Knee Osteoarthritis: Intersections of Obesity, Inflammation, and Metabolic Dysfunction

by

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*"Learn from **yesterday**, live for **today**, hope for **tomorrow**.*
The important thing is not to stop questioning."
-Albert Einstein

To my son, *Hugo*, who is my little sunshine. His love and adorable smile warm my heart and remind me of what is really important. Hugo, I love you higher, taller, longer, prettier, deeper, stronger, mightier, fuller and brighter than I could have ever imagined.

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List of Abbreviations

-2LL	-2 Log-likelihood
AGEs	Advanced glycation end products
BIA	Bioelectrical impedance
BL	Baseline
BMI	Body mass index
BML	Bone marrow lesion
CI	Confidence Interval
cm	Centimeters
COX-2	Cyclooxygenase-2
CRP	C-reactive protein
DAG	Directed acyclic graph
DBP	Diastolic blood pressure
dGEMRIC	Delayed gadolinium-enhanced magnetic resonance imaging of cartilage
FA	Flip angle
FS	Fat saturation
FSE	Fast spin echo
HDL-c	High-density lipoprotein cholesterol
HOMA-IR	Homeostatic model assessment-insulin resistance
HT	Hormone therapy
IL-1	Interleukin-1
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
iNOS	Inducible nitic oxide synthase
IPW	Inverse proportional weighting
kg	Kilograms
kg/m^2	Kilograms per meter squared
K-L	Kellgren-Lawrence
LDL-c	Low-density lipoprotein cholesterol
MA	Massachusetts
mg/dL	Milligrams per deciliter
MI	Michigan
mm	Millimeter
mmHg	Millimeters mercury
MMP	Matrix metalloproteinase
MO	Missouri
MRI	Magnetic resonance imaging
MSM	Marginal structural model

NC	North Carolina
NCHS	National Center for Health Statistics
ng/mL	Nanograms per milliliter
NHANES	National Health and Nutrition Examination Survey
NJ	New Jersey
NO	Nitric oxide
OA	Osteoarthritis
OR	Odds Ratio
PD	Proton density
RIA	Radioimmunoassay
SBP	Systolic blood pressure
SD	Standard deviation
SE	Spin echo
SE	Standard error
SPGR	Spoiled gradient echo
SWAN	Study of Women's Health Across the Nation
TE	Echo time
TNF-α	Tumor necrosis factor-alpha
TR	Repetition time
U.S.	United States
V	Follow-Up Visit
µg/L	Microgram per liter
µIU/mL	Micro-international units per milliliter

ABSTRACT

Knee Osteoarthritis: Intersections of Obesity, Inflammation, and Metabolic Dysfunction

by

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Chair: Siobán D. Harlow

Background: Obesity is a risk factor for osteoarthritis (OA) and may impart joint-damaging effects through dysfunctional metabolic mechanisms associated with increased adipose tissue.

Objective: To evaluate the relationship between cardiometabolic markers and measures of knee OA from radiographs and magnetic resonance imaging (MRI).

Methods: Data from 1,066 National Health and Nutrition Examination Survey (NHANES) III participants (60+ years of age) was used to examine relationships of radiographic knee OA and cardiometabolic measures. Data from Michigan Study of Women's Health Across the Nation (SWAN) participants was used to relate serum leptin levels with prevalent and incident knee OA and to MRI-defined measures of knee joint damage.

Results: The prevalence of knee OA in the NHANES III sample was 34% (average age 70.5 years). The baseline prevalence in Michigan SWAN (average age 46 years) was 18%; at follow-up visit 11, when participants were average age 57 years, the prevalence was 65%. Cardiometabolic biomarkers were associated with knee OA in both populations, independent of body size. Among NHANES III participants, those with knee OA had 35% higher HOMA-IR measures and 52% higher serum leptin levels compared to those without knee OA. The magnitude of the association between HOMA-IR and knee OA was strongest among men whereas leptin was more strongly associated among women. Serum leptin levels were associated with prevalent and incident knee OA in Michigan SWAN. Effect estimates were similar in the two populations; a 5 ng/mL increase in serum leptin was associated with 28% higher odds of knee OA among obese NHANES III women and with 38% higher odds among Michigan SWAN women. In SWAN, serum leptin levels 10 years prior to MRI assessment were associated with more severe cartilage defects, larger bone marrow lesions and osteophytes, meniscal tears, synovitis and joint effusion.

Conclusions: Cardiometabolic dysfunction is associated with knee OA and increased serum leptin levels are associated with prevalent and incident knee OA and MRI-defined knee joint damage among women. These findings support a metabolic role of obesity in knee OA. Management of cardiometabolic dysfunction among obese individuals may be beneficial in forestalling the onset or progression of knee osteoarthritis.

CHAPTER ONE

Introduction

OVERVIEW

The most common type of arthritis is osteoarthritis (OA), a degenerative joint condition characterized by failure of the joint integrity including cartilage degradation, changes in the underlying subchondral bone, development of osteophytes, damage to soft tissues including the meniscus and ligaments, and the presence of synovitis and joint effusion. Osteoarthritis, the leading cause of pain and disability, is highly prevalent and is estimated to affect more than 37% of United States (U.S.) adults over the age of 60 (1).

Due to the strong correlation of age and osteoarthritis (2), OA has commonly been viewed as a part of "normal aging". However, the onset of OA can begin by age 40 (3) and the incidence of disease levels off in older age groups. Thus, OA is not an inevitable consequence of aging (4) but instead, age-related changes may make the joint more vulnerable to joint damage. These factors include decreased repair mechanisms of chondrocytes, greater joint stability due to ligament laxity, and greater muscle weakness leading to greater compressive forces on weight-bearing joints. As shown in Figure 1.1., the interaction of these age-related changes simultaneous with the presence of other risk

factors including genetics, joint mal-alignment and injury, and obesity (5) are predictive of the development and severity of osteoarthritis.

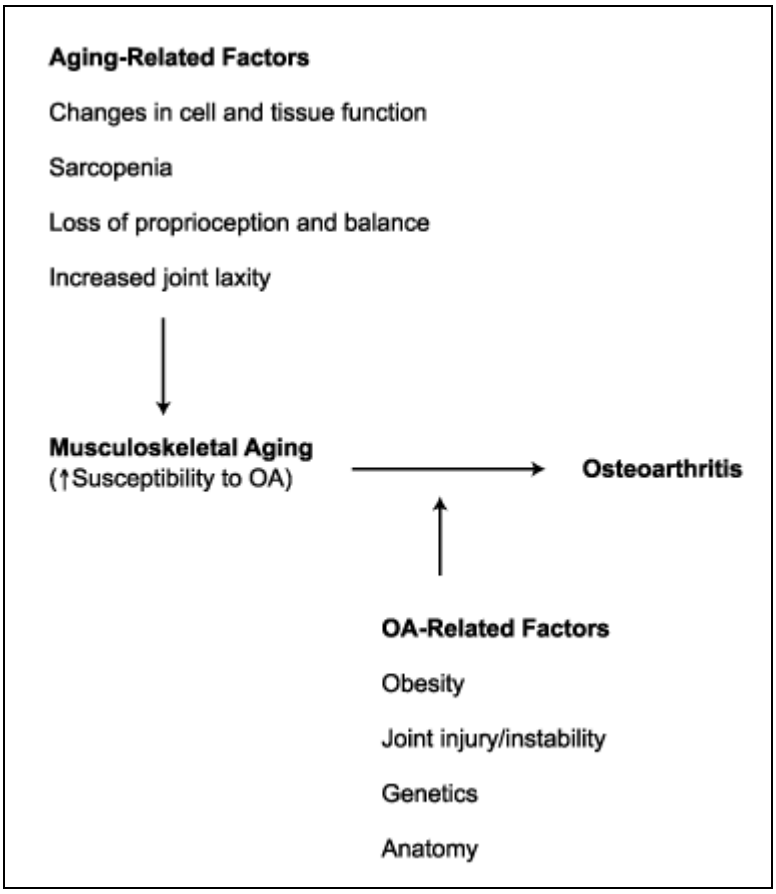

Figure 1.1. Theoretical relation between aging and the development of osteoarthritis (OA).
From Loeser RF. Aging and cartilage in osteoarthritis—what's the link? Sci Aging Knowledge Environ 2004;29:pe31.

Obesity is the strongest and most consistently-reported risk factor for knee OA (6) and is of great interest because it is potentially modifiable (7). The relationship between mid-life obesity and osteoarthritis appears to be particularly important (8,9). This observation was first made more than 50 years ago by Silberberg and colleagues (10). Using tissue samples from 200 male and female cadavers selected from all age decades, he observed

that the majority of obese individuals with osteoarthritic lesions of the sternoclavicular joint were young (10).

The relationship between obesity and osteoarthritis has conventionally been thought to operate through a mechanism of increased mechanical loading across the joint (11). However, not all obese individuals have osteoarthritis nor are all persons with osteoarthritis obese. This, combined with observed associations between obesity and OA in non-weight bearing joints (10,12-15) have prompted new hypotheses about the role of adipose tissue in joint damage..

Osteoarthritis has been associated with many traditional cardiovascular and metabolic risk factors, including inflammatory markers, dyslipidemia, insulin resistance, and blood pressure. Furthermore, those with OA have been found to have greater prevalence of metabolic syndrome (16) as well as decreased survival compared to those without OA in the general population (17,18). Thus, there is significant interest in the consideration of OA as a metabolic disorder.

Despite the recent flurry of opinion articles proposing a potential mechanistic link between the metabolic impact of obesity and osteoarthritis, few epidemiologic studies have investigated such an association. Of the studies that are available, few include a full characterization of relevant biomarkers or have assessment of joint damage using multiple methods including radiographs and magnetic resonance imaging (MRI).

SPECIFIC AIMS

The purpose of this dissertation was (1) to characterize the relative importance of obesity-related cardiovascular and metabolic biomarkers with respect to radiographically-defined knee osteoarthritis among men and women and, (2) to relate serum leptin levels to knee osteoarthritis measures from radiograph and MRI in a longitudinal cohort of women.

Aim 1: To determine whether cardiometabolic biomarker [insulin resistance, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglycerides, waist-to-hip ratio; systolic blood pressure (SBP), diastolic blood pressure (DBP), and leptin] levels are associated with radiographically-defined knee osteoarthritis using data from the National Health and Nutrition Examination Survey III and to evaluate if the magnitude of association differs by gender.

Aim 2: To examine whether serum leptin levels are associated with knee osteoarthritis prevalence and incidence and to determine whether leptin trajectories over time differ by knee OA status using data from the Michigan Study of Women's Health Across the Nation, a population-based longitudinal study of the menopause transition and its health consequences.

Aim 3: To evaluate the relationship between serum leptin levels and the presence of cartilage defects, bone marrow lesions (BML), osteophytes, meniscal abnormalities, synovitis or joint effusion imaged using MRI and to determine whether leptin trajectories

over time differ by severity of the knee MRI features using data from the Michigan Study of Women's Health Across the Nation.

BACKGROUND

Osteoarthritis (OA) is a highly prevalent joint disorder estimated to affect more than 37% of adults over age 60 (1) and it is the leading cause of pain and disability.

Obesity is a well-documented risk factor for prevalent knee osteoarthritis (19-24). Data from the Chingford general population survey (United Kingdom) reported that women in the highest tertile of body mass index (BMI) had 6-fold increased odds of knee OA and nearly 18 times higher odds of bilateral knee osteoarthritis, compared to women in the lowest tertile of BMI (12). This relationship has been confirmed in multiple population-based studies, including the National Health and Nutrition Examination Survey (21), the Baltimore Longitudinal Study of Aging (22), and the Study of Women's Health Across the Nation (25).

Several mechanisms by which obesity can influence osteoarthritis onset and progression have been proposed. Some investigators have focused on the impact of obesity as a sheer mechanical force causing increased joint loading and subsequent damage to the articular cartilage (26-28). However, associations between obesity and osteoarthritis in non-weight bearing joints (12-15) have expanded our conceptualization of the OA-obesity relationship and the role of adipose tissue.

The consideration of adipose tissue as an active endocrine organ contributing to changing cardiovascular and metabolic environments has prompted new hypotheses about the relationship of obesity and OA. A recent report evaluated the relationship of OA to both obesity status and cardiometabolic risk factor clustering in an effort to separate the impact of mechanical loading from the impact of metabolic factors (29). While the co-occurrence of obesity and cardiometabolic clustering was associated with 6-fold increased odds of having prevalent knee OA as compared to the non-obese without cardiometabolic clustering, obesity alone was only associated with a 3-fold increased odds of having prevalent knee OA (29).

Emerging evidence about the active metabolic environment of chrondrocytes (cartilage cells), including glucose transport, cholesterol efflux, and lipid metabolism (30) have lead investigators to consider novel obesity-related biomarkers that may have shared pathophysiology between osteoarthritis and the cardiovascular and metabolic diseases, including inflammatory markers, lipids, insulin resistance and the adipocytokines.

BIOMARKERS ASSOCIATED WITH OBESITY

Obesity is now well-appreciated as a state associated with a systemic inflammatory response. Adipose tissue secretes pro-inflammatory cytokines including interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α) (31). C-reactive protein (CRP) is an acute phase reactant that is synthesized by the liver in response to many inflammatory cytokines, including IL-6. Levels of CRP can be used as a marker of inflammation and have been related to many disease outcomes including cardiovascular

disease (32,33) and diabetes (34-36). Importantly, recent work from the Whitehall II Study demonstrates that CRP and IL-6 measures, both markers of an inflammatory state, increase with increasing body weight over an 11-year period and that the changes were greatest among those who were overweight at baseline (37).

Increased lipids and insulin resistance are often common phenotypic hallmarks of obese individuals. Dyslipidemia, characterized by increased triglyceride and LDL-c levels and decreased HDL-c levels, is often associated with obesity, especially abdominal adiposity (38). Furthermore, increased levels of inflammatory cytokines and non-esterified fatty acids are common with obesity, and these factors are also involved in the development of insulin resistance (39).

Leptin is an adipocytokine that is encoded by an obesity gene (40) and is secreted by adipose tissue in direct proportion to the amount of body fat (41). Although leptin acts to reduce food intake and increase energy expenditure (42), extremely high leptin levels are a hallmark of obesity. Recent work suggests that many obese individuals seem to be resistant to the effects of leptin (43), suggesting a leptin resistance syndrome as a parallel concept to insulin resistance.

BIOMARKERS ASSOCIATED WITH OSTEOARTHRITIS

It was once thought that inflammation was unique to rheumatoid arthritis, but emerging evidence from both epidemiological and basic science research suggests that systemic inflammation may also be important in OA. Circulating levels of CRP are higher in those

with knee OA (44) and are associated with the risk and progression of knee OA (45-47). However, there are questions about whether this relationship is independent of body size (48). Other inflammatory markers have also showed important associations with OA. As recently reviewed by Katz et al. (30), many pro-inflammatory cytokines, including interleukin-1 (IL-1) and TNF-α may be related to OA through the modulation of chondrocytes to secrete proteases which can cause degradation of the cartilage matrix.

The active inflammatory environment associated with OA may result from disordered lipid metabolism, possibly also through inflammatory pathways. While there is conflicting evidence in the epidemiological literature about the relationship of hyperlipidemia and osteoarthritis (12,20,49-51), disordered lipid metabolism among chondrocyte samples from patients with osteoarthritis has been demonstrated. Tsezou and colleagues (52) showed that the expression of genes regulating cholesterol efflux is reduced in osteoarthritic cartilage and that chondrocytes had intracellular lipid deposits. Furthermore, apolipoprotein(a), cholesterol, triglycerides, and HDL-c have been identified in synovial fluid from arthritic joints (53).

Metabolic changes resulting from insulin resistance and increased glucose load are closely related to proinflammatory cytokine production, characteristic of a chronic inflammatory state. Advanced glycation end products (AGEs), the result of the chain of chemical reactions after the initial glycation reaction, may be associated with increased collagen stiffness, alterations in the mechanical properties of the extracellular matrix and decreased proteoglycan synthesis. These combined effects may possibly result in

cartilage degradation (54). Importantly, chondrocytes express the functional receptor for AGEs which, when stimulated with ligands, induces production of pro-inflammatory cytokines (55).

Adipose tissue, once considered a passive storage portal of energy, is now recognized as a highly metabolic endocrine organ with the capacity to secrete active agents including the adipocytokines (leptin, adiponectin, resistin, visfatin, etc). Important findings of different patterns of distribution of the adipocytokines between the joint and the circulating compartment suggest that the joint is a unique area of activity for adipocytokines and that their presence may be related to local joint degradation effects (56,57).

Because of its strong correlation with body size, most efforts to examine the adipocytokines and osteoarthritis have focused on leptin. Leptin levels in synovial fluid are correlated with the severity of knee OA (58) and leptin and its receptor have been identified in many joint tissues, including human chondrocytes, osteophytes (56,59), synovium and infrapatellar fat pad (60). Leptin expression has been directly associated with the degree of cartilage degeneration (56,57) and synergistic relationships of leptin and proinflammatory cytokines have been reported (56). Following administration of leptin, chondrocytes from osteoarthritic patients had increased production of interleukin-1 beta (IL-1β), matrix metallopeptidase 9 (MMP-9) and MMP-13, suggesting that mechanistically, leptin has a direct pro-inflammatory and catabolic role in cartilage metabolism (57).

Leptin is also important with respect to bone health and direct and centrally-mediated effects of leptin are known. Leptin promotes mineralized nodule formation (61,62) and is stimulatory to chondrocytes (63,64). However, centrally-mediated effects of leptin are contradictory; leptin inhibits anabolic neurons in the brain (65) and stimulate bone resorption (66). Additional work needs to be done to better understand the relationship of leptin and bone. Importantly, little is known how this relationship is modified by body size or how these findings are associated with bone-related disease outcomes such as osteoarthritis.

In addition to the potential for local joint degradation caused by leptin, it may also be an important modulator of inflammatory processes within the joint. Leptin enhanced expression of inducible nitric oxide synthase (iNOS) and cyclooxygenase enzymes (COX-2) and production of nitric oxide (NO), IL-6 and IL-8 (67). These products have important proinflammatory properties and have been implicated in the pathogenesis of OA (67).

COMMONALITIES OF OSTEOARTHRITIS, CARDIOVASCULAR DISEASE AND METABOLIC DISEASE

Mechanistically, there are many ways by which osteoarthritis, cardiovascular disease and metabolic diseases may have common physiological links. When considering the pathobiology of these diseases, destruction of collagen is a hallmark of each one, which may be related to their shared biomarker risk factors. Collagens are located throughout

the body and are essential elements in fibrous tissues including tendons, ligaments, cartilage, and blood vessels. Increased collagen stiffness damages the structure of the proteins with ensuing implications for disease outcomes including OA and vascular disease. Possible culprits of collagen damage include advanced-glycation end products whose accumulation have been linked to osteoarthritis and vascular disease and are associated with accompanying inflammatory responses.

PUBLIC HEALTH IMPLICATIONS

Osteoarthritis is a burdensome public health problem given its increasing prevalence, role in functional limitations and disability and high economic costs related to disease management. Currently, nearly 2 of every 5 adults aged 60 and older have osteoarthritis (1) and the frequency of knee OA continues to accelerate, likely because of the aging of the population and the increasing proliferation of the primary risk factor, obesity. Osteoarthritis is often associated with significant pain, disability and functional limitations, thus resulting in loss of productivity and increased health care expenditures. In 2004, osteoarthritis accounted for 97% of the total knee replacements and 83% of total hip replacements in the United States (68). In terms of dollars, the economic cost of arthritis due to medical care expenditures and loss in productivity is estimated to be $128 billion per year (69).

Given that osteoarthritis is a disease which we attempt to *manage* rather than *cure,* the combined impact of the aging of the population, increase in average life expectancy, and the increasing prevalence of obesity suggest that the economic impact of OA will only

exponentiate in the years to come. This reality requires us as public health professionals to consider new treatment modalities and therapies to either reduce the burden of disease among those who are afflicted or postpone or eliminate the onset of disease among those who remain healthy. While current treatment paradigms for osteoarthritis focus on those who are already diseased, consideration of OA in the framework of cardiovascular and metabolic diseases may allow us to consider treatment solutions beyond mere weight reduction.

STUDY POPULATIONS AND DATA

Study populations. Two different study populations were utilized to provide the data necessary to address the aims of this dissertation. Data from the National Health and Nutrition Examination Survey III was used to address Aim 1 and the Michigan Study of Women's Health Across the Nation data was used to address Aims 2 and 3. Details of each study are provided below.

The National Health and Nutrition Examination Survey (NHANES) III is a survey of the civilian non-institutionalized U.S. population conducted by the National Center for Health Statistics (NCHS) from 1988-1994. NHANES III utilized a stratified multistage probability sampling design, including a two-phase survey period. Adults aged 60+ years, African-Americans and Mexican-Americans were oversampled to provide stable estimates of health characteristics for these subgroups.

A subset of the total NHANES III sample was utilized for this investigation, as only adults aged 60+ years were recruited for the radiograph acquisition component and only Phase 2 (1991-1994) knee radiographs have been scored for osteoarthritis. Of these participants, only data from those subjects with morning visits who were fasted and had blood assayed for leptin were included in the analytic subsample. Inclusion in the morning group was randomly assigned and a sample of the specimens was randomly selected for leptin assay. There were no medical, safety, or other exclusions for the radiograph component. All data, including radiographs, specimen collection for assay of cardiometabolic measures, measurement of body size and self-reported demographic information were collected at the same visit. The participation rate in the radiograph component was 93% among eligible individuals. Data from 1,066 NHANES III participants were available for this analysis (Figure 1.2).

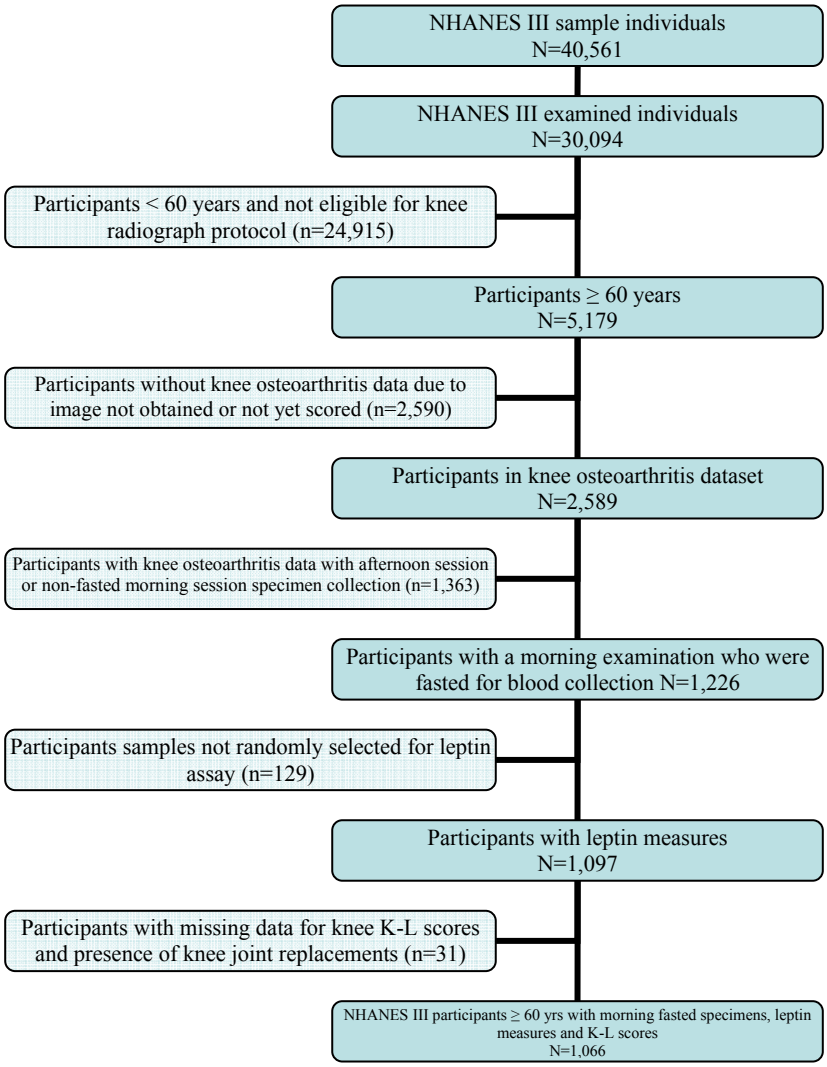

Figure 1.2. Data availability in National Health and Nutrition Examination Survey (NHANES) III sample to provide final analytic sample of 1,066 adults aged 60+ years with morning fasted specimens for assay of cardiometabolic biomarkers including leptin and with Kellgren-Lawrence (K-L) scores for ascertainment of knee osteoarthritis (OA) status.

The Study of Women's Health Across the Nation (SWAN) is a multiethnic longitudinal cohort study of the menopause transition and its associated health consequences. The Michigan site is one of seven clinical sites for SWAN and was established as a population-based sample of eligible women from two Detroit-area communities in 1996.

The Michigan SWAN population was identified with a community census based on the electrical utility listings of the targeted communities, Ypsilanti and Inkster, Michigan. Households were contacted by telephone (if available) or in-person.

A total of 543 eligible women were recruited from the Michigan site to the SWAN Core Longitudinal Study, including 325 African American and 218 Caucasian women (60:40 ratio). Eligibility criterion at baseline included 42-52 years of age, having an intact uterus, having had at least one menstrual period in the previous 3 months, no use of reproductive hormones in the previous 3 months, and self-identification with the site's designated race or ethnic group (either African American or Caucasian at Michigan). Participation in annual assessments has been excellent and is similar among African American and Caucasian women. At follow-up visit 11, 80% of still-living Michigan SWAN participants completed a study visit.

At the 1996 baseline, Michigan SWAN women completed the assessment protocol common to all SWAN sites; then a supplemental protocol including radiographs and functional assessments was implemented. Women were seen for annual follow-up visits, although the supplemental radiograph OA imaging protocol was included only at visits baseline, 2, 4, and 11 and MRIs were only obtained at follow-up visit 11. The timing of relevant measures in the Michigan SWAN study is shown in Table 1.1.

Table 1.1. Relevant Measures Available for Michigan Study of Women's Health Across the Nation Cohort, 1996-2007 (Baseline [BL] – Follow-Up Visit [V] 11)

	1996 BL	1997 V1	1998 V2	1999 V3	2000 V4	2001 V5	2002 V6	2003 V7	2004 V8	2005 V9	2006 V10	2007 V11
Knee X-rays	X		X		X							X
Knee MRIs												X
Leptin	X	X		X	X	X	X	X				
Body size	X	X	X	X	X	X	X	X	X	X	X	X
Menopause status	X	X	X	X	X	X	X	X	X	X	X	X

All Michigan SWAN women were eligible for inclusion in the osteoarthritis protocol; however, women with artificial joints, pacemakers, defibrillators, or other implanted metal considered incompatible with MRI were excluded from the MRI protocol.

MEASURES

Osteoarthritis Imaging in NHANES III. Among NHANES III participants, knee OA was defined using non-weight bearing anterioposterior knee radiographs from a Centrix III x-ray unit with Kodak Lanex double screens and TML film (phototimed with 12:1 stationary grid). Radiographs were scored for osteophytes-defined osteoarthritis severity using the Kellgren-Lawrence (K-L) Atlas of Knee Radiographs of Arthritis (70), where 0=normal, 1=possible osteophyte, 2=definite osteophyte, 3=moderate multiple osteophytes, and 4=large osteophytes, severe sclerosis. Those with a K-L score ≥ 2 or those with knee joint replacements were considered to have osteophytes-defined radiographic OA.

NHANES III knee radiographs were read by one of two radiologists with additional scoring by a second reader if there was evidence of disease. Scores from the two readers were compared and discordant scores were subjected to consensus readings. The quality

control program has been described (71). For the K-L scoring, Kappa statistics for inter-rater agreement were >0.71; for intra-rater agreement the Kappa scores were >0.84 and >0.82 for the primary and secondary reader, respectively.

Osteoarthritis Imaging in Michigan SWAN. Michigan SWAN knee radiographs have been obtained weight-bearing in the anterior-posterior semi-flexed position. Radiographs taken at baseline (1996), follow-up visit 2 (1998), and follow-up visit 4 (2000) were obtained using General Electric radiographic equipment (model X-GE MPX-80; General Electric Medical Systems, Milwaukee, WI); radiographs from follow-up visit 11 (2007) were obtained with the AXIOM Aristos radiographic system with integrated digital flat detector technology (Erlangen, Germany). Knees were scores using the Kellgren and Lawrence (K-L) grading system of the Atlas of Standard Radiographs of Arthritis (70) such that 0=normal; 1=doubtful OA; 2=minimal OA; 3=moderate OA; 4=severe OA. Participants with artificial knee replacements were assigned a K-L score of 4.

All Michigan SWAN knee radiographs have been read by two readers in accordance with a quality control system that includes the utilization of "drift" films; the same set of drift films are used for both readers and have been used for each round of x-ray reading. All knee images are first scored independently by each reader; discordant scores between the two readers are re-evaluated during a consensus session to determine a final score. Knee radiographs from baseline and follow-up visits 2, 4, and 11 were read independently (without knowledge of a participant's previous scores).

Magnetic resonance imaging (MRI) assessment of all eligible Michigan SWAN participants was completed at follow-up visit 11 (2007) so that soft tissue changes and inflammatory processes within the joint could be characterized. Knee joints were imaged using a 3T (Model Achieva, Philips Healthcare, Andover, Massachusetts) or 1.5 T (GE Signa, GE Medical Systems, Milwaukee, WI) MR scanner. Specific sequences included sagittal, coronal, and axial fast spin echo (FSE) proton density (PD) with fat saturation (FS) sequences (repetition time [TR] 4000 msec, echo time [TE] 15 msec, 4 mm thickness), sagittal spin echo (SE) PD (TR 1000 msec, TE 14 msec, 3 mm thickness), and sagittal 3-D spoiled gradient echo (SPGR) with FS (TR 38 msec, TE 6.9 msec, flip angle [FA] 45°, 2 mm effective thickness). The FSE PD FS sequences were chosen to enable tissue contrast between articular cartilage, bone, and fluid, while still maintaining a high signal to noise ratio for evaluation of periarticular soft-tissues (72-75). SPGR FS images are included for additional assessment of articular cartilage.

MR images of each knee were scored by two musculoskeletal radiologists, globally and by compartment for cartilage defects, subchondral bone marrow lesions (BMLs), osteophytes, meniscal abnormalities/tears, joint effusions and synovitis as described below and with the scoring procedures outlined in Table 1.2. To ensure reproducibility, there was an initial calibration session to generate comparable scored values (n=20). A rigorous quality-control program was maintained such that 60% of MRI images were double-read with agreement (kappa statistic) in excess of 90%. Scoring discrepancies were resolved by consensus. The radiologists were blinded to radiographic and clinical findings.

Table 1.2. Description of Michigan SWAN Magnetic Resonance Imaging Scoring Procedures for Cartilage Defects, Bone Marrow Lesions, Osteophytes, Meniscal Tears, Synovitis, and Joint Effusion.

Cartilage Defects	
0	Normal
1	Internal signal alteration only
2	Cartilage defect < 50%
3	Cartilage defect 50-99%
4	Cartilage defect 100% with no bone ulceration
5	Cartilage defect 100% with bone ulceration (visible irregularity in normal contour of the subchondral cortex)
Bone Marrow Lesions	
0	Normal
1	Largest diameter < 1 cm
2	Largest diameter 1.01 to 2.0 cm
3	Largest diameter > 2.0 cm
Osteophytes	
0	No osteophyte
1	Osteophyte ≤ 5 mm
2	Osteophyte > 5 mm ≤ 10 mm
3	Osteophyte > 10 mm
Meniscal Tears	
0	Normal
1	Intrasubstance (Crues Grade 1 and 2) meniscal abnormality only
2	Non-displaced tear (extending to meniscal surface, Crues Grade 3)
3	Displaced or macerated tear
Synovitis	
0	Normal
1	Mild synovitis
2	Moderate-to-marked synovitis
Joint Effusion	
0	Physiologic fluid
1	Small effusion (≤ 10 mm)
2	Moderate and/or large effusion (> 10 mm)

Each knee was evaluated for location, severity, and approximate size of cartilage defects in two compartments (medial femorotibial, lateral femorotibial) and four specific surfaces (medial tibial, medial femoral, lateral tibial, lateral femoral). Cartilage defects were scored for depth on the basis of the classification adopted by Drape et al. (76) and across

multiple compartments with use of the Noyes arthroscopic system, adapted to MR imaging (77).

Bone marrow lesions were also evaluated in two compartments and four specific surfaces. Bone marrow lesions were defined as focal but noncircumscribed areas of abnormal high signal on FSE PD FS images, in a subchondral location. Bone marrow lesions were recorded by site and severity, and were measured perpendicular to the bone cortex (75,78).

Osteophytes were evaluated in the medial and lateral compartments and were defined as abnormal bone growths arising from the margin of the involved compartment. Tibial spine growths were considered as osteophytes in their respective compartments only if there were definite excrescences as opposed to mere "pointing" of the tibial spines (79).

Meniscal abnormalities/tears were evaluated in the medial and lateral compartments. Intrameniscal abnormalities as well as frank tears incorporated a modification of the system of Crues et al. (80-82).

Synovitis was defined as abnormally increased linear or irregular striations within the infrapatellar (Hoffa) fat pad or joint recesses and irregular thickening at the margin of the fat pad with the articular cartilage (83-84). These changes consisted of low signal on SE short TE images and intermediate-to-high signal on FSE PD FS images.

Joint effusions represent substantially greater than expected physiologic amounts of synovial fluid in the lateral or medial patellar recesses exceeding 10 mm in width (85).

Cardiometabolic Measures in NHANES III. Cardiometabolic measures available in the NHANES III dataset included glucose, insulin, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglycerides, blood pressure, waist-to-hip ratio and body mass index (BMI).

Plasma glucose was measured using a modified hexokinase enzymatic method (Cobas Mira), with a lower limit of detection of 2 mg/dL. Serum insulin was measured using commercially-available radioimmunoassay (RIA) kits (Cambridge Laboratories (Cambridge, MA); Ventrex, Inc. (Cambridge, MA); and Pharmacia Diagnostics (Fairfield, NJ)), with a lower limit of detection of 2.5 μIU/mL. Insulin values were adjusted linearly to account for differences in RIA kits across the study period. Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated using the following formula:

$$[\text{Insulin } \mu\text{IU/mL} \times (\text{Glucose mg/dL} \times 0.055) / 22.5]$$

and was used as a proxy measure of insulin resistance. Cholesterol levels were measured using a peroxidase-catalyzed reaction; HDL-c levels were measured following the precipitation of the other lipoproteins with a Hitachi 704 Analyzer. LDL-c was estimated using the Friedewald equation (86). Triglycerides were measured enzymatically using a series of coupled reactions in which triglycerides were hydrolyzed to produce glycerol. Serum leptin was measured using a RIA with a polyclonal antibody raised in rabbits

against highly purified recombinant human leptin (Linco Research, Inc., St. Louis, MO). The minimum detectable concentration of the leptin assay was 0.5 μg/L and the within- and between-assay coefficients of variation ranged from 3.4%-8.3% and from 3.6%-6.2%, respectively (87). The laboratory reference document for NHANES III is available (88).

Blood pressure was measured three times by trained personnel and recorded to the nearest even number, according to a standardized protocol. The average of the three measurements was used in data analysis. Height (cm) was measured using a stadiometer. Weight (kg) was measured using a digital scale. Body mass index was calculated as [weight (kg) / height $(m)^2$]. Each individual was classified as either non-obese (BMI < 30 kg/m^2) or obese (BMI ≥ 30 kg/m^2). Waist and hip circumference (cm) were measured using a nonstretching tape.

Cardiometabolic Measures in Michigan SWAN. At each SWAN annual examination, height (cm) and weight (kg) has been measured using a stadiometer and calibrated balance-beam scale, respectively, while participants wear a single layer of light clothing and no shoes. Body mass index (BMI, kg/m^2) is calculated as weight in kilograms divided by the square of height in meters. Waist (cm) and hip (cm) circumferences have also been measured at each annual examination, using a non-stretchable tape 3 cm above the umbilicus, after a relaxed expiration, and the maximum girth around the buttocks, respectively. Body composition was measured using bioelectrical impedance (BIA). The measures, resistance and reactance, can be used to estimate body composition because the

body is composed of conductive intracellular and extracellular materials separated by insulating layers of materials such as lipids. This is the technique being used in the National Health and Nutrition Examination Survey (NHANES) body composition assessments.

The SWAN specimen collection protocol includes a fasted (minimum 10-hour) blood draw to provide samples for the assay of cardiovascular and metabolic biomarkers. All archival specimens are maintained at -80°C and shipped on dry ice for processing. Serum leptin levels from banked specimens were determined spectrophotometrically using commercially-available colorimetric enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI) and run according to the manufactures' instructions. The coefficient of variation percent for duplicate samples for each subject is 0.4-12.4% and the lower limit of detection is 1 ng/mL. Assays were conducted in the Peter Mancuso laboratory at the University of Michigan School of Public Health.

Other Measures in NHANES III. Age was self-reported and measured in years. Other demographic variables were analyzed as categorical variables, as follows: self-reported race/ethnic group (Non-Hispanic White, Non-Hispanic Black, Mexican American, and Other), gender, marital status (married vs. not married), educational attainment (less than high school vs. high school or more). Referent groups were Non-Hispanic Whites, those not married and those with less than a high school education.

Other Measures in Michigan SWAN. Age at each annual visit was calculated as date of annual visit minus date of birth. Race/ethnicity classification as African American or Caucasian was determined by self-report at baseline. Participants were asked about their current smoking status (yes/no) and about bleeding patterns, current hormone use, hysterectomy and oophorectomy at each annual visit. Participants were categorized as being premenopausal, early perimenopausal, late perimenopausal, postmenopausal, hysterectomy, or unable to determine due to exogenous hormone use for each annual visit. Premenopausal status was defined as regular menses with bleeding in the past three months, early perimenopausal status was defined as bleeding in the past three months but increasing irregularity in menses, late perimenopausal status was defined as bleeding in the past year but not in past three months, and postmenopausal status was defined as no bleeding for 12 months.

STATISTICAL ANALYSIS

Statistical Analysis for Aim 1. Given the complex survey sampling of the NHANES III population, appropriate analytical techniques incorporated stratum, cluster and weight variables for the subsample to account for unequal probability of selection, for non-response, and for post-stratification weighting to the U.S. population estimates from the U.S. Census Bureau. These variables were calculated by and included in the datasets from the National Center for Health Statistics.

NHANES III pseudo-stratum (SDPSTRA6), pseudo-cluster (SDPPSU6) and Phase 2 morning session subsample weight (WTPFSD2) variables were used in all analyses. WTPFSD2 was selected as the sample weight because only knee radiographs from Phase

2 were scored and because leptin was only assayed among participants from the morning session. Missing data was handled using case-wise deletion. Because of the small number of "Other" race/ethnicity, data from these participants were excluded from multivariate analyses. Potential demographic and cardiometabolic variables of interest were selected a priori given the availability of measures in the NHANES III datasets and their known relevance as risk factors for knee OA.

Univariate distributions of the continuous variables of age and the cardiometabolic measures were examined, overall and by osteophytes-defined radiographic knee osteoarthritis status and reported as means and standard errors (SEs). Distributions were examined for normalcy. While leptin and HOMA-IR were not normally distributed, they were modeled on their original scale to ease with interpretation of results. Sensitivity analyses were conducted and demonstrated that utilization of leptin and HOMA-IR measures on their original scale provided similar associations as did those reported on the natural log scale. Frequencies of the categorical variables were examined, overall and by osteophytes-defined radiographic knee osteoarthritis group, to ensure sufficient sample sizes in individual cells for appropriate analyses. Categorical variables are reported as percentages and SEs. SAS PROC SURVEYMEANS and PROC SURVEYFREQ statements, with specification of the strata, cluster and weight variables were used to calculate appropriate univariate statistics. Subgroup analyses were conducted using domain statements.

The unadjusted relationships of the continuous cardiometabolic biomarkers and knee OA were characterized using logistic regression models (SAS PROC SURVEYLOGISTIC). Bivariate (unadjusted) associations of knee OA and categorical independent predictors were evaluated using Rao-Scott Chi-Square tests.

All variables were considered for inclusion in the multivariable analysis; variables were retained in the final models if the adjusted estimates changed by 10% or more as compared to the unadjusted analysis. Some variables were not retained in the final model due to collinearity concerns with other variables. Because HOMA-IR is calculated from glucose and insulin, these measures could not all be included in the final multivariable model. HOMA-IR, a proxy of insulin resistance, was selected for multivariable modeling. Similarly, DBP was not included in the final model due to collinearity concerns with SBP. Given that differences in DBP were relatively small between knee OA groups and not clinically significant, SBP was selected for inclusion in the final model. The two measures of body size, BMI and waist:hip ratio could not be included in the same model.

Covariate-adjusted models were stratified by obesity status and gender because of significant interactions between these variables and the independent cardiometabolic variables of interest. Regression diagnostics were examined for the final multivariable model and there was no evidence of collinearity among the included variables.

A sensitivity analysis was conducted to examine the consistency of the observations from the multivariable models after adjustment for waist:hip ratio instead of BMI within obesity and gender stratum.

Statistical Analysis for Aim 2. Means and standard deviations (SD) or frequencies and percents of leptin, body size variables and relevant covariates were examined overall and by knee OA status at baseline and follow-up visits 4 and 11. The statistical significance of differences by knee OA status were evaluated using t-tests, analysis of variance, or chi-square tests.

To fully utilize the richness of the available data, including multiple measures of knee OA status and annual assessment of leptin through follow-up visit 07, three analytical approaches were employed to relate leptin measures and knee OA status. In the first two approaches, the outcome of interest was knee osteoarthritis whereas in the third approach, leptin was the outcome. First, to determine the association of leptin levels and knee OA prevalence at baseline, we examined the cross-sectional association of baseline leptin and knee OA using multivariable logistic regression analysis. Second, to determine the association of leptin levels and incident knee OA, discrete survival analysis techniques were utilized to model the time to incident OA through follow-up visit 4 as a function of leptin levels. As leptin levels were not available at follow-up visit 2, values from follow-up visit 3 were substituted. An interaction of leptin and time was tested and found to be non-significant.

Third, leptin levels from baseline through follow-up visit 7 were evaluated overall and stratified by prevalent knee OA at baseline; 10-year incident knee OA; or disease free during follow-up. Student's t-tests were used to compare leptin levels at each time point between women with prevalent, incident or no knee OA during follow-up. Then, linear mixed models (PROC MIXED) with random intercepts and slopes for age were used to examine level and change in leptin measures over time. Knee OA status by time interactions in the model evaluated whether the rates of change in leptin measures differed between the three groups. Interactions between knee OA status and race/ethnicity, smoking status and menopause status were considered. SAS PROC SGPLOT was used to graph predicted trajectories of leptin measures with corresponding 95% confidence intervals for each knee OA group.

Statistical Analysis for Aim 3. For each knee MRI feature, the maximum score across knees and compartments was used in analysis. Means and standard deviation (SD) or frequencies and percepts of leptin, body size variables and relevant covariates at baseline were examined overall and by categories of each type of MRI-defined knee joint damage from follow-up visit 11. The statistical significance of differences by knee MRI feature were evaluated using analysis of variance or chi-square tests at the α=0.05 level. Leptin levels were correlated (Spearman correlations) with cartilage defects, bone marrow lesions, osteophytes, meniscal tears, synovitis and joint effusion. Multivariable analyses using ordinal logistic regression analysis were conducted to relate the MRI scores from follow-up visit 11 with baseline leptin measures adjusting for relevant covariates. To examine if severity of cartilage defects, bone marrow lesions, osteophytes, meniscal

tears, synovitis or joint effusions at follow-up visit 11 were associated with different levels and rates of change in leptin levels, linear mixed models (PROC MIXED) with random intercepts and slopes for age were used.

Statistical Adjustment for Body Size in Aims 2 and 3. Due to the collinearity between body size and leptin, all multivariable modeling for Aims 2 and 3 included residuals of BMI as the measure of body size confounding. The BMI residual variable represents the variation in BMI that remains following simple regression of BMI on leptin. Given that leptin represents the metabolic component of body size, the BMI residual represents the association of body size and OA through other pathways, including mechanical loading. Thus, the BMI residual was included as a covariate in all models to assess potential effect modification of the relationship between leptin and knee OA or MRI features by body size.

Model Selection for Aims 2 and 3. Model fit and final model selection was based on the Akaike's information criterion and chi-square tests comparing the log likelihood ratios between candidate models. Models were adjusted for age, race/ethnicity, menopause status (or hysterectomy yes/no), current smoking status, and BMI residuals, as appropriate.

Statistical significance was defined at $\alpha<0.05$ and all analyses were completed using SAS v9.3. (SAS Institute, Cary, NC).

SUMMARY

This dissertation represents an epidemiologic investigation of the relationship between cardiometabolic factors and knee osteoarthritis. Utilization of data from both NHANES III and the Michigan SWAN study allow for investigation of this relationship through multiple lenses.

Chapter 2 examines the relative importance of a constellation of cardiometabolic biomarkers with respect to knee osteoarthritis prevalence among four groups: non-obese men, obese men, non-obese women and obese women. Strengths of the NHANES III sample include large sample size, availability of multiple cardiometabolic biomarkers and inclusion of both men and women. Findings from the NHANES III analysis informed our scientific direction for Chapters 3 and 4 in which the Michigan SWAN dataset was used to relate serum leptin measures to knee osteoarthritis prevalence and incidence and to determine if changes in leptin levels over time were associated with radiographic knee osteoarthritis or knee joint damage imaged using MRI. Chapter 5 concludes the dissertation with a summary of main findings, discussion of the importance of our results in light of emerging work from the basic sciences, suggestions for future studies to expand on the findings of this dissertation, and implications for public health and clinical care with respect to osteoarthritis.

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CHAPTER TWO

Sex Dimorphism in the Association of Cardiometabolic Characteristics and Osteophytes-Defined Radiographic Knee Osteoarthritis Among Obese and Non-Obese Adults: NHANES III

INTRODUCTION

Osteoarthritis (OA) is a highly prevalent disorder, affecting approximately 27 million Americans (1). Estimates from a population-based study predict that 1 of every 2 elderly adults will have knee OA (2). Arthritis generates substantial economic burden as a result of health care expenditures (3), decrements in physical functioning (4,5) and loss in productivity associated with physical disability (6).

Obesity is a widely-acknowledged risk factor for knee OA (7-9). There is a growing appreciation of the need to understand how obesity contributes to OA considering the increasing prevalence of obesity and overweight in the US and world-wide (10,11), the underlying inflammatory component in both obesity and OA (12), and the knowledge that few interventions have been successful without addressing either weight or the inflammatory response (13).

Several mechanisms have been proposed by which obesity can influence OA onset and progression [reviewed by Sowers & Karvonen-Gutierrez (14)]. Some investigations have concluded that the primary role for obesity in OA etiology is as a sheer mechanical force leading to increased joint loading and subsequent articular cartilage damage (15,16). However, this does not explain the associations observed between obesity and OA in non-weight bearing joints (7,17-19) thereby motivating additional and alternative explanations of the OA-obesity relationship. The recognition that adipose tissue can contribute to changing metabolic environments has stimulated the consideration of hypotheses about the relationship of obesity and OA that extend beyond those of biomechanical loading. Emerging evidence about the active metabolic environment of chondrocytes, including glucose transport, cholesterol efflux and lipid metabolism [reviewed by Katz (20)] have led investigators to consider novel obesity-related biomarkers that may reflect underlying pathology between OA and the cardiovascular and metabolic diseases.

Findings from studies that have examined cardiovascular or metabolic risk factors and OA are mixed. Some (21-23), but not all studies (24,25) have found positive associations of OA with cardiovascular risk factors. Importantly, studies of osteoarthritis and obesity that have included cardiometabolic measures have rarely incorporated measures of fat tissue metabolism such as leptin, an adipocytokine that is an important modulator of the inflammatory response (26). Synthesis and secretion of leptin has been demonstrated in osteoblasts and chondrocytes (27,28) and its receptors have been identified in articular

cartilage (29). Importantly, leptin levels are associated with markers of bone formation and leptin receptor is associated with greater cartilage loss (30).

In an effort to further elucidate the role of cardiometabolic dysfunction with respect to OA, we report the relationship of cardiovascular and metabolic risk factors with radiographically-defined knee OA among a sample of men and women aged 60 and over, representative of the United States population. We hypothesized that individuals with greater insulin resistance, poorer lipid profiles, and greater leptin levels would have an increased odds of knee OA. We further hypothesized that the magnitude of these relationships would differ by gender.

METHODS

Data Source and Sample. Data are from the National Health and Nutrition Examination Survey (NHANES) III, a survey of the civilian non-institutionalized U.S. population conducted by the National Center for Health Statistics (NCHS) from 1988-1994. NHANES III utilized a stratified multistage probability sampling design, including a two-phase survey period. Adults aged 60+ years, African Americans and Mexican Americans were oversampled to provide stable estimates of health characteristics for these subgroups.

These analyses address a subset of the total NHANES III sample as only adults aged 60+ years were recruited for the radiograph acquisition component and only Phase 2 (1991-1994) knee radiographs have been scored for osteophytes-defined OA severity. There

were no medical, safety or other exclusions for the radiograph component; the overall completion rate for obtaining radiographs from eligible participants was 93%. Furthermore, only data from those with morning visits who were fasted and had blood assayed for leptin were considered for these analyses. Study subjects were randomly assigned to the morning group and samples were randomly selected for leptin assay. Thus, this report is based upon data from 1,066 adults aged 60 years or greater with knee OA data and fasted blood samples available for assay. Participants with data available to be included in this sample are more likely to be Caucasian but less likely to have a high school education as compared to the total NHANES sample. Further, they have a higher body mass index (BMI) and are older, as is expected given that age was part of the inclusion criterion for the radiograph protocol.

Osteoarthritis Measures. Knee OA was defined using non-weight bearing anterioposterior knee radiographs from a Centrix III x-ray unit with Kodak Lanex double screens and TML film (phototimed with 12:1 stationary grid). Radiographs were scored for osteophytes-defined osteoarthritis severity using the Kellgren-Lawrence (K-L) Atlas of Knee Radiographs of Arthritis (31), where 0=normal, 1=possible osteophyte, 2=definite osteophyte, 3=moderate multiple osteophytes, and 4=large osteophytes, severe sclerosis. Those with a K-L score ≥ 2 or those with knee joint replacements were considered to have osteophytes-defined radiographic OA.

Knee radiographs were read by one of two radiologists with additional scoring by a second reader if there was evidence of disease. Scores from the two readers were

compared and discordant scores were subjected to consensus readings. The quality control program has been described (32). For the K-L scoring, Kappa statistics for inter-rater agreement were >0.71; for intra-rater agreement the Kappa scores were >0.84 and >0.82 for the primary and secondary reader, respectively.

Cardiometabolic Measures. The primary independent variables were cardiometabolic risk factors, including a proxy indicator of insulin resistance (HOMA-IR), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), triglycerides, waist:hip ratio, blood pressure, and leptin. Each individual was classified as either non-obese (BMI<30 kg/m^2) or obese (BMI≥30 kg/m^2). Assay information is available in Chapter One and in the NHANES III reference documents (33,34). Homeostatic model assessment-insulin resistance (HOMA-IR) was calculated using the following formula: [Insulin μIU/mL X (Glucose mg/dL X 0.055) / 22.5].

Blood pressure was measured three times by trained personnel and recorded to the nearest even number, according to a standardized protocol. The average of the three measurements was used in data analysis. Height (cm) was measured using a stadiometer. Weight (kg) was measured using a digital scale. Body mass index was calculated as [weight (kg) / height $(m)^2$]. Waist and hip circumference (cm) were measured using a nonstretching tape.

Other Variables. Age was measured in years. Other demographic variables were analyzed as categorical variables, as follows: self-reported race/ethnic group (Non-

Hispanic White, Non-Hispanic Black, Mexican American, and Other), gender, marital status (married vs. not married), educational attainment (less than high school vs. high school or more). Referent groups were Non-Hispanic Whites, those not married and those with less than a high school education.

All data, including knee radiographs, specimen collection for assay of cardiometabolic measures, measurement of body size and self-reported demographic information were collected at the same visit. NHANES III was approved by the NCHS Institutional Review Board. Written informed consent was obtained from all participants.

Statistical Analysis. NHANES III pseudo-stratum (SDPSTRA6), pseudo-cluster (SDPPSU6) and Phase 2 morning session subsample weight (WTPFSD2) variables were used in all analyses. WTPFSD2 was selected as the sample weight because only knee radiographs from Phase 2 were scored and because leptin was only assayed among participants from the morning session. Missing data was handled using case-wise deletion. Because of the small number of "Other" race/ethnicity, data from these participants were excluded from multivariate analyses. Potential demographic and cardiometabolic variables of interest were selected a priori given the availability of measures in the NHANES III datasets and their known relevance as risk factors for knee OA.

Univariate distributions of the continuous variables of age and the cardiometabolic measures were examined, overall and by osteophytes-defined radiographic knee

osteoarthritis status and reported as means and standard errors (SEs). Distributions were examined for normalcy. While leptin and HOMA-IR were not normally distributed, they were modeled on their original scale to ease with interpretation of results. Frequencies of the categorical variables were examined, overall and by osteophytes-defined radiographic knee osteoarthritis group, to ensure sufficient sample sizes in individual cells for appropriate analyses. Categorical variables are reported as percentages and SEs.

The unadjusted relationships of the continuous cardiometabolic biomarkers and knee OA were characterized using logistic regression models (SAS PROC SURVEYLOGISTIC). Bivariate (unadjusted) associations of knee OA and categorical independent predictors were evaluated using Rao-Scott Chi-Square tests.

All variables were considered for inclusion in the multivariable analysis; variables were retained in the final models if the adjusted estimates changed by 10% or more as compared to the unadjusted analysis. Some variables were not retained in the final model due to collinearity concerns with other variables. Because HOMA-IR is calculated from glucose and insulin, these measures could not all be included in the final multivariable model. HOMA-IR, a proxy of insulin resistance, was selected for multivariable modeling. Similarly, diastolic blood pressure (DBP) was not included in the final model due to collinearity concerns with systolic blood pressure (SBP). Given that differences in DBP were relatively small between knee OA groups and not clinically significant, SBP was selected for inclusion in the final model. The two measures of body size, BMI and waist:hip ratio could not be included in the same model.

Covariate-adjusted models were stratified by obesity status and gender because of significant interactions between these variables and the independent cardiometabolic variables of interest. Regression diagnostics were examined for the final multivariable model and there was no evidence of collinearity among the included variables.

A sensitivity analysis was conducted to examine the consistency of the observations from the multivariable models after adjustment for waist:hip ratio instead of BMI within obesity and gender stratum. SAS Version 9.2 (SAS Institute, Cary, NC) was used for all data analyses.

RESULTS

This sample (N=1,066) with knee radiographs included non-Hispanic Whites (83.2%), non-Hispanic Blacks (7.3%), Mexican Americans (2.3%) and Other race/ethnicity (7.2%). The design-adjusted mean age of the sample was 70.5 years (standard error (se)=0.14) with 57% being female.

After considering the survey sampling design, 34.1% (se=0.60%) of individuals had osteophytes-defined radiographic knee OA as defined as a KL score $\geq$ 2; obese men and obese women had the greatest prevalence of osteophytes-defined radiographic knee OA (Figure 2.1.). Individuals with osteophytes-defined radiographic knee OA were, on average, 2 years older (P<0.0001) and were more likely to be female, non-Hispanic

Black, unmarried, less educated and a never smoker in comparison to those without knee OA (Table 2.1.).

Female participants were 1.1 years older, were less likely to be married but more likely to have completed high school as compared to male participants. Further, more women were never smokers whereas more men were former smokers (Table 2.2.). Women were more likely to be categorized as normal weight or obese as compared to men but there was no difference in the BMI between men and women. Women had lower glucose, triglyceride and diastolic blood pressure levels as compared to men but higher HDL-c, LDL-c, and systolic blood pressure. Notably, design-adjusted average leptin levels were more than 2 times greater among women as compared to men ($P<0.0001$).

Body size, cardiometabolic characteristics and knee OA. The design-adjusted average BMI among those with osteophytes-defined radiographic knee OA was 29.4 kg/m^2 compared to 26.0 kg/m^2 among those without knee OA ($P<0.0001$). While one fourth of the total sample (25.3%) was classified as obese, 40% of those with osteophytes-defined radiographic knee OA were classified as obese.

With the exception of waist:hip ratio, all of the cardiometabolic factors were significantly associated with osteophytes-defined radiographic knee OA status. Those with knee OA had 35% higher HOMA-IR values and 52% higher leptin levels as compared to those without knee OA. While statistically significant, lipids were only 2-7% worse in those

with knee OA while blood pressure levels were 1-3% higher compared to those without knee OA.

Sex dimorphism with respect to cardiometabolic characteristics and OA. After adjustment for BMI, HOMA-IR and leptin levels had the strongest relationships with osteophytes-defined radiographic knee OA in multivariable models that included all of the cardiometabolic measures (leptin, HOMA-IR, LDL-c, SBP, triglycerides) and adjustment for age, race/ethnicity, marital status, educational attainment and smoking status (Table 2.3.). The magnitude of the association between HOMA-IR and knee OA was much greater among men than women. Among non-obese and obese men, respectively, each higher unit in HOMA-IR was associated with 18-34% greater odds [odds ratio (OR)=1.18, 95% confidence interval (CI) 1.15, 1.22 and OR=1.34, 95% CI 1.27, 1.42] of having osteophytes-defined radiographic knee OA. Among non-obese women, a one-unit higher HOMA-IR was associated with only 4% greater odds of knee OA (95% CI 1.01, 1.07) while HOMA-IR was actually associated with decreased odds of knee OA among obese women (Figure 2.2.).

Leptin levels were strongly associated with osteophytes-defined radiographic knee OA. Among obese women, a 5 μg/L higher leptin was associated with 28% greater odds of having knee OA (OR=1.28, 95% CI 1.26, 1.31). Among non-obese women, the magnitude of the association between leptin levels and knee OA was less although statistically significant (OR=1.04, 95% CI 1.01, 1.07). The relationship of leptin and knee OA was reversed in men compared to women. A 5 μg/L higher level of leptin was

associated with 27% decreased odds of having knee OA among obese men, and 39% decreased odds of having knee OA among non-obese men (Figure 2.3.).

LDL-c was significantly and positively associated with osteophytes-defined radiographic knee OA among obese and non-obese men ($P<0.0001$) and negatively associated with knee OA among obese women ($P<0.0001$) (Table 2.3.). For both obese and non-obese men, a 5 mg/dL higher LDL-c was associated with 5-6% greater odds of knee OA. Conversely, for obese women, a 5 mg/dL increase in LDL-c was associated with 6% decreased odds of having knee OA. LDL-c was not associated with knee OA among non-obese women (Table 2.3.).

The relationship of systolic blood pressure (SBP) and osteophytes-defined radiographic knee OA was also different among men and women, regardless of obesity status. A 5 mmHg increase in SBP was associated with 3-12% decreased odds of having knee OA among men but with 9-10% increased odds of having knee OA among women (Table 2.3.).

Body mass index was associated with having osteophytes-defined radiographic knee OA among women. A 1 kg/m^2 increase in BMI was associated with 15-18% increased odds of having knee OA among women. However, among men, the relationship of BMI and knee OA differed based on obesity status. In non-obese men, a 1 kg/m^2 increase in BMI was associated with 30% increased odds of having knee OA (OR=1.33, 95% CI 1.29,1.36). Among obese men, however, BMI was not associated with having knee OA.

When multivariable analyses were adjusted for waist:hip ratio instead of BMI as a sensitivity analysis, estimates for LDL-c and SBP were virtually unchanged in terms of magnitude or direction (Table 2.4.). Odds ratios for HOMA-IR changed very little (<5%) and odds ratios for leptin changed by 3-12% among women and obese men in models adjusted for waist:hip ratio instead of BMI. Changes were more pronounced among non-obese men (+16% change for HOMA-IR and +26% change for leptin).

DISCUSSION

Using data from a nationally-representative sample of the U.S. elderly population, the importance of obesity as a risk factor for osteophytes-defined radiographic knee osteoarthritis was confirmed. The prevalence of obesity was twice as great among those having knee OA as compared to those without knee OA. There were also very strong associations of having osteophytes-defined radiographic knee OA with cardiometabolic characteristics, even following adjustment for body size using BMI. Most importantly, we report compelling and consistent relationships of HOMA-IR and leptin, two obesity-related biomarkers, with knee osteoarthritis. Notably, the overwhelming difference in the magnitude and direction of these associations had to be considered within the context of being male or female. Striking sex differences were observed for HOMA-IR and leptin in relation to having osteophytes-defined radiographic knee OA. Odds ratios for HOMA-IR were 14% greater among non-obese men vs. non-obese women. For those who were obese, the odds of having knee OA increase substantially with increasing HOMA-IR among men; however, among obese women, the odds ratios for HOMA-IR were in the

opposite direction. Conversely, leptin levels were most important with respect to osteophytes-defined radiographic knee OA among women compared to men. We found that higher leptin is associated with increased odds of knee OA among women but the opposite was observed in men. These relationships with cardiometabolic risk factors were observed in multivariable models also adjusting for BMI. Our findings suggest that the impact of leptin has an independent effect on knee OA prevalence among women, over and above the effect of body size alone.

Sex dimorphism with respect to body composition is well-reported in the literature, suggesting that fatness means something different for men versus women. For a given level of BMI, women generally have a larger proportion of body mass that is fat as compared to men and women are more likely to deposit adipose tissue subcutaneously whereas men are more likely to deposit fat viscerally (35). This is particularly relevant to our findings because, although circulating levels of both leptin and insulin are proportional to fat mass (36), the levels of each reflect different fat depots. Leptin levels correlate better with subcutaneous adipose tissue (37-40), which is proportionally larger in women whereas insulin is better correlated with visceral adipose tissue (41), which is proportionally larger in men. Our findings of stronger relationships between osteophytes-defined radiographic knee OA and leptin among women and between osteophytes-defined radiographic knee OA and an index of insulin resistance among men may reflect the predominance of each as the more important obesity-related hormone and fat depot for that sex. This hypothesis is supported in studies using animal models where the brains of male rats are more sensitive to insulin but female rats are more sensitive to

leptin (42). Furthermore, for a given amount of fat mass, women have higher circulating leptin levels (37-40) but increases in body fat among women are associated with smaller decreases in insulin sensitivity as compared to men (43).

The sex dimorphism with respect to leptin and insulin may also be related to differences in sex steroid metabolism and levels among men and women. Estrogen levels are higher in women, even postmenopausal women, and estrogen has been shown to induce leptin secretion in women (44). Further, male rats exposed to exogenous estrogen have increased sensitivity to leptin (42). Excess androgens in men (45) and women (46) are associated with increased insulin resistance but androgens have a negative association with leptin levels in men (47,48).

There have been very few studies of osteoarthritis and cardiometabolic measures that have included both men and women (24,49-52), and few have analyzed their data stratifying by gender. In the AGES Reykjavik Study, carotid plaque severity and coronary calcifications were associated with hand OA (52) and marginally associated with total knee or hip replacements in women (53) but not men. Our finding of a "protective" effect for leptin among men is interesting and has not been reported in any other studies. We hypothesize that our findings may be reflective of the reportedly higher synovial fluid leptin levels within the joint among women (54,55).

Our finding of no association of BMI and osteophytes-defined radiographic knee OA among obese men is interesting, especially given its importance among women and non-

obese men. Given our findings of a stronger relationship of HOMA-IR and knee OA among obese men as compared to non-obese men, we hypothesize that obese men may have proportionally greater depots of visceral fat, which is associated with insulin resistance (41), as compared to non-obese men. This hypothesis is supported by our sensitivity analyses in which waist:hip ratio, a proxy for central adiposity, was associated with greater odds of knee OA among obese men but not among non-obese men. However, we do not have direct measures of visceral fat to test this hypothesis.

This analysis extends previous work by Sowers et al. (21) with regard to cardiometabolic obesity and knee osteoarthritis. That study reported that the odds of knee osteoarthritis were greatest among women who were both obese and had cardiometabolic dysfunction; however, the cardiometabolic measures were not considered individually. We now report that leptin levels and a proxy indicator of insulin resistance, in particular, are strongly and significantly related to knee OA status.

This investigation has substantial strengths. Notably, our analytical approach considered obesity and gender stratified models of the cardiometabolic measures relative to osteophytes-defined radiographic knee OA status. Because many of the cardiometabolic measures vary with respect to obesity, this approach allowed us to examine their relationship with OA, independent of obesity. In doing so, it was observed that, regardless of obesity status, and after adjustment for BMI, cardiometabolic risk factors are associated with OA prevalence. The decision to utilize gender-stratified models allowed us to reveal important patterns in the relationship of osteophytes-defined

radiographic knee OA status and cardiometabolic measures. The nationally-representative sample utilized in this analysis is a strength in that it provides sufficient data to address variation in age, sex, and race/ethnic status.

Limitations of this report are that non-weight bearing radiographs were used to determine osteoarthritis status in the NHANES-III population and so our measure is truly reflective of osteophytes-defined radiographic knee OA status. Usage of non-weight bearing radiographs means that measures of joint space narrowing, a proxy measure of cartilage loss, cannot be ascertained. The impact of cardiometabolic dysfunction may have a different relationship with cartilage loss as compared to measures of bone overgrowth which were described by the K-L scoring system. However, utilization of the non-weight bearing radiographs should not affect the measures of osteophytes and bone sclerosis, reflected in the K-L scoring we report here. The cross-sectional design of NHANES is a limitation of this study so causality between cardiometabolic measures and OA cannot be determined. We used HOMA-IR as a proxy for insulin resistance because more sophisticated estimates such as glucose clamp measures were not available in this large epidemiologic dataset. While some studies have suggested that HOMA-IR may not be a good marker of insulin resistance in older adults (56), a larger study demonstrated high correlation between HOMA-IR and insulin sensitivity among older individuals with and without impaired glucose tolerance (57). Additionally, although the hypothesized relationship between cardiometabolic measures and knee OA operates under a paradigm of increased systemic inflammation, we did not include the measure of C-reactive protein

(CRP), an inflammatory biomarker, due to the known technical limitations of the available CRP assay in this population.

The observations warrant further investigation in other populations with data available on men and women. The NHANES dataset only includes information about osteoarthritis in the knee joints. However, because knee joints may be vulnerable to damage associated with increased mechanical loading due to obesity, confirmation of these findings in populations with information about osteoarthritis in non-weight bearing joints is of high interest. Reproducibility of these findings in other populations and across joint sites would have important implications for intervention and treatment and possibly indicate that effective therapies for osteoarthritis prevention may differ by sex.

CONCLUSION

We have reported consistent, statistically significant associations of cardiometabolic biomarkers and osteophytes-defined radiographic knee OA among a nationally-representative sample of US adults and conclude that cardiometabolic dysfunction is related to knee OA prevalence over-and-above the effect imparted by obesity. Furthermore, we describe a striking sex dimorphism in the pattern (direction and magnitude) of cardiometabolic risk factors relative to osteophytes-defined radiographic knee OA. A proxy measure of insulin resistance is associated with OA prevalence among men, whereas among women, leptin levels appear to be more important. Considering the substantial implications for primary and secondary interventions,

replication of these findings should be pursued in other populations with OA and cardiometabolic data, especially in those populations including both men and women.

Table 2.1. Demographic and Cardiometabolic Features of National Health and Nutrition Examination Survey III (NHANES III) Participants Aged 60+ Years, by Osteophytes-Defined Radiographic Knee Osteoarthritis (OA) Status.

	No knee osteoarthritis N=632	**Knee osteoarthritis** N=434	**P-value OA vs. no OA**
	n (%, se %)	**n (%, se %)**	
Sex			
Male	340 (69.6%, 1.3)	172 (30.4%, 1.3)	**<0.0001**
Female	292 (58.4%, 1.1)	262 (41.6%, 1.1)	
Race/Ethnicity			
Non-Hispanic White	368 (63.5%, 1.0)	242 (36.5%, 1.0)	**<0.0001**
Non-Hispanic Black	99 (52.0%, 2.2)	94 (48.0%, 2.2)	
Mexican American	133 (60.9%, 2.7)	84 (39.1%, 2.7)	
Other	32 (70.7%, 5.0)	14 (29.3%, 5.0)	
Marital Status			
Married	399 (65.3%, 0.6)	235 (34.7%, 0.6)	**<0.0001**
Not Married	232 (59.9%, 1.0)	197 (40.1%, 1.0)	
Educational Attainment			
Less than high school	346 (60.7%, 0.5)	254 (39.3%, 0.5)	**<0.0001**
High school or more	284 (64.9%, 1.9)	179 (35.1%, 1.9)	
Smoking Status			
Current	119 (75.5%, 1.2)	45 (24.5%, 1.2)	**<0.0001**
Former	243 (66.9%, 1.7)	142 (33.1%, 1.7)	
Never	270 (56.5%, 1.2)	247 (43.5%, 1.2)	
BMI Category			
BMI < 25 kg/m^2	253 (78.1%, 1.0)	88 (21.9%, 1.0)	**<0.0001**
BMI 25-29.9 kg/m^2	268 (63.7%, 1.4)	175 (36.3%, 1.4)	
BMI ≥ 30 kg/m^2	110 (41.4%, 1.2)	170 (58.6%, 1.2)	
	Mean (SE)	**Mean (SE)**	
Age	69.8 (0.14)	71.7 (0.07)	**<0.0001**
Body mass index, kg/m^2	26.0 (0.03)	29.4 (0.13)	**<0.0001**
Cardiometabolic factors			
Glucose (mg/dL)	108.3 (0.39)	116.2 (0.41)	**<0.0001**
Insulin (μIU/mL)	10.9 (0.08)	13.9 (0.23)	**<0.0001**
HOMA-IR	3.1 (0.03)	4.2 (0.05)	**<0.0001**
HDL-cholesterol (mg/dL)	52.6 (0.39)	51.7 (0.35)	**0.02**
LDL-cholesterol (mg/dL)	137.2 (0.48)	143.4 (0.80)	**<0.0001**
Triglycerides (mg/dL)	155.1 (1.28)	167.1 (1.43)	**<0.0001**
Waist:hip ratio	0.95 (0.001)	0.96 (0.002)	0.38
Systolic blood pressure	136.8 (0.37)	141.6 (0.19)	**<0.0001**
Diastolic blood pressure	73.6 (0.12)	74.5 (0.08)	**<0.0001**
Leptin (μg/L)	11.8 (0.07)	18.0 (0.28)	**<0.0001**

*All data are design-adjusted (using weights, cluster and stratum variables) to account for the survey sample design.

Table 2.2. Demographic and Cardiometabolic Features of National Health and Nutrition Examination Survey III (NHANES III) Participants Aged 60+ Years by Gender.

	Male N=517	**Female** N=554	**P-value Male vs. Female**
	n (%, se %)	**n (%, se %)**	
Race/Ethnicity			
Non-Hispanic White	275 (83.2%, 1.0)	335 (83.3%, 0.5)	0.50
Non-Hispanic Black	91 (6.9%, 0.3)	102 (7.5%, 0.6)	
Mexican American	126 (2.5%, 0.1)	91 (2.1%, 0.2)	
Other	20 (7.4%, 1.2)	26 (7.1%, 0.8)	
Marital Status			
Married	400 (81.0%, 0.3)	234 (46.9%, 1.0)	**<0.0001**
Not Married	112 (19.0%, 0.3)	317 (53.1%, 1.0)	
Educational Attainment			
Less than high school	307 (43.6%, 1.0)	293 (40.5%, 1.2)	**0.004**
High school or more	205 (56.4%, 1.0)	258 (59.5%, 1.2)	
Smoking Status			
Current	96 (17.1%, 0.8)	68 (13.1%, 0.6)	**<0.0001**
Former	258 (51.3%, 0.7)	127 (26.4%, 0.5)	
Never	158 (31.5%, 0.7)	359 (60.6%, 0.8)	
BMI Category			
BMI < 25 kg/m^2	160 (31.6%, 0.8)	181 (38.0%, 0.7)	**<0.0001**
BMI 25-29.9 kg/m^2	239 (44.5%, 1.0)	204 (36.0%, 0.8)	
BMI ≥ 30 kg/m^2	113 (24.0%, 0.9)	167 (26.0%, 0.8)	
	Mean (SE)	**Mean (SE)**	
Age	69.9 (0.16)	71.0 (0.16)	**<0.0001**
Body mass index, kg/m^2	27.2 (0.05)	27.3 (0.11)	0.49
Cardiometabolic factors			
Glucose (mg/dL)	115.3 (0.90)	108.2 (0.23)	**<0.0001**
Insulin (μIU/mL)	11.4 (0.14)	12.0 (0.16)	0.68
HOMA-IR	3.6 (0.07)	3.4 (0.04)	0.06
HDL-cholesterol (mg/dL)	46.5 (0.65)	56.5 (0.28)	**<0.0001**
LDL-cholesterol (mg/dL)	135.1 (0.88)	142.7 (0.76)	**<0.0001**
Triglycerides (mg/dL)	160.5 (1.59)	158.9 (1.66)	0.39
Waist:hip ratio	1.006 (0.001)	0.92 (0.001)	**<0.0001**
Systolic blood pressure	137.1 (0.46)	139.7 (0.32)	**<0.0001**
Diastolic blood pressure	75.7 (0.17)	72.6 (0.04)	**<0.0001**
Leptin (μg/L)	7.6 (0.08)	18.9 (0.29)	**<0.0001**

*All data are design-adjusted (using weights, cluster and stratum variables) to account for the survey sample design.

Table 2.3. Adjusted Odds Ratios (95% Confidence Intervals) for the Association Between Cardiometabolic Factors and Osteophytes-Defined Radiographic Knee Osteoarthritis Modeled Separately by Gender and Obesity Status, NHANES III.*

Models in the following stratum:	Leptin† OR (95% CI)	HOMA-IR OR (95% CI)	Body Mass Index OR (95% CI)	LDL-c‡ OR (95% CI)	Systolic Blood Pressure‖ OR (95% CI)
Non-obese men (N=372)	**0.61 (0.56,0.67)**	**1.18 (1.15,1.22)**	**1.33 (1.29,1.36)**	**1.05 (1.03,1.06)**	**0.97 (0.96,0.99)**
Obese men (N=102)	**0.73 (0.70,0.77)**	**1.34 (1.27,1.42)**	1.01 (0.96,1.05)	**1.06 (1.04,1.08)**	**0.88 (0.86,0.90)**
Non-obese women (N=356)	**1.04 (1.01,1.07)**	**1.04 (1.01,1.07)**	**1.15 (1.12,1.18)**	1.00 (0.99,1.01)	**1.10 (1.09,1.11)**
Obese women (N=148)	**1.28 (1.26,1.31)**	**0.88 (0.86,0.89)**	**1.18 (1.17,1.20)**	**0.94 (0.93,0.95)**	**1.09 (1.05,1.13)**

* All models are adjusted for age, ethnicity, marital status, educational attainment, $_{log}$triglycerides and smoking status (current, former, never).

† Estimates for leptin reflect a 5 μg/L increase in leptin.

‡ Estimates for LDL-c represent a 5 mg/dL increase in LDL-c.

‖ Estimates for systolic blood pressure represent a 5 mmHg increase in systolic blood pressure.

Table 2.4. Sensitivity Analysis - Adjusted Odds Ratios (95% Confidence Intervals) for the Association Between Cardiometabolic Factors and Osteophytes-Defined Radiographic Knee Osteoarthritis Modeled Separately by Gender and Obesity Status, NHANES III. Adjusted for Waist-to-Hip Ratio Instead of Body Mass Index.*

	Leptin†	HOMA-IR	Waist:Hip Ratio‡	LDL-c‖	Systolic Blood Pressure¶
Models in the following stratum:	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
Non-obese men (N=372)	**0.82 (0.74,0.92)**	**1.41 (1.35,1.48)**	**0.70 (0.63,0.79)**	**1.05 (1.04,1.06)**	**0.96 (0.94,0.98)**
Obese men (N=102)	**0.75 (0.73,0.77)**	**1.33 (1.27,1.39)**	**4.69 (4.36,5.04)**	**1.08 (1.05,1.10)**	**0.84 (0.82,0.85)**
Non-obese women (N=356)	**1.18 (1.15,1.21)**	**1.09 (1.06,1.12)**	1.03 (0.93,1.14)	1.01 (1.00,1.02)	**1.09 (1.08,1.10)**
Obese women (N=148)	**1.39 (1.37,1.41)**	**0.90 (0.89,0.92)**	0.89 (0.65,1.22)	**0.93 (0.92,0.94)**	**1.10 (1.08,1.13)**

* All models are adjusted for age, ethnicity, marital status, educational attainment, $_{log}$triglycerides and smoking status (current, former, never).

† Estimates for leptin reflect a 5 μg/L increase in leptin.

‡ Estimates for waist:hip ratio reflect a 0.1 unit increase in waist:hip ratio.

‖ Estimates for LDL-c represent a 5 mg/dL increase in LDL-c.

¶ Estimates for systolic blood pressure represent a 5 mmHg increase in systolic blood pressure.

Figure 2.1. Prevalence (95% Confidence Interval) of Osteophytes-Defined Radiographic Knee Osteoarthritis by Obesity Status and Gender Among National Health and Nutrition Examination Survey (NHANES) III Sample Aged 60+ Years.

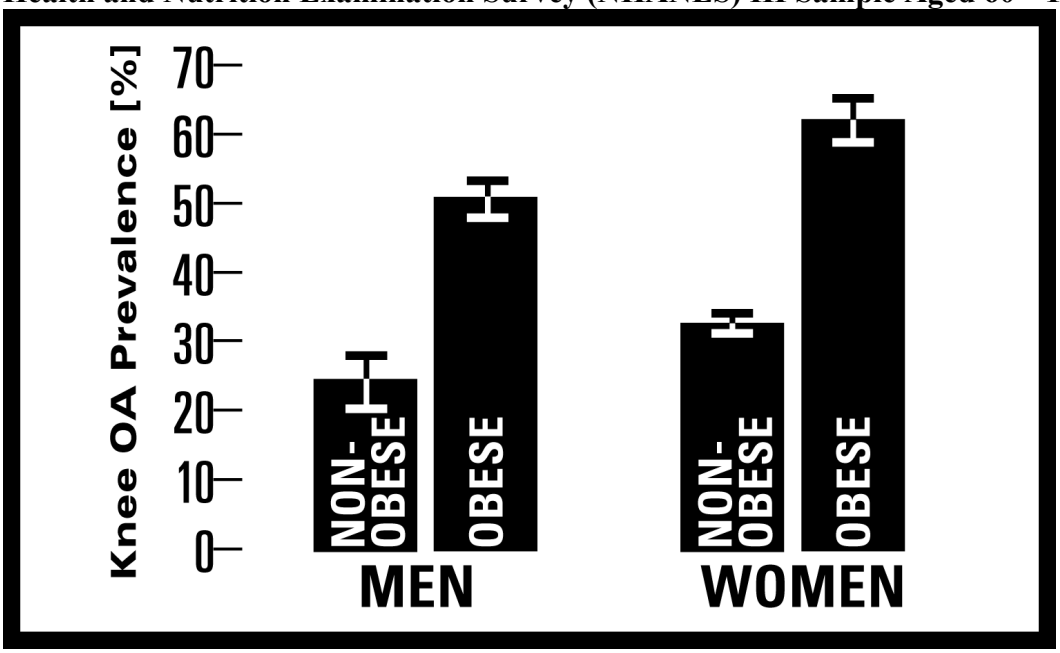


Figure 2.2. Odds Ratio (95% Confidence Interval) of Osteophytes-Defined Radiographic Knee Osteoarthritis Associated with HOMA-IR by Gender and Obesity Status, National Health and Nutrition Examination Survey (NHANES) III. Estimates from Each of the Four Obesity by Sex Models Were Adjusted for Age, Ethnicity, Marital Status, Educational Attainment, Smoking Status, Leptin, Body Mass Index, $_{log}$Triglycerides, LDL-c, and Systolic Blood Pressure.

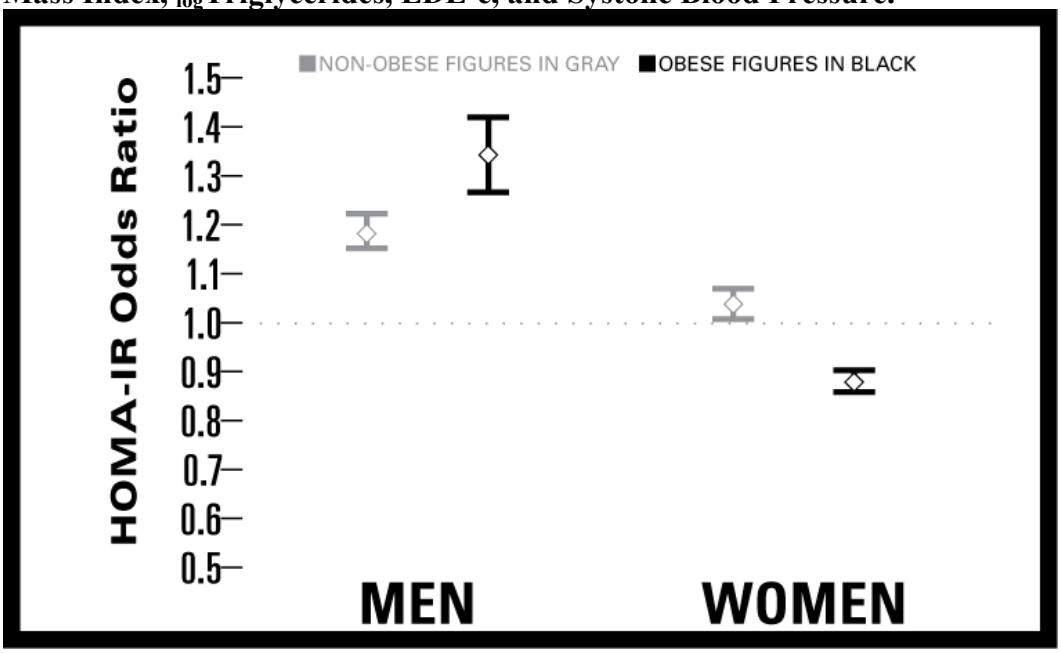
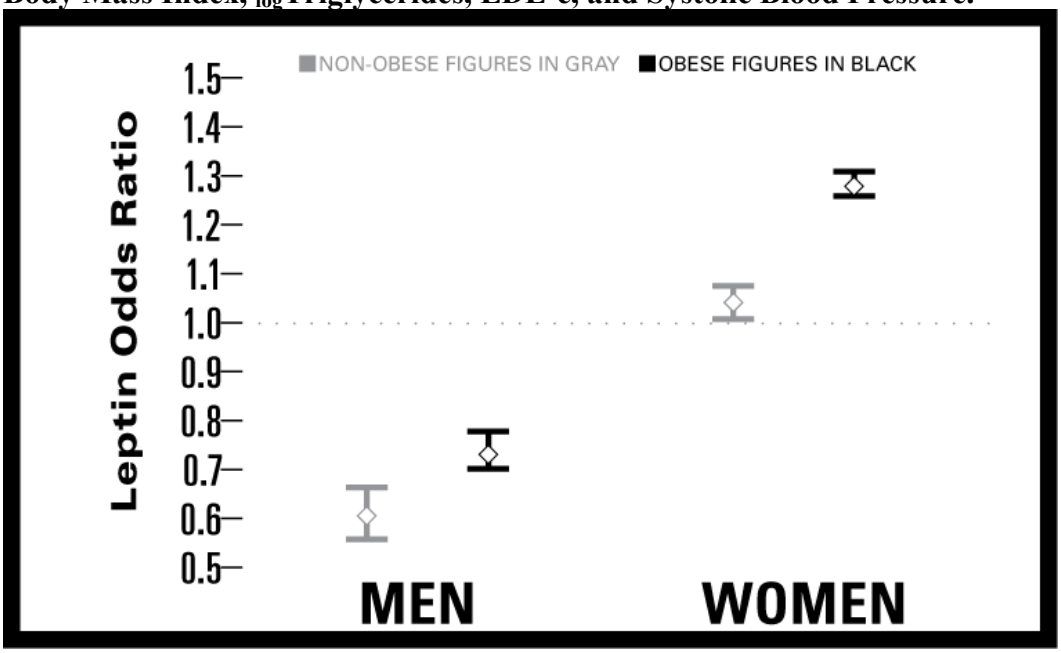


Figure 2.3. Odds Ratio (95% Confidence Interval) of Osteophytes-Defined Radiographic Knee Osteoarthritis Associated with Leptin by Gender and Obesity Status, National Health and Nutrition Examination Survey (NHANES) III. Estimates from Each of the Four Obesity by Sex Models Were Adjusted for Age, Ethnicity, Marital Status, Educational Attainment, Smoking Status, HOMA-IR, Body Mass Index, $_{log}$Triglycerides, LDL-c, and Systolic Blood Pressure.



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CHAPTER THREE

Leptin Levels are Associated with Radiographic Knee Osteoarthritis Among a Cohort of Mid-Life Women

INTRODUCTION

As the leading cause of pain, functional limitations and disability in the United States (1), osteoarthritis is associated with decreased productivity and increased health care expenditures (2). In 2003, the cost of medical care expenditures and earnings losses associated with arthritis and rheumatism was estimated to be $128 billion (3). Osteoarthritis (OA), a joint condition characterized by loss of articular cartilage, subchondral bone remodeling, soft tissue damage and inflammation, is the most common form of arthritis, affecting more than 26 million U.S. adults over the age of 25 (4).

Obesity is a well-documented risk factor for prevalent knee OA (5-11). For example, data from the Chingford general population survey (United Kingdom) reported that women in the highest tertile of body mass index (BMI) had 6-fold increased odds of knee OA and nearly 18 times higher odds of bilateral knee osteoarthritis, compared to women in the lowest tertile of BMI (12).

The impact of obesity on osteoarthritis onset and progression is likely due to several mechanisms. Some investigators have focused on the impact of obesity as a sheer mechanical force causing increased joint loading and subsequent damage to the articular cartilage (13-15). However, associations observed between obesity and OA in non-weight bearing joints such as those in the hands and/or wrists (12,16-18) suggest that alternative mechanisms impact the OA-obesity relationship and emphasize the role of adipose tissue.

There is emerging evidence about the active metabolic environment of chondrocytes, including glucose transport, cholesterol efflux, and lipid metabolism (19). These new findings have prompted the consideration of novel obesity-related biomarkers, such as the adipocytokines, in studies of osteoarthritis and have suggested that there may be shared pathophysiology between osteoarthritis and the cardiovascular and metabolic diseases. Adipose tissue, once considered a passive storage portal of energy, is now recognized as an endocrine organ as adipocytes have the ability to secrete active agents including adipocytokines (20). Efforts to examine the adipocytokines with respect to OA have focused on leptin because of its strong correlation with body size. Leptin levels in synovial fluid are correlated with the severity of knee OA (21) and leptin and its receptor have been identified in many joint tissues including human chondrocytes, osteophytes (22,23), synovium and infrapatellar fat pad (24).

While it has been postulated that leptin might be an important link between obesity and osteoarthritis (19,25-28), epidemiologic evidence of such a relationship is limited,

possibly because few studies of OA have leptin measures available. Further, no studies have evaluated whether changes in leptin are associated with osteoarthritis despite evidence that changes in body size are associated with joint damage (29,30). Thus, the goal of this investigation was to describe the relationship between serum leptin measures and knee OA prevalence and incidence and to determine whether changes in leptin levels over time differed by OA status in a mid-aged population of women.

METHODS

The Michigan Study of Women's Health Across the Nation (SWAN) is one of seven clinical sites for SWAN, a multiethnic cohort study characterizing the endocrinological, physiological, and behavioral changes occurring during the menopausal transition. The Michigan SWAN population, established in 1996, is a population-based sample of eligible women from two Detroit-area communities. The population was identified with a community census based on the electrical utility listings of the targeted communities. Households were contacted by telephone (if available) or in-person. A total of 543 eligible women were recruited from the Michigan site to the SWAN Core Longitudinal Study, including 325 African American and 218 Caucasian women. Eligibility criterion at baseline included 42-52 years of age, having an intact uterus, having had at least one menstrual period in the previous 3 months, no use of reproductive hormones in the previous 3 months, and self-identification with the site's designated race/ethnic group (either African American or Caucasian at Michigan).

At the 1996 baseline, Michigan SWAN women completed the assessment protocol common to all SWAN sites; then a supplemental protocol including radiographs and functional assessments was implemented. Women were seen for annual follow-up visits, although the supplemental osteoarthritis imaging protocol was included only at visits baseline, 2, 4, and 11. Participation at the annual assessments has been excellent, with 80% seen at follow-up visit 11. The University of Michigan Institutional Review Board approved the study protocol, and written informed consent was obtained from each participant.

Osteoarthritis Measures. Anterior-posterior radiographs of the knees have been taken weight-bearing in the semi-flexed position (31). Radiographs taken at baseline, follow-up visit 2 and follow-up visit 4 were obtained using General Electric radiograph equipment (model X-GE MPX-80; General Electric Medical Systems, Milwaukee, Wisconsin). Radiographs from follow-up visit 11 were obtained with the AXIOM Aristos radiographic system with integrated digital flat detector technology (Siemens, Erlangen, Germany). Knees were scored using the Kellgren and Lawrence (K-L) grading system of the Atlas of Standard Radiographs of Arthritis (32) such that 0=normal; 1=doubtful OA; 2=minimal OA; 3=moderate OA; 4=severe OA. Participants with artificial knee replacements were assigned a K-L score of 4. Knee osteoarthritis was defined as at least one knee with a score ≥ 2.

All radiographs were read by two readers in accordance with a quality control system that includes the utilization of "drift" films; the same set of drift films are used for both

readers and have been used for each round of x-ray reading. All knee images were first scored independently by each reader; discordant scores were re-evaluated during a consensus session to determine a final score.

Leptin Assay. The SWAN specimen collection protocol includes a fasted (minimum 10-hour) blood draw to provide samples for a specimen repository that is maintained at -80°C until processing. Serum leptin levels were determined spectrophotometrically using commercially-available colorimetric enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI) and run according to the manufacturer's instructions. The coefficient of variation percent for duplicate samples for each subject is 0.4-12.4% and the lower limit of detection is 1 ng/mL. Banked specimens from baseline and visits 1, 3, 4, 5, 6 and 7 were assayed for leptin.

Other Measures. At each annual examination, height (cm) and weight (kg) was measured using a stadiometer and calibrated balance-beam scale, respectively, while participants wear a single layer of light clothing and no shoes. Body mass index (BMI, kg/m^2) was calculated as weight in kilograms divided by the square of height in meters. Waist (cm) and hip (cm) circumferences have also been measured at each annual examination, using a non-stretchable tape 3 cm above the umbilicus, after a relaxed expiration, and the maximum girth around the buttocks, respectively.

Menopause status was ascertained at each annual exam based on questions about bleeding patterns, current hormone use, hysterectomy and oophorectomy. At each visit,

participants were categorized as being premenopausal, early perimenopausal, late perimenopausal, postmenopausal, hysterectomy, or unable to determine due to exogenous hormone use. Premenopausal status was defined as regular menses with bleeding in the past three months, early perimenopausal status was defined as bleeding in the past three months but increasing irregularity in menses, late perimenopausal status was defined as bleeding in the past year but not in past three months, and postmenopausal status was defined as no bleeding for 12 months.

Participants were asked about their current smoking status (yes/no) at each annual visit. Race/ethnicity classification as African American or Caucasian was determined by self-report at baseline. Age at each annual visit was calculated as date of annual visit minus date of birth.

Statistical Analysis. Means and standard deviations (SD) or frequencies and percents of leptin, body size variables and relevant covariates were examined overall and by knee OA status at baseline and follow-up visits 4 and 11. The statistical significance of differences by OA status were evaluated using t-tests, analysis of variance, or chi-square tests.

To fully utilize the richness of the available data, including multiple measures of knee OA status and annual assessment of leptin through follow-up visit 07, three analytical approaches were employed to relate leptin measures and knee OA status. In the first two approaches, the outcome of interest was knee osteoarthritis whereas in the third approach,

leptin was the outcome. First, to determine the association of leptin levels and knee OA prevalence at baseline, we examined the cross-sectional association of leptin and knee OA using multivariable logistic regression analysis. Second, to determine the association of leptin levels and incident knee OA, discrete-time survival analysis techniques were utilized to model the time to incident OA through follow-up visit 4 as a function of leptin levels. To complete this analysis, a dataset was constructed with multiple observations per participant; each row in the dataset represented a study visit in which the participant was at risk of knee OA onset through follow-up visit 4. Once women developed knee OA, they were censored. The discrete-time survival model was then estimated using logistic regression whereby the odds ratios are the effect estimates of interest. The hazard rate is the probability that a woman has incident knee OA at a particular follow-up visit while at risk for knee OA. As leptin levels were not available at follow-up visit 2, values from follow-up visit 3 were substituted. Third, leptin levels from baseline to follow-up visit 7 were evaluated overall and by prevalent, incident, or no knee OA status through follow-up visit 11. Student's t-tests were used to compare leptin levels at each time point between women prevalent, incident, or no knee OA through follow-up visit 11. Then, linear mixed models (PROC MIXED) with random intercepts and slopes for age were used to examine level and change in leptin measures over time. Osteoarthritis status by time interactions in the model evaluated whether the rates of change in leptin measures differed between women with prevalent, incident or no knee OA through follow-up visit 11. Interactions between the OA groups and race/ethnicity, smoking status and menopause status were considered. SAS PROC SGPLOT was used to graph

predicted trajectories of leptin measures with corresponding 95% confidence intervals for each knee OA group.

Due to the collinearity between body size and leptin ($r=0.73$, $P<0.0001$), all multivariable modeling included residuals of BMI as the measure of body size confounding. The BMI residual variable represents the variation in BMI that remains following simple regression of BMI on leptin. Given that leptin represents the metabolic component of body size, the BMI residual represents the association of body size and OA through other pathways, including mechanical loading. Interactions of the BMI residual and leptin were tested to assess potential effect modification of the relationship between leptin and knee OA by body size.

Model fit and final model selection was based on the Akaike's information criterion and chi-square tests comparing the log likelihood ratios between candidate models. Models were adjusted for age, race/ethnicity, menopause status (or hysterectomy yes/no), current smoking status, and BMI residuals, as appropriate. Statistical significance was defined at $\alpha<0.05$ and all analyses were completed using SAS v9.3 (SAS Institute, Cary, NC).

RESULTS

The prevalence of radiographically-defined knee OA increased over the ten-year follow-up period. At baseline, when participants were 46 years of age on average, 18% of participants had prevalent knee OA; at follow-up visit 11, 65% of women had knee OA

(Table 3.1.). Similarly, the prevalence of moderate to severe OA (K-L score of 3 or 4) changed from 4% of participants at baseline to 28% ten years later.

At baseline, the mean leptin level was 30.6 ng/mL. At follow-up visit 7, the average leptin level was 38.0 ng/mL; we observed a statistically significant increasing trend in leptin levels over time ($P<0.0001$). As shown in Table 3.2., leptin levels were 43% higher at baseline and 50% higher at follow-up visit 04 among women with knee OA as compared to those without knee OA ($P <0.0001$). Leptin levels were not different according to race/ethnicity, age, menopause status, or smoking status.

More than half of all participants were obese at baseline in 1996/7; by follow-up visit 11, the prevalence of obesity increased to 64.6% (Table 3.1.). There was a statistically significant increase in all body size measures with the exception of height over the study period. Body size measures were 15-30% greater among women with knee OA as compared to those without knee OA. At all time points, women with knee OA had statistically significantly greater weight, BMI, waist circumference, hip circumference and waist:hip ratio as compared to those without knee OA.

By design, 60% of participants were African-American and 40% were Caucasian. In accordance with the Study's inclusion criterion, all participants were pre- or early peri-menopausal at baseline and were not using exogenous hormones. By follow-up visit 11, most women (78%) were postmenopausal and only 8% were using exogenous hormones (Table 3.1.). Menopause status differed by OA status at follow-up visit 11 ($P=0.02$);

women with OA were more likely to have had a hysterectomy as compared to women without knee OA. Current use of exogenous hormones did not differ by knee OA status at follow-up visit 4 or visit 11.

The proportion of women that were current smokers declined slightly over time; at baseline, 27% of women were current smokers whereas 23% were current smokers at follow-up visit 11. The proportion of current smokers was similar among women with and without knee OA at baseline; however, at follow-up visit 11, women with knee OA were less likely to be current smokers as compared to women without knee OA (19% vs. 29%, respectively) (P=0.02).

Cross-sectional analysis relating leptin levels to prevalent knee OA. As shown in Table 3.3., greater baseline leptin levels were positively and significantly associated with knee OA at the baseline visit. A 5 ng/mL increase in leptin was associated with 38% greater odds of having knee OA after adjustment for age, race/ethnicity, menopause status, current smoking status, and BMI [odds ratio (OR)=1.38, 95% confidence interval (CI) 1.26, 1.42]. African American women had 2.5 times greater odds of having knee OA at baseline as compared to Caucasian women (OR=2.55, 95% CI 1.46, 4.46).

Serum leptin levels and time to incident knee OA. The two-year incidence of knee OA at follow-up visits 2 and 4 was 19% and 14%, respectively. After adjustment for baseline age, race/ethnicity, menopause status, current smoking status, and BMI, greater leptin levels were associated with incident knee OA (Table 3.4.). A 5 ng/mL increase in leptin

was associated with 37% greater odds of incident knee OA (OR=1.37; 95% CI 1.25, 1.49). African American women had two times greater odds of incident knee OA (OR=1.98, 95% CI 1.12, 3.47) as compared to Caucasian women. Women who had a hysterectomy had three times greater odds of incident knee OA (OR=3.15, 95% CI 1.39, 7.14) as compared to pre-menopausal/early peri-menopausal women. Age and current smoking status were associated with lower odds of incident knee OA. A 1 year increase in baseline age was associated with 8% decreased odds of incident knee OA (OR=0.92, 95% CI 0.90, 0.94). Those who were current smokers had 47% decreased odds of having incident knee OA as compared to those who were not current smokers (OR=0.53, 95% CI 0.29, 0.99).

Trajectories of leptin in relation to knee OA status at follow-up visit 11. There was a statistically significant increase in leptin levels with time; on average, leptin levels increased by 0.78 ng/mL per year ($P<0.0001$). As shown in Figure 3.1., serum leptin levels were highest among women with prevalent knee OA at baseline. After adjustment for age, BMI residuals, race/ethnicity, hysterectomy status and baseline smoking status, women with prevalent knee OA at baseline had serum leptin levels that were 21 ng/mL higher as compared to women that remained knee OA free throughout the 10 years of follow-up. Women who developed incident knee OA during the follow-up had serum leptin levels that were 14 ng/mL higher as compared to those without knee OA. Most notably, the highest leptin levels among those women who never developed knee OA are still lower than those among women with knee OA. Also, the leptin levels among those with incident disease during the follow-up do not overlap with levels among those with

prevalent disease at baseline. While leptin levels differed by knee OA status, there was no statistically significant difference in the slope of the leptin trajectories over time among the groups.

DISCUSSION

While several investigators have speculated that leptin may represent an important link between obesity and knee OA (19,25-28), few epidemiological studies have examined this hypothesis. This study is the first to examine leptin levels with respect to both prevalent and incident knee OA and to relate longitudinal measures of leptin to subsequent OA status. In this population of mid-life women, we found that higher leptin levels were associated with increased odds of both prevalent and incident knee OA but that the rate of change in leptin levels over time did not differ by knee OA status.

Only one other study has related leptin measures to radiographic knee OA status. Ku et al. reported that cross-sectional leptin levels (measured in synovial fluid) were related to knee OA severity among a population of knee surgery patients (21). Unlike our study, however, BMI did not differ among those with and without knee OA, suggesting that our population and the Ku et al. population are very different with respect to body size. Further, the leptin levels among women in our population are much higher than those reported among patients in the Ku et al. study; 74% of women in our study had baseline leptin levels that were higher than the upper range of values reported among patients in Ku et al. (15.8 ng/mL) (21). Data from the National Health and Nutrition Examination Study (NHANES) the average leptin value among women in the United States aged ≥ 20

years is 12.7 ng/mL (33), suggesting that the population in the Ku et al. paper (21) may have leptin levels that are lower than the general population of the United States.

Our data suggest that women with prevalent knee OA at baseline are the most metabolically compromised in terms of high leptin levels as evidenced by the observation that leptin levels were highest among women with prevalent knee OA at baseline as compared to those with 10-year incident knee OA or no knee OA. Individuals with prevalent knee OA at baseline represent those who were relatively young at disease onset and so we hypothesize that metabolic dysfunction may be an important risk factor, particularly among younger individuals.

Mechanistically, leptin may be associated with OA through catabolic or anabolic mechanisms. Leptin has been shown to have an anabolic effect on chondrocytes and osteoblasts (22), which may be associated with repair of damaged cartilage but also increased osteophyte development. Because the Kellgren Lawrence scoring system reflects both joint space width (a proxy of cartilage loss) and the presence of osteophytes, the anabolic impact of leptin with respect to radiographically-defined knee OA can not be differentiated. Leptin may also have a catabolic effect on cartilage due to its pro-inflammatory capabilities. Synergistic relationships of leptin and proinflammatory cytokines including interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), matrix metallopeptidase 9 (MMP-9), MMP-13 and nitric oxide have been reported (22,34,35). Increased local inflammation within the joint has been shown to play a catabolic role in cartilage metabolism (34). It is possible that the proinflammatory impact

of leptin may be detrimental to other collagenous tissues within the joint including the meniscus and ligaments. Future analyses in this study population will examine the relationship of leptin and inflammatory biomarkers with knee joint features imaged in MRI to better describe this potential mechanistic pathway.

This study examined leptin levels with respect to knee OA prevalence and incidence in a non-clinical population. Important strengths unique to this study include longitudinal measures of leptin, repeated assessment of knee OA status, and information about potential confounders including body size, menopause status and smoking. Our analytical approach was complicated by the fact that leptin, being a product of adipose tissue, is highly correlated with all measures of body size and greater body size is a hypothesized risk factor for knee OA through non-metabolic mechanisms such as increased joint loading or poorer muscle strength. Thus, our analyses were adjusted for the residuals of BMI on leptin in an effort to describe the metabolic impact of obesity (i.e., leptin) on knee OA, over-and-above the non-metabolic effect of greater body size. We do not have measures of OA in non-weight bearing joints in this population.

The prevalence of knee OA continues to increase in the study population, likely because of the aging of the population and the increasing proliferation of obesity. While obesity is a known risk factor for osteoarthritis, better characterization of the mechanisms by which greater fat mass are associated with joint damage will provide important information to aid in prevention and intervention strategies. We have reported that leptin, an adipocytokine secreted by adipose tissue is associated with knee OA prevalence and

incidence over-and-above the non-metabolic impact of BMI. These findings support the potential utility of serum leptin as a biomarker for OA risk and suggest that interventions targeting the inflammatory impact of leptin on joint health may forestall disease onset and/or decline.

Table 3.1. Descriptive Characteristics of the Michigan Study of Women's Health Across the Nation (SWAN) Sample at Baseline, Follow-Up Visit 04, and Follow-Up Visit 11.

	Baseline N=542	**Visit 04** N=252	**Visit 11** N=387
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Age (years)	46.1 (2.7)	50.2 (2.7)	56.9 (2.8)
Weight (kg)	85.8 (21.8)	87.9 (22.1)	90.1 (22.2)
Height (cm)	163.5 (6.2)	162.8 (5.8)	162.6 (6.2)
BMI (kg/m^2)	32.1 (8.0)	33.2 (8.3)	34.1 (8.4)
Waist circumference (cm)	94.1 (17.1)	98.5 (17.6)	102.3 (17.6)
Hip circumference (cm)	113.9 (16.2)	115.4 (17.0)	118.1 (16.9)
Waist:hip ratio	0.82 (0.07)	0.85 (0.08)	0.87 (0.08)
Leptin (ng/mL)	30.6 (18.3)	34.4 (20.1)	-----
	n (%)	**n (%)**	**n (%)**
Knee Osteoarthritis	98 (18.1%)	109 (43.3%)	251 (64.9%)
K-L Score			
0	322 (59.4%)	61 (24.2%)	44 (11.4%)
1	122 (22.5%)	82 (32.5%)	92 (23.8%)
2	78 (14.4%)	81 (32.1%)	144 (37.2%)
3	19 (3.5%)	27 (10.7%)	62 (16.0%)
4	1 (0.2%)	1 (0.4%)	45 (11.6%)
Obese (BMI>30 kg/m^2)	301 (56.1%)	153 (61.5%)	250 (64.6%)
Ethnicity			
African-American	324 (59.8%)	181 (71.8%)	238 (61.5%)
Caucasian	218 (40.2%)	71 (28.2%)	149 (38.5%)
Menopause Status			
Premenopausal	271 (50.3%)	11 (4.4%)	0 (0.0%)
Early Perimenopausal	268 (49.7%)	104 (41.4%)	12 (3.1%)
Late Perimenopausal	0 (0.0%)	37 (14.7%)	15 (3.9%)
Postmenopausal	0 (0.0%)	51 (20.3%)	303 (78.3%)
Hysterectomy	0 (0.0%)	22 (8.8%)	52 (13.4%)
Unknown, hormone use	0 (0.0%)	26 (10.4%)	5 (1.3%)
Hormone therapy use	0 (0.0%)	45 (17.9%)	32 (8.3%)
Current Smoker	146 (27.3%)	63 (25.1%)	87 (22.5%)

Table 3.2. Descriptive Characteristics of the Michigan Study of Women's Health Across the Nation (SWAN) Sample at Baseline and Follow-Up Visit 11, by Knee Osteoarthritis (OA) Status.

	Baseline			Visit 04			Visit 11		
	No OA Mean (SD)	**OA Mean (SD)**	**P-value**	**No OA Mean (SD)**	**OA Mean (SD)**	**P-value**	**No OA Mean (SD)**	**OA Mean (SD)**	**P-value**
Age (years)	46.1 (2.8)	46.0 (2.6)	0.73	50.2 (2.8)	50.3 (2.8)	0.74	56.6 (2.8)	57.1 (2.8)	0.11
Weight (kg)	82.2 (20.0)	101.8 (22.5)	**<0.0001**	78.6 (17.6)	100.2 (21.6)	**<0.0001**	75.1 (15.8)	98.2 (21.0)	**<0.0001**
Height (cm)	163.5 (6.2)	163.3 (6.4)	0.85	162.7 (5.8)	163.0 (5.9)	0.65	162.3 (6.4)	162.8 (6.0)	0.47
BMI (kg/m^2)	30.8 (7.3)	38.1 (8.5)	**<0.0001**	29.7 (6.5)	37.8 (8.2)	**<0.0001**	28.5 (5.9)	37.1 (7.9)	**<0.0001**
Waist circumference (cm)	91.5 (16.0)	105.4 (17.5)	**<0.0001**	91.9 (14.8)	107.1 (17.2)	**<0.0001**	91.4 (15.2)	108.2 (16.0)	**<0.0001**
Hip circumference (cm)	111.3 (14.9)	125.5 (17.1)	**<0.0001**	108.0 (12.7)	125.1 (17.0)	**<0.0001**	107.2 (13.0)	124.0 (15.8)	**<0.0001**
Waist:hip ratio	0.82 (0.07)	0.84 (0.07)	**0.02**	0.85 (0.08)	0.86 (0.08)	0.50	0.85 (0.08)	0.87 (0.08)	**0.01**
Leptin (ng/mL)	28.4 (17.2)	40.6 (20.1)	**<0.0001**	28.2 (17.9)	42.2 (20.2)	**<0.0001**	-----	-----	
	n (%)	**n (%)**		**n (%)**	**n (%)**		**n (%)**	**n (%)**	
Obese (BMI≥30 kg/m^2)	15 (15.8%)	80 (84.2%)	**<0.0001**	67 (47.2%)	86 (80.4%)	**<0.0001**	50 (36.8%)	200 (79.7%)	**<0.0001**
Ethnicity									
African-American	249 (56.1%)	75 (76.5%)	**0.0002**	97 (67.8%)	84 (77.1%)	0.11	76 (55.9%)	162 (64.5%)	0.09
Caucasian	195 (43.9%)	23 (23.5%)		46 (32.2%)	25 (22.9%)		60 (44.1%)	89 (35.5%)	
Menopause Status									
Premenopausal	230 (52.2%)	41 (41.8%)	0.06	7 (4.9%)	4 (3.7%)	0.71	0 (0.0%)	0 (0.0%)	**0.02**
Early Perimenopausal	211 (47.9%)	57 (58.2%)		61 (43.0%)	43 (39.5%)		9 (6.3%)	3 (1.2%)	
Late Perimenopausal	0 (0.0%)	0 (0.0%)		20 (14.1%)	17 (15.6%)		6 (4.4%)	9 (3.6%)	
Postmenopausal	0 (0.0%)	0 (0.0%)		29 (20.4%)	22 (20.2%)		107 (78.7%)	196 (78.1%)	
Hysterectomy	0 (0.0%)	0 (0.0%)		9 (6.3%)	13 (11.9%)		12 (8.8%)	40 (15.9%)	
Unknown, hormone use	0 (0.0%)	0 (0.0%)		16 (11.3%)	10 (9.2%)		2 (1.5%)	3 (1.2%)	
Hormone therapy use	0 (0.0%)	0 (0.0%)	N/A	27 (19.0%)	18 (16.5%)	0.61	14 (10.3%)	18 (7.2%)	0.29
Current Smoker	121 (27.8%)	25 (25.5%)	0.65	42 (29.6%)	21 (19.3%)	0.06	40 (29.4%)	47 (18.7%)	**0.02**

Table 3.3. Cross-Sectional Analysis of the Relationship Between Serum Leptin Values and Knee Osteoarthritis Status at Baseline Visit Among Michigan Study of Women's Health Across the Nation (SWAN) Study Participants.

	Leptin*	***Age***	***African American vs. Caucasian Race/Ethnicity***	***Early Peri-menopausal vs. Premenopausal***	***Current Smoker vs. Not Current Smoker***	
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**-2LL**
Unadjusted model	**1.19 (1.12, 1.26)**					454.720
Fully adjusted model[†]	**1.38 (1.26, 1.42)**	1.00 (0.92, 1.10)	**2.55 (1.46, 4.46)**	1.57 (0.95, 2.60)	1.05 (0.58, 1.90)	400.885

*Estimate represents 5 ng/mL change in leptin value.

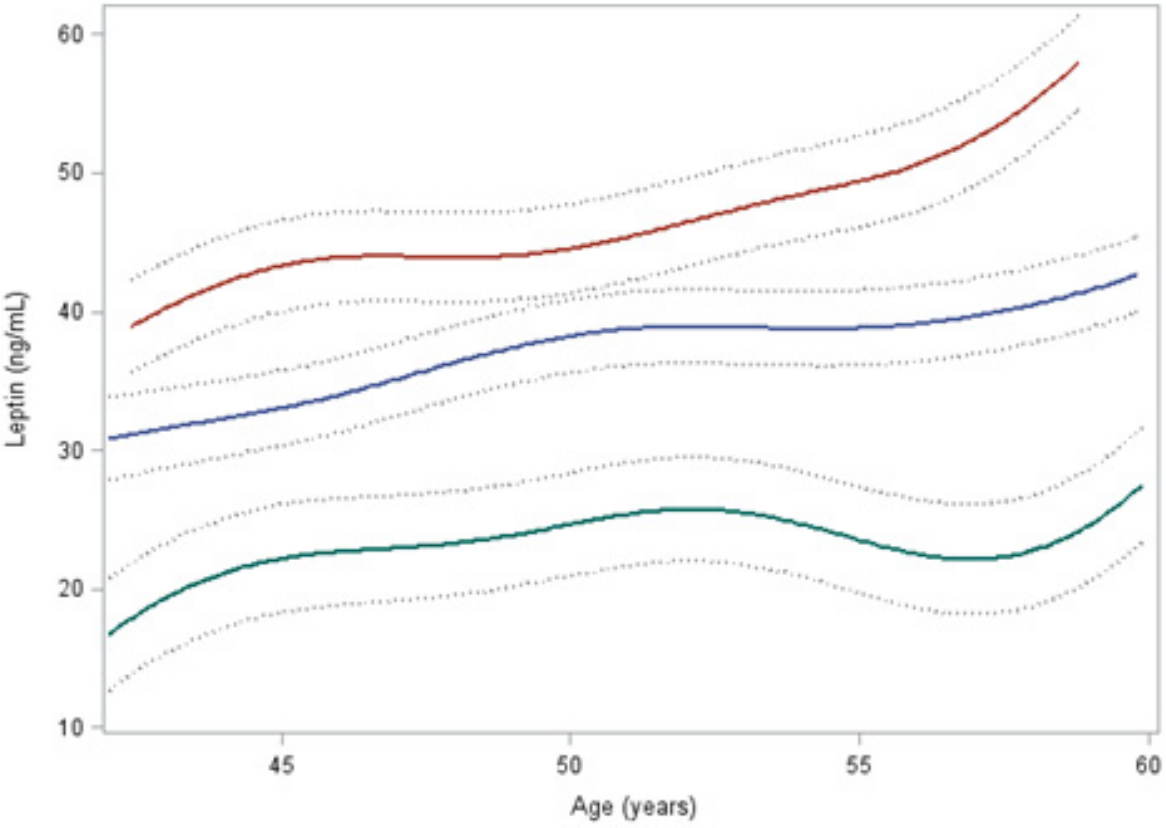
[†] Model included leptin, age, race/ethnicity, menopause status, smoking status and BMI residuals.

Table 3.4. Discrete Survival Time Analysis of Relationship Between Serum Leptin Values and Time to Knee Osteoarthritis Onset Among Michigan Study of Women's Health Across the Nation (SWAN) Participants, Baseline to Follow-Up Visit 4.

	OR (95% CI)
Leptin*†	**1.37 (1.25, 1.49)**
Baseline age	**0.92 (0.90, 0.94)**
Black Race/Ethnicity	**1.98 (1.12, 3.47)**
Menopause status	
Late Peri-/Postmenopause	1.66 (0.98, 2.83)
Hysterectomy	**3.15 (1.39, 7.14)**
Unknown due to exogenous hormone use	1.27 (0.57, 2.84)
Current Smoker	**0.53 (0.29, 0.99)**

* Estimate represents 5 ng/mL change in leptin value.
† Model also adjusted for BMI residuals.

Figure 3.1. Predicted Trajectories of Serum Leptin (ng/mL) by Knee Osteoarthritis Status Among Michigan Study of Women's Health Across the Nation (SWAN) Participants, Adjusted for Age, Body Mass Index Residuals, Race/Ethnicity, Hysterectomy Status and Baseline Smoking Status. Red Line Represents Women with Prevalent Knee Osteoarthritis at Baseline; Blue Line Represents Women with Incident Knee Osteoarthritis Through Follow-up Visit 11; Green Line Represents Women without Knee Osteoarthritis During Follow-up.



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CHAPTER FOUR

The Relationship Between Serum Leptin and Measures of Magnetic Resonance Imaging-Assessed Knee Joint Damage in a Population of Mid-Life Women

INTRODUCTION

Osteoarthritis (OA) is a highly prevalent joint condition, affecting more than 37% of adults over age 60 (1). It is the leading cause of pain and disability among United States adults (2). Initially thought to afflict only elderly populations, research has shown that the onset of OA can begin by age 40 (3) and that the prevalence of MRI-defined knee joint damage is common among mid-life women (4). Sowers and colleagues recently reported that, among a population of mid-aged women, medial compartment full-thickness cartilage defects were present in 15% of knees and synovitis in 25% of knees (4). The presence of large osteophytes, marked synovitis, macerated meniscal tears, and full-thickness cartilage defects were associated with knee pain and decrements in physical functioning (4).

By 2030, nearly one-third of mid-aged adults (45-64 years) will have arthritis (5), given the aging of the population and the increasing prevalence of obesity (6). Obesity is a major risk factor for radiographic knee OA (7-13) and the presence of knee cartilage

defects (14-16) and bone marrow lesions (17,18) observed on magnetic resonance imaging (MRI). However, not all obese persons have knee OA nor are all individuals with knee OA obese. This fact, in addition to the observed association between obesity and OA in non-weight bearing joints (19-22) suggests that the relationship between body size and osteoarthritis extends beyond that of mechanical loading, the commonly conceptualized mechanism through which obesity influences onset and progression of OA.

Most studies of osteoarthritis and obesity use body mass index (BMI) as a marker of body size and proxy for mechanical loading. However, data from the Michigan Bone Health and Metabolism Study suggest that compartmental measures of body composition, including fat mass and skeletal muscle mass are better predictors of radiographic knee osteoarthritis incidence and severity as compared to BMI (23). Greater skeletal muscle mass is associated with increased cartilage volume (24-26) and is protective against cartilage loss (25,27) whereas greater fat mass is a risk factor for cartilage defects (27,28), cartilage loss (29), bone marrow lesions (27) and joint replacement (30).

Consideration of body composition with respect to osteoarthritis recognizes that adipose tissue is a highly metabolic endocrine organ which may be involved in joint damage through mechanisms other than increased mechanical loading. Adipocytokines, metabolically-active agents secreted by adipose tissue have been implicated in a variety of cardiovascular, metabolic, and inflammatory-mediated diseases (31). Discovery that

patterns of distribution of the adipocytokines differ between the joint and the circulating compartment suggest that the joint is a unique area of activity for adipocytokines (32-35).

Most efforts to examine the adipocytokines and osteoarthritis have focused on leptin because of its strong correlation with body size. Leptin levels in synovial fluid are correlated with the severity of knee osteoarthritis (36) and, as shown in Chapter Three, serum leptin levels are associated with prevalent and incident knee OA status among mid-aged women. Greater cartilage degradation, characterized by lower dGEMRIC (delayed gadolinium-enhanced magnetic resonance imaging of cartilage) indices (14) and less cartilage volume (26), is associated with higher serum leptin levels. However, leptin levels did not predict 2-year changes in cartilage volume or cartilage defects (37).

Utilization of MRI technology provides an opportunity to examine the role that obesity plays in joint damage. In addition to providing direct assessment of bone (osteophytes and bone marrow lesions) and cartilage defects, MRI provides imaging of other soft tissues which may be subject to obesity-related damage including the meniscus and ligaments as well as markers of local inflammation including synovitis and joint effusion (38). This paper examines the relationship of baseline and longitudinal serum leptin measures and MRI-assessed cartilage degradation, bone marrow lesions, osteophytes, meniscal abnormalities, joint effusions and synovitis among a cohort of mid-life women.

METHODS

Study population. The Michigan Study of Women's Health Across the Nation (SWAN) is one of seven clinical sites for SWAN, a multiethnic cohort study characterizing the endocrinological, physiological, and behavioral changes occurring during the menopausal transition. The Michigan SWAN population, established in 1996, is a population-based sample of eligible women from two Detroit-area communities. The population was identified with a community census based on the electrical utility listings of the targeted communities. Households were contacted by telephone (if available) or in-person. A total of 543 eligible women were recruited from the Michigan site to the SWAN Core Longitudinal Study, including 325 African American and 218 Caucasian women. Eligibility criterion at baseline included 42-52 years of age, having an intact uterus, having had at least one menstrual period in the previous 3 months, no use of reproductive hormones in the previous 3 months, and self-identification with the site's designated race or ethnic group (either African American or Caucasian at Michigan).

Commencing with the 1996 baseline, Michigan SWAN women have completed annual assessment protocols and specimen collection common to all SWAN sites with 80% of participants seen at follow-up visit 11. At follow-up visit 11 (2007), 387 Michigan SWAN women participated in a supplemental osteoarthritis imaging protocol including knee radiographs and knee magnetic resonance imaging (MRI). Women were not included in the MRI protocol if they were ineligible due to artificial joints, pacemakers, defibrillators, or other implanted metal considered incompatible with MRI (n=10) or if they refused participation (n=15), leaving 364 women available for this analysis. Women

who did have MRI data at follow-up visit 11 did not differ in baseline age, body size, leptin levels, race/ethnicity or menopause status as compared to those with knee MRIs (Table 4.2.) but they were more likely to be current smokers (P=0.02).

The University of Michigan Institutional Review Board approved the study protocol, and written informed consent was obtained from each participant.

Magnetic Resonance Imaging. Knee joints were imaged using a 3T (Model Achieva, Philips Healthcare, Andover, Massachusetts) or 1.5 T (GE Signa, GE Medical Systems, Milwaukee, WI) MR scanner. Specific sequences included sagittal, coronal, and axial fast spin echo (FSE) proton density (PD) with fat saturation (FS) sequences [repetition time (TR) 4000 msec, echo time (TE) 15 msec, 4 mm thickness), sagittal spin echo (SE) PD (TR 1000 msec, TE 14 msec, 3 mm thickness), and sagittal 3-D spoiled gradient echo (SPGR) with FS (TR 38 msec, TE 6.9 msec, flip angle [FA] 45°, 2 mm effective thickness). The FSE PD FS sequences were chosen to enable tissue contrast between articular cartilage, bone, and fluid, while still maintaining a high signal to noise ratio for evaluation of periarticular soft-tissues (39-42). SPGR FS images are included for additional assessment of articular cartilage. MR images of each knee were scored by two musculoskeletal radiologists, globally and by compartment for cartilage defects, subchondral bone marrow lesions (BML), osteophytes, meniscal tears, joint effusions and synovitis as described in Table 4.1.

To ensure reproducibility, following an initial calibration session to generate comparable scored values (n=20), a rigorous quality-control program was maintained such that 60% of MRI images were double-read with agreement (Kappa statistic) in excess of 90%. Scoring discrepancies were resolved by consensus. Radiologists were blinded to radiographic and clinical findings.

Cartilage defects were evaluated with respect to severity and approximate thickness at four specific surfaces (medial tibial, medial femoral, lateral tibial, and lateral femoral). Cartilage defects were scored for depth on the basis of the classification adopted by Drape et al. (43) and across multiple compartments with use of the Noyes arthroscopic system, adapted to MR imaging (44). *Bone marrow lesions* were evaluated at the medial tibial, medial femoral, lateral tibial and lateral femoral surfaces. Bone marrow lesions were defined as focal but non-circumscribed areas of abnormal high signal on FSE PD FS images, in a subchondral location and were measured perpendicular to the bone cortex (42,45). *Osteophytes* were evaluated in the medial and lateral compartments and were defined as abnormal bone growths arising from the margin of the involved compartment (46). Tibial spine growths were considered as osteophytes in their respective compartments only if there were definite excrescences as opposed to mere "pointing" of the tibial spines. *Meniscal abnormalities/tears* were evaluated in the medial and lateral compartments. Intrameniscal abnormalities as well as frank tears incorporated a modification of the system of Crues et al. (47-49). *Synovitis* was defined as abnormally increased linear or irregular striations within the infrapatellar (Hoffa) fat pad or joint recesses and irregular thickening at the margin of the fat pad with the articular cartilage

(50,51). These changes consisted of low signal on SE short TE images and intermediate-to-high signal on FSE PD FS images. *Joint effusions* represent substantially greater than expected physiologic amounts of synovial fluid in the lateral or medial patellar recesses exceeding 10 mm in width (52).

Data used for this analysis represent each participant's maximum score for cartilage defects, BMLs, osteophytes, meniscal tears, synovitis and joint effusion across knees and by compartment, as appropriate.

Body Size Measures. At each annual examination, height (cm) and weight (kg) was measured using a stadiometer and calibrated balance-beam scale, respectively, while participant's wear a single layer of light clothing and no shoes. Body mass index (BMI, kg/m^2) was calculated as weight in kilograms divided by the square of height in meters. Waist (cm) and hip (cm) circumferences have also been measured at each annual examination, using a non-stretchable tape 3 cm above the umbilicus, after a relaxed expiration, and the maximum girth around the buttocks, respectively. Body composition was measured using bioelectrical impedance (BIA). The measures, resistance and reactance, can be used to estimate body composition because the body is composed of conductive intracellular and extracellular materials separated by insulating layers of materials such as lipids. This is the technique being used in the National Health and Nutrition Examination Survey (NHANES) body composition assessments.

Leptin Assay. The SWAN specimen collection protocol includes a fasted (minimum 10-hour) blood draw to provide samples for a specimen repository that is maintained at -80°C until processing. Serum leptin levels were determined spectrophotometrically using commercially-available colorimetric enzyme immunoassay kits (Cayman Chemical, Ann Arbor, MI) and run according to the manufacturer's instructions. The coefficient of variation percent for duplicate samples for each subject is 0.4-12.4% and the lower limit of detection is 1 ng/mL. Banked specimens from baseline and visits 1, 3, 4, 5, 6 and 7 were assayed for leptin.

Other Measures. Participants were asked about their current smoking status (yes/no) at each annual visit. Race/ethnicity classification as African American or Caucasian was determined by self-report at baseline. Age at each annual visit was calculated as date of annual visit minus date of birth.

Menopause status was ascertained at each annual exam based on questions about bleeding patterns, current hormone use, hysterectomy and oophorectomy. At each visit, participants were categorized as being premenopausal, early perimenopausal, late perimenopausal, postmenopausal, hysterectomy, or unable to determine due to exogenous hormone use. Premenopausal status was defined as regular menses with bleeding in the past three months, early perimenopausal status was defined as bleeding in the past three months but increasing irregularity in menses, late perimenopausal status was defined as bleeding in the past year but not in the past three months, and postmenopausal status was defined as no bleeding for 12 months.

Statistical Analysis. The prevalence (frequencies and percents) of cartilage defects, bone marrow lesions, osteophytes, meniscal tears, synovitis and joint effusions were reported overall and by radiographic knee OA status. Means and standard deviation (SD) or frequencies and percents of leptin, body size and relevant covariates at baseline were examined overall and by categories of MRI knee joint damage from follow-up visit 11; the statistical significance of these relationships were assessed using analysis of variance or chi-square tests at the $\alpha=0.05$ level. Multivariable analyses using ordinal logistic regression analysis were conducted to relate each of the knee MRI variables from follow-up visit 11with baseline leptin measures adjusting for relevant covariates. Longitudinal linear mixed models (PROC MIXED) with random intercepts and slopes for age were used to examine the level and rates of change in leptin measures over time, stratified by category of cartilage defects, bone marrow lesions, osteophytes, meniscal tears, synovitis and joint effusions at follow-up visit 11.

Due to the collinearity between body size and leptin, all multivariable modeling included residuals of BMI as the measure of body size confounding. The BMI residual variable represents the variation in BMI that remains following simple regression of BMI on leptin. Given that leptin represents the metabolic component of body size, the BMI residual represents the association of body size and joint damage through other pathways, including mechanical loading. Interactions of the BMI residual and leptin were tested to assess potential effect modification of the relationship between leptin and joint damage by body size.

Model fit and final model selection was evaluated using Akaike's information criterion and chi-square tests comparing the log likelihood ratios between candidate models. Models were adjusted for age, race/ethnicity, menopause status (or hysterectomy yes/no), current smoking status, and BMI residuals, as appropriate. Statistical significance was defined at $\alpha<0.05$ and all analyses were completed using SAS v9.3 (SAS Institute, Cary, NC).

RESULTS

The prevalence of the cartilage defects, bone features and markers of local inflammation observed on the MRIs are reported in Table 4.3. Nearly all participants had cartilage defects; the prevalence of full-thickness defects was 24% among this population of mid-aged women. Bone marrow lesions (BML) were also common; 29% of women had a "small" (≤1 cm) BML and 12% had a large/very large BML (> 1 cm). Only 20% of women had no osteophytes on MRI. Of those women with knee osteophytes, 60% were less than 5 mm, 28% were 5-10 mm and 12% were greater than 10 mm in size. More than half of the sample had meniscal tears; 31% of women had displaced or macerated tears. Knee synovitis was present in 33% of the women and approximately one-third of those women had moderate-to-marked synovitis. Joint effusions were very common and 14% of the participants had moderate-large effusions (more than 10 mm in size).

Women with radiographically-defined knee OA had greater prevalence of cartilage defects, BMLs, osteophytes, meniscal tears, synovitis and effusion as compared to those

without knee OA (Table 4.3.). Notably, however, some women with knee OA had no BMLs, osteophytes, synovitis, or joint effusions and had normal menisci. Similarly, some women without knee OA had joint damage as defined by the presence of these knee MRI features.

Severity of knee cartilage defects increased with greater baseline body size, including greater weight, BMI, waist circumference, hip circumference waist:hip ratio, fat mass and skeletal muscle mass (Table 4.4.) but did not differ by height, demographic characteristics, menopause status or smoking status. Baseline weight and BMI among women with full-thickness cartilage defects was 25% greater as compared to women with cartilage defects < 50% thickness and baseline fat mass was 40% greater. Similar associations with body size were observed for BMLs, osteophytes, meniscal tears, synovitis and joint effusion (see Appendix A-E.).

Leptin levels were strongly associated with having severe cartilage defects, larger bone abnormalities and more meniscal tears, synovitis and effusion (Table 4.5.). At both baseline and follow-up visit 7 (the last year in which leptin measures were available), we observed a statistically significant increasing trend in leptin levels with greater severity of all measures of knee joint damage. Correlations were greatest between leptin levels at baseline and knee osteophytes on MRI at visit 11 (r=0.41), followed by effusion (r=0.34), synovitis (r=0.31), cartilage defects (r=0.28), bone marrow edema (r=0.24) and meniscal abnormalities (r=0.22) (Figure 4.1.). Leptin levels increased from baseline to follow-up visit 7 overall and within each category of cartilage defects, BML, osteophytes, meniscal

tears, synovitis and effusion but the amount of change was not associated with severity of knee joint damage.

As shown in Table 4.6., higher leptin levels at baseline were associated with greater odds of having more severe knee joint damage at follow-up visit 11 after adjusting for age, smoking status, menopause status and BMI residuals. The odds ratios associated with a 5 ng/mL change in baseline leptin ranged from 1.22 to 1.52. The greatest effect was observed for osteophytes; a 5 ng/mL increase in baseline leptin values was associated with 52% higher odds being in the next severity category of osteophytes (95% CI 1.40, 1.65). Baseline age was positively associated with more severe cartilage defects and bone marrow edema in the multivariable models.

Trajectories of leptin levels from baseline through follow-up visit 7 were modeled and stratified by levels of cartilage defects, BMLs, osteophytes, meniscal tears, synovitis and effusion. The greatest differences in leptin levels at baseline and over time were observed with respect to cartilage defects, osteophytes, synovitis and joint effusion. As shown in Figure 4.2., compared to those with no cartilage defects, leptin levels at age 42 years were 12.2 ng/mL higher among women with 50-99% thickness cartilage defects and 21.7 ng/mL higher among women with full-thickness cartilage defects ($P=0.02$ and $P<0.0001$, respectively). Women with full-thickness cartilage defects had higher leptin levels at age 42 as compared to even the highest leptin levels among women with less severe cartilage defects. The rate of change in leptin levels over time was attenuated with

increases in age among those with more severe cartilage defects as compared to those with no cartilage defects ($P<0.05$).

Leptin levels at age 42 years were, on average, 27 ng/mL higher among women with osteophytes > 10 mm; 21 ng/mL higher for those with ostophytes 5-10 mm; and 9 ng/mL higher for those with osteophytes < 5 mm (all $P<0.0001$). As shown in Figure 4.3., leptin levels among women with large (> 5 mm) osteophytes were consistently higher as compared to women with small (≤ 5 mm) or no osteophytes. Women with moderate to marked synovitis at follow-up visit 11 had 16.8 ng/mL higher leptin levels at age 42 years as compared to women with no synovitis ($P<0.0001$) (Figure 4.4.). Similarly, women with moderate to large effusions had leptin levels 20 ng/mL higher on average at age 42 as compared to women with only normal physiologic fluid ($P<0.0001$).

DISCUSSION

Leptin, an adipocytokine secreted by adipose tissue is speculated to be part of the biological mechanism which links obesity and osteoarthritis (53-56). This paper is the first to examine the relationship between leptin levels and multiple characteristics of knee joint damage imaged using MRI. We found that leptin levels ten years prior to MRI assessment were associated with the presence of cartilage defects, bone marrow lesions, osteophytes, meniscal tears, synovitis and effusion among a population of mid-aged women. This study is particularly novel in that it directly addresses an important hypothesis with respect to obesity and OA using epidemiological data from a large longitudinal study of mid-life women. The Michigan SWAN study provides a unique

opportunity to examine the role of leptin with respect to knee osteoarthritis given the availability of longitudinal leptin measures, careful ascertainment of potential confounders including body size, menopause status and smoking, and characterization of knee joint status using both radiographs and magnetic resonance imaging on a large cohort of women.

While leptin levels have been reported to be associated with severity of knee osteoarthritis (36) and we showed that leptin was associated with prevalent and incident knee OA in Chapter Three, questions remain about the mechanism by which leptin may modulate joint damage. Leptin may have both an anabolic and catabolic impact on joint tissues. The anabolic effect of leptin on chondrocytes and osteoblasts may be associated with osteophyte development (32), a hallmark of OA and part of the Kellgren-Lawrence (K-L) based scoring system of radiographs. However, leptin is well-known to exhibit pro-inflammatory properties and is associated with increased production of interleukin-1 beta (IL-1β), matrix metallopeptidase 9 (MMP-9) and MMP-13 (33). Thus, leptin may play a catabolic role in cartilage degradation, another hallmark of osteoarthritis.

Previous studies of leptin and OA, including our analysis in Chapter Three, have utilized radiographs to characterize OA status using K-L scores (36) or have only focused on cartilage when using MRI (14,26). Examination of BMLs, osteophytes, meniscal tears, synovitis and joint effusion in addition to cartilage defects provides an opportunity to learn more about the importance of leptin with respect to anabolic, catabolic and inflammatory mechanisms purported in joint damage. Data from the Michigan SWAN

population demonstrates that leptin is associated with increased severity of all measures of knee joint damage after adjustment for age, menopause status, smoking status and BMI. Leptin measures were most strongly correlated with osteophytes and greatest differences in leptin levels were observed across categories of osteophyte severity. These findings provide preliminary evidence to suggest that the anabolic impact of leptin may be particularly important early in the disease process. This hypothesis is further strengthened by the observation that baseline leptin levels were similar among women with cartilage defects <50% and women without cartilage defects. While it is probable that leptin also plays a catabolic role in cartilage damage, the data suggests that this may occur later in the disease process as leptin is associated with more severe cartilage defects.

Similar to findings reported previously (4), we observed a high prevalence of full-thickness cartilage defects, meniscal tears, synovitis and joint effusion in this population of mid-aged women. We have previously reported the prevalence of these MRI-defined abnormalities on a by-knee basis and across the medial, lateral and patellofemoral compartments, where appropriate. In the current analysis, we chose to analyze our data on a by-woman basis and utilized maximum severity scores across knees and compartments given that it is hypothesized that the effect of serum leptin on the knee joint would be systemic. This difference in analytical approaches explains the slightly higher prevalences reported as compared to our previous paper (4). Other studies have observed cartilage damage, presence of BMLs, and meniscal tears among mid-aged adults (18,57,58). Guymer et al. reported 13% prevalence of BMLs among mid-aged

women without knee OA (18); the prevalence in our non-osteoarthritic population was 17%. Our reported prevalence of meniscal tears (31%) are slightly higher than those reported among mid-aged women from Framingham, Massachusetts (19%), although the prevalence of radiographic knee osteoarthritis is notably different (63% vs. 18%) (58).

Limitations of this analysis include the fact that leptin measures were collected prior to ascertainment of MRI-defined knee joint status, thus, no definitive statement with regard to causality can be made. Further, unlike other studies, MRIs were not scored for cartilage volume but instead for a categorical cartilage defects score which is not necessarily analogous to cartilage volume. Our analytical approach was complicated by the fact that leptin, being a product of adipose tissue, is highly correlated with all measures of body size and greater body size is a hypothesized risk factor for joint damage through non-metabolic mechanisms such as increased joint loading or poorer muscle strength. We adjusted for the residuals of BMI on leptin in an effort to quantify the influence of body size independent of the metabolic effects of leptin.

In conclusion, this paper reports that leptin levels are associated with MRI-defined cartilage defects, bone marrow lesions, osteophytes, meniscal abnormalities, synovitis and joint effusion and that correlations were strongest with osteophytes. These findings suggest that the role of leptin is primarily anabolic in the early stages of the joint degradation process. Replication of these findings in other populations with leptin measures and knee MRI data is needed. Understanding the role that leptin plays in the joint degradation process is critical to develop more targeted interventions for

osteoarthritis with respect to timing of disease onset and mechanisms by which leptin acts on the joint.

Table 4.1. Description of Michigan Study of Women's Health Across the Nation (SWAN) Knee Magnetic Resonance Imaging Protocol for Cartilage Defects, Bone Marrow Lesions, Osteophytes, Meniscal Abnormality/Tears, Synovitis, and Joint Effusion.

Cartilage Defects	
0	Normal, internal signal alteration only
1	Cartilage defect < 50% thickness
2	Cartilage defect 50-99% thickness
3	Cartilage defect 100% thickness with or without bone ulceration
Bone Marrow Lesions	
0	Normal
1	Largest diameter ≤ 1 cm
2	Largest diameter > 1 cm
Osteophytes	
0	No ostephyte
1	Osteophyte ≤ 5 mm
2	Osteophyte > 5 mm ≤ 10 mm
3	Osteophyte > 10 mm
Meniscal Tears	
0	Normal
1	Intrasubstance meniscal abnormality only (Crues Grade 1 and 2)
2	Non-displaced tear extending to meniscal surface (Crues Grade 3)
3	Displaced or macerated tear
Synovitis	
0	Normal
1	Mild synovitis
2	Moderate-to-marked synovitis
Joint Effusion	
0	Physiologic fluid
1	Small effusion (≤ 10 mm)
2	Moderate and/or large effusion (> 10 mm)

Table 4.2. Baseline Characteristics of Michigan Study of Women's Health Across the Nation (SWAN) Women With and Without Knee Magnetic Resonance Imaging (MRI) Assessments at Follow-Up Visit 11.

	No follow-up visit 11 MRI n=179	**Follow-up visit 11 MRI** n=364	
	Mean (SD)	**Mean (SD)**	**P-value**
Age (years)	46.1 (2.7)	46.1 (2.8)	0.85
Height (cm)	163.3 (6.3)	163.5 (6.2)	0.78
Weight (kg)	84.9 (21.5)	86.2 (22.0)	0.53
BMI (kg/m^2)	31.7 (8.0)	32.2 (8.1)	0.46
Waist circumference (cm)	93.8 (17.6)	94.3 (17.0)	0.73
Hip circumference (cm)	113.5 (16.2)	114.0 (16.3)	0.70
Waist-hip ratio	0.82 (0.07)	0.82 (0.07)	0.63
Fat mass (kg)	35.0 (15.7)	37.0 (16.7)	0.20
% Fat mass	39.7 (8.8)	41.1 (9.2)	0.10
Skeletal muscle mass (kg)	21.8 (3.4)	21.7 (3.4)	0.80
Leptin (ng/mL)	29.1 (19.3)	31.5 (18.3)	0.16
	N (%)	**N (%)**	
Obese (BMI ≥ 30 kg/m^2)	98 (56.0%)	203 (56.1%)	0.99
Current Smoker	59 (33.5%)	87 (24.2%)	0.02
Race/ethnicity			
African American	98 (54.8%)	227 (62.4%)	0.09
Caucasian	81 (45.3%)	137 (37.6%)	
Menopause Status			
Premenopausal	90 (50.6%)	182 (50.3%)	0.95
Early peri-menopausal	88 (49.4%)	180 (49.7%)	

Table 4.3. Prevalence of Knee Magnetic Resonance Imaging Findings Overall and by Radiograph-Defined Knee Osteoarthritis (OA) Status at Follow-Up Visit 11 Among 364 Michigan Study of Women's Health Across the Nation (SWAN) Participants.

	Overall (n=364)	No knee OA (n=135)	Knee OA (n=225)
Cartilage defects	**N (%)**	**N (%)**	**N (%)**
Normal, internal signal alteration only	8 (2.2%)	5 (3.7%)	3 (1.3%)
Cartilage defect < 50% thickness	143 (39.3%)	82 (60.7%)	60 (26.7%)
Cartilage defect 50-99% thickness	127 (34.9%)	44 (32.6%)	81 (36.0%)
Cartilage defect 100% thickness	86 (23.6%)	4 (3.0%)	81 (36.0%)
Bone marrow lesions (BML)			
Normal	215 (59.1%)	112 (83.0%)	101 (44.9%)
Largest diameter ≤ 1 cm	104 (28.6%)	21 (15.6%)	81 (36.0%)
Largest diameter > 1 cm	45 (12.4%)	2 (1.5%)	43 (19.1%)
Osteophytes			
None	74 (20.3%)	59 (43.7%)	15 (6.7%)
≤ 5 mm	174 (47.8%)	74 (54.8%)	96 (42.7%)
5-10 mm	82 (22.5%)	2 (1.5%)	80 (35.6%)
> 10 mm	34 (9.3%)	0 (0.0%)	34 (15.1%)
Meniscal abnormalities/tears			
Normal	18 (5.0%)	7 (5.2%)	11 (4.9%)
Intrasubstance meniscal abnormality only	157 (43.1%)	91 (67.4%)	63 (28.0%)
Non-displaced tear	77 (21.2%)	26 (19.3%)	50 (22.2%)
Displaced or macerated tear	112 (30.8%)	11 (8.2%)	101 (44.9%)
Synovitis			
Normal	241 (66.2%)	125 (92.6%)	113 (50.2%)
Mild synovitis	90 (24.7%)	10 (7.4%)	79 (35.1%)
Moderate-to-marked synovitis	33 (9.1%)	0 (0.0%)	33 (14.7%)
Effusion			
Physiologic fluid	61 (16.8%)	37 (27.4%)	24 (10.7%)
Small effusion (≤ 10 mm)	253 (69.5%)	97 (71.9%)	152 (67.6%)
Moderate and/or large effusion (> 10 mm)	50 (13.7%)	1 (0.7%)	49 (21.8%)

Table 4.4. Baseline Characteristics of Michigan Study of Women's Health Across the Nation (SWAN) Women by Knee Cartilage Defect Severity From Magnetic Resonance Imaging at Follow-Up Visit 11.

	Cartilage Defect Score				
	None, internal signal alteration only	Defect < 50% thickness	Defect 50-99% thickness	Defect 100% thickness	P-value
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)	46.0 (3.5)	45.8 (2.6)	46.3 (3.0)	46.2 (2.7)	0.42
Weight (kg)	69.6 (12.7)	80.0 (20.2)	85.0 (21.1)	99.8 (20.8)	<0.0001
Height (cm)	163.9 (4.8)	163.7 (5.6)	163.0 (6.1)	163.9 (7.1)	0.67
BMI (kg/m^2)	25.9 (4.8)	29.8 (7.4)	32.0 (7.5)	37.3 (7.9)	<0.0001
Waist circumference (cm)	81.5 (7.3)	89.3 (15.9)	94.0 (15.6)	104.2 (16.9)	<0.0001
Hip circumference (cm)	102.3 (11.5)	109.6 (14.7)	112.8 (15.4)	124.3 (16.0)	<0.0001
Waist:hip ratio	0.80 (0.07)	0.81 (0.07)	0.83 (0.07)	0.84 (0.07)	0.03
Fat mass (kg)	23.6 (9.2)	33.0 (14.8)	36.2 (15.8)	46.1 (17.8)	<0.0001
Skeletal muscle mass (kg)	20.5 (1.7)	20.9 (3.3)	21.5 (3.3)	23.5 (3.1)	<0.0001
	n (%)	n (%)	n (%)	n (%)	
Obese (BMI≥30 kg/m^2)	1 (12.5%)	63 (44.4%)	69 (54.3%)	70 (82.4%)	<0.0001
Current Smoker	2 (25.0%)	35 (25.0%)	36 (28.4%)	14 (16.7%)	0.28
Ethnicity					
African-American	7 (87.5%)	88 (61.5%)	79 (62.2%)	53 (61.6%)	0.53
Caucasian	1 (12.5%)	55 (38.5%)	48 (37.8%)	33 (38.4%)	
Menopause Status					
Premenopausal	3 (37.5%)	77 (53.9%)	64 (50.8%)	38 (44.7%)	0.51
Early Perimenopausal	5 (62.5%)	66 (46.2%)	62 (49.2%)	47 (55.3%)	

Table 4.5. Serum Leptin Levels at Baseline and Follow-Up Visit 7 According to Magnetic Resonance Imaging-Defined Knee Cartilage Defects, Bone Marrow Lesions, Osteophytes, Meniscal Tears, Synovitis, and Joint Effusions Among Michigan Study of Women's Health Across the Nation (SWAN) Women at Follow-Up Visit 11.

	Baseline Leptin (ng/mL)	Visit 7 Leptin (ng/mL)
	Mean (SD)	Mean (SD)
Cartilage defects		
Normal, internal signal alteration only	14.9 (5.7)	27.0 (9.1)
Cartilage defect < 50% thickness	27.4 (16.1)	33.5 (19.1)
Cartilage defect 50-99% thickness	31.3 (17.4)	37.1 (19.4)
Cartilage defect 100% thickness with or without bone ulceration	40.2 (20.4)	47.1 (22.6)
P-value	<0.0001	<0.0001
Bone marrow lesions (BML)		
Normal	27.6 (16.6)	35.0 (19.8)
Largest diameter ≤ 1 cm	36.6 (19.9)	40.4 (20.5)
Largest diameter > 1 cm	37.4 (17.9)	45.9 (22.8)
P-value	<0.0001	0.005
Osteophytes		
None	21.5 (16.2)	28.8 (17.7)
≤ 5 mm	29.5 (15.1)	34.3 (18.2)
5-10 mm	38.7 (20.6)	46.2 (21.1)
> 10 mm	45.9 (16.6)	56.5 (20.6)
P-value	<0.0001	<0.0001
Meniscal abnormalities/tears		
Normal	27.7 (16.5)	40.8 (19.8)
Intrasubstance meniscal abnormality only	27.4 (17.2)	31.9 (18.2)
Non-displaced tear	33.7 (17.3)	40.1 (21.2)
Displaced or macerated tear	36.3 (19.4)	44.6 (21.7)
P-value	0.0007	<0.0001
Synovitis		
Normal	27.5 (16.3)	34.3 (19.8)
Mild synovitis	37.6 (18.4)	43.3 (19.5)
Moderate-to-marked synovitis	44.0 (21.9)	48.4 (23.9)
P-value	<0.0001	<0.0001
Effusion		
Physiologic fluid	24.5 (15.8)	29.3 (16.5)
Small effusion (≤ 10 mm)	29.6 (15.9)	37.5 (20.8)
Moderate and/or large effusion (> 10 mm)	48.8 (21.7)	50.6 (18.9)
P-value	<0.0001	<0.0001

Table 4.6. Odds Ratios (95% Confidence Intervals) of Baseline Serum Leptin in Relation to Magnetic Resonance Imaging-Defined Knee Cartilage Defects, Bone Marrow Lesions, Osteophytes, Meniscal Tears, Synovitis, and Joint Effusion Among Michigan Study of Women's Health Across the Nation (SWAN) Women at Follow-Up Visit 11.*

	Leptin	
	Odds Ratio[†]	**95% Confidence Interval**
Cartilage defects	1.28	1.18, 1.37
Bone marrow lesions	1.22	1.13, 1.32
Osteophytes	1.52	1.40, 1.65
Meniscal abnormalities/tears	1.24	1.16, 1.34
Synovitis	1.35	1.23, 1.48
Effusion	1.35	1.24, 1.48

*All models adjusted for age, menopause status, smoking status and BMI.
[†] Odds ratio represents 5 ng/mL change in leptin.

Figure 4.1. Spearman Rank Correlations (95% Confidence Intervals) for Magnetic Resonance Imaging-Defined Knee Cartilage Defects, Bone Marrow Lesions, Osteophytes, Meniscal Tears, Synovitis and Joint Effusion with Serum Leptin Levels at Baseline Among Michigan Study of Women's Health Across the Nation (SWAN) Participants.

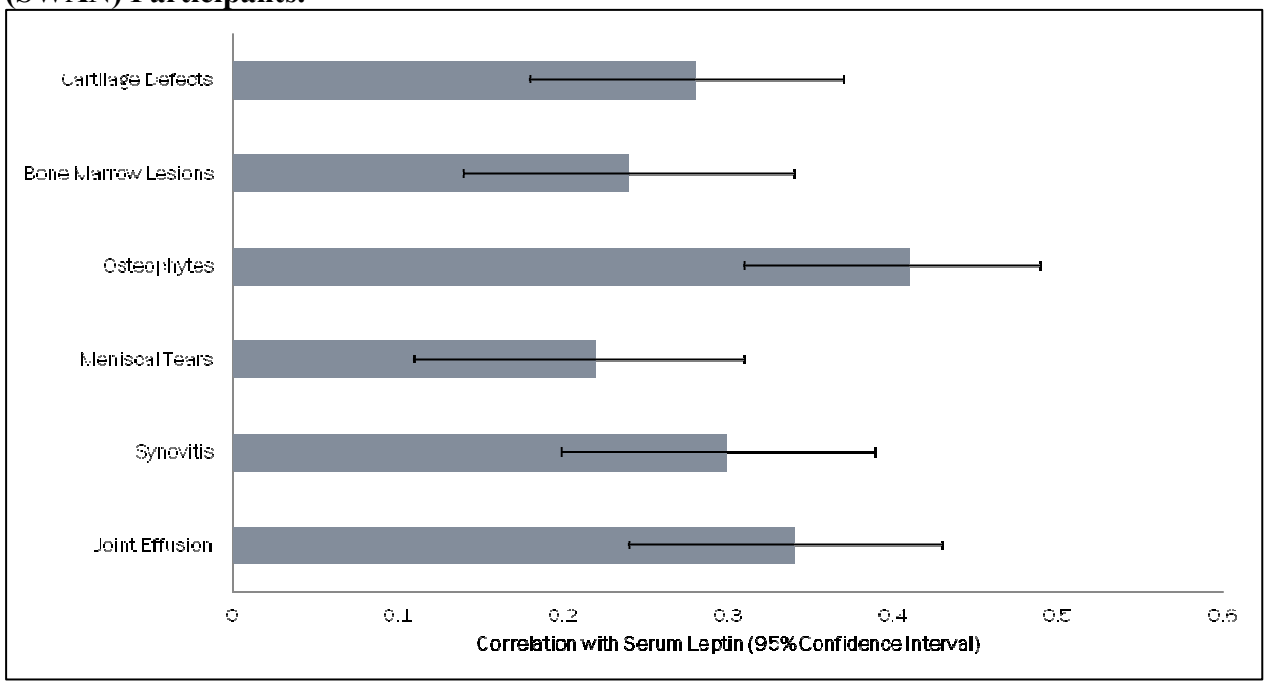


Figure 4.2. Predicted Trajectories of Serum Leptin (ng/mL) by Magnetic Resonance Imaging-Defined Knee Cartilage Defects at Follow-Up Visit 11 Among Michigan Study of Women's Health Across the Nation (SWAN) Participants. Brown Line Represents Women With No Cartilage Defects (Signal Alteration Only); Green Line Represents Women With Cartilage Defects < 50% Thickness; Red Line Represents Women With Cartilage Defects 50-99% Thickness; Blue Line Represents Women With Cartilage Defects 100% Thickness.

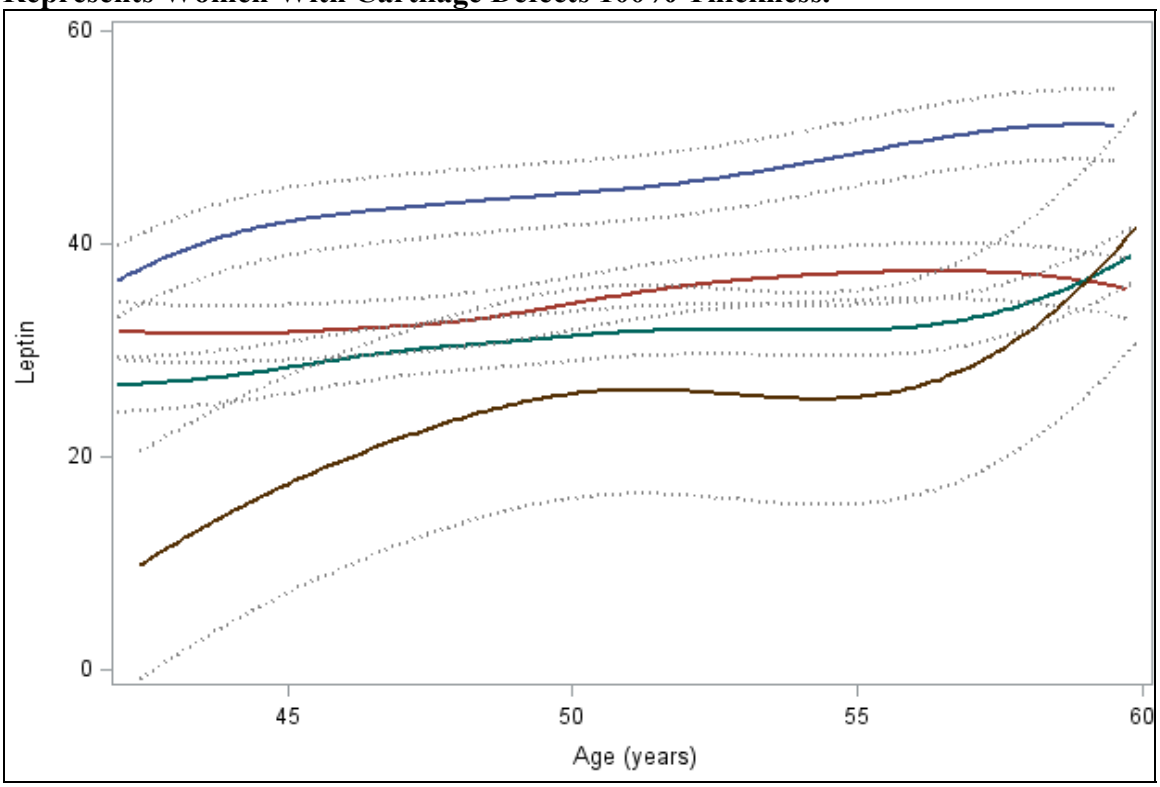


Figure 4.3. Predicted Trajectories of Serum Leptin (ng/mL) by Magnetic Resonance Imaging-Defined Knee Osteophytes at Follow-Up Visit 11 Among Michigan Study of Women's Health Across the Nation (SWAN) Participants. Brown Line Represents Women With No Osteophytes; Green Line Represents Women With Osteophytes ≤ 5 mm; Red Line Represents Women With Osteophytes 5-10 mm; Blue Line Represents Women With Osteophytes > 10 mm.

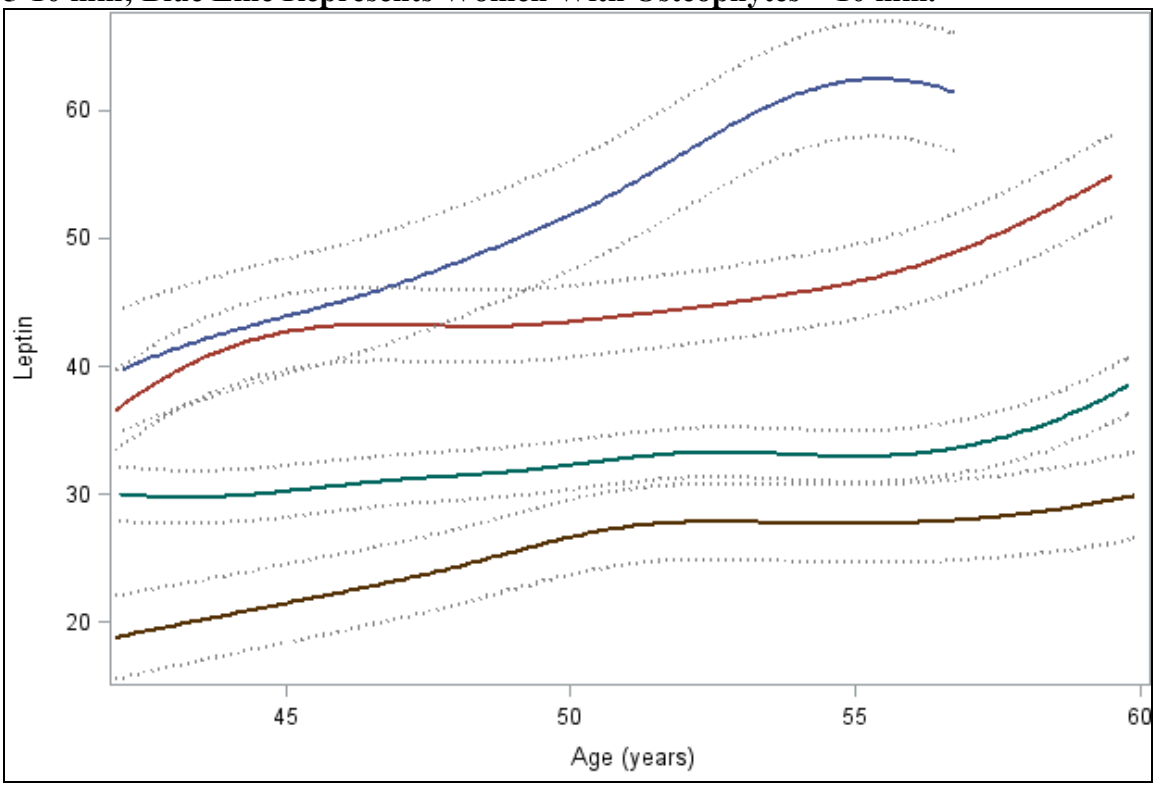
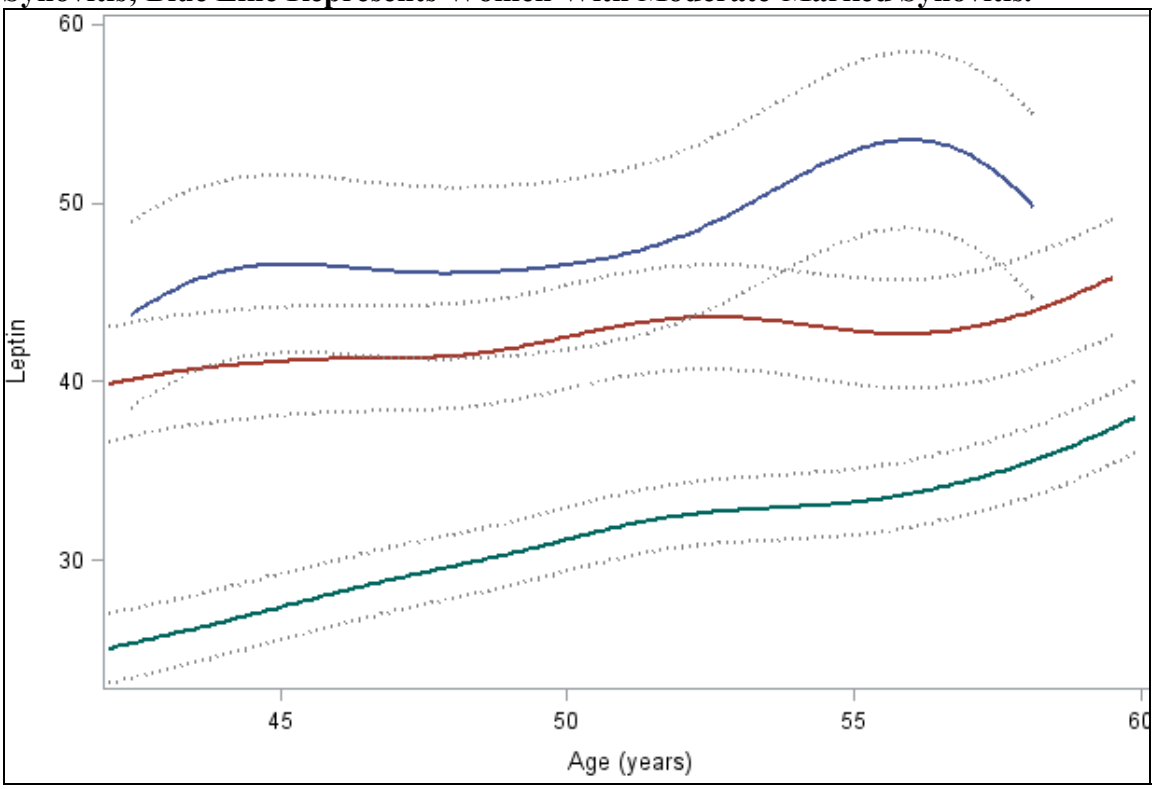


Figure 4.4. Predicted Trajectories of Serum Leptin (ng/mL) by Magnetic Resonance Imaging-Defined Knee Synovitis at Follow-Up Visit 11 Among Michigan Study of Women's Health Across the Nation (SWAN) Participants. Green Line Represents Women With No Synovitis; Red Line Represents Women With Mild Synovitis; Blue Line Represents Women With Moderate-Marked Synovitis.



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CHAPTER FIVE

Discussion

OVERVIEW

This dissertation represents one of the first epidemiologic efforts to examine the association of cardiometabolic biomarkers and knee osteoarthritis beyond the impact of sheer body size.

Osteoarthritis (OA) is a complex disease characterized by failure of the entire joint organ including degradation of cartilage, exposure of underlying subchondral bone, development of osteophytes, damage to soft tissues including the menisci and ligaments and presence of local inflammatory responses characterized by synovitis and joint effusions. Obesity is an important and consistently-reported risk factor for osteoarthritis prevalence and progression, particularly in weight-bearing joints. Logically, greater body mass causing increased mechanical loading across weight-bearing joints such as the knees and hips has been an operative paradigm for many years.

It is estimated that compressive loads across the weight-bearing joints are three times body weight during walking and six times body weight during stair climbing among

normal-weight individuals (1). These compressive forces are exacerbated in conditions including osteoarthritis and obesity. Greater mechanical loading across weight-bearing joints have been associated with joint damage (2-4) and so this has traditionally been the paradigm by which obesity is thought to be associated with osteoarthritis.

Evidence of associations between obesity and osteoarthritis in non-weight bearing joints (5-9), however, raises questions about uniformity of the role of obesity in joint damage. Furthermore, appreciation of the complex role that adipose tissue plays in endocrine pathology has generated new hypotheses about the relationship of obesity and osteoarthritis. Basic science and animal models of osteoarthritis suggest that metabolic dysfunction, including dyslipidemia, disordered carbohydrate metabolism and systemic levels of adipocytokines are independently associated with joint damage. However, little is known about whether these relationships are observed in vivo among population based samples of women and men.

SUMMARY AND SIGNIFICANCE OF FINDINGS

This dissertation examined the metabolic impact of obesity on knee osteoarthritis using data from two population-based cohort studies, the National Health and Nutrition Examination Survey (NHANES) III and the Michigan Study of Women's Health Across the Nation (SWAN).

In Chapter Two, we related multiple measures of metabolic dysfunction to prevalent knee osteoarthritis among male and female participants aged 60 years and older from

NHANES III. Importantly, we observed a sex dimorphism with respect to the magnitude and direction of effect estimates for homeostatic model assessment-insulin resistance (HOMA-IR) and leptin. The relationship of HOMA-IR and knee OA was stronger among men as compared to women while serum leptin levels were associated with increased odds of knee OA in women but not in men. Among obese women, a 5 μg/L increase in serum leptin levels was associated with 28% higher odds of having prevalent knee OA. Knee OA was consistently associated with several markers of cardiometabolic risk including not only insulin resistance and higher levels of serum leptin but also with higher levels of low density lipoprotein-cholesterol (LDL-c) and high systolic blood pressure, even after adjustment for BMI. Our data also confirmed the importance of obesity as a risk factor for knee osteoarthritis as prevalence of obesity was twice as great among those having knee OA as compared to those without knee OA.

Given our findings in Chapter Two that systemic leptin may be an important risk factor for knee osteoarthritis among women, we investigated the relationship between leptin and knee OA among a cohort of mid-aged women from the Michigan SWAN Study. In Chapter Three, we evaluated the association between baseline and longitudinal serum leptin measures and prevalent and incident knee OA status over ten years of follow-up. Higher serum leptin levels were associated with increased odds of both prevalent and two-year incident knee OA; a 5 ng/mL increase in serum leptin levels was associated with 37% higher odds of knee OA. This estimate is similar in magnitude to that observed among obese women from the NHANES III population. In the longitudinal analysis, we reported that serum leptin levels increased with age, independent of increases in body

size. Most notably, however, women with prevalent knee OA at baseline had average serum leptin levels at age 42 that were higher than even the highest serum leptin levels among women who subsequently developed incident knee OA. Further, average serum leptin levels among women who remained disease free during follow-up were relatively low and never reached the average levels observed among women with prevalent or incident knee OA.

In Chapter Four, we extended our assessment of the potential impact of serum leptin on knee OA by evaluating the association between baseline and longitudinal serum leptin levels and knee joint damage assessed using magnetic resonance imaging (MRI) technology. Based on our findings, serum leptin levels ten years prior to MRI assessment, when women were between the ages of 42-52 years, were associated with the presence of more severe cartilage defects, larger bone marrow lesions and osteophytes, meniscal tears, and more marked synovitis and joint effusions. Correlations of serum leptin levels and the measures of joint damage were strongest for MRI-assessed osteophytes, and the largest differences in serum leptin values were observed across categories of MRI-assessed osteophytes. Importantly, our MRI data indicated an astonishingly high prevalence of knee joint damage during the mid-life. Tibio-femoral full-thickness cartilage defects were observed in nearly one-quarter of women and more than 30% of women had macerated meniscal tears.

Knee osteoarthritis is a highly prevalent condition affecting more than one-third of United States adults age 60 years and older. Onset of knee OA begins during middle age

and markers of end-stage joint damage are common during this time with obesity being a well-known and major risk factor for knee OA. This dissertation provides evidence that cardiometabolic dysfunction is associated with knee osteoarthritis, independent of body mass. Notably, we demonstrate that serum leptin levels during the mid-life (5th decade) are associated with knee OA status ten years later. Addressing the cardiometabolic impact of obesity among mid-aged populations may have important ramifications for the prevention or forestalling of incident knee osteoarthritis onset or progression through appropriate treatment and management of metabolic dysfunction.

STRENGTHS AND LIMITATIONS

Knee Radiograph Protocols. Though osteoarthritis was assessed in the NHANES III cohort using radiographs, they were obtained using non-weight bearing images and so the measure reflects osteophytes-defined radiographic knee OA status only and not the contribution of joint space narrowing, a proxy measure of cartilage loss (10,11). Thus, our estimates for cardiometabolic biomarkers as risk factors pertain only to osteophyte-defined radiographic knee OA and do not provide information about the potential impact of metabolic dysfunction on cartilage loss or other MRI-defined parameters of joint abnormality. Further, because the radiographs among Michigan SWAN women were obtained in the weight-bearing position, our estimates of knee OA prevalence in the two populations are not entirely comparable.

Algorithmic Estimation of Insulin Resistance. NHANES III is one of the largest datasets available with full characterization of cardiometabolic biomarkers. However,

because NHANES III was designed as a large epidemiological study, ascertainment of insulin resistance is estimated using an algorithm based on glucose and insulin to provide HOMA-IR, a proxy measure. While HOMA-IR and insulin sensitivity have been shown to be highly correlated, independent of glucose tolerance (12), some still question whether HOMA-IR is a good marker of insulin resistance (13). More sophisticated estimates such as glucose clamp measures are optimal and utilized in diabetic populations but are not appropriate for use in large epidemiologic studies.

Confounding. Residual confounding by body mass is possible. In all analyses the cardiometabolic biomarkers, particularly leptin which is a direct product of adipose tissue, were highly correlated with BMI. However, we adjusted for BMI or the residuals of BMI and the stability of our estimates across study populations and analytic methodologies lends support for the claim that our findings represent a real metabolic impact of obesity on knee OA.

Selection Bias. In all epidemiologic studies, cohort selection is an important potential source of bias. The NHANES III sample was selected and sampled to be representative of the general U.S. population and sampling weights, cluster and stratum variables were utilized in analysis to account for the complex sampling design. However, only adults aged 60 years and older were included in the radiograph protocol. We and others have demonstrated that the onset of radiographic osteoarthritis begins during the 5th decade (5,14) and some evidence suggests that the impact of obesity and cardiometabolic dysfunction may be especially important among those developing OA during mid-age

(5,15,16). Thus, using the NHANES III sample of only those aged 60+ years, our estimates may be conservative.

While the Michigan SWAN study is a population-based cohort study, sampled residents are only from two distinct communities in metro-Detroit, MI. As has been well-reported in the popular media over the past several years, the State of Michigan represents the upper end of the distribution in terms of body size (BMI) nationally. The 2010 obesity rate in Michigan was 30.9%, ranking it 8th overall (17). Among Michigan SWAN women, nearly two-thirds were obese at follow-up visit 11 (in 2007). Thus, obesity is highly prevalent among this population, even compared to State of Michigan statistics, and our observations with respect to leptin as a biomarker represent the impact of very high serum leptin levels on observed incidence and prevalence of knee OA.

Unbalanced Design and Assessment of Causality. In epidemiologic research, we are interested in data analyses that can provide insight into the causes of disease. While there are several definitions of causation, the concept of probabilistic causation, where a cause increases the probability that its effect will occur, is widely used given its practicality and flexibility of use (18). Probabilistic causative theory relies on the consideration of counterfactuals, although they are rarely observed directly. Instead, advanced epidemiological methods have been developed to estimate causal effects and the impact of confounding and effect-measure modification in the absence of direct assessment (19).

In our analysis of the relationship between serum leptin and knee OA, we were interested in the effect of serum leptin independent of BMI, as shown in the directed acyclic graph (DAG) below:

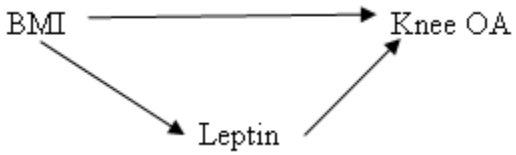

Figure 5.1. Directed Acyclic Graph (DAG) of direct and indirect effect of body size (body mass index, BMI) on knee osteoarthritis (OA) status.

Figure 5.1. indicates that body size (as represented by BMI) has a direct and indirect effect on knee OA status; BMI is (presumably) associated with knee OA through both mechanical loading and through serum leptin. Thus, to estimate the causal effect of serum leptin on OA we must consider BMI as a confounder. However, because of the high correlation of BMI and leptin ($r=0.73$, $P<0.0001$), conventional statistical adjustment in a multivariable model is statistically and biologically inappropriate. Instead, our approach adjusted for the residuals of BMI, which represents the variation in BMI that remains following simple regression of BMI on serum leptin. Given that serum leptin represents a metabolic component of total adiposity, the BMI residual can be interpreted as the association of body mass and OA through other pathways, including mechanical loading. This approach has been utilized previously in an analysis with similar methodological problems (20).

The main limitation of this approach is that the possibility of residual confounding remains and so definitive statements about causality cannot be made unambiguously.

Advanced epidemiologic methods, including marginal structural models (MSMs) with inverse proportional weighting (IPW) have been developed for these types of methodological quandaries, but these approaches are not well-understood by the clinical community (an intended readership of this research). Furthermore, MSMs rely on a hypothesized counterfactual for estimation of the IPW weights (21) and there is truly no counterfactual for the tails of the observed serum leptin distribution. Because leptin is produced by adipose tissue, there are no individuals in this population with very low serum leptin levels that have very high body mass and there are no individuals with very high serum leptin levels with low body mass.

The unbalanced data structure available for serum leptin measures and knee OA status further limited our ability to make causal inferences. Due to the 7-year gap between radiographic knee OA measures in the middle of the study, we were unable to differentiate between leptin as a causal factor for knee OA or as a consequence (through increased body size) of knee OA. Our observation that the estimated average range of serum leptin levels by age were mutually exclusive among women with prevalent, incident and no knee OA over the ten years of follow-up does, however, provide some suggestion that serum leptin may be associated with knee OA onset. Further, the similarity of the change in serum leptin levels over time across all three groups suggests no difference in the rate of change in serum leptin following knee OA onset as compared to expected increases with aging.

As discussed below, future work examining the relationship of leptin levels and osteoarthritis in non-weight bearing joints will allow for more direct assessment of causality because it eliminates the need to consider the impact of mechanical loading on large joints due to increased body mass.

Well-Characterized Datasets with Appropriate Measures. The NHANES III and the Michigan SWAN Study populations are major strengths of this work. Few epidemiologic studies have the breadth of data available for this type of analysis, including a full panel of cardiometabolic biomarkers and osteoarthritis assessment using radiography and MRI. The few studies that have been published on this topic are limited to a heterogeneous sample with respect to body size (22) or were narrow in focus, i.e., examining only cartilage (23-26) or bone marrow lesions (27). In Chapter Two, we utilized data from NHANES III, a sample representative of the U.S. population including both men and women with a wide panel of cardiometabolic biomarkers available for analysis. Our work represents the most comprehensive examination of cardiometabolic risk factors with respect to radiographic knee osteoarthritis to date. In Chapter Three and Chapter Four, data from the Michigan SWAN Study, a bi-racial population-based sample of mid-age women was chosen for investigation because of the availability of longitudinal serum leptin measures and full characterization of OA status through knee radiographs at four time points and knee MRIs at one time point.

Magnetic Resonance Imaging. While osteoarthritis is diagnosed based on radiographic images, it is well-appreciated that the disease is a culmination of joint failure at multiple

levels and tissues including cartilage, bone and soft tissue (28). Although OA is not generally diagnosed using MRI per se, it is accepted as the gold standard in joint imaging for the identification of early anatomical abnormalities. Ours is the first study to relate serum leptin measures to the presence of MRI-defined osteophytes, meniscal tears, synovitis and joint effusion in addition to cartilage defects and bone marrow lesions. Systemic leptin may impart damaging effects to the joint through catabolic (cartilage breakdown), anabolic (bone formation, osteophytes) or inflammatory (damage to soft tissues) mechanisms, so full characterization of the joint using MRI is a major strength of our study.

Retention. Notably, ongoing participation in the Michigan SWAN population has been excellent. At follow-up visit 11, 80% of still-living participants remained active after ten years of study. Further, participation in the MRI protocol was excellent; less than 4% of active participants refused the MRIs and only 2.5% were ineligible. Individuals with MRIs at follow-up visit 11 did not differ with respect to demographic or body size characteristics, thus it is reasonable to assume that missing data is missing at random.

PUBLIC HEALTH AND CLINICAL IMPLICATIONS

Our findings have important clinical ramifications, not the least of which is to bring attention to the need for evaluation of joint status during the mid-life. The high prevalence of knee joint damage among mid-aged women demonstrates that anatomic joint damage may begin earlier than previously thought. An important translational conclusion of these studies is that the current clinical practice of focusing joint health

efforts among a more elderly population is probably inadequate. If we seek to prevent disease or forestall its progression, we must evaluate individuals prior to or earlier in the disease process.

To those experiencing the effects of osteoarthritis, its effects on mobility and quality of life are well known. While our analyses focused on structural joint damage, others have shown that symptomatic osteoarthritis is a debilitating disease commonly associated with pain, stiffness, functional limitations and disability. No cure exists for OA, and current treatment methodologies are inconsistent in providing symptom relief. Persistent and/or progressive symptoms are associated with loss in productivity and increased health care expenditures. In 2004, osteoarthritis accounted for 97% of the total knee replacements and 83% of total hip replacements in the United States (29). In terms of dollars, $10.5 billion in hospital charges was spent on osteoarthritis in 2006, making it a more expensive condition than pneumonia, stroke, or complications from diabetes (29).

The prevalence of knee OA continues to accelerate, likely because of the aging of the population and the increasing proliferation of the primary risk factor, obesity. The proportion of the total population that is over the age of 60 continues to increase and globally, the prevalence of obesity is rapidly increasing. Given that osteoarthritis is a disease in which we merely attempt to *manage* rather than *cure,* the combined impact of the aging of the population and the increasing prevalence of obesity suggest that the economic impact of OA will exponentiately increase in the next two decades. Further, given that OA onset begins during mid-age and that the current economic situation

requires more and more individuals to retire later, the impact of OA on productivity losses and disability claims can be expected to increase.

The financial cost of OA is burdensome to the healthcare system. While joint replacements are considered an effective and cost-effective treatment for knee osteoarthritis by the orthopedic community (30-32), the price tag can be enormous. In 2004, $30 billion in hospital costs was spent on joint replacements (29) and, as shown in Figure 5.2., the number of procedures has steadily increased particularly for knee replacements. This upward trend can only be expected to continue, given the increasing prevalence of obesity, a major risk factor for knee OA. Economic costs of joint replacement have been shown to be higher among obese patients as compared to non-obese patients (33,34). Greater costs among the obese are due to higher joint failure rates (35,) and more adverse events including infection and deep venous thrombosis (37,38).

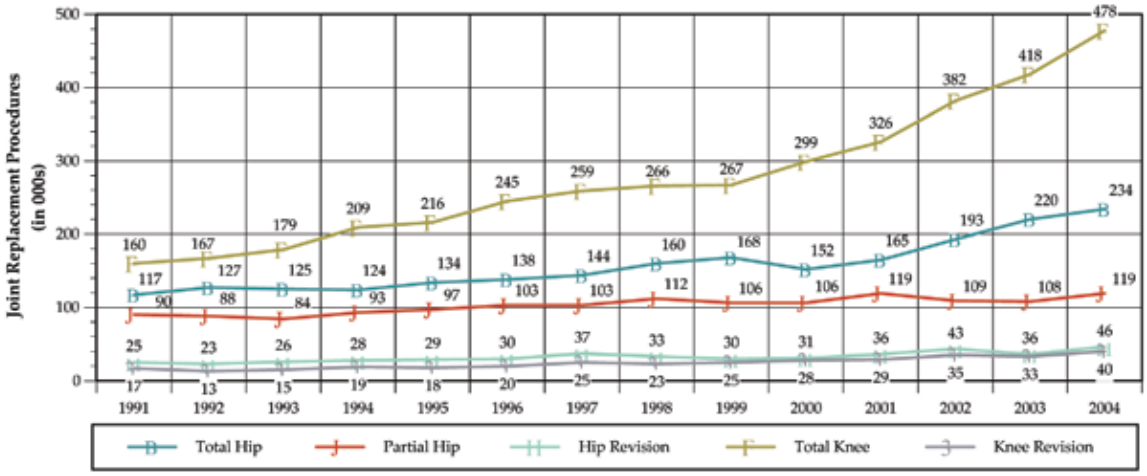

Figure 5.2. Total hip and knee joint replacement procedures in the United States from 1991 to 2004.
From United States Bone and Joint Decade. The Burden of Musculoskeletal Diseases in the United States. Rosemont, IL: American Academiy of Ortopaedic Surgeons; 2008. pp. 71-96.

More important than cost, perhaps, is the observation that patients do not always improve following joint replacement and some, in fact, get worse (39). Postoperative complications following joint replacement include pulmonary embolism, fat embolism syndrome, pneumonia, myocardial infarction, postoperative delirium, urinary tract infections, and deep vein thrombosis (40). While severe complications are more common among elderly and sickly patients, obesity is a major risk factor for post-operative events (37,38). Arthroplasty is not ideal among younger patients, given likelihood of earlier loosening or failure of the artificial joint (41), particularly among obese patients (35,36). Until more effective prevention strategies or therapeutic interventions are identified, many individuals with osteoarthritis will undergo joint replacement (42). Controlling the impact of obesity on osteoarthritis is a promising prevention strategy.

A better understanding of potentially modifiable risk factors for knee osteoarthritis are therefore of great interest to the public health community. While obesity is most commonly cited as a modifiable osteoarthritis risk factor, and studies have shown that weight reduction is effective in symptom management (43), most weight reduction efforts are largely unsuccessful in the long-term due to weight regain (44) and weight regain is often more detrimental than maintenance of high body weight. Targeting the treatable consequences of obesity associated with knee osteoarthritis, including metabolic dysfunction, may be an important new strategy for reducing the additional burden of osteoarthritis. Those individuals with metabolic dysfunction may represent a group that can be identified clinically as being more at risk of osteoarthritis, particularly at younger ages. Our data suggests that controlling metabolic dysfunction even in the presence of high body weight may be protective for knee OA.

Disordered cardiovascular and metabolic status are prominent health concerns among adults and new medications and surgical interventions have extended the lives of individuals to the degree that cardiovascular disease and diabetes are now considered chronic diseases which must be managed. Pharmacologic interventions to treat hyperlipidemia or insulin resistance are available and may provide disease modifying benefits. A recent publication by investigators from the Rotterdam Study reported that statin use was associated with decreased progression of knee but not hip osteoarthritis (45). Despite the limitations of this study, including small cell sizes and the observed effect only among those in the longest-use category of statins, the findings are provocative and suggest that pharmacologic treatments for cardiometabolic dysfunction may be protective for joint health. Learning more about interactions of osteoarthritis, cardiovascular disease and diabetes and their common underlying pathologies is critical to provide coordinated care for individuals with these multiple co-morbidities.

FUTURE RESEARCH DIRECTIONS

Given our findings and the possibility that pharmacological control of metabolic dysfunction may be beneficial for osteoarthritis, there are many exciting avenues for future work in this field. First, these findings need to be replicated in other cohorts, especially in studies that include men and women. The relationship between cardiometabolic dysfunction and knee OA has been examined among women in the United Kingdom Chingford Study (46) where hypertension, hypercholesterolemia and blood glucose levels were positively associated with knee OA. No other major cohort studies of knee osteoarthritis have evaluated the relationship with cardiometabolic

dysfunction or serum leptin. Candidate studies with populations available to replicate our studies may include the Multicenter Osteoarthritis Study, the Johnston County Osteoarthritis Project, the Framingham Osteoarthritis Cohort, the Rotterdam Study, and the Osteoarthritis Initiative. However, cardiometabolic data, including leptin, may not be currently available in these populations.

Second, our findings must be confirmed in relation to osteoarthritis in other joints. Obesity is an important risk factor for knee OA and, arguably, mechanical loading is an important mechanism for this relationship in weight-bearing joints. Our findings support a metabolic impact of obesity on knee osteoarthritis but our analyses were complicated by the need to account for the mechanical impact of body weight. Replication of our findings among non-weight bearing joints would further support the cardiometabolic hypothesis, that obesity imparts damaging effects to the joint through metabolic mechanisms in addition to biomechanical loading. It is possible that these findings may be unique to the knee joint, given the presence of the infrapatellar fat pad, which is a known local source of adipocytokines. We plan to analyze hand OA data from Michigan SWAN with respect to the cardiometabolic hypothesis.

Third, while it has been reported that leptin may be associated with osteoarthritis through catabolic mechanisms (i.e., cartilage degradation associated with a more pro-inflammatory environment) or anabolic mechanisms (i.e., bone build-up and the development of osteophytes), the potential relationship is reportedly more complex. Osteoarthritis pathophysiology is often thought of as a cartilage disease, but changes in

bone, including development of osteophytes and subchondral bone remodeling ultimately resulting in sclerosis are additional hallmarks of the disease. A better understanding of both systemic and local leptin biology and its relationship to bone metabolism and knee osteoarthritis is clearly needed.

During the past decade, leptin has come to the forefront as a major mediator of bone health. However, differences in the central versus peripheral effect of leptin on bone are noteworthy. The net direct effect of leptin on bone is anabolic (47), due to both stimulation of osteoblasts and inhibition of osteoclasts. This dual nature of leptin peripherally is characterized by bone formation through differentiation of bone marrow stromal cells into osteoblasts (48), development of mineralized bone nodules by osteoblasts (49,50) and inhibition of osteoblast apoptosis (49). Leptin also acts peripherally to limit bone resoprtion by inhibiting differentiation of peripheral blood mononuclear cells into osteoclasts (51). The centrally-mediated effects of leptin on bone are thought to act as a brake to the ongoing anabolic processes at the local level (49,52). This was first demonstrated by Ducy et al. (53), who showed that leptin or leptin receptor deficient mice actually had elevated bone mass and that intracerebroventricular infusion with leptin was associated with bone loss. More recent work has identified leptin receptors in the hypothalamus and has confirmed the negative central effect of leptin on bone (54). One possible pathway for this association is through suppression of neuropeptide Y, which is an inhibitor of bone formation (55). In human studies, associations of leptin and measures of bone health, including bone mineral density, are mixed (52).

The integration of this new science into the field of osteoarthritis is just beginning but it represents a very exciting avenue for future research. Mechanistically, several pathways exist through which the impact of leptin on bone may be important in terms of joint health. Emerging evidence about the centrally-mediated role of leptin on bone suggests that leptin may be associated with decreased trabecular bone mass but increased cortical bone mass (47,55), particularly in long bones at the trabecular-cortical interface. This area of the bone, most proximal to the joint, is hypothesized to be associated with joint damage through changes in subchondral bone micro-architecture. Animal models have demonstrated that rapid bone turnover (56,57) and thinning of the subchondral bone (58) is characteristic of early OA. Leptin may be a potential mediator of this accelerated subchondral bone turnover, which has been shown to be associated with cartilage degradation (59,60).

In cases of abnormally high leptin levels, osteoarthritis is associated with decreased bone formation and increased bone resorption (61). These high local levels of leptin may occur when fat is sequestered within bone tissue or, possibly, in joints such as the knee with the local contribution of the infrapatellar fat pad. Subchondral bone resorption within the joint may jeopardize bone integrity, thereby making it less supportive of joint architecture and its ability to absorb mechanical forces. These hypotheses represent cutting-edge thinking in the field and warrant further consideration both in vivo and in vitro with respect to osteoarthritis and joint damage. Given the strong association of leptin with knee osteoarthritis in our study, and given the scientific evidence of a role of

leptin in bone health, consideration of the impact of bone on joint damage may prove to be more efficacious than considering only cartilage.

Fourth, future work should incorporate symptomatic osteoarthritis and consideration of the functional limitations associated with OA as potential outcomes. Osteoarthritis is of great public health importance because of its strong association with outcomes including pain, functional limitations, disability and joint replacement. While body size and obesity are major risk factors for each of these conditions, there is a dearth of literature examining the role of cardiometabolic risk factors as potential modifiers of OA-associated symptoms despite reports from other disciplines that cardiometabolic dysfunction may be associated with more symptom complaint and disability, independent of pathologic disease (62-64). Given the increased inflammatory environment associated with cardiometabolic dysfunction, future work should consider how it relates to osteoarthritis-associated pain, functioning and outcomes.

CONCLUSION

Osteoarthritis and knee joint damage is a highly prevalent condition, with onset of major joint damage occurring during the mid-life. Current paradigms conceptualizing the role of obesity as a risk factor fail to recognize the pathological importance of cardiometabolic dysfunction on joint damage. This dissertation provided evidence of strong relationships between cardiometabolic biomarkers and knee OA using data from two major United States cohort studies. These findings support the need to better characterize the impact of obesity on joint damage and support the hypothesis that the

relative importance of cardiometabolic biomarkers may vary by gender. Notably, our work highlights the high prevalence of joint damage among a cohort of mid-life women and demonstrates that leptin levels early during the mid-life are predictive of later joint damage. This dissertation should encourage researchers and the clinical community to consider metabolic status as an important predictor of knee osteoarthritis and to engage in future work to learn how management of metabolic dysfunction may reduce osteoarthritis onset, progression, and morbidity.

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Appendix A.
Baseline Characteristics of Michigan Study of Women's Health Across the Nation (SWAN) Women by Bone Marrow Lesions from Magnetic Resonance Imaging at Follow-Up Visit 11.

	Bone Marrow Lesions			
	Normal	**Largest diameter ≤ 1 cm**	**Largest diameter > 1 cm**	**P-value**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	
Age (years)	45.9 (2.7)	46.3 (2.8)	46.6 (3.0)	0.23
Weight (kg)	81.3 (21.1)	91.3 (21.0)	97.5 (21.6)	<0.0001
Height (cm)	163.4 (6.0)	163.4 (6.7)	164.1 (6.0)	0.79
BMI (kg/m^2)	30.4 (7.5)	34.3 (8.1)	36.3 (7.9)	<0.0001
Waist circumference (cm)	90.2 (15.9)	99.8 (17.6)	101.3 (14.8)	<0.0001
Hip circumference (cm)	110.3 (15.3)	118.5 (16.0)	121.9 (16.3)	<0.0001
Waist:hip ratio	0.82 (0.08)	0.84 (0.07)	0.83 (0.08)	0.02
Fat mass (kg)	33.3 (15.2)	41.1 (16.4)	44.6 (19.2)	<0.0001
Skeletal muscle mass (kg)	21.3 (3.4)	21.9 (3.1)	23.3 (3.3)	0.001
	n (%)	**n (%)**	**n (%)**	
Obese (BMI≥30 kg/m^2)	99 (46.3%)	70 (68.0%)	34 (75.6%)	<0.0001
Current Smoker	57 (26.8%)	21 (20.6%)	9 (20.5%)	0.40
Ethnicity				
African-American	132 (61.4%)	68 (65.4%)	27 (60.0%)	0.74
Caucasian	83 (38.6%)	36 (34.6%)	18 (40.0%)	
Menopause Status				
Premenopausal	116 (54.2%)	48 (46.6%)	18 (40.0%)	0.15
Early Perimenopausal	98 (45.8%)	55 (53.4%)	27 (60.0%)	

Appendix B.
Baseline Characteristics of Michigan Study of Women's Health Across the Nation (SWAN) Women by Osteophytes from Magnetic Resonance Imaging at Follow-Up Visit 11.

	Osteophytes				
	None	**≤ 5 mm**	**5-10 mm**	**> 10 mm**	**P-value**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	
Age (years)	45.8 (2.9)	46.1 (2.8)	46.3 (2.6)	45.1 (2.7)	0.72
Weight (kg)	72.2 (18.1)	81.2 (17.7)	99.1 (17.9)	111.4 (22.5)	<0.0001
Height (cm)	164.3 (5.2)	162.7 (6.3)	163.9 (6.8)	165.0 (5.4)	0.09
BMI (kg/m^2)	26.7 (6.3)	30.7 (6.5)	37.1 (7.3)	40.8 (7.7)	<0.0001
Waist circumference (cm)	83.9 (14.6)	91.2 (14.2)	103.2 (15.5)	111.4 (16.1)	<0.0001
Hip circumference (cm)	103.6 (13.3)	110.5 (13.1)	124.0 (14.8)	130.9 (15.5)	<0.0001
Waist:hip ratio	0.81 (0.08)	0.82 (0.07)	0.83 (0.07)	0.85 (0.07)	0.03
Fat mass (kg)	26.8 (12.4)	33.7 (13.2)	45.4 (14.4)	56.6 (21.2)	<0.0001
Skeletal muscle mass (kg)	20.3 (3.0)	21.1 (30.0)	23.3 (3.1)	24.7 (3.4)	<0.0001
	n (%)	**n (%)**	**n (%)**	**n (%)**	
Obese (BMI≥30 kg/m^2)	17 (23.0%)	88 (50.9%)	66 (81.5%)	32 (94.1%)	<0.0001
Current Smoker	20 (27.4%)	44 (25.6%)	18 (22.2%)	5 (15.2%)	0.53
Ethnicity					
African-American	45 (60.8%)	104 (59.8%)	52 (63.4%)	26 (76.5%)	0.32
Caucasian	29 (39.2%)	70 (40.2%)	30 (36.6%)	8 (23.5%)	
Menopause Status					
Premenopausal	41 (55.4%)	86 (50.0%)	44 (53.7%)	11 (32.4%)	0.14
Early Perimenopausal	33 (44.6%)	86 (50.0%)	38 (46.3%)	23 (32.4%)	

Appendix C.
Baseline Characteristics of Michigan Study of Women's Health Across the Nation (SWAN) Women by Meniscal Tears from Magnetic Resonance Imaging at Follow-Up Visit 11.

	Meniscal Tear				P-value
	Normal	Intra-substance meniscal abnormality only	Non-displaced tear	Displaced or macerated tear	
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)	45.6 (3.0)	46.0 (2.7)	45.9 (2.9)	46.3 (2.7)	0.57
Weight (kg)	80.0 (25.5)	78.3 (18.9)	90.8 (21.7)	95.1 (21.6)	<0.0001
Height (cm)	162.2 (5.7)	163.0 (5.9)	164.3 (6.2)	163.9 (6.6)	0.32
BMI (kg/m^2)	30.4 (9.7)	29.4 (6.7)	33.6 (7.8)	35.5 (8.2)	<0.0001
Waist circumference (cm)	90.7 (20.7)	88.8 (14.8)	96.9 (16.5)	100.7 (17.2)	<0.0001
Hip circumference (cm)	109.4 (19.1)	108.6 (14.1)	116.5 (15.7)	120.7 (16.5)	<0.0001
Waist:hip ratio	0.83 (0.08)	0.82 (0.07)	0.83 (0.07)	0.83 (0.07)	0.23
Fat mass (kg)	33.9 (18.8)	31.5 (13.5)	37.8 (16.2)	43.3 (18.1)	<0.0001
Skeletal muscle mass (kg)	20.6 (3.4)	20.8 (3.1)	22.5 (3.6)	22.8 (3.2)	<0.0001
	n (%)	n (%)	n (%)	n (%)	
Obese (BMI≥30 kg/m^2)	7 (38.9%)	66 (42.3%)	52 (67.5%)	78 (70.3%)	<0.0001
Current Smoker	2 (22.2%)	47 (30.3%)	15 (19.7%)	21 (19.1%)	0.13
Ethnicity					
African-American	14 (77.8%)	94 (59.9%)	52 (67.5%)	67 (59.8%)	0.33
Caucasian	4 (22.2%)	63 (40.1%)	25 (32.5%)	45 (40.2%)	
Menopause Status					
Premenopausal	12 (66.7%)	80 (51.3%)	38 (49.4%)	52 (46.9%)	0.47
Early Perimenopausal	6 (33.3%)	76 (48.7%)	39 (50.7%)	59 (53.1%)	

Appendix D.
Baseline Characteristics of Michigan Study of Women's Health Across the Nation (SWAN) Women by Synovitis from Magnetic Resonance Imaging at Follow-Up Visit 11.

	Synovitis			P-value
	Normal	Mild	Moderate/ Marked	
	Mean (SD)	Mean (SD)	Mean (SD)	
Age (years)	46.0 (2.8)	46.2 (2.7)	46.5 (2.5)	0.60
Weight (kg)	80.0 (19.9)	94.9 (19.8)	107.2 (21.2)	<0.0001
Height (cm)	163.3 (6.1)	163.4 (6.3)	165.4 (6.2)	0.17
BMI (kg/m^2)	30.0 (7.2)	35.7 (7.8)	39.2 (7.6)	<0.0001
Waist circumference (cm)	89.9 (15.8)	101.7 (16.4)	106.2 (14.8)	<0.0001
Hip circumference (cm)	109.5 (14.6)	121.0 (15.7)	128.1 (15.3)	<0.0001
Waist:hip ratio	0.82 (0.07)	0.84 (0.07)	0.83 (0.07)	0.06
Fat mass (kg)	32.6 (14.6)	43.1 (16.1)	51.5 (19.0)	<0.0001
Skeletal muscle mass (kg)	20.9 (3.1)	22.7 (3.1)	24.7 (3.8)	<0.0001
	n (%)	n (%)	n (%)	
Obese (BMI≥30 kg/m^2)	109 (45.4%)	66 (74.2%)	28 (84.9%)	<0.0001
Current Smoker	65 (27.3%)	19 (21.1%)	3 (9.7%)	0.07
Ethnicity				
African-American	144 (59.8%)	58 (64.4%)	25 (75.8%)	0.18
Caucasian	97 (40.3%)	32 (35.6%)	8 (24.2%)	
Menopause Status				
Premenopausal	119 (49.8%)	52 (57.8%)	11 (33.3%)	0.05
Early Perimenopausal	120 (50.2%)	38 (42.2%)	22 (66.7%)	

Appendix E.
Baseline Characteristics of Michigan Study of Women's Health Across the Nation (SWAN) Women by Joint Effusion from Magnetic Resonance Imaging at Follow-Up Visit 11.

	Joint Effusion			
	Physiologic fluid	**Small effusion (≤ 10 mm)**	**Moderate and/or large effusion (> 10 mm)**	**P-value**
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	
Age (years)	46.0 (2.9)	46.1 (2.8)	45.9 (2.6)	0.86
Weight (kg)	76.9 (18.0)	83.9 (20.0)	109.0 (21.3)	<0.0001
Height (cm)	163.5 (5.6)	163.2 (6.2)	164.9 (6.6)	0.22
BMI (kg/m^2)	28.8 (6.6)	31.5 (7.4)	40.2 (8.0)	<0.0001
Waist circumference (cm)	87.0 (15.3)	93.3 (16.2)	108.1 (15.6)	<0.0001
Hip circumference (cm)	107.4 (13.8)	112.7 (15.2)	129.1 (15.6)	<0.0001
Waist:hip ratio	0.81 (0.08)	0.83 (0.07)	0.84 (0.07)	0.09
Fat mass (kg)	29.9 (14.2)	35.3 (14.5)	54.2 (19.1)	<0.0001
Skeletal muscle mass (kg)	20.8 (2.9)	21.4 (3.2)	24.4 (3.4)	<0.0001
	n (%)	**n (%)**	**n (%)**	
Obese (BMI≥30 kg/m^2)	22 (36.1%)	136 (54.2%)	45 (90.0%)	<0.0001
Current Smoker	22 (36.7%)	58 (23.1%)	7 (14.6%)	0.02
Ethnicity				
African-American	39 (63.9%)	150 (59.3%)	38 (76.0%)	0.08
Caucasian	22 (36.1%)	103 (40.7%)	12 (24.0%)	
Menopause Status				
Premenopausal	32 (52.5%)	128 (51.0%)	22 (44.0%)	0.62
Early Perimenopausal	29 (47.5%)	123 (49.0%)	28 (66.0%)	