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Uncovering the Role of the Infrapatellar Fat Pad in Knee Osteoarthritis and Pain

by
Jessica Grace Wagner

DISSERTATION
Submitted in partial satisfaction of the requirements for degree of
DOCTOR OF PHILOSOPHY

in

Rehabilitation Science

in the

GRADUATE DIVISION
of the
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Approved:

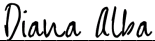
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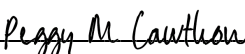
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DEDICATION

This dissertation is dedicated to my family, to my friends, to my community. I will never be able to fully express my gratitude for the endless love and support I have received during this journey. Most importantly, this dissertation is dedicated to the strong women in my life, both in this world and in the next. Lori Tuttle, Cory Schaller, Annabel Grace Wagner, and my incredible mom, Kimberly Ann Wagner. You are my role models.

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I want to thank my advisor, Dr. Kelsey Collins, for taking me on as her first graduate student. I am incredibly grateful that she took a chance on a PT with an interest in biology. Now, I not only have a mentor, but also a role model and friend. Without her unwavering support and guidance, I would not be the young investigator I am today. I want to thank my dissertation committee—Drs. Kristine Godziuk, Peggy Cawthon, Diana Alba, Richard Souza—for their invaluable guidance. Without their support and varied expertise, I wouldn't have been able to achieve this level of interdisciplinary, well-rounded research. Finally, to the many mentors, colleagues, friends, and family that have been my cheerleaders, sounding boards, toughest critics, and shoulders to cry on. You have all set the standard for how to be a good scientist and human, and I will do the same for others.

UNCOVERING THE ROLE OF THE INFRAPATELLAR FAT PAD IN KNEE OSTEOARTHRITIS AND PAIN

Jessica Grace Wagner

Abstract

The infrapatellar fat pad (IFP) has gained considerable attention in research on knee osteoarthritis (OA) for its substantial inflammatory role in knee joint health beyond simple mechanical shock-absorption. Despite increased efforts to investigate the structure, a consensus on whether the IFP contributes a pro- or anti-inflammatory role is lacking. The IFP is a highly innervated structure that contains immune cells, which highlight the candidacy of the IFP to contribute to knee OA pain. However, minimal research has evaluated the relationship between the IFP and knee OA pain. Furthermore, despite evidence that the IFP demonstrates morphological and compositional changes based on BMI-derived obesity and sex in knee OA, investigation into the sensitivity of the IFP to such factors that relate to high-risk of knee OA and greater pain severity have yet to be explored. This dissertation leveraged multi-disciplinary scientific approaches to investigate tissue abnormality at the IFP in relation to knee OA pain across sex and BMI and in response to a key signal involved in fat-cartilage crosstalk in OA. Magnetic resonance imaging (MRI) allows for accessible visualization of morphological tissue changes and was utilized to evaluate abnormality (e.g. inflammation and fibrosis) at the IFP in relation to pain. Greater tissue abnormality at the IFP was found to correlate with worse pain and symptoms in patients with isolated patellofemoral joint OA—a subpopulation of knee OA—and differ by sex and

BMI, in that females and individuals with a BMI ≥ 30 kg/m² demonstrated stronger relationships between the IFP and OA pain. Tissue profiling of the secreted factors from the IFP—termed secretome—revealed sex- and BMI-based differences in protein secretion that significantly correlated with patient-reported outcomes in patients with end-stage knee OA. These findings suggest that the state of the IFP (both through clinical and biological measures) may be related to OA pain, which has been historically challenging to understand given the well-described structure-pain discordance in knee OA. Furthermore, when the IFP secretome was probed with leptin (a key satiety hormone elevated in patients with obesity and females, correlates with pain, and mediates fat-cartilage crosstalk in knee OA degeneration), the IFP demonstrated dysregulation in factors involved in protein kinase signaling. Unlike cartilage and synovium, the IFP was the only tissue to show a unique modulation of leptin response in eicosanoids specifically by sex and BMI, which suggests a modulated pain profile with leptin. These findings offer potential targets for therapeutic intervention of the IFP in knee OA. Differences in how the IFP relates to patient pain across sex and BMI provide an opportunity for patient stratification and prognostic insight into high-risk, high-pain knee OA populations. By connecting molecular, imaging, and clinical characteristics, the IFP may provide the ability to disentangle knee OA pain that has been previously difficult to unravel, and offers an untapped opportunity for precision care in high-risk, high-pain knee OA populations.

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Overview

Chapter 1 serves as a general introduction to the anatomy, epidemiology, pathophysiology, and scientific methods relevant to this dissertation. Chapters 2-4 are three independent scientific studies which include study-specific introduction, methods, results, discussion, and conclusion, and references. Chapter 5 is an integration of the important findings generated from this dissertation and summarizes the clinical implications and future directions of this work.

Chapter 1 Introduction

The Infrapatellar Fat Pad

The infrapatellar fat pad (IFP), or Hoffa's fat pad, is a local fat depot in the knee joint located posterior and inferior to the patella, depicted in Figure 1.1.¹⁻³ It is highly innervated and vascularized, and bordered by synovium, cartilage, and bone.^{2,4} The IFP is present in most vertebrates (humans, mice, rats, goats, sheep, horses, and primates) and its proposed purpose in humans includes mechanical shock absorption via reduced load bearing at the patellar tendon.⁵ Recent research on the complex inflammatory contribution of the IFP highlights a potential multi-faceted and complex role in knee joint health, beyond purely mechanical.^{1,3,6} This section discusses the current state of the literature on anatomy, composition, clinical characteristics, and morphological changes of the IFP with musculoskeletal disease and obesity.

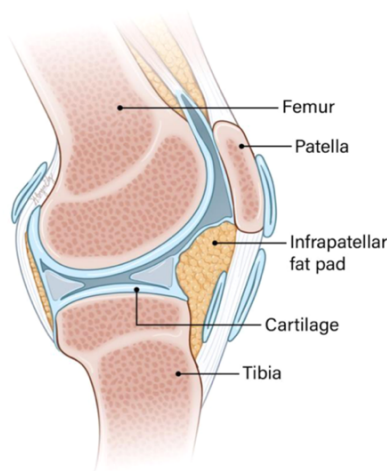


Figure 1.1. The infrapatellar fat pad (IFP) located inferior and posterior to the patella. Bordered posteriorly by the synovium. Figure reproduced with permission of npj (Nature Partner Journals) Aging from The infrapatellar fat pad in inflammaging, knee joint health, and OA.²

Anatomy and Physiology of the IFP

The IFP is primarily composed of white adipocytes organized into discrete lobules with densely woven peripheral nerve fibers throughout, shown in Figure 1.2. The nerve supply stems from the genicular nerves, which are sensory branches originating from the sciatic, femoral, and obturator nerves.⁷ Some studies have found the posterior articular branch of the tibial nerve also provides innervation,⁸ as well as the presence of many free nerve fibers including type VIa and Substance P that sense pain, pressure, and temperature.⁵ Blood vessels are found in tandem to the nerve distribution in the IFP, derived from the medial and lateral genicular arteries.^{8,9}

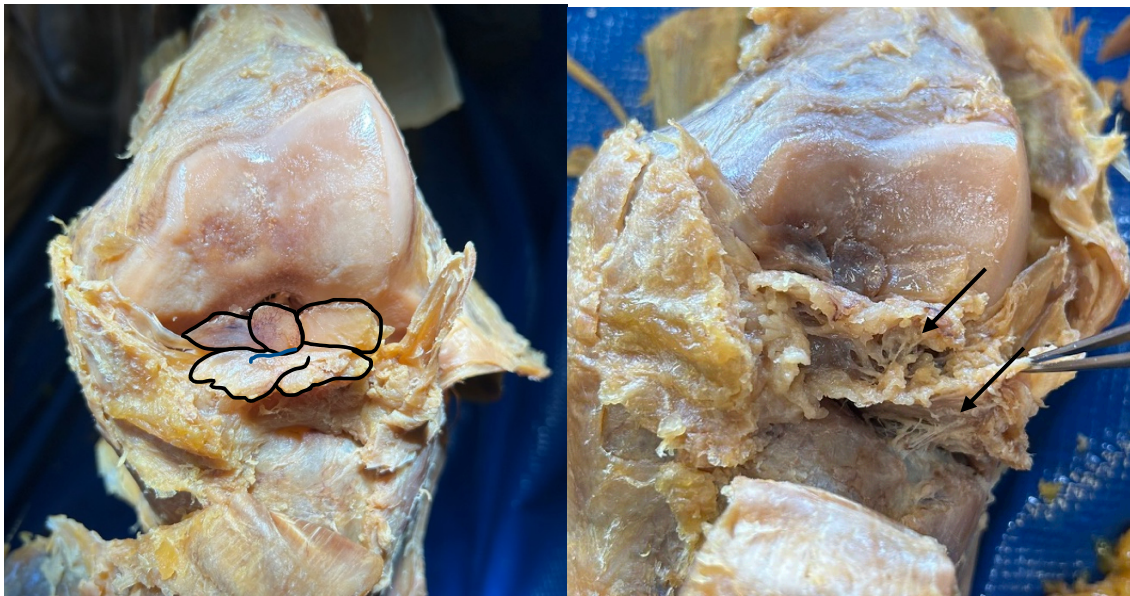


Figure 1.2. Knee cadaver prosection of the IFP and relevant innervation. Left image shows the distinct lobules, which have been traced digitally to emphasize structural organization. Right image shows the dense innervation stemming off the tibia and webbing throughout the IFP. Images captured on protected phone with permission from UCSF Anatomy Learning Center. The author wishes to thank individuals who donate their bodies and tissues for the advancement of education and research.

Adipose tissue is composed of connective tissue and adipocytes that can be classified by morphology (i.e., white, brown, and beige), and by location (e.g., subcutaneous, visceral, local joint).¹⁰ Subcutaneous adipose tissue, or SAT, is primarily made of white adipose located deep to the skin throughout the body, and functions primarily for energy storage in the form of lipids.^{10,11} Visceral adipose tissue, or VAT, surrounds the intra-abdominal organs and plays a more active role in metabolism.^{10,12} Although the IFP is visually similar to SAT, it exhibits a more specialized role that exceeds energy storage. In cases of extreme starvation, the IFP size and lipid storage are preserved while SAT and VAT deplete.^{2,13} When comparing mechanical properties of adipose from humans, the IFP is stiffer than SAT, and able to withstand higher compressive, tensile, and shear stresses.¹⁴ With indentation testing, the IFP demonstrates the ability to withstand greater load compared to other adipose tissues (e.g. SAT, VAT, and plantar fat pad) due to its structural arrangement.¹⁵ The structural organization and mechanical properties of the IFP emphasize that its purpose is unique and separable from systemic adipose.

IFP Dysfunction and Disease

The IFP contains immune cells, e.g. macrophages and T cells, and produces cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF)- α , vascular endothelial growth factor (VEGF), and prostaglandin E2 (PGE2), which have all been linked to cartilage degeneration in knee OA, depicted in Figure 1.3.^{1,2} As an adipose depot, the IFP also secretes adipokines, i.e. cytokines specifically produced by adipocytes, such as adiponectin, and leptin.^{1,16-18} Adiponectin—a hormone that regulates glucose levels,

lipid metabolism, and insulin sensitivity¹⁹—has been shown to exert significant pro-inflammatory effects on chondrocytes through an increased production of IL-6, monocyte chemoattractant protein-1 (MCP-1), MMP-3 and MMP-9.¹⁹⁻²¹ Like the SAT, the IFP is a major source of leptin—a satiety hormone directly related to systemic adiposity—which has gained considerable attention in research on knee OA for its role in fat-cartilage crosstalk and exacerbation of cartilage degeneration.^{1,4,22 23,24} The IFP has the potential to adversely affect the knee joint organ system in disease with an increased production and secretion of pro-inflammatory mediators that have the potential to induce degeneration and inflammation in adjacent tissues.

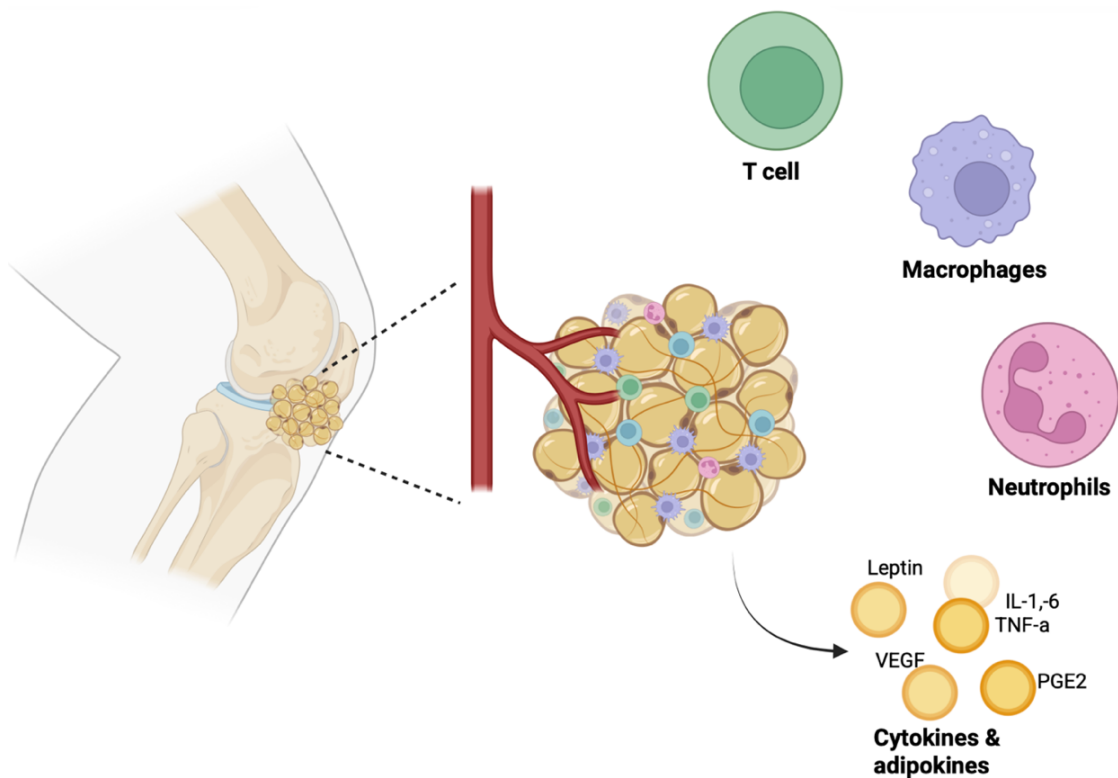


Figure 1.3. The highly vascularized IFP and its resident immune cells. The IFP contains immune cells, e.g. T cells, macrophages, and neutrophils. The adipocytes in the IFP produce and secrete cytokines and adipokines such as leptin, IL-1 and IL-6, TNF-alpha, VEGF and PGE2. Figure created using BioRender.com.

The IFP also demonstrates an anti-inflammatory potential.^{3,25,26} The macrophages in the IFP reside without polarization until activated in response to changes in the surrounding conditions: tissue damage, inflammation, dysfunction.²⁷ Several studies highlight M1 (pro-inflammatory) macrophages produced by the IFP in knee OA, yet others emphasize the presence of M2 (anti-inflammatory) macrophages, including CD14+ and CD206+.²⁸ Moreover, our understanding of the role of the IFP has evolved to an active adipose depot that plays a biphasic role in knee joint health, depicted in Figure 1.4.^{1,27} In a healthy state, there is a balance between M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages in the IFP. An inflamed or diseased state may trigger an imbalance that shifts toward M1 macrophage polarization and secretion of pro-inflammatory cytokines leading to inflammation of other tissues at the knee. The IFP is not an inherently pro-inflammatory tissue. It is unclear what causes an imbalance at the IFP that then leads to inflammation, fibrosis, and increased secretion of pro-inflammatory factors. Further investigation into the biological inflammatory milieu of the IFP is clearly needed, and is a key aim of this dissertation. To probe the molecular properties of the IFP in health and disease, simple biopsy from patients is challenging and not performed at this time—in part due to the high-innervation and densely organized structure of the IFP. Semi-quantitative imaging allows for highly accessible evaluation of inflammation and fibrosis at the IFP, and has been evaluated specifically in the context of knee OA. As such, imaging features of the IFP are an integral component in this dissertation to characterize the IFP in knee disease across research disciplines.

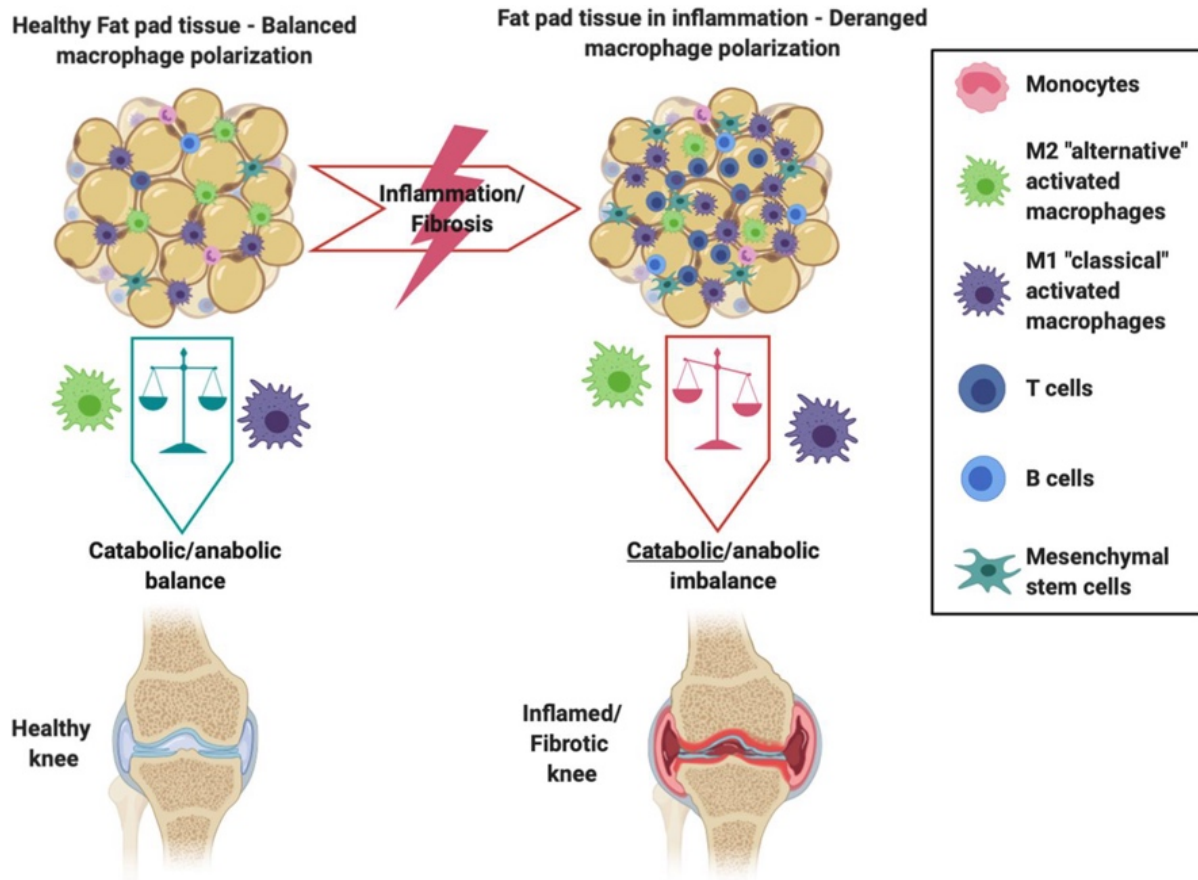


Figure 1.4. IFP macrophage immune balance: tipped scale in an inflamed state. Figure reproduced with permission of Frontiers in Bioengineering and Biotechnology from Infrapatellar Fat Pad/Synovium Complex in Early-Stage Knee Osteoarthritis: Potential New Target and Source of Therapeutic Mesenchymal Stem/Stromal Cells.²⁷

Clinical Evaluation of the IFP

Magnetic resonance (MR) imaging provides excellent contrast and spatial resolution for multiplanar tomographic imaging of tissues not well-visualized on radiographs, e.g. muscle, cartilage, and adipose tissue. MR imaging utilizes magnets and pulsing radiofrequency to align, disrupt, then realign protons to map tissues that are high in protons such as water and fat. Varying contrasts and MR sequences can provide different visualization of tissues depending on the diagnostic goal. T1-relaxation MR images reflect the amount of time taken for protons to realign 63% of their initial longitudinal magnetization, i.e. return to the main magnetic field.²⁹ T2-relaxation MR images reflect the amount of time taken for protons to realign with 37% of their initial longitudinal magnetization.²⁹ T1-weighted MR images enhance the signal of fatty tissue (bright) and suppress the signal of water (dark), while T2-weighted MR images enhance the signal of water/fluid (bright). Tissues with protons that realign faster, such as fat and water, will appear brighter on images. MR imaging protocols of both contrast types are the gold standard to evaluate the morphological features of the IFP.^{30,31} Imaging characteristics of the IFP that are assessed include volume and signal (area and intensity). Figure 1.5 shows a T2-weighted MR image of a healthy IFP.



Figure 1.5. T2-weighted MR image of a normal IFP at the knee in the sagittal plane.

Changes in volume and signal intensity are hallmarks of IFP abnormality on MRI, but a clear correlation with pain and disease is lacking. The majority of the literature supports that individuals with knee OA (both in the tibiofemoral and patellofemoral regions) demonstrate greater IFP volume compared to their healthy counterparts,³² which is positively associated with osteophytes and cartilage lesions.^{32,33} Other evidence shows that greater volume of IFP correlates with reduced medial and lateral tibial cartilage defects,³ suggesting a potential protective effect for cartilage degradation. In the context of obesity, IFP volume on MR imaging appears unrelated to systemic fat mass.³⁴ Hyperintensity refers to areas that appear relatively brighter than the surrounding tissues. In T2-weighted MR images, areas of hyperintensity indicate areas of increased water content from inflammation, scarring, or vascular disease. In the context of knee joint health, hyperintensity at the IFP is positively associated with knee structural abnormalities including bone marrow lesions and tibiofemoral cartilage

defects.³⁵ One study observed that hyperintensity at the IFP on MR imaging is positively associated with the proinflammatory cytokine marker matrix metalloproteinase (MMP)-3 in the synovium after traumatic injury (anterior cruciate ligament tear).³⁶ Of the studies that have utilized imaging to study the IFP in knee joint health, most have related IFP abnormality to knee OA structural change. A small subset of studies on IFP MR features relate to patient symptoms, albeit conflicting results: some find greater IFP volume does not relate to knee pain,³⁷ and others report IFP volume and signal intensity positively relate to knee pain.^{35,38} The conflicting findings may be attributed to differences in MR protocols, methods of pain measure collection (VAS vs. KOOS), and inclusion criteria (BMI, sex, age). The lack of a conclusive relationship between MR characteristics of IFP abnormality with patient symptoms in knee OA may be due to the heterogeneity of patient populations and pain severity in knee OA. Sex- and BMI-based analysis within the relationship between the IFP and pain may reveal unique differences in the tissue across patient populations. MR imaging is a valuable and accessible tool to investigate the IFP, but MRI signal and volume changes at the IFP are surrogate measures for tissue dysfunction that associate with inflammation.³¹ To contextualize pain-related imaging features at the IFP, this dissertation also describes biological changes at the IFP in the context of OA disease, specifically, and its risk factors: sex and BMI.

The Intersection of the IFP, Osteoarthritis, and Risk Factors

Osteoarthritis (OA) is a leading cause of global pain and disability, affecting over 250 million people worldwide.³⁹⁻⁴¹ OA is the degenerative disease of synovial joints, with the highest occurrence at the knee. Diagnostic criteria of knee OA includes radiographic

evidence of marginal osteophytes, joint space narrowing, subchondral sclerosis, and cysts, and the most common symptom of OA is joint pain.⁴² Traditionally, OA has been defined as the loss of cartilage lining articular joints and the above radiographic features.⁴³ OA is more recently defined as a disease of the whole joint involving complex and abnormal remodeling of all joint tissues driven by inflammatory mediators, mechanical factors, and risk factors including age, female sex, and obesity (Figure 1.6).⁴⁴

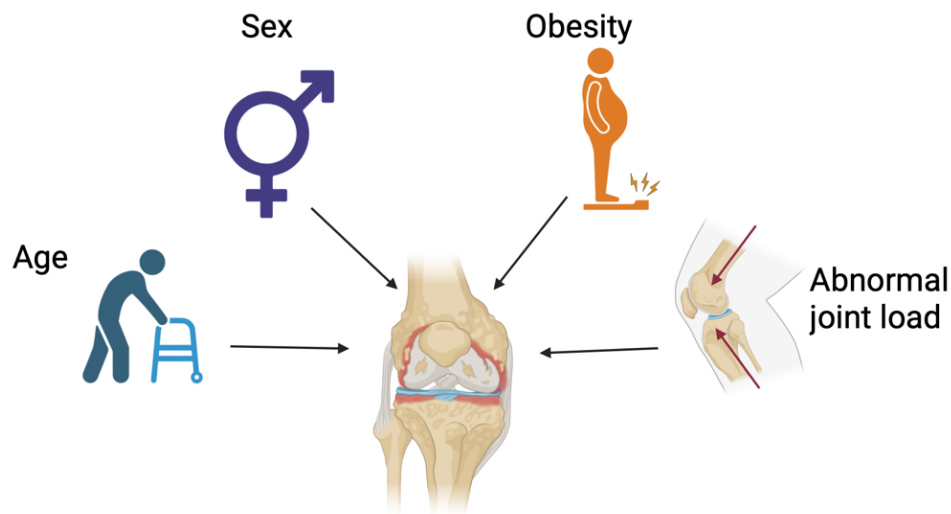


Figure 1.6. Contributing factors involved in knee OA pathology. Non-modifiable risk factors (e.g. age and sex) and modifiable risk factors (e.g. obesity) contribute to knee OA. Figure created using Biorender.

Risk Factors for OA

Patients with obesity (PWO; defined by Body Mass Index, or BMI, $\geq 30 \text{ kg/m}^2$) and females⁴⁵ disproportionately carry the burden of OA with increased prevalence of disease compared to their counterparts (BMI $< 30 \text{ kg/m}^2$ and males), shown in Figure 1.7.^{41,46-48} The current clinical dogma is that loading due to body mass and large body size explains the burden of OA disease in PWO.⁴⁰ However, body mass and BMI

insufficiently describe these relationships.⁴⁹ While increased medial compartment tibiofemoral contact stress and joint space narrowing associated with knee OA may be exacerbated by mechanical load,³⁹ cartilage breakdown at the lateral compartment suggests that breakdown solely due to joint load is not the case.⁵⁰ There lacks conclusive, causative evidence that demonstrates that that increased body size with obesity is the problem in aggravating OA pathogenesis, versus metabolic dysregulation. With regard to sex, females demonstrate greater prevalence and higher risk of developing OA compared to males.^{46,51} Post-menopausal females are also at risk of increased adiposity, and female sex and obesity compounds an even higher risk of knee OA.⁴⁶ Recent studies are linking estrogen signaling and knee OA pathogenesis,⁵² but the connections between adiposity, sex hormones, joint degeneration, and pain remains to be disentangled.

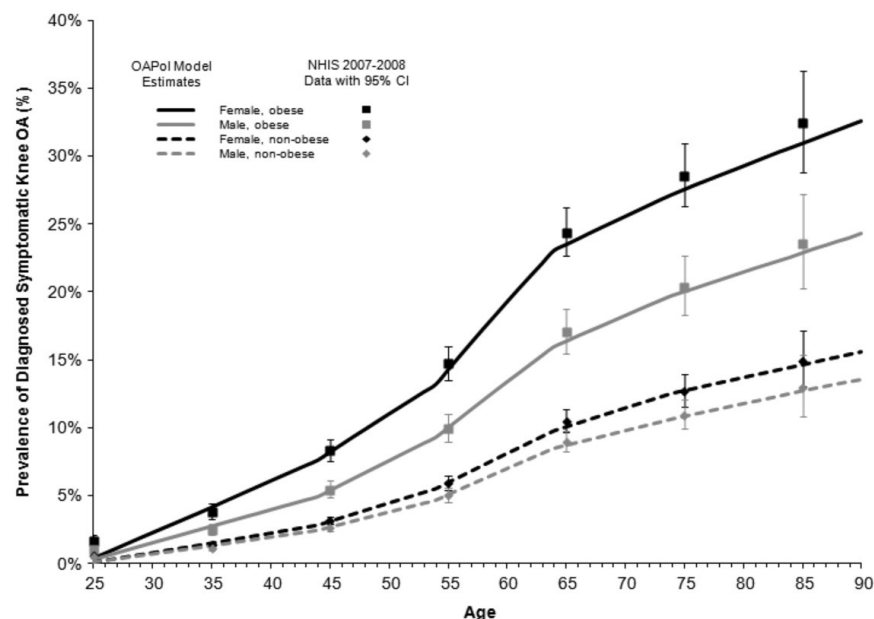


Figure 1.7. Estimated prevalence of diagnosed symptomatic knee OA by age and obesity status in the United States from 2007-08 National Health Interview Survey data. Figure reproduced with permission of Arthritis Care & Research from Lifetime Risk and Age at Diagnosis of Symptomatic Knee Osteoarthritis in the US.⁴⁶

A note on BMI-derived obesity. BMI is a measure of body size: $\text{mass}_{\text{kg}} \div \text{height}_{\text{m}}^2$.

BMI has been the most widely used tool to define obesity in research. However, BMI is not a marker of metabolic disturbance, adiposity, or fat composition—all required to diagnose obesity. Most studies in knee OA and other research fields have utilized BMI to define obesity using the World Health Organization (WHO) BMI cutoff of 30 kg/m² to differentiate individuals with (≥ 30 kg/m²) and without (< 30 kg/m²) obesity. On a population scale, high BMI is associated with increased adiposity and a higher likelihood of obesity and metabolic disturbance.⁵³ Recent literature shows that some patients defined as “obese” by BMI are metabolically healthy when evaluating body composition, insulin resistance and other metabolic markers.⁵⁴ BMI is a highly accessible demographic measure and is included in this dissertation. However, this author acknowledges the limitation of BMI to define obesity on an individual basis and the need to incorporate measures of adiposity and metabolic disturbance to wholly define obesity and contribute meaningfully to obesity-related research and the development of precision treatment for this population.

PWO and females experience more pain compared to other patients (BMI < 30 kg/m² and males) with OA,^{45,55,56} but it is unclear why. PWO are not always appropriate for arthroplasty due to concern for complications,⁵⁷ leading to prolonged conservative management and exhaustion of physical therapy without a clear prognosis. Moreover, there is a known unsolved discordance between degree of structural degeneration and pain severity in knee OA among all populations.^{39,58} The lack of congruency in the explanation for how these major risk factors impact OA pathogenesis and pain calls for

a paradigm shift in the approach to studying OA, starting with thorough characterization of the active tissues involved in knee joint health: the IFP.

Local Adipose Depot as an Immunometabolic Regulator of the Joint in OA

Articular cartilage has long been the focus in knee OA. With evolving research highlighting adipose tissue as an active organ in musculoskeletal health,^{23,59} the role of the IFP—the largest local adipose depot in the joint—in this context remains to be fully characterized. Most studies investigating biological changes in the IFP with OA utilize IFP surgical waste collected during total knee arthroplasty (TKA) for OA IFP; and IFP surgical waste collected during anterior cruciate ligament reconstruction (ACLR), or cadaveric IFP for non-OA IFP. Comparing gene expression of OA versus non-OA IFP (ACLR), increased expression of pro-inflammatory cytokines—interleukin (IL)-6 and vascular endothelial growth factor (VEGF)—and adipokines—adiponectin, leptin, and fatty acid binding protein (FAB)4—are seen in OA IFPs.¹⁶ Activated CD4+ T cells (or helper T cells) are found in abundance in OA IFPs and secrete IL-6 and tumor necrosis factor (TNF)- α ,¹⁷ further demonstrating a hyper-inflammatory profile of the IFP with knee OA. Using single-nucleus (sn)RNA-sequencing, OA IFPs exhibit a higher proportion of macrophage signaling towards fibroblasts, an upregulation of fibronectin (FN)-1 gene expression, and downregulation in Jun gene expression.¹⁸ In the context of cartilage health and OA, FN-1 is an extracellular matrix (ECM) glycoprotein which binds to collagen (COL)II and is essential for cartilage health through chondrocyte (highly specialized cartilage cells) differentiation and structural integrity,⁶⁰ and has been previously established as a key gene functioning in OA.⁶¹ Jun is an age-associated

differentially expressed gene that functions as part of the Activator Protein(AP)-1 complex and plays a role as a “master switch” for turning on genes involved in cell fate. In the cartilage of rats with OA, down-regulation of Jun induced chondrocyte senescence through chromatin destabilization and inhibition of cellular proliferation.⁶² The IFP appears to contribute a pro-inflammatory profile in OA that may be directly linked to cartilage degeneration and OA progression.

Clinically, individuals with knee OA often present with stiffness and pain at the anterior knee, which may be attributed in part to the IFP given the abundance of innervation and free nerve endings. Compared to their healthy counterparts, OA IFPs exhibit increased inflammatory infiltration, vascularization, and fibrosis.¹³ ECM remodeling reduces in the IFP with end-stage OA, shown by a reduction in gene expression of COLI and COLIII compared to non-OA IFPs,¹⁶ which aligns with the fibrotic phenotype of the IFP with OA. IFP adipocytes also increase in size with knee OA, as shown in Figure 1.8.¹⁶ Adipocyte hypertrophy is linked with metabolic dysfunction, where there is a reduction in adiponectin production and subsequently reduced insulin sensitivity.⁶³ Interestingly, adipocyte hypertrophy is largely attributed to excess energy storage with obesity, yet this observation of adipocyte hypertrophy at the IFP with end-stage OA was seen in patients with a BMI ranging from 25.95-32.25 kg/m².¹⁶ This finding highlights the complex, nonlinear interplay of OA and obesity, among other risk factors, that requires deeper exploration.

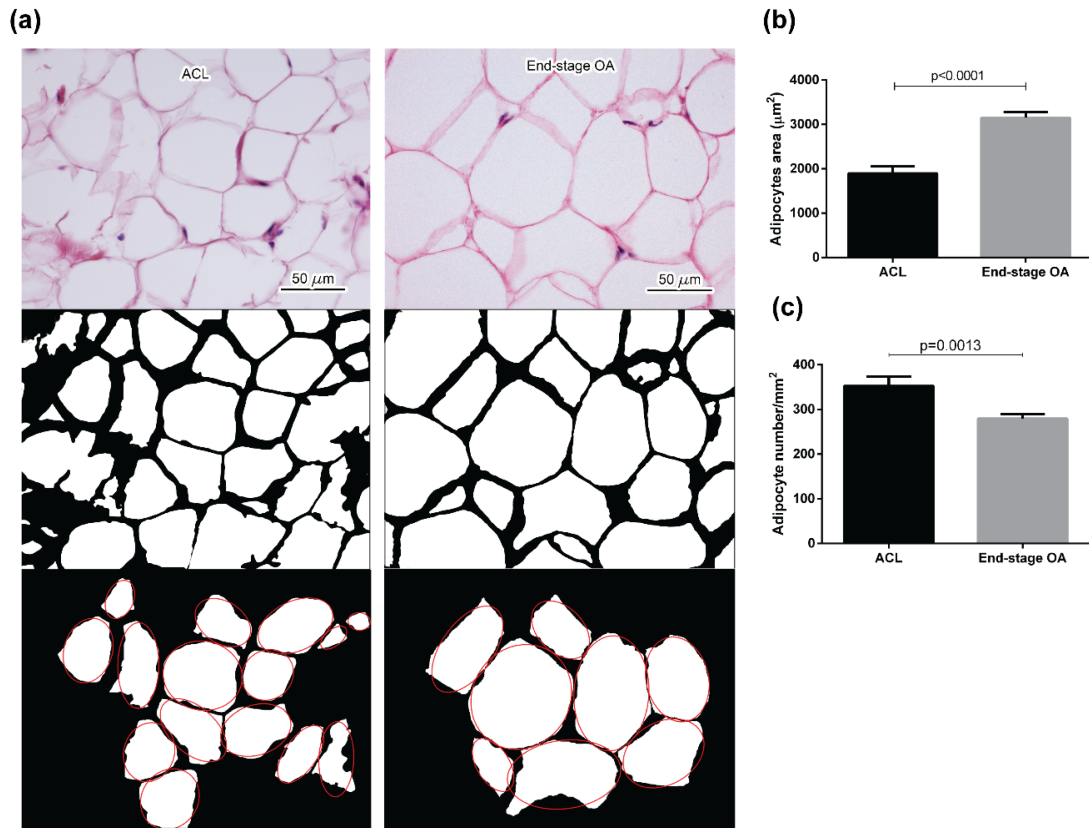


Figure 1.8. Histology of IFP adipocytes from patients with and without end-stage OA. Compared to non-OA (a), OA IFP adipocytes have an increased area (b). ACL, anterior cruciate ligament [repair]; OA osteoarthritis. Figure reproduced from International Journal of Medical Sciences from Infrapatellar Fat Pad Gene Expression and Protein Production in Patients with and without Osteoarthritis.¹⁶

IFP Differs by Obesity Status and Sex in OA

Obesity is characterized by a state of chronic systemic low-grade inflammation and the IFP is highly prone to a pro-inflammatory state. Recent investigation into the IFP has gleaned valuable insight into local joint changes due to systemic factors in the context of OA progression. In OA comorbid with obesity, IFP adipocytes are larger in size compared to IFP adipocytes from individuals with BMI <25 kg/m²,^{16,28} suggesting that while the local adipose depot functions independently of systemic adiposity, it is sensitive to changes with obesity. At the metabolome level, OA IFPs from PWO exhibit

an upregulation in triglyceride (TG) (54:2) and TG (50:2).¹⁸ TGs function in metabolism-related pathways, specifically lipid metabolism, and elevated levels of TG in serum relate to greater incidence and severity of OA⁶⁴ emphasizing an overlap of obesity- and OA-related changes at the IFP. Among females with OA, there is a lack of evidence showing differences in IFP morphological changes (i.e. adipocyte hypertrophy) compared to males, with little to no studies investigating sex-based differences in this variable. At the transcriptome, OA IFPs from females demonstrate upregulation of SOX5 mRNA levels compared to males.¹⁸ The SOX family of transcription factors, especially SOX5, is found upregulated in OA-affected cartilage. The IFP is sensitive to differences in obesity, sex, and OA disease. With the complex interplay of these factors, there is an obvious need to describe the communication and signaling between the IFP and adjacent tissues with OA and across obesity status and sex to fully understand how the IFP changes from knee homeostasis to joint disease. Moreover, characterization of molecular changes at the IFP with OA in relation to other involved tissues and patient-reported outcomes is needed to contextualize how the fibrotic, inflammatory changes the IFP contribute to OA, both in structure and pain.

Obesity and OA: Fat Drives Cartilage Damage

OA is more recently defined as a disease of the whole joint rather than isolated cartilage disease. Others are pushing the definition farther, describing OA as a whole-body disease mediated by systemic metabolic factors.^{23,24,59} This is evidenced by fat-cartilage crosstalk, where the absence of systemic adipose leads to protection from cartilage damage in mice with injury-induced OA.²³ When fat is surgically reintroduced

to fat-free mice, or lipodystrophic (LD), cartilage damage returns, emphasizing that communication between fat and cartilage is detrimental to cartilage health in OA. Other studies delineating the relationship between obesity and OA highlight leptin—a satiety hormone and pro-inflammatory factor secreted by fat, also described as an adipokine, directly related to adiposity—as a key driver of fat-cartilage crosstalk. LD mice implanted with leptin knockout fat were further protected from more severe damage, suggesting that the absence of leptin provides a protective effect against OA disease progression. Complement Factor D (CFD) is a serum factor found downstream of leptin-mediated fat-cartilage crosstalk in mice.²⁴ Knockout of CFD in mice led to similar cartilage protection as leptin knockout, but did not improve knee hyperalgesia—a measure of pain—in mice.⁶⁵ In humans, serum leptin levels are elevated in individuals with obesity, elevated in individuals with knee OA compared to healthy patients, and correlate with radiographic severity of knee OA. This suggests that leptin secreted by adipose may link occurrence of obesity and OA. The role of leptin in pain, however, remains to be probed further. Leptin may be a targetable factor for treatment or prevention of OA cartilage damage. The IFP, being an adipose depot, is also a site of leptin production and secretion. As such, how the IFP responds and contributes to leptin signaling in fat-cartilage crosstalk may uncover an avenue for precision therapeutic intervention in knee OA that has gone untapped.

Consideration for the IFP in the Treatment of OA

Treatment of OA follows a standard course of conservative management (exercise, weight management, physical therapy and bracing), pharmacological

management (non-steroidal anti-inflammatory drugs and opioids), intra-articular injections (corticosteroid, hyaluronic acid, platelet-rich plasma), and potentially surgical treatment for end stage OA (i.e. total knee arthroplasty, or TKA).⁶⁶ The IFP has historically not been considered as a treatment target in OA. More recently, the lack of consistent operative management of the IFP in TKA has gained more attention with respect to influence on post-operative outcomes. Approximately 20% of patients post TKA continue to experience pain over 3 months after surgery, with many patients never seeing resolution of their symptoms.^{67,68} Protocol for IFP handling during TKA has varied throughout the years. Previously, the IFP was routinely resected (excision rates exceeding 80%) during TKA to improve visualization during operation.⁶⁹ However, this posed adverse biomechanical effects through patellar tendon shortening and increased anterior knee pain.⁶⁹ While IFP excision rates have declined, a standard protocol for the preservation of the IFP during TKA does not exist. Rather, it is up to the surgeon's discretion on whether to excise the IFP during TKA. Several randomized controlled trials have shown that preservation of the IFP during TKA leads to better patient-reported outcomes on the Knee Injury and Osteoarthritis Outcome Survey (KOOS).^{25,69} One study followed patients 5 years postop, reporting that patients with an excised IFP had persistent symptoms, compared to resolution in those with their IFP maintained.²⁵ In contrast, a study in female guinea pigs—which develop spontaneous knee OA—showed that removal of the IFP led to improved joint health compared to guinea pigs with their IFP maintained.⁷⁰ Taken together, the clinical and preclinical data suggest that a solution to IFP tissue dysfunction in knee OA is likely not as simple as “remove” or “maintain” the IFP. On the one hand, the IFP undergoes morphological and secretory

changes with OA, obesity, and, to a lesser extent, sex to express a pro-inflammatory role. On the other hand, the IFP may provide a protective effect in progression of pain postoperatively. The solution may lie in targeting a specific component or action of the IFP. The next section provides a focused review of the inflammatory profile of the IFP in its interaction with other tissues to delineate its role in OA and obesity.

The IFP is an Active Communicator in Knee OA

As a whole-organ, whole-body disease, decoding the communication between involved tissues in OA is crucial to understand the initiation and cascade of tissue breakdown and inflammation. Fat-cartilage crosstalk plays a key role in OA pathogenesis, but it is unclear how the IFP contributes to this. The synovium is a hub for inflammatory mediators and borders the IFP, allowing for potential direct communication and transduction of signals contributing to knee health. The current literature on crosstalk between the IFP and adjacent tissues relevant to this dissertation are described in the next sections.

IFP-Cartilage Crosstalk

Articular cartilage degeneration has long been the primary characteristic of OA structural disease. Articular cartilage is a viscoelastic, smooth connective tissue that lines the synovial joints.⁷¹ It is approximately 2 to 4 mm thick and is both avascular and aneural, receiving its nutrients via diffusion from the synovium and subchondral bone.⁷² Cartilage is composed of a dense extracellular matrix (ECM) with a distribution of chondrocytes. Chondrocytes—i.e. specialized cartilage cells that regulate ECM

stability—have limited potential for replication.⁷¹ It is well-known that cartilage is not a regenerative tissue, making repair an unresolved problem in the treatment of OA. The ECM is made of water (up to nearly 80% of its volume) as well as collagen, glycosaminoglycans (GAGs), and proteoglycans.⁷² Cartilage is divided into three distinct zones: superficial, middle, and deep. The superficial zone (~10-20% of the total cartilage volume) protects deeper layers from shear stresses with tightly packed collagen fibers aligned in parallel to the articular surface, and is the first layer to break down with OA.⁷² Chondrocytes are most abundant in the superficial zone⁷³ and express cAMP responsive element binding protein (CREB)⁵, proteoglycan (PRG)⁴, and cartilage oligomatrix protein (COMP), which are all essential for maintaining cartilage ECM.^{73,74} Dysregulation of these factors and others have been well-characterized in the cartilage through several signaling pathways: wntless-like (Wnt), nuclear factor (NF)- κ B, mechanistic target of rapamycin kinase (mTOR), mitogen-activated protein kinases (MAPK), and transforming growth factor (TGF) β , among others.⁷³ With OA, the IFP actively produces and secretes cytokines and adipokines that disrupt or activate these pathways and influence cartilage health, highlighting a potential for IFP-cartilage crosstalk in OA pathogenesis.

As described earlier in this chapter, the IFP produces and secretes various cytokines and adipokines, and many studies show an increase in pro-inflammatory mediators produced at and secreted by the IFP in OA. With OA, the IFP secretes increased levels of TNF- α and IL-1 β , which are prominent activators of the NF- κ B signaling pathway that, in turn, induces the secretion of ECM degrading enzymes (e.g. MMPs) leading to cartilage degeneration.⁷³ OA IFPs also demonstrate a reduction in the

expression of CREB5, which regulates cartilage integrity through Wnt signaling,⁷⁴ and is further reduced in OA IFPs from PWO,¹⁸ suggesting that the IFP affects Wnt signaling in cartilage and is mediated by obesity status.

Culturing tissues or cells in treated or other tissue-derived conditioned media is a valuable and effective method for delineating secreted factors and signaling involved in tissue crosstalk. Specifically, culturing tissues in fat-conditioned media derived from OA IFPs allows for the ability to measure tissue changes in response to OA IFP signaling. One study found that bovine cartilage cultured in human OA IFP-derived FCM exhibited reduced nitric oxide (NO) production and GAG release, and downregulated MMP-1 expression, and further inhibit catabolic processes when stimulated (by IL-1 β)²⁶ suggesting the IFP to play a protective role in cartilage health. In contrast, a study found that OA IFP-derived fat-conditioned media (FCM) induced catabolism and inflammation of chondrocytes, and increased expression of IL-1 β , IL-6, and COX2, and downregulation of SOX9 and COL2A1 (well-characterized chondrogenic-specific genes).²² Additionally, cartilage explants treated with OA IFP-derived FCM exhibit a loss in proteoglycans and increase in the number of MMP and cyclooxygenase (COX)2 positive cells, which are linked to chronic inflammation and pain. Through targeting downstream mediators of classic OA signaling pathways and validation through inhibitory testing, this OA IFP-induced cartilage degradation was attributed to the p38MAPK and ERK1/2 signaling pathways. Interestingly, pathological OA cartilage changes mediated by leptin are attributed in part to activation of p38MAPK signaling.⁷⁵ With leptin largely produced by the IFP and increased with OA, this highlights a potential mechanistic contribution of the IPF in fat-cartilage crosstalk through leptin

signaling. Figure 1.9 shows an overview of the variable effects of interorgan crosstalk between the IFP and cartilage discussed in this section.

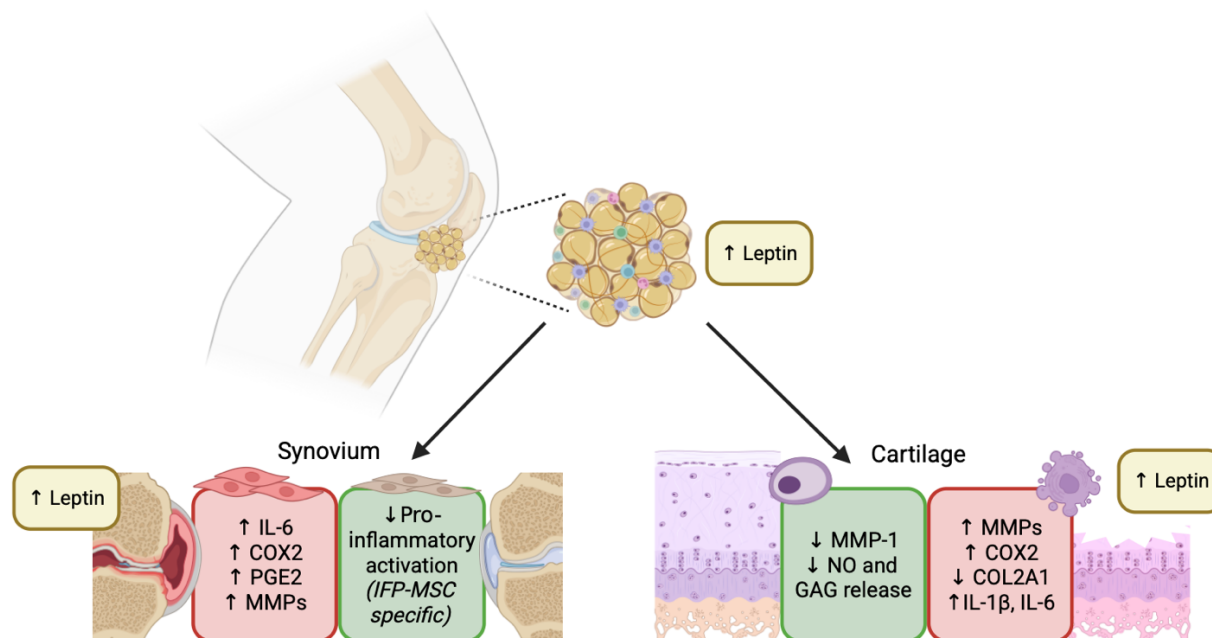


Figure 1.9. Influence of IFP communication with adjacent tissues in OA. IFP produces and secretes leptin in a normal state, and increased leptin secretion has been noted in OA IFP. The IFP has been shown to impart both harmful and beneficial effects on OA synovium, and increased levels of leptin have also been found in OA synovial fluid. The IFP has also been shown to impart both harmful and beneficial effects on OA cartilage, and leptin mediates fat-cartilage crosstalk in OA cartilage degeneration.

IFP-Synovium Crosstalk

Inflammation of the synovium, or synovitis, is another hallmark in knee OA.

Synovium is the soft tissue lining diarthrodial joints and includes a continuous surface layer of cells (intima), the underlying tissue (subintima), and a small amount of fluid rich in hyaluronan between the intimal surfaces that provides joint lubrication.⁷⁶ The intima consists of macrophages and fibroblasts, and the subintima includes resident fibroblasts, infiltrating cells, and blood and lymphatic vessels housed in a collagenous

ECM.⁷⁶ Synoviocytes, or synovium cells, are either macrophage-like or fibroblast-like. Macrophage-like synoviocytes are involved in immunological defense; fibroblast-like synoviocytes (or FLS) secrete hyaluronan to lubricate and nourish joint cartilage and control ECM turnover to maintain synovium matrix structure.⁷⁷ In total, the synovium acts as an active contributor to knee homeostasis and immune regulation. OA synovitis is most typically characterized by thickening of the synovial lining, increased vascularity, and inflammatory cell infiltration,⁷⁸ including FLS.⁷⁷ Excessive proliferation of FLS, ECM synthesis, and increased expression of pro-inflammatory factors (NO, PGE2, IL-6) from FLS have all been noted with OA. The synovium and IFP are active, inflammatory organs, and the synovium borders the IFP posteriorly. DPP4+ has been shown to be a common progenitor of the IFP and synovium highlighting that these structures share both a border and cell lineage.⁷⁹ As such, decoding the communication between these two organs is crucial to understand the inflammatory cascade in the knee joint organ system with OA.

Similar to chondrocytes from cartilage, studying harvested FLS from the synovial membrane of patients with OA that are cultured in OA IFP-derived fat-conditioned media provides insight into the influence of IFP signaling on adjacent tissues. Stimulation of OA FLS with IFP-conditioned media induces increased expression and protein secretion of IL-6, COX-2, PGE2, and MMPs 1, 3, 9, and 13⁸⁰—all well-characterized pro-inflammatory, pro-degradative mediators in knee OA. Further, OA FLS cultured in SAT-derived fat-conditioned media stimulated significantly lower levels of all pro-inflammatory mediators compared to IFP-conditioned media.⁸⁰ This suggests that the IFP imparts a stronger inflammatory influence than systemic adipose in fat-synovium

crosstalk in OA. In contrast, IFP-MSCs demonstrate immunomodulatory effects on synoviocytes including attenuation of pro-inflammatory synoviocyte and macrophage polarization in vitro.⁸¹ Figure 1.9 shows an overview of the variable effects of interorgan crosstalk between the IFP and synovium discussed in this section.

Of note, leptin is found significantly increased in OA synovium and positively correlates with severity of structural damage with OA.⁸² Throughout this chapter, leptin has been noted in several knee tissues: leptin is found elevated in the synovium, imparts degradative effects on cartilage, is produced and secreted by the IFP, and increases with obesity. As such, the next section describes leptin in obesity and OA and poses the IFP as a potential key transducer of leptin signal to the joint.

IFP, Leptin, and OA: Systemic Signal to Local Joint

Leptin is the most consistently increased fat-derived mediator reported in obesity-influenced OA.^{49,83,84} Similar to insulin resistance, leptin resistance is a hallmark of obesity and is characterized by increased circulating leptin with impaired transduction to the hypothalamus via failed binding to leptin receptors at the blood brain barrier, impairing the anorexic effect of the satiety hormone.⁸⁵ Outside of the central nervous system, leptin receptors are found in peripheral tissues including articular cartilage,⁸⁶ but the effect of leptin resistance on peripheral tissues has not been characterized. In PWO, OA, and OA comorbid with obesity, circulating leptin levels significantly increase,^{82,87} which correlate with markers of cartilage degeneration.⁸⁸ Leptin signaling plays a key role in fat-cartilage crosstalk in OA,²³ but it is still unclear how leptin from adipose tissue is transduced into the joint.

Expression and secretion of leptin from the IFP increases with OA and obesity, highlighting that sensitivity of the IFP to musculoskeletal and metabolic disturbance may be measured by this adipokine. Literature supports that leptin secretion from the IFP triggers increased gene expression and protein levels of MMPs, TNF- α , and IL-6 in human OA cartilage and synovium, indicating leptin as a catabolic regulator linking the IFP and adjacent tissues in OA.^{16,89} While the IFP may not have a clear pro- or anti-inflammatory role in knee OA and obesity, leptin signaling through the IFP may be the driver of the pro-inflammatory cascade from local adipose to adjacent tissues.

The IFP demonstrates sensitivity to obesity and female sex—key risk factors in OA—via altered tissue profile and morphology.^{18,28} As an adipokine that relates to inflammatory and pain pathways in PWO and females,^{82,88,90} leptin signaling through the highly innervated local adipose structure may elucidate the painful phenotype seen in these high risk populations. Additionally, leptin serum levels reduce with physical activity and exercise,⁹¹ suggesting that this signal is modifiable as it is influenced by metabolic and environmental factors. Investigation into not only leptin signaling through the IFP in OA, but also across sex- and BMI-based groups is required to fully probe the role of the IFP in OA pathogenesis.

These findings pose the IFP as the key signal transducer of increased circulating leptin to the knee joint that triggers downstream catabolism at the synovium and the cartilage to induce OA pathogenesis that is modulated by sex and obesity. To delineate this, further research is needed to directly test the secretory profile of the IFP, cartilage, and synovium with leptin signaling in OA across BMI and sex. The next section

describes tissue profiling measures relevant to this dissertation to measure the secretome of the IFP.

Defining Tissue Communication Through the Secretome

The secretome, or secretory profile, is a method of advanced tissue profile to specifically measure soluble secreted factors (e.g. proteins, metabolites) from a desired tissue. This is done by culturing collected tissue, in this case surgical waste tissue from humans who have undergone TKA, in conditioned media (for 48 hours) to extract the secreted molecules, or signals, from the tissue. Up to this point, the tissue biological profile of the IFP presented in this dissertation is derived from the intrinsic tissue profile, i.e. transcription factors, proteins, metabolites that reside within the target tissue. In this dissertation, the secretome of the IFP, cartilage, and synovium are measured to describe the extrinsic profile of said tissues and probe the signals transmitted between the tissues. Describing the secretome of the IFP and adjacent knee tissues allows for novel investigation into the extrinsic profile of each of tissue to disentangle secreted signals that are sent (and subsequently received) among the 3 OA tissues, advancing our knowledge of interorgan crosstalk in OA. An additional treatment of these tissues with leptin—via leptin-challenged conditioned media, modeling an influx in circulating leptin—directly measures how each secretome uniquely (or similarly) responds to leptin to delineate leptin-mediated interorgan crosstalk. The next section provides background on secretome analysis via proteomics and metabolomics which are measured via mass spectrometry.

Mass Spectrometry

Mass spectrometry (MS) is a highly effective qualitative and quantitative analytical tool that measures the mass-to-charge ratio (m/z) or property related to m/z of molecules present in a given sample, such as tissue or conditioned media.⁹² MS analyzes molecules in a sample through 3 main components: ionization, mass analyzer, and detection.⁹² In short, MS first converts molecules in a sample into ions, which are separated in the mass analyzer, and the fragments are detected based on their m/z and relative intensity.⁹³

Before MS can occur, the sample must be prepared by converting it into either a gaseous or liquid phase using chromatography.⁹² Liquid chromatography (LC) is the method relevant to this dissertation and, as such, will be the focus in this section. Chromatography separates a given mixture into components based on polarity via allowing a mixture and a mobile phase to flow over a stationary phase, which separates molecules based on the different affinities for these two phases.⁹⁴ The mobile phase in LC is a liquid that carries the sample and propels the compounds through the stationary phase, which are essentially barriers that separate the compounds. The stationary phase is achieved through various methods including, but not limited to, planar, partition, and column.⁹⁴ Column chromatography is most commonly used and is relevant to this dissertation. Column chromatography involves a column of fiberglass or steel with silica particles inside that act as the stationary phase, separating compounds as they pass through the column based on their polarity.⁹⁴ In normal-phase LC, a polar stationary phase separates non-polar compounds as the liquid passes through the

column. In reverse-phase LC, a nonpolar stationary phase separates polar compounds as the liquid passes through the column.

Once the sample has passed through the column and completed the LC portion, the separated compounds in the sample are ionized via an ion source. Compounds are ionized by removing a valence electron, achieving a positive charge that then breaks down the molecule into smaller fragments.^{92,93} Once charged, the ions travel through the mass analyzer where the ions are accelerated, pass through slits in the MS apparatus, and separate based on their m/z , which are lastly quantified by a detector and recorded onto a computer for data processing and analysis.^{92,93,95} This process, LC-MS, is depicted in Figure 1.10.⁹⁵

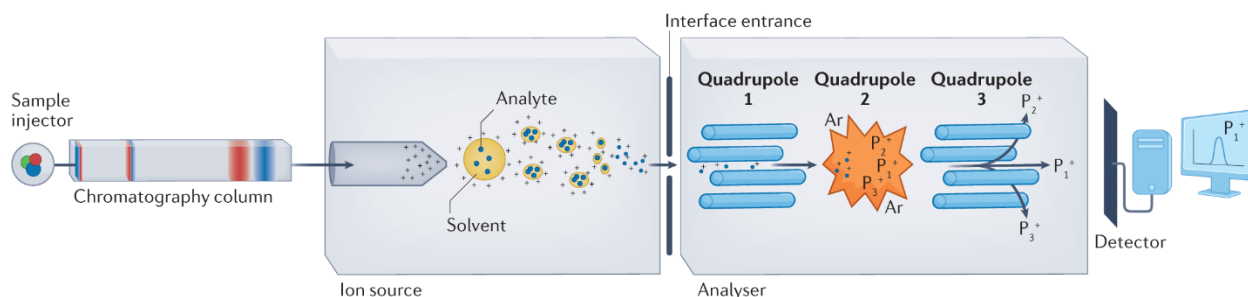


Figure 1.10. Schematic of liquid chromatography-mass spectrometry (LC-MS) workflow. In brief, the liquid sample is injected into the chromatography column, separated compounds are ionized, then ionized molecules are fragmented and detected based on their mass-to-charge ratio (m/z) and recorded based on m/z and measures of abundance. Figure reproduced with permission of Nature Reviews from Liquid chromatography-tandem mass spectrometry for clinical diagnostics.⁹⁵

Mass spectrometry for molecular profiling of biological fluids and tissues has advanced our knowledge of health and disease and bolstered development of pharmaceutical interventions. As such, utilization of this scientific tool for investigation of the IFP secretome is vital to advance our knowledge of secreted molecules by the IFP

in disease. Profiling of the IFP secretome is integral to this dissertation and specifically includes details on the proteomic and metabolomic features of the IFP in knee OA. In brief, the genome contains the complete set of genetic instructions within an organism (DNA), the transcriptome represent gene expression which gets translated from mRNA into proteins which carry out cellular functions,⁹⁶ shown in Figure 1.11. Proteomics is the study of interactions, function, composition, and structures of proteins and their cellular activities.⁹⁷ Metabolomics is similar, but the study of metabolites. Metabolites are metaphorically downstream to proteins (Figure 1.11) such that metabolites are small-molecule intermediates and byproducts resulting from metabolism, such as breaking down food, drugs or chemicals, or the body's own tissues.⁹⁶

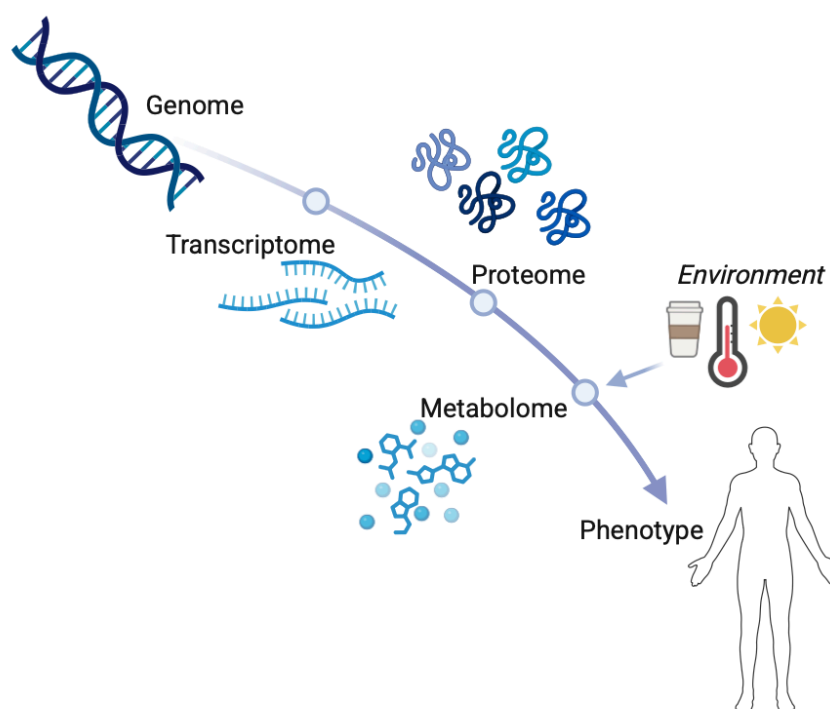


Figure 1.11. Genome to phenotype: Flow of molecular profile in biology. The genome contains the complete set of genetic instructions within an organism (DNA). The transcriptome represent gene expression, which gets translated from mRNA into proteins which carry out cellular functions. Cellular functions and environmental influences converge in metabolism which results in small-molecule byproducts, or metabolites.

Studying the IFP secretome at the proteome and metabolome levels allows for multi-level molecular profiling of secreted signals from the IFP in knee OA disease. This thesis describes changes in the IFP secretome by sex, BMI, in response to leptin signaling and in relation to pain to contribute novel insight into how the IFP differs in molecular composition by sex and BMI in the context of patient pain. Prior to characterization of the profile of the IFP secretome, an investigation into imaging characteristics of the IFP in relation to pain and structure in a subset of knee OA lays the foundation for understanding how the IFP links to pain and characterizing relationships across sex and BMI.

Chapter 2 Relationships Between The Infrapatellar Fat Pad and Patellofemoral Joint Osteoarthritis Differ with Body Mass Index and Sex

This dissertation leverages an interdisciplinary approach to uncover how the IFP relates to OA across structure, pain, sex, and BMI. To begin to unravel IFP dysfunction, Chapter 2 utilizes quantitative imaging, specifically Magnetic Resonance Imaging (MRI), to investigate how tissue abnormality at the IFP relates to structural and functional change with OA in a sub-population of knee OA. This work was published in the *Journal of Orthopaedic Research* on January 20, 2025.⁹⁸

Introduction

Knee osteoarthritis (OA) is a chronic degenerative condition and a leading cause of pain and disability worldwide with no known disease-modifying treatment.⁹⁹ The global prevalence of knee OA increased by 22% between 1990 and 2019, and is expected to grow by 75% by 2050 compared to levels observed in 2020. While traditional measures of knee OA focus on articular cartilage damage, osteophytes, and joint space narrowing, knee OA is now more commonly understood as a whole-joint disease, causing changes in all tissues of the knee joint.¹⁰⁰ These changes include abnormal bone remodeling, synovial effusion, and synovitis. Although the pathogenesis for OA is not fully understood, known risk factors for knee OA include age, previous knee injury, female sex, and obesity.¹⁰¹

In addition to increasing the mechanical load borne by the knee, increased adiposity is thought to contribute to OA risk via systemic inflammatory and metabolic effects that impair cartilage homeostasis.^{102,103} Preclinical studies indicate a causative role for adipose tissue and secreted factors from adipose tissue in OA pathogenesis.²³ Harasymowicz et al. found that adiponectin, a major adipokine, can induce pro-inflammatory gene expression in OA articular chondrocytes.¹⁰⁴ In addition, they found increased gene expression and protein level of adiponectin receptors in chondrocytes from OA knee joints for obese individuals, compared to lean individuals.

While obesity has been linked to OA risk^{105,106}, the role of local adipose structures in the onset and progression of knee OA remains unclear. However, previous studies indicate that the IFP can be altered due to obesity status.²⁸ The infrapatellar fat pad (IFP) is a highly innervated and vascularized adipose structure in the knee joint located directly below the inferior surface of the patella and posteriorly adjacent to the cartilage of the femoral trochlea.^{31,107} The IFP is located closely to synovial layers as well as articular cartilage surfaces.¹⁰⁸

Despite these correlations and relations, mechanistic insight about the IFP is lacking. While the IFP was once considered to only have a buffering and lubricating function in the knee, early studies suggest that the IFP could play an important role in knee joint homeostasis, finding that even in extreme starvation conditions, the IFP is preserved.^{13,31} More recent research suggests that the IFP contributes to the synovial fluid pro-inflammatory profile, secreting cytokines, interleukins, growth factors, and adipokines that can inhibit the production of cartilage matrix proteins.¹⁰⁹

Given the IFP's location directly inferior to the patella, we sought to determine relationships between Magnetic Resonance Imaging (MRI) signal abnormalities in the IFP and OA in the patellofemoral joint (PFJ). PFJOA is increasingly recognized as a more prevalent site of OA, accounting for up to 64% of symptomatic knee OA, and occurring in isolation in up to 20% of people.¹¹⁰ Previous literature has shown an increase in severity of isolated PFJOA on radiographs to be associated with greater levels of pain, stiffness and functional limitation, after adjusting for age, sex and Body Mass Index (BMI).¹¹¹ As with general knee OA, the question surrounding the relationship between IFP and PFJOA includes two key components: structure and symptoms.

This study aimed to leverage existing data previously collected from a cohort with PFJOA to identify relationships between MRI signal abnormalities in the IFP and changes both structurally – via joint synovitis scores and cartilage degeneration measures on MRI – and clinically – via patient-reported outcomes. In utilizing an existing pool of data, we included all MR imaging measurements available to maximize our observation of potential relationships. Our secondary aim was to interrogate these relationships in patients with normal, overweight, and obese BMI groups, and tertiarily characterize the differences in these relationships among males and females. We hypothesized that there is a significant positive relationship between IFP abnormalities and PFJOA disease and functional severity, and that these relationships change with weight-based and sex-based groups.

Methods

Design

Cross-sectional study; Level of evidence, 4.

Study Subjects

Seventy male and female participants aged 28-80 from an ongoing institutional review board-approved study of PFJOA were included. This work is ancillary to the aims of the ongoing study, whose aims are to explore how biomechanics and bone shape mediate the relationship between bone metabolism and cartilage health.¹¹² All participants provided written informed consent prior to participation in the study. MRIs were available in all participants. Those with Whole Organ Magnetic Resonance Imaging Scores¹¹³ (WORMS) > 1 (indicating at least a partial-thickness focal cartilage defect) in the patellar or trochlear cartilage were included. Participants with tibiofemoral cartilage involvement (WORMS > 0) or knee ligament trauma or surgery were excluded.

MRI Examination Method

Participants underwent MRI scan acquisitions in a 3.0T whole-body hybrid PET (Positron Emission Tomography)-MRI scanner (Signa PET-MR, GE Healthcare, Milwaukee, WI, USA) capable of simultaneous acquisition of both time-of-flight (TOF) PET and high resolution MR data.¹¹⁴ Participants were positioned supine, feet-first with two 16-channel medium flex receive-only coils (NeoCoil, Pewaukee, WI, USA) wrapped around each knee. For MRI, bilateral sagittal three-dimensional (3D) proton-density fat-saturated fast-spin-echo (3D PDFS FSE i.e., Cube, whole joint) and bilateral axial

Magnetization-Prepared Angle-Modulated Partitioned k-Space Spoiled Gradient Echo Snapshots (MAPSS, dedicated for PFJ) sequences¹¹⁵ were acquired for morphological assessments of both knees. The detailed scanning protocol can be found in Table 2.1. Only MR images were used in this study and the PET acquisition parameters are detailed further in a separate article from the ongoing study.¹¹²

Table 2.1. Acquisition parameters for simultaneous PET-MRI sequences in this study.

Imaging Sequences		Scan Parameters	Resolution/ Scan Time	Assessment
Sagittal Prescription	3D fat-sat FSE-Cube	FOV=15 × 15 cm ² ; 256 × 256 matrix size; 0.6 mm slice thickness; 600 slices; TR/TE= 1200/26 ms; BW=±62.5 kHz; ETL = 32; ARC AF (k _y × k _z) = 2 × 3	Isotropic 0.6 mm/ 13 mins	Overall morphological assessment of the whole joint, for isolated PFJ-OA confirmation
	3D fat-sat T _{1ρ} and T ₂ MAPSS	FOV = 14 × 14 cm ² ; 256 × 128 matrix size; 4 mm slice thickness; 78 slices; TR/TE = 8.4/2.1ms; BW=±62.5 kHz; T _{rec} =1.2 s; 64 views per preparation; TSL= 0/10/40/80ms; FSL= 500 Hz; TE for T ₂ -preparation = 0/12.8/25.7/51.4 ms; ARC AF (k _y × k _z) = 2 × 3	0.55 × 1.1 × 4 mm ³ / 12 mins	T _{1ρ} and T ₂ quantification of TFJ cartilage (Not Used in this study)
Axial Prescription	3D fat-sat T _{1ρ} and T ₂ MAPSS	FOV = 5.76 × 29.2 cm ² ; 64 × 324 matrix size; 64 slices; 0.9 mm slice thickness; TR/TE = 4.5/2.1ms; BW=±31.25 kHz; T _{rec} =1.2 s; 128 views per preparation; TSL= 0/10/40/80ms; FSL= 500 Hz; TE for T ₂ -preparation = 0/12.8/25.7/51.4 ms; ARC AF (k _y × k _z) = 3 × 1	Isotropic 0.9 mm/ 23 mins	T _{1ρ} and T ₂ quantification of PFJ cartilage
	3D 2-point Dixon Fat/Water GRE	FOV = 40 × 30 cm ² ; 256 × 96 matrix size; 64 slices; 5 mm slice thickness; TR =4ms; 2 TEs; BW= ±83.3 kHz	1.56 × 3.13 × 5 mm ³ / 20 sec	For MR-based PET attenuation correction
	Static PET (Simultaneous with MRI scanning 60 min)	Transverse Filter Cutoff 3 mm, Subset 28, Iterations/Axial Filter 4/ Standard	1.95 × 1.95 × 2.78 mm ³	SUV bone remodeling (Not Used in this study)

MRI Assessment

Abnormalities of the IFP were scored by a radiologist (ZA – 10 years MSK, 18 years radiology experience) using semi-quantitative gradings both in terms of area and signal intensity³⁶. Sample scored images are shown in Figure 3.1. In addition, joint effusion and synovitis were semi-quantitatively assessed with the Anterior Cruciate Ligament OsteoArthritis Score (ACLOAS)¹¹⁶, the MRI Osteoarthritis Knee Score (MOAKS) based on axial fat-suppressed knee images,¹¹⁷ and the synovial proliferation score.³⁶ Patellar and trochlear cartilage abnormalities were semi-quantitatively scored by two radiologists (ZA, CN – 11 years MSK radiology) using the WORMS system for cartilage signal and morphology on images with an eight-point scale.¹¹³ MRI scoring guidelines are outlined in Table 2.2, and example images of normal IFP and abnormal IFP are shown in Figure 2.1.

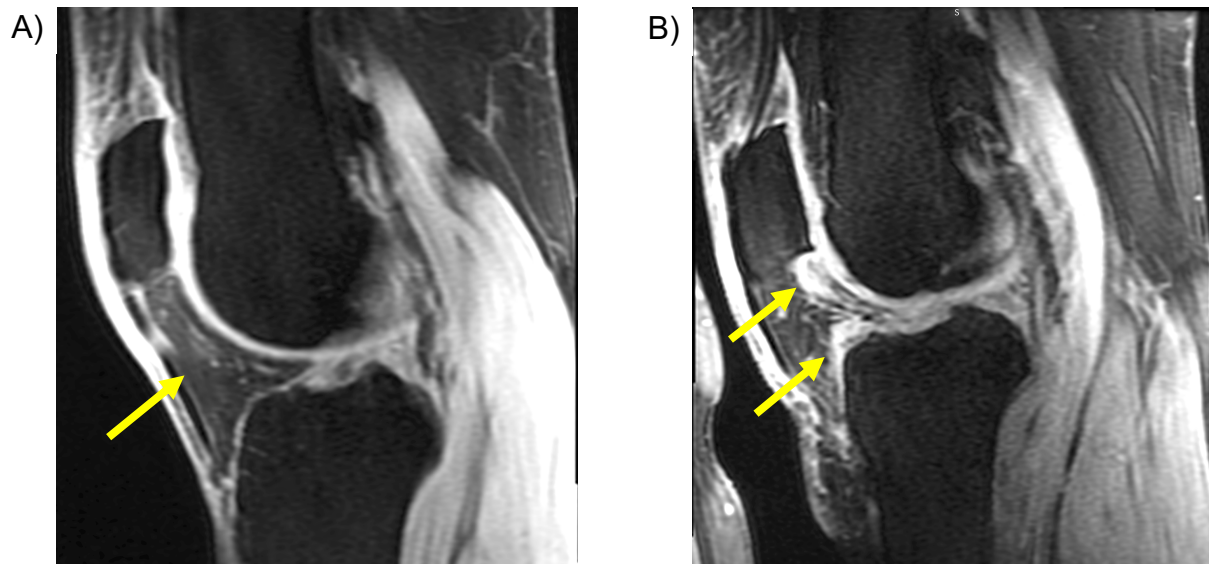


Figure 2.1. Example IFP abnormality scoring on MR images. Yellow arrows indicate location of IFP signal. Note: MR Images were brightened 50% for enhanced viewing in print. A) Grade 0 for abnormal IFP area and signal. B) Grade 2 for abnormal IFP area (>33% but less than 66% of involvement) and Grade 3 for abnormal IFP signal (abnormal signal intensity similar to fluid).

Table 2.2. Semiquantitative scoring of IFP and PFJ cartilage change on MR imaging.

Scoring Item	Grading Scale with description
Abnormal IFP Area ³⁶	<i>Area of abnormal signal region within Hoffa's fat pad</i>
	0: No abnormal signal
	1: ≤33% of Hoffa's fat pad involvement
	2: >33% but less than 66% of Hoffa's fat pad involvement
Abnormal IFP Signal ³⁶	3: ≥66% of Hoffa's fat pad
	<i>Signal intensity of the fat tissue filling up the infrapatellar fat pad on fat-suppressed fluid sensitive sequences</i>
	0: No abnormal signal within Hoffa's fat pad (homogenously suppressed)
	1: Abnormally high signal intensity area/areas within Hoffa's fat pad, that is lower than cartilage signal intensity
Synovial Proliferation Score ³⁶	2: Abnormal signal areas with signal intensity similar to cartilage but lower than fluid signal
	3: Abnormal signal intensity similar to fluid signal intensity
	0: No joint effusion
	1: Smooth synovium without synovial bands or proliferation
ACLOAS Effusion ¹¹⁶	2: Mildly irregular synovium
	3: Extensive synovial proliferation with irregular thickening
	0: <2mm
	1: ≥2 but <5mm
MOAKS Effusion ¹¹⁷	2: ≥5mm but <10mm
	3: ≥10mm
	0: Normal (physiologic amount of fluid)
	1: Small amount of fluid
WORMS ¹¹³ - cartilage signal and morphology	2: Moderate amount of fluid
	3: Large amount of fluid
	0: Normal thickness and signal
	1: Normal thickness but increased signal on T2-weighted images
WORMS ¹¹³ - cartilage signal and morphology	2.0: Partial-thickness focal defect <1 cm in greatest width
	2.5: Full-thickness focal defect <1 cm in greatest width
	3: Multiple areas of partial-thickness (Grade 2.0) defects intermixed with areas of normal thickness, or a Grade 2.0 defect wider than 1 cm but <75% of the region
	4: Diffuse (≥75% of the region) partial-thickness loss
	5: Multiple areas of full-thickness loss (Grade 2.5) or a Grade 2.5 lesion wider than 1 cm but <75% of the region
	6: Diffuse (≥75% of the region) full-thickness loss

ACLOAS, Anterior Cruciate Ligament OsteoArthritis Score; MOAKS, MRI Osteoarthritis Knee Score; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

Self-reported symptoms

Participants completed the self-administrated Knee Injury and Osteoarthritis Outcome Score (KOOS)¹¹⁸ to assess disease burden of OA. We included all five subscales of KOOS (pain, symptoms, function in daily living (ADL), function in sport and recreation, and knee-related quality of life (QOL) as well as the patellofemoral pain and osteoarthritis subscale of KOOS (KOOS-PF)¹¹⁹ to measure pain in PFJOA specifically. Scores range from 0-100 with a score of 100 representing excellent knee health, and lower scores indicating worse knee health.

Statistical Analysis

Data from bilateral images per participant were included for analysis. Repeated measures correlations accounting for within-individual association for bilateral knees was performed between abnormal IFP area and signal, respectively, with KOOS subscale scores, ACLOAS and MOAKS joint effusion scores, synovial proliferation score, and WORMS scores for the patellar and trochlear cartilage. All statistical analyses were conducted using Python (version 3.9.6) and repeated measures correlations were conducted using the `rm_corr` package.^{120,121} For secondary analysis, participants were split into 3 BMI groups: BMI ≥ 30 kg/m² (obesity), BMI 25-29 kg/m² (overweight), BMI < 25 kg/m² (normal, as defined by the World Health Organization classification for overweight and obesity).¹²² One-way ANOVA with Tukey HSD *posthoc* analyses were used to compare abnormal IFP area and signal, all MR imaging outcomes, and KOOS scores across BMI groups and sex-based groups, respectively.

Results

Table 2.3 shows descriptive statistics of the participants included in the study. The mean age of participants with obesity was significantly lower than the mean age of participants with healthy BMI (p -value < 0.01) (Figure 2.2). The mean age of participants who were overweight was lower than that of normal BMI participants, but not significantly different.

Table 2.3. Participant characteristics & mean KOOS subscores.

Characteristic	Value
Total Participants, n	70
Age, years mean (SD)	49.1 (10.3)
Female, n (%)	40 (57%)
Participants with Obese BMI (≥ 30 kg/m ²), n (%)	15 (21%)
Age, years mean	43.4
Participants with Overweight BMI (25.0-29.9 kg/m ²), n (%)	28 (40%)
Age, years mean	48.2
Participants with Normal BMI (<25 kg/m ²), n (%)	27 (39%)
Age, years mean	53.2
KOOS, mean (SD)	
Pain	87.7 (12.9)
Symptom	83.4 (14.8)
Sports	76.1 (23.0)
ADL	92.3 (10.9)
QOL	67.3 (26.2)
PF	77.8 (20.1)

KOOS: Knee Osteoarthritis and Injury Outcome Score. ADL: Activities of Daily Living. QOL: Quality of Life. PF: PatelloFemoral.

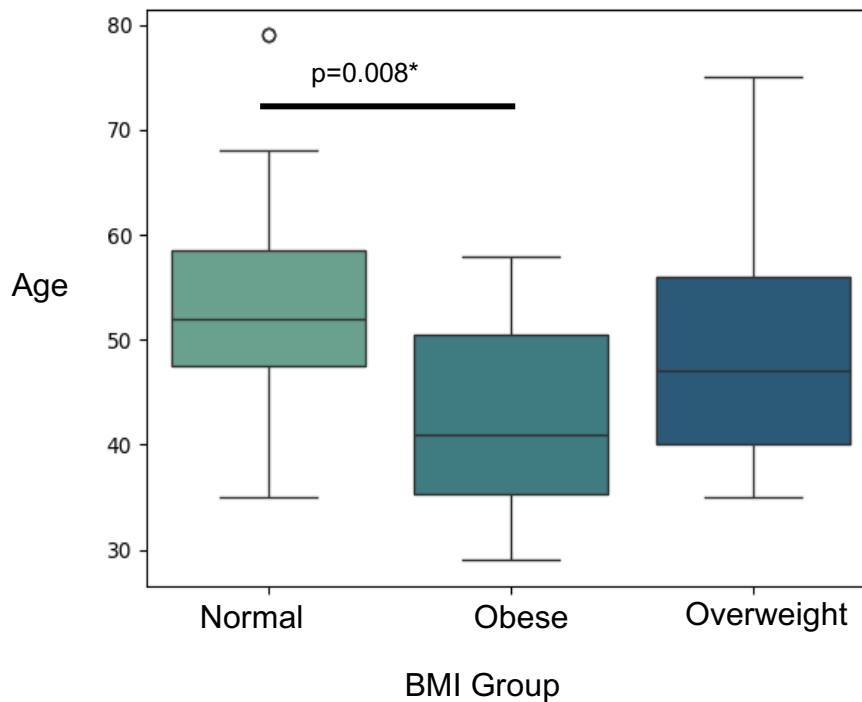


Figure 2.2. PWO are significantly younger than individuals with a normal BMI. ANOVA with Tukey-Kramer *posthoc* analysis of between group differences in mean age across BMI-based groups (normal: <25 kg/m²; obese ≥30 kg/m²; overweight: 25-29.99 kg/m²) showed the obese group to be significantly younger* (p<0.01) than the normal BMI group. This does not include overall mean age.

For simplified result reporting, “IFP” indicates both signal intensity and signal area unless explicitly specified otherwise. No significant differences were found in abnormal IFP when comparing left and right knees. No significant differences were found between weight-based groups and between sex-based groups, respectively, in abnormal IFP and all synovitis measures.

Abnormal IFP is not associated with PFJ structural degeneration

To investigate a relationship between IFP and PFJOA, we assessed correlations between abnormal IFP and measures of PFJ effusion, synovitis, and cartilage

degeneration on MR imaging (ACLOAS effusion, MOAKS effusion, synovial proliferation score, WOMBS patellar cartilage subscore, WOMBS trochlear cartilage subscore). IFP was not found to be significantly associated with any of these measures of PFJ structural degeneration, both overall and in weight-based groups (Table 2.4).

Table 2.4. Repeated measures correlations between IFP area/signal and PFJOA degenerative changes in all data and weight-based groups.

Associations	All Data		Weight-based Groups					
			Normal (BMI <25 kg/m ²)		Overweight (BMI 25-29.99 kg/m ²)		Obese (BMI ≥30 kg/m ²)	
	r	p-value	r	p-value	r	p-value	r	p-value
IFP Area & Outcomes								
ACLOAS Effusion	0.13	0.29	0.08	0.71	0.06	0.78	0.45	0.10
MOAKS Effusion	-0.03	0.79	<1e-6	1.0	-0.07	0.72	<1e-6	1.0
SP	0.11	0.38	-0.08	0.70	0.16	0.41	0.47	0.08
WORMS - Patella Cartilage	0.09	0.44	0.25	0.20	0.06	0.75	0.001	1.0
WORMS - Trochlea Cartilage	0.21	0.09	0.22	0.27	0.28	0.14	<1e-6	1.0
IFP Signal & Outcomes								
ACLOAS Effusion	0.19	0.12	0.23	0.24	0.16	0.42	0.25	0.36
MOAKS Effusion	0.16	0.19	0.14	0.48	0.24	0.21	<1e-6	1.0
SP	0.07	0.59	-0.18	0.37	0.19	0.34	0.13	0.64
WORMS - Patella Cartilage	-0.12	0.32	<1e-6	1.0	-0.18	0.34	-0.09	0.74
WORMS - Trochlea Cartilage	0.04	0.74	0.05	0.80	0.01	0.96	0.14	0.61

ACLOAS: Anterior Cruciate Ligament OsteoArthritis Score. MOAKS: MRI Osteoarthritis Knee Score. SP: Synovial Proliferation score. WOMBS: Whole-Organ Magnetic Resonance Imaging Score.

Size of abnormal IFP area is associated with patient-reported pain and symptoms

We also investigated correlations between IFP and patient functional outcomes by assessing correlations with KOOS sub-scores. We observed significant negative correlations (weak to moderate repeated measures correlation coefficient, or r) between abnormal IFP signal area and all KOOS sub-scores (Table 2.5). IFP signal intensity was significantly associated with lower KOOS QOL scores ($r = -0.30$, $p = 0.02$) but was not associated with any of the other KOOS sub-scores.

Table 2.5. Repeated measures correlations between IFP area/signal and KOOS sub-scores in all data and weight-based groups.

AssociationsAll Data			Weight-based Groups					
			Normal (BMI <25 kg/m ²)		Overweight (BMI 25-29.99 kg/m ²)		Obese (BMI ≥30 kg/m ²)	
	r [95% CI]	p-value	r [95% CI]	p-value	r [95% CI]	p-value	r [95% CI]	p-value
IFP Area & KOOS								
Pain	-0.29 [-0.5, -0.05]	0.02*	-0.02	0.93	-0.35	0.08	-0.45-	0.11
Symptom	-0.43 [-0.61, -0.2]	<0.001*	-0.20	0.34	-0.42 [-0.7, -0.04]	0.03*	-0.65 [-0.88, -0.18]	0.01*
ADL	-0.28 [-0.5, -0.04]	0.02*	-0.03	0.90	-0.31	0.12	-0.48	0.09
Sports	-0.36 [-0.56, -0.12]	0.004*	-0.09	0.64	-0.43 [-0.7, -0.05]	0.03*	-0.57 [-0.84, -0.05]	0.04*
QOL	-0.37 [-0.57, -0.14]	0.003*	-0.15	0.49	-0.41 [-0.69, -0.03]	0.04*	-0.53	0.05
PF	-0.37 [-0.56, -0.13]	0.003*	-0.10	0.63	-0.41 [-0.69, -0.02]	0.04*	-0.55 [-0.84, -0.02]	0.04*
IFP Signal & KOOS								
Pain	-0.22	0.09	-0.32	0.12	-0.12	0.55	-0.34	0.23
Symptom	-0.23	0.07	-0.21	0.32	-0.07	0.72	-0.50	0.07
ADL	-0.14	0.26	-0.20	0.35	-0.05	0.80	-0.40	0.16
Sports	-0.17	0.18	-0.01	0.95	-0.11	0.60	-0.48	0.08
QOL	-0.30 [-0.51, -0.05]	0.02*	-0.28	0.18	-0.17	0.41	-0.55 [-0.84, -0.03]	0.04*
PF	-0.20 [-0.43, 0.05]	0.11	-0.08	0.71	-0.11	0.60	-0.47	0.09

95% Confidence Interval reported for significant correlations. * indicates significance (p-value <0.05). KOOS: Knee Injury and Osteoarthritis Outcome Score; ADL: Activities of Daily Living; QOL: Quality of Life; PF: PatelloFemoral.

Correlations between IFP and KOOS sub-scores differ across weight-based groups

As with measures of joint effusion, synovitis, and cartilage degeneration we were interested in evaluating an effect of BMI on the above relationships. In participants with healthy BMI ($<25 \text{ kg/m}^2$), IFP was not associated with any of the KOOS sub-scores (Table 2.5). In both overweight participants (BMI $25\text{-}29.99 \text{ kg/m}^2$) and participants with obesity (BMI $\geq 30 \text{ kg/m}^2$), abnormal IFP area was significantly negatively associated with KOOS Symptoms, Sports, QOL, and KOOS-PF sub-scores. Strength of weak to moderate significant associations was moderate to strong in the obesity group (r -0.53 to -0.65), and moderate in the overweight group (r -0.41 to -0.42) in each sub-score. IFP signal was not associated with KOOS sub-scores for healthy and overweight BMI groups, but was significantly negatively associated with KOOS QOL in the obesity group ($r = -0.55$, $p = 0.04$).

Correlations between abnormal IFP and KOOS sub-scores differ across sex-based groups

A tertiary aim of this project was to investigate sex-based differences in the relationships between IFP and measures of PFJ structural degeneration, and functional outcomes with the KOOS, respectively. In sex-based analysis, IFP was not significantly associated with PFJ structural outcomes (joint effusion, synovial proliferation, or patellar or trochlear cartilage degeneration) for both the female and male groups (Table 2.6). When reassessing the relationships between IFP and KOOS subscale scores in females, we observed significant moderate negative correlations (r -0.37 to -0.60)

between IFP and KOOS scores across all subcategories (Table 2.7). In male participants, abnormal IFP was not associated with KOOS scores.

Table 2.6. Repeated measures correlations between IFP area/signal and PFJOA degenerative changes in sex-based groups.

Associations	Sex-based Groups			
	Females		Males	
	r [95%CI]	p-value	r [95%CI]	p-value
IFP Area & Outcomes				
ACLOAS Effusion	0.17	0.30	0.07	0.73
MOAKS Effusion	0.16	0.31	-0.32	0.08
Synovial Proliferation Score	0.13	0.43	0.07	0.71
WORMS – Patella Cartilage	0.04	0.80	0.15	0.42
WORMS – Trochlea Cartilage	0.13	0.43	0.30	0.11
IFP Signal & Outcomes				
ACLOAS Effusion	0.13	0.44	0.26	0.16
MOAKS Effusion	0.25	0.13	0.08	0.67
Synovial Proliferation Score	0.03	0.84	0.10	0.58
WORMS – Patella Cartilage	-0.06	0.69	-0.16	0.39
WORMS – Trochlea Cartilage	-0.05	0.30	-0.05	0.78

ACLOAS: Anterior Cruciate Ligament OsteoArthritis Score. MOAKS: MRI Osteoarthritis Knee Score. SP: Synovial Proliferation score. WORMS: Whole-Organ Magnetic Resonance Imaging Score.

Table 2.7. Repeated measures correlations between IFP area/signal and KOOS sub-scores in sex-based groups.

Associations	Sex-based groups			
	Female		Male	
	r [95% CI]	P-value	r [95% CI]	P-value
IFP Area & KOOS				
Pain	-0.50 [-0.71, -0.22]	0.001*	-0.01	0.96
Symptom	-0.54 [-0.73, -0.26]	0.001*	-0.24	0.25
ADL	-0.49 [-0.7, -0.21]	0.002*	0.04	0.86
Sports	-0.44 [-0.66, -0.14]	0.006*	-0.24	0.24
QOL	-0.49 [-0.7, -0.2]	0.002*	-0.27	0.19
PF	-0.51 [-0.71, -0.23]	0.001*	-0.19	0.36
IFP Signal & KOOS				
Pain	-0.60 [-0.77, -0.35]	<0.001*	0.14	0.50
Symptom	-0.55 [-0.74, -0.27]	<0.001*	0.15	0.47
ADL	-0.52 [-0.72, -0.24]	0.001*	0.26	0.20
Sports	-0.37 [-0.61, -0.05]	0.02*	-0.02	0.91
QOL	-0.53 [-0.73, -0.25]	0.001*	-0.13	0.53
PF	-0.49 [-0.7, -0.2]	0.002*	0.06	0.78

95% Confidence Interval reported for significant correlations. * indicates significance (p-value <0.05). KOOS: Knee Injury and Osteoarthritis Outcome Score; ADL: Activities of Daily Living; QOL: Quality of Life; PF: PatelloFemoral.

Discussion

Our findings support our hypothesis that IFP abnormalities from imaging are significantly associated with OA-related patient-reported pain and function scores, but not structural OA pathology in the PFJ. Furthermore, we observe that relationships between IFP and patient-reported symptoms vary across BMI-based and sex-based groups. These data collectively suggest that evaluating the IFP could be critical for understanding OA-related pain phenotypes and comorbid factors of BMI and sex.

Among the full cohort, we observed significant weak to moderate associations between abnormal IFP signal area and worse KOOS scores in all sub-categories. As hypothesized, we observed clear sexual dimorphic relationships in IFP abnormalities. In female participants, we observed moderate to strong significant negative relationships between IFP and all KOOS sub-scores. In contrast, in male participants, we observed no significant relationships among the patient-reported outcomes. Previous studies exploring sex differences in OA and pain have observed higher patient-reported pain scores in females compared to males.^{123,124} The mechanism by which sex-based differences in OA occur, whether through hormonal, biomechanical, or metabolic differences, is not yet fully understood. Our results suggest that patient-reported pain and function in females with PFJOA may be more strongly related to IFP abnormalities. Glass et al. observed that differences between female and male OA-related pain increased in the presence of PFJOA.⁵¹ Isolated PFJOA is a unique sub-group of knee OA that could lend promising insight into sex-based differences in OA.

When interrogated across BMI groups, significant correlations between IFP abnormalities and KOOS Symptoms, Sport, QOL, and PF nearly all increased in strength among those with BMI over 25 kg/m² (i.e. overweight and obese BMI groups), while participants with normal BMI demonstrated no significant relationships. Most notably, the significant negative relationship between IFP area and KOOS Symptom increased when isolating participants with overweight BMI and obese BMI, respectively. This same trend of increased strength of correlation from overweight BMI group to obese BMI group is observed in KOOS Sport and PF. While the relationship between IFP area and KOOS QOL is not significant in participants with obesity, it is worth noting

that the correlation trends toward significance ($p=0.05$) and follows the same pattern observed among other KOOS sub-scores with stronger negative correlations with increasing BMI. This trend could suggest a linear effect between obesity status and strength of relationship between IFP abnormality and OA pain.

While several previous studies focus on IFP signal intensity alteration in knee OA,¹²⁵⁻¹²⁷ we analyzed both abnormal IFP area and signal intensity and observed that abnormal area, rather than signal intensity, was significantly associated with KOOS sub-scores. This highlights abnormal IFP area as a key measure to consider in future investigation on IFP abnormality in OA and in relation to pain.

Adipose tissue plays a role in regulating systemic metabolism, and aging and obesity can lead to dysfunction of adipose tissue.² Our results support our hypothesis that obesity contributes to local adipose pathology in the IFP influencing OA symptom severity. Of note, there are recent data that suggest that the fat pad does not mitigate OA pathogenesis,¹⁰³ and another that suggests it does.⁷⁰ While the consensus on the role of the IFP in OA is still unclear, our findings that individuals at higher risk for adipose dysregulation ($BMI \geq 30 \text{ kg/m}^2$) demonstrate strongest relationships between the IFP and OA symptoms emphasizes further investigation into the mechanistic link between systemic fat and local fat, and in turn, OA pain.

Interestingly, abnormal IFP was not associated with PFJOA pathology in our cohort. Previous research has related IFP abnormalities to structural change and symptoms in general knee (tibiofemoral joint, TFJ) OA. Some of these findings include studies that observe higher IFP signal in knee OA patients compared to healthy controls.¹²⁷⁻¹²⁹ Wang et al. found that IFP signal intensity alteration predicted knee

replacement over 5 years,¹²⁵ and Ruan et al. found significant associations between quantitatively-measured IFP signal intensity alteration and effusion-synovitis in people with knee OA over two years.¹²⁶ Our study is among the first to relate IFP abnormality to isolated PFJOA, and thus warrants further investigation using more nuanced quantitative MR measures to provide a deeper understanding of microstructural and compositional articular changes at the PFJ.

Our study has several limitations. First, our findings are based on an existing cohort of participants with degrees of isolated PFJOA and excludes those with marked cartilage degeneration in the TFJ. Our sample size is small, which limits the complexity of our statistical analysis to repeated measures correlations. A larger sample size would sufficiently power more advanced statistics, like a linear mixed effects model, of outcomes and, as such, offer the ability to adjust for confounding variables – e.g. age. Our analysis of abnormal IFP signal in relation to PFJOA pathology may be affected by the IFP's proximity to the PFJ, and further research is needed to confirm our findings in a larger sample including patients with TFJ OA and patients with damage in both the tibiofemoral and patellofemoral joints. While our study excluded those with traumatic knee injuries or surgeries, our cohort was not screened to exclude participants taking non-steroidal anti-inflammatory drugs or lipid-lowering agents, which could influence IFP metabolism.¹²⁷ Although both WOMS graders in our study were trained by the same radiologist on a semi-quantitative scale that has demonstrated substantial to good agreement for intra-reader reproducibility,³⁶ future studies could use fully quantitative methods, such as standard deviation of IFP signal intensity and ratio of high signal intensity volume to whole IFP volume¹²⁶ to measure alteration in IFP signal. While we

did not aim to assess the predictive value of quantitative IFP imaging in this study, future research into identifying the IFP imaging measurement(s) with the most utility in predicting OA progression would be of great benefit. In addition, our study uses BMI as a proxy for estimating body fat proportion using height and weight, but future studies could obtain more accurate calculations for body fat percentage through evaluations such as skinfold measurement, DXA (Dual Energy X-ray Absorption) scan, and hydrostatic weighing.⁵³

To our knowledge, this study is among the first to relate IFP signal alterations to OA pain for patients with PFJOA. A universal limitation in research into understanding OA pathology is the discordance between structural degeneration and pain severity. These findings highlight the sensitivity of the IFP to differences by sex and obesity-status, and could provide a window into why females and individuals with obesity experience greater OA pain and symptoms. Our findings support that the IFP may be the port through which metabolic disruption from obesity influences OA pain severity. Alterations in IFP signal area could potentially point to systemically mediated changes in the metabolic profile of the IFP that influence knee OA-related pain. Chapter 3 probes the tissue profile of the IFP through evaluation of the secretome to uncover sex- and BMI-differentially secreted signals from the IFP that relate to different pain phenotypes.

Chapter 3 IFP Secretome profile across sex and BMI relates to patient-reported outcomes in patients at end-stage of disease

In Chapter 2, a relationship between infrapatellar fat pad (IFP) tissue abnormality and patient-reported outcomes in patellofemoral OA was established, with observed changes in strength of correlation among females and individuals with BMI ≥ 30 kg/m².⁹⁸ This chapter investigates the tissue biology behind the modulation of IFP-pain relationships by sex and BMI in end-stage knee OA, to fill the gap in our understanding of the vastly heterogeneous and puzzling pain phenotypes in knee OA.

Introduction

Osteoarthritis (OA) is the leading cause of global pain and disability, affecting over 250 million people worldwide and most commonly occurring at the knee.³⁹ Aside from age, sex and Body Mass Index (BMI) are the highest risk factors for OA.¹⁰¹ Females and people with obesity (defined by BMI ≥ 30 kg/m²) are most impacted by the disease, experiencing more pain and severity of knee OA (e.g. Visual Analog Scale, KOOS, radiographic structural change) than their male and “normal” BMI (<25 kg/m²) counterparts, respectively.^{46,51} While there have been major advances in understanding the structural pathology of OA disease in articular cartilage—including mitochondrial oxidation and chondrocyte apoptosis¹³⁰—the well-recognized discordance between structural changes and pain severity within OA remains unresolved. Further, it is well

documented that females and patients with obesity (defined by BMI and metabolic dysfunction) suffer greater pain and symptoms with OA,^{51,55,131} yet the mechanistic insight into this remains to be derived. Some evidence has shown that in females, estrogen—specifically estrogen deficiency with menopause—may induce cartilage degradation in knee OA,¹³² but a causative link between female sex and OA pathogenesis, and more importantly, pain remains to be uncovered. In individuals with obesity, the historical viewpoint of obesity and OA is due to higher body mass or increased body size, which potentially exceeds the loading capacity of the joint resulting in tissue damage.¹³³ Our collective understanding of OA with obesity has evolved into a complex contribution of metabolic disturbance and chronic low-grade inflammation that may perpetuate a pro-inflammatory state at the knee joint.¹³⁴ However, as with female sex, the pathways and signaling cascades that causally link systemic adiposity to OA pathogenesis and pain have not yet been found. The IFP demonstrates altered morphological and compositional qualities by males versus females and different BMI status.²⁸ As such, investigation into the molecular profile of the IFP by sex and BMI may provide valuable insight into the unique mechanisms affected by these populations that link to increased pain severity.

Recent advances in molecular profiling technologies (e.g. metabolomics, proteomics) have enabled detailed characterization of circulating and tissue-level factors, offering a window into the molecular processes occurring with OA.^{45,135} At the tissue-level, most work to-date has been characterizing the tissue intrinsic profile of cartilage and synovium, mainly in animal models.^{74,136} Recently, there has been a shift in focus toward adipose tissue as a key component driving OA pathogenesis through

tissue crosstalk in both animal and human models.^{23,24,59} The IFP has largely been overlooked in studies characterizing tissues affected by OA. The first and only comprehensive profile of the IFP in knee OA was recently published by Peters et al., which has laid foundational insight on cell populations and the tissue intrinsic profile of healthy and diseased IFP.¹⁸ However, there is still a lack of evidence detailing the tissue biology and communication of the IFP in knee OA and pain. Knee OA is a complex whole-organ disease involving altered signaling and dysregulated inflammatory pathways,¹⁰⁰ thus characterization of the signals secreted by joint tissues is crucial to inform understanding of how the knee environment changes in relation to disease. Profiling of the secreted factors, or *secretome*, from a tissue provides key insight into communication between tissues that may be targeted.¹³⁷ As such, this study leverages multi-omic secretome profiling of the IFP examined in relation to pain to delineate the molecular pathways uniquely dysregulated by sex and BMI that may contribute to altered pain phenotypes in specific patient subpopulations. Employing a multi-omic approach across proteomic, metabolomic, and lipidomic profiling maximizes the potential molecular characterization gleaned from this tissue. Lipidomic profiling specifically measured for eicosanoids, which are pain-related metabolites that link to inflammation and have been specifically highlighted in relation to knee OA pain (e.g. prostaglandin E, or PGE).^{65,138}

The Knee Injury and Osteoarthritis Outcome Survey (KOOS) is a widely utilized patient-reported outcome measure validated to capture a patient's perceived pain, symptoms, and functional disability.¹³⁹ Previous studies have identified relationships between MR imaging characteristics of the IFP and KOOS scores,^{35,38} including results

presented in Chapter 2 from this author,⁹⁸ yet the biological underpinnings of these associations remain uncharted. Human serum and urine biomarkers linked to knee OA have been linked to clinical measures of patient symptoms measured via the KOOS, including ATS13—a member of the ADAMTS metalloproteinase family.¹⁴⁰ However, a link between local tissue changes and patient outcomes remains to be fully characterized. The IFP is a strong candidate to influence pain and inflammation given its immune cell residence, high innervation, and previously established link to pain. Despite this, the extent to which the IFP molecular profile and signaling pathways relate to patient clinical outcomes remains insufficiently explored.

To address these key gaps, the objective of this study was to integrate IFP secretome characterization across sex- and BMI-based groups to identify uniquely dysregulated molecules that correlate with patient-reported pain and symptoms. We hypothesized that specific proteomic and metabolomic signatures would correlate with patient-reported outcomes and differ in mechanisms of dysregulation by sex and BMI. Decoding the secretory differences of the IFP by sex and BMI in relation to pain may uncover unique mechanisms of varying pain phenotypes. Mechanistic insight into heightened pain phenotypes in different knee OA subpopulations will identify potential targets for precision therapeutics for patients so heavily impacted by the disease.

Methods

Patient Recruitment

In collaboration with Dr. Mohit Kapoor at the University of Toronto and Schroeder Arthritis Institute, 20 patients undergoing total knee arthroplasty (TKA) for end-stage OA (Kellegren-Lawrence grade III and IV) were recruited for this study between September 2023 and April 2025. Recruitment for the study was intentional to include at least 3 individuals from the following populations for multi-omics group-based comparisons to be sufficiently powered:^{18,24,135} females, males, patients with BMI ≥ 30 kg/m², and patients with a BMI < 25 kg/m². We prespecified selection of patients from categories of BMI ≥ 30 kg/m² (to represent obesity), and BMI < 25 kg/m² (as a comparative group without overweight or obesity).¹²² In this study, patients with a BMI ≥ 30 kg/m² were grouped in High BMI (HBMI) group, and patients with a BMI < 25 kg/m² were grouped in Low BMI (LBMI) group. Table 3.1 shows group demographics across our populations of interest.

Table 3.1. Group demographics at time of TKA.

Demographic	Females	Males	HBMI	LBMI
Participants, n	12	8	14	6
Age, yrs median (range)	71.5 (58-86)	66.5 (50-83)	68.5 (50-86)	74 (52-85)
BMI, median (range)	30.9 (20.7-43.1)	31.1 (21.0-34.5)	31.6 (30.3-43.1)	22.6 (20.7-23.7)
Comorbidities*, n				
Hypertension	6	4	8	2
Diabetes	1	0	0	1
High cholesterol	4	3	5	2
Cardiovascular disease	1	1	2	0

HBMI, High BMI; LBMI, Low BMI; yrs, years; BMI, Body Mass Index. Comorbidites* were collected via self-report.

Patient-Reported Pain and Health

Prior to TKA, all patients completed a demographic assessment questionnaire where they self-reported comorbidities and associated treatments. To assess disease burden of OA, patients also completed the Knee Injury and Osteoarthritis Outcome Survey (KOOS), which includes 5 subscales: Pain, Symptoms, ADL (Activities of Daily Living), Sport (function in Sport and recreation), and QOL (knee-related Quality of Life).¹¹⁸ Scores range from 0-100 in each subscale with a score of 100 representing excellent knee health, and lower scores indicating worse knee health.

Tissue Collection and Conditioned Media Protocol

Surgical waste collected at time of TKA included segments of cartilage, synovium, and IFP from the index surgical joint. Segments of all tissues were labelled and flash frozen in cryovials immediately after surgery and stored at -80°C for future use. Tissues, approximately 250 mg, were collected in 50 mL conical tubes filled with warm DMEM. Each tissue was cut into 6-12 small pieces (20-40 mg/piece). Tissue pieces were washed in 10mL PBS in conical tubes, incubated at 37°C for 5 minutes, followed by disposal of the rinse PBS, and repeat of this wash 2 times. Adequately washed tissue pieces were removed from conical tube, placed in cell insert (1.0 µm) in a 6-well plate along with 500µL of DMEM and placed in the incubator at 37°C for 48 hours. After 48 hours, tissue pieces were removed, placed in cryovials and flash-frozen using liquid nitrogen, and stored at -80°C. The media were collected in cryovials, flash frozen and stored at -80°C until recruitment completed and samples ready for

metabolomic and proteomic analysis. This chapter only includes investigation on the IFP tissue media.

Multi-omic Secretome Profiling

Multi-omic profiling of the conditioned media from IFP tissue samples included targeted metabolomics, targeted lipidomics (i.e. eicosanoid-panel), and untargeted proteomics. Nikita Looby, PhD, of the Kapoor Lab performed targeted metabolomics of all tissue secretome samples (leptin and control) using a combination of direct injection (DI) mass spectrometry (MS) with a reverse-phase liquid chromatography (LC)-MS/MS using the MTX MEGA Assay (Metabolomix, Edmonton, Canada) following previously established methods.¹⁸ In brief, this assay, in combination with QE orbitrap (ThermoFischer Scientific, Waltham, MA) mass spectrometer, was used for targeted identification and quantification of 646 endogenous metabolites including amino acids, or amino acid-related metabolites, biogenic amines, ceramides, cholesterol esters, diacylglycerols, acylcarnitines, glyceophospholipids, sphingomyelins, triacylglycerols, organic acids, and nucleotide/nucleosides.¹⁸

A separate aliquot of IFP conditioned media from each patient that was flash frozen following conditioned media protocol was shipped to the Collins Lab for protein and eicosanoid profiling using a novel dual extraction method that separates proteins and metabolites from a single sample.²⁴ In brief, all samples were extracted with methanol, vortexed, and placed at -20 °C for 30 minutes to promote protein precipitation. Next, the supernatant containing metabolites was collected and dried via vacuum concentration for lipidomic eicosanoid profiling, which included a panel of 35

known eicosanoids. The peptide pellet (leftover after the supernatant containing metabolites was removed) was resuspended in trypsin, incubated overnight for digestion of peptides, and peptide extraction for proteomic analysis was completed using the Thermo Scientific EasyPep MS Sample Prep Kit (ThermoFischer Scientific, Waltham, MA). Prepared samples were analyzed and processed at the University of Reno, Proteomics Center, using LC-MS (Evosep One LC coupled to a Bruker timsTOF Pro2 MS, Bruker, Billerica, MA) for untargeted proteomics. Raw data was processed using Spectronaut (version 19, Biognosys AG, Switzerland) for peak picking, alignment, and quantitation. For eicosanoid profiling, a 5 uL aliquot of each sample was subjected to a targeted metabolomic panel of 40 known eicosanoids, analyzed at the Quantitative Metabolite Analysis Center at the University of California, San Francisco¹³⁸ on a Shimadzu 30-AD UPLC series with a SCIEX 7500 Triple Quadrupole Mass Spectrometer with a reverse-phase liquid chromatography column. Raw data was processed using a built-in SCIEX OS software package (version 2.1.6.59781) for peak picking, alignment, and quantitation. Eicosanoids detected were validated and confirmed with commercially bought standard.

Statistical Analysis

Sex- and BMI-based group differences in KOOS subscale scores were performed by two-way ANOVA. To evaluate differences in IFP secretome profile (metabolomics, proteomics, and eicosanoid-panel metabolomics) between sex- and BMI-based groups, respectively, a general linear model (GLM) via the limma pipeline utilized by Metaboanalyst¹⁴¹ was performed. GLM for sex-based differences included

covariates age and BMI. GLM for BMI-based differences included covariates age and sex. From GLM output, factors found dysregulated by sex and BMI, respectively, were assessed for correlation with KOOS subscale scores via Pearson correlation.¹⁴²

Correlation analysis and visualization of secretome dysregulation—KOOS relationships were performed in Python (version 3.9.6)¹²⁰ using the `scipy.stats.pearsonr` package, `statsmodels.multipletests` package to adjust for multiple testing (e.g. p-FDR), and `matplotlib` to visualize correlation heatmaps.

Results

Sex-Dysregulated Secreted Factors from the OA IFP

62 proteins were significantly dysregulated by sex before and after adjusting for covariates age and BMI, and 30 proteins were significantly dysregulated by sex only after adjusting for covariates, shown in Figure 3.1. Two metabolites were found significantly dysregulated by sex with covariate adjustment: sphingomyelin (SM) ae C16:1 and SM ae C24:1. No eicosanoids were found significantly dysregulated by sex after adjusting for age and BMI.



Figure 3.1. IFP secretome protein dysregulation by sex.

General linear model output for protein dysregulation of IFP secretome. Proteins that maintain significance across adjusted and unadjusted models are shown in blue dots, proteins significant only after adjusting for covariates are shown in red dots. Proteins not significant after adjusting for covariates are shown in green and grey.

BMI-Dysregulated Secreted Factors from the OA IFP

When evaluating BMI-based groups, 197 proteins were significantly dysregulated by obesity status when adjusting for age and sex as covariates, shown in Figure 3.2. Four metabolites were found significantly dysregulated by obesity status after covariate adjustment. No eicosanoids were found significantly dysregulated after covariate adjustment.



Figure 3.2. IFP secretome protein dysregulation by BMI. General linear model of proteins dysregulated by obesity-status in the IFP secretome. Proteins that maintain significance across adjusted and unadjusted models are shown in blue dots, proteins significant only after adjusting for covariates are shown in red dots. Proteins not significant after adjusting for covariates are shown in green and grey.

Females and patients with high BMI experience worse pain and disability

Distribution of KOOS scores in all patients and across sex- and BMI-based groups are shown in Figure 3.3. Females demonstrated lower KOOS scores than males, and the HBMI group demonstrated lower KOOS scores than the LBMI group. Two-way ANOVA revealed a significant difference in KOOS score by sex in Symptom subscale, and by BMI in Symptom and Sport subscales.

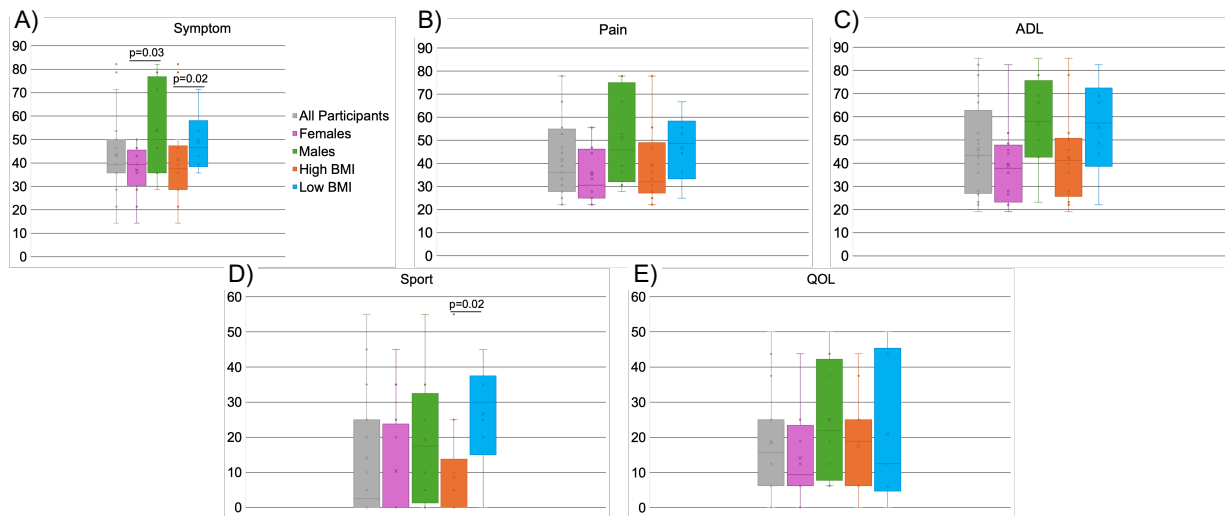


Figure 3.3. Distribution of KOOS scores across sex- and BMI-based groups. Boxplot shows median distribution with orange line in center indicating mean. Females and individuals with a BMI ≥ 30 kg/m² demonstrate the lowest KOOS scores across all subscales. Two-way ANOVA revealed statistically significant differences in KOOS scores between females and males in Symptom subscale (A, pink bar), and between individuals with a BMI ≥ 30 and BMI < 25 in Symptom (A, blue bar) and Sport (D, blue bar) subscales.

Dysregulation of IFP secretome by Sex Correlates with KOOS Subscales

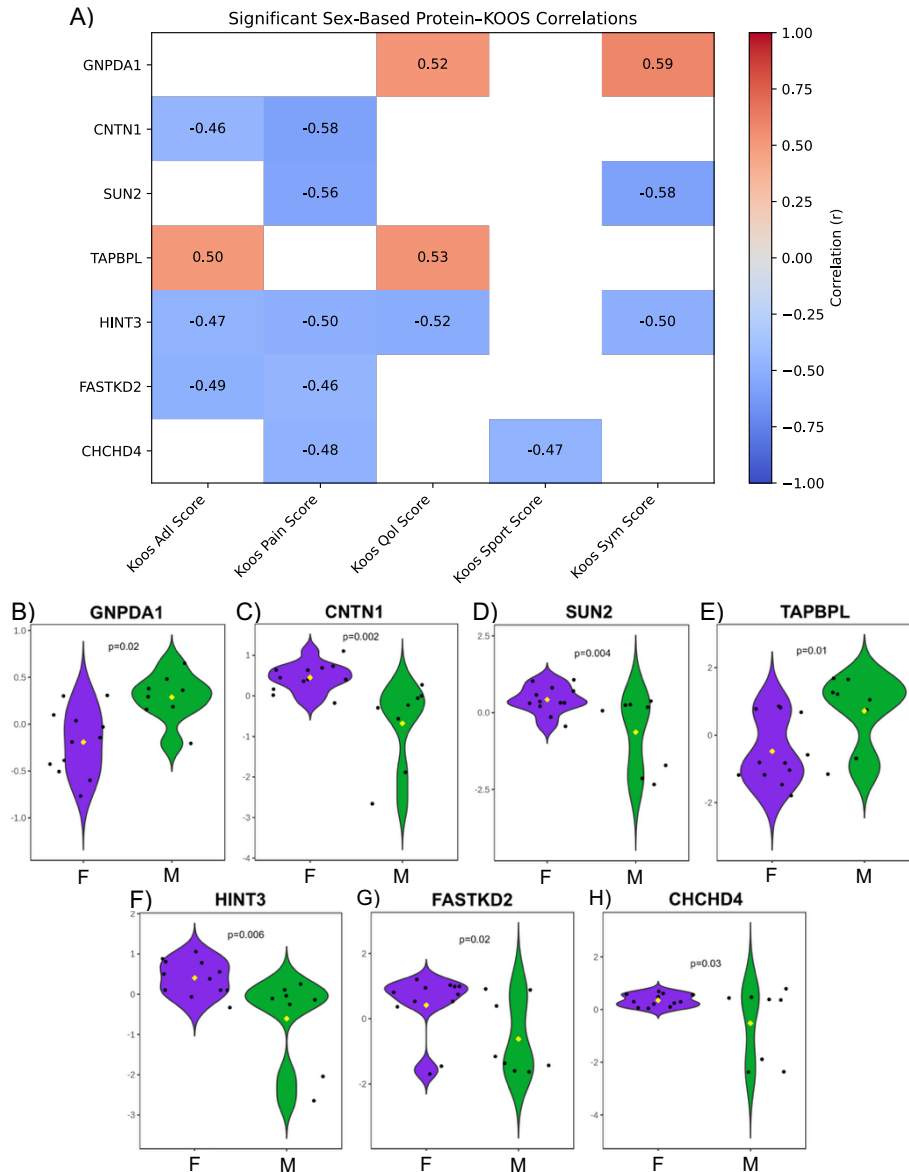
Fifteen proteins that were significantly dysregulated by sex were found significantly correlated across the 5 KOOS subscales, shown in Table 3.2. Seven of the 15 proteins correlated with more than 1 subscale: histidine triad nucleotide-binding protein 3 (HINT3), FAS-induced serine/threonine kinase domain containing protein 2 (FASTKD2), mitochondrial intermembrane space import and assembly protein 40 (CHCHD4), glucosamine-6-phosphate deaminase 1 (GNPDA1), contactin 1 (CNTN1), and SAD1/UNC84 domain protein 2 (SUN2). Figure 3.4 shows a correlation heatmap of the 7 proteins with each KOOS subscale, where relevant. A lower KOOS score indicates worse disability, and a higher KOOS score indicates better health and function. A positive correlation shows that higher protein intensity is associated with better pain and

function, while a negative correlation shows that higher protein intensity is associated with worse pain outcomes.

Table 3.2. Sex-related protein dysregulation correlates with KOOS subscales
Directionality of Pearson correlation coefficients is negative

KOOS Subscale	Protein	r	p-val
KOOS Symptom	ETFA	-0.45	0.045
	GXYLT1	-0.5	0.024
	DNMBP	0.45	0.044
	GNPDA1	0.59	0.0057
	SMPDL3A	-0.48	0.031
	HINT3	-0.5	0.025
	SUN2	-0.58	0.0078
KOOS Pain	CNTN1	-0.58	0.008
	SUN2	-0.56	0.011
	HINT3	-0.50	0.024
	CHCHD4	-0.48	0.032
	FASTKD2	-0.46	0.040
KOOS ADL	TAPBPL	0.50	0.025
	FASTKD2	-0.49	0.028
	HINT3	-0.47	0.036
	CNTN1	-0.46	0.039
KOOS Sport	AAR2	0.45	0.047
	CHCHD4	-0.47	0.038
	ANXA13	0.45	0.045
	BAK1	0.5	0.026
KOOS QOL	GNPDA1	0.52	0.018
	HINT3	-0.52	0.02
	RAB20	-0.47	0.035
	TAPBPL	0.53	0.016

KOOS, Knee Injury and Osteoarthritis Outcome Survey; ADL, Activity of Daily Living; QOL, Quality of Life. ETFA, electron transfer flavoprotein subunit alpha; GXYLT1, glucoside xylotransferase 1; DNMBP, dynamin binding protein; GNPDA1, glucosamine-6-phosphate deaminase 1; SMPDL3A, sphingomyelin phosphodiesterase acid like 3A; HINT3, histidine triad nucleotide binding protein 3; SUN2, Sad1 and UNC84 domain containing 2; CNTN1, contactin 1; CHCHD4, mitochondrial intermembrane space import and assembly protein 40; FASTKD2, FAST kinase domain-containing protein 2; TAPBPL, tapasin-related protein; AAR2, splicing factor 2; ANXA13, annexin A13; BAK1, Bcl-2 homologous antagonist/killer; RAB20, Ras-related protein 20.



I) Top 5 Reactome Pathways

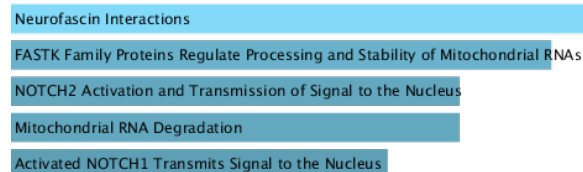


Figure 3.4. Significant Relationships between Sex-Dysregulated Proteins and Pain. A) Correlation heatmap of proteins dysregulated by sex that significantly related to >1 KOOS subscale. The IFP protein intensities that differed between females and males with significant between group (after covariate adjustment) difference include GNPDA1 (B) CNTN1 (C), SUN2 (D), TAPBPL (E), HINT3 (F), FASTKD2 (G), and CHCHD4 (H). The colors correspond to female and male participants: purple – females (F), green – males (M). Top 5 Reactome 2024 pathways of proteins dysregulated by sex in the IFP secretome that relate to pain is shown in I.

Levels of HINT3 in the IFP secretome was negatively correlated with KOOS Symptom, Pain, ADL, and QOL subscales (Table 3.2, Figure 3.4). CHCHD4 expression was negatively correlated with KOOS Pain and Sport subscales. CNTN1 and FASTKD2 were both negatively correlated with KOOS Pain and ADL subscales. SUN2 was negatively correlated with KOOS Symptom and Pain subscales. GNPDA1 was positively correlated with KOOS Symptom and QOL subscales. TAPBPL was positively correlated with KOOS ADL and QOL subscales.

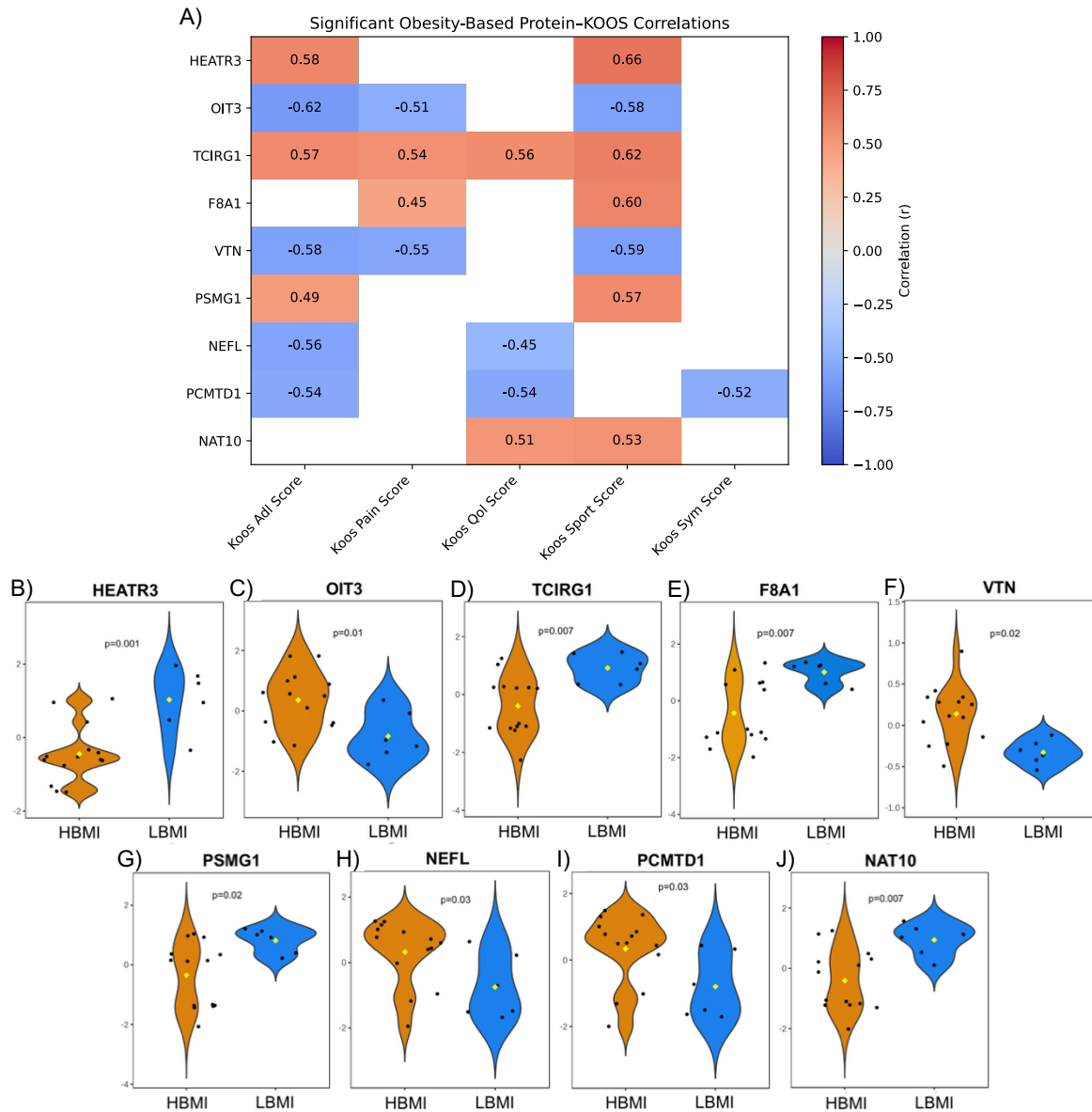
Dysregulation of OA IFP secretome by BMI Correlates with KOOS Subscales

Thirty five proteins and 1 metabolite that were significantly dysregulated by BMI significantly correlated with KOOS subscales, shown in Table 3.3. Nine proteins were significantly correlated with more than 1 KOOS subscale, shown in Figure 3.5.

Table 3.3. Obesity-related protein and metabolite dysregulation correlates with KOOS subscales.

KOOS Subscale	Protein	r	p-val
KOOS Symptom	PCMTD1	-0.52	0.019
	EVI2B	-0.46	0.043
KOOS Pain	F8A1	0.45	0.047
	TCIRG1	0.54	0.013
	OIT3	-0.51	0.022
	VTN	-0.55	0.013
	GPNMB	0.46	0.042
KOOS ADL	VPS13D	-0.55	0.013
	HEATR3	0.58	0.007
	TCIRG1	0.57	0.009
	OIT3	-0.62	0.003
	PSMG1	0.49	0.029
	VTN	-0.58	0.007
	PCMTD1	-0.54	0.013
	NEFL	-0.56	0.009
	NIT1	0.45	0.048
KOOS Sport	SMIM11	0.45	0.045
	AKR7A2	0.52	0.018
	MTMR14	0.49	0.027
	TRAPPC6B	0.53	0.015
	NIT1	0.54	0.013
	ZC2HC1A	0.47	0.036
	MIA	0.51	0.023
	VTN	-0.59	0.006
	MCM4	0.46	0.040
	PSMG1	0.57	0.009
	MED1	0.45	0.046
	OIT3	-0.58	0.007
	F8A1	0.6	0.004
	UBAP1	0.49	0.028
	N-Acetyl-Methionine*	-0.47	0.035
	HEATR3	0.66	0.001
	FGR	0.5	0.024
	NOTUM	0.59	0.006
	AAR2	0.45	0.047
	C1orf50	0.46	0.039
	LCMT1	0.47	0.037
	NUDT16L1	0.5	0.026
	TCIRG1	0.62	0.003
	NAT10	0.53	0.017
	ARIH2	0.54	0.014
	TRAPPC11	0.52	0.018
	RMC1	0.53	0.016
	ISG20	0.51	0.022
KOOS QOL	PCMTD1	-0.54	0.013
	ETV6	-0.49	0.029
	NEFL	-0.45	0.048
	NAT10	0.51	0.021
	TCIRG1	0.56	0.011

*indicates metabolite. PCMTD1, roteine-L-isoaspartate O-methyltransferase domain-containing protein 1; EVI2B, ecotropic viral integration site 2B; F8A1, coagulation factor VIII associated 1; TCIRG1, T cell immune regulator 1; OIT3, oncoprotein-induced transcript-3; VTN, vitronectin; GPNMB, glycoprotein NMB; VPS13D, vacuolar protein sorting 13 homolog D; HEATR3, HEAT repeat-containing protein 3; PSMG1, proteasome assembly chaperone 1; NEFL, neurofilament light polypeptide; NIT1, nitrilase-like protein 1; SMIM11, small integral membrane protein 11; AKR7A2, aldo-keto reductase family 7 member A2; MTMR14, myotubularin related protein 14; TRAPPC6B, trafficking protein particle complex subunit 6B; ZC2HC1A, zinc finger C2HC domain-containing protein 1A; MIA, melanoma inhibitory activity; MCM4, minichromosome maintenance protein; MED1, mediator complex subunit 1; UBAP1, ubiquitin associated protein 1; FGR, Gardner-Rasheed feline protein; C1orf50, chromosome 1 open reading frame 50; LCMT1, leucine carboxyl methyltransferase 1; NUDT16L1, nudix hydrolase 16 like 1; NAT10, N-acetyltransferase 10; ARIH2, Ariadne-2 homolog; RMC1, regulator of MON1-CCZ1; ISG20, interferon-stimulated gene 20 kDa protein; ETV6, ETS transcription factor.



K) Top 5 Reactome Pathways

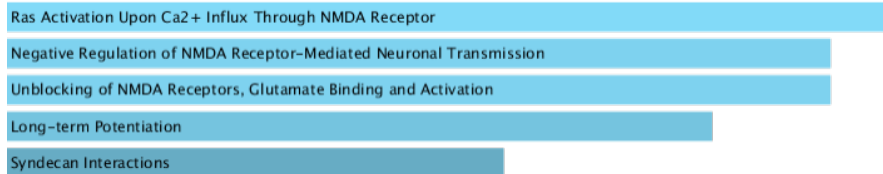


Figure 3.5. BMI-Based Protein Dysregulation Significantly Correlated Across KOOS. Correlation heatmap of proteins dysregulated by BMI that significantly related to >1 KOOS subscale (A). IFP protein intensities that differed between HBMI (High BMI) and LBMI (Low BMI) groups with significant between group difference (after covariate adjustment) include HEATR3 (B), OIT3 (C), TCIRG1 (D), F8A1 (E), VTN (F), PSMG1 (G), NEFL (H), PCMTD1 (I), and NAT10 (J). Colors correspond to BMI-based groups: orange – HBMI, blue – LBMI. Top 5 Reactome 2024 pathways of proteins dysregulated by BMI that relate to pain are shown in K.

TCIRG1, or T cell immune regulator 1, was positively correlated with KOOS ADL, Pain, QOL, and Sport subscales (Figure 3.5). OIT3, oncoprotein-induced transcript-3, and VTN, vitronectin, were both negatively correlated with KOOS ADL, Pain, and Sport subscales. PCMTD1, or protein-L-isoaspartate O-methyltransferase domain-containing protein 1, was negatively correlated with KOOS Symptom, ADL, and QOL subscales. NEFL, neurofilament light polypeptide, was negatively correlated with KOOS ADL and QOL subscales. HEATR3 (HEAT repeat containing 3) and PSMG1 (proteasome assembly chaperone 1) were found positively correlated with KOOS ADL and Sport subscales. F8A1, coagulation factor VIII associated 1, was positively correlated with KOOS Pain and Symptom subscales. NEFL, or neurofilament light polypeptide, was positively correlated with KOOS QOL and Sport subscales. NAT10, or N-acetyltransferase 10, was positively correlated with KOOS QOL and Sport subscales.

Discussion

Dysregulation of IFP secreted factors by sex and BMI relate to knee OA pain through potentially unique mechanisms. Sex-based differences in the IFP-KOOS correlated secretome revealed dysregulation of mitochondrial factors. BMI-based differences in IFP-KOOS correlated secretome revealed dysregulation of immune cell-related factors.

Sex-dysregulated proteins observed at the IFP secretome correlated to pain and disability and revealed proteins involved in mitochondrial regulation. HINT3—correlated with 4 out of 5 KOOS subscales—is located in the cytoplasm and is broadly involved in cell signaling and functions to maintain mitochondrial integrity.^{143,144} FASTKD2 is a

mitochondrial inner membrane protein crucial for maintaining mitochondrial energy balance.¹⁴⁵ Specifically, FASTKD2 is an RNA-binding protein required for mitochondrial RNA processing and translation.¹⁴⁵ CHCHD4 is a mitochondrial protein that resides in the intermembrane space with a role in bioenergetics, regulating mitochondrial oxygen consumption rate and cellular ATP level.¹⁴⁶ The observation of these mitochondrial proteins to be dysregulated by sex in the IFP and correlate across multiple KOOS subscales suggest that mitochondrial dysfunction may be a key component in understanding the link between IFP tissue changes and increased severity of pain experienced by females with knee OA. Mitochondria are essential for energy regulation, metabolism, and programmed cell death in cells and tissues throughout the body. Dysfunctional mitochondria fail to provide adequate energy to neurons, impairing energy, oxidative stress, and calcium balance.¹⁴⁷ Extensive research has explored mitochondrial dysfunction in aging and disease,^{147,148} and in inflammation and pain.¹⁴⁹ In the context of knee OA and cartilage degeneration, excessive level of reactive oxygen species (ROS) can induce mitochondrial damage by disrupting the mitochondrial membrane leading to cytochrome c release into the cytoplasm which triggers chondrocyte cell death.¹⁵⁰ Damaged mitochondria can also trigger immune response through release of ROS, ATP, and mtDNA into the cytoplasm.¹⁴⁷ While the mitochondrial proteins found dysregulated by sex in this study are not sex-specific, estrogen interacts with mitochondria in relation to homeostasis and disease. This and our findings bolster the IFP as a sensitive tissue to sex that links to pain potentially through mitochondrial dysregulation.

BMI-based IFP secretome profile differences correlated with knee OA pain and disability, which revealed dysregulation of proteins involved in immune regulation. TCIRG1 is a membrane protein primarily known for its role in bone metabolism and T cell activation for osteoclast bone resorption.^{151,152} While there is no direct link between TCIRG1 and pain, TCIRG1 in its gene form is pivotal in neutrophil activity.¹⁵³ As an active organ, adipose tissue is a hub for immune mediators including neutrophils and macrophages. In obesity, hypertrophy and hyperplasia of adipocytes kicks off an inflammatory cycle that leads to chronic, low-grade inflammation, production of pro-inflammatory adipokines, and activation of neutrophils and macrophages.¹⁵⁴ This has been well-characterized in subcutaneous adipose tissue, but not in the IFP and not linked with knee OA pain. VTN—found dysregulated by BMI and positively correlated with pain—is a key protein in cell adhesion, tissue remodeling, and, most notably, modulates neutrophil adhesion and chemotaxis.¹⁵⁵ OIT3 demonstrated a similar dysregulation and correlation trend as VTN and is a prominent marker of macrophage polarization.¹⁵⁶ In obesity classified by BMI, research has shown that macrophage activation in adipose tissue changes toward pro-inflammatory polarization and infiltration, contributing to the higher observation of a tendency toward chronic low-grade systemic inflammation in this population, although this is not present in all high BMI individuals.¹⁵⁷ HEATR3 and PSMG1—both negatively correlated with patient pain severity—function to respond to cell damage¹⁵⁸ and regulate abnormal protein degradation.¹⁵⁹ These findings suggest that based on BMI, the IFP differentially secretes factors related to immune response and inflammation that relate to the increased severity of pain experienced by individuals with a high BMI.

To this author's knowledge, this is the first study to investigate the knee OA IFP secretome and demonstrate differences in the OA IFP tissue profile by sex and BMI through unique pathways. Additionally, this study establishes a plausible link between said pathways and pain to uncover why these patient populations experience higher pain with knee OA. This data suggests that irregularities in pain-related secretome signalling occur in the IFP with knee OA, with distinct relevance in females and individuals with a high BMI. While the IFP is not considered an ectopic adipose depot (such as visceral or intramuscular adipose), sex differences have been well-characterized in ectopic fat depots, with significantly different accumulation of liver fat, subcutaneous adipose in the lower extremities, and muscle infiltration of adipose between males and females.^{160,161} The IFP has been historically regarded as a purely mechanical, shock-absorbing structure. Our data suggest a reframing of how the IFP is viewed, as the IFP demonstrates an active role in knee joint symptoms that is influenced by sex and BMI-based differences. The IFP is an active secretory organ with potential distinct signally effects that warrants further investigation.

This study has limitations. IFP tissue was collected from individuals with end-stage knee OA with no controls. As such, we were unable to compare our findings of sex- and BMI-related secretome dysregulation with healthy controls or earlier stage OA. Thus, our findings require further external validation and cannot be generalized. All females are over the age of 50 and reported their last menstrual period more than 1 year prior to time of surgery. While we may infer that the females in this study are either in menopause or post-menopausal, estrogen and progesterone levels were not collected and therefore the role of menopause on this sex-based IFP secretome

dysregulation requires further study. BMI ≥ 30 kg/m² has been widely used as a measure of obesity status.¹⁶² While a high BMI (BMI ≥ 30 kg/m²) is associated with increased adiposity at the population level,¹⁶³ BMI can misdiagnose individuals with obesity as it is not an accurate surrogate marker of metabolic dysfunction or body composition.^{164,165} As such, our results on BMI-based differences cannot be linked directly with fat-tissue related metabolic dysfunction in obesity. To appropriately probe the effect of metabolic disturbance on knee OA IFP and pain, future studies should measure body fat using body composition assessment tools such as Dual-Energy X-ray Absorptiometry (DXA), waist circumference, and blood tests for measures of metabolic health (e.g. albumin, triglycerides, high density lipoprotein or HDL).

This work highlights the value of examining the involvement of the IFP in relation to pain in high-risk, high-pain populations. To build on this and decode the modifiable pathways between the IFP and adjacent tissues that may influence OA disease and pain, Chapter 4 further explores the IFP secretome by probing its response to leptin signal, how response is modulated by sex and BMI, and in relation to adjacent tissues—cartilage and synovium.

Chapter 4 Leptin Signaling Differentially Affects the Secretome of Knee OA Tissues and is Modulated by Sex and BMI

Chapter 4 builds on the profiling of the IFP secretome in Chapter 3 through probing how the IFP, synovium, and cartilage differ in response to leptin signaling (a key mediator of fat-cartilage crosstalk in OA). This chapter leverages multi-omic secretome profiling of multiple knee tissue secretomes and provides novel insight into how the IFP contributes to leptin-mediated fat-cartilage crosstalk in relation to adjacent tissues and how leptin-mediated signals are modulated by sex and BMI in the knee. Chapters 3 and 4 are intended to be published together, and as such, relevant Introduction and Methods sections content is abbreviated in this chapter.

Introduction

Although knee osteoarthritis (OA) is now being recognized as a complex, whole-joint disease, characterization of the coordinated communication and crosstalk between the involved tissues, e.g. cartilage, synovium, and infrapatellar fat pad (IFP), remains unclear. High-throughput molecular profiling has enabled the application of multi-omics approaches to study OA tissues at a systems level.¹⁶⁶ By integrating proteomic and metabolomic data, for example, these approaches can identify coordinated biological pathways across the signaling cascade to uncover mechanisms of tissue dysfunction.¹⁶⁶ Multi-omics analyses are increasingly used in research on circulating (serum) and

tissue-level profiling in knee OA and obesity,¹⁶⁷ but few studies have investigated molecular change and contribution of peripheral tissues, such as the IFP, in OA. Given the multi-tissue nature of OA disease and the varied inflammatory, mechano-regulated, and metabolic tissue communication pathways, there is a critical need for integrative analyses that capture both shared and tissue-specific molecular signatures.

Previous research has highlighted fat-cartilage crosstalk mediated by leptin signaling in OA disease pathogenesis in animal models.^{23,24,59} Leptin—a satiety hormone directly related to adiposity—mediates fat-cartilage crosstalk from adipose outside the knee joint via surgical implantation in animal models.²⁴ In humans, elevated levels of leptin are observed in cartilage, synovium, and IFP and are associated with OA progression.^{2,13,82,87} However, it is unclear how leptin signal is transduced to the knee joint. In high-risk, high-pain OA populations, increased levels of leptin in serum are observed in individuals with obesity due to the increased adiposity, and in females, which also correlates with patient-reported pain.⁹⁰ Adipose tissue in the IFP produces and secretes leptin,^{2,21} and demonstrates an influence on OA-related cartilage and synovium degradation in humans.^{22,27} Thus, the IFP is a prime candidate to transduce leptin signal to adjacent tissues (cartilage and synovium) in the knee joint. Chapters 2 and 3 highlight the sensitivity of the IFP both clinically and biologically to female sex and high BMI, populations that also exhibit increased circulating leptin levels.¹⁶⁸ However, human knee tissue response to leptin signaling of the IFP and adjacent tissues has not yet been explored. Moreover, secretome profiling of cartilage, synovium, and IFP tissues is needed to describe the unique, or overlapping, molecular signature secreted from each knee tissue to decode leptin-mediated interorgan crosstalk in OA.

To address these gaps, we applied an integrative multi-omics approach across IFP, synovium, and cartilage human surgical waste tissues from patients with end-stage OA undergoing total knee arthroplasty (TKA) to characterize the extrinsic molecular profile of knee joint tissues in response to leptin signaling. By performing proteomic and metabolomic profiling (including a targeted eicosanoid panel for investigation of pain-related lipids) of tissue secreted factors, we sought to define shared and tissue-specific response to leptin signaling within diseased tissues to determine how these signatures change when challenged with leptin and how tissue response is modulated by sex and BMI.

We hypothesized that the tissue secretome would differ due to leptin signaling across tissues, and that sex and BMI would significantly influence tissue-specific response to leptin, demonstrating that individual signals secreted by fat may not exert the same influence or signaling on target tissues. We further posited that the IFP would be the most responsive to leptin signal by demonstrating the greatest change in secretome (across proteomic, metabolomic, and eicosanoid profiles) with leptin treatment.

Methods

Chapters 3 and 4 share many of the same methods as the projects analyzed tissues from the same patients (pages 56-57). The key differences in Chapter 4 are that several tissues are included beyond just IFP, e.g. cartilage, synovium, and IFP, and undergo a treatment condition, e.g. leptin, to compare to controls. In light of this, the Patient Recruitment section is not included to reduce redundancy. Sections with

relevant differences (*Tissue Collection & Conditioned Media Protocol, Multi-Omic Profiling, and Statistical Analysis*) are revisited below.

Tissue Collection and Conditioned Media Protocol

Surgical waste collected at time of TKA included cartilage, synovium, and IFP. Segments of all tissues (cartilage, synovium, IFP) were flash frozen in cryovials immediately after surgery and stored at -80°C for future use. Tissues, approximately 250 mg, were collected in 50 mL conical tubes filled with warm DMEM. Each tissue was cut into 6-12 small pieces (20-40 mg/piece) for both the treatment (leptin challenge) and control (no leptin) groups. Tissue pieces were washed in 10mL PBS in conical tubes, incubated at 37°C for 5 minutes, followed by disposal of the rinse PBS, and repeat of this wash 2 times. Adequately washed tissue pieces were removed from conical tube, placed in cell insert (1.0 μ m) in a 6-well plate separately for treatment and control, along with 500 μ L of DMEM, and placed in the incubator at 37°C for 1 hour. Following the 1 hour incubation, the media for treatment tissues was changed out with 500 μ L of 50 nM Leptin-DMEM and returned to incubator at 37°C for 48 hours. After 48 hours, tissue pieces were removed, placed in cryovials and flash-frozen using liquid nitrogen, and stored at -80°C. The media (leptin-conditioned treatment and control) were collected in cryovials, flash frozen and stored at -80°C until recruitment completed and samples ready for metabolomic and proteomic analysis. For all patient except one, there were be 6 conditioned media samples: control media (0 ng/mL leptin) IFP, cartilage, and synovium, and treatment media (50 nM leptin) IFP, cartilage, and synovium. One patient

did not have synovium collected, thus only control media IFP, cartilage, and treatment media IFP, and cartilage were collected and analyzed.

Multi-omic Secretome Profiling

Multi-omic profiling of the conditioned media from tissue samples included targeted metabolomics, eicosanoid-panel targeted metabolomics, and untargeted proteomics. Targeted metabolomics was performed on 1 set of aliquots of all 6 tissues from each patient (cartilage, synovium, IFP; leptin and control) using a combination of direct injection (DI) mass spectrometry (MS) with a reverse-phase liquid chromatography (LC)-MS/MS using the MTX MEGA Assay (Metabolomix, Edmonton, Canada) following previously established methods.¹⁸ In brief, this assay, in combination with QE orbitrap (ThermoFischer Scientific, Waltham, MA) mass spectrometer, was used for targeted identification and quantification of 646 endogenous metabolites including amino acids, or amino acid-related metabolites, biogenic amines, ceramides, cholesterol esters, diacylglycerols, acylcarnitines, glyceophospholipids, sphingomyelins, triacylglycerols, organic acids, and nucleotide/nucleosides.¹⁸

Separate aliquots of all 6 tissues from each patient were flash frozen following conditioned media protocol and shipped to the Collins Lab for protein and eicosanoid profiling using our lab's established, novel dual extraction method that separates proteins and metabolites from a single sample.²⁴ In brief, all samples were extracted with methanol, vortexed, and placed at -20 °C for 30 minutes to promote protein precipitation. Next, the supernatant containing metabolites was collected and dried via vacuum concentration for eicosanoid profiling. The peptide pellet (leftover after the

supernatant containing metabolites was removed) was resuspended in trypsin, incubated overnight for digestion of peptides, and peptide extraction for proteomic analysis was completed using the Thermo Scientific EasyPep MS Sample Prep Kit (ThermoFischer Scientific, Waltham, MA). Prepared samples were analyzed and processed at the University of Reno, Proteomics Center, using LC-MS (Evosep One LC coupled to a Bruker timsTOF Pro2 MS, Bruker, Billerica, MA) for untargeted proteomics. Raw data was processed using Spectronaut (version 19, Biognosys AG, Switzerland) for peak picking, alignment, and quantitation. For eicosanoid profiling, a 5 uL aliquot of each sample was subjected to a targeted metabolomic panel of 40 known eicosanoids, analyzed at the Quantitative Metabolite Analysis Center at the University of California, San Francisco¹³⁸ on a Shimadzu 30-AD UPLC series with a SCIEX 7500 Triple Quadrupole Mass Spectrometer with a reverse-phase liquid chromatography column. Raw data was processed using a built-in SCIEX OS software package (version 2.1.6.59781) for peak picking, alignment, and quantitation. Eicosanoids detected were validated and confirmed with commercially bought standard.

Statistical Analysis

To evaluate differences in the proteomic, metabolomic, and eicosanoid-lipidomic profiles of OA IFP, synovium, and cartilage secretomes, differential expression analysis was run between leptin and control secretomes for each tissue. Starting with 118 samples from 20 unique patients, 6,369 proteins, 445 metabolites, and 35 eicosanoids were measured. After data filtering to remove proteins and eicosanoids with fewer than 20% expression levels above zero and metabolites below the limit of detection, 6,100

proteins, 186 metabolites, and 31 eicosanoids were retained. All data (proteomic, metabolomic, eicosanoid lipidomic) were normalized and scaled per previously established methods.¹⁸ In brief, data were normalized by sum, log-transformed in base 10, and scaled using the Pareto method. Differential expression analysis of proteins and eicosanoids was performed by Kristina Hueniken, and of metabolites by Nikita Looby, via the Limma pipeline (default in Metaboanalyst¹⁴¹). Paired observations were handled using a random intercept for each patient ID. Log₁₀ fold changes (FC) and raw p-values for top differentially expressed proteins, metabolites, and eicosanoids between leptin and control secretomes are presented. Expression levels were considered statistically significant at $p < 0.05$. Differential expression analysis was conducted separately in each tissue. Models were adjusted for batch, leptin treatment (vs control, vehicle), and the interaction between leptin treatment and sex/BMI. A significant p-value on the interaction term indicates that the response to treatment was significantly different between groups.

Results

Leptin drives altered kinase-related protein abundance in the knee OA IFP secretome

In the OA IFP secretome, 288 proteins were significantly dysregulated with leptin treatment: 116 proteins downregulated with leptin and 172 proteins upregulated with leptin (Figure 4.1). Top pathways upregulated with leptin include triglyceride metabolism, and AKT phosphorylates targets in the cytosol shown in figure 4.1. Top proteins upregulated with leptin include A disintegrin and metalloproteinase with thrombospondin motif 14 (ADAMTS14 or ATS14), RAC-gamma serine/threonine-protein kinase (AKT3), RUS family member 1 (RUSF1), Ras GTPase-activating-like protein 3 (IQGA3), and OX-2 membrane glycoprotein (OX2G). Top pathways downregulated with leptin include striated muscle contraction and EPHB-mediated forward signaling. Top proteins downregulated with leptin treatment include Transcription elongation factor A protein-like 5 (TCAL5), MARCKS-related protein (MRP), Discoidin CUB and LCCL domain-containing protein 2 (DCBD2), actin cytoplasmic 1 (ACTB), and Collagen alpha-1(VII) chain (CO7A1).

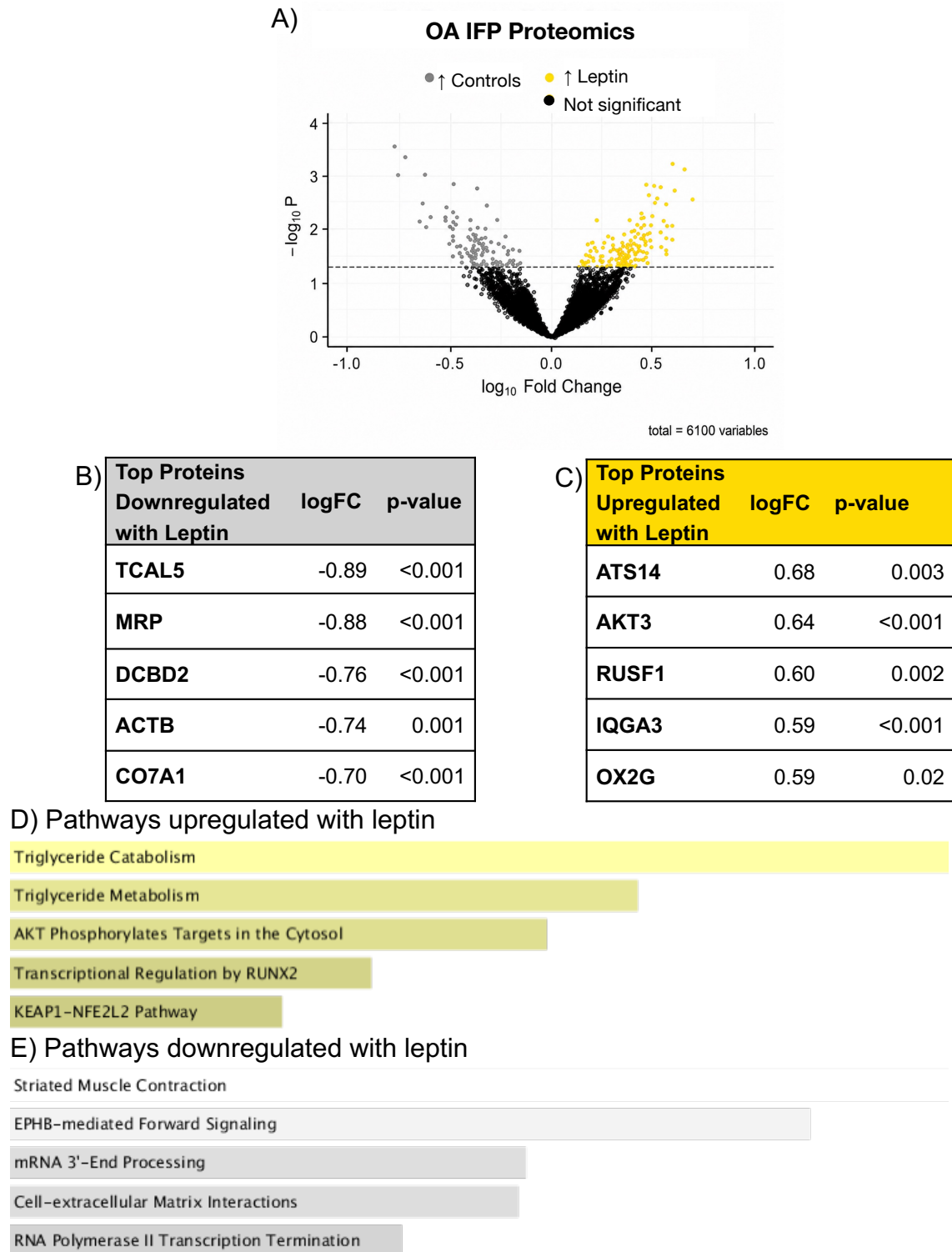


Figure 4.1. Differential protein abundance in leptin versus control IFP secretomes. Volcano plot of upregulated proteins with leptin (right), and downregulated with leptin (left) (A). Top 5 upregulated proteins are shown in C, top 5 downregulated proteins are shown in B. Pathways of upregulated proteins with leptin are shown in D, and pathways of downregulated proteins with leptin are shown in E.

Four metabolites and one eicosanoid were significantly dysregulated with leptin treatment (Figure 4.2). Phosphatidylcholine (PC) ae C36:3 (logFC=0.45) and PC ae C34:2 (logFC=0.46) were upregulated with leptin, and PC ae C36:0 (logFC=-0.53) and methylamine (logFC=-0.30) were downregulated with leptin. Of eicosanoids, oleamide was significantly upregulated (logFC=0.28).

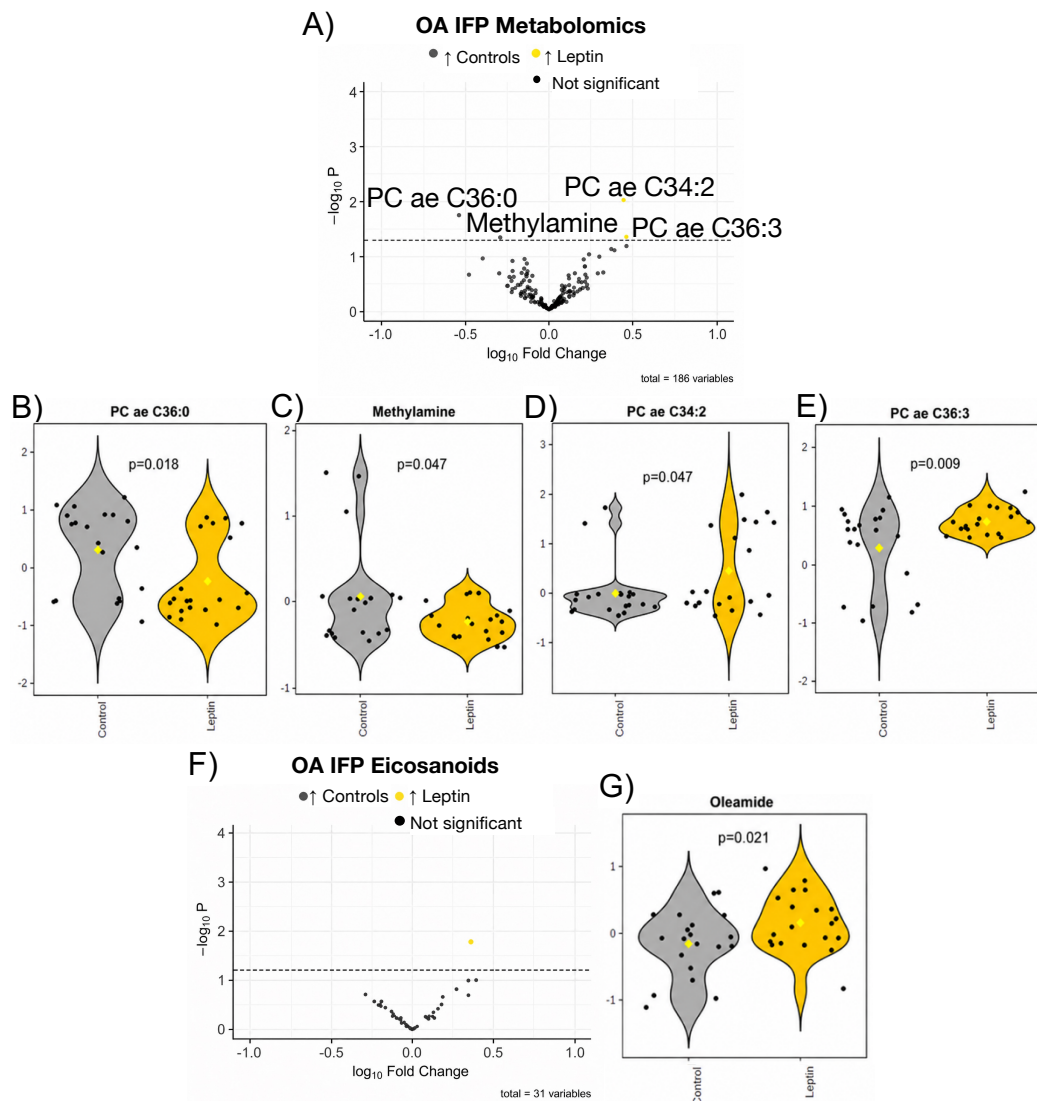


Figure 4.2. Dysregulated metabolites & eicosanoids with leptin in OA IFP secretome. Volcano plot of dysregulated metabolites (A) and eicosanoids (D). Phosphatidylcholine (PC) ae C36:0 (B) and methylamine (C) were downregulated with leptin. PC ae C34:2 (D), PC 36:3 (E), and oleamide (G) were upregulated with leptin.

Leptin drives altered triglyceride metabolism and adipogenesis in OA synovium secretome

In the OA synovium secretome, 202 proteins were dysregulated with leptin treatment: 99 proteins downregulated with leptin, and 103 proteins were upregulated with leptin (Figure 4.3). Top upregulated pathways with leptin treatment include regulation of TP53 activity through acetylation and AKT phosphorylates targets in the nucleus, shown in Figure 4.3. Top proteins upregulated with leptin treatment include Matrix extracellular phosphoglycoprotein (MEPE), guanine deaminase (GUAD), obscurin (OBSCN), vacuolar fusion protein CCZ1 (CCZ1B/CCZ1), and NCK-interacting protein with SH3 domain (SPN90). Top pathways downregulated with leptin treatment include regulation of PTEN gene transcription, regulation of adipocyte proliferation and adipogenesis. Top downregulated proteins with leptin treatment include olfactomedin 4 (OLFM4), serine/threonine MAPK-activated protein kinase (MARK2), E3 ubiquitin-protein ligase (RBBP6), sialidase-4 (NEUR4), and conserved oligomeric golgi complex unit 8 (COG8).

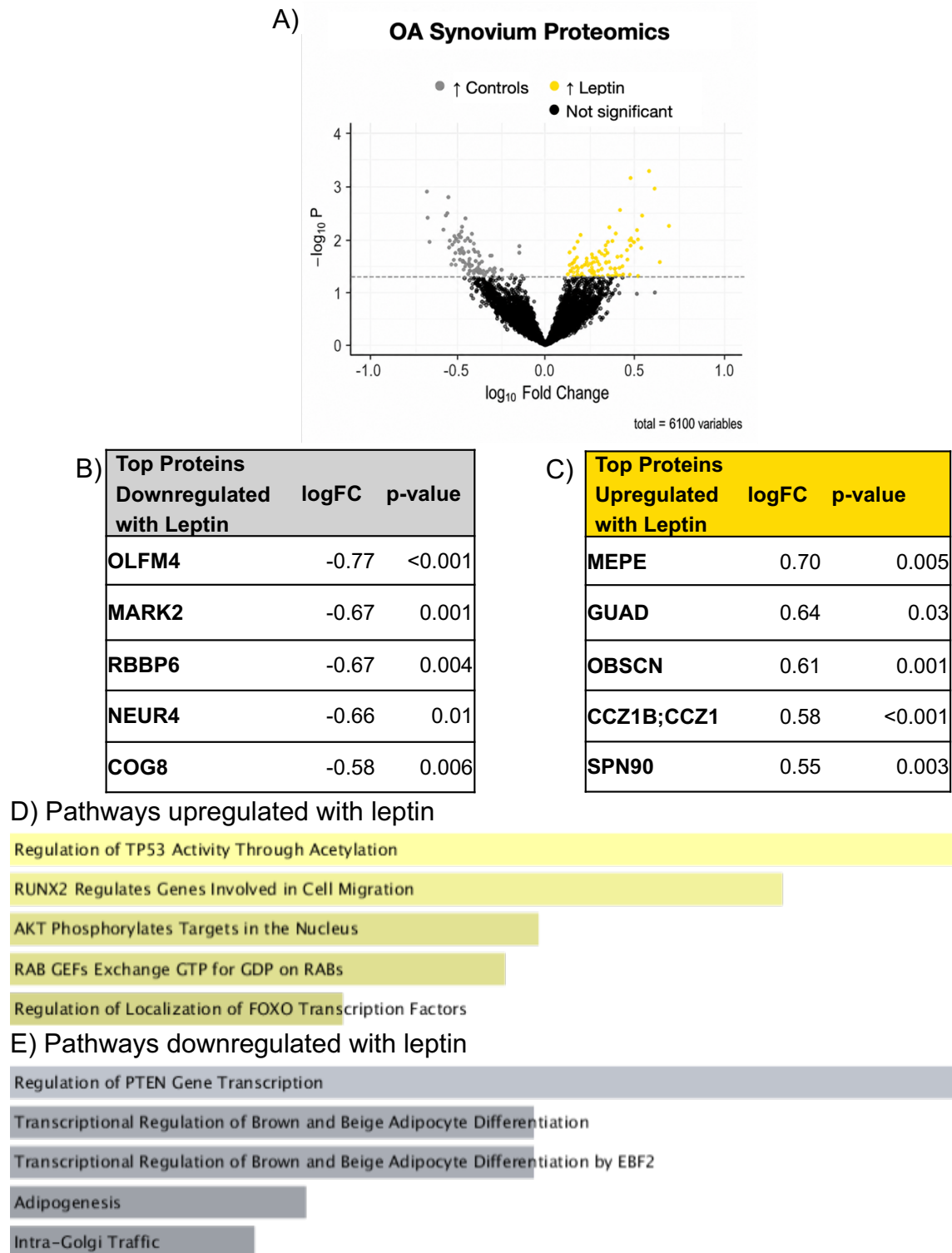


Figure 4.3. Differential protein abundance in leptin versus control synovium secretome. Volcano plot of upregulated proteins (A) with leptin (right), and downregulated with leptin (left). Top 5 upregulated proteins are shown in C, top 5 downregulated proteins are shown in B. Pathways of upregulated proteins with leptin are shown in D, and pathways of downregulated proteins with leptin are shown in E.

Two metabolites were significantly upregulated with leptin treatment: Triglyceride (TG) 50:3 ($\log_{10}FC=0.40$) and TG 54:4 ($\log_{10}FC=0.40$), shown in the volcano plot and violin plots in Figure 4.4. Of eicosanoids, 12,13-diHOME was significantly downregulated ($\log_{10}FC=-0.36$) with leptin, and Resolvin D5 (RvD5) was significantly upregulated ($\log_{10}FC=0.29$).

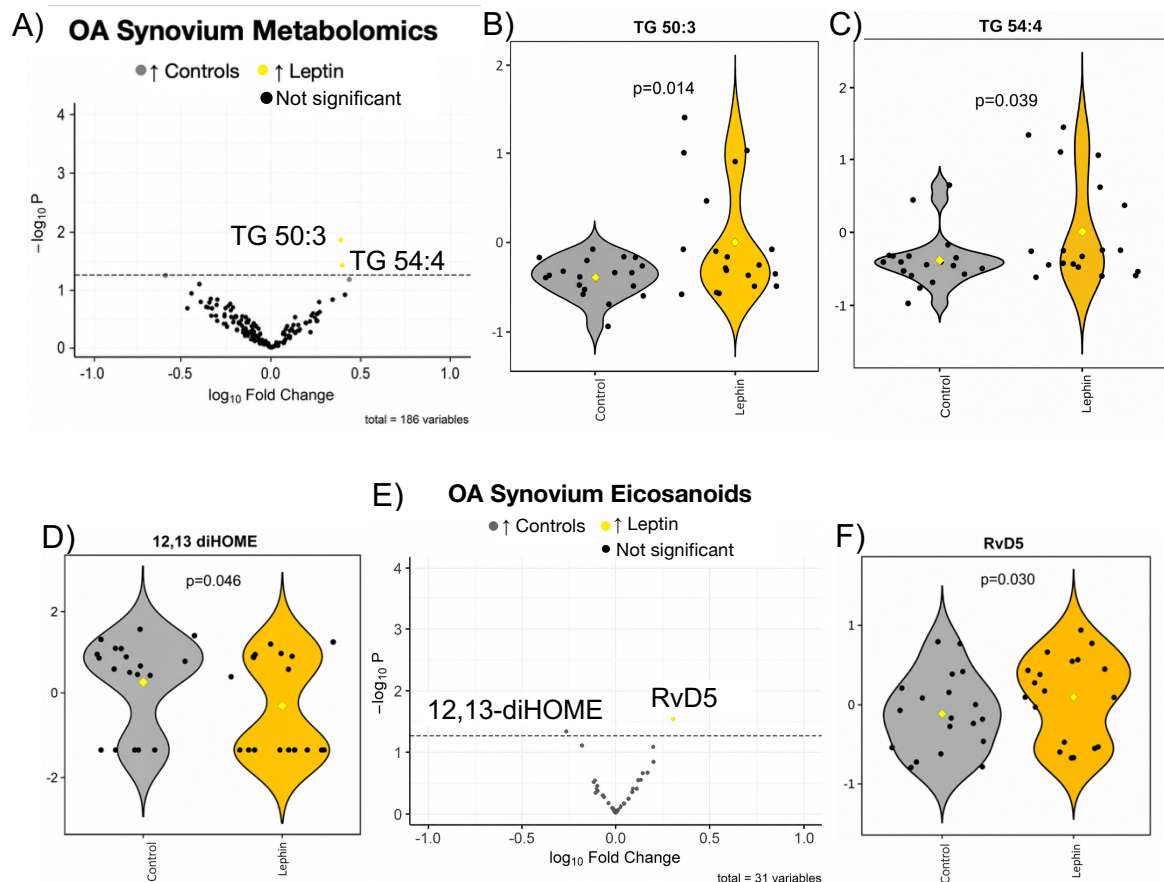


Figure 4.4. Dysregulated metabolites & eicosanoids with leptin in OA synovium secretomes.

Volcano plot of dysregulated metabolites (A) and eicosanoids (E). Triglyceride (TG) 50:3 (B) and TG 54:4 (C) were upregulated with leptin treatment in cartilage secretomes. RvD5 was significantly upregulated with leptin treatment (F), and 12,13-diHOME (D) was significantly downregulated with leptin in the OA cartilage secretome.

Leptin drives altered serine, threonine and amino acid metabolism in the OA cartilage secretome

In the OA cartilage secretome, 919 proteins were dysregulated with leptin treatment: 900 proteins were downregulated with leptin, and 19 proteins were upregulated with leptin (Figure 4.5). Top pathways upregulated with leptin treatment include VLDL assembly and clearance and plasma lipoprotein assembly. Top proteins upregulated with leptin include ATP-dependent RNA helicase (DDX46), filamin A-interacting protein 1 (FLIP1), WD repeat-containing protein 36 (WDR36), and A0AAG2SXZ4 and A0A0J9YWU9 unreviewed Ig-like domain-containing proteins. Top pathways downregulated with leptin treatment include mitochondrial fatty acid oxidation and leucine isoleucine and valine metabolism. Top downregulated proteins include NipSnap homolog 1 (NIPS1), mRNA cap guanine-N(7) methyltransferase (MCES), periodic tryptophan protein 2 homolog (PWP2), transmembrane protein 168 (TM168), and zinc transporter in the endoplasmic reticulum SLC39A7 (S39A7).

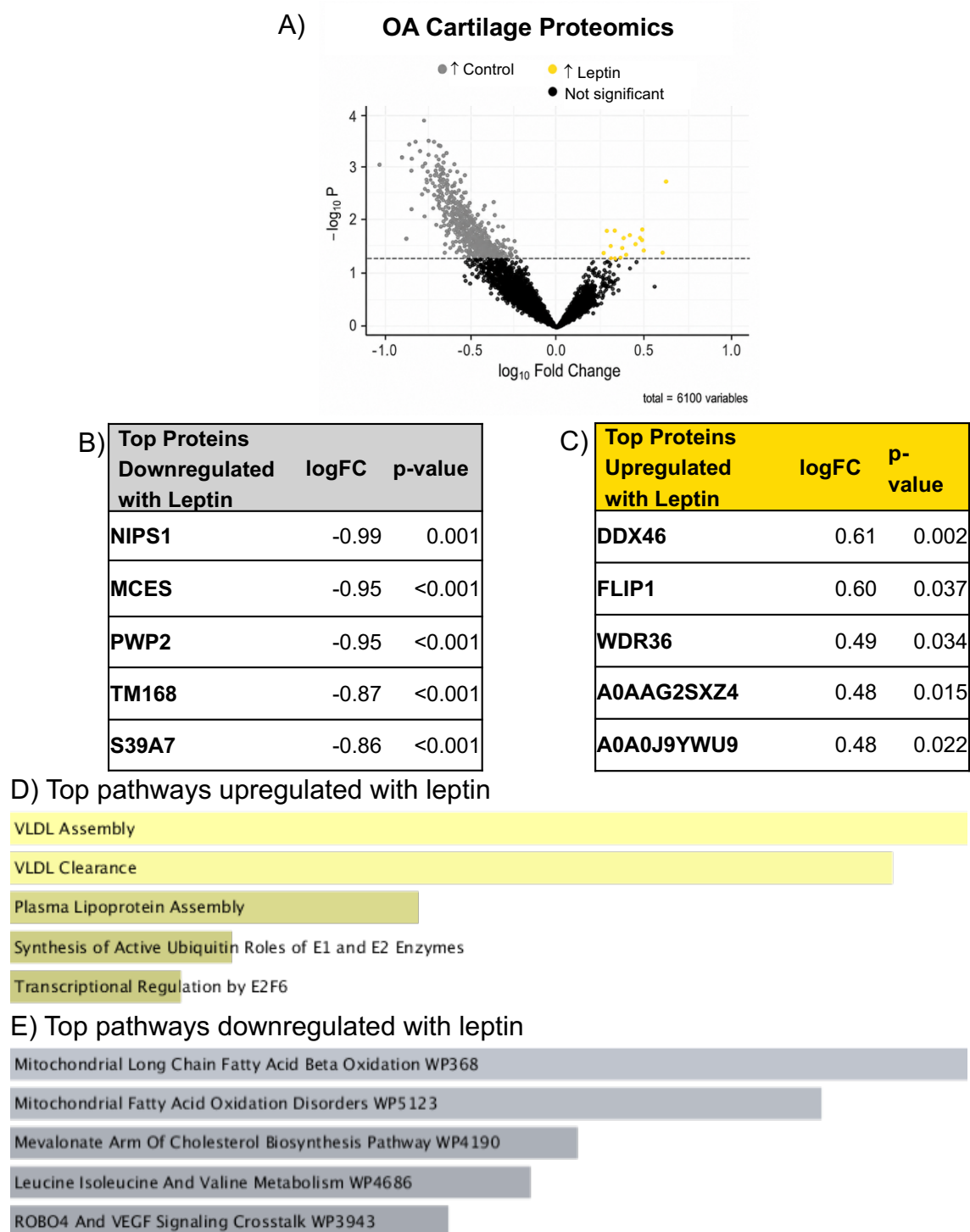


Figure 4.5. Differential protein abundance in leptin versus control OA cartilage secretomes.

Volcano plot of upregulated proteins (A) with leptin (right), and downregulated with leptin (left). Top 5 upregulated proteins are shown in C, top 5 downregulated proteins are shown in B. Pathways of upregulated proteins with leptin are shown in D, and pathways of downregulated proteins with leptin are shown in E.

Twenty-four metabolites were dysregulated with leptin in the cartilage secretome, including 15 metabolites downregulated with leptin and 9 metabolites upregulated with leptin—shown in Figure 4.6. Threonine (logFC=0.23), serine (logFC=0.24), and valine (logFC=0.191) were significantly upregulated with leptin. Of eicosanoids, 9-HODE was significantly upregulated with leptin (logFC=0.22).

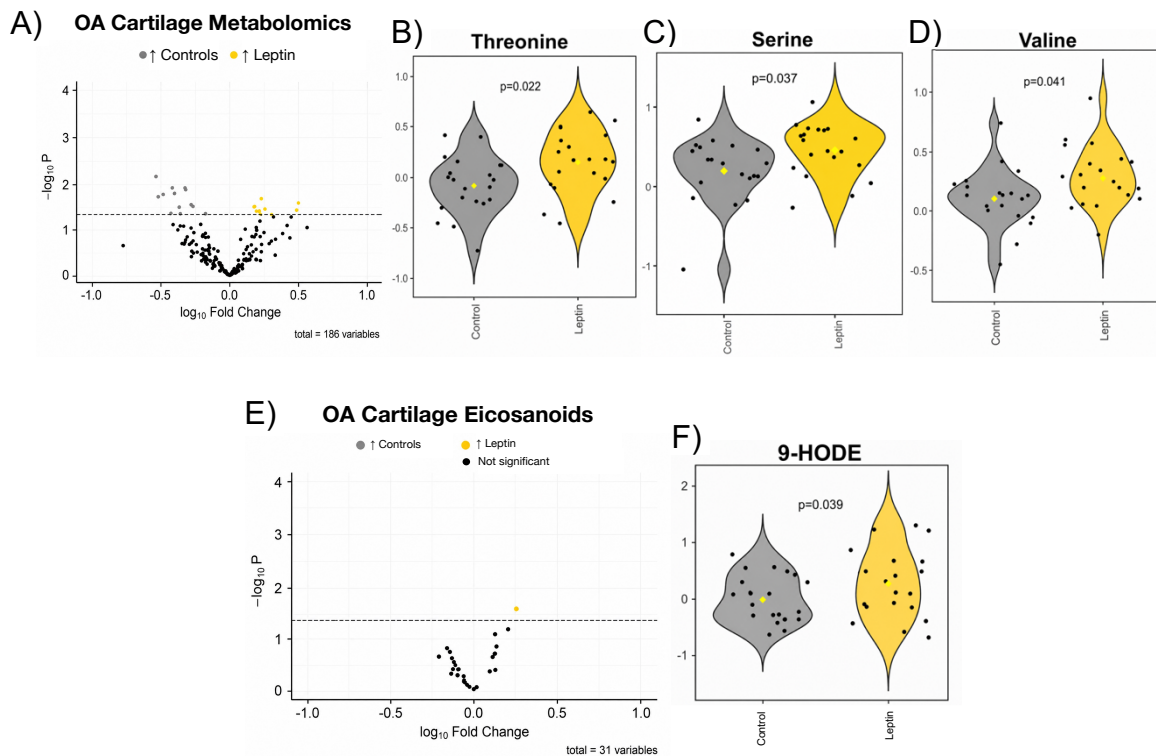


Figure 4.6. Dysregulated metabolites & eicosanoids with leptin in OA cartilage secretome.

Volcano plot of dysregulated metabolites (A) and eicosanoids (E). Threonine (B), serine (C), valine (D), and 9-HODE (F) were all significantly upregulated with leptin.

Sex and BMI modulate secretome response to leptin differently in knee OA tissues

To describe how sex and BMI modulate tissue-specific leptin response, interaction effects of sex and BMI on IFP, synovium, and cartilage secretome change with leptin were analyzed. Figure 4.7 shows boxplot distribution of the significant interaction effect logFC values for proteome response to leptin in cartilage, synovium, and IFP. In the cartilage secretome, sex significantly affected the secretome response to leptin in 72 proteins, with leptin response significantly different in females (median logFC -0.09) compared to males (median logFC -1.06) (Figure 4.7A). BMI significantly affected cartilage secretome response to leptin in 494 proteins, with leptin response significantly different in HBMI (median logFC -0.001) compared to LBMI (median logFC -0.9) (Figure 4.7B). In the synovium secretome, sex significantly affected the secretome response to leptin in 247 proteins, with leptin response significantly different in females (median logFC -0.11) compared to males (median logFC 0.26) (Figure 4.7C). BMI significantly affected synovium secretome response to leptin in 302 proteins, with response to leptin significantly different in HBMI (median logFC -0.13) compared to LBMI (median logFC 0.47). In the IFP secretome, sex significantly affected the secretome response to leptin in 251 proteins, with leptin response significantly different in females (median logFC -0.10) compared to males (median logFC 0.38) (Figure 4.7E). BMI significantly affected IFP secretome response to leptin in 190 proteins, with leptin response significantly different in HBMI (median logFC 0.10) compared to LBMI (median logFC -0.18) (Figure 4.7F).

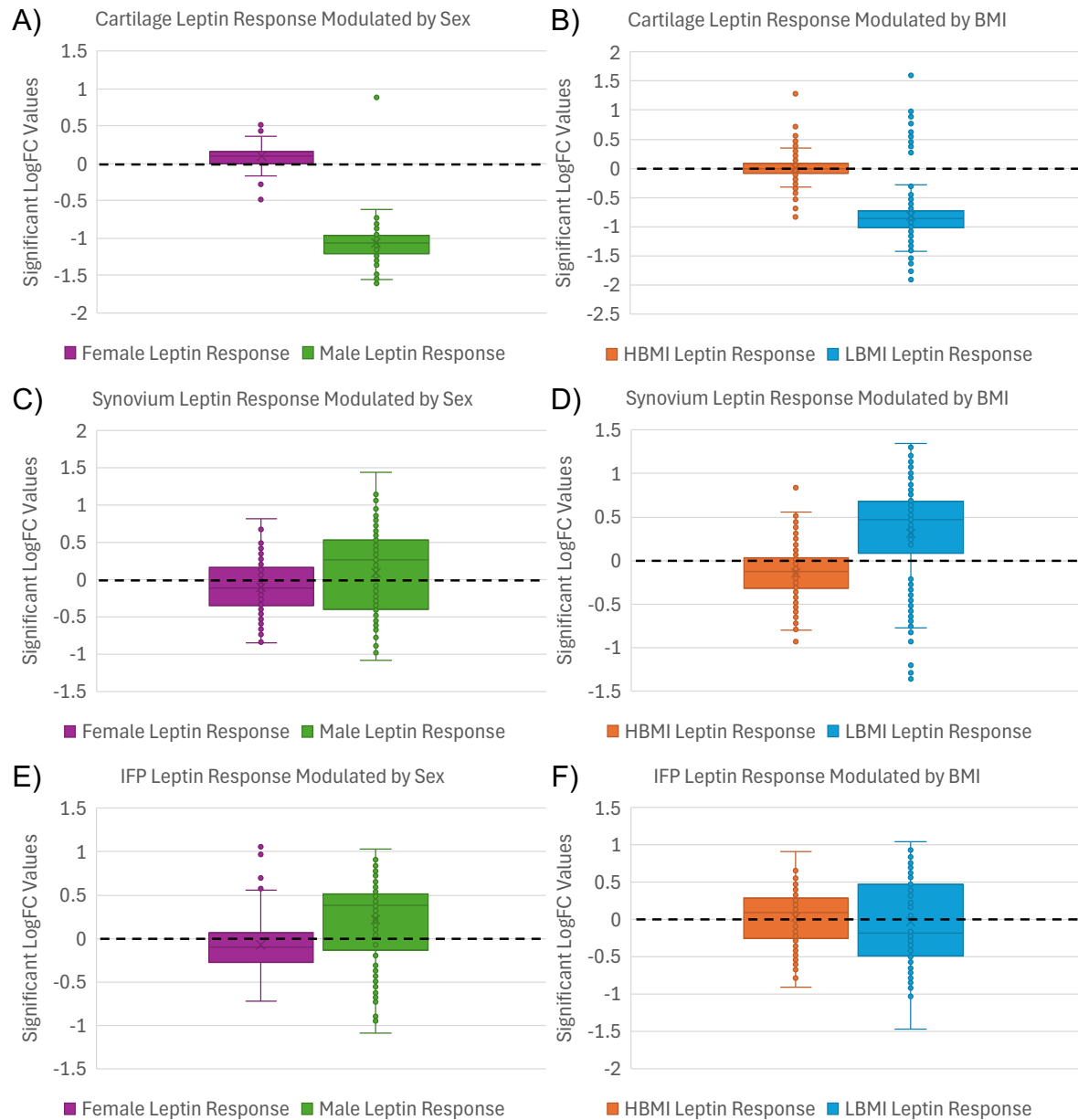


Figure 4.7. Interaction effect of sex and BMI on tissue-specific leptin response. Boxplots of significant logFC values of leptin response in females—colored in purple—versus males—colored in green—are shown in A, C, and E. Boxplots of significant logFC values of leptin response in HBMI (patients with high BMI; BMI ≥ 30 kg/m²)—colored in orange—versus LBMI (patients with low BMI; BMI < 25 kg/m²)—colored in blue—are shown in B, D, and F.

Significant interaction effect of sex on metabolite response to leptin in OA IFP, synovium, and cartilage secretomes is shown in Table 4.1, and significant interaction effect of BMI on metabolite response to leptin in OA secretomes is shown in Table 4.2.

Table 4.1. Significant interaction effect of sex on metabolite changes with leptin in OA tissue secretomes.

Metabolite	Leptin logFC Females	Leptin logFC Males	Interaction p- value
IFP secretome			
Caprylic acid	-0.657	1.038	0.000
Propionic acid	-0.626	0.971	0.002
TG(54:4)	0.251	-0.623	0.008
Caproic acid	-0.394	0.455	0.033
Isovaleric acid	-0.397	0.345	0.033
Kynurenic acid	0.067	0.872	0.033
Urea	-0.087	0.669	0.034
Synovium secretome			
C5	-0.356	0.29	0.013
Cartilage secretome			
C7DC	-0.130	0.550	0.011
PC aa C32:1	0.293	-0.591	0.015
Glyceric acid	-0.026	-0.479	0.019
PC aa C32:0	0.094	-0.817	0.038
Glutamic acid	-0.034	0.627	0.043
N-Acetyl-Tryptophan	-0.187	0.765	0.047

logFC, log base 10 fold change; IFP, infrapatellar fat pad; TG, triglyceride; PC, phosphatidylcholine.

Table 4.2. Significant interaction effect of BMI on metabolite changes with leptin in OA tissue secretomes.

Metabolite	Leptin logFC HBMI	Leptin logFC LBMI	Interaction p- value
IFP secretome			
DOPA	0.463	-0.792	0.003
Xanthosine	-0.124	0.561	0.003
Adenine	0.442	-0.421	0.006
C7DC	0.148	-0.885	0.014
C10	-0.011	-0.695	0.021
Propionic acid	-0.353	0.866	0.031
N-Acetyl-Methionine	-0.141	0.318	0.031
Deoxyguanosine	-0.121	0.773	0.047
PC aa C40:4	-0.088	0.707	0.048
Synovium secretome			
PC ae C34:1	-0.263	0.661	0.001
PC ae C36:2	-0.689	0.583	0.003
PC ae C40:2	0.127	-0.938	0.006
5-Hydroxylysine	0.100	-1.119	0.006
PC ae C36:5	-0.238	0.466	0.010
PC ae C36:0	-0.385	0.838	0.011
PC ae C34:0	-0.318	0.469	0.022
Deoxyinosine	-0.031	-1.107	0.022
PC aa C34:3	-0.404	0.363	0.024
TG(50:3)	0.225	0.895	0.025
Tryptophan	0.005	0.478	0.033
PC aa C32:1	-0.150	0.475	0.034
Xanthosine	0.057	-0.795	0.042
Methylhistidine	-0.102	0.416	0.046
C18	-0.362	0.428	0.046
Cartilage secretome			
Asparagine	0.039	-0.584	0.004
alpha-Ketoglutaric acid	-0.098	0.557	0.007
PC ae C36:3	0.363	-0.460	0.012
SM C16:0	0.080	-0.842	0.024
PC aa C38:3	-0.024	0.451	0.025
TG(48:1)	0.514	-0.446	0.025
LysoPC a C16:0	0.099	-0.471	0.034
PC aa C40:5	0.145	-0.415	0.037

logFC, log base 10 fold change; HBMI, high BMI; LBMI, low BMI; IFP, infrapatellar fat pad; PC, phosphatidylcholine; TG, triglyceride; SM, sphingomyelin.

Sex and BMI modulate eicosanoid leptin response only in the IFP

When evaluating the interaction effect of sex (females vs males) on tissue specific response to leptin in eicosanoid secretome, the IFP was the only tissue to demonstrate a significant interaction effect. Table 4.3 shows significant differences in logFC of eicosanoid abundance with leptin treatment compared to control in females versus males with the associated interaction effect p-value. 11,12-Epoxyeicosatrienoic acid(-EET), 9-Hydroxy-eicosatetraenoic acid(-HETE), 12-HETE, 15-HETE, and 11-HETE were all found significantly downregulated with leptin in females compared to leptin response in males (Table 4.1). Eicosanoid 15-keto Prostaglandin F2 α (PGF2 α) was found significantly downregulated with leptin in males compared to females (Table 4.3).

Table 4.3. Significant interaction effect of eicosanoid response to leptin in females versus males in OA IFP.

Eicosanoid	Leptin logFC Females	Leptin logFC Males	Interaction p- value
11,12-EET	-0.411	0.489	0.005
9-HETE	-0.478	0.431	0.006
12-HETE	-0.475	0.336	0.012
15-HETE	-0.304	0.687	0.016
15-keto PGF2 α	0.192	-0.746	0.018
11-HETE	-0.299	0.384	0.025

logFC, log base 10 fold change; EET, Epoxyeicosatrienoic acid; HETE, Hydroxy-eicosatetraenoic acid; PG, Prostaglandin.

When evaluating the interaction effect of BMI (high BMI vs low BMI) on tissue specific response to leptin in eicosanoid secretome, the IFP was the only tissue to demonstrate a significant interaction effect. Table 4.4 shows significant differences in logFC of eicosanoid abundance with leptin treatment compared to control in patients with HBMI versus patients with LBMI. 15-HETE, 9-Hydroxyoctadecadienoic acid(-

HODE), eicosapentaenoic acid (EPA), adrenic acid, and 9-HETE were all significantly upregulated with leptin in patients with a HBMI compared to LBMI (Table 4.2).

Prostaglandin E2 (PGE2) & PGD2, PGJ2, PGE2, and 15-keto PGF2 α were all significantly downregulated with leptin in patients with a HBMI compared to LBMI (Table 4.4).

Table 4.4. Significant interaction effect of eicosanoid response to leptin in HBMI versus LBMI in OA IFP.

Eicosanoid	Leptin logFC HBMI	Leptin logFC LBMI	Interaction p- value
15-HETE	0.436	-0.709	0.008
9-HODE	0.402	-0.271	0.015
PGE2 & PGD2	-0.527	0.433	0.025
EPA	0.225	-0.319	0.025
PGD2	-0.439	0.484	0.026
PGJ2	-0.456	0.435	0.032
PGE2	-0.261	0.655	0.032
15-keto PGF2 α	-0.438	0.410	0.046
Adrenic Acid	0.297	-0.295	0.047
9-HETE	0.096	-0.606	0.048

logFC, log base 10 fold change; HBMI, high BMI (BMI ≥ 30 kg/m²); LBMI, low BMI (BMI < 25 kg/m²); HETE, Hydroxy-eicosatetraenoic acid; HODE, Hydroxyoctadecadienoic acid; PG, Prostaglandin; EPA, Eicosapentaenoic acid.

Discussion

Leptin drives differently altered secretomes from joint tissues in human knee OA. IFP, synovium, and cartilage secretome changes reveal unique secretory pathways altered by leptin that highlight important areas of leptin-mediated tissue crosstalk. Leptin-induced changes at the OA IFP secretome reveals protein kinase signaling factors. At the OA synovium secretome, leptin treatment revealed dysregulation of glycoproteins and lipid metabolism. At the OA cartilage secretome, leptin treatment

altered amino acid and serine-threonine metabolism. Sex and BMI differentially modulate knee OA tissue response to leptin. Females and patients with a high BMI demonstrated a muted response to leptin via lower logFC values compared to males and patients with a low BMI, respectively, especially evident in OA cartilage secretome. Eicosanoid response to leptin was modulated by sex and BMI only at the OA IFP secretome, highlighting that the pain profile of the IFP is both sensitive to sex and BMI and may be further exacerbated by leptin.

Leptin induced dysregulation of kinase proteins and phosphatidylcholine metabolism in the OA IFP secretome. Marcks-related protein (MRP) was one of the top downregulated proteins with leptin at the OA IFP. MRP is a membrane-associated protein with several roles in regulating cell structure and motility,¹⁶⁹ and is a substrate for protein kinase C (PKC) in that phosphorylation of PKC leads to the migration of MRP from the cell membrane to the cytosol which triggers activation of several immune signal transduction pathways downstream.¹⁶⁹ AT5L1, or ADAMTS14, is a pre-collagen protease of the ADAMTS metalloproteinase family that has been extensively researched in osteoarthritis,¹⁷⁰ and was found upregulated with leptin in the IFP secretome. The ADAMTS family of proteins have been closely linked to cartilage ECM degeneration in OA, but has not been previously characterized in the OA IFP secretome. AKT3, part of the PI3K/AKT signaling pathway previously implicated in regulating OA progression in the cartilage, was also found upregulated with leptin in the OA IFP. Activation of PI3K/AKT signaling has been shown to exhibit a detrimental effect on OA progression, where sustained activation of AKT signaling in articular chondrocytes induces OA in mice,¹⁷¹ and blocking of AKT signaling attenuates cartilage degeneration in mice.¹⁷²

Oleamide, an eicosanoid that suppresses expression of pro-inflammatory mediators by inhibiting NF- κ B and MAPK phosphorylation in macrophages,¹⁷³ was also found upregulated in the OA IFP with leptin. Leptin is a key signal in fat-cartilage crosstalk.²⁴ Leptin treatment of the OA IFP led to dysregulation of secretome factors (at the proteome and eicosanoid levels) related to OA cartilage degeneration that also interact with several protein kinase signaling pathways. This suggests that leptin alters IFP crosstalk through dysregulation of protein kinase signal mediators that may in turn influence cartilage degeneration in knee OA. An additional unique feature of leptin response observed at the IFP secretome was the dysregulation of phosphatidylcholines (PC): PC ae C36:0, PC ae 36:3, and PC ae C34:2. PC abundance, specifically activation of PC to lysoPC, has been associated with OA knee cartilage volume loss over time.¹⁷⁴ Previous work on PC activation, abundance, and dysregulation in knee OA has been in serum, cartilage, and synovium.¹⁷⁴ These findings highlight the need to probe PC activity as a potential downstream target of leptin at the IFP that may influence adjacent tissues in the context of high BMI and obesity.

At the OA synovium secretome, leptin treatment revealed dysregulation of lipid metabolism and adipogenesis. Top dysregulated proteins at the OA synovium secretome with leptin were glycoproteins: MEPE is a glycoprotein involved in osteoclast bone metabolism,^{175,176} and OLFM4 is a glycoprotein that functions in immunity and inflammation and has been previously observed in synovial tissue from patients with rheumatoid arthritis.^{177,178} Additionally, two triglycerides were upregulated with leptin in the OA synovium secretome. Glycoproteins and triglycerides play key roles in lipid and adipose metabolism and regulation,^{179,180} and elevated levels of triglycerides have been

associated with an increased risk of OA.¹⁸¹ The top pathways upregulated in the synovium secretome with leptin treatment included adipocyte differentiation and adipogenesis. An overlapping response to leptin treatment in OA synovium and IFP was alteration of protein kinase-related factors, which supports previous literature that poses the IFP and synovium as a functional unit given their shared anatomical border, cell lineage, and, now, response to leptin.⁴ MARK2, downregulated at the OA synovium secretome with leptin, is a serine/threonine protein kinase activated by MAPK, and its dysregulation has both been highlighted in previous literature in OA synovial fluid,¹⁸² and aligns with pathway analysis of altered p53, PTEN, and AKT activity—also previously described in literature on OA and obesity.¹⁸³ While different factors related to protein kinase signaling were dysregulated by leptin in OA synovium and IFP, an overlap in response to leptin highlights a potential mechanism of IFP-synovium crosstalk mediated by leptin that should be explored.

OA cartilage secretome revealed altered amino acid and serine-threonine metabolism with leptin, and demonstrated a more distinct tissue response to leptin compared to IFP and synovium. Amino acid function has been well studied in cartilage health and OA through the role in mitochondrial function and oxidative stress.¹⁸⁴ Top significant pathways dysregulated with leptin in the OA cartilage secretome revealed isoleucine, valine metabolism, as well as mitochondrial fatty acid oxidation. As a whole, the OA cartilage secretome response was substantially different from the IFP and synovium, with 900 out of 919 proteins significantly downregulated with leptin, spanning a wide variety of protein profiles. OA synovium and IFP secretomes exhibited a lower count of proteins and metabolites dysregulated with leptin (~200), and dysregulation of

several serine/threonine protein kinase signal mediators. Interestingly, serine and threonine were both upregulated at the OA cartilage secretome. Serine and threonine are major protein kinase signaling hubs where the hydroxyl group of these amino acids are phosphorylated to produce a family of serine/threonine-specific protein kinases,¹⁸⁵ including MAPK, AKT—dysregulated at the OA IFP—and MARK2—dysregulated at the OA synovium. The overlap of dysregulation in proteins and metabolites that interact with protein kinase signaling across all 3 tissue secretomes suggest a potential mechanism of leptin-mediated crosstalk among IFP, synovium, and cartilage in OA.

Tissue secretome-specific response to leptin was modulated by sex and BMI and revealed altered leptin response in males versus females and HBMI versus LBMI. Significant logFC with leptin treatment across sex and BMI groups in all 3 tissue secretomes showed that females and HBMI demonstrated a lower response to leptin treatment, with logFC values around 0 (indicating minimal to no change). In the cartilage secretome, males and LBMI demonstrated logFC values close to -1, suggesting a magnified response to leptin in these groups. Pathway analysis of sex-modulated cartilage secretome response to leptin revealed leucine, isoleucine, and valine metabolism, suggesting that OA cartilage tissue in females demonstrates a muted response to leptin in amino acid metabolism. Pathway analysis of BMI-modulated cartilage leptin response revealed regulation of mononuclear cell migration, which has been previously implicated in knee OA cartilage degeneration.¹⁸⁶ This suggests that OA cartilage tissue in individuals with HBMI may be less responsive to leptin through muted response of inflammatory cell signaling. Interestingly, the IFP demonstrated similar logFC distribution to leptin within sex- and BMI-based groups suggesting sex and BMI

to impart less disparate responses to leptin. Pathway analysis of sex-modulated leptin response at the IFP secretome revealed leptin signaling, and BMI-modulated leptin response at the IFP secretome revealed MMP activation and IL-4 and IL-13 signaling—found in OA cartilage tissue.^{187,188} The synovium also demonstrated less disparity in logFC response between males versus females, and between HBMI and LBMI, similar to the IFP. Pathway analysis revealed mRNA processing with sex-modulated leptin response, and regulation of protein transport and signaling with BMI-modulated leptin response. Whether female sex or high BMI results in lower peripheral leptin response and subsequently muted leptin-mediated tissue crosstalk remains to be delineated. This emphasizes the need to not only investigate multiple tissues in knee OA, but the many populations that experience knee OA to thoroughly characterize tissue changes. Previous literature and previous chapters of this dissertation highlight differences in the morphology and secretory profile of the IFP by sex and BMI status.^{28,98} As an adipose depot, the IFP is also a site of leptin production and secretion,² which may explain the less variable magnitude of logFC secretome change with leptin between sex- and BMI-based groups. Circulating leptin levels in patients included in this study were not measured. However, our findings support previous literature that peripheral tissues in the knee may demonstrate leptin resistance in populations (females, patients with obesity) that demonstrate elevated levels of circulating leptin and subsequent leptin resistance.^{90,91,168}

The IFP was the only tissue to demonstrate a significant modulation of leptin response in eicosanoid secretome profile by sex and BMI. Several eicosanoids that were differentially dysregulated with leptin in females versus males and in HBMI versus

LBMI have been reported in OA literature, namely prostaglandins.^{189,190} Eicosanoids are derivatives of arachidonic acid through multiple metabolic pathways including cytochrome P450 (cytP450), cyclooxygenase (COX), and lipoxygenase (LOX).¹³⁸ The majority of eicosanoids differently dysregulated with leptin across sex are activated through the cytP450 pathway,¹³⁸ while the majority of eicosanoids differently dysregulated with leptin by BMI are activated through the COX and LOX pathways.¹³⁸ Sex differences in eicosanoid profiles 12-HETE and 15-HETE in synovial fluid and serum, respectively, have been reported in recent work on Complement Factor D—a downstream factor of leptin involved in fat-cartilage crosstalk in animal models.^{65,191} Observation of these same eicosanoids in human tissue, let alone OA IFP, highlights translation of these factors in sex-differences in knee OA. Overall, the unique effects of sex and BMI on leptin-dysregulated eicosanoid profile offer novel insight into leptin-mediated communication pathways specific to pain. The IFP was the only tissue to exhibit an interaction effect of sex and BMI on eicosanoid profile response to leptin, which further emphasizes the candidacy of the IFP as a unique pain modulator in knee OA. Further research is needed to delineate a causal link between leptin and sex- and BMI-related eicosanoid dysregulation in order to uncover mechanistic targets for future precision care.

This is among the first studies to describe the tissue secretome profiles of the IFP, synovium, and cartilage in OA, and characterize how the secreted profile of these tissues respond to leptin signal. The IFP secretome demonstrated changes in molecules that have been shown to interact with protein kinase signaling in OA. Cartilage and synovium demonstrated modulation of proteins and metabolites that interact with protein

kinase pathways, which could pose a potential mechanism of leptin-mediated tissue crosstalk. Unsurprisingly, tissue response to leptin was modulated by sex and BMI. Circulating leptin is different by sex and BMI, and elevated leptin levels associate with pain. Thus, further interrogation of peripheral tissue response to leptin signal may elucidate different disease phenotypes in knee OA.

This study is not without limitations. We did not have control tissues from healthy individuals without knee OA. This is a common challenge in human tissue biological studies that require larger tissue collection (e.g. cartilage, synovium, IFP) versus a small, less invasive collection (e.g. serum, synovial fluid). As such, the sex- and BMI-based differences reported in this study are specific to the knee OA patient population and cannot be generalized. Future studies including cadaveric human tissues and non-OA related knee surgical intervention (e.g. ACL reconstruction) would be beneficial to compare the secretome profiles reported in this study with other clinical populations. We had lower representation of males and individuals with a low BMI (BMI <25 kg/m²) in our study, further limiting generalizability. Further external validation of these findings is needed in other knee OA cohorts and datasets. This study investigated relationships and modulation of high vs low BMI on tissue responses, but did not include measures of metabolic disturbance, excess fat mass, or fat dysregulation associated with obesity as a chronic disease. This limits our ability to draw connections to only BMI and not obesity. Future studies will need to examine obesity more comprehensively, using body composition and metabolic health assessments, to gain more conclusive insight on peripheral tissue response to leptin in the context of OA and obesity.

This project offers novel insight into unique and overlapping leptin-mediated crosstalk in knee OA tissues. Leptin driving differently dysregulated secretomes in cartilage, synovium, and IFP emphasizes the value of investigating secreted factors from multiple tissues rather than tissues in isolation to define mechanisms of interorgan crosstalk driving OA. Our results help expand our understanding of tissue changes with OA and factors that may initiate or influence a cascade of tissue degeneration and breakdown across the whole joint organ system. Similar dysregulation of protein kinase signalling mediators across IFP, synovium, and cartilage highlight a potential target for future research in understanding whole joint damage in knee OA. The variability in tissue response to leptin signaling by sex and BMI also emphasizes the need to thoroughly consider how high-risk groups—females and individuals with BMI ≥ 30 kg/m²—may respond differently to this signalling cascade. The unique interaction effect of sex and BMI in modulating eicosanoid response to leptin in the IFP further supports the IFP as a valuable tissue in understanding varying pain populations in knee OA.

Chapter 5 Integrated Discussion and Future Directions

The goal of my dissertation was to conduct an interdisciplinary evaluation of IFP tissue dysfunction across knee OA populations in relation to pain using semi-quantitative imaging measures of tissue abnormality and advanced multi-omic profiling of the secretome. This information was deeply needed because the precise role of the IFP remains enigmatic and controversial in the literature, and in clinical practice. To this end, IFP tissue dysfunction evaluated using a multi-disciplinary approach revealed translatable information on morphological and molecular differences at the IFP by patient characteristics in the context of OA pain. The IFP demonstrated imaging features that indicate tissue dysfunction (i.e. signal abnormality) that not only relate to patient-reported pain, but also differ by sex and BMI. The IFP reflects pain severity among high-pain, high-risk patient groups (i.e. females and individuals with high BMI). Profiling the IFP secretome using novel, integrated multi-omic models rather than tissue intrinsic profile revealed key insight into the dysregulated signals communicated from the IFP due to differences in sex and BMI. Modulation of IFP tissue dysfunction and pain by sex and BMI was observed across several scales and in multiple knee OA populations (PFJOA and end-stage TFJOA). This findings emphasizes that the IFP consistently shows differences by sex and BMI in knee OA, despite the varied location, severity, and stage of knee OA disease. The sensitivity of this tissue to sex and BMI status provides insight into the elevated pain profile observed in female and high BMI populations with knee OA, which has been historically difficult to decode. Unique response of OA cartilage, synovium, and IFP secretomes to leptin signaling (a key signal elevated in high-risk, high-pain OA groups) begins to disentangle how systemic

influences may induce local joint changes. Furthermore, the mix of unique and shared responses to leptin across the 3 tissues highlights the necessity to contextualize tissue dysfunction in knee OA to the whole-joint, rather than tissues in isolation. The study design of the projects within this dissertation did not allow for causal analysis to determine whether IFP tissue dysfunction initiates disease onset, progression, or pain. However, the associations observed between various measures of tissue quality (imaging features and secreted molecular signals) and pain severity highlight the IFP as a crucial, active tissue that plays a role in knee OA pain. The translatable findings across imaging and biology provide potential avenues for prognostic insight (through longitudinal imaging studies of the IFP) and precision treatment avenues (through targeting the mechanism of sex- and BMI-based IFP change) for knee OA management.

Gaps Filled

Previous studies have provided limited, isolated information on the IFP in relation to knee OA structural outcomes.³²⁻³⁴ My scientific contribution through this dissertation not only establishes relationships between IFP tissue change and pain, but does so by uniting imaging across biology to provide translational insight into how tissue changes at the IFP relate to heterogeneous patient pain. No study to date has evaluated the relationship between IFP signal abnormality, using both intensity and area submeasures, and pain in patients with PFJOA. Most importantly, the relationship between IFP signal abnormality and pain was observed to be stronger among females and patients with a BMI ≥ 30 kg/m² compared to their counterparts (males and BMI <25

kg/m², respectively). Only one prior study has detailed the molecular and cellular landscape of the IFP in knee OA, which provided valuable insight into the intrinsic profile of the IFP in disease.¹⁸ This dissertation is the first to characterize the extrinsic profile and secretory contribution of the IFP through targeted evaluation of the secretome, establishing a novel approach to investigating tissue crosstalk. Furthermore, decoding the leptin response of the IFP, cartilage, and synovium secretomes in knee OA, as well as how sex and BMI modulate said responses, provides novel insight into how a prominent, systemic signal induces local tissue change. Previous studies have shown that the IFP demonstrates morphological and compositional changes with BMI-derived obesity and knee OA,^{6,15,16,18,22,28} but this is the first study to establish a relationship between sex- and BMI-based IFP molecular features with patient-reported outcomes in knee OA. My interdisciplinary-emphasized dissertation addresses important gaps in our understanding of the vast heterogeneity of patient pain in knee OA by detailing that IFP tissue change indeed reflects how the patient experiences the disease through mechanisms that may prove targetable and treatable.

Overall Findings

The IFP has long been misunderstood and underappreciated. This tissue was regarded as a passive tissue contributing primarily to mechanical shock absorption at the knee.¹⁰⁸ However, to our knowledge, these are the first studies to show that the IFP is associated with patient pain in varying populations of knee OA, from isolated PFJOA to end-stage knee OA at time of TKA. In individuals with PFJOA, not only did the IFP relate to patient pain and functional limitation measured via the KOOS, but relationships

differed by sex and BMI with high-pain groups (females and patients with BMI ≥ 30 kg/m²) exhibiting stronger relationships between IFP tissue abnormality and pain. In individuals at the end stage of TFJ knee OA, the IFP secretome revealed uniquely dysregulated factors by sex and BMI that related to pain on the KOOS. These findings across semi-quantitative imaging and multi-omic profiling show that the composition and molecular contribution of the IFP in knee OA may have distinct phenotypes in patient groups at high-risk for OA disease onset and progression.^{51,64,106,124} Degree of tissue abnormality at the IFP on MR—signal abnormality—was more strongly related with patient pain in females and individuals with a high BMI. Thus, tissue dysfunction at the IFP may be more indicative of patient pain in high-risk, high-pain groups. Sex- and BMI-based differences in IFP secretome profile correlated with patient pain provide molecular insight into why the IFP demonstrates sex- and BMI-specific tissue features.

Sex-dysregulated IFP secretome profiles correlated with multiple KOOS subscales and revealed proteins involved in mitochondrial function and regulation. Mitochondria are integral to energy production and cell health. Mitochondrial dysregulation leads to energy imbalance, chondrocyte apoptosis, and articular cartilage degeneration in knee OA.^{130,150} While directionality and causality remains to be tested, this study highlights a potential mechanism of IFP crosstalk in OA via dysregulation, or miscommunication, of signals that act on mitochondrial function in chondrocytes, thus influencing knee joint health. More specific to pain, dysfunction of mitochondria leads to neuroinflammation through oxidative stress caused by overgeneration of reactive oxygen species and nitric oxide (NO). Mitochondrial dysfunction, resulting in oxidative stress, is a pivotal element described in knee OA. The dysregulation of mitochondrial

proteins from the OA IFP correlated with pain suggests that the IFP is a source of pain and inflammation in knee OA through a mitochondrial mechanism. Specific to sex differences, estrogen interacts with mitochondria through several isoforms,¹⁹² and has been highlighted in aging, disease, and knee OA.^{52,132} Previous literature and the findings in this dissertation support the hypothesis that the IFP secretome differs by sex, and greater severity of pain experienced by females may be attributed in part to dysregulated mitochondrial proteins from the IFP.

BMI-based differences in IFP secretome profiles correlated with multiple KOOS subscales and revealed proteins involved in immune regulation, specifically macrophage polarization and neutrophil activation. Adipose tissue, including the IFP, is a major hub for immune regulators.^{2,10,12,23} Low-grade systemic inflammation is a hallmark of obesity, attributed in part to a chronic state of adipose tissue dysregulation with higher macrophage and neutrophil activity.^{48,49,134,154,157} Our finding that the IFP undergoes similar dysregulation with high BMI (associated with increased adiposity and obesity) aligns with the literature, with the added novel insight that these changes correlate with worsened pain and symptoms in knee OA. While causative links need to be established, these findings contribute novel pathways of sex- and BMI-based tissue dysfunction at the IFP that may begin to disentangle the variable pain phenotypes in knee OA across patient populations and establish avenues of targeted therapeutic intervention.

When probing how the IFP responds to leptin signaling in knee OA relative to adjacent tissues, the IFP exhibited differentially dysregulated proteins, metabolites, and eicosanoids with leptin signaling compared to cartilage and synovium. The proteome

and eicosanoid changes at the IFP in response to leptin highlighted protein kinase signaling mediators. This suggests that the IFP may transduce leptin signaling to adjacent tissues through such signaling pathways. This aligns with literature showing that leptin interacts with AMPK, one study showing that leptin stimulates fatty-acid oxidation by activating AMPK in muscle.¹⁹³ When assessing all 3 knee OA tissues, the IFP and synovium demonstrated overlapping changes with leptin, which aligns with previous literature suggesting that the IFP and synovium are a functional unit with shared progenitors and action in knee OA health.^{4,79} While this study did not aim to investigate the effect of age on secretome profile and leptin response, it is worth noting that protein kinase signaling pathways are involved in the aging process.¹⁹⁴ Deficiency in sensitivity of AMPK activation in aged tissues in mice has been noted and associated with the occurrence of cerebrovascular stroke and metabolic syndrome, both age-associated diseases.¹⁹⁴ Osteoarthritis is categorized as another disease of aging, and dysregulated PI3K/AKT/mTOR signaling has been noted in chondrocytes with OA degeneration.¹⁹⁵ Therapeutic benefits have also been seen with modulation of several protein kinase signaling pathways in human cartilage, where inhibition and regulation of upstream targets of PI3K/AKT/mTOR, and AKT signaling reduce IL-1 β -stimulated inflammation, attenuate chondrocyte apoptosis and ECM degradation, and rescues IL-1 β -induced mitochondrial-related apoptosis.¹⁹⁵⁻¹⁹⁸ Investigation into the mechanism of protein kinase signaling at the IFP and how leptin mediates or alters normal signaling will provide valuable insight into how the IFP influences knee OA disease and pain.

Mechanistic studies should not assume that signals driving OA are homogeneous across age, sex, and BMI groups. Females and individuals with BMI ≥ 30 kg/m² exhibit

elevated levels of circulating leptin compared to males and individuals with BMI <25 kg/m².^{90,91,168} As expected, sex and BMI modulated leptin response. What was not expected was the varying modulation of leptin response across IFP, synovium and cartilage. Cartilage demonstrated the most disparate leptin response between sex-based and BMI-based groups, respectively, followed by synovium, then IFP with the lowest magnitude of different leptin response between groups—females and males, and individuals with high BMI and low BMI, showed a similar spread of logFC around zero. While the IFP demonstrates morphological and compositional differences based on sex and BMI,^{16,32,98} the IFP showed the least disparity in leptin response across sex- and BMI-based groups. The IFP is a source of leptin production and secretion at baseline, which may explain the less disparate response to leptin across sex and BMI compared to the synovium and cartilage secretomes. Females and individuals with a high BMI demonstrated a muted response to leptin across all 3 tissues, with logFC values nearing 0 suggesting minimal secretome change with leptin response. This may be speculated as peripheral tissue leptin resistance. Central leptin resistance has been described at the blood brain barrier, but peripheral tissue leptin resistance remains to be fully explored. As with insulin and the development of insulin resistance, chronic elevated levels of leptin lead to leptin resistance, most commonly seen with increased adiposity and obesity, and observed in females.^{85,91} The muted peripheral tissue leptin response in females and individuals with a high BMI is worthy of consideration in the context of leptin resistance. This remains purely speculative as serum leptin levels were not measured in the cohort studied in this thesis. This study alone cannot establish whether the IFP is a transducer of circulating leptin signal from systemic adipose to knee joint

cartilage tissue. However, the work presented in this dissertation furthers our understanding of how the IFP responds to leptin and is modulated by sex and BMI to uncover the active contribution of the IFP in knee OA joint health mediated by leptin signal.

IFP response to leptin revealed sex- and BMI-modulation of eicosanoids only in the local fat depot. No interaction effects at the cartilage or synovium were observed in eicosanoid response to leptin at the secretomes. This further emphasizes the sensitivity of the IFP's pain-related profile to sex and BMI, with additional insight into leptin-mediated modulation of pain factors. Previous work has shown that eicosanoids, including PGE2 may regulate leptin release and lipolysis by adipose tissue in mice,¹⁹⁹ which begs "the chicken or the egg?" question. Whether leptin induces eicosanoid response or eicosanoids regulate leptin release from the IFP, there is a clear connection between IFP-secreted eicosanoids, sex and BMI modulation, and leptin signal in knee OA. Whether isolated PFJOA or end-stage knee OA, the results presented in this dissertation demonstrate that IFP tissue dysfunction characterized across abnormal quantitative imaging features, multi-omic secretome changes based on sex and BMI, and leptin-mediated secretome changes correlate with pain and link to inflammatory response in knee OA. Overall, these results highlight the IFP as an integral tissue to consider in knee OA research as it may reflect or influence varying pain phenotypes across disease severity and within sex and BMI groups. The IFP also demonstrates a potential role in modulating inflammatory regulation in knee OA through leptin-mediated crosstalk.

Clinical Significance

Overall, the findings of this dissertation connect with clinically relevant issues in knee OA management. The IFP is reflective of OA-related knee pain, especially in populations that experience the greatest severity of pain. The heterogeneity of knee OA pain phenotypes has made understanding pain in knee OA pathophysiology difficult to separate and directly test. These results indicate that the IFP may exhibit unique inflammatory profiles by demographic factors, potentially influencing higher pain perception in females and individuals with a high BMI. These findings in both clinical imaging and molecular characterization suggest that targeting unique tissue dysfunction at the IFP may resolve pain in specific populations in knee OA. Imaging characteristics of the IFP may provide detailed prognostic insight into how high-pain populations—namely females and individuals with a BMI ≥ 30 kg/m²—may progress through disease, or even predict whether an individual will develop more severe pain before symptoms have set in. This work highlights unique sex-differences in mitochondrial dysregulation and BMI-based differences in immune cell dysfunction that related to how the patient experiences the disease. Therapeutic approaches to mitochondria include behavioral (exercise), stem cell therapies, and pharmacological intervention.^{200,201} Immune cells can also be targeted for therapeutics, including macrophages through injected delivery to increase numbers of macrophages, or ex vivo implantation of activated macrophages to stimulate a desired immune response.²⁰² Understanding the biological underpinnings of these findings may uncover targetable therapeutic opportunities at the IFP for high-risk populations, improving precision care approaches for management of knee pain in OA populations. Further research into leptin-mediated IFP crosstalk with adjacent

tissues may uncover an untapped therapeutic opportunity at the IFP through targeting leptin. Exercise has been shown to reduce circulating leptin levels in humans.⁹¹ While the effect of exercise has not been specifically investigated in modulating leptin or other inflammatory molecules at the IFP, investigation into how exercise may modulate peripheral tissue leptin activity may offer an easily accessible treatment avenue for knee OA pathogenesis and pain. Targeting a crosstalk mechanism, e.g. leptin-mediated protein kinase signaling, may lend to a more comprehensive treatment for the whole knee joint in OA. Targeting a signal mechanism may succeed where other disease modifying OA drugs (DMOADs), such as single-targets of IL-1Ra,²⁰³ have fallen short. The overall clinical takeaway I strive to contribute with this dissertation is that the IFP is an active tissue in OA pain and differently reflects important patient characteristics (e.g. sex, BMI). As such, measures of IFP tissue change should be included as a standard measure in knee OA disease. Knee OA is not a “one size fits all disease”, therefore treatment of knee OA should not aim to fit all. The unique mechanisms of tissue dysfunction at the IFP that link to pain and patient features offer an opportunity to approach the treatment of knee OA pain in light of the heterogeneity, rather than trying to fit all populations into the same mold.

Future Directions

This work lays the foundation for a series of future studies that can help understand the role and therapeutic potential of the IFP. With its prevalent immune profile and vast innervation, the IFP is a prime candidate to contribute to knee OA pain, potentially through unique sex- and BMI-dependent mechanisms. Future research

should compare the measures of tissue quality in the IFP secretome outlined in this work between knee OA and normal knee joint health to contextualize these findings to general disease progression, and true sex- and BMI-based differences. As of now, the findings in this dissertation only pertain to the OA population. Comparing sex-based IFP differences in OA with sex-based IFP differences in a healthy state will establish whether mitochondrial dysregulation stems from differences in sex or from a diseased state (or potentially a combination of the two). The same can be said for BMI-based differences, but going even deeper into fat composition (e.g. DXA) and metabolic disturbance (e.g. blood biomarkers—albumin, HDL—and waist circumference) to truly probe obesity in IFP tissue changes with OA. Given its high innervation and densely organized structure, pursuing biopsy of the IFP is not feasible at this time. However, continued leverage of human surgical waste across procedures (at TKA for knee OA; at ACLR, arthroscopies, or other non-OA related knee surgical intervention) allows for thorough molecular characterization of the IFP.

Another avenue of future exploration would be to merge the research disciplines of semi-quantitative imaging studies (e.g. MR, PET), tissue and secretome profiling, and patient-reported outcomes in a longitudinal study. Directly comparing IFP signal abnormality changes with changes in tissue secretory profile would uncover how imaging of the IFP may be utilized as a prognostic marker for OA progression and pain in sub-populations. With a longitudinal study, age may also be evaluated. Age is one of the greatest risk factors for OA and a major piece in the OA puzzle.³⁹ Sex and BMI were a focus of this dissertation, thus age was not a measure or factor investigated, rather comparisons were adjusted for age as a covariate. However, it was observed across all

projects and populations in this dissertation that individuals with a BMI ≥ 30 kg/m² were younger than individuals with a BMI < 25 kg/m². A separate study exploring how age influences the IFP using imaging, biological profiling, and patient outcomes will provide an added layer in understanding the contribution of the IFP in age, and disease.

A direct comparison of IFP tissue with other adipose tissue depots within healthy and knee OA patients would delineate how the IFP differs, aligns, and potentially communicates with other adipose depots throughout the body. Directly comparing the secretory profiles of adipose depots within the same patient would delineate how general adipose tissue communication changes with disease. Using advances in biological research methods, the IFP has the potential to reflect varying pain phenotypes, differentially secrete signals related to patient factors and pain, and modulate communication in response to a systemic signal. The IFP may be the beacon through which genetic factors (e.g. sex), systemic signals, and metabolic dysregulation are transduced to impact the local joint environment and inflammation in knee OA.

My dissertation began as a pursuit to see how many scientific doors I could open. In accomplishing this multi-disciplinary work, I see that my path now crafts new doors through which future generations of researchers will walk. As a clinician scientist, I will merge imaging, biology, and patient outcomes across populations and musculoskeletal disease, for the greatest discoveries are only ever achieved when we as a scientific community collaborate.

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