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The Mediating Effect of Leptin on the Relationship Between Body Weight and Knee Osteoarthritis in Older Adults

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Abstract

Objective—Obesity is associated with increased risk of osteoarthritis (OA) of the knee. Emerging evidence suggests that adipokines, substances produced by adipose tissue, may play a role in the development of knee OA. Our aim was to determine whether the inflammatory adipokine, leptin, partially mediates the relationship between body mass index (BMI) and knee OA.

Methods—We used baseline data from 653 participants aged ≥70 years in the population-based MOBILIZE Boston study. Height and weight were measured and participants were assessed for knee OA using clinical criteria. Serum leptin was measured using a microsphere-based assay.

Results—Average BMI and serum leptin was 27.5kg/m^2 and 589pM, respectively; the prevalence of knee OA was 24.7%. In regression models adjusted for covariates, we found that a 5 kg/m2 increase in BMI was associated with a 32% higher odds of knee OA (OR = 1.32, 95% CI 1.10, 1.58); a 200 pM increase in serum leptin was associated with 11% higher odds of knee OA (OR 1.11, 95% CI 1.05, 1.17). The ratio of the standardized coefficients for the indirect/total effect calculated using the product of coefficients method was 0.49, suggesting that approximately half of the total effect of BMI on knee OA may be mediated by serum leptin. The estimated confidence intervals for the mediated effect suggest that this effect is statistically significant. Similarly, mediation analysis using a counterfactual approach suggested statistically significant mediation effect of leptin.

Conclusions—We found that almost half of the association between elevated BMI and knee OA could be explained by the inflammatory adipokine leptin.

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The public health burden of osteoarthritis is substantial. Arthritis is the most common cause of physical disability; and osteoarthritis is the most common form of arthritis. Osteoarthritis (OA) is estimated to affect more than 27 million adults in the U.S., with the prevalence expected to increase as the baby boomer generation ages.[1,2] Osteoarthritis commonly affects the weight-bearing joints, such as the hip and knee, causing joint pain and stiffness and impaired mobility. In fact, knee OA is the leading cause of mobility disability in older adults.[3] As a consequence, osteoarthritis is associated with significant personal and societal costs related to time lost from work, early retirement, increased health care utilization, and joint replacement surgeries.[4,5]

Despite the substantial financial and personal costs of knee OA, this condition remains incompletely understood. Under normal conditions, articular cartilage provides a near frictionless surface, aiding in the distribution of pressure loads within the joint. In osteoarthritis, there is imbalance of the anabolic and catabolic processes of the chondrocytes, leading to damage of the structural and functional integrity of the cartilage and adjacent bone and other joint tissues. Excess weight has been identified as an important risk factor for OA of the knee. Those with obesity have 1.5 to 2 times the risk of developing knee OA than their leaner counterparts.[6] While the link between excess weight and higher risk of knee OA is well accepted, the mechanism of association is debated. It has been hypothesized that the added axial loading and malalignment of the knee associated with obesity accelerates the age-associated process of cartilaginous degeneration.[7,8] However, others posit that in addition to obesity-induced mechanical impairments, the metabolic, immunologic and biochemical derangements induced by excess adiposity also contribute to the development of knee OA.[7,9,10] Adipocytes and macrophages, two primary constituents of fat tissue, secrete immuno-modulating substances, termed adipokines. Leptin was one of the first adipokines identified.[11] Serum levels of leptin are highly correlated with degree of adiposity. Recent work suggests that leptin may play a role in the development of osteoarthritis.[9,12] There is evidence that leptin is present in synovial fluid; chondrocytes have leptin receptors; and leptin may be a key regulator of chondrocyte metabolism.[9,13–15] The aim of our study was to examine the extent to which serum leptin levels may mediate the greater prevalence of knee OA observed with higher body weight.

Methods

Study population

We used data from the MOBILIZE Boston Study (MBS), a population-based study of older persons residing in the Boston area. MBS is a National Institute of Aging funded study with the primary aim of identifying novel risk factors for falls in the elderly. Eligible participants were 70 years and older, living within a five mile radius of the study coordinating center at the Institute for Aging Research, Hebrew SeniorLife, Boston, Massachusetts. Spouses or companions of participants aged 64 or older were also eligible to join the study. Study recruitment took place from 2005 to 2008; 765 people completed the two-part baseline assessment which included a home interview followed by a study clinic visit at the Institute for Aging Research. The baseline assessments collected information related to a number of domains including demographics, health measures, and measured and self assessed physical

and mental functioning, and phlebotomy. For this cross-sectional analysis, we used the baseline assessment data. Participants eligible for our study had complete information related to body weight, height and serum data available at baseline. (n=653) Details of the study methods and recruitment have been published previously.[16,17]

Weight Status

Weight was measured without shoes using a calibrated balance beam scale; height was measured without shoes using a stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters. BMI was entered in all final models as a continuous variable. Baseline characteristics of the sample are presented in Table 1 by BMI categories in accordance with the NIH classification schema[18] : normal weight – BMI 19-24.9 kg/m^2, overweight – BMI 25-29.9 kg/m^2, obese - BMI ≥30 kg/$m.^2$

Knee Osteoarthritis

Participants underwent musculoskeletal examination by trained research nurses in order to assess the American College of Rheumatology's clinical criteria for knee OA.[19] The examination included observation of movement, tenderness and swelling of the relevant joints. Study staff conducting examinations were trained by physician specialists (rheumatologist and physiatrist) and certified by demonstrating proficiency in conducting the assessments. Assessments of inter-rater reliability for examination measures generally showed good to very good agreement (kappa statistics 0.40 to 0.76).[16]

Serum Leptin

Blood was collected for baseline laboratory tests and samples were stored at -70° until analysis. The plasma concentration of leptin was determined by multiplexed microbead immunoassay used as specified by the manufacturer (Luminex). The technique uses detection antibodies as the immobilized probe on the solid, microbead surface. When mixed with serum, the labeled antibodies allows for quantification of antigen–antibody reactions that occur on the microsphere surface by measurement of the relative fluorescence intensity, thus providing measurement of the analyte of interest. Prior research finds good correlation between the multiplexed assay and conventional enzyme-linked immunosorbent assay for serum leptin measurement.[20] Intra and Inter-assay coefficients of variation for these microbead assays are <15% and <20%, respectively.

Statistical analysis

We used descriptive statistics to characterize participants' demographic and clinical characteristics at baseline. Chi-square tests and one-way analysis of variance were used to examine the unadjusted relationship between categories of weight status and both osteoarthritis and serum leptin.

To understand how much of the measured effect of the independent variable (BMI) on the dependent variable (knee OA) is attributable to a potential mediator variable (serum leptin), we first used sequential regression analyses to examine the relationship between weight status and both osteoarthritis and serum leptin. In our model (Figure 1), the observed effect of the independent variable on the dependent variable is called the total effect (Path C). The

total effects are comprised of a direct effect pathway (Path C') of the independent variable on the dependent variable, and a total indirect pathway (mediated: Path A + B) of the independent variable on the dependent variable through the mediator. Baron and Kenny proposed a four step approach to examine mediation effects in which sequential regression analyses are conducted and the relevant coefficients are examined at each step[21] Using the Baron and Kenny method, mediation can be said to be present when the following conditions hold in sequential regression analyses: 1) The independent variable (BMI) is associated with the dependent variable (knee OA) in the absence of the putative mediator variable (leptin);.2) The independent variable (BMI) is associated with the mediator variable (leptin); 3) The mediator (leptin) is associated with the dependent variable (knee OA); 4) the observed effect of the independent variable (BMI) on the outcome (kneeOA) shrinks when the mediator (leptin) is added to the model. Full mediation occurs if inclusion of the mediation variable shrinks the observed relationship between the independent variable and dependent variable to zero. However, full mediation rarely occurs. Partial mediation occurs when the observed relationship between the independent and dependent variable is weaker with the inclusion of the mediator variable in the model.

We used logistic regression for dichotomous outcomes (i.e. knee OA) and linear regression for continuous outcomes (leptin), thus the regression coefficients for the different outcomes are scaled differently. Using the coefficients of different scales to calculate mediation effect can be misleading, therefore, we used standardized the regression coefficients using the standard errors of the coefficients to assess for statistical significance of the mediation pathway.[22] We estimated the coefficient for the indirect effect by calculating the product of the standardized regression coefficient (Path A) of the independent variable on the mediator with the regression coefficient (Path B) of the mediator variable on the dependent variable (A * B). Thus, the regression coefficient for the indirect effect represents the change in the dependent variable for every unit change in the independent variable that is mediated by the mediator. Bootstrapping methods were used to calculate the 95% confidence intervals for the coefficients for the total, indirect, and direct effect.[21] Coefficients were considered statistically significant if the confidence intervals did not cross zero.

Our primary mediation analysis utilized the product of coefficients method described above. As a secondary analysis, we conducted the same mediation analysis using a newer technique for mediation analysis based on a counterfactual framework which allows for the presence of interaction of the independent variable with the mediator variable.[23] This technique uses parametric regression to estimate two models: a model for the mediator conditional on the independent variable and covariates and also a model of the dependent variable conditional on the independent variable and covariates. The direct and indirect effects are estimated from the models' regression parameters.

All regression models of the mediation analysis controlled for potential confounders of the BMI-knee OA and the leptin-knee OA relationships: age, sex, race (black, white, other), education level (less than high school, high school completion, more than high school), smoking status (never, former, current), angular knee deformity assessed by physical examination (present, not present) and physical activity (Physical Activity Scale for the Elderly, continuous).

All analyses were conducted using Stata V12 (Stata Corporation, College Station, TX, USA).

Results

Characteristics of Study Sample

Our study sample was predominantly female and white. (Table 1) Average BMI and serum leptin of the participants was 27.5 kg/m^2 and 589 pM, respectively. Obese participants were more likely to be female, black and non-smokers. The prevalence of clinical knee osteoarthritis was 30% among obese participants and 31% among those who were overweight, compared to 15% of normal weight participants.

Mediation Analysis using the Baron and Kenny Method

We first used the Baron and Kenny method of sequential regression modeling to assess for the mediating effect of serum leptin. Unadjusted analysis showed a strong association of higher weight category and serum leptin levels. (Path A, Table 1) Multivariable adjusted analyses also showed a similarly strong association of greater BMI with higher serum leptin. For every one point increase in BMI (kg/m2), leptin was estimated to increase by 64 pM in regression models adjusted for covariates ($p<0.001$). (Path A) Multivariable adjusted analyses demonstrated that higher serum leptin level was associated with increased odds of knee OA. (Path B, Table 2) Higher BMI was significantly associated with knee OA in analyses adjusted for covariates other than leptin. (Path C, Table 2) When leptin is included in the model, the observed association between BMI and knee OA was substantially attenuated and becomes statistically insignificant. These data suggest the partial mediation of the observed adverse impact of higher BMI on knee OA by serum. Our sex-stratified analyses, found the mediation effect primarily in women. (Table 2) In older men, neither body mass index nor leptin was significantly associated with knee OA. (Table 2)

Quantification of Direct and Indirect Effects Using the Product of Coefficients Method

We found that the standardized coefficients for both the direct and indirect effects were statistically significant. (Table 3) Our analyses suggest that serum leptin mediates approximately 49% of the total observed adverse effect of higher BMI on knee OA. For women, leptin was found to be a significant mediator of the relationship between body weight and knee OA. Because the association between BMI and knee OA was only observed in women, the percentage of total effect mediated by leptin was not calculated in men. (Table 3)

Mediation Analysis Using the Counterfactual Method

We conducted a mediation analysis using the counterfactual approach, which yielded results similar in interpretation to the results of the product of coefficients method. We estimated the coefficient for the natural direct effect (the change in odds of knee OA if the value of leptin was controlled at 600 pM, while BMI varies from 25 kg/m^2 to 30 kg/m^2) to be 0.140 (95% CI -0.075, 0.354). The coefficient for the natural indirect effect (the change in odds of knee OA if BMI was controlled at 30 kg/m^2, but the value of leptin varied from its estimated value for those with BMI of 25 kg/m^2 to the estimated value of leptin for those with BMI of

30 kg/m^2) to be 0.135 (95%CI 0.031, 0.239). The coefficient for the total effect (the change in odds of knee OA overall if BMI varied from 25 kg/m^2 to 30 kg/m^2) was estimated to be 0.275 (95% CI 0.087, 0.463). In this analysis, the coefficient for the indirect and total effects were statistically significant, but the coefficient for the direct effect was not. The proportion of the observed effect of body mass index on prevalence of knee OA estimated to be mediated by leptin was to be 49%.

Discussion

Our analysis of a population-based sample of community-dwelling older adults confirmed previous findings that greater BMI is associated with higher prevalence of knee OA, and that higher BMI is correlated with higher serum leptin levels. Additionally, we confirmed a previous finding that serum leptin is independently associated with knee OA.[12] To our knowledge, this is the first population-based study to determine the possible mediating role of serum leptin in the relationship between body weight and knee OA.

The suggestion that leptin may be a possible link between obesity and higher risk of knee OA, is supported by several lines of evidence. A study of human knee joints demonstrated the presence of leptin in synovial fluid in concentrations significantly correlated with BMI, suggesting that circulating leptin likely diffuses into the joint space.[9] Additionally, leptin may exert regulatory effects in the articular spaces. Leptin receptors have been identified on human chondrocytes.[24] Leptin has also found to be secreted by human chrondrocytes; with higher levels of secretion among patients with knee OA compared to those without.[9] In mouse models, leptin has been found to influence the intra-articular expression of growth factors, stimulate proteoglycan and collagen synthesis, and stimulate the production of pro-inflammatory cytokines.[13,14,25] Finally, a study of older transgenic mice that lack normal leptin signaling mechanisms (ob/ob – nonfunctional leptin molecule or db/db – lacking the leptin receptor) develop extreme obesity, but do not develop knee OA.[26] In contrast, mice with diet-induced obesity show higher incidence of knee OA, in spite of lower levels of obesity relative to the transgenic mice. These physiologic data suggest that leptin plays an important role in the development of knee OA, perhaps through an immune-mediated or inflammatory process.

We found that serum leptin is positively associated with higher odds of knee OA and that leptin may partially mediate the association between BMI and knee OA. We are not aware of prior work that estimates how much of the adverse impact of higher BMI on knee OA might be mediated by leptin. Our work is consistent with the few epidemiologic studies examining the relationship between leptin and OA. One study of patients with knee OA demonstrated that serum leptin was significantly correlated with the severity of disease.[27] Another recently published study of middle-aged women demonstrated that 5 ng/ml incremental increase in serum leptin levels was associated with greater odds of both prevalent (OR 1.38; 95% CI 1.26, 1.52) and incident (OR 1,31; 95% CI 1.21, 1.41) knee OA after adjustment for covariates, including BMI.[12] Taken together, these data lend support to the theory that the increase in knee OA observed with higher body weight may indeed be at least partially mediated by metabolic factors, including adipokines such as leptin.

When stratified by sex, our analysis demonstrated a strong relationship between body weight and knee OA in women, but not in men. However, our results are in contrast to prior studies that have demonstrating increased risk of knee OA with higher BMI in both men and women.[28–30] For example, one study demonstrated nearly doubling of risk of knee OA associated with increase in BMI of 5 kg/m^2 in men.[28] Our sample size, particularly the male sample, was rather small. While our results could point to the possibility of sex-differences in the risk factors associated with knee OA, it is also possible that our male sample was too small to detect the relationships of interest. Further work should be done to examine for the possibility of differential associations by sex.

A strength of our study was that it analyzed of a multiracial, population-based sample. However, some limitations of our study have to be considered. The cross-sectional design does not allow us to establish temporality of our factors of interest. We postulate that obesity develops initially, which leads to higher serum leptin, which then contributes to the pathogenesis of knee OA. However, in this study we can not confirm this temporal relationship, nor can we establish causality. Prior work has shown that obesity is a risk factor for development of knee OA.[31,32] Because our population was primarily aged 70 and older, we cannot generalize the results to younger populations who may be earlier in their OA disease process. We did not have radiographic assessments of knee OA, which are likely to be more sensitive to the presence of disease. We did not have information about other risk factors for OA such as joint trauma, family history or prior medical conditions that may contribute to OA (i.e. hemochromatosis, septic arthritis, inflammatory arthritis, avascular necrosis, hemophilia, gout). While our data suggests that there may be gender-based differences in the relationship between body weight, leptin and knee OA, our study sample was relatively small which did not allow us to draw definitive conclusions regarding potential differences by sex. Because of the limitation of small sample size, we were also unable to test for potential differences by race. Finally, leptin is highly correlated with body weight, and more specifically with fat mass. Therefore, the mediating effect of leptin can be difficult to interpret. In our analyses, leptin was more highly correlated with knee OA than BMI. This might be observed if knee OA is more closely associated with fat mass than with total mass (or BMI). If it is true that fat mass is more predictive of knee OA than total mass or BMI, this suggests that knee OA associated knee with obesity may not be solely due to a mechanical effect of body weight on the knee joints. We did not have measures of body composition which may have enhanced the understanding and interpretation of our findings. Further targeted studies are needed to explore the interrelationships between body composition, inflammatory adipokines and osteoarthritis.

In conclusion, our work adds to the growing body of scientific evidence supporting the hypothesis that metabolic and/or immunologic factors may underlie the development of obesity-related knee OA. As the prevalence of overweight and obesity is high and the impact of knee OA on mobility and disability is substantial, it is important for scientists and clinicians to have a better understanding of the interaction between obesity, metabolic factors and OA. Better understanding of these relationships may allow for improved identification of which populations are at highest risk of developing obesity-related knee OA and might inform the development of effective prevention and treatment efforts.

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References

1. Lawrence RC, Felson DT, Helmick CG, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. Arthritis Rheum. 2008; 58(1):26–35. doi:10.1002/art.23176. [PubMed: 18163497]
2. Hootman JM, Helmick CG. Projections of US prevalence of arthritis and associated activity limitations. Arthritis & Rheumatism. 2006; 54(1):226–229. doi:10.1002/art.21562. [PubMed: 16385518]
3. Guccione AA, Felson DT, Anderson JJ, et al. The effects of specific medical conditions on the functional limitations of elders in the Framingham Study. Am J Public Health. 1994; 84(3):351–358. [PubMed: 8129049]
4. Kotlarz H, Gunnarsson CL, Fang H, Rizzo JA. Insurer and out-of-pocket costs of osteoarthritis in the US: Evidence from national survey data. Arthritis & Rheumatism. 2009; 60(12):3546–3553. doi:10.1002/art.24984. [PubMed: 19950287]
5. Buckwalter JA, Saltzman C, Brown T. The impact of osteoarthritis: implications for research. Clin Orthop Relat Res. 2004; (427 Suppl):S6–15. [PubMed: 15480076]
6. Felson DT, Anderson JJ, Naimark A, Walker AM, Meenan RF. Obesity and knee osteoarthritis. The Framingham Study. Ann Intern Med. 1988; 109(1):18–24. [PubMed: 3377350]
7. Timothy M Griffin FG. The role of mechanical loading in the onset and progression of osteoarthritis. Exercise and sport sciences reviews. 2005; 33(4):195–200. [PubMed: 16239837]
8. Sharma L, Lou C, Cahue S, Dunlop DD. The mechanism of the effect of obesity in knee osteoarthritis: The mediating role of malalignment. Arthritis & Rheumatism. 2000; 43(3):568–575. doi:10.1002/1529-0131(200003)43:3<568::AID-ANR13>3.0.CO;2-E. [PubMed: 10728750]
9. Dumond H, Presle N, Terlain B, et al. Evidence for a key role of leptin in osteoarthritis. Arthritis & Rheumatism. 2003; 48(11):3118–3129. doi:10.1002/art.11303. [PubMed: 14613274]
10. Sowers M, Karvonen-Gutierrez CA, Palmieri-Smith R, Jacobson JA, Jiang Y, Ashton-Miller JA. Knee osteoarthritis is greatest in obesity with cardiometabolic clustering. Arthritis Rheum. 2009; 61(10):1328–1336. doi:10.1002/art.24739. [PubMed: 19790111]
11. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 372(6505):425–432. doi:10.1038/372425a0. [PubMed: 7984236]
12. Karvonen-Gutierrez CA, Harlow SD, Mancuso P, Jacobson J, De Leon CFM, Nan B. Leptin levels are associated with radiographic knee osteoarthritis among a cohort of mid- life women. Arthritis Care & Research. 2012 n/a–n/a doi:10.1002/acr.21922.
13. Iliopoulos D, Malizos KN, Tsezou A. Epigenetic regulation of leptin affects MMP-13 expression in osteoarthritic chondrocytes: possible molecular target for osteoarthritis therapeutic intervention. Annals of the Rheumatic Diseases. 2007; 66(12):1616–1621. doi:10.1136/ard.2007.069377. [PubMed: 17502362]
14. Vuolteenaho K, Koskinen A, Kukkonen M, et al. Leptin Enhances Synthesis of Proinflammatory Mediators in Human Osteoarthritic Cartilage—Mediator Role of NO in Leptin-Induced PGE2, IL-6, and IL-8 Production. Mediators of Inflammation. 2009; 2009:1–10. doi:10.1155/2009/345838.
15. Ku JH, Lee CK, Joo BS, et al. Correlation of synovial fluid leptin concentrations with the severity of osteoarthritis. Clin Rheumatol. 2009; 28(12):1431–1435. doi:10.1007/s10067-009-1242-8. [PubMed: 19662330]

16. Leveille SG, Kiel DP, Jones RN, et al. The MOBILIZE Boston Study: Design and methods of a prospective cohort study of novel risk factors for falls in an older population. BMC Geriatrics. 2008; 8(1):16. doi:10.1186/1471-2318-8-16. [PubMed: 18638389]
17. Samelson EJ, Kelsey JL, Kiel DP, et al. Issues in Conducting Epidemiologic Research Among Elders: Lessons From The MOBILIZE Boston Study. Am J Epidemiol. 2008; 168(12):1444–1451. doi:10.1093/aje/kwn277. [PubMed: 18953059]
18. U.S. National Institues of Health. Clinical Guidelines for the Identification, Evaluation and Treatment of Overweight and Obesity in Adults. National Institutes of Health; Bethesda, MD: 1998.
19. Altman R, Asch E, Bloch D, et al. Development of criteria for the classification and reporting of osteoarthritis: Classification of osteoarthritis of the knee. Arthritis & Rheumatism. 1986; 29(8): 1039–1049. doi:10.1002/art.1780290816. [PubMed: 3741515]
20. Liu MY, Xydakis AM, Hoogeveen RC, et al. Multiplexed analysis of biomarkers related to obesity and the metabolic syndrome in human plasma, using the Luminex-100 system. Clin Chem. 2005; 51(7):1102–1109. doi:10.1373/clinchem.2004.047084. [PubMed: 15976097]
21. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol. 1986; 51(6): 1173–1182. [PubMed: 3806354]
22. Sobel ME. Asymptotic confidence intervals for indirect effects in structural equation models. Sociological Methodology. 1982; 13:290–312.
23. Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. Psychol Methods. 2013; 18(2):137–150. doi:10.1037/a0031034. [PubMed: 23379553]
24. Figenschau Y, Knutsen G, Shahazeydi S, Johansen O, Sveinbjörnsson B. Human articular chondrocytes express functional leptin receptors. Biochem Biophys Res Commun. 2001; 287(1): 190–197. doi:10.1006/bbrc.2001.5543. [PubMed: 11549273]
25. Bao J, Chen W, Feng J, Hu P, Shi Z, Wu L. Leptin plays a catabolic role on articular cartilage. Molecular Biology Reports. 2009; 37(7):3265–3272. doi:10.1007/s11033-009-9911-x. [PubMed: 19876764]
26. Griffin TM, Huebner JL, Kraus VB, Guilak F. Extreme obesity due to impaired leptin signaling in mice does not cause knee osteoarthritis. Arthritis Rheum. 2009; 60(10):2935–2944. doi:10.1002/art.24854. [PubMed: 19790050]
27. Staikos C, Ververidis A, Drosos G, Manolopoulos VG, Verettas D- A, Tavridou A. The association of adipokine levels in plasma and synovial fluid with the severity of knee osteoarthritis. Rheumatology (Oxford). 2013 doi:10.1093/rheumatology/kes422.
28. Järvholm B, Lewold S, Malchau H, Vingård E. Age, bodyweight, smoking habits and the risk of severe osteoarthritis in the hip and knee in men. Eur J Epidemiol. 2005; 20(6):537–542. [PubMed: 16121763]
29. Lohmander LS, Gerhardsson de Verdier M, Rollof J, Nilsson PM, Engström G. Incidence of severe knee and hip osteoarthritis in relation to different measures of body mass: a population-based prospective cohort study. Ann Rheum Dis. 2009; 68(4):490–496. doi:10.1136/ard.2008.089748. [PubMed: 18467514]
30. Dawson J, Juszczak E, Thorogood M, Marks S- A, Dodd C, Fitzpatrick R. An investigation of risk factors for symptomatic osteoarthritis of the knee in women using a life course approach. J Epidemiol Community Health. 2003; 57(10):823–830. [PubMed: 14573590]
31. Manninen P, Riihimäki H, Heliövaara M, Mäkelä P. Overweight, gender and knee osteoarthritis. Int J Obes Relat Metab Disord. 1996; 20(6):595–597. [PubMed: 8782738]
32. Felson DT, Zhang Y, Hannan MT, et al. Risk factors for incident radiographic knee osteoarthritis in the elderly. The framingham study. Arthritis & Rheumatism. 1997; 40(4):728–733. doi:10.1002/art.1780400420. [PubMed: 9125257]

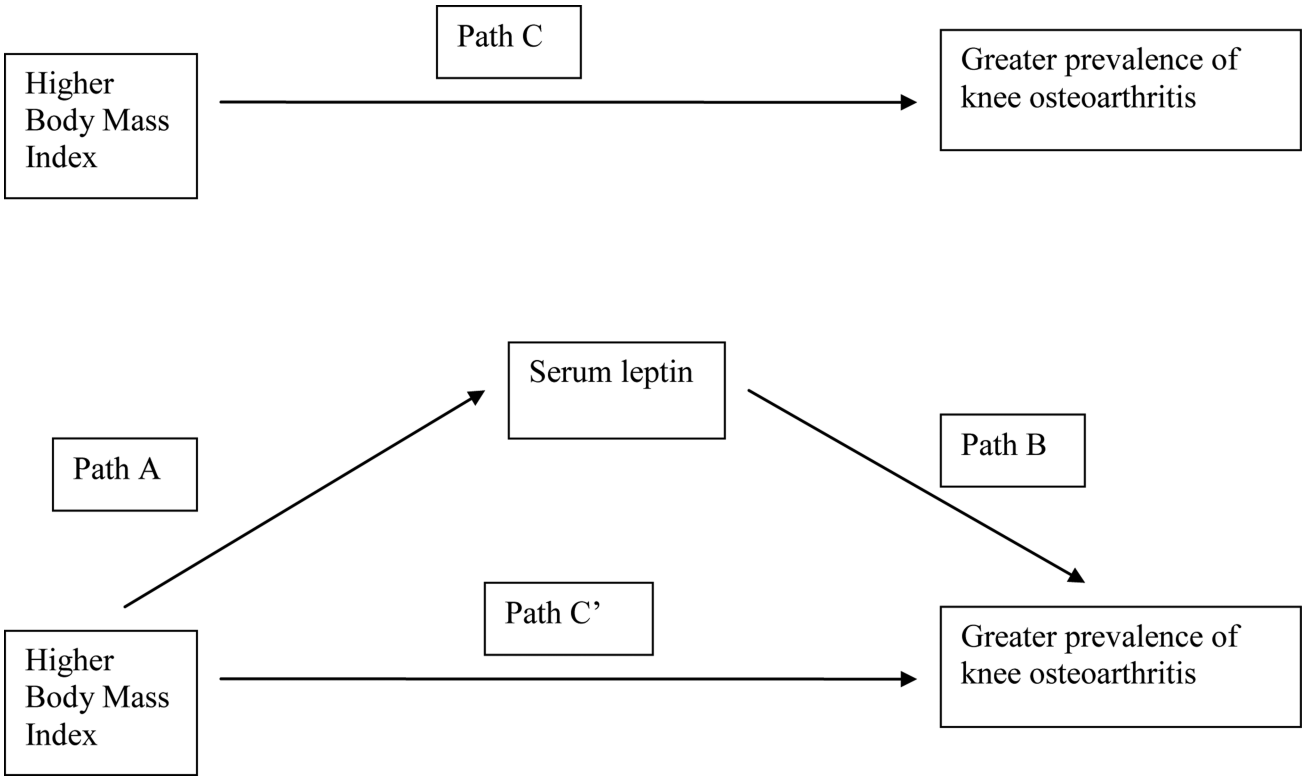

Figure 1.
Model of the Potential Mediating Effect of Serum Leptin on the Relationship between Body Mass Index and Knee Osteoarthritis

Table 1

Sample Characteristics According to Body-Mass Index Categories

	All N=653	Normal Weight N=203	Overweight N=262	Obese N=188	P value *
Age - mean (sd)	78.1 (5.4)	78.8 (5.7)	78.0 (5.5)	77.5 (4.8)	0.037
Female (%)	63	64	55	72	0.001
Race					
White (%)	79	82	81	72	0.053
Black (%)	16	12	14	22	
Other (%)	6	7	5	6	
Education level					
Less than HS (%)	10	9	10	12	0.207
HS (%)	23	24	19	27	
More than HS (%)	67	67	71	61	
Smoking status					
Never (%)	43	42	44	42	0.046
Former (%)	53	50	53	55	
Current (%)	5	9	3	3	
Knee deformity (%)	15	14	17	11	0.205
Physical Activity (mean, SD) †	110.2 (71.0)	112.9 (72.3)	116.1 (77.1)	99.1 (58.8)	0.001
Clinical Knee Osteoarthritis (%)	25	15	31	30	<0.001
Leptin (mean, SD) ‡	593.9 (694.4)	288.6 (328.1)	524.3 (472.0)	1024.5 (983.4)	<0.001

* Between weight group comparisons of categorical variables were tested using Chi-square tests and one-way analysis of variance

† Physical Activity Scale for the Elderly – unit-less measure

‡ Relationship between leptin and weight category – tests Path A, Figure 1

Table 2

Multivariable Adjusted Regression Models for Mediation Analysis*

	Clinical Knee Osteoarthritis (dependent variable) Odds ratio (95% CI)		
	Body Mass Index only (Path C)	**Leptin only (Path B)**	**Body Mass Index and Leptin in the model (Path C')**
Total			
Body Mass Index (kg/m2)†	1.32 (1.10 - 1.58)§	-	1.15 (0.93 - 1.41)
Leptin (pM)‡	-	1.11 (1.05 - 1.17)§	1.08 (1.02 - 1.15)§
Men			
Body Mass Index (kg/m2)†	1.07 (0.75 - 1.52)	-	0.90 (0.60 - 1.35)
Leptin (pM)‡	-	1.15 (0.98 - 1.34)	1.17 (0.98 - 1.40)
Women			
Body Mass Index (kg/m2)†	1.53 (1.22 - 1.94)§	-	1.35 (1.04 - 1.79)§
Leptin (pM)‡	-	1.11 (1.05 - 1.18)§	1.07 (0.99 - 1.14)

*Models adjusted for age, sex, race, education, knee deformity, smoking status and physical activity. BMI is a continuous variable in the models. Sequential models test different components of the mediation pathway (Paths B, C', C). Coefficients are not standardized for this analysis.

†Estimate represents change in odds ratio for a 5kg/m^2 increase in BMI

‡Estimate represents change in odds ratio for a 200 pM increase in serum leptin

§$p<0.05$

Table 3

Coefficients and Confidence Intervals for Product of Coefficients Mediation Analysis*

	Total Effect Direct Effect + Indirect Effect	**Direct effect Path C'**	**Indirect effect Path A + Path B**	**Percentage of total effect of obesity mediated by leptin**
Mediation of knee of osteoarthritis by leptin				
Total	0.149 (0.066, 0.258)†	0.076 (0.008, 0.213)†	0.073 (0.013, 0.095)†	49%
Men	0.023 (−0.183, 0.168)	−0.050 (−0.240, 0.124)	0.073 (−0.018, 0.141)	-
Women	0.242 (0.171, 0.408)†	.172 (0.102, 0.371)†	.070 (0.008, 0.119)†	29%

*Models adjusted for age, sex, race, education, knee deformity, smoking status and physical activity. We calculated the product of the standardized regression coefficients for path A and path B to estimate the coefficient for the indirect effect. We estimated the percent of the total effect mediated by leptin by calculating the ratio of the standardized coefficients for the indirect effect to the total effect.

†p<0.05