Associations between Bone Density and Body Composition in Persons Living with HIV

A thesis submitted by

Rajaa Nahra, MD

In partial fulfillment of the requirements
for the degree of
Master of Science
in
Clinical and Translational Research

TUFTS UNIVERSITY

Sackler School of Graduate Biomedical Sciences

Date
May 2014

Thesis Chair: Christine Wanke, MD
Program Mentor: Lisa Ceglia, MD, MS
Project Mentor: Jessica Paulus, ScD
Statistical Mentor: Farzad Noubary, PhD

Abstract

The prevalence of osteoporosis is higher in persons living with HIV (PLWH) than in HIV-uninfected individuals. PLWH also have an increased risk of developing an adipose redistribution syndrome (HARS) and muscle loss. Data examining the relationships between bone, fat and muscle mass in PLWH are scarce and inconsistent. We examined the association between total body bone mineral density (BMD) with measures of central and appendicular fat and lean body mass in men and women living with HIV.

HIV-positive men (n=466; mean age 46 years) and women (n=153; mean age 43 years) were evaluated for total body BMD by dual energy x-ray absorptiometry (DXA), anthropometric indices of central (waist circumference) and appendicular fat (triceps skin-fold), and DXA-derived measures (trunk-to-extremity fat ratio, trunk fat, appendicular fat, and percent lean body mass). From this cohort, 297 men and 101 women were included in the longitudinal analysis. Multivariable linear regression, separately in each sex, assessed the relationship between total body BMD and each of the body composition measures in the cross-sectional analyses; and between baseline trunk-to-extremity fat ratio and 2-year change in total body BMD.

We found that trunk-to-extremity fat ratio was associated with lower total body BMD in men (β=-0.02, p=0.01). Both higher central and appendicular fat measurements were associated with lower total body BMD in men (p for all <0.05 except appendicular fat by DXA, p=0.1), but not women (β=0.02, p=0.2), after multivariable adjustment. Percent lean body mass was positively associated with total body BMD in men (β=0.004,

p<0.001), and women (β=0.003, p=0.06). Baseline trunk-to-extremity fat ratio and percent lean mass were not significantly associated with 2-year changes in total body BMD in men or women.

In this cohort, there was a positive association between lean mass and total body BMD in both sexes with HIV, implying that lean mass is an important determinant of BMD in this population. Our study also found a negative association between measures of body fat and total body BMD in men with HIV, suggesting that higher fat mass in men with HIV may have an adverse effect on BMD. Baseline body composition measures did not predict change in total body BMD over a 2-year period. Larger and longer-term studies are needed to confirm these findings.

Table of Contents

List of Tables

List of Figures

List of abbreviations

ART antiretroviral therapy
BMD: bone mineral density
BMI body mass index
CT computed tomography
DXA: dual energy x-ray absorptiometry
HARS HIV-associated adipose redistribution syndrome
MRI magnetic resonance imaging
NFHL Nutrition for Healthy Living Cohort
NNRTI non-nucleoside reverse
PI protease inhibitor
PLWH persons living with HIV

Introduction

1.1 Background and Significance

Since the advent of antiretroviral therapy in the management of chronic HIV infection, persons living with HIV (PLWH) are living longer and are increasingly facing chronic and age-related health problems including osteoporosis. PLWH have an increase in fracture risk compared to that of age and gender-matched uninfected patients[1-3]. PLWH also have an increased risk for developing HIV-associated adipose redistribution syndrome (HARS) – a disorder involving central fat deposition and/or peripheral fat atrophy, more frequently occurring as separate disease entities in the non-HIV infected population [4]. Maldistribution of fat may be a risk factor for bone loss as has been suggested by recent observational, mainly cross-sectional, studies in non-HIV infected populations [5-7]. Notably, adipose tissue secretes multiple circulating adipokines, some of which are pro-inflammatory and may adversely affect bone metabolism.

Data examining the relationship between bone and fat mass in PLWH are inconsistent [8-16]. In a cross-sectional study of HIV-infected men, excess trunk fat deposition was associated with lower lumbar spine volumetric bone mineral density [10]. On the other hand, a recent longitudinal study in HIV-infected women, mostly non-White, found total fat mass and trunk fat to be positively associated with total hip and femoral neck bone mineral density [9].

Accelerated loss of muscle mass has been well-described among older non-HIV infected adults and linked to declines in bone mineral density [17-22]. Decreased lean body mass, as a measure of muscle mass, has also been associated with decreased bone

mineral density in younger, middle-aged PLWH on successful antiretroviral therapy in several [23-25] but not all studies [12].

The conflicting data on the association between fat mass and BMD in non-HIV infected as well as HIV infected individuals may be due to differences in measurement of fat and bone mass and the population studied among other factors.

To better understand whether body composition is an important determinant of bone mineral density in middle-aged PLWH, we examined the cross-sectional and longitudinal association between measures of central and peripheral fat mass, lean mass and total body bone mineral density in HIV-infected men and women who participated in the Nutrition for Healthy Living Cohort [4, 26-31].

1.2 Specific Aims and Hypotheses:

Specific Aim 1: To examine the association between trunk to extremity fat ratio, a measure of body fat distribution, and total body BMD in PLWH.

Hypothesis 1: Trunk to extremity fat ratio will be inversely associated with total body bone mineral density.

Specific Aim 2: To examine the association between trunk fat, waist circumference, appendicular fat and triceps-skin fold and total body BMD in PLWH.

Hypothesis 2: Measures representative of central fat mass (e.g., trunk fat and waist circumference) will be inversely associated with total body BMD. Measures of peripheral fat mass (e.g., appendicular fat and triceps-skin fold) will be positively associated with total body BMD.

Specific Aim 3: To assess whether trunk to extremity fat ratio is associated with 2-year changes in total body BMD in PLWH.

Hypothesis 3: A higher trunk to extremity fat ratio will result in a greater decline in total body BMD. Specific Aim 4: To examine the association between lean mass by DXA and total body BMD in PLWH.

Hypothesis 4: Lean mass by DXA *will be positively associated with total body BMD.*

Methods

2.1 Study cohort

Our study population consisted of individuals enrolled in the Nutrition for Healthy Living Cohort (NFHL), an NIH-funded study of the nutritional and metabolic consequences of HIV infection. Recruitment for this study started in 1995. Eligible participants included HIV-seropositive men and women (aged 18 years or older) living in the greater Boston area or Rhode Island. Individuals were excluded if they had any of the following conditions at the time of enrollment: pregnancy, diabetes, thyroid disease, malignancies other than those associated with HIV, or inadequate fluency in English. Study methods and baseline characteristics of the full cohort have been described previously [26]. The parent study was approved by the institutional review boards at Tufts Medical Center (Boston, MA) and Miriam Hospital (Providence, RI), and informed consent was obtained from all participants. For the cross-sectional analysis, we included men and women who had at least one DXA scan performed at the most recent visit. To be included in the longitudinal analysis, participants had to have had at least two DXA scans performed at different visits within a period of two years (± 6 months).

2.2 Data Collection

In brief, all participants were evaluated at semiannual visits by trained study staff. Data collected at each study contact included anthropometric measurements, such as weight, height, triceps skinfold, waist, hip and mid-arm circumference, and dietary intake from 3-day food records. At each visit, participants also completed a detailed questionnaire eliciting information on sociodemographic characteristics, clinical status, health-related quality of life, smoking, and alcohol use. Data on calcium and vitamin D

intake, use of prednisone, hydrocortisone, and anti-retroviral therapy were collected by trained interviewers. Anti-retroviral therapy was defined as use of at least 3 medications from 2 or more classes (nucleoside reverse transcriptase inhibitor, non-nucleoside reverse transcriptase inhibitor, and protease inhibitor). Alcohol use was defined as heavy (more than 2 drinks on 4-7 days/week) vs. non-heavy. Daily caloric intake, protein, calcium, and vitamin D consumption, including supplements, were determined from 3-day food records using the Minnesota Nutrition Data System Version 4.06_34. If a 3-day food record was not kept, a 24-hour food recall was obtained by a trained nutritionist. Strength training over the past 7 days was assessed using the physical activity recall instrument [31].

2.3 Clinical measures

Subjects were weighed (kg) fully dressed, but without shoes, heavy clothing, or objects, before eating or drinking (minimum 5-hour fast). Height (cm) was measured without shoes by stadiometer [31]. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Body circumferences and skin-fold measurements were conducted by trained study personnel. The average of 3 measures within 3 mm was used [4]. Transverse whole-body scans were obtained by 1 of 3 validated technicians using a QDR2000 scanner (Hologic, Waltham, MA) in the array mode. DXA phantoms were scanned daily to minimize instrument drift. DXA was conducted with the subject in a standard supine position, wearing a hospital gown, and after emptying his or her bladder. Hologic 2000 software computed total body bone mineral density (BMD) in g/cm^2. Body composition obtained by DXA, including trunk and appendicular fat, was measured in kilograms [16]. Trunk-to-extremity fat ratio is obtained by dividing trunk fat

in kilograms over appendicular fat in kilograms [32-37]. Percent lean mass for the whole body was obtained by DXA.

Fasting blood was collected and stored at each visit for immunologic, biochemical, and nutritional testing. The nadir CD4+ cell count was determined by either self-report or measured CD4+ cell count, which were highly correlated (r=0.87) [4]. HIV RNA (log10 copies/mL) was measured by the Roche Amplicor Monitor reverse transcriptase–polymerase chain reaction assay (Roche Molecular Systems, Somerville,NJ), with a lower detection limit of 400 copies per milliliter.

2.4 Power calculation

Published data are available describing the correlation between trunk-to-extremity fat ratio and volumetric BMD in g/cm^3 for HIV-infected men (r=-0.44, p=0.044) and amenorrheic female athletes (r=-0.45, p=0.04) (4,29). We are not able to use the regression coefficients from these studies because the outcome in our study is total body areal BMD in g/cm^2.

Given a sample size of 153 women in our cross-sectional analysis, we have 80% power to detect a correlation with absolute value of 0.23 or greater between trunk-to-extremity fat ratio and total BMD. Similarly, given a sample size of 466 men in our cross-sectional analysis, we have 80% power to detect a correlation with absolute value of 0.13 or greater. As for the longitudinal analysis, we have 80% power to detect a correlation between trunk-to-extremity fat ratio and 2-year change in total body BMD with absolute value of 0.28 or greater given our sample size of 101 women, and a correlation with absolute value of 0.17 or greater in our sample size of 297 men. Calculations were performed assuming a two-sided hypothesis test with a significance

level of 0.05 using NCSS/PASS software (NCSS, LLC. Kaysville, Utah, USA. www. ncss.com).

2.5 Statistical Analysis

Statistical analyses were performed using the R statistical package. All baseline characteristics are presented as means ± SDs if normally distributed or as medians and inter-quartile ranges if not normally distributed. Results were considered significant if the 2-tailed *P* value was <0.05.

Our cross-sectional sample was constructed using the last DXA measure from participants enrolled in the NFHL from 1995-2005, in order to use the most recent information available. The visit when the last DXA was performed was referred to as the index visit. The longitudinal sample was constructed using the participant's baseline (first) DXA measure and a follow-up DXA measure over the following 18 to 30 months. The visit when the first DXA was performed was referred to as the index visit in the longitudinal sample. The primary dependent variable in the cross-sectional study was total body BMD at the index visit.

The primary dependent variable in the longitudinal study was the percent change in total body BMD between the index visit and follow-up visit. It was calculated as follows: (total body BMD at follow-up visit minus total body BMD at baseline)/total body BMD at baseline.

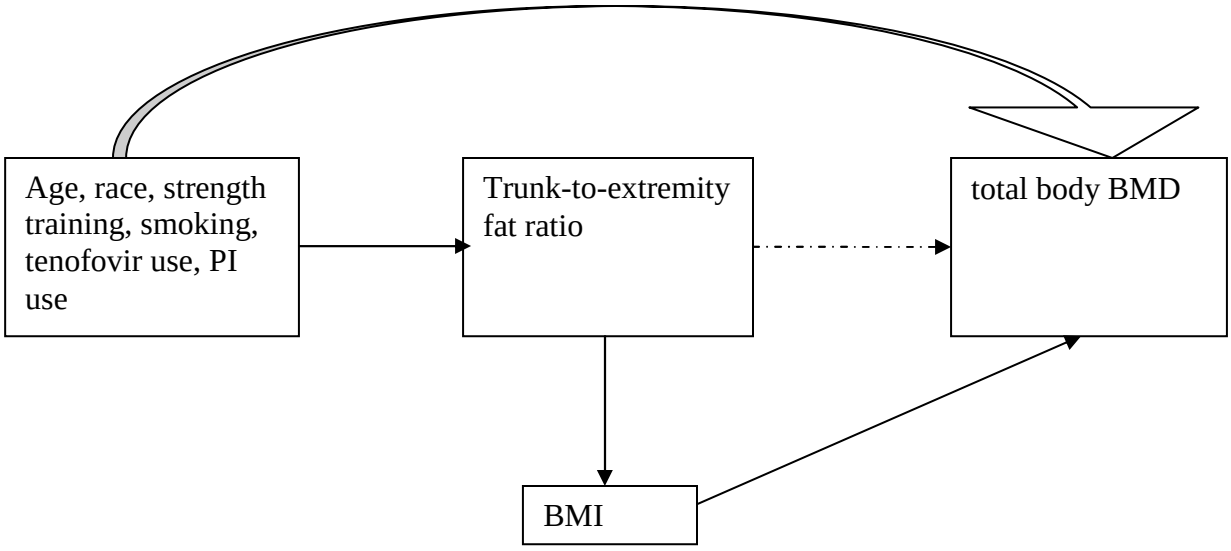
Independent variables of interest included: the primary independent variable of trunk-to-extremity fat ratio and the secondary independent variables of trunk fat and waist circumference (measures of central fat mass), appendicular fat and triceps skinfold (measures of peripheral fat mass) and lean mass (measure of muscle mass). Trunk-to-extremity fat ratio takes into account relative fat loss in the extremities as well as

increased trunk fat and is a good surrogate of visceral adipose tissue and insulin resistance [34, 36, 37]. Waist circumference and triceps-skin fold are anthropometric measures of central and appendicular fat, respectively, and can be implemented in a clinical setting.

Analyses were stratified by sex because of differences in body fat distribution between men and women [4]. Previous literature has examined relationships between measures of body adiposity and lean mass with BMD in men and women separately [9-11, 14, 23, 38]. The assumption of linearity between total body BMD and trunk-to-extremity fat ratio was assessed using a model with a quadratic term for trunk-to-extremity fat ratio.

A priori and based on biological and clinical expertise, we identified age, race, strength training, smoking, tenofovir use, protease inhibitor and BMI at the index visit as potential confounders based on directed acyclic graph (DAG) methods (Figure 1) [13, 31, 39-41].

Figure 1: Directed acyclic graph (DAG) of the association between trunk-to-extremity fat ratio and total body BMD



Since information about menopause status was not collected in the early phase of the NFHL, and most women undergo menopause around 50 years of age, we used age 50 years and over as an indicator of menopause [42]. Univariate regression analyses of all the independent variables with the outcome were performed. We considered tenofovir use to be a potential intermediate variable along the causal pathway between the primary independent variable (trunk-to-extremity fat ratio) and our principal study outcome (total body BMD). Due to a potential bias influencing the use of tenofovir in those who have underlying HARS, we carried out separate models with and without adjusting for tenofovir (data not shown). While it is important to note that BMI is a measure of total body weight (adjusted for height), trunk-to-extremity fat ratio, trunk fat, appendicular fat, waist circumference and triceps skin-fold are all measures of body composition. Previous studies on the association between body composition, lean mass with BMD have adjusted for BMI [10, 16, 43-45]. Therefore, we ran separate models for the various independent variables of trunk-to-extremity fat ratio, trunk fat, appendicular fat, waist circumference, triceps skin-fold, percent lean mass with the principal study outcome (total body BMD) with and without BMI in the model. Adjusted analyses used multivariable linear regression modeling with candidate variable selection based on clinical concepts and consideration of ten observations per predictor of interest. For the longitudinal analysis, we ran two separate models of baseline trunk-to-extremity fat ratio and percent lean mass with percent change in total body BMD adjusting for age, race, strength training, smoking and BMI in each of these models.

Results

3.1 Demographic Data

Men

A total of 466 men were included in our cross-sectional analysis (Table 1). They had a mean age of 46 years. Sixty-seven percent (n=311) of the men were Non-Hispanic White. They had been living with HIV for a mean duration of 11 years. Almost half (n=212) of the men were smokers. Steroid use was reported in less than 5% of the participants. Approximately 25% (n=126) of the men reported regular bouts of strength training. Twenty-seven percent of men (n=127) had a history of tenofovir use. Mean total body BMD was 1.1 g/cm^2 (Table 1).

Table 1: Characteristics of HIV-infected women and men observed at the time of first DXA measurement

	Cross-sectional cohort n=619		Longitudinal cohort n=398	
	Men	Women	Men	Women
n	466	153	297	101
Age (years)	46 (±46)	43 (±7)	43 (±7)	40 (±7)
Time HIV positive (years)	11 (±5)	11 (±5)	8 (±4)	8 (±4)
Race/ethnicity				
Non-Hispanic Black	113 (24%)	79 (52%)	85 (29%)	51 (50%)
Non-Hispanic White	311 (67%)	56 (37%)	184 (62%)	37 (37%)
Hispanic	42 (9%)	18 (11%)	28 (9%)	13 (13%)
Alcohol **				
Non-heavy drinker	337 (95%)	129 (97%)	109 (90%)	38 (93%)
Heavy drinker	24 (5%)	5 (3%)	12 (10%)	3 (7%)
Smoking				
Former/Never	253 (55%)	51 (34%)	163 (55%)	36 (36%)
Current	212 (45%)	101 (66%)	134 (45%)	64 (64%)
Calcium (mg)	1060 (733-1440)	708 (416-1123)	1050 (680-1554)	730(493-1020)
Vit D (mcg)	11(5-16)	7 (3-13)	12 (5-17)	7 (4-14)
HIV RNA log$_{10}$ copies/ml	2.3 (2.3-4.0)	2.3 (2.3-4.5)	2.3 (2.3-3.9)	2.3 (2.3-3.8)
CD4+ cells/mm^3	411 (234-610)	423 (213-661)	350 (214-580)	439 (281-657)
Nadir CD4 cells/mm^3	168 (50-293)	174 (64-300)	216 (92-374)	275 (139-470)
No antiretroviral therapy	89 (19%)	43 (28%)	56 (19%)	22 (22%)
BMI (kg/m2)	24.6 (22.4-27.7)	26.2(22.1-31.5)	24.7 (22.8-27.7)	27.1 (23.9-31.3)
Current steroid use	10 (2%)	6 (4%)	3 (1%)	2 (2%)
Current strength training	126 (27%)	17 (11%)	71 (24%)	9 (9%)
ART regimen				
NNRTI	153 (33%)	39 (26%)	77 (26%)	23 (23%)
PI	246 (53%)	67 (44%)	157 (53%)	43 (43%)
Ever Tenofovir	127 (27%)	42 (28%)	9 (3%)	2 (2%)

	Cross-sectional cohort n=619		Longitudinal cohort n=398	
	Men	Women	Men	Women
Independent variables:				
Trunk: extremity fat ratio	1.3 (0.97-1.85)	0.95 (0.73-1.3)	1.13 (0.78-1.60)	0.92 (0.7-1.2)
Waist circumference (cm)	89.8 (83.4-98.2)	86.2 (75.5-98.4)	89.6 (83.8-97.2)	91 (±15)
Trunk fat (kg)	7.5 (5.0-10.9)	10.5 (6.9-16.1)	7.4 (4.7-11.3)	12.3 (7.9-17.7)
Triceps skin fold (mm)	6.5 (4.7-11.1)	19.2 (11-27.7)	7.3 (5-12)	21 (13.3-29.3)
Appendicular fat (kg)	5.6 (3.8-8.1)	10.1 (7.2-15.3)	6.3 (4.4-9.2)	13.4 (9.0-17.4)
Percent lean mass	81.1 (75.7-85.4)	67.6 (60.6-74.3)	78.7 (73.3-84.1)	64.6 (57.4-72.0)

**Data are percentage of patients or median value (25th-75th percentile) or mean (±SD)*
***There were 105 men and 19 women in cross-sectional cohort, 176 men and 60 women in longitudinal cohort, who were missing data on alcohol use*

Two hundred ninety-seven men were included in the longitudinal analysis (Table 1). They had a mean age of 43 years and a mean duration of HIV infection of 8 years. In addition, 3% of men (n=9) had tenofovir exposure as most of them were enrolled prior to use of tenofovir as antiretroviral therapy. Mean percent change in total body BMD was -1.08 over an average period of 2 years. Median time between the baseline and the second DXA visit was 24 months.

Women

A total of 153 women were included in our cross-sectional analysis (Table 1). They had a mean age of 43 years. Thirty-seven percent (n=56) of the women were non-Hispanic White. They had been living with HIV for a mean duration of 11 years. Sixty-six percent (n=101) of the women were smokers. Steroid use was reported in less than 5% of the participants. Approximately 10% (n=17) of women reported regular bouts of strength training. 28% (n=42) of women had a history of tenofovir use. Mean total body BMD was 1.1 g/cm^2 (Table 1).

One hundred and one women were included in the longitudinal analysis (Table 1). They had a mean age of 40 years and mean duration of HIV infection of 8 years. Two percent of women (n=2) have history of prior tenofovir use. Mean percent change in total body BMD was 0.14 over an average period of 2 years. Median time between the baseline and the second DXA visit was 25 months.

3.2 BMI

BMI was found to be strongly correlated with trunk fat, waist circumference, appendicular fat, triceps skin fold and percent lean mass ($r \geq 0.5$) in both men and women, as evaluated by Scatter plots and correlation matrix (Tables 4-5, Figures 3-7, 9-13).

3.3 Fat and BMD

Men

Median trunk-to-extremity fat ratio was 1.3 in the cross-sectional analysis (Table 1). Descriptive statistics for all measures of central (waist circumference and trunk fat) and appendicular (triceps skin fold and appendicular fat) adiposity are shown in Table 1. The p-value for the quadratic term for trunk-to-extremity fat ratio was 0.4 in the cross-sectional analysis. Therefore, a linear model was deemed adequate. The relationship between total body BMD and trunk-to-extremity fat ratio displayed in our Scatter plot was linear (Figure 14). After controlling for age, race, exercise, smoking, and BMI, in the cross-sectional analysis, higher trunk-to-extremity fat ratio was associated with lower total body BMD (Table 2, Model 1). Similarly, higher individual measures of central and appendicular fat by anthropometry and DXA (except appendicular fat by DXA) were associated with lower total body BMD after multivariable adjustment (Table 2, Models 2-5). In the models without BMI, only waist circumference and appendicular fat were found to be associated with total body BMD. Moreover, higher waist circumference and appendicular fat were associated with higher total body BMD. Protease inhibitor and tenofovir use were found not to be significant in multivariable modeling and therefore, they were not included in the final models.

Table 4: Correlation Matrix of BMI vs body composition measures in men in the cross-sectional analysis:

	Trunk-to-extremity fat ratio	Trunk fat	Waist circumference	Appendicular fat	Triceps skin-fold	Percent lean mass
BMI	0.15	0.8	0.9	0.7	0.5	-0.6

Table 5: Correlation Matrix of BMI vs body composition measures in women in the cross-sectional analysis:

	Trunk-to-extremity fat ratio	Trunk fat	Waist circumference	Appendicular fat	Triceps skin-fold	Percent lean mass
BMI	0.3	0.9	0.9	0.9	0.7	-0.8

Figure 2: Scatter plot of trunk-to-extremity fat ratio and BMI in men in the cross-sectional analysis:

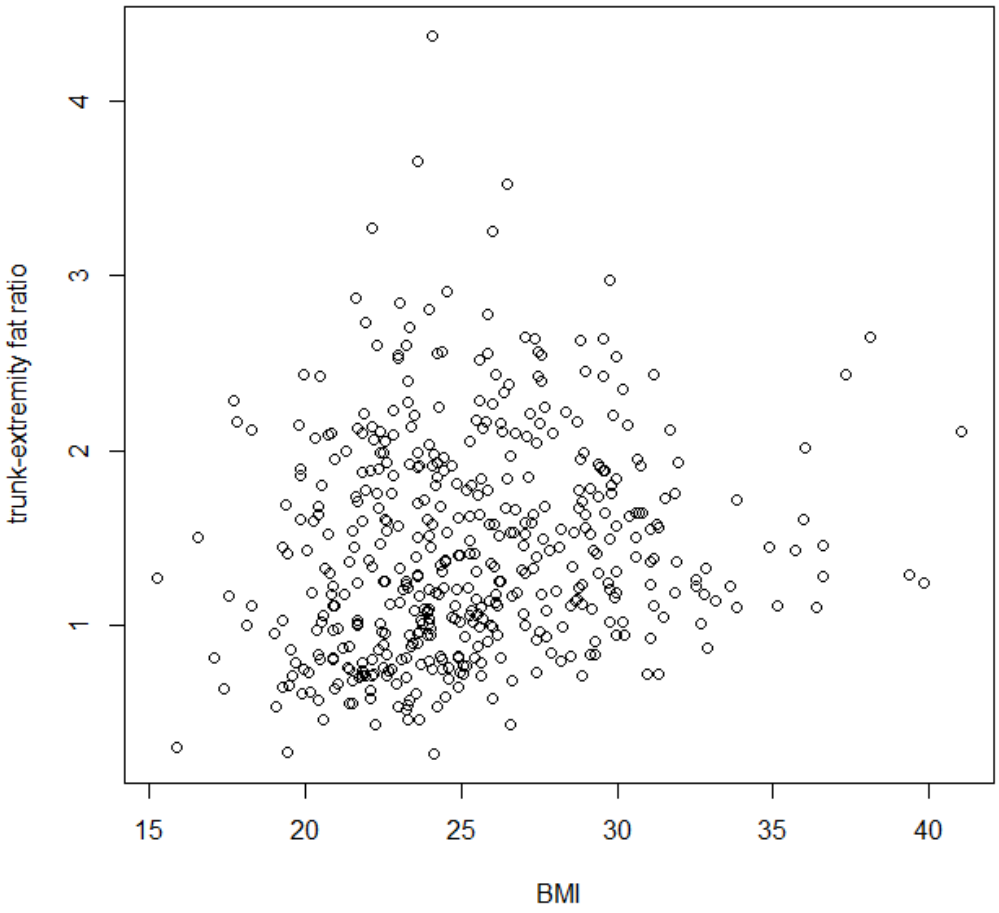


Figure 3: Scatter plot of trunk fat and BMI in men in the cross-sectional analysis:

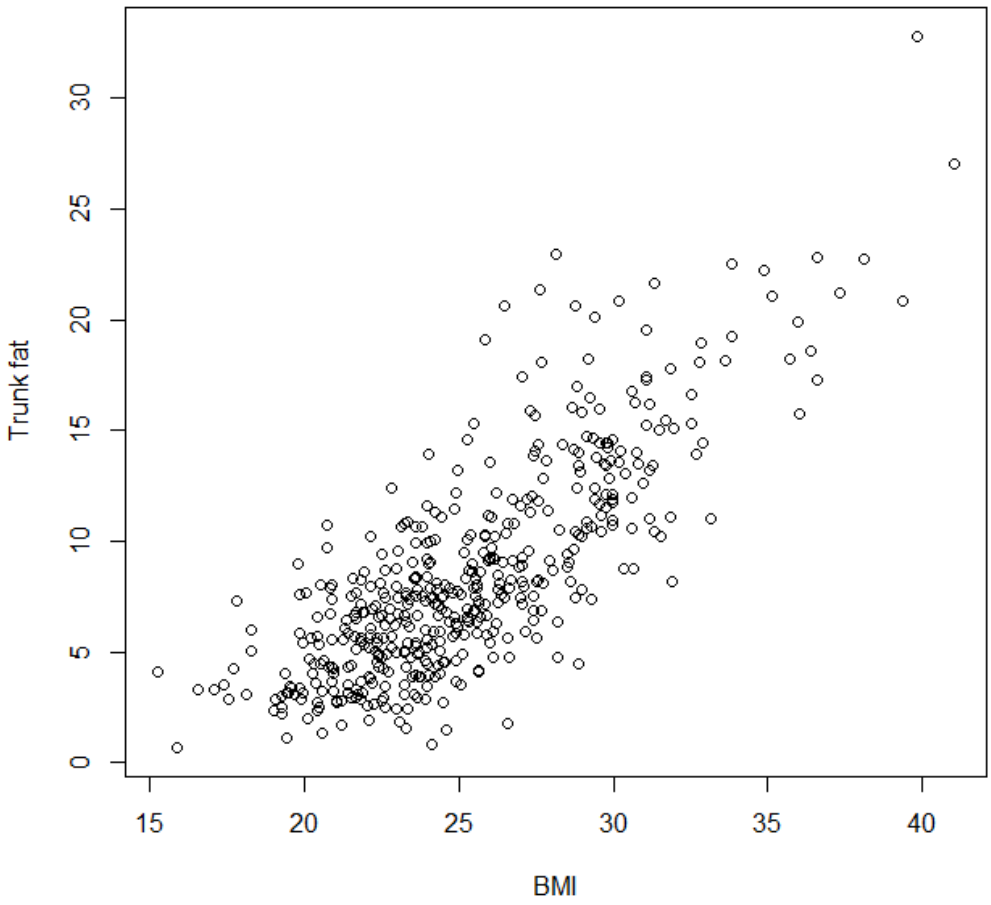


Figure 4: Scatter plot of waist circumference and BMI in men in the cross-sectional analysis:

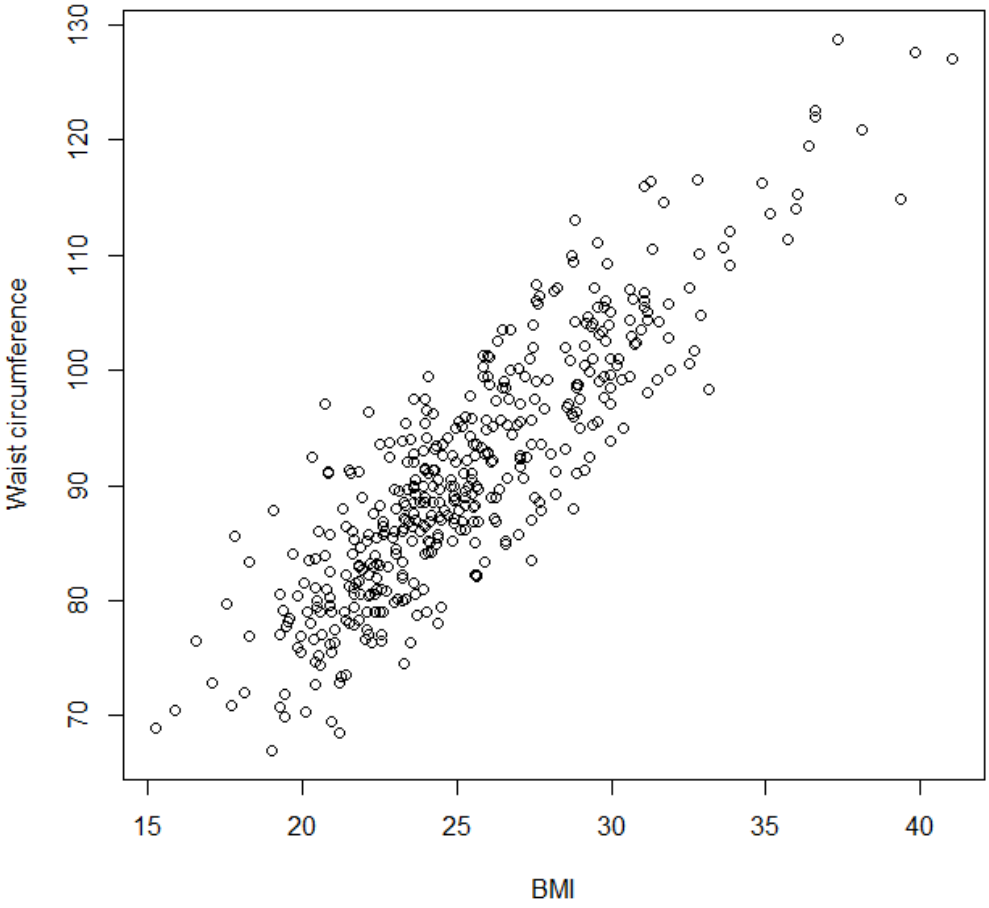


Figure 5: Scatter plot of appendicular fat and BMI in men in the cross-sectional analysis:

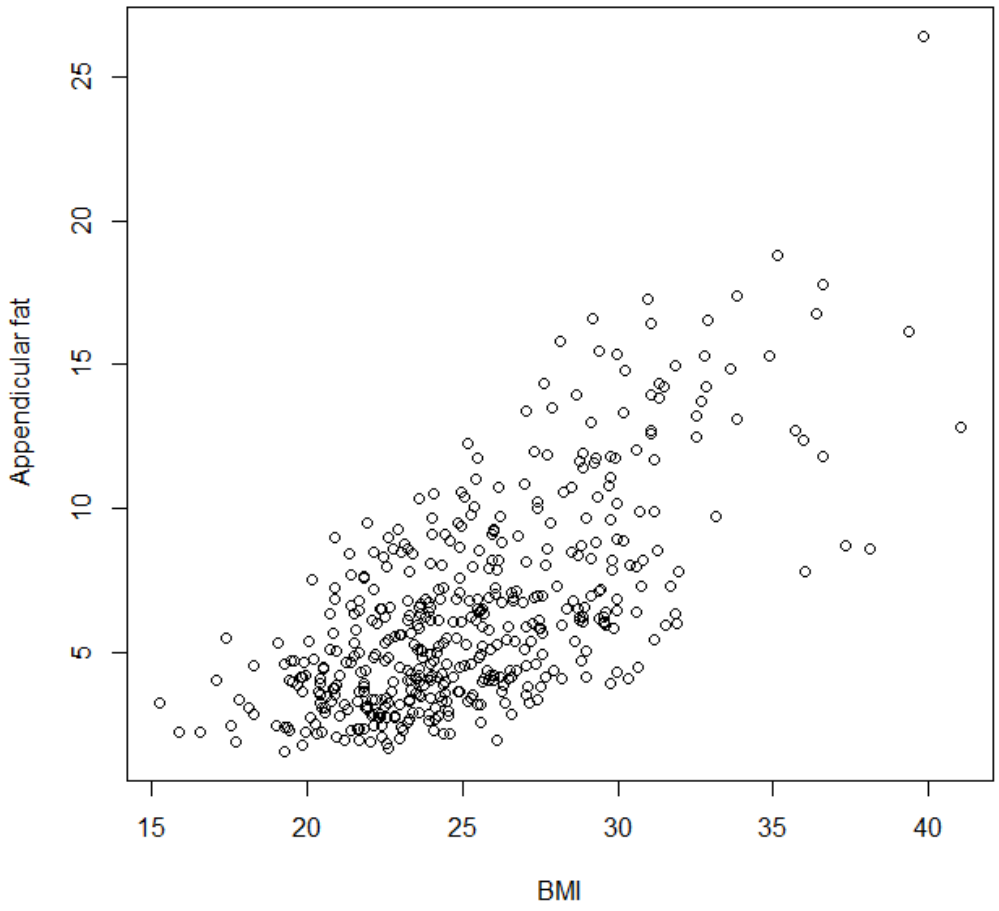


Figure 6: Scatter plot of triceps skin-fold and BMI in men in the cross-sectional analysis:

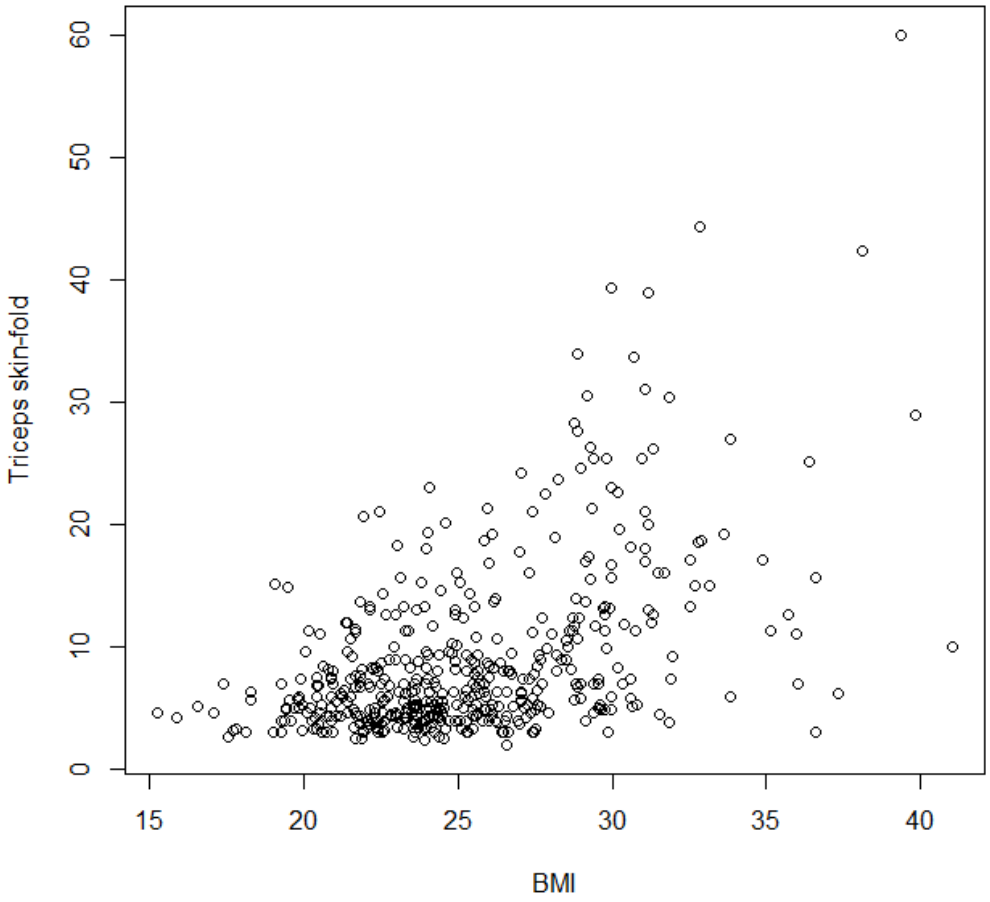


Figure 7: Scatter plot of percent lean mass and BMI in men in the cross-sectional analysis:

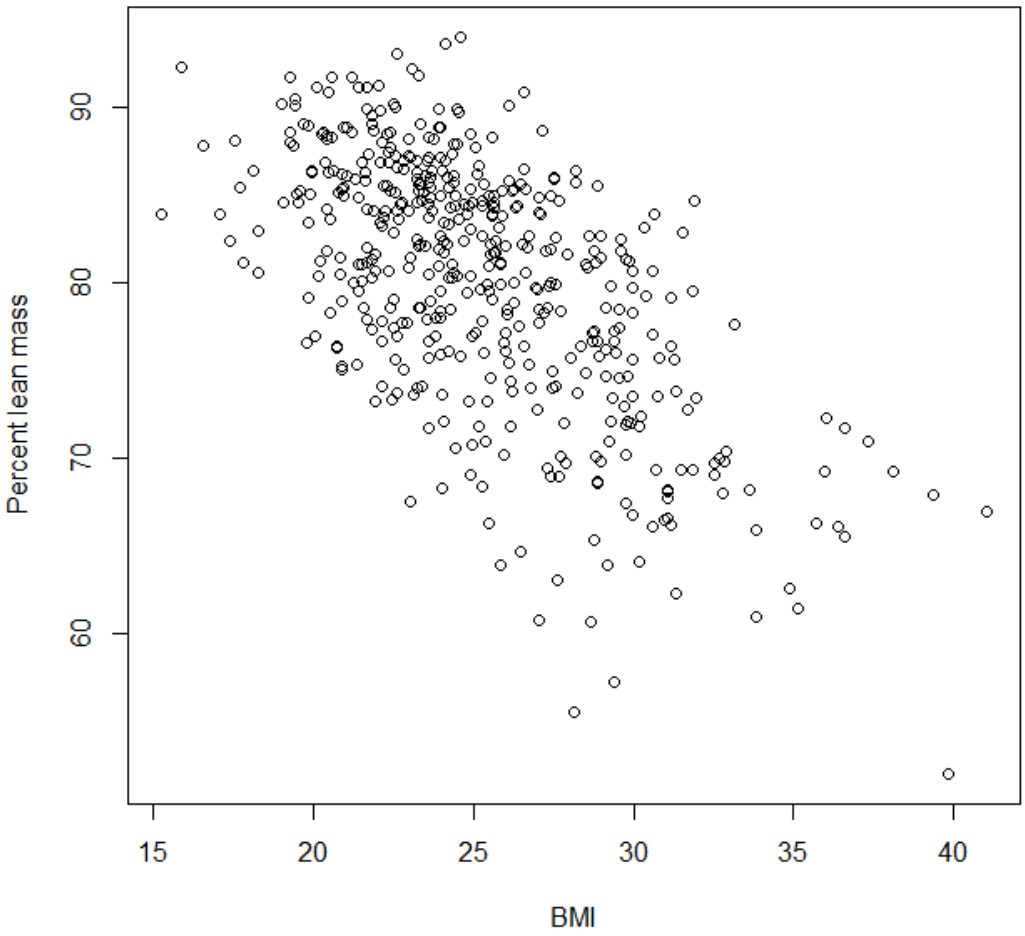


Figure 8: Scatter plot of trunk-to-extremity fat ratio and BMI in women in the cross-sectional analysis:

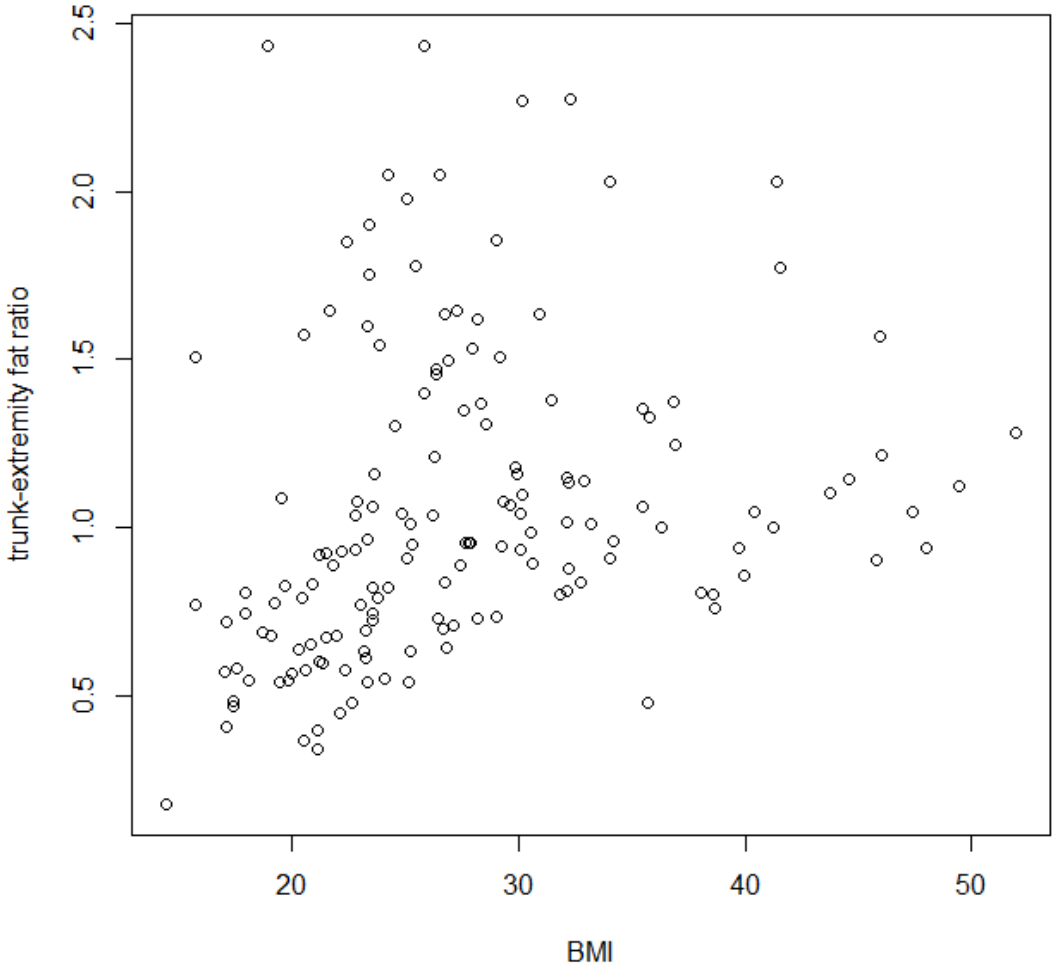


Figure 9: Scatter plot of trunk fat and BMI in women in the cross-sectional analysis:

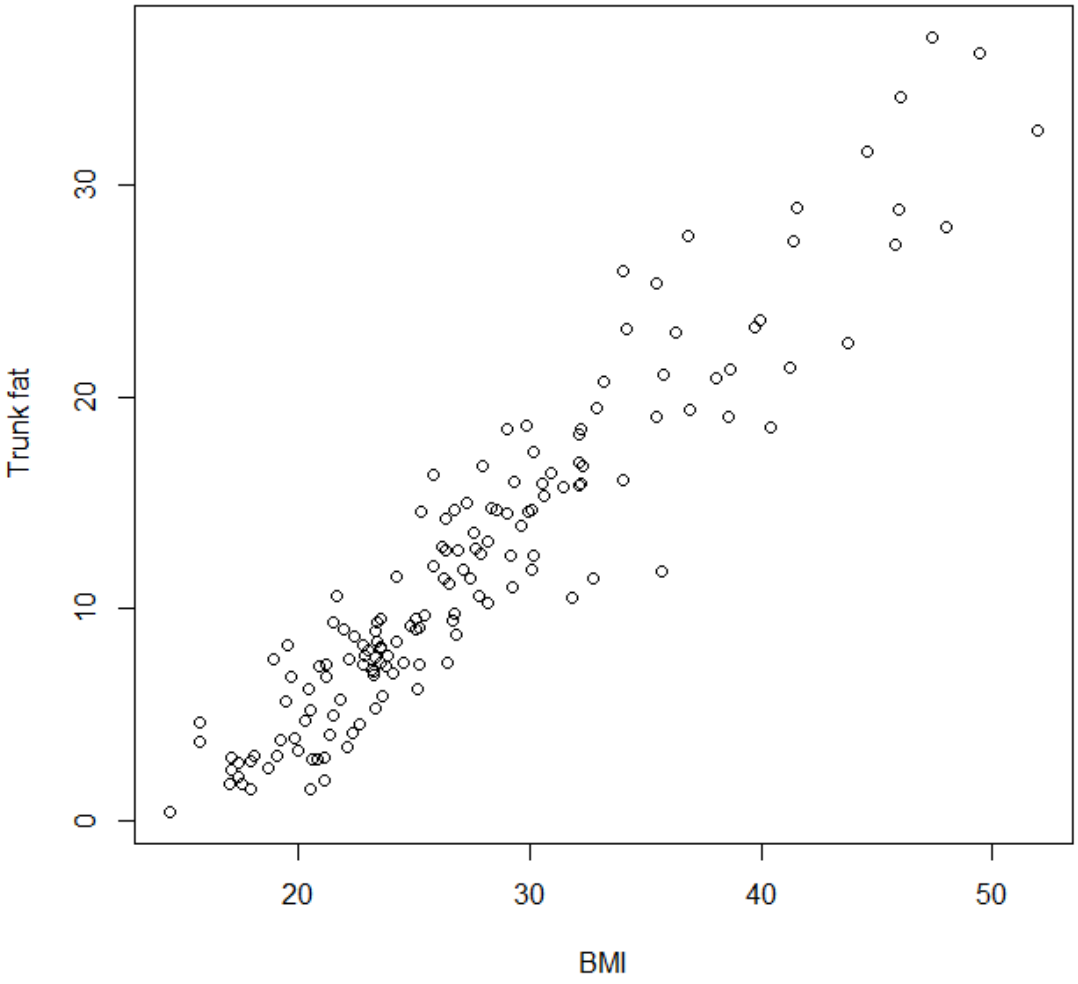


Figure 10: Scatter plot of waist circumference and BMI in women in the cross-sectional analysis:

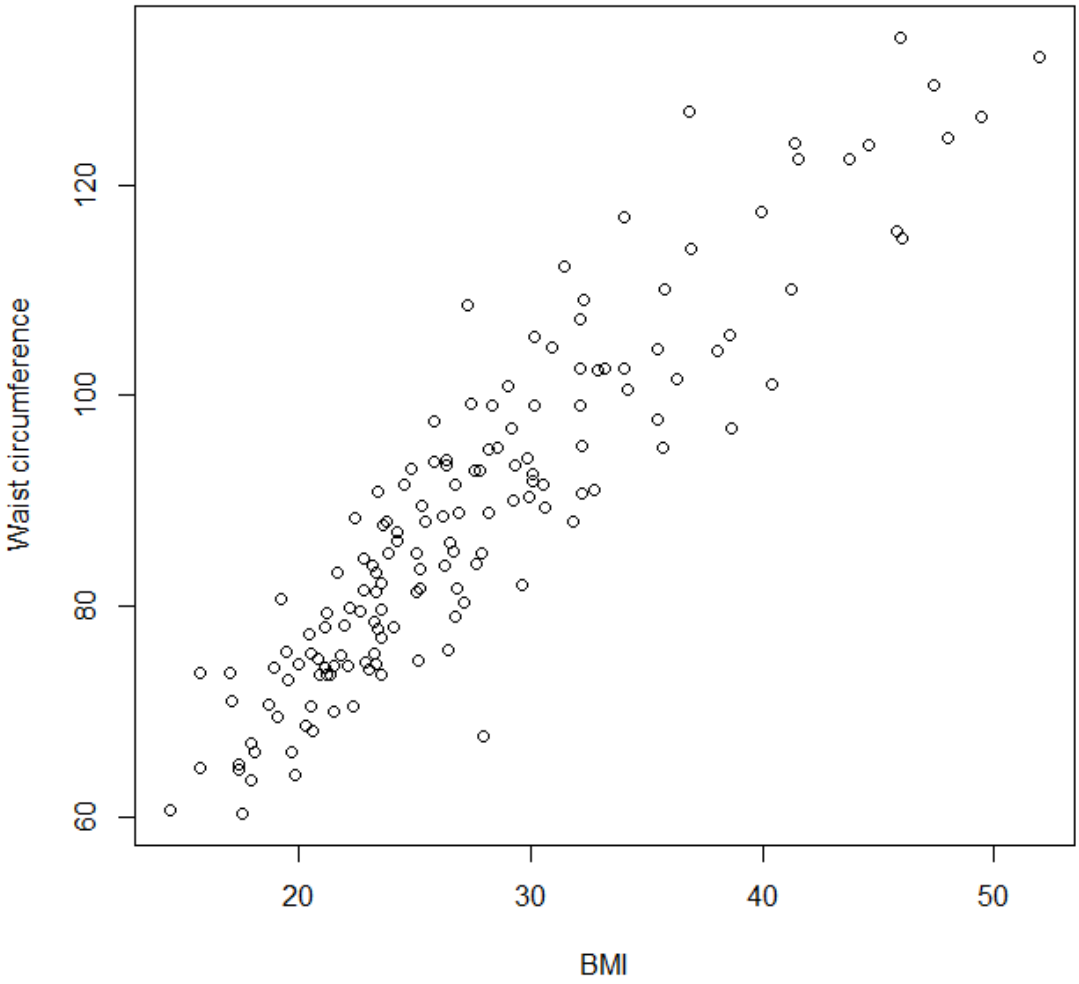


Figure 11: Scatter plot of appendicular fat and BMI in women in the cross-sectional analysis:

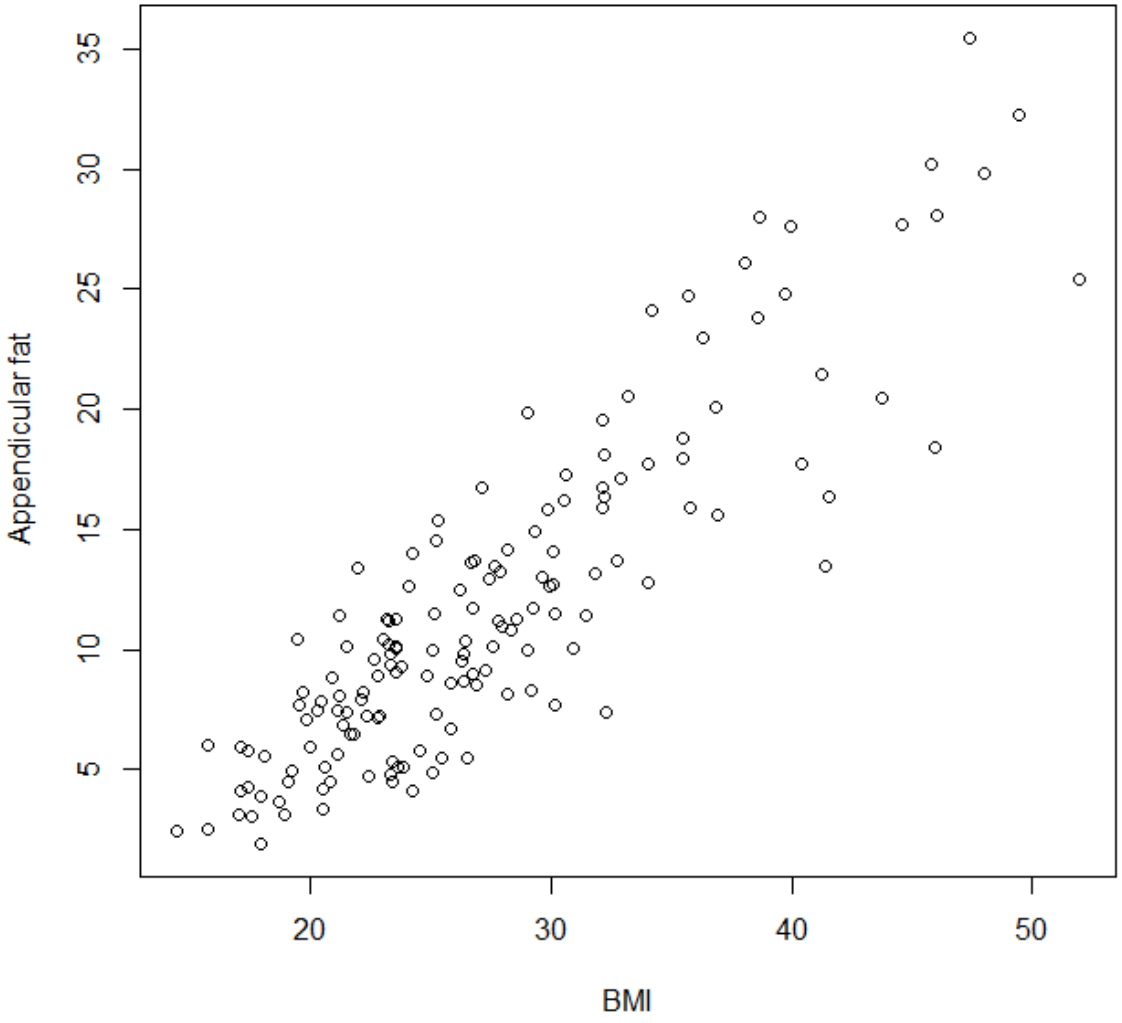


Figure 12: Scatter plot of triceps skin-fold and BMI in women in the cross-sectional analysis:

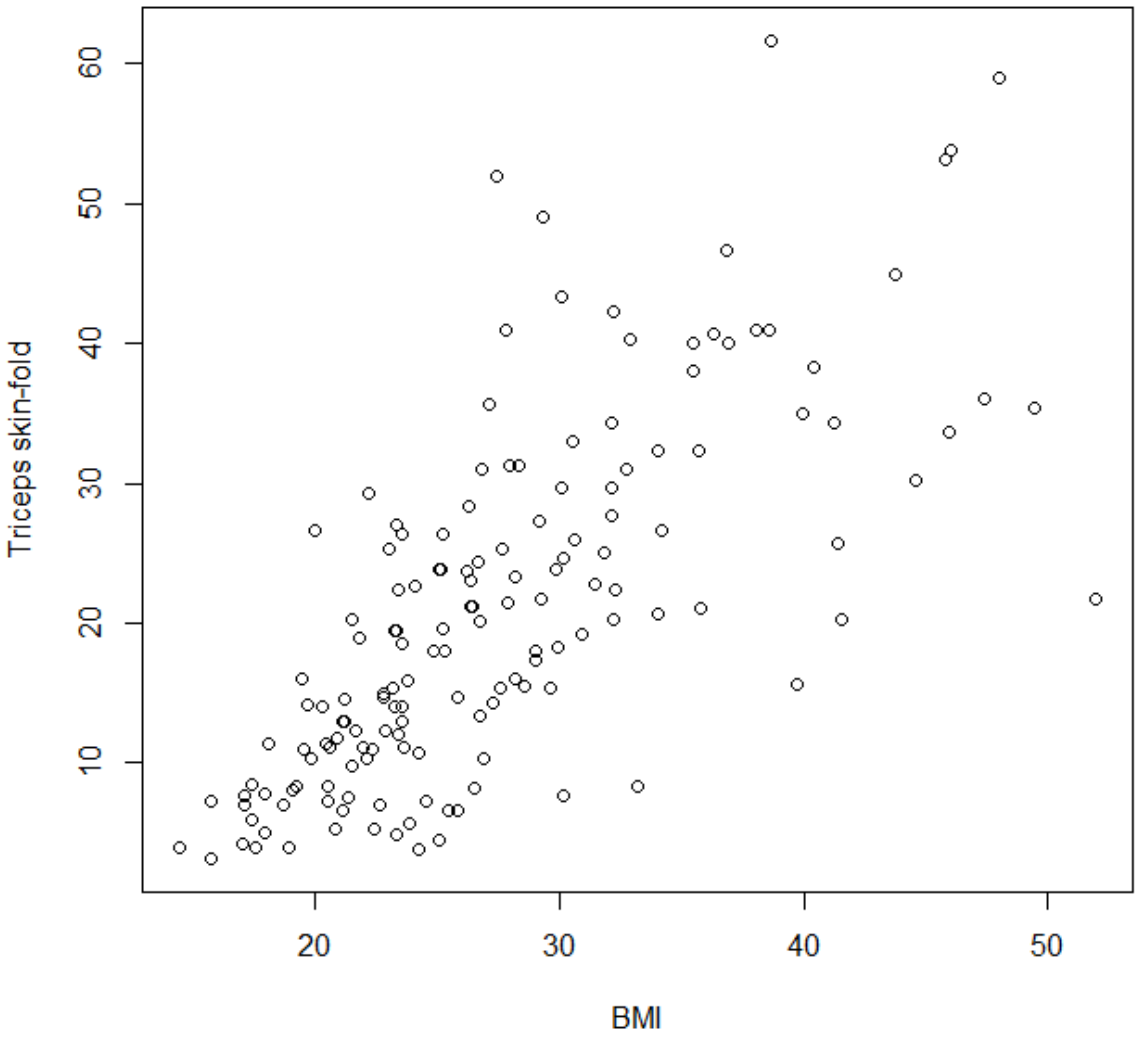


Figure 13: Scatter plot of percent lean mass and BMI in women in the cross-sectional analysis:

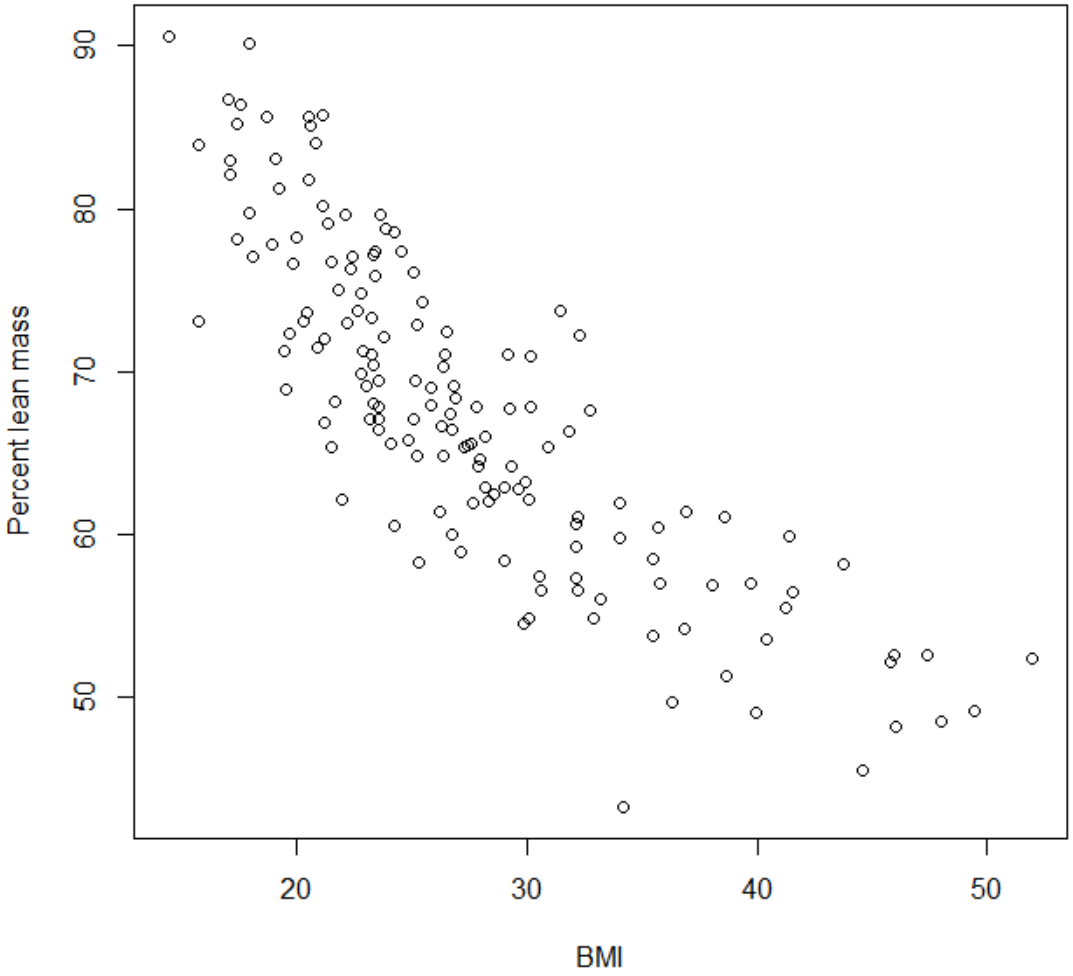
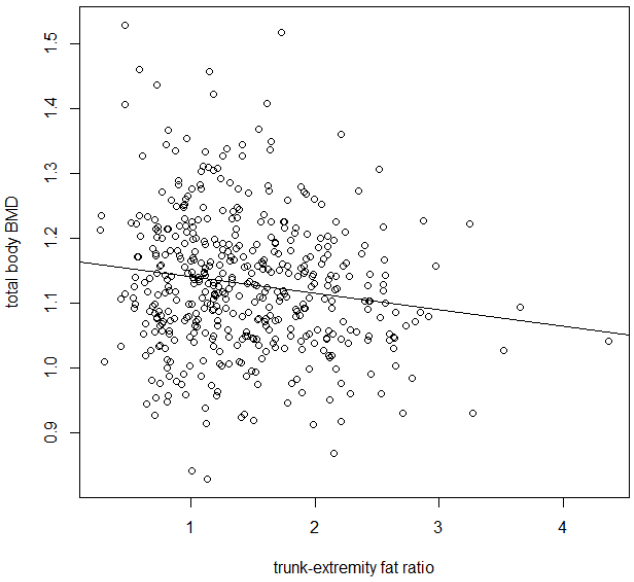
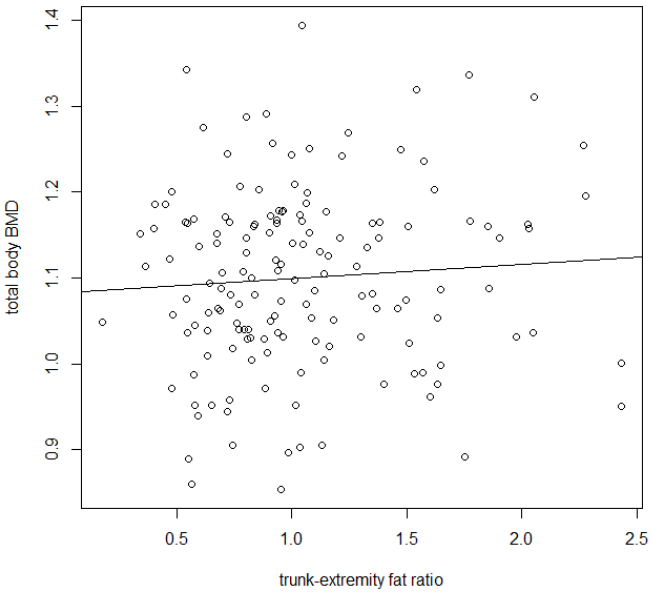


Figure 14: Trunk to extremity fat ratio and total body BMD in HIV-infected men and women
(n=466 men; n=153 women)

Men



Women



Median trunk-to-extremity fat ratio was 1.13 in the longitudinal cohort (Table 1). Baseline trunk-to-extremity fat ratio was not associated with 2-year changes in total body BMD, after controlling for age, race, exercise, smoking, and BMI (Table 3, Model 1).

Women

Median trunk-to-extremity fat ratio was 0.95 in the cross-sectional analysis (Table 1). The quadratic term for trunk-to-extremity fat ratio had a p-value of 0.7 in the cross-sectional analysis. Therefore, the model examining the relation between trunk-to-extremity fat ratio and total body BMD was deemed adequate. After controlling for age, race, exercise, smoking, and BMI, in the cross-sectional analysis, there was no association between trunk-to-extremity fat ratio, each of the measures of central (waist circumference and trunk fat) and appendicular (triceps skin fold and appendicular fat) adiposity with total body BMD (Table 2, Models 1-5). Conversely, in the models without BMI, higher trunk-to-extremity fat ratio and measures of central (waist circumference and trunk fat) were associated with higher total body BMD.

Median trunk-to-extremity fat ratio was 0.92 in the longitudinal cohort (Table 1). Baseline trunk-to-extremity fat ratio was not associated with 2-year changes in total body BMD, after controlling for age, race, exercise, smoking, and BMI (Table 3, Model 1).

3.4 Lean Mass and BMD

Men

Higher percent lean mass by DXA was associated with higher total body BMD, after controlling for age, race, exercise, smoking, and BMI, in the cross-sectional analysis. Conversely, there was no association between percent lean mass by DXA and total body BMD when BMI was taken out of the model (Table 2, Model 6). Moreover,

baseline percent lean mass was not associated with 2-year changes in total body BMD (Table 3, Model 2).

Women

Higher percent lean mass by DXA was associated with higher total body BMD after controlling for age, race, exercise, smoking, and BMI, in the cross-sectional analysis. Conversely, there was no association between percent lean mass by DXA and total body BMD when BMI was taken out of the model (Table 2, Model 6). There was no association between baseline percent lean mass and 2-year changes in total body BMD (Table 3, Model 2).

Table 2: Cross sectional analysis of measures of body fat, lean mass and total body BMD
(n=466 men; n=153, women)

	Unadjusted		Adjusted*		Adjusted**	
	Men	Women	Men	Women	Men	Women
	Beta (SE) P value	Beta (SE) P value	Beta (SE) P value	Beta (SE) P value	Beta (SE) P value	Beta (SE) P value
Model1 Trunk_extremity fat ratio	-0.03 (0.01) *0.001*	0.016 (0.019) 0.38	-0.01 (0.01) 0.2	0.03 (0.02) 0.06	-0.02 (0.01) *0.01*	0.02 (0.02) 0.2
Model 2 Trunk fat kg	0.001 (0.001) 0.4	0.002 (0.001) *0.025*	0.001 (0.001) 0.2	0.002 (0.001) *0.02*	-0.008 (0.002) *<0.001*	0.001 (0.003) 0.6
Model 3 Waist circumference cm	0.001 (0.0004) *0.01*	0.001 (0.0005) *0.02*	0.001 (0.0004) *0.002*	0.001 (0.001) *0.01*	-0.002 (0.001) *0.04*	0.001 (0.001) 0.6
Model 4 Appendicular fat kg	0.004 (0.001) *0.003*	0.003 (0.001) *0.04*	0.003 (0.001) *0.03*	0.002 (0.001) 0.1	-0.003 (0.002) 0.1	-0.003 (0.003) 0.3
Model 5 Triceps skin fold mm	0.0004 (0.0007) 0.5	0.0008 (0.0007) 0.2	6 e-05 (7 e-04) 0.9	0.0007 (0.001) 0.3	-0.002 (0.001) *0.006*	-0.001 (0.001) 0.4
Model 6 Percent Lean mass	0.001 (0.001) 0.4	-0.001 (0.001) 0.3	0.001 (0.001) 0.4	-0.001 (0.001) 0.4	0.004 (0.001) *<0.001*	0.003 (0.001) 0.06

Models 1-6 are all separate models

* Adjusted for age (years), race (White, Black, Hispanic), strength training (current vs. not current), smoking (current vs. former/never)

** Adjusted for age (years), race (White, Black, Hispanic), strength training (current vs. not current), smoking (current vs. former/never) and BMI (kg/m^2)

Table 3: Longitudinal analysis of the association between baseline trunk-extremity fat ratio, lean mass and percent change in total body BMD
(n=297 men; n=101 women)

	Unadjusted		Adjusted*		Adjusted**	
	Men	Women	Men	Women	Men	Women
	Beta (SE) P value	Beta (SE) P value	Beta (SE) P value	Beta (SE) P value	Beta (SE) P value	Beta (SE) P value
Model 1 Trunk-to-extremity fat ratio	0.17 (0.29) 0.6	0.34 (0.94) 0.7	0.02 (0.33) 0.9	0.27 (1.02) 0.8	0.04 (0.34) 0.9	0.14 (1.08) 0.9
Model 2 Percent lean mass	0.01 (0.02) 0.5	0.01 (0.04) 0.8	0.004 (0.02) 0.8	-0.01 (0.04) 0.7	0.005 (0.03) 0.9	-0.002 (0.06) 0.9

Models 1and 2 are separate models.

* Adjusted for age (years), race (Non-Hispanic White, Non-Hispanic Black, Hispanic), strength training (current vs. not current), smoking (current vs. former/never)

** Adjusted for age (years), race (White, Black, Hispanic), strength training (current vs. not current), smoking (current vs. former/never) and BMI (kg/m^2)

Discussion

We found a significant inverse association between both central and appendicular measures of body adiposity with total body BMD after controlling for age, race, smoking, strength training and BMI in a cohort of men living with HIV. Similar associations were not observed in our sample of women living with HIV after similar multivariable adjustment. Lean mass was positively associated with total body BMD in both the men and women in this study in the models that adjusted for BMI.

The associations between measures of body adiposity and lean mass with total body BMD were quite different in both men and women when we did not control for BMI. One conceivable explanation for these

different associations is that the measures of central and appendicular fat mass when adjusted for BMI are better

predictors of HARS and perhaps visceral fat than in the models without BMI.

Recent preclinical and clinical studies indicate a potential adverse effect of excess adipose tissue on bone metabolism [5-7, 17, 19-21, 45-47]. Fat, in particular visceral fat, releases multiple circulating pro-inflammatory factors called adipokines that stimulate osteoclastogenesis and bone resorption [5, 45, 48]. In addition, bone marrow fat can have a lipotoxic effect on bone cells through the secretion of fatty acids which block osteoblast differentiation from mesenchymal stem cells inside the bone marrow [46, 48-50]. The inverse association between central measures of body adiposity and total body BMD in men, in our study, is consistent with other studies examining the association between central fat and BMD in both HIV and non-HIV infected males [7, 10, 15, 47, 51]. Our data also indicate an inverse association between peripheral measures of body adiposity

and total body BMD in men unlike prior studies that showed lower BMD in association with lower appendicular fat in men infected with HIV [11, 14]. Possible explanations for these differing findings are that the prior reports had a much smaller sample size of men and used different criteria for measuring peripheral fat. In addition, the men in the prior reports were much younger and had lower BMD levels than our study sample.

We did not find an association between measures of body adiposity and total body BMD in our sample of women as was seen in the Women's Interagency HIV Study [9]. Our null finding may have been, in part, attributed to the smaller sample size of women in our study. The Women's Interagency HIV Study, which found that increased trunk fat was associated with increased total hip and femoral neck BMD in mostly Black women infected with HIV, had over 300 HIV-infected women. It also differed from our study in that it examined a more specific skeletal site, hip BMD, rather than total BMD and did not control for BMI. As demonstrated in a study in non-HIV infected men and women, relationships between measures of adiposity, such as trunk-to-extremity fat ratio, were positively associated with lumbar spine but not total body BMD [44].

Our findings of a positive association between lean mass and total body BMD in men and women are consistent with prior studies in persons infected with HIV [23-25]. Furthermore, a recent meta-analysis of 44 studies reported a positive correlation between lean mass with total body BMD in both men and women not infected with HIV [52]. Thus, this study lends further support to the concept that lean mass is an important determinant of BMD in both men and women infected with HIV.

In our longitudinal analysis, neither baseline trunk-to-extremity fat ratio nor lean mass was associated with 2-year changes in total body BMD in men or women. This lack

of an association may be due, in part, to the smaller sample size and the relatively short 18- to 30-month follow-up interval, which in a middle-aged cohort of individuals may not be adequate to detect BMD change. Furthermore, the longitudinal cohort included participants who were in the cross-sectional cohort but had baseline data taken at an earlier point in the study. These individuals were less likely to have been on tenofovir compared to our full cohort. Tenofovir use started in 2001 for this group. For the cross-sectional group, 23% had visits before 2001, but for the longitudinal group, 64% had the baseline visit before 2001. Use of tenofovir has been associated with increased bone loss in persons infected with HIV [31, 40, 53]. These findings suggest that the association between measures of central and peripheral fat mass, lean mass and change in BMD over time will need to be further investigated in an older cohort, followed over a longer period of time and accounting for changes in body adiposity and lean mass that may result from change in antiretroviral therapy as well as other interventions.

The strengths of the present study are the inclusion of both women and men infected with HIV who were followed with serial DXA scans as well as the availability of several anthropometric body composition indices (waist circumference and triceps skin-fold) and DXA-derived measures (trunk-to-extremity fat ratio, trunk fat and appendicular fat) collected during the observational study.

However, one limitation is that these measures do not provide as accurate an assessment of visceral versus subcutaneous fat as measurements taken from MRI or CT. Therefore, we used the trunk-to-extremity fat ratio, a commonly-used measure for determining the relationship between these two fat depots and a good predictor of visceral fat [34]. Second, our study was limited by the measures collected during the

observational study which did not include biochemical measures (i.e., calcium, 25-hydroxyvitamin D, testosterone, or markers of bone turnover). We chose a two-year DXA interval as we were limited by loss to follow-up for longer intervals. We do not have data on site-specific BMD but rather total body BMD, but these two measurements are often correlated [54]. Data on bisphosphonate therapy was not collected in this study; however, we anticipate that it was a not a highly prescribed medication in this younger adult population, thus, less likely to influence our results.

Conclusion

In this cohort, there was a positive association between lean mass and total body BMD in both sexes with HIV, implying that lean mass is an important determinant of BMD in this population. Our study also found a negative association between measures of body fat and total body BMD in men with HIV, suggesting that higher fat mass in men with HIV may have an adverse effect on BMD. Baseline body composition measures did not predict change in total body BMD over a 2-year period in our sample. Larger and longer-term studies are needed to confirm these findings. Furthermore, studies that include site-specific BMD together with markers of bone turnover and relevant circulating hormones will be helpful in obtaining a better understanding of the relationship between body composition measures and changes in BMD.

References

1. Prieto-Alhambra, D., et al., *"HIV infection and its association with an excess risk of clinical fractures and : a nation-wide case-control study.* J Acquir Immune Defic Syndr, 2014.
2. Triant, V.A., 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**(9): p. 3499-504.
3. Young, B., et al., *Increased rates of bone fracture among HIV-infected persons in the HIV Outpatient Study (HOPS) compared with the US general population, 2000-2006.* Clin Infect Dis, 2011. **52**(8): p. 1061-8.
4. Jacobson, D.L., et al., *Prevalence of, evolution of, and risk factors for fat atrophy and fat deposition in a cohort of HIV-infected men and women.* Clin Infect Dis, 2005. **40**(12): p. 1837-45.
5. Gilsanz, V., et al., *Reciprocal relations of subcutaneous and visceral fat to bone structure and strength.* J Clin Endocrinol Metab, 2009. **94**(9): p. 3387-93.
6. Choi, H.S., et al., *Relationship between visceral adiposity and bone mineral density in Korean adults.* Calcif Tissue Int, 2010. **87**(3): p. 218-25.
7. Bhupathiraju, S.N., et al., *Centrally located body fat is associated with lower bone mineral density in older Puerto Rican adults.* Am J Clin Nutr, 2011. **94**(4): p. 1063-70.
8. Yao, J., et al., *The Pilot Study of DXA Assessment in Chinese HIV-Infected Men With Clinical Lipodystrophy.* Journal of Clinical Densitometry, 2011. **14**(1): p. 58-62.
9. 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.* J Acquir Immune Defic Syndr, 2012. **61**(4): p. 469-76.
10. Huang, J.S., et al., *Increased abdominal visceral fat is associated with reduced bone density in HIV-infected men with lipodystrophy.* AIDS, 2001. **15**(8): p. 975-82.
11. Huang, J.S., R.V. Mulkern, and S. Grinspoon, *Reduced intravertebral bone marrow fat in HIV-infected men.* AIDS, 2002. **16**(9): p. 1265-9.
12. Degris, E., et al., *Longitudinal study of body composition of 101 HIV men with lipodystrophy: dual-energy X-ray criteria for lipodystrophy evolution.* J Clin Densitom, 2010. **13**(2): p. 237-44.
13. 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 Infect Dis, 2013. **13**(1): p. 293.
14. Rosenthall, L. and J. Falutz, *Bone mineral and soft-tissue changes in AIDS-associated lipoatrophy.* J Bone Miner Metab, 2005. **23**(1): p. 53-7.
15. Brown, T.T., 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, 2004. **89**(3): p. 1200-6.
16. McDermott, A.Y., et al., *Effect of highly active antiretroviral therapy on fat, lean, and bone mass in HIV-seropositive men and women.* Am J Clin Nutr, 2001. **74**(5): p. 679-86.

17. Nguyen, T.V., et al., *Bone mass, lean mass, and fat mass: same genes or same environments?* Am J Epidemiol, 1998. **147**(1): p. 3-16.
18. Douchi, T., et al., *Relative contribution of lean and fat mass component to bone mineral density in males.* J Bone Miner Metab, 2003. **21**(1): p. 17-21.
19. Hsu, Y.H., et al., *Relation of body composition, fat mass, and serum lipids to osteoporotic fractures and bone mineral density in Chinese men and women.* Am J Clin Nutr, 2006. **83**(1): p. 146-54.
20. Reid, I.R., et al., *Determinants of total body and regional bone mineral density in normal postmenopausal women--a key role for fat mass.* J Clin Endocrinol Metab, 1992. **75**(1): p. 45-51.
21. Reid, I.R., *Relationships among body mass, its components, and bone.* Bone, 2002. **31**(5): p. 547-55.
22. Reid, I.R., et al., *Determinants of the rate of bone loss in normal postmenopausal women.* J Clin Endocrinol Metab, 1994. **79**(4): p. 950-4.
23. Buehring, B., et al., *The frequency of low muscle mass and its overlap with low bone mineral density and lipodystrophy in individuals with HIV--a pilot study using DXA total body composition analysis.* J Clin Densitom, 2012. **15**(2): p. 224-32.
24. Brown, T.T., et al., *Body composition, soluble markers of inflammation, and bone mineral density in antiretroviral therapy-naive HIV-1-infected individuals.* J Acquir Immune Defic Syndr, 2013. **63**(3): p. 323-30.
25. Erlandson, K.M., et al., *Functional impairment is associated with low bone and muscle mass among persons aging with HIV infection.* J Acquir Immune Defic Syndr, 2013. **63**(2): p. 209-15.
26. Shevitz, A.H., et al., *Elevated resting energy expenditure among HIV-seropositive persons receiving highly active antiretroviral therapy.* AIDS, 1999. **13**(11): p. 1351-7.
27. Silva, M., et al., *The effect of protease inhibitors on weight and body composition in HIV-infected patients.* AIDS, 1998. **12**(13): p. 1645-51.
28. Wilson, I.B., et al., *Relation of lean body mass to health-related quality of life in persons with HIV.* J Acquir Immune Defic Syndr, 2000. **24**(2): p. 137-46.
29. Forrester, J.E., et al., *Weight loss and body-composition changes in men and women infected with HIV.* Am J Clin Nutr, 2002. **76**(6): p. 1428-34.
30. Jones, C.Y., et al., *Insulin resistance in HIV-infected men and women in the nutrition for healthy living cohort.* J Acquir Immune Defic Syndr, 2005. **40**(2): p. 202-11.
31. Jacobson, D.L., et al., *Evolution and predictors of change in total bone mineral density over time in HIV-infected men and women in the nutrition for healthy living study.* J Acquir Immune Defic Syndr, 2008. **49**(3): p. 298-308.
32. Freitas, P., et al., *Fat mass ratio: an objective tool to define lipodystrophy in hiv-infected patients under antiretroviral therapy.* J Clin Densitom, 2010. **13**(2): p. 197-203.
33. Rosenthall, L. and J. Falutz, *Estimation of Total-Body and Regional Soft Tissue Composition From DXA Bone Densitometry of the Lumbar Spine and Hip.* Journal of Clinical Densitometry, 2010. **13**(3): p. 263-266.

34. Savgan-Gurol, E., et al., *Waist to hip ratio and trunk to extremity fat (DXA) are better surrogates for IMCL and for visceral fat respectively than for subcutaneous fat in adolescent girls.* Nutr Metab (Lond), 2010. **7**: p. 86.
35. Hadigan, C., et al., *Fasting hyperinsulinemia and changes in regional body composition in human immunodeficiency virus-infected women.* J Clin Endocrinol Metab, 1999. **84**(6): p. 1932-7.
36. Asha, H.S., et al., *Human immunodeficiency virus-associated lipodystrophy: an objective definition based on dual-energy x-ray absorptiometry-derived regional fat ratios in a South Asian population.* Endocr Pract, 2012. **18**(2): p. 158-69.
37. Ackerman, K.E., et al., *DXA surrogates for visceral fat are inversely associated with bone density measures in adolescent athletes with menstrual dysfunction.* J Pediatr Endocrinol Metab, 2011. **24**(7-8): p. 497-504.
38. Dolan, S.E., S. Carpenter, and S. Grinspoon, *Effects of weight, body composition, and testosterone on bone mineral density in HIV-infected women.* J Acquir Immune Defic Syndr, 2007. **45**(2): p. 161-7.
39. Greenland, S., J. Pearl, and J.M. Robins, *Causal diagrams for epidemiologic research.* Epidemiology, 1999. **10**(1): p. 37-48.
40. Powderly, W.G., *Osteoporosis and bone health in HIV.* Curr HIV/AIDS Rep, 2012. **9**(3): p. 218-22.
41. Kelsey, J.L., *Risk factors for osteoporosis and associated fractures.* Public Health Rep, 1989. **104 Suppl**: p. 14-20.
42. Forman, M.R., et al., *Life-course origins of the ages at menarche and menopause.* Adolesc Health Med Ther, 2013. **4**: p. 1-21.
43. Berentzen, T.L., et al., *Waist circumference adjusted for body mass index and intra-abdominal fat mass.* PLoS One, 2012. **7**(2): p. e32213.
44. Zillikens, M.C., et al., *The role of body mass index, insulin, and adiponectin in the relation between fat distribution and bone mineral density.* Calcif Tissue Int, 2010. **86**(2): p. 116-25.
45. Cohen, A., et al., *Abdominal fat is associated with lower bone formation and inferior bone quality in healthy premenopausal women: a transiliac bone biopsy study.* J Clin Endocrinol Metab, 2013. **98**(6): p. 2562-72.
46. Fazeli, P.K., et al., *Marrow fat and bone--new perspectives.* J Clin Endocrinol Metab, 2013. **98**(3): p. 935-45.
47. Katzmarzyk, P.T., et al., *Relationship between abdominal fat and bone mineral density in white and African American adults.* Bone, 2012. **50**(2): p. 576-9.
48. Ofotokun, I., E. McIntosh, and M.N. Weitzmann, *HIV: inflammation and bone.* Curr HIV/AIDS Rep, 2012. **9**(1): p. 16-25.
49. Wiercinska-Drapalo, A., et al., *The possible association between serum cholesterol concentration and decreased bone mineral density as well as intravertebral marrow fat in HIV-1 infected patients.* Infection, 2007. **35**(1): p. 46-8.
50. Gunaratnam, K., et al., *Mechanisms of palmitate-induced lipotoxicity in human osteoblasts.* Endocrinology, 2014. **155**(1): p. 108-16.
51. Pasco, J.A., et al., *Musculoskeletal deterioration in men accompanies increases in body fat.* Obesity (Silver Spring), 2014. **22**(3): p. 863-7.

52. Ho-Pham, L.T., U.D. Nguyen, and T.V. Nguyen, *Association between lean mass, fat mass, and bone mineral density: a meta-analysis.* J Clin Endocrinol Metab, 2014. **99**(1): p. 30-8.
53. Stellbrink, H.J., et al., *Comparison of changes in bone density and turnover with abacavir-lamivudine versus tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study.* Clin Infect Dis, 2010. **51**(8): p. 963-72.
54. Franck, H. and M. Munz, *Total body and regional bone mineral densitometry (BMD) and soft tissue measurements: correlations of BMD parameter to lumbar spine and hip.* Calcif Tissue Int, 2000. **67**(2): p. 111-5.