Novel Risk Factors for Clinical Vertebral Fracture in Women

NOVEL RISK FACTORS FOR CLINICAL VERTEBRAL FRACTURE IN WOMEN

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Abstract

Vertebral fracture (VF) is the most common type of fracture. Vertebral fractures are associated with significant disability, poorer quality of life, high cost, increased morbidity, higher mortality, and are predictive of future fracture risk. However, little is known about modifiable VF risk factors, which may be inherently different than for fractures at other sites due to different microarchitecture, biomechanics, and compressive loading.

We therefore investigated the prospective association between several potential novel risk factors and risk of incident clinical vertebral fracture in women participating in the Nurses' Health Study without a prior history of any fracture. The risk factors were: 1) diuretic use, including thiazides and loop diuretics; 2) proton pump inhibitors (PPI) and histamine-2-receptor antagonists (H_2RA); and 3) body size, as measured by body mass index and waist circumference. Self-reported vertebral fracture was confirmed by medical record review. Cox proportional-hazards models were used to simultaneously adjust for potential confounders.

We found that thiazide diuretics and loop diuretics are each independently associated with increased risk of vertebral fracture in women. We found that PPI use is independently associated with a modestly higher risk of VF and the risk increases with longer duration of use. There was no statistically significant association between H_2RA use and VF risk. Larger waist circumference, a measure of central obesity, was independently associated with higher risk of VF in women. Greater lean body mass was independently associated with lower risk of VF in women. These findings suggest new potentially modifiable risk factors for clinical vertebral fracture, and underscore that risk factors for fractures are fracture site-specific.

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INTRODUCTION

The vertebra is the most common fracture site in adults, making vertebral fractures (VF) a major public health problem with 700,000 new cases in the United States each year.[1] Vertebral fractures are associated with significant disability,[2,3] poorer quality of life,[4-6] high cost (over $1 billion annually),[7-9] increased morbidity,[10,11] higher mortality (~23%),[12-15] and are predictive of future fracture risk,[16,17] including at the spine[18] and hip.[19] The incidence of vertebral fracture increases with age for both women[20] and men,[21] and appears to be rising dramatically, especially after age 75 years.

However, little is known about modifiable VF risk factors, which may be inherently different than for fractures at other sites due to different microarchitecture,[22-24] biomechanics,[25] and compressive loading.[26,27] The spine is particularly susceptible to microdamage (e.g. linear microcracks and diffuse damage)[28] in a way that other fracture sites are not because of the vertebra's different biomechanics, loading parameters, microarchitecture, composition, geometry, and structural integrity,[29-31] and the microdamage accumulates with aging.[32] In fact, most vertebral fractures are not due to trauma, but precipitated by routine everyday activities.[33] The unexpected development of a vertebral fracture during seemingly benign activities (e.g. bending or lifting light objects) speaks to the underlying fragility of the vertebra and vulnerability to fracture that is not necessarily captured by bone densitometry testing.[34] However, if modifiable risk factors for vertebral fracture were better understood, we could likely better identify patients at risk and create targeted interventions to prevent vertebral fracture.

Therefore, the purpose of this research is to prospectively examine the association between several potential modifiable risk factors and the development of clinical vertebral fracture in women. The potential risk factors are the following:

1) Diuretic use (thiazides and loop diuretics). We hypothesized that thiazide diuretic use will be associated with a decreased risk of clinical vertebral fracture and loop diuretic use will be associated with an increased risk of clinical vertebral fracture.

2) Proton pump inhibitor and histamine-2-receptor antagonist use. We hypothesized that these medications will be associated with an increased risk of clinical vertebral fracture.

3) Body size, assessed by body mass index and waist circumference. We hypothesized that increased adiposity, in particular abdominal adiposity as assessed by waist circumference, will be associated with an increased risk of clinical VF.

We studied the association between these potential risk factors and development of clinical vertebral fracture in the Nurses' Health Study, a large, ongoing prospective cohort study of women. The conceptual model underlying our analyses is described in **Figure 1**. The overarching hypothesis driving these analyses is that these potential risk factors disrupt the balance between bone breakdown and repair, thus impairing remodeling of microdamage that accumulates over time, and increasing the risk of clinical vertebral fracture. Our findings from these analyses were the following:

1) Diuretic use (thiazides and loop diuretics). Thiazide diuretics and loop diuretics are each independently associated with increased risk of vertebral fracture in women. This analysis included 420 incident vertebral fracture cases documented between 2002 and 2012. The multivariate-adjusted relative risk of clinical vertebral fracture for women taking thiazides compared with women not taking thiazides was 1.47 (95% CI 1.18 to 1.85). The multivariate adjusted relative risk of vertebral fracture for women taking loop diuretics compared with women not taking loop diuretics was 1.59 (95% CI 1.12 to 2.25).

2) Proton pump inhibitor and histamine-2-receptor antagonist use. PPI use is independently associated with a modestly higher risk of VF and the risk increases with longer duration of use. There was no statistically significant association between H_2RA use and VF risk. Our analysis included 547 incident VF cases (2002-2014). The multivariate adjusted relative risk (MVRR) of VF for women taking PPIs was 1.29 (95%CI 1.04-1.59) compared with non-users. Longer duration of PPI use was associated with higher VF risk (MVRR 1.16 [0.90-1.49] for <4 years; 1.27 [0.93-1.73] for 4-7.9 years; 1.64 [1.02-2.64] for ≥ 8 years; p_{trend}=0.01). The MVRR of VF for women taking H_2RAs was 1.22 (0.90-1.67) compared with non-users. Longer duration of H_2RA use was not associated with VF risk (MVRR 1.16 [0.88-1.53] for <4 years; 0.98 [0.60-1.59] for ≥ 4 years; p_{trend}=0.72).

3) Body size, assessed by body mass index and waist circumference. Larger waist circumference, a measure of central obesity, was independently associated with higher risk of VF in women. Greater lean body mass was independently associated with lower risk of VF in women. Our analysis included 536 incident VF cases (2002-2014). Compared with women with BMI 21.0-24.0 kg/m^2, the multivariable-adjusted relative risk (MVRR) of VF for women with BMI ≥ 32.0 kg/m^2 was 0.84 (95% CI 0.61, 1.14; p_{trend}=0.09), and after further adjusting for waist circumference was 0.70 (95% CI 0.49, 0.98; p_{trend}=0.004). Compared with women with waist circumference < 71.0 cm, the multivariate-adjusted relative risk (MVRR) of VF for women with waist circumference ≥ 108.0 cm was 1.77 (95% CI 1.06, 2.93; p_{trend}=0.02), and after further adjusting for BMI was 2.50 (95% CI 1.44, 4.34; p_{trend}<0.001).

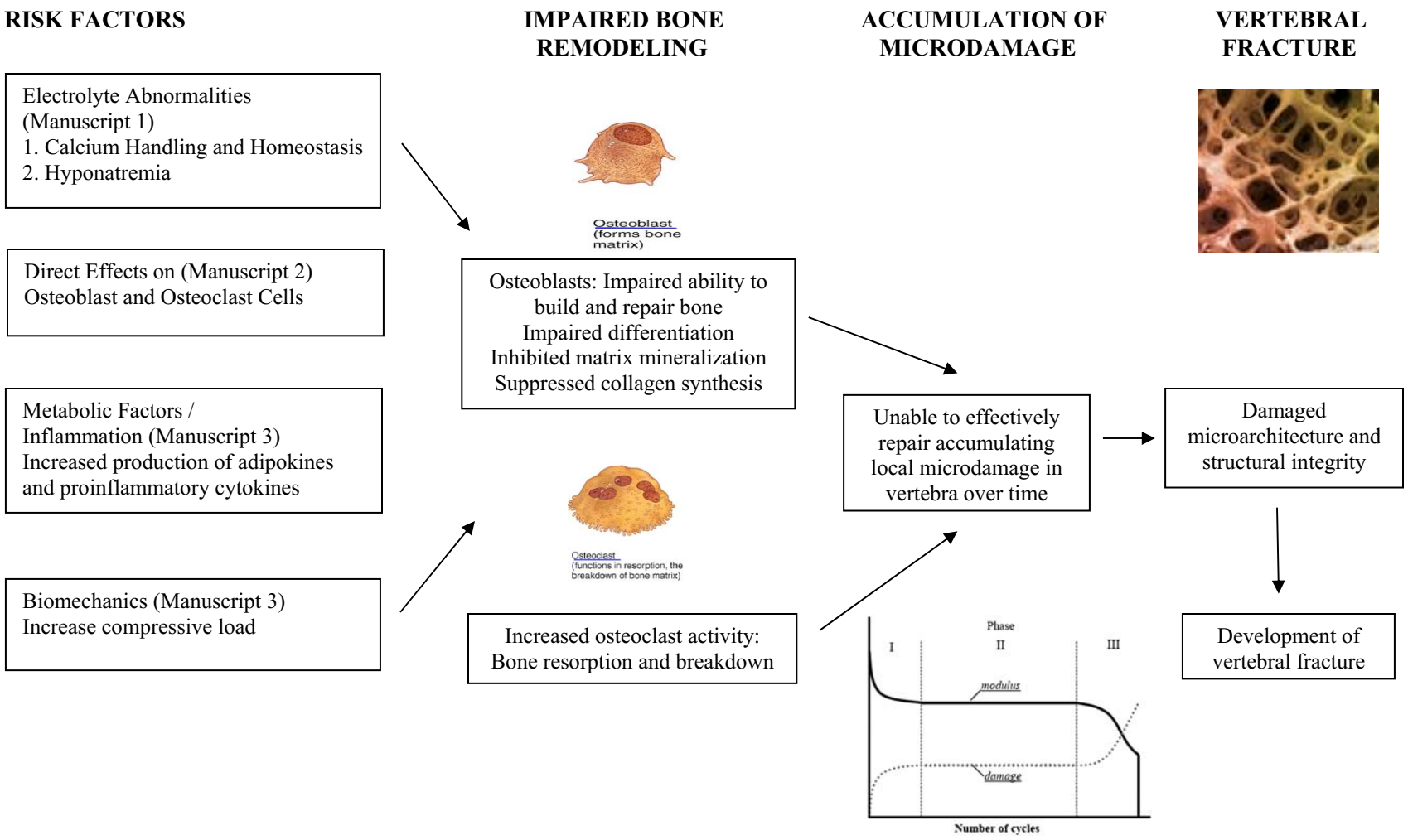
Several themes and insights regarding risk factors for clinical vertebral fracture emerged from the results of these analyses. First, our findings underscore that risk factors for fracture are

site-specific. In other words, a given risk factor can be associated with an increased risk of clinical vertebral fracture but associated with a decreased risk of hip fracture.

Second, our findings suggest that the pathways in the development of vertebral fracture are complex and dynamic, and traditional osteoporosis screening methods such as bone densitometry testing, may not necessarily fully capture the risk for vertebral fracture. Bone mineral density testing only assesses one component of bone, but does not capture other important components such as bone quality, microarchitecture, or the dynamic process of bone modeling and repair occurring at the micro-level. These components and processes may be particularly important for the development of vertebral fracture compared with other fracture sites due to the vertebra's susceptibility to and accumulation of microdamage over time.

Third, it is therefore important to study the association between risk factors and development of vertebral fracture, rather than the association with an intermediate outcome such as bone mineral density, because there are multiple pathways to fracture development beyond that captured by bone mineral density testing. Our findings highlight the need for a deeper understanding of the complex mechanisms and systems involved in the development of vertebral fracture, and suggest future avenues of research to better characterize risk factors and potential areas for targeted intervention for this common and costly disease.

FIGURE 1. Conceptual Model for Risk Factors and Development of Vertebral Fracture



I. Diuretic Use and Risk of Vertebral Fracture in Women

ABSTRACT

Background: Vertebral fracture is the most common type of osteoporotic fracture. While thiazide diuretics, which are commonly prescribed for the treatment of hypertension, decrease calciuria, they may also induce hyponatremia, which has been associated with increased vertebral fracture risk. Loop diuretics increase calciuria, which would reduce bone mineral density and increase vertebral fracture risk, but they rarely cause hyponatremia. Recent studies on diuretics and fractures did not include or specifically examine vertebral fracture. The few studies of diuretics and vertebral fracture have been limited by cases defined by self-report or administrative data, relatively small number of cases, study design that was not prospective, and lack of long-term follow-up with updated information on diuretic use.

Methods: We conducted a prospective cohort study of thiazide diuretic use, loop diuretic use, and risk of incident clinical vertebral fracture in 55,780 women, 55-82 years of age, participating in the Nurses' Health Study, without a prior history of any fracture. Diuretic use was assessed by questionnaire every four years. Self-reported vertebral fracture was confirmed by medical record review. Cox proportional-hazards models were used to simultaneously adjust for potential confounders.

Results: Our analysis included 420 incident vertebral fracture cases documented between 2002 and 2012. The multivariate-adjusted relative risk of clinical vertebral fracture for women taking thiazides compared with women not taking thiazides was 1.47 (95% CI 1.18 to 1.85). The multivariate adjusted relative risk of vertebral fracture for women taking loop diuretics compared with women not taking loop diuretics was 1.59 (95% CI 1.12 to 2.25).

Conclusion: Thiazide diuretics and loop diuretics are each independently associated with increased risk of vertebral fracture in women.

INTRODUCTION

Vertebral fracture is the most common type of osteoporotic fracture[1] and is associated with significant disability,[2] morbidity [11] and mortality.[13] Twenty-five percent of postmenopausal women in the United States are estimated to have a vertebral fracture[35] and the prevalence increases with advancing age.[20] In recent years, the incidence of vertebral fracture appears to be rising dramatically in men and women, especially after age 75 years, whereas the incidence of hip fracture is declining.[36] Risk factors for vertebral fracture may differ from those for fractures at other sites due to different microarchitecture,[22-24] biomechanics,[25] and compressive loading.[26,27]

Thiazide diuretics, which are associated with decreased cardiovascular events and mortality,[37,38] are commonly prescribed for treating hypertension.[39,40] In the U.S., they are the fourth most frequent prescription drug and tenth most used drug overall.[41] In older U.S. adults, hydrochlorothiazide is the second most commonly used prescription or over-the-counter drug.[42] Thiazides decrease urinary calcium excretion[43] and improve calcium balance,[44] which preserve bone mineral density at the vertebral body,[45,46] but thiazides can also induce hyponatremia and several recent studies suggest that hyponatremia is associated with increased fracture risk.[47-52] While thiazide use has been found to be protective for hip fracture in multiple studies,[53-58] the two studies of thiazide use and vertebral fracture[59, 60] found no statistically significant association.

Loop diuretics increase urinary calcium excretion,[61] which could lower bone mineral density, a known risk factor for vertebral fracture,[60] but they rarely cause hyponatremia. However, results have been inconsistent on the association between loop diuretics and bone

mineral density.[62-65] The two studies to date on the association between loop diuretics and vertebral fracture[66,67] found no statistically significant association.

The few studies to date on the association between diuretic use and vertebral fracture have been limited by cases that were defined by self-report or administrative data rather than medical record review, relatively small number of cases, study design that was not prospective, and lack of long-term follow-up with updated exposure information on diuretic use. Therefore, we studied the prospective association between thiazide diuretic use, loop diuretic use, and risk of incident clinical vertebral fracture over a 10-year period in the Nurses' Health Study.

METHODS

Study Population

The Nurses' Health Study (NHS) is an ongoing, prospective cohort study which began in 1976, enrolling 121,700 female registered nurses 30-55 years of age. The cohort is followed with biennial mailed questionnaires that ask about lifestyle practices, medications and newly diagnosed diseases. The follow-up rate has been >90% of the eligible person-time. Deaths are confirmed through the National Death Index. Approximately 97% of the cohort is white.

This analysis includes 55,780 women who answered the 2012 questionnaire, which included the question on lifetime history of vertebral fracture, and who also answered the 2002 questionnaire, which included questions on thiazide and loop diuretic use, and serves as the baseline year for this analysis. Updated information on diuretic use was obtained every four years during the follow-up period. The study protocol was approved by the Brigham and Women's Hospital Institutional Review Board.

Assessment of Diuretic Use

On the 2002, 2006 and 2010 questionnaires, nurses were asked if they regularly took "Thiazide diuretic" or "Lasix" (the brand name for a loop diuretic) in the past two years. The question did not ask about diuretic dose or frequency. Thiazide use and loop diuretic ("Lasix") use were categorized as binary variables (yes/no) in our analyses.

Assessment of Covariates

Potential confounders included age, body mass index (BMI) (<22 kg/m^2, 22-24.9 kg/m^2, 25-29.9 kg/m^2, and ≥30 kg/m^2), race (white or non-white), smoking status (never, past, current), physical activity (quintiles of metabolic equivalent task scores), self-reported history of falls, hypertension, diabetes, osteoporosis, postmenopausal hormone use, anti-hypertension medication use, anti-depressant medication use, bisphosphonate use, oral steroid use, and physical exam during the previous two years, ascertained from the questionnaires. Self-reported weight was highly reliable (r=0.97) among a subset of participants who underwent direct measurement of their weight.[68] Physical activity reported on the questionnaires has been previously validated in a similar cohort when compared with physical activity diaries (r=0.79).[69] Self-reported hypertension[70] and diabetes[71] were previously validated in this cohort. Race was self-reported and categorized in this analysis as white and non-white.

Diet was assessed by extensively validated[72] semiquantitative food-frequency questionnaires that inquired about the average intake of over 130 individual food items and 22 individual beverages as well as vitamins and supplements during the previous year. The participants were asked to complete food frequency questionnaires in 2002, 2006, and 2010. The variables considered in our models were alcohol intake (none, 0.1-4.9 g/day, 5-14.9 g/day, ≥ 15

g/day), caffeine intake, supplemental calcium intake (none, 1-500 mg/day, >500 mg/day), supplemental vitamin D intake (none, 1-400 IU/day, >400 IU/day), and quintiles of dietary intakes of calcium, vitamin D, vitamin A, protein, phosphorus, and magnesium.

Ascertainment of Vertebral Fracture

Participants were first asked about lifetime history of a diagnosis of vertebral fracture on the 2012 questionnaire. They were asked whether they had ever had a clinician-diagnosed "vertebral (spine) fracture, x-ray confirmed" and the year of first diagnosis. We mailed a supplemental questionnaire to nurses who reported a vertebral fracture in 2002 or afterwards and asked permission to obtain their medical records related to the vertebral fracture. Among the participants who gave consent to obtain their medical records and for whom we were able to obtain the medical records that contained sufficient information to make a diagnosis, we confirmed cases of vertebral fracture by radiology report (e.g. x-ray, computed tomography scan, or magnetic resonance imaging) or medical report (e.g. clinic visit note, operative note, hospital discharge summary).

A self-reported vertebral fracture was confirmed as a case if the radiology or medical report contained the word "fracture" (e.g. "vertebral fracture", "spine fracture", "compression fracture", "wedge fracture") or language to suggest a vertebral fracture (e.g. "severe wedge compression", "vertebral collapse", "acute compression"). Medical records that contained less definitive language for a vertebral fracture were adjudicated by an International Society for Clinical Densitometry-trained expert on the reading and interpretation of vertebral fractures, who was blinded to exposure status. We coded participants as "probable" cases when a diagnosis of

vertebral fracture was less certain (e.g. “mild compression deformity”, “stable” or “chronic” “compression deformity”); “probable” cases were not included in our analysis.

We included vertebral fractures that were related to low or moderate trauma (e.g. tripping, slipping, falling from the height of a chair or lower). We excluded vertebral fractures due to high trauma (e.g. fall from a ladder, fall down a flight of stairs), motor vehicle accidents, bicycle accidents, or horseback riding accidents. We also excluded cases of cervical or sacral fracture. We included in the analysis only cases of vertebral fracture that were confirmed by medical record review and diagnosed during the 10 years of follow-up between 2002 and May 31, 2012. Participants with a history of hip, wrist, or vertebral fracture at baseline were excluded from the analysis.

Statistical Analyses

The study design was prospective; information on thiazide and loop diuretic use was collected before the diagnosis of clinical vertebral fracture. For each participant, person-time of follow-up was counted from the date on which the 2002 questionnaire was returned to 1) the date on which the vertebral fracture was diagnosed, 2) death, or 3) May 31, 2012, whichever occurred first. We allocated person-time of follow-up according to the updated exposure status at the start of each follow-up period. We used Cox proportional-hazards models to simultaneously adjust for potential confounders as listed above. All P values are two-tailed.

RESULTS

Thiazide Use and Vertebral Fracture Risk

During 543,209 person-years of follow-up over a 10-year period, there were 420 confirmed cases of incident vertebral fracture. The characteristics of the cohort according to thiazide use in 2002 are shown in **Table 1.1**. For our analyses, however, the updated responses to thiazide use were included for each time period.

In 2002, 14.8% of the participants were taking thiazides and in 2010 it was 19.4%. Those taking thiazides had higher BMI, less physical activity, and were more likely to have hypertension and diabetes. In 2002, 99.5% of the women were postmenopausal and there were relatively similar rates of postmenopausal hormone use amongst the thiazide and non-thiazide users.

After adjusting for age, thiazide use was associated with an increased risk of clinical vertebral fracture (RR 1.44, 95% CI 1.16 to 1.80) (**Table 1.2**). The multivariable-adjusted results were materially unchanged (RR 1.47, 95% CI 1.18 to 1.85).

Loop Diuretic Use and Vertebral Fracture Risk

The characteristics of the cohort according to loop diuretic use in 2002 are shown in **Table 1.3**. For our analyses, however, the updated responses to loop diuretic use were included for each time period. In 2002, 2.8% of the participants were taking a loop diuretic and in 2010 it was 6.5%. Compared with the women not taking a loop diuretic, the women taking a loop diuretic were slightly older, had higher BMI, less physical activity, and were more likely to have diabetes and hypertension. The loop diuretic users had slightly lower supplemental calcium intake and lower alcohol intake.

Table 1.1 Age-Standardized Baseline Characteristics of Women According to Thiazide Use in 2002

	Thiazide Use	
	No (n=47,520)	Yes (n=8,260)
Age, years[*]	66.1 (6.6)	67.5 (6.6)
Body Mass Index, kg/m²	26.5 (5.1)	28.8 (5.7)
Physical Activity (METS/week)[†]	18.9 (22.5)	16.6 (19.9)
Dietary Calcium (mg/day)[#]	857.3 (320.4)	850.6 (312.9)
Calcium Supplement (mg/day)	630.4 (527.3)	630.7 (523.7)
Calcium Supplement Use (yes/no), %	80	81
Total (Dietary and Supplemental) Vitamin D Intake (IU/day)[#]	567.7 (336.8)	572.0 (330.5)
Phosphorus Intake (mg/day)[#]	1,254 (257)	1,256 (254)
Magnesium Intake (mg/day)[#]	374.0 (123.2)	373.7 (119.9)
Total Protein Intake (gm/day)[#]	69.9 (13.0)	71.3 (13.1)
Animal Protein Intake (gm/day)[#]	46.1 (13.6)	47.7 (13.5)
Total (Dietary and Supplemental) Vitamin A Intake (mcg/day)[#]	1,931 (1,403)	1,911 (1,297)
Alcohol Intake (gm/day)	6.2 (10.7)	5.8 (10.4)
Smoking status		
Never smoker, %	47	45
Past smoker, %	46	50
Current smoker, %	7	5
History of Falls	8	10
History of Diabetes, %	7	12
History of Hypertension, %	43	94
Self-Reported Osteoporosis, %	16	14
Postmenopausal Hormone Use, %	38	41
Bisphosphonate Use, %	13	10
Oral Steroid Use, %	2	3

[*] Value is not age adjusted.
[†] Physical activity and history of falls was not asked about in 2002 so data is from the 2000 questionnaire.
[#]Energy adjusted.

Table 1.2 Age- and Multivariable-Adjusted Relative Risks for Incident Vertebral Fracture According to Diuretic Use*

	Thiazide Use	
	No	Yes
Cases of Vertebral Fracture (n)	316	104
Person-years (n)	451,178	92,032
Age-adjusted Relative Risk (95% CI)	1.0	1.44 (1.16, 1.80)
Multivariate Relative Risk (95% CI) †	1.0	1.47 (1.18, 1.85)

	Furosemide Use	
	No	Yes
Cases of Vertebral Fracture (n)	382	38
Person-years (n)	521,248	21,961
Age-adjusted Relative Risk (95% CI)	1.0	1.63 (1.16, 2.28)
Multivariate Relative Risk (95% CI) †	1.0	1.59 (1.12, 2.25)

*Thiazide and furosemide use were updated throughout the analysis period (2002-2012). Relative risks are for the risk of vertebral fracture compared with the group that did not use diuretics.

† The multivariate model includes: body mass index, race, physical activity, history of falls, smoking status, alcohol intake, supplemental calcium intake, quintiles of diet calcium intake, total vitamin D intake, vitamin A intake, total protein intake, history of diabetes or self-reported osteoporosis, history of beta-blocker use, bisphosphonate use, oral steroid use, or postmenopausal hormone use, and recent physical exam.

Table 1.3 Age-Standardized Baseline Characteristics of Women According to Furosemide Use in 2002

	Furosemide Use	
	No (n=54,208)	Yes (n=1,572)
Age, years[*]	66.2 (6.6)	68.8 (6.7)
Body Mass Index, kg/m²	26.7 (5.1)	31.4 (7.3)
Physical Activity (METS/week)[†]	18.7 (22.2)	13.7 (20.8)
Dietary Calcium (mg/day)[#]	856.1 (319.1)	864.8 (328.4)
Calcium Supplement (mg/day)	632.4 (526.7)	569.0 (526.5)
Calcium Supplement Use (yes/no), %	80	76
Total (Dietary and Supplemental) Vitamin D Intake (IU/day)[#]	568.6 (335.3)	561.3 (360.4)
Phosphorus Intake (mg/day)[#]	1,254 (256)	1,276 (260)
Magnesium Intake (mg/day)[#]	374.0 (122.6)	374.4 (128.6)
Total Protein Intake (gm/day)[#]	70.0 (13.0)	72.6 (13.6)
Animal Protein Intake (gm/day)[#]	46.2 (13.6)	49.3 (14.0)
Total (Dietary and Supplemental) Vitamin A Intake (mcg/day)[#]	1,926 (1,382)	2,010 (1,634)
Alcohol Intake (gm/day)	6.2 (10.7)	4.3 (9.4)
Smoking status		
Never smoker, %	47	43
Past smoker, %	46	51
Current smoker, %	7	6
History of Falls	8	12
History of Diabetes, %	8	25
History of Hypertension, %	50	83
Self-Reported Osteoporosis, %	15	16
Postmenopausal Hormone Use, %	38	38
Bisphosphonate Use, %	13	10
Oral Steroid Use, %	2	6

[*] Value is not age adjusted.

[†] Physical activity and history of falls was not asked about in 2002 so data is from the 2000 questionnaire.

[#]Energy adjusted.

After adjusting for age, loop diuretic use was associated with an increased risk of clinical vertebral fracture (RR 1.63, 95% CI 1.16 to 2.28) (**Table 1.2**) and the multivariable-adjusted results were similar (RR 1.59, 95% CI 1.12 to 2.25).

Additional Analyses

In secondary analyses, we attempted to examine the association between duration of thiazide or loop diuretic use and risk of clinical vertebral fracture, but there were too few cases with longer duration of diuretic use to draw meaningful conclusions. We adjusted for other classes of anti-hypertensive medications, including angiotensin-converting enzyme inhibitors, calcium channel blockers, and beta-blockers and the results were not substantially changed. When we restricted the analysis to participants with hypertension, the point estimates were not substantially changed. When we further adjusted for the use of antidepressants, including selective serotonin reuptake inhibitors and tricyclic antidepressants, the results were materially unchanged. There was no statistically significant interaction for either diuretic with age (p for interaction $\geq$0.34) or history of osteoporosis (p for interaction $\geq$0.17).

DISCUSSION

In this large, prospective study of women, we observed an independent increased risk of incident clinical vertebral fracture for thiazide use and loop diuretic use. Our study has several strengths that distinguish it from other reports, including the large number of incident clinical vertebral fracture events that were confirmed by medical record review and repeated assessment of diuretic use over time.

The higher risk of vertebral fracture with thiazide use was unexpected. Our ascertainment of thiazide use appears to be reasonably accurate. A prior study of thiazide use and hip fracture risk in the Nurses' Health Study[53] reported a lower risk, findings consistent with other studies in the literature.[54-58]

This is the first prospective study to report an increased risk of clinical vertebral fracture with thiazide use in women. The Danish population-based case-control study,[59] which used national registry data for thiazide use and fracture cases, did not observe a statistically significant association between former or current thiazide use and vertebral fracture. However, after age stratification, there was an increased odds for current thiazide users among those >65 years of age (OR 1.20, 95% CI 1.04 to 1.39). Of note, the validity of fracture reports from the national registry was based on a random sample of diagnoses (n=35) and the authors did not specify how many of the 35 reports were vertebral fractures versus fractures at other sites.[73] The prospective Study of Osteoporotic Fractures[60] did not find a statistically significant association between thiazide use and risk of vertebral fracture. While the study was prospective and vertebral fractures were defined radiographically, there were only 181 incident vertebral fractures among the women who did not have a baseline prevalent fracture, thiazide use was ascertained only at baseline, and follow-up averaged less than 4 years.

In contrast to the consistently reported protective relation between thiazides and hip fracture,[53-58] the higher risk of vertebral fracture with thiazide use might be mediated by hyponatremia (information on serum sodium in NHS was not available), which directly affects bone mainly via activation of osteoclasts.[74] With raised activity of osteoclasts, bone resorption is increased without concomitant bone formation, impairing local repair of microdamage and decreasing bone quality, thereby increasing fracture risk, all of which is not captured by bone

mineral density measurements.[75,76] Skeletal bone is a rich reservoir of sodium[77] and may play a key role in maintaining sodium homeostasis,[78] but possibly at the expense of the bone's structural integrity. Two recent studies, one in rats[79] and the other in cell culture,[80] support this possibility. The spine is particularly susceptible to microdamage (e.g. linear microcracks and diffuse damage)[28] compared with other fracture sites, given its different biomechanics, loading parameters, microarchitecture, composition, geometry, and structural integrity,[31,75,76] and the microdamage accumulates with aging.[32] The potential harmful effect of hyponatremia on the spine's ability to repair local microdamage may explain the increased vertebral fracture risk seen with thiazide use.

The association between thiazide use and hyponatremia is well-established[81,82] with an estimated 3 in 10 patients exposed to thiazides expected to develop hyponatremia.[83] Our study's finding of a positive association between thiazide use and clinical vertebral fracture is consistent with two recent prospective reports demonstrating the possible role of even mild hyponatremia increasing the risk of developing both vertebral and non-vertebral fractures, independent of falls and bone mineral density.[47,49] The Rotterdam study[47] reported an association between hyponatremia and higher prevalence of vertebral fracture (OR 1.78, 95% CI 1.04 to 3.06), combined prevalent and incident vertebral fracture (OR 1.61, 95% CI 1.00 to 2.59), but not incident vertebral fracture alone.[47] However, there were only 8 incident vertebral fracture cases among the 136 participants with hyponatremia. More compelling data come from the prospective Osteoporotic Fractures in Men Study,[49] where hyponatremia was associated with an increased risk of both prevalent (OR 2.46, 95% CI 1.22 to 4.95) and incident (OR 3.53, 95% CI 1.35 to 9.19) vertebral fractures, with the results unchanged even after adjusting for bone mineral density.

Although our findings of an association between furosemide use and vertebral fracture was in the expected direction,[84,85] this is also the first prospective study to report an increased risk of clinical vertebral fracture with loop diuretic use in women. The mechanisms by which loop diuretic use increase vertebral fracture risk are likely different than the mechanisms for thiazide diuretics. Possible mechanisms include the calciuric effect of loop diuretics,[61] subsequent increase in plasma parathyroid hormone level,[63] and decreased bone mineral density.[63,64] In contrast to prior studies, we confirmed vertebral fractures by medical record review rather than by self-report or from administrative data. In the Danish population-based case-control study[66] that defined loop diuretic use and vertebral fractures from registry data, the association between loop diuretics and vertebral fracture in adjusted models was not statistically significant. In the prospective Women's Health Initiative (WHI),[67] no statistically significant association was found between ever use of loop diuretics and clinical vertebral fracture. However, vertebral fracture was ascertained by self-report and a prior validation study in WHI found a relatively lower rate of agreement between self-report and medical records for clinical vertebral fracture (51%), compared with 78% for hip and 81% for forearm/wrist fractures.[86]

There are several limitations to our observational study. There is the possibility of residual or unmeasured confounding. For example, we did not have data on bone mineral density or morphometric fracture assessment in our participants. Our definition of clinical vertebral fracture was based on medical record review, which means that some participants who might have had a vertebral fracture could not be confirmed. In these cases, we did not have permission or were not able to obtain their medical record, or there was insufficient evidence in the medical record to make a definitive diagnosis of vertebral fracture. We recognize that the incidence rate therefore is likely low because of our method of case ascertainment. However, this

misclassification would likely be non-differential since we would not expect vertebral fracture cases who were exposed to diuretics to be identified differently from cases who were not exposed to diuretics so the findings presented are internally valid. We were unable to assess adherence to diuretic use. We were unable to examine a dose-response relation between diuretic use and vertebral fracture risk. While we postulate that hyponatremia, even mild, may be responsible for the association between thiazide use and vertebral fracture risk, we did not have information on serum sodium concentration for our participants. Finally, since our study population was female and almost entirely white, our findings are not necessarily generalizable to men or other races.

In this large prospective cohort study, thiazides and loop diuretics were independently associated with an increased risk of clinical vertebral fracture in women. Further research is warranted on diuretics and vertebral fracture risk, especially since thiazides are so commonly prescribed for the treatment of hypertension.

II. Proton Pump Inhibitor Use, H_2-Receptor Antagonist Use and Risk of Vertebral Fracture in Women

ABSTRACT

Background: The few prospective studies examining the relation between proton pump inhibitor (PPI) use and risk of vertebral fracture (VF) suggest a higher risk, but the magnitude of the association has been inconsistent. There is very limited prospective data on the association between histamine-2 receptor antagonist (H_2RA) use and VF risk. Our objective was to determine the association between PPI use, H_2RA use, and incident clinical VF in women.

Methods: We conducted a prospective study in 55,545 women participating in the Nurses' Health Study. PPI and H_2RA use was assessed by questionnaire every four years. Self-reports of VF were confirmed by medical record.

Results: Our analysis included 547 incident VF cases (2002-2014). The multivariate adjusted relative risk (MVRR) of VF for women taking PPIs was 1.29 (95%CI 1.04-1.59) compared with non-users. Longer duration of PPI use was associated with higher VF risk (MVRR 1.16 [0.90-1.49] for <4 years; 1.27 [0.93-1.73] for 4-7.9 years; 1.64 [1.02-2.64] for ≥ 8 years; p_{trend}=0.01). The MVRR of VF for women taking H_2RAs was 1.22 (0.90-1.67) compared with non-users. Longer duration of H_2RA use was not associated with VF risk (MVRR 1.16 [0.88-1.53] for <4 years; 0.98 [0.60-1.59] for ≥ 4 years; p_{trend}=0.72).

Conclusions: PPI use is independently associated with a modestly higher risk of VF and the risk increases with longer duration of use. There was no statistically significant association between H_2RA use and VF risk. Our findings add to the growing evidence suggesting caution with PPI use, particularly with longer duration of use.

INTRODUCTION

Proton pump inhibitors (PPI) and histamine-2 receptor antagonists (H_2RA), treatments for acid-related upper gastrointestinal disorders, are among the most commonly used medications in the United States. PPIs have been available over-the-counter since 2003,[87] with numerous over-the-counter options now available in addition to prescription options. The prevalence of PPI use increased from 15.7% in 2005 to 18.5% in 2011.[88]

Vertebral fracture (VF) is the most common type of osteoporotic fracture[1] and is associated with significant disability,[2] morbidity[11] and mortality.[13] Twenty-five percent of postmenopausal women in the United States are estimated to have a vertebral fracture[35] and the prevalence increases with advancing age.[20] In recent years, the incidence of vertebral fracture has risen dramatically in men and women, especially after age 75 years.[36] Risk factors for vertebral fracture may differ from those for fractures at other sites due to different microarchitecture,[22-24] biomechanics,[25] and compressive loading.[26,27]

Prior studies suggest an increased risk of osteoporotic fracture with PPI use,[89-98] but the findings have not been consistent.[99,100] The few prospective studies to date examining the relation between PPI use and risk of VF also suggest an increased risk,[100-102] but the magnitude of the association has been inconsistent. There have been very limited studies on duration of PPI use and risk of VF.[95,100] There are also limited data on the association between H_2RA use and risk of VF.[95,100] The one prospective study of the association between H_2RA use and VF risk suggested no association.[100]

Potential etiologies for increased fracture risk from the use of these acid-suppressing agents include decreased calcium absorption,[103,104] upregulation of osteoclast activity,[105] impaired bone resorption[106,107] resulting in altered bone remodeling, or hypergastrinemia

resulting in parathyroid hyperplasia and decreased bone mineral density.[108] H_2RAs could potentially act through similar mechanisms but are less potent acid-suppressants than PPIs.

Therefore, we studied the prospective association between PPI use, H_2RA use, and risk of incident clinical VF in 55,545 women over a 12-year period in the Nurses' Health Study.

METHODS

Study Population

The Nurses' Health Study (NHS) is an ongoing, prospective cohort study which began in 1976, enrolling 121,700 female registered nurses 30-55 years of age. The cohort is followed with biennial mailed questionnaires that ask about lifestyle practices, medications and newly diagnosed diseases. The follow-up rate has been >90% of the eligible person-time. Approximately 97% of the cohort is white.

This analysis included 55,545 women who answered the 2012 or 2014 questionnaire, which included questions on history of vertebral fracture, and who also answered the 2002 questionnaire, which first included questions on PPI and H_2RA use, and serves as the baseline year for this analysis. Participants were excluded if they had a prior history of hip or wrist fracture, or history of cancer (other than non-melanoma skin cancer). Updated information on PPI and H_2RA use was obtained every four years during the follow-up period. The study protocol was approved by the Brigham and Women's Hospital Institutional Review Board.

Assessment of Proton Pump Inhibitor and H_2-Receptor Antagonist Use

On the 2002 questionnaire, nurses were asked whether they regularly took "Prilosec or Prevacid" (the only two PPIs available at that time) or an "H_2 blocker (e.g. Zantac, Pepcid,

Tagamet)" in the past two years. On the 2006 and 2010 questionnaires, nurses were asked whether they regularly took "Prilosec, Nexium, Prevacid (lansoprazole), Protonix, Aciphex" or an "H_2 blocker (e.g. Pepcid, Tagamet, Zantac, Axid)" in the past two years. The questions did not ask about dose or frequency of PPI or H_2RA use.

Assessment of Covariates

Potential confounders included age, body mass index (BMI) (<22 kg/m^2, 22-24.9 kg/m^2, 25-29.9 kg/m^2, and ≥30 kg/m^2), race (white or non-white), smoking status (never, past, current), physical activity (quintiles of metabolic equivalent task scores), self-reported history of falls, history of hypertension, diabetes, osteoporosis, postmenopausal hormone use, diuretic use, bisphosphonate use, and physical examination during the previous two years, ascertained from the questionnaires. Self-reported weight was highly reliable (r=0.97) among a subset of participants who underwent direct measurement of their weight.[68] Physical activity reported on the questionnaires has been previously validated in a similar cohort when compared with physical activity diaries (r=0.79).[69] Self-reported hypertension[70] and diabetes[71] were previously validated in this cohort. Race was self-reported and categorized in this analysis as white and non-white.

Diet was assessed by extensively validated[72,109] semi-quantitative food-frequency questionnaires that inquired about the average intake of over 130 individual food items and 22 individual beverages, as well as vitamins and supplements during the previous year. The participants were asked to complete food frequency questionnaires in 2002, 2006, and 2010. The variables considered in our models were alcohol intake (none, 0.1-4.9 g/day, 5-14.9 g/day, ≥ 15 g/day), supplemental calcium intake (none, 1-500 mg/day, >500 mg/day), supplemental vitamin

D intake (none, 1-400 IU/day, >400 IU/day), and quintiles of dietary intakes of calcium, vitamin D, vitamin A, protein, phosphorus, magnesium and caffeine.

Ascertainment of Clinical Vertebral Fracture

Participants were asked about lifetime history of a clinician-diagnosed "vertebral (spine) fracture, x-ray confirmed" on the 2012 questionnaire and the year of first diagnosis. They were asked again about a diagnosis of vertebral fracture on the 2014 questionnaire. We mailed a supplemental questionnaire to nurses who reported a vertebral fracture in 2002 or afterwards and asked permission to obtain their medical records related to the vertebral fracture. Among the participants who gave consent to obtain their medical records and for whom we were able to obtain medical records that contained sufficient information to make a diagnosis, we confirmed cases of vertebral fracture by radiology report (e.g. x-ray, computed tomography scan, or magnetic resonance imaging) or medical report (e.g. clinic visit note, operative note, hospital discharge summary).

A self-reported vertebral fracture was confirmed as a case if the radiology or medical report contained the word "fracture" (e.g., "vertebral fracture", "spine fracture", "compression fracture", "wedge fracture") or language to suggest a vertebral fracture (e.g., "severe wedge compression", "vertebral collapse", "acute compression"). Medical records that contained less definitive language for a vertebral fracture were adjudicated by an International Society for Clinical Densitometry-trained expert on the reading and interpretation of vertebral fractures, who was blinded to exposure status. We coded participants as "probable" cases when a diagnosis of vertebral fracture was less certain (e.g. "mild compression deformity", "stable" or "chronic" "compression deformity"); "probable" cases were not included in our analysis.

We included vertebral fractures that were related to low or moderate trauma (e.g. tripping, slipping, falling from the height of a chair or lower). We excluded vertebral fractures due to high trauma (e.g., fall from a ladder, fall down a flight of stairs), motor vehicle accidents, bicycle accidents, or horseback riding accidents. We also excluded cases of cervical or sacral vertebral fracture. We included in the analysis only cases of vertebral fracture that were confirmed by medical record review and diagnosed during the 12 years of follow-up between 2002 and May 31, 2014.

Statistical Analyses

The study design was prospective; information on PPI use, H_2RA use and the covariates of interest were collected before the diagnosis of clinical vertebral fracture. For each participant, person-time of follow-up was counted from the date the 2002 questionnaire was returned to: 1) the date the vertebral fracture was diagnosed, 2) death, or 3) May 31, 2014, whichever occurred first. Participants were censored if they developed a hip fracture or any cancer (other than non-melanoma skin cancer) during the follow-up period. Information on PPI use, H_2RA use and other covariates was collected from the baseline questionnaire and updated on subsequent questionnaires. We allocated person-time of follow-up according to the updated exposure status at the start of each follow-up period. Period-specific categories of PPI use, H_2RA use and other covariates were used in the analysis. We used Cox proportional-hazards models to simultaneously adjust for potential confounders as listed above. All P values are two-tailed.

RESULTS

Proton Pump Inhibitor Use and Vertebral Fracture Risk

During 606,848 person-years of follow-up over a 12-year period, there were 547 confirmed cases of incident vertebral fracture. The characteristics of the cohort according to PPI use in 2002 are shown in **Table 2.1**. For our analyses, however, the updated information on PPI use and covariates was used for each time period.

In 2002, 6.2% of the participants were taking PPIs and the percentage increased over time (16.8% in 2006 and 19.1% in 2010). Compared with women not taking a PPI, women who were taking a PPI had higher BMI, were less physically active, and were more likely to have diabetes, hypertension, or osteoporosis. The PPI users had slightly higher calcium supplement intake and lower alcohol intake. In 2002, 99.3% of the women were postmenopausal and PPI users had higher postmenopausal hormone use compared with non-users.

After adjusting for age, PPI use was associated with an increased risk of clinical vertebral fracture (RR 1.44, 95%CI 1.17 to 1.77) (**Table 2.2**). After multivariable adjustment, the relative risk of clinical vertebral fracture was 1.36 (95%CI 1.11 to1.68) for PPI users compared with non-users. After further adjustment for history of osteoporosis, the relative risk of clinical vertebral fracture was 1.29 (95%CI 1.04 to 1.59).

H_2-Receptor Antagonist Use and Vertebral Fracture Risk

The characteristics of the cohort according to H_2RA use in 2002 are shown in **Table 2.1**. For our analyses, however, the updated responses to H_2RA use as well as covariate information were included for each time period. In 2002, 6.2% of the participants were taking an H_2RA and the percentage remained stable over time (6.1% in 2006 and 6.1% in 2010). Compared with

Table 2.1. Age-Standardized Baseline Characteristics of Women According to PPI and H_2-Receptor Antagonist Use in 2002

	PPI Use		H_2-Receptor Antagonist Use	
	No (n=52,078)	Yes (n=3,467)	No (n=52,084)	Yes (n=3,461)
Age, years[*]	65.9 (6.5)	66.2 (6.6)	65.9 (6.5)	66.3 (6.5)
Body Mass Index (kg/m²)	26.8 (5.2)	28.6 (5.6)	26.9 (5.2)	28.4 (5.5)
Physical Activity (METS/week)[†]	18.8 (22.6)	15.1 (18.3)	18.8 (22.5)	15.6 (18.7)
Dietary Calcium (mg/day)[#]	855.3 (320.1)	848.3 (314.3)	855.8 (319.8)	841.5 (318.6)
Calcium Supplement (mg/day)	620.2 (523.4)	657.5 (543.5)	622.1 (524.5)	631.9 (529.7)
Calcium Supplement Use (yes/no), %	80	81	80	81
Total (Dietary and Supplemental) Vitamin D Intake (IU/day)[#]	562.4 (334.3)	582.4 (337.2)	563.1 (334.5)	573.2 (335.7)
Phosphorus Intake (mg/day)[#]	1,253 (257)	1,260 (250)	1,254 (256)	1,249 (254)
Magnesium Intake (mg/day)[#]	373.3 (122.6)	373.1 (121.8)	373.6 (122.8)	369.3 (119.1)
Total Protein Intake (gm/day)[#]	70.1 (13.0)	71.1 (12.8)	70.1 (13.0)	70.6 (13.0)
Animal Protein Intake (gm/day)[#]	46.3 (13.7)	47.7 (13.2)	46.3 (13.7)	47.3 (13.4)
Total (Dietary and Supplemental) Vitamin A Intake (mcg/day)[#]	1,918 (1,382)	1,908 (1,354)	1,917 (1,379)	1,921 (1,402)
Alcohol Intake (gm/day)	6.3 (10.76)	4.7 (9.1)	6.3 (10.7)	5.0 (9.5)
Smoking status				
Never smoker, %	47	46	47	44
Past smoker, %	46	50	46	50
Current smoker, %	7	4	7	7
History of Falls	8	12	8	11
History of Diabetes, %	8	11	8	11
History of Hypertension, %	49	67	49	67
Self-Reported Osteoporosis, %	14	17	14	16
Postmenopausal Hormone Use, %	38	48	38	47
Bisphosphonate Use, %	11	11	11	11

[*] Value is not age adjusted.
[†] Physical activity and history of falls was not asked about in 2002 so data are from the 2000 questionnaire.
[#] Energy adjusted.

Table 2.2. Age- and Multivariable-Adjusted Relative Risks for Clinical Vertebral Fracture According to PPI and H_2-Receptor Antagonist Use*

	PPI Use	
	No	Yes
Cases of Vertebral Fracture (n)	426	121
Person-years (n)	523,600	83,248
Age-adjusted Relative Risk (95% CI)	1.0	1.44 (1.17, 1.77)
Multivariate Relative Risk (95% CI) † (Model without osteoporosis)	1.0	1.36 (1.11, 1.68)
Multivariate Relative Risk (95% CI) †† (Model with osteoporosis)	1.0	1.29 (1.04, 1.59)
	H_2-Receptor Antagonist Use	
	No	Yes
Cases of Vertebral Fracture (n)	503	44
Person-years (n)	569,267	37,580
Age-adjusted Relative Risk (95% CI)	1.0	1.34 (0.98, 1.82)
Multivariate Relative Risk (95% CI) † (Model without osteoporosis)	1.0	1.26 (0.92, 1.72)
Multivariate Relative Risk (95% CI) †† (Model with osteoporosis)	1.0	1.22 (0.90, 1.67)

*PPI and H_2-Receptor Antagonist use were updated throughout the analysis period (2002-2014). Relative risks are for the risk of vertebral fracture compared with the group that did not use PPIs or H_2-Receptor Antagonists.

† The multivariate model includes body mass index, race, physical activity, history of falls, smoking status, alcohol intake, supplemental calcium intake, quintiles of diet calcium intake, total vitamin D intake, vitamin A intake, total protein intake, history of diabetes, postmenopausal hormone use, diuretic use and recent physical exam.

†† The multivariate model includes body mass index, race, physical activity, history of falls, smoking status, alcohol intake, supplemental calcium intake, quintiles of diet calcium intake, total vitamin D intake, vitamin A intake, total protein intake, history of diabetes, self-reported osteoporosis, postmenopausal hormone use, diuretic use and recent physical exam.

women not taking an H_2RA, women taking an H_2RA had higher BMI, were less physically active, and were more likely to have a history of diabetes or hypertension, and slightly more likely to have a history of osteoporosis. The participants using a H_2RA had slightly lower alcohol intake and were more likely to be on postmenopausal hormone therapy compared with participants not using a H_2RA.

After adjusting for age, H_2RA use was not associated with a statistically significant risk of clinical vertebral fracture (RR 1.34, 95%CI 0.98 to 1.82) (**Table 2.2**). After multivariable adjustment, the relative risk was attenuated and not statistically significant (RR 1.26, 95%CI 0.92 to 1.72). After further adjustment for history of osteoporosis, the relative risk of vertebral fracture was further attenuated (RR 1.22, 95%CI 0.90 to 1.67).

Duration of PPI Use, H_2-Receptor Antagonist Use, and Risk of Vertebral Fracture

We examined the association between duration of PPI or H_2RA use and risk of clinical vertebral fracture over the study period. We categorized duration of PPI use into none, <4 years, 4-7.9 years, and ≥ 8 years. Longer duration of PPI use was associated with higher risk of vertebral fracture (**Table 2.3**). After multivariable adjustment, compared with participants not taking PPIs, the relative risk of vertebral fracture was 1.22 (95%CI 0.95 to 1.56) for participants with <4 years of PPI use, 1.35 (95%CI 0.99 to 1.85) for participants with 4-7.9 years of PPI use, and 1.75 (95%CI 1.09 to 2.81) for participants with ≥ 8 years of use (p for trend=0.003). After further adjustment for history of osteoporosis, the relative risk of vertebral fracture was slightly attenuated (RR 1.16 [95%CI 0.90 to 1.49] for <4 years of PPI use, 1.27 [95%CI 0.93 to 1.73] for 4-7.9 years of PPI use, and 1.64 [95%CI 1.02 to 2.64] for ≥ 8 years of use; p for trend=0.01).

Table 2.3. Age-Adjusted and Multivariate Relative Risks for Clinical Vertebral Fracture According to Duration of PPI or H_2-Receptor Antagonist Use*

PPI Use	None	< 4 years	4 – 7.9 years	≥ 8 years	P for Trend
Cases of Vertebral Fracture (n)	396	78	48	19	
Person-years (n)	488,126	64,894	25,970	6,458	
Age-adjusted Relative Risk (95% CI)	1.0	1.28 (1.00, 1.63)	1.48 (1.09, 2.01)	1.93 (1.21, 3.08)	0.001
Multivariate Relative Risk (95% CI) † (Model without osteoporosis)	1.0	1.22 (0.95, 1.56)	1.35 (0.99, 1.85)	1.75 (1.09, 2.81)	0.003
Multivariate Relative Risk (95% CI) †† (Model with osteoporosis)	1.0	1.16 (0.90, 1.49)	1.27 (0.93, 1.73)	1.64 (1.02, 2.64)	0.01

H_2-Receptor Antagonist Use	None	< 4 years	≥ 4 years	P for Trend
Cases of Vertebral Fracture (n)	464	60	17	
Person-years (n)	526,606	47,448	11,394	
Age-adjusted Relative Risk (95% CI)	1.0	1.32 (1.01, 1.73)	1.10 (0.68, 1.79)	0.21
Multivariate Relative Risk (95% CI) † (Model without osteoporosis)	1.0	1.19 (0.91, 1.58)	0.99 (0.61, 1.61)	0.63
Multivariate Relative Risk (95% CI) †† (Model with osteoporosis)	1.0	1.16 (0.88, 1.53)	0.98 (0.60, 1.59)	0.72

*PPI and H_2-Receptor Antagonist use were updated throughout the analysis period (2002-2014). Relative risks are for risk of vertebral fracture compared with the group that did not use PPIs or H_2RAs. This analysis includes 541 cases since 6 cases were missing information on duration of PPI and H_2RA use.

† The multivariate model includes BMI, race, physical activity, history of falls, smoking status, alcohol intake, supplemental calcium intake, quintiles of diet calcium intake, total vitamin D intake, vitamin A intake, total protein intake, self-reported diabetes, postmenopausal hormone use, and recent physical exam.

†† The multivariate model includes body mass index, race, physical activity, history of falls, smoking status, alcohol intake, supplemental calcium intake, quintiles of diet calcium intake, total vitamin D intake, vitamin A intake, total protein intake, history of diabetes, self-reported osteoporosis, postmenopausal hormone use, diuretic use and recent physical exam.

Because there were very few cases with longer duration of H_2RA use, we categorized H_2RA use into none, <4 years and ≥ 4 years. The multivariable-adjusted relative risk of vertebral fracture was 1.19 (95%CI 0.91 to 1.58) for <4 years of H_2RA use and 0.99 (95%CI 0.61 to 1.61) for ≥ 4 years of use (p for trend=0.63). After further adjustment for history of osteoporosis, the relative risk of vertebral fracture remained non-significant (RR 1.16 [95%CI 0.88 to 1.53] for <4 years of H_2RA use and 0.98 [95%CI 0.60 to 1.59] for ≥ 4 years of H_2RA use; p for trend=0.72).

Additional Analyses

We performed a mediation analysis to quantify the proportion of the association between PPI use and risk of vertebral fracture explained by osteoporosis as an intermediate condition. Osteoporosis explained 17.3% (8.0% - 33.4%; $p < 0.001$) of the association between PPI use and vertebral fracture.

We examined whether the associations with vertebral fracture for PPI use and H_2RA use varied with age (above and below age 70 years), history of osteoporosis, and bisphosphonate use. There was no statistically significant interaction for PPI use with age (p for interaction > 0.88), history of osteoporosis (p for interaction > 0.26), or bisphosphonate use (p for interaction > 0.36). There was no statistically significant interaction for H_2RA use with age (p for interaction 0.59), history of osteoporosis (p for interaction > 0.63), or bisphosphonate use (p for interaction > 0.08).

DISCUSSION

In this prospective study of over 55,000 women, we observed an independent higher risk of incident clinical vertebral fracture with PPI use. We also observed an independent higher risk

of vertebral fracture with longer duration of PPI use. We did not observe a statistically significant association between H_2RA use and VF risk. Our study has several strengths that distinguish it from other reports, including the large number of incident clinical vertebral fracture events that were confirmed by medical record review, repeated assessment of PPI and H_2RA use over time, as well as information on duration of PPI and H_2RA use over a longer period of time than prior studies.

Few prospective cohort studies have examined the association between PPI use and vertebral fracture and reported findings were inconsistent.[100-102] Some of these differences could be related to different methods of ascertaining the outcome of vertebral fracture, including self-report,[100] follow-up x-ray,[101] and claims data[102] as well as varying sample sizes. We confirmed cases of vertebral fracture through medical record review. Our findings are consistent with the other prospective studies that reported an increased risk of vertebral fracture with PPI use, although our magnitude of risk is lower compared with the other studies, which ranged from 1.47 to 3.50.

Emerging research has raised concerns over long-term use of PPIs due to adverse outcomes.[110] The only prospective study to date examining the association between duration of PPI use and VF risk, the Women's Health Initiative,[100] looked at duration of 3 or more years (duration of PPI use was categorized into none, <1 year, 1-3 years, and > 3 years), whereas our study examined substantially longer duration of PPI use. While the Women's Health Initiative reported an increased risk of vertebral fracture with longer duration of PPI use, this risk was attenuated with longer duration of use (<1 year: 1.67, 95%CI 1.22-2.27; 1-3 years: 1.40, 95%CI 1.02-1.92; and > 3 years: 1.11, 95%CI 0.59-2.07). Given the wide confidence interval for the longest duration group (> 3 years), it is likely that there were few cases in that category, and the

follow-up was too short to draw definitive conclusions about long-term use. Our results might differ from the WHI study because our shortest duration category (< 4 years) encompasses the duration examined by the WHI study. Our vertebral fracture cases were also confirmed by medical record review, whereas the WHI used self-reports.

While the mechanism for the association between longer duration of PPI use and vertebral fracture is not clearly understood, studies suggest that it is less likely to be completely mediated through changes in bone mineral density.[92,100,111-113] In our mediation analysis, osteoporosis accounted for less than 20% of the association between PPI use and vertebral fracture. For this reason, we created multivariate models with and without a history of osteoporosis. Inclusion of osteoporosis attenuated the association between PPI use and vertebral fracture, but the results remained statistically significant. Inclusion of osteoporosis in our multivariate model also attenuated the association between H_2RA use and vertebral fracture.

Our study did not find a statistically significant association between H_2RA use and clinical vertebral fracture. It is postulated that H_2RAs act through similar mechanisms as PPIs, but with less potency. Our findings are consistent with the WHI study,[100] the only other prospective study to date on the association between H_2RA use and vertebral fracture, which reported no significant association between H_2RA use and vertebral fracture, as well as no significant association between duration of H_2RA use and vertebral fracture.

There are several limitations to our study that merit discussion. Given the observational design, there is the possibility of residual confounding. For example, although we did have information on self-reported osteoporosis, we did not have data on actual bone mineral density or morphometric fracture assessment. Our definition of clinical vertebral fracture was based on medical record review, so for some participants who self-reported a vertebral fracture, we did not

have permission or were not able to obtain their medical record, or there was insufficient evidence in the medical record to make a definitive diagnosis of vertebral fracture. We recognize that the observed incidence rate therefore is lower because of our method of case ascertainment. We were unable to assess adherence to PPI or H_2RA use. Finally, since our study population was female and almost entirely white, our findings are not necessarily generalizable to men or other races.

In this large prospective cohort study, PPI use was independently associated with a higher risk of clinical vertebral fracture in women and the risk increases with longer duration of PPI use. There was no statistically significant association between H_2RA use and vertebral fracture. Our findings add to the growing observational evidence suggesting caution with PPI use, particularly with longer duration of use. The risks and benefits of PPI use should be taken into consideration when starting or with continued use of PPIs in older women. Further research is warranted on PPI and H_2RA use and vertebral fracture risk, particularly with longer duration of use, since the use of these acid-suppressing agents is so common amongst older women who are at the highest risk for vertebral fracture.

III. Body Mass Index, Waist Circumference, and Risk of Incident Vertebral Fracture in Women

ABSTRACT

Background: Recent evidence suggests that the relation between obesity and fracture risk may be site-specific. Previous studies on the association between body mass index (BMI) and risk of vertebral fracture (VF) have been inconsistent, and there have been no prospective studies to date on the relation between waist circumference, a measure of central obesity, and vertebral fracture risk.

Methods: We conducted a prospective study in 54,940 women participating in the Nurses' Health Study with no previous history of vertebral fracture. BMI was assessed biennially. Waist circumference was assessed in the year 2000. Self-reports of vertebral fracture were confirmed by medical record review.

Results: Our analysis included 536 incident VF cases (2002-2014). Compared with women with BMI 21.0-24.0 kg/m^2, the multivariable-adjusted relative risk (MVRR) of VF for women with BMI ≥ 32.0 kg/m^2 was 0.84 (95% CI 0.61, 1.14; p_{trend}=0.09), and after further adjusting for waist circumference was 0.70 (95% CI 0.49, 0.98; p_{trend}=0.004). Compared with women with waist circumference < 71.0 cm, the multivariate-adjusted relative risk (MVRR) of VF for women with waist circumference ≥ 108.0 cm was 1.77 (95% CI 1.06, 2.93; p_{trend}=0.02), and after further adjusting for BMI was 2.50 (95% CI 1.44, 4.34; p_{trend}<0.001).

Conclusion: Larger waist circumference, a measure of central obesity, was independently associated with higher risk of VF in women. Greater lean body mass was independently associated with lower risk of VF in women.

INTRODUCTION

Vertebral fracture (VF) is the most common type of fracture and is associated with significant disability,[2] morbidity[11] and mortality.[13] Twenty-five percent of postmenopausal women in the United States are estimated to have a vertebral fracture[35] and the prevalence increases with advancing age.[20] In recent years, the incidence of vertebral fracture has risen dramatically in men and women, especially after age 75 years.[36] Risk factors for vertebral fracture may differ from those for fractures at other sites due to different microarchitecture,[22-24] biomechanics,[25] and compressive loading.[26,27,114]

Obesity has traditionally been thought to be protective for fractures[115,116] because obesity has been associated with higher bone mineral density (BMD). However, emerging evidence is challenging the notion that obesity is protective for fractures,[117-120] especially given our evolving understanding of the complex pathophysiology underlying the relation between obesity and fractures. Moreover, the distribution of body fat, particularly abdominal obesity, may affect bone differently[121] and potentially affect fracture risk.[122] The relation between obesity and fractures also appears to be fracture site-specific.[119,123] In a meta-analysis on body mass index (BMI) and fracture risk, after adjusting for BMD, BMI was protective for hip fractures but was a risk factor for other fractures, fractures of the tibia and fibula, distal forearm, and upper arm.[124] However, this meta-analysis did not examine the association between BMI and vertebral fracture.

We recently conducted a meta-analysis of prospective studies on the association between BMI and vertebral fracture risk in men and women and found no statistically significant association in women.[125] However, we found substantial heterogeneity among the studies in women,[60,126-130] and the studies did not include waist circumference, a measure of central obesity,

in the multivariable models. No published study to date has examined the independent association between waist circumference and vertebral fracture risk in women.

Therefore, we studied the prospective association between BMI, waist circumference, and risk of incident clinical vertebral fracture over a 12-year period in 54,940 women in the Nurses' Health Study.

METHODS

Study Population

The Nurses' Health Study (NHS) is an ongoing, prospective cohort study which began in 1976, enrolling 121,700 female registered nurses 30-55 years of age. The cohort is followed with biennial mailed questionnaires that ask about lifestyle practices, medications and newly diagnosed diseases. The follow-up rate has been >90% of the eligible person-time. Approximately 97% of the cohort is white.

This analysis includes 54,940 women who answered the 2012 or 2014 questionnaire, which included questions on history of VF, and who also had available information on BMI or waist circumference. Participants were excluded if they had a prior history of hip or wrist fracture, or history of cancer (other than non-melanoma skin cancer). Updated information on BMI was obtained throughout the follow-up period. The study protocol was approved by the Brigham and Women's Hospital Institutional Review Board.

Assessment of Body Mass Index and Waist Circumference

Weight has been queried on every biennial questionnaire in NHS. Self-reported weight was highly reliable (r=0.97) among a subset of participants who underwent direct measurement

of their weight.[68] Body mass index (kg/m^2) was calculated from the information provided by participants on weight and height. We ultimately categorized BMI in our models as <21.0 kg/m^2, 21.0-24.9 kg/m^2, 25.0-29.9 kg/m^2, 30.0-31.9 kg/m^2 and ≥32.0 kg/m^2.

Waist circumference was queried among NHS participants in 1986, 1996, and 2000. Self-reported waist circumference was highly reliable (r=0.88) among a subset of NHS participants who underwent direct measurement of their waist circumference.[68] For participants who did not provide waist circumference information in 2000, we carried forward information provided on waist circumference in 1996 if available.

Assessment of Covariates

Potential confounders ascertained from the questionnaires included age, race (white or non-white), smoking status (never, past, current), physical activity (quintiles of metabolic equivalent task scores), self-reported history of falls, history of hypertension, diabetes, self-reported osteoporosis, postmenopausal hormone use, bisphosphonate use, diuretic use, and proton-pump inhibitor use, and physical examination during the previous two years. Race was self-reported and categorized in this analysis as white and non-white. Physical activity reported on the questionnaires has been validated previously in a similar cohort when compared with physical activity diaries (r=0.79).[69] History of hypertension[70] and diabetes[71] were previously validated in this cohort. Self-reported osteoporosis has been validated recently in a similar cohort.[131]

Diet was assessed by extensively validated[72,109] semi-quantitative food-frequency questionnaires that inquired about the average intake of over 130 individual food items and over 20 individual beverages, as well as vitamins and supplements during the previous year. The

participants were asked to complete food frequency questionnaires in 2002, 2006, and 2010. The variables considered in our models were alcohol intake (none, 0.1-4.9 g/day, 5-14.9 g/day, ≥ 15 g/day), supplemental calcium intake (none, 1-500 mg/day, >500 mg/day), supplemental vitamin D intake (none, 1-400 IU/day, >400 IU/day), and quintiles of dietary intakes of calcium, vitamin D, vitamin A, protein, phosphorus, magnesium and caffeine.

Ascertainment of Clinical Vertebral Fracture

Participants were asked about lifetime history of a clinician-diagnosed "vertebral (spine) fracture, x-ray confirmed" on the 2012 questionnaire and the year of first diagnosis. They were asked again about a diagnosis of vertebral fracture on the 2014 questionnaire. We mailed a supplemental questionnaire to participants who reported a vertebral fracture in 2002 or afterwards and asked permission to obtain their medical records related to the vertebral fracture. Among the participants who gave consent to obtain their medical records and for whom we were able to obtain medical records that contained sufficient information to make a diagnosis, we confirmed cases of vertebral fracture by radiology report (e.g. x-ray, computed tomography scan, or magnetic resonance imaging) or medical report (e.g. clinic visit note, operative note, hospital discharge summary).

A self-reported vertebral fracture was confirmed as a case if the radiology or medical report contained the word "fracture" (e.g., "vertebral fracture", "spine fracture", "compression fracture", "wedge fracture") or language to suggest a vertebral fracture (e.g., "severe wedge compression", "vertebral collapse", "acute compression"). Medical records that contained less definitive language for a vertebral fracture were adjudicated by an International Society for Clinical Densitometry-trained expert on the reading and interpretation of vertebral fractures, who

was blinded to exposure status. We coded participants as "probable" cases when a diagnosis of vertebral fracture was less certain (e.g. "mild compression deformity", "stable" or "chronic" "compression deformity"); "probable" cases were not included in our analysis. This approach has been used in previous studies of clinical vertebral fracture in NHS.[132]

We included vertebral fractures that were related to low or moderate trauma (e.g. tripping, slipping, falling from the height of a chair or lower). We excluded vertebral fractures due to high trauma (e.g., fall from a ladder, fall down a flight of stairs), motor vehicle accidents, bicycle accidents, or horseback riding accidents. We also excluded cases of cervical or sacral vertebral fracture. We included in the analysis only cases of vertebral fracture that were confirmed by medical record review and diagnosed during the 12 years of follow-up between 2002 and May 31, 2014.

Statistical Analyses

The study design was prospective; information on BMI, waist circumference and the covariates of interest was collected before the diagnosis of clinical vertebral fracture. For each participant, person-time of follow-up was counted from the date the 2002 questionnaire was returned to: 1) the date the vertebral fracture was diagnosed, 2) death, or 3) May 31, 2014, whichever occurred first. Participants were censored if they developed a hip fracture or any cancer (other than non-melanoma skin cancer) during the follow-up period. Information on BMI and other covariates was collected from the baseline questionnaire and updated on subsequent questionnaires. We allocated person-time of follow-up according to the updated exposure status at the start of each follow-up period. Period-specific categories of BMI and other covariates were used in the analysis. We used Cox proportional-hazards models to simultaneously adjust for

potential confounders as listed above. All P values are two-tailed. Data analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC). When BMI and waist circumference are included in the same multivariable model, the interpretation of BMI is altered and reflects lean body mass to a greater degree,[133] and the interpretation of waist circumference reflects abdominal adiposity.

RESULTS

Body Mass Index and Vertebral Fracture Risk

During 597,363 person-years of follow-up over a 12-year period, there were 536 confirmed cases of incident clinical vertebral fracture. The characteristics of the cohort according to BMI in 2002 are shown in **Table 3.1**. For our analyses, however, the updated information on BMI and covariates was used for each time period. In 2002, the median BMI was 26.0 kg/m^2. By 2012, the median BMI was 25.7 kg/m^2. The Spearman correlation coefficient for the association between BMI and waist circumference was 0.71 ($p<0.0001$) in 2002 and decreased to 0.62 ($p<0.0001$) by 2010. In 2002, women with higher BMI were slightly younger, less physically active, less likely to be taking a calcium supplement, had lower total vitamin D intake, higher protein intake, lower alcohol intake, and were more likely to have a history of falls, diabetes, or hypertension. They were less likely to report a history of osteoporosis or to be taking bisphosphonates. In 2002, 99.3% of the women were postmenopausal.

After adjusting for age, compared with women with BMI 21.0-24.9 kg/m^2, higher BMI was not significantly associated with risk of clinical vertebral fracture (RR 0.84, 95% CI 0.63, 1.12) (**Table 3.2**). After multivariable adjustment, compared with women with BMI 21.0-24.9 kg/m^2, the relative risk of clinical vertebral fracture was 0.84 (95% CI 0.61, 1.14) for women

Table 3.1. Age-Adjusted Baseline Characteristics of Participants in 2002 by Body Mass Index (kg/m²)

	<21.0 (n=4,963)	21.0 - 24.9 (n=17,592)	25.0 - 29.9 (n=19,490)	30.0 - 31.9 (n=4,591)	≥ 32.0 (n=8,304)
Age, years*	67.0 (6.8)	66.3 (6.6)	66.0 (6.5)	65.6 (6.4)	64.4 (6.0)
Body Mass Index (kg/m²)	19.8 (1.1)	23.2 (1.1)	27.3 (1.4)	30.9 (0.6)	36.2 (4.1)
Waist Circumference (cm) †	72.7 (7.7)	80.0 (8.9)	89.2 (10.0)	97.0 (10.3)	104.5 (12.9)
Physical Activity (METS/week) †	25.6 (27.1)	22.0 (23.3)	17.7 (20.5)	13.8 (20.2)	11.4 (18.7)
Dietary Calcium (mg/day) #	861 (331)	860 (323)	855 (316)	847 (308)	846 (320)
Calcium Supplement (mg/day)	715 (522)	680 (527)	605 (517)	563 (516)	510 (517)
Calcium Supplement Use (yes/no), %	84.6	83.1	79.3	77.6	72.1
Total Vitamin D Intake (IU/day) #	609 (353)	587 (338)	555 (329)	540 (329)	516 (321)
Total Protein Intake (g/day) #	67.5 (12.9)	69.1 (12.7)	70.6 (12.9)	71.5 (13.2)	72.5 (13.4)
Animal Protein Intake (g/day) #	42.8 (13.7)	45.0 (13.3)	47.0 (13.4)	48.2 (13.8)	49.4 (14.0)
Total Vitamin A Intake (mcg/day) #	2,066 (1,539)	1,961 (1,410)	1,891 (1,347)	1,873 (1,358)	1,821 (1,277)
Alcohol Intake (g/day)	8.1 (12.5)	7.7 (11.6)	5.9 (10.4)	4.5 (8.9)	3.2 (7.6)
Smoking status					
Never smoker, %	48.5	46.5	46.4	48.0	47.4
Past smoker, %	40.4	45.5	46.8	46.2	48.1
Current smoker, %	11.1	8.0	6.8	5.8	4.5
History of Falls, %	6.5	7.3	8.4	9.1	10.5
History of Diabetes, %	2.7	3.7	7.6	11.9	19.5
History of Hypertension, %	29.2	39.7	52.6	63.5	71.3
Self-Reported Osteoporosis, %	23.2	16.7	12.2	9.7	8.3
Postmenopausal Hormone Use, %	44.7	44.7	40.7	38.2	33.7
Current Bisphosphonate Use, %	19.4	14.8	9.4	6.3	5.0

* Value is not age adjusted.
† Waist circumference, physical activity and history of falls were not asked about in 2002 so data are from the 2000 questionnaire.
# Energy adjusted.

Table 3.2. Body Mass Index and Risk of Clinical Vertebral Fracture

	Body Mass Index (kg/m^2)					
	<21.0	21.0 - 24.9	25.0 - 29.9	30.0 – 31.9	≥ 32.0	P for trend
Cases of Vertebral Fracture (n)	83	192	161	39	61	
Person-years	61,456	190,054	208,456	48,856	88,541	
Age-adjusted Relative Risk (95% CI)	1.15 (0.89, 1.48)	1.0	0.81 (0.66, 1.00)	0.88 (0.62, 1.24)	0.84 (0.63, 1.12)	0.02
Multivariate Relative Risk (95% CI) †	1.08 (0.84, 1.41)	1.0	0.84 (0.68, 1.04)	0.90 (0.63, 1.29)	0.84 (0.61, 1.14)	0.09
Multivariate Relative Risk + Waist Circumference (95% CI)	1.22 (0.93, 1.60)	1.0	0.77 (0.61, 0.96)	0.78 (0.54, 1.13)	0.70 (0.49, 0.98)	0.004

Body mass index was updated throughout the analysis period (2002-2014).

† The multivariate model includes race, physical activity, history of falls, smoking status, alcohol intake, supplemental calcium intake, quintiles of intake of dietary calcium, total vitamin D, vitamin A, and total protein, history of diabetes, hypertension, self-reported osteoporosis, postmenopausal hormone use, thiazide and furosemide use, proton pump inhibitor use, and recent physical exam.

with BMI ≥32 kg/m^2. After further adjusting for waist circumference, the relative risk of clinical vertebral fracture was 0.70 (95%CI 0.49, 0.98) for women with BMI≥32 kg/m^2 compared with women with BMI 21.0-24.9 kg/m^2. The results were similar when we examined the association with baseline BMI (without updating of BMI) and BMI updated every two years.

Waist Circumference and Vertebral Fracture Risk

The characteristics of the cohort according to waist circumference in 2002 are shown in **Table 3.3**. The median baseline waist circumference was 86.4 cm. In 2002, women with larger waist circumference were slightly younger, less physically active, less likely to be taking a calcium supplement, had lower total vitamin D intake, higher protein intake, lower alcohol intake, and were more likely to have a history of falls, diabetes, or hypertension. They were also less likely to report a history of osteoporosis or to be taking bisphosphonates.

After adjusting for age, compared with women with waist circumference < 71.0 cm, women with waist circumference ≥ 108.0 cm had an increased risk of clinical vertebral fracture (RR 1.72 , 95% CI 1.05, 2.80) (**Table 3.4**). After multivariable adjustment, compared with women with waist circumference < 71.0 cm, the relative risk was 1.77 (95%CI 1.06, 2.93) for women with waist circumference ≥108.0 cm. After further adjusting for BMI, the relative risk of clinical vertebral fracture was 2.50 (95%CI 1.44, 4.34) for women with waist circumference ≥108.0 cm, compared with < 71.0 cm.

Interaction Analyses

We examined whether the association between BMI and vertebral fracture risk varied by waist circumference, and whether the association between waist circumference and vertebral

Table 3.3. Age-Adjusted Baseline Characteristics of Participants in 2002 by Waist Circumference (cm)

	<71.0 (n=3,449)	71.0 – 79.9 (n=10,920)	80.0 – 88.9 (n=12,558)	89.0 – 98.9 (n=7,435)	99.0 – 107.9 (n=5,439)	≥108.0 (n=2,984)
Age, years[*]	64.8 (6.4)	65.5 (6.5)	66.5 (6.6)	67.0 (6.6)	67.2 (6.6)	66.3 (6.6)
Body Mass Index (kg/m²)	21.3 (2.6)	23.3 (2.7)	25.8 (3.3)	28.2 (3.9)	30.6 (4.5)	34.7 (5.9)
Waist Circumference (cm)[†]	66.3 (2.8)	75.2 (2.8)	84.9 (2.8)	93.7 (2.1)	102.4 (2.8)	116.3 (7.9)
Physical Activity (METS/week)[†]	27.4 (28.4)	23.7 (24.3)	19.2 (21.0)	16.6 (19.2)	13.8 (20.4)	11.2 (16.8)
Dietary Calcium (mg/day)[#]	871 (339)	865 (321)	861 (321)	847 (309)	853 (315)	849 (324)
Calcium Supplement (mg/day)	723 (529)	690 (523)	642 (519)	600 (520)	587 (519)	553 (534)
Calcium Supplement Use (yes/no), %	84.2	84.2	81.7	79.1	78.9	75.5
Total Vitamin D Intake (IU/day)[#]	607 (360)	590 (334)	575 (332)	554 (326)	554 (324)	540 (335)
Total Protein Intake (g/day)[#]	68.6 (13.3)	69.1 (12.5)	69.7 (12.7)	70.4 (13.0)	70.9 (13.0)	72.4 (14.0)
Animal Protein Intake (g/day)[#]	43.9 (14.2)	44.8 (13.2)	46.0 (13.3)	46.7 (13.5)	47.6 (13.5)	49.1 (14.5)
Total Vitamin A Intake (mcg/day)[#]	2,089 (1,565)	2,006 (1,441)	1,935 (1,392)	1,884 (1,364)	1,895 (1,302)	1,896 (1,387)
Alcohol Intake (g/day)	7.4 (10.9)	7.5 (11.5)	6.7 (10.8)	5.7 (10.3)	4.8 (9.9)	3.7 (8.9)
Smoking status						
Never smoker, %	49.1	47.5	47.5	47.2	46.0	46.1
Past smoker, %	41.8	45.3	45.4	46.8	48.4	49.1
Current smoker, %	9.1	7.2	7.1	6.0	5.6	4.9
History of Falls, %	6.4	7.2	8.2	8.4	9.8	11.1
History of Diabetes, %	2.2	2.9	5.2	8.6	12.9	21.5
History of Hypertension, %	29.9	37.6	47.7	56.4	63.6	70.6
Self-Reported Osteoporosis, %	20.0	17.6	14.6	12.9	12.2	10.1
Postmenopausal Hormone Use, %	45.9	46.3	42.6	41.4	38.4	34.6
Current Bisphosphonate Use, %	16.8	16.1	12.5	9.2	8.9	6.6

[*] Value is not age adjusted
[†] Waist circumference, physical activity and history of falls were not asked about in 2002 so data are from the 2000 questionnaire.
[#] Energy adjusted.

Table 3.4. Waist Circumference* and Risk of Clinical Vertebral Fracture

	Waist Circumference (cm)						
	<71.0	71.0 – 79.9	80.0 - 88.9	89.0 – 98.9	99.0 – 107.9	≥108.0	P for trend
Cases of Vertebral Fracture (n)	26	113	129	95	64	42	
Person-years	38,298	120,407	137,390	81,287	58,819	32,485	
Age-adjusted Relative Risk (95% CI)	1.0	1.32 (0.86, 2.02)	1.24 (0.81, 1.89)	1.47 (0.95, 2.28)	1.37 (0.87, 2.17)	1.72 (1.05, 2.80)	0.03
Multivariate Relative Risk (95% CI) †	1.0	1.33 (0.87, 2.04)	1.30 (0.85, 1.98)	1.59 (1.02, 2.46)	1.44 (0.90, 2.29)	1.77 (1.06, 2.93)	0.02
Multivariate Relative Risk + Body Mass Index (95% CI)	1.0	1.45 (0.94, 2.23)	1.56 (1.00, 2.42)	2.04 (1.28, 3.26)	1.94 (1.17, 3.19)	2.50 (1.44, 4.34)	<0.001

* Waist circumference was assessed in 2000. Waist circumference data from 1996 was used for participants who did not provide waist circumference data in 2000.

† The multivariate model includes race, physical activity, history of falls, smoking status, alcohol intake, supplemental calcium intake, quintiles of intake of dietary calcium, total vitamin D, vitamin A, and total protein, history of diabetes, hypertension, self-reported osteoporosis, postmenopausal hormone use, thiazide and furosemide use, proton pump inhibitor use, and recent physical exam.

fracture varied by BMI. There was no statistically significant interaction between BMI and waist circumference (p for interaction > 0.44). We examined whether the associations with vertebral fracture for BMI and waist circumference varied by age (above and below age 70 years), history of osteoporosis, or bisphosphonate use. There was no statistically significant interaction between BMI and age (p for interaction > 0.97), history of osteoporosis (p for interaction > 0.25), or bisphosphonate use (p for interaction > 0.27). There was no statistically significant interaction between waist circumference and age (p for interaction > 0.65), history of osteoporosis (p for interaction > 0.33), or bisphosphonate use (p for interaction > 0.71).

We also performed a mediation analysis to quantify the proportion of the association between BMI, waist circumference and risk of vertebral fracture that could be explained by osteoporosis as an intermediate condition. Osteoporosis explained 39.6% (11.5% - 76.7%, $p < 0.0001$) of the association between BMI and vertebral fracture, and 33.3% (1.3% - 94.9%, $p < 0.0001$) of the association between waist circumference and vertebral fracture.

DISCUSSION

In this prospective study of over 54,000 women, larger waist circumference was independently associated with higher risk of clinical vertebral fracture. Higher lean body mass was also associated with lower risk of vertebral fracture. When BMI and waist circumference are included in the same model, the interpretation of BMI is altered and reflects lean body mass to a greater degree, whereas waist circumference reflects abdominal adiposity. These findings suggest that the distribution of fat is an important predictor of vertebral fracture. Our study has several strengths that distinguish it from other reports, including the large number of incident

clinical vertebral fracture events that were confirmed by medical record review, assessment of waist circumference, and repeated assessment of BMI over time.

The inverse association between lean body mass and risk of vertebral fracture was only revealed after the addition of waist circumference to the multivariable model. In our recent meta-analysis on the association between BMI and vertebral fracture, we did not find a statistically significant association between BMI and vertebral fracture in women (RR = 0.98, 95%CI 0.81-1.20, n=79,512 participants, n=1,296 vertebral fracture events).[125] However, none of the prior studies examining the association between BMI and vertebral fracture risk included waist circumference in their models. There was also substantial heterogeneity across the previous studies in women (I^2=90.1%, p<0.001), which could have been due to adjustment for BMD, as well as methodological differences, including different definitions of vertebral fracture as an outcome, paucity of vertebral fracture cases, measurement of obesity and body mass index, lack of inclusion or updating of important covariates during study follow-up, length of study follow-up, and choice of study population.

Moreover, BMI alone in a model may also be a less reliable marker of body fatness in older adults compared with younger populations.[134,135] The components of BMI, fat mass and lean body mass, vary by sex and age. For a given BMI, women have a higher percentage of body fat than men.[136] BMI as a measure of adiposity has limitations in older adults because aging is associated with changes in body composition, including loss of muscle mass and increase in fat mass.[136] Moreover, aging is also associated with changes in the distribution of fat. Therefore, the collection of information on body fat distribution, such as waist circumference, a measure of central adiposity, can provide additional insights on body fat distribution in older adults.[133] Our study underscores the importance of assessing BMI as well as waist circumference when

studying the association between adiposity and vertebral fracture risk. None of the prior studies examining the association between BMI and vertebral fracture risk included waist circumference in their models or studied the independent association between waist circumference and vertebral fracture risk.

Our evolving understanding of the complex pathophysiology underlying the dynamic relation between fat, bone, and fracture risk is also challenging the notion that obesity is protective against fractures. Different mechanisms have been suggested to explain the relation between fat mass, bone metabolism, and resulting fracture risk.[137,138] Interestingly, bone and fat cells are derived from the same progenitor cells and adipose tissue is recognized as a metabolically active endocrine organ which can exert complex effects on bone mass, strength and quality. Adipose tissue can exert hormonal effects on bone through the increased production of adipokines, such as adiponectin, a hormone linking bone and fat metabolism, as well as proinflammatory cytokines, all of which can promote bone resorption and inhibit bone formation.[137,138] Higher adipokine levels were associated with lower bone mineral density[139] and increased risk of incident fractures in men.[140] There have been no studies to date on the association between adipokine levels and fracture risk in women. Increased production of insulin-like growth factor (IGF-1) may also disrupt bone homeostasis, thereby playing a role in mediating the harmful effects of visceral adiposity on bone health.[141] The relation between obesity and vertebral fracture risk may also be related to impaired biomechanical factors. Obesity, in particular abdominal obesity, could exert a negative effect on the bone's mechanical properties[142] as well as place an undue compressive load burden on the spine,[143] thereby predisposing the vertebrae to fracture.

The site of fat tissue may also exert differing effects on bone (e.g. visceral fat versus subcutaneous fat).[144] In the Nurses' Health Study, abdominal adiposity was associated with increased hip fracture risk in women with low physical activity.[122] Both total and visceral adiposity were associated with higher prevalent vertebral fracture in women.[145] Several studies have found that higher abdominal fat is associated with lower bone mineral density.[141,146]

In addition to the negative effects on bone as measured by bone mineral density testing, visceral abdominal fat could also have detrimental effects on bone "quality" (e.g. bone microarchitecture, cortical porosity, bone matrix, mineralization, collagen deposition, geometry, and three-dimensional connectivity of bone) that is independent of bone mineral density. This negative effect of adipose tissue on bone quality may be more local or paracrine, rather than systemic.[147] A recent study of healthy pre-menopausal women examined the association between trunk fat, as measured by dual-energy x-ray absorptiometry and helical quantitative computed tomography, and bone quality, as measured through transiliac crest bone biopsies, and found that women in the highest tertile of trunk fat had inferior bone quality, as evidenced by lower trabecular bone volume fraction, fewer and thinner trabeculae, lower trabecular stiffness, and higher cortical porosity, as well as decreased bone formation.[121]

We also found that BMI, interpreted as greater lean body mass when in the same model as waist circumference, was independently associated with lower risk of vertebral fracture. Studies suggest that skeletal mass is positively associated with bone mineral density[148,149] as well as trabecular bone geometry and microarchitecture.[150] Aging is associated with loss of both lean muscle mass (sarcopenia) and muscle function (dynapenia).[151] Loss of trunk musculature can affect vertebral strength as well as place undue compressive loading on vertebra.[114,152] Therefore, sarcopenia[153] or sarcopenic obesity[154,155] could potentially be associated with higher fracture risk.

However, further research is needed not only on the role of overall lean body mass, but specifically the role of trunk muscle strength and function and their association with vertebral fracture risk.

There are several limitations to our study that merit discussion. Given the observational design, there is the possibility of residual confounding. For example, although we did have information on self-reported osteoporosis, we did not have data on actual bone mineral density or morphometric fracture assessment. Our definition of clinical vertebral fracture was based on medical record review; some participants who self-reported a vertebral fracture did not have permission or we were not able to obtain their medical records to make a definitive diagnosis of vertebral fracture. We recognize that the observed incidence rate therefore is lower because of our method of case ascertainment, but the associations should be valid as the ability to obtain records was not related to the exposures being studied. Finally, since our study population was female and almost entirely white, our findings are not necessarily generalizable to men or other races.

In this large prospective cohort study, larger waist circumference, a measure of abdominal adiposity, was associated with higher risk of clinical vertebral fracture in women. Moreover, higher lean body mass was associated with lower risk of clinical vertebral fracture. These results suggest that avoiding central adiposity as well as maintaining muscle strength may potentially reduce the risk of vertebral fracture in older women. Further research is warranted on body composition, particularly central adiposity, lean body mass, and vertebral fracture risk.

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