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Exploratory analysis of the potential relationship between urinary molybdenum and bone mineral density among adult men and women from NHANES 2007–2010

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Abstract

Human exposure to molybdenum (Mo) may play a role in reducing bone mineral density (BMD) by interfering with steroid sex hormone levels. To begin to address gaps in the literature on this topic, the potential relationship between urinary Mo (U-Mo) and BMD at the femoral neck (FN-BMD) and lumbar spine (LS-BMD) was explored in a sample of 1,496 adults participating in the 2007–2010 cycles of the National Health and Nutrition Examination Survey. Associations were assessed using multiple linear regression models stratified on sex and age. In adjusted models for 50–80+ year-old women, there was a statistically significant inverse relationship between natural log-U-Mo and LS-BMD (p-value: 0.002), and a statistically significant dose-dependent decrease in LS-BMD with increasing U-Mo quartiles (trend p-value: 0.002). A suggestive (trend p-value: 0.08), dose-dependent decrease in FN-BMD with increasing U-Mo quartiles was noted in this group of women as well. All other adjusted models revealed no statistically significant or suggestive relationships between U-Mo and FN-BMD or LS-BMD. Bone health is important for overall human health and well-being and, given the exploratory nature of this work, additional studies are needed to confirm the results in other populations, and clarify the potential underlying mechanisms of Mo on BMD.

Keywords

Molybdenum; steroid sex hormones; bone mineral density; biomarkers; epidemiology

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CONFLICT OF INTEREST

All other authors declare no conflict of interest.

1. INTRODUCTION

Bone mineral density (BMD) is a noninvasive measure of bone health that depends on peak bone mass reached in adolescence and subsequent bone loss (Small, 2005; Surgeon General 2004). BMD is lower in women than men and decreases substantially with age in both sexes, leading to complications such as osteoporosis, which increases the risk of subsequent fractures (Burger et al., 1998; Bonnick and Shulman, 2006; Kanis, 2002; Small, 2005). It was estimated that in 2010 over half of the U.S. adult population aged 50 years and older had osteoporosis or low bone mass, and the prevalence is projected to grow by nearly 20% over the next decade (Wright et al., 2014). The morbidity and mortality of osteoporosis and its related complications are significant. Bone fractures (all types) are associated with higher mortality rates in both men and women, and adversely influence quality of life through increased chronic pain and disability (Nelson et al., 2010). Moreover, by 2025, it is predicted that the economic burden of osteoporosis will cost the U.S. over \$25 billion each year (Burge et al., 2007).

Steroid sex hormones are integral in promoting bone growth and maintaining bone density (Compston, 2001; Manolagas, 2013; Manolagas et al., 2002). The role of estrogen in stimulating bone growth and maturation and inhibiting bone resorption is well-documented (Karsenty, 2012; Riggs, 2000). Androgens also influence bone metabolism and are essential for promoting skeletal integrity, particularly in men (Sinnesael et al., 2011; Vanderschueren et al., 2004). Testosterone reduces bone resorption – perhaps through the aromatization of estrogen – and enhances bone formation (Falahati-Nini et al., 2000; Riggs et al., 2013). The effects of testosterone on BMD are evident in studies conducted among hypogonadal men (abnormally low testosterone levels) who were found to have significantly reduced BMD compared to age-matched controls (Riggs et al., 2013). Furthermore, long-term treatment of these patients with androgen therapy has been shown to increase BMD independent of aging, suggesting that testosterone is important for promoting bone formation (Behre et al., 1997).

Molybdenum (Mo) is a naturally-occurring element found in low levels in various foods (e.g., legumes, grains, organ meats) and drinking water, and is essential for human health (CDC, 2013; WHO, 2011). Mo also is present in nutritional supplements and has important industrial applications (CDC, 2013; WHO, 2011). In humans, this essential trace element functions a co-factor for three enzymes that play an integral role in metabolism (Kisker et al., 1997). While deficiency in Mo itself is rare in humans, insufficient or nonexistent levels of Mo-containing enzymes have been associated with seizures, mental retardation, and early death (Kisker et al., 1997; Schwarz and Belaidi, 2013). Previous human health studies have shown that Mo has the potential to alter levels of serum testosterone (Lewis and Meeker 2015; Meeker et al. 2010). Based on these findings, it is possible that through the testosterone-disrupting effects of Mo, this trace metal may influence BMD in adults.

Currently, there are no published studies that have explored the potential relationship between biomarkers of Mo exposure and bone health. To build upon previous research showing the potential androgen-disrupting effects of Mo and to fill apparent data gaps in the literature, we investigated the relationship between levels of urinary Mo (U-Mo) and BMD

in men and women participating in the National Health and Nutrition Examination Survey (NHANES).

2. MATERIALS AND METHODS

2.1. Study Population

This analysis used publicly-available data that were derived from NHANES 2007–2010. NHANES is a cross-sectional survey administered by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). NHANES collects nationally representative data on the health and nutritional status of non-institutionalized civilian residents, and conducts more detailed laboratory analyses on a subset of the participants (Johnson et al., 2013). Data were analyzed for a subset of men and women that were at least 20 years old and had data on U-Mo and BMD at the femoral neck (FN-BMD) and lumbar spine (LS-BMD) ($n=2,472$). Men and women with missing data on select biomarkers [urine: cadmium (U-Cd) and creatinine (U-CR); blood: lead (B-Pb) and total mercury (B-Hg); serum: cotinine (S-COT) and total 25-hydroxyvitamin D2 and D3 (S-D2D3)], race/ethnicity, body mass index (BMI), alcohol and milk consumption, and recreational activity were excluded ($n=976$), resulting in a final sample size of 1,496 men ($n=830$) and women ($n=666$). A large number of participants had missing data on alcohol consumption. Missing data on the other variables of interest was much less prevalent, accounting for only 0–9% of the eligible participants. These variables were selected *a priori*. With the exception of U-CR, which was necessary to account for variability in urine output (Barr et al., 2005), all other aforementioned variables are risk factors (or surrogates for them) for reduced BMD (Akesson et al., 2006; Burger et al. 1998, Hannan et al., 2000; Hukkanen et al., 2005; Khalil et al., 2008, 2016; Pollack et al., 2013; Staessen et al., 1999; Tsai et al., 2015) and, consequently, were considered for inclusion in the fully-adjusted statistical models. For example, we considered cadmium, lead, and mercury as potential covariates as these metals have been linked to osteotoxicity (Akesson et al., 2006; Pollack et al., 2013; Staessen et al., 1999; Tsai et al., 2015). While several of these variables were collected across multiple NHANES cycles, there was complete overlap only in 2007–2008 and 2009–2010, which is why this analysis was limited to those years.

NHANES received approval from the NCHS Ethics Review Board, and informed consent was obtained for all participants. The methods used by CDC to collect data on the variables of interest in the present analysis are described briefly in the subsequent sections. Additional details are provided on the NHANES website (<http://www.cdc.gov/nchs/nhanes.htm>).

2.2 Biomarkers

Whole venous blood and urine were collected from participants in a Mobile Examination Center (MEC), which were then processed, stored, and shipped to labs at the CDC or University of Minnesota (U-CR only) for analysis. Metals were quantified with inductively coupled plasma-mass spectrometry (CDC, 2004, 2006), whereas S-COT and S-D2D3 were measured with high performance liquid chromatography (CDC, 2008, 2010) and U-CR was assessed using an enzymatic method (CDC, 2011). Biomarker concentrations that were

below the below analytical limit of detection (LOD) were assigned a value of $\text{LOD} \div 2$. LODs are reported in the footnotes section of Table 1.

BMD was calculated by CDC using the bone mineral content and bone area data that was collected via dual-energy x-ray absorptiometry (CDC, 2007). While BMD was also calculated by CDC for several other regions of the femur and spine, FN-BMD and LS-BMD (L1–L4), in particular, are recommended for evaluating osteoporosis and low BMD (Khalil et al., 2016; Looker et al., 2012; NOF, 2014), which is why this analysis specifically focused on these regions of the body.

2.3 Demographics

Information on age and race/ethnicity was collected from participants in their homes by a trained interviewer that used a Computer-Assisted Personal Interviewing (CAPI) system. Interviews were in English or Spanish, or with the assistance of an interpreter, if necessary.

2.4 Body Measures

Height and weight of participants were measured in the MEC by health technicians with the assistance of a recorder. These measurements were used by CDC to calculate BMI.

2.5 Dietary Intake

Dietary information was collected from participants in the MEC by trained interviewers using the CAPI system. In particular, frequency of alcohol consumption during the previous year, and prevalence of regular milk drinking (defined by CDC as milk consumption at least 5 times/week) during one's lifetime, including childhood, were selected as surrogates for alcohol and calcium consumption, respectively (Campbell and Auinger, 2007; Khalil et al., 2016).

2.6 Physical Activity

Information on physical activity was collected from participants in their homes by trained interviewers using the CAPI system and a questionnaire that was modeled after the World Health Organization's Global Physical Activity Questionnaire (<http://www.who.int/chp/steps/GPAQ>). The prevalence of moderate-intensity (e.g., brisk walking, bicycling, swimming, or golf) or vigorous-intensity (e.g., running or basketball) recreational activity was selected to characterize overall physical activity level among the participants (Khalil et al., 2016).

2.7 Statistical Analysis

Statistical analysis was performed using SAS 9.3 for Windows (SAS Institute, Cary, NC, USA). Previous research suggests that BMD differs by sex and age (Burger et al., 1998) and the same may be true for U-Mo as well. Consequently, the sample was first stratified on sex (male or female) and age groups (20–50 years old or 50–80+ years old) in an attempt to separate potential sex-age effects in subsequent analyses. Biomarkers quantified in urine were expressed as raw and, to adjust for urine output (Barr et al., 2005), CR-corrected values. Mean BMD across L1–L4 was calculated as an estimate of the average LS-BMD (Khalil et al., 2016). The frequency that alcohol was consumed in the past year (1 day/

week vs. <1 day/week), regular milk intake (yes vs. no), and moderate/vigorous-intensity recreational activity (yes vs. no) were dichotomized. All other variables (i.e., BMI, other biomarkers, and race/ethnicity) remained unchanged. Following these data modifications, sex-age-specific descriptive statistics were then calculated.

Sex-age-specific bivariate relationships between participant characteristics and U-Mo, FN-BMD, and LS-BMD were assessed by calculating Pearson correlation coefficients (continuous variables) or performing t-tests or ANOVA (categorical variables). Statistical tests relied on CR-corrected biomarkers and natural log (ln)-transformed variables where appropriate.

Sex-age-specific associations between U-Mo and FN-BMD and LS-BMD were initially assessed in unadjusted linear regression models. Multivariable linear regression models were then created to evaluate adjusted relationships. All multivariable models were adjusted for U-CR and age, but the selection of additional covariates was sex-age-specific and based on the relevant results of the bivariate analyses. In particular, for each model additional covariates were included if they were identified in the relevant bivariate analyses to be related to the outcome only (i.e., variables that increase the precision of the effect estimates when added to the model) or both the outcome and U-Mo (i.e. potential confounders). U-Mo and other selected covariates were ln-transformed to improve model fit. FN-BMD and LS-BMD followed normal distributions, so they were modeled untransformed. These unadjusted and adjusted models were also re-run with U-Mo categorized as quartiles (with the lowest quartile as the referent) to explore potential non-linear relationships with FN-BMD and LS-BMD. Sampling weights were not used in the present analysis because inclusion of weights in addition to adjusting for variables used in the calculation of weights leads to losses in the precision of effect estimates (Korn and Graubard, 1991; Johnson et al., 2013; Silver et al., 2013). Nevertheless, use of weights in stratified approaches, such as in this analysis, is not possible. Statistical significance was defined as a p -value <0.05

3. RESULTS

Table 1 reports the characteristics of this subset men and women. Across all sex and age categories, participants were predominantly non-Hispanic White with comparable BMIs. Both alcohol and milk consumption frequencies were greater for men relative to women, but the frequencies were comparable within sexes when stratified by age. The prevalence of moderate or vigorous recreational activity decreased with age for both men and women. Younger men were more active relative to their female counterparts, but similar patterns of recreational activity were noted for older adults. The detection frequency for U-Mo was 100%. The remaining biomarkers were detected in 68% (S-COT) to 100% (S-D2D3, B-Pb, and U-CR) of all samples. Relative to women, men had lower concentrations of U-Mo when corrected for urine output, but had higher values for FN-BMD and LS-BMD. There was also a decrease with age in FN-BMD within both men and women and LS-BMD within women only.

Statistically significant or suggestive relationships were observed for both continuous and categorical characteristics with U-Mo, FN-BMD, and LS-BMD (Supplementary Material:

Tables S1–S4). There were few differences in the findings when comparing the associations for men and women in the same age category. Relationships for the same variables noted across multiple sex-age groups were consistently in the same direction. There was evidence for a positive association for S-D2D3 and milk consumption with U-Mo and an inverse relationship for S-COT, B-Pb, and alcohol consumption with U-Mo. Both BMI and recreational activity were positively related to FN-BMD, whereas S-D2D3, U-Cd, and B-Pb were inversely associated with FN-BMD. For LS-BMD, positive relationships were noted for S-COT, BMI, alcohol consumption, and recreational activity, and negative associations were noted for U-Cd and B-Pb. There were differences in U-Mo, FN-BMD, and LS-BMD by race/ethnicity categories as well.

Shown in Table 2 are the results of the models where FN-BMD and LS-BMD were regressed on either ln-U-Mo or quartiles of U-Mo. No relationships were observed in any of the unadjusted models (results not shown). However, in multivariable models there was a statistically significant inverse association between ln-U-Mo and LS-BMD among 50–80+ year-old women [β (95% CI): -0.041 (-0.067 , -0.016)]. Similarly, there was a statistically significant dose-dependent decrease in LS-BMD with increasing U-Mo quartiles among 50–80+ year-old women [β (95% CI) from lowest to highest quartile: -0.055 (-0.109 , -0.000), -0.074 (-0.133 , 0.016), -0.105 (-0.169 , -0.040)]. Although the trend was suggestive, a dose-dependent decrease in FN-BMD with increasing U-Mo quartiles was observed in this group of women as well [β (95% CI): -0.041 (-0.085 , 0.002), -0.045 (-0.092 , 0.002), -0.051 (-0.103 , 0.001)]. All other adjusted models revealed no statistically significant or suggestive relationships between U-Mo and FN-BMD and LS-BMD.

4. DISCUSSION

The potential relationship of U-Mo with FN-BMD and LS-BMD was explored using data collected from a sample of adult men and women that were participants in NHANES 2007–2010. In adjusted models for older women, there was a significant inverse association between U-Mo and LS-BMD, as well as suggestive evidence for an inverse association between U-Mo and FN-BMD. However, in other sex-age strata, no other relationships were noted. While this analysis serves as the first evidence of its kind, caution should be used when interpreting the health significance of the reported findings. The results of this analysis should be used as motivation for future research in humans and experimental models on this topic.

The potential biological mechanisms through which Mo may act to influence BMD have not been identified or even largely explored, but it is plausible that this trace element may disrupt levels of steroid sex hormones, which are necessary for promoting and maintaining bone health (Compston, 2001; Manolagas et al., 2013). It is well-established that estrogen directly affects bone cells and is fundamental to skeletal growth and bone homeostasis in both men and women (Weitzmann and Pacifici, 2006; Khosla et al., 1998). Androgens additionally influence skeletal health, albeit to a lesser degree than estrogen, via direct interactions with bone cell receptors (Clarke and Khosla, 2009). Specifically, androgens may stimulate longitudinal bone growth and have also been found to decrease osteoblast and osteocyte apoptosis (Clarke and Khosla, 2009).

Several studies have reported that Mo may alter hormone levels in animals and humans (Haywood et al., 1998, 2004; Roychoudhury et al., 2014; Meeker et al., 2010; Lewis and Meeker, 2015). In sheep treated with tetrathiomolybdenum (TTM), Mo was found to accumulate in the pituitary, brain, and adrenal glands; exposure to Mo was accompanied by histopathological changes in these target organs as well (Haywood et al., 1998, 2004). Moreover, Mo significantly reduced trophic hormone release and subsequent reproductive activity in treated sheep compared with controls (Haywood et al., 2004). Additionally, an *in vitro* study reported that Mo induced a significant dose-dependent decrease in the release of estrogen in ovarian fragments of rats (Roychoudhury et al., 2014). While no human studies have explored the potential relationship of Mo with estrogen, a few studies have demonstrated that increased exposure to Mo is associated with decreased levels of serum testosterone in adult men (Meeker et al., 2010; Lewis and Meeker, 2015). Additional studies are required to: (1) affirm the specific potential effects of Mo on the endocrine system, especially in females; (2) determine the extent to which potential Mo-induced hormonal fluctuations are related to low BMD; and (3) to identify other potential biological pathways involved in the action of Mo on BMD.

One of the primary limitations of this analysis was the cross-sectional nature of the data, which limited conclusions of causality due to temporal ambiguity. The rate that BMD changes over the life course is gradual and varies by genetic and environmental factors (Emaus et al., 2005; Berger et al., 2010). Thus, longitudinal studies in qualitatively disparate populations are required to capture the temporal relationship between Mo exposure and BMD, and to determine the contribution of Mo exposure on the rate of bone loss over time. Also, it is possible that U-Mo fluctuates to some degree within individuals over time (Wang et al., 2015), but it is expected that error related to U-Mo would be random respected to FN-BMD and LS-BMD, which would bias the results toward the null. There was concern that excluding a large number of participants due to missing data on alcohol consumption may have biased the findings. As a sensitivity analysis, the models were re-run including these individuals ($n=2,224$). As with the original analysis, there was a statistically significant inverse relationship between U-Mo and LS-BMD (p-values for ln-U-Mo and the trend for U-Mo quartiles were 0.02 and 0.01, respectively), but the relationship between U-Mo and FN-BMD was no longer suggestive (results not shown). Because sampling weights were not incorporated into this analysis, the results also may not be fully generalizable to adults of the same age range from the general U.S. population. This study also was potentially limited by the lack of data on serum copper levels in this population, which may modify the Mo-BMD relationships observed in this investigation. Notably, early animal studies have shown that Mo may influence normal bone development through its antagonistic relationship with copper (Comar et al., 1949; Pitt, 1976). Additionally, we cannot rule out reverse causation as a possible explanation for our findings. Since bone tissue serves as a reserve for minerals and heavy metals such as Mo (Kubaszewski et al., 2014), it is possible that our results observed in older women may be due to the release of Mo from bone demineralization associated with aging (Lauretani et al., 2008). Additional human health studies, principally longitudinal in design, are necessary to resolve the direction of these associations observed in our study. Finally, due to the exploratory nature of this analysis, additional alternative explanations, such as statistical chance and residual confounding cannot be ruled out as well.

5. CONCLUSIONS

U-Mo was inversely associated with BMD among older women from NHANES. Because bone health is critical to overall human health and well-being, additional research is needed in other populations to confirm the results of the present analysis and clarify the potential underlying mechanisms of Mo on BMD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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R.C.L. works for Exponent, Inc. (Exponent), a company that provides consultation on potential human health risks posed by exposure to environmental agents, including metals. The opinions expressed in this manuscript are those of the authors only and not necessarily those of Exponent or other scientists that work for the company.

ABBREVIATIONS

BMD	bone mineral density
B-Hg	blood total mercury
B-Pb	blood lead
CAPI	Computer-Assisted Personal Interviewing
CDC	Centers for Disease Control and Prevention
FN-BMD	femoral neck bone mineral density
LS-BMD	lumbar spine bone mineral density
Mo	molybdenum
MEC	Mobile Examination Center
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
S-COT	serum cotinine
S-D2D3	total 25-hydroxyvitamin D2 and D3
TTM	tetrathiomolybdenum
U-Cd	urinary cadmium
U-CR	urinary creatinine
U-Mo	urinary Mo

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HIGHLIGHTS

- Mo may affect bone health through altering levels of steroid sex hormones.
- U-Mo and femoral neck and lumbar BMD were explored among adults from NHANES.
- U-Mo was inversely associated with femoral neck and lumbar BMD in older women only.
- Research is needed to confirm these results and clarify the underlying mechanism.

Table 1

Characteristics of men ($n=830$) and women ($n=666$) from NHANES 2007–2010

Variable	Men: 20–50 years old ($n=541$)		Men: 50–80+ years old ($n=289$)		Women: 20–50 years old ($n=459$)		Women: 50–80+ years old ($n=207$)	
	P50 (P25, P75)	%	P50 (P25, P75)	%	P50 (P25, P75)	%	P50 (P25, P75)	%
U-Mo ($\mu\text{g/L}$) ^a	49.8 (28.7, 78.9)		48.4 (24.6, 75.6)		45.4 (23.6, 78.7)		33.7 (15.2, 62.0)	
U-Mo ($\mu\text{g/g}$)	38.8 (26.3, 55.6)		38.2 (24.7, 56.1)		44.9 (32.1, 63.8)		42.9 (27.0, 68.6)	
FN-BMD (g/cm^2)	0.93 (0.83, 1.03)		0.83 (0.74, 0.93)		0.85 (0.77, 0.93)		0.75 (0.66, 0.83)	
LS-BMD (g/cm^2)	1.05 (0.98, 1.13)		1.07 (0.97, 1.19)		1.04 (0.97, 1.13)		0.95 (0.85, 1.03)	
S-COT (ng/mL) ^a	0.49 (0.03, 107)		0.05 (0.02, 5.62)		0.06 (0.02, 67.6)		0.03 (0.01, 0.10)	
S-D2D3 (nmol/L) ^a	59.9 (47.1, 74.7)		64.4 (46.1, 78.6)		60.9 (43.5, 80.6)		69.2 (46.1, 88.2)	
U-Cd ($\mu\text{g/L}$) ^a	0.18 (0.09, 0.32)		0.35 (0.21, 0.62)		0.23 (0.11, 0.42)		0.32 (0.16, 0.59)	
U-Cd ($\mu\text{g/g}$)	0.14 (0.09, 0.22)		0.30 (0.20, 0.49)		0.23 (0.14, 0.37)		0.44 (0.29, 0.71)	
B-Pb ($\mu\text{g/dL}$) ^a	1.33 (0.93, 2.00)		2.00 (1.36, 2.70)		0.83 (0.59, 1.22)		1.53 (1.15, 2.20)	
B-Hg ($\mu\text{g/L}$) ^a	0.92 (0.48, 1.88)		1.19 (0.64, 2.32)		0.88 (0.52, 1.56)		1.16 (0.70, 2.13)	
U-CR (mg/dL) ^a	136 (83, 195)		130 (75, 179)		104 (59, 163)		70 (43, 120)	
Race/ethnicity								
Mexican American	23		17			16		14
Other Hispanic	12		7			14		11
Non-Hispanic White	44		50			48		58
Non-Hispanic Black	17		21			18		15
Other	4		5			4		2
BMI (kg/m^2)	26.9 (23.9, 30.2)		28.0 (25.3, 31.3)		26.2 (23.2, 31.7)		27.1 (24.2, 31.5)	
Drink alcohol								
<1 day/week	43		41			63		60
1 day/week	57		59			37		40
Regular milk drinker								
Yes	49		43			37		38
No	51		57			63		62
Moderate/vigorous-intensity recreational activity								

Variable	Men: 20–50 years old (n=541)		Men: 50–80+ years old (n=289)		Women: 20–50 years old (n=459)		Women: 50–80+ years old (n=207)	
	P50 (P25, P75)	%	P50 (P25, P75)	%	P50 (P25, P75)	%	P50 (P25, P75)	%
Yes		64		45		53		45
No		36		55		47		55

^aLODs: U-Mo=0.92 µg/L, S-COT=0.015 ng/mL, S-D2D3=2.05 nmol/L (D2) and 2.23 nmol/L (D3), U-Cd=0.20 µg/L, B-Pb=0.25 µg/dL, B-Hg=0.33 µg/L, and U-CR=1 mg/dL.

Association between U-Mo and FN-BMD and LS-BMD among men ($n=830$) and women ($n=666$) from NHANES 2007–2010

Table 2

BMD	U-Mo	Men: 20–50 years old ($n=541$)			Men: 50–80+ years old ($n=289$)			Women: 20–50 years old ($n=459$)			Women: 50–80+ years old ($n=207$)		
		β_{adjusted}	(95% CI)	p -value	β_{adjusted}	(95% CI)	p -value	β_{adjusted}	(95% CI)	p -value	β_{adjusted}	(95% CI)	p -value
FN-BMD	In-variable	-0.006	(-0.024, 0.012)	0.52 ^a	0.009	(-0.013, 0.030)	0.43 ^b	-0.001	(-0.018, 0.018)	0.97 ^c	-0.016	(-0.037, 0.005)	0.13 ^d
	Q1	—			—			—			—		
	Q2	-0.015	(-0.050, 0.019)	0.39 ^a	0.021	(-0.020, 0.062)	0.31 ^b	0.019	(-0.014, 0.051)	0.26 ^c	-0.041	(-0.085, 0.002)	0.06 ^d
	Q3	-0.011	(-0.048, 0.027)	0.58 ^a	0.023	(-0.021, 0.068)	0.30 ^b	-0.005	(-0.043, 0.033)	0.80 ^c	-0.045	(-0.092, 0.002)	0.06 ^d
	Q4	-0.009	(-0.050, 0.032)	0.67 ^a	0.008	(-0.040, 0.057)	0.73 ^b	0.013	(-0.028, 0.055)	0.53 ^c	-0.051	(-0.103, 0.001)	0.05 ^d
LS-BMD	Trend			0.85 ^a			0.78 ^b			0.82 ^c			0.08 ^d
	In-variable	-0.009	(-0.026, 0.008)	0.29 ^e	-0.008	(-0.036, 0.019)	0.55 ^f	-0.010	(-0.029, 0.009)	0.29 ^g	-0.041	(-0.067, -0.016)	0.002 ^h
	Q1	—			—			—			—		
	Q2	-0.021	(-0.054, 0.011)	0.20 ^e	-0.024	(-0.076, 0.028)	0.36 ^f	-0.006	(-0.041, 0.028)	0.72 ^g	-0.055	(-0.109, -0.000)	0.05 ^h
	Q3	-0.030	(-0.066, 0.005)	0.09 ^e	0.004	(-0.053, 0.060)	0.90 ^f	-0.028	(-0.069, 0.012)	0.17 ^g	-0.074	(-0.133, -0.016)	0.01 ^h
Trend	Q4	-0.027	(-0.066, 0.011)	0.16 ^e	-0.052	(-0.112, 0.009)	0.09 ^f	-0.027	(-0.071, 0.017)	0.22 ^g	-0.105	(-0.169, -0.040)	0.002 ^h
				0.19 ^e			0.18 ^f			0.17 ^g			0.002 ^h

^a Adjusted for ln-U-Cd, ln-B-Pb, ln-BMI, race/ethnicity, moderate/vigorous-intensity recreational activity, ln-age, and ln-U-CR.^b Adjusted for ln-U-Cd, ln-BMI, race/ethnicity, ln-age, and ln-U-CR.^c Adjusted for ln-S-D2D3, ln-U-Cd, ln-B-Pb, ln-BMI, race/ethnicity, ln-age, and ln-U-CR.^d Adjusted for ln-S-D2D3, ln-U-Cd, ln-BMI, race/ethnicity, ln-age, and ln-U-CR.^e Adjusted for ln-B-Pb, ln-BMI, race/ethnicity, moderate/vigorous recreational-intensity activity, ln-age, and ln-U-CR.^f Adjusted for ln-U-Cd, ln-BMI, race/ethnicity, drink alcohol, ln-age, and ln-U-CR.^g Adjusted for ln-S-COT, ln-B-Pb, ln-BMI, race/ethnicity, ln-age, and ln-U-CR.^h Adjusted for ln-U-Cd, ln-BMI, race/ethnicity, ln-age, and ln-U-CR.