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Overweight/Obesity and HIV Disease Progression in HIV+ Adults in Botswana

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OVERWEIGHT/OBESITY AND HIV DISEASE PROGRESSION IN HIV+ ADULTS IN BOTSWANA

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by

Sabrina Sales Martinez

2015

To: Interim Dean Mark Williams
R.Stempel College of Public Health and Social Work

This dissertation, written by Sabrina Sales Martinez, and entitled Overweight/Obesity and HIV Disease Progression in HIV+ Adults in Botswana, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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DEDICATION

First and foremost, I thank my parents for their love and support throughout my life and during my journey in completing my doctoral degree. To my loving husband, many thanks for supporting me in any outrageous endeavor I have ever wanted to embark on, including a PhD.

I also dedicate this dissertation to my uncle and godfather who passed away too young from HIV and was not able to live to see and take advantage of the progress and wonderful discoveries made in HIV research. His memory will always drive my passion for research in this field.

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ABSTRACT OF THE DISSERTATION

OVERWEIGHT/OBESITY AND HIV DISEASE PROGRESSION IN HIV+ ADULTS IN BOTSWANA

by

Sabrina Sales Martinez

Florida International University, 2015

Miami, Florida

Professor Adriana Campa, Major Professor

Studies indicate that overweight and obesity protect against HIV-disease progression in antiretroviral therapy (ART)-naïve patients. We examined retrospectively the relationship of overweight/obesity with HIV-disease progression in ART-naïve HIV+ adults in Botswana in a case-control study with 18-month follow-up, which included 217 participants, 139 with BMI 18.0-24.9 kg/m^2 and 78 with BMI ≥25 kg/m^2. Archived plasma samples were used to determine inflammatory markers: leptin and bacterial endotoxin lipopolysaccharide (LPS), and genotype single nucleotide polymorphisms (SNPs) of the Fat Mass and Obesity Associated Gene (FTO).

At baseline, BMI was inversely associated with risk for AIDS-defining conditions (HR=0.218; 95%CI=0.068, 0.701, *P*=0.011), and higher fat mass was associated with reduced risk of the combined outcome of CD4+cell count ≤250/μL and AIDS-defining conditions, whichever occurred earlier (HR=0.918; 95%CI=0.847, 0.994, *P*=0.036) over 18 months, adjusting for age, gender, marriage, children, and baseline CD4+cell count and HIV-viral load.

FTO-SNP rs17817449 was associated with BMI (OR=1.082; 95%CI=1.001, 1.169; *P*=0.047). Fat mass was associated with the risk alleles of rs1121980 (OR=1.065; 95%CI=1.009, 1.125, *P*=0.021), rs8050136 (OR=1.078; 95%CI=1.021, 1.140; *P*=0.007),

and rs17817449 (OR=1.086; 95%CI=1.031, 1.145; *P*=0.002), controlling for age, gender, tribe, total energy intake, and activity. There were no associations of SNPs with markers of disease progression.

Leptin levels were positively associated with BMI (β=1.764; 95%CI=0.788, 2.739; *P*=0.022) and fat mass (β=0.112; 95%CI=0.090, 0.135; *P*<0.001), but inversely with viral load (β=-0.305; 95%CI=-0.579, -.031; *P*=0.030). LPS levels were inversely associated with BMI (OR=0.790, 95%CI=0.630, 0.990; *P*=0.041), and fat mass (OR=0.852, 95%CI=0.757, 0.958; *P*=0.007) and directly with viral load (OR=2.608, 95%CI=1.111, 6.124; *P*=0.028), adjusting for age, gender, smoking and %fat mass.

In this cohort, overweight/obesity predicted slower HIV-disease progression. Obesity may confer an advantage in maintaining fat stores to support the overactive immune system. FTO-SNPs may contribute to the variation in fat mass; however, they were not associated with HIV-disease progression. Our findings suggest that the obesity paradox may be explained by the association of increased LPS with lower BMI and higher viral load; while viral load decreased with increasing leptin levels. Studies in African populations are needed to clarify whether genetic variation and inflammation mediate the obesity paradox in HIV-disease progression.

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ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
BIA	Bioelectrical impedance
BMI	Body mass index
CRP	C-reactive protein
FTO	Fat mass and obesity associated gene
HALS	HIV associated lipodystrophy syndrome
HIV	Human Immunodeficiency Virus
IL-6	Interleukin 6
LAL	Limulus amebocyte lysate
LPS	Lipopolysaccharide
SNP	Single nucleotide polymorphism
TB	Tuberculosis
TNF-α	Tumor necrosis factor alpha

CHAPTER I: INTRODUCTION

Statement of Problem

The prevalence of obesity is increasing worldwide, including developing African nations.[1,2] Botswana is experiencing an increase in obesity, which is also observed among the HIV+ adults.[3] At the same time, Botswana is experiencing one of the worst epidemics of HIV with a prevalence rate of 23.0% among those between the ages 15 to 49 years.[4] An obesity paradox has been documented in many conditions including HIV, where those who are obese may have a survival advantage or improved disease outcomes.[5] It is described as paradox in HIV since obesity has been associated with higher CD4 cell counts and delayed time to AIDS defining conditions.[6-9] Studies on HIV and obesity are mainly conducted in settings where the patients are treated with antiretroviral therapy (ART), and where being or becoming obese is a disadvantage for physical and mental reasons, as obesity increases the risk for comorbidities with confounding findings and interpretations.[10,11] In addition, inflammation accompanies both obesity and HIV disease, and its role in HIV disease progression has not been described in the ART naïve population, specifically as related to markers of inflammation such as Leptin, C-reactive protein and immune activation and bacterial lipopolysaccharide (LPS) endotoxin.

Genetics has a strong influence on obesity[12] which has been estimated to account for 40-70% of the variation in human adiposity.[13] The association of the Fat Mass and Obesity Associated (*FTO)* gene has been reported to be strong with obesity, however, it has not been replicated in all populations and currently there are no published studies on the *FTO* gene variants in HIV disease or in a population in Botswana.

The objective of this study was to examine the relationship between overweight/obesity and HIV disease progression, inflammatory markers, and SNPs of the *FTO* gene in HIV+ adults who are not on ART in Botswana.

Significance of Study

The proposed research is important since there are several studies in the literature indicating that overweight or obesity provides some protection from accelerated HIV disease progression,[6-9] but the potential mechanisms for such relationships are lacking. This study design allows for the examination of the effects of HIV before ART initiation to obtain a better understanding of how obesity and genetic factors are interrelated and affect HIV disease progression. In addition, as HIV becomes a chronic disease with the advent of more effective and accessible treatments, and the risks for obesity and its comorbidities increase, recognizing genetic susceptibility for obesity will help design interventions that are tailored to an individual's genetic variations.[14]

The evidence from the literature generates the following questions:

- Is the "obesity advantage" related to the genetically acquired energy efficiency, regardless of fat accumulation, or it is associated with fat accumulation that provides a protection to rapid wasting, which is one of the most powerful signs of HIV-disease progression and associated with shorter time to death?
- Could it be the result of both factors?
- How does the interaction of obesity and HIV affect inflammation and the immune response?
- Could those without a genetic risk for obesity benefit from early aggressive nutrition intervention and ART to prevent wasting?

Botswana is one of the first resource-limited countries to provide ART on a sizeable scale,[15] however, many countries, especially in sub-Saharan Africa, still have challenges in providing ART and maintaining adherence.[16] Of the 21.2 million people in Africa eligible for ART under the 2013 WHO guidelines, only 7.6 million are receiving ART.[17] Therefore, acquiring information on factors that may delay disease progression and possible mechanisms related to these risks is significant and timely. Moreover, the findings from this study could be translated and contribute towards clinically relevant recommendations.

Innovation

This is the first study exploring the role of genetic susceptibility to obesity as a positive factor to delay HIV disease progression in ART naïve patients.

In the following three studies, hypotheses were tested and results presented as research papers.

Specific Aims and Hypothesis

CHAPTER III: EFFECT OF BMI AND BODY COMPOSITION ON HIV DISEASE PROGRESSION IN HIV INFECTED ART NAÏVE ADULTS IN BOTSWANA

***Specific Aim 1*:** To determine the relationship between being overweight/obese (BMI ≥ 25 kg/m^2) and HIV disease progression (CD4 cell count, HIV viral load and AIDS defining conditions) in HIV+ asymptomatic adults in Botswana not on antiretroviral therapy (ART) over 18 months.

> ***Hypothesis 1a*:** (Primary outcome) The overweight/obese group will present less clinically significant CD4 cell count decline from Baseline (≥ 25%) over 18 months than the normal weight group.
>
> ***Hypothesis 1b*:** The overweight/obese will have significantly lower levels of HIV viral load over 18 months than the normal weight group.
>
> ***Hypothesis 1c:*** Significantly less AIDS defining conditions will occur over 18 months in the overweight/obese group compared to the normal weight group.

CHAPTER IV: PRELIMINARY ASSOCIATION STUDY OF THE FAT MASS AND OBESITY GENE POLYMORPHISMS WITH HIV DISEASE PROGRESSION

Specific Aim 2: To determine the interrelationships among being overweight/obese, markers of genetic propensity for obesity (single nucleotide polymorphisms (SNPs) in *FTO*) and HIV disease progression (CD4 cell count, HIV viral load and AIDS defining conditions) in HIV+ asymptomatic adults in Botswana not on ART over 18 months.

> ***Hypothesis 2a:*** The *FTO* gene SNPs will be associated with being overweight/obese.
>
> ***Hypothesis 2b:*** The *FTO* gene SNPs will be associated with delayed HIV disease progression over 18 months.
>
> > ***Hypothesis 2b1:*** The *FTO* gene SNPs will be associated with significantly higher CD4 cell count.
> >
> > ***Hypothesis 2b2:*** The *FTO* gene SNPs will be associated with significantly lower HIV viral load.
> >
> > ***Hypothesis 2b3:*** The *FTO* gene SNPs will be associated with significantly less AIDS-defining conditions.

CHAPTER V: ASSOCIATION OF INFLAMMATORY MARKERS WITH BODY COMPOSITION AND HIV DISEASE PROGRESSION IN ART NAÏVE HIV+ ADULTS IN BOTSWANA

***Specific Aim 3*:** To evaluate the relationship between the inflammatory markers (leptin, C-reactive protein and bacterial lipopolysaccharide) and HIV disease progression in HIV+ asymptomatic adults not on ART in Botswana over 18 months.

> ***Hypothesis* 3a**: Higher levels of leptin, C-reactive protein and bacterial lipopolysaccharide will be associated with significantly faster HIV disease progression.
>
> > ***Hypothesis 3a1***: Higher levels of inflammatory markers will be associated with lower levels of CD4 cell count.
> >
> > ***Hypothesis 3a2***: Higher levels of inflammatory markers will be associated with higher HIV viral load.
> >
> > ***Hypothesis 3a3***: Higher levels of inflammatory markers will be associated with greater AIDS-defining conditions.

Table 1: Statistical Analysis of Hypotheses

Hypothesis	Dependent Variable (Outcome Variable)	Measurement of Dependent Variable	Type of Dependent Variable	Independent Variable	Measurement of Independent Variable	Type of Independent Variable	Statistical Analysis
1a	CD4 cell count (decrease of ≥ 25%)	CD4 cell count in blood	Dichotomous variable (0=no decrease ≥ 25% and 1=yes decrease ≥ 25%)	Body Mass Index (BMI)	BMI measured as weight in kg/ height in meters squared	Dichotomous variable (0=BMI 18.0-24.9kg/m^2 and 1=BMI ≥ 25 kg/m^2)	Proportional Hazard Survival Model will be performed to compare hazard ratios on time to event (CD4 cell count decrease ≥ 25%) over 18 months.
1b	HIV viral load	HIV viral load in blood	Continuous variable	Body Mass Index (BMI)	BMI measured as weight in kg/ height in meters squared	Dichotomous variable (0=BMI 18.0-24.9kg/m^2 and 1=BMI ≥ 25 kg/m^2)	Pearson and Spearman correlations, as deemed appropriate and scatterplots will be performed to evaluate the relationship between HIV viral load and BMI groups. Linear regression will be used to examine the relationship between HIV viral load, at baseline and 18 months, and BMI groups
1c	AIDS Defining Conditions	Presence of AIDS Defining Condition Recorded by Study Physician	Dichotomous variable (0=no and 1=yes)	Body Mass Index (BMI)	BMI measured as weight in kg/ height in meters squared	Dichotomous variable (0=BMI 18.0-24.9kg/m^2 and 1=BMI ≥ 25 kg/m^2)	Proportional Hazard Survival Model will be performed to compare hazard ratios on time to event (AIDS defining conditions) over 18 months.
2a	Body Mass Index (BMI)	BMI measured as weight in kg/ height in meters	Dichotomous variable (0=BMI 18.0-24.9kg/m^2 and 1=BMI ≥ 25	Genotypes for *FTO* SNPs	5 Genotypes for *FTO* SNPs determined by TaqMan	Dichotomous variable (0=non-risk alleles and	Logistic Regression will be performed to see the relationship between *FTO* SNPs and BMI groups.

		squared	kg/m^2)		Discrimination Assay	1=risk allele)	Cox Proportional Hazard Survival Model will be performed to compare hazard ratios on time to event (BMI ≥ 25 kg/m^2) over 18 months.
2b1	CD4 cell count	CD4 cell count in blood	Continuous variable	Genotypes for *FTO* SNPs	5 Genotypes for *FTO* SNPs determined by TaqMan Discrimination Assay	Dichotomous variable (0=non-risk alleles and 1=risk allele)	Pearson and Spearman correlations, as deemed appropriate, will be performed to evaluate the relationship between markers of disease progression (CD4 cell count and HIV viral load) and *FTO* SNPs. Logistic regression will be used to examine the relationship between markers of disease progression and *FTO* SNPs over 18 months.
2b2	HIV viral load	HIV viral load in blood	Continuous Variable	Genotypes for *FTO* SNPs	5 Genotypes for *FTO* SNPs determined by TaqMan Discrimination Assay	Dichotomous variable (0=non-risk alleles and 1=risk allele)	Pearson and Spearman correlations, as deemed appropriate, will be performed to evaluate the relationship between markers of disease progression (CD4 cell count and HIV viral load) and FTO SNPs. Logistic regression will be used to examine the relationship between markers of disease progression and FTO SNPs over 18 months.
2b3	AIDS	Presence of	Dichotomous	Genotypes	5 Genotypes	Dichotomous	Logistic Regression will

	Defining Conditions	AIDS defining conditions recorded by study physician	variable (0=no and 1=yes)	for *FTO* SNPs	for *FTO* SNPs determined by TaqMan Discrimination Assay	variable (0=non-risk alleles and 1=risk allele)	be performed to see the relationship between AIDS defining conditions and *FTO* SNPs. Cox Proportional Hazard Survival Model will be performed to compare hazard ratios on time to event (AIDS defining condition) over 18 months.
3	Leptin, CRP and LPS	Plasma leptin and LPS measured with ELISA and plasma high sensitivity CRP provided from parent grant	Continuous Variable	CD4 cell count, HIV viral load and AIDS Defining Conditions	CD4 cell count and HIV viral load in blood and presence of AIDS defining conditions recorded by study physician	Continuous variable for CD4 cell count and HIV viral load Dichotomous variable for AIDS Defining Conditions (0=no and 1=yes)	Pearson and Spearman correlations, as deemed appropriate, will be performed to evaluate the relationship between inflammatory markers and markers of disease progression (CD4 cell count and HIV viral load). Student's t-test will be performed to compare the differences in inflammatory markers and presence of AIDS defining conditions. Linear and logistic regressions will be used to examine the relationship between inflammatory markers and markers of disease progression over 18 months.

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CHAPTER II: LITERATURE REVIEW

Obesity Paradox

Gruberg and colleagues[1] coined the term "obesity paradox" after rejecting their original hypothesis and finding that those who were overweight (BMI =25-30 kg/m^2) or obese (BMI >30 kg/m^2) had lower mortality rates 1 year after undergoing percutaneous coronary intervention. However, as early as 1982 this paradox was documented in dialysis patients by Degoulet et al.,[2] which also showed a protective effect of BMI on overall and cardiovascular related mortality. This paradox has been documented in cardiovascular and non-cardiovascular diseases with mortality and other disease-related outcomes, especially in diseases associated with cachexia and wasting.[3]

The mechanisms of the obesity paradox are not well understood. Valentijn et al.,[4] described several causes and their overlapping hypotheses to try to explain the obesity paradox in the surgical population, however the hypotheses can also be extended to other conditions, including HIV. These hypotheses are shown below in Figure1.

Figure 1: Possible Causes of the Obesity Paradox and its' Multifactorial Origin with Overlapping Hypotheses[4]

Potential Mechanisms for the Obesity Paradox

BMI does not differentiate between fat and lean body mass and cannot account for the quantity of adiposity of an individual.[5] Some investigators have suggested the use of waist circumference as a measurement of body fat distribution instead of BMI since it has been shown to be associated with all-cause mortality[6] and inflammatory markers[7,8] independent of BMI. Visceral or abdominal adiposity has been shown to be associated with an inflammatory state,[9] however, BMI does not indicate fat distribution in visceral, peripheral or lower body. Therefore, the positive outcomes in the overweight/obese populations that have been observed may be due to variations of body fat mass and presence of a higher proportion of lean body mass.

Bacterial lipopolysaccharide or LPS is an endotoxin and a stimulator of inflammatory cytokine production and lipoproteins can bind LPS. Models of endotoxaemia in mice and rats have shown that lipoproteins such as LDL, VLDL, HDL, triglycerides and chylomicrons can affect LPS bioactivity.[10-13] Although obesity is associated with an increase in inflammation, there may be an obesity paradox present in some conditions where a reduced inflammatory response is observed. This hypothesis has been coined the "endotoxin-lipoprotein hypothesis." [14] The theory is that LPS binds to lipoproteins through the generation of micelles, therefore reducing cytokine proliferation and inflammation.[15] Rauchhaus et al.[10] proposed that higher levels of cholesterol may be beneficial since lipoproteins can moderate the inflammatory immune response.

Valentijn et al.,[4] also considers genetics as a possible explanation for the survival advantage in overweight/obese. Genetic polymorphisms may have variable effects on body composition through its effect on food intake, energy expenditure and BMI.[16] These variable effects may produce differing survival effects in the overweight/obese populations.

This study examined the obesity paradox in a Botswana population with HIV and ART naïve. The impact of obesity on HIV disease progression over time were explored. The relationship between being overweight/obese and HIV disease progression and its interrelationship between genetic propensity for obesity and inflammatory markers was investigated.

Obesity in Botswana

Growth in economy, urbanization, and diminished physical activity is contributing to a rise in obesity in African countries, such as Botswana.[17] The prevalence of overweight and obesity in 2011 were different throughout sub-Saharan Africa and generally was higher in women compared to men.[17] Botswana has the following rates of overweight (18.5-24.9 kg/m^2): 53.5% in women and 41.6% in men, and obesity (>25 kg/m^2) 17.7% in women and 6.9% in men.[18] A nationally representative study[19] showed that approximately 23.3% of women were overweight compared to 12.9% in men, and 15.7% of women compared to 3.2% of men were obese. The mean BMI for women and men were 24.4 kg/m^2 and 21.7 kg/m^2 respectively. Increasing age and education was associated with higher BMI. BMI increased in women with high parity and remained constant for men. Data on overweight and obese and its relationship to health outcomes are lacking in the general and HIV populations in Botswana.

HIV and Obesity

In 2008, an estimated 1.4 billion people around the world were obese and about 65% of the population in the world live in countries where more people die from being overweight and obese than from being underweight.[20] An increase of obesity has also been observed in HIV+ adults, especially when on antiretroviral treatment (ART),[21] and

rates of obesity are more common now than the occurrence of wasting in developed countries.[22,23] It's been estimated that in sub-Saharan Africa 10% of women had a BMI of less than 18.5 kg/m^2 with wasting still prevalent in some areas.[24] However, sub-Saharan Africa is experiencing a nutrition transition with a rapid rise in overweight and obesity.[25] Studies conducted to investigate obesity and its effect on HIV disease progression have so far been inconclusive, with some studies reporting an association and others reporting no association between CD4 cell counts, a measure of HIV disease progression, and obesity. [21, 26-29]

HIV Disease Progression and Obesity

Studies have shown that obesity may be protective of further HIV disease progression before initiation of ART. Jones et al.[27] showed in 871 women who were recruited from a multicenter prospective cohort in the United States (US) that a higher baseline BMI was associated with a lower chance of reaching CD4 cell count <200 cells/μL for the first time. All women were ART naïve at baseline. A higher BMI was also negatively associated with experiencing HIV-related events, with a 3.1 times greater odds of HIV-related death in the lowest BMI category compared to those in the highest category (95% CI, 1.2–8.1). An average change in CD4 cell count of 1.65 cells/μL per unit change in BMI (95% CI, 0.05–3.25) was experienced and the authors indicated that, positive changes in BMI tended to be associated with positive changes in CD4 cell count, even controlling for previous CD4 cell count and BMI, albeit not significant. Shor-Posner and colleagues[26] studied 125 HIV+ ART naïve adults in Miami, Florida. They followed over 18 months HIV+ obese (BMI >27kg/m^2) adults. Patients had proportionally lower clinically significant decrease in CD4 cell count (decrease of > 25% cells from baseline) in obese than nonobese HIV+ adults (18.8% vs.60.5%, p=0.004). In addition,

BMI during the 18 months was negatively associated with death, even after adjusting for CD4 cell count of < 200 cells/μL at baseline. Shuter et al.[28] through a retrospective review of 189 medical records from New York, showed that being overweight was independently associated with lack of an AIDS diagnosis before clinic enrollment (OR=3.16; CI: 1.44, 6.96). In addition, lower baseline BMI and decreasing BMI during follow-up were independently predictive of advancement to AIDS.

Currently, only one study has shown that immune markers (CD3, CD8, and total lymphocyte counts) were associated with obesity in HIV+ ART treated adults with undetectable viral loads. Adeyemi et al.[29] conducted a study with 216 HIV+ patients with diabetes in Chicago and demonstrated that obese patients (BMI 30-39.9kg/m^2) had CD3 counts 26% higher than patients with normal BMI (p=0.004) and CD8 counts 28% higher (p=0.01), after controlling for age, sex, ethnicity, renal function, A1C, and duration since HIV diagnosis. The result may also be evaluated in a dose-response manner as an absolute increase in CD3 counts of 27 and CD8 of 16 for every increase of 1 kg/m^2 in BMI. There was also a 17% higher total lymphocyte count among the obese patients due to the higher CD3 and CD8 cells after controlling for the same covariates (p=0.04).

Availability of longitudinal studies on BMI and HIV disease progression are lacking. A study conducted by Crum-Cianflone et al.[30] compared immune cell counts and BMI in 1,097 HIV+ patients in seven centers throughout the US in the pre-ART and ART eras. They showed that, at the time of HIV diagnosis, white blood cell count and immune counts (CD4, CD8, CD4/CD8 ratio, CD3, CD19 and CD56) were not statistically significant by BMI categories. In a longitudinal analysis those diagnosed in the pre-ART era had less mean postdiagnosis decreases in white blood cell count, total lymphocyte count, CD4 cell count, CD4%, and CD4/CD8 ratio as BMI category increased (all with $P<0.05$). Obese participants who were diagnosed in the ART era had significantly higher

increases in CD4 cell counts, CD4% and CD4/CD8 ratio (all with $P<0.05$) compared to normal weight participants. A longitudinal retrospective study in HIV+ ART naïve individuals in South Carolina who were starting ART was conducted by Johnson et al.[31] They demonstrated that when comparing normal weight individuals, those who were obese had larger increases in CD4 cell count (5.5 cells/uL, $P<0.001$). Mean CD4 cell count in the obese group was higher at diagnosis (611.2 cells/μL) than the normal weight group (550.5 cells/μL, $P<0.019$). Excess weight in the pre-ART era and even after initiation of ART seems protective of disease progression and contributes to immune recuperation, possibly by preserving energy and nutritional stores in order to deal with HIV and AIDS-related illnesses.

Factors associated with weight gain were investigated in a study conducted by Tate et al.[32] The participants included in the study were from Alabama, ART naïve and initiating ART. Over two years, the prevalence of overweight/obesity increased from 45% to 56%. Those with a BMI of <18.5 kg/m^2 had lower CD4 cell counts and higher viral load at baseline ($P<0.05$) at baseline than the rest. Greater increases in BMI were observed in patients with baseline CD4 count <50 cells/μL and boosted protease inhibitor use, but did not account for all of the variation observed in weight change. Obesity was highly prevalent, however, and the study did not examine how weight gain affected disease progression.

The protective effect provided by obesity has been hypothesized to be related to extra available energy in the form of fat available for use in time of crisis during the HIV disease that would spare protein's use[33] and also help to preserve the immune system, especially innate immunity that is not directly affected by the infection.[28,34] HIV and its secondary infections are known to generate a hypermetabolic state that is sustained throughout the disease.[35] The HIV patient is susceptible to infections, in which the body

will require energy to battle continuous infections and immune activation. Those who are obese may also have a genetic advantage in maintaining fat stores and utilizing energy more efficiently to fuel the immune system. Mechanistic studies in this area are needed.

HIV Disease Progression and Body Composition

Although BMI can provide a simple measure of obesity, its association with body fat mass can vary by age, gender, race/ethnicity, and degree of fitness.[36,37] Measuring body composition to be able to differentiate between body compartments such as lean body mass and fat mass can provide useful information and can be easily completed with the use of bioelectrical impedance (BIA). A safe electric current can be sent through the body to measure resistance and body fat will provide more resistance than lean mass. With the use of equations body fat can be estimated.[38] In addition, BIA can provide an estimate of the amount of intracellular and extracellular water and metabolically active tissues, known as body cell mass. In HIV, BIA has been shown to be an effective approach to detect total and area specific fat mass.[39] While alterations in body composition, also known as HIV associated lipodystrophy syndrome (HALS)[40], have been well-documented in patients taking ART, alterations in body composition and its association to HIV disease progression in ART naïve patients are not as well known.

Some studies have shown significant associations between body composition measurements and HIV disease progression in ART naïve adults. In a study conducted by Swanson et al.,[41] in 56 HIV infected women in Chicago, bioelectrical impedance was used to characterize body composition and examine its relationship with measures of HIV disease. Correlations were found with positive associations with CD4 cell count and fat-mass adjusted for height ($r=0.32$, $P=0.01$). Body cell mass adjusted for height was

also correlated with viral load (r=0.32, P=0.05). Visnegarwala and colleagues[42] analyzed gender differences in body composition and stage of HIV disease in 422 HIV+ ART naïve adults from patients recruited in 15 US cities. In men, body cell mass was inversely associated with prior AIDS defining conditions (coefficient -1.7kg, *P*<0.005) and HIV viral load (-0.92 kg, *P*=0.02). In women, those with prior AIDS defining conditions had lower total body fat (-9.54 kg, *P*<0.005) and total body fat was also associated with CD4 cell count (+1.64 kg, *P*=0.02). Hamill et al.[43] compared body composition in South African women with CD4 cell count >350 cells/μL (n=74) and low CD4 cell count (~200 cells/μL) (n=74). Those with CD4 cell count >350 cells/μL had significantly higher fat-mass in kg (*P*<0.001) and lean body mass in kg (*P*=0.005). In addition, the group with higher CD4 cell count had a higher fat mass squared/lean body mass squared ratio (P=0.002). They concluded that biological variations between men and women may have contributed to the different findings with the different body compartments and disease progression parameters. The studies conducted on body composition in ART naïve HIV infected adults had a cross-sectional design and included populations in the US. Changes may occur through time in body composition that may ultimately have an effect on HIV disease progression.

The *Fat Mass and Obesity Associated (FTO)* Gene in African Populations

The Fat Mass and Obesity Associated (FTO) gene was first identified in 2007[44] and has been associated with obesity and obesity related traits.[45,46] The gene was originally found in mice and was responsible for the fused toe (Ft) phenotype and also provided the gene its name.[47] The hypothalamus is the site where the *FTO* gene is mainly expressed[48] and may be involved in energy homeostasis,[49] and fat cell lipolysis which may regulate body fat.[50] Specifically, the single nucleotide polymorphism (SNP) of

the *FTO* gene rs9939609 A allele is most commonly associated with obesity.[44,46,51] Five of the SNPs previously shown to be associated with BMI were included in this study (rs9939609, rs1421085, rs8050136, rs17817449, and rs1121980). Currently, there are no published studies on the *FTO* gene and its association to obesity and adipokines in HIV+ patients or in a population in Botswana.

Henning and colleagues[52] were the first to investigate the polymorphisms of the *FTO* gene in an African population. The effect of the *FTO* gene on BMI was assessed in 2208 slender Gambian children and adults. In adults, BMI and weight for height was not associated with any of the genotypes of the *FTO* gene. In addition, the 95% confidence interval of the effect size for variant rs9939609 did not correspond to that of Europeans reported in other research studies. The findings of this study may not be relevant to other populations where obesity is prevalent or an abundance of food is available.

Adeyemo et al.[53] conducted a study that assessed the genetic variation in the *FTO* gene in 517 West Africans and 968 African Americans and its association to obesity as measured by BMI, waist circumference and fat mass percentage. The variant of the *FTO* gene rs9939609 did not show any associations with obesity in the two populations, however two other gene variants rs1121980 and rs7204609 were associated with obesity in the West African participants ($p<0.05$).

A genetic association study completed by Lombard et al,[54] assessed the association of SNPs from *FTO* and other obesity related genes with BMI in 990 black adolescent South Africans. Seven *FTO* SNPs were included; rs9939973, rs9940128, rs1421085, rs1121980, rs17817449, rs8050136, and rs9939609. The *FTO* SNP rs1781749, the minor allele or G, was associated with increased BMI (effect size 1.9, $p<0.05$) after adjusting for sex, age, and pubertal age. Sex specific associations were not found with SNPs of the *FTO* gene. In a model that included the SNPs for *FTO*

rs1781749, *LEP* (leptin) rs10954174, *LEP* rs6966536, and *MCAR* (melanocortin 4 receptor) rs17782313 the effect size for the FTO SNP was 2.2, p=0.007. This finding was adjusted for age, sex and sex-specific pubertal age. The effect corresponded to the estimated percent change in BMI of each allele independent of the covariates. Based on the available data from studies on *FTO,* the most common SNP rs9939609 was not associated with BMI in the African populations but concluded that more studies are needed to investigate these relationships in other African populations. The results from this study are relevant to Botswana, who are closely related culturally and genetically to South Africans, with whom they share borders, language and ancestors.[55] However, Botswana and other African nations are known to be a heterogeneous population with many ethnic groups.[56] Replication studies in diverse African populaces are need to identify the SNPs that may cause the variations seen in obesity relevant to the pertaining population. GWAS studies in African populations are lacking to identify novel SNPs that are appropriate to the population is of importance.

The *Fat Mass and Obesity Associated (FTO)* Gene and HIV Disease Progression

Currently, in the literature there are no know studies evaluating markers of HIV disease progression and SNPs of the *FTO* gene in an HIV mono-infected population. Recently, Pineda-Tenor et al.[57] carried out a cross-sectional study in 261 HIV/HCV co-infected participants in Spain. Those with the rs9939609 AA genotype had higher levels of BMI (p=0.016) when compared to the AT/TT genotypes remained significant after adjusting for age, gender, markers of HIV disease progression, markers of HCV disease progression, liver fibrosis and ART. However, the genotypes for rs9939609 were not associated with CD4 cell count or HIV viral load.

Obesity and Inflammation

Inflammation is a condition in which the homeostasis between pro and anti-inflammatory markers in the body are out of balance and may lead to a prolonged stage of inflammation which in turn may affect the progression or outcomes of many conditions and/or diseases.[58] Obesity is known to be associated with a chronic state of low grade inflammation. Inflammation in obesity is due to many mechanisms which include disordered secretion of adipokines within adipose tissue resulting in insulin resistance and metabolic disorders.[59] Adipokines can affect many processes in the body such as metabolism, growth, blood pressure, vascularization, coagulation and inflammation and a variation in fat mass can change their production.[60] It has also been shown that an accumulation of macrophages occurs in obesity and this affects the increased release of TNF-α, a pro-inflammatory cytokine.[61] TNF-α release, mainly derived from macrophages, initiates a pro-inflammatory cascade of events and can block insulin activity leading to insulin resistance.[62] Obesity may also reduce the production of other inflammatory products as a result of the "endotoxin-lipoprotein hypothesis."[14] Higher levels of lipoproteins are usually found in obesity which can bind bacterial endotoxin such as LPS therefore affecting the release of pro-inflammatory cytokines. There is conflicting information in the literature regarding obesity and inflammation, which requires further research.

HIV Disease Progression and Inflammation

Diminishing CD4 cells is one of the main features of HIV progression and eventually the diagnosis of AIDS. There are many mechanisms described as to why a loss of immune related cells occur in HIV and inflammation is a key culprit. Loss of immune function in HIV may be due to a persistent immune activation. Inflammation is

involved in the depletion of T-cells, and chronic immune activation drives the general and HIV-specific losses of immune function.[63] Continuous inflammation induces pro-inflammatory cytokines which has been shown to produce thymic and T cell progenitor dysfunction.[64] It has also been shown that triggered or activated CD4 cells are a favored target for viral infection and replication. Inflammation may also set up non-infected CD4 cells and other immune cells for death by programmed cell death or apoptosis.[65] This constant state of inflammation also predisposes the patients to the occurrence of non-HIV related complications such as cardiovascular disease.[66] The role of obesity in inflammation in HIV infection is not clear and the literature is contradictory, therefore, it is important to examine the interrelationships that exist between inflammation, obesity, and HIV disease progression to elucidate the potential mechanisms involved and how it could later be translated into clinically relevant recommendations.

Pro-Inflammatory Markers

Leptin

Adipocytokines or adipokines are biologically active molecules that are secreted by adipocytes.[67] Adipose tissue, now deemed to be an endocrine organ,[68] secretes hormones such as leptin that may play an important role in body weight regulation.[69] Leptin was the first adipokine to be isolated and it is involved in weight, inflammatory, and immune regulation. Leptin is considered to be pro-inflammatory and can increase T cell activation and cytokine release proliferation thereby promoting a Th1 response.[69] Leptin levels are increased in obesity and have been shown to also act upon monocytes and prompt the release of cytokines such as TNF-α or IL-6.[70] Serum levels of leptin are proportional to body fat mass.[71] Females tend to have higher levels of leptin due to higher body fat mass, and sex steroids may also increase leptin mRNA levels[72,73] The

adipose tissue of obese individuals contain a larger amount of macrophages that when activated will secrete cytokines such as TNF-α and IL-6.[74]

Leptin as a proinflammatory cytokine may have effects on the innate and adaptive immune responses. It has been suggested that leptin does not begin an immune response but instead may impact the outcome by inducing proinflammatory cytokines from different cell types, such as T cells.[75] Inflammatory stimuli such as lipolysaccharide (LPS), a bacterial endotoxin, increases leptin gene expression and its concentration rapidly increases after the first stimulus. LPS binds to a Toll-like receptor on adipose cells and expression of other toll-like receptors occurs, which then increases the release of leptin and other inflammatory cytokines.[76] Leptin also binds to CRP during an immune response.[77] Leptin deficient mice have shown impaired responses to microbial pathogens such as tuberculosis.[75,78] Therefore, leptin plays an important role in the immune system and may respond differently depending on the stimuli.

Leptin is considered to be a pleiotropic molecule with not only metabolic roles but also endocrine and immune regulation.[79] Higher levels of leptin in some studies have been shown during infection and inflammation, however the results in human studies have been inconsistent in various diseases, including HIV.[79] Leptin is involved in the proliferation of T- cells including CD4 and CD8 cells.[80] In addition, leptin has anti-apoptotic properties for many cell types including lymphocytes.[81] Higher levels of leptin found in obese individuals[82] may enhance the immunity through its role in proliferation and anti-apoptotic effects on lymphocytes. The interplay between leptin and the immune response is an area of importance in HIV as it may assist in determining possible mechanisms for the protective effect of obesity often seen in HIV.

HIV Disease Progression and Leptin in HIV ART Naïve

Most of the research on adipokines, like leptin, and HIV have been conducted in ART treated patients and information on how leptin affects HIV disease progression in HIV ART naïve is lacking. Below are a few of the studies conducted on ART naïve participants, however, some of the findings still remain inconclusive.

Changes in body fat distribution such as, lipodystrophy, is common in ART treated HIV+ persons. This side effect is closely associated with the use of Protease Inhibitors (PIs).[83] Leptin levels are decreased in HIV associated lipodystrophy and the metabolic or immunological effect is only shown once the patients become hypolipidemic.[84] The action of leptin is affected by ART.[83]

Prabha and colleagues[85] investigated the role of leptin levels in the coinfection of HIV and tuberculosis (TB) in 20 HIV infected ART naïve participants, 20 HIV and TB infected ART naïve participants and 20 healthy controls in India. Mean serum levels of leptin in the group with HIV and TB was significantly lower than that of the control HIV group and healthy control group ($p<0.01$). Women had higher levels of leptin than men in all groups, which included the HIV control group (14 ± 4 vs. 5 ± 1, $p<0.01$). In all groups leptin increased consistently with BMI and in all groups combined for every unit increase in BMI a 1.8 fold increase was observed in leptin. CD4 cell counts and leptin were not statistically correlated in any group, even after adjusting for BMI.

A study conducted in Nigeria by Onyemelukwe et al.[86] examined serum leptin levels in ART naïve HIV infected adults in relation to BMI, CD4 cell count, asymptomatic and symptomatic HIV and AIDS. Groups for this study included 26 healthy HIV negative controls, 20 normal weight participants, and 20 underweight participants. The median serum leptin levels was lower in the normal weight group and underweight group compared to the control group ($Z=-2.26$, $p=0.024$ and $Z=-2.56$, $p=0.009$ respectively).

The median leptin level of symptomatic participants were lower compared to asymptomatic participants (27.9 ng/mL IQR=4.9-36.4 vs. 43.7 ng/mL IQR=11.8-48.2, Z=-2.07, p=0.038). Leptin levels were lower in the AIDS cases compared to the non-AIDS cases (22 ng/mL, IQR=3.8-40 vs. 35 ng/mL, IQR=25.9-45.6) but was not statistically significant. Female HIV+ participants had lower median leptin levels as compared to male HIV+ participants (7.42 ng/mL IQR=3.2-44.3 vs. 35.6 ng/mL IQR=27.7-43.2, p=0.05). In this study, HIV+ participants with normal weight had similar leptin levels as the healthy HIV negative controls unless there was a difference in weight or symptomatic HIV related conditions.

Azzoni et al,[87] investigated the association between HIV viral load, cellular activation, and adipose tissue related measures in 83 HIV infected ART naïve South African women with a CD4+ cell count of 200-350 cells/μL. The results showed that women with high viral load (> study median) had a higher BMI, waist circumference, subcutaneous abdominal fat, and trunk fat mass. Leptin levels were associated with BMI ($r = 0.699$, $p<0.001$), visceral abdominal fat ($r = 0.542$, $p<0.0001$) and total fat mass, ($r = 0.764$, $p<0.001$). In a multivariate regression model the relationship between leptin levels and $\log_{10}$ viral load remained (effect estimate = -0.0186653, $p = 0.03$), even after controlling for subcutaneous abdominal fat area. These results support a direct association between leptin levels and HIV viral replication, independent of the amount of adipose tissue. Since these women were ART naïve, they may have been newly infected which would explain the higher viral load and the reason they may be healthier suggested by the higher BMI and fat mass. The data may also suggest that the cytokine dysregulation produced by obesity and related to inflammation might not be the source of the protective effect of obesity previous to ART. Instead, the energy advantage may delay wasting and maintain an active innate immune response. The studies discussed

above have limitations that warrant further research, including small sample sizes, inconsistent findings, and cross-sectional design. Although, some studies include African populations, heterogeneity in Africans is present and including other nations would provide relevant information to their respective regions.

The *Fat Mass and Obesity Associated (FTO)* Gene and Leptin

Obesity affects the release of adipokines such as adiponectin, leptin, cytokines IL-6 and TNF-α from adipose tissue which may further lead to fat deposits.[88] Contradictory evidence of an association with the *FTO* gene and adipokines and inflammatory markers exists in the literature. Zimmermann and colleagues[89] did not show an association across *FTO* gene SNP rs9939609 genotypes and inflammatory markers including TNF-α, IL-6 and leptin in healthy Danish middle-aged men. A study by Zabena et al,[90] investigated the association of rs9939609 SNP of the FTO gene with obesity-related measures, to assess the *FTO* gene expression in subcutaneous and visceral adipose fat in morbidly obese patients in Spain and the relationship with adipocytokine gene expressions. The rs9939609 A allele was more frequent in the morbidly obese patients as compared to the control group, who had no diabetes and were not obese, (0.52 and 0.34), even after controlling for age and sex (OR 2.26; 95% CI 1.19–4.30, p=0.013). *FTO* mRNA expression was associated with leptin (r=0.695, p<0.001). Qi et al, [91] investigated the longitudinal relationships of the *FTO* gene variant rs9939609 with obesity and plasma adiponectin and leptin levels in men and women from prospective studies, The Nurses' Health Study and the Health Professional Follow-Up Study. The AA genotype of the SNP rs9939609 was associated with BMI in a 26-year follow-up in women (p<0.05) and 16-year follow-up in men (p<0.05) after adjusting for age and diabetes status and correlated with greater obesity risk during follow-up in

both women and men in contrast to genotypes TA and TT. A trend was shown in the allele A between higher leptin levels when adjusted for age (p=0.06). No significant associations were seen in men with the *FTO* gene polymorphism and leptin levels. Terra et al.[92] demonstrated that the *FTO* gene is associated with an anti-inflammatory profile in adipose tissue of morbidly obese (BMI >40 kg/m^2) women. Adiponectin expression was greater than any other cytokine measured in both subcutaneous and visceral fat and decreased when comparing morbidly obese women to a control group (BMI<25 kg/m^2) (4.03±0.47 vs.10.50±1.74, p=0.019). In subcutaneous fat, *FTO* expression was negatively associated with BMI (r=-0.457, p=0.004) and leptin (r=-0.475, p=0.026). Inconsistent findings were present in the studies on *FTO* and leptin and between genders. The *FTO* SNP rs9939609 was most commonly researched in the above studies. Studies with African populations on possible relationships between *FTO* and leptin are currently not available.

C-Reactive Protein

C-reactive protein or CRP is considered a sensitive systemic marker of inflammation and an acute phase reactant. CRP is produced and released mainly by hepatocytes in response to proinflammatory cytokines, such as IL-1 and IL-6.[93,94] Within adipose tissue, adipocytes and monocyte-derived macrophages may release proinflammatory cytokines boosting hepatic production of CRP.[95] Higher levels of CRP have also been associated with obesity and specifically abdominal obesity.[96] High sensitivity CRP (hsCRP) allows for a lower limit of detection and a value of > 10 mg/L may indicate the presence of a subclinical infection or inflammation.[97] CRP is currently used in many diagnostic, clinical, and research settings as an acute and chronic measure of inflammation.[98] CRP is considered to be a stable molecule to measure and

many inexpensive methodologies are available to determine CRP.[99] Such measures are attractive for resource limited settings, like many countries in Africa. A recent study showed that CRP >1.2 mg/L had a specificity of 68.87% for predicting CD4 cell counts of <200 cell/μL in HIV adults, which shows that CRP may be a sensitive biomarker beyond cardiovascular disease.[100]

HIV Disease Progression and C-Reactive Protein in HIV ART Naïve

Lau et al.[101] examined the association between CRP and immune suppression and progression to AIDS in 513 HIV-infected men recruited into a multicenter cohort in 6 US cities who were ART naïve at baseline. CRP was negatively correlated with CD4 cell count ($r=-0.17$, $p<0.001$) and positively correlated with HIV RNA levels ($r=0.20$, $p<0.001$). Those with higher concentrations of CRP had shorter times to AIDS (overall log-rank test, $P<0.001$). Median times to AIDS were 5.07 years (95% CI: 4.15-7.26 years) for those with CRP levels of 1.3 – 2.3 mg/L and 4.48 years (95% CI: 3.17, 5.61) for those with CRP levels more than 2.3 mg/L. Accelerated progression to AIDS was only seen in those with CRP levels of >1.2 mg/L. Using Cox proportional hazard models, it was observed that increased hazards for the development of AIDS in those with higher levels of CRP remained after adjusting for CD4 cell counts and $\log_{10}$ HIV viral load. CRP increased over time for participants with and without AIDS, however, those who progressed to AIDS had more of a rapid increase in CRP concentrations than those without progression to AIDS. Using a random-effects model, those that progressed to AIDS had 8.5% change per year in CRP concentrations compared to those that did not progress to AIDS, which had a 4.5% change per year. This study demonstrated a steady increase in CRP concentrations before participants' progress to AIDS.

Guimarães et al.[102] compared hsCRP between 42 HIV+ ART naïve patients and 129 HIV treated patients in Brazil. They investigated the relationship between hsCRP and cardiovascular risk factors with HIV disease progression. ART treated participants had higher levels of hsCRP compared to ART naïve participants (1.14±1.17 mg/l vs. 0.3±1.16 mg/L; p<0.001). The ART treated participants had a higher percentage with values >3 mg/dL, associated with a higher cardiovascular risk, compared to the ART naive group (56% vs. 26%: OR=3.56; 95% CI: 1.55-8.29, p=0.001). There were no statistically significant correlations between CRP and measures of HIV disease progression, CD4 cell counts and HIV viral load.

Chaudhary and colleagues[103] explored possible associations of CRP with CD4 cell counts and survival in 119 HIV infected ART naïve adults and 33 healthy individuals in India. CRP was negatively correlated with CD4 cell counts (r=-0.148, p=0.045). Using a Cox proportional hazard model, higher CRP category (>12mg/dL) was associated with AIDS related mortality (RH=1.23, 95% CI: 1.12, 1.25, p=0.0047) after 1 year, albeit only 8 participants were included in this analysis. CRP was suggested by the authors as another measure of disease progression in a resource limited setting.

A retrospective study by Baker et al. [104] compared markers of inflammation and coagulation in 254 ART naïve or not receiving ART for 6 months to 128 patients who initiated ART from various centers in the US during a 6 months follow-up. At baseline there were no statistical differences in CRP between the ART naïve or the ART initiated group. In addition, CRP was not associated with baseline CD4 count or HIV RNA level. After initiating ART, CRP did not significantly change from baseline.

A published study of inflammatory markers in 546 HIV pre-ART participants by Ledwaba and colleagues[105] looked at whether elevated levels of inflammatory markers would be associated with increased risk of death after initiation of ART in South African

participants. Prior to commencing ART, median hsCRP was significantly higher in the case group that included those who died during a median follow-up period of 24 months compared to a control group containing 80 uninfected adults (11.25 IQR=2.90-51.90 vs. 3.6 IQR=1.50-11.30, *P*=<0.001). At baseline participants with advanced HIV infection had lower levels of hsCRP compared to patients with early HIV (3.05 IQR=1.2-6.2 vs. 4.30 IQR=1.95-12.3, *P*=0.002). Advanced disease, which was accompanied by higher levels of CRP, was related to death after starting ART. The studies investigating the relationship between CRP and HIV disease progression have inconsistent results as some did not show significant findings. Since CRP is affected by ART, studies that include ART naïve are of importance to see the true associations with HIV disease progression. Studies among African populations are limited in this area of research.

The Fat Mass and Obesity Associated (FTO) Gene, and CRP

Adipose tissue may also determine levels of circulating CRP through IL-6, as its presence in adipose tissue affects production of CRP by the liver.[106] FTO seems to affect the amount of adipose tissue and therefore levels of CRP. In a recent study completed by Fall et al.,[107] over ninety-one thousand adults of European descent contributed to an analysis used to confirm causal effects of adiposity, the *FTO* gene variant rs9939609 and cardiometabolic traits, including CRP. Both BMI and *FTO* SNP were associated with CRP (*P*<0.05). Fisher et al.[108] studied the association of the *FTO* rs9939609 SNP and CRP levels in 2,415 adult men and women in Germany and found that using a regression model that *FTO* SNP was associated with higher CRP levels in men (~14% higher) and women (~12%, *P*=0.02) for the A allele. This model was adjusted for age, BMI, waist-to-hip ratio, common diseases (myocardial infarction, stroke, and diabetes), education, engagement in sports, and smoking. Zimmerman and

colleagues[89] also looked at the association of CRP and *FTO* rs9939609 SNP. No statistically significant results analyzing CRP were observed. Studies on FTO SNPs and its relationships with adipokines and markers of inflammation such as CRP that were discussed above have been mainly conducted using European populations. Studies with African populations are lacking. Overall, studies in this subject are limited and more research is warranted to examine possible pleiotropic effects of *FTO*.

Lipopolysaccharide (LPS)

Microbial translocation or the movement of bacterial products from the intestinal lumen into systemic circulation is measured by bacterial products such as lipopolysaccharide (LPS).[109] Microbial translocation may have a role in persistent immune activation and can provoke pro-inflammatory reactions.[110] During HIV, microbial translocations may occur due to epithelial damage to the intestine, loss of T-helper-17 cells and a reduced removal of microbial products by phagocytes are observed.[110] Increased secretion of pro-inflammatory cytokines can also contribute to increased T cell activation and specifically can increase CD8 T cell activation and depletion of CD4 T cells.[111] Conversely, a theory for the obesity paradox includes the endotoxin-lipoprotein hypothesis.[14] This theory states that during obesity there are a large number of lipoproteins circulating and LPS binds to lipoproteins through the formation of micelles in the gut. Lower circulating LPS would reduce the inflammatory response. This hypothesis merits investigation in a HIV infected population that is ART naïve that may be protective of further disease progression by obesity.

HIV Disease Progression and Lipopolyssacharide (LPS) in HIV Infection without ART

Redd et al.[112] observed in 107 HIV+ ART naïve adults the longitudinal relationship between microbial translocation, which included LPS, and inflammatory cytokine response in a cohort with varying rates of HIV disease progression in Uganda. Markers of microbial translocation were also compared to 24 HIV uninfected adults in the US. At baseline there were no statistical differences in the levels of LPS between the HIV infected group in Uganda and the US uninfected group. When comparing groups stratified by HIV disease progression there were still no differences at baseline or over time in LPS. The authors proposed that the different levels of microbial translocation markers such as LPS, may be a symptom but not a direct cause of HIV disease progression.

Marchetti and authors[113] investigated microbial translocation, as measured by LPS, in 379 HIV ART naïve participants in Italy. Participants were recent seroconverters with high CD4 cell counts, and who were followed for an average of 3 years. Participants were separated into two groups based on the median LPS level (110 pg/mL) and there were no differences at baseline in CD4 cell counts and HIV viral load between the groups. In a subset that included 17 participants, LPS did not change significantly over time (13-25 months). The group above the LPS median had a shorter median time to event (AIDS defining condition, death, CD4 cell count <200 cells/μL or start of ART, whichever happened first) of 1.5 years (95% CI: 1.0, 2.0) as compared to those below the median who had an average time to event of 4 years (95% CI: 3.1, 5.6), P=0.0002. This association remained significant after controlling for proinflammatory cytokines, age, CD4, viral load, HCV, HBV and duration of HIV (RH=1.85, 95% CI: 1.32, 2.58, p<0.001). In this study LPS was a strong predictor of HIV disease progression

independent of CD4 cell count and HIV viral load. In summary, the evidence points towards an association between LPS levels and disease progression, but is weak in elucidating whether the relationship is of cause-and-effect or mediated by other factors. Additionally, few studies are available on LPS in HIV ART naïve participants and the sample size over time quite small. Currently, there are no published literature examining the relationship between *FTO* SNPs and LPS in humans.

Summary

There is evidence in the literature of an obesity paradox, where higher BMI seems to be protective of mortality and disease-related outcomes in several conditions, including HIV.[3] Obesity may boost the innate immune system even in ART, which is probably why it is protective before ART. Fat available for use in time of crisis in HIV disease may spare protein's use.[33] However, factors generating this paradox are not well understood and the mechanism for the associated benefits of higher BMI are lacking. The prevalence of overweight and obesity are increasing globally, including sub-Saharan countries like Botswana.[17] Botswana also has the second highest prevalence rate of HIV in the world.[114] Before the initiation of ART, BMI and body composition measures such as fat mass and body cell mass seem to be protective of HIV disease progression, however, longitudinal studies in African populations are lacking.

Studies that examine the relationships between being overweight/obese and HIV disease progression and its interrelationship between genetic propensity for obesity and inflammatory markers, such as the study reported in this dissertation, are needed. The studies conducted on body composition in HIV infected, ART naïve patient populations were cross-sectional and included populations in the US. Changes may occur through time in body composition that may ultimately have an effect on HIV disease progression.

Replication studies in diverse African populations are needed to identify the SNPs that may cause the variations seen in obesity that is relevant pertaining to the population. Most of the studies discussed above have limitations that warrant further research, including small sample sizes, inconsistent findings, and cross-sectional design. In addition, studies that include ART naïve patients are of importance in determining the true associations with HIV disease progression. Although, some studies include African populations, heterogeneity among Africans is present and including other ethnicities is likely to provide relevant information. The role of obesity in inflammation in HIV infection is not clear and the literature is contradictory, therefore, it is important to examine the interrelationships that exist between inflammation, overweight/obesity, and HIV disease progression to elucidate the potential mechanisms involved and how it could later be translated into clinically relevant recommendations.

Table 1: Studies Conducted in Obesity and HIV Disease Progression

Author/Study Design	Study Purpose	Population	Main Outcome(s)	Study Findings
Johnson et al, 2014 *Retrospective Longitudinal Cohort Study*	To investigate the longitudinal association of BMI at HIV diagnosis with disease progression as evaluated by CD4 cell counts obtained during routine medical care.	396 ART naïve and had one follow-up visit	CD4 cell counts	Longitudinally, the mean CD4 count was 611.2 cells/mm^3 for obese individuals, 598.1 cells/mm^3 for overweight individuals and 550.5 cells/mm3 for normal weight individuals. When compared to the normal weight category, the obese category had significantly larger increases in CD4 count (5.5 cells/mm^3, $P < 0.001$) versus the overweight category (-2.1 cells/mm^3, $P < 0.001$).
Tate et al., 2012 *Prospective Longitudinal Cohort Study*	To evaluate factors associated with change among patients receiving ART.	681 ART treatment naïve patients who maintained ART therapy for 2 years.	BMI	At 24 months, 20% of patients moved from normal to overweight/obese or overweight to obese BMI categories. Underweight participants had lower CD4 counts and Higher viral load at ART initiation. Greater increases in BMI were observed in patients with baseline CD4 count <50 cells/μL and boosted protease inhibitor use, but did not account for all of the variation observed in weight change.
Crum-Cianflone et al., 2011 *Retrospective Longitudinal Cohort Study*	To investigate relationship between body weight and a variety of immune cell counts at diagnosis and over the course HIV infection among a cohort of documented HIV seroconverters who had serial immune cell measurements and longitudinal weight data.	1097 HIV seroconverters who were participants in the US Military HIV Natural History Study (NHS) (96% male).	Immune cell counts	Immune cell counts at HIV diagnosis did not significantly differ by BMI category. In the longitudinal models for those diagnosed before the advent of the highly active antiretroviral therapy (HAART) era, mean postdiagnosis decreases in the white cell count, total lymphocyte count, CD4 count, CD4 percentage, and CD4/CD8 ratio were less as the BMI category increased (all with P values of <0.05). Among HIV seroconverters diagnosed in the HAART era, obese compared to normal-weight patients had

				significantly smaller increases in CD4 counts, CD4 percentages, and the CD4/CD8 ratio (all with P values of <0.05). Similar findings were also noted among underweight versus normal-weight patients.
Adeyemi et al., 2009 *Cross-sectional Study*	To explore the effects of obesity on lymphocyte subsets in HIV-positive patients with diabetes.	216 HIV+ patients with Diabetes on HAART and undetectable viral load.	White blood cell count and the following lymphocyte subgroups, defined by surface antigens: CD3 (T cells), CD4 (helper T cells), CD8 (cytotoxic or suppressor T cells), CD19 (B cells), and CD56 (natural killer cells).	As BMI increased, there were significant increases in CD3, CD8, and total lymphocyte counts. For total white blood cell count and the other lymphocyte subgroups (CD4, CD19, and CD56), the relationship with BMI was not significant. Obese patients had CD3 counts 26% higher than patients with normal weight and CD8 counts 28% higher, controlling for age, sex, ethnicity, renal function, HbA1c, and duration since HIV diagnosis.
Jones et al., 2003 *Longitudinal Prospective Cohort Study*	To examine the association of BMI with HIV disease outcomes.	871 HIV+ women 16-55 years of age with history of IDU or high-risk sexual contact without prior AIDS-defining clinical illness and ART naïve at baseline	Time to first CD4 cell count <200 cells/mm3, first CD4 cell count <100 cells/mm3, opportunistic infection and HIV-related death	Mean CD4 cell count at baseline progressively higher and mean log viral load progressively lower as BMI increased. A higher baseline BMI was associated with a lower rate of occurrence of the first CD4 <200 cells/mm3. In analysis using time-varying BMI, underweight and normal women had increased risk of clinical AIDS and underweight women had increased risk of HIV-related death, compared with obese women.

Shuter et al., 2001 *Retrospective Case-Control Study*	To determine the prevalence and predictive value of overweight in an urban HIV clinic.	189 HIV+ patients from Jacobi Medical Center HIV Clinic	Progression to AIDS and CD4 cell count	Female gender and lack of AIDS diagnosis were independently associated with overweight. Among patients without AIDS, there was a trend towards slower disease progression and lower viral load in overweight patients, despite similar baseline CD4 cell count and similar time to initiation of HAART. In multivariate proportional hazards analyses, lower baseline BMI and falling BMI during follow-up were independently predictive of progression to AIDS.
Shor-Posner et al, 2000 *Longitudinal Prospective Cohort Study*	To compare immune measures and disease progression in obese and nonobese HIV-1– seropositive men and women in the pre-HAART era.	125 HIV-1–infected and 148 seronegative male and female drug.	HIV-1 related mortality, CD4 cell count <200 cells/μL and 25% decline in CD4 cell count	Over an 18-month period, 60.5% of the nonobese HIV-1–seropositive patients exhibited a 25% decline in CD4 cell count, compared with 18% of the obese patients. During the follow-up period, 38% of the lean and 13% of the nonobese study subjects died of HIV-1–related causes. Measurements of BMI were inversely associated with progression to death, independent of CD4 count <200 cells/mm3.

Table 2: Genetic Association Studies on the *FTO* Gene in African Populations

Author/Study Design	Study Purpose	Population	FTO SNPs Analyzed	FTO Results with BMI	Other Results
Lombard et al., 2012 *Genetic Association Study (Cross-sectional)*	To assess the association of candidate loci with BMI in black South Africans. The authors focused on SNPs in the *FTO, LEP, LEPR, MC4R, NPY2R and POMC* genes.	990 randomly selected adolescents from the larger Birth to Twenty cohort (a longitudinal birth cohort study of health and development in Africans) born in the metropolitan area of Soweto, Johannesburg,	rs9939973 rs9940128 rs1421085 rs1121980 rs17817449 rs8050136 rs9939609	FTO rs17817449, the minor allele (G in both cases) is associated with increased BMI. The seven FTO SNPs in this study explain 0.6% of variation in log(BMI) after adjusting for age-, gender- and sex-specific pubertal stage and 1.4% unadjusted. FTO rs17817449 (p=0.022) associated with BMI after adjustment for above factors.	Sex, sex-specific pubertal stage and exact age together explain 14.3% of variation in log(BMI). Each risk allele was associated with an estimated average increase of 2.5% BMI.
Adeyemo et al., 2010 *Genetic Association Study (Cross-sectional)*	To evaluate genetic variation in the FTO gene and investigate associations with obesity in West Africans and African Americans.	Two independent subsets: 1) 968 unrelated African Americans in Washington, D.C., and 2) 517 unrelated West Africans enrolled as control subjects as part of the Africa-America Diabetes Mellitus (AADM) Study.	262 tag single nucleotide polymorphisms (SNPs) across the entire gene.	The combined effect size for BMI for the top SNPs from meta-analysis was 0.77 kg/m^2 (P = 0.009, rs9932411) and 0.70 kg/m^2 (P =0.006, rs7191513). Two previously reported associations with intron 1 SNPs (rs1121980 and rs7204609, r^2 = 0.001) were replicated among the West Africans.	Both African-ancestry samples showed weaker linkage disequilibrium (LD) patterns compared to other continental (e.g., European) populations. Several intron 8 SNPs, in addition to intron 1 SNPs, showed significant associations in both study samples.
Hennig et al., 2009	To assess the effect of 16 FTO SNPs on	2208 Lean Gambians (Children and Adults)	rs6499642	FTO SNPs not associated with birth weight (BWT), early	Not Applicable

Genetic Association Study (Cross-sectional)	body mass in a large population of predominantly lean Gambians participating in a long-term surveillance program providing contemporary and early-life anthropometric measurements.		rs6499643 rs7206790 rs9940646 rs12447107 rs17817288 rs16945088 rs17817449 rs8063946 rs8063946 rs3751812 rs3751813 rs9939609 rs9931494 rs7190492 rs72046609 rs9935403	weight gain in 1–2 year olds, BMI in adults (≥ 18 y), or weight-for-height (WFH) z-score across all ages. No association was seen between genotype and WFH z-score or other measures of body mass.	

Table 3: Studies Conducted on Leptin and HIV Disease Progression in ART Naïve

Author/Study Design	Study Purpose	Population	Main Outcome(s)	Study Findings
Azzoni et al., 2010 *Cross-sectional Study*	To assess the relationship of fat or markers related to glucose and lipid metabolism with viral load in a cross-sectional sample of 83 ART-naïve HIV-1 infected South African women.	83 ART naïve South African women	Cell activation, innate immunity effector levels and adipose tissue-related measures including leptin	Women with viral load greater than the population median had lower serum leptin. Leptin levels were inversely associated with viral replication, independent of the amount of adipose tissue.
Onyemelukwe et al., 2009 *Cross-sectional Study*	To determine serum leptin levels in ART naïve HIV-1 infected adults in relation to BMI, CD4 cell count and presence or absence of symptomatic HIV disease or features of AIDS.	40 consecutive matched, HIV infected adults (20 normal weight and 20 underweight) and 26 sex matched HIV negative, healthy normal weight controls in Nigeria	Leptin	Female patients tended to have lower leptin than males. Median leptin was lower in underweight when compared to normal weight patients and also lower in symptomatic patients when compared to asymptomatic patients but not significantly different between AIDS and non-AIDS cases. Among healthy controls, leptin levels positively correlated with CD4 T counts but in HIV/AIDS patients the correlation was not significant.
Prabha et al., 2005 *Cross-sectional Study*	To determine the leptin levels in HIV and HIV with active pulmonary TB and to see whether leptin levels are altered with co-infection.	20 asymptomatic, ART naïve HIV infected patients, 20 asymptomatic ART naïve HIV infected patients with TB, 20 healthy control subjects (All participants from India)	Leptin	Serum leptin levels and BMI were significantly lower in the patients with HIV-TB than control and HIV subjects. Multivariate regression analysis showed that serum leptin was significantly dependent on BMI and sex but not on age and the disease groups. Leptin did not correlated with either with CD4 cell count so with any of the serum cytokines in HIV and HIV-TB patients.

Table 4: Studies Conducted on C-Reactive Protein and HIV Disease Progression in ART Naïve

Author/Study Design	Study Purpose	Population	Main Outcome(s)	Study Findings
Ledwaba L et al., 2012 *Nested Case-Control Substudy*	To assess in an ART naïve group of patients with advanced HIV infection whether pre-ART levels of inflammatory and coagulation biomarkers are associated with mortality. In addition, to assess whether initiation of ART lowered levels of these biomarkers, and compared pre-ART biomarker levels among patients with early versus late HIV infection and HIV uninfected patients.	187 HIV infected ART-naïve patients with CD4 cell count <200 cells/μL who died 359 HIV infected ART-naïve patients with CD4 cell count <200 cells/μL matched controls 80 uninfected (South African Cohort)	Mortality	Median baseline biomarkers levels for cases and controls, respectively, were 11.25 vs. 3.6 mg/L for hsCRP, HIV viral load was correlated with hsCRP. Adjusted odds ratios for the highest versus lowest quartile of baseline biomarker levels were 3.5 (95% CI: 1.9–6.7) for hsCRP, 2.6 (95%CI 1.4–4.9) for D-dimer, and 3.8 (95% CI: 1.8–7.8) for IL-6. These associations were stronger for deaths that occurred more proximal to the biomarker measurements. Levels of D-dimer and IL-6, but not hsCRP, were significantly lower at month 6 after commencing ART compared to baseline.
Baker et al., 2011 *Retrospective Longitudinal Study*	To determine whether ART initiation in ART naïve or those off of ART (≥6 months) reduced markers of inflammation.	254 HIV+ ART naïve or off of ART (≥6 months) at baseline (126 differed initiation of ART (DC) and 128 had immediate initiation of ART (VS)	D-dimer, IL- and CRP	At month 6, 62% of VS group had HIV RNA <400copies/mL and median CD4 count was 190 cells/mm3 higher than for the DC group (590 vs. 400 cells/mm3). Compared with DC, the VS group had 32% lower D-dimer levels at month; differences were not significant for hsCRP or IL-6 levels.
Chaudhary et al., 2008	To elucidate association of CRP with CD4 counts and survival in HIV-1 infected	119 HIV-infected ART naïve patients	AIDS related mortality and CD4 cell count	CRP was correlated with CD4 count and mortality at 1 year. CRP was negatively correlated with CD4 counts with levels of CRP being highest in the group with CD4 cell

Prospective Longitudinal Study	individuals	33 healthy individuals		counts below 200 cells/µL. Higher CRP category (>12 mg/dL) was associated with AIDS-related deaths in a Cox proportional Hazard Model after adjusting for CD4 cell count.
Guimaraes et al., 2008 *Cross-sectional Study*	To compare hsCRP in HIV-infected patients treated or not with ART and to correlate hsCRP levels with traditional cardiovascular risk factors and to factors related to the HIV infection.	129 HIV+ ART-treated 42 HIV+ ART naive	CRP	hsCRP levels were higher in ARV-treated compared to ARV-naive patients. Seventy-two (56%) ARV-treated patients and 11 (26%) ARV-naıve patients had hsCRP concentrations >3 mg/dl (high risk for cardiovascular complications). No correlation was found between hsCRP levels and CD4 cell counts and HIV-viral load. Independent factors associated with hsCRP levels were therapy with current non-nucleoside reverse transcriptase inhibitors (NNRTI), waist-to-hip ratio, fasting glucose and glucose levels 2 h after load in multivariate analysis model 1 and current NNRTI therapy, protease inhibitor therapy and cardiometabolic syndrome in multivariate analysis model.
Lau et al., 2006 *Retrospective Longitudinal Cohort Study*	To elucidate the relationship between HIV disease and the concentration of CRP.	513 HIV serpopostive men randomly chosen MACS participatns ART naïve at baseline	Time to AIDS	The cross-sectional associations between log10 CRP were correlated inversely with CD4 lymphocyte counts and directly with log10 HIV RNA levels. Levels of CRP of more than 2.3 mg/L were associated with a decreased time to the development of AIDS (relative time to AIDS, compared with individuals with CRP levels of 1.2 mg/L or less, which remained significant after adjustment for CD4 lymphocyte counts and HIV RNA and hemoglobin concentrations. Levels of CRP

				significantly increased over time with mean slopes of 8.5% and 4.5% per year for individuals with and without progression to AIDS, respectively. Individuals had a geometric mean CRP level of 2.5 mg/L in the 6-month interval before progression to AIDS, which was an increase from a nadir of 1.0 mg/L at 6.5 years before progression to AIDS.

Table 5: Studies Conducted on LPS and HIV Disease Progression in ART Naïve

Author/Study Design	Study Purpose	Population	Main Outcome(s)	Study Findings
Marchetti et al., 2011 *Retrospective Longitudinal Study*	To evaluate the role of immune activation and microbial translocation in HIV infected ART naïve patients with high CD4 cell count in the first years of chronic infection in predicting HIV disease progression. Also, to cross-sectionally evaluate the correlations between markers of immune activation, microbial translocation, and CD4 cell count and HIV viraemia and their modifications during untreated infection.	379 HIV+ patients ART naive with CD4>200 cells/ μL	AIDS defining condition, death, CD4 cell count of <200 cells/μL or start of ART	A median of 3.1 years after the estimated serconversion date median LPS marker 110 pg/mL (IQR 75-215). LPS was the only biomarker associated with primary composite outcome independently of age, HIV-RNA and CD4 RR=1.04 per $\log_e$ higher, 95% CI: 1.18-1.66, p<0.001).
Redd et al., 2009	To examine the longitudinal	107 HIV+ ART	LPS, soluble	Multiple markers for microbial translocation

Retrospective Longitudinal Study	relationship between microbial translocation and circulatory inflammatory cytokine responses in a cohort of people with varying rates of HIV-1 disease progression in Rakai, Uganda.	naïve Ugandan patients and 24 HIV uninfected US adults	CD14, LPS, EndoCAb, LPS binding protein and inflammatory cytokines	(lipopolysaccharide, endotoxin antibody, and sCD14) did not change significantly during HIV-1 disease progression. Moreover, circulating immunoreactive cytokine levels either decreased or remained virtually unchanged throughout disease progression.

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CHAPTER III: EFFECT OF BMI AND BODY COMPOSITION ON HIV DISEASE PROGRESSION IN HIV INFECTED ART NAÏVE ADULTS IN BOTSWANA

Abstract

Background: An obesity paradox has been documented in many conditions including HIV. Several studies indicate that overweight or obesity provides some protection from accelerated HIV disease progression in patients not receiving antiretroviral therapy (ART). Studies conducted to investigate obesity and its effect on HIV disease progression have been inconclusive and are lacking in African settings. This research investigates the relationship between overweight and obesity and HIV disease progression in HIV+ asymptomatic adults not on ART in Botswana over a period of 18 months.

Methods: A retrospective, cohort study in asymptomatic, ART naïve, HIV+ adults was conducted that included 217 participants, 139 with BMI 18.0-24.9 kg/m^2 and 78 participants with BMI ≥ 25 kg/m^2. The primary outcome was time to event (25% or greater decrease in CD4 cell count) during 18 months of follow-up and secondary outcomes were time to event of CD4 cell count <250 cells/μL, and AIDS defining conditions. Proportional survival hazard models were used to compare hazard ratios on time to events of HIV disease progression between BMI groups and BMI and body composition as continuous variables over 18 months.

Results: Using Cox Proportional Hazards model, higher baseline BMI was associated with significantly lower risk of having an AIDS defining condition over an 18 months follow-up (HR, 0.218; 95% CI, 0.068-0.701, P=0.011). Higher fat mass at baseline was also significantly associated with a decreased risk of having AIDS defining conditions (HR, 0.855; 95% CI, 0.741-0.987, P=0.033) and the combined outcome of having CD4 cell count ≤ 250/μL and AIDS defining conditions, whichever occurred earlier (HR, 0.918;

95% CI: 0.847-0.994, P=0.036). All models were adjusted for age, gender, marital status, children (yes or no) and baseline CD4 cell count and baseline HIV viral load. There was no significant effect of BMI on HIV viral load over 18 months.

Conclusions: Higher BMI and fat mass were associated with slower disease progression in HIV infected ART naive adults in Botswana. Mechanistic research is needed to evaluate the association of obesity and HIV disease progression.

Keywords: HIV infection, ART naïve, BMI, disease progression, body composition, BIA

Introduction

The importance of good nutrition in maintaining good health and delaying disease progression for the HIV patient has been well documented in the literature.[1,2] At the beginning of the HIV epidemic, wasting was one of the main nutritionally related concerns, however, today rates of obesity are more common than the occurrence of wasting, particularly in developed countries,[3-5] especially with antiretroviral treatment (ART).[6]

An obesity paradox has been documented in many conditions including HIV infection, where those who are obese may have a survival advantage or improved disease outcomes.[7] The protective effect of obesity has been hypothesized to be related to extra available energy in the form of available fat for use in time of crisis during the HIV disease that would spare protein use,[8] and also help to preserve the immune system response. Studies on HIV and obesity were mainly conducted in settings where the patients are treated with ART, which may confound some of the findings and interpretations, as ART has been associated with lipodystrophy and obesity.[5] Increased obesity has been documented in countries with limited resources,[9] however, studies conducted in African settings on the relationship between HIV and obesity are lacking. It has been estimated that in sub-Saharan Africa, 10% of women have a BMI of less than 18.5 kg/m^2, with wasting still prevalent in some areas.[10] However, sub-Saharan Africa is experiencing a nutrition-related transition with a rapid rise in overweight and obesity.[11] Botswana is also experiencing an increase in obesity.[12] Growth in economy, urbanization, and diminished physical activity is contributing to the rise in obesity in Southern Africa.[13] Studies conducted to investigate obesity and its effect on HIV disease progression have so far been inconclusive, with some studies reporting an association and others reporting no association between CD4 cell counts and obesity.[6,14-17]

Botswana is also experiencing one of the worst epidemics of HIV with a prevalence rate of 23.4%, among those in the range between 15 and 49 years of age in men and women.[18] Botswana has been one of the first developing countries to provide universal access to ART.[19] Currently, HIV+ adults in Botswana can receive ART once their CD4 cell counts are less than 500 cells/μL. Countries especially in sub-Saharan Africa are still having many challenges in providing ART and maintaining adherence.[20] Therefore, having information on delaying disease progression, and its relationships to nutrition-related measures, in those regions where the two epidemics are co-occuring, is timely.

The objective of this study was to examine the relationship between overweight and obesity and HIV disease progression in HIV+ asymptomatic adults not on ART in Botswana over 18 months. The primary outcome was time to the first occurrence of a 25% or greater decrease in CD4 cell count during the course of the study and the secondary outcomes were time to reaching CD4 cell count <250 cells/μL, AIDS defining conditions, and changes in HIV viral load. This study was conducted in HIV+ adults who were ART naïve and had CD4 cell counts >350 cells/μL.

Methods

Study Design and Participants

A retrospective, cohort study analyzing data from 219 HIV+ men and women from the placebo group of a clinical trial in Botswana was conducted. This trial was a multifactorial, randomized, double-blinded, placebo-controlled clinical trial which investigated whether supplementation with multivitamins and selenium could improve immune function and prolong time to AIDS in ART naïve HIV+ adults.[21] The study was conducted between December 2004 and July 2009. The placebo group was used to avoid any confounding effects from the micronutrient supplementation on the outcome measures. Participants were eligible for the parent study with documentation of HIV

seropositive test results, CD4 cell count was >350 cells/μL, BMI was >18 kg/m^2 for women and 18.5 kg/m^2 for men, age ≥ 18 years, lack of a past history of AIDS-defining conditions, participation in another clinical trial and taking ART. Women were excluded if pregnant or had intention to become pregnant. All participants provided informed consent and were recruited from the Botswana-Harvard AIDS Initiative Partnership (BHP) in Gaborone, Botswana. The study protocol was approved by the Florida International University Institutional Review Board (IRB), the Harvard School of Public Health IRB and the Botswana Health Research Unit of the National Ministry of Health. Appropriate informed consent was obtained and clinical research was conducted in accordance with guidelines for human experimentation as specified by the US Department of Health and Human Services, the researchers' institutions, or both.

Clinical Data

At baseline and every three months, physical examination and medical history were performed by a trained nurse or a physician. Anthropometrics were also obtained and body mass index (BMI) was calculated by dividing the weight in kilograms by height in meters squared. Waist and hip circumference was measured using a tape measure. Waist circumference was measured at the narrowest part of the waist between the lowest rib and the iliac crest and the hip circumference at the widest portion of the buttocks. Bioelectrical impedance analysis (BIA) using the Biodynamics body composition analyzer (model BIA-310; Biodynamics Corp., Seattle, WA), determined impedance and calculated body composition. Subjects were measured without shoes and socks, and electrodes were placed on the participant's right hand and wrist and right foot and ankle. Blood pressure was measured in the left arm with the elbow flexed to heart level. Morbidity information including AIDS-defining conditions, were collected by

questionnaires at screening, and at every monthly visit, and confirmed by documentation in the medical chart.

Laboratory Data

At baseline and every three months blood was drawn for CD4 and CD8 cell counts. Every six months, blood was also drawn to evaluate HIV viral load, lipid panel, and blood chemistry. Lymphocyte phenotype was determined with a four-color immunophenotyping panel of monoclonal antibodies. Differential counts were determined using a Coulter MaxM hematology instrument and corroborated with cytocentrifuge smears. HIV-viral load was determined using an in-vitro nucleic acid amplification test (Amplicor reagents and protocol, Roche-Diagnostics, Branchburg, NJ). The lipid panel was measured using Roche reagent kits (Branchburg, NJ) on a COBAS Integra 400 plus auto-analyzer using enzymatic methods according to standard operating procedures.

Nutritional Data

Twenty-four hour recalls were conducted at baseline and every 3 months by trained clinical staff. Macronutrient and fiber intakes were calculated using the NutriBase Professional, V.9 (Cybersoft, Phoenix, AZ) program modified by the South Africa database, including native foods for which information was available. An average of at least 3 dietary recalls were used to obtain their estimated energy, carbohydrate, protein, fat and fiber intakes.

Statistical Analysis

BMI was stratified into two groups: 1. normal weight (BMI=18.0-24.9 kg/m^2) and 2. overweight/obese (BMI ≥ 25 kg/m^2). The overweight and obese BMI categories were combined to increase the sample size for the analyses. Laboratory markers of HIV infection and disease progression included in the analyses were CD4 cell count, CD4%,

CD8 cell count, CD8%, CD4/CD8 ratio and HIV viral load. Descriptive statistics such as frequencies, percentages, medians and interquartile ranges were used to characterize the total population, the two groups stratified by BMI and the study variables (primary and secondary outcomes) at baseline. Mann Whitney U and Chi-square tests were used to determine differences in HIV disease progression markers and markers of nutritional status between the BMI groups and by gender. Linear and logistic regressions at baseline and 18 months were conducted to observe the relationship between BMI and HIV disease progression and body composition measures from BIA using BMI as a continuous variable and dichotomous variable. The multiple regression analysis also included a measure to check for multicollinearity known as variance inflation factor (VIF). All models had a VIF <5, therefore multicollinearity was not a problem. Proportional survival hazard models were used to compare hazard ratios on time to events of HIV disease progression outcomes with BMI groups, BMI as a continuous variable and body composition over 18 months. The primary outcome was time to the first occurrence of a 25% or greater decrease in CD4 cell count during the course of the study and secondary outcomes were time to CD4 cell count <250 cells/μL, and AIDS defining conditions. These events were documented from the time of inclusion into the study to the date of the visit in which the event was recorded. All models were adjusted for covariates. For all analyses, a two sided test was used and $P<0.05$ was considered statistically significant. Statistical analyses were completed using SPSS version 21.

Results

Demographics and Clinical Characteristics

A total of 219 participants were assigned randomly into a placebo group by the parent study.[21] Two participants had missing data and were excluded from the final analysis. Seventy-eight participants at baseline had BMI ≥ 25 kg/m^2 and 139 had

BMI=18.0-24.9 kg/m^2. BMIs were stable throughout the 18 months of follow-up and did not statistically change within the normal and overweight/obese groups (Table 1). Most of the participants were female (75.1%) with a median age of 33 years, reflecting the characteristics of the epidemic in Botswana.[22] Almost half (45.2%) of the participants had at least a secondary education level and most had children (85.7%).

There were differences in demographic factors among the BMI groups. Those with BMI ≥ 25 kg/m^2 were older and more likely female, married and have children (Table 1). There were no differences in laboratory markers of HIV infection at baseline between the BMI groups. As shown on Table 2, those with BMI ≥ 25 kg/m^2 had a larger waist circumference and higher levels of total cholesterol, triglycerides, LDL cholesterol, glucose, and blood pressure. Albumin levels were higher in those with BMI 18-24.9 kg/m^2. Dietary intake was statistically different between the BMI groups. The overweight/obese group had higher basal metabolic rate and fat mass, but lower lean body mass, body cell mass and extracellular mass. Physical activity was not statistically different between the BMI groups (data not shown).

Correlations between Laboratory Markers of HIV Infection and Body Composition

BMI was significantly correlated with measures of body composition but not with the markers of HIV infection (Table 4). Waist circumference was associated with CD4% (r=-.17, *P*<.05) and CD4/CD8 ratio (r=-.19, *P*=.05). Waist-to-hip ratio showed a correlation with CD4% (-.33, p<0.01), CD8 cell count (r=.22, *P*<.01), and CD4/CD8 ratio (r=-.26, *P*<.01). Both fat mass and lean mass were associated with CD4 cell count (r=-.14, r=.14, *P*<.05, respectively).

Effect of BMI on Laboratory Markers of HIV Infection and Disease Progression

Table 5 displays the results from logistic regression analysis that compared the BMI groups at baseline, 18 months and the change over 18 months in the laboratory

markers of HIV infection and disease progression. No significant associations were found with viral load, CD4 cell count, CD4%, CD8 cell count, CD8%, and CD4/CD8 ratio. However, there was a trend where CD4% showed those with BMI ≥ 25 kg/m^2 to be more likely to have a higher change in CD4% over 18 months (OR=1.076, 95% CI=.988,1.166, *P*=.077). All models were controlled for age, gender, marital status, and children (whether they had children, yes or no).

Linear regressions using BMI as a continuous variable were also conducted to compare with measures of HIV disease progression at baseline, 18 months, and the change over 18 months (Table 6). There was a trend in the association between CD8 cell count and BMI at 18 months (β=.002, 95% CI= .000, .004, *P*=.082). The change in CD4% between baseline and 18 months was significantly associated with BMI, showing that for every unit increase in BMI there was .214 increase in the difference in CD4% (β=.214, 95% CI= .051, .376, *P*=0.011). All models were adjusted for age, gender, marriage and children. No significant differences were found with CD4 cell count, CD8%, and CD4/CD8 ratio.

Table 7 shows that after controlling for age, gender, marital status, children, baseline CD4 cell count and viral load, there were no significant differences between BMI groups in the main outcome of 25% decline in CD4 cell count over 18 months (HR= .744; 95% CI= .489, 1.131; *P*=.166). No significant differences were also observed in the secondary outcomes of CD4 cell count ≤ 250/μL, AIDS defining conditions, or the combined outcome of CD4 cell count ≤ 250/μL, AIDS defining conditions, whichever occurred first. As shown in Table 8, using continuous baseline BMI, higher BMI was associated with a protective effect on the risk of having an AIDS defining condition (HR= .218; 95% CI= .068, .781; *P*=.011) after adjusting for age, gender, marriage, children and baseline CD4 cell count and viral load. Significant associations were not found

between continuous BMI and CD4 cell count ≤250/μL, AIDS defining conditions and the combined outcome of CD4 cell count ≤250/μL, AIDS defining conditions, whichever occurred first.

Effect of Body Composition on Laboratory Markers of HIV Infection and Disease Progression

There were no significant associations using linear regression between measures of HIV disease progression and fat mass, lean body mass, and body cell mass at baseline, 18 months and the difference between these visits (Table 5).

A trend towards significance was seen with the outcome of 25% decline in CD4 cell count and fat mass (HR= .974; 95% CI= .945, 1.003, *P*=.083) (Table 9). Higher fat mass was protective of having an AIDS defining condition (HR= .855; 95% CI= .741, .987; *P*=.033). Higher fat mass was protective against the combined outcome of CD4 cell count ≤ 250/μL and AIDS defining conditions, whichever occurred first (HR= .918; 95% CI= .847, .994; *P*=.036). The effect of lean body mass on disease progression was similar to fat mass, as shown on Table 10. As lean body mass increased there was a higher risk of having ≥ 25% decline in CD4 cell count during the 18 months of follow-up but was trending towards significance (HR= 1.027; 95% CI= .997, 1.059, *P*=.082). Higher lean body mass was associated with higher risk of having an AIDS defining condition (HR= 1.169, 95% CI= 1.013, 1.349; *P*=.033), and higher risk of CD4 cell count ≤ 250/μL and AIDS defining condition albeit not significant (HR= 1.075; 95% CI= .995, 1.161; *P*=.068). These models were also adjusted for age, gender, marital status, having children and baseline CD4 count and viral load.

Discussion

This study showed that BMI and fat mass were protective by delaying the time to the first AIDS defining condition in HIV+ ART naïve adults in Botswana during 18 months

of follow-up. The fat stores would have provided the extra energy needed to maintain the innate immune system responding to protect against opportunistic infections and delaying wasting. There were no significant findings over 18 months when comparing the main outcome of a clinically significant decline of CD4 cell count (25%) and BMI stratified into two groups, BMI=18.0-24.9 kg/m^2 and BMI ≥ 25 kg/m^2. This finding suggests that the protective effect of fat stores are not enough to counteract the focused viral attack to the specific immune response, a suggestion supported by the results that show no association between BMI and HIV viral load. Shor-Posner et al.[17] found a significant difference between obese and nonobese when comparing clinically significant 25% decline in CD4 cell count over 18 months. However, these participants were drug users and may have been in worse nutritional status and have had HIV for longer duration and were in more advanced stage. BMI at baseline as a continuous variable was associated with 78% lower risk of having an AIDS defining condition for every one unit increase in BMI. Past incidence of >1 AIDS defining condition was associated with 1.3 fold higher risk of having BMI <20 kg/m^2 and AIDS defining conditions were also associated with a higher risk of wasting.[23]

Higher BMI was also associated with a higher CD4% after 18 months of follow-up. CD4% is considered to be more steady than absolute CD4 cell counts and provide a better prognostic information before ART is initiated.[24,25] Recently, it was shown to be a good predictor of disease progression for those starting ART with a CD4 cell count of >350 cells/μL.[26] Other investigators have also found that immune counts are affected by weight and/or BMI in HIV.[14,27] In a cross-sectional study[14] with patients on ART, an absolute increase of 16 units of CD8 cell count was seen for every 1kg/m^2 increase of in BMI. Crum-Cianflone and colleagues[27] compared normal weight to obese HIV+ adults at diagnosis and through HAART or ART initiation and concluded that those who were

obese had smaller reductions in CD4% through time, regardless of whether diagnosis took place in the pre-HAART era or after. They also found similar results with CD4/CD8 counts and only significant differences between normal weight and overweight in the HAART era with CD8 counts. Our findings in ART-naïve population, early in HIV disease showed that there were no significant effects of BMI with CD4/CD8 or CD8 cell count. Interestingly, Crum-Cianflone and colleagues[27] also showed that weight did not have an effect on immune cells at HIV diagnosis, but instead the effect of weight on immune cells was seen as the disease progressed. This study also did not have any significant differences at baseline between the normal weight and overweight/obese groups, because the great majority started their participation early in the disease at diagnosis.

This is the first study that demonstrated a longitudinal association between reduced AIDS defining conditions with higher fat mass. Higher fat mass was also associated with the combined outcome of CD4 cell count ≤ 250/μL and AIDS defining conditions. On the outcome of a decline in the CD4 cell count of 25% or greater, the survival analysis showed a positive trend association with fat mass and negative association with lean body mass, a finding supported by cross-sectional significant findings in our larger (N=878) cohort in Botswana.[28] Supporting this relationship over time, Visnegarwala and colleagues[29] demonstrated in a cross-sectional study that women with a prior AIDS defining condition had lower total body fat in kilograms. Maintaining a large lean mass has higher energy costs. Stored protein is less available than dietary protein and provides less energy than the same weight in fat,[30] this might be one of the reasons why lean mass was not protective, but might be even counterproductive to maintain adequate immune response before ART.

Findings from this study indicate that higher fat mass may provide protection from advanced HIV disease. This protective effect has been hypothesized to be related to extra available energy for use in times of emergency during the HIV disease that would reduce protein's use[8] and also help to preserve the immune system, especially the innate immunity that is not directly affected by the infection.[16,31] HIV and its secondary infections are known to generate a hypermetabolic state that is sustained throughout the disease.[32] The HIV patient is susceptible to infections, in which the body will require energy to battle continuous infections and immune activation. Those who are obese may have a genetic advantage in maintaining fat stores and utilizing that energy more efficiently to fuel the immune system.

Although there seems to be an obesity paradox in HIV, being obese is a risk factor for mortality and chronic conditions or diseases such as cardiovascular diseases, diabetes, hypertension, and cancer.[33-36] The participants of this study in the overweight/obese group had higher levels of total cholesterol, triglycerides, glucose, although not clinically significant, and higher LDL and blood pressure. These differences could over a period of time potentially increase their risks for chronic conditions. Evidence from the literature supports that once a patient receives ART, their cardiovascular and liver risks increases, and obesity becomes an adverse factor for health and survival.[5] Stevens and colleagues[37] have stated concerns over providing confusing messages when discussing the obesity paradox and the possible harm it will have on public health efforts related to reducing obesity rates to lower the risk of chronic diseases. Conversely, other researchers[38] theorize that our view of normal or optimal weight or BMI categories are too strict and may be biologically unsuitable. They also argue that those with an established disease diagnosis may benefit from an emphasis

on lifestyle recommendations regarding physical activity and nutrition rather than intentional weight loss or gain.

The limitations of this study include the sample size, not having sufficient numbers of participants to be able to examine differences between overweight and obese groups separately, and the predominance of women among the participants. The exact time since HIV diagnosis was not available, however, it was known that the participants were asymptomatic, never had a diagnosis of AIDS and did not qualify for ART. The majority were referred from the clinics where they were diagnosed. Participants with BMI in the range of underweight category were not included in the parent study as those ranges are strong prognostic indicators of AIDS.[21] This HIV population was in the early stage of the disease in order to study ART naïve individuals and the finding cannot be generalized to all HIV seropositive patients. The early stage of disease may be one of the reasons why we did not see a significant decline of CD4 as a main outcome. In HIV, bioimpedance (BIA) has been shown to be an effective method for detecting fat mass,[39] however, BIA have limitations, since its values can be influenced by body arrangement, hydration, ingestion of food and drinks, air and body temperature and exercise.[40] Our BIA measurement protocol provided for these limitations by giving detailed instructions to the participants on their preparation for their visit, and standardizing the measurement parameters. The strengths of this study consists of capturing and analyzing longitudinal data from HIV+ participants in Botswana, that we collected from early asymptomatic stage through defined health outcomes, and that this type of information is lacking in African populations.

In summary, the results of this study demonstrate that higher BMI and fat mass were protective in delaying HIV disease progression in this group of HIV+, ART naïve adults in Botswana. BMI separated into two groups, normal weight and

overweight/obese groups, did not show a significant effect on decline of CD4 cell count over 18 months and may have been due to limited sample size and to the participants' early stage of the disease. Mechanistic studies on the relationship between BMI and body composition on disease progression are needed to clarify the obesity paradox in HIV.

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Table 1: Change in BMI* from Baseline to 18 Months by BMI Groups

Groups	Baseline BMI	18 Months BMI	*P*-value
Overall	22.8 (20.7-27.2)	24.1 (20.4-27.2)	.511
Normal BMI	21.2 (19.9-22.6)	20.9 (19.3-22.5)	.338
Overweight/Obese	28.5 (26.6-30.1)	28.1 (26.1-30.4)	.466

*Reported as median (interquartile range); BMI expressed as kg/m^2

Table 2: Demographic Characteristics by BMI Group at Baseline

Variable	Total (N=217)	Normal BMI (N=139)	Overweight/ Obese BMI (N=78)	*P*-value
Age (years)[a]	33 (28-39)	31 (28-38)	35 (30-43)	.005[b]
Gender				
Male	54 (24.9)	44 (31.7)	10 (12.8)	.002[b]
Female	163 (75.1)	95 (68.3)	68 (87.2)	
Marital Status				
Single	124 (59.0)	86 (65.2)	38 (48.7)	.010[b]
Married	24 (11.4)	9 (6.8)	15 (19.2)	
Other	62 (29.5)	37 (28.0)	25 (32.1)	
Education				
None	13 (6.2)	9 (6.8)	4 (5.1)	.984
Primary	83 (39.5)	52 (39.4)	31 (39.7)	
Secondary	95 (45.2)	59 (44.7)	36 (46.2)	
Tertiary	19 (9.0)	12 (9.1)	7 (9.0)	
Income (pula)				
None	48 (22.9)	31 (23.5)	17 (21.8)	.849
<300	20 (9.5)	12 (9.1)	8 (10.3)	
300-600	41 (19.5)	26 (19.7)	15 (19.2)	
601-1000	51 (24.3)	33 (25.0)	18 (23.1)	
1001-6000	43 (20.3)	27 (20.5)	16 (20.5)	
>6000	6 (2.9)	2 (1.5)	4 (5.1)	
Unsure	1 (0.5)	1 (0.8)	0 (0.0)	
Electricity				
Yes	83 (39.5)	49 (37.1)	34 (43.6)	.354
No	127 (60.5)	83 (62.9)	44 (56.4)	
Children				
Yes	180 (85.7)	107 (81.1)	73 (93.6)	<.001[b]
No	30 (14.3)	25 (18.9)	5 (6.4)	
BMI (kg/m^2)[a]	22.8 (20.7-27.2)	21.2 (19.9-22.6)	28.5 (26.6-30.1)	.012[b]
CD4 Cell Count (cells/μL)[a]	411.3 (326.3-546.7)	415.9 (324.7-548.9)	401.2 (331.2-545.2)	.908
CD8 Cell Count (cells/μL)[a]	838.6 (617.5-1194.9)	804.1 (588.8-1229.0)	891.3 (669.0-1177.8)	.251
CD4/CD8 Ratio[a]	0.5 (0.3-0.7)	0.5 (0.4-0.7)	0.5 (0.3-0.7)	.375
HIV Viral Load (Log_{10} copies/mL)[a]	4.3 (3.5-4.8)	4.3 (3.6-4.8)	4.2 (3.3-4.8)	.525

[a]Reported as median (interquartile range); all other variables reported as n (%)
[b]Statistically significant, *P*<.05

Table 3: Nutrition Related Characteristics by BMI Group at Baseline

Variable[a]	Total (N=217)	Normal BMI (N=139)	Overweight/Obese BMI (N=78)	*P*-value
Waist (inches)	32.3 (29.9-35.7)	30.7 (29.1-32.3)	37.6 (34.8-39.3)	<.001[b]
Waist-to-hip Ratio	0.8 (0.8-0.9)	0.8 (0.8-0.9)	0.8 (0.8-0.9)	.017[b]
Total Cholesterol (mmol/L)	3.7 (3.0-4.3)	3.5 (2.9-4.0)	4.0 (3.5-4.7)	<.001[b]
Triglycerides (mmol/L)	0.8 (0.6-1.0)	0.7 (0.5-0.9)	0.8 (0.6-1.2)	<.001[b]
HDL (mmol/L)	1.1 (0.9-1.3)	1.1 (0.9-1.4)	1.1 (0.9-1.2)	.301
LDL (mmol/L)	2.4 (2.0-2.9)	2.4 (1.8-2.7)	2.6 (2.2-3.0)	.043[b]
Glucose (mmol/L)	4.5 (4.3-4.8)	4.5 (4.2-4.7)	4.6 (4.4-5.1)	.002[b]
Hemoglobin (g/dL)	12.9 (11.8-14.0)	12.7 (11.4-14.1)	13.0 (12.2-14.0)	.251
Hematocrit (%)	39.4 (36.3-42.3)	39.0 (35.4-42.6)	40.0 (37.3-4.9)	.359
Albumin (g/L)	41.0 (39.3-43.4)	41.6 (39.0-44.5)	39.8 (37.7-42.6)	.010[b]
Iron (µmol/L)	9.3 (6.3-13.0)	9.5 (6.0-13.2)	8.8 (6.6-12.7)	.959
Systolic Blood Pressure (mm Hg)	119 (110-130)	114 (108-128)	122 (111-139)	<.001[b]
Diastolic Blood Pressure (mm Hg)	76 (69-82)	72 (65-80)	80 (70-88)	<.001[b]
Total Energy Intake (kcal)	1507.1 (1177.5-1902)	1496.4 (1178.6-1933.2)	1568.0 (1175.6-1890.7)	.749
Protein Intake (%)	16.3 (14.0-19.6)	16.9 (14.1-20.7)	15.9 (14.0-18.6)	.959
Carbohydrate Intake (%)	65.6 (57.1-71.8)	65.6 (57.3-71.4)	65.9 (56.6-72.7)	.202
Fat Intake (%)	17.4 (12.4-22.9)	17.4 (12.1-22.8)	17.4 (12.7-23.2)	.524
Fiber Intake (grams)	16.0 (12.0-21.6)	16.1 (12.1-21.4)	15.6 (11.8-22.2)	.871
Basal Metabolic Rate (kcal)	1401 (1268-1564)	1338 (1209-1498)	1532 (1382-1696)	<.001[b]
Fat Mass (%)	29.0 (23.9-33.9)	25.1 (19.9-29.3	34.9 (32.5-37.9)	<.001[b]
Lean Mass (%)	71.0 (66.1-76.1)	74.9 (70.6-80.1)	65.1 (62.1-67.5)	<.001[b]
Body Cell Mass (%)	34.1 (31.6-37.3)	35.9 (33.4-39.5)	31.2 (30.-33.2)	<.001[b]
Extracellular Mass (%)	36.8 (33.7-40.0)	38.9 (36.2-41.0)	33.3 (31.8-35.4)	<.001[b]

[a]All variables reported as median (interquartile range)
[b]Statistically significant, *P*<.05

Table 4: Spearman Correlation Coefficients at Baseline

	BMI	Waist	Waist-to-hip	Fat mass	Lean mass	CD4 cell count	CD4%	CD8 cell count	CD8 %	CD4/ CD8 ratio	Viral Load
BMI	1.00										
Waist	.82**	1.00									
Waist-to-hip	.16*	.53**	1.00								
Fat mass	.72**	.58**	.03	1.00							
Lean mass	-.72**	-.58**	.03	-1.00**	1.00						
CD4 cell count	-.03	-.08	-.04	-.14*	.14*	1.00					
CD4%	-.09	-.24**	-.32**	-.08	.08	.50**	1.00				
CD8 cell count	.06	.14	.22**	-.03	.03	.26**	-.61**	1.00			
CD8%	-.05	.04	.11	-.01	.01	.29**	.72**	.73**	1.00		
CD4/ CD8 ratio	-.05	-.19*	-.26**	-.06	.06	.44**	.94**	-.71**	-.89**	1.00	
Viral load	-.08	-.06	.10	-.02	.02	-.23**	-.40**	.29**	.42**	-.43**	1.00

*Statistically significant, *P*<0.05, **Statistically significant, *P*<0.01

Table 5: Logistic Regression at Baseline, 18 Months and the Change Over 18 Months by BMI Groups*

	Baseline			18 Months			Change Over 18 Months		
Variable	OR	95% CI	P-value	OR	95% CI	P-value	OR	95% CI	P-value
CD4 cell count	1.000	.999, 1.002	.603	1.000	.999, 1.002	.640	1.001	.998, 1.004	.399
CD4%	.984	.946, 1.024	.424	.994	.952, 1.038	.783	1.076	.988, 1.166	.077
CD8 cell count	1.000	.999, 1001	.966	1.000	1.000, 1.001	.433	1.000	1.000, 1.001	.381
CD8%	.991	.964, 1.019	.536	.993	.962, 1.026	.691	.976	.910, 1.046	.486
CD4/CD8 ratio	.767	.286, 2.056	.598	.554	.139, 2.215	.554	.781	.308, 1.983	.603
HIV viral load	1.054	.748, 1.485	.764	.886	.590, 1.330	.558	.900	.519, 1.561	.707

*Dependent variable = BMI groups; reference category=BMI ≥25kg/m^2; Individual models analyzed for each independent variable and adjusted for age, gender, marriage, and children.

Table 6: Linear Regression at Baseline, 18 Months and the Change Over 18 Months by Continuous BMI*[a]

	Baseline			18 Months			Change Over 18 Months		
Variable	Slope	95% CI	P-value	Slope	95% CI	P-value	Slope	95% CI	P-value
CD4 cell count	.001	-.002, .005	.469	.003	-.002, .008	.240	-.004	-.010, .002	.165
CD4%	-.036	-.116, .043	.371	.052	-.063, .168	.374	.214	.051, .376	.011[b]
CD8 cell count	<.001	-.001, .011	.938	.002	.000, .004	.082	.001	-.001, .002	.399
CD8%	-.031	-.087, .024	.270	-.069	-.155, .017	.116	-.076	-.225, .073	.371
CD4/CD8 ratio	-.818	-2.878, 1.243	.435	-3.258	-7.323, .807	.115	-.673	-2.613,1.266	.494
HIV viral load	.007	-.715, .728	.986	.204	-.789, 1.196	.686	.625	-.609, 1.859	.318

*Individual models analyzed for each independent variable and adjusted for age, gender, marriage, and children

[a]Dependent variable for baseline analysis included continuous BMI at baseline, dependent variable for 18 months analysis included continuous BMI at 18 months, and dependent variable for difference between visits analysis included continuous BMI at baseline.

[b]Statistically significant, *P*<.05

Table 7: Adjusted Hazard Ratios on the Effect of BMI Groups on HIV Disease Progression Outcomes in HIV+ Adults in Botswana During a Follow-up Period of 18 Months*

Outcome	HR[b]	95% CI[c]	*P*-value
≥ 25% decline in CD4 cell count	.744	.489, 1.131	.166
CD4 cell count ≤ 250/μL	1.021	.381, 2.740	.966
AIDS Defining Conditions	.500	.047, 4.465	.500
CD4 cell count ≤ 250/μL & AIDS Defining Conditions	1.089	.453, 2.619	.849

*Cox proportional hazards model were used to examine the effect of BMI groups (0=BMI 18.0-24.9 kg/m^2 and 1=BMI ≥25kg/m^2) on individual HIV disease progression outcomes. All individual HIV disease progression outcomes were analyzed as separate models and adjusted for age, gender, marriage, children and baseline CD4 count and viral load.
[a]Statistically significant, *P*<.05
[b]HR= Hazard Ratio
[c]CI=Confidence Interval

Table 8: Adjusted Hazard Ratios on the Effect of Baseline BMI on HIV Disease Progression Outcomes in HIV+ Adults in Botswana During a Follow-up Period of 18 Months*

Outcome	HR[b]	95% CI[c]	*P*-value
≥ 25% decline in CD4 cell count	.963	.919, 1.009	.113
CD4 cell count ≤ 250/μL	1.043	.932, 1.167	.462
AIDS Defining Conditions**	.218	.068, .701	.011[a]
CD4 cell count ≤ 250/μL & AIDS Defining Conditions	.904	.796, 1.028	.124

*Cox proportional hazards model were used to examine the effect of baseline continuous BMI on individual HIV disease progression outcomes. All individual HIV disease progression outcomes were analyzed as separate models and adjusted for age, gender, marriage, children and baseline CD4 count and viral load.
**Model adjusted for age, gender, children and baseline CD4 cell count and viral load
[a]Statistically significant, *P*<.05
[b]HR= Hazard Ratio
[c]CI=Confidence Interval

Table 9: Adjusted Hazard Ratios on the Effect of Baseline Fat Mass on HIV Disease Progression Outcomes in HIV+ Adults in Botswana During a Follow-up Period of 18 Months*

Outcome	HR[b]	95% CI[c]	*P*-value
≥ 25% decline in CD4 cell count	.974	.945, 1.003	.083
CD4 cell count ≤ 250/μL	.984	.909, 1.065	.793
AIDS Defining Conditions	.855	.741, .987	.033[a]
CD4 cell count ≤ 250/μL & AIDS Defining Conditions	.918	.847, .994	.036[a]

*Cox proportional hazards model were used to examine the effect of baseline continuous fat mass % on individual HIV disease progression outcomes. All individual HIV disease progression outcomes were analyzed as separate models and adjusted for age, gender, marriage, children and baseline CD4 count and viral load.
[a]Statistically significant, *P*<.05
[b]HR= Hazard Ratio
[c]CI=Confidence Interval

Table 10: Adjusted Hazard Ratios on the Effect of Baseline Lean Mass on HIV Disease Progression Outcomes in HIV+ Adults in Botswana During a Follow-up Period of 18 Months*

Outcome	HR[b]	95% CI[c]	*P*-value
≥ 25% decline in CD4 cell count	1.027	.997, 1.059	.082
CD4 cell count ≤ 250/μL	1.008	.939, 1.100	.692
AIDS Defining Conditions	1.169	1.013, 1.349	.033[a]
CD4 cell count ≤ 250/μL & AIDS Defining Conditions	1.075	.995, 1.161	.068

*Cox proportional hazards model were used to examine the effect of baseline continuous lean mass % on individual HIV disease progression outcomes. All individual HIV disease progression outcomes were analyzed as separate models and adjusted for age, gender, marriage, children and baseline CD4 count and viral load.
[a]Statistically significant, *P*<.05
[b]HR= Hazard Ratio
[c]CI=Confidence Interval

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CHAPTER IV: PRELIMINARY ASSOCIATION STUDY OF THE FAT MASS AND OBESITY ASSOCIATED GENE POLYMORPHISMS WITH HIV DISEASE PROGRESSION

Abstract

Objective: Being overweight or obese in HIV has been shown to be protective in delaying disease progression. The objective of this study was to assess whether polymorphisms of the fat mass and obesity associated gene (*FTO*) are associated with body composition and HIV disease progression in HIV+, ART naïve adults in Botswana.

Methods: A retrospective study was completed with 215 participants from the placebo group of a randomized controlled trial previously conducted and published. Five SNPs of the *FTO* gene were examined for their association with body composition and measures of HIV disease progression.

Results: A total of 137 normal weight and 78 overweight/obese participants were studied. Only rs17817449 was associated with BMI in the dominant genetic risk model. *FTO* SNPs were not associated with laboratory markers of HIV infection at baseline. Fat mass and lean mass both were associated with rs1121980, rs8050136, and rs17817449 with the risk alleles having a higher percentage of fat mass and lower lean body mass compared to the non-risk homozygous allele. Over 18 months, *FTO* SNPs were not associated with outcomes of HIV disease progression.

Conclusions: *FTO* SNPs may contribute to the variation in body fat mass and lean mass in adults in Botswana. *FTO* SNPs were not associated with a protective effect on HIV disease progression. Further studies including GWAS are needed in African populations to clarify whether genetic variation mediates the obesity paradox.

Keywords: HIV infection, ART naïve, BMI, disease progression, *FTO* gene

Introduction

Studies have shown that obesity may be protective of further HIV disease progression before initiation of antiretroviral therapy (ART).[1-4] Obesity was even found to be protective after initiation of ART.[5] Higher CD4 cell counts and delayed diagnosis of AIDS have been found in those who were overweight or obese.[1-3,6,7] Those who are obese may have a genetic advantage in maintaining fat stores and utilizing energy to fuel the immune system. Conversely, obesity may also increase one's risk for developing chronic conditions such as cardiovascular diseases, diabetes and cancer.[8-10] It is also a concern for the HIV patient that is now living longer due to increasing use of antiretrovirals. Information on mechanisms for the potential benefits of obesity in delaying HIV disease progression are lacking.

Obesity has a strong genetic background[11] which has been estimated to account for 40-70% of the variation in human adiposity.[12] The *Fat Mass and Obesity Associated (FTO)* gene was first identified in 2007[13] and has been associated with obesity and obesity related traits.[14,15] The hypothalamus is the site where the FTO gene is mainly expressed[16] and may be involved in energy homeostasis,[17] and fat cell lipolysis which may regulate body fat.[18] Specifically, the single nucleotide polymorphism (SNP) of the *FTO* gene rs9939609 (A allele) is most commonly associated with obesity;[13,15,19] however this relationship has not been replicated in an African population. Currently, there are no published studies on the *FTO* gene and its association with obesity in HIV+ patients or in a population in Botswana.

Botswana is experiencing one of the worst epidemics of HIV, with a prevalence rate of 23.4% among those who are 15 and 49 years of age.[20] Botswana has the first successfully implemented ART program in Africa and provides universal access to ART

when CD4 cell counts reach < 350 cells/μL.[21] Botswana, like other African countries, is also experiencing a lifestyle transition that includes increasing rates of obesity.[22-24]

The aim of this preliminary study was to determine the interrelationships among being overweight/obese, markers of genetic propensity for obesity (SNPs in FTO) and HIV disease progression in HIV+ asymptomatic adults in Botswana not on ART over 18 months.

Methods

Study Design and Participants

We completed a retrospective study analyzing frozen laboratory specimens from 215 HIV-positive men and women from the placebo group of a multifactorial, randomized, double-blinded, and placebo-controlled clinical trial in Botswana.[25] This parent study originally included 219 participants in the placebo group, however only 215 participants had sufficient samples for laboratory analysis. The parent study investigated whether supplementation with multivitamins and selenium can improve immune function and prolong time to AIDS in ART naïve HIV infected adults.[25] The study was performed between December 2004 and July 2009. Confounding effects from the micronutrient supplementation on the outcome measures were avoided by using the participants from the placebo group for the current study. Participants were eligible for the parent study with documentation of HIV seropositive test results, CD4 cell count > 350 cells/μL, BMI > 18 kg/m^2 for women and > 18.5 kg/m^2 for men, age ≥ 18 years, lack of a past history of AIDS-defining conditions, lack of current participation in another blinded clinical trial and still ineligible for receiving ART in Botswana during the study period. Women were excluded if pregnant or had intention to become pregnant. All participants provided informed consent and approval to use their stored blood for future studies. Participants were recruited from the Botswana-Harvard AIDS Initiative

Partnership (BHP) in Gaborone, Botswana. We obtained approval to use their stored blood for genotyping SNPs from the *FTO* gene from the Florida International University Institutional Review Board (IRB), the Harvard School of Public Health IRB, and the Botswana Health Research Unit of the National Ministry of Health.

Clinical Data

At baseline and every three months, physical examination and medical history were performed by a trained nurse or a physician. Anthropometrics were also obtained and body mass index (BMI) was calculated by dividing the participants' weight in kg by their height in meters^2.

Waist and hip circumference was measured using a non-stretchable tape measure. Waist circumference was measured at the narrowest part of the waist between the lowest rib and the iliac crest and the hip circumference at the widest portion of the buttocks. Bioelectrical impedance analysis (BIA) was performed using the Biodynamics body composition analyzer (model BIA-310; Biodynamics Corp., Seattle, WA), which provides a print-out of measured impedance and calculated body fat and lean mass. Subjects were measured fasting, without shoes and socks, and electrodes were placed on the participant's right hand and wrist and right foot and ankle. Medical history included intercurrent health events and currently prescribed medications; a review of records was used to verify prescriptions and determine changes in health status. Morbidity information was collected by questionnaires at screening, and at every monthly visit, and confirmed by documentation in the medical chart. Participants were followed medically by the research physicians at the same facilities where the research was conducted to improve compliance with study visits.

Twenty-four hour recalls were conducted at baseline and every 3 months by trained clinical staff. Macronutrient and fiber intakes were calculated using the NutriBase

Professional, V.9 (Cybersoft, Phoenix, AZ) program modified by the South Africa database, including native foods for which information was available. An average of at least 3 dietary recalls were used to obtain their estimated energy, carbohydrate, protein, fat and fiber intakes. A questionnaire on activity patterns to obtain data on physical activity was completed. Time spent walking was used a measure of physical activity.

Laboratory Data

At baseline and every six months, blood was also drawn to evaluate HIV viral load, lipid panel, and blood chemistry. Every three months blood was drawn for CD4 and CD8 cell counts. Lymphocyte phenotype was determined with a four-color immunophenotyping panel of monoclonal antibodies. Differential counts were determined using a Coulter MaxM hematology instrument and corroborated with cytocentrifuge smears. HIV-viral load was determined using an *in-vitro* nucleic acid amplification test (Amplicor reagents and protocol, Roche-Diagnostics, Branchburg, NJ).

Single Nucleotide Polymorphisms for the FTO gene

The Qiagen QIAamp DNA Blood kit was used to isolate total DNA from plasma samples stored at -80° C. The DNA purification procedure was done using a standard microcentrifuge and QIAamp Mini spin columns. A minimum of 200 µL of plasma was used to isolate sufficient DNA for analysis.

Polymorphism for the SNPs rs9939609, rs1421085, rs8050136, rs17817449, and rs1121980 of the FTO gene that have previously been associated with BMI were genotyped by TaqMan allelic discrimination assays from Life Technologies Inc. (Carlsbad, CA). Fluorescence was visualized through a real-time PCR system, Bio-Rad CFX96 real-time PCR machine (Hercules, CA). Bio-Rad SsoFast Supermix was used with the TaqMan assay. PCR amplification was completed using 10 µL volume. PCR thermal cycling included enzyme activation at 95°C for 2 minutes, 49 cycles of

denaturation at 95°C for 5 seconds and annealing and extension at 61°C for 5 seconds. A random 10% of samples were re-genotyped to assess genotyping reproducibility.

Statistical Analysis

BMI was stratified into two groups: 1. normal weight (BMI=18.0-24.9 kg/m^2) and 2. overweight/obese (BMI= ≥ 25 kg/m^2). Laboratory markers of HIV infection and disease progression included in the analyses were CD4 cell count, CD4%, CD8 cell count, CD8%, CD4/CD8 ratio and HIV viral load. Descriptive statistics such as frequencies, percentages, medians and interquartile ranges were used to characterize the two stratified BMI groups at baseline and its relation to the *FTO* SNPs. Kruskal-Wallis test was used to determine differences in body composition and HIV disease progression markers between the *FTO* SNP alleles. Chi-square tests were used to determine differences in gender, AIDS defining conditions, BMI groups and *FTO* SNPs. Logistic regression was used to assess the associations between the BMI groups and the presence of the *FTO* SNPs. Logistic regression was also used for the dominant genetic risk model in which the subjects homozygous and heterozygous for the variant allele were compared to the wild-type homozygous group for measures of body composition and HIV disease progression. Proportional survival models were used to compare hazard ratios on time to HIV disease progression outcomes between *FTO* alleles, using the dominant model over 18 months. HIV disease progression outcomes included time to ≥ 25% decline in CD4 cell count, CD4 cell count ≤ 250 and/or AIDS defining conditions over 18 months. Models were adjusted for covariates that included age, gender, tribes, baseline CD4, baseline HIV viral load, total dietary kcal intake, and activity. For all analyses, a two sided test was used and $P<0.05$ was considered statistically significant. Statistical analyses were completed using SPSS version 21.

Results

Genotype Frequencies and Demographics

The genotype and allele frequencies of all SNPs are shown in Table 1. Overall, 99% of the samples were successfully genotyped. Ten percent of the samples were re-genotyped with 96% concordance. All of the SNPs, except for rs1421085, were in Hardy-Weinberg equilibrium, (*P*>.01). Further analysis with SNP rs1421085 was not performed since the minor allele frequency was less than 5% in this sample and it was not in Hardy-Weinberg equilibrium.

Baseline description of the study participants separated by BMI groups is provided in Table 2. A total of 215 participants, 137 in the normal BMI group and 78 in the overweight/obese BMI were included in the analysis, as four participants were excluded for having missing data. The overweight/obese group was older (median (IQR)) (35.00 (30.00-43.00)) than the normal BMI group (31.00 (28.00-38.00), *P*<.004) and had a greater proportion of women (87.20% vs. 69.30, *P*<.001). There were no statistically significant differences between these groups in laboratory markers of HIV infection at baseline.

Association of FTO SNPs, Body Composition and Laboratory Markers of HIV Infection

SNPs rs1121980, rs8050136, and rs9939609 genotypes were not associated with measures of body composition and laboratory markers of HIV infection at baseline (Tables 3-6). As shown in Table 6, the SNP rs17817449 had a marginal additive model association with BMI (*P*=.088), fat mass (*P*=.088), and lean mass (*P*=.085) reflecting a possible gene-dosage effect. Subjects with the GG genotype had higher BMI and fat mass and lower lean mass compared to the other genotypes, albeit not statistically significant.

Regression analysis was completed using the dominant model for all SNPs for examination of their relationships with measures of body composition and laboratory markers of HIV infection (Tables 7-10). A significant relationship with rs8050136 was seen with fat mass (OR=1.078; 95% CI= 1.021, 1.140; *P*=.007) and lean mass (OR=.928; 95% CI= .878, .981; *P*=.008). SNP rs17817449 was the only SNP associated with BMI (OR=1.082; 95%CI= 1.001, 1.169; *P*=.047). SNP rs17817449 was also associated with fat mass (OR=1.086; 95% C=, 1.031, 1.145; *P*=.002) and lean mass (OR=.921; 95% CI= .874, .971; *P*=.002). Similarly, rs1121980 had significant relationships with fat mass (OR=1.065; 95% CI= 1.009, 1.125, *P*=.021) and lean mass (OR=.939; 95% CI= .889, .991; *P*=.022).

Separate analyses were conducted by gender using logistic regression models to examine relationships between *FTO* SNPs, body composition and laboratory markers of HIV infection. In females, rs8050136 in the dominant model showed a statistically significant association with fat mass (OR=1.102; 95% CI= 1.029, 1.179; *P*=.005) and lean mass (OR=.909; 95% CI= .849, .973; *P*=.006). The SNP rs17817449 was also significantly associated with fat mass (OR=1.099; 95% CI= 1.031, 1.171; *P*=.008) and lean mass (OR=.911; 95% CI =.855, .971; *P*=.004). No other SNPs had significant results for gender-specific associations.

Although none of the SNPs had significant findings using the dominant model with laboratory markers of HIV infection, rs8050136 did have a trend relationship with HIV viral load (OR=.705; 95% CI= .481, 1.035; *P*=.074), showing a protective effect. As shown in Tables 12-15, the *FTO* SNPs were not associated with HIV disease progression outcomes of 25% decline in CD4, CD4 ≤ 250 cells/μL and/or AIDS defining conditions over 18 months using Cox proportional hazard models.

Discussion

This study provides preliminary data on the influence of *FTO* variants on the risk of being overweight/obese in HIV+ adults in Botswana. No significant associations were found with the SNP variants and waist or waist-to-hip ratio. The variant rs17817449 showed a trend towards higher BMI with the risk allele (GG) compared with the non-risk allele (TT). This variant also had a significant association with BMI, showing a greater odds of having higher BMI when the risk allele is present, after controlling for age, gender, tribe, total kcal intake and activity. In a study conducted in South African adolescents, rs17817449 was also associated with BMI after adjusting for age, gender and pubertal stage.[26] They showed a 1.9% effect size increase in BMI for each risk allele. The relationship between measures of obesity and FTO variants has been contradictory among indigenous African populations. In West Africans,[27] several SNP*s* were associated with BMI, waist circumference and percent fat mass; however, no association was found with rs9939609, which is the most replicated SNP in the literature. A replication study completed with lean Gambian adults did not show any associations between 16 *FTO* SNPs and measures of body mass, but phenotypic comparisons of obesity could not be evaluated since participants that were obese were not part of the study.[28]

Fat mass and lean mass were associated with rs1121980, rs8050136, and rs17817449 with the risk alleles having a higher percentage of fat mass and lower lean body mass compared to homozygosity for the non-risk allele. Adeyemo et al.[27] also investigated percent body fat in West Africans and showed significant relationships with *FTO* SNPs; however, the investigators did not report significant findings with any of the SNPs included in our study. Although the exact mechanism for the effect of FTO on weight is unknown, it is hypothesized that it may affect food intake and energy

expenditure.[29] FTO is highly expressed in the hypothalamus region of the brain, where food intake is controlled.[16] In several studies, rs9939609 was shown to be associated with higher food intake in children and adults.[30-33] Greater levels of *FTO* mRNA have also been found in the adipose tissue of obese persons.[34-35] Although, *FTO* may regulate adiposity, factors that influence mobilization of adipose tissue such as leptin, have not been consistently demonstrated.

This is the first study that examined *FTO* SNPs in HIV+ adults in Africa. Botswana is known to be a heterogeneous population with many ethnic groups.[36] All of the SNPs were in Hardy Weinberg equilibrium, except for rs1421085, which also had a minor allele frequency of less than 5% in this sample. The SNP rs1421085 was excluded from the analyses; however, similar genotype frequencies for this SNP were also seen in the reference population of the Yoruba tribe from Africa.[37] The heterogeneity present in this population may also be a limitation for this study.

Studies have shown that obesity may be protective of further HIV disease progression.[4,38] We showed in this same cohort that higher fat mass was associated with a decreased risk of AIDS defining conditions and CD4 cell count <250 cells/μL (unpublished data). This protection provided by fat may be explained partially through its association with leptin, an adipokine secreted by adipose tissue. Higher fat mass is associated with higher levels of leptin, which has been shown to be associated with proliferation and reduced apoptosis of immune cells such as CD4+ T-cells.[41,42] In the current analysis, we did not find any significant effects of *FTO* variants on laboratory markers of HIV infection and disease progression over time. However, our findings suggest that *FTO* SNPs may indirectly influence HIV disease progression through their effect on fat mass before ART initiation.

Based on our analyses, further studies should include a larger sample size to be able to examine the associations with the SNPs of the *FTO* gene, BMI and HIV disease progression with greater statistical power. A larger sample size would have allowed for the examination of the relationship of the *FTO* variants and body mass in further stratified subgroups of overweight and obese separately. Including other candidate SNPs of the *FTO* gene would also provide a better understanding of the role of the variants in the *FTO* gene on BMI or obesity in this population. Controlling for other variable that might affect BMI such as medications, time of HIV diagnosis, and co-morbidities may also provide a better grasp on the effect of the gene on the phenotypic outcomes.

The *FTO* still remains the gene most consistently associated with obesity in various populations,[43] but its associations have not been replicated in many African populations. GWAS studies in African populations are lacking, and identifying SNPs that are relevant to this population is of importance since the SNPs showing associations in European and Asian populations may be different from SNPs that will show associations in African populations. In this study, the SNPs chosen were based on those most associated with BMI in the literature; however, only rs17817449 had a significant relationships with BMI. Significant associations were seen with percent body fat mass and lean mass which have been essential factors in HIV disease progression.[44,45] A recent publication by Monda et al.[46] identified new loci within the *FTO* gene that was associated with BMI in individuals of African ancestry; however, the authors concluded that SNPs were not likely to contribute to population differences in obesity, highlighting the importance of further studies.

This preliminary study did have limitations which may affect the interpretation of our findings. Our study only included 215 participants and may not have had sufficient

statistical power, as genetic association studies usually require a large number of participants. This study was retrospective in design. No underweight participants were included in the parent study and only a few cases of morbid obesity were present, which did not allow for the full spectrum of BMI to be examined and lowered the statistical power. The findings of this study are pertinent to this specific population and not generalizable to all African populations.

In summary, our data indicate that *FTO* SNPs may contribute to the variation in body fat mass and lean mass in adults in Botswana. *FTO* SNPs were not directly associated with a protective effect on HIV disease progression. Additional loci within the *FTO* gene should be examined in African populations to enhance our comprehension of their influence on obesity and its benefits and risk factors.

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Table 1: *FTO* SNP Allele and Genotype Frequencies in HIV+ Adults in Botswana

	Major allele	Minor allele	Genotype frequencies			Allele frequencies	
dbSNP ID	(A)	(B)	(AA)	(AB)	(BB)	Major	Minor
rs1121980	T	C	0.241	0.474	0.274	0.516	0.484
rs1421085	T	C	0.917	0.056	0.019	0.953	0.047
rs8050136	A	C	0.239	0.452	0.300	0.530	0.470
rs9939609	A	T	0.247	0.493	0.252	0.502	0.498
rs17817449	T	G	0.366	0.412	0.213	0.577	0.423

Table 2: Demographic Characteristics by BMI Group (N=215)

Variable	Normal BMI (N=137)	Overweight/ Obese BMI (N=78)	*P*-value
Age (years)	31.00 (28.00-38.00)	35.00 (30.00-43.00)	.004[b]
Gender [a]			
Male	42 (30.70)	10 (12.80)	.003[b]
Female	95 (69.30)	68 (87.20)	
Waist (inches)	30.71 (29.13-32.28)	37.64 (34.84-39.27)	<.001[b]
Waist-to-hip ratio	.80 (.78-.85)	.85 (.79-.88)	<.013[b]
Lean mass (%)	74.90 (70.40-80.00)	65.10 (62.10-67.50)	<.001[b]
Fat mass (%)	25.10 (20.00-25.10)	34.90 (32.50-37.90)	<.001[b]
CD4 cell count (cells/μL)	415.87 (324.02-546.99)	401.10 (331.24-545.24)	.935
CD4 %	27.02 (20.19-31.19)	23.66 (18.88-30.07)	.151
CD8 cell count (cells/μL)	797.05 (588.47-1225.48)	891.36 (669.20-1177.81)	
CD8 %	52.24 (44.18-58.29)	49.92 (43.40-59.28)	.228
CD4/CD8 Ratio	.54 (.37-.67)	.49 (.32-.71)	.624
HIV viral load (Log_{10} copies/mL)	4.33 (3.60-4.82)	4.22 (3.30-4.82)	.586

[a]Reported as n (%); all other variables reported as median (interquartile range)
[b]Statistically significant, *P*<.05

Table 3: FTO rs1121980: Association with Body Composition and Measures of HIV Disease Progression

Variable	TT	TC	CC	P-value
Age (years)	33 (29-39)	33 (28-39)	32 (28-42)	.829
BMI (kg/m^2)	21.60 (28.83-25.95)	23.60 (20.48-27.70)	22.60 (20.70-26.60)	.614
Waist (inches)	31.10 (29.53-34.65)	33.27 (29.92-37.30)	32.28 (29.92-35.04)	.425
Waist-to-hip ratio	.84 (.78-.88)	.80 (.79-.85)	.82 (.78-.88)	.146
Lean mass (%)	72.25 (65.65-77.28)	70.25 (64.28-75.90)	71.10 (65.48-76.03)	.273
Fat mass (%)	27.75 (22.73-34.35)	29.45 (24.10-35.73)	28.90 (23.98-33.53)	.278
CD4 cell count (cells/μL)	409.03 (329.59-566.49)	413.58 (324.17-544.17)	417.66 (325.80-560.00)	.901
CD4%	25.19 (19.32-31.58)	25.68 (16.69-30.56)	26.45 (19.58-30.61)	.967
CD8 cell count (cells/μL)	905.58 (650.25-1297.91)	839.66 (619.65-1159.03)	789.35 (604.56-1110.60)	.627
CD8%	52.45 (44.11-60.19)	51.14 (42.80-58.55)	50.88 (43.50-58.54)	.769
CD4/CD8 ratio	.51 (.33-.65)	.53 (.34-.72)	.51 (.36-.64)	.886
HIV viral load (log_{10} copies/mL)	4.11 (3.28-4.92)	4.34 (3.42-4.81)	4.37 (3.71-4.82)	.507

Table 4: FTO rs8050136: Association with Body Composition and Measures of HIV Disease Progression

Variable	AA	AC	CC	P-value
Age (years)	34 (29-39)	32 (28-39)	32 (28-43)	.343
BMI (kg/m^2)	23.10 (21.30-27.10)	23.65 (20.35-27.33)	22.4 (20.45-25.95)	.641
Waist (inches)	32.09 (29.92-36.81)	33.46 (30.12-36.32)	32.28 (29.52-34.45)	.272
Waist-to-hip ratio	.84 (.79-.84)	.81 (.79-.85)	.82 (.78-.88)	.396
Lean mass (%)	71.40 (66.88-75.55)	70.05 (64.95-75.88)	71.70 (67.50-79.85)	.162
Fat mass (%)	28.60 (24.45-33.13)	29.95 (24.13-35.05)	28.30 (20.15-32.50)	.159
CD4 cell count (cells/μL)	407.53 (325.55-580.64)	403.33 (318.04-532.34)	415.87 (343.72-545.17)	.533
CD4%	26.35 (19.60-31.95)	27.36 (19.58-30.56)	24.80 (19.74-30.24)	.794
CD8 cell count (cells/μL)	845.60 (586.65-1249.86)	773.38 (597.30-1120.43)	960.91 (667.27-1249.21)	.280
CD8%	52.28 (42.66-57.92)	48.59 (42.96-58.55)	53.54 (46.33-59.40)	.217
CD4/CD8 ratio	.54 (.36-.71)	.56 (.34-.69)	.44 (.35-.63)	.474
HIV viral load (log_{10} copies/mL)	4.13 (3.63-4.71)	4.34 (3.23-4.90)	4.35 (3.67-4.78)	.577

Table 5: FTO rs9939609: Association with BMI, Body Composition and Measures of HIV Disease Progression

Variable	AA	AT	TT	P-value
Age (years)	31 (28-43)	33 (28-39)	34 (29-40)	.656
BMI (kg/m^2)	22.25 (20.05-25.08)	24.20 (20.83-27.80)	22.80 (21.25-26.60)	.105
Waist (inches)	32.28 (29.53-34.25)	33.27 (29.92-37.30)	31.99 (29.92-35.91)	.266
Waist-to-hip ratio	.82 (.79-.88)	.81 (.78-.87)	.83 (.78-.88)	.478
Lean mass (%)	71.70 (67.50-79.70)	70.55 (65.65-75.90)	70.40 (65.25-76.00)	.507
Fat mass (%)	28.30 (20.30-32.50)	29.45 (24.10-34.35)	29.60 (24.00-34.75)	.500
CD4 cell count (cells/μL)	414.54 (342.38-537.99)	421.13 (319.79-550.40)	397.88 (324.59-537.28)	.908
CD4%	25.21 (19.20-28.68)	27.19 (19.52-31.78)	26.42 (19.65-31.50)	.516
CD8 cell count (cells/μL)	933.94 (613.96-1296.52)	838.63 (629.54-1171.28)	813.47 (586.34-1117.45)	.532
CD8%	54.01 (47.92-60.58)	48.76 (43.91-59.09)	52.57 (42.49-56.23)	.187
CD4/CD8 ratio	.48 (.34-.60)	.56 (.33-.71)	.50 (.36-.71)	.349
HIV viral load (log_{10} copies/mL)	4.39 (3.68-4.80)	4.34 (3.41-4.92)	4.19 (3.35-4.74)	.605

Table 6: FTO rs17817449: Association with Body Composition and Measures of HIV Disease Progression

Variable	TT	GT	GG	P-value
Age (years)	31 (28-40)	33 (28-39)	35 (29-39)	.343
BMI (kg/m^2)	22.20 (20.43-25.83)	23.60 (20.25-27.38)	23.95 (21.58-27.85)	.088
Waist (inches)	31.69 (29.53-34.65)	33.27 (29.92-36.37)	32.29 (30.09-34.93)	.222
Waist-to-hip ratio	.82 (.78-.88)	.80 (.79-.87)	.82 (.77-.88)	.808
Lean mass (%)	73.60 (66.38-80.00)	70.30 (65.40-76.40)	68.75 (65.08-73.70)	.085
Fat mass (%)	26.40 (20.00-33.63)	29.70 (23.60-34.60)	31.25 (26.30-34.93)	.088
CD4 cell count (cells/μL)	407.04 (323.34-532.84)	442.65 (334.44-558.80)	397.94 (330.16-513.81)	.517
CD4%	25.29 (18.92-30.09)	26.59 (19.73-30.88)	25.93 (19.60-31.88)	.640
CD8 cell count (cells/μL)	866.85 (616.07-1115.62)	839.66 (623.67-1263.82)	783.42 (584.47-1150.51)	.628
CD8%	52.33 (46.42-58.45)	51.28 (42.57-59.50)	51.18 (43.37-56.76)	.447
CD4/CD8 ratio	.49 (.34-.62)	.55 (.34-.68)	.55 (.36-.72)	.432
HIV viral load (log_{10} copies/mL)	4.38 (3.66-4.81)	4.35 (3.41-4.950	4.10 (3.46-4.65)	.360

Table 7: Association of FTO rs1121980 with Body Composition and HIV disease Progression in a Dominant Model*

Variable	OR[a]	95% CI[b]	*P*-value
BMI groups**	1.549	.754, 3.182	.234
BMI (kg/m^2)	1.057	.974, 1.147	.183
Waist (inches)	1.049	.951, 1.157	.338
Waist-to-hip ratio	.144	.001, 134.902	.574
Lean mass (%)	.939	.889, .991	.022[c]
Fat mass (%)	1.065	1.009, 1.125	.021[c]
CD4 cell count (cells/μL)	1.001	.999, 1.003	.492
CD4%	1.006	.963, 1.050	.804
CD8 cell count (cells/μL)	1.001	.999, 1.001	.397
CD8%	.993	.963, 1.023	.625
CD4/CD8 ratio	1.443	.463, 4.495	.527
HIV viral load (log_{10} copies/mL)	.736	.498, 1.087	.124

**Dominant model: homozygous and heterozygous for the variant were compared to the wild-type homozygous. Models adjusted for age, gender, tribes, total kcal dietary intake and activity

**BMI groups: 0=BMI 18.0-24.9 kg/m^2 and 1=BMI ≥ 25 kg/m^2

[a]OR=Odds Ratio

[b]CI=Confidence Intervals

[c]Statistically significant, $P < .05$

Table 8: Association of FTO rs8050136 with Body Composition and HIV disease Progression in a Dominant Model*

Variable	OR[a]	95% CI[b]	*P*-value
BMI groups**	1.584	.776, 3.231	.207
BMI (kg/m^2)[a]	1.071	.988, 1.162	.097
Waist (inches) [a]	1.084	.980, 1.199	.117
Waist-to-hip ratio	1.641	.002, 1142.635	.882
Lean mass (%)	.928	.878, .981	.008[c]
Fat mass (%)	1.078	1.021, 1.140	.007[c]
CD4 cell count (cells/μL)	1.001	.998, 1.002	.825
CD4%	1.013	.972, 1.057	.534
CD8 cell count (cells/μL)	1.001	.999, 1.001	.390
CD8%	.981	.952, 1.010	.190
CD4/CD8 ratio	1.347	.473, 3.835	.577
HIV viral load (log_{10} copies/mL)	.705	.481, 1.035	.074

**Dominant model: homozygous and heterozygous for the variant were compared to the wild-type homozygous. Models adjusted for age, gender, tribes, total dietary kcal, and activity

**BMI groups: 0=BMI 18.0-24.9 kg/m^2 and 1=BMI ≥ 25 kg/m^2

[a]OR=Odds Ratio

[b]CI=Confidence Intervals

[c]Statistically significant, $P < .05$

Table 9: Association of FTO rs9939609 with Body Composition and HIV disease Progression in a Dominant Model*

Variable	OR[a]	95% CI[b]	*P*-value
BMI groups**	.956	.455, 2.007	.904
BMI (kg/m^2)	.991	.918, 1.070	.818
Waist (inches)	1.501	.056, 39.884	.808
Waist-to-hip ratio	1.018	.926, 1.118	.716
Lean mass (%)	1.029	.975, 1.087	.294
Fat mass (%)	.971	.920, 1.025	.290
CD4 cell count (cells/μL)	1.001	.998, 1.002	.868
CD4%	.982	.940, 1.026	.424
CD8 cell count (cells/μL)	1.001	1.000, 1.001	.159
CD8%	1.013	.983, 1.043	.405
CD4/CD8 ratio	.861	.310, 2.394	.774
HIV viral load ($\log_{10}$ copies/mL)	1.204	.811, 1.788	.357

**Dominant model: homozygous and heterozygous for the variant were compared to the wild-type homozygous. Models adjusted for age, gender, tribes, total dietary kcal and activity

**BMI groups: 0=BMI 18.0-24.9 kg/m^2 and 1=BMI ≥ 25 kg/m^2

[a]OR=Odds Ratio

[b]CI=Confidence Intervals

Table 10: Association of FTO rs17817449 with Body Composition and HIV disease Progression in a Dominant Model*

Variable	OR[a]	95% CI[b]	*P*-value
BMI groups**	1.732	.885, 3.388	.109
BMI (kg/m^2)	1.082	1.001, 1.169	.047[c]
Waist (inches)	1.093	.954, 1.203	.067
Waist-to-hip ratio	.132	.000, 59.237	.516
Lean mass (%)	.921	.874, .971	.002[c]
Fat mass (%)	1.086	1.031, 1.145	.002[c]
CD4 cell count (cells/μL)	1.002	1.001, 1.004	.136
CD4%	1.013	.979, 1.060	.360
CD8 cell count (cells/μL)	1.001	.999, 1.001	.862
CD8%	.986	.959, 1.013	.290
CD4/CD8 ratio	1.500	.552, 4.076	.426
HIV viral load ($\log_{10}$ copies/mL)	.781	.550, 1.111	.169

*Dominant model: homozygous and heterozygous for the variant were compared to the wild-type homozygous. Models adjusted for age, gender, tribes, total dietary kcal and activity

**BMI groups: 0=BMI 18.0-24.9 kg/m^2 and 1=BMI ≥ 25 kg/m^2

[a]OR=Odds Ratio

[b]CI=Confidence Intervals

[c]Statistically significant, $P < .05$

Table 11: Adjusted Hazard Ratios on the Effect of rs1121980 Using the Dominant Model on HIV Disease Progression Outcomes in HIV+ Adults in Botswana During a Follow-up Period of 18 Months*

Outcome	HR[b]	95% CI[c]	*P*-value
≥ 25% decline in CD4 cell count	1.326	.837, 2.100	.230
CD4 cell count ≤ 250/μL	1.875	.544, 6.469	.320
AIDS Defining Conditions**	1.536	.165, 14.264	.706
CD4 cell count ≤ 250/μL & AIDS Defining Conditions	1.203	.416, 3.475	.733

*Cox proportional hazards model were used to examine the effect of rs1121980 on individual HIV disease progression outcomes. Dominant model used for rs1121980: homozygous and heterozygous for the variant were compared to the wild-type homozygous. All individual HIV disease progression outcomes were analyzed as separate models and adjusted for age, gender, tribe, baseline CD4 count, baseline viral load, total dietary kcal, and activity.

**Models adjusted for age, gender and baseline CD4 cell count, baseline viral load, total dietary kcal and activity

[a]Statistically significant, $P<.05$

[b]HR=Hazard Ratio

[c]CI=Confidence Interval

Table 12: Adjusted Hazard Ratios on the Effect of rs8050136 Using the Dominant Model on HIV Disease Progression Outcomes in HIV+ Adults in Botswana During a Follow-up Period of 18 Months*

Outcome	HR[b]	95% CI[c]	*P*-value
≥ 25% decline in CD4 cell count	.946	.610, 1.468	.805
CD4 cell count ≤ 250/μL	1.269	.394, 4.082	.690
AIDS Defining Conditions**	.279	.042, 1.854	.187
CD4 cell count ≤ 250/μL & AIDS Defining Conditions	.842	.303, 2.339	.742

*Cox proportional hazards model were used to examine the effect of rs8050136 on individual HIV disease progression outcomes. Dominant model used for rs8050136: homozygous and heterozygous for the variant were compared to the wild-type homozygous. All individual HIV disease progression outcomes were analyzed as separate models and adjusted for age, gender, tribe, baseline CD4 count, baseline viral load, total dietary kcal, and activity.

**Models adjusted for age, gender and baseline CD4 cell count, baseline viral load, total dietary kcal, and activity

[a]Statistically significant, $P<.05$

[b]HR=Hazard Ratio

[c]CI=Confidence Interval

Table 13: Adjusted Hazard Ratios on the Effect of rs9939609 Using the Dominant Model on HIV Disease Progression Outcomes in HIV+ Adults in Botswana During a Follow-up Period of 18 Months*

Outcome	HR[b]	95% CI[c]	*P*-value
≥ 25% decline in CD4 cell count	1.039	.631, 1.709	.881
CD4 cell count ≤ 250/μL	1.141	.350, 3.712	.827
AIDS Defining Conditions**	-	-	-
CD4 cell count ≤ 250/μL & AIDS Defining Conditions	1.399	.443, 4.412	.567

*Cox proportional hazards model were used to examine the effect of rs9939609 on individual HIV disease progression outcomes. Dominant model used for rs9939609: homozygous and heterozygous for the variant were compared to the wild-type homozygous. All individual HIV disease progression outcomes were analyzed as separate models and adjusted for age, gender, tribe, baseline CD4 count, baseline viral load, total dietary kcal, and activity.

**Model did not converge all AIDS defining conditions occurring in group with risk alleles

[a]Statistically significant, $P<.05$

[b]HR=Hazard Ratio

[c]CI=Confidence Interval

Table 14: Adjusted Hazard Ratios on the Effect of rs17817449 Using the Dominant Model on HIV Disease Progression Outcomes in HIV+ Adults in Botswana During a Follow-up Period of 18 Months*

Outcome	HR[b]	95% CI[c]	*P*-value
≥25% decline in CD4 cell count	.977	.641, 1.489	.913
CD4 cell count ≤ 250/μL	1.517	.520, 4.421	.445
AIDS Defining Conditions**	.387	.060, 2.490	.317
CD4 cell count ≤ 250/μL & AIDS Defining Conditions	1.205	.468, 3.101	.700

*Cox proportional hazards model were used to examine the effect of rs17817449 on individual HIV disease progression outcomes. Dominant model used for rs17817449: homozygous and heterozygous for the variant were compared to the wild-type homozygous. All individual HIV disease progression outcomes were analyzed as separate models and adjusted for age, gender, tribe, baseline CD4 count, baseline viral load, total dietary kcal, and activity.

**Models adjusted for age, gender and baseline CD4 cell count, baseline viral load, total dietary kcal, and activity

[a]Statistically significant, $P<.05$

[b]HR=Hazard Ratio

[c]CI=Confidence Interval

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CHAPTER V: ASSOCIATION OF INFLAMMATORY MARKERS WITH HIV DISEASE PROGRESSION IN ART NAÏVE HIV+ ADULTS IN BOTSWANA

Abstract

Objective: This study evaluated the relationship between inflammatory markers including C-reactive protein, leptin, and bacterial endotoxin lipopolysaccharide (LPS) and body composition and HIV disease progression in HIV+ asymptomatic adults not on ART in Botswana over 18 months.

Methods: This is a retrospective analysis of data and specimens from a nutritional study, henceforth called parent study. Current study was conducted in 144 HIV+ ART naïve adults who were in the early stages of HIV disease in Botswana. CRP was measured by the parent study and plasma leptin and LPS were determined in a subset of 60 participants at baseline and 18 months. Linear and logistic multivariable regression analyses were conducted and adjusted for age, gender, smoking and body fat mass%.

Results: CRP was positively associated with BMI and waist circumference and inversely with CD4%. Leptin had significant relationships with BMI, fat mass%, and waist circumference. Higher HIV viral load was significantly associated with lower levels of leptin. LPS was inversely associated with BMI and fat mass%. Higher levels of LPS were also associated with higher viral load.

Conclusions: HIV disease progression was predictive of inflammation and found to be independent of body fat. These findings suggest that at least one explanation for the obesity paradox may be accounted for by increased LPS and lower leptin among those with lower BMI which was associated with higher HIV viral load. The mechanisms

affecting HIV disease progression through inflammation are complex and still not entirely understood, which warrants further prospective investigation.

Keywords: HIV infection, ART naïve, disease progression, CRP, leptin, LPS, inflammation

Introduction

Chronic inflammation and immune activation are related to HIV disease progression as documented in the literature.[1-3] Inflammation is also associated with other HIV co-morbidities and non-HIV related complications such as cardiovascular and metabolically-related diseases/conditions.[4-6] Inflammation is involved in the depletion of T-cells, and chronic immune activation drives the general and HIV-specific losses of immune function.[7] Continuous inflammation induces pro-inflammatory cytokines which has been shown to produce thymic and T cell progenitor dysfunction.[8] It has also been shown that triggered or activated CD4 cells are favored targets for viral infection and replication. Inflammation can also set up non-infected CD4 cells and other immune cells for death by programmed cell death or apoptosis.[9] The biomarkers of inflammation examined in this study were C-reactive protein, leptin, and lipopolysaccharide (LPS).

Obesity is known to be associated with a chronic state of low grade inflammation. Inflammation in obesity is due to many mechanisms which include disordered secretion of adipokines within adipose tissue, resulting in insulin resistance and metabolic disorders.[10] There seems to be controversy in the literature regarding obesity and inflammation and its relationship to HIV disease, which requires further research. A nutrition transition in sub-Saharan Africa is occurring with an increase in obesity,[11] along with being the most affected region by the HIV epidemic.[12] The interplay of body composition, inflammation and its effect on HIV disease progression warrant investigation to provide relevant clinical treatment for patients.

C-reactive protein or CRP is considered a sensitive systemic marker of inflammation and an acute phase reactant.[13] Higher levels of CRP have also been associated with obesity and specifically abdominal obesity.[14] CRP > 3 mg/dL has been associated with a higher risk for inflammation and mortality.[15] In HIV, CRP has been

shown to be a sensitive marker beyond cardiovascular disease;[16] it has been demonstrated to be an important predictor of higher risk of AIDS and mortality after ART initiation in ART naïve patients.[17]

Leptin is considered to be a pleiotropic molecule, with not only metabolic roles but also endocrine and immune regulation.[18] In some studies, higher levels of leptin were found to be present during infection and inflammation; however, the results in human studies have been inconsistent in various diseases, including HIV.[18] Leptin is involved in the proliferation of T- cells, including CD4 and CD8 cells.[19] Higher levels of leptin have been found in obese individuals[20] which may enhance the immunity through its role in proliferation and anti-apoptotic effects on lymphocytes. Leptin has been inversely associated with HIV viral load, independent of adipose tissue.[21]

Microbial translocation or bacterial products that crossed the gastrointestinal mucosa into circulation, is thought to have a role in persistent immune activation in HIV infection and can provoke pro-inflammatory reactions.[22] Lipopolysacchride (LPS), an endotoxin, is a constituent of Gram-negative bacterial cell walls and known to produce proinflammatory responses.[23] During HIV, microbial translocations may occur due to epithelial damage to the intestine, loss of T-helper-17 cells and a reduced removal of microbial products by phagocytes.[22] Increased secretion of pro-inflammatory cytokines can also contribute to increased T cell activation and specifically can increase CD8 T cell activation and depletion of CD4 T cells.[22] LPS has also been implicated in obesity and insulin resistance and high levels may affect inflammation and promote weight gain.[25] Although microbial translocation is associated with immune activation and disease progression in developed countries,[26-28] whether it contributes to HIV disease progression in African HIV+ individuals is not well understood. Sequestration of LPS by

higher circulating lipoproteins in obesity has been suggested as mechanism for the obesity paradox[29] and warrants further investigation in HIV.

Most of the studies looking at the relationship between inflammation and HIV have been conducted in settings where patients are taking ART, and mostly in developed nations. Both of these conditions may confound findings. Few longitudinal studies are available in African populations. The objective of this study was to evaluate over 18 months the relationship of inflammatory markers (C-reactive protein, leptin, and LPS) with body composition and laboratory markers of HIV disease infection and progression in HIV+ asymptomatic adults who are not receiving ART in Botswana.

Methods

Study Design and Participants

A retrospective study was conducted to examine data and specimens from 144 HIV-positive men (n=34) and women (n=110) from the placebo group of a multifactorial, randomized, double-blinded, placebo-controlled clinical trial in Botswana, which investigated whether supplementation with multivitamins and selenium could enhance immune function and delay the time to AIDS in ART naïve HIV+ adults.[30] The study was conducted between December 2004 and July 2009. This parent study originally included 219 participants in the placebo group, however our retrospective study only included 144 participants with available data on high sensitivity C-reactive protein (hs-CRP). The placebo group was used to avoid any confounding effects from the micronutrient supplementation on the outcome measures. Participants were eligible for the study with confirmation of HIV status, CD4 cell count >350 cells/μL, BMI >18 kg/m^2 for women and 18.5 kg/m^2 for men, age ≥ 18 years, lack of a past history of AIDS-defining conditions, and were not participating in another blinded clinical trial or taking ART. Women were excluded if pregnant or had intention to become pregnant. All

participants provided informed consent and approval to use their stored blood for future studies. Participants were recruited from the Botswana-Harvard AIDS Initiative Partnership (BHP) in Gaborone, Botswana. We obtained approval to use their stored blood for leptin and LPS from the Florida International University Institutional Review Board (IRB), the Harvard School of Public Health IRB and the Botswana Health Research Unit of the National Ministry of Health.

Clinical Data

At baseline and every three months, physical examination and medical history were performed by a trained nurse or a physician. Anthropometrics were also obtained and body mass index (BMI) was calculated by dividing the participants' weight in kg by their height in meters^2. Waist and hip circumference was measured using a non-stretchable tape measure and following standardized procedures. Waist circumference was measured at the narrowest part of the waist between the lowest rib and the iliac crest and the hip circumference at the widest portion of the buttocks. Bioelectrical impedance analysis (BIA) was measured using the Biodynamics body composition analyzer (model BIA-310; Biodynamics Corp., Seattle, WA), which provides a print-out of measured impedance and calculated body fat and lean mass. Subjects were measured without shoes and socks, and electrodes were placed on the participant's right hand and wrist and right foot and ankle. Morbidity information was collected by questionnaires at screening, and at every monthly visit, and confirmed by documentation in the medical chart. Participants received their medical care in the same facilities to improve retention.

Laboratory Data

At baseline and every six months, blood was also drawn to evaluate HIV viral load, lipid panel, and blood chemistry. Every three months blood was drawn for CD4 and CD8 cell counts. The remaining plasma was centrifuged, aliquoted into cryovials and

stored at -80° C for future studies. Lymphocyte phenotype was determined with a four-color immunophenotyping panel of monoclonal antibodies. Differential counts were determined using a Coulter MaxM hematology instrument and corroborated with cytocentrifuge smears. HIV-viral load was determined using an in-vitro nucleic acid amplification test (Amplicor reagents and protocol, Roche-Diagnostics, Branchburg, NJ).

Inflammatory Markers

A total of 144 participants had high sensitivity-C reactive protein (hs-CRP) levels from the parent study that was analyzed using protein-latex assay (Roche, Basel, Switzerland) in the Botswana-Harvard HIV Reference Laboratory in Gaborone, Botswana. A subset of 60 participants were randomly selected with available samples to measure plasma leptin and LPS levels at baseline and 18 months. Plasma leptin levels were measured using the Quantikine Human Leptin Immunoassay kit by R&D systems (Minneapolis, MN, USA), a sandwich ELISA designed to measure soluble human leptin in plasma, according to manufacturer's instructions. Plasma LPS or endotoxin levels were measured using the Limulus Amebocyte Lysate (LAL) by Lonza (Walksersville, MD, USA), a chromogenic quantitation of bacterial endotoxin, according to manufacturer's instructions with the following modifications: Samples were diluted 1:20 with endotoxin-free water to avoid interference with background color and preheated to 70°C for 10 minutes prior to analyses to inactivate plasma proteins. Duplicates were assessed for each sample, for both leptin and LPS. The coefficient of variation or CV% was calculated and CVs greater than 10% were reanalyzed.

Statistical Analysis

Laboratory markers of HIV infection and disease progression included in the analyses were CD4 cell count, CD4%, CD8 cell count, CD8%, CD4/CD8 ratio and HIV viral load. Descriptive statistics such as frequencies, percentages, medians and

interquartile ranges were used to characterize the participants at baseline and its relation between laboratory markers of HIV infection and inflammatory markers. Mann Whitney U was used to determine differences in laboratory markers of HIV infection and body composition between the BMI groups and by inflammatory marker groups. BMI was stratified into two groups: Group 1: normal weight (BMI=18.0-24.9 kg/m^2) and Group 2: overweight/obese (BMI=≥ 25 kg/m^2) for the statistical analyses. Viral load was also stratified into <4 $\log_{10}$ copies/mL or 10,000 copies/mL and ≥ 4 $\log_{10}$ copies/mL as a measure of further disease progression and infectivity.[31, 32] Linear and logistic regressions were used to assess the relationship between body composition, laboratory markers of HIV infection, and inflammatory markers. The linear multivariable regression analysis also included a measure to check for multicollinearity known as variance inflation factor (VIF). All models had a VIF <5, therefore multicollinearity was ruled out. Multicollinearity was also checked for the logistic regression models and variables with correlations r >.75 were not included. Models were adjusted for covariates that included age, gender, smoking and fat mass%. Logistic regression models included dependent inflammatory markers at the following cut-offs: hs-CRP < or ≥ 3mg/L; leptin< or ≥ median and LPS < or ≥ median. Smoking tobacco was used a covariate since it has immunomodulatory effects and may affect T-cell activation.[33,34] Variables that were not normally distributed were natural log transformed, except for CD4 and CD8 cell counts that were square-root transformed. For all analyses, a two sided test was used and $P<0.05$ was considered statistically significant. Statistical analyses was completed using SPSS version 21.

Results

Demographics

Table 1 displays the demographics of the population included for the analyses conducted below. In summary, the median age was 33 years with an IQR of 29-39 years and 76.40% were women. The median CD4 cell count and HIV viral load were 409.03 (IQR: 329.79-552.58) and 4.33 (IQR:3.58-4.83), respectively. The inter-assay CV for Leptin was 7.45 and for LPS 4.38%. The intra-assay CV for leptin ranged between 6.76-8.69% and for LPS between 2.41-5.87%.

Spearman Correlations

A positive correlation was seen between hs-CRP and leptin (r=.404, *P*=.010) (Table 2). Leptin was also correlated with LPS (r=.357, *P*=.005). There was no significant correlation between hs-CRP and LPS. Hs-CRP was not correlated with measures of body composition and HIV disease progression. However, trends were seen between hs-CRP and BMI, waist circumference, lean body mass and fat mass. Strong positive correlations were observed between leptin and BMI (r=.734, *P*<.001), waist circumference (r=.603, p<.001) and fat mass % (r=.838, *P*<.001). LPS was inversely correlated with BMI (r=.288, *P*=.025), waist circumference (r=.493, P=.003) and fat mass % (r=.362, *P*=.004). Lean mass % was inversely correlated with leptin (r=-.836, *P*<.001) and LPS (r=-.361, *P*=.005). No correlations were observed between inflammatory markers and laboratory markers of HIV infection. The correlations between LPS and CD8% and viral load were approaching significance at r=.253, *P*=.052 and r=.246, *P*=.060, respectively.

Relationship between BMI and Inflammatory Markers

BMI was categorized into two groups: normal BMI (18.0-24.9 kg/m^2) and overweight/obese BMI (≥ 25 kg/m^2) and its association with inflammatory markers were

examined. Those who were overweight/obese had higher median (IQR) levels of hs-CRP (*P*=.028) and leptin (*P*<.001) compared to the normal BMI group, (Table 3). Conversely, lower levels of LPS were seen in the overweight/obese group compared to the normal BMI group (*P*=.001).

Inflammatory Markers, Body Composition and Laboratory Markers of HIV Infection

Table 4 demonstrates that having a CRP at or above 3 mg/L is associated with higher median (IQR) of waist circumference (*P*=.036), and CD8 cell count (*P*=.030) and lower levels of CD4% (*P*=.013), and CD4/CD8 ratio (*P*=.015). A trend was observed in the relationship of having a hs-CRP ≥ 3 mg/L and higher CD8% (*P*=.078). As shown on Table 5, those who had higher median levels of leptin (≥ 13,304 pg/mL) had higher median (IQR) BMI (*P*<.001), waist circumference (*P*=.003), and fat mass (*P*<.001). Levels below the median of leptin, were associated with higher lean body mass (*P*<.001). None of the parameters for HIV disease progression were significantly associated with median levels of leptin. Having LPS levels below the median (<.0582 EU/mL) were significantly related to higher BMI (*P*=.001), waist circumference (*P*=.005), and fat mass% (*P*=.002) (Table 6). Levels above the median were associated with higher viral load (*P*=.023) and lean body mass % (*P*=.002).

Regression Analyses of Inflammatory Markers and Laboratory Markers of HIV Infection and Disease Progression

Linear regression models were conducted to examine the relationship of body composition and HIV disease progression markers with hs-CRP, leptin, and LPS at baseline, 18 months and the change over 18 months. All models were adjusted for age, gender, smoking and fat mass%. Log transformed BMI at baseline was the only measure of body composition associated with continuous hs-CRP at baseline (β=1.905; 95% CI= .281, 3.530; *P*=.022) (Table 7). No markers of HIV infection were associated

with continuous hs-CRP at baseline or 18 months. Log transformed leptin was associated with continuous BMI at baseline (β=1.764; 95% CI= .788, 2.739; *P*=.022) and 18 months (β=2.739; 95% CI= 1.133, 3.075, *P*<.001) (Table 8). Higher leptin was also associated with higher fat mass% at baseline (β=.112; 95% CI= .090, .135; *P*<.001) and 18 months (β=.403; 95% CI= .017, .069; *P*=.002). For every unit increase in transformed leptin there was a significant 2.103 unit increase in waist circumference at 18 months (β=2.103; 95% CI= .806, 3.400; *P*=.002). Having a viral load of <4 $\log_{10}$ copies/mL was associated with higher leptin levels (β= -.305; 95% CI= -.579, -.031; *P*=.030) at baseline. Only waist circumference at baseline had a significant relationship with log transformed LPS (β=-1.940; 95% CI= -3.397, .483; *P*=.011) (Table 9).

A separate linear regression analysis stratified by BMI groups was conducted. In those who had a BMI ≥ 25kg/m^2, higher log transformed leptin was associated with lower viral load (β=-.205; 95% CI= -3.94, -.016; *P*=.035) at baseline.

Hs-CRP ≥ 3 mg/L was significantly related to higher BMI at baseline (OR=60.960; 95% CI= 2.784, 1334.723; *P*=.009), waist circumference at 18 months (OR=98.811; 95% CI= 1.697, 5755.086; *P*=.027), CD4% at baseline (OR=.949; 95% CI= .902, .998; *P*=.042) (Table 10) and waist circumference over 18 months (OR=65.078; 95% CI: 1.490, 2842.157; *P*=.030). Lower CD8 count change over 18 months was associated with CRP ≥ 3 mg/L (OR=.906; 95% CI=.831, .988; *P*=.025). A trend association was seen in the relationship between hs-CRP ≥ 3 mg/L and BMI ≥ 25 kg/m^2 (OR=2.407; 95% CI= .941, 6.156; *P*=.067). Leptin above the median (13,304 pg/mL) was related to several body composition measures at baseline and 18 month; however, there were no association with measures of HIV disease progression (Table 11). Leptin above the median was associated with a higher odds of having higher BMI at baseline (OR=2.073; 95% CI= 1.194, 3.601; *P*=.010) and 18 months (OR=1.554; 95% CI= 1.125,

2.146; *P*=.008). Overweight/obese BMI category was also associated with leptin above the median at baseline (OR=58.572, 95% CI= 2.187, 1568.688; *P*=.015) and 18 months (OR=79.634; 95% CI= 4.607, 1376.659; *P*=.003). Additionally, a higher odds of having more fat mass% was seen in the group that had leptin levels at or above the median at baseline (OR=1.580, 95% CI= 1.214, 2.055; *P*=.001) and 18 months (OR=1.231; 95% CI= 1.070, 1.416; *P*=.004). The likelihood of higher leptin increased with higher waist circumference (OR=1.267; 95 CI= 1.016, 1.580; *P*=.036). Leptin above the median was also associated with having an increase in CD4% over 18 months (OR1.456; 95% CI=1.456, 1.025, 2.069; *P*=.036). Lastly, Table 12 displays the relationships between body composition and laboratory markers of HIV infection and disease progression for those at or above the median of LPS (.0582 EU/mL). BMI and having BMI ≥ 25 kg/m^2 was protective of having LPS above the median at baseline (OR=.790, 95% CI= .630, .990; *P*=.041; and OR=.035, 95% CI= .004, .283; *P*=.002, respectively) and BMI at 18 months (OR=.799, 95% CI= .655, .976; *P*=.028). Fat mass% was also shown to be protective of having higher LPS at baseline (OR=.852, 95% CI= .757, .958; *P*=.007). At baseline LPS at or above the median was associated with higher viral load (OR=2.608, 95% CI= 1.111, 6.124; *P*=.028) and having a viral load ≥ 4 $\log_{10}$ copies/mL (OR=8.005, 95% CI= 1.762, 36.357; *P*=.007). The relationships between LPS as a continuous variable and viral load ≥ 4 $\log_{10}$ copies/mL was approaching significance (OR=3.776, 95% CI= .959, 14.869; *P*=.057).

Discussion

The results presented here suggest a strong association between inflammatory markers and body composition and HIV disease progression in ART naïve adult participants in Botswana. To our knowledge, no study has assessed leptin or LPS in a population from Botswana with or without HIV infection.

CRP has previously been shown to be associated with CD4 cell count and HIV viral load in ART naïve HIV+ adults.[35-37] Our study did not confirm these associations with measures of HIV disease progression, except for CD4%. CRP levels ≥ 3 mg/L were associated with lower CD4%. CD4% is considered to be a more stable measure than absolute CD4 cell counts and is thought to provide a better prognostic information before ART is initiated in HIV infection.[38,39] Thus, this study showed that CD4%, as a measure of HIV disease progression, was an independent predictor of higher CRP levels after adjusting for age, gender, smoking and fat mass%.

CRP and its association with mortality were investigated in HIV+ populations in Botswana;[40,41] however, these previous reports did not asses its relationship with parameters of disease progression such as CD4 cell count or viral load. CRP levels were found to be higher 4 weeks after initiation of ART in patients who died compared to those who survived, in another HIV+ adult cohort in Botswana.[40] Baseline levels of CRP were also higher in those who died after ART initiation compared to matched controls in Botswana.[41]

Azzoni et al.[21] was the first to show that leptin levels were inversely associated with viral replication independent of the amount of adipose tissue. Their cohort of exclusively ART naïve women from South Africa also demonstrated an association between viral load and immune activation. We confirmed an inverse relationship between leptin levels and viral load at baseline, independent of body fat. These associations were found in HIV+ male and female participants. These results are in agreement with those of Azzoni and colleagues[21] who hypothesized that high viral replication and immune activation could result in chronic inflammation that would cause lipoatrophy, therefore, lowering leptin levels.

Leptin is involved in the proliferation of CD4 and CD8 cells,[42] however, we did not find any significant relationships between leptin and CD4 or CD8 cell counts, even while adjusting for fat mass%. However, leptin above the median was associated with a greater increase in CD4% over 18 months. Other researchers[21,43,44] examining leptin in HIV+ ART naïve individuals did not find significant relationship with CD4 cell counts. The lack of an association of CD4 cell count and leptin may be due to the early stage of the disease and healthier status in these participants. The parent study's enrollment criteria included having CD4 ≥ 350 cells/μL and BMI >18 kg/m^2, which may have precluded the chance of examining the associations with lower levels of CD4 counts and BMI in the range of undernutrition at baseline. Additionally, ART-naïve participants usually present uncontrolled viral loads.

Previous studies evaluating LPS and HIV disease progression in ART naïve HIV+ adults have shown conflicting findings. In a cohort of HIV-positive, ART-naïve adults, Marchetti el al.[28] reported a significant relationship between LPS and disease progression as a composite outcome that included, AIDS defining conditions, death, CD4 cell counts < 200 cells/μL, or start of ART in HIV+ ART naïve adults, who were followed for approximately 3 years. This relationship was independent of CD4 cell counts and HIV viral load. Conversely, a study examining LPS and HIV disease progression in ART naïve Ugandans and Americans was not able to detect sufficient LPS levels in their samples for analyses.[45] A recent study conducted in Kenyan women demonstrated that LPS levels were associated with chronic HIV infection, whether treated or not with ART; however, no direct associations with CD4 cell count or viral load was found.[46] This present study did not find a significant association between LPS and CD4 cell count, but LPS was significantly and inversely associated with viral load. LPS levels above the median were associated with higher viral load and those with LPS levels ≥ the median

were 8 times more likely to have viral load > $4\log_{10}$ copies/mL or 10,000 copies/mL. Interestingly, LPS was also inversely associated with measures of body composition, therefore being overweight or obese was protective of having higher levels of LPS. Timmons et al.[47] did not show significant association between LPS and body composition; however, they did report relationship with sCD14. Soluble CD14 is a measure of microbial translocation, which is freed from monocytes by LPS stimulation.[48] There was an inverse association between sCD14 and limb and trunk fat in 178 HIV+ adults that included ART treated and non-treated patients. The authors suggest that microbial translocation may be involved in muscle and fat loss during HIV disease. Unfortunately, the result could not be separated from the effect of ART treatment since the analyses included both ART treated and non-treated. In animals, it has been demonstrated that the quality of diet, rather than weight, was a better predictor of the composition of intestinal bacteria.[49] A high fat diet has been shown to be associated with higher LPS levels.[50] Diet composition of those with a higher BMI may have affected inflammation and should be researched further.

A possible explanation for the lower levels of LPS observed in those with higher BMI might be the endotoxin lipoprotein hypothesis. Rauchhaus et al.[29] were the first to describe the endotoxin-lipoprotein hypothesis as a way to explain the paradox of higher cholesterol levels being beneficial in congestive heart failure. Higher circulating lipoproteins may modulate inflammation by binding to lipopolyssachrides and reduce their effect on release of proinflammatory cytokines. LPS is sequestered during micelle creation under the direction of lipoproteins. Since higher BMI is associated with higher risk of elevated cholesterol, triglycerides and LDL,[51,52] more circulating lipoproteins may be available to capture LPS.

The limitations of our study include the study design, which was retrospective and observational. A larger sample size may have also showed significant relationships between inflammatory markers and disease progression. A selection bias may also have been present since we chose to complete leptin and LPS in a random subset that had samples available at baseline and 18 months. In HIV, bioimpedance (BIA) has been shown to be an effective method for detecting fat mass;[53] however, BIA has limitations, since its values can be influenced by body arrangement, hydration, ingestion of food and drinks, air and body temperature and exercise.[54] Our BIA measurement protocol countered these limitations by giving detailed instructions to the participants on their preparation for their visit, and standardizing the measurement parameters.

Conclusions

Body composition and HIV disease progression affected inflammation in this HIV+ adult population in Botswana, early in their disease. Laboratory markers of HIV infection and disease progression was predictive of inflammation, independent of body fat. Higher LPS was associated with lower BMI and higher viral load, while higher leptin was associated with higher fat mass and lower viral load. These are new and thought-provoking findings that might help explain the obesity paradox often observed in HIV+ ART adults. The mechanisms affecting HIV disease progression through inflammation are complex and still not entirely understood, which warrants further prospective investigation before and after initiation of ART.

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of the participants in the study without whom advancement in the nutritional management of the HIV disease are not possible.

Table 1: General Characteristics of the Sample Population

Variable	Total (N=144)
Age (years)	33.00 (29.00-39.00)
Gender [a]	
Male (%)	34 (23.60)
Female (%)	110 (76.40)
BMI (kg/m^2)	22.70 (20.70-26.93)
Waist (inches)	32.28 (29.92-35.81)
Waist to hip ratio	.82 (.79-.88)
Lean mass (%)	71.30 (66.30-76.00)
Fat mass (%)	28.70 (24.00-33.70)
CD4 cell count (cells/μL)	409.03 (329.79-552.58)
CD4 %	26.35 (19.64-31.04)
CD8 cell count (cells/μL)	844.35 (621.96-1189.17)
CD8 %	51.70 (43.58-58.30)
CD4/CD8 Ratio	.53 (.36-.68)
HIV viral load (Log_{10} copies/mL)	4.33 (3.58-4.83)
CRP (mg/L)	1.69 (.80-4.74)
Leptin (pg/mL)[b]	13,304.75 (5649.13-24,430.50)
LPS (EU/mL) [b]	.058 (.053, .068)

[a]Reported as n (%); all other variables reported as median (interquartile range)
[b]Completed in a subset of 60 participants

Table 2: Baseline Spearman Correlation Coefficients and *P*-values

Variables	CRP	Leptin[a]	LPS[a]
CRP (mg/L)	-	r=.404 p=.010**	r=-.042 p=.796
Leptin (pg/mL)	r=.404 p=.010**	-	r=-.357 p=.005**
LPS (EU/mL)	r=-.042 p=.796	r=-.357 p=.005**	-
BMI (kg/m^2)	r=.150 p=.073	r=.734 p<.001**	r=-.288 p=.025*
Waist (inches)	r=.179 p=.072	r=.603 p<.001**	r=-.493 p=.003**
Waist-to-hip ratio	r=.151 p=.129	r=-.023 p=.895	r=.082 p=.638
Lean body mass (%)	r=-.154 p=.066	r=-.836 p<.001**	r=.361 p=.005**
Fat mass (%)	r=.155 p=.065	r=.838 p<.001**	r=-.362 p=.004**
CD4 cell count (cells/μL)	r=-.083 p=.647	r=.005 p=.969	r=.015 p=.912
CD4%	r=-.144 p=.175	r=-.044 p=.740	r=-.134 p=.308
CD8 cell count (cells/μL)	r=.088 p=.294	r=-.090 p=.496	r=.157 p=.232
CD8%	r=.076 p=.365	r=-.240 p=.065	r=.253 p=.052
CD4/CD8 Ratio	r=-.104 p=.216	r=.096 p=.464	r=-.134 p=.308
HIV viral load (log_{10} copies/mL)	r=.092 p=.271	r=-.145 p=.273	r=.246 p=.060

[a]Completed in a subset of 60 participants
*P<0.05, **P<0.01

Table 3: Relationship Between BMI Groups and Measures of Inflammatory Markers at Baseline*

Inflammatory Markers	BMI (18-24.9 kg/m^2)	BMI >25kg/m^2	*P*-value
CRP (mg/L)[a]	1.41 (.70-4.15)	2.37 (1.20-5.89)	.028[c]
Leptin (pg/mL) [b]	5,687.05 (2,799.63-11,366.73)	22,894.90 (14,147.43-33,943.33)	<.001[c]
LPS (EU/mL)[b]	.068 (.061-.079)	.055 (.053-.058)	.001[c]

*Data reported as median (interquartile range)
[a]BMI group; BMI (18-24.9 kg/m^2) included 95 participants and BMI group >25kg/m^2 included 49
[b]BMI group; BMI (18-24.9 kg/m^2) included 30 participants and BMI group >25kg/m^2 included 30
[c]Statistically significant, *P*<.05

Table 4: Relationship Between CRP Groups and Body Composition and Measures of Inflammatory Markers at Baseline*

Variables	CRP<3 mg/L	CRP ≥3 mg/L	*P*-value
BMI (kg/m^2)	22.40 (20.50-25.90)	24.40 (20.70-28.95)	.094
Waist (inches)	31.79 (29.92-34.44)	33.86 (30.71-38.29)	.036[a]
Waist to hip ratio	.81 (.78-.88)	.83 (.80-.89)	.139
Lean mass (%)	71.70 (67.20-75.90)	69.25 (64.25-79.53)	.254
Fat mass (%)	28.30 (24.10-32.80)	30.75 (20.48-35.78)	.248
CD4 cell count (cells/μL)	419.05 (332.48-553.63)	401.30 (323.92-555.39)	.591
CD4 %	27.56 (22.02-31.56)	22.95 (18.42-29.85)	.013[a]
CD8 cell count (cells/μL)	773.28 (586.65-1163.89)	925.36 (712.48-1213.74)	.030[a]
CD8 %	48.98 (42.91-57.64)	54.67 (44.68-61.18)	.078
CD4/CD8 Ratio	.56 (.39-.71)	.41 (.29-.62)	.015[a]
HIV viral load (Log_{10} copies/mL)	4.14 (3.47-4.82)	4.57 (3.66-5.08)	.141

*Data reported as median (interquartile range)
[a]Statistically significant, *P*<.05

Table 5: Relationship Between Median Groups of Leptin and Body Composition and Measures of Inflammatory Markers at Baseline*

Variables	Leptin<13,304 pg/mL	Leptin ≥13,304 pg/mL	*P*-value
BMI (kg/m^2)	21.30 (20.00-23.60)	27.10 (25.60-31.10)	<.001[a]
Waist (inches)	30.51 (29.82-35.04)	36.24 (32.87-38.39)	.003[a]
Waist to hip ratio	.83 (.79-.90)	35.24 (32.87-38.39)	.804
Lean mass (%)	75.50 (69.85-80.40)	64.40 (62.40-66.00)	<.001[a]
Fat mass (%)	24.50 (19.60-29.75)	35.60 (32.00-37.60)	<.001[a]
CD4 cell count (cells/μL)	394.41 (318.82-478.95)	393.17 (316.36-543.87)	.842
CD4 %	24.74 (19.22-28.60)	23.60 (18.92-30.04)	.988
CD8 cell count (cells/μL)	941.90 (677.67-1255.15)	889.52 (693.89-1271.36)	.912
CD8 %	54.75 (47.93-61.33)	48.37 (41.06-61.06)	.108
CD4/CD8 Ratio	.44 (.32-.57)	.47 (.30-.75)	.589
HIV viral load (Log_{10} copies/mL)	4.31 (3.64-4.87)	4.06 (3.50-4.60)	.275

*Data reported as median (interquartile range)
[a]Statistically significant, *P*<.05

Table 6: Relationship Between Median Groups of LPS and Body Composition and Measures of Inflammatory Markers at Baseline*

Variables	LPS<.0582 EU/mL	LPS ≥.0582 EU/mL	*P*-value
BMI (kg/m^2)	26.70 (25.05-31.15)	22.40 (20.70-25.00)	.001[a]
Waist (inches)	36.32 (34.35-39.37)	31.50 (29.92-35.42)	.005[a]
Waist to hip ratio	.80 (.79-.87)	.82 (.78-.90)	.748
Lean mass (%)	64.70 (62.55-70.08)	71.90 (67.03-80.03)	.002[a]
Fat mass (%)	35.30 (29.93-37.45)	28.10 (19.98-32.98)	.002[a]
CD4 cell count (cells/μL)	424.03 (336.23-545.24)	392.09 (311.12-459.95)	.301
CD4 %	24.14 (19.16-30.34)	23.75 (18.49-28.42)	.433
CD8 cell count (cells/μL)	848.30 (688.51-1276.73)	961.51 (685.78-1253.53)	.412
CD8 %	48.13 (42.73-59.26)	54.81 (47.65-62.10)	.089
CD4/CD8 Ratio	.53 (.32-.67)	.44 (.31-.61)	.375
HIV viral load (Log_{10} copies/mL)	3.93 (3.15-4.46)	4.36 (3.92-4.82)	.023[a]

*Data reported as median (interquartile range)
[a]Statistically significant, P<.05

Table 7: Linear Regression at Baseline, 18 Months and the Change Over 18 Months by CRP*

	Baseline			18 Months			Change Over 18 Months		
Variable	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
BMI[b]	1.905	.281, 3.530	.022[d]	.646	-1.839, 3.131	.603	.949	-2.579, 4.477	.983
BMI > 25 kg/m^2	.398	-.143, 939	.148	.665	-.353, 1.683	.195	-	-	-
Fat mass%[a]	.020	-.009, .050	.177	.010	-.043, .062	.709	.014	-.030, .057	.536
Waist[b]	-.175	-2.316, 1.965	.871	2.491	-1.108, 6.090	.170	1.736	-.145, 3.617	.070
CD4 cell count[c]	-.002	-.057, .053	.947	.016	-.078, .111	.730	-.027	-.114, .060	.536
CD4%	-.013	-.041, .015	.368	-.016	-.066, .034	.520	.035	-.024, .093	.243
HIV viral load[b]	.084	-.151, .320	.480	.296	-.165, .756	.203	.103	-.226, .432	.620
HIV viral load ≥ 4log$_{10}$ copies/mL	.091	-.342, .523	.679	.709	-.111, 1.529	.089	-	-	-
CD8 cell count[c]	.008	-.019, .034	.565	.025	-.027, .077	.335	-.037	-.085, .012	.137
CD8%	.004	-.017, .024	.709	.008	-.029, .046	.650	-.046	-.098, .006	.085
CD4/CD8 Ratio	-.169	-.952, .613	.669	-.581	-2.080, .917	.438	.737	-1.214, 2.688	.455

*Models adjusted for age, gender, smoking, and fat mass percentage
[a]Model adjusted for age, gender, and smoking
[b]Log transformed
[c]Square root transformed
[d]Statistically significant, *P*<.05

Table 8: Linear Regression at Baseline, 18 Months, and the Change Over 18 Months by Leptin*

	Baseline			18 Months			Change Over 18 Months		
Variable	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
BMI[b]	1.764	.788, 2.739	.001[d]	2.104	1.133, 3.075	<.001[d]	.493	-1.463, 2.450	.615
BMI > 25 kg/m^2	.584	.260, .908	.001[d]	.864	.498, 1.230	<.001[d]	-	-	-
Fat mass%[a]	.112	.090, .135	<.001[d]	.043	.017, .069	.002[d]	-.016	-.057, .025	.437
Waist[b]	.394	-1.546, 2.333	.681	2.103	.806, 3.400	.002[d]	.247	-1.073, 1.567	.704
CD4 cell count[c]	.027	-.010, .065	.151	-.016	-.062, .030	.495	.026	-.036, .089	.402
CD4%	.006	-.014, .026	.551	-.003	-.028, .022	.816	.020	-.010, .049	.194
HIV viral load[b]	-.145	-.311, .022	.087	-.129	-.335, .078	.217	.107	-.177, .331	.344
HIV viral load ≥ 4log$_{10}$ copies/mL	-.305	-.579, -.031	.030[d]	-.173	-.541, .194	.348	-	-	-
CD8 cell count[c]	.005	-.014, .024	.599	-.008	-.031, .014	.459	-.010	-.040, .019	.493
CD8%	-.002	-.015, .010	.685	.001	-.017, .017	.990	-.019	-.044, .006	.140
CD4/CD8 Ratio	.156	-.429, .740	.595	-.117	-.950, .716	.780	.509	-.451, 1.470	.292

*Models adjusted for age, gender, smoking, and fat mass percentage
[a]Model adjusted for age, gender, and smoking
[b]Log transformed
[c]Square root transformed
[d]Statistically significant, *P*<.05

Table 9: Linear Regression at Baseline, 18 Months, and the Change Over 18 Months by LPS*

	Baseline			18 Months			Change Over 18 Months		
Variable	β	95% CI	*P*-value	β	95% CI	*P*-value	β	95% CI	*P*-value
BMI[b]	-.483	-1.208, .243	.188	-.023	-.399. .354	.904	-2.979	-1.771, .822	.466
BMI < 25 kg/m^2	-.273	-.506, -.040	.022[d]	-.017	-.160, .127	.818	-	-	-
Fat mass%[a]	.001	-.014, .016	.892	.001	-.008, .009	.950	.004	-.013, .020	.673
Waist[b]	-1.940	-3.397, -.483	.011[d]	-.005	-.477, .467	.982	.051	-1.088, 1.190	.927
CD4 cell count[c]	.009	-.017, .034	.509	-.004	-.019, .011	.569	.003	-.038, .045	.876
CD4%	.003	-.011, .017	.648	-.006	-.014, .002	.165	-.010	-.030, .010	.319
HIV viral load[b]	.054	-.059, .168	.337	.047	-.020, .113	.168	.012	-.135, .159	.869
HIV viral load ≥ 4log$_{10}$ copies/mL	.121	-.067, .309	.201	.064	-.056, .183	.289	-	-	-
CD8 cell count[c]	.003	-.010, .016	.660	.004	-.003, .011	.290	.006	-.013, .026	.512
CD8%	.003	-.005, .011	.432	.001	-.004, .007	.643	-.002	-.019, .015	.809
CD4/CD8 Ratio	.019	-.377, .415	.924	-.193	-.458, .072	.150	-.115	-.757, .526	.719

*Models adjusted for age, gender, smoking, and fat mass percentage
[a]Model adjusted for age, gender, and smoking
[b]Log transformed
[c]Square root transformed
[d]Statitically significant, *P*<.05

Table 10: Logistic Regression at Baseline, 18 Months, and Change Over 18 Months by CRP Groups (< and ≥ 3 mg/L)*

	Baseline			18 Months			Change Over 18 Months		
Variable	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
BMI[b]	60.960	2.784, 1334.723	.009[d]	8.685	.550, 137.236	.125	1.012	.002, 213.705	.599
BMI ≥ 25 kg/m^2	2.407	.941, 6.156	.067	1.719	.623, 4.746	.296	-	-	-
Fat mass%[a]	1.023	.973, 1.076	.366	1.010	.960, 1.063	.697	1.006	.935, 1.083	.868
Waist[b]	.223	.006, 7.968	.411	98.811	1.697, 5755.086	.027[d]	65.078	1.490, 2842.157	.030[d]
CD4 cell count[c]	.971	.881, 1.071	.560	.976	.874, 1.090	.976	.909	.786, 1.052	.201
CD4%	.949	.902, .998	.042[d]	.990	.937, 1.047	.732	1.066	.965, 1.179	.209
HIV viral load[b]	1.289	.851, 1.951	.231	1.231	.785, 1.999	.400	1.100	.630	
HIV viral load ≥ 4log10 copies/mL	1.568	.732, 3.359	.247	1.681	.652, 4.334	.282	-	-	-
CD8 cell count[c]	1.030	.984, 1.078	.207	.993	.935, 1.054	.811	.906	.831, .988	.025[d]
CD8%	1.023	.988, 1.060	.203	1.010	.970, 1.052	.633	.933	.852, 1.022	.137
CD4/CD8 Ratio	.292	.063, 1.363	.117	.621	.102, 3.787	.605	3.544	.142, 88.638	.441

*Models adjusted for age, gender, smoking, and fat mass percentage
[a]Model adjusted for age, gender, and smoking
[b]Log transformed
[c]Square root transformed
[d]Statistically significant, *P*<.05

Table 11: Logistic Regression at Baseline, 18 Months, and the Change Over 18 Months by Leptin Groups (< and ≥ Median)*

	Baseline			18 Months			Change Over 18 Months		
Variable	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
BMI	2.073	1.194, 3.601	.010[d]	1.554	1.125, 2.146	.008[d]	1.438	.819, 2.524	.206
BMI ≥ 25 kg/m^2	58.572	2.187, 1568.688	.015[d]	79.634	4.607, 1376.659	.003[d]	-	-	-
Fat mass%[a]	1.580	1.214, 2.055	.001[d]	1.231	1.070, 1.416	.004[d]	.985	.887, 1.093	.775
Waist	<.001	<.001,<.001	.998	1.267	1.016, 1.580	.036[d]	.967	.000, -	1.000
CD4 cell count[b]	1.074	.833, 1.385	.581	1.168	.864, 1.581	.313	1.279	.837, 1.956	.256
CD4%	1.022	.902, 1.157	.737	1.110	.971, 1.270	.127	1.456	1.025, 2.069	.036[d]
HIV viral load[c]	.654	.231, 1.850	.424	.615	.209, 1.812	.378	.855	.186, 3.928	.840
HIV viral load ≥ 4log10 copies/mL	.308	.039, 2.408	.261	.180	.018, 1.816	.146	-	-	-
CD8 cell count[b]	1.013	.878, 1.169	.861	.960	.848, 1.088	.522	.872	.698, 1.089	.228
CD8%	.982	.913, 1.057	.628	.961	.886, 1.042	.338	.867	.709, 1.061	.165
CD4/CD8 Ratio	5.405	.124, 235.292	.381	73.701	.393, 13808.169	.107	133.567	.116, 154108.571	.174

*Models adjusted for age, smoking, and fat mass percentage
[a]Model adjusted for age and smoking
[b]Square root transformed
[c]Log transformed
[d]Statistically significant, *P*<.05

Table 12: Logistic Regression at Baseline, 18 Months, and the Change Over 18 Months by LPS Groups (< and ≥ Median)*

	Baseline			18 Months			Change Over 18 Months		
Variable	OR	95% CI	*P*-value	OR	95% CI	*P*-value	OR	95% CI	*P*-value
BMI	.790	.630, .990	.041[d]	.799	.655, .976	.028[d]	.875	.603, 1.270	.482
BMI ≥ 25 kg/m^2	.035	.004, .283	.002[d]	.321	.076, 1.351	.121	-	-	-
Fat mass%[a]	.852	.757, .958	.007[d]	.932	.854, 1.017	.113	1.046	.944, 1.158	.391
Waist	.727	.446, 1.187	.203	.907	.778, 1.056	.209	.795	.555, 1.140	.212
CD4 cell count[b]	.915	.763, 1.098	.339	.915	.763, 1.098	.339	1.092	.825, 1.444	.538
CD4%	.971	.888, 1.061	.971	.942	.861, 1.029	.185	.905	.784, 1.045	.173
HIV viral load[c]	2.608	1.111, 6.124	.028[d]	1.691	.782, 3.656	.182	.637	.247, 1.638	.349
HIV viral load ≥ 4log10 copies/mL	8.005	1.762, 36.357	.007[d]	3.776	.959, 14.869	.057	-	-	-
CD8 cell count[b]	1.003	.925, 1.087	.941	1.038	.956, 1.126	.376	1.124	.982, 1.266	.089
CD8%	1.025	.972, 1.081	.362	1.038	.977, 1.103	.228	1.061	.941, 1.197	.330
CD4/CD8 Ratio	.630	.051, 7.857	.720	.120	.005, 2.860	.190	.032	.000, 3.912	.161

*Models adjusted for age, gender, smoking, and fat mass percentage
[a]Model adjusted for age, gender, and smoking
[b]Square root transformed
[c]Log transformed
[d]Statistically significant, *P*<.05

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CHAPTER VI: SUMMARY AND CONCLUSIONS

This study investigated the obesity paradox in HIV+ ART naïve adults who were in early stage of HIV disease in Botswana. The impact of obesity on HIV disease progression over time was explored. An obesity paradox has been documented in many conditions including HIV, where those who are obese may have a survival advantage or improved disease outcomes.[1] The genetic propensity for being overweight or obese was examined using SNPs of the *FTO* gene, which is reported to be most associated with BMI, and its association to HIV disease progression. In addition, inflammatory markers and their relationship to body composition and HIV disease progression were investigated.

Studies on HIV and obesity are mainly conducted in settings where the patients are treated with ART, which may confound some of the findings and interpretations, as ART has been associated with lipodystrophy and obesity.[2] Rising rates of obesity have been documented in countries with limited resources;[3] however, studies conducted in African settings on the relationship between HIV and obesity are lacking. Studies conducted to investigate obesity and its effect on HIV disease progression have so far been inconclusive, with some studies reporting an association and others reporting no association between CD4 cell counts and obesity.[1, 4-7] Studies on the relationship between BMI and body composition on HIV disease progression are needed to reveal possible mechanisms for the obesity paradox in HIV. The examination of such interrelationships is important for translation of results into clinically relevant recommendations for people living with HIV.

Our results demonstrated a protective effect of BMI and fat mass in delaying the time to the first AIDS defining condition in HIV+ ART naïve adults in Botswana during 18 months of follow-up. Higher BMI was also associated with a higher CD4%, another

marker of HIV disease progression over 18 months. In addition, higher fat mass was also associated with the combined outcome of CD4 cell count ≤ 250/μL and AIDS defining conditions. Survival analysis showed a positive trend with the outcome of a decline in the CD4 cell count of 25% or greater and association with fat mass and negative association with lean body mass. Findings from these analyses indicate that higher fat may provide protection from advanced HIV disease. The fat stores would have provided the extra energy needed to maintain the innate immune system responding to protect against opportunistic infections and delaying wasting. Extra available energy for use in times of emergency during the HIV disease may reduce the use of protein for energy[8] and also assist to conserve the immune system, especially the innate immunity that is not directly affected by the infection.[9,10]

This study provides preliminary data on the genetic association between *FTO* SNPs on the genetic risk of being overweight/obese and HIV disease progression in HIV+ adults in Botswana. There were no significant findings between the *FTO* SNPs and markers of HIV disease progression; however, a trend between the risk allele for rs8050136 was protective of having higher HIV viral load. The variant rs17817449 had a significant association with BMI, showing a greater odds of having higher BMI when the risk allele was present. The risk alleles for rs1121980, rs8050136, and rs17817449 were associated with fat mass and lean mass. A higher percentage of fat mass and lower lean body mass were associated with these risk alleles compared to homozygosity for the non-risk allele. Although the exact mechanism for the effect of *FTO* on weight is unknown, it is hypothesized that it may affect food intake and energy expenditure.[11] *FTO* is highly expressed in the hypothalamus region of the brain, where food intake is controlled.[12,13] The *FTO* still remains the gene most consistently associated with obesity in various populations,[14] but its associations have not been replicated in many African

populations. GWAS studies in African populations are lacking, and identifying SNPs that are relevant to this population is of importance since the SNPs showing associations in European and Asian populations may be different from SNPs that will show associations in African populations.

The relationship of inflammatory markers such as C-reactive protein, leptin, and bacterial endotoxin lipopolysaccharide (LPS) with body composition and HIV disease progression in HIV+ asymptomatic adults not on ART in Botswana over 18 months was also evaluated. To our knowledge, no study has assessed leptin or LPS in a population from Botswana with or without HIV infection. The results from this study suggest a strong association of inflammation markers with body composition and HIV disease progression in ART naïve adult participants in Botswana. Higher levels of inflammation as measured was associated with a more progressed state of the disease. The relationships were found to be independent of body fat. Our findings support the hypotheses in the literature that high viral replication and immune activation could result in chronic inflammation that would cause lipoatrophy, therefore, lowering leptin levels.[15] LPS levels above the median were associated with higher viral load and those with LPS levels ≥ the median were 8 times more likely to have viral load > $4\log_{10}$ copies/mL or 10,000 copies/mL. In addition, LPS was also inversely associated with measures of body composition, therefore being overweight or obese was protective of having higher levels of LPS. The endotoxin lipoprotein hypothesis or theory may provide a possible explanation for the lower levels of LPS observed in those with higher BMI. Higher circulating lipoproteins may modulate inflammation by binding to lipopolyssachrides and reduce their effect on release of proinflammatory cytokines.[16]

Current study demonstrated that an obesity paradox may be present in early HIV infection. We investigated and suggest several mechanisms for this paradox. Fat

accumulation was found to be protective against further HIV disease progression in HIV+ adults who were early in the disease state and ART naïve. Higher levels of leptin and lower levels of LPS were associated with being overweight/obese and higher amounts of body fat mass percentage. Concurrently, lower leptin and higher LPS levels had significant relationships to higher HIV viral load, a marker of HIV disease progression. Additionally, a trend association was demonstrated with one of the *FTO* SNPs and HIV viral load. The risk allele was associated with lower HIV viral load, therefore having a protective effect. Establishment of mechanisms for this paradox are needed in order to allow for translation of results into meaningful clinical recommendations and care guidelines for resource limited settings.

Table 1: Study Hypotheses and Results

Hypothesis #	Hypothesis	Study Results
1a	(Primary outcome) The overweight/obese group will present less clinically significant CD4 cell count decline from Baseline (≥ 25%) over 18 months than the normal weight group.	There were no significant differences between BMI groups in the main outcome ≥ 25% decline in CD4 cell count over 18 months (HR= .744; 95% CI= .489, 1.131; *P*=.166) after controlling for age, gender, marital status children (yes/no), baseline CD4 cell count and baseline HIV viral load.
1b	The overweight/obese will have significantly lower levels of HIV viral load over 18 months than the normal weight group.	BMI groups at baseline, 18 months and the change over 18 months were not statistically associated with HIV viral load.
1c	Significantly less AIDS defining conditions will occur over 18 months in the overweight/obese group compared to the normal weight group.	There were no significant differences between BMI groups in the outcome of AIDS defining conditions (HR= .500; 95% CI= .047, 4.465; P=.500) after controlling for age, gender, marital status, children (yes/no), baseline CD4 cell count and baseline HIV viral load. However, continuous BMI was associated with AIDS defining conditions (HR=.218, 95% CI=.068, .701, *P*=.011).
2a	The FTO gene SNPs will be associated with being overweight/obese.	The *FTO* SNP rs17817449 was associated with BMI (OR=1.082; 95%CI= 1.001, 1.169; *P*=.047).
2b1	The FTO gene SNPs will be associated with significantly higher CD4 cell count.	There were no significant associations with between *FTO* SNPs and CD4 cell count.
2b2	The FTO gene SNPs will be associated with significantly lower HIV viral load.	None of the *FTO* SNPs were significantly associated with HIV viral load. However, there was a trend towards significance with rs8050136 and HIV viral load in a dominant model (OR=.705; 95% CI=.481, 1.035; *P*=.074) after controlling for age, gender, tribes, total dietary kcal, and activity.
2b3	The *FTO* gene SNPs will be associated with significantly less AIDS-defining conditions.	There were no significant associations between *FTO* SNPs and AIDS-defining conditions.
3a1	Higher levels of inflammatory markers will be	There were no significant associations with between

	associated with lower levels of CD4 cell count.	inflammatory markers and CD4 cell count.
3a2	Higher levels of inflammatory markers will be associated with higher HIV viral load.	Leptin was associated with HIV viral load >4 log_{10} copies/mL (OR=-.305; 95%CI=-.579, -.031; *P*=.030) at baseline. LPS above the median was associated with HIV viral load (OR=2.608, 95% CI=1.111, 6.124, *P*=.028) and HIV viral load >4 log_{10} copies/mL (OR=-8.005; 95%CI= 1.762, 36.357; *P*=.007) at baseline and HIV viral load >4 log_{10} copies/mL (OR=-3.776; 95%CI= .959, 14.869; P=.057) at 18 months.
3a3	Higher levels of inflammatory markers will be associated with greater AIDS-defining conditions.	There were no significant associations with between inflammatory markers and AIDS-defining conditions.

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CHAPTER VII: STRENGTHS AND LIMITATIONS

The results from this study could be translated into clinically relevant nutrition and care recommendations for HIV+ adults who are antiretroviral (ART) naïve in limited resource settings. This is the first study to examine the effect of the genetic propensity to become overweight/obese and inflammatory markers on HIV disease progression in an HIV+ population in Botswana early in their disease. Both inflammatory markers, leptin and LPS, have not previously been investigated in Botswana, with or without HIV. Additionally, the *FTO* gene has never been genotyped in an HIV -infected population and its association to HIV disease progression examined.

The strengths of this study consists of capturing and analyzing longitudinal data from HIV+ participants in Botswana, that was collected from the early asymptomatic stage through later stages with well-defined health outcomes. This type of information is lacking in African populations. Considering that most of the HIV+ cases are within sub Saharan Africa,[1] more research in this area is warranted. This was also the first known study to assess the role of *FTO* in the obesity paradox that often occurs in HIV+ adults. Trends were noted in the relationship of some of the *FTO* SNPs with markers of HIV disease progression, which may suggest a role in the obesity paradox and needs further investigation in a larger cohort with adequate sample size.

Sample Size

The limitations of this study may have contributed to some of the null findings observed. The sample size was insufficient to be able to examine differences between overweight and obese groups separately. A larger sample size would have allowed for the examination of the relationship of the *FTO* SNPs and body mass in further stratified subgroups of overweight and obese separately. BMI in the range of underweight category was not studied since underweight individuals were excluded from participation

in the parent study, as those ranges are strong prognostic of AIDS.[2] Inadequate sample size may have also contributed to find non-significant associations between inflammatory markers and CD4 cell count. In addition, there was a predominance of women among the participants. Botswana is known to be a heterogeneous population with many ethnic groups.[3] The heterogeneity present in this population may also be a limitation for this study.

Study Design

Study limitations also include a weaker study design, which was retrospective and observational. The exact time since HIV diagnosis or infection were not available; however, it was known that the participants were asymptomatic, never had a diagnosis of AIDS and did not qualify for ART. The majority were referred from the clinics where they were diagnosed. This HIV population was in the early stage of the disease and its finding cannot be generalized to all HIV patients, and may be one of the reasons why we did not see a significant decline of CD4 as a main outcome. Findings from this study cannot be generalized to other HIV+ populations, including ART-treated patients, but may be used in resource limited settings were ART is being slowly rolled out.

Methods

In HIV, bioimpedance (BIA) has been shown to be an effective method for detecting fat mass; however, BIA have limitations, since its values can be influenced by body arrangement, hydration, ingestion of food and drinks, air and body temperature and exercise.[4] Our BIA measurement protocol minimized the potential for variability by providing detailed instructions to the participants on their preparation for their visit, and standardizing the measurement parameters.

Selection bias may have contributed to null findings since we chose to complete leptin and LPS in a subset that had samples available at baseline and 18 months.

Participants who had both samples were those who maintained their CD4 cell counts above the cut-off for eligibility for ART and were still followed by the parent study. Therefore, those excluded may have been rapid progressors, with different genetic makeup. Interestingly, despite these limitations, we had findings with CD4% and CRP and leptin and LPS with viral load.

LPS measured by the Limulus amebocyte lysate (LAL) method is the most widely used method; however, it may be affected by inhibition or interference of other compounds.[5] To prevent interference, dilution and heating methods were incorporated into the LPS assay methodology for this study. The LAL method for measuring LPS is currently only recommended for research purposes and not for a clinical setting.

The obesity paradox is present in the literature in a variety of diseases that include HIV.[6] Controversies exist on both sides of the dispute on whether this paradox is real, a query that has implications for HIV management and public health efforts. Equipoise in the literature regarding obesity paradoxes should drive the need for more research to provide the appropriate care and advice for patients. The results from this study may be used as preliminary data for future research in this topic.

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CHAPTER VIII: FUTURE RESEARCH

The results from this study demonstrated that fat may provide protection against further HIV disease progression in HIV+ adults who are early in the disease and ART naïve. Higher levels of leptin and lower levels of LPS were associated with being overweight/obese and higher amounts of body fat mass percentage. At the same time, higher leptin and lower LPS levels had significant relationships to higher HIV viral load, a marker of HIV disease progression. There was a trend association with one of the *FTO* SNPs and HIV viral load. However, genetic association studies generally require large sample sizes.[1] It seems there is an obesity paradox present in the HIV+ ART naïve cohort we investigated. An obesity paradox is present in several diseases and conditions[2] and mechanistic studies are needed in this area of obesity and HIV to establish the nature and cause for this paradox in order to develop clinical recommendations. The preliminary results from the analyses conducted with *FTO* SNPs, inflammatory markers, and measures of HIV disease progression will be useful to calculate sample size for future studies. Further examination of the mechanisms and best approaches for translation of findings into recommendations into care of people living with HIV are needed.

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