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The Association of an Increasing Waist Circumference and Risk of Incident Low Physical Function in Adults with Knee Osteoarthritis

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Abstract

Objective: To investigate an eight-year change in waist circumference (WC) with the risk of incident low physical function over one-year in adults with or at risk of knee osteoarthritis (OA).

Methods: Data from the Osteoarthritis Initiative (OAI) were used. Change in WC was measured from study enrollment (0-month) to the 96-month visit and classified as Increase (≥ 5cm gain) or Maintain (< 5cm gain). We identified WHO risk category based on WC at study enrollment as Large WC (males ≥ 102cm, females ≥ 88cm) or Small WC (males < 102cm, females < 88cm). The outcome was incident low physical function (≥ 28 WOMAC physical function subscale) at the 108-month visit. To investigate the association of the 8-year change in WC with the risk of low physical function, we calculated risk ratios 95% confidence intervals [95%CI and adjusted for potential confounders. We repeated the analyses stratified by the WHO disease risk category.

Results: The Increase WC group had 1.43 [1.04, 1.96] times the risk of incident low physical function compared to adults in the Maintain WC group. Adults with a Large at baseline WC, who increased WC, had 1.55 [1.00, 2.37] times the risk of incident low physical function compared to those who maintained WC. Adults with a Small WC at baseline, who increased WC, had 1.97 [0.84, 4.63] times the risk compared to the who maintained WC.

Conclusions: Increasing WC increases the risk of incident low physical function in the following year. Maintaining WC may mitigate developing low physical function.

Key Indexing Terms:

knee osteoarthritis; waist circumference; physical function

Introduction

Knee osteoarthritis (OA) is a chronic disease that ranks 11th among conditions contributing to years-lived with a disability, and the prevalence of knee OA is expected to increase with the aging global population.¹ Adults with knee OA are at risk for functional limitations such as reduced physical function.^{2,3} Functional limitations, in turn, may lead to persistent disabilities such as difficulty accessing services outside of the home and fulfilling roles in society, e.g., continuing to work.² Therefore, understanding factors associated with an increased burden of disease, specifically by maintaining physical function, is critical in this population.

Obesity is a risk factor for the development of knee OA by increasing joint loading, systemic inflammation, and altering body composition.⁴ These physiologic processes amplify knee pain, reduce physical activity and muscle strength, and predispose adults with knee OA to functional limitations.⁴ Obesity is typically assessed by the total amount of body weight, but the distribution of body weight, i.e., waist circumference (WC), is also important to consider.⁵ In particular, older adults with OA who have a large WC had low physical function compared to those with a healthy WC.⁶ Since WC is another tool to assess physical function and it is easy to measure, it is important to include WC when evaluating adults with knee OA. Furthermore, having a measure of functional status in adults with knee OA is important to identify those at risk of disability and who may need additional healthcare services to minimize disability.

Body weight and WC are not static and typically increase during mid-life.⁷ Increasing body weight may amplify the risk of functional limitation in adults with knee OA, more than current obesity status alone.⁸ Riddle et al. observed that an increase in body weight of more than 10% was associated with a decline in physical function in adults with knee OA.⁸ Gaining body weight increased the risk of functional decline and disability more than just being obese.⁸ It is unknown if an increasing WC, e.g., gaining weight in the abdominal region, also increases the risk of developing low physical function in adults with knee OA. It is critical to study and measure the risk of developing low physical function to prevent OA related-disability.

The World Health Organization (WHO) has sex-specific WC cutoffs to identify individuals with higher disease risk.⁹ Males with a WC ≥ 102 cm and females with a WC ≥ 88 cm have a higher risk of diabetes, dyslipidemia, hypertension, and cardiovascular disease.⁹ The WHO cut-points are also associated with walking difficulty among adults with or at risk of knee OA.¹⁰ Adults with a WC above the WHO disease risk threshold, who also increase their WC beyond this cut-point are possibly at a higher risk than those who maintain their WC above the WHO high-risk threshold. Conversely, adults below the WHO high-risk threshold who increase their WC may still be at risk of low physical function compared to those who maintain their WC below the threshold.

The purpose of our study was to investigate an eight-year change in WC with the risk of incident low physical function in the following year among adults with or at risk of knee OA. Also, to investigate an eight-year change in WC with the risk of low physical function

in the following year among two groups of adults with knee OA stratified by the WHO disease risk category. We hypothesized those who increased WC would have a higher risk of developing low physical function in the following year compared to those who maintained their WC.

Methods:

The study has Institutional Review Board approval from the University of Delaware (approval #1125357) and all Osteoarthritis Initiative (OAI) research site. All participants provided written informed consent before enrollment in the OAI study.

Study sample:

We used participant data from the study enrollment (0-month), 96-month, and 108-month visits of the OAI cohort, a large prospective, longitudinal study of adults with or at risk for knee OA. Study enrollment began in 2004, and participants between the ages of 45–79 were monitored annually for the development or progression of knee OA. Adults with rheumatoid or inflammatory arthritis, a bilateral end-stage disease defined as severe joint space narrowing or total knee replacements in both knees, and those who used ambulatory aids other than a cane were excluded from the OAI study enrollment. Participants were recruited from four study locations in Baltimore, Maryland; Columbus, Ohio; Pittsburgh, Pennsylvania; Pawtucket, Rhode Island. The OAI has Institutional Review Board approval, and each study location required participants to sign an informed consent before enrollment. Additional information on the specific objectives and study protocols is available online at (<https://oai.epiucsf.org/datarelease/>).

Exposure Measurement:

WC was measured around the participant's mid-torso near the umbilicus, between the lower rib and the iliac crest, at the largest circumference using a standardized tape measure. WC was measured three times, and the largest circumference was recorded in centimeters to the nearest 0.1 cm. This method is a valid measure of WC and has high test-retest reliability (ICC = 0.99 for males and ICC = 0.99 for females) and intra-rater reliability (ICC = 0.95 for males and ICC = 0.89 for females).¹¹ We used WC data from OAI measured at study enrollment (0-month) and the 96-month visit.

Study Exposure – 8-year Change in WC:

A change in WC was measured as the difference in WC between the study enrollment (0-month) and 96-month visits. An 8-year time period was used in the study because it was the longest duration in the OAI dataset to capture changes in WC. We categorized an eight-year change in WC as 1) Increase (≥ 5 cm) and 2) Maintain (< 5 cm). We chose a 5 cm increase in WC, as it is associated with an increased risk of mortality and disability in the general population, with a similar risk observed in both males and females.^{12,13} Participants who decreased WC during the 8-year follow-up were included in the Maintain group. We did not investigate a decrease in WC since we were interested in studying the association of increasing WC with low physical function.

Stratified Analysis - WHO Disease Risk Category:

WC at study enrollment (0-month) was stratified into Large and Small using the World Health Organization (WHO) cut-points for disease risk:¹¹ 1) Large WC (males ≥ 102 cm, females ≥ 88 cm) and 2) Small WC (males < 102 cm, females < 88 cm).

Study Outcome – Incident Low Physical Function in the Following Year:

Physical function was measured using the Western Ontario and McMaster Universities of Osteoarthritis (WOMAC) physical function subscale.^{3,14} The WOMAC is a patient-reported questionnaire that assesses disease progression in adults with knee or hip OA and includes three subscales pain, stiffness, and physical function.¹⁵ There are 17 questions related to the physical function subscale, e.g., What degree of difficulty do you have with going upstairs? Responses are in a Likert format from none to extreme difficulty, scores range from 0 to 68, and higher scores indicate worse physical function.¹⁵ The WOMAC physical function subscale is both a valid and reliable measure of physical function in adults with knee OA.^{14,15} A score of ≥ 28 on the WOMAC physical function subscale defined low physical function because it is a threshold that has been previously used to identify low physical function in adults with knee OA.¹⁶ Participants who were classified as having low physical function at the 96-month visit were removed from the final analysis to determine incidence at 108-month visit, i.e., at one-year follow-up. Specifically, our primary outcome of incident low physical function was defined as a participant progressing from no physical function limitations (WOMAC < 28) at the 96-month visit to low physical function (WOMAC ≥ 28) at the 108-month visit.

Potential confounders:

We adjusted for several potential confounders based on their association with WC and physical function that have been identified in previous studies. Variables used at study enrollment, i.e., 0-month OAI visit, were measured by interview or questionnaire and included the following: sex (female/male)¹⁷, race (white/other)¹⁸, and education (college degree/no college degree)¹⁹. Variables used at 96-month visit included the following: radiographic knee OA described as Kellgren-Lawrence grade ≥ 2 on x-ray in one or both knees (yes/no)²¹, age (years)²², presence of a comorbidity (score on the modified Charleston Comorbidity index)²³, body mass index (BMI) computed from weight and height assessments (kg/m^2)²⁴, knee pain (score on the WOMAC pain subscale)²⁵, and presence of depressive symptoms (score on the Center for Epidemiological Studies Depression Scale)²⁶.

Statistical analysis:

We calculated means, standard deviations, minimum, and maximum for continuous variables and percentages for categorical variables of participant characteristics at study enrollment, 96-month, and 108-month by the following exposure groups Maintain WC and Increase WC, and stratified by WHO disease risk threshold, e.g., Large WC and Small WC. To examine the association of an 8-year change in WC with the risk of incident low physical function in the following year, we calculated risk ratios (RR) and 95% confidence intervals

(95%CI). Maintain WC as the reference group. To investigate the generalizability of study findings in adults with a Large and Small WC at study enrollment, we repeated the analyses in these stratified samples. We calculated both unadjusted RR and RR adjusted for potential confounders using Poisson regression models. Specifically, we used PROC genmod with a Poisson distribution and a log-link function in SAS 9.4 (Cary, NC).²⁷

Results:

Of the 4796 participants from OAI, 2.3% (109/4796) had missing WC data at study enrollment (Figure 1). Of those participants with study enrollment data, 36.4% (1706/4687) of the participants had missing WC or WOMAC data, and 5.1% (241/4687) of the participants had a prevalent low physical function (≤ 28 on the WOMAC physical function subscale) at the 96-month visit (Figure 1). Of those participants free of the outcome at 96 months, 6.7% (184/2740) had missing WOMAC data at the 108-month visit (Figure 1). A total of 2556 participants were included in the analytic sample (Figure 1).

Participants in the analytic sample were on average (SD) 68 years of age (8.7) and overweight with a BMI of 28.5 (4.9). 56% were women, and 84% were white (Table 1). Participant characteristics were similar between the Maintain WC and Increase WC (Table 1). When the sample was stratified, there were differences in participant characteristics, specifically regarding gender and education (Table 2).

In the overall sample of adults without low physical function at 96 months, 5.7% (145/2556) developed low physical function in the following year. Adults in the Increase WC group had (adjusted RR [95%CI]) 1.43 [1.04, 1.96] times the risk of developing low physical function compared to those in the Maintain WC group (Table 3). Within individuals with Large WC in the stratified analysis, the Increase WC group had 1.55 [1.00, 2.37] times the risk of developing low physical function compared to those in the Maintain WC group (Table 3.) Within the individuals with Small WC at baseline in the stratified analysis, the Increase WC group had a higher risk of developing low physical function compared to the Maintain WC group. However, the effect did not reach statistical significance when adjusting for potential confounders 1.97 [0.84, 4.63] (Table 3).

Discussion:

Adults with an increasing WC over eight years had a higher risk of developing low physical function in the following year compared to adults who maintained their WC. In adults with a large WC at baseline, increasing WC over eight years remained a risk factor for developing low physical function in the following year compared to maintaining WC. Within adults with a small WC at baseline, those who increased their WC over eight years had a higher risk of developing low physical function in the following year than those who maintained WC, but the risk as attenuated when adjusting for confounders.

Our results suggest that adults with or at risk of knee OA, increasing WC over eight years, regardless of the baseline WHO disease risk category, heightens the risk of low physical function in the following year. However, increasing WC starting below the WHO high-risk threshold may still be relevant to physical function. In our study, the majority of participants

72.3% (13/18) with a Small WC and incident low physical function increased their WC above the Large WC category. While the adjusted RR for adults who increased their WC in small WC stratum compared to those who maintained WC was not statistically significant, the unadjusted RR there was a risk of low physical function 1.97 [0.84, 4.63]. Thus, it still may be important to continue to advise adults with or at risk of knee OA to avoid increasing WC above the WHO high-risk threshold.

Other authors have reported findings related to physical function and a large WC in adults with knee OA. For example, adults in the OAI cohort with a WC above the WHO disease risk threshold had 2.4 times the risk of developing a slow walking speed, e.g., a performance-based measure of physical function, in four years.¹⁰ Similarly, participants in the higher WC quartiles demonstrated lower self-reported physical function on health-related quality of life scale over two years.⁵ The absolute risk of developing low physical function in the large and maintain WC (5.8%) was slightly higher than the small and increase WC (5.5%) [risk difference of 0.3%], indicating that the absolute risk of low physical function may be related to a large WC, which is a similar finding in another OAI study.¹⁰ The objective of the study was to investigate relative risk, and our results indicate that the risk of low physical function in the following year is also high among those with an increasing WC. Perhaps maintaining WC over eight years is also a method to reduce the risk of future functional limitations in adults with or at risk of knee OA.

Our findings further highlight the importance of measuring WC in addition to body weight measured by BMI in adults with knee OA.^{6,10,28–29} The distribution of body mass around the abdominal region is associated with the progression of knee OA and an independent predictor of functional outcomes.^{6,30,31} In our analysis, we observed a higher risk of low physical function in the following year after adjusting for BMI, indicating that a large WC in itself is related to functional limitation. While obesity measured by BMI is a known risk factor for functional outcomes, researchers and clinicians should measure and track WC to identify adults with additional risk.

A strength of our study is that we used a large prospective data set. We used a 5 cm change in WC, which is a meaningful change for the risk of disease, disability, and all-cause in both males and females.¹³ WC changes throughout adulthood, and having an eight-year exposure period provided an extended time frame to observe both changes in WC.³³ Lastly, we selected a threshold of ≥ 28 on the WOMAC – physical function subscale instead of an absolute difference to better discriminate adults with low physical function.

However, our study is not without limitations. We were unable to determine a causal relationship between WC and incident low physical function because we were unable to account for other variables such as knee OA progression that may have occurred during the exposure period and attributed to the development of low physical function. Further, body weight and physical function can fluctuate over time, and our analyses did not account for the dynamic changes that may have occurred in the 8-year exposure period. Also, we stratified the sample at study enrollment (0-month), which may have resulted in a sample selection bias since we did not account for participants who crossed-over between the Large WC and Small WC groups during the 8-year exposure period. We did not account for

missing data with techniques such as imputation in our analysis. Also, less than 7% of participants developed low physical function during the study, which limits our ability to estimate the relationship precisely. Lastly, the OAI cohort is demographically homogenous and has diminished generalizability to the overall population of adults with knee OA. For example, most participants in OAI have more than a college education, a household income \$50,000, and are white; therefore, applying the results of our study to different groups of adults with knee OA should be done with caution.

Conclusion:

Clinicians should encourage adults with or at risk of knee OA to avoid increasing their WC to reduce the risk of developing a future functional limitation. Researchers and clinicians should continue to measure and monitor WC over time, in addition to BMI, since an increasing WC is also associated with the future risk of developing low physical function.

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Figure 1. Flow diagram for sample size reduction

Table 1.

Characteristics of study participants by 8-year change in WC (study enrollment to 96-month visit) (n=2556)

	All	I ₁ Maintain WC	I ₂ Increase WC
Total Sample (n=number of participants if data is missing)	2556	1775	801
Age ^o (years) [mean (sd) min, max]	68.4 [(8.7) 53, 87]	69.0 [(8.7) 53, 87]	67.1 [(8.4) 53, 87]
Gender [^] (% female) (n)	56.3% (1439)	56.7% (995)	55.4% (444)
Race [^] (% white) (n)	83.9% (2145)	85.1% (1493)	81.4% (652)
Education [^] (n=2554) (% college) (n)	67.0% (1706)	67.5% (1180)	66.1% (526)
BMI ^o (kg/m ²) (n=2555) [mean (sd) min, max]	28.5 [(4.9) 15.5, 53.5]	27.8 [(4.6) 15.5, 50.4]	29.9 [(5.3) 18.4, 53.5]
Knee pain ^o (n=2551) [mean (sd) min, max]	2.5 [(4.1) 0, 24.0]	2.4 [(4.0) 0, 24.0]	2.7 [(4.2) 0, 23.4]
Comorbidity ^o (n=2539) [mean (sd) min, max]	0.6 [(1.1) 0, 8.0]	0.6 [(1.1) 0, 8.0]	0.6 [(1.0) 0, 7.0]
Depression ^o (n=2531) [mean (sd) min, max]	6.1 [(6.6) 0, 51.0]	6.0 [(6.5) 0, 47.0]	6.5 [(7.0) 0, 51.0]
Radiographic knee OA ^o (% with) (n)	64.4% (1645)	64.0% (1122)	65.3% (523)
WC at study enrollment [^] (cm) [mean (sd) min, max]	101.5 [(12.5) 62.8, 149.3]	103.3 [(11.8) 70.5, 147.3]	97.6 [(13.1) 62.8, 149.3]
WC at 96-month ^o (cm) [mean (sd) min, max]	102.9 [(12.8) 62.8, 163.5]	100.5 [(11.8) 62.9, 145.4]	108.4 [(13.3) 72.0, 163.5]
8-year change in WC (cm) [mean (sd) min, max]	1.5 [(8.6) -37.9, 45.8]	-2.7 [(6.1) -37.9, 4.9]	10.7 [(5.5) 5.0, 45.8]
WOMAC - PF at 96-month ^o [mean (sd) min, max]	6.9 [(8.0) 0, 28.0]	6.6 [(7.8) 0, 28]	7.6 [(8.2) 0, 28]
WOMAC - PF at 108-month [*] [mean (sd) min, max]	8.4 [(9.9) 0, 68]	8.1 [(9.8) 0, 68]	9.2 [(10.3) 0, 46.0]

I₁ Maintain WC were participants who changed their WC < 5 cm over 8 years

I₂ Increase WC were participants who had a change in WC ≥ 5 cm over 8 years

[^] Data collected at study enrollment

^o Data collected at 96-month visit

^{*} Data collected at 108-month visit

WOMAC - PF = Western Ontario and McMaster University Osteoarthritis Index (WOMAC) - Physical Function Subscale

Knee pain = WOMAC Pain Subscale score

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Comorbidity = Charlson Comorbidity Index score
Depression = Center for Epidemiologic Studies Depression Scale score
WC = Waist circumference

Table 2.

Characteristics of study participants by 8-year change in WC (study enrollment to 96-month visit) stratified by WHO disease risk category at study enrollment (n=2556)

	All	Small WC		Large WC	
		¹ Maintain WC	² Increase WC	³ Maintain WC	⁴ Increase WC
Total Sample (n=number of participants if data is missing)	2556	469	326	1286	475
Age ^o (years) [mean (sd) min, max]	68.4 [(8.7) 53, 87]	67.2 [(9.2) 53, 87]	66.4 [(8.5) 53, 87]	69.6 [(8.5) 53, 87]	67.5 [(8.3) 53, 87]
Gender [^] (% female) (n)	56.3% (1439)	20.7% (97)	42.3% (138)	69.8% (898)	64.74% (306)
Race [^] (% white) (n)	83.9% (2145)	86.8% (407)	82.8% (270)	84.5% (1086)	80.4% (382)
Education [^] (n=2554) (% college) (n)	67.0% (1706)	79.2% (369)	76.9% (249)	63.3% (811)	58.7% (277)
BMI ^o (kg/m ²) (n=2555) [mean (sd) min, max]	28.5 [(4.9) 15.5, 53.5]	24.6 [(2.9) 15.5, 32.4]	26.2 [(3.3) 18.4, 35]	30.0 [(4.6) 17.4, 50.4]	32.4 [(4.8) 21.5, 53.5]
Knee pain ^o (n=2551) [mean (sd) min, max]	2.5 [(4.1) 0, 24.0]	2.0 [(3.9) 0, 23.0]	2.4 [(4.1) 0, 23.4]	2.5 [(4.1) 0, 24.0]	2.9 [(4.3) 0, 23.4]
Comorbidity ^o (n=2539) [mean (sd) min, max]	0.6 [(1.1) 0, 8.0]	0.5 [(1.1) 0, 8.0]	0.4 [(0.9) 0, 6.0]	0.7 [(1.1) 0, 8.0]	0.7 [(1.1) 0, 7.0]
Depression ^o (n=2531) [mean (sd) min, max]	6.1 [(6.6) 0, 51.0]	5.2 [(6.4) 0, 47.0]	5.7 [(6.2) 0, 37.0]	6.3 [(6.5) 0, 44.0]	7.1 [(7.4) 0, 51.0]
Radiographic knee OA ^o (% with) (n)	64.4% (1645)	53.6% (251)	54.0% (176)	67.8% (871)	73.0% (347)
WC at study enrollment [^] (cm) [mean (sd) min, max]	101.5 [(12.5) 62.8, 149.3]	91.6 [(7.1) 70.5, 101.9]	87.2 [(8.8) 62.8, 101.9]	107.5 [(10.2) 88.0, 147.3]	104.7 [(10.6) 88.0, 149.3]
WC at 96-month ^o (cm) [mean (sd) min, max]	102.9 [(12.8) 62.8, 163.5]	91.5 [(8.1) 66.1, 106.4]	98.0 [(8.6) 72, 128.3]	103.9 [(11.2) 62.8, 145.4]	115.4 [(11.0) 94.1, 163.5]
8-year change in WC (cm) [mean (sd) min, max]	1.5 [(8.6) -37.9, 45.8]	-0.1 [(3.9) -17.5, 4.9]	10.8 [(5.7) 5.0, 42.5]	-3.6 [(6.5) -37.9, 4.9]	10.7 [(5.4) 5.0, 45.8]
WOM/AC - PF at 96-month ^o [mean (sd) min, max]	6.9 [(8.0) 0, 28.0]	4.9 [(7.1) 0, 28.0]	6.3 [(7.7) 0, 27.6]	7.2 [(8.0) 0, 28.0]	8.5 [(8.4) 0, 28.0]
WOM/AC - PF at 108-month ^o [mean (sd) min, max]	8.4 [(9.9) 0, 68]	6.2 [(8.8) 0, 48]	7.5 [(9.3) 0, 46]	8.7 [(10.0) 0, 68]	10.4 [(10.7) 0, 68]

¹ Participants with a small WC (male <102 cm, female <88 cm) at study enrollment who maintained their WC (< 5 cm) over 8 years

² Participants with a small WC (male <102 cm, female <88 cm) at study enrollment who increased their WC (> 5 cm) over 8 years

³ Participants with a large WC (male 102 cm, female 88 cm) at study enrollment who maintained their WC (< 5 cm) over 8 years

⁴ Participants with a large WC (male 102 cm, female 88 cm) at study enrollment who increased their WC (> 5 cm) over 8 years

[^] Data collected at study enrollment

^o Data collected at 96-month visit

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* Data collected at 108-month visit

WOMAC - PF = Western Ontario and McMaster University Osteoarthritis Index (WOMAC) - Physical Function Subscale Knee pain = WOMAC Pain Subscale score
Comorbidity = Charlson Comorbidity Index score
Depression = Center for Epidemiologic Studies Depression Scale score
WC = Waist circumference

Table 3.**Risk Ratios for Incident Low Physical Function**

		Incident Outcome/Total [*]	%	Unadjusted RR (95%CI)	Adjusted RR ^{**} (95%CI)
8-year Change in WC [^]					
	Maintain WC	86/1755	4.9	REFERENCE	REFERENCE
	Increase WC	59/801	7.4	1.50 (1.09, 2.07)	1.43 (1.04, 1.96)
Stratified by WHO Disease Risk Category [°]					
Small WC	Maintain WC	12/469	2.6	REFERENCE	REFERENCE
	Increase WC	18/326	5.5	2.16 (1.05, 4.42)	1.97 (0.84, 4.63)
Large WC	Maintain WC	74/1286	5.8	REFERENCE	REFERENCE
	Increase WC	41/475	8.6	1.50 (1.04, 2.16)	1.55 (1.00, 2.37)

[^] Change in WC from study enrollment to 96-month visit (8 years) categorized by Maintain WC (< 5cm) or Increase WC (> 5cm)

[°] WHO disease risk category at study enrollment Small WC (male <102 cm, female <88 cm) or Large WC (male ≥ 102 cm, female ≥ 88 cm) by change in WC from study enrollment to 96-month visit (8 years) Maintain (< 5 cm) or Increase (> 5 cm)

^{*} Incident outcome: low physical function defined as WOMAC ≥ 28 on the physical function subscale at 108-month visit

^{**} Adjusted for sex (female/male), race (white/other), education (college/< college) at 0-month visit; ROA (yes/no), age (years), comorbidity, BMI (kg/m²), WOMAC pain subscale, depression at 96-month visit

WHO = World Health Organization

statistically significant at alpha level < 0.05

95% CI = 95% confidence interval