HIV Infection Is Associated With Worse Bone Material Properties, Independently of Bone Mineral Density

Robert Güerri-Fernández, MD, PhD,† Daniel Molina, MD,‡† Judit Villar-García, MD,*† Daniel Prieto-Alhambra, MD, PhD,§ Leonardo Mellibovsky, MD, PhD,‡ Xavier Nogués, MD, PhD,‡ Alicia González-Mena, MD,* Ana Guelar, MD,* Marta Trenchs-Rodríguez, MD,‖ Sabina Herrera-Fernández, MD,* Juan Pablo Horcajada, MD, PhD,* Adolfo Díez-Pérez, MD, PhD,*‡ and Hernando Knobel, MD, PhD**

Abstract: Low bone mineral density (BMD) in HIV-infected individuals has been documented in an increasing number of studies. However, it is not clear whether it is the infection itself or the treatment that causes bone impairment. Microindentation measures bone material strength (Bone Material Strength index) directly. We recruited 85 patients, 50 infected with HIV and 35 controls. Median Bone Material Strength index was 84.5 (interquartile range 83–87) in HIV-infected patients and 90 (88.5–93) in controls ($P < 0.001$). No significant differences in BMD between cases and controls at any of the sites examined (total hip, femoral neck, and lumbar spine). HIV infection is associated with bone damage, independently of BMD.

Key Words: HIV, bone material properties, BMD, microindentation

(*J Acquir Immune Defic Syndr* 2016;72:314–318)

INTRODUCTION

Low bone mineral density (BMD) and increased fracture risk in HIV-infected individuals have been documented in an increasing number of studies.[1–7] However, the mechanisms behind these associations are not clear: both the HIV infection itself and/or the toxicity of antiretroviral therapy (ART) toxicity have been implicated, but without conclusive evidence.

BMD and fracture risk have a close inverse correlation in the general population, particularly in untreated individuals.[8] However, although ~50% of women with nonvertebral fractures have a BMD T-score that exceeds the diagnostic threshold for osteoporosis (−2.5), less than half of patients who meet the criteria for osteoporosis will suffer a fracture.[9] Therefore, dual-energy x-ray absorptiometry (DXA) provides limited information on bone health, and no information about other key components of bone quality, such as bone strength, composition, or microarchitecture, which are obtained through expensive or invasive methods.[10,11]

Microindentation is a technique that quantifies a patient's bone material properties at the tissue level and can be performed in the clinical setting. This technique discriminates between patients with and without fractures,[4,12] and has been shown to detect bone tissue alterations in other situations where BMD is relatively preserved despite increased fracture risk, such as fragility fractures in patients with osteopenia,[13] diabetes mellitus,[14] or who are undergoing glucocorticoid treatment.[15]

Because the underlying mechanisms of the bone abnormalities observed in HIV-infected patients are not yet well characterized, we performed a bone microindentation study in a cohort of HIV-positive patients and a sample of age- and sex-matched controls. We hypothesized that HIV-infected patients have poorer bone tissue mechanical properties—Bone Material Strength index (BMSi) measured by microindentation. Our secondary objectives were to test for differences in BMD, markers of inflammation, and bone turnover between these groups, as well as the general safety of microindentation in this series.

PATIENTS AND METHODS

Patients

We conducted a cross-sectional study of outpatients of the Infectious Diseases Department at Hospital del Mar, Barcelona, Spain, and controls were healthy volunteers recruited from a Primary Care Center in Barcelona. The study was approved by the local Clinical Research Ethics Committee, and all participants gave written informed consent (Ethical Committee number 2013/5250/I).

All HIV-infected patients were viremic (HIV >40 copies/mL) and had no previous history of ART. Individuals were excluded from the study if they had a history of treatment with bone-active drugs, or if they had any condition that could interfere with bone metabolism, such as liver disease, alcoholism, malignancy, Cushing syndrome, hypogonadism,

Received for publication January 11, 2016; accepted February 4, 2016.

From the *Infectious Diseases Department, Hospital del Mar, Hospital del Mar Research Institute (IMIM), Barcelona, Spain; †Department of Medicine, Autonomous University of Barcelona, Barcelona, Spain; ‡Internal Medicine Department, Hospital del Mar, Hospital del Mar Research Institute, Barcelona, Spain; §Oxford NIHR Musculoskeletal Biomedical Research Unit, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, United Kingdom; and ‖Primary Care, ABS Passeig de Sant Joan, Barcelona, Catalan Institute of Health (ICS), Spain.

Supported by a grant of Fondos FEDER and Fondo de Investigaciones Sanitarias (PI13/00589) of the ISCIII-Spanish Ministry of Health.

The authors have no funding or conflicts of interest to disclose.

Correspondence to: Robert Güerri-Fernández, MD, PhD, Infectious Diseases Department, Hospital del Mar, Passeig Marítim 25-29, 08003 Barcelona, Spain (e-mail: rguerri@imim.es).

hyperthyroidism, hypopituitarism, hyperparathyroidism, chronic kidney disease, chronic obstructive pulmonary disease, hepatitis C or B, diabetes, neuropathic disease, and use of glucocorticoids, opioids, or intravenous drugs.

During the inclusion visit, we obtained a full medical and medication history, blood samples, hip and spine BMD by DXA [DXA-Hologic QDR4500SL (S/N 45329)], lateral x-ray (to rule out previous fractures) of the thoracic and lumbar spine, and microindentation test. The coefficient of variation (CV) for DXA measurements is 1.7% at the femoral neck and 1% in the spine.

Bone Microindentation Testing

Microindentation testing was performed using a handheld OsteoProbe Reference Point Indenter (Active-Life-Scientific, Santa Barbara, CA), which consists of a head unit with a displacement transducer, and an impact mechanism. After applying local anaesthesia (2% mepivacaine), a sterilized stainless steel probe with a 90° conical tip (375um diameter; <4 μm tip sharpness radius) is placed on the midpoint of the midshaft anterior tibial plateau and inserted through the soft tissue until it makes contact with the bone surface. Holding the device perpendicular to the bone surface, the operator displaces the device until, after a preload force of 10 N, the device's trigger mechanism releases a 30 N impact force. This displaces the test probe into bone, and the displacement transducer measures the indentation distance. After 8 repeated indentations separated by approximately 2 mm, the operator performs 5 additional indentations with the same probe on a cube of polymethylmethacrylate (PMMA) considered outliers, and ruled out, those measurements that were 1.5-fold the interquartile range (IQR) below or above the limits of the IQR. The software provides the BMSi, defined as 100 times the ratio between the harmonic mean indentation distance of the 5 impacts into the calibration phantom (PMMA) and that of the 8 impacts into the bone. This technique has previously been validated in humans.[12–15] The procedure takes less than 5 minutes, causes minimal discomfort to the patient, and no complications have been observed in published studies.[12–15] The coefficient of variation for microindentation is 3%.

Laboratory Tests

All measurements were performed at 8:00 AM and while fasting in all patients, to avoid variations due to circadian rhythms.

Serum concentration of specific markers was analyzed using the following assays: calcium, phosphate [Roche Diagnostics, Interassay CV (iCV) <10%]; bone turnover markers Amino propeptide of type 1 collagen (P1NP) and collagen type I cross-linked C-telopeptide (CTX) [Immunoenzymatic electrochemiluminescence, ECLIA (Roche Diagnostics) iCV <10%]; intact parathyroid (iPTH); Chemiluminescent immunoassay (CLIA) [iCV <10% (Siemens)], Serum 25-hydroxy-vitamin D (CLIA) [iCV <10%; Elecsys (Roche)]. High sensitivity C-reactive protein {CLIA [Immulite 2000 (Siemens)]}, erythrocyte sedimentation rate, beta-2 microglobulin (CLIA) [Immulite 2000 (Siemens)]. D-dimer [immunoturbidimetry (ACL TOP300)] and fibrinogen [Clauss method (ACL TOP300)].

Statistical Analysis

We compared quantitative and categorical variables between HIV-infected and control groups using 2-sample t tests and χ^2 test, respectively. We performed univariate and multivariate linear regression to test for association between BMSi and HIV status. Factors with a univariate P value of <0.20 were included in the multivariable model. We explored interactions in the HIV group between baseline BMSi and sex, age, nadir CD4 count, and time since diagnosis.

Assuming a type I and II error of 0.05 and 0.2 in a 2-sided test, respectively, 15 controls and 22 patients with HIV were required to detect a statistically significant difference of ≥5 units of BMSi. The common standard deviation was assumed to be 5, and we expected a drop-out rate of 10%. Results with $P < 0.05$ (2-tailed) were considered statistically significant. Analyses were performed using Stata/IC 13.1.

RESULTS

We recruited a total of 85 study subjects (50 HIV-infected patients and 35 controls) between January and October 2014. The time since diagnosis of infection ranged from 0 to 19 years (mean age at diagnosis, 34 years). We observed no remarkable difference in smoking, alcohol habits, and physical activity between cases and controls. Baseline characteristics of the sample are presented in Table 1.

We observed no differences in bone turnover markers. Patients with HIV had significantly lower levels of 25-hydroxy-vitamin D than controls (20.9 ng/mL vs 33.3 ng/mL; $P = 0.015$).

In contrast, levels of high sensibility C-reactive protein (0.48 mg/dL vs 0.12 mg/dL; $P = 0.005$) and erythrocyte sedimentation rate (20 mm/h vs 3 mm/h; $P = 0.0001$) were significantly higher in HIV-infected individuals than in controls; similarly for D-dimer (289.6 ng/mL vs 137.2 ng/mL; $P = 0.018$), fibrinogen (400 mg/mL vs 317 mg/mL; $P = 0.037$), and beta-2 microglobulin (2.48 mg/L vs 1.438 mg/L; $P = 0.0001$), respectively.

Compared with controls, patients with HIV had significantly lower BMSi, and thus worse bone material properties at the tissue level. Median BMSi was 84.5 (IQR 83–87) in HIV-infected patients and 90 (88.5–93) in controls ($P < 0.001$). Women had significantly lower BMSi in HIV population [85 (83–87) vs 80 (77–83); $P = 0.0004$]. These differences were not significant in controls 92 (88–96 in men) vs 89 (86–93 in women); $P = 0.07$.

The associations observed between HIV and BMSi in the univariate analyses were maintained in multivariable analysis (Table 2). In contrast, we observed no significant differences in BMD between cases and controls at any of the sites examined (total hip, femoral neck, and lumbar spine; Table 1).

We tested for prespecified interaction between HIV and microindentation with age, sex, nadir CD4 count, and time since diagnosis but found no interactions.

There were no complications in any patient during or after the microindentation procedure.

TABLE 1. Characteristics of Study Population

	HIV Negative	HIV Positive	*P*
N	35	50	
Age	33.9 (27.6 to 53.8)*	36.7 (31.7 to 46.2)	0.49
Male, n (%)	24 (68.5)	35 (70)	0.12
Weight, kg	70 (60 to 78)	68 (63 to 75)	0.48
Body mass index, kg/m^2	22.9 (20.5 to 24.6)	23.8 (21.7 to 24.9)	0.06
Smoking, n (%)			
Never	16 (45.7)	24 (48)	0.18
Former	3 (8.5)	3 (6)	0.44
Current	16 (45.8)	23 (46)	0.49
Cigarettes (pack-year)	12.8 (14.2)	10.1 (14)	0.19
Alcohol (>20 g/d)	0	0	
Family history of fracture, n (%)	4 (11)	4 (8)	0.46
Prevalent spine fractures, n (%)	0	2 (4)	0.07
Years since HIV diagnosis		2 (1 to 4)	
Nadir CD4 count, per mL		380 (267 to 480)	
Viral load at baseline, $\log_{10}$		4.2 (1.2)	
Ever met AIDS criteria, %		2 (4)	
Bone mineral density			
Femoral neck BMD, g/cm^2	0.792 (0.733 to 0.957)	0.807 (0.769 to 0.88)	0.393
Femoral neck T-score	−0.65 (−1.1 to 0.3)	−0.65 (−1.1 to −0.2)	0.502
Femoral neck Z-score	−0.6 (−0.98 to 0.4)	−0.33 (−0.93 to 0.07)	0.883
Femoral total hip BMD, g/cm^2	0.884 (0.833 to 1.06)	0.936 (0.875 to 1.056)	0.749
Femoral total hip T-score	−0.6 (−1.1 to 0.7)	−0.25 (−0.9 to 0.2)	0.840
Femoral total hip Z-score	−0.44 (−1 to 0.82)	−0.05 (−0.56 to 0.57)	0.923
Lumbar spine BMD, g/cm^2	0.980 (0.894 to 1.06)	0.978 (0.908 to 1.079)	0.413
Lumbar spine T-score	−0.65 (−1.3 to −0.2)	−0.8 (−1.3 to 0.1)	0.338
Lumbar spine Z-score	−0.53 (−1.08 to −0.08)	−0.43 (−1.08 to 0.47)	0.872
Bone microindentation (BMSi)	90 (88.5 to 93)	84.5 (83 to 87)	0.001
Bone turnover			
Amino propeptide of type 1 collagen, ng/mL	53.01 (34 to 63.86)	52.27 (39.3 to 59.73)	0.453
Bone alkaline phosphatase, μg/mL	11.8 (7.7 to 15.7)	13.4 (10.2 to 16.7)	0.145
C-telopeptide, ng/mL	0.307 (0.168 to 0.415)	0.267 (0.210 to 0.382)	0.480
Calcium metabolism			
Parathormone, pg/mL	23 (15 to 33)	22.5 (19 to 38)	0.193
25-OH vitamin D, ng/mL	33.3 (15.3 to 42.9)	20.9 (11.67 to 28.58)	0.015
Calcium, mg/dL	9.5 (9.3 to 9.8)	9.45 (9.2 to 9.6)	0.132
Phosphorus, mg/dL	3.45 (3.1 to 3.8)	3.2 (2.8 to 3.5)	0.132
Inflammation and coagulation			
High sensitivity C-reactive protein, mg/dL	0.04 (0.002 to 0.19)	0.22 (0.08 to 0.6)	0.005
Erythrocyte sedimentation rate, mm/h	3 (2 to 5)	20 (11 to 25)	0.000
D-dimer, ng/mL	137.2 (126.3 to 144.1)	289 (207 to 440)	0.018
Fibrinogen, mg/dL	317 (205 to 345)	400 (317 to 470)	0.037
Beta-2 microglobulin, mg/L	1.4 (1.36 to 1.47)	2.48 (2.03 to 2.83)	0.000

*Results are shown as median (IQR), unless indicated otherwise.

DISCUSSION

In this study, we observe poorer bone material properties in HIV-infected patients than in controls, as measured by in vivo microindentation; this effect is independent of BMD and ART. Moreover, HIV-infected patients present higher levels of markers of inflammation, and this persistent inflammatory status has previously been found to be associated with bone fragility in other conditions.[16,17]

We found that bone density (by DXA) in our series of treatment-naive patients with HIV was similar to that in age-matched, uninfected controls after adjustment for confounding factors. The main difference between ours and previous studies is that we did not observe differences between these groups in their profile of traditional risk factors (i.e., alcohol consumption, corticosteroid treatment, body mass index, tobacco use, intravenous drug use, and malnutrition). This

TABLE 2. Results of Linear Regression Analysis

	Controls	HIV+
BMSi, mean (SD)	90.99 (4.67)	85.03 (5.42)
Unadjusted*	Ref	−5.96 (−8.25 to −3.65)†
Age- and sex-adjusted	Ref	−6.53 (−8.69 to −4.37)†
Fully adjusted‡	Ref	−6.91 (−9.21 to −4.61)†

*Results are shown as β coefficient (95% CI), unless indicated otherwise.
†$P < 0.001$.
‡Adjusted for sex, age, body mass index, 25-OH vitamin, high sensitivity C-reactive protein, and fibrinogen.

absence of differences may be because the profile of patients with HIV has changed over time, in that HIV is now mainly transmitted through sexual activity, whereas in the past, the transmission mechanism was mainly drug use.[2,18–22]

Grijsen et al[20] found that HIV infection was not associated with BMD, which suggests that bone loss in HIV may begin before the HIV infection itself, because of previous exposure to risk factors.

However, although BMD is considered the gold standard for evaluating bone health, in specific clinical situations, impaired quality of bone material is the most prominent feature rather than the amount of mineral,[13–15] and bone fragility is only partially assessed by densitometry.[9] Until recently, direct mechanical testing of bone strength has been cumbersome, requiring an invasive procedure to extract bone samples for ex vivo laboratory testing. Actually, microindentation has permitted direct measurement of the tissue's mechanical performance (i.e., strength vs fragility). As reported, microindentation is able to measure some changes in material properties that are not detected by DXA.

We also found no differences in bone turnover markers despite that HIV-infected patients showed lower BMSi values, independently of BMD. Microindentation induces the separation of mineralized collagen fibrils and initiates microcracks, which are likely to be the mechanism of fracture initiation.[12] Hence, this technique measures the mechanical competence of bone tissue to resist the initiation and propagation of fractures,[4,12,13] that is independent of bone turnover. This indicates that the material properties are impaired, although the amount of mineral, as measured by BMD, and also bone turnover markers, remain at a similar level than controls.

We might speculate with the hypothesis that exists an association between bone metabolism and the immune system, which could ultimately explain our findings. HIV infection results in extensive damage to the immune system, with continuous immune activation leading to chronic inflammation.[23,24] The complex regulatory signaling network at the immuno–skeletal interface, with the potential impact of inflammatory changes, may alter bone mechanical properties by affecting bone tissue quality firstly, bone turnover, BMD, and ultimately bone fragility.[25]

Our results represent a new line of research into bone disease in the context of HIV infection. Because we can quantify a distinct dimension of bone strength, the mechanisms that underlie this phenomenon can be explored in depth and, more importantly, treatments or strategies to prevent bone loss and fractures can be developed. The long-term survival of HIV-infected individuals increases their exposure to complications such as fragility fractures. Similarly, further studies of bone material properties after initiating ART are also required.

This study has some limitations that must be taken into account. First, this is a single-center study, with a relatively limited number of patients. Second, although this technique is well validated and is receiving increasing interest in many areas, it is not yet possible to make clinical decisions based on its findings.

This study also has various strengths, such as the fact that we have been able to better isolate the impact of the infection itself, without the potential interference of ART or confounding due to lifestyle factors or concomitant diseases. The use of the microindentation technique is also an important advantage, in that it allows us to measure bone material properties directly and to capture a new aspect of bone strength.

CONCLUSIONS

In summary, for the first time, we describe in vivo measurement of bone material properties in HIV-infected patients. Microindentation captures changes in bone properties that are not measured by BMD. We found that HIV infection is associated with impaired bone material properties, independently of BMD.

REFERENCES

1. Grund B, Peng G, Gibert CL, et al. Continuous antiretroviral therapy decreases bone mineral density. *AIDS*. 2009;23:1519–1529.
2. Knobel H, Guelar A, Vallecillo G, et al. Osteopenia in HIV-infected patients: is it the disease or is it the treatment? *AIDS*. 2001;15:807–808. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11371701. Accessed February 28, 2014.
3. Brown TT, Ruppe MD, Kassner R, et al. Reduced bone mineral density in human immunodeficiency virus-infected patients and its association with increased central adiposity and postloadhyperglycemia. *J Clin Endocrinol Metab*. 2004;89:1200–1206. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15001610. Accessed February 28, 2014.
4. Güerri-Fernández RC, Nogués X, Quesada Gómez JM, et al. Microindentation vivo measurement bone tissue material properties atypical femoral fracture patients controls. *J Bone Miner Res*. 2013;28:162–168. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22887720. Accessed August 3, 2013.
5. Prieto-Alhambra D, Güerri-Fernández R, De Vries F, et al. HIV infection and its association with an excess risk of clinical fractures and : a nationwide case-control study. *J Acquir Immune Defic Syndr*. 2014. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24457634. Accessed February 28, 2014.
6. Triant VA, Brown TT, Lee H, et al. Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large U.S. healthcare system. *J Clin Endocrinol Metab*. 2008;93:3499–3504. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2567857&tool=pmcentrez&rendertype=abstract. Accessed February 24, 2014.
7. Bedimo R, Maalouf NM, Zhang S, et al. Osteoporotic fracture risk associated with cumulative exposure to tenofovir and other antiretroviral agents. *AIDS*. 2012;26:825–831. Available at: http://www.ncbi.nlm.nih.gov/pubmed/22301411. Accessed February 22, 2014.
8. Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *BMJ*. 1996;312:1254–1259. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2351094&tool=pmcentrez&rendertype=abstract. Accessed March 27, 2015.

9. Cummings SR, Bates D, Black DM. Clinical use of bone densitometry: scientific review. *JAMA*. 2002;288:1889–1897. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12377088. Accessed July 5, 2015.
10. Amin S, Kopperdhal DL, Melton LJ, et al. Association of hip strength estimates by finite-element analysis with fractures in women and men. *J Bone Miner Res.* 2011;26:1593–1600. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3201782&tool=pmcentrez&rendertype=abstract. Accessed July 5, 2015.
11. Silva BC, Leslie WD, Resch H, et al. Trabecular bone score: a non-invasive analytical method based upon the DXA image. *J Bone Miner Res.* 2014;29:518–530. Available at: http://www.ncbi.nlm.nih.gov/pubmed/24443324. Accessed February 27, 2015.
12. Diez-Perez A, Güerri R, Nogues X, et al. Microindentation for in vivo measurement of bone tissue mechanical properties in humans. *J Bone Miner Res.* 2010;25:1877–1885. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3153354&tool=pmcentrez&rendertype=abstract. Accessed May 14, 2015.
13. Malgo F, Hamdy NAT, Papapoulos SE, et al. Bone material strength as measured by microindentation in vivo is decreased in patients with fragility fractures independently of bone mineral density. *J Clin Endocrinol Metab.* 2015;100:2039–2045. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25768670. Accessed July 5, 2015.
14. Farr JN, Drake MT, Amin S, et al. In vivo assessment of bone quality in postmenopausal women with type 2 diabetes. *J Bone Miner Res.* 2014;29:787–795. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3961509&tool=pmcentrez&rendertype=abstract. Accessed June 29, 2015.
15. Mellibovsky L, Prieto-Alhambra D, Mellibovsky F, et al. Bone tissue properties measurement by reference point indentation in glucocorticoid-induced osteoporosis. *J Bone Miner Res.* 2015. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25736591. Accessed July 5, 2015.
16. Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med.* 2001;344:907–916. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11259725. Accessed June 15, 2015.
17. Hoes JN, Bultink IEM, Lems WF. Management of osteoporosis in rheumatoid arthritis patients. *Expert Opin Pharmacother.* 2015;16:559–571. Available at: http://www.ncbi.nlm.nih.gov/pubmed/25626121. Accessed Jul 5, 2105.
18. Güerri-Fernandez R, Vestergaard P, Carbonell C, et al. HIV infection is strongly associated with hip fracture risk, independently of age, gender, and comorbidities: a population-based cohort study. *J Bone Miner Res.* 2013;28:1259–1263. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23362011. Accessed February 28, 2014.
19. Womack JA, Goulet JL, Gibert C, et al. Increased risk of fragility fractures among HIV infected compared to uninfected male veterans. *PLoS One*. 2011;6:e17217. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3040233&tool=pmcentrez&rendertype=abstract. Accessed February 16, 2014.
20. Grijsen ML, Vrouenraets SME, Wit FWNM, et al. Low bone mineral density, regardless of HIV status, in men who have sex with men. *J Infect Dis.* 2013;207:386–391. Available at: http://www.ncbi.nlm.nih.gov/pubmed/23148289. Accessed May 20, 2015.
21. Bolland MJ, Grey AB, Gamble GD, et al. CLINICAL Review #: low body weight mediates the relationship between HIV infection and low bone mineral density: a meta-analysis. *J Clin Endocrinol Metab.* 2007;92:4522–4528. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17925333. Accessed February 25, 2014.
22. Stall R, Paul JP, Greenwood G, et al. Alcohol use, drug use and alcohol-related problems among men who have sex with men: the Urban Men's Health Study. *Addiction*. 2001;96:1589–1601. Available at: http://www.ncbi.nlm.nih.gov/pubmed/11784456. Accessed July 5, 2015.
23. Vikulina T, Fan X, Yamaguchi M, Roser-Page S, et al. Alterations in the immuno-skeletal interface drive bone destruction in HIV-1 transgenic rats. *Proc Natl Acad Sci.* 2010;107:13848–13853. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2922243&tool=pmcentrez&rendertype=abstract. Accessed July 5, 2015.
24. Moir S, Ho J, Malaspina A, et al. Evidence for HIV-associated B cell exhaustion in a dysfunctional memory B cell compartment in HIV-infected viremic individuals. *J Exp Med.* 2008;205:1797–1805. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2525604&tool=pmcentrez&rendertype=abstract. Accessed April 1, 2015.
25. Walker Harris V, Brown TT. Bone loss in the HIV-infected patient: evidence, clinical implications, and treatment strategies. *J Infect Dis.* 2012;205(suppl 3):S391–S398. Available at: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3415950&tool=pmcentrez&rendertype=abstract. Accessed July 5, 2015.