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Relation of adipokines to pain sensitization and pain patterns in patients with knee osteoarthritis

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RELATION OF ADIPOKINES TO PAIN SENSITIZATION AND PAIN PATTERNS IN PATIENTS WITH KNEE OSTEOARTHRITIS

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DEDICATION

I dedicate this thesis to my parents, Julia and Carlos Bomfim, and my siblings for their endless love, support, and encouragement.

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RELATION OF ADIPOKINES TO PAIN SENSITIZATION AND PAIN PATTERNS IN PATIENTS WITH KNEE OSTEOARTHRITIS

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ABSTRACT

INTRODUCTION: Pain sensitization, a process whereby nociceptive processing pathways are altered, leading to enhanced pain perception, is associated with pain severity in knee osteoarthritis (OA) and the progression in patterns of pain symptoms from being presently only intermittently to eventually becoming constant. What contributes to pain sensitization in knee OA is not clear. Obesity and low-grade inflammation, both of which are common in knee OA, play a role in the pain experience in knee OA. Whether pain sensitization in knee OA is induced by nociceptive input from systemic pro-inflammatory cytokines produced and released by adipose tissue is not known. Heightened pain as a result of systemic cytokines could be one mechanism by which obesity contributes to pain in knee OA. Characterizing the relation of systemic inflammatory cytokines to pain sensitization and pain pattern progression could help identify potential targets for the development of disease-modifying therapies and may provide additional rationale for weight-related interventions among patients with knee OA.

OBJECTIVES: The goal of this study was to examine the contributions of leptin and TNF-α, two systemic pro-inflammatory proteins produced by adipocytes, to pain sensitization and pain pattern progression in people with knee OA.

METHODS: Data were obtained from the Multicenter Osteoarthritis (MOST) Study, a longitudinal cohort of older adults with or at risk of knee OA. Leptin and TNF-α levels were measured at baseline. Sensitization measures, pressure pain threshold (PPTs) and temporal summation (TS), were completed at baseline and 24 months later. Pain patterns were assessed as frequency and severity of intermittent pain and the severity of constant pain using the Intermittent and Constant OA Pain (ICOAP) questionnaire. We examined the relation of cytokine levels, analyzed as sex-specific tertiles, to PPT and TS at baseline and to changes in PPT and TS over two years. We also evaluated the relation of cytokines to the following pain patterns, assessed by the ICOAP instrument: (1) no pain; (2) intermittent pain only; (3) constant +/- intermittent pain. Finally, to test our hypothesis that higher cytokine levels increase the risk of pain pattern progression, we defined a binary outcome as the development of more frequent intermittent or constant pain.

RESULTS: Among 739 participants (1478 knees) that met the inclusion criteria, the mean age was 61, mean BMI 30, and 58% were female. At baseline, higher leptin levels were associated with lower PPT (greater sensitivity or lower threshold for pain) at the knee (adjusted ß = -0.44 [95% confidence interval (CI) -0.86, -0.02]) while higher TNF-α levels were associated with lower PPT at the wrist (adjusted ß = -0.28 [95% CI -0.54, -0.01]). There were no significant associations with temporal summation at baseline, nor with change in PPT or temporal summation over two years. There was no significant difference in the odds of having intermittent pain (odds ratio (OR) 0.53 [95% CI 0.11, 2.63]) nor in the odds of having constant pain +/- intermittent pain (OR 0.40 [95% CI 0.06, 2.46] compared with no pain between those in highest versus lowest tertiles of

leptin. Similarly higher baseline levels of TNF-α were not significantly associated with the odds of having intermittent pain (OR 0.1.72 [95% CI 0.40, 7.36]) nor in the odds of having constant pain +/- intermittent pain (OR 3.21 [95% CI 0.68, 15.00] compared with no pain. Lastly, belonging to the highest tertile of leptin (OR 0.70 [95% CI 0.37, 1.33]) or TNF-α (OR 1.14 [95% CI 0.71, 1.85]) did not significantly increase the odds of developing more frequent pain when compared to their lowest tertile counterparts.

CONCLUSION: PPT had a small association with both leptin and TNF-α after adjusting for pertinent confounders. A lack of an association with change in PPT or TS over two years suggests these cytokines may only have a transient effect on nociceptive neurons. Similarly, leptin and TNF-α were not associated with pain patterns or with the likelihood of developing more frequent pain, which has been linked to sensitization. Given this study did not track longitudinal changes to cytokine levels, it provides an incomplete assessment of the potential role leptin and TNF-α may have in generating and maintaining chronic pain states. Nonetheless, in this sizable cohort, these pro-inflammatory cytokines did not appear to have an appreciable contribution to pain sensitization or pain patterns in knee OA.

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LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
BU	Boston University
CES-D	Center for Epidemiologic Studies Depression Scale
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CSQ	Coping Strategies Questionnaire
DRG	Dorsal Root Ganglion
ETDA	Ethylenediamine Tetraacetic Acid
FDA	Food and Drug Administration
GEE	Generalized Estimating Equation
ICOAP	Intermittent and Constant Osteoarthritis Pain Questionnaire
IL-6	Interleukin-6
IRB	Institutional Review Board
KL	Kellgren-Lawrence
MOST	Multicenter Osteoarthritis Study
MRI	Magnetic Resonance Imaging
OA	Osteoarthritis
OR	Odds Ratio
PPT	Pressure Pain Threshold
NHANES	National Health and Nutrition Examination
NIH	National Institute of Health

NRS	Numerical Rating Scale
NSAID	Non-Steroidal Anti-Inflammatory Drug
TKR	Total Knee Replacement
TNF-α	Tumor Necrosis Factor Alpha
TS	Temporal Summation
US	United States
WAT	White Adipose Tissue

INTRODUCTION

Brief Overview of Osteoarthritis

Osteoarthritis (OA) is the most common form of arthritis (joint disease) worldwide, affecting approximately 500 million people[1] and increases in prevalence with age. It is generally characterized by the slow, progressive damage to and loss of articular cartilage, which is the tissue that encapsulates the ends of the bones that make up most joints in the human body, as well as changes in the bone and other tissues within the joint. OA is incorrectly considered a disease of 'wear and tear', whereas it involves active biologic processes that leads to joint pathology.

The most common sites for OA to occur are in the hand, knee, and hip, with the knee being the most symptomatic joint. OA can also occur in other joints, often related to prior injury[2]. It is also not uncommon for multiple joints to be involved.

OA is a highly complex, heterogenous musculoskeletal disease. Even though the exact etiology and pathophysiology are not fully elucidated, it is understood that the development of OA requires input from a combination of both systemic and local factors. The main known systemic risk factors include older age, female sex, obesity, genetics/family history, and high bone mineral density[2]. In contrast, local risk factors, which may relate more to the mechanical aspects of the joint, include joint injury, abnormal bone morphology, poor muscle strength, high mechanical joint load, malalignment, and repetitive use[2]. The relative contribution of each risk factor can vary greatly depending on the joint. For example, obesity is a major risk factor for knee OA but its contribution towards hand OA is less clear. Similarly, hormonal status is thought

to have a prominent role in hand OA[3,4] but is generally not a distinguishing factor for those with knee OA.

The main symptoms of OA are pain, stiffness, tenderness, and swelling though not everyone with OA becomes symptomatic. In terms of symptomatic disease, the knees are the most commonly affected site[5]. Therefore, most OA research has been focused on investigating the causes of OA-related pain in people with knee OA. This is also the case for this thesis.

Definitions of Knee Osteoarthritis

Knee OA is most commonly defined based on radiographic (x-ray) findings. The Kellgren-Lawrence (KL) system is the most widely used method for assessing OA severity based on radiographic features of OA[6]. This scoring system assesses the degree of joint space narrowing (JSN) which roughly correlates to articular cartilage loss, and marginal osteophytes as the primary contributors to KL grade, as well as other findings such as subchondral sclerosis and cysts. A minimum KL grade of two out of four is considered to be the standard for defining definite radiographic OA[7]. A major benefit of this approach is that it provides a standardized method of identifying knee OA while also enabling easy monitoring of structural progression. However, it does not measure symptoms, and does not necessarily correlate well with symptoms due to the multifactorial nature of pain. For example, among the 319 participants with radiographic knee OA in NHANES I, only 47% reported knee pain[8]. Nonetheless, symptomatic knee

OA (SKOA) is defined as presence of radiographic OA (KL ≥ 2) accompanied by symptoms of pain, aching, and/or stiffness in the same knee.

Pain in Knee Osteoarthritis

Pain is the primary symptom in knee osteoarthritis (OA) and the leading cause of disability among adults in the United States (US)[9]. In the US alone, the total number of people with painful knee OA ranges from 27 million to 31 million[2,5] and the lifetime risk of developing SKOA is estimated to be approximately forty percent and even higher among those with obesity[10]. The number of people with SKOA is expected to rise due to an aging population and the high preponderance of obesity in the US, a major risk factor for knee OA[11]. Without a cure for OA, managing pain is essential to preserving joint function and improving quality of life. Despite this, the exact causes of OA pain are unknown and there are very few effective treatments available that can provide substantial pain relief without risking serious side effects. Elucidating the etiology and pathophysiology of pain in OA remains an urgent and unmet societal need.

Scope of the Problem

Impact of Knee OA Pain on Physical Health

Physical disability or functional limitation is one of the most important sequalae of SKOA. Daily activities like walking, climbing stairs, getting out of bed, using the restroom, getting in and out of a car and bathing/showering are

exceedingly more difficult for people with SKOA compared to pain-free controls[12]. Likewise, SKOA has also been associated with a reduced capacity to participate in certain recreational activities (i.e., running, jogging, bicycling, weightlifting) which are deemed essential for disease prevention and health maintenance. OA-related pain often leads to severe functional limitations which tends to promote increased sedentary behavior. Consequently, knee OA has been linked to the development of several comorbidities such as cardiovascular disease, obesity, diabetes, and depression[13–17], thereby contributing greatly to morbidity and mortality rates in the U.S.

Impact of Knee OA Pain on Mental Health

As mentioned earlier, the physical limitations of knee OA impose significant restriction on people's lives. One consequence of functional decline is that it limits people's ability to participate in social gatherings, thereby making it harder to maintain relationships with others. Consequently, this can lead to increased social isolation which tends to promote depressive symptoms. Longitudinal data from the Osteoarthritis Initiative study revealed people with SKOA had greater odds of developing depressive symptoms compared to those without knee OA (odds ratio (OR): 1.43; 95% confidence interval (CI): 1.03, 1.98)[17]. An odds ratio measures the likelihood of achieving an outcome (in this case the development of depressive symptoms) given a specific exposure (in this case SKOA) relative to the odds of achieving that same outcome in the absence of

that exposure. Similarly, SKOA has also been associated with greater odds of suicidal ideation (OR: 1.27, 95% CI 1.09-1.48)[18].

As mentioned previously, OA pain is primarily activity-related and typically abates with rest during its early stages. However, in later stages, it tends to assume a more chronic pain pattern punctuated with intermittent increase in pain which often impacts sleep[19]. The effects of OA pain on sleep quality have been widely documented and extensively studied[20,21]. One study showed that as many as one third of people with SKOA have trouble initiating sleep and approximately 80% experience difficulty maintaining sleep[21].

Economic Impact of Knee OA Pain

Pain is the main reason with knee OA people seek out medical care. Given the nature of the disease, its high prevalence, and lack of effective treatment, OA is responsible for a large portion of the annual national and individual healthcare expenditure. According to a 2020 report from the United States Bone and Joint Initiative, OA accounted for approximately 10% of all hospitalizations and 2% of all ambulatory visits in the U.S. between 2008-2014. During this period, the average national OA-related expenditure was estimated to be $136.8 billion annually. This included $65.5 billion in direct costs (medical expenditures) and another $71.3 billion in indirect costs (earning losses)[22]. OA limits people's capacity to maintain gainful employment and promotes absenteeism.

On an individual level, U.S. adults spent an average of $2,000 dollars on OA-related medical care annually while accruing an additional $4,000 dollars in lost wages due to increased absenteeism. It is estimated that the lifetime cost associated with knee OA is approximately $140,000, the majority of which comes from the cost of total knee replacement (TKR).

The Structure-Symptom Discordance of OA

Traditionally, OA was thought to be primarily a disease involving articular cartilage, but it now understood to be a disease of the whole joint[23]. Magnetic resonance imaging (MRI) enables visualization of the remodeling of the subchondral bone, hypertrophy of the joint capsule, and inflammation of the synovial lining as being common features of OA. One of the ways that researchers have attempted to investigate the causes of OA pain is by evaluating its relation to structural pathology. However, so far these studies have shown only a modest association between structural pathologies and pain severity[5,24]. This phenomenon is widely acknowledged in OA literature as the structure-symptom discordance of OA. It is now understood that structural pathology, alone, cannot explain the variation in pain experienced by people with knee OA exhibit as will be discussed in the following section.

Knee OA Pain Patterns

Qualitative studies by Hawker et. al[19] on OA pain have revealed unique clinical patterns that advanced our understanding of the pain experience caused by OA. These studies showed that at the very early stages of knee OA, knee pain typically occurs infrequently and when it does, it's usually short-lasting, of mild severity, and largely related to joint movement or joint loading[19]. This activity-related pain usually responds well to rest. One can imagine how mechanical injury and inflammation may be exacerbated by weight-bearing activities, thereby eliciting pain. However, as the disease progresses, OA pain tends to become more intense, more frequent, increasingly present at rest, and increasingly refractory to analgesics and other types of therapies. Once these later stages are reached, significant physical impairment is noted, and total knee replacement usually follows. It is much less clear how structural pathologies could account for this transition in the pain experience.

Pain Sensitization

Until recently, it was unclear what biological factors or processes might be responsible for this progression from early symptomatic disease, dominated by a pattern of acute, intermittent activity-related pain, to its later stages defined as a chronic, persistent pain state. Recent investigations into the neuronal mechanisms of pain have implicated pain sensitization for the increased severity and frequency of pain and the transition in the pain experience in people with knee OA from intermittent to constant pain.

The knee joint is innervated by afferent neurons, though healthy cartilage is aneural. When tissue damage occurs, pro-inflammatory cytokines, released by damaged cells, bind to free nerve endings triggering action potentials that transmit pain signals first to the dorsal horn of the spinal cord where it is then processed and propagated to the pain center located in the somatosensory cortex. Under physiological conditions, pain is protective and evoked to help avoid further tissue damage.

Pain sensitization describes a pathological, neurological process whereby both peripheral (joint afferents) and central nociceptive neurons (located within the dorsal root of the spinal cord) become increasingly more responsive to sub-threshold or non-noxious stimuli, leading to an enhanced pain facilitation. While peripheral sensitization refers to this phenomenon at the level of peripheral nociceptors and is generally restricted to site of injury and typically requires ongoing input for its maintenance, central sensitization can potentially lead to painful sensations in distant, otherwise healthy tissue and persistent hypersensitive state even in the absence of peripheral pathology[25]. Under these conditions, pain is no longer protective and extremely disruptive in daily life. Pain sensitization has been associated with greater pain severity in knee OA[26] and more recently with pain patterns such as the transition from intermittent to more constant pain[27]. Yet what causes pain sensitization in knee OA is not well-understood.

Systemic Inflammation as a Potential Contributor to Sensitization

Understanding the causes of pain sensitization is crucial for developing more effective therapies that can ultimately prevent the progression to a chronic pain state. Local inflammation is one possible contributor of OA pain as synovitis (inflammation of the synovial lining) has been shown to correlate with pain as well as pain sensitization in people with knee OA[28]. The link between local articular inflammation and pain raises a question of whether systemic inflammation may play a role in knee OA pain. Heightened pain as a result of systemic cytokines could be one mechanism by which obesity, which itself is associated with low-grade systemic inflammation, contributes to pain in knee OA.

Weight loss is one of the most effective methods to alleviate pain and slow down the progression of knee OA[29]. Weight loss is strongly recommended by the American College of Rheumatology (ACR) for individuals with knee OA who are overweight or obese[30]. This recommendation is made despite the fact that we still have a poor understanding of how obesity contributes to the pathogenesis of the disease or to the generation or maintenance of pain once the disease is established. One way that obesity is believed to impact knee OA is by serving as a proxy for weight. Increased mechanical joint load has been shown to hasten the degradation of cartilage[6]. However, this mechanical explanation is only valid for cases of disease in weight-bearing joints like the knees. This does not account for the increased risk for hand OA among individuals with obesity[11], which may point to the metabolic effects of obesity.

Obesity is characterized as a state of excessive accumulation of white adipose tissue (WAT). Apart from its well-known role as an energy reservoir[31], WAT also behaves as an endocrine organ, producing and releasing factors that help to regulate physiological and pathological processes. When an imbalance between energy intake and expenditure occurs (positive energy balance), adipocytes (fat cells) hypertrophy and become dysfunctional. This increase in size leads to an abnormal overproduction of inflammatory mediators such as adipokines and other pro-inflammatory cytokines which are released into the bloodstream and contribute to a low-grade systemic inflammatory state[32]. This accumulation of fat and subsequent rise in inflammation is linked to the development of several chronic health conditions like diabetes mellitus, hyperlipidemia, hypertension, insulin resistance, cardiovascular disease, and chronic pain conditions[32].

Systemic cytokines may contribute to sensitization by binding directly to nociceptors or indirectly by increasing other inflammatory mediators that then act directly on neurons. Given a lack of a blood-synovial barrier[33] and evidence surrounding the beneficial effects of weight loss, it is plausible that local infiltration by systemic, pro-inflammatory cytokines produced in adipose tissue, known as adipokines, but also from other tissues contribute to pain and pain sensitization. This possibility is supported by a study in which higher amounts of visceral fat were associated with worsening knee pain[34] and by animal models demonstrating that systemic inflammation can lead to sensitization[35–43]. Further, inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), have been shown to act directly on nociceptors within the dorsal root ganglion, thereby potentially contributing to pain sensitization[44].

Among the numerous systemic, pro-inflammatory cytokines implicated in the pathophysiology of OA, leptin and TNF-α stand out as two potentially important contributors to pain sensitization. Leptin is a 16 kilodalton peptide hormone produced nearly exclusively in adipose tissue[45]. Its most well-known role is its involvement in appetite regulation and energy storage and its levels correlate strongly with the amount of white adipose tissue present in the body[46]. One of leptin's lesser-known roles is its promotion of cartilage metabolism. The presence of leptin receptors on the surface of chondrocytes coupled with data showing an increase in production of cartilage-degrading enzymes after an injection of leptin into the knees of mice models suggests that leptin potentiates cartilage catabolism and promotes inflammation. This is further corroborated by studies which have shown higher than normal expression of leptin in serum and in synovial fluid of people with knee OA compared to healthy controls[44]. Thus, it is plausible that leptin may act to also promote pain by directly or indirectly acting on peripheral or central nociceptors.

In contrast, TNF-α is a pro-inflammatory cytokine that promotes cartilage catabolism by impeding the production of components of the cartilage matrix and promotes the synthesis and release of inflammatory mediators (iNOS, COX-2, PGE2)[47,48] TNF-α is produced by nearly all cells including adipose cells and articular chondrocytes. Studies have found increased concentration of TNF-α within the synovial fluid, the synovial membrane, articular cartilage, and the subchondral bone of people with knee OA.

Characterizing the relation of systemic inflammatory cytokines to pain sensitization and pain patterns may help to clarify the impact of obesity on knee OA pain and could also provide additional rationale for weight-related interventions among patients with knee OA. This thesis aims to investigate the roles of systemic inflammation on pain sensitization and pain patterns by determining the relation between leptin and TNF-α to pain sensitization and pain patterns.

SPECIFIC AIMS & HYPOTHESES

Overall Objective

The overall objective of this thesis was to assess the role of pro-inflammatory cytokines on knee OA pain.

Specific Aims & Hypotheses

Aim 1: To determine the relation of cytokine levels (leptin, TNF-α) to pain sensitization

Hypotheses:

H1a. Higher leptin levels will be associated with lower PPTs (greater pain sensitivity) cross-sectionally, and longitudinally with worsening sensitization.

H1b. Higher TNF-α levels will be associated with lower PPTs (greater pain sensitivity) cross-sectionally, and longitudinally with worsening sensitization.

Aim 2: To determine the relation of cytokine levels (leptin, TNF-α) to the pain experience in OA.

Hypotheses:

H2a. Higher baseline leptin levels will be associated with later stage pain patterns cross-sectionally (constant +/- intermittent pain > intermittent pain only > no pain), and with an increased risk of developing more frequent pain or persistent pain compared to those with lower baseline leptin levels.

H2b. Higher TNF-α levels will be associated with later stage pain patterns cross-sectionally (constant +/- intermittent pain > intermittent pain only > no pain), and with an increased risk of developing more frequent pain or persistent pain compared to those with lower baseline TNF-α levels.

METHODS

Overview of Parent Study

The present study leverages prospective data obtained from the Multicenter Osteoarthritis (MOST) Study[49]. MOST is an observational, longitudinal, NIH-funded cohort of community-dwelling male and female adults, aged 50-79 years at baseline, either with or at risk of knee OA at the time of enrollment. At-risk individuals were defined as those who were either overweight, experiencing knee symptoms, or had a history of knee injury or surgery at the time of initial contact. The primary aim of this original study was to track the time course of OA and identify new risk factors that contribute to the development and progression of knee OA.

A total of 3026 participants (6054 knees) were enrolled in MOST. Participant recruitment and enrollment occurred at two sites: Birmingham, Alabama and Iowa City, Iowa from 2003-2005. After an initial assessment (MOST baseline visit), participants were reassessed at 15-months, 30-months, 60-months, 72-months, and 84-months. With the exception of the 72-month follow-up visit which was a telephone interview, all study visits were conducted in-person. At each in-person visit, clinical and imaging assessments (knee x-rays) were obtained.

Participant retention in the MOST Study was high. The total attrition rate for the study, between baseline and the 84-month visit, was approximately ten percent. By the 60-month visit, 2882 participants remained in the study, corresponding to a five percent reduction from the initial 3026 participants. Another five percent loss occurred between

the 60-month and 84-month visits. Loss-to-follow-up occurred primarily due to moving out of the area or death.

A more detailed overview of the study protocol can be found elsewhere[50]. MOST was reviewed and approved separately by the institutional review boards (IRB) of each involved institution (Boston University School of Medicine, University of Alabama at Birmingham, University of Iowa, and University of California, San Francisco). Written and informed consent was obtained from all participants in MOST prior to conducting any study-related assessments.

Study Sample

The focus of aim one was to investigate the relation of cytokines to sensitization measures. We used data from a random sample of MOST participants in whom cytokines (leptin, TNF-α) were assayed at the 60-month visit, and in whom sensitization measures (PPT, temporal summation) were obtained at both the 60- month and 84-month visits. Data from prior visits were not included because sensitization measures were not obtained, which were introduced into the MOST protocol beginning at the 60-month visit. Participants who had a history of bilateral knee replacement prior to the 60-month visit were excluded from the study sample, as knee replacement can affect measures of sensitization[51]. Data derived from the native knee in those with history of only unilateral knee replacement was included.

The focus of the second aim was to relate cytokine levels to knee OA pain. We included data from MOST participants who had 60-month cytokine data (leptin and TNF-α) and knee pain data available at the 60- month and 84-month visits. Participants who had bilateral knee replacement prior to the 60-month visit were excluded from this analysis since it would affect their knee pain data. Again, data from the native knee was included in cases of only unilateral knee replacement.

We will refer to the 60-month and 84 month MOST visits as the baseline and two-year follow-up visits, respectively.

Exposure Measures

Baseline leptin and TNF-α levels were the exposures in the present study. Ethylenediamine tetraacetic acid (EDTA) plasma blood samples were collected and stored at the 60-month visit of the MOST study (i.e., baseline for the current study). Plasma was analyzed at University of Vermont Laboratory for Clinical Biochemistry Research using enzyme-linked immunoassay kits from R&D Systems (leptin: DLP00; TNF-α: HADK2M- Millipore Systems).

Outcome Measures

Aim One: Sensitization

Pressure Pain Threshold

Pressure pain threshold (PPT) is a measure of pain sensitivity that is defined as the minimum amount of pressure required to elicit a slightly painful response at a particular site in the body[52]. When PPT is measured at the knee, the site of pathology in knee OA, it reflects peripheral sensitization. When assessed at a distal, healthy site, it is thought to reflect central sensitization. PPT measures were obtained during the baseline and follow-up visits using a pressure algometer (1 cm^2 rubber tip, FDIX25; Wagner). The instrument was applied at a constant rate of 0.5 kg/s to the center of the patella of each knee and at the wrist (control site; right side unless contraindicated). **Figure 1 provides** a picture of the pressure algometer device used to obtain PPT measurements. Pressure algometry was chosen because it is a reliable measure of PPT in people with knee OA[53]. PPT was defined as the point at which participants first noticed a change from pressure to slight pain[26] . Three trials were conducted for each anatomical site; PPT was calculated as the average of these three trials. Lower PPT values represent heightened pain sensitivity.

Figure 1. FDIX25 pressure algometer from Wagner Instruments[54]

Temporal Summation

Temporal summation (TS) is an enhanced response to repetitive stimuli. Presence of TS indicates central sensitization. Mechanical TS was assessed in the MOST Study, using a 60g monofilament (Aarhus, Denmark). Participants were asked to rate any pain experienced using a 0 to 10 numerical rating scale (NRS) upon application of the mechanical stimulus probe to the skin over the right wrist. The probe was then applied at a constant rate of 1 Hz for 30 seconds (30 touches) on the same wrist. TS was calculated as the difference in pain ratings between the end and the beginning of the trial. The greater the difference in pain ratings, the greater the degree of TS.

Aim Two: Knee Pain

Knee pain was assessed using several instruments in the MOST study. To address our aim of understanding the relation of inflammatory cytokines to the changes in pain patterns in OA, we focused on the Intermittent and Constant Osteoarthritis Pain (ICOAP) questionnaire. The ICOAP is an 11-item survey designed to semi-quantitatively assess intermittent and constant pain over the prior 7 days in individuals with hip or knee pain. Five questions focus on constant pain while the other six relate to intermittent pain. Participants were asked to rate the severity of each pain type on a 5-point Likert scale, with 0 indicating no pain and 4 indicating extreme pain. Frequency of intermittent pain was similarly captured with 0 indicating never and 4 indicating very often. Psychometric testing has shown the ICOAP questionnaire to be a valid and reliable way to measure pain[55,56]. The questionnaire was administered at both timepoints in a knee-specific manner. This method has been previously validated by others[27].

Potential Confounders

The potential confounders included age, sex, BMI, race (white vs. other), Kellgren-Lawrence (KL) grade, depressive symptoms (yes/no), and pain catastrophizing (yes/no). Although race itself is not a confounding variable, it reflects a number of constructs, such as socioeconomic status and health disparities. Therefore, race is being used as proxy for differences in sociocultural life experiences that may impact both the exposure (cytokine levels) and the outcomes (sensitization and pain) in this study.

The KL classification system[6] is widely used, validated[57] method of defining knee OA severity on a 0-4 scaled based on the presence of specific pathological features (i.e. joint space narrowing, osteophytes, subchondral sclerosis, and bony cysts) visible on plain radiographs (x-rays). Higher KL scores indicate more severe disease while KL 0 represents no radiographic OA. In MOST, bilateral knee x-rays were obtained at each visit. All x-rays were independently reviewed for KL determination by two readers blinded to pain status. Disputed readings were resolved by a panel of 3 readers.

Presence of depressive symptoms was defined using the Center for Epidemiologic Studies Depression Scale (CES-D)[58]. The CES-D is a 20-item measure that asks individuals to rate how often they experience symptoms associated with depression such as restless sleep, poor appetite, and loneliness. Each item is scored on a scale from 0 to 3 and summed to produce the CES-D score (0-60 scale). Participants with a CES-D score $\geq$ 16 at baseline were classified as having depressive symptoms, a cutoff that has been shown to have good sensitivity, specificity, and internal validity[59]. Studies have shown an independent association between depressive disorders and reduced pain thresholds[60,61] therefore it was appropriate to adjust for it in our models.

Pain catastrophizing refers to a person's psychological tendency to fixate on and magnify pain and has been associated with pain sensitization and with more intense pain in the future among people with knee OA[62–64]. Pain catastrophizing was measured using an abbreviated version of the Coping Strategies Questionnaire (CSQ), which has good validity and reliability[65,66].

Statistical Analysis

Aim One

Aim one focused on evaluating the cross-sectional and longitudinal associations of each cytokine (Leptin, TNF-α) with measures of sensitization.

Cytokine exposures were analyzed as continuous measures - with leptin evaluated per 100-unit increments while TNF-α was kept at per unit change - and as sex-specific tertiles to consider dose-response threshold effects. The outcomes in this analysis were measures of pain sensitization: PPT in the knee, PPT in the wrist, and TS, which were all analyzed as continuous variables.

We evaluated the relation of cytokines to sensitization measures using linear regression with generalized estimating equation (GEE) to account for correlations between two knees within an individual when PPT at the patella was the outcome. GEE was not used for PPT at the wrist or for TS since those are person-based outcomes. Each analysis was adjusted for potential confounders as listed in a previous section.

Results of this analysis are reported as beta estimates - which indicate the degree of change in the outcome per each unit of change (or specified increment of change) in the exposure - accompanied with their respective 95% confidence interval (CI). The 95% CI reflects the following: if the experiment were to be replicated an infinite number of times, in the absence of bias, the 95% CI will contain within it the true value in 95% of those trials. If the value ‘0’ is found within this range, it is interpreted as reflecting no

association between the exposure and the outcome. Additionally, CI's provide insight into the precision of an estimate, which reflects the study's sample size.

Aim Two

The goal of aim two was to investigate the relation of baseline cytokines to the pain experience in OA.

In the cross-sectional analysis, we examined the relation of each cytokine – analyzed as a continuous variable and as sex-specific tertiles - to total ICOAP score using linear regression with GEE to account for correlations between two knees within an individual. Total ICOAP was calculated by summing the individual components of the Intermittent Pain ICOAP subscale as well as the Constant Pain ICOAP subscale. To avoid selection bias, we did not exclude participants unless they did not respond to the entire ICOAP questionnaire; the total score was computed as a weighted sum of those questions that did have responses. Results of this analysis are reported as beta estimates with their respective 95% CIs.

We also evaluated the relation between cytokines, analyzed as sex-specific tertiles, to pre-specified ICOAP pain patterns: (1) no pain; (2) intermittent pain only; (3) constant +/- intermittent pain using multinomial logistic regression with GEE. The results of this analysis are presented as odds ratios with their respective 95% CIs. Odds ratios indicate the likelihood of achieving an outcome given a specific exposure relative to the odds of achieving that same outcome in the absence of that exposure. An odds ratio >1

indicates an increased likelihood of the outcome with a given exposure whereas an odds ratio <1 indicates a decreased likelihood. An odds ratio = 1 indicates that the exposure is not associated with the outcome.

Lastly, to test our hypothesis that higher cytokine levels result in increased odds of developing more frequent pain, we used logistic regression with GEE with the outcome defined as the development of more frequent intermittent or constant pain for the whole sample. Participants who underwent any knee replacement between baseline and follow-up were treated as having met the outcome. Similarly, the results of this analysis are presented as odds ratios accompanied with their respective 95% CIs.

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, North Carolina) and statistical significance was determined based on alpha set to 0.05.

RESULTS

Cohort Characteristics

At baseline for this study, a random sample of 750 participants had their biospecimens assayed. Of those, 739 participants (1478 knees) had all available data necessary for analyses. **Figure 2** shows how the current sample was obtained.

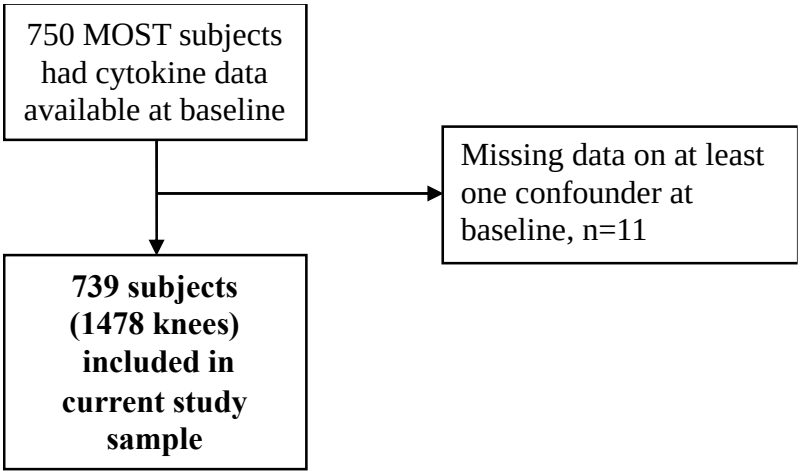

Figure 2. Study sample selection

The mean age of participants was 61 years. Other baseline cohort characteristics can be found listed in **Table 1**. Briefly, a majority of participants were white (84%) and female (58%). The mean BMI was 30 kg/m^2. The KL grade distribution was as follows: 39.7% KL=0, 17.6% KL=1, 17.6% KL=2, 17.7% KL=3, and 7.4% KL=4. Approximately 57% had no radiographic OA (KL=0 or 1) Nearly two-thirds of all knees (962 out of 1478 total) belonged to participants who were determined to be frequent users of oral

anti-inflammatory medication. **Table 2** provides the mean and distribution of baseline leptin and TNF-α levels for each sex-specific tertile.

Participant characteristics	**N= 739 participants (1478 knees)**
Age, years (mean (SD))	61.1 (7.6)
Female, N (%)	436 (58.1)
BMI, kg/m^2 (mean (SD))	30.3 (5.8)
Race (self-reported), N (%)	
White	633 (84.4)
Black	111 (14.8)
Other	6 (0.8)
Radiographic severity by KL grade, N (%)	
KL0	587 (39.7)
KL1	259 (17.6)
KL2	260 (17.6)
KL3	262 (17.7)
KL4	109 (7.4)
Depressive symptoms, N (%)	82 (11.0)
Oral anti-inflammatory medication use, N (%)	962 (64.2)
leptin, pg/mL (mean (SD))	18803.5 (27730.5)
TNF-α, pg/mL (mean (SD))	3.2 (1.8)
PPT range, kg/cm^2	
Patella, median (IQR)	4.7 (2.8)
Wrist, median (IQR)	3.2 (1.9)
Temporal summation, N (%)	300 (42.3)

SD, standard deviation; BMI, body mass index; KL Grade, Kellegren-Lawrence Grade; TNF-α, tumor necrosis factor alpha; PPT, pressure pain threshold; IQR, interquartile range

Table 1. Baseline participant characteristics.

	Sex	Tertile	N	Mean	Std. Dev.	Min.	Max.
leptin, pg/mL (n=1488)	Female	Lowest	290	12822.6	5000.9	2176.0	20878.1
		Middle	292	29983.0	5129.0	21089.1	38521.6
		Highest	290	62060.8	20606.4	38732.3	120847.8
	Male	Lowest	204	4116.3	1314.8	1369.3	6143.2
		Middle	206	9349.8	2051.1	6167.5	12868.1
		Highest	206	28721.4	17721.0	12888.6	102166.1
TNF-α, pg/mL (n=1500)	Female	Lowest	290	1.9	0.5	0.5	2.6
		Middle	292	3.2	0.4	2.6	3.8
		Highest	290	5.3	1.7	3.8	12.3
	Male	Lowest	208	2.0	0.5	0.7	3.7
		Middle	210	3.1	0.3	2.7	3.7
		Highest	210	5.4	4.2	3.7	44.2

leptin estimated normal range: 2205-11,149 pg/mL (males), 3877 - 77,273 pg/mL (females); TNF-α estimated normal range: 0.5-5.0 pg/mL

Table 2. Cohort baseline leptin and TNF-α levels as sex-specific tertiles.

Aim One: Relation of Cytokines to Sensitization Measures

Leptin

A 100-unit increase in leptin corresponded with a 0.0012 unit lower PPT at the knee cross-sectionally (adjusted β = -0.0012 [95% CI: -0.00020, -0.0004]). (**Table 3**). The same unit increase in leptin was not significantly associated with PPT at the wrist or TS cross-sectionally. It was also not associated with longitudinal change in PPT or TS over 24 months.

Assessing leptin levels as tertiles revealed similar results. Being in the highest tertile was significantly associated with lower PPT at the knee (adjusted β = -0.44 [95%

CI: -0.86, -0.02]) but not with the change in PPT over two years at the knee (**Table 4**). Leptin was not associated with PPT at the wrist or with TS cross-sectionally or longitudinally.

	PPT- Patella			
	Baseline PPT	P-value	Change in PPT	P-value
leptin, pg/mL **per 100 unit increase** (n=1466 knees, 733 participants)	**-0.0012 (-0.0020, -0.0004)**	**0.003**	0.0003 (-0.0005, 0.0010)	0.5
	PPT- Wrist			
	Baseline	P-value	Change in PPT	P-value
	-0.0002 (-0.0010, 0.0006)	0.6	0.0000 (-0.0006, 0.0007)	0.9
	Temporal Summation (TS)			
	Baseline TS	P-value	Change in TS	P-value
	-0.0003 (-0.0009, 0.0004)	0.4	0.0004 (-0.0005, 0.0012)	0.4

Values indicate adjusted β (95% confidence interval) calculated per 100 unit increase in leptin using linear regression models (with GEE for PPT at the knees). Models were adjusted for age, sex, body mass index, race, radiographic severity (KL grade), pain catastrophizing, and depressive symptoms; PPT, pressure pain threshold. **Bolded values indicate a significant result at alpha set to 0.05.**

Table 3. Relation of leptin to PPT and TS cross-sectionally, and to change in PPT and TS over 24 months.

Cytokine	Tertile	PPT- Patella			
		Baseline PPT	P-value	Change in PPT	P-value
leptin, pg/mL n= 1466 knees	Lowest	ref	-	ref	-
	Middle	-0.14	0.4	0.16	0.3
		(-0.49, 0.21)		(-0.15, 0.47)	
	Highest	**-0.44**	**0.04**	-0.03	0.9
		(-0.86, -0.02)		(-0.42, 0.36)	
		PPT - Wrist			
	Tertile	Baseline PPT	P-value	Change in PPT	P-value
leptin, pg/mL n= 733 participants	Lowest	ref	-	ref	-
	Middle	-0.00	0.9	-0.02	0.8
		(-0.27, 0.27)		(-0.28, 0.24)	
	Highest	-0.17	0.3	-0.07	0.6
		(-0.51, 0.17)		(-0.39, 0.24)	
		Temporal Summation (TS)			
	Tertile	Baseline TS	P-value	Change in TS	P-value
leptin, pg/mL n= 733 participants	Lowest	ref	-	ref	-
	Middle	-0.05	0.6	0.04	0.7
		(-0.24, 0.14)		(-0.16, 0.25)	
	Highest	0.01	0.9	0.13	0.5
		(-0.25, 0.27)		(-0.21, 0.47)	

Values indicate adjusted β (95% confidence interval) calculated using linear regression models (with GEE for PPT at the knees). The lowest tertile was defined as the referent group. Models were adjusted for age, sex, body mass index, race, radiographic severity (KL grade), pain catastrophizing, and depressive symptoms; PPT, pressure pain threshold. **Bolded values indicate a significant result at alpha set to 0.05.**

Table 4. Relation of leptin tertiles to PPT and TS, and to change in PPT and TS over 24 months.

TNF-α

A single unit increase in TNF-α was not significantly associated with PPT at the knee, PPT at wrist, or with TS at baseline (**Table 5)**. Similarly, a single unit increase was not associated with any changes in PPTs or TS over 2 years.

When grouped into tertiles, the highest and middle tertiles of TNF-α at baseline were significantly associated with lower PPTs only at the wrist, (adjusted β = -0.28 [95% CI: -0.54, -0.01] and adjusted β = -0.28 [95% CI: -0.54, -0.02], respectively). TNF-α was not associated with change in PPT over two years at the wrist or at the knee, with associations close to the null for the highest compared with lowest tertiles. There were no significant associations between TNF-α and TS.

TNF-α, pg/mL **per unit increase** n= 1478 knees, 739 participants	PPT- Patella			
	Baseline PPT	P-value	Change in PPT	P-value
	-0.0246 (-0.1083, 0.0592)	0.6	-0.0050 (-0.0636, 0.0536)	0.9
	PPT- Wrist			
	Baseline PPT	P-value	Change in PPT	P-value
	-0.0314 (-0.0825, 0.0198)	0.2	-0.0082 (-0.0519, 0355)	0.7
	Temporal Summation (TS)			
	Baseline TS	P-value	Change in TS	P-value
	-0.0271 (-0.0555, 0.0012)	0.06	0.0121 (-0.0215, 0.0457)	0.5

Values indicate adjusted β (95% confidence interval) calculated as per unit change in TNF-α using linear regression models (with GEE for PPT at the knees). Models were adjusted for age, sex, body mass index, race, radiographic severity (KL grade), pain catastrophizing, and depressive symptoms; PPT, pressure pain threshold.

Table 5. Relation of TNF-α to PPT and TS cross-sectionally, and to change in PPT and TS over 24 months.

Cytokine	Tertile	PPT- Patella			
		Baseline PPT	P-value	Change in PPT	P-value
TNF-α, pg/mL n=1478 knees	Lowest	ref	-	ref	-
	Middle	0.05	0.8	0.15	0.3
		(-0.29, 0.39)		(-0.15, 0.44)	
	Highest	-0.23	0.2	-0.02	0.9
		(-0.57, 0.11)		(-0.32, 0.28)	
		PPT - Wrist			
	Tertile	Baseline PPT	P-value	Change in PPT	P-value
TNF-α, pg/mL n= 739 participants	Lowest	ref	-	ref	-
	Middle	**-0.28**	**0.03**	0.15	0.2
		(-0.54, -0.02)		(-0.10, 0.40)	
	Highest	**-0.28**	**0.04**	0.02	0.9
		(-0.54, -0.01)		(-0.24, 0.28)	
		Temporal Summation (TS)			
	Tertile	Baseline TS	P-value	Change in TS	P-value
TNF-α, pg/mL n= 739 participants	Lowest	ref	-	ref	-
	Middle	-0.12	0.2	0.04	0.7
		(-0.30, 0.07)		(-0.16, 0.25)	
	Highest	-0.11	0.3	-0.01	0.9
		(-0.32, 0.10)		(-0.24, 0.23)	

Values indicate adjusted β (95% confidence interval) calculated using linear regression models (with GEE for PPT at the knees). The lowest tertile was defined as the referent group. Models were adjusted for age, sex, body mass index, race, radiographic severity (KL grade), pain catastrophizing, and depressive symptoms; PPT, pressure pain threshold. **Bolded text indicates significant result at alpha set to 0.05.**

Table 6. Relation of TNF-α tertiles to PPT and TS, and to change in PPT and TS over 24 months.

Aim Two: Relation of Cytokines to ICOAP Pain

Baseline Total ICOAP Score

A 100-unit increase in leptin was associated with a 0.0006 unit increase in total ICOAP cross-sectionally (adjusted β = -0.0006 [95% CI: -0.0013, 0.0024]). Similarly, a t a single unit increase in TNF-α was associated with a 0.08 unit increase in total ICOAP cross-sectionally (adjusted β = -0.0822 [95% CI: -0.0416, 0.2061]), though these differences were not statistically significant (**Table 7**).

Assessment of leptin and TNF-α as tertiles to assess for dose-dependent effects produced similar results. Participants in the highest tertile of leptin had a baseline total ICOAP score that was 0.85 units higher than those in the lowest tertile, though this difference was not statistically significant (**Table 8)**. Similarly, participants in the highest tertile of TNF-α had a baseline total ICOAP score that was 0.45 units higher than those in the lowest tertile, though this relationship was also not statistically significant.

Cytokine	Baseline Total ICOAP Score β Est. (95% CI)	P-value
leptin, pg/mL **per 100 unit increase** n = 817 knees, 408 participants	0.0006 (-0.0013, 0.0024)	0.5
TNF-α, pg/mL **per unit increase** n= 826 knees, 413 participants	0.0822 (-0.0416, 0.2061)	0.1

Values indicate adjusted β (95% confidence interval) calculated using linear regression models with GEE. Models were adjusted for age, sex, body mass index, race, KL grade, pain catastrophizing, and depressive symptoms; ICOAP, Intermittent and Constant Osteoarthritis Pain Questionnaire.

Table 7. Relation of leptin and TNF-α to total ICOAP pain cross-sectionally.

Cytokine	Tertile	Baseline Total ICOAP Score β Est. (95% CI)	P-value
leptin, pg/mL n= 817	Lowest	ref	-
	Middle	0.21 (-0.52, 0.93)	0.5
	Highest	0.85 (-0.04, 1.74)	0.06
TNF-α, pg/mL n= 826	Lowest	ref	-
	Middle	-0.57 (-1.25, 0.11)	0.09
	Highest	0.45 (-0.29, 1.19)	0.2

Values indicate adjusted β (95% confidence interval) calculated using linear regression models with GEE. The lowest tertile was defined as the referent group. Models were adjusted for age, sex, body mass index, race, KL grade, pain catastrophizing, and depressive symptoms; ICOAP, Intermittent and Constant Osteoarthritis Pain Questionnaire.

Table 8. Relation of leptin and TNF-α tertiles to total ICOAP pain cross-sectionally.

Baseline ICOAP Pain Pattens

At baseline, participants were grouped into the following ICOAP pain patterns: (1) no pain; (2) intermittent pain only; (3) constant pain with or without intermittent pain. There were no significant associations between those in the highest versus lowest tertile of either cytokine for the odds of having intermittent or constant pain compared with no pain. (**Table 9)**.

Cytokine	Tertile	Intermittent Pain Only (N=428) vs. No Pain (N=25) OR (95% CI)	Constant Pain +/- Intermittent Pain (N=93) vs. No Pain (N=25) OR (95% CI)
leptin, pg/mL	Lowest	ref	ref
	Middle	0.55 (0.15, 2.00)	0.86 (0.20, 2.26)
	Highest	0.53 (0.11, 2.63)	0.40 (0.06, 2.46)
TNF-α, pg/mL	Lowest	ref	ref
	Middle	0.49 (0.17, 1.39)	0.46 (0.14, 1.59)
	Highest	1.72 (0.40, 7.36)	3.21 (0.68, 15.00)

Values indicate adjusted odds ratios (95% confidence interval) calculated using multinomial logistic regression with GEE. The lowest tertile was defined as the referent group. Models were adjusted for age, sex, body mass index, race, KL grade, pain catastrophizing, and depressive symptoms; ICOAP, Intermittent and Constant Osteoarthritis Pain Questionnaire.

Table 9. Relation of leptin and TNF-α tertiles to categorical ICOAP pain patterns cross-sectionally.

Change in ICOAP

Out of a total of 1462 knees included in this analysis, only 152 (11.6%) knees met our pre-specified criteria for pain progression. Worsening was defined as any increase ≥ 1 in frequency or severity of intermittent pain or development of any severity of constant pain between baseline and the two-year followup quantified using the ICOAP questionnaire. Being in the highest tertile of leptin or TNF-α was not significantly associated with the risk of developing more frequent or persistent pain compared to those in the lowest tertiles (**Table 10**).

Cytokine	Tertile	Likelihood of Developing More Frequent Pain OR (95% CI)	P-value
leptin, pg/mL n= 1462 knees	Lowest	ref	-
	Middle	0.84 (0.49, 1.45)	0.5
	Highest	0.70 (0.37, 1.33)	0.2
TNF-α, pg/mL n= 1462 knees	Lowest	ref	-
	Middle	0.80 (0.49, 1.31)	0.3
	Highest	1.14 (0.71, 1.85)	0.5

Pain was assessed using the Intermittent and Constant Osteoarthritis Pain (ICOAP) Questionnaire. Values indicate adjusted odds ratio (95% confidence interval) calculated using binary logistic regression with GEE. Lowest tertile was defined as the referent group. Models were adjusted for age, sex, body mass index, race, KL grade, pain catastrophizing, and depressive symptoms.

Table 10. Relation of leptin and TNF-α to likelihood of developing more frequent or persistent pain.

DISCUSSION

Given the growing body of evidence supporting a metabolic role of obesity and of pain sensitization in patients with knee OA[67], this thesis sought to assess whether systemic pro-inflammatory cytokines, produced mainly by adipocytes but also by other cells, contribute to pain sensitization in patients with knee OA and influence the progression of pain patterns.

Leptin has been shown to promote systemic inflammation[68–70] and to be higher in systemic circulation and in the synovial fluid of OA compared with healthy controls [44,71–73]. Because of this, we hypothesized that higher levels of leptin in circulation would be associated with lower PPT values at baseline (indicating greater pain sensitization) and with worsening sensitization over two years. In our study, higher leptin levels were associated with lower PPT values but only at the knee and only at baseline. However, the beta estimates were very small (close to zero) and potentially clinically insignificant. Based on our data, it appears leptin alone may have only a minimal effect, if any, on pain sensitization.

There are three potential reasons that could account for these results. The first is the possibility of residual confounding due to the observational nature of this study. Despite adjusting for potential confounders there is still a chance that other, unknown variable(s) is/are contributing to sensitization while causing leptin levels to remain low, or *vice versa*. Secondly, leptin may be but one of many other pro-inflammatory cytokines such as interleukin-1-beta, interleukin-6, interleukin-8, nitric oxide, and prostaglandin E_2 that are more important, many of which have been shown to be involved in both

peripheral and central sensitization in animal models[74–76]. These cytokines are typically expressed concurrently, making it difficult to assess their relative contributions under physiological conditions. Lastly and perhaps most importantly, the effects of pro-inflammatory cytokines such as leptin may be attenuated by the reciprocal or opposing effects of anti-inflammatory cytokines such as adiponectin, another adipokine.

A similar trend was found for TNF-α. Higher levels of TNF-α at baseline were associated with greater pain sensitization (lower PPT) at the wrist, albeit also of a small magnitude of unclear clinical relevance.

A previous study found that higher levels of sensitization (lower PPT) were associated with higher total ICOAP scores and a greater likelihood of constant +/- intermittent pain compared with intermittent pain only[27]. Since the reason why people with knee OA transition from intermittent, weight-bearing activity related pain to more constant pain even at rest is not known, we hypothesized that inflammatory cytokines levels may play a role. While our study showed no significant relation between leptin levels and the pain pattern experience in OA, it did find that participants in the highest TNF-α tertile at baseline had numerically greater odds of having intermittent pain vs. no pain cross-sectionally (OR 1.72), of having constant +/- intermittent pain cross-sectionally (OR 3.42), and of developing more persistent pain longitudinally. However, none of these findings were statistically significant and the confidence intervals were wide; thus, no conclusions of an association can be made.

To our knowledge, our study is the first to assess the relation of leptin and TNF-α to pain sensitization and to the progression from early to late-stage pain patterns. While

most studies have traditionally focused on the relation between inflammatory cytokines and knee OA pain intensity, none have examined their impact on pain frequency and their relation to specific OA pain patterns. This study attempted to provide insights into associations with pain patterns by incorporating data from the ICOAP questionnaire which enables classification of participants into different pain pattern groups that are thought to reflect the two mechanistically distinct types of OA pain (activity-related, intermittent pain vs. activity-independent, chronic or persistent pain). Doing so allows for a more comprehensive assessment of the OA pain experience. Furthermore, our study relies on cytokine data from a large sample size of 739 participants to evaluate leptin and TNF-α.

Despite these substantial strengths, our study does have several limitations that should be noted. Firstly, a majority of the participants were white (84%); consequently, our data are not fully representative of those who are impacted by knee OA. Secondly, leptin levels depend on total body fat and it's likely that given the mean BMI of our cohort (30.3) was at the threshold of obesity, participants in this study may not have had sufficiently high leptin levels to enable proper evaluation of its relation to pain sensitization. Indeed, other studies have reported higher levels of leptin among knee OA participants with similar average BMI[71,77]. Nonetheless, our leptin values spanned a wide range, and thus provided sufficient ability to assess the relation of leptin to sensitization and pain patterns within these particular ranges of leptin. Finally, we did not evaluate cytokine levels at the 24-month time point. It is plausible, though unlikely in two years,

that our longitudinal findings may reflect participants with markedly different inflammatory profiles than what was present at the baseline visit in this study.

CONCLUSION & FUTURE DIRECTION

In conclusion, higher levels of leptin and TNF-α were only very weakly associated with lower PPT cross-sectionally after adjusting for important confounders, calling into question as to whether these small associations have any clinical relevance. Further, leptin and TNF-α were not associated with TS cross-sectionally nor with the change in PPT or with change in TS in 24 months. Based on these findings, we conclude that although leptin and TNF-α levels are well-established pro-inflammatory mediators of inflammation in OA, these cytokines by themselves do not appear to be directly involved in substantially promulgating pain sensitization. Similarly, high levels of leptin and TNF-α were not associated with the increased risk of developing more frequent or persistent pain.

Future may consider evaluating other systemic pro-inflammatory cytokines that have also been associated with sensitization such as interleukin-6 or evaluate the combined effects of relevant cytokines as there may be an additive or synergistic effect that is missed by assessing individual cytokines. Lastly, while the focus of the present study was on pro-inflammatory cytokines, it may be beneficial to characterize the relation between anti-inflammatory cytokines like adiponectin to pain sensitization and similarly to the OA pain experience to determine if those have a greater impact. Evaluating the role

of other pro-inflammatory systemic and anti-inflammatory cytokines jointly, especially in the context of obesity, remains an important area of investigation that could provide new insights into pain management in knee OA.

APPENDIX

Appendix A: Intermittent and Constant Osteoarthritis Pain (ICOAP) Questionnaire

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

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

A) CONSTANT PAIN

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

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

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

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

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

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

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

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

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

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

☐0	☐1	☐2	☐3	☐4
Not at all/ No constant knee pain	Mildly	Moderately	Severely	Extremely

B) PAIN THAT COMES AND GOES

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

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

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

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

☐0	☐1	☐2	☐3	☐4
Never/ No knee pain that comes and goes	Rarely	Sometimes	Often	Very Often

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

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

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

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

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

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

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

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

THANK YOU!

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