range for serum uric acid was 0.2–200 mg/dL. Plasma C-reactive protein (CRP) was measured using a latex immunoassay with the assay range of 0.2–4,000 mg/L. Estimated GFR was calculated from age, sex, and serum creatinine [27].

Subjects with a reported history of diabetes mellitus, fasting glucose of 126 mg/dL or higher, or glycosylated hemoglobin levels at 6.5 % or higher were classified as diabetic. Those with a reported history of hypertension, systolic blood pressure of 140 mmHg or higher, or diastolic blood pressure of 90 mmHg or higher were classified hypertensive.

Bone mineral density measurements

BMD of the lumbar spine was measured by DXA using a GE Lunar Prodigy. A standard quality control program included daily calibrations with machine-specific phantoms to ensure machine accuracy of greater than 98 %.

Statistical analysis

Uric acid becomes insoluble and supersaturated in bodily fluids above a concentration of about 7 mg/dL. The nonparametric locally weighted scatterplot smoothing (LOESS) method was used to determine whether the saturation point affects the functional form of the association between uric acid and BMD. The LOESS method generated a smooth curve of BMD as a function of uric acid. Visual inspection of the LOESS plot indicated that the relationship between BMD and uric acid was piecewise linear with an inflection (change of slope) at the uric acid value of 4.8, above which the slope appeared steeper (Fig. 1). We then fitted piecewise linear spline models to BMD as a function of uric acid with a fixed knot at 4.8. We also employed generalized additive models to examine the shape of the association between uric acid and BMD accounting for other covariates. The generalized additive model is an extension of the generalized linear model in which one or more independent variables can be modeled with nonparametric smooth functions [28].

The model was initially adjusted for age and BMI (model 1). Covariates for lifestyle risk factors for osteoporosis including physical activity; smoking and drinking habit; years after menopause (coded as 0 if subject had not experienced menopause) (model 2); comorbidity including diabetes mellitus and hypertension (model 3); and serum calcium, alkaline phosphatase (ALP), estimated GFR, and log (CRP) (model 4) were successively added to regression models. The selection of covariates was based on the literature review on factors affecting BMD [29–35].

There were missing values for physical activity in 180 women (29.3 %), years after menopause in 140 women (22.8 %), and drinking habit in 1 woman (0.2 %). These were imputed using the expectation–maximization (EM) algorithm [36].

Statistical analyses were performed using SAS, version 9.2 (SAS Institute, Inc., Cary, NC, USA) and R statistical software

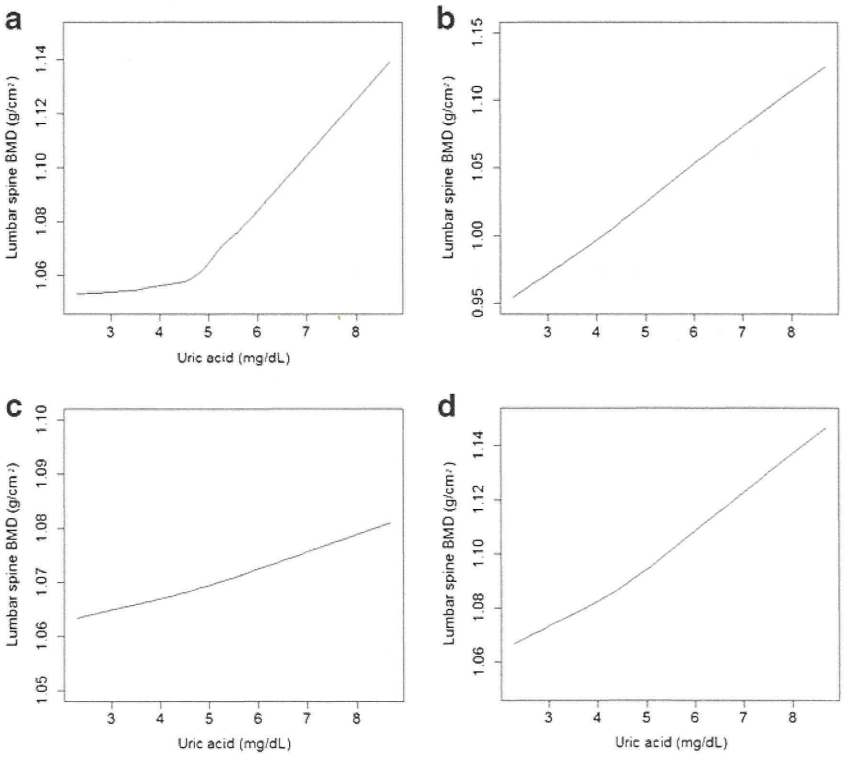

Fig. 1 Plots of lumbar spine bone mineral density against uric acid level. **a** The LOESS plot. **b–d** The plots generated using generalized additive models accounting for age (**b**), body mass index (**c**), or estimated glomerular filtration ratio (**d**). The values of the covariates were fixed at their mean when the association between lumbar spine BMD and uric acid were plotted. *BMD* bone mineral density

version 2.15.2 (R Foundation, Vienna, Austria). All statistical tests were two-sided, and a p value less than 0.05 was considered statistically significant.

Results

Characteristics of study participants are shown in Table 1. Women included in the analysis were similar to those excluded from the analysis with respect to major characteristics. Of the 615 women included in the analysis, serum uric acid had a mean value of 4.7 mg/dL with standard deviation of 1.0 mg/dL. Only 12 women (2.0 %) had hyperuricemia (i.e., uric acid level higher than 7.0 mg/dL), and 19 (3.1 %) women were obese (i.e., BMI equal to or higher than 30 kg/m^2).

Table 1 Characteristics of participants

	Participants (n=615)
Uric acid (mg/dL)	4.7±1.0
Lumbar spine bone mineral density (g/cm^2)	1.06±0.18
Age (years)	57.6±6.4
Log (CRP in mg/L)[a]	0.12±0.17
BMI (kg/m^2)	22.2±3.5
Smoking	
Current	19 (3.1)
Ex	53 (8.6)
Never	543 (88.3)
Drinking[b]	
Abstainer	219 (35.7)
Infrequent	188 (30.6)
Light	171 (27.9)
Moderate to heavy	36 (5.9)
Activity[b]	
Sedentary	283 (65.1)
Active	152 (34.9)
Postmenopausal[b]	373 (78.5)
Age at menopause in postmenopausal women (years)	50.9±3.8
Diabetes	44 (7.2)
Hypertension	114 (18.5)
Serum calcium (mg/dL)	9.3±0.3
Estimated GFR (mL/min/1.73 m^2)	97.8±21.6
ALP (IU/L)	223.5±66.3

For continuous variables, the mean is shown with standard deviation. For categorical variables, the number (percentage) is shown. Percentages may not add up to 100 because of rounding errors

BMD bone mineral density, *CRP* C-reactive protein, *BMI* body mass index, *GFR* glomerular filtration rate, *ALP* alkaline phosphatase, *IU* international unit

[a] The natural log (base e) was taken for CRP due to skewed distribution

[b] There were missing values for physical activity in 180 women (29.3 %), years after menopause in 140 women (22.8 %), and drinking habit in 1 woman (0.2 %)

Association between BMD and uric acid

In piecewise linear regression of BMD as a function of uric acid with a fixed knot at uric acid level of 4.8 mg/dL, the change in slope at the knot was not statistically significant in univariate analysis and all four models of multivariate analyses (p values=0.31–0.79). The generalized additive models also demonstrated that uric acid was approximately linearly associated with BMD when accounting for each of age, BMI, or estimated GFR (Fig. 1). Therefore, the knot was subsequently dropped. The resulting multiple linear regression models fitted simple linear relationship between uric acid and BMD. Serum uric acid levels were significantly and positively associated with lumbar spine BMD adjusting for age and BMI (model 1, Table 2). The association between uric acid and BMD remained significant after successively adjusting for lifestyle risk factors and years after menopause (model 2); comorbidity (model 3); and serum calcium, estimated GFR, log (CRP), and ALP (model 4). Serum uric acid levels explained 0.48–0.63 % of variance in BMD (R^2=0.187–0.258).

Effect modification

One of the presumed mechanisms of the association between BMD and uric acid is the antioxidant property of uric acid. Considering the complicated and interrelated relationship between oxidative stress and inflammation, we postulated that the degree of inflammation modifies the association between BMD and uric acid. To test this hypothesis, we examined the interaction between log (CRP) and uric acid, but it was not significant (p=0.22).

Table 2 Adjusted associations of serum uric acid with lumbar spine bone mineral density (n=615)

	Beta[a]	%V[b]	p	R^2
Model 1	0.084	0.63	0.03	0.187
Model 2	0.081	0.57	0.04	0.199
Model 3	0.084	0.61	0.03	0.206
Model 4	0.078	0.48	0.049	0.258

Model 1—adjusted for age, BMI; model 2—adjusted for age, BMI, smoking, drinking, physical activity, and years after menopause; model 3—adjusted for age, BMI, smoking, drinking, physical activity, years after menopause, diabetes, and hypertension; model 4—adjusted for age, BMI, smoking, drinking, physical activity, years after menopause, diabetes, hypertension, serum calcium, estimated GFR, log (CRP), and ALP. Abbreviations are as in Table 1

[a] Standardized beta coefficient

[b] Variance of lumbar spine bone mineral density explained by uric acid

Sensitivity analysis

Previous studies have demonstrated that menopause is associated with changes in both BMD and uric acid. Women have a minimal decline in BMD until 1–2 years prior to the final menstrual period when they begin to experience a rapid decline in BMD. The decline in BMD decelerates 1–2 years after the final menstrual period, but continues [37]. On the other hand, postmenopausal status was associated with higher levels of uric acid [38, 39]. Therefore, the associations of age with BMD and uric acid in this study sample of peri- and postmenopausal women may not be linear. The LOESS plots of BMD and uric acid as a function of age demonstrated that both of the relationships were piecewise linear, with an inflection at around the age of 60 (Fig. 2a, b). Uric acid rapidly increased with increasing age until age 60 years, then decelerated but continued to increase. Similarly, lumbar spine BMD declined rapidly with increasing age, but the rate of decline slowed down at the age of 60 years but continued to decline. As a sensitivity analysis, we examined the association between uric acid and BMD after excluding 177 women older than 60. The analysis demonstrated significant and positive associations between BMD and uric acid in all models, with effect sizes slightly larger than those observed in the main analyses, supporting the robustness of our scientific conclusion (Table 3).

We also conducted another sensitivity analysis after excluding 281 women with any missing values in covariates. This sensitivity analysis revealed slightly larger effect sizes of the association between UA and BMD than those in the main analyses, but the associations failed to reach statistical significance (data not shown).

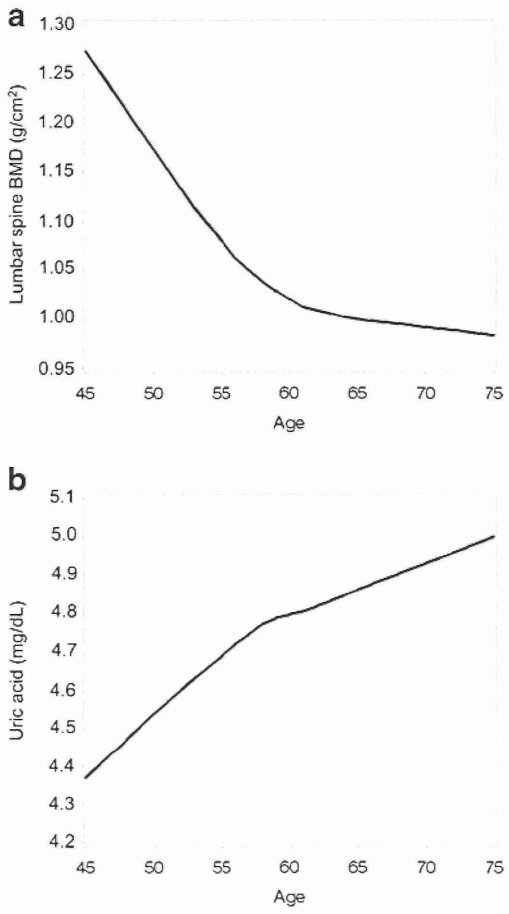

Fig. 2 LOESS plots of bone mineral density and uric acid against age. *BMD* bone mineral density

Discussion

In this cross-sectional analysis of 615 peri- and postmenopausal women aged between 45 and 75 years, higher serum levels of uric acid were significantly associated with higher values of BMD in the lumbar spine, independent of covariates including years after menopause. One standard deviation (1.0 mg/dL in this study population) increment in uric acid was associated with an approximately 0.08 standard deviation increase in lumbar spine BMD. We also demonstrated rapid changes in uric acid and BMD with increasing age until the age of 60, and the rate of changes slowed down thereafter. The positive association between BMD and uric acid remained significant after excluding women older than 60 years.

Our study confirms and extends a previous study that has demonstrated a positive association between BMD and uric acid in peri- and postmenopausal women [15, 16]. We showed that uric acid was positively and linearly associated with lumbar spine BMD, and therefore not only the presence of hyperuricemia but also the magnitude of uric acid elevation plays an important role. Addition of years after menopause did not significantly affect the uric acid–BMD association. We did not observe any sharp inflection point (i.e., change of slope) in the association between uric acid and BMD, incongruent with our hypothesis that the association between uric acid and

Table 3 Adjusted associations of serum uric acid with lumbar spine bone mineral density after excluding 177 women older than 60 years (n=438)

	Beta[a]	%V[b]	p	R^2
Model 1	0.103	0.96	0.02	0.284
Model 2	0.091	0.73	0.04	0.294
Model 3	0.101	0.89	0.02	0.304
Model 4	0.107	0.91	0.02	0.359

Model 1—adjusted for age and BMI; model 2—adjusted for age, BMI, smoking, drinking, physical activity, and years after menopause; model 3—adjusted for age, BMI, smoking, drinking, physical activity, years after menopause, diabetes, and hypertension; model 4—adjusted for age, BMI, smoking, drinking, physical activity, years after menopause, diabetes, hypertension, serum calcium, estimated GFR, log (CRP), and ALP. Abbreviations are as in Table 1

[a] Standardized beta coefficient

[b] Variance of lumbar spine bone mineral density explained by uric acid

BMD becomes inverse in the hyperuricemic range. However, it should be noted that only a small portion of women in this study had hyperuricemia, and further study is needed to determine if the association between BMD and uric acid in the hyperuricemic range may differ from that in the physiologic range.

We also demonstrated that there was a period of rapid increase in uric acid until the age of 60 years when the rate of increase slowed. The observed trajectory of uric acid is consistent with menopause-related changes, rather than changes secondary to chronological aging. This is congruent with previous studies showing that uric acid levels were higher in postmenopausal women compared with pre- or perimenopausal women [38, 39]. We observed a similar menopause-related change in BMD, consistent with previous studies [37]. However, the inflection (i.e., change of slope) was observed at around the age of 60 for both uric acid and BMD in the present study, which appears too far apart from the mean age at menopause of approximately 51 years. The possible explanations for the discrepancy include reporting error and the nature of cross-sectional data, which are predisposed to recall bias and are unable to separate the effects of aging from secular trend. Hence, a longitudinal study is warranted to determine the precise trajectory of uric acid during the menopause transition.

This study has several limitations. First, the study design was cross-sectional and did not allow us to infer a cause–effect relationship between uric acid level and BMD. However, one previous longitudinal study demonstrated that higher serum uric acid levels were associated with slower annual decline in BMD in peri- and postmenopausal women [16]. Second, we employed an EM algorithm to impute missing values in covariates. Missing values occurred mostly in two variables—physical activity and age at menopause. Sensitivity analysis excluding women with any missing values in covariates yielded similar, albeit not significant, effect sizes of the association between BMD and uric acid, indirectly supporting the robustness of the approach. The association failed to reach statistical significance due to the reduced number of women included in the sensitivity analysis. Third, the data were obtained from the medical records of female teachers who had received health checkup annually and were therefore expected to be generally in good health and health conscious. In fact, the prevalence of comorbidity such as hypertension and diabetes, and the smoking rate were lower than those in the general population [40–42]. In addition, the women in this study had lower weight compared with peri- and postmenopausal Australian women in a previous study on uric acid and BMD [16]. Thus, the observed associations of uric acid with menopause and BMD were less likely to be confounded by obesity and other comorbidity, but the generalizability of the findings to other populations may be limited. In addition, BMD measurement was performed voluntarily, which could introduce selection bias. However, women in the analysis were comparable to those excluded from the analysis, most of whom had not had BMD measurement and excluded. Fourth, the observed association was marginally significant. We speculate that it is mostly likely due to relatively small sample size because the finding was consistent throughout various models. Lastly, any observational studies like this one cannot be free of possible confounding due to uncontrolled or unmeasured variables. Several important variables such as bone turnover markers, PTH, and serum 25-hydroxyvitamin D were not measured or available for the analysis.

Despite these limitations, the study has several strengths. Even though this was a retrospective analysis, the data were drawn from medical records for health checkup, which were in general free of missing values except for a few measurements. These measurements were performed voluntarily or as a part of optional examinations. The main finding was robust to the inclusion of a variety of covariates including years after menopause and exclusion of older women.

In conclusion, the present study showed that higher uric acid levels in the physiologic range of uric acid are linearly associated with higher lumbar spine bone mineral density in peri- and postmenopausal Japanese women. Further research is needed to elucidate the precise underlying mechanism of the association between uric acid and bone mineral density and to determine if the positive association between BMD and uric acid is still observed in the hyperuricemic range.

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Conflicts of interest None.

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ORIGINAL ARTICLE

Development of a simple screening test for sarcopenia in older adults

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Aim: To develop a simple screening test to identify older adults at high risk for sarcopenia.

Methods: We studied 1971 functionally independent, community-dwelling adults aged 65 years or older randomly selected from the resident register of Kashiwa city, Chiba, Japan. Data collection was carried out between September and November 2012. Sarcopenia was defined based on low muscle mass measured by bioimpedance analysis and either low muscle strength characterized by handgrip or low physical performance characterized by slow gait speed.

Results: The prevalence of sarcopenia was 14.2% in men and 22.1% in women. After the variable selection procedure, the final model to estimate the probability of sarcopenia included three variables: age, grip strength and calf circumference. The area under the receiver operating characteristic curve, a measure of discrimination, of the final model was 0.939 with 95% confidence interval (CI) of 0.918–0.958 for men, and 0.909 with 95% CI of 0.887–0.931 for women. We created a score chart for each sex based on the final model. When the sum of sensitivity and specificity was maximized, sensitivity, specificity, and positive and negative predictive values for sarcopenia were 84.9%, 88.2%, 54.4%, and 97.2% for men, 75.5%, 92.0%, 72.8%, and 93.0% for women, respectively.

Conclusions: The presence of sarcopenia could be detected using three easily obtainable variables with high accuracy. The screening test we developed could help identify functionally independent older adults with sarcopenia who are good candidates for intervention. **Geriatr Gerontol Int 2014; 14 (Suppl. 1): 93–101.**

Keywords: disability, rehabilitation, sarcopenia, screening, sensitivity and specificity.

Introduction

Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal mass and strength with aging.[1] A recent realization that sarcopenia is associated with a risk of adverse events, such as physical disability, poor quality of life and death, has provided significant impetus to sarcopenia research.[1] Effective interventions have been vigorously sought and some interventions, such as resistance training in combination with nutritional supplements, appear promising.[2–4] It is also becoming apparent that interventions might be more effective early rather than late in the course when patients develop physical disability or functional dependence.[4,5] The early stage in the course of sarcopenia (i.e. without loss of physical or functional independence) might therefore represent a valuable opportunity to carry out interventions to decelerate the progress of sarcopenia and prevent physical disability.

However, patients with sarcopenia are generally unaware of their sarcopenic state until the gradual decline in muscle function becomes severe enough to be pathological, resulting in physical and functional dependence.[4,6] As patients are unlikely to seek medical

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attention for their sarcopenic state, population screening to detect sarcopenia before the occurrence of physical disability could improve the chance of intervention.

Currently, the recommended criteria for the diagnosis of sarcopenia require the documentation of low muscle mass and either low muscle strength or low physical performance.[1] Muscle mass is commonly assessed by dual energy X-ray absorptiometry (DXA) or bioimpedance analysis (BIA), muscle strength with handgrip strength, and physical performance with Short Physical Performance Battery or usual gait speed.[1,7] Unfortunately, the feasibility of applying the recommended diagnostic algorithm in the setting of population screening is limited by the need for special equipment and training. Hence, a screening test for sarcopenia simple enough to be carried out on a large scale is required.

Using baseline data from the Kashiwa study on functionally independent, community-dwelling older adults, we designed an analysis to develop a simple screening test for sarcopenia and examine its ability to estimate the probability of sarcopenia.

Methods

Participants

The Kashiwa study is a prospective cohort study designed to characterize the biological, psychosocial and functional changes associated with aging in community-dwelling older adults. In 2012, a total of 12 000 community-dwelling, functionally independent (i.e. not requiring nursing care provided by long-term care insurance) adults aged 65 years or older were randomly drawn from the resident register of Kashiwa city, a commuter town for Tokyo in Chiba prefecture, Japan, and asked by mail to participate in the study. A total of 2044 older adults (1013 men, 1031 women) agreed to participate in the study and comprised the inception cohort. The sample reflected the distribution of age in Kashiwa city for each sex.

Baseline examinations were carried out between September and November 2012 at welfare centers and community centers close to the participants' residential area, to obviate their need to drive. A team consisting of physicians, nurses, physical therapists, dentists and nutritionists carried out data collection. To standardize data collection protocol, they were given the data collection manual, attended two sessions for training in the data collection methods and carried out a rehearsal of data collection. A total of 73 participants who did not undergo BIA, usual gait speed or handgrip strength measurements were excluded, leaving an analytic sample of 1971 older adults (977 men, 994 women).

The study was approved by the ethics committee of the Graduate School of Medicine, The University of Tokyo. All participants provided written informed consent.

Sarcopenia classification and measurement of each component of sarcopenia

We followed the recommendation of the European Working Group on Sarcopenia in Older People (EWGSOP) for the definition of sarcopenia.[1] The proposed diagnostic criteria required the presence of low muscle mass plus the presence of either low muscle strength or low physical performance.

Muscle mass measurement

Muscle mass was measured by BIA using an Inbody 430 machine (Biospace, Seoul, Korea).[8] Appendicular skeletal muscle mass (ASM) was derived as the sum of the muscle mass of the four limbs. ASM was then normalized by height in meters squared to yield skeletal muscle mass index (SMI) (kg/m^2).[1] SMI values lower than two standard deviations below the mean values of young male and female reference groups were classified as low muscle mass (SMI <7.0 kg/m^2 in men, <5.8 kg/m^2 in women).[9]

Muscle strength measurement

Muscle strength was assessed by handgrip strength, which was measured using a digital grip strength dynamometer (Takei Scientific Instruments, Niigata, Japan). The measurement was carried out twice using their dominant hand, and the higher of two trials (in kilograms) was used for the present analysis. Handgrip strength values in the lowest quintile were classified as low muscle strength (cut-off values: 30 kg for men, 20 kg for women).

Physical performance measurement

Physical performance was assessed by usual gait speed. Participants were instructed to walk over an 11-m straight course at their usual speed. Usual gait speed was derived from 5 m divided by the time in seconds spent in the middle 5 m (from the 3-m line to the 8-m line). Good reproducibility of this measurement was reported previously.[10] Usual gait speed values in the lowest quintile were classified as low physical performance (cut-off values: 1.26 m/s for each sex).

Other measurements

Demographic information and medical history of doctor-diagnosed chronic conditions were obtained

using a standardized questionnaire. Physical activity was assessed using Global Physical Activity Questionnaire and Metabolic Equivalent minutes per week was computed.[11] Serum albumin was measured at the time of the visit. Anthropometric measurements were obtained with the participants wearing light clothing and no shoes. Height and weight were measured with a fixed stadiometer, and a digital scale and used to compute body mass index (BMI). Upper arm, thigh and calf circumferences were measured to the nearest 0.1 cm directly over the skin using a measuring tape with the participant sitting. Upper arm circumference was measured at the mid-point between the olecranon process and the acromion of the non-dominant arm with the participant's arm bent 90° at the elbow. Calf circumference measurement was made at the maximum circumference of the lower non-dominant leg with the participant's leg bent 90° degrees at the knee. Thigh circumference was measured 15 cm above the upper margin of the patella of the dominant leg.

Statistical analysis

All analyses were stratified by sex. Differences in participant characteristics between those with and without sarcopenia were examined using Student's *t*-test or Wilcoxon rank–sum test. To develop a statistical model to estimate the probability of sarcopenia, candidate variables were selected by experts based on cost, ease of measurement and availability of equipment to measure them. The candidate variables included age, sex, BMI, grip strength, and thigh, calf and upper arm circumferences. Pearson's correlation between each component of sarcopenia and the candidate variables was first computed. We then examined the functional form of the relationships between the variables, and the logit of sarcopenia probability using restricted cubic spline plots and the Wald test for linearity.[12] We considered dichotomization, square and logarithmic transformations if the Wald test for linearity was statistically significant, rejecting the assumption of linearity.[12] A multivariate logistic regression model including all the candidate variables ("full model") was constructed. Variable selection with Bayesian Information Criteria was carried out to make the model parsimonious, and a multivariate logistic regression model including the variables selected ("restricted model") was made.[13] A bootstrapping procedure was used to obtain estimates of internal validity of the model[14] and to derive the final models by correcting the regression coefficients for overoptimism.[15] The final model was presented as a score chart to facilitate clinical application.[15] The score chart was created based on rounded values of the shrunken regression coefficients.

The ability of each model to correctly rank order participants by sarcopenia probability (discrimination ability) was assessed by the area under the receiver operator characteristic (ROC) curve.[16,17] The model fit was verified using the Hosmer–Lemeshow goodness-of-fit test.[18]

There were no missing values of any variable in the entire analytic sample.

All analyses were carried out using SAS version 9.3 (SAS Institute, Cary, NC, USA) and R statistical software version 2.15.2 (R Foundation, Vienna, Austria). Two-sided $P < 0.05$ was considered statistically significant.

Results

There were 32.2% of men and 48.9% of women classified as having low muscle mass, and 14.2% of men and 22.1% of women were classified as having sarcopenia. The participant characteristics by the sarcopenia status in each sex are shown in Table 1. Those with sarcopenia were older and had smaller body size compared with those without sarcopenia in each sex (all $P < 0.001$). Those with sarcopenia were physically less active in each sex. Chronic medical conditions were in general more prevalent in those with sarcopenia, and a statistically significant difference was observed for hypertension in women, stroke in men and osteoporosis in both sexes. Serum albumin was significantly lower in those with sarcopenia in each sex.

Table 2 shows the correlation between each component of sarcopenia and the candidate variables. SMI was correlated with all the variables, with the highest correlation coefficient observed with calf circumference in each sex. Usual gait speed was most highly correlated with age, followed by grip strength and calf circumference in the order of the magnitude of correlation, and this finding was consistent in both sexes.

Visual inspection of the restricted cubic spline plots and the Wald test for linearity suggested that the variables were linearly associated with the logit of sarcopenia probability, except for grip strength in both sexes and upper arm circumference in women (data not shown). However, neither dichotomization nor transformation improved the model fit, and we decided to use linear terms of these variables in the development of statistical models.

Table 3 shows the unadjusted and adjusted associations between sarcopenia and the variables. In bivariate analysis, all the variables were significantly associated with sarcopenia. In multiple logistic regression with all the variables (full model), age was positively, and grip strength and calf circumference were inversely associated with sarcopenia, whereas BMI, thigh circumference and upper arm circumference were not significantly associated. Variable selection resulted in the selection of age, grip strength and calf circumference, and the three selected variables were significantly associated with

Table 1 Characteristics of study participants

	Men			Women		
	Sarcopenia (n = 139)	No sarcopenia (n = 838)	P	Sarcopenia (n = 220)	No sarcopenia (n = 774)	P
Age (years)	78.4 ± 5.5	72.2 ± 5.0	<0.001	76.2 ± 5.8	71.8 ± 4.9	<0.001
Height (cm)	160.0 ± 5.6	164.9 ± 5.5	<0.001	148.2 ± 5.6	152.3 ± 5.1	<0.001
Weight (kg)	54.1 ± 7.2	64.3 ± 8.0	<0.001	46.4 ± 5.7	52.9 ± 7.6	<0.001
BMI (kg/m^2)	21.1 ± 2.5	23.6 ± 2.6	<0.001	21.1 ± 2.6	22.8 ± 3.2	<0.001
Grip strength (kg)	27.5 ± 4.3	36.0 ± 5.3	<0.001	18.4 ± 3.2	23.6 ± 3.3	<0.001
Thigh circumference (cm)	38.8 ± 3.5	42.4 ± 3.3	<0.001	38.9 ± 3.4	41.7 ± 4.0	<0.001
Calf circumference (cm)	32.8 ± 2.3	36.3 ± 2.5	<0.001	32.1 ± 2.1	34.5 ± 2.7	<0.001
Upper arm circumference (cm)	25.7 ± 2.5	28.4 ± 2.4	<0.001	25.7 ± 2.3	27.3 ± 2.9	<0.001
SMI (kg/m^2)	6.34 ± 0.48	7.44 ± 0.58	<0.001	5.25 ± 0.41	6.02 ± 0.60	<0.001
Usual gait speed (m/s)	1.28 ± 0.24	1.51 ± 0.24	<0.001	1.26 ± 0.26	1.51 ± 0.23	<0.001
Physical activity (MET-minutes/week)	1813 (720, 3504)	2540 (1200, 4746)	0.008	1341 (33, 3209)	2587 (1092, 4824)	<0.001
Chronic conditions (%)						
Hypertension	51.1	46.5	0.32	45.9	38.1	0.04
Diabetes mellitus	18.0	14.9	0.36	8.2	8.9	0.73
Stroke	12.2	6.4	0.01	5.9	4.4	0.35
Osteoporosis	4.3	1.4	0.02	32.7	16.6	<0.001
Use of medications (%)						
Statins	18.7	17.4	0.71	29.1	30.6	0.66
Antihypertensives	53.2	45.1	0.08	42.7	36.2	0.08
Albumin (g/dL)	4.37 ± 0.26	4.43 ± 0.23	0.005	4.39 0.23	4.43 ± 0.22	0.04

Values are shown as mean ± standard deviation except for physical activity which was not normally distributed and therefore the mean value and inter-quartile range were shown. BMI, body mass index; MET, Metabolic Equivalent; SMI, skeletal muscle mass index.

Table 2 Pearson correlations between components of sarcopenia and six candidate variables

	Age	BMI	Grip strength	Thigh circumference	Calf circumference	Upper arm circumference
Men						
SMI	−0.33***	0.70***	0.49***	0.70***	0.78***	0.69***
Grip strength	−0.46***	0.21***	1	0.27***	0.35***	0.35***
Usual gait speed	−0.35***	0.007	0.29***	0.06	0.13***	0.10**
Women						
SMI	−0.24***	0.69***	0.50***	0.67***	0.75***	0.65***
Grip strength	−0.36***	0.16***	1	0.22***	0.33***	0.21***
Usual gait speed	−0.42***	−0.08**	0.36***	0.01	0.12***	−0.02

*, **, ***Significance at 0.1%, 1%, 5% level, respectively. BMI, body mass index; SMI, skeletal muscle mass index.

sarcopenia in multiple logistic regression (restricted model). These findings were consistent in both sexes. The area under the ROC curve of the full model was 0.940 (95% confidence interval [CI] 0.920–0.959) for men and 0.910 (95% CI 0.888–0.932) for women, showing excellent discriminative ability. The area under the ROC curve of the restricted model (0.939 with 95% CI 0.918–0.958 for men and 0.909 with 95% CI 0.887–0.931 for women) was not significantly different from that of the full model in both sexes ($P = 0.71$ for men, 0.43 for women). Assessment of internal validity showed that discriminative ability of the restricted model is expected to be good in similar populations (area 0.937 for men, 0.907 for women).

The final model was presented as a score chart in each sex (Table 4). The use of the score chart with two hypothetical patients is shown in Table S1. The discriminative ability of the score chart was comparable with those of the full and restricted models in each sex (area 0.935 for men, 0.908 for women; Fig. S1).

Figure 1 shows the estimated probabilities corresponding to the sum scores as calculated with the score chart in Table 4, and the sensitivity and specificity using the sum scores as cut-off values. The sum score that maximized the sum of sensitivity and specificity was 105 for men and 120 for women. The corresponding sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios were 84.9%, 88.2%, 54.4% and 97.2%, and 7.19 and 0.17 for men, and 75.5%, 92.0%, 72.8% and 93.0%, and 9.44 and 0.27 for women, respectively.

Sensitivity analysis

Because there are no established reference cut-off values for grip strength and usual gait speed in Japanese older adults, we used the lowest quintiles of the observed distributions to classify low muscle strength and low physical performance. As sensitivity analysis, we used the lowest deciles of grip strength and usual gait speed to capture participants with more severely impaired muscle function (i.e. strength or performance), and defined them as having sarcopenia, with the same cut-off values for muscle mass as in the main analysis. We then examined the model performance with all six variables and with the same set of three variables as selected in the main analysis (age, grip strength and calf circumference). The cut-off value of grip strength was 27 kg for men and 17 kg for women, and that of usual gait speed was 1.16 m/s for men and 1.13 m/s for women. The prevalence of sarcopenia was 9.6% in men and 12.7% in women. Both models performed well (area of the full model: 0.932 for men, 0.919 for women; area for the restricted model; 0.931 for men, 0.918 for women; Figure S2).

Discussion

To estimate the probability of sarcopenia in functionally independent, community-dwelling Japanese older adults, we created multivariate models based on the three selected variables (age, grip strength and calf circumferences), and found excellent discrimination ability of the models: the area under the curve was 0.939 for men and 0.909 for women. We constructed a score chart in each sex so that the approximate probability of sarcopenia could be easily obtained from the values of the three variables, and confirmed that the score charts also had excellent discrimination.

Although our multivariate models had excellent discrimination capacity, the model's sensitivity and specificity at candidate diagnostic thresholds must be assessed to judge the model's clinical usefulness.[18] Higher sensitivity can be achieved at the expense of lower specificity and vice versa. For example, if higher sensitivity was desired; for example, 90%, then the cut-off score would be 101 for men and 104 for women, and the specificity would be lower at 82.2% for men and 70.4% for women. Higher specificity, 90%, could be achieved with the higher cut-off score of 107 for men

Table 3 Unadjusted and adjusted associations between sarcopenia and the variables

Variables	Men					Women				
	Bivariate		Multivariate (full model)		Multivariate (restricted model)	Bivariate		Multivariate (full model)		Multivariate (restricted model)
	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI) *P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI) *P*
Age	1.21 (1.17–1.26)	<0.001	1.07 (1.02, 1.12)	0.008	1.07 (1.02, 1.12) 0.008	1.16 (1.13, 1.20)	<0.001	1.10 (1.05, 1.14)	<0.001	1.09 (1.04, 1.13) <0.001
BMI	0.68 (0.63–0.74)	<0.001	0.96 (0.78, 1.18)	0.69		0.82 (0.78, 0.87)	<0.001	0.86 (0.74, 1.00)	0.05	
Grip strength	0.71 (0.67, 0.75)	<0.001	0.73 (0.68, 0.78)	<0.001	0.73 (0.68, 0.79) <.001	0.57 (0.53, 0.62)	<0.001	0.58 (0.53, 0.64)	<0.001	0.59 (0.55, 0.65) <0.001
Thigh circumference	0.73 (0.69, 0.78)	<0.001	1.05 (0.91, 1.21)	0.53		0.82 (0.78, 0.86)	<0.001	0.94 (0.85, 1.04)	0.24	
Calf circumference	0.57 (0.52, 0.63)	<0.001	0.62 (0.53, 0.73)	<0.001	0.62 (0.56, 0.69) <.001	0.68 (0.64, 0.74)	<0.001	0.80 (0.69, 0.91)	<0.001	0.71 (0.65, 0.78) <0.001
Upper arm circumference	0.63 (0.57, 0.68)	<0.001	0.97 (0.82, 1.15)	0.71		0.80 (0.75, 0.85)	<0.001	1.15 (0.98, 1.35)	0.10	

BMI, body mass index; CI, confidence interval; OR, odds ratio.

and 118 for women, resulting in lower sensitivity of 77.7% for men and 76.8% for women (Fig. 1). The trade-off between sensitivity and specificity depends on the cost of incorrect classification of those with sarcopenia relative to the cost of incorrect classification of those without sarcopenia. The cost of incorrect answers would vary according to the clinical or research scenario and personal preferences.[16,17]

Several observations suggested that the selection of three variables (age, grip strength and calf circumference) was not based on chance. First, sarcopenia was classified based on muscle mass, muscle strength and physical performance, all of which were significantly correlated with the three variables. Calf circumference was used to represent muscle mass, considering the highest correlation between SMI and calf circumference among the variables considered. A strong correlation between calf circumference and muscle mass was previously shown in Caucasian older women who were on average more obese than women in the present.[19] Grip strength was used as an indicator of muscle strength. Usual gait speed, a measure of physical performance, was significantly correlated with each of the three variables. Second, sarcopenia was associated with each of the three variables in both bivariate and multivariate analyses in each sex, and *P*-values for these findings were comfortably below 0.01. Third, the models with the three variables had excellent discrimination for sarcopenia based on more stringent cut-off levels for grip strength and usual gait speed.

There have been several prior attempts at estimating the quantity of muscle mass using a variety of variables with varying degrees of accuracy.[20–23] Although these studies were inspired by the desire to facilitate the diagnosis of sarcopenia, recently developed definitions of sarcopenia entail the presence of low muscle function, as well as muscle mass.[1,24] The present study developed statistical models with high accuracy for sarcopenia, which was defined based on muscle mass and muscle function.

This study had several limitations. First, the measurement method of usual gait speed was different from those used by the majority of previous studies.[25] The measurement method used in the present study required the participant to walk 3 m before the measurement started. An attribute of this method is that it is less affected by the gait initiation phase where age-related changes independent of gait speed occur.[26,27] This method has been widely used in Japan,[9,28] and has been shown to be reliable,[10] but because it starts measuring after the gait initiation phase, it tends to yield higher values than those obtained with other measurement methods, such as usual gait speed over a 4- or 6-m course,[25] making direct comparison difficult. Second, the current analysis was carried out on data from Japanese older adults, and our findings therefore might not

Table 4 Score charts for estimated probability of sarcopenia

Variables	Value													
Men														
Age			<66	66	68	70	72	74	76	78	80	82	84	86≦
Score			0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10	+11
Grip strength		<20	20	23	26	29	32	35	38	41	44	47	50≦	
Score		+99	+90	+81	+72	+63	+54	+45	+36	+27	+18	+9	0	
Calf circumference	<26	26	28	30	32	34	36	38	40	42≦				
Score	+81	+72	+63	+54	+45	+36	+27	+18	+9	0				
Estimated individual probability of sarcopenia														
Sum score	70	80	90	95	100	105	110	115	120	125	130	135	140	145
Probability (%)	1	2	5	8	13	19	28	39	51	64	74	83	89	93
Women														
Age			<66	66	68	70	72	74	76	78	80	82	84	86≦
Score			0	+2	+4	+6	+8	+10	+12	+14	+16	+18	+20	+22
Grip strength		<14	14	16	18	20	22	24	26	28	30	32	34≦	
Score		+110	+100	+90	+80	+70	+60	+50	+40	+30	+20	+10	0	
Calf leg circumference			<26	26	28	30	32	34	36	38	40	42≦		
Score			+63	+56	+49	+42	+35	+28	+21	+14	+7	0		
Estimated individual probability of sarcopenia														
Sum score	80	90	95	100	105	110	115	120	125	130	135	140	145	150
Probability (%)	1	3	5	8	12	19	28	39	51	63	74	82	88	93

Values for each variable are given with such intervals that the scores show small steps, and scores for intermediate values can be estimated by linear interpolation. The exact formula to calculate the scores are as follows: score in men, $0.62 \times (\text{age} - 64) - 3.09 \times (\text{grip strength} - 50) - 4.64 \times (\text{calf circumference} - 42)$; score in women, $0.80 \times (\text{age} - 64) - 5.09 \times (\text{grip strength} - 34) - 3.28 \times (\text{calf circumference} - 42)$. The corresponding probabilities of sarcopenia are calculated with the following formulae: probability in men, $1 / [1 + e^{-(\text{sum score} / 10 - 11.9)}]$; probability in women, $1 / [1 + e^{-(\text{sum score} / 10 - 12.5)}]$.

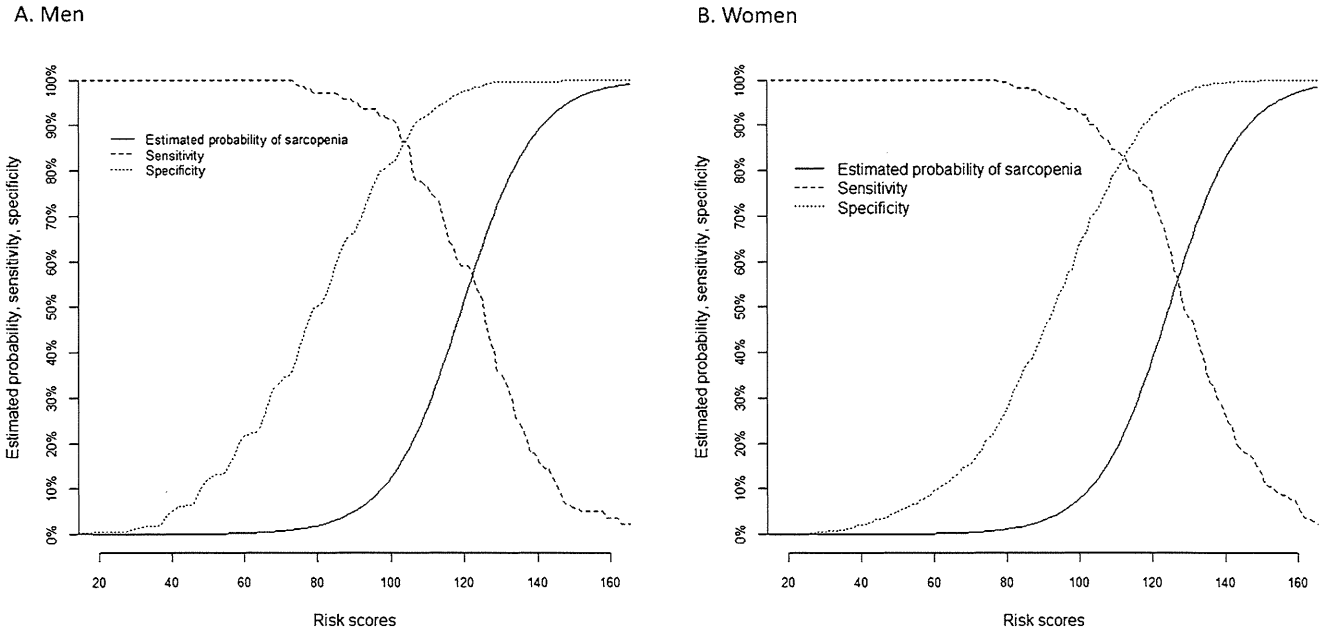

Figure 1 Estimated probabilities, sensitivity and specificity corresponding to sum scores. The sum scores and corresponding estimated probabilities are read from Table 3.

be applicable to populations of other race/ethnicity or in other countries. Similarly, caution should be exercised in projecting beyond the range of our data. For example, the obese were underrepresented in our data, and the performance of our models was not assessed for the obese. However, the present findings suggest that three variables, namely age, grip strength and calf circumference, should be considered for inclusion in the development of sarcopenia screening in other populations. Third, although the internal validity was good (i.e. the models would perform well in a similar population), assessment of external validity is still warranted to determine whether the results can be extended to other Japanese populations. Finally, we could not exclude the possibility of the healthy volunteer effect (i.e. volunteers for clinical studies tend to be healthier than the general population). Although participants were randomly selected from the resident register, participation was voluntary and the response rate was approximately 17%. However, the sensitivity analysis showed that the models' ability to estimate the probability of sarcopenia remained excellent when participants with more severely impaired muscle function were categorized as having sarcopenia.

In conclusion, we showed that the presence of sarcopenia in older adults could be detected with high accuracy using three easily obtainable variables. Importantly, we derived the models from a functionally independent, community-dwelling population. Functionally independent older adults with sarcopenia are good candidates for interventions to prevent further physical limitations, given their potential for regaining muscle mass and restoration of muscle function. The score charts we developed can be used as an effective screening tool and help identify functionally independent older adults with sarcopenia.

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Disclosure statement

The authors declare no conflict of interest.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Receiver operating characteristic curves of models estimating the probability of sarcopenia.
Figure S2 Receiver operating characteristic curves of models estimating the probability of sarcopenia based on different cut-off values for grip strength and usual gait speed.
Table S1 Application of Score Chart in two hypothetical patients.

ORIGINAL ARTICLE

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Pleiotropic Effects of Obesity on Fracture Risk: The Study of Women's Health Across the Nation

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ABSTRACT

Some aspects of an obese body habitus may protect against fracture risk (higher bone mineral density [BMD] and greater tissue padding), while others may augment that risk (greater impact forces during a fall). To examine these competing pathways, we analyzed data from a multisite, multiethnic cohort of 1924 women, premenopausal or early perimenopausal at baseline. Obesity was defined as baseline body mass index (BMI) $> 30\ kg/m^2$. Composite indices of femoral neck strength relative to fall impact forces were constructed from DXA-derived bone size, BMD and body size. Incident fractures were ascertained annually during a median follow-up of 9 years. In multivariable linear regression adjusted for covariates, higher BMI was associated with higher BMD but with lower composite strength indices, suggesting that although BMD increases with greater skeletal loading, the increase is not sufficient to compensate for the increase in fall impact forces. During the follow-up, 201 women had fractures. In Cox proportional hazard analyses, obesity was associated with increased fracture hazard adjusted for BMD, consistent with greater fall impact forces in obese individuals. Adjusted for composite indices of femoral neck strength relative to fall impact forces, obesity was associated with decreased fracture hazard, consistent with a protective effect of soft tissue padding. Further adjustment for hip circumference, a surrogate marker of soft tissue padding, attenuated the obesity–fracture association. Our findings support that there are at least three major mechanisms by which obesity influences fracture risk: increased BMD in response to greater skeletal loading, increased impact forces, and greater absorption of impact forces by soft tissue padding. © 2014 American Society for Bone and Mineral Research.

KEY WORDS: OBESITY; OSTEOPOROSIS; FRACTURE RISK; STRENGTH RELATIVE TO LOAD; FALL IMPACT FORCES; SOFT TISSUE PADDING

Introduction

Obesity has long been thought to protect against osteoporosis [(1)] and fragility fractures,[(2)] primarily because greater skeletal loading in obese individuals leads to increased bone mineral density (BMD)[(2,3)] and more favorable bone geometry.[(4,5)] However, impact forces in a fall are also greater in obese individuals because body weight is greater. Thus, for obesity to reduce fracture risk, the increase in BMD stimulated by greater skeletal loading has to compensate for greater impact forces. Unfortunately, both chronic inflammation and type 2 diabetes are also more prevalent in obese individuals, and both have deleterious influences on fracture risk;[(6–9)] therefore the BMD advantage from greater skeletal loading may not be sufficient to reduce one's fracture risk.

In fact, multiple recent studies report that fractures are no less common in obese individuals than in the non-obese,[(10,11)] and that fracture risk in some body sites (eg, extremities) is actually increased with obesity,[(11–15)] challenging the conventional assumption that obesity protects against fractures. There does, however, appear to be some protection conferred by obesity against fractures of the hip and pelvis in these studies.[(12–15)] Greater absorption of impact forces by soft tissue padding around the hip may underlie this relative reduction in hip and pelvic fracture risk in obese women;[(4,11,12,14)] however, the role of soft tissue padding in obesity–fracture associations has not been empirically examined in longitudinal studies.

To disentangle these disparate effects of obesity and fracture risk, we tested a series of hypotheses aimed at isolating different components in the obesity–fracture relationship. We hypothesized that:

1. Obesity would be associated with increased BMD, reflecting the increased bone mass stimulated by greater skeletal loading.

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2. Obesity would be associated with reduced bone strength relative to load (ie, that the BMD benefit of obesity would be insufficient to compensate for greater fall impact forces).
3. Adjusted for BMD (thus, removing the effects of body weight on BMD), obesity would be associated with increased fracture risk, reflecting greater fall impact forces (ie, we hypothesized that, of the remaining pathways, the influence of body weight on fall impact forces would dominate).
4. Adjusted for bone strength relative to load (thus, removing the effects of body weight on both BMD and fall impact forces), obesity would be associated with decreased fracture risk, reflecting the protective role of soft tissue padding (ie, we hypothesized that, of the remaining pathways, the protection provided by soft tissue padding would dominate).

We used data from the Hip Strength Across the Menopause Transition study to test these hypotheses.

Materials and Methods

Study design and population

The Study of Women's Health Across the Nation (SWAN) is a multicenter, multiethnic longitudinal study to characterize the biological and psychosocial changes that occur during the menopausal transition. Between 1995 and 1997, a screening survey to assess study eligibility was conducted in women using either community-based or population-based sampling frames at each of seven participating clinical sites.[16] Briefly, cohort entry criteria were as follows: age 42 to 53 years, with intact uterus and at least one ovary, not using sex steroid hormones at enrollment, had at least one menstrual period in the 3 months prior to screening, and self-identified as either white, African American, Hispanic, Chinese, or Japanese. Each site recruited at least 450 eligible women into the cohort in 1996 and 1997, resulting in an inception cohort of 3302 women.[17,18]

Five sites (Boston, Detroit, Pittsburgh, Los Angeles, and Oakland) collected DXA scans of the hip and lumbar spine in all but 46 participants who weighed more than 136 kg (the scanner weight limit); they constituted the SWAN bone cohort. All five sites enrolled whites, and each site also enrolled women belonging to one prespecified minority ethnic group: African American in Boston, Detroit, and Pittsburgh and Japanese and Chinese in Los Angeles and Oakland, respectively. The Hip Strength Across the Menopause Transition study, the focus of this report, measured femoral neck size using archived hip DXA scans from the 1960 women in the SWAN bone cohort who had a baseline and two or more follow-up scans by follow-up visit 10 (2006–2007). At baseline, 36 women did not get either bone size or body size measurements, leaving 1924 women in the analytic sample (963 white, 503 African American, 238 Japanese, and 220 Chinese). All protocols were approved by Institutional Review Boards at each site and all participants gave written informed consent.

Assessment of BMD and bone strength relative to load

Using the OsteoDyne Hip Positioner System (Osteodyne Inc.), DXA scans of the posterior–anterior lumbar spine and total hip were acquired at the baseline visit. Hologic QDR 4500 models were used in Boston, Detroit, and Los Angeles, and QDR 2000 scanners were used in Pittsburgh and Oakland (Hologic Inc., Waltham, MA, USA). A standard quality-control program, conducted in collaboration with Synarc, Inc. (Newark, CA, USA), included daily phantom measurements, 6-month cross-calibration with a circulating anthropomorphic spine standard, local site review of all scans, central review of scans that met problem-flagging criteria, and central review of 5% random sample of scans. The 2D-projected (areal) BMD in the femoral neck and lumbar spine were recorded.

As markers of relative bone strength, we employed composite indices of femoral neck strength relative to load that integrate BMD, bone size, and (in light of the "supply and demand balance") body size.[19] They have been shown to predict fracture risk in white women[19] and in women from a multi-ethnic cohort,[20] and unlike BMD, they do not require race/ethnicity information to do so.[20] Also unlike BMD, differences in the composite strength indices between diabetics and nondiabetics are consistent with known differences in fracture risks between these groups.[7] In addition, unlike BMD, the composite strength indices are inversely associated with serum C-reactive protein (CRP) levels, a marker of chronic inflammation, and partially explain the increased fracture risk associated with inflammation.[6]

Two bone-size measurements were made on archived baseline hip scans using pixel dimensions provided by the manufacturer: femoral neck axis length (FNAL) and femoral neck width (FNW) (Fig. 1). The composite indices of femoral neck strength relative to load were computed using height, weight, FNAL, FNW, and femoral neck BMD (Fig. 1).[19] Compression strength index (CSI) reflects the ability of the femoral neck to withstand axial compressive loads proportional to body weight, bending strength index (BSI) reflects its ability to withstand bending forces proportional to body weight, and impact strength index (ISI) reflects its ability to absorb the energy of impact in a fall from standing height (which is proportional to the product of body height and weight).[19] To examine reproducibility of the composite strength indices, 20 women were scanned twice after repositioning; the intraclass correlation coefficient for each index was greater than 0.98.

Incident fracture ascertainment and classification

During each of nine annual follow-up visits, fractures since the previous visit were self-reported using a standardized interviewer-administered questionnaire. In all years, the number of fractures, body site(s), and how fractures occurred were recorded. SWAN initiated collection of the date of fracture at follow-up visit 6. Because dates of fractures were not collected in the first six follow-ups, they were imputed using the midpoint between the participant's index and previous visits. Fractures reported at visit 6 and later were confirmed by reviewing medical records. Medical records were available for 85% of fractures and of these, only four fractures (3.8%) could not be confirmed. We excluded from all analyses factures not typically associated with osteoporosis, in particular fractures of the face, skull, fingers, and toes.[21,22] We created two categories of fractures: all fractures and minimum trauma fractures. Minimum trauma fractures excluded those that occurred as a result of a fall from a height greater than 6 inches, in a motor vehicle accident, while moving fast (eg, bicycling or skating), while playing sports, or from impact with heavy or fast-moving projectiles.

Measurement of obesity

At the baseline and each of nine follow-up visits, height and weight were measured using a fixed stadiometer and a digital scale with the participants wearing light clothing and no shoes. The maximum hip circumference was measured over

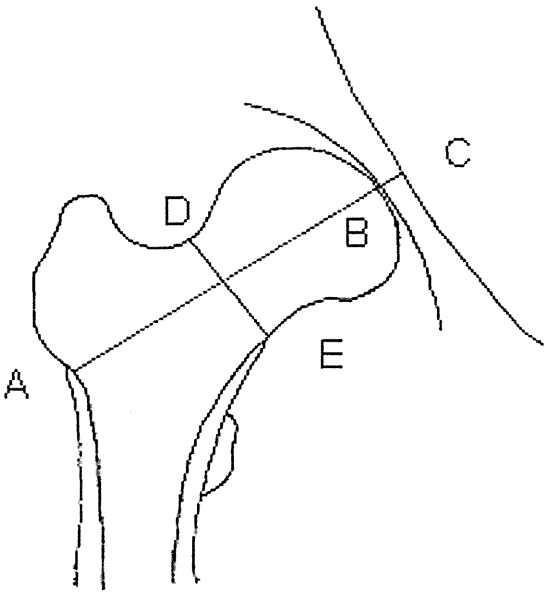

Fig. 1. Femoral neck size measurements and formulae to compute composite femoral neck strength indices. AB is the femoral neck axis length (FNAL), the distance from the base of the greater trochanter to the apex of the femoral head, and DE is the femoral neck width (FNW), the smallest thickness of the femoral neck along any line perpendicular to the femoral neck axis. C is where the femoral neck axis meets the inner pelvic rim. Composite femoral neck strength indices were computed using the following formulae, where BMD refers to the areal (projected 2D) bone mineral density in the femoral neck obtained from DXA:

$$\text{Compression Strength Index (CSI)} = \frac{\text{BMD}^{*}\text{FNW}}{\text{Weight}}$$

$$\text{Bending Strength Index (BSI)} = \frac{\text{BMD}^{*}\text{FNW}^{2}}{\text{FNAL}^{*}\text{Weight}}$$

$$\text{Impact Strength Index (ISI)} = \frac{\text{BMD}^{*}\text{FNW}^{*}\text{FNAL}}{\text{Height}^{*}\text{Weight}}$$

All three indices were recorded in units of g/kg-m. With BMD measured in g/cm^2, FNW and FNAL in cm, weight in kg, and height in meters, CSI and BSI were scaled by 100 to get values in units of g/kg-m.

undergarments (if a participant refused, over light clothing) using a measuring tape with the participants standing with their feet together. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2). Previous studies suggest nonlinear relationships between BMI and fracture risk. Therefore, participants were classified into four mutually exclusive BMI categories: low weight (BMI $<$ 22 kg/m^2), normal weight (22 kg/m^2 $\leq$ BMI $<$ 25 kg/m^2), overweight (25 kg/m^2 $\leq$ BMI $<$ 30 kg/m^2), and obese (30 kg/m^2 $\leq$ BMI). Only 39 women (2.0%) had BMI $<$ 18.5 kg/m^2 and therefore women with BMI $<$ 22 kg/m^2, which accounted for 22.5% of the analytic sample, were categorized as low weight.

Other measurements

Participants provided the following information at baseline: age (years), race/ethnicity, menopause transition stage (premenopause or early perimenopause: no changes versus some changes in regularity of menses but with no gaps of $\geq$ 3 months), physical activity level (summary score combining intensity with frequency of active living, home, and recreational physical activity from modified Baecke interview[23]), prescription medications used, vitamin D and calcium supplement use, alcohol consumption (abstainer; infrequent: not abstainer, but $\leq$ 1 drink per week; light to moderate: $>$ 1 drink per week, but $\leq$ 1 per day; and heavy: $>$ 1 drink per day), smoking history, and comorbidities. Women who reported use of diabetes medications or had fasting serum glucose $\geq$126 mg/dL were classified as diabetic. Serum glucose was measured from blood drawn after an overnight fast, using a hexokinase-coupled reaction (Roche Molecular Biochemicals Diagnostics, Indianapolis, IN, USA). Serum CRP level was measured at Medical Research Laboratories (Highland Heights, KY, USA), using an ultrasensitive rate immunonephelometric method with a lower limit of detection of 0.3 mg/L (BN100; Dade-Behring, Marburg, Germany). The CRP assay within-run coefficient of variation (CV) at CRP concentrations of 0.5 and 22.0 mg/L were 10%–12% and 5%–7%, respectively. During each of the follow-up visits, information on use of medications was collected using interviewer-administered questionnaires.

Statistical analysis

The first set of analyses was designed to examine the effect of obesity on BMD and bone strength relative to load, and examined cross-sectional associations at the baseline SWAN visit. We first compared the means of the five bone strength estimates (BMD in the lumbar spine and femoral neck, and three composite indices of femoral neck strength relative to load) across BMI categories. Although we had expected to see a J-curve relationship between BMI and bone strength or fracture hazard, the preliminary analysis found a graded relationship between BMI categories and each of the bone strength estimates and fracture hazard, and we decided to set the low weight group as the reference group. The linear relationships between BMI and bone strength estimates were also tested by entering BMI into the models as a continuous variable rather than a categorical variable.

Multiple linear regression was used to adjust for the following potential confounders, which were also measured at baseline: age (continuous); race/ethnicity; menopause transition stage (premenopause versus early perimenopause); smoking status (never, past, current); alcohol use categories (abstainer, infrequent, light to moderate, heavy); level of physical activity (above median versus below median); current use (yes versus no) of medications from the following four classes (one indicator variable for each): supplementary vitamin D, supplementary calcium, other bone-active medications (oral steroids, chemotherapy for breast cancer, aromatase inhibitors, antiepileptics), and central nervous system active medications (tranquilizers, antidepressants, sedatives, sleeping pills); ever/previous use (yes versus no) of oral steroids; ever/previous use of sex steroids (oral estrogen/progesterone, estrogen patches, birth control pills); history of prior fracture as an adult (after age 20 years); and study site. Use of osteoporosis medications (bisphosphonates, selective estrogen receptor modulators, calcitonin, parathyroid hormone, or vitamin D in pharmacological doses) at baseline was reported by only one participant, and therefore the osteoporosis medications variable was not included in the models. Dunnett's method was used to adjust for multiple comparisons between the low weight (reference category) and the three higher BMI categories.

To statistically eliminate the (potentially negative) effects of chronic inflammation and diabetes from the association between BMI and bone strength estimates, we added diabetes status (yes/no) and serum CRP level (which was log-transformed to minimize its skew) to the base models, in a second step.

The second set of analyses examined obesity associations with incidence of fracture over 9 years, and employed Cox proportional hazard regression with time to first fracture as the dependent variable, and baseline BMI as primary predictor. The models were initially adjusted for the same set of baseline covariates as in the cross-sectional base model plus use in at least two consecutive visits during follow up of medications from the following four classes (using one yes/no indicator variable for each class): sex steroid hormones; osteoporosis medications; oral steroids; and other bone-active medications (chemotherapy, aromatase inhibitors, antiepileptics) as time-invariant covariates. This initial model (the longitudinal base model) estimated the overall effect of obesity on fracture risk. In subsequent models, we added select covariates representing one or more hypothesized obesity–fracture pathways in order to statistically eliminate the effects of the hypothesized pathway(-s), and estimate the obesity–fracture association independent of the selected pathway(s). We first added BMD to the model to control for the skeletal loading effect of body weight on BMD. Next, we separately added each composite index of femoral neck strength relative to load to the longitudinal base model to control for the effects of body weight on both bone mass and fall impact forces. We then further adjusted for log(CRP) and diabetes to remove any residual effects of diabetes and inflammation (which are more prevalent in obese individuals), and thus better isolate the protective effect of soft tissue padding on fracture risk. In the final model, we added a surrogate marker of soft tissue padding around the hip, derived from hip circumference to test if it explained away any remaining protective effect of obesity on fracture risk. The surrogate hip soft tissue measure was created as the residual from race/ethnicity-specific linear regressions of hip circumference on body height.

We conducted all the time-to-event analyses for each of two event types: all fractures and minimum trauma fractures. For analyses of time to first minimum-trauma fractures, follow-up time was censored at the time of the first trauma-associated fracture.[24]

A total of 96 (5.0%) women had one or more covariates missing and the missing values were imputed by single imputation using the expectation maximization (EM) algorithm.[25] All analyses were conducted using SAS version 9.3 (SAS Institute, Inc., Cary, NC, USA). Two-sided $p < 0.05$ was considered statistically significant.

Results

Participants were classified into four BMI categories: 432 (22.5%) were categorized as low weight (BMI $< 22\ kg/m^2$), 455 (23.6%) as normal weight ($22\ kg/m^2 \leq BMI < 25\ kg/m^2$), 469 (24.4%) as overweight ($25 kg/m^2 \leq BMI < 30\ kg/m^2$), and 568 (29.5%) as obese ($30\ kg/m^2 \leq BMI$). Distributions of characteristics across BMI categories are shown in Table 1. African American women and women in early perimenopause were more likely to be in higher BMI categories. Diabetes, history of previous fracture, less healthier habits (current smoking and less physically active), and use of central nervous system (CNS)-active medications were also more prevalent in higher BMI categories, whereas Chinese and Japanese women and use of supplementary vitamin D and calcium were more common in lower BMI categories.

Cross-sectional associations between BMI and estimates of bone strength

Multivariable-adjusted means of femoral neck BMD and lumbar spine BMD increased significantly with increasing BMI categories (Table 2, base model). In contrast, the adjusted means of all three composite indices of femoral neck strength relative to load decreased significantly with increasing BMI (all $p < 0.001$). Consistent with the graded increase in BMD and graded decrease in composite strength indices with increasing BMI categories, BMI as a continuous predictor was also linearly and positively associated with BMD, and linearly and negatively associated with the composite strength indices. Adjusting for diabetes and log(CRP) only slightly diminished the magnitude of the associations between BMI and the composite indices of strength relative to load (Table 2). Adjusting for diabetes and log(CRP) had virtually no impact on the associations between BMI and either lumbar spine BMD or femoral neck BMD.

Association between baseline BMI and incident fracture

After median follow-up of 9.0 (interquartile range, 8.9–9.1) years, 201 women (10.5%) had at least one fracture, at a rate of 12.6 per 1000 person-years. Foot (non-toe) and ankle were the most common locations for first incident fracture. In Cox proportional hazard regression, adjusted for age, race/ethnicity, menopause transition stage, smoking status, alcohol use, level of physical activity, use of medications at baseline and during follow-up, history of prior fracture as an adult, and study site, fracture hazard was not significantly associated with BMI (Table 3, base model). After additional adjustment for femoral neck BMD, obesity was significantly associated with increased fracture hazard: relative increment in fracture hazard in obese relative to low weight women: 89% (95% confidence intervals (CI), 14% to 214%) (Table 3, model 2a). The relative increment in fracture hazard in obese women compared to normal weight women was also statistically significant: 78% (95% CI, 13% to 181%, $p = 0.01$). In stark contrast, obesity was significantly associated with decreased fracture hazard when adjusted instead for any of the composite indices of femoral neck strength relative to load: relative decrement in fracture hazard in obese relative to low weight women was 57% (95% CI, 24% to 76%) after adjusting for CSI, 41% (95% CI, 1% to 65%) after adjusting for BSI, and 53% (95% CI, 16% to 74%) after adjusting for ISI (Table 3, model 3). The relative decrement in fracture hazard in obese women relative to normal weight women after adjusting for CSI was 39% ($p = 0.053$). Addition of diabetes and log(CRP) to the model with CSI minimally affected the associations between BMI and fracture hazard (Table 3, model 4). Further adjustment for hip soft tissue attenuated the associations between BMI and fracture hazard and made them statistically nonsignificant (Table 3, model 5). Similar results were observed when diabetes, log(CRP), and hip soft tissue were added to Models 3b and 3c (the models with BSI and ISI)—data not shown.

Consistent with the graded associations between BMI categories and fracture hazard in models 2 through 4 (Table 3), BMI as a continuous predictor was linearly and positively associated with fracture hazard after adjusting for femoral neck or lumbar spine BMD (Table 3, models 2a and 2b), but linearly and negatively associated with fracture hazard after adjusting for composite

Table 1. Participant Characteristics at the Baseline Visit in the Complete Study Sample and by Body Mass Index Categories[a]

Characteristics	All ($n = 1924$)	Low weight[b] ($n = 432$)	Normal[b] ($n = 455$)	Overweight[b] ($n = 469$)	Obese[b] ($n = 568$)	p for trend[c]
Age (years)	45.9 (2.7)	45.7 (2.7)	45.9 (2.7)	46.1 (2.7)	45.8 (2.7)	0.47
Height (cm)	162.3 (6.5)	161.0 (6.8)	161.8 (6.5)	163.2 (6.4)	162.8 (6.2)	<0.001
Weight (kg)	72.6 (19.3)	52.7 (5.4)	61.6 (5.5)	72.9 (6.9)	96.3 (14.6)	<0.001
BMI (kg/m^2)	27.5 (6.9)	20.3 (1.3)	23.5 (0.9)	27.3 (1.4)	36.3 (5.1)	<0.001
Hip circumference (cm)	105.3 (13.9)	91.2 (4.3)	97.7 (4.5)	105.5 (5.4)	122.0 (11.3)	<.0001
Waist circumference (cm)	84.6 (15.2)	68.9 (4.0)	76.0 (4.4)	85.3 (6.7)	103.1 (11.3)	<0.001
Race/ethnicity, n (%)						<0.001
White	963 (50.1)	185 (42.8)	236 (51.9)	248 (52.9)	294 (51.8)	
African American	503 (26.1)	38 (8.8)	69 (15.2)	141 (30.1)	255 (44.9)	
Chinese	220 (11.4)	100 (23.2)	72 (15.8)	39 (8.3)	9 (1.6)	
Japanese	238 (12.4)	109 (25.2)	78 (17.1)	41 (8.7)	10 (1.8)	
Menopause transition stage, n (%)[d]						0.009
Premenopausal	1087 (56.5)	260 (60.2)	266 (58.5)	264 (56.3)	297 (52.3)	
Early perimenopausal	837 (43.5)	172 (39.8)	189 (41.5)	205 (43.7)	271 (47.7)	
Smoking Status, n (%)						<0.001
Never smoked	1142 (59.8)	280 (65.1)	287 (63.2)	266 (57.2)	309 (55.0)	
Ex-smoker	486 (25.4)	92 (21.4)	112 (24.7)	128 (27.5)	154 (27.4)	
Current smoker	283 (14.8)	58 (13.5)	55 (12.1)	71 (15.3)	99 (17.6)	
Alcohol consumption, n (%)[e]						<0.001
Abstainer	992 (51.6)	199 (46.3)	218 (48.0)	235 (50.1)	340 (59.9)	
Infrequent	179 (9.3)	47 (10.9)	37 (8.2)	41 (8.7)	54 (9.5)	
Light to moderate	489 (25.5)	112 (26.1)	125 (27.5)	121 (25.8)	131 (23.1)	
Heavy	261 (13.6)	72 (16.7)	74 (16.3)	72 (15.4)	43 (7.6)	
Physical activity level, n (%)						<0.001
Above median	945 (50.6)	247 (59.1)	257 (57.8)	243 (53.3)	198 (36.2)	
Below median	921 (49.4)	171 (40.9)	188 (42.3)	213 (46.7)	349 (63.8)	
History of prior fracture as adult, n (%)	353 (18.4)	57 (13.2)	72 (15.8)	101 (21.5)	123 (21.7)	<0.001
Current use of medications, n (%)						
Supplementary vitamin D	742 (38.6)	177 (41.2)	175 (38.6)	182 (38.8)	208 (36.6)	0.17
Supplementary calcium	863 (44.9)	218 (50.7)	212 (46.7)	208 (44.4)	225 (39.6)	<0.001
CNS active medications[f]	199 (10.3)	39 (9.0)	37 (8.1)	47 (10.0)	76 (13.4)	0.01
Other bone-active medications[g]	44 (2.3)	8 (1.9)	7 (1.5)	13 (2.8)	16 (2.8)	0.17
Previous use of medications, n (%)						
Sex steroid hormones	1419 (73.8)	287 (66.4)	330 (72.5)	357 (76.1)	445 (78.4)	<0.001
Oral steroids	92 (4.8)	13 (3.0)	20 (4.4)	21 (4.5)	38 (6.7)	0.008
Diabetes mellitus, n (%)[h]	88 (4.6)	3 (0.7)	7 (1.5)	10 (2.1)	68 (12.0)	<0.001
FNAL (cm)	8.97 (0.51)	8.98 (0.54)	8.98 (0.51)	9.01 (0.51)	8.91 (0.48)	0.09
FNW (cm)	2.75 (0.20)	2.73 (0.20)	2.73 (0.20)	2.75 (0.20)	2.78 (0.20)	<0.001
Lumbar spine BMD (g/cm^2)	1.07 (0.13)	1.00 (0.11)	1.04 (0.12)	1.09 (0.13)	1.13 (0.13)	<0.001
Femoral neck BMD (g/cm^2)	0.84 (0.13)	0.75 (0.10)	0.80 (0.10)	0.85 (0.11)	0.95 (0.13)	<0.001
CSI (g/kg-m)	3.31 (0.64)	3.90 (0.55)	3.54 (0.47)	3.20 (0.43)	2.76 (0.45)	<0.001
BSI (g/kg-m)	1.02 (0.22)	1.19 (0.21)	1.08 (0.19)	0.98 (0.16)	0.87 (0.16)	<0.001
ISI (g/kg-m)	0.18 (0.04)	0.22 (0.03)	0.20 (0.03)	0.18 (0.02)	0.15 (0.03)	<0.001

Abbreviations: BMI, body mass index; CNS, central nervous system; FNAL, femoral neck axis length; FNW, femoral neck width; CSI, compression strength index; BSI, bending strength index; ISI, impact strength index.

[a]Mean and standard deviation shown for continuous variables and number of participants and percentage shown for categorical variables.

[b]BMI categories: low weight: BMI < 22, normal: $22 \leq$ BMI < 25, overweight: $25 \leq$ BMI < 30, and obese: $30 \leq$ BMI.

[c]p value for trend across increasing BMI categories was calculated using the Jonckheere-Terpstra test.

[d]Women were classified as premenopausal if they had experienced at least one menstrual period in the last 3 months with no change in the regularity of their menstrual bleeding during the last year and early perimenopausal if they had experienced at least one menstrual period in the last 3 months with some change in the regularity of their menstrual bleeding during the last year.

[e]Women were classified as abstainer if they consumed no alcohol, infrequent if they consumed less than one drink per week, light to moderate if they consumed more than one drink per week but less than one drink per day, and heavy if they consumed more than one drink per day.

[f]CNS active medications include tranquilizers, antidepressants, sedatives, and sleeping pills.

[g]Other bone-active medications include oral steroids, chemotherapy for breast cancer, aromatase inhibitors, and antiepileptics.

[h]Women who reported use of diabetes medications or had fasting serum glucose ≥ 126 mg/dL were classified as diabetic.

Table 2. Adjusted* Means of Bone Mineral Density and Indices of Bone Strength Relative to Load as Function of Body Mass Index[a]

	Reference (BMI < 22)	Normal (22 ≤ BMI < 25)		Overweight (25 ≤ BMI < 30)		Obese (30 ≤ BMI)		BMI as continuous predictor	
	Adjusted mean	Adjusted mean difference from the reference group	*p*	Adjusted mean difference from the reference group	*p*	Adjusted mean difference from the reference group	*p*	Adjusted increment in mean per unit BMI increment (kg/m^2)	*p*
Femoral neck BMD (g/cm^2)									
Base model[b]	0.76	0.04 (0.03, 0.06)	<0.001	0.08 (0.06, 0.09)	<0.001	0.17 (0.15, 0.18)	<0.001	0.0078 (0.0069, 0.0087)	<0.001
+ DM and CRP[c]	0.78	0.04 (0.03, 0.06)	<0.001	0.08 (0.06, 0.09)	<0.001	0.16 (0.14, 0.19)	<0.001	0.0080 (0.0069, 0.0091)	<0.001
Lumbar spine BMD (g/cm^2)									
Base model	1.01	0.04 (0.02, 0.06)	<0.001	0.08 (0.06, 0.10)	<0.001	0.13 (0.11, 0.15)	<0.001	0.0097 (0.0089, 0.0105)	<0.001
+ DM and CRP	1.03	0.04 (0.02, 0.06)	<0.001	0.08 (0.06, 0.10)	<0.001	0.13 (0.11, 0.16)	<0.001	0.0099 (0.0090, 0.0108)	<0.001
CSI (g/kg-m)									
Base model	3.88	−0.33 (−0.41, −0.26)	<0.001	−0.66 (−0.73, −0.58)	<0.001	−1.07 (−1.14, −0.98)	<0.001	−0.062 (−0.066, −0.059)	<0.001
+ DM and CRP	3.81	−0.30 (−0.37, −0.23)	<0.001	−0.60 (−0.68, −0.53)	<0.001	−0.95 (−1.04, −0.86)	<0.001	−0.059 (−0.063, −0.055)	<0.001
BSI (g/kg-m)									
Base model	1.17	−0.09 (−0.12, −0.07)	<0.001	−0.18 (−0.21, −0.15)	<0.001	−0.28 (−0.31, 0.25)	<0.001	−0.016 (−0.017, −0.015)	<0.001
+ DM and CRP	1.15	−0.09 (−0.11, −0.06)	<0.001	−0.16 (−0.19, −0.13)	<0.001	−0.24 (−0.28, −0.21)	<0.001	−0.015 (−0.017, −0.014)	<0.001
ISI (g/kg-m)									
Base model	0.22	−0.020 (−0.024, −0.016)	<0.001	−0.039 (−0.043, −0.035)	<0.001	−0.063 (−0.068, −0.059)	<0.001	−0.0037 (−0.0039, −0.0035)	<0.001
+ DM and CRP	0.21	−0.018 (−0.022, −0.014)	<0.001	−0.035 (−0.040, −0.031)	<0.001	−0.055 (−0.061, −0.050)	<0.001	−0.0034 (−0.0037, −0032)	<0.001

Abbreviations: BMI, body mass index; DM, diabetes mellitus; CRP, C-reactive protein; CSI, compression strength index; BSI, bending strength index; ISI, impact strength index; BMD, bone mineral density

[a]Results of multiple linear regression analysis with BMI as categorical predictor (reference group: low weight category, BMI < 22kg/m^2) and as continuous predictor in separate models.

[b]Base model: Adjusts for age, menopause transition stage, race/ethnicity, study site, physical activity, smoking status and alcohol consumption, history of fracture since age 20, baseline use of medications (supplementary vitamin D, supplementary calcium, bone-active medications, central nervous system active medications, ever/previous use of oral steroids, and ever/previous use of sex steroids).

[c]DM and CRP were added in step 2.