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# Change in circulating undercarboxylated osteocalcin (ucOCN) is associated with fat accumulation in HIV-seropositive women

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## Abstract

**Background:** Bone mineral density (BMD) loss and fat accumulation are common in people living with HIV (PLWH). The bone-derived hormone, undercarboxylated osteocalcin (ucOCN) regulates fat metabolism. We investigated the relationship between ucOCN change and body fat change among perimenopausal/postmenopausal HIV-seronegative and HIV-seropositive women on long-term antiretrovirals.

**Methods:** Perimenopausal and postmenopausal women enrolled in the Women's Interagency HIV Study (WIHS) MSK sub-study underwent trunk and total fat assessment via dual x-ray absorptiometry (DXA) at study enrollment (index visit) and again two years later. Circulating ucOCN and carboxylated osteocalcin (cOCN) were also measured at the index and two-year visits. The correlation between the two-year change in ucOCN and cOCN and change in trunk and total fat was assessed as a function of HIV-serostatus using linear regression modeling. Multivariate linear regression assessed the association between ucOCN and cOCN change and total and trunk fat change after adjusting for sociodemographic variables. Linear regression models restricted to

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HIV-seropositive women were performed to examine the contributions of HIV specific factors (index CD4 count, viral load, and cART use) on the associations.

**Results:** Increased ucOCN over the two-year follow-up was associated with less trunk and total fat accumulation in models adjusting for HIV-serostatus and participants sociodemographics, while there was no association with cOCN and the fat parameters. None of the HIV-specific factors evaluated influenced the association between ucOCN and fat parameters.

**Conclusion:** The current study suggests that increases in ucOCN are associated with decreased fat accumulation in HIV-seronegative and HIV-seropositive postmenopausal women on long-term antiretroviral therapy.

### Keywords

Osteocalcin; Bone; Body Composition; HIV; cART

## Introduction

The advent of combined antiretroviral therapy (cART) has improved the life expectancy of people living with HIV (PLWH)[1]. However, cART treated PLWH are at an increased risk for many comorbidities including osteoporosis, or low bone mineral density (BMD)[2,3]. PLWH on cART are also at risk for the development of obesity, abnormal body fat redistribution, and visceral or central fat accumulation[4-6]. These body composition changes increase the risk of a variety of adverse health outcomes, including heart disease, stroke, and diabetes for PLWH[7-9]. The high prevalence and consequences of these comorbidities has led to increased efforts to understand the pathogenesis of these diseases in PLWH[10].

Evidence exists for a hormonal cross-talk between bone and fat organ systems[11,12], suggesting a link between osteoporosis development and body composition changes. For example, greater fat mass is associated with greater BMD, particularly in postmenopausal women[13]. Also, body composition is an independent predictor of BMD in a cohort that included women living with HIV[14]. Bone has also been shown to directly influence fat metabolism through the production of osteocalcin[15,16], a bone-derived protein that circulates either as γ-carboxylated vitamin K dependent calcium binding protein (cOCN) or undercarboxylated osteocalcin (ucOCN). ucOCN specifically has hormonal functions including the regulation of fat metabolism directly by altering adipocyte signaling and indirectly by stimulating insulin secretion in pancreatic β-cells[17,18]. In preclinical mouse models, administration of recombinant ucOCN leads to reduced fat mass accumulation and attenuated weight gain in response to a high fat diet[18]. While the association between circulating ucOCN and body fat has been conflicting in postmenopausal women[19,20], ucOCN has been associated with lower percent body fat in individuals with both type I[21] and type II diabetes mellitus,[22] and with lower body mass index (BMI)[23] and waist circumference[24] in aging cohorts. Despite, the established association between bone-derived ucOCN and fat metabolism, the relationship between change in ucOCN and fat accumulation in PLWH on long-term cART is not known.

Several studies have reported changes in osteocalcin levels in PLWH after seroconversion and cART treatment[25-29], however they have primarily focused on total osteocalcin, which includes both cOCNand ucOCN isoforms. Total osteocalcin is generally used as a marker of bone turnover rather than an indicator of hormonal function. Hirakawa et al demonstrated that ucOCN levels increase following cART initiation[30], but did not assess body fat. Therefore, the goal of this study was to determine the association between changes in ucOCN and changes in body fat among a cohort of peri- and post-menopausal HIV-seropositive and seronegative women and to determine whether this association varies according to HIV-serostatus.

Both body fat redistribution and increased bone remodeling are common during the menopausal transition[31,32] and ucOCN is released into circulation during bone remodeling[17,18] Therefore, to take advantage of the longitudinal study design and to reduce confounding bias associated with between subjects correlation[33,34], we evaluated the effect of the two-year within subjects change of both ucOCN and trunk and total fat. Due to the associations between body composition and bone health and the role of ucOCN in fat and energy metabolism in a variety of cohorts, we hypothesize that ucOCN change is prognostic of changes in body fat in both HIV-seronegative and HIV-seropositive women.

## Methods

### Study Participants:

Participants were enrolled at one of three study locations within the Women's Interagency HIV study (WIHS); Bronx, San Francisco, or Chicago. WIHS is an ongoing multisite longitudinal cohort study of women with and at risk for HIV infection that has been recruited in waves since its initiation in 1993. More information on the WIHS can be found here[35]. A total of 244 women (152 HIV-seropositive and 92 HIV-seronegative) were enrolled in the present musculoskeletal (MSK) sub-study. The MSK cohort included HIV-seropositive women on long-term cART (at least 2 years) and a matched cohort of HIV-seronegative women. The eligibility criteria for HIV-seropositive women included having a CD4 count >100 cells/ μLmeasured prior to enrollment in the MSK sub-study, and consistent ART use for at least 12 months without missing more than 2.5 months of therapy for the prior year. Participants had to be less than 264 pounds with a height of 6'1'' or below as per DXA manufacture's criteria for weight and height. Of the total 244 women initiatlly enrolled, we included 46 HIV-seronegative and 76 seropositive women that completed both a MSK enrollment, here after defined as (index visit), and 2 year follow-up study visit and had accompanied repository serum aliquots to test for biomarkers. At the index visit, study participants were between 40-60 years of age. The reproductive stage was categorized based on self-reported responses to the Study of Women's Health Across the Nation (SWAN) study questions with definitions as follows: early perimenopause (at least 1 menstrual period in the last 3 months with some changes in the regularity over the last 12 months), late perimenopause (no bleeding in between 3-11 of the last 12 months), or early postmenopausal (no bleeding for > 1 but < 5 years)[36]. The substudy design was limited to women self-reporting as either perimenopausal or postmenopausal at the index visit. Therefore, as described previously[37], we categorized study participants into early

perimenopause and postmenopause (including late perimenopause and early postmenopause). During the index visit, viral load was determined by circulating HIV RNA and categorized as either undetected (≤ 20 copies/mL) or detected (> 20 copies/mL). Index visit CD4 count was measured based on cell/mL. cART use was categorized based on class of self-reported antiretroviral drugs; nucleoside reverse transcriptase inhibitors; (NRTI), protease inhibitors (PI), and integrase strand transfer inhibitor (INSTI).

### Bone Mineral Density Measurements:

All participants underwent whole body dual energy x-ray absorptiometry (DXA) scans using Lunar Prodigy densitometers (GE Medical Systems, Madison WI) at the index visit and again two years later. Scans were read centrally at the Image Analysis Lab (New York, NY) and included total and trunk fat, as well as, regional BMD at the lumbar spine, total hip, femoral neck, distal and ultradistal radius. Height and weight were measured using a stadiometer and balance beam scale at each WIHS research visit and BMI was calculated from these metrics.

### Blood Biomarkers:

Blood samples were collected at the index visit and again two years later using sodium citrate coated tubes and separated into plasma aliquots and then stored at −80 °C. Measurements of N-terminal propeptide of procollagen type 1 (P1NP; RIA; IDS, Scottsdale, AZ [<3.3% intra and < 5.1 inter-assay CV]) and C-telopeptide of type 1 collagen (CTX, ELISA, IDS Scottsdale, AZ [<2.9% intra and <11% intra-assay CV]) at the index visit were made at the Columbia University Irving Medical Center Biomarker Laboratory. Index and follow-up measurements of undercarboxylated (ucOCN) and carboxylated osteocalcin (cOCN) were batch-analyzed at the Chicago WIHS/Hektoen Institute of Medicine Laboratory using enzyme-linked immunosorbent assay (ELISA, Takara Shuzo Co., Otsu, Shiga, Japan, ucOCN [<5% intra and <10% inter-assay CV] and uOCN [<5% intra and <3% inter-assay CV]).

### Statistical Analysis:

The primary outcomes of interest were two-year change in trunk and total fat, and the primary exposure of interest was two-year change in circulating ucOCN. To understand the contribution of skeletal changes to ucOCN change, we also assessed the change in cOCN and the DXA-derived BMD as a secondary outcome. Sociodemographic and clinical covariates including age (continuous), race (Black, White, Other), WIHS site (Bronx, San Francisco, Chicago), menopause status (early perimenopause, postmenopause) hepatitis C virus (HCV) status (categorized as positive by either antibody or RNA), smoking status at index visit (current, former/never), and HIV-serostatus were collected at the index visit. Changes in ucOCN, cOCN and in trunk and total fat were the arithmetic difference from subtracting the index visit measure from the two-year follow-up measure. Change in ucOCN and cOCN, trunk and total fat, and regional BMD were compared using rank tests. Linear regression models tested the associations between two-year change in ucOCN and cOCN with two-year change in trunk and total fat and regional BMD as a function of HIV-serostatus. Models were also run to test the ucOCN and cOCN change by HIV-serostatus interaction. Multivariate linear models tested the associations between two-year change in

ucOCN and cOCN and two-year change in both trunk and total fat after adjusting for sociodemographic and clinical covariates. Separate models were further adjusted for the index measures of trunk and total fat. To test the effects of HIV-specific covariates at the index visit among HIV seropositive women; CD4, viral load, and cART class (testing cART, PI, and INSTI use separately) on the associations between ucOCN and cOCN change and trunk and total fat change, linear regression models with change in ucOCN as the independent variable and change in trunk and total fat as the dependent variable were run.

## Results

Study participants had a mean age of 48.7 (±5) years and BMI of 29.1 (±5.7) kg/m$^2$. 76% were Black, 12% White, and 12% identified as other (Table 1). 39% were categorized as early perimenopausal and the remaining 61% were characterized as late perimenopause or early postmenopause based on self-reported criteria. Enrollment across the three sites was relatively balanced but reflected differences in parent study size; 38% from the Bronx site, 33% from San Francisco, and 30% from Chicago. Within the HIV-seropositive cohort, the median index CD4 count was 578 (439, 748) cells/mL. Nearly all women (97%) were taking cART at the index visit, with the remaining 3% on either monotherapy or unknown. Within the cART-treated cohort, 96% received a regimen that included an NRTI, 49% included an NNRTI, 49% a PI, and 11% an INSTI, and 64% were undetectable for HIV virus (Table 1). ucOCN levels at the index and follow-up visit were not significantly different between HIV-seronegative and HIV-seropositive women (Table 2). The two-year change in ucOCN was slightly greater in HIV-seropositive women (1.94±2.32 ng/mL at the index visit to 2.32±3.04 ng/mL at the two-year follow up) compared to HIV-seronegative women (2.06±2.42 ng/mL to 2.24±2.94 ng/mL) but the difference was not statistically significant. cOCN was greater in HIV-seropositive women when compared to HIV-seronegative women at the baseline (4.94±4.93 vs. 3.39±1.75 ng/mL; p=0.02) and follow-up visits (5.37±5.25 vs. 3.93±2.29 ng/mL; p=0.04). However, the two-year change in cOCN was not different between HIV-seropositive and seronegative women. Index and follow-up measures of trunk and total fat did not differ by HIV-serostatus, nor did the two-year change in trunk and total fat (Table 2). The two-year loss in BMD at the ultradistal radius was significantly greater in HIV-seropositive (0.46±0.08 g/cm$^2$ to 0.45±0.05 g/cm$^2$) compared to HIV-seronegative women (0.44±0.09 g/cm$^2$ to 0.41±0.10 g/cm$^2$, p=0.011). There were no qualitatively or statistically significant differences in the BMD change at all other skeletal sites (Supplemental Table 1).

The two-year increase in ucOCN was associated with a two-year decrease in both trunk (p=0.012) and total fat (p=0.008) accumulation in HIV-seropositive women. A similar relationship was observed among the HIV-seronegative women, although it did not reach statistical significance (Table 2). There was no association between two-year cOCN change and trunk or total fat change in either HIV-seropositive or seronegative groups (Table 2). The two-year change in ucOCN and the two-year change in BMD at any of the skeletal sites were not statistically significant in either HIV-seropositive or seronegative women (Supplemental Table 1). After adjusting for sociodemographic and clinical variables and HIV-serostatus, the increase in ucOCN was a significantly associated with reduced accumulation of both total (p=0.050) and trunk fat (p=0.030) (Table 3). Specifically, each one-unit (ng/mL) increase in ucOCN over the two-year follow-up was associated with

0.58kg less total fat and 0.36kg less trunk fat gain. Despite the qualitatively greater association noted in HIV-seropositive women, HIV-serostatus was not statistically significant in these fully adjusted models, nor was the HIV-serostatus by ucOCN change interaction, suggesting that the association did not vary significantly by HIV-serostatus (data not shown). In parallel analysis, cOCN change was not associated with trunk or total fat change in adjusted models (Supplemental Tables 3). The relationship between ucOCN change trunk and total fat gain remained significant in models adjusting for trunk and total fat measures at the index visit (Supplemental Table 3). In models including only HIV-seropositive women, the associations between the two-year change in ucOCN and the two-year change in trunk and total fat were not affected by any of the HIV-specific potential confounding variables, including both PI and INSTI use (Supplemental Table 4).

## Discussion

The current study investigated the relationship between changes in circulating ucOCN and fat in HIV-seronegative and HIV-seropositive perimenopause/postmenopausal women on long-term cART. We observed that the two-year increase in ucOCN was negatively associated with the two-year decrease in both trunk and total fat gain. This relationship was qualitatively greater in HIV-seropositive women. However, the association between two-year change in ucOCN and that for both trunk and total fat remained statistically significant in multivariate linear models including all women and adjusting for HIV-serostatus, along with a variety of sociodemographic and clinical variables that have been implicated with body composition changes, such as age, smoking, and menopause status. The associations of change in ucCON with those of trunk and total fat were unaffected by the addition of HIV-specific variables such as viral load, CD4 count, and the class of cART used. These results suggest an important role for bone as an endocrine organ in the regulation of fat gain in HIV-seropositive women.

Total fat and lean mass are associated with BMD in cohorts including PLWH[14], and early fat gain after cART initiation is associated with BMD loss[38], suggesting a bone-fat cross talk in PLWH. Bone has an endocrine function with the ability to directly regulate fat tissue through the production of osteocalcin[12]. Osteocalcin is the most abundant non-collagen protein in bone and is produced almost exclusively by osteoblasts[39]. Circulating osteocalcin levels are thus commonly used as a biomarker of bone remodeling in the diagnosis of osteoporosis and in the monitoring of osteoporosis therapy response[40]. During bone formation, osteocalcin is carboxylated via a vitamin K-dependent mechanism, which increases the binding hydroxyapatite bone specificity of osteocalcin[41]. The carboxylated isoform of osteocalcin (cOCN), is therefore generally reflective of the amount of active bone formation[42]. In the current study we assessed the circulating levels of cOCN and found elevated levels in HIV-seropositive women when compared to HIV-seronegatives at both study visits, which is likely reflective of an overall increase in skeletal remodeling as evidenced by the reduced BMD in HIV-seropositive women at both index and follow-up visits, as well as the greater BMD loss over the two-year follow-up noted in the ultradistal radius. Despite the evidence of an active bone remodeling process, the associations between osteocalcin and fat were only present when the undercarboxylated isoform (ucOCN) was used as the independent variable. In addition to its role in the development and maintenance

of the extracellular matrix[43], recent data has identified undercarboxylated isoform of osteocalcin, ucOCN, as a mediator of fat metabolism[17,18,44].

The preponderance of data demonstrating a link between ucOCN and body fat is derived from mouse studies, where osteocalcin gain of function mice or obese mice treated with recombinant ucOCN are protected from fat accumulation[45-47]. The data in human subjects have been mixed, with some evidence that the associations are affected by the race, sex, baseline BMI, and the metabolic status of the study cohort[48-50]. For example, while the circulating levels of ucOCN are negatively associated with body fat in both type 1[21] and type 2 diabetes mellitus[22], the associations in postmenopausal women have been mixed. Specifically, Centi et al[19] found no association between ucOCN and body fat in a group of postmenopausal women undergoing a weight loss program, while Schafer et al[20] found a negative association between the three-month change in ucOCN and the 12-month change in body fat in a cohort of postmenopausal women undergoing osteoporosis treatment. In the current study, we report a significant negative association between the two-year change in ucOCN and body fat that is at least qualitatively greater in HIV-seropositive perimenopause/post-menopausal women when compared to women who are HIV-seronegative.

The qualitatively greater association between change in ucOCN and change in trunk and total fat in HIV-seropositive women on cART may point to HIV-specific factors influencing bone-fat cross talk. cART initiation is one potential driver of both bone loss[51] and fat gain[52]. However, the eligibility for the current study was limited to women with HIV on cART treatment for at least two years prior to enrollment and hence we were not able to look at changes due to cART initiation and early usage. Furthermore, body composition stabilizes in women on long-term cART treatment[14]. Similarly, the rate of BMD decline appears to slow after the first two-years of cART initiation[53], which is consistent with the current study wherein we report largely no difference in the two-year change in BMD according to HIV-serostatus.

The influence of cART on both bone and fat is dependent on the class of antiretroviral used. Specifically, both PI and INSTI use are associated with the largest fat changes in PLWH[52] and, while PIs exhibit deleterious effects on bone, INSTIs appear to be more bone neutral[54]. Further, ucOCN concentrations have been reported to differ between PI and INSTI-treated patients, with PI initiation leading to significantly greater ucOCN levels after 1 year when compared to the INSTI, raltegravir[55]. Yet in the current study, the addition of either PI or INSTI use as covariates did not influence the associations between ucOCN change and trunk and total fat, which may be due to the relatively long duration of cART use (>two-years) of participants under study. Further, HIV-seropositive women in the current study had relatively stable HIV infection; with high CD4 cell counts (compared to historical levels among HIV-seropositive women) at the index visit, and 65% had undetectable viral loads. Future work is needed to determine whether the qualitatively larger associations between ucOCN and fat parameters noted in HIV-seropositive women are statistically significant in larger cohorts to determine whether the difference in the associations are due to factors related to ongoing HIV infection or due to cART.

As described above, the carboxylation of osteocalcin is a vitamin K-dependent process[41]., therefore several studies have aimed to manipulate osteocalcin carboxylation state via dietary supplements. Vitamin K supplementation[56,57], calcium supplementation[58], and a green leafy diet intervention[59] have all been reported to increase the carboxylation state of circulating osteocalcin. Yet despite these effects on osteocalcin carboxylation, only vitamin K2 supplementation through menaquinone-7 treatment, has been reported to reduce body fat[57]. It is unclear why various dietary supplementations have not improved body composition, but it is worth noting that the three studies that have investigated body fat in response to osteocalcin carboxylation alterations have recruited community dwelling elderly participants[56,5758]. To date, it is unclear whether any of these interventions would affect body fat in PLWH specifically and further research aimed at understanding whether the associations between ucOCN and body fat in PWLH is affected by either vitamin K or diet is necessary.

As far as we know, the current study is the first to describe the inverse relationship between change in circulating ucOCN and change in body fat in HIV-seropositive women on cART. Among its strengths were the well-matched cohort of HIV-seropositive and seronegative women and the longitudinal (two-year) assessments of body composition and ucOCN. Among its weaknesses are a low number of study participants which was due to a limited number of participants who completed both index visit measurements as well as a second measure at the two year follow up visit. The study also focused only on a single sex cohort of peri- or post-menopausal HIV-seropositive women on long term cART; these findings may not be generalizable to men or younger pre-menopausal women.

## Conclusions:

The current study demonstrates that the two-year increase in circulating ucOCN is associated with less fat accumulation in both HIV-seronegative and HIV-seropositive women on long-term antiretroviral therapy. The associations were qualitatively greater in HIV-seropositive women and robust to a variety of socio-demographic and clinical confounding variables, including age, menopausal stage, and smoking status. Our findings suggest a potentially novel bone-fat hormonal crosstalk in women living with HIV on cART.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Acronyms:

| | |
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| **PLWH** | People living with HIV |
| **BMD** | bone mineral density |
| **PI** | protease inhibitors |
| **INSTI** | integrase strand transfer inhibitor |
| **NRTIs** | nucleoside reverse transcriptase inhibitors |
| **NNRTIs** | non-nucleoside reverse transcriptase inhibitors |
| **P1NP** | Procollagen type 1 N-terminal propeptide |
| **CTX** | C-telopeptide of type I collagen |

## References

1. Smit M et al. Future challenges for clinical care of an ageing population infected with HIV: a modelling study. The Lancet.Infectious diseases 15, 810–818, doi:S1473-3099(15)00056-0 [pii] (2015). [PubMed: 26070969]
2. McComsey GA et al. Bone disease in HIV infection: a practical review and recommendations for HIV care providers. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 51, 937–946, doi:10.1086/656412 (2010). [PubMed: 20839968]
3. Compston J HIV infection and osteoporosis. BoneKEy reports 4, 636, doi:10.1038/bonekey.2015.3 [doi] (2015). [PubMed: 25709813]
4. Debroy P et al. Progressive increases in fat mass occur in adults living with HIV on antiretroviral therapy, but patterns differ by sex and anatomic depot. The Journal of antimicrobial chemotherapy 74, 1028–1034, doi:10.1093/jac/dky551 [doi] (2019). [PubMed: 30668716]
5. Koethe JR et al. Rising Obesity Prevalence and Weight Gain Among Adults Starting Antiretroviral Therapy in the United States and Canada. AIDS Research and Human Retroviruses 32, 50–58, doi:10.1089/aid.2015.0147 [doi] (2016). [PubMed: 26352511]
6. Grant PM et al. Long-term body composition changes in antiretroviral-treated HIV-infected individuals. Aids 30, 2805–2813, doi:10.1097/qad.0000000000001248 (2016). [PubMed: 27662545]
7. Stanley TL & Grinspoon SK Body composition and metabolic changes in HIV-infected patients. The Journal of infectious diseases 205 Suppl 3, S383–S390, doi:10.1093/infdis/jis205 (2012). [PubMed: 22577212]

8. Nansseu JR, Bigna JJ, Kaze AD & Noubiap JJ Incidence and Risk Factors for Prediabetes and Diabetes Mellitus Among HIV-infected Adults on Antiretroviral Therapy: A Systematic Review and Meta-analysis. Epidemiology (Cambridge, Mass.) 29, 431–441, doi:10.1097/EDE.0000000000000815 [doi] (2018).
9. Falutz J Management of fat accumulation in patients with HIV infection. Current HIV/AIDS reports 8, 200–208, doi:10.1007/s11904-011-0087-3 [doi] (2011). [PubMed: 21739217]
10. Lerner AM, Eisinger RW & Fauci AS Comorbidities in Persons With HIV: The Lingering Challenge. Jama 323, 19–20, doi:10.1001/jama.2019.19775 (2020).
11. Kirk B, Feehan J, Lombardi G & Duque G Muscle, Bone, and Fat Crosstalk: the Biological Role of Myokines, Osteokines, and Adipokines. Current osteoporosis reports, doi:10.1007/s11914-020-00599-y (2020).
12. Guntur AR & Rosen CJ Bone as an endocrine organ. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists 18, 758–762, doi:10.4158/EP12141.RA [doi] (2012).
13. Ho-Pham LT, Nguyen UDT & Nguyen TV Association Between Lean Mass, Fat Mass, and Bone Mineral Density: A Meta-analysis. The Journal of Clinical Endocrinology & Metabolism 99, 30–38, doi:10.1210/jc.2013-3190 (2014). [PubMed: 24384013]
14. Sharma A et al. Association of regional body composition with bone mineral density in HIV-infected and HIV-uninfected women: women's interagency HIV study. Journal of acquired immune deficiency syndromes (1999) 61, 469–476, doi:10.1097/QAI.0b013e31826cba6c (2012). [PubMed: 22895436]
15. Kanazawa I Osteocalcin as a hormone regulating glucose metabolism. World J Diabetes 6, 1345–1354, doi:10.4239/wjd.v6.i18.1345 (2015). [PubMed: 26722618]
16. Moser SC & van der Eerden BCJ Osteocalcin-A Versatile Bone-Derived Hormone. Frontiers in endocrinology 9, 794–794, doi:10.3389/fendo.2018.00794 (2019). [PubMed: 30687236]
17. Guedes JAC, Esteves JV, Morais MR, Zorn TM & Furuya DT Osteocalcin improves insulin resistance and inflammation in obese mice: Participation of white adipose tissue and bone. Bone 115, 68–82, doi:S8756-3282(17)30439-8 [pii] (2018). [PubMed: 29183784]
18. Ferron M, Hinoi E, Karsenty G & Ducy P Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proceedings of the National Academy of Sciences of the United States of America 105, 5266–5270, doi:10.1073/pnas.0711119105 [doi] (2008). [PubMed: 18362359]
19. Centi AJ et al. Osteocalcin carboxylation is not associated with body weight or percent fat changes during weight loss in post-menopausal women. Endocrine 50, 627–632, doi:10.1007/s12020-015-0618-6 (2015). [PubMed: 25963022]
20. Schafer AL et al. Change in undercarboxylated osteocalcin is associated with changes in body weight, fat mass, and adiponectin: parathyroid hormone (1-84) or alendronate therapy in postmenopausal women with osteoporosis (the PaTH study). J Clin Endocrinol Metab 96, E1982–1989, doi:10.1210/jc.2011-0587 (2011). [PubMed: 21994958]
21. Takashi Y et al. Circulating osteocalcin as a bone-derived hormone is inversely correlated with body fat in patients with type 1 diabetes. PloS one 14, e0216416, doi:10.1371/journal.pone.0216416 [doi] (2019). [PubMed: 31050684]
22. Kanazawa I et al. Serum undercarboxylated osteocalcin was inversely associated with plasma glucose level and fat mass in type 2 diabetes mellitus. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 22, 187–194, doi:10.1007/s00198-010-1184-7 [doi] (2011).
23. Saleem U, Mosley TH Jr. & Kullo IJ Serum osteocalcin is associated with measures of insulin resistance, adipokine levels, and the presence of metabolic syndrome. Arteriosclerosis, Thrombosis, and Vascular Biology 30, 1474–1478, doi:10.1161/ATVBAHA.110.204859 [doi] (2010).
24. Yeap BB et al. Reduced serum total osteocalcin is associated with metabolic syndrome in older men via waist circumference, hyperglycemia, and triglyceride levels. European journal of endocrinology 163, 265–272, doi:10.1530/EJE-10-0414 [doi] (2010). [PubMed: 20501596]

25. Brown TT et al. Reduced bone mineral density in human immunodeficiency virus-infected patients and its association with increased central adiposity and postload hyperglycemia. J Clin Endocrinol Metab 89, 1200–1206, doi:10.1210/jc.2003-031506 (2004). [PubMed: 15001610]
26. Slama L et al. Changes in bone turnover markers with HIV seroconversion and ART initiation. The Journal of antimicrobial chemotherapy 72, 1456–1461, doi:10.1093/jac/dkx011 [doi] (2017). [PubMed: 28175307]
27. de Menezes Barbosa EGM et al. Impact of antiretroviral therapy on bone metabolism markers in HIV-seropositive patients. Bone 57, 62–67, doi:10.1016/j.bone.2013.07.019 (2013). [PubMed: 23891908]
28. van Vonderen MG et al. First line zidovudine/lamivudine/lopinavir/ritonavir leads to greater bone loss compared to nevirapine/lopinavir/ritonavir. AIDS (London, England) 23, 1367–1376, doi:10.1097/QAD.0b013e32832c4947 [doi] (2009).
29. Shiau S et al. Bone turnover markers in children living with HIV remaining on ritonavir-boosted lopinavir or switching to efavirenz. Bone 138, 115500, doi:10.1016/j.bone.2020.115500 (2020). [PubMed: 32590137]
30. Hirakawa H et al. Antiretroviral Therapy Containing HIV Protease Inhibitors Enhances Fracture Risk by Impairing Osteoblast Differentiation and Bone Quality. The Journal of infectious diseases 215, 1893–1897, doi:10.1093/infdis/jix246 [doi] (2017). [PubMed: 28525596]
31. Ji M-X & Yu Q Primary osteoporosis in postmenopausal women. Chronic Dis Transl Med 1, 9–13, doi:10.1016/j.cdtm.2015.02.006 (2015). [PubMed: 29062981]
32. Karvonen-Gutierrez C & Kim C Association of Mid-Life Changes in Body Size, Body Composition and Obesity Status with the Menopausal Transition. Healthcare (Basel) 4, 42, doi:10.3390/healthcare4030042 (2016).
33. Hoover DR, Shi Q, Burstyn I & Anastos K Repeated Measures Regression in Laboratory, Clinical and Environmental Research: Common Misconceptions in the Matter of Different Within- and between-Subject Slopes. Int J Environ Res Public Health 16, 504, doi:10.3390/ijerph16030504 (2019).
34. Scott AJ & Holt D The Effect of Two-Stage Sampling on Ordinary Least Squares Methods. Journal of the American Statistical Association 77, 848–854, doi:10.2307/2287317 (1982).
35. Barkan SE et al. The Women's Interagency HIV Study. WIHS Collaborative Study Group. Epidemiology (Cambridge, Mass.) 9, 117–125 (1998).
36. Finkelstein JS et al. Bone mineral density changes during the menopause transition in a multiethnic cohort of women. The Journal of clinical endocrinology and metabolism 93, 861–868, doi:jc.2007-1876 [pii] (2008). [PubMed: 18160467]
37. Ross RD et al. Vol. 12 100279 (2020).
38. Bonnet E et al. Early loss of bone mineral density is correlated with a gain of fat mass in patients starting a protease inhibitor containing regimen: the prospective Lipotrip study. BMC infectious diseases 13, 293-2334–2313-2293, doi:10.1186/1471-2334-13-293 [doi] (2013). [PubMed: 23809140]
39. Hauschka PV, Lian JB, Cole DE & Gundberg CM Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. Physiol Rev 69, 990–1047 (1989). [PubMed: 2664828]
40. Garnero P Biomarkers for osteoporosis management: utility in diagnosis, fracture risk prediction and therapy monitoring. Mol.Diagn.Ther 12, 157–170 (2008). [PubMed: 18510379]
41. Gundberg CM, Lian JB & Booth SL Vitamin K-dependent carboxylation of osteocalcin: friend or foe? Advances in nutrition (Bethesda, Md.) 3, 149–157, doi:10.3945/an.112.001834 (2012).
42. Kuo TR & Chen CH Bone biomarker for the clinical assessment of osteoporosis: recent developments and future perspectives. Biomarker research 5, 18, doi:10.1186/s40364-017-0097-4 (2017). [PubMed: 28529755]
43. Poundarik AA et al. Dilatational band formation in bone. Proc.Natl.Acad.Sci.U.S.A 109, 19178–19183 (2012). [PubMed: 23129653]
44. Moser SC & van der Eerden BCJ Osteocalcin—A Versatile Bone-Derived Hormone. Frontiers in Endocrinology 9, doi:10.3389/fendo.2018.00794 (2019).
45. Lee NK et al. Endocrine regulation of energy metabolism by the skeleton. Cell 130, 456–469, doi:S0092-8674(07)00701-5 [pii] (2007). [PubMed: 17693256]

46. Ferron M, Hinoi E, Karsenty G & Ducy P Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci U S A 105, 5266–5270, doi:10.1073/pnas.0711119105 (2008). [PubMed: 18362359]
47. Ferron M, McKee MD, Levine RL, Ducy P & Karsenty G Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. Bone 50, 568–575, doi:10.1016/j.bone.2011.04.017 (2012). [PubMed: 21550430]
48. Levinger I et al. Multifaceted interaction of bone, muscle, lifestyle interventions and metabolic and cardiovascular disease: role of osteocalcin. Osteoporos Int 28, 2265–2273, doi:10.1007/s00198-017-3994-3 (2017). [PubMed: 28289780]
49. Kord-Varkaneh H, Djafarian K, khorshidi M & Shab-Bidar S Association between serum osteocalcin and body mass index: a systematic review and meta-analysis. Endocrine 58, 24–32, doi:10.1007/s12020-017-1384-4 (2017). [PubMed: 28822067]
50. Liu X et al. Osteocalcin and measures of adiposity: a systematic review and meta-analysis of observational studies. Archives of osteoporosis 15, 145–145 (2020). [PubMed: 32945990]
51. Yin MT & Overton ET Increasing Clarity on Bone Loss Associated With Antiretroviral Initiation. The Journal of infectious diseases 203, 1705–1707, doi:10.1093/infdis/jir184 (2011). [PubMed: 21606527]
52. Koethe JR et al. HIV and antiretroviral therapy-related fat alterations. Nature Reviews Disease Primers 6, 48, doi:10.1038/s41572-020-0181-1 (2020).
53. Grant PM et al. Long-term Bone Mineral Density Changes in Antiretroviral-Treated HIV-Infected Individuals. The Journal of infectious diseases 214, 607–611, doi:10.1093/infdis/jiw204 (2016). [PubMed: 27330053]
54. Brown TT et al. Changes in Bone Mineral Density After Initiation of Antiretroviral Treatment With Tenofovir Disoproxil Fumarate/Emtricitabine Plus Atazanavir/Ritonavir, Darunavir/Ritonavir, or Raltegravir. The Journal of infectious diseases 212, 1241–1249, doi:10.1093/infdis/jiv194 (2015). [PubMed: 25948863]
55. Hirakawa H et al. Antiretroviral Therapy Containing HIV Protease Inhibitors Enhances Fracture Risk by Impairing Osteoblast Differentiation and Bone Quality. The Journal of infectious diseases 215, 1893–1897, doi:10.1093/infdis/jix246 (2017). [PubMed: 28525596]
56. Shea MK, Dawson-Hughes B, Gundberg CM & Booth SL Reducing Undercarboxylated Osteocalcin With Vitamin K Supplementation Does Not Promote Lean Tissue Loss or Fat Gain Over 3 Years in Older Women and Men: A Randomized Controlled Trial. J Bone Miner Res 32, 243–249, doi:10.1002/jbmr.2989 (2017). [PubMed: 27604070]
57. Knapen MHJ, Jardon KM & Vermeer C Vitamin K-induced effects on body fat and weight: results from a 3-year vitamin K2 intervention study. European Journal of Clinical Nutrition 72, 136–141, doi:10.1038/ejcn.2017.146 (2018). [PubMed: 28952607]
58. Lewis JR et al. Effects of calcium supplementation on circulating osteocalcin and glycated haemoglobin in older women. Osteoporosis International 30, 2065–2072, doi:10.1007/s00198-019-05087-3 (2019). [PubMed: 31342138]
59. Sim M et al. The effects of vitamin K-rich green leafy vegetables on bone metabolism: A 4-week randomised controlled trial in middle-aged and older individuals. Bone reports 12, 100274–100274, doi:10.1016/j.bonr.2020.100274 (2020). [PubMed: 32455149]

**Table 1:**

Characteristics of HIV seronegative and seropositive women at the index visit.

| | HIV-seronegative (n=46) | HIV seropositive (n=76) | P-value |
|---|---|---|---|
| Age (yr, Mean [SD]) | 48.2 (5.03) | 48.9 (4.89) | 0.53 |
| Height (m, Mean [SD]) | 1.6 (0.07) | 1.6 (0.07) | 0.75 |
| Weight (kg, Mean [SD]) | 75.3 (13.9) | 77.9 (15.3) | 0.38 |
| BMI (kg/m2, Mean [SD]) | 28.4 (5.53) | 29.4 (5.83) | 0.46 |
| Race (n, %) | | | |
| White | 4 (8%) | 11 (14%) | 0.63 |
| Black | 35 (77%) | 58 (75%) | |
| Others | 6 (13%) | 8 (10%) | |
| Menopause Status (n, %) | | | |
| Early perimenopause | 18 (40) | 30 (38%) | 0.91 |
| Late perimenopause/Post-menopausal | 27 (60%) | 47 (61%) | |
| Enrollment Site (n, %) | | | |
| Bronx/Manhattan | 18 (40%) | 28 (36%) | 0.92 |
| San Francisco | 14 (31%) | 26 (33%) | |
| Chicago | 13 (28%) | 23 (29%) | |
| Smoking Status (n, %) | | | |
| Former/Never | 16 (35%) | 32 (41%) | 0.51 |
| Current | 29 (64%) | 45 (58%) | |
| Hepatitis C Virus Infection (n, %) | | | |
| Negative | 39 (86%) | 61 (79%) | 0.30 |
| Ab+RNA-/RNA unknow+RNA+ | 6 (13%) | 16 (20%) | |
| Bone Turnover Markers (ng/ml) | | | |
| P1NP (Mean [SD]) | 53.64 (17.6) | 55.05 (26.2) | 0.71 |
| CTX (Mean [SD]) | 0.32 (0.2) | 0.33 (0.18) | 0.32 |
| HIV Viral load | | | |
| Detected (n, %) | | 27 (35%) | |
| Undetected (n, %) | | 49 (64%) | |
| CD4 count at index (Mean [SD]) | | 594.2 (269.2) | |
| Nadir CD4 count at index (Mean [SD]) | | 302.4 (192.1) | |
| ART use at Index (n, %) | | 74 (97%) | |
| PI use at Index (n, %) | | 37 (49%) | |
| INSTI use at Index (n, %) | | 68 (89%) | |
| NNRTI use at Index (n, %) | | 37 (49%) | |

**Table 2:**

Two-year change in ucOCN and cOCN and trunk and total fat and univariate linear regression analysis for the association between the two-year change ucOCN and the two-year change in fat variables stratified by HIV-serostatus.

| | HIV-seronegative (n=46) | | | | | HIV-seropositive (n=76) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Index Visit [Mean (SD)]** | **Follow Up Visit [Mean (SD)]** | **Two-year change [Mean (SD)]** | **Association with ucOCN [β (SE), p-value[1]]** | **Association with cOCN [β (SE), p-value[2]]** | **Index Visit [Mean (SD)]** | **Follow Up Visit [Mean (SD)]** | **Two-year change [Mean (SD)]** | **Association with ucOCN [β (SE), p-value[1]]** | **Association with cOCN [β (SE), p-value[2]]** |
| ucOCN (ng/mL) | 2.06 (2.42) | 2.35 (2.94) | 0.29 (1.66) | NA | N/A | 1.94 (2.32) | 2.57 (3.04) | 0.62 (2.11) | N/A | N/A |
| cOCN (ng/mL) | 3.49 (1.75) ≠ | 3.93 (2.29) ≠ | 0.44 (2.15) | N/A | N/A | 4.94 (3.82) | 5.37 (5.25) | 0.43(2.36) | N/A | N/A |
| Total Fat (kg) | 29.8 (10.6) | 33.0 (10.9) | 3.21 (6.20) | −0.26 (0.56), 0.65 | −0.43(0.42), 0.30 | 31.9 (11.4) | 32.9 (12.3) | 1.0 (6.3) | −0.86 (0.33) **0.012** | −0.35 (0.30), 0.25 |
| Trunk Fat (kg) | 15.1 (5.49) | 17.1 (5.82) | 2.01 (3.60) | −0.14 (0.32), 0.67 | −0.17 (0.24), 0.49 | 16.5 (6.0) | 17.4 (6.7) | 0.87 (3.39) | −0.49 (0.18), **0.008** | −0.27 (0.12), 0.10 |

≠Indicates significant difference between HIV-seronegative and HIV-seropositive women. Bolded text indicates p<0.05.

1. For association of two-year change in ucOCN with two-year change in the row variable.

2. For association of two-year change in cOCN with two-year change in the row variable.

**Table 3:**

Two-year change in ucOCN and change in total fat and trunk fat adjusting for confounding variables from multivariate linear regression in the entire group of participants (n=122).

| | Total Fat Change Estimate (kg) (95% CI) | p-value[1] | Trunk Fat Change Estimate (kg) (95% CI) | p-value[2] |
|---|---|---|---|---|
| **ucOCN change** (ng/mL) | −0.58 (−1,16, 0.0005) | **0.050** | −0.36 (−0.68, 0.03) | **0.030** |
| **HIV-serostatus:** Negative vs. Positive | −1.73 (−3.95, 0.48) | 0.13 | −0.85 (−2.09, 0.38) | 0.17 |
| **Menopausal Status:** Early peri vs. late peri/post meno | 1.44 (−1.40, 4.31) | 0.32 | 0.65 (−0.94, 2.23) | 0.42 |
| **Age:** At index visit | −0.13 (−0.42, 0.15) | 0.35 | −0.07 (−0.23, 0.08) | 0.38 |
| Change (index to second visit) | −0.11 (−2.99, 2.77) | 0.98 | 0.24 (−1.36, 1.85) | 0.83 |
| **Race:** White vs. Black | −0.04 (−3.37, 3.27) | 0.63 | −0.20 (−2.05, 1.64) | 0.77 |
| Other vs. Black | 0.87 (−2.70, 4.44) | 0.12 | 0.29 (−1.69, 2.28) | 0.18 |
| **HCV:** Ab+ RNA unknown/RNA+ vs. Negative | −2.25 (−5.11, 0.59) | 0.62 | −1.09 (−2.69, 0.49) | 0.94 |
| **Smoking**: Current vs. Former/Never | −0.57 (2.81, 0.16) | 0.99 | −0.52 (−1.30, 1.19) | 0.73 |
| **Enrollment Site**: San Francisco vs. Bronx | −0.01 (−2.69, 2.67) | 0.53 | −0.26 (−1.76, 1.23) | 0.65 |
| Chicago vs. Bronx | −0.91 (−3.78, 1.94) | 0.94 | −0.36 (−1.95, 1.23) | 0.76 |

Data include all participants within both sero-positive (n=46) and sero-negative (n=76) groups. All row variables included in the multivariate linear model. Bolded text indicates $p<0.05$

1. For multivariate association of two-year change in the row variable with two-year change in total fat

2. For multivariate association of two-year change in the row variable with two-year change in trunk fat