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Structural Bone Deficits in HIV/HCV-Coinfected, HCV-Monoinfected, and HIV-Monoinfected Women

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Background. Coinfection with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) is associated with reduced bone mineral density (BMD) and increased fracture rates, particularly in women. The structural underpinnings for skeletal fragility in coinfected women have not been characterized. We used tibial peripheral quantitative computed tomography to evaluate skeletal parameters in women, by HIV/HCV status.

Methods. We conducted a cross-sectional study among 50 HIV/HCV-coinfected, 51 HCV-monoinfected, and 50 HIV-monoinfected women. Tibial volumetric BMD and cortical dimensions were determined with peripheral quantitative computed tomography. Race-specific *z* scores for age were generated using 263 female reference participants without HIV infection or liver disease.

Results. Coinfected participants had lower mean *z* scores for trabecular volumetric BMD (−0.85), cortical volumetric BMD (−0.67), cortical area (−0.61), and cortical thickness (−0.77) than reference participants (all $P < .001$). The smaller cortical dimensions were due to greater mean *z* scores for endosteal circumference (+0.67; $P < .001$) and comparable *z* scores for periosteal circumference (+0.04; $P = .87$). Trabecular volumetric BMD was lower in coinfected than in HCV- or HIV-monoinfected participants. HCV-infected women with stage 3–4 liver fibrosis had lower mean *z* scores for trabecular volumetric BMD, cortical thickness, and total hip BMD those with stage 0–2 fibrosis.

Conclusions. Compared with healthy reference patients, HIV/HCV-coinfected women had decreased tibial trabecular volumetric BMD, diminished cortical dimensions, and significant endocortical bone loss.

Keywords. bone; hepatitis C virus; HIV; coinfection; peripheral quantitative computed tomography.

Low bone mineral density (BMD) is a recognized metabolic complication of human immunodeficiency virus (HIV) [1, 2] and chronic hepatitis C virus (HCV) infection [3, 4]. Among HIV-infected patients, BMD further decreases 2%–6% during the first 2 years of antiretroviral therapy (ART) [5–7]. Each of these chronic infections has been associated with an increased risk of fracture compared with uninfected persons [8–10]. HCV coinfection is associated with further reductions in BMD in HIV-infected patients [11–14], particularly women [11, 12, 14]. Rates of hip fracture are also significantly higher in HIV/HCV-coinfected than in HIV-monoinfected, HCV-monoinfected, and uninfected persons [10].

Various factors related to HIV and chronic HCV have been hypothesized to contribute to skeletal fragility. HIV-related inflammation, ART-related toxicity, increased prevalence of tobacco or alcohol use, and established osteoporosis risk factors exacerbated by HIV contribute to

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low BMD and increased fracture risk in persons with HIV infection [15]. Inflammatory cytokines associated with chronic HCV, particularly tumor necrosis factor (TNF) α, interleukin-1 (IL-1), and interleukin-6 (IL-6), might inhibit bone formation and increase bone resorption [16, 17]. The development of HCV-related hepatic decompensation might further contribute to a decrease in BMD by impairing the production of factors (eg, 25-hydroxyvitamin D [25(OH)D], insulinlike growth factor 1) that promote bone formation and mineralization [3, 4].

Despite the observed decrements in BMD [11–14] and increased fracture risk among HIV/HCV-coinfected individuals [10], the structural underpinnings and mechanisms for the skeletal fragility in these patients have not been established. Studies of BMD in coinfected patients have relied exclusively on dual-energy x-ray absorptiometry (DXA), a 2-dimensional projection technique that summarizes total trabecular and cortical bone mass within the projected bone area. DXA is therefore unable to identify distinct deficits within trabecular and cortical bone. Peripheral quantitative computed tomography (pQCT) provides 3-dimensional estimates of trabecular and cortical volumetric BMD and cortical geometry in the appendicular skeleton that are highly correlated with failure load [18, 19]. Characterization of trabecular and cortical bone deficits and associated metabolic and body composition abnormalities in HIV/HCV-coinfected patients can suggest potential mechanisms of bone loss and inform future studies of bone therapies in this at-risk and aging population. We performed tibial pQCT and whole-body DXA in ART-treated HIV/HCV-coinfected women compared with HCV-monoinfected women, ART-treated HIV-monoinfected women, and healthy women without a history of HIV infection or viral hepatitis. Our analyses focused on women because associations between HIV/HCV coinfection and low BMD have been stronger in women than in men [12, 14]. We hypothesized that coinfected women would have lower trabecular volumetric BMD and cortical thinning due to endocortical bone loss, a pattern observed in other chronic inflammatory diseases [20–22], compared with HCV- or HIV-monoinfected women and those without a history of either infection.

METHODS

Study Design and Setting

We performed a cross-sectional study among ART-treated HIV/HCV-coinfected, HCV-monoinfected, and ART-treated HIV-monoinfected women recruited from infectious diseases and hepatology practices at the Hospital of the University of Pennsylvania, Penn Presbyterian Medical Center, and Jonathan Lax Center, 3 centers affiliated with the University of Pennsylvania in Philadelphia. These practices provide HIV and HCV care to all 3 patient groups. The study was approved by the institutional review boards of the University of Pennsylvania and Jonathan Lax Center. Informed consent was obtained from all participants.

Study Participants

All study participants were aged ≥18 years. Other eligibility criteria were as follows: HIV-monoinfected women, (1) stable ART regimen for 3 months, (2) HIV RNA < 1000 copies/mL (suggesting controlled HIV infection), and (3) negative results for HCV antibody; HCV-monoinfected women, (1) detectable HCV RNA, (2) HCV genotype 1 (predominant genotype in the United States and western Europe [23, 24]), (3) hepatic fibrosis staging by liver biopsy or noninvasive test within 2 years before enrollment, and (4) negative results for HIV antibody; HIV/HCV-coinfected women, criteria 1–2 for HIV-monoinfected and 1–3 for HCV-monoinfected participants. Liver fibrosis assessment was required for HCV-infected participants because we wanted to assess bone and body composition measurements by hepatic fibrosis stage.

Exclusion criteria were as follows: (1) active opportunistic infection, malignancy, hepatic decompensation, or substance abuse within 3 months of enrollment (conditions that can alter body composition and/or bone mass [3, 4, 25]); (2) previous HCV therapy (which can affect BMD [26]); or (3) pregnancy or breastfeeding (to avoid radiation exposure). We targeted a sample size of 50 participants per group to provide 80% power to detect a difference in z score of 0.5 between the groups, which we considered clinically significant.

Reference data were generated in 263 women (aged 21–78 years) in a prior study of bone and body composition using the same pQCT and DXA machines and methods [27]. These participants were recruited from Hospital of the University of Pennsylvania and Penn Presbyterian Medical Center clinics and the surrounding community, as described elsewhere [27]. Exclusion criteria included a reported history of chronic diseases or medications known to affect nutrition or bone health, including HIV infection and chronic liver disease.

Assessment of Demographic, Clinical, and Anthropometric Data

Demographic and clinical data were collected from coinfected, HCV-monoinfected, and HIV-monoinfected participants using a structured questionnaire. Data included age; race and ethnicity; current smoking status; alcohol use, determined with the Alcohol Used Disorders Identification Test questionnaire [28, 29]; diabetes; postmenopausal status (absence of menstrual periods for >12 months in a woman aged >45 years); and calcium and vitamin D supplement use. Physical activity was measured with the Multi-Ethnic Study of Atherosclerosis (MESA) physical activity questionnaire [30]. Minutes of activity in a typical week were summed for each activity type, converted to hours, and multiplied by metabolic equivalent (MET) level [30, 31]. Total MET-hours per week of activity and intentional exercise were determined.

For HIV-infected participants, records were reviewed to determine HIV transmission risk factors and most recent CD4 cell count, HIV RNA, hepatitis B surface antigen (HBsAg), serum

creatinine, and ART regimen. For chronically HCV-infected participants, we collected the most recent HBsAg, HCV RNA, creatinine, and METAVIR stage of hepatic fibrosis by liver biopsy [32], HCV FibroSure test (Laboratory Corporation of America, Burlington, North Carolina) [33], or Hepascore test (Quest Diagnostics, San Juan Capistrano, California) [34]. For the healthy female reference group, data were available on age, race, ethnicity, current smoking, physical activity (based on MESA questionnaire), and postmenopausal status. Body weight and height were measured using a digital scale (Scaltronix) and stadiometer (Holtain), respectively. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

pQCT Measurements

Bone measurements in the left tibia were obtained by means of pQCT (Stratec XCT2000 12-detector unit; Orthometrix) with a voxel size of 0.4 mm, and were analyzed with Stratec software (version 6.00). A scout view was obtained to place the reference line at the medial distal end-plate border. Bone measurements were obtained at 3% and 38% of tibia length proximal to the reference line. At the 3% metaphyseal site, scans were analyzed for trabecular volumetric BMD (in milligrams per cubic centimeter). At the 38% diaphyseal site, scans were analyzed for cortical volumetric BMD (milligrams per cubic centimeter), cortical cross-sectional area (square millimeters), and cortical thickness, periosteal circumference, and endosteal circumference (all in millimeters). Quality control was monitored daily using a hydroxyapatite phantom. The coefficient of variation (CV) was 0.5%–1.6%.

Whole-Body DXA Measurements

Areal BMD at the total hip and femoral neck and whole-body fat and lean mass were assessed by means of DXA using a Hologic densitometer (Delphi/Discovery Systems; Hologic). Participants with BMD below the expected range for age and sex were identified by z scores of −2.0 or less [35]. Measurements were performed in the array mode using standard positioning techniques. Quality control was monitored daily using a phantom. The CV was <1% [36]. Appendicular lean mass, a measure of skeletal muscle [37], and whole-body fat mass were converted to appendicular lean mass index and fat mass index (both in kilograms per square meter) using height.

Laboratory Data

Blood samples were collected for determination of serum calcium and phosphate, measured using standard clinical methods with CVs of 1.3% and 2.1%, respectively [38]. Intact parathyroid hormone (PTH) was measured using an automated immunochemical assay with a CV of 3%–6%. Serum 25(OH)D and 1,25-dihydroxyvitamin D (1,25$[OH]_2$D) were measured using immunoaffinity enrichment and liquid chromatography-tandem mass spectrometry with interassay CVs of 3%–5% and 10%–11%, respectively [39–41]. Intact fibroblast growth factor 23 (FGF23), which regulates 1,25$(OH)_2$D and phosphate levels [42], was measured using enzyme-linked immunosorbent assay (Kainos Laboratories; CV, 6%–11%). TNF-α, IL-1β, and IL-6 were measured using a high-sensitivity multiplex assay on an electrochemiluminescence platform (Meso Scale Discovery), with intra-assay CVs of 10.5%, 7.2%, and 5.9%, respectively.

Statistical Analysis

We first converted pQCT and DXA measurements to sex- and race-specific (black vs nonblack) z scores relative to age to facilitate comparisons across the age range and between racial groups. The z scores were generated using the LMS method [43] based on curves generated with the data from the 263 reference participants. The LMS method accounts for the nonlinearity, heteroscedasticity, and skew of bone and body composition data with age [37]. Cortical dimension z scores were further adjusted for tibia length [44]. The age and race distributions of the reference participants were selected for the purpose of generating reference curves, and differed in comparisons with the 3 infected groups. Sex, race, and age-specific z scores were generated for all bone and body composition outcomes.

We determined differences in body composition between coinfected, HCV-monoinfected, HIV-monoinfected, and reference participants using whole-body DXA. We used multivariable linear regression to determine whether appendicular lean mass index and whole-body fat mass index z scores were associated with pQCT and DXA bone measurements within each group.

Next, we determined differences in mean pQCT and DXA bone z scores in (1) coinfected versus reference participants, (2) coinfected versus HCV-monoinfected, (3) coinfected versus HIV-monoinfected, and (4) HCV- and HIV-monoinfected versus reference participants. We evaluated postmenopausal status as an effect modifier, given its impact on bone health. We also determined whether coinfected patients with a history of injection drug use more commonly had bone abnormalities than those without this history. Because Baker et al [27] had reported that lean mass was positively associated with trabecular volumetric BMD and cortical area and negatively associated with cortical volumetric BMD in the reference group, we performed multivariable linear regression, adjusting for appendicular lean mass index and whole-body fat mass index z scores. Analyses were also adjusted for smoking and physical activity, because we observed significant differences in these variables across the groups.

Among chronically HCV-infected patients, we used multivariable linear regression to determine whether advanced hepatic fibrosis/cirrhosis (METAVIR stage 3–4 fibrosis) and high HCV RNA levels (≥800 000 IU/mL) were associated with larger mean differences in pQCT and DXA bone z scores, after adjustment for appendicular lean mass index and fat mass index z scores, smoking, and physical activity.

Analyses were performed using Stata 13.0 software (Stata Corp). Although multiple comparisons were performed across numerous correlated bone measurements, Bonferroni correction was not appropriate because it assumes that these measurements are independent. We interpreted findings with caution and examined the consistency of results.

RESULTS

Participant Characteristics

50 HIV/HCV-coinfected, 51 HCV-monoinfected, and 50 HIV-monoinfected participants were enrolled. Their characteristics, along with those of the 263 reference participants, are summarized in Table 1. HIV-monoinfected patients were younger and less commonly reported current smoking than coinfected or HCV-monoinfected participants. No differences were observed across the 3 infected groups in alcohol use, postmenopausal status, diabetes, HBsAg, or calcium/vitamin D use. Median HCV RNA levels and prevalence of advanced hepatic fibrosis/cirrhosis were similar between coinfected and HCV-monoinfected participants. Coinfected patients were less commonly black and more frequently reported injection drug use and blood transfusion as HIV risk factors than HIV-monoinfected participants. No differences in HIV suppression, median CD4 cell count, or use of antiretrovirals, including tenofovir, were observed between coinfected and HIV-monoinfected participants. The reference group was more commonly white and had higher median total physical activity (in MET-hours per week) than the 3 infected groups.

Group Differences in Body Composition

HIV/HCV-Coinfected Versus Reference Participants

In comparisons stratified on race, coinfected and reference participants had comparable BMI (Table 1). Race-specific appendicular lean mass index and whole-body fat mass index z scores also did not differ (Table 2).

HIV/HCV-Coinfected Versus HCV- and HIV-Monoinfected Participants

Coinfected participants had lower mean appendicular lean mass index and whole-body fat mass index z scores than HCV- and HIV-monoinfected participants (Table 2). In analyses stratified on race, coinfected patients had a lower median BMI, but group differences were not statistically significant.

HCV- and HIV-monoinfected Versus Reference Participants

Mean appendicular lean mass index and fat mass index z scores were higher in HCV- and HIV-monoinfected than in reference participants (Table 2). In analyses stratified on race, HCV- and HIV-monoinfected patients had higher median BMIs than reference participants ($P < .05$).

Associations Between Body Composition and Bone Measurements

Within each of the 4 groups, appendicular lean mass index z scores were positively associated with all pQCT and DXA bone measurements ($P < .01$), independent of fat mass index z scores. No associations were observed between fat mass index z scores and pQCT and DXA bone measurements, after controlling for appendicular lean mass index z scores.

Group Differences in Bone Measurements

HIV/HCV-Coinfected Versus Reference Participants

With pQCT, mean trabecular volumetric BMD, cortical volumetric BMD, cortical area, and cortical thickness z scores were lower in coinfected than in reference participants (Table 2). The smaller cortical dimensions were due to significantly greater z scores for endosteal circumference, with comparable z scores for periosteal circumference. Adjustment for appendicular lean mass index and fat mass index z scores, smoking, and total physical activity did not change results (Supplementary Table 1). Postmenopausal status increased differences between coinfected and reference participants in z scores for cortical volumetric BMD (−1.01 vs −0.16) and cortical area (−0.89 vs 0.02; $P < .05$ for both interactions).

Mean total hip and femoral neck BMD z scores at DXA were lower in coinfected participants (Table 2). Higher proportions of coinfected women had low BMD for age (z score, −2.0 or less) at the total hip (9 of 50 [18.0%] vs 6 of 263 [2.3%]; $P < .001$) and femoral neck (5 of 50 [10.0%] vs 5 of 263 [1.9%]; $P < .001$). There were no differences in pQCT or DXA bone measurements between those who reported a history of injection drug use and those who did not (data not shown).

HIV/HCV-Coinfected Versus HCV- and HIV-Monoinfected Participants

Mean trabecular volumetric BMD (Figure 1) and cortical thickness z scores at pQCT were lower in coinfected than in HCV- and HIV-monoinfected participants (Table 2). After adjustment for the appendicular lean mass index and fat mass index z scores, smoking, and physical activity, differences in trabecular volumetric BMD were attenuated and differences in cortical thickness were no longer significant in the comparison with HCV-monoinfected (Supplementary Table 2) and HIV-monoinfected (Supplementary Table 3) participants.

Mean total hip and femoral neck BMD z scores at DXA were similar between coinfected and HCV-monoinfected patients (Table 2). Similar proportions of coinfected and HCV-monoinfected patients had low BMD for age at the total hip (9 of 50 [18.0%] vs 6 of 51 [11.7%]; $P = .40$) and femoral neck (5 of 50 [10.0%] vs 4 of 51 [7.8%]; $P = .75$).

Coinfected participants had lower mean total hip and femoral neck BMD z scores than HIV-monoinfected participants (Table 2). There were no significant differences between

Table 1. Characteristics of HIV/Chronically HCV-Coinfected, Chronically HCV-Monoinfected, HIV-Monoinfected, and Healthy Female Reference Participants

Characteristic	HIV/HCV-Coinfected (n = 50)	HCV-Monoinfected (n = 51)	HIV-Monoinfected (n = 50)	Healthy Reference (n = 263)	*P* Value: HIV/HCV vs HCV	*P* Value: HIV/HCV vs HIV	*P* Value: HIV/HCV vs Reference
Age, median (IQR), y	51 (46–56)	55 (50–60)	47 (40–52)	47 (36–60)	.01	.004	.1
Race, No. (%)					.2[a]	.02[a]	<.001[a]
Black	39 (78)	43 (84)	42 (84)	112 (42)			
White	11 (22)	6 (12)	4 (8)	136 (52)			
Other	0 (0)	2 (4)	4 (8)	15 (6)			
Hispanic, No. (%)	1 (2)	1 (2)	4 (8)	4 (2)	>.99[a]	.4[a]	.6[a]
BMI, median (IQR), kg/m^2							
Black	29.2 (25.5–33.0)	31.3 (27.7–35.3)	31.9 (26.4–36.8)	28.6 (24.8–33.6)	.1	.2	.5
White	24.1 (22.2–27.7)	29.8 (26.7–34.9)	31.0 (24.3–36.7)	23.3 (20.6–27.2)	.08	.2	.9
Current smoker, No. (%)	40 (80)	25 (49)	16 (32)	35 (13)	.001	<.001	<.001
Physical activity, mean (SD), MET-h/wk							
Total	171.0 (127.6)	180.9 (104.4)	203.7 (108.3)	268.7 (197.5)	.7	.2	<.001
Intentional exercise	21.5 (28.3)	21.7 (24.2)	24.1 (37.7)	46.6 (72.7)	.9	.7	<.001
Alcohol use, median (IQR), AUDIT score	0 (0–2)	0 (0–3)	1 (0–2)	. . .	.6	.2	. . .
Postmenopausal, No. (%)[b]	33 (66)	38 (75)	24 (48)	125 (48)	.3	.1	.01
Type 2 diabetes mellitus, No. (%)	9 (18)	17 (33)	4 (8)	. . .	.08	.2[a]	. . .
Receiving calcium, No. (%)	10 (20)	16 (31)	11 (22)	. . .	.2	.8	. . .
Receiving vitamin D, No. (%)	12 (24)	18 (35)	12 (24)	. . .	.2	>.99	. . .
Serum creatinine, median (IQR), mg/dL	0.82 (0.70–0.92)	0.77 (0.68–0.96)	0.82 (0.75–0.95)	. . .	.7	.4	. . .
HBsAg positive, No. (%)	3 (6)	5 (10)	1 (2)	. . .	.6	.3	. . .
HCV RNA, median (IQR), log IU/mL	6.3 (5.9–6.8)	6.4 (5.8–6.8)	. . .	. . .	.8	. . .	. . .
METAVIR fibrosis stage, No. (%)					.8	. . .	. . .
0–2	38 (76)	40 (78)	. . .	. . .			
3–4	12 (24)	11 (22)	. . .	. . .			
METAVIR inflammation grade, No. (%)					.7	. . .	. . .
0–1	32 (64)	30 (59)	. . .	. . .			
2–3	18 (36)	21 (41)	. . .	. . .			
HIV RNA <75 copies/mL, No. (%)	43 (86)	. . .	37 (74)	. . .	. . .	.1	. . .
CD4 cell count, median (IQR), cells/mm^3	605 (451–805)	. . .	635 (458–830)	. . .	. . .	.8	. . .
HIV risk factor, No. (%)							
Injection drug use	17 (34)	. . .	0 (0)	. . .	. . .	<.001	. . .
Unprotected sex	34 (68)	. . .	47 (94)	. . .	. . .	.002	. . .
Transfusion	3 (6)	. . .	0 (0)	. . .	. . .	.08	. . .
Other	2 (4)	. . .	3 (6)	. . .	. . .	.5[a]	. . .
ART use, No. (%)							
NRTI	44 (88)	. . .	50 (100)	. . .	. . .	.03[a]	. . .
NNRTI	16 (32)	. . .	20 (40)	. . .	. . .	.4	. . .
Protease inhibitor	18 (36)	. . .	27 (54)	. . .	. . .	.07	. . .
Integrase inhibitor	10 (20)	. . .	6 (12)	. . .	. . .	.3	. . .
Tenofovir	35 (70)	. . .	39 (78)	. . .	. . .	.4	. . .

Abbreviations: ART, antiretroviral therapy; AUDIT, Alcohol Use Disorders Identification Test-Consumption; BMI, body mass index; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IQR, interquartile range; MET-h, metabolic-equivalent hours; NNRTI, nonnucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse-transcriptase inhibitor; SD, standard deviation.

[a] *P* value determined by Fisher exact test.

[b] Postmenopausal status was defined as the absence of menstrual periods for >12 months in a woman aged >45 years.

Table 2. DXA and Tibial pQCT Measurements in HIV/HCV-Coinfected, HCV-Monoinfected, HIV-Monoinfected, and Healthy Female Reference Participants

Bone Measurement	*z* Score, Mean (SD)				*P* Value		
	HIV/HCV Coinfected (n = 50)	HCV Monoinfected (n = 51)	HIV Monoinfected (n = 50)	Healthy Reference (n = 263)	HIV/HCV vs HCV	HIV/HCV vs HIV	HIV/HCV vs Reference
pQCT							
Trabecular vBMD	−0.85 (1.11)	−0.21 (1.03)	0.06 (1.36)	−0.01 (1.01)	.004	<.001	<.001
Cortical vBMD	−0.67 (1.10)	−0.36 (1.07)[a]	−0.40 (1.12)[a]	0.00 (1.00)	.16	.24	<.001
Cortical area	−0.61 (1.51)	−0.12 (1.07)	0.04 (0.99)	0.03 (0.93)	.07	.01	<.001
Cortical thickness	−0.77 (1.39)	−0.26 (1.07)	−0.22 (1.04)	0.00 (0.99)	.04	.03	<.001
Periosteal circumference	0.04 (1.07)	−0.03 (1.20)	0.31 (0.78)[a]	0.01 (0.89)	.77	.15	.87
Endosteal circumference	0.67 (1.37)	0.25 (0.95)	0.45 (0.94)[a]	0.07 (0.96)	.08	.37	<.001
DXA							
Total hip BMD	−0.89 (1.27)	−0.62 (1.12)[a]	−0.18 (1.18)	0.00 (1.00)	.27	.005	<.001
Femoral neck BMD	−0.81 (1.10)	−0.56 (1.17)[a]	−0.23 (1.15)	0.00 (1.01)	.30	.01	<.001
Appendicular lean mass index	0.01 (1.11)	0.57 (1.01)[a]	0.55 (1.40)[a]	0.00 (1.01)	.01	.04	.91
Whole-body fat mass index	0.00 (1.26)	0.56 (1.03)[a]	0.52 (1.36)[a]	0.00 (1.00)	.02	.05	.99

Abbreviations: BMD, bone mineral density; DXA, dual-energy x-ray absorptiometry; HCV, hepatitis C virus; HIV, human immunodeficiency virus; pQCT, peripheral quantitative computed tomography; SD, standard deviation; vBMD, volumetric BMD.

[a] $P < .05$ for comparison with healthy female reference participants.

coinfected and HIV-monoinfected participants in the prevalence of low BMD for age at the total hip (9 of 50 [18.0%] vs 4 of 50 [8.0%]; $P = .14$) or femoral neck (5 of 50 [10.0%] vs 4 of 50 [8.0%]; $P = .73$).

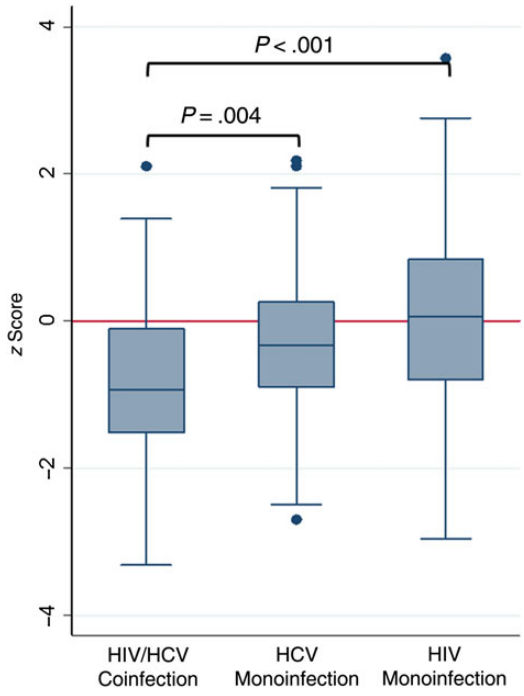

Figure 1. Mean trabecular volumetric bone mineral density *z* scores for antiretroviral-treated human immunodeficiency virus (HIV)/hepatitis C virus (HCV)–coinfected, HCV-monoinfected, and antiretroviral-treated HIV-monoinfected female participants.

HCV- and HIV-Monoinfected vs Healthy Reference Participants

HCV-monoinfected participants had lower mean cortical volumetric BMD, total hip BMD, and femoral neck BMD *z* scores than the reference group. After adjustment for appendicular lean mass index and fat mass index *z* scores, smoking, and physical activity, trabecular volumetric BMD, cortical area, cortical thickness, periosteal circumference, total hip BMD, and femoral neck BMD *z* scores remained lower in HCV-monoinfected patients (Supplementary Table 4). Postmenopausal status did not increase differences in pQCT measurements between HCV-monoinfected and reference participants.

HIV-monoinfected patients had lower cortical volumetric BMD and higher mean periosteal and endosteal circumference *z* scores than reference participants. After adjustment for appendicular lean mass index and fat mass index *z* scores, smoking, and physical activity, HIV-monoinfected patients had lower cortical thickness, higher endosteal circumference, and lower total hip and femoral neck *z* scores (Supplementary Table 5). Postmenopausal status increased differences between HIV-monoinfected and reference participants only in cortical thickness *z* scores (−0.61 vs −0.03; $P < .05$ for interaction).

Inflammatory Cytokines and Vitamin D–Related Metabolism

TNF-α levels were significantly higher in coinfected than in HCV- or HIV-monoinfected participants. There were no differences

Table 3. Mineral Metabolism and Inflammatory Cytokine Levels in HIV/HCV-Coinfected, HCV-Monoinfected, and HIV-Monoinfected Women

Laboratory Measurement	Median Value (IQR)			*P* Value	
	HIV/HCV Coinfected (n = 50)	HCV Monoinfected (n = 51)	HIV Monoinfected (n = 50)	HIV/HCV vs HCV	HIV/HCV vs HIV
Vitamin D metabolism					
Serum calcium, mg/dL	10.0 (9.5–10.4)	10.3 (9.8–10.8)	10.1 (9.5–10.6)	.22	.25
Serum phosphate, mg/dL	3.6 (3.3–3.9)	3.7 (3.3–4.1)	3.7 (3.1–4.3)	.29	.85
PTH, pg/mL	36.1 (28.3–47.2)	38.6 (27.2–50.2)	40.9 (31.5–64.8)	.45	.03
25(OH)D, ng/mL	23.5 (13.6–35.8)	24.7 (16.8–36.5)	25.4 (13.4–35.9)	.45	.95
$1,25(OH)_2D$, ng/mL	51.7 (33.7–72.6)	46.3 (37.3–67.4)	52.8 (39.4–71.3)	.81	.53
FGF23, pg/mL	35.4 (25.0–43.9)	41.3 (25.0–54.3)	28.9 (20.5–37.7)	.22	.13
Inflammatory cytokines					
IL-1β, pg/mL	0.12 (0.12–0.31)	0.12 (0.12–0.18)	0.12 (0.12–0.18)	.34	.25
IL-6, pg/mL	1.45 (1.15–2.06)	1.65 (0.92–2.25)	1.06 (0.76–1.67)	.99	.06
TNF-α, pg/mL	3.11 (2.53–3.85)	2.64 (2.10–3.46)	2.05 (1.66–2.73)	.04	<.001

Abbreviations: $1,25(OH)_2D$, 1,25-hydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; FGF23, fibroblast growth factor 23; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IL-1β, interleukin 1β; IL-6, interleukin 6; IQR, interquartile range; PTH, parathyroid hormone; TNF, tumor necrosis factor.

in median serum calcium, phosphate, PTH, 25(OH)D, $1,25(OH)_2D$, FGF23, IL-1β, and IL-6 levels between coinfected, HCV-monoinfected, and HIV-monoinfected participants (Table 3).

Bone Measurements in HCV-Infected Patients, by Liver Fibrosis Stage and HCV RNA Level

In multivariable linear regression analyses among chronically HCV-infected patients, those with stage 3–4 liver fibrosis had lower mean trabecular volumetric BMD, cortical thickness, and total hip BMD *z* scores than those with stage 0–2 fibrosis (Table 4). Serum $1,25(OH)_2D$ levels were lower in patients with fibrosis stage 3–4 than in those with stage 0–2 (53.0 vs 40.7 ng/mL; $P = .03$), but there were no differences in calcium, phosphate, PTH, 25(OH)D, FGF23, IL-1β, IL-6, or TNF-α levels (data not shown). Mean cortical thickness *z* scores were lower and mean endosteal circumference *z* scores higher in participants with HCV RNA levels ≥800 000 IU/mL (Table 4).

DISCUSSION

Our study found that ART-treated HIV/HCV-coinfected women had substantially lower tibial trabecular volumetric BMD and diminished cortical dimensions with significant endocortical bone deficits, independent of appendicular lean mass

Table 4. Differences (95% CIs) in Mean Tibia pQCT and DXA Bone *z* scores in Chronically HCV-Infected Female Participants by HCV RNA Level and METAVIR Fibrosis Stage

Bone Measurement	Difference in Mean *z* Score (95% CI) for High vs Low HCV RNA[a]	*P* Value	Difference in Mean *z* Score (95% CI) for Fibrosis Stage 3–4 vs 0–2[a]	*P* Value
pQCT				
Trabecular vBMD	−0.09 (−.54 to .35)	.67	−0.46 (−.88 to −.02)	.04
Cortical vBMD	−0.40 (−.88 to .08)	.11	−0.32 (−.82 to .18)	.20
Cortical area	−0.29 (−.78 to .20)	.25	−0.49 (−.99 to .02)	.06
Cortical thickness	−0.62 (−1.11 to −.13)	.01	−0.64 (−1.14 to −.14)	.01
Periosteal circumference	0.30 (−.19 to .79)	.22	−0.26 (−.77 to .24)	.31
Endosteal circumference	0.76 (.26 to 1.28)	.004	0.47 (−.07 to 1.01)	.10
DXA				
Total hip BMD	−0.12 (−.57 to .33)	.61	−0.46 (−.92 to −.01)	.04
Femoral neck BMD	−0.01 (−.45 to .44)	.99	−0.28 (−.73 to .18)	.23

Abbreviations: BMD, bone mineral density; CI, confidence interval; DXA, dual-energy x-ray absorptiometry; HCV, hepatitis C virus; pQCT, peripheral quantitative computed tomography; vBMD, volumetric BMD.

[a] Differences were adjusted for appendicular lean mass index and fat mass index *z* scores, current smoking, and physical activity. High HCV RNA levels were defined as ≥800 000 IU/mL; low levels, as <800 000 IU/mL.

and fat mass, compared with healthy reference participants. The mean trabecular volumetric BMD z score of −0.85 and mean cortical volumetric BMD and endosteal circumference z scores of −0.67 (Table 1) indicate that the average female coinfected participant had results at the 20th and 25th percentiles for these parameters, respectively, for age, sex, and race. Furthermore, tibial trabecular volumetric BMD was lower and median TNF-α levels higher in coinfected women than in either HCV- or HIV-monoinfected women. Finally, chronically HCV-infected women with stage 3–4 liver fibrosis had lower mean trabecular volumetric BMD, cortical area, and cortical thickness z scores than HCV-infected women with stage 0–2 fibrosis.

The findings of decreased trabecular volumetric BMD and reduced tibial cortical dimensions with increased endosteal, but preserved periosteal, circumferences among coinfected women, in the absence of hepatic decompensation, support the hypothesis that HIV- and HCV-mediated chronic inflammation might contribute to the structural bone deficits observed in this group. Studies of patients with other chronic inflammatory conditions, particularly inflammatory bowel disease [20, 21] and rheumatoid arthritis [22], have demonstrated a similar pattern of trabecular bone loss and endocortical thinning. The higher levels of TNF-α among coinfected participants further suggests the contribution of chronic inflammation. This cytokine can reduce trabecular and cortical bone formation by inhibiting osteoblast differentiation, inhibiting osteoblast collagen secretion, and inducing osteoblast apoptosis [16, 17]. TNF-α can also promote accelerated trabecular and cortical bone resorption by inducing expression of receptor activator of nuclear factor kappa-B ligand (RANKL), which stimulates osteoclast activation and inhibits osteoblast apoptosis [45, 46].

Our results extend the observations of prior DXA studies in HIV/HCV patients [47, 48]. One study evaluated 179 HIV/HCV patients (35% female; 85% black) at Johns Hopkins University [47]. The prevalence of low BMD at the total hip, femoral neck, or lumbar spine among coinfected females was 19.1%, similar to that observed in the present analysis. A subsequent study at Johns Hopkins evaluated bone strength, measured by DXA-derived hip structural analysis, in 88 coinfected men compared with 289 age- and race-matched uninfected male controls [48]. Coinfected men had compromised bone strength at the narrow neck and shaft of the proximal femur, and the smaller cortical area of the shaft coupled with normal bone width (ie, periosteal circumference) was consistent with endocortical bone deficits, similar to our findings with pQCT. Finally, 2 studies used high-resolution pQCT to evaluate bone microstructure in ART-treated HIV-infected premenopausal women [49] and young men [50], and both identified trabecular and cortical bone deficits compared with HIV-uninfected controls.

Appendicular lean mass was strongly associated with all pQCT measurements. Lean mass confers beneficial effects on BMD [51]. Lower lean mass was a determinant of regional bone mineral content via DXA in ART-treated HIV-infected men [52] and was associated with reduced total hip BMD in HIV/HCV-coinfected men [48]. Reductions in lean mass may be partially reversible with ART [53], but may persist after HIV suppression, possibly owing to HIV-related inflammation [54].

We observed that HCV-infected women with advanced hepatic fibrosis/cirrhosis had lower mean trabecular volumetric BMD, cortical area, and cortical thickness than those with stage 0–2 liver fibrosis. These results suggest that advanced hepatic fibrosis, in the absence of hepatic decompensation, negatively affects bone mass and quality. This finding might reflect the prolonged impact of chronic HCV-associated systemic inflammation on bone.

Our study had several limitations. First, the cross-sectional design did not allow us to evaluate changes in pQCT measurements and other osteoporosis risk factors over time. Second, our analyses did not account for durations of HIV and chronic HCV, duration of ART use, history of injection drug/alcohol abuse, or poor nutrition, and these factors might be important contributors to bone deficits. Third, we did not have laboratory data to confirm the absence of HIV infection or viral hepatitis among reference participants. Finally, our results are generalizable only to women, but bone deficits with HIV/HCV coinfection have also been described in men [47, 48]. Future studies should examine pQCT measurements in coinfected men.

In conclusion, ART-treated HIV/HCV-coinfected women had decreased tibial trabecular volumetric BMD, significant endocortical bone loss, and increased TNF-α levels. Future studies should evaluate HIV/HCV-coinfected men, examine changes in bone structure over time, and determine whether virologic cure after HCV therapy can improve BMD and reverse bone deficits.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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