female adults (>18 years old) were eligible if they had self-reported, physician diagnosed knee OA and a screening Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain score of >125. Exclusion criteria included other systemic or rheumatic arthritis, concomitant inflammatory processes, upcoming knee replacement surgery, chemical, radiologic, or surgical synovectomy in any large joint within the previous 3 months,
gastrointestinal ulcers, clinically significantly or uncontrolled cardiovascular, hepatic, or renal disorder, any serious medical condition within 6 months such as heart attack, stroke, cancer, or diabetes, smoking, participation in a clinical trial involving an investigational or marketed drug within the previous 6 months, and pregnancy (or intention to become pregnant), less than 6 months post-partum, lactating or less than 6 months post-lactation.

**Blood collection and analysis**

Participants provided a non-fasted blood sample with instructions to avoid alternative therapies such as acupuncture, massage therapy, or strenuous physical activity or consume pain medications for 48 hours prior. A 5 mL sample of blood was collected in a standard serum separating tube (SST™, BD, Mississauga, Canada) by a qualified medical technician via venipuncture. Samples were allowed to sit at room temperature for 30 minutes prior to centrifugation (2500 rpm and 4 °C for 15 minutes) and immediately stored at -80°C until analysis.

Serum C-reactive protein (CRP) was analysed in duplicate by Lifelabs® Medical Laboratory Services (Guelph, Canada) using latex-enhanced immunoturbidimetric assay. Serum biomarker quantification was performed in duplicate using enzyme-linked immunosorbent assay (ELISA) for COMP, HA, MMP-3, interleukin 6 (IL-6), and nitric oxide (NO) (R&D systems, MN, USA), and PIIANP (EMD Millipore, MO, USA) according to the manufacturers’ protocols. The assay detection limits were 0.010 ng/mL for COMP, 0.068 ng/mL for HA, 0.009 ng/mL for MMP-3, 0.70 pg/mL for IL-6, 0.25 μmol/L for NO, and 30 ng/mL for PIIANP. Inflammatory cytokines interleukin 18 (IL-18) and leukemia inhibitory factor (LIF) were measured in duplicate using a custom Bio-Plex Pro cytokine kit (Bio-Rad Laboratories, CA, USA) according to the manufacturer’s instructions. The lower limit of quantification was 1.8 pg/mL for IL-18 and 12 pg/mL for LIF.
Anthropometric measurements & clinical characteristics

Height was measured to the nearest millimeter using a stadiometer (Model 217, SECA®, Hanover, USA) and body weight was measured to the nearest 0.05 kg on a digital scale (SVI-200F, Acculab®, Barrie, Canada).

The WOMAC was used to evaluate pain, stiffness, and physical function associated with OA with a higher score indicating greater pain and stiffness, and worse physical functioning (Bellamy et al 1988). The visual analog scale (VAS) version of the questionnaire was completed in the presence of a study coordinator and scored according to the WOMAC® Osteoarthritis Index User Guide IX (Bellamy 2011).

The 6-minute walk test (6MWT) and stair climb test (SCT) are performance-based measures administered to assess physical function. Briefly, the 6MWT was conducted by marking off a 20 m distance in a straight and flat interior hallway and asking participants to walk as quickly as possible for 6 minutes, with standardized, verbal encouragement given at every minute, as adapted from the American Thoracic Society (2002) and Enright (2003). The total distance walked was determined to the nearest cm. There are no standard guidelines or current standards for conducting a SCT and the feasibility of the test parameters are dependent on the environmental setting (Dobson et al 2013). However, when permitted, an ascent and descent of 9 stairs test without external distractions is recommended (Dobson et al 2013). In this study, the SCT involved participants ascending and descending a flight of 7 stairs as quickly and safely as possible, while holding on to the handrail. Assistive devices were not used by any participants and no verbal encouragement was provided. The time required for the total ascent and descent was recorded to the nearest millisecond (ms).

Statistical Analysis

Since serum biomarker data were not normally distributed (determined by the Shapiro-Wilk test), Spearman’s coefficients were used to examine correlations with clinical characteristics, controlling for age, sex, and body mass index (BMI). There were no significant correlations
between serum biomarkers and disease duration (data not shown), so disease duration was not included in the final correlation analysis. Biomarker levels were compared between females and males using an unpaired Student’s t-test. All statistical analyses were performed using the Statistical Analysis System (version 9.3; Cary, NC, USA) with \( p \leq 0.05 \) considered statistically significant. Unless otherwise indicated, data are presented as mean ± standard deviation.

4.4 Results

Participant characteristics

Participant characteristics are summarized in Table 4.1. Participants had a mean age of 60 ± 11.8 years, mean BMI of 32.6 ± 7.5 kg/m\(^2\), and were predominately female. In this cohort of adults with knee OA, WOMAC scores revealed a mild to moderate level of pain, and moderate levels of stiffness and physical functioning. Physical function as assessed with the SCT and 6MWT was worse than Canadians of a similar age group, but similar to obese and persons with knee OA (Enright 2003; Hill et al 2011).
Table 4.1 Participant characteristics, WOMAC scores and physical function tests (n=54)*

<table>
<thead>
<tr>
<th>Participant Characteristics</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>60.0 ± 11.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.6 ± 7.5</td>
</tr>
<tr>
<td>Disease duration (months)</td>
<td>86.6 ± 112.0</td>
</tr>
<tr>
<td>Males/Females (N)</td>
<td>16/38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>WOMAC Scores</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain score (mm)</td>
<td>193.4 ± 94.4</td>
</tr>
<tr>
<td>Stiffness score (mm)</td>
<td>104.4 ± 45.1</td>
</tr>
<tr>
<td>Physical function score (mm)</td>
<td>651.0 ± 329.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physical Function Tests</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SCT (seconds)</td>
<td>12.1 ± 3.6</td>
</tr>
<tr>
<td>6MWT (meters)</td>
<td>445.6 ± 76.3</td>
</tr>
</tbody>
</table>

*Mean ± standard deviation.
BMI = body mass index; 6MWT = 6-minute walk test; SCT = stair climb test.

Serum Biomarkers

Mean levels of the biomarkers detected in the serum samples are presented in Table 4.2. There were no significant differences between females and males, with the exception of MMP-3 (p=0.01). LIF was not detected (data not shown). Serum HA was significantly associated with longer 6MWT distance (r=0.33, p=0.02) and less time taken in the SCT (r= -0.38, p=0.006) in all participants (Figure 1). Serum MMP-3 was significantly associated with higher pain score (r=0.30, p=0.03) in all participants (Figure 2A). Serum IL-6 was significantly associated with higher pain score (r=0.31, p=0.03) (Figure 2B), higher stiffness score (r=0.30, p=0.03) more time taken in the SCT (r=0.32, p=0.02) (Figure 3), and non-significantly with decreased 6MWT distance (r= -0.24, p=0.09). Serum COMP, PIIANP, CRP, NO, and IL-18 were not significantly associated with any clinical characteristics (data not shown).
Table 4.2 Serum biomarker levels

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>All Participants (N=54) Mean ± SD (Range)</th>
<th>Females (N=38) Mean ± SD</th>
<th>Males (N=16) Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMP (ng/mL)</td>
<td>309.7 ± 123.6 (118.9 – 810.7)</td>
<td>301.7 ± 130.5</td>
<td>329.9 ± 105.7</td>
</tr>
<tr>
<td>PIIANP (ng/mL)</td>
<td>2541.6 ± 1311.0 (1004.0 – 6979.0)</td>
<td>2594.6 ± 1268.8</td>
<td>2412.3 ± 1194.1</td>
</tr>
<tr>
<td>HA (ng/mL)</td>
<td>96.6 ± 80.9 (14.2 – 421.8)</td>
<td>94.9 ± 14.0</td>
<td>100.6 ± 84.7</td>
</tr>
<tr>
<td>MMP-3 (ng/mL)</td>
<td>16.4 ± 9.4 (4.3 – 45.7)</td>
<td>14.3 ± 8.4\textsuperscript{a}</td>
<td>21.1 ± 10.2\textsuperscript{b}</td>
</tr>
<tr>
<td>IL-18 (pg/mL)</td>
<td>62.1 ± 31.4 (23.4 – 156.5)</td>
<td>57.9 ± 28.8</td>
<td>72.1 ± 35.9</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>3.4 ± 5.5 (0.7 – 11.5)</td>
<td>3.8 ± 6.4</td>
<td>2.4 ± 1.5</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>4.3 ± 5.1 (0.5 – 22.0)</td>
<td>5.1 ± 5.8</td>
<td>2.6 ± 2.2</td>
</tr>
<tr>
<td>NO (μmol/L)</td>
<td>24.6 ± 12.2 (3.7 – 69.9)</td>
<td>25.6 ± 14.0</td>
<td>22.24 ± 11.1</td>
</tr>
</tbody>
</table>

\textsuperscript{a,b} Levels of MMP-3 were significantly different between males and females (p=0.01)

COMP = cartilage oligomeric matrix protein; CRP = C-reactive protein; HA = hyaluronic acid; IL-6 = interleukin-6; IL-18 = interleukin-18; MMP-3 = matrix metalloproteinase -3; NO = nitric oxide; PIIANP = type-IIA collagen N-propeptide; SD = standard deviation.

Figure 4.1 Significant associations between serum HA and physical function tests in all participants. Serum HA was measured by ELISA in 54 participants with knee OA and correlated with performance in the 6MWT (p=0.02) (A) and SCT (p=0.006) (B) as objective measures of physical function.
Figure 4.2 Significant associations between serum MMP3 and IL-6 and WOMAC pain score in all participants. Serum MMP3 was measured by ELISA in 54 participants with knee OA and correlated with WOMAC pain score (A). Serum IL-6 was measured by a ELISA in 54 participants with knee OA and correlated with WOMAC pain score (B).

Figure 4.3 Significant associations between serum IL-6 and stair climb test (SCT). Serum IL-6 was measured by ELISA in 54 participants with knee OA and correlated with performance in the SCT as an objective measure of physical function.

4.5 Discussion

This study examined the relationships between serum biomarkers and OA clinical characteristics in participants with mild to moderate painful knee OA. Participants in the study represented a
typical population of adults with knee OA, with a mean age of 60 years, a mean BMI in the obese category, and 70% female. WOMAC scores revealed a mild to moderate level of pain and moderate levels of stiffness and physical function. This study correlates a panel of joint and inflammatory serum biomarkers to WOMAC scores and performance-based measures of physical function (i.e. 6MWT and SCT) to investigate the relationship of biomarkers with OA pain and physical function in order to advance the use of biomarkers in the diagnosis, study and treatment of OA.

Serum HA was significantly associated with longer 6MWT distance and faster SCT time in all participants. To our knowledge, this is the first time serum HA has been correlated with objective measures of physical function, although variations in HA levels during the day are activity and movement related, not strictly diurnal (Criscione et al 2005). HA is a glycosaminoglycan produced by cells in the synovial membrane and found in cartilage and synovial fluid (Criscione et al 2005). It has a structural role in the connective tissue matrix and is involved in joint lubrication (Elliott et al 2005; Turan et al 2007). Serum HA increases with age and disease duration and is higher in OA patients compared to controls (Elliott et al 2005; Turan et al 2007). Serum HA has also been positively associated with radiographic OA in several (Elliott et al 2005; Golightly et al 2011; Sasaki et al 2013), but not all (Turan et al 2007), studies. Joint movement pumps excess HA and water out of the joint and into the lymphatic system and circulation (Criscione et al 2005; Goldberg et al 1991). It is possible that individuals with better physical functioning are more active and therefore have more HA in the circulation. The association of HA with measures of physical function in this study are reasonable as HA plays a role in joint lubrication (Elliott et al 2005) and serum levels have been found to change with movement (Criscione et al 2005), but more research into the relationship between serum HA and physical functioning is required.

Serum MMP-3 was significantly associated with higher WOMAC pain scores. MMPs are produced by chondrocytes and synovial fibroblasts and are responsible for hydrolyzing macromolecules in the extracellular matrix (ECM) (Lohmander et al 2005). MMP-3, specifically, plays an important role in the destruction of the ECM in OA and is higher in the serum and synovial fluid of OA patients than healthy controls (Lohmander et al 2005; Manicourt et al
Serum MMP-3 has not previously been found to correlate with WOMAC scores or pain scores, but has been associated with changes in joint space narrowing (JSN) and cartilage volume (Lohmander et al 2005; Pelletier et al 2010). In one randomized controlled trial in adults with knee OA, serum MMP-3 decreased in the treatment group, which also experienced increased function and decreased pain scores (Manicourt et al 2006). MMP-3 was the only biomarker that was significantly different between males and females in the current study. This trend for sex-related differences was also reported by Manicourt et al. (1994).

Serum IL-6 was significantly associated with higher WOMAC pain score, stiffness score and worse performance on the SCT and non-significantly in the 6MWT. IL-6 is a pro-inflammatory cytokine that plays a role in cartilage destruction in OA (Shimura et al 2013; Stannus et al 2010). It can stimulate synovial proliferation, osteoclast activation, and MMP production (Park et al 2007). In rheumatoid arthritis it has been correlated with laboratory and clinical characteristics and blocking IL-6 can be an effective therapy (Ding et al 2006; Park et al 2007). Interestingly, it was reported that IL-6 levels above 2.5 pg/mL increase the risk of mobility disability in metabolic syndrome (Esposito et al 2004). This is a population that presents with relatively high BMIs and unfavourable inflammatory profiles, similar to OA. In the current study, the mean level of IL-6 in all participants was 3.4 ± 5.5 pg/mL and IL-6 correlated with decreased distance in the 6MWT and slower time in the SCT, demonstrating a significant relationship between serum IL-6 and physical function in this sample of OA patients. Penninx et al. (2004) also found that serum IL-6 levels were associated with poorer performance in the 6MWT and higher IL-6 levels were associated with fewer steps taken per day in individuals with OA (Stannus et al 2010). Serum IL-6 levels were not previously found to be associated with WOMAC scores (Bas et al 2014; Brenner et al 2004; Penninx et al 2004), but significantly associated with pain severity on a VAS scale (Shimura et al 2013). Levels have been found to decrease alongside decreases in pain score and improvements in physical function, in randomized controlled clinical trial (Abou-Raya et al 2014; Messier et al 2013). Similarly, baseline IL-6 levels have been associated with change in pain while standing (Stannus et al 2013), also supporting the connection between IL-6, pain, and physical function.
Both MMP-3 and IL-6 were correlated with pain in this study, and although MMP-3 has not been previously correlated with pain scores, IL-6 has been shown to up-regulate MMP-3 levels, suggesting that the relationship between MMP-3 and pain could be mediated through IL-6. Inflammation is a key component of OA and is associated with knee pain and progression of cartilage degeneration (Bas et al 2014). Inflammatory mediators can activate and sensitize afferent nociceptive sensory fibers, causing hyperalgesia and altered pain processing seen in OA (Bellucci et al 2013).

Despite the significant associations discussed above, there were a number of biomarkers that did not correlate with any clinical characteristics. Serum COMP, although a well-studied OA biomarker (Verma et al 2013), was not significantly associated with any of clinical characteristics in the current study. COMP is a non-collagenous glycoprotein that binds to collagen to stabilize the articular cartilage collagen fiber network and is released as bound proteins are cleaved from the cartilage matrix in OA (Wisłowska et al 2005; Clark et al 1999). It is been shown to predict structural progression of OA in several studies (Blumenfeld et al 2013; Clark et al 1999; Golightly et al 2011; Sharif et al 2004; Valdes et al 2014; Verma et al 2013). However, the association of serum COMP with pain and physical function is not clearly established. Serum COMP has been associated with non-standardized pain scores in two studies (Sowers et al 2009; Verma et al 2013) and with WOMAC pain score in 30 adults with knee OA (Wisłowska et al 2005). However, in other reports, serum COMP was not associated with WOMAC scores or other clinical characteristics (El-Arman et al 2010) and in a 3-year study of OA progression, changes in serum COMP were not associated with changes in WOMAC scores (Vilim et al 2002). COMP has also been reproducibly associated with Kellgren-Lawrence (KL) grade and JSN from radiographs and loss of cartilage from MRI (Blumenfeld et al 2013; Clark et al 1999; Valdes et al 2014). We hypothesize that, since COMP is a component of cartilage, it may be a structural marker, but not a marker of pain or physical function in OA, explaining the absence of correlations in the current study.

Serum PIIANP was also not associated with any of the clinical characteristics investigated. Serum PIIANP is thought to indicate the rate of type II collagen synthesis as the cartilage attempts repair to damaged cartilage (Aigner et al 1999). Type II collagen is synthesized as a
procollagen molecule, with type IIB in normal adult cartilage and type IIA procollagen expressed in early cartilage and at the onset of cartilage hypertrophy (Garnero et al 2002; Rousseau et al 2004). Studies have not confirmed a correlation between serum PIIANP and KL score, pain on a VAS, or function as measured by Lequesne's index (Garnero et al 2002; Quintana et al 2008). It was observed that patients with lower serum PIIANP had worse radiographic progression after 1 year (Garnero et al 2002). Similar to COMP, PIIANP is a marker of collagen synthesis in cartilage, and it is reasonable that it is an indication of structural damage, but not clinical symptoms, in adults with knee OA.

Serum CRP also did not significantly correlate with any of the clinical characteristics studied. CRP is a marker of general inflammation and has been investigated for its role in OA pathogenesis, with conflicting evidence (Pelletier et al 2010). A meta-analysis of 32 studies in OA patients found that CRP levels were moderately correlated with increased pain and decreased physical function, but not with radiographic evidence (Jin et al 2013). However, the high correlation between CRP and obesity may limit the utility of CRP as a biomarker in OA (Garnero et al 2001; Sowers et al 2002). For example, in this study, CRP was significantly associated with a number of clinical characteristics only before BMI was accounted for in the model (data not shown).

Serum IL-18 was also not significantly associated with any clinical characteristics in this study. IL-18 is well known for its role in rheumatoid arthritis and induction of cartilage degradation through production of MMPs (Futani et al 2002). It is reported to be higher in the plasma and synovial fluid of OA patients relative to healthy controls and correlates with radiographic severity (Wang et al 2013). However, this is the first study to examine serum IL-18 in relation to OA clinical characteristics. In adults with OA, IL-18 was higher in synovial fluid than plasma (Wang et al 2013) and, it is possible that synovial fluid levels better correlate with clinical characteristics in OA.

Serum NO was also not significantly associated with any clinical characteristics measured. NO is a marker of oxidative stress and, along with its redox derivatives, appears to have a number of different functions in both normal and diseased joints (Abramson 2008). Nitrate and nitrite levels
have been found to be higher in plasma and synovial fluid of OA patients compared to healthy adults (Karan et al 2003). However, similar to the present findings with serum, synovial fluid NO was not previously related to WOMAC score in adults with knee OA (Brenner et al 2004).

This exploratory investigation has some noteworthy strengths and limitations. One limitation is that sufficient synovial fluid samples were not available from this group of adults. Since several studies have reported that inflammatory cytokines are higher in synovial fluid than serum (Wang et al 2013), examination of both would have provided a more complete picture of the synovial-serum concentration relationship. In addition, the joint and inflammatory biomarkers examined in this study are not specific to the knee and their levels may not represent changes occurring only in the knee joint, but rather reflect whole body changes in cartilage or inflammation. OA in other joints was not examined or accounted for in this study, and this could also account for the lack of association between some biomarker levels and knee specific symptoms (Bijlsma et al 2011; Felson et al 2014; Kraus et al 2011). However, whole body joint deterioration is difficult to account for and remains an important limitation in OA biomarker research (Bijlsma et al 2011; Felson et al 2014; Kraus et al 2011). The inclusion of participants in this study was made on the basis of self-reported physician diagnosed knee OA, which has shown good agreement with medical records (March et al 1998; Cheng et al 2000; Ho-Pham et al 2014). A similar approach has been taken in previous OA studies (Parazzini et al 2003; Hootman et al 2003; Miller et al 2008). In addition, the timing of blood sampling was not controlled. However, diurnal variation of biomarkers is not completely understood or explored. Examinations into HA, PIIANP, and COMP revealed that differences in serum levels only occur between before arising from bed and 1 hour following usual morning activities, with levels staying consistent for the reminder of the day (Criscione et al 2005; Quintana et al 2008; Kong et al 2006). Strengths of this study were the use of common and validated measures of pain and other symptoms of OA with the WOMAC and objective measures of physical function in the 6MWT and SCT. In addition, a panel of biomarkers was measured, representing different joint tissues and inflammation. A sample size of 54 was sufficient to detect significant differences and is comparable to similar cross-sectional analyses of pain and biomarkers in OA (Ishijima et al 2011; Orita et al 2011). However, future OA biomarker investigations should consider including larger sample sizes with repeated measurements.
4.6 Conclusion

This study found significant correlations between serum biomarkers and clinical characteristics in a population with mild to moderate OA knee pain. Specifically, serum HA was correlated with physical function, serum MMP-3 was correlated with pain, and serum IL-6 was correlated with pain and physical function. The markers of collagen metabolism, COMP and PIIANP, were not associated with any clinical characteristics. Just as the diagnosis of OA is based on a combination of structural and symptomatic features (Zhang et al 2010), a combination of structural and symptomatic biomarkers may be required to fully characterize OA status.

4.7 Acknowledgments

The research was sponsored by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA #200121). We thank all the study participants as well as Natasha Sheikh and Svitlana Yurchenko, for their assistance in data collection. Additionally, we acknowledge Premila Sathasivam, and Mehrnoosh Kashani for their assistance with sampling, Anna Deboer, Laelie Snook, and Jessica Ralston for their technical support, and the many undergraduate research students and volunteers whom helped with the study.
4.8 Bridge to Chapter 5

OA pain was examined in relation to lifestyle factors and serum biomarkers in Studies 1 and 2, with the goal of gaining a deeper understanding of OA pain in order to treat and manage it. Reducing OA pain could improve physical function and quality of life for adults suffering with OA. Study 3 will now investigate a novel treatment in a randomized, double-blind clinical trial with the primary outcome of pain reduction. Secondary outcome measures include the same clinical characteristics examined in Study 2; physical function and stiffness scores, 6-minute walk test performance, and stair climb task performance. In addition, evaluation of the treatment with change in biomarker levels is presented in Appendix 15.
Chapter 5: High-Rosmarinic Acid Spearmint Tea in the Management of Knee Osteoarthritis Symptoms

As published with revisions for thesis:
5.1 Abstract

Individuals with medically diagnosed knee osteoarthritis (OA) participated in a randomized, double-blind study to investigate the effects of a high-rosmarinic acid (rosA) spearmint tea. Sixty-two participants were randomized by gender and screening pain score to consume tea brewed from a high-rosA spearmint variety or a commercially available spearmint twice daily for 16 weeks. Pain, quality of life, and physical function at baseline and Week 16 were assessed using the Western Ontario and McMaster Osteoarthritis Index (WOMAC), Short-form 36-item Health Survey (SF-36), 6-Minute Walk Test (6MWT), and Stair Climb Test (SCT). Data from 46 participants (mean age=60.7; BMI=32.9 kg/m²) were analyzed. Pain score significantly decreased from Week 0 to 16 for the high-rosA group but not for the control group and scores for stiffness and physical disability significantly decreased from Week 0 to 16 for both groups. Increased quality of life score on the bodily pain index in the SF-36 was observed at Week 16 within the high-rosA group only, although no significant differences were observed between the groups. A non-significant improvement was observed in the 6MWT at Week 16 in the high-rosA group only. There were no changes in the SCT for either group. Therefore, 16-week daily consumption of the high-rosA and commercial spearmint teas significantly improved stiffness and physical disability scores in adults with knee OA, but only the high-rosA tea significantly decreased pain. Consumption of high-rosA tea warrants further consideration as a potential complementary therapy to reduce pain in OA. Clinical Trial Registration Number: NCT01380015.

5.2 Introduction

Osteoarthritis (OA) is a disease characterized by the degeneration of articular cartilage, manifesting as joint pain, stiffness, and impaired function leading to physical disability and decreased quality of life (Martel-Pelletier and Pelletier 2010). Knee OA is one of the most common chronic diseases with an estimated prevalence of 24% among North America adults (Bijlsma et al 2011). The pathogenesis of OA is complex and not fully understood, but is associated with an imbalance of anabolic and catabolic activities in the cartilage in which degenerative processes prevail, leading to the loss of cartilage (Loeser 2009). There is no
treatment that slows cartilage breakdown, so treatment is focused on decreasing pain and increasing function with non-pharmacological interventions like exercise and assistive devices, as well as non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen (Zhang et al 2008). While these pharmaceuticals offer acute pain management for some individuals, serious gastrointestinal and cardiac side effects limit their long-term use (Pincus et al 2001). It is reported that 30–45% of individuals with OA also use supplements to manage the disease (Lapane et al 2012; Lawson et al 2004; Singh et al 2006), with glucosamine being the most common. However, even for glucosamine, controversy remains surrounding efficacy, after a large National Institute of Health, randomized, double-blind glucosamine trial failed to show any significant improvements in OA patients (Clegg et al 2006). Therefore, further research into safe, complementary therapies for OA is required.

Rosmarinic acid (rosA) is a polyphenolic compound naturally present in spearmint, peppermint, fennel, and other species of the Lamiaceae family and which has been hypothesized to be of potential benefit for OA (Fletcher et al 2005; Pearson et al 2010; Petersen et al 2003; Youn et al 2003). In vitro, rosA has been shown to have anti-inflammatory, anti-oxidant, immunosuppressant, and anti-bacterial activities (Youn et al 2003). Through selective breeding techniques, a spearmint plant containing approximately 20 times more rosA than native spearmint was developed (high-rosA spearmint plant clone 700B) (Fletcher et al 2005). In porcine cartilage explants, a high-rosA spearmint extract reduced the expression of lipopolysaccharide (LPS)-induced prostaglandin E₂ (PGE₂) and nitric oxide release and also inhibited glycosaminoglycan (GAG) release, suggesting a potential chondroprotective effect (Pearson et al 2010). Also, healthy, mature standardbred horses were fed hay and sweet feeds containing either no (n=4) or 54 mg/kg body weight of the high-rosA spearmint (n=4) for 24 days (Pearson et al 2012). Following intercarpal LPS injections to induce inflammation, the horses consuming high-rosA spearmint had reduced synovial fluid PGE₂ and GAG levels compared to the control horses. These results demonstrate the anti-inflammatory and potential chondroprotective actions of the high-rosA spearmint and support its use as a complementary therapy for the management of knee OA. Therefore, a study was conducted to examine the effects of daily consumption of tea brewed from the high-rosA spearmint plant on measures of pain, stiffness, quality of life, and physical function in adults with OA of the knee.
5.3 Methods

Study Design

This randomized, parallel-arm, double-blind study was conducted at the Human Nutraceutical Research Unit of the University of Guelph. The University of Guelph Human Research Ethics Board approved the study protocol (REB#11JA040), which was registered in the NIH clinical trial registry (Protocol No. NCT0138001) and all participants provided written consent.

Recruitment and Screening

Adult (>18 years old) males and females were recruited from Guelph, ON and the surrounding communities from June 2011 to June 2012 and were eligible for study inclusion if they were non-smokers, had medically diagnosed OA of the knee and a screening Western Ontario and McMaster University Osteoarthritis Index (WOMAC) pain score of >125. Exclusion criteria included other systemic or rheumatic arthritis, concomitant inflammatory processes, upcoming knee replacement surgery, chemical, radiologic, or surgical synovectomy in any large joint within the previous 3 months, gastrointestinal ulcers, clinically significant, uncontrolled cardiovascular, hepatic, or renal disorder, any serious medical condition within 6 months such as heart attack, stroke, cancer, or diabetes, known allergy or hypersensitivity to mint or other food allergies, smoking, alcohol consumption >14 drinks per week, recreational drug use, participation in a clinical trial within the previous 6 months, and pregnancy (or intention to become pregnant), less than 6 months post-partum, lactating or less than 6 months post-lactation.

Study Treatment Tea and Tea Protocol and Blinding

The study treatment tea was brewed from the high-rosA spearmint plant (Clone 700B) (Fletcher et al 2005) and the control tea was brewed from commercially available spearmint tea (Distinctly Teas Inc., Stratford, ON). Dried spearmint leaves were blended on low in a food-grade blender for 30 seconds. Three grams of plant material were transferred into individual Teeli®flip tea bags (Riensch & Held, Germany), which were stapled. The identical control and high-rosA tea
bags were placed in vacuumed sealed bags, coded by a person external to the study team, and stored at room temperature until use. All participants and study team members were blinded throughout data collection and analysis.

Participants were instructed to consume 2 cups of tea per day for a 16-week period in a provided 300 mL study mug. Brewing instructions detailed that one tea bag was to be steeped in boiling water for 5 minutes with occasional stirring and the addition of milk, cream, sugar or sweetener was not allowed. Three grams of high-rosA spearmint in 300 mL of water for 5 minutes was verified by high-performance liquid chromatography to provide 130-150 mg of rosA per cup compared with ~13 mg rosA from the control tea (data not shown). Therefore, participants in the control and high-rosA groups consumed 26 versus 280 mg rosA per day, respectively. Analysis of brewed tea samples by Maxxam Analytics (Mississauga, ON) ensured pesticide, microbiological and mycotoxin levels were below the NHP Directorate Accepted Tolerance Limits (NHPD 2012). Participants were asked to not consume any herbal teas for the duration of the study. Consumption of coffee as well as black and green tea was allowed. Participants recorded the times at which they consumed the study tea in a daily study diary as a measure of compliance.

Treatment Randomization

Participants were block randomized into treatment groups based on sex and screening WOMAC pain score. Covariate adaptive randomization (Kang et al 2008) was performed by an individual not associated with the study. Sequentially numbered sheets of paper were used to implement the allocation sequence.

Outcome Measures

Outcome measures were assessed at baseline, Week 8, and 16. At baseline and after completion of the 16-week protocol, anthropometric measurements were taken. Phone check-in conversations occurred at weeks 2, 6, 10 and 14 to discuss compliance, medications, physical activity, and any other issues. Safety of tea consumption was assessed by recording adverse
events. Participants were asked to maintain their regular medication and supplement use throughout the study period but were also advised that they could increase or decrease their use of pain medication as they felt necessary. They were asked to track all medications and supplements in their daily study diary. Changes in pain medication consumption were quantified by calculating total number of tablets taken per week (Constant et al 1997).

Height was measured to the nearest millimeter using a stadiometer (Model 217, SECA®, Hanover, USA) and body weight was measured to the nearest 0.05 kg on a digital scale (SVI-200F, Acculab®, Barrie, Canada). Two measurements were averaged for each of waist and hip circumference determined over clothing with a tape measure (Model 201, SECA®, Hanover, USA). Blood pressure and body composition was determined with an Omron® digital blood pressure monitor and bioelectrical impedance analysis machine (Bodystat®1500, Bodystat®, Isle of Man, UK), respectively. All anthropometric measurements were completed according to standard operating procedures and completed by the same trained study coordinator (AEC).

The WOMAC is a validated, standardized 24-item questionnaire that assesses pain, disability and joint stiffness associated with OA (Bellamy et al 1988). In the presence of a study coordinator, the 100 mm visual analog scale (VAS) version of the WOMAC was administered and scored according to the WOMAC® Osteoarthritis Index User Guide IX (Bellamy 2009).

The Medical Outcomes Study 36-item Short-Form General Health Survey (SF-36) is a self-administered questionnaire that assesses 8 components of health-related quality of life (QoL) (i.e. physical function, physical role, bodily pain, vitality, social functioning, role emotional, mental health, and general health) and 2 composite scores (i.e. physical component score and mental composite score) (Maruish et al 2009). In the presence of a study coordinator, participants completed the questionnaire which was scored using Medical Outcomes software.

The 6-minute walk test (6MWT) and 7-stair climb test (SCT) are performance based measures used to assess physical function. Briefly, the walk test was conducted by marking off a 20 m distance in a straight and flat interior hallway and asking participants to walk as quickly as possible for 6 minutes, with standardized, verbal encouragement given at every minute, as
adapted from the American Thoracic Society (2002) and Enright (2003). Participants were permitted to rest, as necessary, and to use any mobility aids, as per their normal use. The total distance walked was determined to the nearest cm. The SCT involved participants ascending and descending a flight of 7 stairs as quickly as was safely possible. The time required for the total ascent/descent was recorded to the nearest millisecond (ms). The 6MWT and SCT were administered by the same trained study coordinator (NS).

A non-fasted 5 mL blood sample was collected in a standard serum separating tube (SST™, BD, Mississauga, Canada) tube by a qualified medical technician via venipuncture at baseline and Week 16. Samples were allowed to sit at room temperature for 30 minutes prior to centrifugation (2500 rpm and 4 °C for 15 minutes). For 48 hours before the blood samples were being taken, participants were instructed to not participate in alternative therapies such as acupuncture, massage therapy, or strenuous physical activity. Serum was sent to Lifelabs® Medical Laboratory Services (Guelph, Canada) for analysis of C-reactive protein (CRP) by latex-enhanced immunoturbidimetric assay. Serum biomarker quantification was performed in duplicate using enzyme-linked immunosorbent assay (ELISA) on duplicate samples for COMP, HA, MMP-3, and nitric oxide (NO) (R&D systems, MN, USA), and PIIANP (EMD Millipore, MO, USA) according to the manufacturers’ protocols. The assay detection limits were 0.010 ng/mL for COMP, 0.068 ng/mL for HA, 0.009 ng/mL for MMP-3, 0.25 μmol/L for NO, and 30 ng/mL for PIIANP. Inflammatory cytokines interleukin 18 (IL-18) and leukemia inhibitory factor (LIF) were measured in duplicate using a custom Bio-Plex Pro cytokine kit (Bio-Rad Laboratories, CA, USA) according to the manufacturer’s instructions. The lower limit of quantification was 1.8 pg/mL for IL-18 and 12 pg/mL for LIF.

Statistical Analysis

All statistical analyses were performed using the Statistical Analysis System (version 9.3; Cary NC, USA) with $P<0.05$ considered statistically significant. A sample size calculation based on the outcome of total WOMAC score, using a significance level of 0.05 and power of 80%, indicated that 25 participants per group were required. Individual WOMAC pain, stiffness and function subscales were converted to normalized units (NU, 0 - 100) (Bellamy 2009). Data was
examined for normality using stem leaf diagrams and box plots. Baseline anthropometric data and outcome scores were compared between the high-rosA and control groups using unpaired t-tests. Analysis for efficacy was done using the per protocol population. Baseline data for all outcome measures were compared against Weeks 8 and 16 within the high-rosA and control groups using unpaired Student’s t-tests. Between high-rosA and control group differences (Week 0 to Week 8 and Week 0 to Week 16) were analyzed for all outcome measures using repeated measures analysis of variance (ANOVA). Differences in pain medication intake between groups was assessed using repeated measures ANOVA. As biomarker data was not normal, the log of values was taken and differences between groups over time was examined with repeated measures ANOVA.

5.4 Results

Participant Flow, Withdrawals and Exclusions

A total of 411 individuals completed the phone screening questionnaire, 191 individuals completed the in-person screening questionnaire, and 62 individuals were randomized to treatment (Figure 5.1). Twenty-eight participants in each group received the intervention, and 2 participants in the high-rosA group withdrew due to time commitment issues and 1 due to a previously unidentified mint sensitivity. One participant in the control group withdrew due to a diagnosis of gout, 1 due to knee replacement surgery, 1 from prolonged non-related food poisoning illness, and 1 participant was excluded because they began smoking. In the high-rosA group, data from 2 participants was removed from analysis due to arthroscopic surgery after 12 weeks participation in the study and 1 for non-compliance with study protocols. Complete data was collected and analyzed for the remaining 46 participants.
Figure 5.1 Participant flow through the trial (CONSORT diagram)
Participant Characteristics

Participant characteristics and baseline outcome measures were not significantly different between the high-rosA (n=22) and control (n=24) groups at baseline (Table 5.1). Participant characteristics and anthropometric measurements were not significantly different between groups at any time during the study (data not shown). Tea consumption compliance was 96.8 and 94.8% for the high-rosA and control tea groups, respectively, as self-reported in daily study diaries. This was consistent with study coordinator tracking of dispensed and returned tea bags. There were no significant differences in pain medication tablets consumed per week between the groups or over time (data not shown).
Table 5.1 Participant Characteristics and Outcome Measures at Baseline

<table>
<thead>
<tr>
<th>Participant characteristics</th>
<th>High-rosA (n=22)a</th>
<th>Control (n=24)a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participant characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>60.5 ± 11.1</td>
<td>60.8 ± 12.1</td>
</tr>
<tr>
<td>Sex (n) (males/females)</td>
<td>8/14</td>
<td>6/18</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.8 ± 9.4</td>
<td>164.3 ± 8.8</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.8 ± 28.3</td>
<td>85.2 ± 19.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.3 ± 8.6</td>
<td>31.5 ± 6.9</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>65.6 ± 18.2</td>
<td>58.4 ± 13.0</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>49.9 ± 13.2</td>
<td>45.0 ± 9.8</td>
</tr>
<tr>
<td>Blood pressure SBP/DBP (mmHg)</td>
<td>135.4/83.8</td>
<td>132.3/80.7</td>
</tr>
<tr>
<td>Pulse (bpm)</td>
<td>73.9 ± 11.5</td>
<td>73.8 ± 12.2</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>37.8 ± 12.0</td>
<td>38.0 ± 9.6</td>
</tr>
<tr>
<td>Lean body weight (kg)</td>
<td>57.0 ± 16.1</td>
<td>51.8 ± 10.9</td>
</tr>
<tr>
<td><strong>WOMAC Scores</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain</td>
<td>39.6 ± 13.9</td>
<td>36.1 ± 22.2</td>
</tr>
<tr>
<td>Stiffness</td>
<td>54.9 ± 19.6</td>
<td>52.5 ± 23.2</td>
</tr>
<tr>
<td>Physical disability</td>
<td>36.8 ± 17.7</td>
<td>39.0 ± 22.1</td>
</tr>
<tr>
<td>Total score</td>
<td>131.3 ± 42.4</td>
<td>127.5 ± 60.5</td>
</tr>
<tr>
<td><strong>SF-36 Scores</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mental component summary score</td>
<td>56.4 ± 9.0</td>
<td>52.0 ±10.4</td>
</tr>
<tr>
<td>Physical component summary score</td>
<td>38.6 ± 6.6</td>
<td>39.3 ± 7.3</td>
</tr>
<tr>
<td><strong>Physical Function Tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6MWT (m)</td>
<td>448.4 ± 73.8</td>
<td>487.1 ± 87.2</td>
</tr>
<tr>
<td>SCT (seconds)</td>
<td>11.4 ± 3.0</td>
<td>12.9 ± 4.2</td>
</tr>
</tbody>
</table>

aValues are mean ± standard deviation.

DBP = diastolic blood pressure; SBP = systolic blood pressure; 6MWT = six-minute walk test; SCT = stair climb task.

**Adverse Events**

There were no serious adverse events reported during the study. Participants in the high-rosA group reported headache (n=2), constipation (n=3), and loose bowel movements (n=1). Participants in the control group reported dry mouth (n=1), itchy skin (n=1), and staining of dentures (n=1). All reported adverse events were transient and short-term.

**Outcome measures**

WOMAC pain score significantly decreased from baseline within the high-rosA group at Week 16 (p=0.002) (Figure 5.2A). In the control group, WOMAC pain score also decreased from
baseline; however, it was significant at Week 8 (p=0.04) but not at Week 16 (p=0.07). There were no significant differences in WOMAC pain score between the groups at any time points in the study. WOMAC stiffness significantly decreased from baseline to Week 16 within the high-rosA (p=0.004) and control (p=0.04) groups, although there was no difference between groups (p=0.37) (Figure 5.2B). WOMAC physical disability score significantly decreased from baseline to Week 16 within the high-rosA (p=0.02) and the control (p=0.03) groups but no significant differences were observed between the groups at any time point (Figure 5.2C). Generally, WOMAC scores on all scales did not significantly differ from Week 8 to Week 16 within or between the treatment groups.

**Figure 5.2** Mean scores and standard error are presented for (A) WOMAC pain scores, (B) WOMAC stiffness scores, (C) WOMAC physical function score and (D) WOMAC total scores for the high-rosA group (circles) and the control group (triangles). The change within groups from baseline to week 16 was examined; significant differences within the high-rosA group are indicated with a cross (+) and significant differences within the control group are indicated with an asterisk (*). rosA, rosmarinic acid; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.
In the SF-36, the only significant change observed was in the QoL score for bodily pain, with an increase observed from baseline to Week 16 within the high-rosA group (Table 5.2). There were no other significant changes observed for SF-36 scores either within or between the rosA and control groups (Table 5.2).

Table 5.2 SF-36 Scores for Weeks 0 and 16 for the High-rosA and Control Groups

<table>
<thead>
<tr>
<th></th>
<th>High-rosA&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Control&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Canadian Norm&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 16</td>
<td>Week 0</td>
</tr>
<tr>
<td>Physical Function</td>
<td>55.7 ± 19.8</td>
<td>59.8 ± 23.5</td>
<td>55.4 ± 20.9</td>
</tr>
<tr>
<td>Physical Role</td>
<td>63.1 ± 19.9</td>
<td>76.1 ± 24.1</td>
<td>61.2 ± 27.7</td>
</tr>
<tr>
<td>Bodily Pain</td>
<td>46.6 ± 7.6</td>
<td>58.1 ± 22.1&lt;sup&gt;*&lt;/sup&gt;</td>
<td>49.7 ± 17.8</td>
</tr>
<tr>
<td>General Health</td>
<td>70.7 ± 12.7</td>
<td>70.8 ± 18.4</td>
<td>69.3 ± 19.0</td>
</tr>
<tr>
<td>Vitality</td>
<td>60.5 ± 15.6</td>
<td>63.1 ± 19.9</td>
<td>51.9 ± 19.6</td>
</tr>
<tr>
<td>Social Functioning</td>
<td>86.9 ± 21.0</td>
<td>89.2 ± 22.9</td>
<td>76.0 ± 25.3</td>
</tr>
<tr>
<td>Role Emotional</td>
<td>86.4 ± 19.0</td>
<td>87.9 ± 23.5</td>
<td>78.8 ± 24.8</td>
</tr>
<tr>
<td>Mental Health</td>
<td>80.0 ± 13.4</td>
<td>80.2 ± 23.4</td>
<td>76.8 ± 19.3</td>
</tr>
<tr>
<td>Physical Component score</td>
<td>38.6 ± 6.6</td>
<td>42.5 ± 9.0</td>
<td>39.3 ± 7.3</td>
</tr>
<tr>
<td>Mental Component score</td>
<td>56.4 ± 8.9</td>
<td>55.8 ± 13.0</td>
<td>51.4 ± 10.9</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are mean ± standard deviation  
<sup>b</sup>For healthy Canadians aged 55-64 years (Hopman et al 2000)  
<sup>*</sup>Change from baseline to Week 16, p<0.05

The 6MWT total distance travelled did not significantly differ from baseline to Week 16 either within or between (p=0.12) the high-rosA (p=0.94) or control groups (p=0.96) (Table 5.3). The total time taken to complete the SCT was not significantly different from baseline to Week 16 either within or between (p=0.8) the high-rosA (p=0.43) or the control group (p=0.44).
**Table 5.3** Six-Minute Walk Test distances (m) for Weeks 0, 8, and 16 for the high-rosA and control groups

<table>
<thead>
<tr>
<th></th>
<th>High-RosA(^a) (m)</th>
<th>Change from baseline(^b) (m)</th>
<th>Control(^a) (m)</th>
<th>Change from baseline(^b) (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 0</td>
<td>451.5 ± 73.6</td>
<td></td>
<td>439.4 ± 87.2</td>
<td></td>
</tr>
<tr>
<td>Week 8</td>
<td>451.7 ± 92.9</td>
<td>+ 0.2</td>
<td>438.3 ± 84.5</td>
<td>- 1.1</td>
</tr>
<tr>
<td>Week 16</td>
<td>473.8 ±72.3</td>
<td>+22.3</td>
<td>439.5 ± 88.2</td>
<td>+0.1</td>
</tr>
</tbody>
</table>

\(^a\)Values are mean ± standard deviation

\(^b\)Difference from Week 0

There were no significant differences in the levels of any biomarkers between groups at any point in the study (Table 5.4).

**Table 5.4** Biomarker levels in the high-rosA and control groups in Week 0 and Week 16*  

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Treatment (mean ± SD)</th>
<th>Control (mean ± SD)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 16</td>
<td>Week 0</td>
</tr>
<tr>
<td>HA (ng/mL)</td>
<td>1.85 ± 0.30</td>
<td>1.85 ± 0.28</td>
<td>1.86 ± 0.32</td>
</tr>
<tr>
<td>MMP-3 (ng/mL)</td>
<td>1.11 ± 0.21</td>
<td>1.13 ± 0.22</td>
<td>1.16 ± 0.22</td>
</tr>
<tr>
<td>PIIANP (ng/mL)</td>
<td>3.35 ± 0.14</td>
<td>3.37 ± 0.14</td>
<td>3.33 ± 0.23</td>
</tr>
<tr>
<td>COMP (ng/mL)</td>
<td>1.49 ± 0.12</td>
<td>1.50 ± 0.15</td>
<td>1.45 ± 0.20</td>
</tr>
<tr>
<td>IL-18 (pg/mL)</td>
<td>1.70 ± 0.20</td>
<td>1.73 ± 0.20</td>
<td>1.79 ± 0.20</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>1.34 ± 0.27</td>
<td>1.31 ± 0.19</td>
<td>1.30 ± 0.21</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.44 ± 0.43</td>
<td>0.36 ± 0.47</td>
<td>0.38 ± 0.50</td>
</tr>
</tbody>
</table>

*Values are log transformed.

COMP = cartilage oligomeric matrix protein; CRP = C-reactive protein; HA = hyaluronic acid; IL-6 = interleukin-6; IL-18 = interleukin-18; ln – log; MMP-3 = matrix metalloproteinase -3; NO = nitric oxide; PIIANP = type-IIB collagen N-propeptide; SD = standard deviation.

**5.5 Discussion**

This was the first human intervention study to examine the effects of daily consumption of tea brewed from a novel high-rosA spearmint plant on markers of pain, stiffness, and physical function in adults with knee OA. The study was also unique in that it utilized a pragmatic design by allowing participants to maintain their normal pain medication routine. This was intended to explore the potential of the high-rosA tea as a complementary OA therapy.
Anthropometric measurements did not change over the treatment period, which is important, as weight loss is known to improve OA symptoms (Gudbergsen et al 2012; Riddle and Stratford 2013). Although the pain scores were never significantly different between groups, the significant decrease during the treatment period within the high-rosA group is an important finding. In the control group, pain scores only significantly decreased from Week 0 to Week 8 with no further significant decreases, suggesting a placebo effect. Placebo analgesia is a well-known phenomenon in OA and pain research (Doherty and Dieppe 2009), which possibly contributed to the lack of significant difference between the groups. Because of this known placebo effect, we prioritized running a blinded study. Therefore, a commercial spearmint tea, identical in appearance and flavor to the high-rosA tea but which contained a small amount of rosA (approximately 15 mg per 300 mL), was used as a control. As the decreases in WOMAC scores ceased at Week 8 in the control group, but continued to decline in the high-rosA group, lengthening the treatment period may result in significant difference between the groups.

The SF-36 questionnaire was created to address the importance of patient perspective in the assessment of health care outcomes (Maruish et al 2009). The significant increase in QoL bodily pain score in high-rosA group suggests that reduced pain led to an increase in QoL in this group. This is in agreement with the decrease in WOMAC pain score observed for the high-rosA group. As expected, the SF-36 scores for physical function, physical role, bodily pain, and physical component score were all below the Canadian norms at all time points studied and in both groups (Table 5.2) (Hopman et al 2000). Individuals with musculoskeletal disorders tend to report lower physical functioning scores compared to the general population and of those patients, OA patients generally have worst pain and physical functioning scores (Dawson et al 2004; Kosinski et al 1999; Picavet and Hoeymans 2004).

The study participants demonstrated impaired physical function compared with average healthy Canadians of comparable age in the 6MWT. In the current study (mean age = 61 years), the average distance walked in the 6MWT at baseline was 445.5 m (range; 312.0 – 611.9 m), which falls below the average distance of 672 m for males and 611 m for females (range; 416 to 888 m) for healthy Canadians with an average age of 65 (Hill et al 2011). This is not surprising, as individuals with arthritis, as well as overweight/obese individuals have been documented to walk
shorter distances in the 6MWT (Enright 2003). Although no significant differences were observed in the 6MWT, the high-rosA group walked 22 meters further at Week 16 than at baseline. The control group only walked 0.1 meters further at Week 16 compared with baseline. We postulate that, at Week 16 the high-rosA group was starting to experience improvements in physical function related to their decreased pain. While not statistically significant, the improvement in walk distance could be meaningful as even small increases in walking distance are important to individuals with OA (Frestedt et al 2009). The SCT is useful as it measures the ability to negotiate stairs and this is a common challenge for individuals with OA (Bennell et al 2011a). Heiberg et al. (2012) reported that individuals with OA completed an 8-stair climb test within the range of 10-14 seconds. This is similar to the range of 10-12 seconds recorded in the present study where 7 stairs were used.

There were no significant changes in any of the biomarkers measured in either treatment group. The data presented in Table 5.4 is included for reference and design of future studies.

In the current study, compliance to drinking the tea was high, which reflects our participants’ willingness to consume spearmint tea as a treatment for OA. This is important, given the lack of effective OA treatment options (Zhang et al 2008) and reliance on pain medications to which adherence rates are low (Sale et al 2006). Failure to adequately manage OA pain and symptoms can negatively impact social interactions and quality of life and lead to increased personal, healthcare, and economic costs (Davis et al 2002).

5.6 Conclusion

In this study, individuals with OA of the knee who consumed 600 mL of high-rosA spearmint tea daily for 4 months showed significant improvements in pain scores and physical function. Therefore, adults with knee OA may benefit from including high-rosA spearmint tea in their daily routine. Larger and longer-term studies are required to validate the high-rosA tea as a complementary OA treatment.
5.7 Acknowledgments

The research was sponsored by the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA #200121). We would like to thank all the study participants. Additionally, we thank Dr. Andrew Chow for his insights during the study design and recruitment stages, Dr. Forrest Caldwell, Premila Sathasivam, and Mehrnoosh Kashani for their assistance with sampling, and the many undergraduate research students and volunteers whom helped with the study.
Chapter 6. Summary, General Discussion, and Future Directions

6.1 Summary of Results

The overall aims of this thesis were to investigate associations in pain and physical function and explore the effect of a novel dietary treatment for knee OA. These aims were addressed by conducting two cross-sectional investigations (Study 1 and 2) and one human clinical trial (Study 3). Together, these studies explore associations in OA symptoms, and managing these symptoms with a novel product.

Pain was significantly higher with the use of OA medications and higher BMI category and significantly lower with use of supplements and meeting physical activity guidelines in 197 adults with knee OA. Examining differences between 3 pain categories revealed that stiffness and physical function scores, bilateral knee OA, BMI category, and OA medication use were significantly higher in the higher pain categories, whereas self-reported health, servings of fruit, supplement use, and meeting physical activity guidelines was significantly lower with higher pain category. These results provide additional information on the complex associations of OA pain and reinforced that modifiable factors should be considered in the management of knee OA.

In 54 adults with knee OA, HA, a marker of synovial membrane metabolism, was significantly associated with performance-based measures of physical function. MMP-3, an enzyme involved in tissue degradation in OA, was significantly associated with higher pain score. The inflammatory marker, IL-6, was significantly associated with higher pain score and worse physical function. Cartilage biomarkers COMP and PIIANP and inflammatory markers IL-18, CRP, and NO were not associated with any clinical variables. The use of serum biomarkers to characterize OA status is important for understanding OA and testing response to novel treatments in OA.

Based on promising pre-clinical antioxidant and anti-inflammatory effects of a high-rosA spearmint plant, study 3 examined this novel therapeutic product for the first time in adults with knee OA. Participants (n=46) were randomized to consume tea brewed from the high-rosA spearmint plant or a commercially available spearmint tea twice daily for 16 weeks in a
randomized, double-blind study. In both groups, stiffness and physical disability scores significantly decreased from Week 0 to Week 16. Pain score significantly decreased from Week 0 to 16 for the high-rosA group only. A non-significant increase in distance walked was observed in the 6MWT at Week 16 in the high-rosA group only. The results from this study warrant further exploration of this novel high-rosA spearmint tea and regular consumption of a high-rosA spearmint tea represents a potential complementary therapy to reduce pain in OA patients.

6.2 General Discussion

OA pain is multifactorial and treating it is complicated. Previous work and findings in this thesis have demonstrated that OA pain is modifiable and, importantly, through lifestyle and dietary strategies (Chapter 5). Unfortunately, many older adults with OA believe that pain is a normal part of aging and that adaptation and/or avoidance of painful activities are the only available options (Ross et al 2001; Davis et al 2002; Sale et al 2006; Hawker 2009). Limitation of activities can lead to social isolation and decreased movement and deconditioning, potentially leading to more pain (Davis et al 2002). In study 1, a number of modifiable factors were significantly associated with OA pain. Similar to other studies, BMI was associated with lower pain (Elbaz et al 2011; Riddle and Stratford 2013; Lee et al 2013b). Also as previously shown (Roddy et al 2005), higher levels of physical activity were associated with decreased pain. Interestingly, in an 18-month RCT in overweight and obese adults with knee OA, weight loss from diet plus exercise resulted in better outcomes in pain and physical function, as well as greater decreases in IL-6 levels, than weight loss from diet or exercise alone (Messier et al 2004; Messier et al 2013). These results demonstrate the importance of exercise and diet in weight loss and the management of knee OA. Continuing to identify modifiable factors of OA pain and investigating their relationship is important for managing the disease.

In study 3, pain decreased with consumption of a bioactive enriched spearmint tea. Dietary constituents, especially ones with anti-inflammatory and antioxidant activities, have potential for modifying pain in OA (Khanna et al 2007). Pain has a complex relationship with inflammation (Omoigui et al 2007), and inflammatory mediators play an important role in OA disease pathology (Loeser et al 2012; Lee et al 2013a). In study 3, both intervention groups had decreased pain, but the decrease was only significant in the high-rosA spearmint group at the end.
of the study. Arguably, the higher level of the rosA in the tea contributed to the significance in pain relief. In animal models, oral administration of rosA has shown anti-nociceptive behavioural effects in thermal and chemical induced noxious laboratory tests, as well as reductions in chemically induced paw edema (Guginski et al 2009; Boonyarikpuncheai et al 2014; Hasanein and Zaheri 2014). In addition, rosA exhibited anti-nociceptive effects in tests examining peripheral (i.e. glutamate injection, acetic acid-induced writhing test) and central pain mechanisms (i.e. hot-plate test, formalin test) (Boonyarikpuncheai et al 2014). Significant reductions in OA pain have been reported from RCTs with consumption of anti-inflammatory products and NHPs derived from fruit extracts (Farid et al 2010), plants (Cameron and Chrubasik 2014), spices (Nakagawa et al 2014) and propriety mixtures of fruit, plant, and spice extracts (Nieman et al 2013). Further, NHP use was predictive of a lower pain score in study 1, providing further support for their role in management of OA pain. In addition, participants in the mild OA pain category consumed more servings of fruit than the moderate or severe pain categories. It has been suggested that NHPs and supplements may be of most benefit for the management of OA pain in adults with deficiencies (Cao et al 2013). Study 1 and study 3 were samples from the same population (healthy adults with knee OA living in Guelph and surrounding communities), and fruit and vegetable consumption was low in study 1, suggesting that additional antioxidants or supplements could be beneficial in this group.

In study 3, participants maintained their normal routines, including pain medications. Consumption of the high-rosA spearmint tea is meant to be a complementary to other OA management techniques. As OA is complex and influenced by many factors, there may never be one method that completely treats OA. Even total joint replacement must be followed by pharmaceuticals and physical therapy (Greene and Harwin 2011). Personalized, integrated management approaches should be used in OA treatment (Fernandes et al 2013; McAlindon et al 2014). Indeed, data from study 1 and 3 support previous research that persons with OA use a combination of medications, supplements, physical therapies, and other strategies to manage OA (Lapane et al 2012; Kingsbury 2014).

In the same vein, there may never be one method to completely measure and describe all aspects of OA. The complex and multi-faceted nature of the disease suggests that a combination of pain
measurements, imaging measurements, and biomarker measurements will be necessary to fully characterize OA status. In research settings, OA can be defined by radiographic or symptomatic definitions, or by self-reported physician diagnosed. In a review examining the effect of OA definition on prevalence and incidence estimates, Pereira et al (2011) concluded that estimates of incidence of OA are highest and probably overestimated when radiographic definitions are used and similar when symptomatic or self-report definitions are used. In this thesis, knee OA was self-reported physician diagnosed. Self-reported physician diagnosed knee OA has previously been used in various types of OA research including larger epidemiological studies (Cheng et al 2000; Parazzini 2002; Rogers et al 2002; Hootman et al 2003; da Costa DiBoniventura et al 2012) as well as smaller intervention (Callahan et al 2008; Miller et al 2008) and cross-sectional (Keefe et al 2000). In a validation study, March et al (1998) reported that 81% of OA self-reported physician diagnosed was confirmed when using the ACR clinical criteria strictly. When the pain question was used more loosely, 100% of the diagnoses were confirmed. Good agreement between self-reported physician diagnosis and medical records has been reported for other chronic diseases as well (Katz et al 1996). Self-reporting of chronic diseases also has been shown to be fairly accurate in a Canadian population (Robinson et al 1997). There is no gold standard for identifying cases of OA, with 25 different methods described in the literature using radiographic endpoints and ACR criteria (Busija et al 2010). Self-reported physician diagnosed OA is noted as an acceptable method of identifying cases, and the sensitivity of the method is increased when a symptom question (pain or stiffness) was included in the definition (Busija et al 2010).

People cope with OA pain in different ways. In OA, passive coping has been associated with higher pain and lower physical functioning (Benyon et al 2010). Passive coping strategies like worrying, resting, and retreating generally involve giving up control and allowing external factors to influence outcomes, while active coping involves adapting to situations to try to control the outcome (Hampson et al 1996). Another psychological variable implicated in OA pain is self-efficacy (Marks 2012). With regards to OA, self-efficacy is the belief in one’s capability to manage the symptoms and consequences of the disease (Marks 2012). It has been shown to be a strong predictor of health-related behaviors and play a role in OA pain and function (Maly et al 2007; Marks 2012). In a review, interventions that improve self-efficacy
also improved OA pain and physical function (Marks 2012). Although these variables were not measured or investigated in this thesis, they can be indirectly explored in study 1 and 3. Meeting physical activity guidelines was predictive of a lower pain score in study 1. Exercise has psychological benefits and mood boosting effects that may play a role in pain reduction (Bennell et al 2011b). In addition, being physically active could give individuals a sense of control and stronger self-efficacy that could improve OA symptoms. Similarly, it is possible that in study 3, the action of joining an intervention study gave participants a feeling of control and management over their OA and increased their confidence in managing OA. In addition, the Hawthorne effect, which is the observation that simply participating in a study improves disease condition, could have played a role in disease improvement (Braunholtz et al 2001) and in addition to the benefits of the spearmint tea, each participant had to take 10-20 minutes/day to make and drink the 2 cups of spearmint tea. In a follow-up questionnaire to the study, many participants reported that they enjoyed taking time to themselves each day, and although not measured, the additional relaxation could have had an effect on pain reduction. Measuring psychological variables in OA clinical trials will add an interesting and important component to aid in management of the disease.

6.3 Future Directions

Many research themes have been identified in this thesis, all of which hold exciting potential for future studies. Promising results from pre-clinical work and study 3 indicate that a larger, longer clinical trial with the high-rosA spearmint tea should be undertaken as it has anti-inflammatory and antioxidant actions, which has implications in joint tissue deterioration and alterations in pain processing. OA is a slowly progressing disease, so a trial lasting at least 12 months would be ideal. In addition, in study 3, the control group did not consume a true placebo since there was rosA in the commercial tea, as a blinded trial was prioritized. In the future, the use of a blinded spearmint tasting tea that does not contain rosA would allow for comparison to a placebo group. In future RCTs, joint and inflammatory biomarkers should be included in the outcome measures. The primary outcome should be pain reduction, but assessment of knee structure, stiffness, physical function, self-efficacy and psychological aspects of pain should be included.
The modifiable lifestyle factors identified in study 1 are also relevant to the risk of other chronic progressive diseases, especially a healthy BMI and being physically active. It is generally known and appreciated that a healthy weight, balanced diet and regular exercise are important for all areas of health. Investigations into how to teach that information in a way that leads to changes in behavior of OA patients and those at risk for OA should follow. It would be beneficial to discover the key messages, information, and interventions that will get people with OA to exercise, eat a healthy diet, keep a healthy BMI and maintain those practices for a lifetime.

The continued study of OA biomarkers is also crucial, as it will provide information on the disease and well as help to improve methods to measure and monitor OA. Using an integrated approach where biomarkers are examined in relation to structural outcomes as well as symptomatic outcomes will help provide a more complete picture. The kinetics of biomarkers is a specific area of research that should be examined in order to further this work. Examining the movement of fragments of joint tissues from synovial fluid to circulation to removal from circulation would greatly improve our understanding of serum, plasma, and urine measurements.

The kinetics of potential treatments is also important to examine, especially in regards to NHP and supplement products. The original rationale for the use of glucosamine as a treatment for OA was flawed in that researchers believed that the cartilage could take exogenous glucosamine from the circulation and use it to synthesize new GAG side chains. That has not been demonstrated and it is unknown, and probably unlikely, that any products consumed orally can be used by the cartilage as building blocks for ECM in adults with OA. However, anti-inflammatory and antioxidant products can be used by body tissues and potentially synovial cells. Measuring levels of the product in the circulation, urine, body tissues, and synovial fluid could help to provide an understanding the movement of an oral product that could give powerful insight into treatment options.

Finally, as results from this thesis have demonstrated, OA is a complex disease that is affected by many factors and that management of the disease requires a multi-component, integrated approach. Clinical trials involving comprehensive education and interventions for diet, exercise,
BMI, and self-efficacy with inclusion of education on medication and supplements would be important to systematically examine effect of integrated treatment on OA outcomes.

6.4 Concluding Remarks

There is much to be learned in the OA field. The studies in this thesis highlight the importance of modifiable lifestyle factors in OA management, including high-rosA spearmint tea, BMI, exercise, medications, and supplements. Consumption of a high-rosA spearmint tea represents a potential complementary therapy to manage OA pain. Furthermore, serum biomarkers of joint metabolism and inflammation were associated with symptoms of OA. Taken together, the results from this research have identified modifiable lifestyle factors for managing pain in adults with knee OA, while also demonstrating the complexity of clinical symptoms in OA and the need for further research in this field.
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Appendices

Appendix 1. A table displaying the range and diversity of diseases for which rosA has been examined for its potential therapeutic effects

Table 14.1 A sample of diseases explored with rosA treatment

<table>
<thead>
<tr>
<th>Disease</th>
<th>Experimental Model</th>
<th>Proposed Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer</td>
<td>C6 and HeLa cell lines</td>
<td>Anti-oxidant, anti-proliferative</td>
<td>Erenler et al 2015</td>
</tr>
<tr>
<td></td>
<td>A549 cell line</td>
<td>COX-2 inhibition</td>
<td>Tao et al 2014</td>
</tr>
<tr>
<td></td>
<td>1,2-dimethylhydrazine induced colon cancer in rats</td>
<td>Anti-inflammatory, anti-proliferative</td>
<td>Karthikkumar et al 2014</td>
</tr>
<tr>
<td>Colitis</td>
<td>Dextran sulfate sodium-induced colitis in rats</td>
<td>Anti-inflammatory</td>
<td>Urushima et al 2014</td>
</tr>
<tr>
<td>Alzheimer’s Disease</td>
<td>Aβ25-35 induced mice</td>
<td>Anti-oxidant</td>
<td>Alkam et al 2007</td>
</tr>
<tr>
<td></td>
<td>Neuronal SH-SY5Y cells</td>
<td>Mitochondria protection</td>
<td>Camilleri et al 2013</td>
</tr>
<tr>
<td>Amyotrophic Lateral Sclerosis</td>
<td>Mouse</td>
<td>Decrease levels of COX-2 and free radicals</td>
<td>Shimojo et al., 2010</td>
</tr>
<tr>
<td>Dementia</td>
<td>Scopolamine-induced rats</td>
<td>Inhibition of acetylcholinesterase in the brain</td>
<td>Ozarowski et al 2013</td>
</tr>
<tr>
<td>Encephalitis</td>
<td>Mouse</td>
<td>Anti-inflammatory</td>
<td>Swarup et al., 2007</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>Pentylenetetrazole induced seizures in mice</td>
<td>Anti-oxidant</td>
<td>Coelho et al 2015</td>
</tr>
<tr>
<td>Neuro-diabetic complications</td>
<td>Streptozotocin induced rats</td>
<td>Prevention of lipid peroxidation and increased acetylcholinesterase activity in the brain</td>
<td>Mushtaq et al 2014</td>
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<tr>
<td>Ischemic diabetic stroke</td>
<td>Cerebral artery occlusion to diabetic rats</td>
<td>Anti-inflammatory</td>
<td>Luan et al 2013</td>
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<tr>
<td>Diabetic-induced neuropathy</td>
<td>Streptozotocin induced rats</td>
<td>Anti-inflammatory, anti-oxidant, analgesic</td>
<td>Hasanein and Zaheri 2014</td>
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<td>Diabetes-induced vascular dysfunction</td>
<td>Streptozotocin induced rats</td>
<td>Anti-oxidant, anti-inflammatory</td>
<td>Sotnikova et al 2013</td>
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<tr>
<td>Diabetic-induced</td>
<td>Streptozotocin induced</td>
<td>Reduced connective</td>
<td>Jiang et al</td>
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</tbody>
</table>

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<table>
<thead>
<tr>
<th>nephropathy</th>
<th>rats</th>
<th>tissue growth factor levels</th>
<th>2012</th>
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<tbody>
<tr>
<td>Nephrotoxicity</td>
<td>Gentamicin sulphate-induced renal oxidative damage in rats</td>
<td>Anti-oxidant</td>
<td>Tavafi and Ahmadvand 2011</td>
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<td>Liver injury</td>
<td>Carbon tetrachloride induced liver damage in mice</td>
<td>Anti-oxidant, anti-inflammatory</td>
<td>Domitrovic et al 2012</td>
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<tr>
<td></td>
<td>Induced ischemia/reperfusion injury in rats</td>
<td>Anti-oxidant, anti-inflammatory</td>
<td>Ramalho et al 2014</td>
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<tr>
<td></td>
<td>tert-butyl hydroperoxide induced oxidative liver damage</td>
<td>Increased glutathione and decreased lipid peroxidation</td>
<td>Yang et al 2013</td>
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<tr>
<td>Lung injury</td>
<td>DEP-induced lung injury in mice</td>
<td>Anti-inflammatory</td>
<td>Sanbongi et al 2003</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>T-cells from RA patients</td>
<td>Apoptosis of T-cells</td>
<td>Hur et al 2006</td>
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<tr>
<td></td>
<td>Collagen-induced arthritis in mice</td>
<td>Decreased COX-2 levels</td>
<td>Youn et al 2003</td>
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<tr>
<td>Glaucoma</td>
<td>Trabeculectomy in rabbits</td>
<td>Short-term anti-angiogenic effect</td>
<td>Ferreira et al 2014</td>
</tr>
<tr>
<td>Allergy</td>
<td>OVA-sensitized mice</td>
<td>Anti-inflammatory</td>
<td>Oh et al 2011</td>
</tr>
<tr>
<td></td>
<td>Bloma tropicalis mite induced in mice</td>
<td>Decrease in leukocytes and eosinophils</td>
<td>Costa et al 2012</td>
</tr>
<tr>
<td>Anti-viral</td>
<td>MT-4 cells</td>
<td>Anti-viral</td>
<td>Dubois et al 2008</td>
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<tr>
<td></td>
<td>Herpes simplex virus type 1 (HSV-1) acyclovir-sensitive and clinical isolates of acyclovir-resistant strains</td>
<td>Inhibition of HSV-1 attachment to host cells</td>
<td>Astani et al 2014</td>
</tr>
<tr>
<td>Anti-microbial</td>
<td>Cells exposed to Staphylococcus aureus</td>
<td>Anti-microbial</td>
<td>Slobodnikova et al 2013</td>
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<tr>
<td></td>
<td>Bacillus licheniformis, Pseudomonas vulgaris, Shigella boydii, Salmonella typhi, Staphylococcus aureus, Listeria monocytogenes and Escherichia coli</td>
<td>Anti-microbial</td>
<td>Hakkim et al 2012</td>
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Appendix 2. Certificate of approval from University of Guelph Human Research Ethics’ Board

<table>
<thead>
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<tbody>
<tr>
<td>REB-NPES</td>
</tr>
<tr>
<td>Certification of Ethical Acceptability of Research</td>
</tr>
<tr>
<td>Involving Human Participants</td>
</tr>
</tbody>
</table>

| APPROVAL PERIOD: | October 22, 2013 to October 22, 2014 |
| REB NUMBER:      | 13OC002                                |
| TYPE OF REVIEW:  | Delegated Type 1                       |
| RESPONSIBLE FACULTY: | Wright, A.J. (ajwright@uoguelph.ca) |
| DEPARTMENT:      | Human Health & Nutritional Sciences    |
| SPONSOR(S):      | OMAFRA                                 |
| TITLE OF PROJECT:| Factors affecting self-reported pain, stiffness, and physical function in adults with knee osteoarthritis |
| CHANGES:         | 15 Nov 13: B.14 Compensation           |

The members of the University of Guelph Research Ethics Board have examined the protocol which describes the participation of the human subjects in the above-named research project and considers the procedures, as described by the applicant, to conform to the University’s ethical standards and the Tri-Council Policy Statement, 2nd Edition.

The REB requires that you adhere to the protocol as last reviewed and approved by the REB. The REB must approve any modifications before they can be implemented. If you wish to modify your research project, please complete the Change Request Form. If there is a change in your source of funding, or a previously unfunded project receives funding, you must report this as a change to the protocol.

Unforeseen events or incidental findings must be reported to the REB as soon as possible with an indication of how these events affect, in the view of the Responsible Faculty, the safety of the participants, and the continuation of the protocol.

If research participants are in the care of a health facility, a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and approvals of those facilities or institutions are obtained and filed with the REB prior to the initiation of any research protocols.

The Tri-council Policy Statement, 2nd Edition, requires that ongoing research be monitored by, at a minimum, a final report and, if the approval period is longer than one year, annual reports. Continued approval is contingent on timely submission of reports.

Membership of the Research Ethics Board: B. Beresford, Community Member; F. Caldwell, Physician; K. Cooley, Alt. Health Care; D. Dyck, CBS; D. Emslie, Physician (alt); S. Gregori, CPES; G. Holloway, CBS (alt); J. Knapman, Grad Rep (alt); S. Logan, Grad Rep (alt); A. Niel, OVC (alt); B. Nonnecke, CPES; A. Papadopoulos, OVC; L. Peterson, Community Member (alt); B. Power, Community Member; R. Regan, Legal (alt); L. Spreet, CBS (alt); J. Srbely, CBS (alt); D. Stacey, CPES (alt); J. Sutherland, Legal; L. Vailis, CBS (alt); K. Wendling, Ethics; J. Whitfield, Graduate Rep.

Approved:
Chair, REB-NPES

Date: ________________________
Appendix 3. Participant informed consent form

Date: Protocol & Version Number: 1 Participant Initials:

Factors affecting self-reported pain, stiffness, and physical function in adults with knee osteoarthritis.

You are being asked to participate in a participant questionnaire at the Human Nutraceutical Research Unit (HNRU) in the Department of Human Health & Nutritional Science (HHNS) at the University of Guelph. This research is being conducted under the supervision of Amanda Wright, Ph.D. (Director of the HNRU and Associate Professor in HHNS), Alison Duncan, Ph.D. (Associate Director of the HNRU and Associate Professor in HHNS) and Amy Tucker, Ph.D. (Manager at HNRU). The Ontario Ministry of Agriculture, Food and Rural Affairs is funding this project.

Contact Information
If you have any questions or concerns, please contact:

A. Erin Connelly, B.Sc.  
(Ph.D. candidate, HHNS)  
phone: (519) 824-4120 x56314 or email: teastudy@uoguelph.ca

Amy Tucker, Ph.D. (Manager, HNRU)  
phone: (519) 824-4120 x53749 or email: aboardland@uoguelph.ca

Amanda Wright, Ph.D. (Co-Principal Investigator, HNRU Director, Associate Professor, HHNS)  
phone: (519) 824-4120 x54697 or email: ajwright@uoguelph.ca

Alison Duncan, Ph.D., R.D. (Co-Principal Investigator, HNRU Associate Director, Associate Professor, HHNS)  
phone: (519) 824-4120 x53416 or email: amduncan@uoguelph.ca

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PURPOSE OF THE STUDY
The purpose of this study is to examine the relationships between osteoarthritis symptoms and a number of lifestyle factors in adults with osteoarthritis of the knee. For this visit, you will arrive at the HNRU and complete the Informed Consent process. Should you choose to participate, you will be asked to complete detailed questionnaires regarding your health history and medication use and to assess your level of disease activity using the Western Ontario and McMaster Universities Arthritis Index (WOMAC) questionnaire. The WOMAC is a standardized questionnaire used in the assessment and monitoring of osteoarthritis symptoms.

POTENTIAL RISKS AND DISCOMFORTS
There are minimal risks associated with participation in this visit.

POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY
You will benefit from participating in this visit by gaining the experience as a study participant.

PAYMENT FOR SCREENING VISIT PARTICIPATION
You will be financially compensated for your time and effort for this screening process with at $10 gift certificate to a local coffee retailer.

COSTS FOR SCREENING VISIT PARTICIPATION
There is no direct cost for participating in this screening visit. You will only be responsible for covering any costs related to attending this screening visit (i.e. gas money, public transportation fees, child care fees, etc.).

CONFIDENTIALITY
Every effort will be made to ensure confidentiality of any identifying information that is obtained in connection with this study. All participants will be assigned a number and a study code will be used. Your name will never be used in communicating results of the study. Records will be kept on a password-protected computer and/or in a locked file cabinet in a locked office. All data will be kept indefinitely. In following these guidelines, participants’ confidentiality will be maintained to the best of our ability. Results from the study may be published, but will be presented as group data.

If requested, direct access to your research records for this study will be granted to study monitors, auditors, the University of Guelph Research Ethics Board, and regulatory authorities for verification of study procedures and/or data. Your confidentiality as a study participant will not be violated during this process, to the extent permitted by applicable laws and regulations. By signing this written informed consent form, you are agreeing to authorize such access.

PARTICIPATION AND WITHDRAWAL
You can choose whether to participate in the visit or not. If you agree to participate, you may withdraw at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also refuse to answer any questions you don’t want to answer and still remain in the study. The investigator may withdraw you from this research if circumstances arise that warrant doing so.
RIGHTS OF RESEARCH PARTICIPANTS
You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. This study has been reviewed and received ethics clearance through the University of Guelph Research Ethics Board. If you have questions regarding your rights as a research participant, contact:

Sandy Auld
Research Ethics Coordinator
Room 437, University Centre
University of Guelph
Telephone: (519) 824-4120, ext. 56606
E-mail: sauld@uoguelph.ca

SIGNATURE OF RESEARCH PARTICIPANT/LEGAL REPRESENTATIVE
I have read the information provided for the study “Investigating health history and osteoarthritis symptoms in individuals with osteoarthritis of the knee” as described herein. My questions have been answered to my satisfaction, and I agree to participate in the screening visit. I have been given a copy of this form.

<table>
<thead>
<tr>
<th>NAME OF PARTICIPANT (PLEASE PRINT)</th>
<th>SIGNATURE OF PARTICIPANT</th>
<th>DATE</th>
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</table>

<table>
<thead>
<tr>
<th>NAME OF WITNESS (PLEASE PRINT)</th>
<th>SIGNATURE OF WITNESS</th>
<th>DATE</th>
</tr>
</thead>
</table>
Appendix 4. Lifestyle Questionnaire

IN-PERSON HEALTH HISTORY QUESTIONNAIRE

STUDY TITLE:
Factors affecting self-reported pain, stiffness, and physical function in adults with knee osteoarthritis.

HNRU Coordinator:

Date: ___________________________ Time: ___________________________

Participant ID: ___________________________

Thank you very much for your interest in this study. The purpose of this questionnaire is to gather more information about you and your knee osteoarthritis.

Please feel free to NOT answer any questions that you are uncomfortable with answering. Please feel free to ask the study coordinator any questions you might have.

All information provided in this questionnaire will be kept strictly confidential.
HEALTH HISTORY QUESTIONNAIRE

1. When were you first diagnosed with osteoarthritis of the knee? ________________

2. Who first diagnosed you with osteoarthritis of the knee? (i.e. family doctor, rheumatologist) ________________

3. (a) Which knee do you have osteoarthritis in? BOTH RIGHT LEFT
   (b) Which knee is most problematic? RIGHT LEFT

4. Have you ever had knee replacement surgery? YES NO

5. Are you planning on having knee replacement surgery between now and May 2014? YES NO

6. Have you ever had a synovectomy before? (i.e. surgical removal of inflamed joint tissue) YES NO

7. Do you currently smoke? YES NO
   (a) If NO, have you ever smoked? YES NO
   (b) If YES to (a), how long since you have quit? ________________

8. Approximately how many alcoholic drinks do you consume per week? (1 drink = 12 oz beer, 5 oz wine, or 1.5 oz hard liquor) ________________

9. Are you currently participating in any other research studies? YES NO
   (a) If YES, please describe:

10. How would you describe your general health? POOR GOOD VERY GOOD EXCELLENT
11. Do you have any of the following diagnosed medical conditions:

(a) Rheumatoid arthritis?  YES  NO
(b) Cancer?  YES  NO
(c) Pre-diabetes?  YES  NO
(d) Diabetes?  YES  NO
(e) High cholesterol?  YES  NO
(f) High blood pressure?  YES  NO
(g) Impaired liver function?  YES  NO
(h) Impaired kidney function?  YES  NO
(i) Gastrointestinal ulcer?  YES  NO
(j) Autoimmune disease?  YES  NO
(k) Chronic infection?  YES  NO
(l) Depression?  YES  NO

(m) Are there any other medical conditions that might affect your participation in the study?  YES  NO

(i) If YES, please describe:

12. Are you currently taking any medication and/or over-the-counter drugs? (i.e. Tylenol, Advil, Claritin, Sudafed, etc.)  YES  NO

(a) If YES, please provide the study coordinator with your current medication. The study coordinator will help you complete the following table:
13. Are you currently taking any dietary supplements? (i.e. multivitamin, omega-3, glucosamine) YES NO

(a) If YES, please provide the study coordinator with your dietary supplements. The study coordinator will help you complete the following table:

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>REASON FOR USE</th>
<th>MEDICINAL INGREDIENTS</th>
<th>DOSE</th>
<th>HOW OFTEN</th>
<th>HOW LONG</th>
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14. Are you currently taking any medication for your osteoarthritis? YES NO

(a) If YES, please provide the study coordinator with your current osteoarthritis medication. The study coordinator will help you complete the following table:
<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>REASON FOR USE</th>
<th>MEDICINAL INGREDIENTS</th>
<th>DOSE</th>
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15. Do you see the following health professionals for your osteoarthritis: (make sure it is clear that these are all related to osteoarthritis)

(a) Family doctor or rheumatologist?  YES          NO  If YES, how often? _____________

(b) Physiotherapy?  YES          NO  If YES, how often? _____________

(c) Acupuncture?  YES          NO  If YES, how often? _____________

(d) Massage therapy?  YES          NO  If YES, how often? _____________

(e) Nerve stimulation?  YES          NO  If YES, how often? _____________

(f) Other? (please list)  YES          NO  How often? ____________________

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16. Do you participate in any of the following types of exercises:

(a) Weight training?  YES          NO  If YES, how often? _____________

(b) Stretching?  YES          NO  If YES, how often? _____________

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(c) Aerobics?  YES  NO  If YES, how often? ______________

(d) Underwater?  YES  NO  If YES, how often? ______________

(e) Other? (please list)  YES  NO

_________________________________________________________________________  How often? ______________

_________________________________________________________________________  How often? ______________

_________________________________________________________________________  How often? ______________

17. Do you have ANY allergies? (i.e. food, medications, rag weed or pollen)  YES  NO

(a) If YES, please list:
_________________________________________________________________________

(b) Please describe your type of allergic reaction: (i.e. anaphylaxis, difficulty breathing, swelling, etc.)
_________________________________________________________________________

18 (a). What is your current height? ________________

18 (b). What is your current body weight? ________________

Question #19 to be completed by females only
Hormone levels can affect pain sensation and perception. Since pain is a large part of osteoarthritis, we wish to gather some information about your hormone levels.

19. Are you PRE- or POST-menopausal?  PRE  POST
(a) If POST-menopausal, do you use hormone replacement therapy?  
YES  NO

(b) If YES to using hormone replacement therapy, please specify:

(c) If PRE-menopausal, do you have regular menstrual cycles?  
YES  NO

(d) If NO to having regular menstrual cycles, please describe the irregularities:

(e) Do you currently use oral contraceptives (or other hormonal birth control)?  
YES  NO

(f) If YES to using oral contraceptives or hormonal birth control, please specify:

________________________________

HNRU Coordinator Signature

NB: Provide the WOMAC to the participant and have them complete the form. Complete the 24-hr food recall with the participant. Provide the participant with a coffee voucher. Fill out the In-person Screening Tracking Log.
Appendix 5. Sample of 24-hour food recall sheet

24 HOUR DIET RECALL

Which day of the week does this record? SUN MON TUE WED THU FRI SAT

Was this a typical day? YES NO

*If not, if you wish, you may give an example of a typical day on the back of this page after you entered yesterday’s record.*

Please enter all the food and drink that you consumed over the previous 24 hours. Be detailed and specific.

<table>
<thead>
<tr>
<th>Time</th>
<th>Quantity Eaten</th>
<th>Details of Food or Drink</th>
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<tbody>
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Appendix 6. Certificate of approval from University of Guelph Human Research Ethics’ Board

RESEARCH ETHICS BOARD
Certification of Ethical Acceptability of Research Involving Human Participants

APPROVAL PERIOD: April 12, 2011 to April 12, 2012
REB NUMBER: 11JA040
TYPE OF REVIEW: Full Board
RESPONSIBLE FACULTY: A.J. WRIGHT
DEPARTMENT: Food Science
SPONSOR: OMAFRA

TITLE OF PROJECT: Human Clinical Trial to Investigate the Effects of a High Rosmarinic Acid Spearmint Tea on Measures of Disease Activity and Cartilage Degradation In Osteoarthritis of the Knee

The members of the University of Guelph Research Ethics Board have examined the protocol which describes the participation of the human subjects in the above-named research project and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement.

The REB requires that you adhere to the protocol as last reviewed and approved by the REB. The REB must approve any modifications before they can be implemented. If you wish to modify your research project, please complete the Change Request Form. If there is a change in your source of funding, or a previously unfunded project receives funding, you must report this as a change to the protocol.

Adverse or unexpected events must be reported to the REB as soon as possible with an indication of how these events affect, in the view of the Responsible Faculty, the safety of the participants, and the continuation of the protocol.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and approvals of those facilities or institutions are obtained and filed with the REB prior to the initiation of any research protocols.

The Tri-council Policy Statement requires that ongoing research be monitored by, at a minimum, a final report and, if the approval period is longer than one year, annual reports. Continued approval is contingent on timely submission of reports.

Membership of the Research Ethics Board: M. Bowring, CME; F. Caldwell, Physician (alt); J. Clark, PolSci (alt); J. Dwyer, FRAN; M. Dwyer, Legal; D. Dyck, CBS; D. Emslie, Physician; M. Fairburn, Ext.; J. Hacker-Wright, Ethics; G. Holloway, CBS (alt); V. Kanetkar, CME (alt); L. Kurczynski, FRAN (alt); S. Lachapelle, COA; L. Mann, Ext.; J. Minogue, EHS; P. Saunders, Alter Health Care; S. Singer, COA (alt); L. Son Hing, Psychology; V. Shalla, SOAN (alt); L. Spriet, CBS; L. Trick, Chair; T. Turner, SOAN; L. Vallis; CBS (alt).

Approved: __________________________ Date: __________________________
Chair, Research Ethics Board
Appendix 7. Participant informed consent

CONSENT TO PARTICIPATE IN RESEARCH
Human clinical trial to investigate the effects of high rosmarinic acid spearmint tea on markers of pain, physical function and disease activity in osteoarthritis of the knee

You are being asked to participate in a clinical research study at the Human Nutraceutical Research Unit (HNRU) in the Department of Human Health & Nutritional Science (HHNS) at the University of Guelph. This research is being conducted under the supervision of Amanda Wright, Ph.D. (Director of the HNRU and Associate Professor in HHNS), Alison Duncan, Ph.D. (Associate Director of Research at the HNRU and Associate Professor in HHNS) and Hilary Tulk, M.Sc. (Clinical Trials Manager at HNRU). This project is funded by the Ontario Ministry of Agriculture, Food and Rural Affairs Food Research Program.

Contact Information
If you have any questions or concerns, please contact:

A. Erin Connelly, B.Sc.
M.Sc. graduate student, HHNS
phone: (519) 824-4120 x56314 or email: teastudy@uoguelph.ca

Hilary Tulk, M.Sc.
Clinical Trials Manager, HNRU
phone: (519) 824-4120 x53749 or email: htulk@uoguelph.ca

Amanda Wright, Ph.D.
Co-Principal Investigator, HNRU Director, Associate Professor, HHNS
phone: (519) 824-4120 x54697 or email: ajwright@uoguelph.ca

Alison Duncan, Ph.D., R.D.
Co-Principal Investigator, HNRU Associate Director, Associate Professor, HHNS
phone: (519) 824-4120 x53416 or email: amduncan@uoguelph.ca

PURPOSE OF THE STUDY
The purpose of the study is to test if daily consumption of a spearmint tea, that is high in rosmarinic acid, improves disease activity, functional status, and/or cartilage degradation in adults with osteoarthritis of the knee. The spearmint tea was developed at the University of Guelph using selective breeding methods and has 15 – 20 times more rosmarinic acid than
regular spearmint. Rosmarinic acid has demonstrated antioxidant and anti-inflammatory potential. This study will allow us to investigate if the high rosmarinic acid spearmint tea can be used as a complement to traditional osteoarthritis therapies. Results of this study may also help the commercialization and marketing of the high rosmarinic acid spearmint.

*Drs. Kott and Fletcher in the Department of Plant Agriculture at the University of Guelph have a pending patent application for the high rosmarinic acid spearmint line and are collaborators on the project*

**PROCEDURES**
This research study involves consuming two 300 mL cups of spearmint tea per day for a 4-month period. Approximately 50 individuals will be invited to participate in this study. Half (50%) of the participants will be randomly assigned to consume either the high rosmarinic acid spearmint tea or a commercial spearmint tea. Participants will be asked to keep a daily study record of the time of tea consumption, any medication or natural health products used, any physical activity or therapies, and other significant dietary or lifestyle changes. There will be 9 visits to the HNRU, located in room 144 of the Food Science-Guelph Food Technology Centre Building, 88 McGilvray St. at the University of Guelph. In addition, there will be 4 check-in phone calls made to each participant. The following describes the procedures prior to and during each study visit.

**STUDY TEST PRODUCTS**
Throughout the treatment period you will be asked to consume two cups of spearmint tea per day (300 mL in the morning and 300 mL in the evening). You will be consuming either a commercially available spearmint tea or the spearmint tea with a high rosmarinic acid content that was developed at the University of Guelph through selective breeding techniques. Both teas are naturally caffeine free. The teas will be packaged under food grade conditions and will undergo microbiological and toxicological testing through a certified, independent laboratory prior to being provided to you.

**STUDY DIARY**
- You will be provided with a 4-week supply of tea along with detailed written instructions for how to prepare the tea.
- You will be asked to record the date and time of consumption for each cup of tea.
- You will be asked to record any medication and natural health product usage.

**STUDY VISITS**
In total, you will be asked to attend 9 study visits and to participate in 4 check-in telephone calls. Table 1 summarizes the activities you will be asked to participate in as part of each study visit or phone call. Each activity is explained following Table 1.

Table 1: Activities associated with each study visit and check-in phone calls.

<table>
<thead>
<tr>
<th>STUDY VISIT</th>
<th>TYPE OF APPOINTMENT</th>
<th>APPOINTMENT LENGTH</th>
<th>ACTIVITIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Orientation</td>
<td>HNRU</td>
<td>1 ½ hours</td>
<td>• Meet all the study personnel&lt;br&gt;• Review the study design, timeline, and protocols</td>
</tr>
</tbody>
</table>
- Learn about and taste the high rosmarinic acid mint tea
- Ask questions about any study details
- Sign the Study Consent Form

| Week 0 | HNRU | 2 hours | • Tea distribution  
|        |      |         | • Physical measurements  
|        |      |         | • WOMAC questionnaire  
|        |      |         | • SF-36 questionnaire  
|        |      |         | • 6-min walk  
|        |      |         | • Stair climb  
|        |      |         | • Blood sample  
|        |      |         | • SF sample (consenting participants only) |

| Week 1 | HNRU | 30 minutes | • On-site check-in with study coordinator |

| Week 2 | Phone Call | 10 minutes | • Check-in call from study coordinator |

| Week 4 | HNRU | 30 minutes | • Tea distribution  
|        |      |         | • On-site check-in with study coordinator |

| Week 6 | Phone Call | 10 minutes | • Check-in call from study coordinator |

| Week 8 | HNRU | 1 ½ hours | • Tea distribution  
|        |      |         | • WOMAC questionnaire  
|        |      |         | • SF-36 questionnaire  
|        |      |         | • 6-min walk  
|        |      |         | • Stair climb |

| Week 10 | Phone Call | 10 minutes | • Check-in call from study coordinator |

| Week 12 | HNRU | 30 minutes | • Tea distribution  
|        |      |         | • On-site check-in with study coordinator |

| Week 14 | Phone Call | 10 minutes | • Check-in call from study coordinator |

| Week 16 | HNRU | 2 hours | • Physical measurements  
|        |      |         | • WOMAC questionnaire  
|        |      |         | • SF-36 questionnaire  
|        |      |         | • 6-min walk  
|        |      |         | • Stair climb  
|        |      |         | • Blood sample  
|        |      |         | • SF sample (consenting participants only) |

| Week 20 | HNRU | 1 ½ hours | • WOMAC questionnaire |
Physical Measurements
In privacy, a trained study coordinator will measure your body weight, height, waist and hip circumferences, and blood pressure.

- All physical measurements will be made in privacy by a trained study coordinator.
- Body weight will be measured on a digital scale.
- Height will be measured using a standard stadiometer.
- Waist and hip circumference will be measured over light clothing using a standard inelastic tape measure.
- Blood pressure will be measured using a portable digital blood pressure monitor.

Body Composition measured by Bioelectrical Impedance Analysis (BIA):
In privacy, a trained study coordinator will measure your body composition.
BIA will not be performed on any participant with an artificial pacemaker, an implantable cardioverter-defibrillator (ICD), or similar. Please inform the study coordinator if you have any such device.

- BIA is a very common technique used to determine body composition.
- You will be asked to lie comfortably on a medical bed in a private sampling area.
- The study coordinator will enter your age, sex, height and weight, and waist and hip circumference into a handheld BIA device.
- Two electrodes will be placed on your right foot and two electrodes will be placed on your right hand. These electrodes will be connected to the handheld BIA device.
- The measurement then begins and lasts for approximately 5 seconds.
- During the measurement, you will remain lying down with the researcher standing alongside of you.
- Your body’s opposition to a weak electric current is measured and body water content, lean mass and body fat are estimated.
- You will not feel the very weak electric current during the measurement and the process is completely painless.

Western Ontario & McMaster Arthritis Index (WOMAC): The WOMAC is a standardized 24-item questionnaire that assesses pain, disability and joint stiffness due to osteoarthritis.

36-Item Short-Form Health Survey (SF-36): The SF-36 is a standardized 36-item health questionnaire that assesses overall health including: (1) physical functioning; (2) limitations due
to physical health; (3) bodily pain; (4) general health; (5) vitality, energy, and fatigue; (6) social functioning; (7) limitations due to emotional problems; and (8) mental health.

**6-Minute Walk Test (6-min walk):** The 6-min walk is a timed walking test that measures the total distance traveled in 6 minutes. You will be asked to walk as quickly as you can over a flat surface for 6 minutes. You may rest when necessary and can use any mobility aids, such as canes or walkers which you normally would use.

**Stair Climbing Task (stair climb):** The stair climb is a timed stair climbing task that measures the total time required to climb up a flight of 7 steps, turn around and then climb down 7 steps. You may use a handrail for help.

**Blood Sample:** In the privacy of the HNRU sampling area, a qualified medical technician will collect a blood sample (two 5 mL tubes) from your arm using standard clinical methods. Blood samples will be taken once at the beginning and once at the end of the 4-month tea consumption period, as well as at the 1-month follow-up visit.

**Synovial Fluid:** You will be invited to provide synovial fluid from your knee that is affected by osteoarthritis. Providing a synovial fluid sample is an optional procedure in this study. Synovial fluid samples will be taken once at the beginning and once at the end of the 4-month tea consumption period. If you agree, you will be asked to sign a separate informed consent form specific to the synovial fluid sampling procedure. The study physician will examine the knees of participants who agree to give a synovial fluid sample to ensure that the joint is fit for this procedure. Please remember that you may withdraw your consent at any time.

Collection of synovial fluid is a common diagnostic and therapeutic procedure in arthritis and will be performed by a qualified physician.

- You will be asked to lie comfortably on a medical bed in a private sampling area.
- A physician will examine your knee and determine if it is suitable for synovial fluid sampling.
- The skin on your knee will be washed with an alcohol swab.
- A sterile needle will then be inserted through the skin into the joint space and the fluid then drawn through the needle into the syringe and the needle removed from the knee.
- The physician will apply gentle pressure to the joint.
- The skin will then be cleansed and a bandage will be applied to the needle insertion site.
- Synovial fluid samples will be analyzed for: cartilage degradation with sulphated glycosaminoglycan and cartilage oligomeric matrix protein; oxidative stress with nitric oxide; and inflammation with prostaglandin E2. These are markers used in assessing disease activity in osteoarthritis and have been used in previous studies of rosmarinic acid.

**Study Sample Laboratory Analysis**

One tube of blood (5 mL) will be processed at the HNRU and then sent to an independent medical laboratory (LifeLabs® Medical Laboratory Services) for same-day analysis of C-reactive protein, a marker of inflammation.

The second tube of blood (5 mL) will be processed at the HNRU and a small amount of serum (2 mL) will be frozen at -80°C and stored in the HNRU Wet laboratory (room 143, building #88). Upon completion of the clinical trial, the serum samples will be analyzed at the University of
Guelph for biomarkers of inflammation and osteoarthritis including markers of cartilage degradation (cartilage oligomeric matrix protein), oxidative stress (nitric oxide), and inflammation (prostaglandin E₂). These are the same biomarkers of inflammation and osteoarthritis that will be analyzed in the synovial fluid.

Synovial fluid samples will be stored at -80°C and, upon completion of the clinical trial, the synovial fluid samples will be analyzed at the University of Guelph for markers of cartilage degradation (glycosaminoglycan, cartilage oligomeric matrix protein), oxidative stress (nitric oxide), and inflammation (prostaglandin E₂).

All samples (i.e. serum and synovial fluid) will be stored using unique identifiers (i.e. a participant number and study code). In this way, your confidentiality will be maintained.

Study Results Publication
Results from this study may be published, but will always be presented as group data and with no ability to link data back to an individual (i.e. data will always remain confidential). Your decision to be a participant in this study is voluntary and you are free to withdraw from the study at any time. Following completion of the study analyses, a summary of the research results (both group data and your individual data) will be mailed to you.

POTENTIAL RISKS AND DISCOMFORTS

Every effort to ensure your comfort and safety will be made during the course of this study. In the unlikely event of a study-related injury, study staff will engage appropriate emergency response services to assist in your care. A study coordinator certified in Standard First Aid and CPR will always be present.

There are minimal risks associated with participation in this study. The following summarizes the potential risks:

- If you are currently unaware of any allergies or hypersensitivities to mints, you might experience an allergic reaction after consuming either the high rosmarinic acid or the commercial spearmint tea.
- At 4 study visits (study visits weeks 0, 8, 16, and 20), a qualified and trained medical technician will draw a blood sample from your forearm using a needle. There is a chance that this process could cause you some slight discomfort as the needle is inserted and, as with any venipuncture, there may be some minimal bruising afterwards. These risks and potential discomforts from the blood draws will be managed by having a qualified and experienced medical technician taking your blood. In addition, consuming plenty of water the night before and the morning of can facilitate blood sampling. Also, applying compression to the blood draw site will help to minimize bruising.

The following describes the potential risks and discomforts related to the synovial fluid sampling. Providing a synovial fluid sample is optional and this procedure will only be performed on participants who provide additional consent and whose joints are deemed fit by the study physician.
In those individuals deemed suitable by a physician and who provide consent, synovial fluid samples will be taken from the knee joint using a syringe at 2 study visits (weeks 0 and 16). This is a common procedure and a trained physician will perform the synovial fluid sample collection. However, as with any needle puncture, there is a minimal chance of an infection or bleeding into the joint. Ice or cold packs will be placed on the joint after the procedure.

If unusual pain or swelling occurs in the knee after the synovial fluid sampling procedure and persists for more than 5 days, participants should contact the study investigators. If a participant experiences any unusual pain or swelling of the knee from which synovial fluid was sampled AND a fever develops within 5 days of the synovial fluid sampling, they should proceed to the nearest emergency room.

If a serious adverse event occurs during a study visit at the HNRU, an ambulance will be called. The student investigator will stay with the participant through to the hospital, if necessary. All costs arising from a research related injury will be born by the University of Guelph and the study researchers.

POTENTIAL BENEFITS TO PARTICIPANTS AND/OR TO SOCIETY
If you participate in this research, you will have benefit of gaining experience and skills in self-tracking and self-monitoring of your osteoarthritis symptoms. In addition, you will receive a written summary of your individual study data. Some of this data (i.e. WOMAC Index scores) can provide insight into your arthritis symptoms and, when interpreted by a medical professional, may be useful in guiding clinical decisions related to your disease management.

In this study, 50% of the participants will be assigned to the control commercial spearmint tea group. Although we do not expect immediate clinical benefits in individuals who are consuming the control commercial spearmint tea, your participation will help us to answer our research question and you will still receive all of your individual data.

Your involvement in the study will contribute valuable insights into the health benefits of using a spearmint tea as a complement to traditional drug-based treatment for osteoarthritis. The knowledge generated from this study will be useful to individuals suffering from osteoarthritis and health care professionals seeking to make recommendations of evidence-based complementary therapies. The results may also help the University of Guelph commercialize the high rosmarinic acid spearmint tea plant.

PAYMENT FOR PARTICIPATION
You will be financially compensated for your time and effort at an amount of $200, plus $100 extra for participation in synovial fluid sampling ($50 per sample provided). An additional $50 for completion of the study will be given (for a total of $250 without synovial fluid sampling and $350 with synovial fluid sampling). If you withdraw from the study before completion, your compensation will be pro-rated for your involvement.

COSTS FOR PARTICIPATION
There are no direct costs for participating in this study. You will only be responsible for covering any costs related to ensuring you are able to attend your scheduled study visits (i.e. gas money,
public transportation fees, child care fees, etc.). It is our intention that, through the financial compensation we provide for your time and effort participating in this study, we partially reimburse you for some of the costs you may incur.

CONFIDENTIALITY
Every effort will be made to ensure confidentiality of any identifying information that is obtained in connection with this study. All participants will be assigned a number, and a study code will be used. Your name will never be used in communicating results of the study. Records will be kept on a password-protected computer and/or in a locked file cabinet in a locked office. All data will be kept for up to 25 years, as required by applicable regulations. In following these guidelines, participants’ confidentiality will be maintained to the best of our ability. Results from the study may be published, but will be presented as group data.

If requested, direct access to your research records for this study will be granted to study monitors, auditors, the University of Guelph Research Ethics Board, and regulatory authorities for verification of study procedures and/or data. Your confidentiality as a study participant will not be violated during this process, to the extent permitted by applicable laws and regulations. By signing this written informed consent form you are agreeing to authorize such access.

PARTICIPATION AND WITHDRAWAL
You can choose whether to be in this study or not. If you volunteer to be in this study, you may withdraw at any time without consequences of any kind. You may exercise the option of removing your data from the study. You may also refuse to answer any questions you don’t want to answer and still remain in the study.

If, at any point during the study, new study-related information becomes available that might change your willingness to continue participating in the study (i.e. new safety information, a significant change in the study protocol, etc.), you will be provided with this information in a timely manner. You can then choose whether to continue participating in this study or not.

The investigator may withdraw you from this research if circumstances arise that warrant doing so. Some examples of reasons why your participation may be withdrawn include:
- There is reasonable concern about your continuation in the study.
- You miss an excessive number of study visits or doses of tea and/or study diary entries;
- You no longer meet the required study eligibility criteria;

RIGHTS OF RESEARCH PARTICIPANTS
You may withdraw your consent at any time and discontinue participation without penalty. You are not waiving any legal claims, rights or remedies because of your participation in this research study. This study has been reviewed and received ethics clearance through the University of Guelph Research Ethics Board. If you have questions regarding your rights as a research participant, contact:
Sandy Auld
Research Ethics Coordinator
Room 437, University Centre
University of Guelph
Telephone: (519) 824-4120, ext. 56606
E-mail: sauld@uoguelph.ca

**SIGNATURE OF RESEARCH PARTICIPANT/LEGAL REPRESENTATIVE**
I have read the information provided for the study “**Human clinical trial to investigate the effects of high rosmarinic acid spearmint tea on markers of pain, physical function and disease activity in osteoarthritis of the knee**” as described herein. My questions have been answered to my satisfaction, and I agree to participate in this study. I have been given a copy of this form.

<table>
<thead>
<tr>
<th>NAME OF PARTICIPANT (PLEASE PRINT)</th>
<th>SIGNATURE OF PARTICIPANT</th>
<th>DATE</th>
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<table>
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<tr>
<th>NAME OF WITNESS (PLEASE PRINT)</th>
<th>SIGNATURE OF WITNESS</th>
<th>DATE</th>
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</table>
Healthy, non-smoking males and females (18 years and older) who have osteoarthritis of the knee are needed to participate in a human nutrition study at the University of Guelph. This research will study the health effects of spearmint tea on osteoarthritis.

- Interested individuals will be asked to:
  - Complete a screening questionnaire by telephone or email.
  - Visit the University of Guelph for one 1½ hour screening appointment to assess eligibility for study.

If eligible to participate, individuals will be asked to:

- Visit the University of Guelph on 9 occasions over four months for:
  - One 1½ hour study orientation visit.
  - Two 2-hour study visits and two 1½ hour study visits
    - Four of these study visits will involve a blood sample and assessments of disease severity, pain and physical function.
    - Participants may also choose to allow a medical doctor to draw a synovial fluid sample from a knee joint at 2 of the study visits.
  - Three 30-minute check-up visits and one 1½ hour follow-up visit.
- Consume 2 cups of mint tea per day for 4 months.
- Keep regular records of medication and dietary supplement use.

This study has been reviewed and has received clearance through the University of Guelph Human Research Ethics Board (REB#11JA040) and will be conducted at the Human Nutraceutical Research Unit in the Department of Human Health and Nutritional Sciences

*Financial Compensation Provided*

To find out more about the study and your suitability as a participant please contact Erin at 519-824-4120 x56314 or teastudy@uoguelph.ca
Appendix 8. Telephone participant eligibility questionnaire

Appendix 9: Phone Screening Questionnaire

PHONE PRE-SCREENING PARTICIPANT ELIGIBILITY QUESTIONNAIRE

STUDY TITLE:
Human clinical trial to investigate the effects of high rosmarinic acid spearmint tea on markers of pain, physical function and disease activity in osteoarthritis of the knee.

HNRU Coordinator:

Date: 

Time: 

Thank you for your interest in the mint tea study. I am going to provide you with some brief details about the study to ensure your interest, and then ask you some questions to confirm that you are eligible for the study. This will take approximately 10 minutes. Please feel free to ask me questions at any time.

The purpose of the study is to test if daily consumption of a new spearmint tea for 4 months can improve symptoms in adults with osteoarthritis of the knee. The new spearmint tea was developed at the University of Guelph and has 15-20 times more rosmarinic acid than regular spearmint. Rosmarinic acid is a plant extract that has been shown to have antioxidant and anti-inflammatory effects. Approximately 50 individuals will be invited to participate in this study. Half of the participants will be randomly assigned to consume the high rosmarinic acid spearmint tea and the other half will be randomly assigned to consume a commercial spearmint tea. This study will allow us to investigate if the high rosmarinic acid spearmint tea is an effective complement to traditional osteoarthritis therapies.

If the participants asks: Study participants will be paid $200, plus $100 extra for participation in synovial fluid sampling ($50 per sample provided). An additional $50 for completion of the study will be given. The total reimbursement is therefore $250 WITHOUT synovial fluid sampling and $350 WITH synovial fluid sampling.

1. How did you hear about this study?

______________________
2. Do you have osteoarthritis in your knee?  
   YES   NO

3. Which knee do you have osteoarthritis in?  
   RIGHT  LEFT  BOTH

4. When were you first diagnosed with osteoarthritis? (mm/yyyy) 

5. Who diagnosed your osteoarthritis? (i.e. family doctor, rheumatologist) 

6. Do you smoke?  
   YES   NO

7. Do you have ANY other types of arthritis or inflammatory disease?  
   YES   NO

   (a) If YES, please specify:

8. Do you have any other medical conditions?  
   YES   NO

   (a) If YES, please specify:

9. Have you had a synovectomy in the past 3 months? (i.e. surgical removal of inflamed joint tissue)  
   YES   NO

   (a) If YES, when? (dd/mm/yyyy) 

10. Are you currently taking any medications for osteoarthritis?  
    YES   NO

    (a) If YES, please specify:

11. Are you currently taking any other medications including prescription or any over the counter medications?  
    YES   NO

    (a) If YES, please specify:
12. Are you currently taking any vitamins, minerals, herbal medicines or dietary supplements?  
   YES  NO  

   (a) If YES, please specify:  

13. What are you doing to manage your osteoarthritis?  

Questions #14, #15 and #16 for females only  

14. Are you currently pregnant or planning on becoming pregnant?  YES  NO  

15. Have you recently given birth?  YES  NO  

16. Are you currently breastfeeding?  YES  NO  

17. Do you have any specific allergies/hypersensitivity to mint?  YES  NO  

18. Do you have any food or anaphylactic allergies?  YES  NO  

   (a) If YES, please specify:  

   (b) If YES, please describe what type of allergic reactions you have experienced (i.e. hive, rash, swelling, anaphylaxis):  

19. Are you comfortable providing blood samples?  YES  NO  

20. Have you ever provided a synovial fluid sample? (also called arthrocentesis or joint aspiration, where a needle removes fluid from the joint)  YES  NO  

21. Participants will be required to visit the University of Guelph for one 1 ½ hr screening appointment, one 1 ½ hr study orientation visit, and if eligible to participate in the clinical trial, you will be asked to attend 9 separate study visits over a 4 month period.
Study visits will range from 30 minutes to 2 hours and be separated by at least 2 weeks, with flexibility in scheduling these visits. Would your schedule accommodate these visits? 

YES  NO

NB: If the individual meets the pre-screening eligibility requirements:

- Set up an in-person screening visit
- Enter the scheduled date and time of the screening visit into the Google calendar
- Fill out the Phone Screening Tracking Log
- Arrange to send the potential participant a map to the HNRU and provide parking information
- **Be sure to ask the potential participant to bring with them to the screening visit any current medication and dietary supplements in their original packaging**

If you are unsure whether the individual meets the pre-screening requirements, thank them for their time and tell them that you will discuss their eligibility for the study with the other study investigators and call them back by the end of the next business day.

<table>
<thead>
<tr>
<th>Participant Name:</th>
<th>Screening ID:</th>
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</thead>
<tbody>
<tr>
<td>Telephone #: Home -</td>
<td>Cell -</td>
</tr>
<tr>
<td>Email:</td>
<td>Preferred method of contact?</td>
</tr>
<tr>
<td>Date of Birth (dd/mm/yyyy):</td>
<td>Age:</td>
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</tbody>
</table>

In-person screening visit scheduled for:

<table>
<thead>
<tr>
<th>DATE:</th>
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<tbody>
<tr>
<td>TIME:</td>
</tr>
<tr>
<td>COORDINATOR SIGNATURE:</td>
</tr>
</tbody>
</table>

Appendix 10. In-person participant eligibility questionnaire

IN-PERSON SCREENING PARTICIPANT ELIGIBILITY QUESTIONNAIRE

STUDY TITLE:
Human clinical trial to investigate the effects of high rosmarinic acid spearmint tea on markers of pain, physical function and disease activity in osteoarthritis of the knee.

HNRU Coordinator:

<table>
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<tr>
<th>Date:</th>
<th>Time:</th>
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Participant Name:  Eligibility ID:

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<tr>
<th>Telephone #: Home -</th>
<th>Cell -</th>
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</table>

Email:  Preferred method of contact?

Date of Birth (dd/mm/yyyy):  Age:

Thank you very much for your interest in this study. The purpose of this questionnaire is to gather more information about you and to ensure your safety as a participant in this study.

Please feel free to NOT answer any questions that you are uncomfortable with answering. Please feel free to ask the study coordinator any questions you might have.

All information provided in this questionnaire will be kept strictly confidential.
HEALTH HISTORY QUESTIONNAIRE

1. When were you first diagnosed with osteoarthritis of the knee? __________________________

2. Who first diagnosed you with osteoarthritis of the knee?
   (i.e. family doctor, rheumatologist) __________________________

3. (a) Which knee do you have osteoarthritis in? BOTH  RIGHT  LEFT

   (b) Which knee is most problematic? RIGHT  LEFT

4. Have you ever had knee replacement surgery? YES  NO

5. Are you planning on having knee replacement surgery between now and February 2012? YES  NO

6. Have you ever had a synovectomy before? (i.e. surgical removal of inflamed joint tissue) YES  NO

7. Do you currently smoke? YES  NO

   (a) If NO, have you ever smoked? YES  NO

   (b) If YES to (a), how long since you have quit? __________________________

8. Approximately how many alcoholic drinks do you consume per week? (1 drink = 12 oz beer, 5 oz wine, or 1.5 oz hard liquor) __________________________

9. Are you currently participating in any other research studies? YES  NO

   (a) If YES, please describe:

10. How would you describe your general health?

    POOR  GOOD  VERY GOOD  EXCELLENT
11. Do you have any of the following diagnosed medical conditions:

<table>
<thead>
<tr>
<th>Condition</th>
<th>YES</th>
<th>NO</th>
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<tbody>
<tr>
<td>(a) Rheumatoid arthritis?</td>
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<tr>
<td>(b) Cancer?</td>
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<td>(c) Pre-diabetes?</td>
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<tr>
<td>(d) Diabetes?</td>
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<td>(e) High cholesterol?</td>
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<tr>
<td>(f) High blood pressure?</td>
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<td></td>
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<td>(g) Impaired liver function?</td>
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<td></td>
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<td>(h) Impaired kidney function?</td>
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<td></td>
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<tr>
<td>(i) Gastrointestinal ulcer?</td>
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<td></td>
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<td>(j) Autoimmune disease?</td>
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<td></td>
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<tr>
<td>(k) Chronic infection?</td>
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<tr>
<td>(l) Depression?</td>
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(m) Are there any other medical conditions that might affect your participation in the study?  

(i) If YES, please describe:
12. Are you currently taking any medication and/or over-the-counter drugs? (i.e. Tylenol, Advil, Claritin, Sudafed, etc.)

   YES    NO

   (a) If YES, *please provide the study coordinator with your current medication.*
   The study coordinator will help you complete the following table:

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>REASON FOR USE</th>
<th>MEDICINAL INGREDIENTS</th>
<th>DOSE</th>
<th>HOW OFTEN</th>
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13. Are you currently taking any dietary supplements? (i.e. multivitamin, omega-3, glucosamine)
   YES    NO

   (a) If YES, *please provide the study coordinator with your dietary supplements.*
   The study coordinator will help you complete the following table:

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>REASON FOR USE</th>
<th>ACTIVE INGREDIENTS</th>
<th>DOSE</th>
<th>HOW OFTEN</th>
<th>HOW LONG</th>
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14. Are you currently taking any medication for your osteoarthritis?  

YES  NO

(a) If YES, please provide the study coordinator with your current osteoarthritis medication. The study coordinator will help you complete the following table:

<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>REASON FOR USE</th>
<th>MEDICINAL INGREDIENTS</th>
<th>DOSE</th>
<th>HOW OFTEN</th>
<th>HOW LONG</th>
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15. Do you see the following health professionals for your osteoarthritis: (make sure it is clear that these are all related to osteoarthritis)

(a) Family doctor or rheumatologist?  

YES  NO  
If YES, how often? ________________

(b) Physiotherapy?  

YES  NO  
If YES, how often? ________________

(c) Acupuncture?  

YES  NO  
If YES, how often? ________________

(d) Massage therapy?  

YES  NO  
If YES, how often? ________________

(e) Nerve stimulation?  

YES  NO  
If YES, how often? ________________

(f) Other? (please list)  

YES  NO  
How often? ________________

How often? ________________

How often? ________________
16. Do you participate in any of the following types of exercises:

<table>
<thead>
<tr>
<th>Exercise</th>
<th>YES</th>
<th>NO</th>
<th>How often?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight training?</td>
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<td>Stretching?</td>
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<td>Aerobics?</td>
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<td>Underwater?</td>
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<tr>
<td>Other? (please list)</td>
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</table>

17. Are you satisfied with your current body weight? YES NO

18. Has your body weight changed in the past:

<table>
<thead>
<tr>
<th>Time Period</th>
<th>YES</th>
<th>NO</th>
<th>How much?</th>
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<tr>
<td>2 months?</td>
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<td>1 year?</td>
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19. This study requires that participants maintain their body weight for the 4-month duration of the study. *Would you be comfortable with this?* YES NO

20. Do you have ANY allergies? (i.e. food, medications, rag weed or pollen) YES NO

<table>
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<tr>
<th>If YES, please list:</th>
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</table>
(b) *Please describe* your type of allergic reaction: (i.e. anaphylaxis, difficulty breathing, swelling, etc.)

21. Do you regularly consume fresh or dried mint, or mint products?  
   YES  NO

   (a) If YES, how often?  

   (b) *Please provide specific examples* of mint products that you consume:

22. Have you ever had a reaction or hypersensitivity to mint or mint products?  
   YES  NO

23. Do you consume fresh or dried herbs at an amount above typical flavoring levels for the specific purpose of improving your general health:

   (a) Basil?  
       YES  NO

   (b) Rosemary?  
       YES  NO

   (c) Sage?  
       YES  NO

   (d) Marjoram?  
       YES  NO

   (e) Oregano?  
       YES  NO

   (f) Thyme?  
       YES  NO

   (g) Lavender?  
       YES  NO

   (h) Lemon balm?  
       YES  NO

   (i) Fennel?  
       YES  NO
Question #24 to be completed by females only
Hormone levels can affect pain sensation and perception. Since pain is a large part of osteoarthritis, we wish to gather some information about your hormone levels.

24. Are you PRE- or POST-menopausal? PRE POST

(a) If POST-menopausal, do you use hormone replacement therapy? YES NO

(b) If YES to using hormone replacement therapy, please specify:

(c) If PRE-menopausal, do you have regular menstrual cycles? YES NO

(d) If NO to having regular menstrual cycles, please describe the irregularities:

(e) Do you currently use oral contraceptives (or other hormonal birth control)? YES NO

(f) If YES to using oral contraceptives or hormonal birth control, please specify:

25. This study involves blood draws. Are you comfortable having your blood taken for this study? YES NO

26. Are you a regular blood donor or have you donated blood in the last two months? YES NO

27. Have you ever fainted from having your blood taken? YES NO
28. Have you ever provided a synovial fluid sample before? (Also called arthrocentesis or joint aspiration, where a needle removes fluid from the joint) YES NO

29. In this study, participants will be asked to consider providing a synovial fluid sample. Participants may still continue to participate in the study even if they choose not to do so. Would you be comfortable having a doctor take a synovial fluid sample for this study? YES NO

30. This study involves a 3-day diet history. Would you be comfortable completing a detailed record of all the foods you have eaten over a 3-day period? YES NO

31. This study requires that participants DO NOT consume any herbal teas (other than provided by the study team) for the 4-month duration of the study. Would you be comfortable with this? YES NO

32. This study requires that participants continue their usual dietary supplement routine (i.e. dosage and frequency) for the 4-month duration of the study. Would you be comfortable with this? YES NO

33. This study requires that participants keep a daily record of ALL medications and dietary supplements used (i.e. dosage and frequency) for the 4-month duration of the study and for a 1-month follow-up period. Would you be comfortable with this? YES NO

34. This study requires that before four of the study visits (once a month), participants DO NOT participate in any complementary therapies (i.e. physiotherapy, massage therapy, acupuncture, nerve stimulation) or take any anti-inflammatory medications for 2 days (48 hours). Would you be comfortable with this? YES NO

35. This study requires 9 study visits to the HNRU at the University of Guelph as follows:
- Three ½ hour visits
- Three 1½ hour visits
- Two 2 hour visits
- Possibly 2 additional visits, both less than ½ hour

Study visits will be separated by at least 2 weeks and there is flexibility in their scheduling.
(a) Can your schedule accommodate these visits?  

YES  NO

(b) Are there any particular days of the week that you would prefer the study visits?  

YES  NO

MONDAY  TUESDAY  WEDNESDAY  THURSDAY  FRIDAY

(c) Is there a particular time of day that you would prefer?

MORNING  AFTERNOON  NO PREFERENCE

________________________________
HNRU Coordinator Signature

NB: Provide the WOMAC to the participant and have them complete the form.
Complete the 24-hr food recall with the participant.
Provide the participant with a coffee voucher.
Fill out the In-person Screening Tracking Log.
Appendix 11. Participant tea brewing instructions

Instructions for Brewing the Spearmint Tea

- Please drink one cup of mint tea in the morning and one in the afternoon or evening every day.
- Boil water.
- Place one mint tea bag in the study mug provided.
- Carefully pour 300 mL of boiling water over the tea bag.
- Agitate the tea bag by stirring every 2 minutes during steeping.
- Allow tea to steep for 5 minutes in total.
- Gently press the tea bag against the mug with a spoon to squeeze out any remaining liquid.
- Dispose of tea bag.
- Drink tea black. Please DO NOT add any milk or cream. Sugar may be added.
- Record in your Study Diary that you consumed the mint tea and indicate at what time.

Mint Tea Storage Instructions

- Store the mint tea at standard room temperature, i.e. in a cupboard.
- Keep the mint tea away from moisture and inside the sealed plastic bags and container provided.
- Keep the mint tea bags out of direct sunlight.
**Appendix 12. Example study diary sheet**

**FEBRUARY 17, 2012 - STUDY REMINDERS**

Thank you for your time & participation in our study! Questions about the study? Contact our Team!

**Week: 3 ◊ Thank Study Day: 21◊**

**Mint Tea**

<table>
<thead>
<tr>
<th>Time</th>
<th>Cup1 AM</th>
<th>Cup2 PM</th>
<th>Comments (e.g. missed tea, changes in preparation, etc.)</th>
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**Over-the-Counter, Medications/Dietary Supplements/Natural Health Products**

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Quantity</th>
<th>Taken</th>
<th>Comments (e.g. missed supplements, extra over-the-counter pain medication, etc.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>Yes</td>
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<td>No</td>
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</table>

**Prescribed Medications**

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Quantity</th>
<th>Taken</th>
<th>Comments (e.g. missed prescribed medication, started new prescription, etc.)</th>
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<tbody>
<tr>
<td></td>
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<td>Yes</td>
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**Other Comments (e.g. unusual circumstances, injuries or illness)**

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Appendix 13. Anthropometric case report form

HNRU CASE REPORT FORM
STUDY VISIT #1: BASELINE

Date of Visit: ________________ Date of Birth: ________________ Age: ______

ANTHROPOMETRIC MEASUREMENTS

(A) Body Height Did not complete height Reason:

(1) Body height: ________________ cm

(B) Body Weight Did not complete weight Reason:

(2) Body weight: ________________ kg

C) Body Mass Index (BMI) Did not complete BMI Reason:

(3) Body mass index (BMI): \[ \frac{\text{Weight (kg)}}{\text{Height (m)}^2} = \] ________________ kg/m^2

(D) Waist Circumference Did not complete waist Reason:

Trial #1: ________________ cm Trial #2: ________________ cm

(4) Waist circumference: ________________ cm

(E) Hip Circumference Did not complete hip Reason: __

Trial #1: ________________ cm Trial #2: ________________ cm

(5) Hip circumference: ________________ cm

HNRU Study Coordinator Initials: _____
HNRU CASE REPORT FORM
STUDY VISIT #1: BASELINE

(F) **Blood Pressure (BP)**

Did not complete BP  Reason:

____________________

Trial #1 (Diastolic / Systolic): _______________mm/Hg  Trial #1 (Pulse): _______ bpm

Trial #2 (Diastolic / Systolic): _________________mm/Hg  Trial #2 (Pulse): _______ bpm

(6) Blood pressure (Diastolic / Systolic): _______________mm/Hg

(7) Pulse: ______ bpm

(G) **Body Composition (BIA)**

Did not complete BIA  Reason:

____________________

BIA Test Number: _____________  Did participant void bladder? ______________

(8) BIA Body fat: ______________ %  (9) BIA Lean mass: _____________ %

**BIOLOGICAL SAMPLE ANALYSES**

(L) **C-Reactive Protein (CRP) Analysis**

Did not complete CRP  Reason:

Time of collection: ______________  am / pm

(10) C-reactive protein (CRP): ______________ μg/ml

HNRU Study Coordinator Initials: _____
Appendix 14. Physical function test case report form

HNRU CASE REPORT FORM
STUDY VISIT #1: BASELINE

Date of Visit: ______________

PHYSICAL FUNCTION TESTS

(A) Stair Climbing Task (SCT) Did not complete STR Reason:
1. Ensure participant is wearing comfortable clothes and shoes.
2. Use of walking aids (i.e. cane, walker) is permitted during task administration.
3. Give the following instructions:
The object of this task is to climb up 7 stairs as quickly and as safely as you can. Please begin by standing at the bottom of the stairs with your left hand on the handrail and your toes on the line on the floor. At the word ‘go’, please climb the stairs to the top so that both feet are on the top platform. Without any hesitation, turn around and climb down using the same handrail. You must use the handrail and climb only one step at a time. Now I’m going to show you. Please watch the way I turn without hesitation. Demonstrate by climbing one flight. Are you ready to do that? Remember that the object is to climb AS QUICKLY AS POSSIBLE, but don’t skip any steps.
4. Allow the participant to practice one flight.
5. Position the participant at the start line. Say GO and begin the timer as soon as they go. Stop the timer once both feet are back on the floor at the bottom of the stairs.
6. Record the use of aids and the stepping pattern.
7. Record the time to complete the task.

(5) Did the participant use a walking aid (i.e. walker, cane, etc.)? Yes ☐ No ☐

(6) Did the participant use a foot-over-foot stepping pattern? Yes ☐ No ☐

(7) Did the participant use a foot-beside-foot stepping pattern? Yes ☐ No ☐

(8) Time to complete: ____________ s

HNRU Study Coordinator Initials: _____

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(A) 6-Minute Walk Test (6MWT) Did not complete 6MWT  Reason:

(1) Measured track distance: __________ m

1. Do not administer task if SBP >180 and DBP > 100 mmHg.
2. Use of walking aids (i.e. cane, walker) is permitted during task administration.
3. Give the following instructions:

   The object of this task is to walk as far as possible for 6 minutes. You will walk back and forth in this hallway. 6 minutes is a long time to walk, so you will be exerting yourself. You may 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. You will be walking back and forth around the cones. You should pivot briskly around the cones and continue back the other way without hesitation. Now I’m going to show you. Please watch the way I turn without hesitation. Demonstrate by walking one lap. Are you ready to do that? Remember that the object is to walk AS FAR AS POSSIBLE for 6 minutes, but don’t run or jog.

4. Allow the participant to practice one lap.
5. Position the participant at the start line. Say GO and begin the countdown timer as soon as they go.
6. Do not walk with the participant. You may choose to follow at a distance behind them.
7. Provide standard reinforcement (no other words or body language) at 1 minute intervals as follows:
   - 5 min left: You are doing well. You have 5 minutes to go.
   - 4 min left: Keep up the good work. You have 4 minutes to go.
   - 3 min left: You are doing well. You are halfway done.
   - 2 min left: Keep up the good work. You have only 2 minutes to go.
   - 1 min left: You are doing well. You have only 1 minute to go.
   - 15 sec left: In a moment I’m going to tell you to stop. When I do, just stop right where you are and I will come to you.
8. If the participant stops: You can lean against the wall if you would like, then continue walking whenever you feel able. If necessary, provide a wheelchair for them to sit in.

(2) Did the participant use a walking aid (i.e. walker, cane, etc.) Yes ☐ No ☐

(3) Did the participant stop to rest? Yes ☐ No ☐

(3a) If yes, how many times did the participant stop? _________

(4) Check off the number of laps completed

188
(5) Total distance: # of laps ________(x ________m) +_______ partial  = ______m

HNRU Study Coordinator Initials: _____
**Appendix 15.** Biomarker results from clinical trial

**Table 15.2** Pooled Biomarker results in Week 0 and Week 16 analyzed with student’s t-test (SAS 9.2)

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Week 0</th>
<th>Week 16</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA (ng/mL)</td>
<td>1.86</td>
<td>1.90</td>
<td>0.56</td>
</tr>
<tr>
<td>MMP-3 (ng/mL)</td>
<td>1.13</td>
<td>1.17</td>
<td>0.47</td>
</tr>
<tr>
<td>PIIANP (ng/mL)</td>
<td>3.34</td>
<td>3.33</td>
<td>0.79</td>
</tr>
<tr>
<td>COMP (ng/mL)</td>
<td>1.47</td>
<td>1.49</td>
<td>0.64</td>
</tr>
<tr>
<td>IL-18 (pg/mL)</td>
<td>1.75</td>
<td>1.75</td>
<td>0.90</td>
</tr>
<tr>
<td>NO (µmol/L)</td>
<td>1.32</td>
<td>1.32</td>
<td>0.92</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>0.41</td>
<td>0.40</td>
<td>0.96</td>
</tr>
</tbody>
</table>

COMP = cartilage oligomeric matrix protein; CRP = C-reactive protein; HA = hyaluronic acid; IL-6 = interleukin-6; IL-18 = interleukin-18; MMP-3 = matrix metalloproteinase -3; NO = nitric oxide; PIIANP = type-IIA collagen N-propeptide.