



## Thiazide use and skeletal microstructure: Results from a multi-ethnic study

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### ABSTRACT

**Background:** Thiazide diuretics, a commonly used class of anti-hypertensives, have been associated with increased areal bone mineral density (aBMD). Data regarding effects on fracture are conflicting and no information is available regarding effects on skeletal microstructure and mechanical competence.

**Methods:** We compared skeletal microstructure, volumetric BMD (vBMD), stiffness and prevalent fractures in current thiazide diuretic users and non-users from a population-based multiethnic cohort of elderly adults age  $\geq 65$  years ( $N = 599$ ) with high resolution peripheral quantitative computed tomography (HR-pQCT) and micro-finite element analysis.

**Results:** Female current thiazide diuretic users had higher weight and BMI and were more likely to be non-Caucasian compared to non-users. There were no differences in age, historical fractures or falls between female users and non-users. Female thiazide users tended to have lower calcium and vitamin d intake compared to non-users. After adjusting for age, weight, race and other covariates, 1/3-radius mean aBMD by dual energy x-ray absorptiometry (DXA) was 3.2% ( $p = 0.03$ ) higher in female users vs. non-users. By HRpQCT, adjusted mean cortical vBMD was 2.4% ( $p = 0.03$ ) higher at the radius in female users vs. non-users, but there was no difference in stiffness. DXA results were similar in the subset of Black females. There was no difference in any adjusted aBMD or cortical skeletal parameters by DXA or HRpQCT respectively in males.

**Conclusions:** Thiazide use was associated with a modestly higher aBMD at the predominantly cortical 1/3-radius site and radial cortical vBMD by HRpQCT in females. The effect on cortical bone may offer skeletal benefits in women taking thiazides for other indications such as hypertension, hypercalciuria or recurrent nephrolithiasis.

### 1. Introduction

Thiazide diuretics are one of the most commonly used initial monotherapies for mild hypertension (Griebeler et al., 2016; Anon, 2002). Thiazides increase renal tubular reabsorption of calcium, decrease urinary calcium excretion and favor positive calcium balance which may result in higher areal bone mineral density (aBMD) or a decrease in age-related bone loss (Lamberg and Kuhlback, 1959; Heshmati et al., 1998; Wasnich et al., 1983, 1986; Cauley et al., 1993; Morton et al., 1994). In a large observational study, female thiazide users had significantly higher bone mass than women who had never used thiazide diuretics at the distal radius and calcaneus (Cauley et al., 1993). Limited randomized clinical trial (RCT) data are available. One such study suggests a potential preferential effect of hydrochlorothiazide (HCTZ) upon aBMD at skeletal sites enriched with cortical versus trabecular bone, including the arm and leg, but not at other sites such as the total hip or spine (Reid

et al., 2000). A larger, three-year RCT also found a beneficial effect at the hip and spine, while the forearm was not evaluated (LaCroix et al., 2000). Not all RCTs have shown a benefit of thiazides on aBMD (Cheng et al., 2018).

There is conflicting observational data regarding the effect of thiazides on fracture risk (Paik et al., 2016; Aung and Htay, 2011; Charkos et al., 2019; Yang et al., 2018; Rejnmark et al., 2005; LaCroix et al., 1990). Some, but not all, studies suggest a reduction in risk of hip fracture with thiazide therapy (Aung and Htay, 2011; Charkos et al., 2019; Yang et al., 2018). RCT data evaluating the effects of thiazides on fractures are limited. The Analysis of Medicare claims data in participants from the Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) study in which participants were randomized to chlorthalidone or other types of antihypertensives, showed that participants randomized to chlorthalidone had a lower risk of hip and pelvic fracture (Puttnam et al., 2017).

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Other studies suggest a possible negative effect of thiazides on fracture risk by inducing hyponatremia or falls leading to fractures (Yang et al., 2018). Thus, it remains unclear whether thiazides have a beneficial effect on skeletal health or may affect other aspects of bone quality in addition to BMD. High resolution peripheral quantitative computed tomography (HR-pQCT) can provide non-invasive measurements of volumetric BMD (vBMD) and skeletal microstructure and stiffness, but this modality has not been used to evaluate the effects of thiazides.

Many patients have become reluctant to use approved therapies for osteoporosis or its prevention given the potential for serious, albeit rare, side effects (Khosla et al., 2007; Shane et al., 2014; Khosla and Shane, 2016). Identifying safe medications that have beneficial skeletal effects is, therefore, clinically important. HCTZ and other thiazides have been proposed as one such preventative therapy for post-menopausal osteoporosis by slowing the rate of bone loss in older adults (Wasnich et al., 1983). In order to delineate potential mechanisms of thiazides on bone health and effects on the cortical versus the trabecular compartments of the skeleton, we aimed to compare vBMD, skeletal microstructure, bone mechanical competence, falls and prevalent fractures in thiazide users and non-users. We hypothesized that thiazide use would be associated with a beneficial effect on skeletal microstructure, particularly in the cortical compartment.

## 2. Methods

### 2.1. Design

This analysis cross-sectionally compared skeletal health in elderly female and male current thiazide users compared to those not currently taking thiazides (non-users) who were participating in a population-based longitudinal cohort study of aging. The Columbia University Irving Medical Center (CUIMC) Institutional Review Board approved this study and all participants provided written informed consent.

### 2.2. Study population

The Washington Heights Hamilton Heights Inwood Community Aging Project (WHICAP) is an NIH-funded community-based prospective cohort study of aging among elderly, African-American, Caribbean Hispanic, and Caucasian urban-dwelling ambulatory residents (age > 65) living in Northern Manhattan. The design and recruitment for the study have previously been reported (Tang et al., 2001; Agarwal et al., 2020). Briefly, a probability sample of Medicare recipients, age  $\geq 65$  without dementia from 3 zip codes in Northern Manhattan was invited to participate. The original cohort was recruited beginning in 1992 and enriched with further recruitment subsequently. Returning participants ( $N = 1949$ ) who were able to consent were invited to this ancillary study assessing bone health. Those who agreed to participate underwent a one-time evaluation with DXA, HR-pQCT, and a questionnaire regarding their health and fracture history. This cross-sectional analysis includes those who were enrolled ( $n = 599$ ) to the ancillary study and underwent imaging between 1/2019 and 11/2021: 401 women (299 thiazide non-users and 102 thiazide users) and 198 men (168 thiazide non-users and 30 thiazide users). The demographics of the sample included in this analysis are similar to those that declined participation though patients who declined participation were slightly older ( $80.7 \pm 7.8$  vs.  $76.8 \pm 5.9$ ,  $p < 0.05$ ) than those who agreed to participate. Participants were not excluded on the basis of conditions or medications affecting bone health to maintain the integrity of the population-based sampling.

### 2.3. Dual energy X-ray absorptiometry (DXA)

#### 2.3.1. Areal BMD

Areal BMD was measured with a QDR Discovery or Horizon

instrument (Hologic Inc., Waltham, MA). BMD measurements were obtained at the lumbar spine (LS; L1–L4), femoral neck (FN), total hip (TH), and 1/3 radius. T-scores were obtained using the manufacturer's Caucasian reference norms. Participants were scanned at all three skeletal sites unless hardware precluded the analysis of BMD at a given site(s), in which case the site(s) with hardware were omitted. We excluded vertebra with hardware or other artifacts from the analysis of aBMD at the spine. In vivo precision, determined according to the standard method at this facility, is 1.28% at the LS, 1.36% at the hip, and 0.70% for the distal radius (1/3 site) (Bonnick et al., 2001).

#### 2.3.2. Spine trabecular bone score (TBS) and lateral vertebral fracture assessment (VFA)

Spine trabecular bone score (TBS) and lateral vertebral fracture assessment (VFA) was calculated from subjects' spine DXA image using TBS iNsight software as previously described (version 3.0.3.0; Medimaps, Geneva, Switzerland) (Hans et al., 2011) if the spine image was evaluable. Lateral VFA was acquired from T4 to L5. Participants were categorized as having VF(s) in the imaged spine based on an International Society for Clinical Densitometry (ISCD)-certified densitometrist's reading of the interpretable image using the Genant semi-quantitative method: mild, moderate and severe compression fractures were defined as a 20–25%, 26–40% or >40% reduction in vertebral height, respectively (Genant et al., 1993). The Genant visual semi-quantitative method is the current recommended clinical technique for diagnosing vertebral fracture with VFA.

### 2.4. HR-pQCT

HR-pQCT was performed with an XtremeCT II scanner (Scanco Medical, Brüttisellen, Switzerland) which uses a microfocus x-ray source (68 kVp voltage, 900  $\mu$ A current, 43 sec integration time) scanning a region 10.2 mm long along the axis of the long bone resulting in VOI of 60.7  $\mu$ m isotropic voxel size. The non-dominant distal radius and tibia were scanned unless there was a contraindication (prior fracture or metal implant), in which case the contralateral limb was scanned. The region of interest was defined on a 2-D scout view by placing a reference line at the endplate: proximal endplate for radius and distal endplate for tibia. Images were acquired using a relative offset from the reference line; radius scans at 4% of limb length and tibia at 7.3%. We also scanned the tibia at a more proximal diaphyseal region at 30%, which is composed almost entirely of cortical bone. A single highly trained operator acquired and analyzed all scans. Scans were scored for motion on a scale of 1–5 and scans with a motion score > 3 were excluded from the analysis. We used the manufacturer's standard method to filter and binarize the HR-pQCT images. An automated segmentation algorithm was used to segment the cortical and trabecular regions. We assessed standard HR-pQCT morphological microstructure outcomes, including area; density - total, trabecular (Tb) and cortical (Ct) volumetric BMD (vBMD); microstructure - trabecular number (Tb.N), thickness (Tb.Th), and separation (Tb.Sp), cortical thickness (Ct.Th), and cortical porosity (Ct.Po) (Bouxsein et al., 2010). In vivo short-term reproducibility (CV) for HR-pQCT measures at our center is between 0 and 5% for all measures except Ct.Po.

### 2.5. FEA

Bone strength (stiffness) was estimated from the HR-pQCT images using micro-finite element analysis ( $\mu$ FEA) based on a voxel conversion approach. We simulated a uniaxial compression on each radius and tibia model up to 1% strain using a homogeneous Young's modulus of 10 GPa and Poisson's ratio of 0.3. We used a  $\mu$ FEA solver provided by the manufacturer (Scanco Medical FE-software v1.13, Scanco Medical, Brüttisellen, Switzerland) to solve the models. We estimated whole bone stiffness (N/mm).

## 2.6. Questionnaire and clinical evaluation

Information regarding past medical history, lifestyle, and medications was collected by interview/questionnaire. Thiazide use was assessed by reviewing participants' current prescription medications. A current thiazide user was defined as a participant currently taking a thiazide diuretic based on the study staff's review of medication and confirmation of use by the participant. Fall recall was assessed by questionnaire by asking participants if they had fallen in the last 12 months and the number of falls they sustained. Daily dietary calcium and vitamin D intake was assessed with a validated standardized food frequency questionnaire as previously described (Walker et al., 2009). Total daily calcium and vitamin D intake from diet and supplements is reported. Physical activity was assessed with the physical activity scale for the elderly (PASE) (Washburn et al., 1993). Weight and height were measured by balance beam and a wall-mounted, calibrated Harpenden stadiometer, respectively.

## 2.7. Statistics

Descriptive statistics were expressed as means and standard deviations or absolute (n) and relative (%) frequency. Between-group differences in demographic and skeletal indices were evaluated with Student's *t*-test or Fisher's exact test as appropriate. Values were expressed as mean  $\pm$  standard deviation or percentages. Adjusted analyses were conducted with general linear models (GLM) or logistic regression. Confounders were selected for adjustment in sequential models on the basis of prior associations, known biological mechanisms, and/or between-group differences between thiazide users and non-users. Model "a" included age and weight; model "b" included age, weight and race; and model "c" included age, weight, race, presence of chronic obstructive pulmonary disease (COPD), alcohol use,  $\beta$ -blocker use and current osteoporosis therapy. In the subset of Black women, ethnicity was included rather than race. All analyses were performed using SAS Version 9.4 (Cary, NC). A two-tailed *p*-value  $< 0.05$  was considered statistically significant.

## 3. Results

As shown in Table 1, at baseline female thiazide users had 11.3% higher weight ( $p < 0.0001$ ) and 11.8% higher BMI ( $p < 0.0001$ ) compared to non-users. There was a tendency for calcium and vitamin D intake to be lower in thiazide users than non-users. Thiazide users were more likely to be non-Caucasian (82.4 vs. 68.9%,  $p = 0.01$ ) and Hispanic. They also tended to have a higher frequency of COPD and  $\beta$ -blocker use, but less alcohol consumption. There was no significant difference in age, historical fracture, falls, years menopausal, physical activity score, nor frequency of current smoking, diabetes, cancer, nephrolithiasis, kidney disease, liver disease, current steroid use or osteoporosis therapy.

Unadjusted aBMD by DXA (Table 2) ranged from 5.3–8.8% higher at the femoral neck, total hip and 1/3-radius in female thiazide users compared to non-users, but did not differ at the lumbar spine. After adjusting for age and weight or age, weight and race, or age, weight, race and other covariates, aBMD remained higher at the 1/3-radius in female thiazide-users. aBMD at the 1/3-radius was 2.4% ( $p < 0.05$ ) higher in the fully adjusted model (Table 2) in thiazide users versus non-users. There were no between-group differences in TBS before or after adjustment for covariates. Prevalent compression fractures based on VFA were more common in thiazide users vs. non-users (20.9% vs. 9.3%) before ( $p = 0.006$ ) and after (all  $p < 0.05$ ) adjustment for covariates. Among women with vertebral compression fractures, there was no difference in the number of compression fractures between thiazide users and non-users (Table 2).

As shown in Table 3, before adjustment for covariates, cortical vBMD and stiffness by HR-pQCT were 3.2% and 8.2% higher respectively in

**Table 1**

Comparison of demographic, lifestyle factors, co-morbidities and concomitant medications by thiazide status in women.

	Non-users (N = 299)	Thiazide users (N = 102)	p-Value
Age (years)	76.9 $\pm$ 5.8	76.7 $\pm$ 6.1	0.73
Weight (pounds)	151.9 $\pm$ 32.3	169.1 $\pm$ 37.7	<0.0001
Height (inches)	61.9 $\pm$ 3.1	61.6 $\pm$ 2.7	0.43
BMI (kg/m <sup>2</sup> )	27.9 $\pm$ 5.5	31.2 $\pm$ 6.1	<0.0001
Race (%)			
White	31.10	17.65	0.06
Black	31.10	39.22	
Asian	1.34	0	
American Indian/Alaska Native	0.33	0	
Native Hawaiian/Pacific Islander	0.33	0	
Mixed	35.79	43.14	
Non-Caucasian (%)	68.9	82.4	0.01
Ethnicity (% Hispanic)	45.2	57.8	0.03
Historical fracture (%)	48.2	39.2	0.28
Falls (%)	28.1	34.3	0.26
Historical fall number in last year	1.8 $\pm$ 2.3	1.7 $\pm$ 1.0	0.78
Historical fracture number	1.7 $\pm$ 1.1	1.6 $\pm$ 1.0	0.46
Years menopausal	28.8 $\pm$ 10.0	29.6 $\pm$ 11.3	0.48
Lifestyle factors			
Daily calcium intake (mg/day)	1281 $\pm$ 603	1104 $\pm$ 578	0.01
Vitamin D intake (IU/day)	1967 $\pm$ 3491	1451 $\pm$ 1918	0.06
Physical activity score	82.8 $\pm$ 44.8	81.5 $\pm$ 43.6	0.80
Current smoker (%)	3.3	4.9	0.68
Alcohol use (drinks/week)	1.6 $\pm$ 3.8	0.9 $\pm$ 2.6	0.03
Co-morbidities			
Diabetes (%)	25.8%	33.3%	0.16
Cancer (%)	3.7	2.0	0.10
COPD (%)	23.8	34.3	0.05
Current nephrolithiasis (%)	1.7	2.9	0.46
Kidney disease (%)	3.7	3.9	1.0
Liver disease (%)	6.0	2.9	0.31
Medications			
Current steroid use (%)	0.7	1.0	0.42
Current $\beta$ -blocker use (%)	21.5	34.0	0.03
Current osteoporosis therapy (%)	10.4	8.8	0.78

BMI = body mass index; Values presented as mean  $\pm$  SD or percentages.

female thiazide users vs. non-users at the radius (both  $p < 0.05$ ). After adjustment for covariates, the difference in cortical vBMD at the radius in women remained significant in all of the models (Table 3). After adjustment for age, weight, race, COPD, alcohol use, and medications, adjusted mean cortical vBMD was 2.4% higher in thiazide users ( $p = 0.03$ ). In contrast, the difference in stiffness at the radius was attenuated and no longer significant. There were no differences in other density or microstructural features at the radius. At the 7.3% tibial site, unadjusted cortical density, thickness and stiffness were higher in female thiazide users vs. non-users (all  $p < 0.05$ ), but after adjustment for covariates the differences were attenuated and not significant in any model. There were no differences in any trabecular indices at the 7.3% tibia in fully adjusted models. There were also no differences at the cortical 30% tibia site after adjustment for covariates.

In the subset of Black women, thiazide users ( $N = 40$ ) had higher aBMD at the 1/3-radius compared to non-users ( $n = 40$ ) after adjustment for age, weight and ethnicity (mean  $\pm$  SE: 0.672  $\pm$  0.01 vs. 0.640  $\pm$  0.01 g/cm<sup>2</sup>.  $p < 0.05$ ). The difference was of borderline significance when including COPD, alcohol use,  $\beta$ -blockers and current osteoporosis therapy ( $p = 0.07$ ). There were no differences in cortical parameters or stiffness by HR-pQCT (data not shown). Among men, there were no differences in areal BMD, TBS, prevalent vertebral fractures, skeletal microstructure, or stiffness before or after adjustment for covariates (data not shown).

**Table 2**  
Comparison of areal bone mineral Density (aBMD), trabecular bone score (TBS) and vertebral fracture assessment by thiazide status in women.

	Non-users	Current thiazide users	p-Value	Adjusted p-value <sup>a</sup>	Adjusted p-value <sup>b</sup>	Adjusted p-value <sup>c</sup>
<b>aBMD</b>						
LS T-score	-0.7 ± 1.7	-0.4 ± 1.6	0.08	0.85	0.99	0.81
TH T-score	-1.2 ± 1.0	-0.8 ± 1.1	<b>0.001</b>	0.20	0.18	0.30
FN T-score	-1.6 ± 1.0	-1.2 ± 1.1	<b>0.008</b>	0.54	0.48	0.52
1/3 radius T-score	-1.5 ± 1.4	-0.8 ± 1.4	<b>&lt;0.0001</b>	<b>0.02</b>	<b>0.01</b>	<b>0.03</b>
<b>TBS and VFA</b>						
TBS score	1.2193 ± 0.1220	1.1978 ± 0.1259	0.13	0.59	0.66	0.77
Compression fracture on VFA (%)	9.3	20.9	<b>0.006</b>	<b>0.006</b>	<b>0.008</b>	<b>0.04</b>
Vertebral compression fracture number on VFA	1.3 ± 0.8	1.3 ± 0.5	0.77	0.48	0.49	0.52

LS = lumbar spine; TH = total hip; FN = femoral neck; TBS = trabecular bone score; VFA = vertebral fracture assessment. Bolded type indicates significant p-values.

<sup>a</sup> Adjusted for age and weight.

<sup>b</sup> Adjusted for age, weight and race.

<sup>c</sup> Adjusted for age, weight, race, alcohol use, COPD,  $\beta$ -blocker use and osteoporosis therapy.

**Table 3**  
Comparison of volumetric BMD, skeletal microstructure and finite element analysis by thiazide status in women.

	Non-users	Thiazide users	p-Value	Adjusted p-value <sup>a</sup>	Adjusted p-value <sup>b</sup>	Adjusted p-value <sup>c</sup>
<b>4% Radius</b>						
Total vBMD (mgHA/ccm)	229.5 ± 61.0	242.2 ± 55.5	0.07	0.63	0.58	0.75
Tb. vBMD (mgHA/ccm)	126.0 ± 39.8	127.9 ± 37.6	0.64	0.38	0.45	0.36
Ct. vBMD (mgHA/ccm)	786.3 ± 76.4	811.2 ± 67.6	<b>0.005</b>	<b>0.03</b>	<b>0.03</b>	<b>0.03</b>
Tb. Number (1/mm)	1.240 ± 0.244	1.263 ± 0.263	0.43	0.50	0.53	0.44
Tb. Thickness (mm)	0.226 ± 0.022	0.227 ± 0.015	0.62	0.96	0.83	0.86
Tb. Spacing (mm)	0.816 ± 0.251	0.801 ± 0.222	0.60	0.51	0.56	0.48
Ct. Thickness (mm)	0.768 ± 0.389	0.819 ± 0.166	0.08	0.60	0.58	0.10
Ct. Porosity (%)	1.2 ± 1.0	1.1 ± 0.7	0.50	0.37	0.41	0.49
Stiffness (kN/mm)	44.9 ± 14.6	48.6 ± 13.2	<b>0.03</b>	0.71	0.55	0.64
<b>7.3% Radius</b>						
Total vBMD (mgHA/ccm)	229.8 ± 55.3	239.3 ± 50.1	0.13	0.94	0.89	0.98
Tb. vBMD (mgHA/ccm)	143.0 ± 40.6	142.4 ± 38.0	0.88	0.18	0.19	0.25
Ct. vBMD (mgHA/ccm)	773.6 ± 81.2	793.2 ± 83.8	<b>0.04</b>	0.14	0.13	0.25
Tb. Number (1/mm)	1.202 ± 0.244	1.201 ± 0.273	0.96	0.19	0.21	0.30
Tb. Thickness (mm)	0.249 ± 0.020	0.252 ± 0.019	0.24	0.52	0.55	0.49
Tb. Spacing (mm)	0.844 ± 0.250	0.875 ± 0.382	0.45	<b>0.04</b>	<b>0.049</b>	0.08
Ct. Thickness (mm)	1.134 ± 0.297	1.246 ± 0.266	<b>0.0009</b>	0.07	0.06	0.12
Ct. Porosity (%)	4.1 ± 1.6	4.1 ± 1.8	0.92	0.84	0.80	0.69
Stiffness (kN/mm)	142.0 ± 35.5	152.4 ± 32.3	<b>0.01</b>	0.82	0.72	0.68
<b>30% Tibia</b>						
Ct. vBMD (mgHA/ccm)	1002.0 ± 43.9	1002.7 ± 45.4	0.90	0.71	0.72	0.91
Ct. Thickness (mm)	4.2 ± 0.9	4.3 ± 0.7	0.07	0.67	0.65	0.60
Ct. Porosity (%)	1.6 ± 1.2	1.4 ± 1.4	0.40	0.65	0.67	0.77
Stiffness (kN/mm)	216.1 ± 40.1	226.3 ± 36.9	<b>0.03</b>	0.95	0.91	0.69

Values represent mean ± SD or percentages. Tb = trabecular; Ct = cortical; vBMD = volumetric bone mineral density; mgHA = milligrams of hydroxyapatite; ccm = cubic centimeters; mm = millimeters; kN = kilo Newton; bolded type indicates significant values.

<sup>a</sup> Adjusted for age and weight.

<sup>b</sup> Adjusted for age, weight and race.

<sup>c</sup> Adjusted for age, weight, race, alcohol use, COPD,  $\beta$ -blocker use and osteoporosis therapy.

#### 4. Discussion

To our knowledge, this is the first study using HR-pQCT to assess the effects of thiazide use on vBMD, bone microstructure or stiffness. In our analysis, we detected a modest benefit of thiazide use on aBMD at the predominantly cortical 1/3-radius and cortical vBMD at the radius as measured by HR-pQCT in women. Prior studies have only assessed aBMD, rather than vBMD or microstructure, but some have also suggested a possible preferential benefit at skeletal sites that are rich in cortical bone. Bolland et al. showed an improvement in aBMD over 4 years of HCTZ use at sites rich in cortical bone such as the mid-forearm and legs (Bolland et al., 2007). Similar to some other studies, we saw an effect of thiazide use on aBMD by DXA at the forearm (Lamberg and Kuhlback, 1959; Heshmati et al., 1998; Wasnich et al., 1983, 1986; Cauley et al., 1993; Cheng et al., 2018; Bolland et al., 2007; Solomon

et al., 2016). We found no benefit on the predominantly trabecular spine or trabecular indices by either HRpQCT or TBS. This may suggest that thiazides have a greater effect in the cortical compartment. Only one other study has assessed TBS. Similar to our study, it did not find an association between thiazide use and spine TBS, though this study did report higher spine aBMD in thiazide users, as have some other studies (Morton et al., 1994; LaCroix et al., 2000; van der Burgh et al., 2020). The modest association between thiazide and cortical vBMD was observed in women only. While we did not detect this effect in men, the subset of male thiazide users was small and may have limited our ability to find differences in this subgroup. Some sex-dependent thiazide effects have been demonstrated in other studies with greater effects in women than men (Morton et al., 1994; LaCroix et al., 2000). The reasons for this are unclear but could be related to lower adherence in men or greater negative calcium balance in women (Morton et al., 1994; LaCroix et al.,

2000).

In addition to their potential benefit on skeletal health and a reduction in urinary calcium, some thiazides have been shown to reduce cardiovascular mortality and risk of heart failure (Officers et al., 2002). Given this, thiazides may be particularly useful to prevent bone loss in women with osteopenia (in whom a bisphosphonate may not be indicated due to low risk) and other conditions such as hypertension, hypercalciuria, and nephrolithiasis. Because of the modest effect size and lack of fracture data, their role as a sole therapy in osteoporosis appears limited.

A preferential effect on cortical rather than trabecular bone might have been anticipated as thiazides increase renal calcium re-absorption as well as serum calcium and decrease serum parathyroid hormone (PTH) levels (Wasnich et al., 1983; Cheng et al., 2018; Zaheer et al., 2016). Elevated PTH causes preferential loss of aBMD at sites rich in cortical bone (Silverberg et al., 1989). Unfortunately PTH levels were not available in our study. There are, however, multiple potential mechanisms by which thiazides might affect skeletal metabolism. Thiazides have been reported to stimulate calcium uptake into bone cells and promote osteoblastic differentiation and bone formation (Barry et al., 1997; Hall and Schaublin, 1994; Lajeunesse et al., 2000; James et al., 2014; Dvorak et al., 2007). Thiazides may also induce metabolic alkalosis in some, which decreases osteoclast activity in vitro (Arnett and Spowage, 1996).

On the other hand, there are possible negative consequences of thiazide use on bone health. We found that thiazide use was associated with a greater frequency of vertebral fractures despite similar adjusted spine aBMD and TBS scores. This finding was somewhat unexpected, though an increased risk of incident vertebral fractures has been reported in women taking thiazides in the Nurses' Health Study (Paik et al., 2016). This may be related to the development of hyponatremia, which has been associated with an increased risk of fracture. While some studies have suggested that osteoporosis and falls may mediate the relationship between hyponatremia and fractures, others indicate that the association is independent of falls and decreased BMD (Upala and Sanguaneko, 2016; Hoorn et al., 2011a,b; Kann et al., 2014; Zang, 2013). Neither falls nor adjusted spine aBMD was associated with thiazide use in our study. We were not, however, able to assess for hyponatremia. The association with vertebral fractures requires further study.

We found skeletal site-specific effects – the cortical benefits were apparent at the radius, but not the tibia. The reasons for this cannot be directly addressed by our study. We speculate that a thiazide-induced reduction in PTH may have a greater effect on the radius than the tibia. Work in primary hyperparathyroidism suggests that PTH affects the radius to a greater extent than the tibia (Stein, 2013). Our study has several limitations. This was a cross-sectional analysis and we do not have information regarding the dose, duration, adherence to thiazides or recent changes in thiazide use. Thus, we cannot derive a definitive temporal/causal link between thiazide use and bone indices nor can we examine the effects of drug dosage, recency of use, length of treatment or adherence, which might be helpful in understanding effects. Some data indicate skeletal benefits may require long-term thiazide use (Kruse et al., 2016). Additionally, the overall study size and number of male thiazide users was small. Thus, it is possible that our analysis may have been biased against finding a benefit of thiazides. We may have detected more or larger effects or effects in the cohort and men, in particular, if we were able to limit our analysis to long-term users or more male thiazide users were available, respectively. Additionally, healthy volunteer bias is possible and participants were slightly younger than those who refused to participate. Thus, results may not be reflective of or generalizable to all thiazide users/non-users. Despite this, the results do provide some important preliminary insight regarding the microstructural effects of this valuable category of drugs. There are no other published studies assessing microstructural effects of thiazides utilizing HRpQCT to our knowledge.

Our study also has several strengths. Participants were enrolled from a racially diverse population-based cohort, which limits selection bias and increases the generalizability of these findings. We were able to assess thiazide effects specifically in the subset of Black women to determine if effects vary by race. Further, our analysis was conducted in those most at risk for fracture, elderly adults in whom thiazide use is common. We had comprehensive information on skeletal covariates and thoroughly assessed musculoskeletal health with multiple modalities. We also assessed HR-pQCT using a relative offset to ensure the same region of interest was assessed across the cohort to account for differences in height and limb length. Additionally, we also scanned participants at a proximal tibial site, providing further insight into effects on cortical bone.

In conclusion, thiazide use was associated with a modest effect on aBMD at the predominantly cortical 1/3-radius site and cortical vBMD by HR-pQCT in women. We believe these results provide insight into the potential mechanism by which thiazides affect bone health. The effect on cortical bone may offer skeletal benefits in women with osteopenia taking thiazides for other indications such as hypertension, hypercalciuria or recurrent nephrolithiasis.

#### CRediT authorship contribution statement

**Hoang-Long Huynh:** Writing – original draft. **Lena Fan:** Writing – original draft. **Carmen Germosen:** Data curation, Writing – review & editing. **Mariana Bucovsky:** Project administration, Supervision, Writing – review & editing. **Ivelisse Colon:** Data curation, Writing – review & editing. **Nayoung Kil:** Data curation. **Sanchita Agarwal:** Data curation, Formal analysis, Writing – review & editing. **Marcella Walker:** Conceptualization, Writing – review & editing, Funding acquisition.

#### Declaration of competing interest

All authors declare no conflict of interest.

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