UNIVERSITY OF CALGARY

Exercise and dietary interventions in a rat model of metabolic knee osteoarthritis

by

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A THESIS

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Abstract

Osteoarthritis is a debilitating chronic disease which has no cure or effective treatment. If no changes are made in prevention and treatment, osteoarthritis will continue to represent a significant economic burden to patients and society. The goal of this thesis was to determine the effects of moderate aerobic exercise and prebiotic fibre supplementation on the onset and progression of the metabolic knee osteoarthritis phenotype in rats exposed to a high-fat/high-sucrose diet. This study was divided into three phases. Phase 1: we evaluated the effects of a 12-week aerobic exercise program of varying duration on healthy rat knee cartilage. We determined that moderate, high and extra-high duration treadmill exercise has no detrimental effects on knee joint health, function and integrity. Therefore, we concluded that treadmill exercise at any tested duration was a safe exercise for rats in terms of knee osteoarthritis-like damage, and therefore, could be used as a safe exercise intervention in a pre-clinical rat model of knee osteoarthritis. Phase 2: we evaluated the effects of moderate aerobic exercise, prebiotic fibre supplementation, and the combination of exercise and fibre over a 12-week intervention in rats exposed to a high-fat/high-sucrose diet that has been shown to cause knee joint damage. Our findings indicated that prebiotic fibre and aerobic exercise prevented knee joint damage in this model. Phase 3: we evaluated if the damaging effects of the high-fat/high-sucrose diet could be delayed/reversed. We determined that neither prebiotic fibre supplementation nor aerobic exercise were able to stop the progression of existing knee osteoarthritis-like damage induced by a high-fat/high-sucrose diet. In summary, this thesis provides insight into two different approaches to prevent the development of metabolic osteoarthritis phenotype. However, the studies presented in this thesis were not able to show an effective way to stop the progression of the disease.

Preface

Chapters 2 - 4 and Appendix A are based on scientific manuscripts:

Chapter 2 is based on:

Rios, J. L., Boldt, K. R., Mather, J. W., Seerattan, R. A., Hart, D. A., & Herzog, W. (2018). Quantifying the effects of different treadmill training speeds and durations on the health of rat knee joints. Sports Med Open, 4(1), 15. doi:10.1186/s40798-018-0127-2*

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Chapter 3 is based on:

Rios, J. L., Bomhof, M. R., Reimer, R. A., Hart, D. A., Collins, K. H., & Herzog, W. (2019). Protective effect of prebiotic and exercise intervention on knee health in a rat model of diet-induced obesity. Sci Rep, 9(1), 3893. doi:10.1038/s41598-019-40601-x*

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Chapter 4 is based on:

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Dedication

To my beloved Mom and Dad, who believed in my dreams.

Para minha amada Mãe e amado Pai, que acreditaram em meus sonhos.

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List of Symbols, Abbreviations, & Nomenclatures

ACL: Anterior Cruciate Ligament

ACLT: Anterior Cruciate Ligament Transection

ADAMTS: A Disintegrin and Metalloproteinase with Thrombospondin Motifs

AGE: Advanced Glycation End-Products

BC: Before Christ

BMP: Bone Morphogenetic Protein

CAR: Carrageenan

CCL: CC-Chemokine Ligands

CGP-39: Cartilage Glycoprotein-39

Col-1: Type I Collagen

Col-3: Type II Collagen

COX-2: Cyclooxygenase -2

CRP: C-Reactive Protein

DXA: Dual X-ray Absorptiometry

EGF: Epidermal Growth Factor

EHD: Extra High Duration

G-CSF: Granulocyte Colony Stimulating Factor

GI: Gastrointestinal

GM-CSF: Granulocyte Macrophage Colony Stimulating Factor

GRO/KC: CXCL1 chemokine, Growth-related Oncogene Keratinocyte Chemotractant

HD: High Duration

HDL: High-Density Lipoprotein

HFS: High-Fat/High-Sucrose

ICAM-1: Intercellular Adhesion Molecule 1

IFN-γ: Interferon Gamma

iNOS: Inducible Nitric Oxide Synthase

IGF-1: Insulin-Like Growth Factor 1

IL: Interleukin

IL-1Ra: Interleukin 1 Receptor Antagonist

IP-10/CXCL10: Interferon Gamma-Induced Protein 10

LDL: Low-Density Lipoprotein

LIF: Leukemia Inhibitory Factor

LIX: CXCL5 Chemokine

LPS: Lipopolysaccharide

LOX: Lectin-like oxidized low-density lipoprotein receptor

MCP-1: Macrophage Chemoattractant Protein-1

MD: Moderate Duration

MIA: Monosodium Iodoacetate

MIP: Macrophage Inflammatory Protein

MMP: Matrix Metalloproteinases

MMT: Medial Meniscal Tear

MNX: Meniscectomy

MRI: Magnetic Resonance Imaging

mRNA: Messenger Ribonucleic Acid

NFκB: Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells

NGF: Nerve Growth Factor

NO: Nitric Oxide

OA: Osteoarthritis

OARSI: Osteoarthritis Research Society International (Histology Scoring System)

PGE2: Prostaglandin E2

PPAR-γ: Peroxisome Proliferator-Activated Receptor Gamma

PTOA: Post-Traumatic Osteoarthritis

RAGE: Receptor for Advanced Glycation End-Products

RANTES: Regulated on Activation, Normal T Cell Expressed and Secreted

RNA: Ribonucleic Acid

ROS: reactive oxygen species

RT-qPCR: Real Time Quantitative Polymerase Chain Reaction

SASP: Senescence-Associated Secretory Phenotype

TF: Tissue Factor

TFPI: Tissue Factor Pathway Inhibitor

TGF-β: Transforming Growth Factor-Beta

Th1: Type-1 T Helper Cells

TJ: Tight Junction

TNF: Tumor Necrosis Factor

VCAM-1: Vascular Cell Adhesion Molecule 1

VEGF: Vascular Endothelial Growth Factor

Epigraph

"…faith in new possibilities, and courage to advocate them."

-Jane Addams

Chapter One: Introduction and review of selected literature

1.1 Rationale

Osteoarthritis (OA) is a debilitating chronic disease which has no cure or effective treatment. If no dramatic changes are made in the prevention and treatment of OA, it is estimated that OA will cost Canadians $550 billion in health care expenses by 2040 (Bombardier, Hawker, & Mosher, 2011). Obesity (a marker of metabolic disturbance) has been identified as a primary risk factor for the development of knee OA (Courties, Gualillo, Berenbaum, & Sellam, 2015; Courties, Sellam, & Berenbaum, 2017), and obesity is thought to be a modifiable risk factor (Powell, Teichtahl, Wluka, & Cicuttini, 2005). Metabolic disturbance related to OA has been termed "metabolic OA" (Zhuo, Yang, Chen, & Wang, 2012). Metabolic OA is associated with large fat deposits (Ouchi, Parker, Lugus, & Walsh, 2011), and its inappropriate storage (Thomas, Browne, Mobasheri, & Rayman, 2018), that release inflammatory cytokines/adipokines causing low-level chronic systemic inflammation (Hotamisligil, 2006; Wang, Hunter, Xu, & Ding, 2015). Exposing rats to a high-fat/high-sucrose (HFS) diet leads to metabolic disturbance, and consequently to metabolic knee OA-like damage in rats within 12 weeks (Collins, Reimer, Seerattan, Leonard, & Herzog, 2015c). Exercise and prebiotic fibre supplementation are known to be effective modulators of metabolic disturbance (Beecher, Martin, Pedersen, Heiner, & Buckwalter, 2007; Cluny, Eller, Keenan, Reimer, & Sharkey, 2015; Conti *et al.*, 2015; Earnest *et al.*, 2013; Jeffery *et al.*, 2000; Nicolucci *et al.*, 2017; Pedersen, 2006). However, there is no systematic evidence of the effects of exercise and prebiotic dietary intervention on knee OA. Therefore, the main purpose of this thesis was to determine the effect of aerobic exercise and

prebiotic fibre supplementation on the onset and rate of progression of knee OA in a diet-induced model of metabolic disturbance.

This thesis is divided into five chapters. Chapter one is a review of selected literature. This review covers osteoarthritis phenotyping, animal models of osteoarthritis, metabolic disturbance, inflammation, and introduces the literature on prebiotic fibre and exercise. Chapter two presents the effects of treadmill exercise on knee joint health of CD-Sprague-Dawley rats. In chapter three, we used a rat-model of diet-induced obesity and metabolic disturbance that has been shown to cause reliable and consistent knee OA (Collins, Hart, Reimer, Seerattan, & Herzog, 2015a, 2016b; Collins *et al.*, 2015b; Collins *et al.*, 2015c), and evaluated the effects of an aerobic exercise and a prebiotic fibre supplementation intervention on the development of knee OA. In chapter four, we determined the effects of prebiotic fibre supplementation and aerobic exercise on the progression of existing knee OA induced by metabolic disturbance. In chapter five, we integrate the series of studies presented in this thesis, and raise questions and identify gaps in this research area, as well as acknowledge the limitations of our studies. Finally, in the appendix, there are a series of observations made during the studies that were not displayed in the main chapters of this thesis.

1.2 Osteoarthritis

Osteoarthritis is the most common form of arthritis (Altman, 1995; Davis, Ettinger, Neuhaus, & Mallon, 1991) and is often associated with a low-level grade of inflammation (Berenbaum, 2013; Hotamisligil, 2006; Lumeng, Maillard, & Saltiel, 2009; Sokolove & Lepus, 2013; Zhuo *et al.*, 2012). Classically, osteoarthritis has been defined as the clinical and pathological outcome of a range of disorders that results in structural and functional failure of synovial joints. In the past, it has typically been considered a disease of the articular cartilage

(Nuki, 1999). However, current concepts hold that osteoarthritis involves the entire joint as an organ system, including the subchondral bone, menisci, ligaments, periarticular muscles, capsule, and synovium (Frank, Shrive, Boorman, Lo, & Hart, 2004; Hunter & Felson, 2006; Loeser, Goldring, Scanzello, & Goldring, 2012; Martel-Pelletier, Wildi, & Pelletier, 2012).

Table 1.1. Most commonly used factors allowing for differentiation of clinical phenotypes of osteoarthritis, and animal models used to study these phenotypes. AGE: advanced glycation end products. Adapted from Bijlsma, Berenbaum, & Lafeber (2011). Used with permission.

	Post-traumatic	**Metabolic**	**Ageing**	**Genetic**	**Pain**
Age	< 45 years	45–65 years	> 65 years	Variable	Variable
Main causative effects	Mechanical stress	Mechanical stress, adipokines, hyperglycaemia, estrogen/ progesterone imbalance	AGE, chondrocyte	Gene related	Inflammation, bony changes, aberrant pain perception
Main site	Knee, thumb, ankle, shoulder	Knee, hand, generalized	Hip, knee, hand	Hand, hip, spine	Hip, knee, hand
Widely accepted models	Invasive surgical post traumatic model/ non-invasive post traumatic models	Diet-induced obesity model	Genetically-modified model/ Spontaneous model	Genetically-modified models	Chemical model

OA has been typically classified as primary (idiopathic) when there is no obvious predisposing cause, and secondary when there is some clearly defined predisposing pathology (Altman, 1995). However, based on studies demonstrating different OA pathophysiology onset, OA has now been classified into five distinguishable phenotypes: 1) post-traumatic, 2) metabolic, 3) ageing, 4) genetic, and 5) pain phenotype (Table 1.1) (Bijlsma *et al.*, 2011). Understanding the different clinical and structural OA phenotypes is essential for the development of targeted treatments in those in whom structural changes in either cartilage, bone, or synovial tissue dominate the disease (Bijlsma *et al.*, 2011).

1.2.1 Animal models of osteoarthritis

Osteoarthritis models have been developed using both large and small animals. Typically, large animal models involve the dog, goat, sheep, and horse while small animal models involve the mouse, rat, rabbit, and guinea pig. The choice of which animal to use depends on several factors including, but not limited to, the type of experiment/study, the length of time to develop the disease, husbandry costs, ease of handling, and outcome measurements (Kuyinu, Narayanan, Nair, & Laurencin, 2016). Each animal has its advantages and disadvantages (Table 1.2), some models are better suited to study the disease processes of OA, while others are designed to investigate treatment techniques, which will be discussed below.

1.2.1.1 Spontaneous models of osteoarthritis

OA models have been categorized into spontaneous (primary) and induced (secondary) models. Spontaneous models are based on the occurrence of slowly progressing OA in certain animals (mouse, guinea pig, dog, rabbit, sheep, and horse) that closely simulate the natural progression of human primary OA (Bendele, 2001; Kuyinu *et al.*, 2016; Lampropoulou-Adamidou *et al.*, 2014; McCoy, 2015). These models are good in mimicking the aging OA phenotype. Transgenic mouse models (genetically-modified models) have been developed in which OA develops without any other intervention. Genetically-modified models have played a crucial role in understanding specific genetic contributions to the pathogenesis of OA (Little & Zaki, 2012; Miller, Lu, Tortorella, & Malfait, 2013; Pest, Pest, Bellini, Feng, & Beier, 2015; Staal, Williams, Beier, Vande Woude, & Zhang, 2014).

Table 1.2. Advantages and disadvantages of osteoarthritis animal models of osteoarthritis. Adapted from Gregory et al. (2012). GI: gastrointestinal; MRI: magnetic resonance imaging. Used with permission.

Animal	Advantages	Disadvantages	Models used
Mouse	Low cost Ease of use Genome sequenced Can view whole knee on slides	Thin cartilage Post-operative management difficult	Genetic Meniscal destabilization Chemical Diet-induced obesity
Rat	Low cost Genome sequenced Ease of use Thicker cartilage than mouse Can view whole knee on slides	Small joints Post-operative management difficult	Medial meniscus tear Partial medial meniscectomy ACL transection with partial medial meniscectomy Chemical Diet-induced obesity
Rabbit	Ease of use Genome sequenced	Knee biomechanics Cartilage capable of regeneration Different histology from human Post-operative management difficult	ACL transection Meniscectomy Chemical
Guinea pig	Similar histopathology to human Prone to spontaneous OA	Sedentary lifestyle Arthroscopy not possible	Spontaneous Meniscectomy Chemical
Dog	Prone to spontaneous OA Arthroscopy feasible MRI feasible GI physiology Genome sequenced Validated outcome measures	Cost Public perception	Spontaneous ACL transection Meniscal release Focal cartilage defect
Sheep/goat	Genome sequenced Large joint Ease of use Arthroscopy feasible MRI feasible	Cost GI physiology	Partial/total meniscectomy
Horse	Genome sequenced Spontaneous OA Can induce OA without instability Arthroscopy feasible MRI feasible	Cost Anatomy	Spontaneous Osteochondral-fragment exercise model

An advantage of using spontaneous models is that they ensure the results closely mimic the slow progressive changes noted in human primary OA (Lampropoulou-Adamidou *et al.*, 2014). However, a major disadvantage is the long time period required for the joint damage to develop, which makes it difficult to conduct short-term studies, and increases the costs of housing and animal maintenance significantly (Kuyinu *et al.*, 2016).

It has been reported that the Dunkin Hartley guinea pig model provides the closest representation of primary human OA, and as such has been one of the most widely used animal models to study naturally occurring OA (Jimenez, Glasson, Trubetskoy, & Haimes, 1997; Kuyinu *et al.*, 2016; Lampropoulou-Adamidou *et al.*, 2014; Yan *et al.*, 2014). Guinea pigs rapidly grow to maturity, which is an advantage over larger animal models (Poole *et al.*, 2010), while also developing lesions similar to human patients, further strengthening their use as an animal model in therapeutic and pathogenesis studies (Jimenez *et al.*, 1997). Additionally, the guinea pig is also a natural model to study inflammation in the joint (Huebner & Kraus, 2006). Mice and rabbits have also been used as spontaneous models to study OA disease pathogenesis (Kyostio-Moore *et al.*, 2011), and the development of bioengineered treatment of cartilage defects (Arzi *et al.*, 2012a; Arzi *et al.*, 2012b).

When considering models using large animals, horse articular cartilage has been reported to be the most comparable to humans. These animals provide a naturally occurring model to study bone remodeling, which leads to bone cyst and osteophyte formation (Drum *et al.*, 2007; Olive, D'Anjou, Girard, Laverty, & Theoret, 2009). Horse cartilage has also been used to study repair and osteochondral defects (Frisbie *et al.*, 1999; McCoy, 2015; McIlwraith, Fortier, Frisbie, & Nixon, 2011; Salonius *et al.*, 2019), thus the horse has been said to contain several important factors that are related to the pathogenesis of primary human OA. The sheep knee model has been said to also

be anatomically similar to the human knee, and has been used successfully in studying early cartilage changes in OA (Drum *et al.*, 2007). Furthermore, preclinical trial with dogs are a good model for the study of therapeutic interventions (McDevitt & Muir, 1976; Moreau *et al.*, 2014; Moreau *et al.*, 2013). However, working with large animals usually requires Food and Drug Administration approval of diagnostics, biologics, and devices. Gender and reproductive status must also be considered in OA research due to possible hormonal influences on physiology, response to treatments, and various side effects (Sniekers, Weinans, Bierma-Zeinstra, van Leeuwen, & van Osch, 2008). All of these factors, in addition to the housing of animals and salaries of personnel involved in animal care and assessments, have a direct impact on the costs of OA research (Ishimoto *et al.*, 2013). Another disadvantage of working with large animals is public perception. The public has become increasingly concerned about the humane treatment of animals in research, particularly dogs and horses (Gregory *et al.*, 2012).

Baboons, rhesus macaque, and cynomolgus macaque share several biological and behavioral similarities to humans, and the development of OA in these animals reflects this (Carlson *et al.*, 1994; Chateauvert, Pritzker, Kessler, & Grynpas, 1989; Lankau, Turner, Mullan, & Galland, 2014; Macrini *et al.*, 2013). However, similarities with humans have also been cited as reasons for their exclusion from research (Bradshaw, Capaldo, Lindner, & Grow, 2008). For example, chimpanzees used in experiments often exhibit depression and post-traumatic stress disorder similar to the humans (Ferdowsian *et al.*, 2011). These factors present ethical issues, and in conjunction with the high costs of care, are huge obstacles to their widespread application and use of these animals to develop models to simulate human OA (Conlee & Rowan, 2012; McCoy, 2015).

1.2.1.2 Genetically-modified models of osteoarthritis

Mice have been used extensively to study OA, due to the ease of genetically modifying them, or breed specific strains particularly susceptible to OA (Kuyinu *et al.*, 2016). In mice, gene mutations are used to either protect the animal from OA or intensify a structural change in the disease, which can help to establish the molecular basis of OA including the effects of proinflammatory cytokines on OA development (Little & Hunter, 2013).

For example, genetically-modified models of obesity using leptin knockout and leptin receptor knockout models indicate that mice deficient in leptin that are fed a high-fat diet do not develop OA (Griffin, Huebner, Kraus, & Guilak, 2009). Another example is ADAMTS5 (disintegrin and metalloproteinase with thrombospondin-like motifs -5) knockout mice, in which the deletion of active ADAMTS5 prevents cartilage degradation in a model of OA (Glasson *et al.*, 2005). Genetically-modified models have contributed immensely to the understanding of specific genetic contributions to the pathogenesis of OA (Little & Zaki, 2012; Miller *et al.*, 2013). However, therapeutic interventions targeting these specific genes do not consider other contributing genes which may participate in the pathogenesis of the disease which is a significant disadvantage as it reduces the ability of such results to be translated to human clinical trials (McCoy, 2015).

1.2.1.3 Induced models of osteoarthritis

Induced models of OA mimic secondary OA in humans, i.e. the presence of specific causes or risks which contribute to the pathogenesis of OA. While these causes include congenital disorders, deregulation of calcium deposition, bone remodeling deficiency, joint (e.g., rheumatoid

arthritis) and metabolic disorders, post-traumatic OA (PTOA) models are the most widely studied (Little & Hunter, 2013).

1.2.1.3.1Preclinical post traumatic knee osteoarthritis models

Preclinical models of post traumatic knee OA can be studied by direct (invasive models) or indirect (non-invasive models) injury to the joint. Invasive models can be classified as either surgically or chemically induced. The rapid pathogenesis of OA in induced models is an advantage and ensures that the study can be completed in a short time frame. These models have been used to study the effects of drugs (Kim *et al.*, 2014) on the pathogenesis of PTOA (Pauly *et al.*, 2015). However, one disadvantage of invasive models is that they have no correlation to the natural, degenerative changes seen in human OA (Lampropoulou-Adamidou *et al.*, 2014).

There are several ways to surgically induce OA. The anterior cruciate ligament transection (ACLT) model is the most widely used and well-known model (Adams, 1983; Johnson, 1986; Lampropoulou-Adamidou *et al.*, 2014; McCoy, 2015; Myers, Brandt, O'Connor, Visco, & Albrecht, 1990b). Anterior cruciate ligament (ACL) injury results in joint destabilization, which subsequently leads to OA. This model imitates the degradation of articular cartilage following ACLT. Although it has been used extensively, and in different animal species, the best species for this model are sheep and goats as their stifle joints are sufficiently large for easy replication of the procedure (Frisbie, Cross, & McIlwraith, 2006; Little *et al.*, 2010; Proffen, McElfresh, Fleming, & Murray, 2012). The goat knee has been said to most closely represent the anatomy of the human knee due to medial and lateral meniscus similarity. Furthermore, the insertion sites of the lateral meniscus on the tibia are thought to be the most comparable to those of the human knee (Proffen *et al.*, 2012).

As in humans, animal meniscectomies lead to osteoarthritic changes in the joint (McDermott & Amis, 2006; Roos *et al.*, 1998). A partial meniscectomy causes destabilization of the joint leading to rapid degeneration (Karahan, Kincaid, Kammermann, & Wright, 2001). Due to differences in load transmission between the medial and lateral side of the knee in different species, the site for the surgical procedure varies by animal model. Humans and guinea pigs typically load the medial side of the knee more than the lateral side (Sharma *et al.*, 2013), while rabbits load the lateral side more than the medial (Bendele, 2001; Kuyinu *et al.*, 2016). This finding may explain why rabbits develop more severe lateral OA following surgery on the lateral meniscus compared to the same procedure on the medial meniscus. Extensive research has been performed using meniscectomy in dogs (Arnoczky & Warren, 1983; Intema *et al.*, 2010; Johnson, Francis, Manley, Chu, & Caterson, 2004; Kuyinu *et al.*, 2016).

An advantage of working with the Beagle dog model is that when mature, Beagles have spontaneous superficial degenerative changes on the medial tibial plateau in the area not protected by the meniscus. These lesions show cell and proteoglycan loss, focal loss of the tangential layer, and sometimes have deep fibrillation, or evidence of cell cloning. Beagle knee joint cartilage is relatively thick, and thus, from a histological perspective, offers opportunities to identify and characterize deep lesions. Furthermore, lesions in the Beagle seem to progress more slowly than those induced by meniscectomy in rodents and rabbits (Bendele, 2001; Lindhorst *et al.*, 2000). Limitations of using Beagles include housing the animals in large rooms to allow abundant opportunity for exercise, the long period of time required to achieve maturity (2 years or greater for female Beagles), and the lengthy period to develop moderate degenerative changes on the tibial and femoral cartilage after the surgery (on average 1-3 months) (Bendele, 2001; Lindhorst *et al.*, 2000).

Medial meniscal tears in humans result in joint instability and cartilage degradation (Edd, Giori, & Andriacchi, 2015). To mimic this in an animal model, typically, a transection of the medial collateral ligament is performed, and then the meniscus is cut, with progressive degenerative changes occurring 3-6 weeks post-surgery (Bendele, 2001; McCoy, 2015). Rats and guinea pigs are the most studied examples of animals using this model (Bendele, 2001). In rats, there is a rapid progression of cartilage degeneration, making the meniscal tear model an extremely difficult model for detecting protective effects. The knee joint lesions are morphologically similar to the pathogenesis to human disease, but occur much more rapidly (Bendele, 2001).

The ovariectomy OA model is based on the observation that post-menopausal individuals develop OA due to a reduction in estrogen levels, which is believed to be a factor of the pathogenesis of OA in humans (Martin-Millan & Castaneda, 2013). Mice, rats, guinea pigs, and sheep are often used as ovariectomy models (Cake *et al.*, 2005; Dai, Wang, Li, Liu, & Liu, 2006; Ma *et al.*, 2007; Sniekers, Weinans, van Osch, & van Leeuwen, 2010). However, New Zealand rabbits have also been widely used to study the direct effects of estrogen deficiency on the development of OA (Castaneda *et al.*, 2008; Castaneda *et al.*, 2010; Qin, He, Xia, Guo, & He, 2013).

Chemically induced models involve the injection of a toxic or inflammatory compound directly into the knee joint. They are typically used to study the effects of drugs on the inflammation and the pain caused by these substances. Example of chemicals employed to induce OA in animals are papain, sodium monoiodoacetate, quinolone, and collagenase. Chemically induced models are less invasive than surgically induced models, however, their pathophysiology is not reflective of the mechanisms underlying PTOA. For this reason, chemically induced models are mainly used to study the mechanisms of pain and as target studies for drug therapies (Kuyinu

et al., 2016; Lampropoulou-Adamidou *et al.*, 2014). Animals typically used in chemical models are mice (Daans, Luyten, & Lories, 2011), rats (Kobayashi *et al.*, 2003; Marker & Pomonis, 2012), rabbits (Huh, Baek, Lee, Choi, & Park, 2008; Muehleman *et al.*, 2002), and guinea pigs (Teeple *et al.*, 2007; Williams & Thonar, 1989; Yamada, Uegaki, Nakamura, & Ogawa, 2000). Chemically induced models will be discussed further in the context of pain models of OA.

Invasive models require the use of aseptic techniques to avoid infection, since infection will lead to inflammation, which may affect the outcomes of experiments (Lampropoulou-Adamidou *et al.*, 2014). Non-invasive PTOA models may be one solution to this problem. Non-invasive models produce an external insult to the joint of interest using machines which cause injury through mechanical impact, without causing a lesion on the skin of the animal. The injury then leads to osteoarthritic changes in the joint, similar to the invasive models (Christiansen *et al.*, 2015). Advantages and disadvantages of both invasive and non-invasive models of OA are presented in Table 1.3.

Table 1.3. Advantages and disadvantages of invasive versus non-invasive animal models of osteoarthritis. Adapted from Kuyinu et al. (2016). Used with permission.

	Induced/Invasive	**Induced/Non-invasive**
Pros	Easily reproducible, material readily available, multiple studies in the literature present	Easily reproducible, minimal infection risk, used to study early changes and the effect of early therapeutic intervention
Cons	Possibility of infection, relies on expertise of surgeon, induction too rapid to study early changes or early drug therapy in some animals	Equipment not universally available, relies on proficiency of technician/investigator, minimal literature on application

The most widely used non-invasive OA models cited in the literature are: (1) intra-articular tibial plateau fracture, which reproduces PTOA from high energy impact and is useful to study early OA changes after acute injuries. However, it is not useful for chronic injuries (Christiansen

et al., 2015; Furman *et al.*, 2007; Kuyinu *et al.*, 2016); (2) cyclic articular cartilage tibial compression, which reproduces chronic joint overuse (Kuyinu *et al.*, 2016; Poulet, Hamilton, Shefelbine, & Pitsillides, 2011); (3) tibial compression overload, which reproduces PTOA from low energy impact and the resulting injury pathology generated is analogous to the animal ACLT model but without the need for invasive surgery (Kuyinu *et al.*, 2016; Lockwood, Chu, Anderson, Haudenschild, & Christiansen, 2014); and (4) trans-articular impact, where typically one single load is required to reproduce PTOA, which is a good model to study surgical knee replacement (Kuyinu *et al.*, 2016). All of the above models are PTOA non-invasive models, and I would like to add one non-invasive model which is not related to PTOA - (5) the diet induced model of metabolic OA. This model will be discussed later in this thesis.

Some of the tools/techniques used to measure outcome measures for non-invasive mouse models of OA include micro-computed tomography (μ-CT) scanning which provides a visual representation of the fracture and Safranin-O staining for proteoglycan content (Christiansen *et al.*, 2015; Poulet *et al.*, 2011), in addition to the use of in vivo fluorescence reflectance imaging to quantify inflammation (Satkunananthan *et al.*, 2014). The most described methods for inducing OA in non-invasive mouse model are intra-articular tibial plateau fracture (Furman *et al.*, 2007), cyclic articular cartilage tibial compression (De Souza *et al.*, 2005), and ACL rupture via tibial compression overload (Christiansen *et al.*, 2015).

Additionally, various non-invasive canine models have been developed to investigate OA (Lahm *et al.*, 2005; Mrosek *et al.*, 2006; Thompson, Oegema, Lewis, & Wallace, 1991), and several breeds such as the Labrador, Golden Retriever, German Shepherd and Beagle have been used (Kuyinu *et al.*, 2016). The Beagle trans-articular impact model is the most widely used. A dropping tower with a load of approximately 2000N is used to create an impact on the

patellofemoral joint of the immobile knee, without damaging the skin. This model has been used to test early changes of osteoarthritis which occur in articular cartilage due to joint impact trauma (Lampropoulou-Adamidou *et al.*, 2014; Mrosek *et al.*, 2006), specifically osteochondral lesions (Lahm *et al.*, 2005; Mrosek *et al.*, 2006; Thompson *et al.*, 1991). Impact models also provide a good platform for studying cartilage healing and surgical joint replacement (Kuyinu *et al.*, 2016). Immunofluorescence unfixed cryosections are one of the tools used in this model to assess the degenerative changes of OA (Mrosek *et al.*, 2006), in addition to MRI (Lahm, Uhl, Erggelet, Haberstroh, & Mrosek, 2004; Thompson *et al.*, 1993).

Rabbits and rats have also been used to investigate non-invasive OA models. Rabbit trans-articular mechanical impact models involve a sub-fracture impact toward the patellofemoral joint leading to osteoarthritic changes (Ewers, Newberry, & Haut, 2000; Haut, Ide, & De Camp, 1995; Rundell, Baars, Phillips, & Haut, 2005). However, recent literature on rabbit trans-articular mechanical impact models (Alexander *et al.*, 2012) involve invasive surgery which may lead to undesired effects, as discussed previously for invasive models. There are a limited number of studies which have utilized non-invasive loading protocols to induce OA in rats. Rotational mechanical loads have been used to characterize proinflammatory cytokine and proteinase expression following non-invasive ACL rupture in rats (Tang *et al.*, 2009; Xue *et al.*, 2010). While in another study, the biomechanical characterization of a traumatic ACL injury in rats was performed (Maerz *et al.*, 2015).

1.2.1.3.2Diet-induced model of osteoarthritis

To date, there is sufficient evidence suggesting that metabolic disturbance (such as obesity) is associated with a significant increase in the risk of development of knee (Felson et al., 1988;

Felson & Chaisson, 1997; Hart & Spector, 1993; Manninen, Riihimaki, Heliövaara, & Suomalainen, 2004), hip (Felson & Chaisson, 1997), and hand OA (interphalangeal joints) in humans (Bijlsma et al., 2011; Felson & Chaisson, 1997). Furthermore, it has been suggested that increased mechanical loading in weight bearing joints cannot completely explain OA damage observed in the knee, and that systemic inflammatory mediators have a strong relationship with the damage observed (Collins, Hart, et al., 2015; Collins, Paul, et al., 2015; Collins, Reimer, et al., 2015; Griffin et al., 2010; Griffin, Huebner, Kraus, Yan, & Guilak, 2012), suggesting a new OA phenotype, metabolic OA (Bijlsma *et al.*, 2011; Zhuo *et al.*, 2012). In order to test this new phenotype, and to understand the underlying mechanics behind OA in people with metabolic disturbance, diet-induced models of OA (as called metabolic model of OA) have been developed.

One of the first studies of knee OA caused by a high-fat diet was published by Griffin et al. (2010). They exposed mice to either a low- or high-fat diet (10% and 45% kcal from fat, respectively) from 9 until 54 weeks of age. Interestingly, it was possible to delineate mice on the high-fat diet into 'resistant' (low body mass and fat) and 'prone' (high body mass and fat) populations depending on the extent of their weight gain. The prone mice showed an increased incidence of knee OA, mainly indicated by loss of cartilage proteoglycans. The authors suggested that high susceptibility to obesity was associated with OA-like changes in the knee joint. The same study reported that prone mice were associated with impaired musculoskeletal force generation and motor function compared to the control group. Furthermore, the high-fat diet also induced symptoms characteristically displayed by sufferers of OA, including hyperalgesia and anxiety-like behaviors. Additionally, percentage body fat was significantly associated with knee OA severity and serum levels of leptin, adiponectin, and IL-1α. The same model was also used to study short-term wheel-running exercise in mouse obesity-induced knee OA (Griffin, Huebner, Kraus, Yan,

& Guilak, 2012). It was found that exercise in this cohort improved glucose tolerance and disrupted the co-expression of proinflammatory cytokines, suggesting that increased aerobic exercise may act independently of weight loss in promoting joint health as the mice did not lose body mass or body fat when compared to the sedentary group.

In 2015, a high-fat/high-sucrose model was successfully used to induce OA in CD-Sprague-Dawley rats (Collins *et al.*, 2015a; Collins *et al.*, 2015b; Collins *et al.*, 2015c). This model was also associated with obesity prone and obesity resistant phenotypes, suggesting that individual animals tend to respond differently to the same diet challenge. Furthermore, a high-fat diet resulted in altered chondrocyte function in rabbits, independent of animal weight, and was associated with changes in rabbit cartilage in vivo (Brunner *et al.*, 2012).

1.2.1.3.3Preclinical pain osteoarthritis model

Animals which are preyed on in the wild, such as rats, mice, and guinea pigs, tend to hide their pain as a natural survival instinct (National Research Council, 2003; Peterson, 2004; Stasiak, Maul, French, Hellyer, & VandeWoude, 2003). However, the same behavior is not observed in dogs and cats (Little & Zaki, 2012). When dogs are in distress, they typically express pain by inactivity, whining, and licking. In contrast, cats hiss and hide the injured or painful site. Consequently, pain in dogs and cats can be studied more easily than in prey animals (Bufalari, Adami, Angeli, & Short, 2007). Despite their marked differences in behavior when in pain, prey animals are still widely used to study OA-related pain, more so than dogs, cats, and sheep (Kuyinu *et al.*, 2016; Piel, Kroin, van Wijnen, Kc, & Im, 2014). One reason for this is the sizable cost of working with large animals due to factors such as housing, maintenance, and in some cases, ethical concerns (Kuyinu *et al.*, 2016), as previously discussed.

Models based on mechanical, thermal, anatomical, and chemical changes have been reported for both smaller (prey), as well as larger (predator) animal pain models of OA (Kuyinu *et al.*, 2016). OA induced via surgical, chemical, and mechanical means are commonly used (Piel *et al.*, 2014). Table 1.4 summarizes the most commonly used models. As the other methods have been described in detail previously, only chemically induced models will be discussed below**.**

Table 1.4. Commonly used animal models and outcome measures for pain in osteoarthritis. Adapted from Kuyinu et al. (2016). Used with permission.

Induction method	Species	Changes observed/outcome measures
Monosodium iodoacetate-induced OA (MIA)	Rat, mouse	Thermal and mechanical analgesia, mechanical sensitivity and changes in the gait, hyperalgesia and allodynia, hind limb grip force test
Carrageenan-induced OA (CAR)	Rat Rabbit Guinea pig	Mechanical allodynia, gait, limited locomotion Hind limb weight distribution, mechanical hyperalgesia Thermal hyperalgesia
Anterior cruciate ligament transection (ACLT)	Rat, rabbit Dog	Mechanical allodynia, gait analysis Gait analysis and altered mobility
Meniscectomy (MNX)	Mice	Mechanical allodynia, mechanical and thermal sensitivity
Medial meniscal tear (MMT)	Rat Sheep	Hind paw weight, allodynia, mechanical sensitivity, decreased paw withdrawal Hind paw weight

Chemically induced models of OA include sodium monoiodoacetate-induced OA (MIA) (Ferreira-Gomes, Adaes, & Castro-Lopes, 2008; Little & Zaki, 2012; Liu *et al.*, 2011; Tremoleda *et al.*, 2011), which is the most widely reported method (around 50 % of pain OA studies) (Kuyinu *et al.*, 2016), carrageenan (CAR) (Achari, Reno, Frank, & Hart, 2012), and papain (Laurent *et al.*, 2001). The assessment of pain in small animals with OA is commonly performed using techniques such as the rotarod test (Ruan, Patel, Dawson, Jiang, & Lee, 2013), incapacitance test (Kim, Uchimoto, Duellman, & Yang, 2015), gait analysis (Coulthard, Pleuvry, Brewster, Wilson, & Macfarlane, 2002), spontaneous behavior (Millecamps *et al.*, 2005), mechanical sensitivity (von

Frey test) (Chaplan, Bach, Pogrel, Chung, & Yaksh, 1994; Osikowicz, Mika, Makuch, & Przewlocka, 2008), thermal sensitivity (Hargreaves Test) (Piel *et al.*, 2014), hind limb (paw) withdrawal test (Piel *et al.*, 2014), and vocalizations evoked by extension of the knee (Im *et al.*, 2010). In large animal models, outcome measures such as gait analysis and lameness, are preferred (Kuyinu *et al.*, 2016).

In conclusion, there are numerous ways to test pain in both small and large animal models. Unfortunately, similarly to the fact that no animal model perfectly mimics the complex processes involved in the initiation and progression of OA in humans, no single method of pain assessment perfectly mimics the complex pain processes observed in humans (Piel *et al.*, 2014).

1.2.1.4 Animal Model Summary

One of the main limitations of animal models is the inability to directly translate the findings to humans. However, animal models remain a powerful tool to study disease processes similar to those observed in humans, as long as limitations are acknowledged, and animals are not viewed as representing the human animal species (Quinn, 2005). Differences in anatomy, physiology, and developmental biology must be considered when analyzing results across animal species, including humans.

There is no perfect animal model that mimics human OA in all its facets. Therefore, different animal models combined may provide insights into the human OA phenotype of interest. For example, a pre-clinical PTOA model may contain facets of the post-traumatic human OA phenotype caused by acute or repetitive injury to the joint. A diet-induced metabolic disturbance model may represent aspects of increased joint loading caused by joints from obesity, and local/systemic inflammation associated with the metabolic OA phenotype. The aging OA

phenotype may be studied best through genetically-modified and spontaneous pre-clinical models. Finally, pre-clinical models of OA pain may help understand mechanisms of OA pain in humans and may allow for testing of interventions aimed at pain reduction. Understanding the specific aetiologies of OA phenotypes may help in treatment decisions, such as exercise interventions, drug interventions, or hormone therapies.

We investigated treatment interventions for a metabolic OA phenotype that was induced through exposure to a high-fat/high-sucrose diet in CD-Sprague-Dawley rats. The general question we had was: "Do moderate exercise and dietary fibre supplementation reduce the rate of progression of knee OA in a pre-clinical model of metabolic knee OA?". The rat model proposed by Collins and colleagues demonstrated that exposure to a high-fat/high-sucrose diet leads to damage in the knee (Collins *et al.*, 2015a; Collins *et al.*, 2015c), shoulder (Collins, Hart, Seerattan, Reimer, & Herzog, 2018a), muscles (Collins *et al.*, 2016a), and changes to the gut microbiota, and increased local and systemic inflammation (Collins *et al.*, 2015b). The findings reported in this thesis may help in explaining some of the potential mechanisms underlying the metabolic OA phenotype.

1.3 Osteoarthritis and Metabolic Disturbance

Joints experience a complex set of mechanical loads every day. It has been argued that mechanical loading may all lead to the development of OA (Gabay, Hall, Berenbaum, Henrotin, & Sanchez, 2008). However, joint loading cannot explain the correlation between OA and obesity in non-weight bearing regions/joints such as those of the hand. To date, there is evidence suggesting that obesity is associated with a significant increase in risk of developing OA in weight-bearing joints including the knee (Felson, Anderson, Naimark, Walker, & Meenan, 1988; Felson

& Chaisson, 1997; Hart & Spector, 1993; Manninen, Riihimaki, Heliovaara, & Suomalainen, 2004); and possibly the hip (Felson & Chaisson, 1997), and hand (Bijlsma *et al.*, 2011; Felson & Chaisson, 1997). Moreover, it has been suggested that mechanical loading in weight-bearing joints cannot explain OA damage observed in the non-weight bearing sites of the knee, and that systemic inflammatory mediators have a strong relationship with the damage observed (Collins *et al.*, 2015a; Collins *et al.*, 2015b; Collins *et al.*, 2015c; Griffin *et al.*, 2010; Griffin *et al.*, 2012). Systemic markers, such as adipose factors, so called adipokines, have been suggested to explain metabolic OA in non-weight-bearing regions (Pottie *et al.*, 2006). Therefore, adipokines may offer a metabolic link between obesity and OA (Collins *et al.*, 2015b; Pottie *et al.*, 2006).

OA that is linked to metabolic factors has been called metabolic OA (Zhuo *et al.*, 2012). Metabolic OA may result in the bursting of adipocytes, which then release and promote the release of inflammatory agents, such as macrophages. These macrophages then infiltrate tissues, resulting in a chronic low-level inflammatory profile that is associated with metabolic disease (Hotamisligil, 2006). And inflammation has been implicated in the onset and the rate of development of OA (Hotamisligil, 2006; Lumeng *et al.*, 2009; Zhuo *et al.*, 2012).

The discovery of leptin (an adipokine) was a key breakthrough that furthered our understanding of the importance of adipose tissue (Zhang *et al.*, 1994). Among the adipokines, adiponectin and resistin are associated with leptin and play key roles in the regulation of the action of insulin, inflammation, homeostasis, and other pathologic events. In spite of the fact that relatively little is known about their contribution to the development of OA, leptin, resistin and adiponectin are detected in the synovial fluid of osteoarthritic joints (Conde *et al.*, 2011; Pottie *et al.*, 2006; Simopoulou *et al.*, 2007). Leptin is found in osteophytes and in cartilage of osteoarthritic joints and may have biological activity on chondrocytes (Pottie *et al.*, 2006). It has been suggested

that adipokines may have an active role in the pathogenesis of chronic inflammatory joint diseases, such as rheumatoid arthritis (Pottie *et al.*, 2006). Moreover, the expression of growth factors, stimulates proteoglycan and collagen synthesis, and increases the stimulatory effects of pro-inflammatory cytokines on nitric oxide production in chondrocytes (Aspden, Scheven, & Hutchison, 2001). Additionally, genetic models of metabolic disturbance, using leptin knockout and leptin receptor knockout models, indicate that mice deficient in leptin that are fed a high fat diet do not develop OA (Griffin, Huebner, Kraus, & Guilak, 2009). Additionally, it has been shown that another adipokine, visfatin, is expressed in OA cartilage, and might play a pro-inflammatory role in OA (Gosset *et al.*, 2008).

Intestinal permeability may also play a role in metabolic OA (Szychlinska, Di Rosa, Castorina, Mobasheri, & Musumeci, 2019). It has been demonstrated that a gut microbiota–nutrient interaction increases acetate production in rodents on a high-fat diet, leading to activation of the parasympathetic nervous system, which in turn promotes increased glucose-stimulated insulin secretion, ghrelin secretion, hyperphagia and obesity (Perry *et al.*, 2016). Additionally, the low-grade levels of chronic inflammation observed in metabolic OA may result from the absorption of endotoxin across the intestinal tract (Brun *et al.*, 2007; Collins *et al.*, 2015b; Creely *et al.*, 2007; Harte *et al.*, 2010; Metcalfe *et al.*, 2012), which has been positively correlated with obesity (Cani *et al.*, 2007a; Cluny *et al.*, 2015; Ley *et al.*, 2005) and type 2 diabetes (Harte *et al.*, 2012; Pussinen, Havulinna, Lehto, Sundvall, & Salomaa, 2011). Griffin et al. (2012) reported that OA-inducing effects of a high-fat diet in mouse models were correlated with increased levels of inflammatory cytokines. Additionally, rats fed a high-fat/high-sucrose diet also had increased levels of inflammatory cytokines, which the authors related to endotoxins (Collins *et al.*, 2015b).

Therefore, endotoxins may be a contributing factor to the onset and progression of OA in patients with obesity, and modulating inflammatory levels may be a strategy to prevent and treat OA.

1.4 Inflammation

Inflammation has been described as the principal response of the body to cope with injuries. It includes the five cardinal signs: swelling (*tumor*); redness (*rubor*); pain (*dolor*); fever (*calor*) (Larsen & Henson, 1983); which together lead to loss of function (*laesa*) (Rather, 1971). Inflammation is a beneficial reaction to injury, as it allows for the rapid transport of immune cells to the site of injury, promoting repair (Larsen & Henson, 1983; Liew, 2003). However, the long-term consequences of inflammation are not always beneficial (Liew, 2003) and may lead to metabolic diseases (Hotamisligil, 2006), rheumatoid arthritis (Larsen & Henson, 1983), and osteoarthritis (Edd *et al.*, 2015).

Inflammation can be classified as acute or chronic (Table 1.5). Acute inflammation is characterized by vasodilatation, fluid and plasma protein exudation, and an accumulation of predominantly neutrophilic leukocytes (Liew, 2003). The onset of acute inflammation is rapid and usually of short duration – a few minutes to a few days (Kumar, Abbas, Aster, & Robbins, 2013; Splettstoesser & Schuff-Werner, 2002). In contrast, chronic inflammation is often more insidious, has a longer duration than acute inflammation (days to years) (Kumar *et al.*, 2013), and is characterized by infiltration of mononuclear cells and fibrotic tissue (Davies, Wicks, Powell, Puddicombe, & Holgate, 2003).

Table 1.5 Features of acute and chronic inflammation (Kumar *et al.*, 2013). Used under Fair Dealing Policy.

Feature	Acute	Chronic
Onset	Fast: minutes or hours	Slow: days
Cellular infiltrate	Mainly neutrophils	Monocytes/macrophages and lymphocytes
Tissue injury, fibrosis	Usually mild and self-limited	Often severe and progressive
Local and systemic signs	Prominent	Less prominent; may be subtle

In general, acute inflammatory reactions are triggered by infections (bacterial, viral, fungal, parasitic), trauma (blunt or penetrating), physical or chemical agents (e.g., thermal injury, such as burns or frostbite; irradiation; toxicity from environmental chemicals), tissue necrosis, foreign bodies (splinters, dirt, sutures, crystal deposits), or immune reactions (also called hypersensitivity reactions), which are evoked to counter threats from either the environment or the body's own tissues (Kumar *et al.*, 2013; Sherwood & Toliver-Kinsky, 2004). As the stimuli for an inflammatory response of the immune system often cannot be avoided, such reactions tend to persist, and the features of chronic inflammation may present (Kumar *et al.*, 2013; Lawrence & Gilroy, 2007).

1.4.1 Inflammation and Osteoarthritis

Osteoarthritis has primarily been characterized as a degenerative disorder of articular cartilage in which chondrocytes respond to biomechanical loading and biologic stress in a way that results in breakdown of the extracellular matrix (Berenbaum, 2013; Goldring & Otero, 2011; Kapoor, Martel-Pelletier, Lajeunesse, Pelletier, & Fahmi, 2011; Kumar *et al.*, 2013; Loeser *et al.*, 2012). Recent progress in molecular biology has modified how OA is characterized (Felson, 2006; Kapoor *et al.*, 2011). An inflammatory theory has been suggested following the discovery that

many mediators such as cytokines and prostaglandins may increase the production of matrix metalloproteinases (MMPs) by chondrocytes. Some studies have reported that inflammation is an important driver of the OA process. Recent experimental data have shown that subchondral bone may have a substantial role as a mechanical damper (shock absorber) in the OA process, in addition to being a possible source of inflammatory mediators which have been implicated in pain and the degradation of the deep layer of cartilage (Berenbaum, 2013). Additionally, it has been suggested that the source and type of inflammatory mediators may differ by OA phenotype (Bijlsma *et al.*, 2011). Therefore, the role of inflammation in OA will be discussed in the context of the following phenotypes: post-traumatic OA (PTOA), aging OA, and metabolic OA.

1.4.1.1 Inflammation and post-traumatic osteoarthritis

Post-traumatic OA often leads to synovial inflammation (synovitis), which is associated with increased pain and dysfunction (Scanzello *et al.*, 2011). Synovitis can be characterized by several features including: clinical (Ayral, 1999; Krasnokutsky, Attur, Palmer, Samuels, & Abramson, 2008); imaging (Ayral, Pickering, Woodworth, Mackillop, & Dougados, 2005; Ayral *et al.*, 1999; D'Agostino *et al.*, 2005; Fernandez-Madrid *et al.*, 1995; Keen *et al.*, 2008; Loeuille *et al.*, 2005; Ostergaard *et al.*, 1997); histological (Alsalameh *et al.*, 1990; Ayral *et al.*, 2005; Myers *et al.*, 1990a; Nakamura, Yoshino, Kato, Tsuruha, & Nishioka, 1999; Shibakawa *et al.*, 2003; Walsh *et al.*, 2007); molecular (Benito, Veale, FitzGerald, van den Berg, & Bresnihan, 2005; Brentano *et al.*, 2007; Farahat, Yanni, Poston, & Panayi, 1993; Furuzawa-Carballeda & Alcocer-Varela, 1999; Niissalo, Hukkanen, Imai, Tornwall, & Konttinen, 2002; Presle *et al.*, 2006; Raychaudhuri & Raychaudhuri, 2009; Scanzello *et al.*, 2009; Shibakawa *et al.*, 2003; Smith, Triantafillou, Parker, Youssef, & Coleman, 1997; Yuan *et al.*, 2004), and biological biomarkers

(Conrozier *et al.*, 2000; Masuhara, Nakai, Yamaguchi, Yamasaki, & Sasaguri, 2002; Pearle *et al.*, 2007), as described in Table 1.6. The underlying mechanisms describing why synovia become inflamed in OA remains controversial (Sellam & Berenbaum, 2010). The most widely accepted hypothesis suggests that once degraded, cartilage fragments are released into the joint and contact the synovium. The fragments are then considered foreign bodies, synovial cells react by producing inflammatory mediators, leading to synovial angiogenesis and an increase in the synthesis of inflammatory cytokines and MMPs by synovial cells, thus perpetuating the cartilage degradation (Berenbaum, 2013; Sellam & Berenbaum, 2010). A more recent theory suggests that immunological cells may play a role in the initiation of synovial inflammation (Hussein *et al.*, 2008). Hussein and colleagues (2008) reported that cytokine levels correlated positively with pathological OA features. They also suggested that these immunologic changes may have prognostic potential for the detection and monitoring of OA. Synovial inflammation may lead to synovial angiogenesis mediated by macrophage activation, which, in turn, has been linked to OA pain (Haywood *et al.*, 2003; Mapp & Walsh, 2012). Furthermore, molecular markers for dendritic cells were detected in lymphoid aggregations and perivenular infiltration areas in the synovium in a PTOA rabbit model (Xiaoqiang *et al.*, 2012). Large numbers of dendritic cells were observed in the synovium in the early stages (2 or 4 weeks) after surgical induced OA, and the number of dendritic cells increased with the progression of inflammatory markers (IL-1β and TNF- α) in the synovium between weeks 2-4 following surgical intervention (Xiaoqiang *et al.*, 2012). Furthermore, inflammation of the synovium at early time points following anterior cruciate ligament reconstruction surgery in animal models have been reported (Heard, Achari, Chung, Shrive, & Frank, 2011; Heard *et al.*, 2013; Huebner, Shrive, & Frank, 2013). Upregulated mRNA levels for degradative proteinases in the cartilage at 2 weeks post surgical induced OA have also

been reported (Heard *et al.*, 2011), suggesting that inflammation may play a role not only in the progression, but also in the initiation of PTOA.

Beyond synovitis, joint injuries may set off an acute inflammatory response that includes pro and anti-inflammatory cytokines, as well as innate pathways. Early anti-inflammatory factors (such as IL-10 and IL-1RA) may allow for resolution of inflammation and reduce the risk of progressing to PTOA. The perpetuation of inflammation via continued activation of innate inflammatory pathways may promote progression to PTOA in an injured joint. A number of factors may perpetuate inflammation after joint injury. These include the severity of the initial injury, the balance of pro and anti-inflammatory factors produced in the acute and subacute post-injury periods, and repeated macro- or micro-injury which promote cycles of inflammatory activation involving innate pathway (Lieberthal, Sambamurthy, & Scanzello, 2015).

1.4.1.2 Inflammation and aging osteoarthritis

An increase in both mean life span and life expectancy, in combination with a reduction in early mortality, has led to a large increase in the number of elderly people in modern societies, which has been paralleled by a dramatic increase in chronic diseases, which are often associated with advancing age (Vasto & Caruso, 2004), such as OA (Loeser, 2011). Aging is accompanied by an upregulation of inflammatory responses due to chronic antigenic stresses that challenge the immune system throughout life, and potentially trigger the onset of inflammatory diseases (Franceschi *et al.*, 2000).

Table 1.6. Evidence of the role of synovitis in OA. Adapted from Sellam & Berenbaum (2010). Used with permission.

Features	Observation
Clinical	Effusion, joint swelling, or palpable synovitis Local signs of inflammation Sudden increase in pain Night pain and morning stiffness
Imaging	Gadolinium-enhanced synovium and increased synovial volume detected by MRI Correlation between MRI and histological observations Synovitis seen using ultrasonography of symptomatic joints Association between ultrasound-detected synovitis and clinical symptoms of synovitis Macroscopic synovial changes detected by arthroscopy in about half of patients with knee OA Arthroscopic synovitis associated with progression of knee OA
Histological	Synovial hypertrophy and hyperplasia Infiltration of mononuclear cells (monocytes/macrophages, activated B cells and T cells) Adaptive immune T-cell and B-cell responses to fragments of extracellular matrix Increased angiogenesis Synovitis in the vicinity of degenerative cartilage
Molecular	Production and/or release of proinflammatory cytokines (TNF, IL-1β, IL-6, IL-8, IL-15, IL-17, IL-21) Increased production of PGE2 and nitric oxide Increased expression of adhesion molecules (ICAM-1, VCAM-1) in the synovium Increased activity of MMPs (MMP-1, MMP-3, MMP-9, MMP-13) and ADAMTS Production of adipokines (visfatin, leptin, adiponectin) Release of EGF and VEGF Involvement of macrophages in osteophyte formation via BMPs Insufficient release of anti-inflammatory cytokines (IL-4, IL-10, IL-13, IL-1Ra) Release of proinflammatory and pain neurotransmitters (substance P, NGF)
Biological markers	Increased levels of CRP (detected by ultrasensitive assay) Increased levels of MMP-3 and MMP-9 in synovial cells of patients with rapidly destructive hip arthropathy Other potential surrogate biomarkers of inflammation include CGP-39 and fragments of type II collagen and aggrecan

ADAMTS: a disintegrin and metalloproteinase with thrombospondin motifs; BMP: bone morphogenetic protein; CGP-39: cartilage glycoprotein-39 (also known as chitinase-3-like protein 1 and YKL-40); CRP: C reactive protein; EGF: endothelial growth factor; ICAM-1: intercellular adhesion molecule 1; IL: interleukin; IL-1Ra: interleukin 1 receptor antagonist; MMP: matrix metalloproteinase; NGF: nerve growth factor; PGE2: prostaglandin E2; TNF: tumor necrosis factor; VCAM-1: vascular cell adhesion molecule 1; VEGF: vascular endothelial growth factor.

Although OA is considered to be an age-related disease, the mechanisms underlying age-related OA onset and progression remain largely unknown (Berenbaum, 2013; Loeser, 2011). At the cellular level, senescence can be divided into two main categories: replicative and secretory. Throughout life, normal growth and repair is achieved by cell replication. However, human cells have a limited proliferative capacity. After a period of vigorous proliferation in vitro, the rate of cell division declines, a process known as replicative senescence. Furthermore, some cell types (such as chondrocytes) have a low capacity to divide, leaving little room for replicative senescence. Nevertheless, cells with a low capacity to divide have a high capacity to synthesize soluble mediators, and thus, secretory senescence may be prevalent with aging. Secretory senescence has been called the senescence-associated secretory phenotype (SASP) and includes several inflammatory and pro-degradative mediators driven by oxidative stress (Coppe, Desprez, Krtolica, & Campisi, 2010). Moreover, SASP is primarily a delayed response to epigenomic damage (Campisi, Andersen, Kapahi, & Melov, 2011). Indeed, IL-1β-stimulated MMP-13 chondrocyte production increases with age, suggesting that aging chondrocytes acquire a SASP (Forsyth *et al.*, 2005).

Another theory gaining traction in the literature which relates inflammation, aging and OA, is based on the discovery of advanced glycation end-products (AGEs). AGEs are produced by a non-enzymatic process in aging tissues and weaken cartilage by modifying its mechanical properties. Additionally, AGEs can trigger chondrocyte activation by binding to receptors for AGE (RAGE) which are present at the surface of chondrocytes, leading to an overproduction of proinflammatory cytokines and MMPs (Lotz & Loeser, 2012; Rasheed, Akhtar, & Haqqi, 2011).

1.4.1.3 Inflammation and metabolic osteoarthritis

It is widely known that societies in which the typical diet is rich in fat and sugar are facing a health care crisis of epidemic proportions, due in part to the fact that the number of people with metabolic syndrome is rising drastically (O'Neill & O'Driscoll, 2015). Metabolic syndrome constitutes a challenge for preventive medicine. For example, in 2005 40% of U.S. adults were classified as having metabolic syndrome (Ford, 2005a) with this number expected to increase in the future (O'Neill & O'Driscoll, 2015).

Metabolic syndrome is diagnosed by the presence of any three of the following five features: (1) insulin resistance, fasting glucose equal or higher than 5.6 mmol/l; (2) obesity, waist circumference higher than 94 cm for men and higher than 80 cm for women; (3) dyslipidemia - TG, triglycerides higher than 1.7 mmol/l; (4) dyslipidemia -HDL-C, HDL cholesterol lower than 1.03mmol/l for men and lower than 1.29 mmol/l for women; and (5) hypertension, systolic and diastolic blood pressure higher than 130 and 85 mmHg, respectively (Grundy *et al.*, 2005). Furthermore, metabolic syndrome has been reported to be related to a low-grade, systemic, inflammatory condition (Das, 2004; Kereiakes & Willerson, 2003; Tracy, 2003). Specifically, inflammation is associated with elevated plasma levels of C-reactive protein (CRP), IL-6, and tumor necrosis factor-alpha (TNF-α) (Das, 2004), generating a low-grade systemic inflammation that has been linked to OA (Zhuo *et al.*, 2012).

Insulin resistance is a key feature in the pathogenesis of type 2 diabetes and may precede the onset of hyperglycaemia and subsequent clinical manifestation of type 2 diabetes by 10–20 years. Recent data suggest that a defect in insulin activation, and subsequent glucose transportation into muscle cells, could be induced by serine kinase cascade activation, which is a down-stream mediator of tissue inflammation factors (Perseghin, Petersen, & Shulman, 2003). It has been

suggested that hyperglycaemia may be an independent risk factor for the development of OA (Cimmino & Cutolo, 1990; Hart, Doyle, & Spector, 1995b; Schett *et al.*, 2009). In vitro and in vivo experimental studies have linked hyperglycaemia to local and systemic toxicities relevant to OA, caused by high glucose concentration (Berenbaum, 2011). At the joint, hyperglycaemia induces deleterious effects on cartilage that are mediated by oxidative stress and AGEs, leading to matrix stiffness, subchondral bone destruction, and chondrocyte dysfunction. Specifically, hyperglycaemia decreases the transport of dehydroascorbate into chondrocytes, compromising the synthesis of type II collagen, and increasing levels of reactive oxygen species (ROS) and inflammatory mediators, which results in cartilage destruction (Henrotin, Bruckner, & Pujol, 2003; Hiraiwa *et al.*, 2011; McNulty, Stabler, Vail, McDaniel, & Kraus, 2005; Nah *et al.*, 2008; Nah *et al.*, 2007). AGEs can also bind to their receptor, RAGE, on chondrocyte membranes to trigger the overexpression of proinflammatory and pro-degradative mediators (Loeser *et al.*, 2005; Rasheed *et al.*, 2011; Steenvoorden *et al.*, 2006; Yammani, Carlson, Bresnick, & Loeser, 2006).

Furthermore, motor and sensory dysfunction in muscle, caused by glucose accumulation, may be a systemic factor in the pathogenesis of hyperglycaemia-induced OA (Hurley, 1999). It has been reported that patients with OA often have muscle weaknesses, loss of vibratory sense in the symptomatic joint (Slemenda *et al.*, 1997), and joint laxity, which may be related to alterations in the peripheral nervous system caused by diabetic neuropathy (Shakoor, Lee, Fogg, & Block, 2008).

Obesity is associated with an increased accumulation of adipose tissue, resulting in the increased expression of TNF-α by adipocytes (Hotamisligil, Shargill, & Spiegelman, 1993). TNF-α expression has been reported to decrease after weight loss (Kern *et al.*, 1995). Additionally, adipose tissue releases IL-6, CRP (Mohamed-Ali *et al.*, 1997; Yudkin, Stehouwer, Emeis, &

Coppack, 1999), and plasminogen activator inhibitor-1 (PAI-1) (Lundgren, Brown, Nordt, Sobel, & Fujii, 1996), which all play an important role in low level, chronic inflammation. Furthermore, it has been reported that adipose tissue releases a number of adipokines, including leptin, adiponectin, visfatin and resistin (Gomez, Lago, Gomez-Reino, Dieguez, & Gualillo, 2009). It has been suggested that leptin plays a key role in the pathophysiology of OA development (Aspden, 2011; Dumond *et al.*, 2003; Pottie *et al.*, 2006).

Leptin may exhibit biological activity on chondrocytes by inducing the expression of a series of proinflammatory factors such as nitric oxide synthase (iNOS), insulin-like growth factor 1 (IGF-1), transforming growth factor β (TGF-β) and IL-8, in addition to degenerative enzymes (MMP9–13) (Faggioni, Feingold, & Grunfeld, 2001; Iliopoulos, Malizos, & Tsezou, 2007; Otero, Gomez Reino, & Gualillo, 2003; Sanna *et al.*, 2003). Leptin also induces the production of IL-8 in synovial fibroblasts via a mechanism involving activation of the leptin receptor and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) (Gomez *et al.*, 2011). Additionally, there is an abnormal increase in the production of leptin by OA subchondral osteoblasts, which is involved in altering the secretion of alkaline phosphatase, TGF-β, osteocalcin and type I collagen (Mutabaruka, Aoulad Aissa, Delalandre, Lavigne, & Lajeunesse, 2010). Furthermore, an increased local leptin concentration may directly influence osteoblast proliferation and differentiation, whereas a high systemic leptin level may indirectly suppress bone formation through the hypothalamic relay (Ducy *et al.*, 2000; Motyl & Rosen, 2012). The role of adiponectin, visfatin, and resistin in the pathogenesis of OA remain unclear.

The exact mechanisms linking dyslipidemia to OA are unknown, and a number of hypotheses have been described. It has been suggested that lipid deposition in the joint may trigger the pathophysiological process of OA development due to substantial stores of lipid deposits in

osteoarthritic cartilage, especially in chondrocytes, at the early stages of the disease, prior to histological changes becoming apparent (Gkretsi, Simopoulou, & Tsezou, 2011). Additionally, a substantial proportion of proteins related to lipid metabolism, such as peroxisome proliferator-activated receptors (PPAR), and apolipoproteins, which are differentially expressed in osteoarthritic tissue, have been demonstrated in osteoarthritic cartilage and isolated chondrocytes (Gobezie *et al.*, 2007; Iliopoulos, Malizos, Oikonomou, & Tsezou, 2008; Ruiz-Romero, Lopez-Armada, & Blanco, 2005; Wu *et al.*, 2007). Furthermore, lectin-like oxidized low-density lipoprotein receptor 1 (LOX-1) is expressed in cartilage in weight-bearing and non-weight-bearing regions of patients with OA, but it is not expressed in healthy cartilage (Simopoulou *et al.*, 2007). The presence of LOX-1 in cartilage indicates that chondrocytes are capable of internalizing lipids (Simopoulou *et al.*, 2007). Additionally, there is a deregulation of the expression of cholesterol influx genes in human osteoarthritic chondrocytes (Collins-Racie *et al.*, 2009; Tsezou, Iliopoulos, Malizos, & Simopoulou, 2010). Finally, dyslipidemia in patients with OA may modify the biosynthetic activity of mesenchymal precursor cells, disrupting the homeostasis between adipogenesis, osteogenesis, and chondrogenesis (Aspden *et al.*, 2001).

There are several possible explanations for the link between OA and hypertension (Conaghan, Vanharanta, & Dieppe, 2005; Engstrom, Gerhardsson de Verdier, Rollof, Nilsson, & Lohmander, 2009; Marks & Allegrante, 2002; Puenpatom & Victor, 2009). Firstly, it is possible that the presence of OA decreases the activity level of a person due to the exacerbation of pain with movement, thus increasing the risk of cardiovascular disease as a consequence of OA (Zhuo *et al.*, 2012). Secondly, the commonality of obesity (highlighted by an increased BMI) between OA and hypertension may be responsible (Brown *et al.*, 2000; Zheng & Chen, 2015; Zhuo *et al.*, 2012). Finally, and possibly the most plausible explanation, is the subchondral ischaemia theory

(Felmeden *et al.*, 2003; Karter *et al.*, 2004; Kiefer *et al.*, 2003). The subchondral ischaemia theory suggests that hypertension-induced narrowing of blood vessels over time reduces blood flow, especially of small subchondral vessels in the epiphysis of long bones. Furthermore, occlusion of the vessel lumen may result in reduced blood flow and subsequent stasis, which may in turn lead to the development of micro-emboli in subchondral vessels (Ghosh & Cheras, 2001). Subchondral ischaemia may also compromise nutrient and gas exchange between the articular cartilage and bone, initiating degradative changes in the cartilage (Imhof *et al.*, 2000). It has also been suggested that apoptosis of osteocytes in the ischaemic regions of subchondral bone may trigger remodeling, specifically due to osteoclastic activity, thus reducing bone support for the overlying cartilage (Berger, Kroner, Minai-Pour, Ogris, & Engel, 2003; Kerachian *et al.*, 2011; Shibahara *et al.*, 2000).

Furthermore, intestinal permeability may play a role in metabolic OA. It has been suggested that low-grade chronic inflammation in metabolic OA can result from the absorption of endotoxin across the intestinal tract (Brun *et al.*, 2007; Collins *et al.*, 2015b; Creely *et al.*, 2007; Harte *et al.*, 2010; Metcalfe *et al.*, 2012). Additionally, endotoxin absorption has been positively correlated with obesity (Cani *et al.*, 2007a; Cluny *et al.*, 2015; Ley *et al.*, 2005). Griffin et al. (2012) described the OA-inducing effects of a high-fat diet in mouse models, with concomitantly increased levels of inflammatory cytokines. Additionally, rats fed with high-fat/high-sucrose diet also had increased levels of inflammatory cytokines, which the authors related to the endotoxin (Collins *et al.*, 2015b). Therefore, endotoxin may be a contributing factor to the onset and progression of OA in patients with obesity.

In addition, there is evidence suggesting that Bifidobacterium spp. reduces plasma lipopolysaccharide (LPS) and inflammatory markers besides improving tight junction integrity in

murine models (Cani *et al.*, 2007a). In humans, endotoxin levels are increased in sedentary males relative to those performing regular high intensity exercise (Lira *et al.*, 2010). Additionally, there is a positive correlation between endotoxin and plasminogen activator inhibitor type-1 (PAI-1), endotoxin and total cholesterol, endotoxin and LDL cholesterol, and endotoxin and triglycerides levels (Lira *et al.*, 2010).

It has been reported that endotoxemia in obesity may be associated with diet, which may in turn impair intestinal barrier function through effects on intestinal flora or motility. Specifically, colonization by a greater proportion of LPS-containing bacteria occurs in the intestines of both mice fed on a high-fat diet (Cani *et al.*, 2007a), and rats fed with a high-fat/high-sucrose diet (Collins *et al.*, 2015b). Additionally, endotoxemia may potentially be mediated by both the hyperglycaemic and hyperinsulinemia states (Cuoco *et al.*, 2002). Hyperglycaemia increases gut mucosal permeability in LPS-treated rats independent of the plasma insulin concentration. Insulin may also act directly on the intestine increasing gut absorption (Pussinen *et al.*, 2011).

In summary, obesity is associated with insulin resistance, which is associated with intestinal mucosae dysfunction (Caballero *et al.*, 2008; Shi *et al.*, 2006), resulting in the translocation of LPS into the circulation, as well as endotoxemia and increased serum titres of proinflammatory cytokines (Brun *et al.*, 2007; Creely *et al.*, 2007; Harte *et al.*, 2012). Finally, proinflammatory cytokines have a detrimental effect on articular cartilage and thus may cause, precipitate, or promote knee OA in patients with obesity (Metcalfe *et al.*, 2012).

1.5 Prebiotic Fibre

Dietary fibre is defined as “nondigestible carbohydrates and lignin that are intrinsic and intact in plants” (Trumbo *et al.*, 2002). While prebiotic fibre is defined as “a substrate that is

selectively utilized by host microorganisms conferring a health benefit" (Gibson *et al.*, 2017). It is important to state that not all fibre is a prebiotic, although, all prebiotics are fibre (Slavin, 2013) – see Table 1.7 for the evolution of prebiotic definitions. For a food ingredient to be identified as a prebiotic, it is required to: 1) resist hydrolysis and absorption in the upper gastrointestinal tract; 2) be fermented by the intestinal microflora; 3) selectively stimulates the growth and/or activity of intestinal bacteria potentially associated with health and well-being; and 4) induce either luminal or systemic effects that are beneficial to the host health (Gibson & Roberfroid, 1995).

Table 1.7. Evolution of the prebiotic definition from 1995 until 2017. Used with permission.

Year	Definition	Reference
1995	A nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health.	Gibson, and Roberfroid (1995)
2003	Nondigestible substances that provide a beneficial physiologic effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria.	Reid, Sanders, and Gaskins (2003)
2004	A selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora that confers benefits upon host well-being and health.	Gibson et al. (2004)
2007	A selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microflora, that confer benefits upon host well-being and health.	Roberfroid (2007)
2008	A nonviable food component that confers a health benefit on the host associated with the modulation of the microbiota.	Pineiro et al. (2008)
2010	A selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefits upon host health.	Gibson et al. (2010)
2015	A nondigestible compound that, through its metabolization by microorganisms in the gut, modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiologic effect on the host.	Bindels et al. (2015)
2017	A substrate that is selectively utilized by host microorganisms conferring a health benefit.	Gibson et al. (2017)

Some examples of the health benefits of prebiotics include a reduction in the prevalence and duration of infectious and antibiotic-associated diarrhea; a reduction in the inflammation and

symptoms associated with inflammatory bowel disease; a protection against colon cancer; an enhancement of the bioavailability and uptake of minerals, including calcium, magnesium, and possibly iron; lowering some risk factors for cardiovascular disease; the promotion of satiety, weight loss, and the prevention of obesity (Carlson, Erickson, Lloyd, & Slavin, 2018).

Prebiotics occur naturally in foods, such as artichokes-globe (content: 2.5-9.5% of fresh weight), asparagus, banana (0.3-0.7% of fresh weight), barley (0.5-1.5% of fresh weight), chicory (15-20% of fresh weight), dandelions (12-15% of fresh weight), garlic (9-16% of fresh weight), Jerusalem artichokes (16-20% of fresh weight), leeks (3-10% of fresh weight), onions (1.1-7.5% of fresh weight), rye-flour (0.5-1% of fresh weight), and wheat (1-4% of fresh weight) (van Loo, Coussement, de Leenheer, Hoebregs, & Smits, 1995).

There are various sources/categories of prebiotic fibre, with different health benefits (Gibson *et al.*, 2017). The eight categories of prebiotic fibre that have sufficient evidence in promoting digestive health are: 1) Beta-glucans; 2) Fructan compounds (fructooligosaccharides, oligofructose, and inulin); 3) Galactooligosaccharides; 4) Isomaltooligosaccharides; 5) Guar gum; 6) Lactulose; 7) Resistant starch and maltodextrin; and 8) Xylooligosaccharides and arabinooligosaccharides (Gibson *et al.*, 2017). For the purpose of this thesis we will focus on the fructans, more specifically, oligofructose.

1.5.1 Fructan: Oligofructose

Fructan compounds are found in a wide range of structures. For example, inulin typically has a degree of polymerization of between 3 and 60 fructan monomers (long-chain fructan), while oligofructose is yielding a product with a degree of polymerization of 2–20 fructan monomers (short-chain fructan). All of these compounds exert strong bifidogenic effects, although the length

of polymerization influences these effects (Carlson *et al.*, 2018; van de Wiele, Boon, Possemiers, Jacobs, & Verstraete, 2007).

It has been shown that oligofructose significantly increases calcium absorption (Abrams *et al.*, 2005; Griffin, Davila, & Abrams, 2002); promotes selective microbiota change (e.g.: Bifidobacterium spp), thus improving gut barrier functions, reduces plasma LPS, reduces inflammation; and promotes satiety in humans (Cani, Joly, Horsmans, & Delzenne, 2006; Dehghan, Pourghassem Gargari, & Asghari Jafar-abadi, 2014; Knaapen *et al.*, 2013; Nakamura & Omaye, 2012). In rodents, oligofructose reduces diet-induced obesity, diabetes, hepatic steatosis by mechanisms linked with changes in specific gut microorganisms and metagenomic functions of bacteria (Everard *et al.*, 2014); and it has also been suggested that oligofructose might normalize the metabolomic signature of insulin resistant rats and reduce obesity in offspring (Paul, Bomhof, Vogel, & Reimer, 2016).

1.5.2 Osteoarthritis, Dysbiosis, and Prebiotic Fibre

Osteoarthritis is a disease with a multifactorial etiology. Regarding the metabolic OA phenotype, there is evidence suggesting that chronic low-grade systemic inflammation links the metabolic abnormalities to OA onset (Collins *et al.*, 2015b; Schott *et al.*, 2018). Gut microbial dysbiosis (impaired gut microbiota) may be such a link, which in addition to OA (Szychlinska *et al.*, 2019), has also been associated with the onset of inflammatory bowel syndrome (Wlodarska, Kostic, & Xavier, 2015), obesity (Lee & Hase, 2014), cancer (Yoshimoto *et al.*, 2013), and rheumatoid arthritis (Liao, Alfredsson, & Karlson, 2009). However, the exact mechanism and the contribution of intestinal microbiota metabolites to most of these diseases remain to be elucidated.

There are several hypotheses linking dysbiosis to OA pathogenesis, including aging, diet, obesity, and sex (Szychlinska *et al.*, 2019). It has been demonstrated that old people have significantly greater proportions of Bacteroides, and distinct abundance pattern of Clostridium groups compared with young people (Claesson *et al.*, 2011; O'Toole & Jeffery, 2015). Additionally, the gut microbiota is characterized by a reduced bacterial diversity, and decline in those beneficial ones with advanced age (Nagpal *et al.*, 2018; Salazar *et al.*, 2014). These variations within the gut microbiome with aging may regulate some aspects of age-related physiology, such as immune responses, metabolic alterations, and organ disorders (Nagpal *et al.*, 2018).

Quality and quantity of nutrient intake have been reported to be involved in the pathogenesis of OA (Szychlinska *et al.*, 2019). It has been shown that dietary nutrients alter the microenvironment of the gut microbiome and affect its digestion capacity, structure and immunological reactions, suggesting that nutrients affect host physiological functions depending on the gut microbiome (Asano, Yoshimura, & Nakane, 2013; Tan, Liu, Guo, Applegate, & Eicher, 2014). Therefore, modification in nutrient intake (e.g., oligofructose supplementation), and associated changes to the gut microbiome, have been used as a strategy to prevent OA in rodent models (Rios *et al.*, 2019a; Schott *et al.*, 2018). Obesity is clearly linked to disturbances in nutrient intake and as a result, dietary modulation may provide a first line of defense in managing obesity and its associated comorbidities (Chaudhri, Salem, Murphy, & Bloom, 2008), such as inflammation.

The prevalence and severity of OA is higher in women than in men, and this disparity between men and women increases with menopause (Hanna, Wluka, Bell, Davis, & Cicuttini, 2004; Musumeci *et al.*, 2015), suggesting that hormonal factors, such as high estrogen levels, may produce differences in cartilage volume and bone/muscle strength (Szychlinska *et al.*, 2019). Not

surprising, sex is an important variable affecting the gut microbiota (Kim, Unno, Kim, & Park, 2019). It is known that the bacterial diversity differs significantly between males and females after puberty (Yurkovetskiy *et al.*, 2013), but the reason for these sex-related differences, and how they may affect OA onset and progression, remain unknown.

Since dysbiosis seems to play an important role in OA pathogenesis, prebiotic fibre might be a good candidate for metabolic OA management. Research using prospective cohorts has shown that a diet rich in fibre lowers the risks of moderate or severe knee pain regardless of radiographic knee osteoarthritis status (Dai, Niu, Zhang, Jacques, & Felson, 2017). Moreover, there is evidence suggesting that oligofructose supplementation prevents the development of OA in rodent models of metabolic OA (Rios *et al.*, 2019a; Schott *et al.*, 2018).

1.6 Osteoarthritis and Exercise

It is known that exercise reduces body fat, vascular inflammation (Arjunan *et al.*, 2015; Pinto *et al.*, 2012), brain inflammation (Chennaoui *et al.*, 2015), and systemic inflammation (Conti *et al.*, 2015; Pedersen, 2006). However, the link between OA and exercise is controversial. (Cheng *et al.*, 2000; Marti, Knobloch, Tschopp, Jucker, & Howald, 1989; McDermott & Freyne, 1983). In a prospective survey examining the relationship of self-reported physical activity and physician-diagnosed OA from 1970 to 1995, an association between OA and physical activity was found. For example, running more than 20 miles per week in men younger than 50 years led to high hazard ratios for the potential risk factors to OA (Cheng *et al.*, 2000). On the other hand, other studies have refuted such a link (Chakravarty, Hubert, Lingala, Zatarain, & Fries, 2008; Lane, Oehlert, Bloch, & Fries, 1998; Miller, Edwards, Brandon, Morton, & Deluzio, 2014; Panush *et al.*, 1986). A longitudinal, 18 year study in-long-distance masters runners and non-running control

subjects showed that knee OA was not associated with long-distance running (Chakravarty *et al.*, 2008). Furthermore, when compared to walking, running does not increase the risk of knee OA development (Miller *et al.*, 2014). Griffin and Guilak (2012) demonstrated that wheel running exercise has a protective effect in knee joints of mice fed a high-fat diet for four weeks. Moreover, exercise in mice improved glucose tolerance and disrupted the co-expression of pro-inflammatory cytokines, suggesting that aerobic exercise may promote joint health independent of weight loss. If exercise indeed does not lead to OA, and reduces inflammation, it may be an effective intervention for the prevention and treatment of metabolic OA.

Aerobic exercise has also been shown to be an effective treatment for individuals with knee OA, improving pain and function (Messier, 2010; Roddy, Zhang, & Doherty, 2005). For example, overweight and obese older individuals with knee OA that received exercise combined with a dietary intervention (aimed to achieve and maintain a 5% body weight reduction) over 18 months had significant improvements in knee pain and function, which were partly mediated by changes in inflammatory markers, such as IL-6, TNF-a, IL-1sR and CRP (Runhaar *et al.*, 2019). Furthermore, exercise has also been shown to improve knee function within the first 6 months of intervention, and then maintained these improvements for an additional 12 months (Messier *et al.*, 2004). Thus, exercise seems to be a possible intervention for the prevention and treatment of knee OA in specific circumstances.

1.7 Thesis Purpose

The purpose of this thesis was to determine the effect of prebiotic fibre supplementation and treadmill exercise on the onset and rate of progression of knee OA in a diet-induced model of metabolic disturbance. Therefore, we designed three studies:

The purpose of the first study, chapter two, was to determine the effects of a stepwise increase in speed and duration of treadmill training on knee joint integrity, and to identify the potential treadmill training threshold for the development of joint damage in the rat knee. We hypothesized that excessive chronic treadmill training leads to the development of OA-like changes in the knee, while moderate levels of treadmill training maintain cartilage integrity and induce positive adaptive responses.

The purpose of the second study, chapter three, was to determine the effects of prebiotic fibre supplementation, aerobic exercise, and the combination of prebiotic fibre and aerobic exercise on the onset of metabolic knee OA in rats fed a HFS diet. We hypothesized that prebiotic fibre supplementation, an aerobic exercise regime, and the combination of prebiotic fibre and aerobic exercise prevent OA-like damage in the knee of rats fed a HFS diet.

The purpose of the third study, chapter four, was to determine the effects of prebiotic fibre supplementation, aerobic exercise, and the combination of prebiotic fibre and aerobic exercise on the progression of metabolic knee OA in rats fed a HFS diet. We hypothesized that Prebiotic fibre supplementation, an aerobic exercise regime, and the combination of prebiotic fibre and aerobic exercise, introduced 12 weeks after the onset of HFS diet exposure, reduce the progression of OA-like damage in the knee of rats fed a HFS diet.

Chapter Two: Quantifying the effects of different treadmill training speeds and durations on the health of rat knee joints

Exercise has been suggested to be a potentially useful intervention for the prevention and treatment of metabolic knee OA. However, the effects of a treadmill exercise on the knee joint of CD-Sprague-Dawley rats (rat model of metabolic OA) is not known. Therefore, the first study of this thesis was aimed at determining the effects of a chronic treadmill exercise on adaptations to the knee, by using different exercise durations of exposure, and determining their effects on knee joint integrity. My role is this study was in designing the study, execution of the study, data collection, data analysis, interpretation of data, drafting the manuscript, revising the manuscript, and approving the final submitted version.

2.1 Introduction

Exercise has been used to promote health and fitness as far back as 2500 BC (Albert & Petrucelli, 1978). However, in the past years, exercise has been postulated to act like a drug, (Vina, Sanchis-Gomar, Martinez-Bello, & Gomez-Cabrera, 2012), and as such, provides advantages and risks to an individual's health and fitness. Specifically, aerobic exercise has been shown to lead to increased bone mineral density, insulin sensitivity, high-density lipoprotein cholesterol levels, resting and maximal stroke volume, maximal oxygen uptake, and basal metabolism (Bassi *et al.*, 2015; Pollock & Evans, 1999). Exercise has also been shown to reduce body fat, fasting blood insulin levels, low-density lipoprotein cholesterol levels, resting heart rate, and systolic and diastolic blood pressure (Bassi *et al.*, 2015; Pollock & Evans, 1999). However, the effects of exercise on joint health remain controversial (Chakravarty *et al.*, 2008; Cheng *et al.*, 2000; Lane *et al.*, 1998; Miller *et al.*, 2014).

In a prospective survey, aimed at examining the relationship of self-reported physical activity and physician-diagnosed osteoarthritis (OA) from 1970 to 1995, a positive association between OA incidence and physical activity was found. Running was suggested to lead to an increased risk for developing OA in some studies (Cheng *et al.*, 2000), but not in others (Chakravarty *et al.*, 2008; Lane *et al.*, 1998; Miller *et al.*, 2014). Chakravarty et al. (2008) did not find an increased risk for knee OA in masters' runners compared to controls over an 18 year period (Chakravarty *et al.*, 2008), and Miller et al. (2014) suggested that running does not increase the risk for knee OA compared to walking. However, Cheng et al. (Cheng *et al.*, 2000) reported a positive association between running more than 32 km per week and clinician diagnosed knee OA.

If exercise is thought to act like a drug preventing joint disease, the dosage may be critical to its success. It has been shown that moderate exercise, such as walking and running, exerts added loading to the knee joint, and cyclical loading is thought to be vital for maintaining cartilage integrity/homeostasis and healthy joints (Eckstein, Hudelmaier, & Putz, 2006; Hunter *et al.*, 2015; Maly & Robbins, 2014). In contrast, in vivo and vitro studies with excessive repetitive joint loading has been implicated with the development of OA (Hart, Frank, & Bray, 1995; Madden, Han, & Herzog, 2015). Joints are thought to be designed to operate within a "physiological window" to maintain proper function and allow for positive adaptations (Hart et al., 1995). Loading outside this window may put the joint, and specifically the cartilage, at risk for degeneration (Hart & Scott, 2012; Natsu-Ume et al., 2005).

In rodent model of exercise, for example, Galois et al. (Galois *et al.*, 2004) suggested that different levels of treadmill training may have different influences on the severity of chondral lesions in anterior cruciate ligament transection (ACLT) model of osteoarthritis in Wistar rats; while slight to moderate levels seemed to be beneficial to the knee cartilage health, more strenuous

exercise may be detrimental. Controversially, Yang et al. (Yang, Wang, Kong, Zhang, & Bai, 2017) showed that treadmill exercise up to one hour per day, five days a weeks, for 8 weeks, in CD-Sprague-Dawley rat model of monosodium iodoacetate-induced OA, has a chondroprotective effect, and this effect was more prominent in rats that fulfill in three times per day the one hour treadmill exercise, suggesting that an adaptive phase training may be an important factor in protecting the knee joint from OA-like changes.

Wistar rats, without previous lesion in the knee, have also been randomly assigned to a sedentary control group, a low-intensity running, a medium-intensity running, and a high-intensity running (Ni *et al.*, 2013). As result, rats in the high-intensity running demonstrated OA-like changes in their knees, while rats in the other running groups did not. Moreover, study in rodent models where the animals were allowed minimum (Beckett *et al.*, 2012) or not allowed an adaptive phase (Ni *et al.*, 2013), they have demonstrated OA-like changes in their knees. However, it is unknown how much exercise is too little or too much. Therefore, the purpose of this study was to determine the effects of a stepwise increase in speed and duration of treadmill training on knee joint integrity, and to identify the potential treadmill training threshold for the development of joint damage in the rat knee. We hypothesized that excessive chronic treadmill training leads to the development of OA-like changes in the knee, while moderate levels of treadmill training maintain cartilage integrity and induce positive adaptive responses. The key outcome measures were joint integrity, body composition, gene expression patterns, and blood-based and synovial fluid-based biomarkers.

2.2 Methods

2.2.1 Animals

Twenty-four 10 to 14-week old male CD-Sprague-Dawley rats were housed individually and randomized into four groups: moderate duration exercise (MD, n=6), high duration exercise (HD, n=6), extra high duration exercise (EHD, n=6), or no exercise (control, n=6). Rats were fed (*ad-libitum*) a standard chow-diet (Diet #5001, Lab Diet, USA). A minimum sample size of 5 rats per group is based on the ability to detect a minimal meaningful difference in histological scoring of the knee joint to provide α=0.05 and power of 80%. Calculation of sample size was performed using G*Power Software (version 3.0.10, Germany) (Faul, Erdfelder, Lang, & Buchner, 2007). Data for sample size calculations were obtained from a previous study (Collins *et al.*, 2015c). All experiments were approved by the University of Calgary Life and Environmental Sciences Animal Care Committee, and all methods were conducted in accordance with the animal welfare regulations and guidelines at the University of Calgary.

2.2.2 Exercise training protocol

Following one week of acclimatization to the housing environment, rats were exposed to their respective exercise programs (Table 2.1 for details of the exercise programs) on a Columbus Instruments Exer-3R treadmill (Columbus, OH, USA) for 12 weeks. The moderate duration group progressively built up to thirty minutes of treadmill training each day, five times per week at 25m/min. The high duration group built up to sixty minutes of treadmill training per day for 5 days per week at 25m/min. Rats in the extra high duration protocol reached sixty-minute training sessions seven days per week at 25m/min. In weeks 10, 11, and 12, these rats trained twice, three times, and four times for one hour daily, respectively. This last training intervention has previously

been used to elicit overtraining in Wistar rats (Hohl *et al.*, 2009). Animals in the control group were placed on the treadmill five days a week and completed fifteen minutes of exercise at 10m/min once per week. This was done to account for the stress of handling, and avoiding confounding results. A shock grid at the back of the treadmill was used to prevent animals from falling behind the pace of the treadmill.

2.2.3 Body Composition

Body mass was measured at the beginning of each week. Body mass for each animal was normalized to that of week 1 (familiarization week) and was expressed as the percent increase in body mass from that initial value. One week after completing the 12-week training protocol, and immediately prior to sacrifice, rats were lightly anaesthetized with isoflurane and body composition was measured using Dual X-ray absorptiometry (DXA) with software for small animals (Hologic ODR 4500; Hologic, MA, United States). An average of three scans for each animal was used for analysis.

2.2.4 Blood analysis

Following 12 h of fasting, rats were anaesthetized with isoflurane, and a blood sample was collected by cardiac puncture. Blood was centrifuged at 3000rpm for 15 min at 4°C, and serum stored in aliquots at -80ºC until analyzed. Rats were sacrificed by heart excision. Serum cytokines and adipokines were quantified using a Rat 27 Multiplex Discovery Assay with Luminex®xMAP technology (Eotaxin, EGF, Fractalkine, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IL-13, IL-17A, IL-18, IP-10/CXCL10, GRO/KC, IFN-γ, TNF-α, G-CSF, GM-CSF, MCP-1, leptin, LIX, MIP-1α, MIP-2, RANTES, VEGF; Eve Technologies, Calgary, AB, Canada).

Table 2.1. Twelve-Week Treadmill Training Programs for Group of Rats. Note that the extra high duration group had an increase in session number per day on weeks 10, 11, and 12. Rats in the control group walked per 15 min once a week at 10 m/min. The experimental week for rats in the moderate duration, and high duration groups, consisted of five consecutive days of training sessions, with 24 h of recovery between sessions, followed by 2 days of recovery. The experimental week for rats in the extra high duration group consisted of daily training sessions with 24 h recovery between sessions from week 2 to week 9. On weeks 10, 11, and 12, they had 6, 4, and 2 h recovery, respectively, between training sessions on the same day. Week 1 = familiarization week.

Week	Speed (m/min)	Moderate Duration			High Duration			Extra High Duration		
		Session/ week	Session/ day	Duration (min)	Session/ week	Session/ day	Duration (min)	Session/ week	Session/ day	Duration (min)
1	0	5	1	10	5	1	10	5	1	10
2	15	5	1	20	5	1	20	7	1	20
3	20	5	1	30	5	1	30	7	1	30
4	22.5	5	1	30	5	1	45	7	1	45
5	25	5	1	30	5	1	60	7	1	60
6-9	25	5	1	30	5	1	60	7	1	60
10	25	5	1	30	5	1	60	7	2	60
11	25	5	1	30	5	1	60	7	3	60
12	25	5	1	30	5	1	60	7	4	60
Total travelled distance		37.9 km 23.6 miles			69.6 km 43.2 miles			162.8 km 101.2 miles		

2.2.5 Knee joints

Knee joints were collected from both hind limbs. The left knee was harvested by cutting the femur and tibia/fibula 2 cm above and below the joint line. Excess muscles were removed and joints were fixed in a 10% neutral buffered formalin solution (Thermo Fisher Scientific, MA, USA) for 14 days at room temperature. Knees were then decalcified at room temperature, using Cal-Ex II solution (10% formic acid in formaldehyde, Thermo Fisher Scientific). The decalcification solution was changed daily. The end of decalcification was determined by chemical testing with a 5% ammonium oxalate solution (Thermo Fisher Scientific) until no precipitate was detected for 5 days (on average 21 days). Samples were then processed using an automatic paraffin processor (Leica TP 1020, Leica Microsystems Inc., Concord, Ontario, Canada). They were

dehydrated in a graded series of alcohols, cleared in xylene, and infiltrated with 50% Paraplast X-TRA® wax (Thermo Fisher Scientific), and 50% Paraplast Plus® wax (Thermo Fisher Scientific). Further, the left knee joints were embedded in paraffin wax and stored at room temperature until sectioning. Serial, sagittal plane sections of 10 µm thickness were obtained using a Leica RM 2165 microtome (Leica Biosystems, Nussloch, Germany). Sections were mounted onto Super Frost plus slides (Thermo Fisher Scientific) and allowed to dry at 40°C for 7 days. Alternate slides were stained sequentially with haematoxylin, fast green and safranin-O (Thermo Fisher Scientific) using an auto stainer (Leica ST 5010, Leica Biosystems). Sections were then dehydrated in a graded series of alcohols, cleared in xylene, and mounted with Cytoseal 60 mounting media (Thermo Fisher Scientific) using an auto cover slipper (Leica CV 5030, Leica Biosystems). Slides were dried at room temperature for seven days before being evaluated using a light microscope (Zeiss Axiostar plus, Carl Zeiss Inc., Ontario, Canada). Two independent graders scored all histological sections in a blinded manner using a Modified Mankin Histology Scoring System (Mankin, Dorfman, Lippiello, & Zarins, 1971). Osteoarthritis Research Society International (OARSI) histologic (Gerwin, Bendele, Glasson, & Carlson, 2010) subscores for bone changes, synovial thickening, and meniscus were also determined for each joint. The total Modified Mankin Score for each animal represents the sum of all Mankin Scores and OARSI subscores. Tibial and femoral cartilage thickness were determined from histological slides.

The right knee joints were opened to collect the synovium, menisci, fat pad, and the patellar tendon. The tissues were snap-frozen in liquid nitrogen and stored at -80°C for RNA isolation and subsequent RT-qPCR analysis. Genes analyzed for the patellar tendon and synovium were: Col-1, Col-3, iNOS, COX-2, IGF-1, IL-1, IL-6, and TGF-β. Genes analyzed for the fat pad were: iNOS, PPAR-γ, COX-2, IL-1B, IP-10, Leptin, TF, TFPI, and VEGF. Genes analyzed for the menisci

were: Col-1, Col-3, iNOS, PPAR-γ , COX-2, IGF-1, IL-1, IL-6, TGF-β, IP-10, Leptin, TF, TFPI, VEGF, and PRG4 (Table 2.2 for details). Synovial fluid was also collected from the right knee shortly after sacrifice using the Whatman chromatography paper method (Seifer et al., 2008). Samples were weighed, diluted 1:50, stored at 4°C overnight. After 24 hours, samples were centrifuged, and stored at -80°C until analysis. Synovial fluid cytokines and adipokines were quantified using a Rat 27 Multiplex Discovery Assay with Luminex®xMAP technology (Eotaxin, EGF, Fractalkine, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IL-13, IL-17A, IL-18, IP-10/CXCL10, GRO/KC, IFN-γ, TNF-α, G-CSF, GM-CSF, MCP-1, leptin, LIX, MIP-1α, MIP-2, RANTES, VEGF; Eve Technologies).

The right tibia was stored at -20°C until assessment of the biomechanical properties of cartilage was performed. A spherical indenter (r = 175 ± 2.5 μm), made of a stainless steel 316L shaft and a spherical glass bead, was installed under the multiaxial load cell (force resolution: Fz = 3.5 mN and Fx = Fy = 2.5 mN) of a 3-axis mechanical tester (Mach-1 v500css, Biomomentum, QC, Canada). The tibia was fixed in a sample holder using dental cement. The sample was then immersed into a testing chamber that contained PBS and was equipped with a camera-registration system (Biomomentum). A position grid was superimposed on the image of the tibia articular surface for a mechanically-controlled surface mapping (Sim *et al.*, 2017). Stress-relaxation tests for cartilage properties were performed on 11 sites each for the medial and lateral tibial plateau using the automated mapping system, and Young's modulus were calculated for each test site.

Table 2.2. Primer sequence used for RT-qPCR. *18S (human).

PRIMER	Forward Sequence	Reverse Sequence	Source
18S*	TGG TCG CTC GCT CCT CTC C	CGC CTG CTG CCT TCC TTG G	NR_003286
Col I	CAG ACG GGA GTT TCA CCT C	GAC ATG TAG ACT CTT TGC GGC	J04464
Col III	CTA ACC AAG GCT GCA AGA TG	ATC TGT CCA CCA GTG CTT CC	AJ005395
COX-2	CAG TAC ACT ACA TCC TGA CC	CGT CAA CAC GTA TCT CAT GG	S67722
IGF-1	GCA TTG TGG ATG AGT GTT GC	GGT CTT GTT TCC TGC ACT TC	X06043
IL-1A	CAG GCA TCC TCA GCA GCA GA	GGC TCC TAA GAA CAA GAA TG	NM_017019
IL-1b	AAC CTG CTG GTG TGT GAC GTT C	CAG CAC GAG GCT TTT TTG TTG T	NM_008361
IL-6	TCA CAG AAG GAG TGG CTA AG	ACC ACA GTG AGG AAT GTC CA	NM_012589
iNOS	AAG GCA CAA GAC TCT GAC AC	GGA TCG CAC TTC TGT CTC TC	AB250951
IP10	CAA GGC TTC CCA ATT CTC	ACC TGG ACT GCA TTT GA	U22520
Leptin	CCT GTG GCT TTG GTC CTA TCT G	CTG CTC AAA GCC ACC ACC TCT G	NM_013076
PPAR-γ	CTT GGC CAT ATT TAT AGC TGT CAT TAT T	TGT CCT CGA TGG GCT TCA C	NM_013124
PRG4	AGG GCG TTG CAT CCA AGA A	ATA ATC ACA GTT GCA GGT GGC	NM_001105962
TF	GAC AAT CTT GGA GTG GCA AC	GCT TCA TAG GTC CAG TTC AC	NM_013057
TFPI	CCG AGG AAG CTA TGT GTA AG	AGC CAG TGT AGG TGA AGG TC	NM_173141
TGFB	ACG GCA GCT GTA CAT TGA CT	CAG GAG CGC ACG ATC ATG TT	NM_021578
VEGF	TTA CTG CTG TAC CTC CAC CA	ATA GTG CAG TTG CTC TCC GA	NM_031836

2.2.6 Statistical Analysis

Non-parametric Kruskal-Wallis testing was used to determine differences between the four animal groups for all variables (RT-qPCR analyses, body fat, body mass, cartilage thickness, Mankin/OARSI Score, equilibrium Young's modulus, and protein assays). If significant ($p < 0.05$), post hoc testing using the Mann-Whitney U-Test was used to indicate differences between groups. Further, estimates of effect size were calculated using the ANOVA univariate approach on SPSS (version 22). Partial eta squared (partial $\eta 2$) were reported to provide small (partial $\eta 2 = 0.01$), medium (partial $\eta 2 = 0.06$), and large (partial $\eta 2 = 0.14$) effects (Cohen, 1988).

2.3 Results

2.3.1 Training distances

Rats in the control group travelled a distance of 150 m per week from week 2 to week 12, with a total distance of 1.65 km at the end of the experimental protocol. Rats in the MD and HD groups had a gradual increase in speed and duration from week 2 to week 5 (4 weeks of adaptation), them they ran in a constant speed and duration from week 6 to week 12, leading to a total distance traveled of 37.9 km and 69.9 km, respectively (Figure 2.1, Table 2.1). Rats in the EHD group have a stepwise increase in distance traveled from week 2 to week 5, then a constant distance traveled from week 6 to week 9, and another stepwise increase in distance traveled from week 10 to week 12, leading to a total distance traveled of 162.8 km by the end of the exercise protocol.

2.3.2 Body composition

Rats in all groups exhibited a gradual increase in body mass during the intervention period. However, body fat was significantly reduced by 4.5% ($p = 0.046$), 6.9% ($p = 0.032$), and 9.4% ($p = 0.003$) in the MD, HD, and EHD group rats, respectively, compared to that in the control group rats at the end of the exercise intervention period. Body fat was also significantly reduced by 4.9% ($p = 0.025$) in the EHD rats compared to that in the MD group rats (Figure 2.1B, Table 2.3).

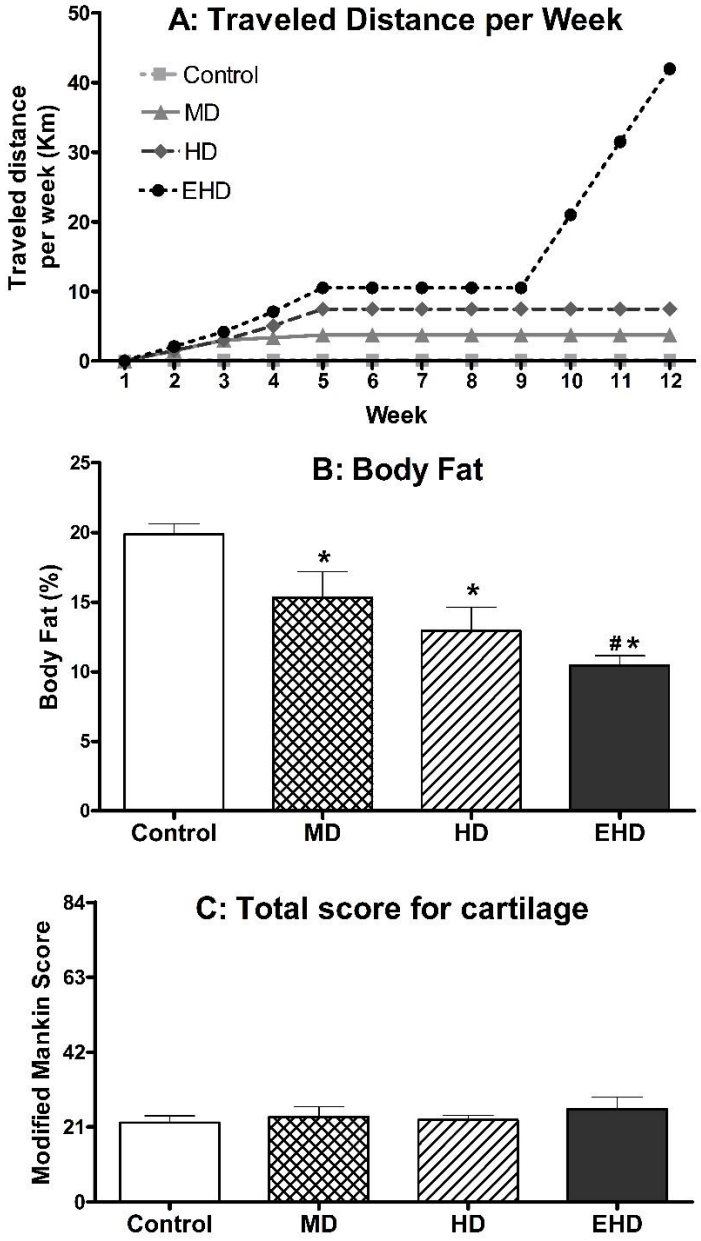

Figure 2.1. A) Travel distance per week per group. Notice, the control group was exposed to a constant amount of exercise throughout the protocol, 150 m per week. For the other groups, there was a gradual increase in the amount of exercise from week 2 to week 5. From week 5 to the end of the intervention period, the levels of exercise were constant for rats in the MD and HD groups. For rats on the EHD group, the exercise distance doubled, tripled, and quadrupled in weeks 10, 11, and 12 compared to weeks 5–9, respectively. B) Body fat. % body fat measured in the end of the experimental protocol. C) Total Modified Mankin Scores: No significant differences were found between groups. MD: moderate duration exercise, HD: high duration exercise, EHD: extra high duration exercise. * indicates significant difference when compared to control group. # indicates significant difference when compared to moderate duration group.

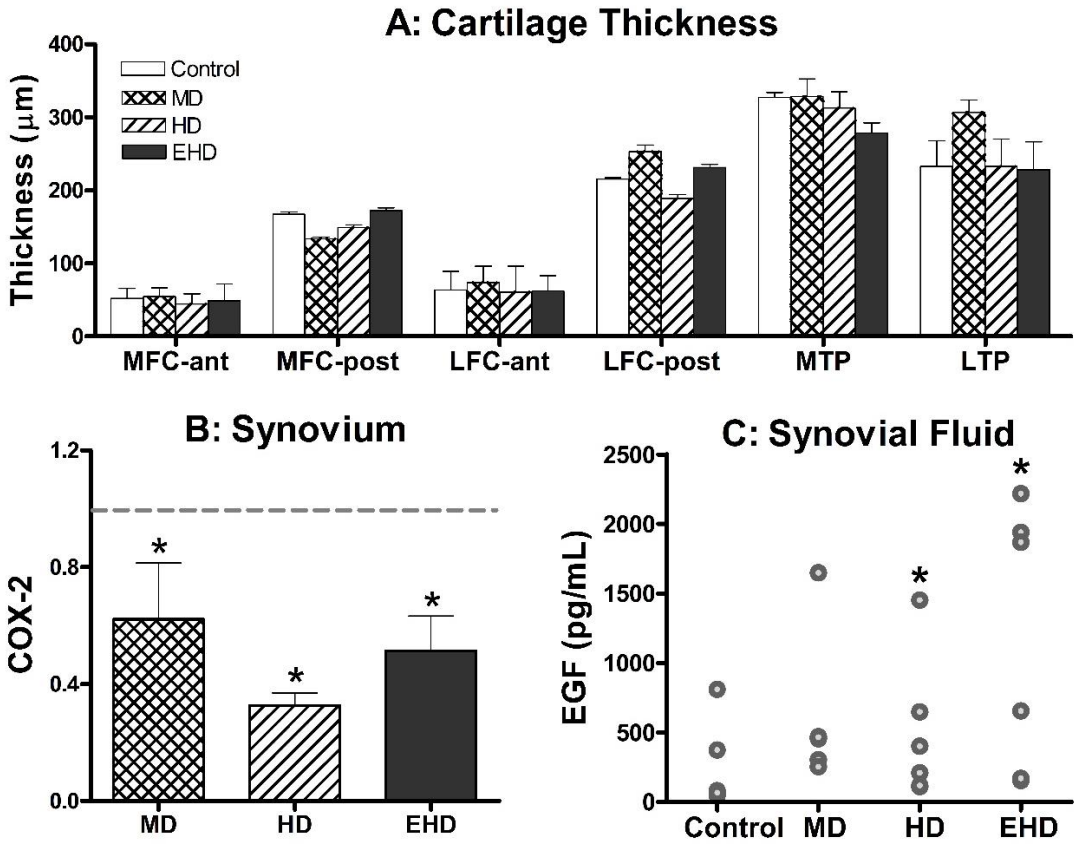

Figure 2.2. A) Cartilage thicknesses across six sites on the knee joint measured through histology. No significant differences were found between groups. B) COX-2 mRNA levels for the synovium expressed as fold change from control group (dashed gray line). C) EGF exhibiting significant differences between groups for synovial fluid; 27 proteins were analyzed with a Luminex-based protein assay. MD: moderate duration exercise, HD: high duration exercise, EHD: extra high duration exercise, MFC-ant: anterior medial femoral condyle, MFC-post: posterior medial femoral condyle, LFC-ant: anterior lateral femoral condyle, LFC-post: posterior lateral femoral condyle. * indicates a significant difference when compared to control group values.

2.3.3 Knee joint integrity and inflammatory markers

There were no differences in cartilage integrity (Figure 2.1C, Table 2.3), mechanical properties, and cartilage thicknesses (Figure 2.2A, Table 2.3) between groups across all joint locations. There were also no differences between groups in gene expression levels for the fat pad, patellar tendon, and menisci. Cyclooxygenase 2 (COX-2) levels in the synovium were lower for the 3 exercise group animals compared to the control group animals ($p=0.010$) (Figure 2.2B).

Epidermal growth factor (EGF) levels in the synovial fluid were significantly greater in EHD and HD group animals than those obtained in control group animals (p=0.039, p=0.014, respectively; Table 2.4, Figure 2.2C). Serum interleukin-12 (IL-12) levels were significantly higher for the EHD group animals than the control group (p=0.015) and the HD (p=0.008) group animals (Table 2.4).

Table 2.3. Estimates of effect size calculated using ANOVA univariate approach. Cohen (1988) has provided point of reference to define small (partial $\eta^2 = 0.01$), medium (partial $\eta 2 = 0.06$), and large (partial $\eta 2 = 0.14$) effects.

Variable	partial η^2
% body fat	0.578
Knee joint	
Total Score	0.062
MTP	0.025
MFC	0.111
LTP	0.033
LFC	0.114
Pat	0.209
Grv	0.255
Synovium	0.128
Menisci	0.075
Bone	0.197
Cartilage thickness	
MFC-ant	0.206
MFC-post	0.154
LFC-ant	0.131
LFC-post	0.143
MTP	0.204
LTP	0.177
Synovium: COX-2	0.679

MTP: medial tibial plateau, MFC: medial femoral condyle, LTP: lateral tibial plateau, LFC: lateral femoral condyle, Pat: patella, Grv: grove, ant: anterior, post: posterior

Table 2.4. Kruskal-Wallis p-values for serum and SF for the 27-protein analyzed using Luminex-based protein assay after 12-weeks on exercise.

Protein	Serum	SF	Protein	Serum	SF
G-CSF	0.310	0.246	IL-5	0.124	0.542
Eotaxin	0.293	0.65	IL-17A	0.467	0.051
IL-1α	0.742	0.216	IL-18	0.960	0.317
Leptin	0.130	0.209	MCP-1	0.666	0.151
MIP-1α	0.647	0.203	IP-10	0.820	0.435
IL-4	0.569	0.181	VEGF	0.398	0.486
IL-1β	0.640	0.255	Fractalkine	0.475	0.111
IL-2	0.364	0.116	LIX	1.000	0.715
IL-6	0.352	0.222	MIP-2	0.588	*
EGF	0.374	**0.027****	TNFα	0.080	0.166
IL-13	0.229	0.168	RANTES	0.983	0.961
IL-10	0.499	0.503	GM-CSF	*	*
IL-12	**0.014****	0.487	GRO/KC	*	*
IFNy	0.203	0.483			

*No statistics were computed due to extrapolated values and/or values out of validated range.

** Indicates $p < 0.05$.

SF: synovial fluid.

2.4 Discussion

Inactivity is the fourth leading risk factor for global mortality (Kohl *et al.*, 2012). A physically inactive lifestyle is associated with the development of non-communicable diseases (de Souto Barreto, 2015), including OA (Shrier, 2004). In contrast, exercise is reported to be a strong factor in health promotion and the prevention or delay of many non-communicable diseases (de Souto Barreto, 2015). However, poor health outcomes have been reported in athletes exposed to extra high levels of exercise (O'Keefe, Franklin, & Lavie, 2014; Plews, Laursen, Kilding, &

Buchheit, 2012), and it has been reported that top-level athletes have an increased risk of developing knee OA (Kujala *et al.*, 1995). Consequently, exercise can be beneficial or detrimental to the general health. Mechanical loading of joints due to exercise may be minimal but sufficient to maintain tissue homeostasis, it may be within the optimal physiological window and help maintain tissue homeostasis, and produce positive tissue adaptations, or it may be strenuous, thus exceeding the physiological window and lead to disruptions of tissue homeostasis, leading to joint disease. In this study, we investigated the effects of a stepwise increase in speed and duration of treadmill training on knee joint health in CD-Sprague-Dawley rats.

We randomized 24 male CD-Sprague-Dawley rats into four groups: moderate duration exercise (MD), high duration exercise (HD), extra high duration exercise (EHD), or no exercise (control). We did not detect changes in cartilage structure, mechanical properties, or thickness between groups and across joint locations. Therefore, the speed and duration of even the most strenuous treadmill exercise protocol used here (up to four hours of exercise daily) was not detrimental to the knee joint cartilage in these animals. Thus, the load applied to the knee through the exercise intervention protocol provided here was likely within the physiologic loading window that has been suggested to allow for adaptation, remodeling, and the proper functioning of cells and tissues (Eckstein *et al.*, 2006; Hart *et al.*, 1995a; Hart & Scott, 2012; Hunter *et al.*, 2015; Maly & Robbins, 2014).

An aggressive endurance exercise protocol in 16-18-week old Wistar rats has been shown to lead to OA-like changes in the knee (Beckett *et al.*, 2012). These rats were allowed one week of familiarization to the exercise protocol, followed by exercising twice a day, 5 days per week, for 3 and 6 weeks, for a total of 30 and 55 km of distance traveled (Beckett *et al.*, 2012). In our study, rats were allowed a prolonged adaptation period and were introduced gradually to the

increasing speed and duration of the exercise protocol, a factor which may be critical for joint health outcomes. The gradual increase in speed and duration of the exercise sessions may have allowed the cartilage to adjust gradually to the increasing load requirements, operating within the physiological window. The adaptive training phase in our study consisted of 4 weeks, and has been suggested to trigger increases in fitness and health in Wistar rats (Hohl *et al.*, 2009), and may be an important factor in the protection of the knee joint. It has been shown that a gradual increase in running volume in Wistar rats results in bone and cartilage remodeling by reducing catabolic genes and increasing aggrecan expression (Rahnamay Moshtagh *et al.*, 2017). However, to our knowledge, there is no study systematically investigating adaptive training phase in order to protect the knee joint, and this is a limitation of our study. Additionally, in the present study, rats in the EHD group doubled, tripled and quadrupled the amount of exercise in week 10, 11, and 12, respectively, relative to weeks 5-9. If these levels of exercise were sustained for an extended period, they may cause damage to the knee. Additionally, the physiological window for knee health is thought to be highly individual, and may be influenced by genetics, sex, lifetime loading history, presence of prior injury/scar tissue, systemic pathology, and local anatomy (Hart & Scott, 2012).

Rats from all groups exhibited a gradual increase in body mass during the exercise intervention period. This increase was consistent with the expected increase in body mass of laboratory rats with age (Sengupta, 2013). However, the MD, HD and EHD group rats were leaner than the control group rats, and the EHD group rats were leaner than the MD group rats at the end of the intervention period. Body fat has been shown to decrease with aerobic exercise in humans (Bond Brill, Perry, Parker, Robinson, & Burnett, 2002) and mice (Pereira *et al.*, 2014). Since body fat was reduced in the exercise group animals, but body weight was similar across all groups, the lean body mass (muscle mass) must have increased in the exercise group animals compared to the

control group animals (not evaluated in our study). Results similar to ours have been reported for human studies where strength and endurance exercise did not produce differences in body mass, but resulted in a reduction in body fat for individuals in an exercise program compared to individuals in a non-exercising group (Sipila, Multanen, Kallinen, Era, & Suominen, 1996). A reduction in body fat is considered a positive outcome for overall health, and has been shown to reduce risks for diabetes, cardiovascular disease, metabolic syndrome, and knee OA (Christensen, Astrup, & Bliddal, 2005; Phelan *et al.*, 2007; Pi-Sunyer, 1996; Toda *et al.*, 1998).

Exercise induces multiple biochemical changes that may affect gene expression (Powers & Lennon, 1999; Ren, Semenkovich, Gulve, Gao, & Holloszy, 1994). In the present study, markers for oxidative stress (iNOS), collagen (Col-I and Col-III), pro-inflammation (IL-1β, COX-2, IL-6, leptin, IGF-1, IP-10), and anti-inflammation (TF, TFPI, VEGGF, TGF-β) were evaluated for the knee joint tissues. Gene expression in the fat pad, patellar tendon, and menisci were similar across all groups. However, COX-2 mRNA levels in the synovium were reduced for all animals in exercise groups compared to animals in the control group. A reduction in COX-2 expression is thought to be positive for the joint environment, since drugs that inhibit COX-2 have been associated with a reduction of OA-like histological changes and suppressed chondrocyte apoptosis (Mastbergen *et al.*, 2006; Ou *et al.*, 2012). Indeed**,** it has been demonstrated that exercise is a robust approach to preserve healthy cartilage (Blazek *et al.*, 2016). Specifically, low intensity treadmill walking for 2, 5, or 15 days has been demonstrated to regulate metabolic responses at the cellular and systemic level, protecting cartilage against OA by altering gene expression of markers involved in OA onset (Blazek *et al.*, 2016). Our findings indicate that all levels of exercise used in our study led to a reduction in COX-2 mRNA in the synovium, suggesting a potential protective effect of exercise for the knee, even in the lowest end highest exercise programs. It is important to

highlight that at the end of the 12-week treadmill training many molecular changes may have occurred to adapt to the new loading conditions, and had already plateaued and normalized to the new exercise threshold by the time tissue was taken. Additionally, we did not study the protein and activity levels, this need to be done in future studies.

Synovial fluid EGF levels were significantly higher in the EHD and HD group animals compared to the control group animals. It has been suggested that EGF is produced by the synovium in the initial stages of rheumatoid arthritis (Kusada *et al.*, 1993), and that activation of EGF receptor signaling may be a causal factor in OA (Appleton, McErlain, Henry, Holdsworth, & Beier, 2007; Jakob *et al.*, 2001). However, more recent studies indicated anabolic effects of EGF receptor signal activation in articular cartilage, suggesting that EGF may promote the expansion and/or activity of an endogenous EGF receptor responsive cell population within the articular cartilage (Shepard, Jeong, Maihle, O'Brien, & Dealy, 2013). Moreover, the EGF receptor is an important signaling molecule in bone development and remodeling, and plays an anabolic role in bone metabolism (Zhang *et al.*, 2011). Overall, the finding of elevated synovial fluid EGF levels in the EHD and HD group animals in the absence of OA-like changes suggests that EGF was not detrimental to knee joint health.

Serum IL-12 levels were significantly higher for the EHD group compared to control group and HD group rats. This result agrees with findings from in vitro studies. Exercise has been reported to increase IL-12 production by macrophages following lipopolysaccharide stimulation (Itoh *et al.*, 2004). IL-12 is believed to be essential for the clearance of bacterial infections (Mattner *et al.*, 1996), and is thought to be an important pathogenetic factor in Th1-type-mediated autoimmune disease. IL-12 deficient mice have been found to be resistant to collagen-induced inflammatory arthritis (McIntyre *et al.*, 1996), while transgenic over-expression of IL-12

exacerbates the course of this disease (Parks *et al.*, 1998). However, the role of IL-12 in maintaining knee joint integrity, and its role in the pathogenesis of osteoarthritis, is poorly understood. It should be noted that the over-expression of serum IL-12 may represent responses to exercise unrelated to the knee joint, as serum may represent input from a number of sources, i.e. muscle, vasculature, etc. However, in combination with the other findings in our study, IL-12 may be playing a protective role for knee joint cartilage. Further mechanistic studies are required to elucidate the role of IL-12 in this model of exercise-induced changes in knee joint health, as well as follow the EHD rats for longer period of time than the 12 weeks of this protocol.

2.5 Conclusion

In summary, a stepwise increase in the speed and duration of a 12-week chronic treadmill exercise program did not lead to OA-like changes in the rat knee, but appeared to produce a potential protective effect through a reduction in COX-2 mRNA levels in the synovium. Further studies aimed at elucidating the preventive and potentially harmful effects of repetitive chronic exercise need to be performed to better understand the effects of joint loading on joint health above or below the physiological window. Working within an optimal physiological exercise window is beneficial for general and for joint health across the life span. It is also important in the context of recreational and elite sports, where the optimal window may be altered following joint injury or disease, and may thus affect the return to sport.

Chapter Three: Protective effect of prebiotic and exercise intervention on knee health in a rat model of diet-induced obesity

In this study, we used a CD-Sprague-Dawley rat-model of diet-induced metabolic disturbance that has been shown to result in reliable and consistent knee OA-like damage, and evaluated the effects of aerobic exercise and prebiotic fibre supplementation on the development of knee OA. The interventions used in this study were based on the findings presented in chapter two regarding exercise, and previous work outlined in chapter one, section 1.5, regarding fibre. Both interventions were shown to be safe when administrated to CD-Sprague-Dawley rats.

My role is this study was in designing the study, execution of the study, data collection, data analysis, interpretation of data, drafting the manuscript, revising the manuscript, and approving the final submitted version.

3.1 Introduction

Obesity, and associated metabolic syndrome, have been identified as primary risk factors for the development of knee osteoarthritis (OA) (Courties *et al.*, 2015; Courties *et al.*, 2017). OA linked to obesity has been defined as a separate "metabolic OA" phenotype (Zhuo *et al.*, 2012), and this phenotype represents nearly 60% of the OA patient population (Singh, Miller, Lee, Pettitt, & Russell, 2002). Metabolic OA is associated with large visceral fat depots (Ouchi *et al.*, 2011) that release inflammatory cytokines/adipokines resulting in low-level systemic inflammation (Hotamisligil, 2006; Wang *et al.*, 2015). Exposing rats to a high-fat/high-sucrose (HFS) diet leads to dysbiosis (impaired gut microbiota) (Collins *et al.*, 2016d), increased body fat, systemic inflammation, knee joint inflammation (Collins *et al.*, 2015c),

intramuscular fat deposition (Collins *et al.*, 2016c; Collins *et al.*, 2016d), and knee damage in rats within 12 weeks (Collins *et al.*, 2015c) (for a detailed review see Collins et al. (2018b), and Courties et al. (2017)).

In many cases, obesity is considered a modifiable risk factor (Powell *et al.*, 2005). Exercise is known as an effective modulator of body fat, systemic inflammation, and blood lipid levels (Conti *et al.*, 2015; Earnest *et al.*, 2013; Jeffery *et al.*, 2000; Pedersen, 2006). Similarly, dietary prebiotic fibre supplementation has been shown to decrease body fat and increase metabolic health in rodents (Cluny *et al.*, 2015; Parnell & Reimer, 2009) and humans (Nicolucci *et al.*, 2017). For example, exercise has been suggested to enhance the ability of skeletal muscles to use lipids, thus allowing for better control of plasma lipid levels (Earnest *et al.*, 2013); while the fermentation of oligofructose prebiotic fibre, a non-digestible plant-derived carbohydrate (Gibson *et al.*, 2017), by the gut microbiota leads to the production of short-chain fatty acids in the colon, which bind to fatty acid receptors and stimulate hormones associated with a reduction in appetite, delay of gastric emptying, and improvement of insulin sensitivity (Cani *et al.*, 2007a; Nakamura & Omaye, 2012). However, there are no studies to date that have examined the effects of exercise and dietary intervention in the knee joint during obesity development (spontaneous knee OA in the metabolic OA phenotype). We hypothesize that prebiotic fibre supplementation, an aerobic exercise regime, and the combination of prebiotic fibre and aerobic exercise will prevent OA-like damage in the knee of rats fed a HFS diet by controlling inflammation, dysbiosis, and metabolic syndrome.

In this study, we determined the effects of prebiotic fibre supplementation, aerobic exercise, and the combination of prebiotic fibre and aerobic exercise on the development of metabolic knee OA in a HFS diet-induced rat model of obesity. Our findings, confirmed our

hypotheses, indicating that prebiotic fibre, aerobic exercise, and the combination of prebiotic fibre and aerobic exercise prevented the development of OA-like damage that is observed in rats on a HFS diet that are not receiving these interventions. We defined OA-like damage as a disorder that results in structural changes to joint integrity involving cartilage, subchondral bone, menisci, and/or synovium.

3.2 Methods

3.2.1 Animals and intervention protocol

Forty-eight male, 12-week old, Sprague Dawley rats, fed a high-fat/high-sucrose diet (HFS, 20% of total weight as fat, 50% sucrose, 20% protein, and 10% from fibre and micronutrients; custom Diet #102412, Dyets, United States) were randomized into a sedentary (HFS, n=12), aerobic exercise (HFS+E, n=12), prebiotic fibre supplementation (HFS+F, n=12), or aerobic exercise combined with prebiotic fibre supplementation (HFS+F+E, n=12) group for 12 weeks. Eight chow-fed (standard chow diet control group, 5% of total weight as fat, 47.5% carbohydrates (only 4% from sucrose), 25% protein, 12.5% from fibre and micronutrients, and 10% moisture; Lab Diet 5001, United States, n=8), age- and sex-matched animals were included as controls. The aerobic exercise intervention consisted of a progressive moderate treadmill training program for 12 weeks, up to 30 minutes per day, 5 days a week as described previously (Table 3.1) (Boldt, Rios, Joumaa, & Herzog, 2018; Rios *et al.*, 2018). The prebiotic fibre was supplemented in the diet at a dose of 10% (wt/wt, Orafti P95, BENEO-Orafti, Germany) (Bomhof, Saha, Reid, Paul, & Reimer, 2014; Cluny *et al.*, 2015). Calculation of sample size was performed using G*Power Software (version 3.0.10, Germany) (Faul *et al.*, 2007). Data for sample size calculations were based on a previous study with HFS and control rats (Collins *et al.*, 2015c). All experiments were

approved by the University of Calgary Life and Environmental Sciences Animal Care Committee, and all methods were conducted in accordance with the animal welfare regulations and guidelines at the University of Calgary.

3.2.2 Body composition

Three days after completing the 12-week intervention protocol, rats were lightly anaesthetized with isoflurane and body composition was measured using Dual X-ray absorptiometry (DXA) with software for small animals (Hologic ODR 4500; Hologic, Bedford, MA, USA). The mean of three repeat scans for each animal were used for analysis.

Body mass was measured at the beginning of each week. Body mass for each animal was normalized to that of week 1 (familiarization week) and was expressed as the percent increase in body mass from that initial value.

Table 3.1. Twelve Week Progressive Treadmill Training Program. Week 1 was a familiarization week. Rats were placed on the treadmill for 10 minutes, with the grid shock turned on, but with the treadmill belt not running. HFS diet and fiber supplementation started on week 1, after the body mass measurement.

Week	**Speed**	**Moderate Duration**		
	m/min	**Sessions/week**	**Sessions/day**	**Training Time (min)**
1	0	5	1	10
2	15	5	1	20
3	20	5	1	30
4	22.5	5	1	30
5-12	25	5	1	30
Total travelled distance		37.9 km 23.6 miles		

3.2.3 Blood glucose and insulin

One day after the end of the intervention period, following 16 h of food deprivation, rats were given an oral gavage of 2 g/kg glucose. Blood was collected at 0, 15, 30, 60, and 120 min post-gavage via tail nick in a chilled tube for insulin analysis. Blood glucose was measured immediately with a blood glucose meter (OneTouch Verio and Blood Glucose Monitoring System, Lifescan, Switzerland). Whole body insulin sensitivity was determined using proxy measures from the glucose tolerance tests (composite insulin sensitivity index – CISI) (Matsuda & DeFronzo, 1999).

3.2.4 Blood lipid profile and endotoxin

One week prior to the start of the 12-week intervention protocol (acclimatization week), following 16 h of food deprivation, blood was collected via tail nick to evaluate the baseline values for cytokines/adipokines (details below). Four days after completing the 12-week intervention protocol, and following 16 h of food deprivation, rats were anaesthetized with isoflurane and cardiac blood samples were collected. Serum was analyzed for lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides) using colorimetric assays (Calgary Lab Service, Calgary, AB, Canada), cytokines/adipokines (details below), and for endotoxin. Concentration of blood endotoxin was measured using the Pierce LAL Chromogenic Endotoxin Quantification assay (Thermo Fisher Scientific, MA, USA) according to manufacturer directions.

3.2.5 Blood and synovial fluid cytokines and adipokines

The right knee joints were opened to collect synovial fluid shortly after sacrifice using the Whatman chromatography paper method (Seifer *et al.*, 2008). Samples were weighed, diluted and centrifuged (Rios *et al.*, 2018). Serum and synovial fluid cytokines and adipokines were quantified using a Rat 27 Multiplex Discovery Assay with Luminex®xMAP technology (Eotaxin, EGF, Fractalkine, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12(p70), IL-13, IL-17A, IL-18, IP-10/CXCL10, GRO/KC, IFN-γ, TNF-α, G-CSF, GM-CSF, MCP-1, leptin, LIX, MIP-1α, MIP-2, RANTES, VEGF; Eve Technologies, AB, Canada).

3.2.6 Cecal matter microbiota

Microbiota analysis was carried out using 16rRNA qPCR as per our previous work (Bomhof, Paul, Geuking, Eller, & Reimer, 2016). Cecal samples were collected at euthanasia for cecal microbiota analysis. DNA was extracted from the cecal samples using the MP Biomedicals Fast DNA Spin Kit for Feces (MP Biomedicals, Lachine, QC, Canada). Amplification and detection were conducted in 96-well plates with SYBR Green 2× qPCR Master Mix (BioRad). Purified template DNA from reference strains was used to generate standard curves for each primer set using 10-fold serial dilutions of DNA. Standard curves were normalized to copy number of 16S rRNA genes using reference strain genome size and 16S rRNA gene copy number values. Total bacteria were also measured. Primer selection for qPCR was based on providing broad coverage of the total microbial signal in rats and those microbes of relevance to an obese rat model (Bomhof *et al.*, 2016).

3.2.7 Knee joint histology

The left knee was harvested by cutting the femur and tibia/fibula 2 cm above and below the joint line, respectively. Muscles were removed and joints were fixed in a 10% neutral buffered formalin solution for 14 days at room temperature. Knees were then decalcified, dehydrated in a graded series of alcohols, embedded in paraffin wax, and stored at room temperature until sectioning (Rios *et al.*, 2018). Serial sagittal plane sections of 10 µm thickness were obtained, and mounted. Subsequently, every second section was stained sequentially with haematoxylin, fast green and safranin-O (Rios *et al.*, 2018). Two independent, blinded graders scored all histological sections using a Modified Mankin Histology Scoring System (Mankin *et al.*, 1971), and the Osteoarthritis Research Society International's (OARSI) histologic (Gerwin *et al.*, 2010) sub-scores for bone changes, synovial thickening and menisci for each joint. Only the worst defect quantified per joint was used for the statistical analyses. The total Modified Mankin Score for each animal was defined as the sum of all individual OARSI sub-scores and the Modified Mankin Score.

3.2.8 Statistical analysis

All data are presented as means ± 1 SEM. Levene's test for equality of variance was conducted on all outcomes. A one-way ANOVA with the five experimental groups as the main factor was performed using Bonferroni post hoc testing to determine differences between groups. A 2-way ANOVA was used to determine the main effects of prebiotic fibre supplementation, aerobic exercise, and their interaction, on the HFS diet. Changes in serum cytokines and adipokines from pre- to post-intervention were determined using a repeated measures ANOVA with time as the within-subject factor and experimental grouping as the between-subject factor. To further explore the changes in serum cytokines and adipokines pre- and post-intervention, data were

normalized relative to the pre-intervention values, and a one-way ANOVA with experimental grouping as the main factor was performed using Bonferroni post hoc testing. To measure the association between knee joint damage, the metabolic variables, and cecal microbiota data, the Pearson Correlation Coefficient was calculated. Analyses were done using SPSS V22.0 software (SPSS, Chicago, IL, USA). Data were considered statistically significant at $p < 0.05$, two-tailed.

3.3 Results

3.3.1 Prebiotic fibre and aerobic exercise prevent OA-like damage to the knee of rats exposed to a HFS diet

The HFS obesity-inducing diet disrupts knee joint homeostasis of male Sprague Dawley rats resulting in structural damage to the joint ($F_{(4,51)}= 22.387$, $p<0.001$). Our most prominent finding is that the subchondral bone in rats fed a HFS diet was replaced by a fibrotic tissue with consequent collapse of the adjacent cartilage, which is a novel contribution to the literature in this area (Figure 3.1). The thick arrow on Figure 1b indicates that normal bone has been replaced by fibrotic tissue in rats fed a HFS diet. The fibrous tissue extends to the tidemark in some places (Figure 1b, thin arrow), and the loss of subchondral bone likely leads to the collapse of the bone and the adjacent cartilage. These changes were observed in the medial tibial plateau, and medial/posterior femoral condyle of 75% (9 out 12) of rats fed a HFS diet. The other 3 rats presented changes in the subchondral bone, with thickening of the cartilage, but without the collapse (Figure 3.2.a). We have also observed thickening of the synovium in HFS fed rats when compare to chow rats (Figure 3.2.b and c).

We found that the prebiotic and exercise interventions, and the combination of the two, fully protected the knee from joint damage (all $p<0.001$), despite the intake of a HFS diet (Figure 3.1, Table 3.1).

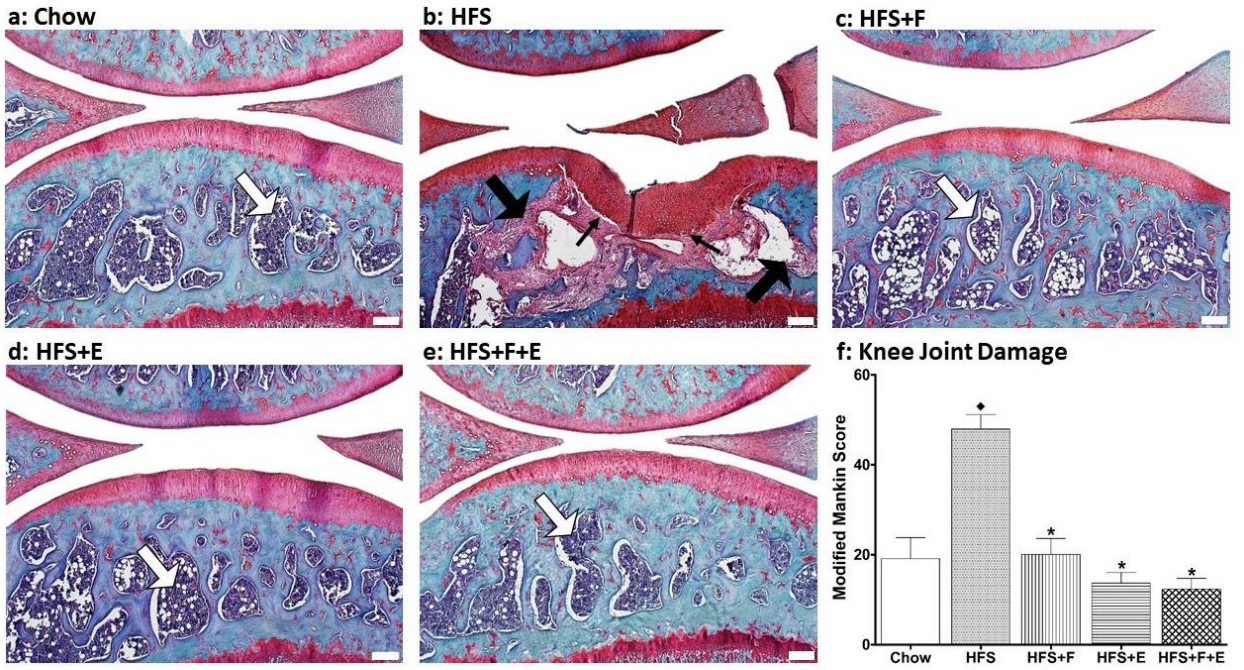

Figure 3.1. Knee joint integrity. White thick arrows indicate healthy bone marrow. Black thick arrows indicate that the normal marrow has been replaced with a fibrotic marrow. Black thin arrows indicate that the fibrous and granulation tissue extended to the subchondral bone and to the tidemark. ♦ Indicates difference from chow group, while * Indicates difference from HFS group. Chow: chow control diet; HFS: high-fat/high-sucrose diet; F: prebiotic fibre; E: aerobic exercise. Values are means ± 1 SEM (white line, bar = 200 μm).

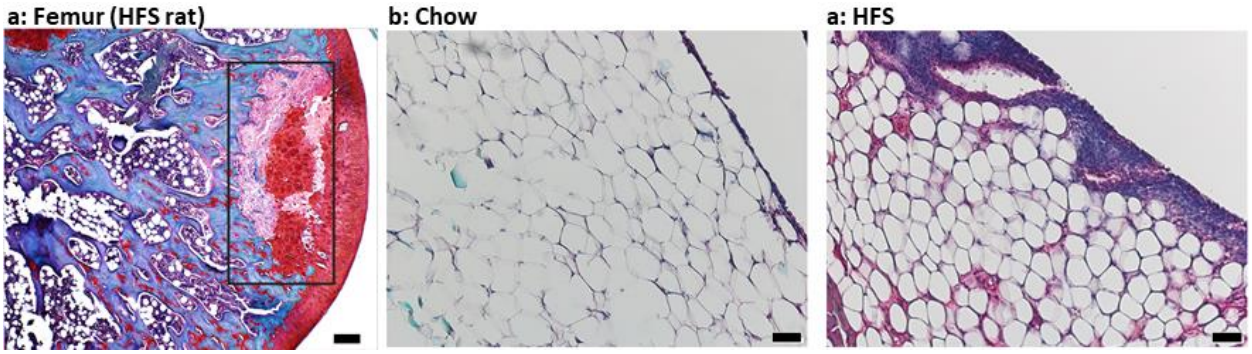

Figure 3.2. a: Knee joint: medial/posterior femur. This image represents the knee joint of the 3 rats fed a HFS diet that did not have the collapse of the cartilage. Inside the rectangle you can notice changes in the subchondral bone that might predispose the further cartilage collapse (black line, bar = 200 μm). b and c: Thickening of synovium was presented in HFS sedentary rats but not in Chow rats (black line, bar = 50 μm).

3.3.2 The combination of prebiotic fibre and aerobic exercise prevents increases in percent body fat in rats exposed to a HFS diet

Absolute percent body fat in rats in the HFS group was 11% higher than rats fed a standard chow control diet (p=0.002, Figure 3.3), and 8% higher than HFS fed rats that received prebiotic fibre supplementation and undertook aerobic exercise at the same time (HFS+F+E; p=0.013). There were no statistically significant differences for percent body fat between the chow, HFS combined with prebiotic fibre supplementation (HFS+F), HFS combined with aerobic exercise (HFS+E), and HFS+F+E group animals (Figure 3.3, Table 3.2). Additionally, percent body fat had a positive association with knee joint damage (Table 3.4).

On average, body mass in the beginning of the experimental protocol was 469g per rat, and it increased for all groups of rats during the experimental period. HFS fed rats had an increase in body mass of 56% from the baseline measurements, while chow, HFS+F, HFS+E, and HFS+F+E rats increased 36%, 41%, 34%, and 32% , respectively ($F_{(4,51)}$= 13.830, p<0.001).

3.3.3 Prebiotic fibre and aerobic exercise prevent increases in local and systemic leptin concentration levels in rats exposed to a HFS diet

Rats in the HFS group had higher leptin levels in knee synovial fluid than did rats in the chow, HFS+F, HFS+E, and HFS+F+E groups (p=0.001, p=0.034, p=0.040, p= 0.014, respectively), indicating that the interventions prevented the increases in synovial fluid leptin levels detected in the untreated obese animals (Table 3.2). Serum leptin levels exhibited the same pattern as the synovial fluid leptin. The HFS group animals had higher serum leptin levels than did the chow group rats (p=0.006). Prebiotic fibre, aerobic exercise, and the combination

of both prevented increases in serum leptin in rats on the HFS diet (p=0.010, p=0.038, and p=0.019, respectively, Table 3.3). Additionally, synovial fluid and serum leptin exhibited a positive association with knee joint damage (Table 3.4).

Overall, the other 26 biomarkers assessed in the synovial fluid and serum did not differ between groups (Table 3.3).

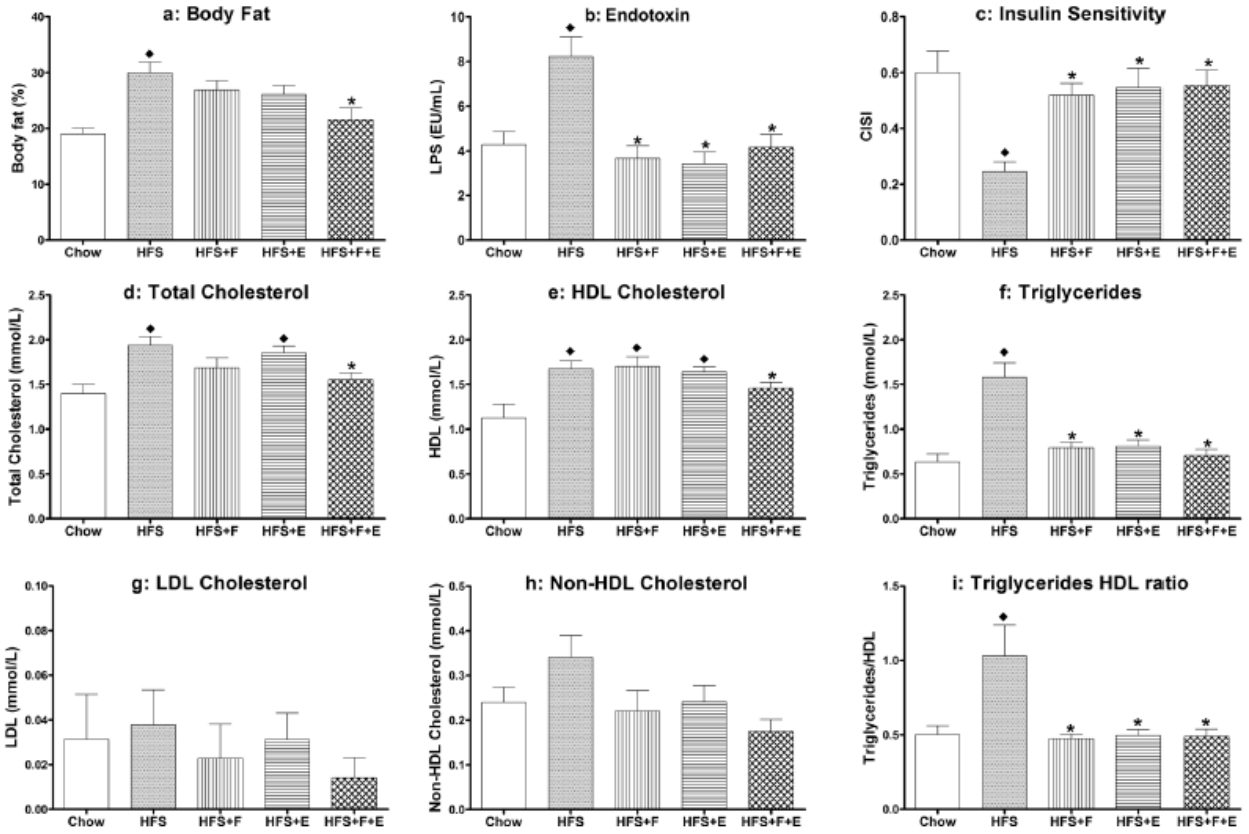

Figure 3.3. Metabolic profile. Body fat, endotoxin, composite insulin sensitivity index, serum lipid profile for rats in the chow, HFS, HFS + F, HFS + E, HFS + F + E groups at the end of the experimental protocol. ♦ Indicates difference from chow group, while *indicates difference from HFS group. Chow: chow control diet; HFS: high-fat/high-sucrose diet; F: prebiotic fibre; E: aerobic exercise. Values are means ± 1 SEM.

Table 3.2. Knee joint damage, percent body fat, synovial fluid leptin, endotoxin, composite insulin sensitivity index, serum lipid profiles, and cecal microbiota for rats treated in the chow, HFS, HFS + F, HFS + E, HFS + F + E groups at the end of the experimental protocol. Values are means ± SEM. Cecal microbiota data are shown as means ± SEM expressed as the relative abundance (%) of bacterial taxa per total bacteria (16S rRNA gene copies of microbial group/total bacteria 16S rRNA gene copies). Chow: chow control diet; HFS: high-fat/high-sucrose diet; F: prebiotic fibre; E: aerobic exercise. HDL: high density lipoprotein; LDL: low density lipoprotein; Chol: cholesterol. Trig: triglycerides.

						Two-way ANOVA							One-way ANOVA		
						F		E		F * E					
	Chow	HFS	HFS+F	HFS+E	HFS+F+E	p-value	partial η^2	p-value	partial η^2	p-value	partial η^2	Adjusted R^2	p-value	partial η^2	Observed Power
Knee joint integrity	19.1 ± 4.7	47.9 ± 3.2	20.1 ± 3.5	13.6 ± 2.4	12.3 ± 2.4	**<0.001**	0.361	**<0.001**	0.539	**<0.001**	0.318	0.666	**<0.001**	0.637	1.000
Percent body fat, %	19.0 ± 1.1	29.9 ± 1.9	26.9 ±1.6	26.1 ± 1.6	21.6 ± 2.0	**0.045**	0.088	**0.016**	0.125	0.690	0.004	0.141	**0.001**	0.299	0.962
SF: leptin, pg/mL	63.8 ± 8.5	251.8 ± 48.7	130.5 ± 20.7	125.4 ± 24.1	113.1 ±18.3	**0.045**	0.092	**0.019**	0.125	0.098	0.064	0.188	**0.001**	0.304	0.959
Endotoxin, EU/ML	4.3 ± 0.6	8.2 ±0.9	3.7 ± 0.6	3.4 ±0.5	4.2 ± 0.6	**0.006**	0.161	**0.002**	0.200	**<0.001**	0.272	0.441	**<0.001**	0.432	0.999
CISI	0.60 ± 0.08	0.25 ±0.04	0.52 ± 0.04	0.54 ± 0.07	0.56 ± 0.06	**0.011**	0.153	**0.003**	0.203	**0.016**	0.14	0.315	**0.001**	0.343	0.976
Total Cholesterol (mmol/L)	1.40 ± 0.10	1.94 ± 0.10	1.69 ±0.11	1.85 ± 0.08	1.56 ± 0.08	**0.005**	0.179	0.244	0.033	0.792	0.002	0.156	**0.001**	0.36	0.956
HDL Cholesterol (mmol/L)	1.12 ± 0.16	1.67 ± 0.09	1.70 ± 0.11	1.64 ± 0.05	1.46 ± 0.06	0.359	0.019	0.108	0.058	0.208	0.036	0.044	**0.001**	0.297	0.96
LDL Cholesterol (mmol/L)	0.03 ± 0.02	0.04 ± 0.02	0.02 ± 0.02	0.03 ±0.01	0.01 ±0.01	0.230	0.033	0.567	0.008	0.930	<0.001	0.026	0.768	0.035	0.148
Non-HDL Cholesterol (mmol/L)	0.24 ± 0.03	0.34 ± 0.05	0.22 ± 0.05	0.24 ± 0.04	0.18 ± 0.03	**0.031**	0.160	0.091	0.102	0.512	0.016	0.183	0.052	0.248	0.67
Triglycerides (mmol/L)	0.64 ±0.09	1.58 ± 0.16	0.79 ± 0.11	0.81 ± 0.07	0.71 ± 0.07	**<0.001**	0.310	**<0.001**	0.291	**0.002**	0.207	0.501	**<0.001**	0.543	1.000
Ratio: Total Chol/HDL	1.10 ± 0.03	1.17 ± 0.04	1.05 ± 0.01	1.13 ± 0.02	1.06 ± 0.02	**0.003**	0.195	0.545	0.009	0.345	0.022	0.158	**0.031**	0.198	0.741
Ratio: Tryg/HDL	0.50 ±0.06	1.03 ± 0.21	0.48 ± 0.03	0.50 ± 0.04	0.49 ± 0.05	**0.022**	0.122	**0.032**	0.107	**0.025**	0.117	0.240	**0.002**	0.296	0.941
Cecal Microbiota															
Bacteroides/ Prevotella	11.91 ± 1.48	4.26 ± 0.39	13.75 ± 2.46	5.95 ± 0.49	12.78 ± 2.26	**<0.001**	0.344	0.835	0.001	0.437	0.014	0.306	**<0.001**	0.346	0.988
Bifidobacterium	0.08 ± 0.02	0.26 ± 0.05	11.01 ± 1.91	0.21 ± 0.05	10.87 ± 1.40	**<0.001**	0.649	0.934	<0.001	0.973	<0.001	0.625	**<0.001**	0.680	1.000
Enterobacteriaceae	0.16 ± 0.03	0.85 ± 0.19	0.82 ± 0.13	0.52 ± 0.10	0.34 ± 0.08	0.412	0.015	**0.003**	0.178	0.551	0.008	0.139	**0.001**	0.292	0.956
Lactobacillus	3.65 ± 0.63	0.36 ± 0.11	3.07 ± 0.56	0.26 ± 0.07	2.37 ± 0.32	**<0.001**	0.552	0.224	0.033	0.368	0.018	0.532	**<0.001**	0.569	1.000

C. coccoides (cluster XIV)	7.20 ± 0.43	7.99 ± 0.63	8.07 ± 1.12	9.97 ± 0.67	8.03 ± 1.02	0.301	0.024	0.279	0.027	0.260	0.029	0.013	0.256	0.097	0.397
C. leptum (cluster IV)	14.08 ± 0.97	15.36 ± 1.70	3.49 ± 1.04	12.58 ± 1.14	4.27 ± 0.72	**<0.001**	0.615	0.410	0.015	0.147	0.047	0.598	**<0.001**	0.640	1.000
C. cluster I	1.23 ± 0.18	1.19 ± 0.24	0.50 ± 0.04	0.86 ± 0.07	0.44 ± 0.04	**<0.001**	0.296	0.141	0.049	0.309	0.024	0.285	**<0.001**	0.360	0.992
C. cluster XI	0.11 ± 0.01	0.30 ± 0.07	0.18 ± 0.05	0.28 ± 0.07	0.08 ± 0.02	**0.006**	0.157	0.264	0.028	0.499	0.010	0.128	**0.017**	0.208	0.810
Roseburia	0.66 ± 0.32	0.24 ± 0.08	1.98 ± 0.24	0.14 ± 0.05	1.91 ± 0.32	**<0.001**	0.624	0.679	0.004	0.936	<0.001	0.599	**<0.001**	0.577	1.000
Methanobrevibacter	0.01 ± 0.00	0.01 ± 0.00	<0.001	0.01 ± 0.00	0.00 ± 0.00	**<0.001**	0.592	0.980	<0.001	0.111	0.057	0.575	**<0.001**	0.594	1.000
Akkemansia muciniphila	1.08 ± 0.50	1.25 ± 0.26	0.21 ± 0.11	1.21 ± 0.25	0.14 ± 0.06	**<0.001**	0.407	0.786	0.002	0.934	<0.001	0.367	**0.001**	0.300	0.963
Faecalibacterium prausnitzii	0.31 ± 0.05	0.95 ± 0.23	0.21 ± 0.08	0.52 ± 0.11	0.23 ± 0.05	**<0.001**	0.248	0.139	0.049	0.107	0.058	0.26	**0.001**	0.314	0.973
Collinsella aerofaciens	0.02 ± 0.00	0.02 ± 0.00	0.04 ± .0.01	0.02 ± 0.00	0.01 ± 0.01	0.301	0.024	0.200	0.037	0.118	0.055	0.047	0.185	0.112	0.462

Table 3.3. Serum 27 cytokine/adipokine profile for rats in the Chow, HFS, HFS+F, HFS+E, HFS+F+E groups before and at the end of the experimental period. Values are means ± SEM. Chow: chow control diet; HFS: high-fat/high-sucrose diet; F: prebiotic fibre; E: aerobic exercise. GM-CSF, IL-6, IFNy, GRO/KC, MIP-2, and EGF were excluded from analysis due to extrapolated values and/or values out of validated range.

											Repeated measures ANOVA					
	Chow		HFS		HFS+F		HFS+E		HFS+F+E		Time		Group		Time *Group	
(unit)	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	p-value	partial η^2	p-value	partial η^2	p-value	partial η^2
G-CSF	44.8 ±4.2	34 ±8.4	40.5 ±3.1	40.6 ±4.3	41.6 ±3.3	33.2 ±6.0	41.8 ±3.9	36.7 ±5.5	40.2 ±4.3	33.7 ±4.2	0.051	0.084	0.855	0.029	0.82	0.034
Eotaxin	5.6 ±0.5	5.4 ±0.9	5.4 ±0.5	6.0 ±0.8	3.9 ±0.3	4.4 ±1.0	4.5 ±0.6	4.4 ±0.8	5.6 ±0.5	4.7 ±0.4	0.905	<0.001	0.221	0.133	0.873	0.030
IL-1a	50.8 ±10.7	36.6 ±15.8	70.1 ±12.2	31.6 ±9.0	129.1 ±66.5	31.3 ±7.5	57.9 ±11.2	28.4 ± 9.5	57.5 ±16.6	16.0 ±3.7	**0.008**	0.289	0.832	0.065	0.953	0.031
Leptin	5288 ±787	8692 ±1308	6358 ±810	33005 ±4849	9202 ±1042	26926 ±5103	11827 ±1586	21002 ±3212	8617 ±2232	15278 ±2182	<**0.001**	0.524	**0.004**	0.258	<**0.001**	0.328
MIP-1a	11.3 ±1.7	7.5 ±0.7	12.9 ±1.4	9.3 ±0.7	14.7 ±1.8	10.2 ±1.1	13.1 ±1.3	7.6 ±1.1	11.4 ±1.2	7.4 ±0.7	<**0.001**	0.380	0.101	0.141	0.933	0.016
IL-4	3.3 ±0.6	4.1 ±1.5	4.4 ±0.7	10.4 ±4.1	2.7 ±0.5	7.4 ±3.3	4.6 ±2.0	4.2 ±1.0	2.9 ±0.6	2.3 ±0.6	0.107	0.061	0.238	0.121	0.235	0.121
IL-1B	69.5 ±37.8	37.2 ±8.0	94.2 ±24.3	153.0 ±42.2	86.7 ±29.1	67.3 ± 25.6	93.1 ±36.8	122.3 ±44.9	55.8 ±14.1	42.4 ±13.7	0.721	0.003	0.240	0.100	0.144	0.123
IL-2	46.0 ±5.1	50.9 ±6.9	49.9 ±3.7	66.0 ±6.2	47.7 ±7.0	55.8 ±9.9	49.6 ±5.7	46.1 ±7.8	54.2 ±4.4	45.8 ±4.2	0.256	0.026	0.628	0.050	0.124	0.132
IL-13	5.6 ±1.6	8.4 ±2.9	6.4 ±1.4	6.5 ±1.6	3.0 ±1.0	5.1 ± 1.9	4.4 ±1.2	5.9 ±2.1	5.6 ±1.1	2.7 ±0.7	0.888	0.001	0.340	0.149	0.555	0.102
IL-10	55.0 ±29.7	35.7 ±10.2	75.0 ±14.3	118.8 ±27.7	68.8 ±18.2	61.2 ±20.5	77.8 ±23.5	90.2 ±30.8	49.0 ±10.4	38.2 ±12.0	0.667	0.004	0.210	0.107	0.157	0.120
IL-12	151.2 ±15.2	176.8 ±20.8	175.3 ±8.6	200.0 ±10.9	141.6 ±13.6	162.6 ±14.0	152.1 ±19.7	141.9 ±20.4	161.3 ±8.7	153.7 ± 14.6	0.231	0.028	0.097	0.140	0.502	0.062
IL-5	36.5 ±3.6	38.1 ±2.8	43.1 ±1.8	44.9 ±2.6	39.4 ±2.6	35.7 ±3.7	41.7 ±3.4	34.1 ±2.8	42.6 ±2.7	36.5 ±2.2	0.138	0.043	0.114	0.133	0.365	0.08
IL-17A	10.1 ±2.0	11.9 ±2.0	11.1 ±1.4	18.4 ±2.8	9.7 ±1.3	10.6 ±2.0	11.1 ±1.4	9.2 ±1.5	12.3 ±1.4	10.4 ±1.0	0.207	0.031	0.095	0.141	**0.021**	0.199
IL-18	274.7 ±32.8	133.4 ±14.3	236.7 ±17.9	168.4 ±19.8	301.9 ±45.5	215.3 ±57.2	237.7 ±25.2	124.1 ±26.8	225.1 ±22.2	109.3 ±15.9	<**0.001**	0.408	0.095	0.141	0.752	0.036
MCP-1	508.6 ±79.1	459.0 ±50.3	602.4 ±56.8	603.4 ±53.9	586.5 ±52.3	468.0 ± 41.6	558.6 ±48.1	351.8 ±51.2	586.1 ±41.1	485.1 ±45.0	**0.001**	0.197	0.158	0.124	0.14	0.129
IP-10	188.5 ±17.0	150.5 ±13.6	226.9 ±12.7	206.0 ±15.6	248.5 ±19.3	194.0 ±18.0	248.2 ±21.2	196.6 ±26.7	210.0 ±16.2	162.7 ± 22.0	<**0.001**	0.28	0.119	0.132	0.778	0.033
VEGF	18.3 ± 5.2	14.5 ±3.4	21.4 ±2.7	24.8 ±2.8	28.8 ±4.3	25.7 ±3.8	26.0 ±4.0	19.3 ±3.2	21.3 ±2.7	14.4 ±2.7	**0.048**	0.086	0.076	0.171	0.296	0.104
Fractalkine	32.9 ± 4.3	30.1 ±1.5	39.1 ±2.5	36.6 ±3.0	48.0 ±3.8	41.4 ±3.1	43.3 ±5.1	30.4 ±4.4	37.6 ±2.8	30.9 ±2.8	**0.001**	0.204	**0.044**	0.172	0.332	0.085
LIX	1879 ± 258	1298 ±182	2108 ±158	1701 ±204	2122 ±262	1291 ±165	2334 ±306	1219 ±218	1976 ±171	1266 ±178	<**0.001**	0.370	0.654	0.046	0.496	0.063
TNFa	9.9 ± 2.0	11.8 ±2.2	10.8 ±1.1	14.3 ±1.5	8.0 ±1.1	10.2 ±1.7	9.3 ±1.3	6.8 ±1.1	11.3 ±1.1	9.5 ±1.2	0.669	0.004	**0.048**	0.178	0.102	0.146
RANTES	647 ±117.1	396.9 ±69.6	1282 ±298	1145 ±318	1376 ± 381	550 ±91	1604 ± 468	810 ±284	889 ±197	487 ±82	**0.002**	0.179	0.192	0.111	0.422	0.072

3.3.4 Prebiotic fibre and aerobic exercise prevent decreases in insulin sensitivity of rats exposed to a HFS diet

HFS fed rats had decreased whole body insulin sensitivity compared to rats fed a chow control diet (p=0.001). Prebiotic fibre, aerobic exercise, and the combination of the two normalized insulin sensitivity to levels found in the chow group rats (p=0.004, p=0.011, p=0.003, compared to HFS group, respectively; Figure 3.2, Table 3.1). Insulin sensitivity exhibited a negative association with knee joint damage (Table 3.2).

Table 3.4. Relationship between knee joint damage (Total Modified Mankin Score), rat metabolic profile and cecal microbiota. r: Pearson's correlation coefficient; SF: synovial fluid; CISI: composite insulin sensitivity index; HDL: high density lipoprotein; LDL: low density lipoprotein; Chol: cholesterol. Trig: triglycerides.

	Knee Joint Damage			Knee Joint Damage	
	r	p-value		r	p-value
Percent body fat	0.327	**0.014**	*Bacteroides/Prevotella*	-0.328	**0.013**
SF: leptin	0.451	**0.001**	*Bifidobacterium*	-0.320	**0.016**
Serum: leptin	0.389	**0.003**	*Enterobacteriaceae*	0.210	0.121
Endotoxin	0.455	**<0.001**	*Lactobacillus*	-0.125	0.359
CISI	-0.327	**0.010**	*C. coccoides* (cluster XIV)	-0.020	0.885
Total cholesterol	0.343	**0.012**	*C. leptum* (cluster IV)	0.436	**0.001**
HDL-cholesterol	0.230	0.087	C. cluster I	0.226	0.094
LDL-cholesterol	0.264	0.052	C. cluster XI	0.206	0.127
Non-HDL cholesterol	0.399	**0.015**	*Roseburia*	-0.294	**0.028**
Triglycerides	0.546	**<0.001**	*Methanobrevibacter*	0.237	0.079
Ratio: Total Chol/HDL	0.220	0.116	*Akkemansia muciniphila*	0.307	**0.021**
Ratio: Trig/HDL	0.330	**0.017**	*Faecalibacterium prausnitzii*	0.457	**<0.001**
			Collinsella aerofaciens	0.008	0.953

3.3.5 Prebiotic fibre and aerobic exercise improve serum lipid profile of rats fed a HFS diet

The HFS diet led to increased serum total cholesterol, HDL-cholesterol, triglycerides, and the triglycerides to HDL-cholesterol ratio compared to rats fed a chow diet (Figure 2). The combined intervention of prebiotic fibre and exercise prevented these increases in serum total

cholesterol and HDL-cholesterol levels (Table 3.1, Figure 3.3). Prebiotic fibre supplementation, exercise, and the combination of the two prevented increases in serum triglyceride levels and increases in the serum triglycerides to HDL-cholesterol ratio (Figure 3.3, Table 3.1). Triglycerides levels exhibited a strong positive association with knee joint damage, while total cholesterol and non-HDL-cholesterol exhibited a moderate positive association with knee joint damage, and HDL- and LDL-cholesterol exhibited weak positive associations with knee joint damage (Table 3.2).

3.3.6 Prebiotic fibre and aerobic exercise prevent increases in serum endotoxin in rats exposed to a HFS diet

The HFS diet led to increased serum endotoxin levels ($p=0.002$) compared to chow fed rats. Prebiotic fibre, aerobic exercise, and the combination of exercise and prebiotic fibre prevented these increases ($p<0.001$, $p<0.001$, and $p<0.001$, respectively), maintaining values similar to those in chow fed control rats (Figure 3.3.b, Table 3.2). Endotoxin levels exhibited a positive association with knee joint damage (Table 3.4).

3.3.7 Prebiotic fibre, but not exercise, prevents microbial dysbiosis in rats fed a HFS diet

The cecal microbiota composition of the HFS rats showed a decrease in *Bacteroides/Prevotella* ($p=0.048$), *Lactobacillus* ($p<0.001$), and *Faecalibacterium prausnitzii* ($p=0.024$) compared to chow fed rats that was prevented by prebiotic fibre supplementation, but not by exercise; and an increase in *Enterobacteriaceae* ($p=0.010$), that was prevented by the combination of prebiotic fibre and exercise (Figure 3.4, Table 3.2). Prebiotic fibre supplementation, and the combination of prebiotic fibre and aerobic exercise, shifts the

microbiota towards a decrease in *Clostridium leptum (cluster IV)*, *Clostridium cluster I*, *Methanobrevibacter*, and *Akkemansia muciniphila*; and an increase in *Bifidobacterium* and *Roseburia* compared to the cecal microbiota of HFS rats ($p<0.05$, Figure 3, Table 3.2). *Bacteroides/Prevotella, Bifidobacterium and Roseburia* exhibited a negative association with knee joint damage, while *Clostridium leptum (cluster IV)*, *Akkemansia muciniphila* and *Faecalibacterium prausnitzii* exhibited a positive association with knee joint damage (Table 3.4).

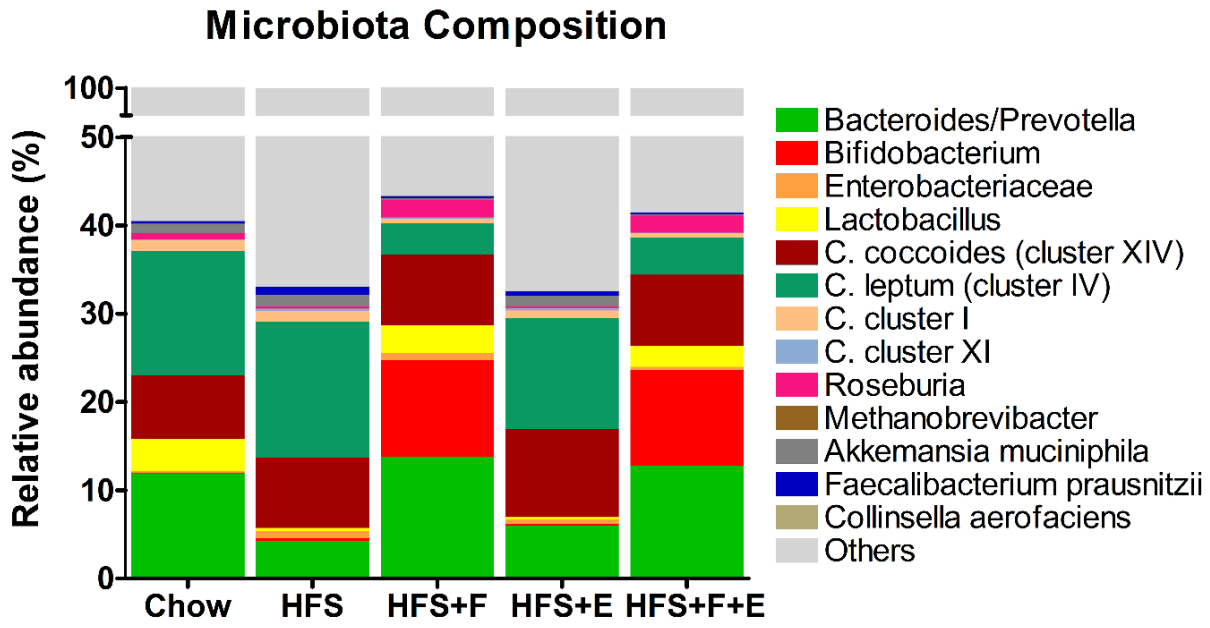

Figure 3.4. Microbiota composition. Cecal matter microbiota composition at the end of the experimental protocol. Values are mean relative abundance of cecal microbiota. Microbial abundance was measured as 16S rRNA gene copies per 20 ng DNA and reported here as relative abundance (%) of bacterial taxa per total bacteria. Chow: chow control diet; HFS: high fat/high sucrose diet; F: prebiotic fibre; E: aerobic exercise.

3.4 Discussion

We showed that prebiotic fibre supplementation and aerobic exercise protect against the development of knee joint damage in rats fed a HFS diet. The maintenance of knee joint

integrity achieved with prebiotic fibre and aerobic exercise was accompanied by a maintenance of metabolic and gut microbiota homeostasis. It appears that the obesity-related metabolic dysregulation and gut microbiota dysbiosis might be strongly linked to the metabolic OA phenotype in the Sprague Dawley rat model (see schematic in Figure 3.5).

Majority of the damage detected in the knee joint of HFS fed rats occurred in the subchondral bone, and the structural changes observed in this study are similar to changes reported in an emu femoral head model of osteonecrosis (Conzemius, Brown, Zhang, & Robinson, 2002), suggesting a potential initiating role for subchondral bone changes in OA pathogenesis associated with the HFS diet. Such a role has been advanced previously in the literature as a result of reduced blood supply and compromised nutrient exchange to the bone (Courties *et al.*, 2017; Zhuo *et al.*, 2012).

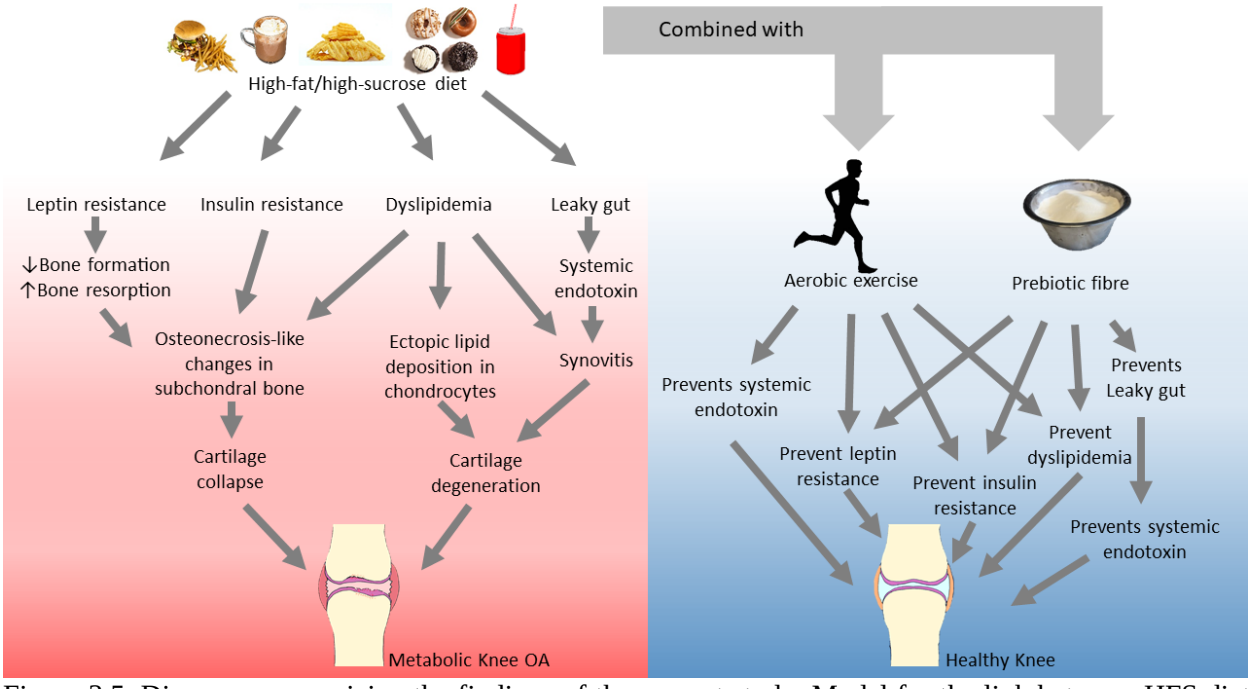

Figure 3.5. Diagram summarizing the findings of the present study. Model for the link between HFS diet and metabolic knee OA, and the pathways which prebiotic fibre and aerobic exercise might have activated to prevent damage of the knee joint in Sprague Dawley rats fed a HFS diet.

The metabolic OA phenotype is associated with hyperglycaemia, insulin resistance, and leptin resistance, which in turn are linked to bone formation/structure (Courties *et al.*, 2017; Klein, 2014; Shanbhogue, Finkelstein, Bouxsein, & Yu, 2016; Srikanthan *et al.*, 2014; Yan, Zhang, Yang, & Sun, 2018; Zhuo *et al.*, 2012). Hyperglycaemia and insulin resistance combined with increased leptin levels may have resulted in some of the changes observed in the subchondral bone of the HFS animals. Additionally, altered lipid metabolism in patients has also been associated with OA development in metabolic disease (Baudart, Louati, Marcelli, Berenbaum, & Sellam, 2017; Zhuo *et al.*, 2012). Rats fed the HFS diet had an altered lipid metabolism, as indicated by the increase in serum triglyceride levels and serum triglycerides to HDL-cholesterol ratio, and we detected a strong association between triglycerides levels and knee joint damage (Table 3.2).

The prebiotic oligofructose fibre supplementation used in our study prevented dyslipidemia, thereby potentially contributing to the maintenance of knee joint homeostasis. It has been shown that drugs that lower lipid levels inhibit OA development in a mouse model of atherosclerosis (Gierman *et al.*, 2014). Elevated plasma cholesterol levels trigger oxidative stress in mitochondria and degradative changes in chondrocytes that, in combination, are thought to lead to OA development and progression (Farnaghi *et al.*, 2017). Moreover, mouse models of hypercholesterolemia-induced OA are characterized by changes in subchondral bone architecture that are rescued when lipid-lowering drugs are administrated (Farnaghi *et al.*, 2017). It has been suggested that hypercholesterolemia induces oxidation and deposition of lipids in tissues, resulting in damage to cartilage similar to that seen in atherosclerosis (Sevin, Yasa, Akcay, Kirkali, & Kerry, 2013), while osteonecrosis might occur because of a narrowing of the vasculature by lipids.

Obesity has been associated with increased gut permeability, allowing bacterial lipopolysaccharides from Gram-negative bacteria to translocate into the blood (endotoxemia), triggering systemic inflammation and insulin resistance (Cani *et al.*, 2007a; Cani *et al.*, 2009). It has been shown that improvements in glucose tolerance in rodents after prebiotic fibre supplementation are related to changes in the microbiota (Cani *et al.*, 2007b; Parnell & Reimer, 2012). We made similar observations regarding changes in microbiota. Increases in the inflammation-associated *Enterobacteriaceae (Lupp et al., 2007)* were prevented by the combination of prebiotic fibre and exercise only. Prebiotic fibre supplementation also increased *Bifidobacterium* compared to HFS fed rats. *Bifidobacterium* has been shown to decrease intestinal permeability, upregulate expression of tight junction proteins and reduce IL-6 and TNF-α in cell lines and in rodents (Ling, Linglong, Weixia, & Hong, 2016). Given that oligofructose is known to increase the abundance of *Bifidobacterium*, it is possible that the prebiotic fibre reduced serum endotoxin levels via this mechanism. Furthermore, certain lactobacilli strains have been associated with health-promoting properties, including attenuated weight gain and improved gut barrier function (Plaza-Diaz, Gomez-Llorente, Fontana, & Gil, 2014), which suggest that the HFS fed animals may have increased gut permeability due in part to the decrease in lactobacilli abundance.

Moreover, in agreement with our finding, it has been shown in a mouse model of high-fat diet–induced obesity that prebiotic fibre supplementation has a protective effect against trauma- induced OA. Manipulation of the gut microbiota is thought to represent a viable disease-modifying therapeutic strategy for OA associated with obesity (Schott *et al.*, 2018).

The aerobic exercise used in this study consisted of a progressive treadmill training of up to 150 minutes/week. This amount of exercise corresponds to the recommended minimal

physical activity guidelines for humans (de Souto Barreto, 2015), and has been shown to be safe for Sprague Dawley knees (Rios *et al.*, 2018). Aerobic exercise in our rats was found to modulate insulin and leptin levels, similar to what has been observed for the prebiotic fibre supplementation. Aerobic exercise in humans is also associated with decreased plasma leptin and insulin levels (Essig, Alderson, Ferguson, Bartoli, & Durstine, 2000). Thus, maintenance of insulin homeostasis in the exercising rats may have prevented increases in leptin and leptin resistance in rats fed a HFS diet, despite similar body fat percentages in HFS+E and HFS rats. Therefore, aerobic exercise may have helped maintain bone turnover homeostasis, thereby preventing the development of OA-like damage in the knees of rats fed the HFS diet.

Aerobic exercise also prevented increases in serum triglyceride levels and serum triglycerides to HDL-cholesterol ratio observed in rats exposed to a HFS diet. These results agree with findings from human studies. For example, it has been shown that there is a dose-response relationship between physical activity and improvements in triglycerides and HDL-cholesterol levels in previously sedentary people (Aadahl, Kjaer, & Jorgensen, 2007). However, the mechanisms underlying these improvements are unclear. By preventing dyslipidemia, aerobic exercise may have contributed to the maintenance of knee joint integrity in the HFS rats.

Aerobic exercise also prevented endotoxemia in rats fed a HFS diet. However, the pathway might not be the same as that suggested for the prebiotic fibre intervention. Endotoxin, an enteric bacteria-derived molecule, is recognized by Toll-like receptors on immune cells, adipocytes, and cells in several organs, such as Kupffer cells in the liver , and when in excess, triggers an inflammatory response (Takeuchi & Akira, 2010). The capacity of Kupffer cells to remove endotoxin might be important in the prevention of metabolic knee OA. Aerobic

exercise has been shown to improve the removal of endotoxins by Kupffer cells (Komine *et al.*, 2017), thereby contributing to the reduction of endotoxemia in rats fed a HFS diet, and possibly preventing the OA-like damage observed in the rat knee.

While prebiotic fibre supplementation and aerobic exercise prevented the development of knee damage, and affected the metabolic outcome variables to a similar extent, exercise did not affect the gut microbiota, while the prebiotic fibre intervention did. Therefore, it appears that the prebiotic intervention affected the microbiota, and as a consequence, the host secondarily via the fermentation of the prebiotic fiber, short chain fatty acids (Cani *et al.*, 2007a) (not assessed in our study). In contrast, the exercise protocol primarily affected host parameters to diminish the impact of the HFS diet and the altered microbiota. Thus, the exercise protocol appeared to impact the metabolic profile and the gut leaky syndrome by increasing the uptake of endotoxins by Kupffer cells (Komine *et al.*, 2017). Aerobic exercise combined with prebiotic fibre supplementation was not more effective as each intervention alone (Figure 3.5). Therefore, it appears that the mechanisms involved for each intervention complement each other but are not additive. Therefore, the impact of the HFS diet on a sedentary host is different than its impact on a host that is exercising and enhancing the resistance of host systems. Exercise is expect to contribute to tissue repair, enhance the metabolism of glucose and fat, and enhance the release of exercise-associated regulatory molecules from the brain to counteract diet-induced factors (Phillips, Baktir, Srivatsan, & Salehi, 2014). It is known that mechanical loading of tissues, such as cartilage, ligaments, tendons, and skin, can lead to resistance to catabolic mediators (Ballyns & Bonassar, 2011) or their expression (Natsu-Ume *et al.*, 2005) . Therefore, exercise-induced tissue loading may result in the maintenance of tissue integrity. The non-exercised rats in this study had reduced tissue loads compared to exercised rats, and

their systems might have been at an increased risk for alteration caused by the HFS diet. It appears that exercise and prebiotic fibre supplementation impact HFS fed-animals differently. Providing both interventions simultaneously may lead to a reinforcement of resistance to the adverse effects of the HFS diet over 12 weeks.

In summary, a HFS diet led to development of knee joint damage in rats that was associated with changes in the metabolic profile in these animals. We provide novel information on the prevention of metabolic OA using a prebiotic fibre and/or an aerobic exercise intervention (Figure 3.5). We suggest that the preventive effects of the prebiotic fibre and aerobic exercise interventions are associated with the leptin and insulin pathways, as well as with lipid metabolism and the leaky gut syndrome. Effectively addressing the prevention of metabolic osteoarthritis will require the engagement of public health programmes. Low cost, non-invasive treatments strategies, such as prebiotic fibre supplementation and aerobic exercise should be a priority.

Chapter Four: Prebiotic and exercise do not stop the progression of osteoarthritis in a rat-model of metabolic disturbance

Results from chapter three demonstrated that prebiotic fibre supplementation, aerobic exercise, and the combination of prebiotic fibre supplementation and moderate exercise prevented knee joint damage that was otherwise observed in this model of metabolic disturbance. In a next step, we wanted to investigate if these same interventions were also able to stop or slow down the progression of existing knee OA induced by a high-fat/high-sucrose diet. In this study we attempted to address this question in part by introducing prebiotic fibre supplementation, aerobic exercise, and the combination of prebiotic fibre supplementation and aerobic exercise at a time point when metabolic disturbance and knee OA-like damage had already progressed to a certain degree. My role is this study was in designing the study, execution of the study, data collection, data analysis, interpretation of data, drafting the manuscript, revising the manuscript, and approving the final submitted version.

4.1 Introduction

Metabolic syndrome is a complex disorder and is defined by the World Health Organization as a pathologic condition characterized by abdominal obesity, insulin resistance, hypertension, and dyslipidemia (low HDL-cholesterol and high triglyceride levels) (Alberti & Zimmet, 1998). Metabolic disturbances are known risk factors for cardiovascular disease (Ford, 2005b; Hu *et al.*, 2004), and metabolic disturbance has also been identified as a risk factor for the development of knee osteoarthritis (OA) (Courties *et al.*, 2015; Courties *et al.*, 2017), and has been recognised as a separate "metabolic OA" phenotype (Zhuo *et al.*, 2012). Moreover,

nearly 60% of the OA patient population present signs of metabolic disturbance (Singh *et al.*, 2002).

Exposing CD-Sprague-Dawley rats to a high-fat/high-sucrose (HFS) diet leads to increased body fat, systemic inflammation, knee joint inflammation (Collins *et al.*, 2015c), intramuscular fat deposition (Collins *et al.*, 2016c; Collins *et al.*, 2016d), and knee damage in rats within 12 weeks (Collins *et al.*, 2015c) (for a detailed review see Collins et al. (2018b), and Courties et al. (2017)). It has been shown that prebiotic fibre supplementation, aerobic exercise, and the combination of prebiotic fibre and aerobic exercise prevent the development of OA-like damage that is observed in rats on a HFS diet, when the interventions were started at the onset of the HFS diet exposure (Rios *et al.*, 2019a). Additionally, clinical trials using elderly knee OA patients with overweight and obesity demonstrated that exercise improved self-reported measures of knee function and pain (Messier *et al.*, 2004), while data from prospective cohort studies showed that a diet rich in fibre lowers the risk of moderate or severe knee pain regardless of radiographic knee osteoarthritis status (Dai *et al.*, 2017).

While there is evidence that early exercise and prebiotic fibre supplementation can prevent the development of OA in a rat-model of diet-induced metabolic disturbance, and that exercise and fibre consumption improve functional outcomes in human OA studies, it remains unknown if these interventions remain successful when started at time points after the introduction of a HFS diet and once knee OA-like damage has begun to emerge. Specifically, it would be crucial to know the threshold in time when exercise and prebiotics are effective, when they become neutral, and possibly if they become destructive and might accelerate knee OA progression. Therefore, the purpose of this study was to determine the effects of prebiotic fibre supplementation, aerobic exercise, and the combination of prebiotic fibre and aerobic

exercise on the progression of metabolic knee OA in a HFS diet-induced rat-model of metabolic disturbance. We hypothesized that prebiotic fibre supplementation, an aerobic exercise regime, and the combination of prebiotic fibre and aerobic exercise, introduced 12 weeks after the onset of HFS diet exposure, reduce the progression of OA-like damage in the knee of rats fed a HFS diet by reducing the metabolic disturbance caused by the HFS diet.

4.2 Methods

4.2.1 Animals and intervention protocol

Seventy male, 12-week old, CD-Sprague-Dawley rats were housed individually and either fed a standard chow diet (control group, 5% of total weight as fat, 47.5% carbohydrates (only 4% from sucrose), 25% protein, 12.5% from fibre and micronutrients, and 10% moisture; Lab Diet 5001, United States, n=11), or a high-fat/high-sucrose diet (HFS, 20% of total weight as fat, 50% sucrose, 20% protein, and 10% from fibre and micronutrients; custom Diet #102412, Dyets, United States, n=59). After 12 weeks on diets, rats consuming the high-fat/high-sucrose diet were randomized (stratified randomization based on percent body fat) into four sub-groups: a sedentary (HFS, n=15), an aerobic exercise (HFS+E, n=14), a prebiotic fibre supplementation (HFS+F, n=15), and an aerobic exercise combined with prebiotic fibre supplementation (HFS+F+E, n=15) group (Figure 4.1). The aerobic exercise intervention consisted of a progressive moderate treadmill training program for 12 weeks, up to 30 minutes per day, 5 days a week as described previously (Boldt *et al.*, 2018; Rios *et al.*, 2018). The prebiotic fibre intervention consisted of supplementing the HFS diet with 10% prebiotic fibre by weight (wt/wt, Orafti P95, BENEO-Orafti, Germany) (Bomhof *et al.*, 2014; Cluny *et al.*, 2015; Rios *et al.*, 2019a). Calculation of sample size was performed using G*Power Software (version 3.0.10, Germany) (Faul *et al.*, 2007) and was based

on a previous study with HFS and control rats (Collins *et al.*, 2015c). All experiments were approved by the University of Calgary Life and Environmental Sciences Animal Care Committee.

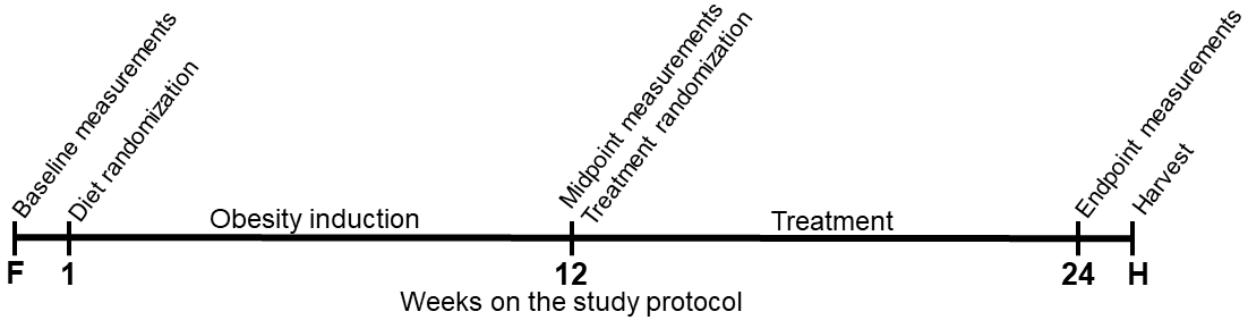

Figure 4.1. Study design. Measurements performed at baseline: Body mass, blood glucose, blood insulin, and blood lipid profile. Measurements performed at midpoint: Body mass, blood glucose, blood insulin, blood lipid profile, and body composition (DXA scan). Measurements performed at endpoint: Body mass, blood glucose, blood insulin, and body composition (DXA scan). Measurements performed at harvesting: blood lipid profile. Body mass was measured at the beginning of each week in the study protocol.

4.2.2 Body composition

Twelve weeks after the diets started (midpoint) and at the end of the intervention protocol, rats were lightly anaesthetized with isoflurane and body composition (for percent body fat and bone mineral density calculations) was measured using Dual X-ray absorptiometry (DXA) with software for small animals (Hologic ODR 4500; Hologic, Bedford, MA, USA). The mean of two repeat scans for each animal was used for analysis. Body mass was measured at the beginning of each week.

4.2.3 Blood glucose and insulin

During the familiarization week (week F), after 12 weeks on their respective diet (midpoint), and at the end of the intervention period (endpoint), rats were given an oral gavage of 2 g/kg glucose following a 16 h food deprivation period. Blood was collected at 0, 15, 30, 60, and 120 min post-gavage via tail nick in a chilled tube for insulin analysis. Blood glucose was

measured immediately with a blood glucose meter (OneTouch Verio and Blood Glucose Monitoring System, Lifescan, Switzerland). Whole body insulin sensitivity was determined using proxy measures from the glucose tolerance tests (composite insulin sensitivity index – CISI) (Matsuda & DeFronzo, 1999).

4.2.4 Blood lipid profile

During the familiarization week (week F – via tail nick: 800 µl), after 12 weeks on their respective diet (midpoint – via tail nick: 800 µl), and at the end of the experimental protocol (via cardiac puncture: 7-10 ml), blood was collected following a 16 h food deprivation period. Serum was analyzed for lipid profiles (total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides) using colorimetric assays (Calgary Lab Service, Calgary, AB, Canada),

4.2.5 Knee joint histology

The left knee was harvested by cutting the femur and tibia/fibula 2 cm above and below the joint line, respectively. Muscles were removed, and joints were fixed in a 10% neutral buffered formalin solution for 14 days at room temperature. Knees were then decalcified, dehydrated in a graded series of alcohols, embedded in paraffin wax, and stored at room temperature until sectioning (Rios *et al.*, 2018). Serial sagittal plane sections of 10 µm thickness were obtained and mounted. Subsequently, every second section was stained sequentially with haematoxylin, fast green, and safranin-O (Rios *et al.*, 2018). Histological sections were quantified using a Modified Mankin Histology Scoring System (Mankin *et al.*, 1971), and a Modified Osteoarthritis Research Society International (OARSI) histologic (Gerwin *et al.*, 2010) sub-score for bone changes, synovial thickening and meniscal damage for each joint. The worst defect for tibia, femur,

synovium, and meniscus for each compartment (medial and lateral) were quantified per joint. The sum of the all the scores per compartment was defined as the Total Modified Mankin Score for each animal (Table 4.1) and were used for the statistical analyses (Rios *et al.*, 2019a).

Table 4.1. The total Modified Mankin Score for each animal was defined as the sum of the worst defect per compartment evaluated using a Modified Mankin Histology Scoring System (for cartilage) (Mankin, Dorfman et al. 1971) and Modified OARSI sub-scores (for bone changes, synovial thickening, and meniscal damage) (Gerwin, Bendele et al. 2010).

Tissue	Score (min-max) per compartment	Number of compartments: Compartments Scored
Cartilage	0-24	4: Medial Tibia, Medial Femur, Lateral Tibia, Lateral Femur
Subchondral Bone	0-13	4: Medial Tibia, Medial Femur, Lateral Tibia, Lateral Femur
Synovium	0-4	1: Synovium
Meniscus	0-4	1: Meniscus
Total Modified Mankin Score (maximal possible score including all compartments): 156		

4.2.6 Statistical analysis

All data are presented as means ± 1 SEM. Non-parametric Kruskal-Wallis testing with pairwise comparisons was used to determine differences between the five animal groups for all variables. We further investigated differences between time-points within groups using Wilcoxon test (percent body fat and bone mineral density), and Friedman test with pairwise comparisons (body mass, CISI, blood lipid profile). To measure the association between knee joint damage, body mass, percent body fat, and metabolic profile at week 24 in the experimental protocol, the Spearman Correlation Coefficient was calculated. Analyses were done using SPSS V25.0 software (SPSS, Chicago, IL, USA). Data were considered statistically significant at $p < 0.05$, two-sided-test. Significance values were adjusted using Bonferroni corrections for multiple tests.

4.3 Results

4.3.1 Knee Joint Histology

Prebiotic fibre supplementation, moderate aerobic exercise, and the combination of prebiotic fibre supplementation and moderate aerobic exercise did not prevent the progression of knee joint damage observed in rats fed a HFS diet. These interventions also did not accelerate the progression of knee joint damage (Total Modified Mankin Score: HFS = 50 ± 7.6, HFS+F = 47 ± 6.3, HFS+E = 50 ± 7.2, HFS+F+E= 53 ± 6.7 – Figure 4.2). Interestingly, the age-matched (36 weeks) chow fed rats had similar joint damage scores as the HFS fed rats (Chow 51 ±7.2).

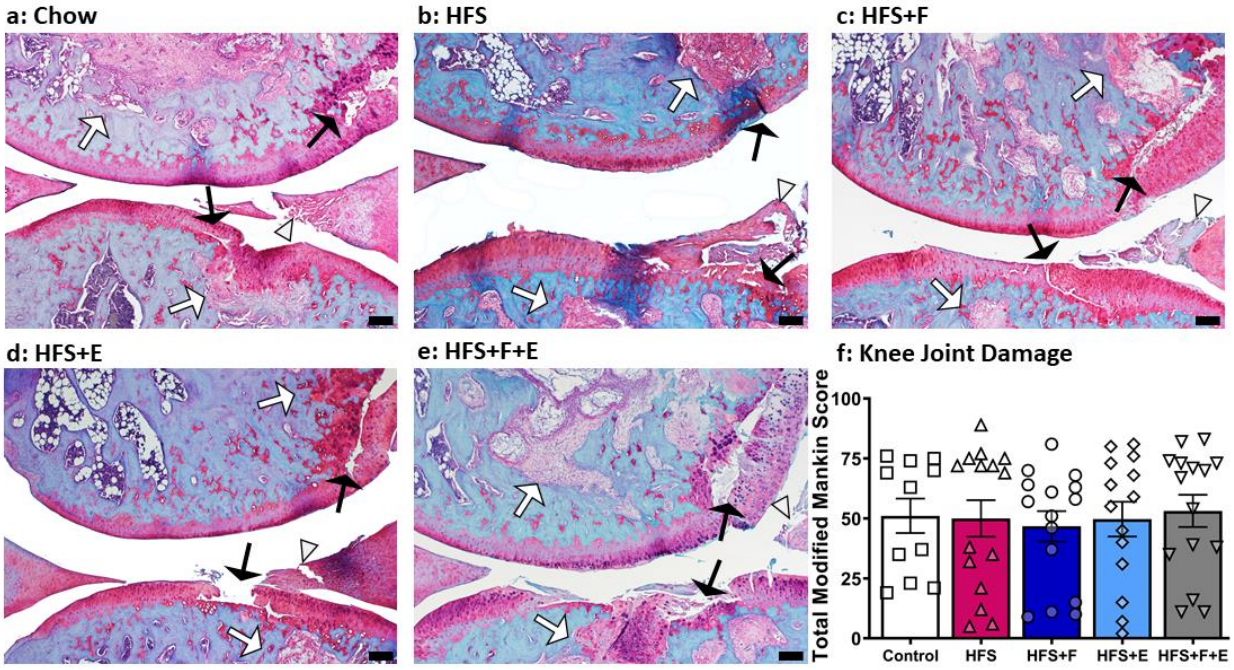

Figure 4.2. Knee joint damage. Images represent the mean rat joints for each experimental group. White thick arrows indicate subchondral bone damage. Black thin arrows indicate cartilage defect. White arrow heads indicate meniscal damage. Chow: chow control diet; HFS: high-fat/high-sucrose diet; F: prebiotic fibre; E: aerobic exercise. Black bar = 200 μm). Values are means ± 1 SEM.

4.3.2 Body Mass and Body composition

The mean body mass at the beginning of the experimental protocol was 489 g (SEM: ± 4.7). Body mass increased for all groups from week 1 to week 12 (before the intervention started). At week 12 of the experiment protocol, the HFS fed rats were heavier (795 g ± 11.0) than rats fed the control-chow diet (652 g ± 15.6; $p<0.05$). From week 12 to 13, there was a slight decrease in body mass, likely due the midpoint measurements performed at the end of week 12. In week 13, the exercise and fibre interventions started, and rats in the control-chow, HFS, and HFS+F group continued to gain weight, while rats in the HFS+E and HFS+F+E groups did not (Figure 4.3a). At week 24, rats in the HFS+E and HFS+F+E had similar body mass as rats fed a control-chow diet ($p>0.05$; 796 g ± 25, 756 g ± 25, and 731 g ± 19, respectively), while rats in the HFS (943 g ± 24) and HFS+F (893 g ± 32) groups were heavier than control-chow fed rats ($p<0.05$).

At week 12 (prior to the interventions), the percent body fat for the rats fed a HFS diet was higher than that measured for the chow fed rats ($p<0.05$, 33.5% and 15.5% respectively, Figure 4.3b). At week 24, rats in the HFS+E and HFS+F+E groups a had lower percent body fat when compared to HFS group animals ($p<0.05$). Rats on the control-chow diet, HFS diet, and in the HFS+F group had a significant increase in percent body fat from week 12 to week 24 ($p<0.05$), while rats in the HFS+E did not have a change in percent body fat ($p=0.572$), and rats in the HFS+F+E had a decrease in percent body fat ($p=0.003$) from week 12 to week 24.

There were no differences in bone mineral density at week 12 (Figure 4.3c). At week 24, there were no differences in bone mineral density between control-chow and HFS group rats. However, HFS+F group rats had a higher bone mineral density than HFS and HFS+E group

rats ($p<0.05$, figure 4.3c). Rats in all groups had an increase in bone mineral density from week 12 to week 24 ($p<0.05$).

There was no association between knee joint damage, percent body fat and body weight at week 24 (Table 4.2).

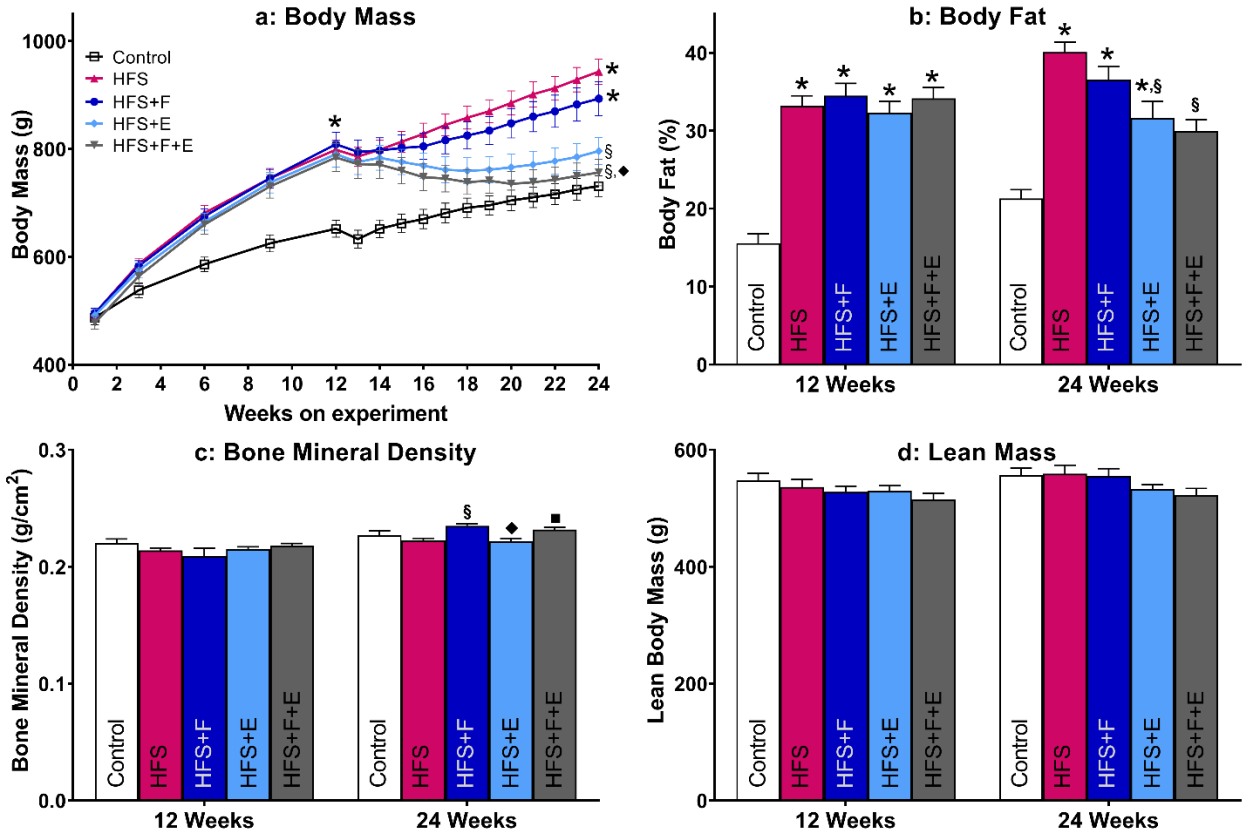

Figure 4.3. Body composition. a: Change in body mass during the experiment protocol. b: percent body fat, c: bone mineral density, and d) lean body mass at week 12 and 24 in the experimental protocol. * Indicates difference from chow group, while § indicates difference from HFS group, ♦ indicates difference from HFS+F; and ■ indicates difference from HFS+E. HFS: high-fat/high-sucrose diet; F: prebiotic fibre; E: aerobic exercise. Values are means ± 1 SEM.

Table 4.2. Relationship between knee joint damage (Total Modified Mankin Score), body mass, percent body fat and rat metabolic profile ate week 24 in the experimental protocol. r_s: Spearman's correlation coefficient; CISI: composite insulin sensitivity index; HDL: high density lipoprotein; LDL: low density lipoprotein.

	Knee Joint Damage	
	r_s	**p-value**
Body mass	-0.094	0.440
Percent body fat	-0.092	0.449
CISI	0.102	0.403
Total cholesterol	0.027	0.823
HDL-cholesterol	0.030	0.806
LDL-cholesterol	0.027	0.823
Non-HDL-cholesterol	0.420	0.732
Triglycerides	-0.060	0.623

4.3.3 Systemic Metabolic Alterations

4.3.3.1 Blood Glucose and Serum Insulin

At baseline, insulin sensitivity was similar across the 5 experimental groups, ranging from 1 to 1.5 ($p > 0.05$, Figure 4.4a). At week 12, there was a significant decrease in insulin sensitivity for all groups fed a HFS diet when compared to rats fed a chow control diet ($p<0.05$). At week 24, HFS group rats had lower insulin sensitivity than rats in the chow-control group ($p=0.004$). No differences where detected between HFS+F, HFS+E, HFS+F+E group animals when compared to chow fed rats ($p > 0.05$); and when compared to the HFS group rats ($p > 0.05$).

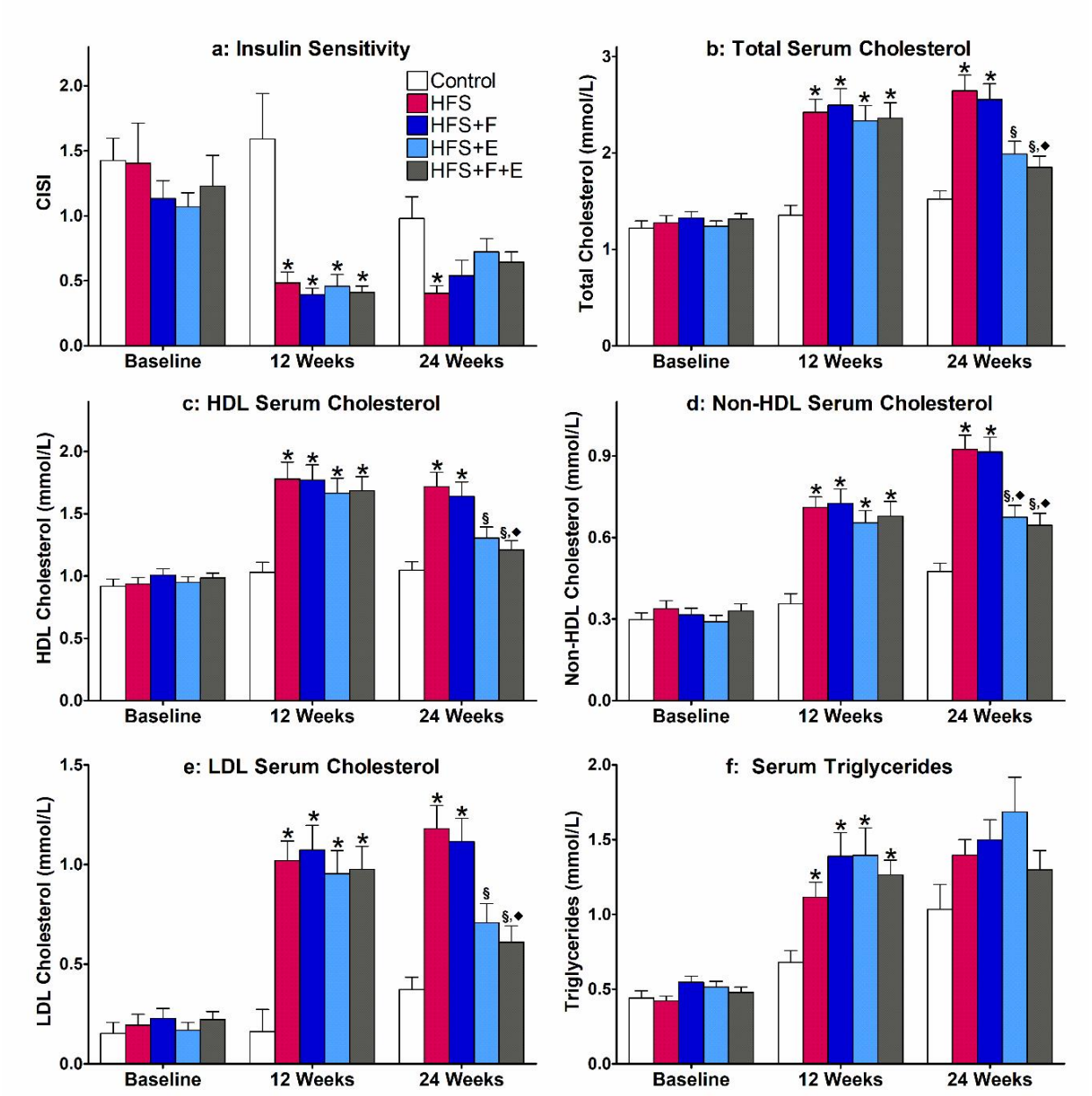

Figure 4.4. Metabolic profile. Composite insulin sensitivity index, and serum lipid profile for rats in the control, HFS, HFS + F, HFS + E, HFS + F + E groups at baseline, week 12, and week 24. * Indicates difference from chow group, while § indicates difference from HFS group, and ♦ indicates difference from HFS+F. HFS: high-fat/high-sucrose diet; F: prebiotic fibre; E: aerobic exercise. Values are means ± 1 SEM.

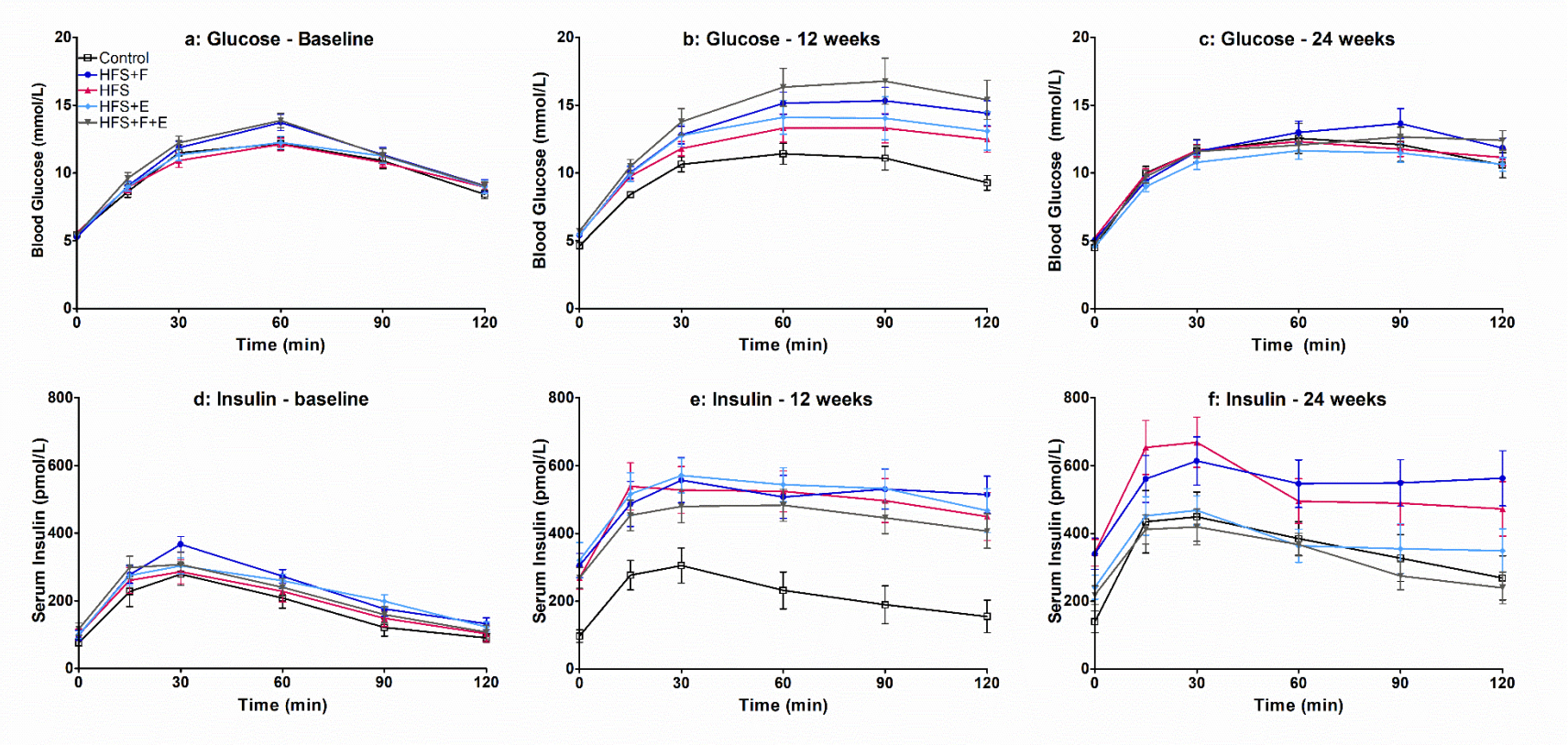

Figure 4.5. Glucose and insulin curves during glucose challenge at baseline, week 12, and week 24. HFS: high fat/high sucrose diet; F: prebiotic fibre; E: aerobic exercise. Values are means ± 1 SEM.

Further analyses revealed that the rats fed the chow diet had the same insulin sensitivity at baseline and week 12 ($p=0.99$), but their insulin sensitivity decreased from week 12 to week 24 ($p = 0.005$). Rats fed a HFS diet had a significant decrease in insulin sensitivity from baseline to week 12 ($p<0.05$). Rats in the HFS, and HFS+F, HFS+E, and HFS+F+E groups had similar insulin sensitivity at weeks 12 and 24 ($p > 0.05$).

Baseline values for blood insulin and glucose were similar for all experimental group animals (Figure 4.5). At week 12, the blood insulin and glucose values were different for animals in the HFS group. Specifically, the glucose and insulin values for the HFS group animals did not recover to fasted values (time 0 min) at the end of the 120 minutes, while control group rats showed a recovery within 60 and 90 min. At week 24, blood glucose levels were similar for all groups. Insulin levels at the week 24 remained increased compared to baseline (week F) measurements. Additionally, at week 24, insulin levels for chow fed rats did not recover within the 120 minutes testing period to pre-glucose challenge levels (Figure 4.5c and 4.5f). There was no association between knee joint damage and CISI at week 24 (Table 4.2).

4.3.3.2 Serum Lipid Profile

At baseline, there were no differences in serum lipid profiles between groups. At week 12, the HFS fed rats had an increase in total serum cholesterol, HDL cholesterol, non-HDL cholesterol, LDL cholesterol, and triglyceride levels compared to the control-chow fed rats ($p<0.05$, Figure 4.4). At week 24, with the exception of triglycerides, the HFS and HFS+F group rats had higher serum lipid levels than the control-chow fed rats; while rats in the HFS+E and HFS+F+E groups had similar serum lipid levels as the chow-control rats ($p > 0.05$, Figure 4.4). There were no differences in triglyceride levels between groups at week 24.

Total serum cholesterol, HDL cholesterol, LDL cholesterol, and triglyceride levels increased from baseline to week 12 for the HFS fed groups ($p > 0.05$); while only HDL cholesterol levels were increased from baseline to week 12 for control-chow fed rats. From week 12 to week 24, the total serum cholesterol did not change for rats in the control-chow fed, HFS, HFS+F, and HFS+E group animals, and decreased for the HFS+F+E group animals ($p = 0.019$). However, total serum cholesterol was increased for all experimental groups from baseline to week 24 ($p < 0.05$).

From week 12 to week 24, HDL serum cholesterol levels did not change for rats in the control-chow fed, HFS, and HFS+F groups, and decreased for rats in the HFS+E ($p = 0.024$) and HFS+F+E ($p = 0.006$) groups. From week 12 to week 24, total triglyceride levels did not change for rats in any of the five experimental groups ($p > 0.05$), but from baseline to week 24, triglyceride levels increased in all groups ($p <0.05$).

There were no changes from baseline to week 12 for non-HDL serum cholesterol and LDL serum cholesterol for the chow-fed rats. The non-HDL serum cholesterol and LDL cholesterol were increased for the four HFS group rats from baseline to week 12. From week 12 to week 24, the non-HDL serum cholesterol did not change for rats in the HFS+E and HFS+F+E groups, while it increased for control, HFS, and HFS+F groups. From week 12 to week 24, total LDL serum cholesterol did not change for rats in the control-chow fed, HFS, HFS+F, and HFS+E groups, and decreased for HFS+F+E group rats. LDL serum cholesterol increased from baseline to week 24 for control-chow fed rats. There was no association between knee joint damage and lipid profile at week 24 (Table 4.2).

4.4 Discussion

While there is evidence that early exercise and prebiotic fibre supplementation can prevent the development of OA in a rat-model of diet-induced metabolic disturbance (Rios *et al.*, 2019a), it remains unknown if these interventions remain successful in preventing or slowing the progression of OA when started at time points after the introduction of the HFS diet and once knee OA-like damage has been established to a certain degree. Therefore, the purpose of this study was to determine the effects of prebiotic fibre supplementation, aerobic exercise, and the combination of prebiotic fibre and aerobic exercise on the progression of knee OA-like damage in a HFS diet-induced rat-model of metabolic disturbance. We used a CD-Sprague-Dawley-model of diet-induced metabolic disturbance, that has been shown to produce reliable and consistent knee OA-like damage as early as four weeks following HFS diet exposure (Collins *et al.*, 2019), with consistent increase in OA severity at 12 (Collins *et al.*, 2015c; Rios *et al.*, 2019a) and 28 weeks following HFS diet exposure (Collins *et al.*, 2015b). We exposed this model to an aerobic exercise intervention and a prebiotic fibre supplementation at 12 weeks following HFS diet exposure to evaluate the effects of these interventions on the progression of knee OA-like damage. We showed that neither prebiotic fibre supplementation nor aerobic exercise changed the progression of existing knee OA-like damage in this pre-clinical model, despite improving selected markers of metabolic disturbance for some of the interventions. However, these results need to be considered in view of the fact that the chow fed control rats had similar knee OA-like damage as all the experimental HFS fed rats, suggesting that factors other than diet played a role in the progression of the knee OA-like damage observed in this study. Furthermore, none of the metabolic markers measured in this study showed an association with the observed knee joint damage, thus excluding the possibility that differences in

metabolic related factors could explain the findings of this study. These findings are insofar of interest since all experimental groups showed a dichotomy in the knee OA outcomes: some animals showed severe bone marrow lesions and had high damage scores, while other animals did not show such lesions, and had low damage scores. However, none of our outcome measures was able to predict this dichotomy.

It has been reported previously that approximately 30% of the chow fed control group rats (36-40 weeks old) have similar knee joint damage as HFS fed rats (Collins *et al.*, 2016b). The authors attributed the similarities between control and HFS group rats to meniscal damage of the chow fed rats via unknown trauma. In our study, rats (36 weeks old) in the control-chow fed group had similar mean Total Modified Mankin Scores for the knee joint as the HFS fed group rats. Rats exposed to a HFS diet for 12 weeks, and at an age of 24 weeks during tissue harvest, show significantly greater knee joint damage than the corresponding control-diet animals. Therefore, it appears that the HFS diet accelerates knee joint damage in Sprague-Dawley rats through pathways identified earlier (Collins *et al.*, 2015c; Rios *et al.*, 2019a) in the short term, but does not seem to affect the knee joint damage scores in older (36 weeks) rats.

Our 36-week old rats fed a chow diet display signs of metabolic disturbance, exhibiting decreased insulin sensitivity and increased non-HDL cholesterol levels compared to when they were 24 weeks old. Triglyceride levels in 36-week old chow fed rats are similar to those of 24-week old HFS fed rats. Therefore, one might hypothesize that CD-Sprague-Dawley rats have age-related signs of metabolic disease that may lead to OA-like damage. However, markers of metabolic disturbance did not correlate with the damage observed in the knee joint. The metabolic disturbance in the chow fed rats may be related to the sedentary behavior imposed by the laboratory environment. Laboratory rats presented with a running wheel are highly

active, and they will exercise voluntarily (Leasure & Jones, 2008; Yancey & Overton, 1993), running at good speeds (40-50 m/min) for long distances ranging from 1.5 to 2.5 km per day.

Rats in the HFS+E and HFS+F+E groups had a better metabolic profile at week 24 compared to HFS group rats. However, metabolic markers did not return to their baseline values, suggesting that these rats still have some metabolic disturbance. It has been shown that elevated plasma cholesterol levels, as seen in our study, trigger oxidative stress in mitochondria and degradative changes in chondrocytes that, in combination, are thought to lead to OA development and progression (Farnaghi *et al.*, 2017). Additionally, drugs that lower lipid levels inhibit OA development in mouse models of atherosclerosis (Gierman *et al.*, 2014). Perhaps, combining the exercise intervention, or the exercise plus fibre intervention, with a change in diet (e.g.: switch to a chow diet), may have allowed for a return to the basal metabolic profile levels, and may have prevented the progression of knee joint damage in this rat model of metabolic OA.

In summary, moderate aerobic exercise, and the combination of prebiotic fibre supplementation and moderate aerobic exercise improved markers of metabolic disturbance. However, the interventions did not slow/stop, nor did they accelerate, the progression of knee OA-like damage. Therefore, exercise and prebiotic fibre supplementation may be safe strategies for populations with metabolic knee OA who chose exercise and/or prebiotic to alleviate metabolic disturbances. However, these conclusions need to be considered in view of the fact that the chow fed rats had similar knee OA-like damage as the HFS fed rats, and that there was a distinct dichotomy in knee joint damage scores across all experimental groups without a satisfactory explanation why some animals were well protected from damage while others were not.

Chapter Five: Integration, Limitations, and Opportunities

The purpose of this chapter is to integrate the series of studies presented in this thesis, raise further gaps in this research area, as well as to acknowledge the limitations of our studies. This thesis was aimed at determining the effects of aerobic exercise and prebiotic fibre supplementation on the onset and rate of progression of knee OA in a diet-induced model of metabolic disturbance. We hypothesized that prebiotic fibre supplementation, and/or an aerobic exercise regime, will prevent, slow down, and/or stop the progression of OA-like damage in the knees of rats fed a high-fat/high sucrose diet. However, before testing our hypotheses, we needed to know if exercise and prebiotic fibre supplementation were safe strategies for CD-Sprague-Dawley rats.

In the literature, we found evidence that prebiotic fibre supplementation was a safe intervention for CD-Sprague-Dawley rats (Cluny *et al.*, 2015; Parnell & Reimer, 2009), therefore, there was no need to re-test this intervention again. Furthermore, prebiotic fibre supplementation has been shown to be a safe intervention for humans (Nicolucci *et al.*, 2017), thereby ensuring that this intervention could potentially be used to translate findings to humans. However, to our knowledge, there was no literature on the safety of treadmill exercise to the knee joint of otherwise healthy CD-Sprague-Dawley rats. Furthermore, the literature for other rat strains was controversial. For example, when Wistar rats, without previous lesions in the knee, were randomly assigned to a sedentary control group, and either a low-intensity, medium-intensity, or a high-intensity treadmill training group, the knee joint damage was variable: rats in the high-intensity treadmill training group had OA-like changes in their knees, while rats in the other running groups did not (Ni *et al.*, 2013). With this controversy in mind, we designed the study presented in chapter two to test for the effects of treadmill exercise in the CD-Sprague-Dawley rat. We determined that

moderate, high and extra-high duration treadmill exercise had no detrimental effects on knee joint health, function and integrity. Therefore, we concluded that treadmill exercise at any tested duration was a safe intervention for CD-Sprague-Dawley rats, and could be used as a safe exercise intervention in a pre-clinical rat model of knee OA. We believe that the load applied to the knee through the exercise intervention protocol presented in chapter two was likely within the physiologic loading window that has been suggested to allow for adaptation, remodeling, and the proper functioning of cells and tissues (Eckstein *et al.*, 2006; Hart *et al.*, 1995a; Hart & Scott, 2012; Hunter *et al.*, 2015; Maly & Robbins, 2014). Another aspect of our study was that rats were allowed a prolonged adaptation period, and were introduced progressively to the increasing speed and duration of the exercise protocol (Table 2.1), a factor which we believe to be critical for the joint health outcomes. The gradual increase in speed and duration of the exercise sessions may have allowed the cartilage to adjust gradually to the increasing load requirements, operating within the physiological window. The adaptive training phase in our study was 4 weeks, and has been suggested to trigger increases in fitness and health in Wistar rats (Hohl *et al.*, 2009), and may be an important factor in the protection of the knee joint from exercise-induced damage. However, it was not known at the start of my thesis work, if treadmill exercise at higher intensities (higher speeds/ longer durations/ shorter rest intervals) was detrimental to knee joint integrity. We also did not know if the specific treadmill exercise protocol used by us might be detrimental to other joints, as we did not evaluate other joints specifically, which is one of the limitations of our study. Moreover, experiments were performed in 12-week old male CD-Sprague-Dawley rats, and it is unknown if the effects observed by us would be the same in female rats, and rats at different ages. These limitations should be addressed in future studies.

Even though treadmill exercise has been used in other rat strains, such as in Wistar rats, the intensities and adaptation periods were not the same as those used by us. These methodological differences might be one of the reasons why our results differ from others. To our knowledge, there is no study in which an adaptive training phase was used in order to protect the knee joint from OA-like damage. I hypothesise that a stepwise increase in the speed and duration of a treadmill exercise program does not lead to OA-like damage in the rat knee (for any rat strain), and that treadmill exercise programs provide a protective effect to knee joints if the program is designed to expose the animals (including humans) to an optimal physiological exercise window.

Once we established that prebiotic fibre supplementation and aerobic treadmill exercise programs were safe intervention strategies, we could use them as interventions aimed at preventing/treating joint damage in a CD-Sprague-Dawley rat-model of high-fat/high-sucrose diet. Therefore, we felt confident proceeding with the study presented in chapter three. In chapter three, we used a CD-Sprague-Dawley rat-model of high-fat/high-sucrose diet that has been shown to cause reliable and consistent knee OA (Collins *et al.*, 2015a, 2016b; Collins *et al.*, 2015b; Collins *et al.*, 2015c), and evaluated the effects of prebiotic fibre supplementation, aerobic exercise, and the combination of prebiotic fibre supplementation and aerobic exercise on the development of knee OA. Although several aspects of joint and metabolic health had been investigated in this CD-Sprague-Dawley rat-model of high-fat/high-sucrose diet, it was not known how this high-fat/high-sucrose diet affects insulin sensitivity, cholesterol (total, HDL, LDL, non-HDL), and triglycerides when rats were exposed to this diet over a 12 or 24 week period. It was also not known if insulin sensitivity, and changes in cholesterol, and/or triglyceride levels are associated with knee joint damage. Also, we hypothesized that these metabolic changes might directly affect joint health, and therefore incorporated them into the studies presented in chapters three and four.

Findings presented in chapter three indicated that prebiotic fibre supplementation, moderate aerobic exercise, and the combinations of prebiotic fibre and exercise prevented knee joint damage that was otherwise observed in this model. Furthermore, we found that the preventive effects of the prebiotic fibre and aerobic exercise interventions were associated with serum leptin, glucose/insulin, lipid, and LPS levels. These results were exciting, because prebiotic fibre supplementation and aerobic exercise are low cost, non-invasive strategies, and they can be readily implemented into clinical trials. I believe that prebiotic fibre supplementation and aerobic exercise interventions should be a priority in public health programmes, and should be implemented as a matter of course in schools in order to prevent not only metabolic disturbances, but also musculoskeletal diseases in children and adolescents. Given the efficacy of prebiotic fibre supplementation and aerobic exercise in preventing metabolic disturbance-related knee OA, the findings of this study should be tested in clinical trials, targeting knee OA patients with signs of metabolic disturbance. However, it is important to highlight that more studies are needed to fill the gap between metabolic disease and OA pathogenesis. In this thesis, we showed statistical associations between markers of metabolic disturbance and knee OA. In a next step, it is necessary to study mechanistic pathways that may relate these metabolic disturbances with the onset and progression of OA. However, caution should be exercised when interpreting results from chapter three, and the findings presented for chow-fed rats in chapter four must be considered in this context.

We do not know the exact mechanism by which prebiotic fibre and/or aerobic exercise prevented OA-like damage in this model. However, it has been show that the oligofructose fibre supplementation used in our study prevents dyslipidemia, thereby potentially contributing to the maintenance of knee joint homeostasis. For example, drugs that lower lipid levels inhibit

OA development in a mouse model of atherosclerosis (Gierman *et al.*, 2014). Elevated plasma cholesterol levels trigger oxidative stress in mitochondria and degradative changes in chondrocytes that, in combination, are thought to lead to OA development and progression (Farnaghi *et al.*, 2017). Moreover, mouse models of hypercholesterolemia-induced OA are characterized by changes in subchondral bone architecture that are rescued when lipid-lowering drugs are administrated (Farnaghi *et al.*, 2017). It has been suggested that hypercholesterolemia induces oxidation and deposition of lipids in tissues, resulting in damage to cartilage similar to that seen in atherosclerosis (Sevin *et al.*, 2013), while osteonecrosis might occur because of a narrowing of the vasculature by lipids. Additionally, improvements in glucose tolerance in rodents after prebiotic fibre supplementation are related to changes in the microbiota (Cani *et al.*, 2007b; Parnell & Reimer, 2012).

Aerobic exercise in our rats was found to modulate insulin and leptin levels, similar to what has been observed for the prebiotic fibre supplementation. Aerobic exercise in humans is also associated with decreased plasma leptin and insulin levels (Essig *et al.*, 2000). Thus, maintenance of insulin homeostasis in the exercising rats may have prevented increases in leptin and leptin resistance in rats fed a HFS diet. Therefore, aerobic exercise may have helped maintain bone turnover homeostasis, thereby preventing the development of OA-like damage in the knees of rats fed the HFS diet. Aerobic exercise also prevented increases in serum triglyceride levels and serum triglycerides to HDL-cholesterol ratio observed in rats exposed to a HFS diet. And by preventing dyslipidemia, aerobic exercise may have contributed to the maintenance of knee joint integrity in the HFS rats.

While prebiotic fibre supplementation and aerobic exercise prevented the development of knee damage, and affected the metabolic outcome variables to a similar extent, exercise did

not affect the gut microbiota, while the prebiotic fibre intervention did. Therefore, it appears that the prebiotic intervention affected the microbiota, and as a consequence, the host secondarily via the fermentation of the prebiotic fiber, short chain fatty acids (Cani *et al.*, 2007a) (not assessed in our study). In contrast, the exercise protocol primarily affected host parameters to diminish the impact of the HFS diet and the altered microbiota. Thus, the exercise protocol appeared to impact the metabolic profile and the gut leaky syndrome by increasing the uptake of endotoxins by Kupffer cells (Komine *et al.*, 2017). Aerobic exercise combined with prebiotic fibre supplementation was not more effective as each intervention alone. Therefore, it appears that the mechanisms involved for each intervention complement each other but are not additive. Therefore, the impact of the HFS diet on a sedentary host is different than its impact on a host that is exercising and enhancing the resistance of host systems. Exercise is expected to contribute to tissue repair, enhance the metabolism of glucose and fat, and enhance the release of exercise-associated regulatory molecules from the brain to counteract diet-induced factors (Phillips *et al.*, 2014). It is known that mechanical loading of tissues, such as cartilage, ligaments, tendons, and skin, can lead to resistance to catabolic mediators (Ballyns & Bonassar, 2011) or their expression (Natsu-Ume *et al.*, 2005) . Therefore, exercise-induced tissue loading may result in the maintenance of tissue integrity. The non-exercised rats in this study had reduced tissue loads compared to the exercised rats, and their systems might have been at an increased risk for alteration caused by the HFS diet. It appears that exercise and prebiotic fibre supplementation impact HFS fed-animals differently. Providing both interventions simultaneously may lead to a reinforcement of resistance to the adverse effects of the HFS diet over 12 weeks.

Once the efficacy of prebiotic fibre supplementation and aerobic exercise in preventing metabolic disturbance-related knee OA was demonstrated in this particular animal model, it was important for us to test if these interventions might also be useful in slowing down or stopping knee OA that was already progressing. Therefore, in chapter four, we tested this idea by introducing the fibre/exercise intervention strategies 12 weeks after first exposure to the high-fat/high-sucrose diet. We determined that neither prebiotic fibre supplementation nor aerobic exercise were able to stop the progression of existing knee OA induced (or that was thought to be induced) by metabolic disturbance. However, the interventions did not accelerate the rate of progression of knee OA, suggesting that exercise and prebiotic fibre supplementation may be safe strategies for populations with metabolic knee OA, who may want (or need) to exercise to alleviate other health related problems, such as diabetes or cardiovascular problems. Interestingly, in our study, 36-week old rats fed a standard diet displayed signs of metabolic disturbance, as well as OA-like damage in their knee joints. Thus, the question arose: is the knee joint damage in these groups of rats caused by aging, metabolic disturbance, or other factors? One way to try to address this question would be to exercise chow-fed rats to try to keep them metabolically healthy, and to test if their knees are protected from OA-like damage.

Although, there was no evidence that the 12-week prebiotic fibre and/or exercise intervention performed in the study presented in chapter four reduced the rate of OA-like damage progression in our Sprague-Dawley-model of metabolic-OA, it might have improved function and pain in our cohort. We did not measure pain or function, and it is a limitation of our study. For example, clinical trials have demonstrated that exercise improves self-reported measures of knee function and pain (Messier *et al.*, 2004), while data from prospective cohorts have

shown that a diet rich in fibre lowers the risks of moderate or severe knee pain regardless of radiographic knee osteoarthritis status (Dai *et al.*, 2017).

In summary, this thesis provides insight into two different ways to prevent the development of metabolic osteoarthritis phenotype: moderate treadmill exercise, and prebiotic fibre supplementation. However, the experiments described in this thesis have limitations. As this thesis represented an effort to describe and characterize the effects of moderate exercise and prebiotic fibre supplementation on the knee joint of rats fed a high-fat/high-sucrose diet, sample sizes were calculated based on the primary knee joint outcomes, and might not be properly powered for secondary outcomes. Moreover, the primary knee joint outcomes in this thesis were cross-sectional, and considering serial time-course measurements (e.g.: µ-CT) would be optimal to describe the progression of the disease. Additionally, it would be important to describe and characterize the effect of moderate exercise and prebiotic fibre supplementation on other joints (such as shoulders and hips) and tissues (muscles and tendons). In appendix A we explored the mechanical and biochemical properties of tail tendons in this rat model of high-fat/high-sucrose diet, and we also studied the effects of moderate exercise and prebiotic fibre supplementation. To our surprise, a high-fat/high-sucrose diet challenge for 12 weeks did not alter the mechanical and biochemical properties of the rat tail tendon when compared to age-matched, chow fed rats.

Another limitation of the studies presented in this thesis is that only male CD-Sprague-Dawley rats were evaluated. Due to the higher incidence of OA in women, future studies should consider incorporating females. Additionally, other animal models should be evaluated, as we do not know if the changes observed in the knee joint induced by a 12-week exposure to a high-fat/high-sucrose diet are exclusive to CD-Sprague-Dawley rats. Moreover, based on finding presented in chapter four, the high-fat/high-sucrose diet seems to accelerate the natural occurrence

of knee OA-like damage in the CD-Sprague-Dawley rats. Thus, knee OA-like damage observed in rats fed a high-fat/high-sucrose diet does not seem to be caused solely by the diet, and caution should be taken when designing future studies to investigate the metabolic OA phenotype.

Based in some of the findings presented in this thesis, I speculate that:

1) CD-Sprague-Dawley rats, when restricted to cage activity only, and fed a standard chow diet, will show signs of metabolic disturbance and knee joint damage (between 24 and 36 weeks old, as shown in chapter four), which might be attributed to sedentary behaviour, and/or aging.
2) CD-Sprague-Dawley rats that are fed a standard chow diet and are exposed to exercise (within a physiological window) early in life, or receive a prebiotic fibre supplementation, might have a delay in knee joint damage (not investigated in this thesis).
3) A HFS diet might accelerate knee joint damage and induce metabolic disturbance in CD-Sprague-Dawley rats that are restricted to cage activity when the diet is introduced at week 12 of life (as shown in chapter three). Knee damage has been reported to be present as early as four weeks into the HFS diet challenge (Collins *et al.*, 2019).
4) Prebiotic fibre supplementation and aerobic exercise, when started at the same time as the HFS diet exposure, will delay knee damage in CD-Sprague-Dawley rats (as shown in chapter three). However, if these interventions start at a time point after the introduction of the HFS diet, and once knee damage has been established to an advanced degree, these interventions will not prevent or slow the progression of joint damage (as shown in chapter four), although they will help to improve signs of metabolic disturbance.

Finally, can findings from this preclinical model be extended to humans? As discussed in chapter one, section 1.2.1, one of the main limitations of animal models is the inability to directly translate the knowledge acquired in animal studies to humans. However, animal models can still be useful and may represent aspects of disease processes in humans (Quinn, 2005). Thus, differences in anatomy, physiology, and developmental biology between species must be considered when attempting to translate the results presented in this thesis to humans. Specifically regarding rat models, one need to consider that rats maintain a growth plate into old age (Roach, Mehta, Oreffo, Clarke, & Cooper, 2003), which might interfere with joint disease onset and progression, making it difficult to translate some of our findings presented in this thesis to humans.

5.1 Conclusion

In summary, findings of this thesis indicate that prebiotic fibre and/or aerobic exercise prevented knee joint damage in a rat model of metabolic disturbance. However, neither prebiotic fibre supplementation nor aerobic exercise were able to stop the progression of existing knee OA induced (or thought to be induced) by metabolic disturbance. However, the interventions did not accelerate the rate of progression of knee OA, suggesting that exercise and prebiotic fibre supplementation may be safe strategies for populations with metabolic knee OA, who may want (or need) to exercise or supplement their diet with prebiotics to alleviate other health related problems, such as diabetes or cardiovascular problems. Therefore, such interventions should be a priority in public health programmes, and prebiotic fibre supplementation and aerobic exercise should be inserted in schools in order to prevent not only metabolic disturbance, but also musculoskeletal diseases. Future studies are needed to elucidate the pathways that may be involved

in the knee joint damage onset in chow and high-fat/high-sucrose fed CD-Sprague-Dawley rats when they are restricted to cage activities (sedentary behavior).

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Appendix A: The mechanical and biochemical properties of tail tendon in a rat model of obesity: effect of moderate exercise and prebiotic fibre supplementation

In this chapter we used a CD-Sprague-Dawley rat-model of diet-induced metabolic disturbance to determine the effects of a HFS diet in a non weight bearing tendon (tail tendon). This study was a further analysis of tissues harvested from rats used on the study presented in chapter there of this thesis. We also evaluated the effects of aerobic exercise, prebiotic fibre supplementation, and the combination of prebiotic fibre and exercise on the rat tail tendon when rats were exposed to a HFS. My role is this study was in designing the study, execution of the study, data collection, data analysis, interpretation of data, drafting the manuscript, revising the manuscript, and approving the final submitted version.

7.1 Introduction

Obesity is associated with a variety of co-morbidities and chronic diseases, including cardiovascular disease (Nangia, Singh, & Kaur, 2016), impaired fasting glucose (Vaidya *et al.*, 2016), cancer (Renehan, Zwahlen, & Egger, 2015; Soguel, Durocher, Tchernof, & Diorio, 2016), and a variety of musculoskeletal diseases (Castro, Skare, Nassif, Sakuma, & Barros, 2016; Pottie *et al.*, 2006; Stenholm *et al.*, 2008).

Tendinopathy in obesity has gained special attention. Plantar fasciitis is a commonly observed tendinopathy in overweight and obese subjects, and has been linked to an increased stress on the passive structure of the foot due to overloading (Frey & Zamora, 2007; Irving, Cook, Young, & Menz, 2007). However, tendinopathies related to obesity have also been observed in non-weight bearing tendons such as the rotator cuff (Gumina *et al.*, 2014) and elbow tendons (Titchener *et al.*,

2013). Features associated with tendinopathy have also been reported in the tail tendon of obese animals. For example, the tail tendon in obese mice that were fed a high fat diet, was found to be softer than in lean mice (Eriksen *et al.*, 2014). Additionally, ultrastructural analysis of the deep digital flexor tendon of rats showed differences in collagen fibril diameter distribution and mass-average diameter between obese (genetically obese (*fa/fa*) male Zucker rats) and lean (*Fa/Fa* or *Fa/fa* male Zucker rats) animals (Biancalana, Veloso, & Gomes, 2010). These structural modifications affected the mechanical properties leading to a significant increase in maximum displacement and strain to failure in obese animals (Biancalana *et al.*, 2010).

Tendon damage has been associated with metabolic disorders resulting from obesity (Abate, Salini, & Andia, 2016; Castro *et al.*, 2016). Adipose tissue releases bioactive peptides and hormones (e.g., cytokines, prostanoids, and metalloproteinases) (Coelho, Oliveira, & Fernandes, 2013; Coppack, 2001; Guerre-Millo, 2002), which can influence tendon structure via activities on mesenchymal cells, leading to a systemic state of chronic, sub-clinical, low-grade inflammation that may damage tendons. Furthermore, cytokines released by visceral fat have been linked to tendon-bone health. Several pro-inflammatory cytokines are increased in animals with greater visceral fat, including interleukin-6 (IL-6), tumour necrosis factor alpha (TNFα), and C-reactive protein. TNFα has been reported to play a role in the production of pain and tendon pathology, and is thought to be a key cytokine in systemic spondyloarthropathy, a bone-tendon junction disease (Anttonen, Orpana, Leirisalo-Repo, & Repo, 2006; Briot, Garnero, Le Henanff, Dougados, & Roux, 2005).

CD-Sprague-Dawley rats fed a high-fat/high-sucrose (HFS) diet are a well-established model of obesity. HFS fed rats have increased body fat and body mass, and have systemic inflammation and local inflammation within the knee (Collins *et al.*, 2015c). Furthermore, HFS

fed rats show structural changes in glycolytic skeletal muscles (Collins *et al.*, 2016c; Collins *et al.*, 2016d), and present with an increased rate of onset and progression of knee osteoarthritis (Collins *et al.*, 2015c) compared to lean control animals fed a regular diet. Intramuscular fat invasion, fibrosis, and increased numbers of pro-inflammatory cells have been observed as rapidly as 3-days following the onset of a HFS diet (Collins *et al.*, 2016d). However, tendinopathy has not been systematically studied in this model of obesity.

Exercise and dietary fibre supplementation are common countermeasures to combat obesity. Exercise reduces body fat (Donnelly *et al.*, 2003; Jeffery *et al.*, 2000), vascular inflammation (Pedersen, 2006; Pinto *et al.*, 2012), brain inflammation (Chennaoui *et al.*, 2015; Gomes da Silva *et al.*, 2013), and systemic inflammation (Conti *et al.*, 2015; Pedersen, 2006; Pinto *et al.*, 2012). Furthermore, it has been suggested that physical activity provides protection against the detrimental effects of obesity on the musculoskeletal system. For example, four weeks of wheel-running exercise reduced the progression of osteoarthritis in obese mice fed a high-fat diet, and also disrupted the co-expression of pro-inflammatory cytokines (Griffin *et al.*, 2012).

Similar to exercise interventions, dietary oligofructose fibre supplementation has been shown to attenuate increases in body fat, and is thought to improve metabolic health in a HFS diet rat model (Cluny *et al.*, 2015; Parnell & Reimer, 2009), and has been shown to have beneficial effects in humans (Hume, Nicolucci, & Reimer, 2015; Nicolucci *et al.*, 2017). Therefore, it appears reasonable to assume that exercise and oligofructose interventions might protect tendons from structural and mechanical damage in a diet-induced obesity (DIO) model. However, this has never been shown.

Therefore, the purpose of this study was (1) to determine the effects of a HFS diet on the mechanical and biochemical properties of the rat tail tendon, and (2) to evaluate the effects of

moderate exercise and fibre supplementation on the mechanical and biochemical properties of the tail tendon in adult rats fed a HFS diet. We hypothesized (1) that tendons of rats on a HFS diet have an increased content of collagen, and a higher failure strain compared to lean animals fed a regular diet, and (2) that exercise and fiber supplementation prevents the structural and mechanical changes induced by the HFS diet.

7.2 Methods

Thirty male, 10 to 12-week old CD-Sprague-Dawley rats, were housed individually and randomized into either a high-fat/high-sucrose (HFS, 20% of total weight as fat, 50% sucrose, 20% protein, and 10% from fibre and micronutrients; custom Diet #102412, Dyets, United States) diet-induced obesity group (HFS, n=24) or a lean age-matched control group (standard chow low fat diet group – LFD, 5% of total weight as fat, 47.5% carbohydrates (only 4% from sucrose), 25% protein, 12.5% from fibre and micronutrients, and 10% moisture; Lab Diet 5001, United States, n=6). Rats in the HFS group were divided into four subgroups: moderate exercise (DIO+E, n=6), dietary fibre supplementation (DIO+F, n=6), dietary fibre supplementation combined with moderate exercise (DIO+F+E, n=6), and sedentary (DIO, n=6). The moderate exercise intervention consisted of a progressive treadmill training program for 12 weeks, up to 30 minutes per day, 5 days a week (Rios *et al.*, 2018); which corresponds to the recommended minimal physical activity guidelines in humans (de Souto Barreto, 2015). The fibre supplementation diet consisted of 10% oligofructose (Orafti P95, BENEO-Orafti, Germany) (Cluny *et al.*, 2015). Four days following the intervention protocol, rats were lightly anaesthetized, body composition was measured via Dual X-ray absorptiometry (DXA), rats were then euthanized, and the tail harvested and frozen at -80°C. Body mass was measured at the beginning of each week, and was normalized

to the body mass measured in week one. All experiments were approved by the University of Calgary Life and Environmental Sciences Animal Care Committee, and all methods were conducted in accordance with the animal welfare regulations and guidelines at the University of Calgary.

7.2.1 Mechanical tests

On the day of mechanical testing, a tail was thawed at room temperature and individual tail tendons were dissected. Two tendons were tested from each tail. Dissected tendons were gripped by two clamps: one end was clamped to a load cell (100 N) of an MTS machine (Bionix, MTS Systems, Eden Prairie, MN, USA), the other end was clamped to the actuator piston for stretching of the tendon in a controlled manner. Tendons were irrigated with physiological saline and kept wrapped in a saline-saturated cloth between tests. Tendons were then stretched to a force of 1.5N. The length, width and thickness in the middle, and at the proximal and distal ends of the tendon were measured using calipers. Following a preconditioning protocol consisting of twenty stretch-shortening cycles of 0.5% strain at a speed of 2.5% strain per minute, tendons were subjected to two stress-relaxation tests. The stress-relaxation tests consisted of a stretch of 2% and 3% strain at a speed of 10% and 15% strain per minute, respectively. The tendon was then allowed to stress-relax for 2 minutes at the final length. The tendon was then stretched to failure at a constant speed of 12% strain/minute. Following the mechanical testing, tendons were kept at -80°C until hydroxyproline content determination.

Stress (σ) was calculated by dividing the force by the cross-sectional area, assuming that the tendon has a rectangular cross sectional shape (Seto, Gatt, & Dunn, 2009). The peak stress obtained during stretching, the steady-state stress after stress-relaxation, the maximal stress and

strain, the yield stress and strain, and the Young's modulus (Figure 7.1) were compared between groups. All mechanical tests were performed within three months of harvest.

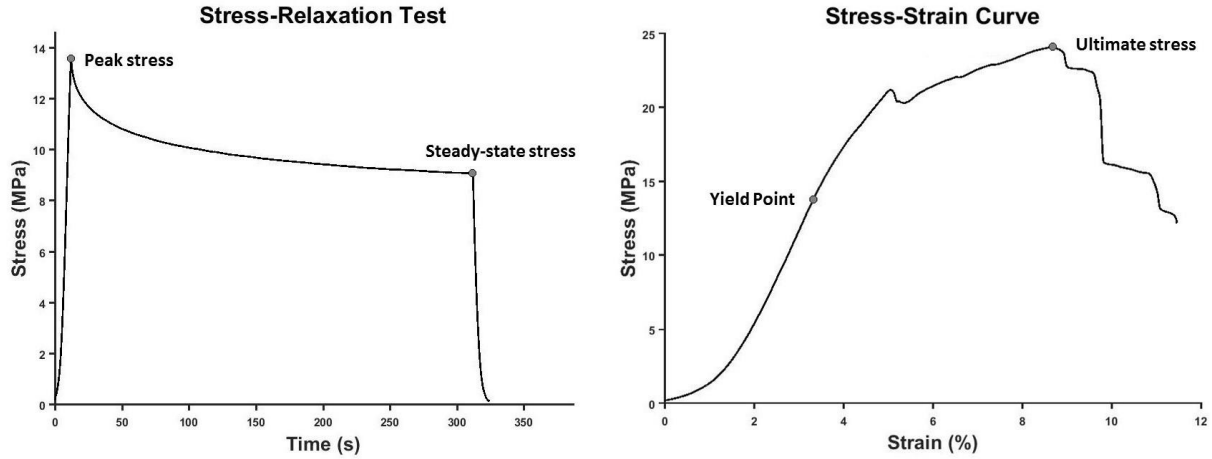

Figure 7.1. Stress-relaxation (left) and stress-strain (right) graphs from one representative tail tendon. Tendons were stretched to a force of 1.5 N. Following a preconditioning protocol consisting of twenty stretch-shortening cycles of 0.5% strain at a speed of 2.5% strain per minute, tendons were subjected to two stress-relaxation tests. The stress relaxation tests consisted of a stretch of 2% and 3% strain at a speed of 10% and 15% strain per minute, respectively. The yield point was defined as the point when the slope decreases by one unit from the maximum slope.

7.2.2 Hydroxyproline (Collagen) Content

Tendons were powdered using a cooled crusher in liquid nitrogen and lyophilized for 24h under vacuum. Lyophilized tendons were then incubated with 6N HCl for 18h at 105°C for hydrolysis. Samples were then cooled at room temperature, centrifuged, and hydroxyproline concentration was determined according to a colorimetric method (Woessner 1961) using 4-(Dimethylamino)benzaldehyde (Sigma Aldrich, hydroxyproline assay kit, MAK008).

7.2.3 Statistical Analysis

Kruskal-Wallis test was used to assess differences between groups for all variables (body mass, body fat, and mechanical and biochemical properties). If significant ($p < 0.05$), post hoc Mann-Whitney U-Test was used to indicate which groups differed. Body mass was normalized by

that measured at week 1 of the experimental protocol and expressed as a percent change in body mass.

7.3 Results

7.3.1 Body mass

Body mass increased for all groups of rats during the experimental period. Body mass increased less in LFD, DIO+E, and DIO+F+E group rats compared to DIO group rats, starting from week 3 of the experimental protocol ($p<0.05$, Figure 7.2). While rats in the DIO+F group exhibited a trend towards a reduced increase in body mass compared to DIO animals, starting at week 3 of the experimental protocol ($p<0.10$), this difference only became statistically significant ($p<0.05$) in week 9 (Figure7. 2).

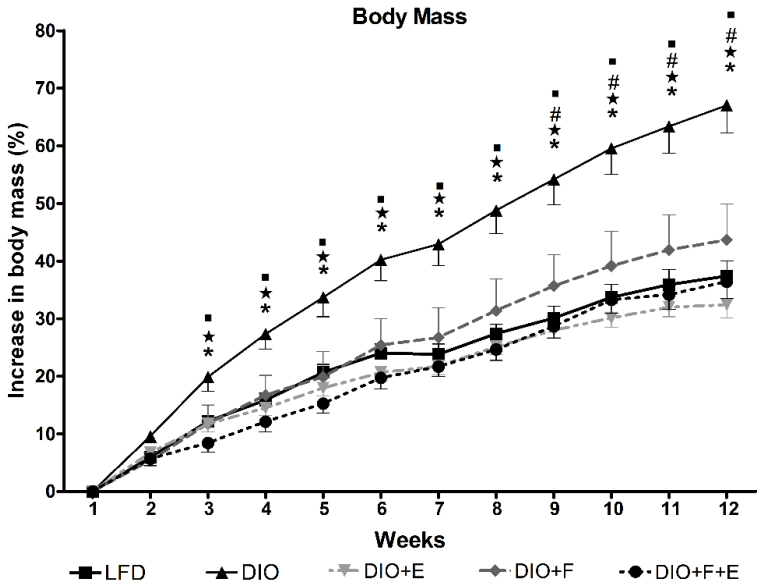

Figure 7.2. Mean (±1 SEM) percent increase in body mass from the body mass measured in week 1. * indicates a significant difference from LFD group rats. ★ indicates a significant difference from DIO+E group rats. # indicates a significant difference from DIO + F group rats. ■ indicates a significant difference from DIO+F+E group rats. LFD: low fat diet group; DIO: diet induced-obesity group; DIO+E: diet induced-obesity combined with moderate exercise group; DIO+F: diet induced obesity combined with fiber supplementation group; DIO+F+E: diet induced obesity combined with fiber supplementation and exercise group.

7.3.2 Body fat composition

DIO, DIO+E, and DIO+F group rats had 16%, 7%, and 8% more body fat than LFD group rats ($p<0.05$), respectively. DIO+E+F group rats had the same percentage of body fat as the LFD group rats, and a lower percentage of body fat than the DIO, DIO+E, and DIO+F group rats ($p<0.05$, Figure 7.3).

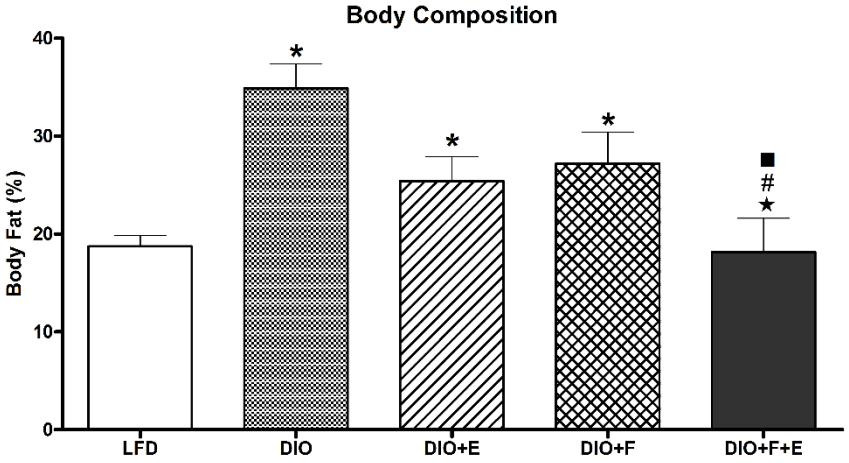

Figure 7.3. Mean (+ 1SE) percent body fat measured at the end of the experimental protocol. *indicates a significant difference from LFD group rats. ■ indicates a significant difference from DIO group rats. ★ indicates a significant difference from DIO+E group rats. # indicates a significant difference from DIO+F group rats. LFD: low fat diet group; DIO: diet induced-obesity group; DIO+E: diet induced-obesity combined with moderate exercise group; DIO+F: diet induced-obesity combined with fiber supplementation group; DIO+F+E: diet induced-obesity combined with fiber supplementation and exercise group.

7.3.3 Mechanical and biochemical properties of tail tendons

Steady-state and peak stresses in the 2% and 3% stress relaxation tests were similar between experimental groups (Figure 7.4), as were the maximal stress, maximal strain, yield stress and yield strain in the failure test protocol (Figure 7.5). The Young's modulus was the same in LFD and DIO group rats, while the Young's modulus was smaller in DIO+E and DIO+E+F than LFD group rats ($p= 0.002$ and $p<0.000$, respectively; Figure 7.6). Hydroxyproline content was similar across groups (Figure 7.7).

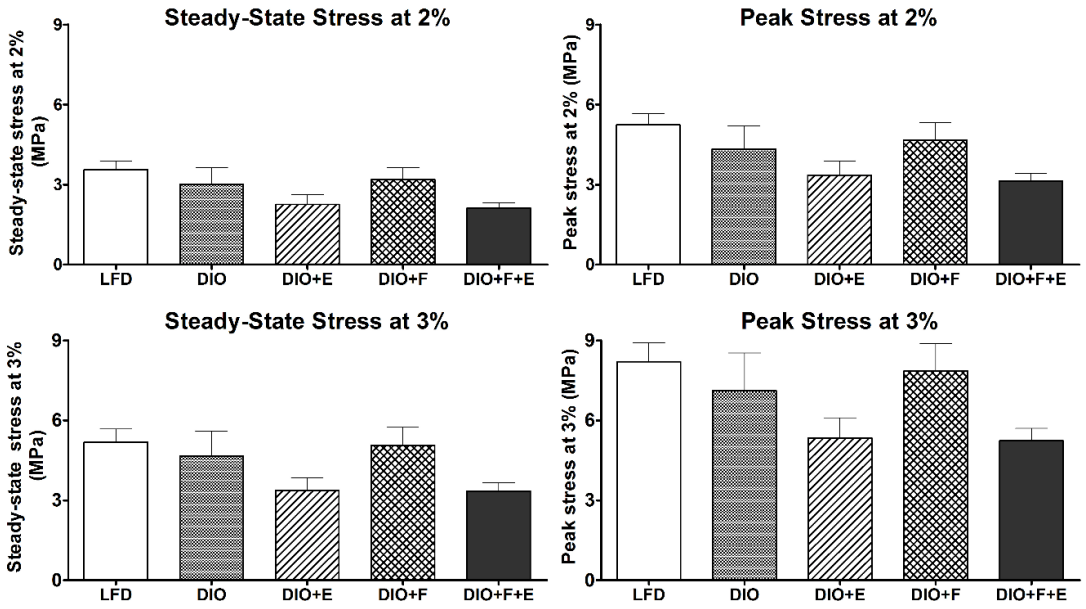

Figure 7.4. Mean (+ 1 SEM) static and dynamic stress at 2% and 3%. There was no difference between groups for the steady-state and peak stress at either 2% and 3%. LFD: low fat diet group; DIO: diet induced-obesity group; DIO+E: diet induced-obesity combined with moderate exercise group; DIO+F: diet induced-obesity combined with fiber supplementation group; DIO+F+E: diet induced-obesity combined with fiber supplementation and exercise group.

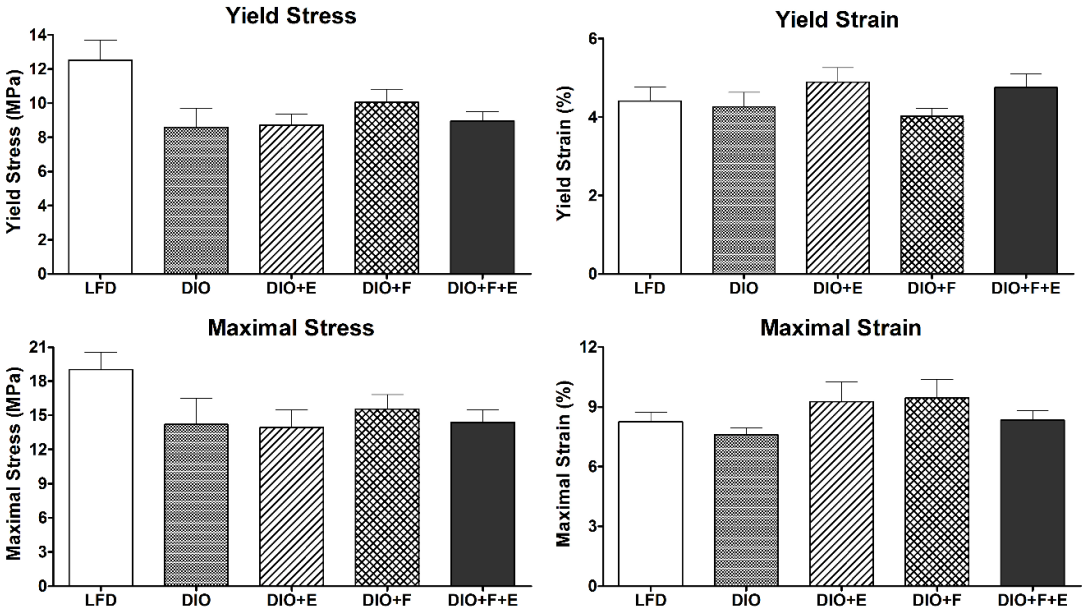

Figure 7.5. Mean (+ 1 SEM) yield stress and strain (top), maximal stress and strain (bottom). There was no difference between groups for yield stress, yield strain, maximal stress, and maximal strain. LFD: low fat diet group; DIO: diet induced-obesity group; DIO+E: diet induced-obesity combined with moderate exercise group; DIO+F: diet induced-obesity combined with fiber supplementation group; DIO+F+E: diet induced-obesity combined with fiber supplementation and exercise group.

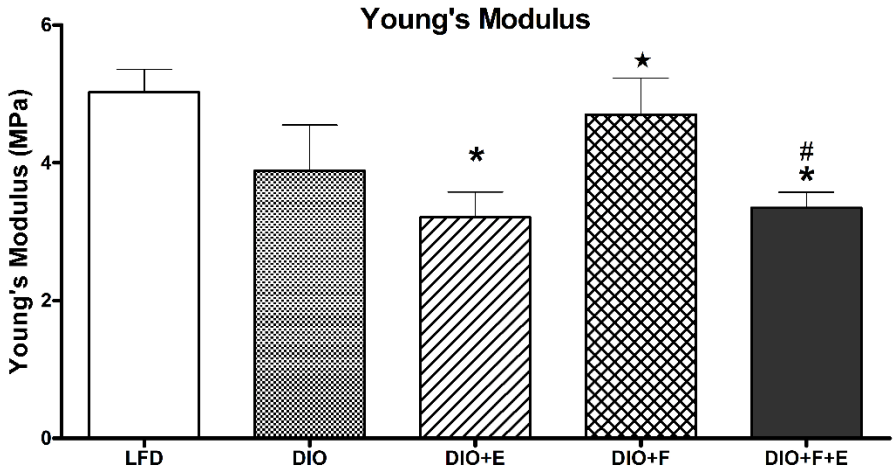

Figure 7.6. Mean (+ 1 SEM) Young's Modulus. * indicates differences from LFD group rats. ★ indicates difference from DIO+E group rats. # indicates difference from DIO+F group rats. LFD: low fat diet group; DIO: diet induced-obesity group; DIO+E: diet induced-obesity combined with moderate exercise group; DIO+F: diet induced-obesity combined with fiber supplementation group; DIO+F+E: diet induced-obesity combined with fiber supplementation and exercise group.

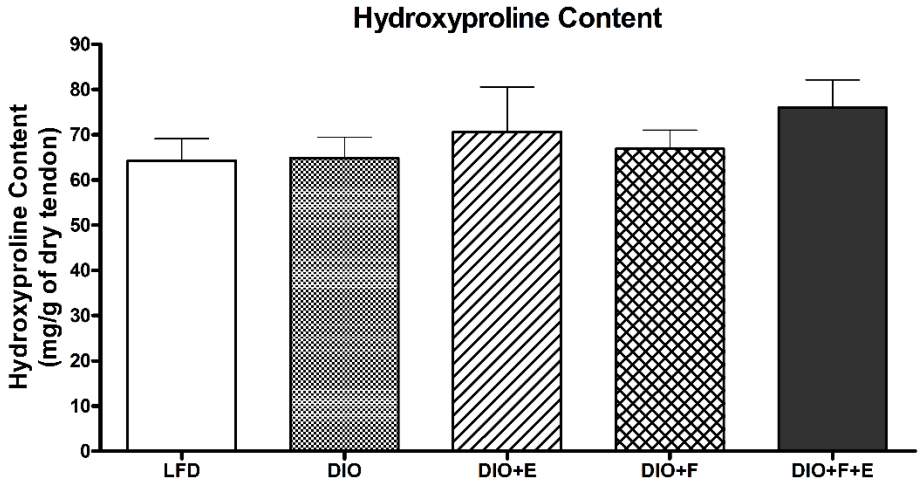

Figure 7.7. Mean (+ 1 SEM) hydroxyproline content LFD: low fat diet group; DIO: diet induced-obesity group; DIO+E: diet induced-obesity combined with moderate exercise group; DIO+F: diet induced-obesity combined with fiber supplementation group; DIO+F+E: diet induced-obesity combined with fiber supplementation and exercise group.

7.4 Discussion

The main findings of this study were that 1) DIO and DIO combined with fibre supplementation did not alter the mechanical and biochemical properties of the rat tail tendons and that 2) DIO combined with moderate exercise, and DIO combined with exercise and fibre supplementation reduced the elastic modulus, but not the collagen content of the tail tendon in rats when compared to LFD rats.

There is a significant positive association between musculoskeletal disorders and the level of obesity (Kortt & Baldry, 2002). Knee, hip and hand osteoarthritis, low back pain, diffuse idiopathic skeletal hyperostosis, carpal tunnel syndrome, plantar fasciitis, osteoporosis, rheumatoid arthritis, and fibromyalgia are some examples of these disorders (Anandacoomarasamy, Caterson, Sambrook, Fransen, & March, 2008). Specifically, tendinopathy has received focussed attention due to its high incidence rate in subjects with obesity compared to non-obese subjects (Albers, Zwerver, Diercks, Dekker, & Van den Akker-Scheek, 2016; de Jonge *et al.*, 2011).

Mice, and CD-Sprague-Dawley and Wistar rats fed with a high-fat/high-sucrose (HFS) diet are a well-established model of diet-induced obesity (Collins *et al.*, 2016d; Collins *et al.*, 2015c; Lorincz, Reimer, Boyd, & Zernicke, 2010; Rosas-Villegas *et al.*, 2017), and have been found to have increased systemic and local inflammation in the knee of CD-Sprague-Dawley rats (Collins *et al.*, 2015c). Furthermore, a HFS diet has been shown to result in metabolic disorders in rats and mice, compromising skeletal muscle structure and function, and knee cartilage, meniscus, and bone integrity (Collins *et al.*, 2016d; Collins *et al.*, 2015c; Lorincz *et al.*, 2010; Rios *et al.*, 2019a).

Contrary to our hypothesis, and in spite of being a well-established model for musculoskeletal disorder (Collins *et al.*, 2016a; Collins *et al.*, 2015a; Collins *et al.*, 2016d; Collins

et al., 2015b; Collins *et al.*, 2015c; Rios *et al.*, 2019a), the HFS diet does not appear to affect the mechanical and biochemical properties of rat tail tendons after 12 weeks on diet, and thus, diet-induced alterations to tendon integrity appears to follow a different time course than those other components of the musculoskeletal system. This result does not agree with previous studies showing alterations in the mechanical and structural properties of tail tendons of obese animals. There are a few possible explanations that may account for this discrepancy between our results and those obtained by others.

Tendinopathies in tail tendons of rats and mice have typically been found at time points greater than the 12 week period used in this study. For example, Eriksen et al. (2014) found a 13% decrease in plateau modulus and a 12% decrease in total modulus in mice fed with a high-fat diet for 32 weeks compared with mice fed a chow diet (Eriksen *et al.*, 2014). In the model used in this study, an induction period of 10-12 weeks is typically required for animals to reach "heterostasis" and demonstrate increased mass and increased metabolic disorders (Collins *et al.*, 2015c; Rios *et al.*, 2019a). Thus, it seems that although a 10-12 week period protocol of HFS diet is sufficient to induce increases in body mass and body fat, it is not enough to alter the mechanical and biochemical properties of the tail tendons, but might have affected other tendons, for example, weight bearing tendons (not evaluated in the present studies). Similar results were obtained by David et al. (2014) who found no differences in tendon mechanical properties in mice after 12 weeks on a high-fat diet. Carroll et al. (2006) and Silva et al. (2014) did not find changes in cardiac collagen content at 12-15 weeks of obesity induction between lean and obese rats, supporting our findings in tail tendons.

Previous studies have reported model-specific adaptations to obesity in animals. It has been suggested that there are differences between models of obesity and associated effects on

musculoskeletal tissues (Li *et al.*, 2008). Tendon mechanical and biochemical alterations have been observed in the monogenic Zucker rat model of obesity compared to control (Biancalana *et al.*, 2010). Genetically obese Zucker rats bear a mutation in the leptin receptor gene (Phillips et al., 1996), thus the changes in tendon properties might be related to the leptin receptor deficiency rather than the obesity itself.

Aging affects the mechanical properties of tendons and the turnover rate of collagen fibres from tail tendons (Everitt, Seedsman, & Jones, 1980; Everitt, Wyndham, & Barnard, 1983). Therefore, the age of the animals may influence tendon adaptations in models of obesity and metabolic disorder. Tendons from old animals compared to young have increased stiffness, increased elastic modulus, increased peak tension (Wood, Arruda, & Brooks, 2011), and the collagen turnover rate is up to 30 times lower (Karsdal, Genovese, Madsen, Manon-Jensen, & Schuppan, 2016). Our rats were 23-25 weeks old when tissue was collected, while in comparable studies, tail tendons were harvested at an older age, for example, at 40 weeks of age by Eriksen et al. (2014). Therefore, our relatively young animals might have had a greater potential to renew collagen, which might also have inhibited the onset of the detrimental effects of obesity on the tendons of our animals compared to the older animals used in previous studies.

However, the HFS diet combined with exercise was associated with a reduction in the Young's modulus of the tail tendon compared to that obtained in LFD control animals. Exercise in normal weight animals has beneficial effects on tendon morphology and function (Kubo, Kanehisa, & Fukunaga, 2001; Langberg, Rosendal, & Kjaer, 2001; Langberg, Skovgaard, Petersen, Bulow, & Kjaer, 1999; Narici *et al.*, 1996). It has been shown that chronic exercise induces collagen fiber formation (Langberg *et al.*, 2001; Langberg *et al.*, 1999), and an increased cross sectional area and stiffness in weight bearing tendons of humans (Kubo *et al.*, 2001; Narici

et al., 1996), horses (Birch, McLaughlin, Smith, & Goodship, 1999), and pigs (Woo *et al.*, 1981). However, little is known about the effects of chronic exercise on non-weight bearing tendons in young and old animals. Viidik et al. (1996) reported a decrease in tail tendon Young's modulus in older animals as we observed here in the present study. But the mechanism behind this change is not well understood. In the current study we did not evaluate the effects of exercise and/or prebiotic fibre on tendons in chow fed rats, therefore we cannot provide a direct evidence on the effects of exercise or prebiotic fibre on tendons in normal weight animals. This is a limitation of our study.

In this study, exercise prevented weight and fat gains typically associated with HFS diets, and was associated with a decrease in the Young's modulus in tail tendons without modifying its collagen content when compared to LFD rats. The lack of correlation between the Young's modulus and collagen has been found before (Oxlund, 1980), since the Young's modulus is also determined by the structural arrangement of collagen and the cross-linking between collagen fibres (Depalle, Qin, Shefelbine, & Buehler, 2015; Guthold *et al.*, 2007). The reduction in Young's modulus in our DIO+E and DIO+E+F rats could be due to changes in cross linking between collagen fibres and their size and structural arrangement within the tail tendon (Biancalana *et al.*, 2010; Derwin & Soslowsky, 1999). However, it is not clear whether the reduction in Young's modulus is beneficial or a sign of degeneration in non-weight bearing tendons.

Dietary modulation may protect against metabolic disease. Previous studies have shown that a dietary fibre intervention was effective in reducing body mass and in increasing metabolic health in rats (Cluny et al., 2015; our study), and humans (Hume et al., 2015; Parnell & Reimer, 2009). A HFS diet combined with fibre supplementation did not show a difference in the mechanical and biochemical properties of the rat tail tendon compared to either the LFD or the DIO groups, suggesting that either there is no effect of this intervention or a longer period of

exposure is required to observe the effects. Further studies with longer periods of exposure to the DIO, exercise, and fibre supplementation than used here, and chow fed animals subjected to exercise and fibre supplementation may be needed to help understand the systemic effect of obesity, exercise, and fibre supplementation on the non- weight bearing tendons of animals exposed to a HFS diet.

7.5 Conclusion

DIO and DIO combined with fibre supplementation did not lead to detectable differences in the mechanical and biochemical properties of the rat tail tendon compared to LFD rats. However, DIO combined with moderate exercise, and DIO combined with moderate exercise and fibre supplementation reduced the Young's modulus when compared to LFD rats, but not the collagen content of the tail tendon in rats. Based on these results, a high-fat/high-sucrose diet by itself has no effect on tail tendon. Longer periods of exposure to the high-fat/high-sucrose diet, may be needed to alter the mechanical and biochemical properties of non-weight bearing tendons.

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