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Menopause is associated with bone loss, particularly at the distal radius, in black South African women: Findings from the Study of Women Entering and in Endocrine Transition (SWEET)

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ABSTRACT

Menopause transition is associated with accelerated bone loss, though data are limited from sub-Saharan African (SSA). Our objective was to describe bone density, geometry and estimated strength in women by menopause status and to explore whether patterns differed within those living with HIV.

Methods: Radius and tibia peripheral QCT data were collected for Black South African women (n = 430) aged 40–61 years with verified menopause and HIV status. pQCT outcomes were distal 4 % radius and tibia total cross-sectional area (CSA), total volumetric bone mineral density (vBMD), and compressive bone strength (BSIc); proximal 66 % radius and 38 % tibia cortical vBMD, total CSA, cortical thickness, and Stress-strain Index (SSI). Linear regression assessed associations between pre, peri-, and postmenopausal groups and pQCT outcomes adjusting for age, height, and weight, and then stratified by HIV status. Mean [95%CI] and tests for trend (*p-trend*) across menopausal groups are presented.

Results: Women were mean (SD) age 49.2 (5.3) years, with a body mass index (BMI) of 32.4 (6.3) m/kg^2, and 18 % were living with HIV. After adjustment, later menopause stage was associated with lower 4 % radius total mean [95%CIs] vBMD (premenopause: 345.7 [335.8,355.5] vs. postmenopause: 330.1 [322.7,337.6] mg/cm^3, *p-trend* = 0.017) and BSIc (premenopause: 0.39 [0.37,0.41] vs. postmenopause: 0.36 [0.35,0.37] g^2/cm^4; *p-trend* = 0.012). Similar trends were observed at the 66 % radius for cortical vBMD (premenopause: 1146.8 [1138.9,1154.6] vs. postmenopause: 1136.1 [1130.1,1142.0] mg/cm^3; *p-trend* = 0.028) and cortical thickness (premenopause: 2.01 [1.95,2.06] vs. postmenopause: 1.93 [1.89,1.98] mm; *p-trend* = 0.036). After stratification by HIV status a similar patten was observed in women with HIV (cortical vBMD premenopause: 1152.9 [1128.5,1177.2] mg/cm^3 vs. postmenopause: 1123.6 [1106.0,1141.2] mg/cm^3, *p-trend* = 0.048). Total CSA varied little by menopause or HIV status at either radius sites; few differences were found at the tibia.

Conclusion: In black South African women, menopause is associated with lower bone density and strength at the distal radius, a common site of osteoporotic fracture, in addition to lower cortical density and thickness at the proximal radius. Although the sample size was small, following stratification by HIV, women living with HIV had evidence of lower cortical density across menopause stages, unlike those without HIV. These findings raise

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concern for the incidence of Colles' fractures in postmenopausal women in South Africa; longitudinal studies of fracture incidence and implications of living with HIV are required.

1. Introduction

The impact of menopausal transition on bone health in North America and other high income country (HIC) populations has been well-documented [1–8]; by contrast, few data are available from sub-Saharan Africa (SSA) [9–11]. Most available studies have used Dual Energy X-ray Absorptiometry (DXA), which does not distinguish between trabecular and cortical bone compartments. Peripheral quantitative computed tomography (pQCT) and high resolution pQCT (HR-pQCT) have become increasingly available in research settings, enabling compartment-specific measures of volumetric bone mineral density (vBMD) within the appendicular skeleton. In both the axial (DXA) and appendicular (pQCT) skeleton, age-related deficits in trabecular bone have been reported in the years preceding menopause, generally followed by accelerated trabecular bone loss through the stages of menopause [12–15]. Recent longitudinal high resolution peripheral quantitative computed tomography (HR-pQCT) data also suggests that in the appendicular skeleton a substantial proportion (~80 %) of bone lost in the transition from perimenopause to postmenopause is from the cortical compartment [16]. Cross-sectional data from the Canadian Multicentre Osteoporosis Study (CaMos) also suggested that a lack of periosteal expansion, more porous cortices, and a greater percentage of load carried at the distal radius in women may underpin sex differences in forearm fracture risk [17]. While these techniques have greatly improved our understanding of compartment specific bone changes across menopause their use has thus far been confined to high income countries (HICs) [18–21], with no HR-pQCT data to date published for women living in SSA.

Studies from SSA that have measured bone density in women at midlife have almost exclusively been from South African urban populations [9–11,22–24]. While a number of these DXA studies included women at different stages of menopause, determining BMD differences by menopause stage was not the primary study objectives [9–11]. Beksinska and colleagues used forearm DXA to describe aBMD in a cohort of predominately Black pre- and perimenopausal women (aged 40–49 years) living in Durban who were long-term users of hormonal contraception and reported no impact of menopausal status on forearm aBMD at baseline or after 2.5 years of follow-up [11]. Another study from the Western Cape included black and white pre- and postmenopausal women (aged 23–82 years) reported that most ethnic differences in aBMD were explained by differences in body weight, and that older black postmenopausal women had greater femoral neck aBMD [10]. In a cross-sectional study by Jaff and colleagues DXA whole body less head (WBLH) aBMD was lower in post- compared to premenopausal women living in Soweto, though this analysis was not adjusted for weight [9]. Of these three studies which included women at two or more menopause stages, only Jaff et al. investigated a population with a prevalence of HIV infection (21.3 %) comparable to that of the national prevalence in South Africa, where approximately one in four women aged 15–49 years live with HIV infection [25]. HIV infection, its treatment, and related sociodemographic risk factors have all been suggested to negatively influence bone health [22,23,26–29]. Previous research in Soweto demonstrated that premenopausal women living with HIV had BMD deficits (lower hip and WBLH aBMD), which despite subsequent gains in weight and fat mass after ART initiation, remained 24 months later compared to women without HIV [22]. If such deficits cannot be prevented, attenuated or reversed, it may disadvantage women living with HIV as they then begin to transition into menopause putting them at greater risk of fracture. Longitudinal data suggest that postmenopausal bone loss in women living with HIV exceeds that of women without [30], in keeping with data from premenopausal women where differences were seen based on ART exposure [22].

The current study using pQCT aimed to assess trabecular and cortical bone compartments in women at different stages of menopausal transition. Our primary hypothesis was that differences between pre- and perimenopausal women would be primarily seen in the trabecular compartment (i.e. lower trabecular vBMD), with postmenopausal women having deficits in both trabecular and cortical compartments compared to pre- and perimenopausal groups. Our secondary hypothesis was that women living with HIV would have pre-existing deficits in bone density and strength (resulting from a combination of HIV infection, exposure to anti-retroviral therapy (ART), and other sociodemographic factors) and as such may be at greater risk of fracture after menopause even if the patterns and magnitude of bone loss were similar to those without HIV. Our objectives were to 1) determine bone density, geometry, and estimated strength by menopause status, and 2) explore whether HIV status modifies the association between menopause and bone outcomes menopause on bone outcomes.

2. Methods

2.1. Recruitment

The Study of Women Entering and in Endocrine Transition (SWEET) was a cross-sectional study based in Soweto a township of the City of Johannesburg Metropolitan Municipality in Gauteng, South Africa [9,31]; participants are the biological mothers and/or caregivers of the children in the Birth to Twenty Plus (BT20) cohort, the largest and longest-running longitudinal birth cohort study of child health and development in Africa [32]. Briefly, recruitment into SWEET took place 21 years after the start of BT20, at which time 2200 of these women were still in contact with the study [31]. A convenience sample of 902 black African women aged 40–60 years were recruited; 200 refused to participate [31]. In addition, those pregnant were excluded. Between March 2011 and May 2013 pQCT (XCT 2000) scans of the radius and tibia were obtained on a subset of 634 women. All women provided signed informed consent. The Human Research Ethics Committee (Medical) of the University of the Witwatersrand approved the protocol (ref: M090620).

2.1.1. HIV status

All women were offered a voluntary HIV combined antibody/antigen test; Alere Determine™ HIV-1/2 (Alere San Diego, Inc. San Diego, CA). Women found to be positive were referred to a local HIV clinic for confirmatory serological testing and onward management [9]. Since 5 years later all women were recruited into a separate follow-up study [33] which also tested for HIV (but did not include pQCT), we were able to impute missing HIV status in some women (Fig. 1). Negative status 5 years later allowed the imputation of negative SWEET study HIV status. A positive status 5 years later coupled with antiretroviral treatment for >5 years allowed us to impute a positive SWEET Study HIV status.

2.2. Questionnaires and menopause staging

Questionnaires were administered in English by a single researcher with members of the research team, whose first language matched that of participants, available to assist if a question was not understood [31]. Reproductive health, menstrual history, educational level and tobacco and smokeless tobacco (snuff) use were determined by interviewer-administered questionnaire, validated for use in this setting [34]. Participants were classified into one of three menopause categories based on self-reported last menstrual period (LMP) [35,36]. Those currently

having regular periods were classified as premenopausal, women having irregular periods were classified as perimenopausal, and women who had had amenorrhea for >12 months were classified as postmenopausal [9,31,36,37].

2.3. Anthropometry

Trained technicians performed all anthropometric measurements using a calibrated electronic scale and a fixed-wall stadiometer (Holtain, Crymych, UK) [9]. Waist and hip circumferences were measured with a soft measuring tape to the nearest 5 mm; the former at the smallest girth above the umbilicus and the latter at the greatest circumference of the hips [9]. Body mass index (BMI, kg/m^2) was calculated by dividing weight (kg) by height (m) squared.

2.4. pQCT

Scans were acquired on a XCT 2000 (Stratec Medizintechnik, Pforzheim, Germany). All scans were obtained, with a voxel size of 0.4 × 0.4 mm and slice thickness of 2 mm, at the radius (at 4 % and 66 % of the limb length proximal to the distal endplate) and tibia (at 4 %, and 38 % of the limb length proximal to the distal endplate). CT scan speed was 30 mm/s and scout view scan speed 40 mm/s. pQCT scans were processed using the manufacturer's software (Stratec XCT version 6.2). At distal 4 % sites, CALCBD analysis was used to calculate total cross-sectional area (CSA) and total and trabecular vBMD. CALCBD contour mode 1 (i.e., the threshold algorithm) was used to exclude pixels in the defined region of interest (ROI) that fell below the default threshold of 180 mg/cm^3; peel mode 1 (i.e., concentric peel) peeled away the outer 55 % of the total CSA of the bone, leaving an inner 45 % CSA considered to be trabecular bone. At proximal cortical-rich sites (66 % and 38 %), CORTBD was used to define cortical vBMD and area. The algorithm removes all voxels within the ROI that have an attenuation coefficient below the threshold. The default threshold of 710 mg/cm^3 was used with separation mode 1. Total CSA was defined at proximal sites using an edge detection threshold of 280 mg/cm^3. Cortical thickness, endosteal circumference, and periosteal circumference were calculated using a circular ring model. Stress-strain Index (SSI), an estimate of bone strength, was obtained at a threshold of 280 mg/cm^3 using cortmode 1. Bone Strength Index of Compression (BSIc, g^2/cm^4) was derived at distal sites as the product of the total vBMD (g/cm^3) squared and total CSA (cm^2). Scans were qualitatively graded by visual inspection to assess their suitability for analysis: scan slices with excessive movement or other artefacts, and scout views with incorrect reference line placement were excluded. Calibration of the XCT 2000 system was performed using the manufacturer's phantom: daily QA scans and weekly QC scans were performed throughout the study period to test scanner performance. Previous studies at this center have reported inter-operator precision as a co-efficient of variation <1 % [38].

2.5. Statistical analysis

All statistical analyses were performed using R version 4.0.3 "Bunny-Wunnies Freak Out" (R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org/) with dplyr (version 1.0.2) for data manipulation [39]. pQCT outcomes of interest at the 4 % distal radius/tibia were total CSA, total vBMD, trabecular vBMD, and BSIc. Proximal sites were the 66 % radius and 38 % tibia and outcomes of interest were cortical vBMD, BMC, cortical CSA, total CSA, cortical thickness, and SSI (stress-strain index). Continuous data are presented as mean (SD), categorical data as counts and percentages (n[%]). The exposure, menopause status, was considered an ordered categorical variable. Linear regression was used to investigate the associations between menopause status and pQCT outcomes, testing for trend (i.e. with menopause as a ordered categorical variable), estimating mean [with 95 % confidence interval] bone outcomes by menopausal stage after adjusting for centered age (i.e. mean age subtracted from participant age), height, and weight. Data were then stratified by HIV status (as HIV is a potential effect modifier), to determine differences in the associations between menopause stage and bone outcomes, adjusting for age, height, and weight, in women with and without HIV. Tests for interaction between HIV status (binary variable) and menopause status (continuous variable) were performed.

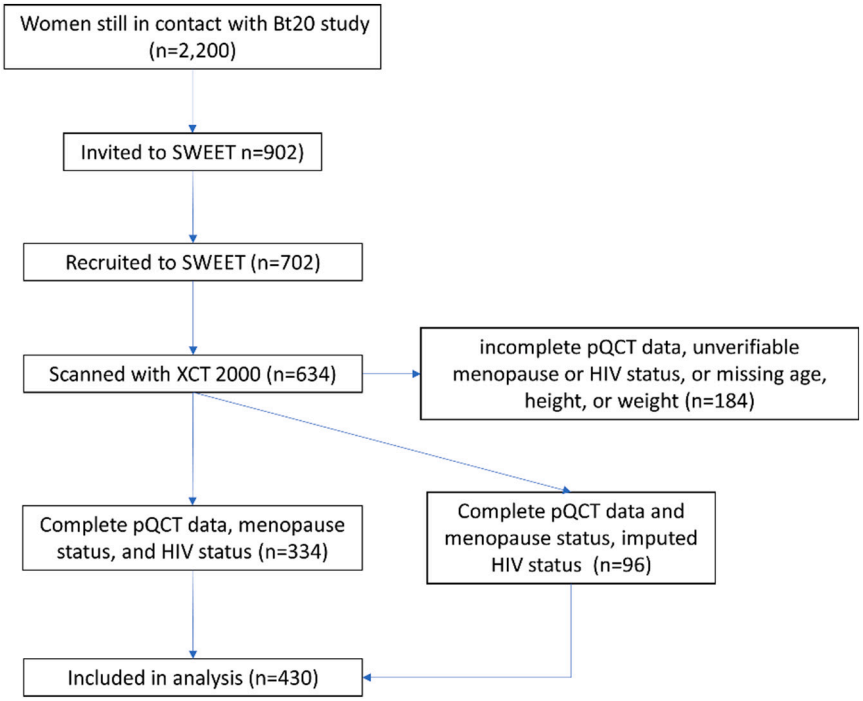

Fig. 1. Flow diagram of showing the number of women from the Birth to 20 (Bt20) study approached who took part in The Study of Women Entering and in Endocrine Transition (SWEET) study, received pQCT scans and were subsequently included in final analyses.

3. Results

3.1. Study population

Of the 634 women who had pQCT performed, 430 (68 %) had complete radius and tibia data, height and weight, as well as a verified menopause and HIV status (Fig. 1). Women, with complete data, were marginally less obese than those without complete data, who were excluded from analyses (Table S1). These 430 women were mean (SD) age 49.2 (5.3) years, with BMI 32.4 (6.3) kg/m^2; 77/430 (18 %) were living with HIV, 29 % had matriculated from high school, and 58 % were currently employed. Of the WLWH 61 % were receiving ART, with use highest in those in the premenopausal group (Table 1.). Menopause hormone therapy (MHT) use was only reported by three of the 430 women, one in the premenopausal group and two in the postmenopausal group. Hysterectomy was reported by 22 women: three were premenopausal, one perimenopausal, and 18 postmenopausal. Those who were premenopausal were on average 1.3 years younger than those who were perimenopausal, who were 2.1 years younger than those who were postmenopausal. Across the three menopause groups, anthropometry measures were similar, as were the proportions of women living with HIV (Table 1).

3.2. Unadjusted associations between menopausal stage and bone outcomes

At the distal radius premenopausal women had greater bone density (total and trabecular vBMD) and strength (BSIc) compared to perimenopausal women, who in turn had higher values than postmenopausal women ($p < 0.01$ for trend for all, Table 1). However, bone size (total CSA) at the distal radius did not differ between the groups. At the proximal radius, similar negative associations were seen across the menopause stages for bone density (cortical vBMD), mass (BMC) and cortical geometry (cortical CSA and thickness) ($p < 0.05$ for trend for all, Table 1). Unlike the distal radius, at the proximal radius total CSA increased across the menopause stages (p for trend = 0.031). Although similar but weaker patterns were observed at the tibia for total vBMD, BSIc, and cortical vBMD, measures of bone size and geometry did not differ at either the distal or proximal tibia by menopause status (Table 1).

3.3. Adjusted associations between menopausal stage and bone outcomes

Age, weight, and height adjusted mean bone outcomes, by menopausal stage, are presented in Table 2. At the distal radius, total and trabecular vBMD, in addition to BSIc remained lowest in postmenopausal women, and highest in premenopausal women (Table 2, Fig. 2). While total CSA at the distal radius varied little across the three groups. Both cortical vBMD and cortical thickness at the proximal radius were similar in pre- and perimenopausal women, but were lower in postmenopausal women (Table 2, Fig. 2). Following adjustment, proximal radius total and cortical CSA were similar across the three groups. At the tibia, only cortical vBMD at the 38 % proximal site differed across the menopause stages, being lower in those who were postmenopausal

Table 1
Characteristics of the study population and descriptive statistics for the pQCT bone outcomes at the radius and tibia, by menopause status.

Menopause status		Premenopause	Perimenopause	Postmenopause	*p* value for trend
		n = 129	*n* = 77	*n* = 224	
Participant characteristics					
Age (years)		46.7 (4.9)	48.0 (4.4)	51.1 (5.1)	<0.001
Weight (kg)		80.8 (17.3)	81.9 (17.4)	80.2 (16.6)	0.674
Height (m)		1.58 (0.05)	1.57 (0.05)	1.58 (0.06)	0.383
BMI (kg/m^2)		32.2 (6.7)	33.0 (6.4)	32.2 (6.1)	0.857
HIV infection (n (%))		26 (20 %)	14 (18 %)	37 (17 %)	0.690[a]
ART (n (%))		19 (73 %)$^{(n=26)}$	4 (29 %)$^{(n=14)}$	24 (65 %)$^{(n=37)}$	0.018[a]
Education	None	10 (8 %)	6 (8 %)	26 (12 %)	0.307[a]
(High school)	Did not finish	76 (59 %)	43 (56 %)	138 (62 %)	
	Matriculated	41 (32 %)	27 (35 %)	55 (25 %)	
Currently employed (n (%))		83 (64 %)	43 (55 %)	125 (56 %)	0.249[a]
Radius pQCT outcomes					
4 %	Total vBMD (mg/cm^3)	350.6 (61.6)	341.8 (60.0)	326.0 (52.7)	<0.001
	Trabecular vBMD (mg/cm^3)	182.7 (44.5)	179.2 (45.9)	169.7 (39.1)	0.004
	Total CSA (mm^2)	326.1 (43.2)	331.7 (49.3)	329.1 (44.5)	0.605
	BSIc (g^2/cm^4)	0.40 (0.11)	0.39 (0.12)	0.35 (0.10)	<0.001
66 %	Cortical vBMD (mg/cm^3)	1153.2 (46.1)	1153.0 (36.5)	1131.5 (47.3)	<0.001
	Cortical BMC (mg/mm)	81.7 (13.0)	81.7 (11.4)	78.0 (13.1)	0.004
	Cortical CSA (mm^2)	70.8 (10.4)	70.8 (9.2)	68.7 (10.2)	0.046
	Cortical thickness (mm)	2.03 (0.31)	2.02 (0.28)	1.91 (0.32)	<0.001
	Total CSA (mm^2)	127.6 (22.3)	128.3 (18.4)	132.5 (22.8)	0.031
	SSI (mm^3)	272.5 (62.0)	272.6 (49.3)	275.3 (54.8)	0.601
Tibia pQCT outcomes					
4 %	Total vBMD (mg/cm^3)	287.4 (42.8)	292.0 (44.0)	276.1 (40.2)	0.007
	Trabecular vBMD (mg/cm^3)	208.8 (36.1)	211.9 (43.1)	202.5 (35.7)	0.100
	Total CSA (mm^2)	1012.1 (117.2)	1000.2 (99.1)	1011.9 (123.0)	0.994
	BSIc (g^2/cm^4)	0.84 (0.22)	0.87 (0.26)	0.78 (0.21)	0.005
38 %	Cortical vBMD (mg/cm^3)	1197.1 (28.1)	1195.4 (28.5)	1178.5 (36.7)	<0.001
	Cortical BMC (mg/mm)	315.2 (42.6)	316.4 (44.7)	308.6 (44.4)	0.137
	Cortical CSA (mm^2)	263.2 (34.5)	264.7 (36.7)	261.6 (34.9)	0.610
	Cortical thickness (mm)	4.28 (0.48)	4.30 (0.55)	4.27 (0.54)	0.816
	Total CSA (mm^2)	410.2 (50.9)	415.0 (56.1)	411.2 (46.7)	0.943
	SSI (mm^3)	1634.5 (300.8)	1631.4 (268.3)	1607.5 (259.6)	0.310

vBMD = volumetric bone mineral density, CSA = cross-sectional area, BSIc = bone strength index of compression, BMC = bone mineral content, SSI = stress strain index. Mean (SD) shown unless otherwise indicated. Education status available for 127, 76, 219, pre-, peri- and postmenopausal women respectively.

[a] Pearson's Chi-squared test.

Table 2
pQCT bone outcomes at the radius and tibia by menopause status following adjustment for age, height, and weight.

Menopause stage		Premenopause	Perimenopause	Postmenopause	p value for trend
		n = 129	n = 77	n = 224	
Radius					
4 %	Total vBMD (mg/cm^3)	345.7 [335.8, 355.5]	338.0 [325.7, 350.4]	330.1 [322.7, 337.6]	0.017
	Trabecular vBMD (mg/cm^3)	179.7 [172.4, 187.0]	176.5 [167.3, 185.7]	172.3 [166.7, 177.8]	0.121
	Total CSA (mm^2)	326.0 [318.4, 333.6]	333.0 [323.5, 342.6]	328.7 [322.9, 334.5]	0.656
	BSIc (g^2/cm^4)	0.39 [0.37, 0.41]	0.38 [0.36, 0.40]	0.36 [0.35, 0.37]	0.012
66 %	Cortical vBMD (mg/cm^3)	1146.8 [1138.9, 1154.6]	1150.4 [1140.6, 1160.2]	1136.1 [1130.1, 1142.0]	0.028
	Cortical BMC (mg/mm)	80.5 [78.4, 82.7]	81.2 [78.5, 83.9]	78.9 [77.2, 80.5]	0.205
	Cortical CSA (mm^2)	70.1 [68.4, 71.8]	70.5 [68.3, 72.6]	69.2 [67.9, 70.5]	0.389
	Cortical thickness (mm)	2.01 [1.95, 2.06]	2.00 [1.94, 2.07]	1.93 [1.89, 1.98]	0.036
	Total CSA (mm^2)	128.5 [124.7, 132.4]	128.9 [124.1, 133.7]	131.8 [128.9, 134.7]	0.178
	SSI (mm^3)	272.8 [263.0, 282.6]	273.3 [261.0, 285.6]	274.9 [267.5, 282.4]	0.731
Tibia					
4 %	Total vBMD (mg/cm^3)	284.4 [278.0, 290.8]	287.7 [279.7, 295.8]	279.3 [274.4, 284.2]	0.189
	Trabecular vBMD (mg/cm^3)	207.1 [201.0, 213.1]	209.0 [201.4, 216.6]	204.5 [199.9, 209.1]	0.483
	Total CSA (mm^2)	1009.6 [991.2, 1027.9]	1004.0 [981.0, 1027.0]	1012.0 [998.1, 1025.9]	0.805
	BSIc (g^2/cm^4)	0.82 [0.79, 0.85]	0.85 [0.81, 0.89]	0.80 [0.77, 0.82]	0.184
38 %	Cortical vBMD (mg/cm^3)	1191.6 [1186.0, 1197.2]	1192.7 [1185.7, 1199.7]	1182.6 [1178.3, 1186.8]	0.011
	Cortical BMC (mg/mm)	311.9 [305.1, 318.6]	314.4 [306.0, 322.8]	314.4 [306.2, 316.3]	0.849
	Cortical CSA (mm^2)	261.6 [256.3, 266.8]	263.5 [256.9, 270.0]	262.9 [259.0, 266.9]	0.712
	Cortical thickness (mm)	4.26 [4.17, 4.35]	4.28 [4.17, 4.40]	4.29 [4.22, 4.36]	0.607
	Total CSA (mm^2)	409.8 [402.4, 417.2]	415.0 [405.7, 424.3]	411.4 [405.8, 417.0]	0.789
	SSI (mm^3)	1624.9 [1582.2, 1667.7]	1631.4 [1577.9, 1685.0]	1613.0 [1580.6, 1645.4]	0.646

vBMD = volumetric bone mineral density, CSA = cross-sectional area, BSIc = bone strength index of compression, BMC = bone mineral content, SSI = stress strain index. Data expressed as Mean [95%CI].

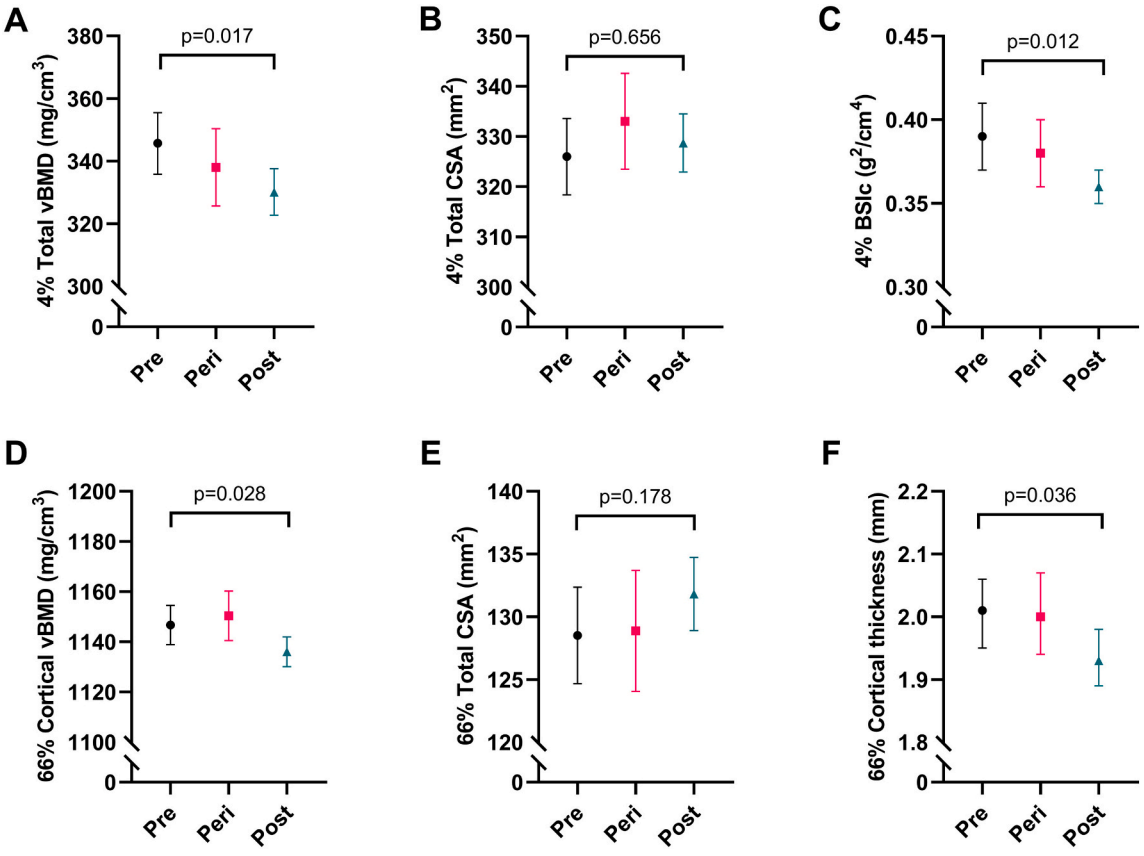

Fig. 2. Radius pQCT total vBMD (A), distal total CSA (B), BSIc (C), cortical vBMD (D), proximal total CSA (E), and cortical thickness (F) by menopause status adjusted for age, height, and weight. Data expressed as Mean [95%CI]. Significance shown from p-value for trend. vBMD = volumetric bone mineral density, CSA = cross-sectional area, BSIc = bone strength index of compression, SSI = stress strain index. *P*-value for trend from regression models with menopause as a continuous variable.

(Table 2, Fig. S2).

3.4. Findings according to HIV status

In the 353 women who were HIV negative, after adjusting for age, weight and height, total vBMD and BSIc at the distal radius were lower in postmenopausal women compared to the women in the earlier menopausal stages (Fig. 3, Table S2). Among the 77 women living with HIV, advancing menopausal stage was associated with lower cortical vBMD at the proximal radius (Fig. 3, Table S2). These trends were not apparent in the Tibia (Fig. S2, Table S3). No indication of an interaction between HIV status and menopause was found (Figs. 3 and S2).

4. Discussion

These novel pQCT data suggest that in this population of urban South African women, menopause is associated with loss of bone density and estimates of bone strength. This was particularly apparent at the distal radius, a common site of osteoporotic fracture, where total vBMD and bone strength (BSIc) values were highest before menopause yet lowest post menopause. Similarly at the cortical-rich proximal radius cortical vBMD and cortical thickness were found to be lowest in postmenopausal women. Furthermore, we found evidence of compartment-specific deficits in vBMD that appear to be associated with HIV status as cortical vBMD in women living with HIV was sequentially lower across the perimenopause and postmenopausal groups.

Our finding of lower bone strength (BSIc) at the distal radius, exactly the site of fragility fractures which classically characterize the early postmenopausal period (i.e., Colles fractures), is of particular clinical relevance. Recent published data highlight how fracture risk has been underappreciated in black African populations [40]. These findings at the distal radius concur with patterns seen in HIC populations [41]. We also identified deficits in cortical bone at the radius in WLWH across the menopause stages, compared to those without HIV. For women in peri- and post-menopause groups, mean bone measures (adjusted for age, height, and weight) were lower in WLWH with little evidence of an interaction between HIV and menopause status (all $p > 0.1$, except cortical vBMD $p = 0.08$). While the underlying mechanisms of potential interactions between HIV infection and menopause remain unclear, the loss of anabolic estrogen coupled with the chronic inflammatory effects of HIV may both promote excess bone resorption and net bone loss. Greater postmenopausal bone loss at the lumbar spine and forearm in 218 Hispanic and African-American women WLWH (vs those without HIV) has been reported, though a formal interaction was not explored [30]. Tenofovir Disoproxil Fumarate (TDF) forms part of first-line ART therapy in South Africa [42,43] (as it does across SSA), and is associated with greater bone loss than other formulations such as Tenofovir alafenamide (TAF) [44]. TDF use may predispose WLWH to mineral loss, particularly affecting the cortical compartment, such as seen at the wrist [45,46]. The mechanism of TDF's deleterious effects may relate to impaired renal tubular function to cause hypophosphaturia and reduced skeletal mineralization [40]. However, large longitudinal studies with prospective fracture reporting are needed. Understanding patterns of bone loss is important when considering interventions to help promote bone health in aging women, particularly those living with HIV.

Our data add mechanistic understanding; we observed patterns across menopause that suggest in this population, mineral loss at trabecular-rich sites precedes that at cortical-rich sites. At the trabecular-rich distal radius deficits in unadjusted and adjusted total vBMD and BSIc were apparent as women transition from pre- to peri-menopause. Although, the strong association between menopausal stage and trabecular vBMD was attenuated following adjustment for age,

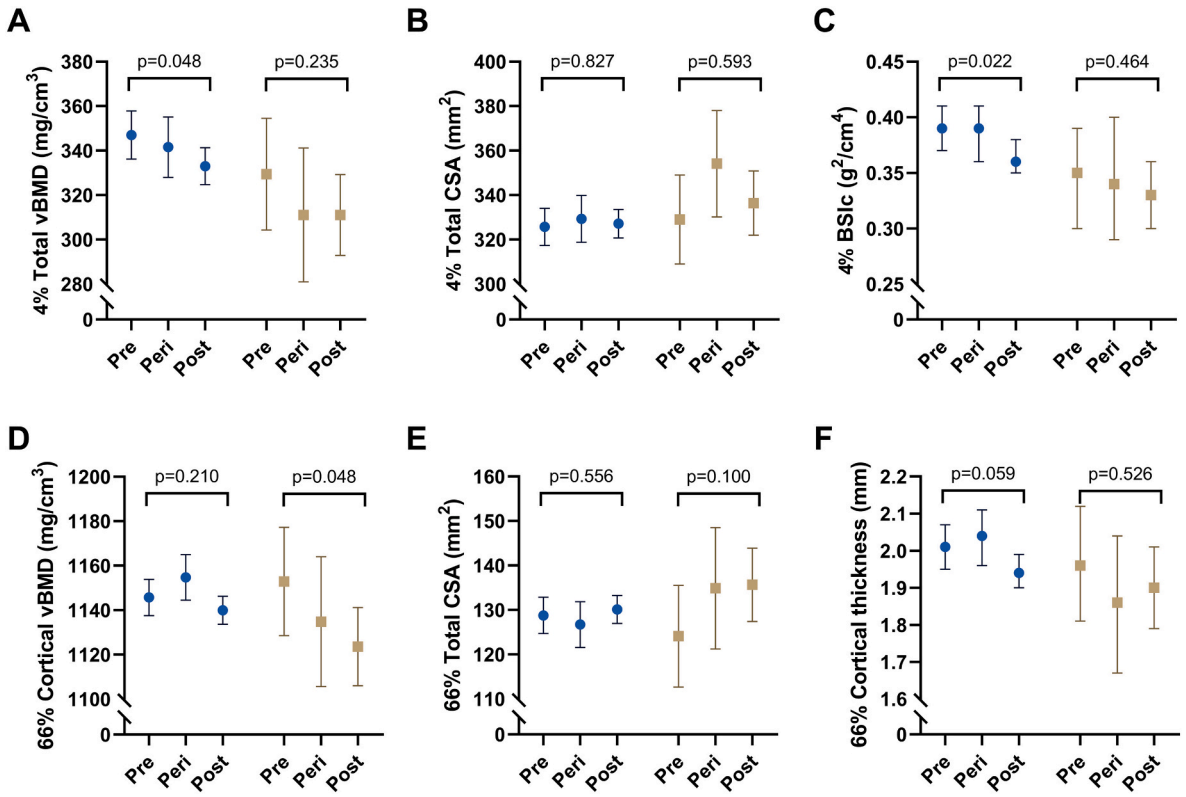

Fig. 3. Radius pQCT total vBMD (A), distal total CSA (B), BSIc (C), cortical vBMD (D), proximal total CSA (E), and cortical thickness (F) in HIV negative women (blue) and women living with HIV (WLWH, gold) by menopause status adjusted for age, height, and weight. Data expressed as Mean [95%CI]. Significance shown from *p*-value for trend. Test for interaction all $p > 0.1$. vBMD = volumetric bone mineral density, CSA = cross-sectional area, BSIc = bone strength index of compression, *P*-value for trend from regression models with menopause as a continuous variable. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

height, and weight, trabecular vBMD remained lower, if not statistically significant, in perimenopausal than premenopausal women, and in postmenopausal than perimenopausal women, and the *p*-value for trend for total vBMD remained significant. This may in part, be explained by technological limitations as single-slice pQCT reports a "trabecular" vBMD based on a pre-defined percentage of total CSA (inner 45 %) and as such does not provide a measure of all trabecular material within a given scan. In the context of decreasing total vBMD across menopause, at this predominantly trabecular site, we cannot fully discount changes occurring in the entirety of the trabecular compartment as this is not measured by this technique due to the lack of resolution to discern cortical from trabecular bone and the trabecular-cortical junction. In contrast at the proximal radius, deficits in cortical vBMD and thickness only became apparent later in menopausal transition, between peri- and post menopause. We noted a trend at the 66 % radius where total CSA appeared to be higher with increasing menopause stage, consistent with other populations where compensatory increases in bone size have been reported as women age [47] and transition from pre- to post menopause [5]. Associations between menopausal stage and bone density and strength were greater at the non-load bearing radius, than the load-bearing distal tibia. Given high rates of obesity in this population (mean BMI 32.4 kg/m^2), this may suggest that routine loading of the tibia provides a degree of protection against menopause-related bone loss.

After stratification by HIV status both groups displayed similar patterns in lower radial total vBMD and BSIc, through the stages of menopause. This was more clearly seen in HIV negative women but is likely due to the smaller sample of women living with HIV rather than a group difference in etiology.

For each menopause stage, women living with HIV had lower estimates of density and strength compared to the HIV negative women, though due to numbers this wasn't formally tested as HIV may be an effect-modifier for menopause related bone-loss. At the proximal radius HIV negative women did not show evidence of lower cortical vBMD across menopause, whereas, in women living with HIV cortical vBMD was lower across the stages of menopause similar to the pooled data (Fig. 2). This may be indicative of an early aging phenotype whereby once trabecular bone is already lost at an earlier stage, deficits in cortical bone begin to become apparent. Interestingly, after stratification by HIV status the pattern of increasing proximal radius total CSA was only apparent in WLWH (although this did not reach the predefined level of statistical significance). Evidence suggests ART, in particular TDF, may cause bone loss at cortical-rich skeletal sites [45,48] though further research is required to describe the sequence of compartmental bone loss in WLWH and whether ART use is an effect modifier for menopause-related bone loss.

This research has several strengths including our large sample-size, verifiable HIV and menopause status, and the availability of data at both the radius and tibia. However, there are several limitations to this work. Our analysis is cross-sectional, so we lacked longitudinal pQCT scans through the stages of menopause. Additionally, evidence suggests that perimenopause can begin when women still have regular cycles [49], and we did not include measured hormone levels to elucidate menopausal stage. We lacked detailed data on weight-bearing physical activity, dietary intake (particularly calcium and vitamin D intake), contraceptive use, parity and breastfeeding history. Relatively low numbers of women living with HIV plus incomplete data on ART regimen, duration of ART treatment, and indicators of HIV disease severity such as CD4 count and viral load limited our ability to test for differences between groups. As such we were unable to determine whether the patterns seen in in women with HIV are related to menopause, HIV infection, TDF exposure or a combination of all three.

5. Conclusions

Transitioning through menopause in this population of urban-dwelling Black South African women was associated with lower bone density and estimates of bone strength at the distal radius, a site that comprises predominantly trabecular bone. Deficits were also evident in cortical density and cortical thickness at the proximal radius. Fewer differences were found by menopause status at the load-bearing tibia apart from cortical vBMD. At all menopause stages women living with HIV had lower absolute density and strength values than women without HIV. Deficits in cortical vBMD were particularly seen by menopause stage in women living with HIV. Taken together this may indicate that women living with HIV might exhibit an early aging phenotype, having already depleted the trabecular compartment, cortical bone becomes compromised earlier because of the impact of their disease or its treatment. Our data suggest that in this population the potential impact of menopause is most pronounced at the distal radius, where lower bone density and strength may predispose to fragility fracture. Longitudinal studies to assess fracture incidence are needed.

CRediT authorship contribution statement

Study design: SN, NJ, NC, LM.
Investigation: SN, NJ, NC, LM.
Resources: SN, NJ, NC, LM.
Formal analysis: MÓB, CLG, TM, and KAW.
Writing - Original Draft: MÓB, CLG, TM, and KAW.
Writing - Review & Editing: MOB, CLG, SN, NJ, NC, LM, and KAW.
Visualization: MÓB.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bone.2022.116543.

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