

Insulin Resistance is Associated with Higher Plasma Viral Load Among HIV-Positive Adults Receiving Longer-Term (1 Year) Combination Antiretroviral Therapy (ART)

LB Mulenga^{1,2,3,4,9*}, P Musonda¹, L Chirwa², M Siwingwa^{1,2}, A Mweemba^{1,2}, S Suwilanji^{1,2}, S Fwoloshi^{1,2}, H Phiri³, D Phiri³, PL Mulenga³, T Chisenga³, R Nsakanya³, A Shibemba^{1,2,3}, J Todd⁵, S Nzala¹, T Kaile¹, C Kankasa^{1,2}, L Hachaambwa^{1,2,6}, C Claassen^{1,2,6}, I Sikazwe^{1,8}, JR Koethe^{4,9}, E Sinkala^{1,2}, DC Heimburger⁴ and CW Wester^{4,9}

¹University of Zambia, School of Medicine, Division of Infectious Diseases, Internal Medicine, Lusaka, Zambia

²University Teaching Hospital, Adult Infectious Diseases Center, Lusaka, Zambia

³Ministry of Health, Ndeke House, Lusaka, Zambia

⁴Vanderbilt University Medical Center (VUMC), Department of Medicine, Division of Infectious Diseases, Nashville, TN, USA

⁵London School of Hygiene and Tropical Medicine, London, United Kingdom

⁶University of Maryland, Baltimore, MD, USA

⁷University of Alabama at Birmingham, Birmingham, AL, USA

⁸Centre for Infectious Diseases Research, Lusaka, Zambia

⁹Vanderbilt Institute for Global Health (VIGH), Nashville, TN, USA

*Corresponding author: Dr Lloyd Mulenga, Division of Infectious Diseases, Internal Medicine, School of Medicine, University of Zambia, Lusaka, Zambia, E-mail: lbmulenga@yahoo.com

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Abstract

Background: As HIV-positive persons survive longer due to the success of combination antiretroviral therapy (ART) in decreasing mortality, the burden of non-communicable diseases including diabetes mellitus (DM) is anticipated to rise. HIV is characterized by systemic inflammations, markers of which decrease quickly following ART initiation, but typically do not completely normalize. Inflammation may be accompanied by insulin resistance (IR), and both are implicated in the pathogenesis of DM in HIV-positive individuals. Sub-Saharan Africa accounts for almost two-thirds of the global HIV burden but there are few reports of IR, DM and HIV in this region. We assessed the relationship between IR and viral suppression among HIV-positive adults in the Zambian national ART program.

Methods: We conducted a cross-sectional survey evaluating HIV-positive adults that had received first line ART (usually TDF/FTC/EFV) for 12 months (\pm 3 months). Twenty clinics were sampled systematically based on the random starting-point, sampling interval and cumulative population size. Eligible patients had plasma viral load (VL), fasting insulin, and glucose performed. Insulin resistance was determined using Homeostatic model assessment (HOMA). We determined proportions for each outcome using linearized standard error 95% confidence intervals and summary estimates. Viral suppression was defined according to the detection threshold of <20 copies/mL and treatment failure was defined as $VL > 1,000$ copies/mL.

Results: Of 473 patients enrolled, 46.8% were male and 53.2% were female. 142 (30%) [95% CI: 0.26-0.34] had IR. Among those with IR, 55 (38.7%) were male whereas 87 (61.3%) were female (p value=0.104). 19% of individuals with IR had treatment failure compared to 5.7% without IR (p value <0.0001). 427 (90.3%) participants had treatment success ($VL < 1,000$ copies/mL), and this was associated with a lower likelihood of IR (odds ratio (OR)=0.26 [0.14, 0.48], p value <0.0001). In addition, a significantly lower proportion of patients with IR were virologically suppressed at one-year compared to individuals without IR, 58% [0.54-0.70] versus 70% [0.65-0.75], respectively (p value=0.042).

Conclusion: In Zambian adults on ART for a year, the development of insulin resistance was strongly associated with suboptimal HIV outcomes, specifically non-viral suppression and treatment failure. Further investigations are warranted to determine if this positive association between IR and VL is causally related, and if so in which direction.

Keywords: Insulin resistance; Combination antiretroviral therapy; Viral suppression; Treatment failure; Zambia

Introduction

HIV continues to be a major public health concern around the globe especially within sub-Saharan Africa where an estimated 25.6 million of the global 36.7 million people living with HIV reside [1]. Over the past 10+ years, there has been an unprecedented scale-up in the provision of combination antiretroviral therapy (ART) to persons

living with HIV; including the disproportionately affected region of sub-Saharan Africa.

This unprecedented scale-up of ART has been a major contributing factor to the 48% decline in AIDS-related deaths witnessed between 2005 and 2016 (UNAIDS 2017). However, as life expectancy increases, HIV-positive individuals are increasingly experiencing co-morbid medical conditions; which are age-related [2,3]. These age-related co-morbid medical conditions are anticipated to rise dramatically over the next 20 years, particularly in Sub-Saharan Africa where communicable diseases are being surpassed by non-communicable diseases (NCDs) as causes of premature mortality [2-10]. Furthermore, these NCDs due to their intersection with HIV, if not controlled in resource-constrained settings of the world, would have not only individual health consequences but also significant deleterious economic implications [7,9].

Diabetes mellitus is one of the most prevalent NCDs globally and the number of individuals developing diabetes mellitus in Africa is anticipated to increase substantially by 2030 [11,12]. Data from the USA and Europe shows that HIV-positive, ART-treated men have a higher incidence of DM when compared to match HIV-negative counterparts [13-15]. A higher prevalence of DM (18%) has also been reported among HIV-positive, ART-naïve adults in Tanzania, compared to HIV-negative adults (5.2%) residing in the same locality.

HIV infection itself may be associated with DM as HIV-positive individuals can develop metabolic syndrome, altered glucose metabolism, dyslipidaemias and lipodystrophy[16]. Furthermore, exposure to ART including; protease inhibitors (PIs) [e.g. indinavir (IDV), lopinavir (LPV)], and some nucleoside reverse transcriptase inhibitors (NRTIs) [e.g. zidovudine (AZT), stavudine (d4T), didanosine (ddI)] may also be contributing factors[17]. Other studies have implicated ART via autoimmune mediated immune reconstitution as an indirect cause of DM [18-20].

Insulin resistance (IR), rather than insulin deficiency is thought to be the main driver of DM among HIV-positive adults, a mechanism also observed in persistent Hepatitis C virus (HCV) infection associated DM [21,22]. In HIV-positive adults, IR may occur independently of ART exposure, but is likely due to persistent immune activation/inflammation [21-24].

Understanding the burden of IR among HIV-positive individuals, and its associated immunologic and virologic factors is critically important within sub-Saharan Africa, where the vast majority of HIV-positive individuals on ART reside. Such an understanding of these adverse metabolic effects including insulin resistance, dyslipidemia, and inflammation would provide invaluable information informing the design of effective interventions to prevent the development of diabetes mellitus in this aging “at risk” population. In this study, we assessed the relationship between IR and viral suppression among HIV-positive adults on ART in the Zambian HIV program.

Materials and Methods

Methodology

We conducted a cross-sectional study evaluating HIV-positive adults that had received first line ART for twelve (12) months with a margin of ± 3 months added to the time point to improve feasibility. A two-stage cluster design was employed with i) 20 clinics being randomly sampled from all clinics dispensing ART in the country and ii) patients being enrolled if they were on ART for 12 months.

In brief, sampling of clinics was performed using systematic sampling to generate probability proportional to proxy size samples. The probability proportional to size (PPS) sampling was used in order to have patients in large sites have the same probability of being selected as patients in smaller sites, and vice versa.

Eligible patients on ART for 12 months were recruited from each of the selected clinics and had fasting blood specimens obtained for CD4 cell count, plasma HIV-1 RNA (viral load), high sensitivity C-Reactive Protein (hsCRP), Tumor Necrosis Factor-Alpha (TNF- α), insulin, glucose, electrolytes including kidney function (chemistry), serum lipids and Complete Blood Count (CBC) testing. Specimens having plasma VL levels $\geq 1,000$ copies/mL were genotyped to determine HIV drug resistance (HIVDR) status.

The collected blood was delivered to the University Teaching Hospital (UTH) laboratory for VL, hsCRP, TNF- α , Insulin, chemistry and serum lipid testing. CD4 cell count, glucose and complete blood counts (CBC) were performed at the local testing labs. No dried blood spots were collected due to lack of expertise to analyze these samples.

Viral loads were measured by polymerase chain reaction (PCR) using the COBAS[®] AmpliPrep/TaqMan[®] HIV-1 Qualitative Test, version 2.0 (TaqMan 96) assay. Virologic definitions included the following: i) viral suppression [10] was defined as having a VL < 20 copies/mL; ii) non-viral suppression (NVS) defined as having a VL value ≥ 20 copies/mL; iii) treatment failure (TF) as having a VL value $\geq 1,000$ copies/mL; and iv) treatment success (TS) being defined as having a VL < 1,000 copies/mL.

Insulin was measured using the human insulin (Hu Insulin[™]) ELISA Kit and IR was measured using the Homeostasis Model Assessment of IR (HOMA-IR) test, a test widely accepted as a surrogate of IR in numerous populations[25,26]. IR was defined as having a HOMA ≥ 2 as reported best HOMA-IR cut-off levels range from 1.85 to 2.07. The Biosource Sensitivity Immunoassay (EASIA)[™] for TNF- α was used and performed on a microtiter plate [27].

All analyses were performed using Stata Statistical Software: Release 15. College Station, TX (StataCorp LLC) to determine proportions for each outcome including linearized standard error 95% confidence intervals and summary estimates. Associations of insulin resistance, inflammatory markers, and treatment failure were measured using the chi-square or fishers exact test. A multivariable logistic regression analysis was used to predict factors associated with insulin resistance.

Results

Four hundred and seventy-three (473) individuals were recruited in the study from all twenty facilities of which 46.8% were male and 53.2% were female. The study design-weighted mean time on ART was 12.2 months (95% CI: 11.8-12.6).

Thirty percent (30%), (95% CI: 26%-34%) of individuals had IR and there was no age difference between patients with and without IR. Patients with IR had increased waist circumference, waist-to-hip circumference ratio and increased visceral fat (Table 1). Other characteristics that varied between the two groups were fasting glucose, viral load, BMI, HDL cholesterol, LDL cholesterol, triglycerides, hsCRP and TNF- α levels/values (Table 2).

Characteristics	Insulin Resistance		p value
	No N=331 (70%)	Yes N=142 (30%)	
Continuous variables (Median, IQR)			
Age (years)	37 (32,44)	36 (29,44)	0.064W
Waist circumference (cm)	81 (74,88)	87 (78.5,94)	<0.0001W
Hip circumference (cm)	94 (88,100)	90 (79,97)	0.0002W
Waist to Hip circumference ratio	0.85 (0.8,0.92)	0.99 (0.88,1.10)	<0.0001W
Visceral Fat Level	5 (3,8)	11 (4,14)	<0.0001W
CD4+ cell count (cells/ μ L)	346 (221,547)	391 (264,593)	0.097W
Hemoglobin (g/dl)	13.0 (11.8,14.2)	12.8 (11.5, 14.3)	0.349W
Fasting Blood Glucose (mmol/l)	5.40 (5.00, 6.00)	5.90 (5.40, 6.50)	<0.0001W
Viral load (copies/mL)	20 (20,38)	20 (20,176)	0.011W
Body Mass Index (kg/m ²)	21.8 (19.7,24.7)	22.7 (20.2,25.6)	0.046W
Total Cholesterol (mmol/l)	4.2 (3.49,4.80)	4.42 (3.64,5.19)	0.085W
High Density Lipoprotein (mmol/l)	1.99 (1.32,2.51)	1.00 (0.74,1.43)	<0.0001W
Low Density Lipoprotein (mmol/l)	1.90 (1.30,2.60)	2.24 (1.71,2.92)	0.0003W
Triglycerides (mmol/l)	0.95 (0.58,1.67)	2.16 (0.94,2.99)	<0.0001W
C-reactive protein (hsCRP) (mg/L)	0.79 (0.47,1.58)	2.38 (1.40,3.49)	<0.0001W
Tumor necrosis factor alpha (TNF- α)(pg/mL)	21.7 (14.9, 29.9)	33.9 (27.5,42.2)	<0.0001W
Insulin (mIU/L)	4.64 (2.81, 6.50)	34.5 (22.8,32.2)	<0.0001W
Categorical variables			
Type of facility			
Health center	196 (59.2)	85 (59.9)	0.490C
Hospital	135 (40.8)	57 (40.1)	
Sex			
Male	155 (46.8)	55 (38.7)	0.104C
Female	176 (53.4)	87 (61.3)	
Marital status (N=462)			
Single	43 (13.2)	20 (14.6)	0.033E
Cohabiting	7 (2.2)	2 (1.5)	
Married	204 (62.8)	77 (56.2)	
Divorced	25 (7.7)	24 (17.5)	
Widowed	46 (14.2)	14 (10.2)	
Employment status			
Unemployed	189 (57.1)	90 (63.4)	0.203C
Employed	142 (42.9)	52 (36.6)	
Treatment Supporter available			
No	50 (15.1)	10 (7.0)	0.016C
Yes	281 (84.9)	132 (93.0)	

WHO clinical stage	294 (92.5)	128 (94.1)	0.708E
1	17 (5.4)	6 (4.4)	
2	6 (1.9)	1 (0.7)	
3	1 (0.3)	1 (0.7)	
4			
ART Regimen	5 (1.5)	1 (0.7)	0.899C
ABC/3TC/EFV	1 (0.3)	0 (Stein Schalkwijk)	
TDF/XTC/ATV	323 (97.6)	140 (98.6)	
TDF/XTC/EFV	2 (0.6)	1 (0.7)	
TDF/XTC/NVP			
Hepatitis B surface antigen	317 (95.8)	138 (97.2)	0.462C
Negative	14 (4.2)	4 (2.8)	
Positive			
Rapid Plasma Reagin (Syphilis)	301 (90.9)	126 (88.7)	0.458C
Negative	30 (9.1)	16 (11.3)	
Positive			
Non-Viral Suppression (NVS) (≥ 20 copies/mL HIV RNA)	232 (70.1)	82 (58.0)	0.042C
No	99 (29.9)	54 (42.0)	
Yes			
W: Wilcoxon/Mann-Whitney Test Rank-Sum Test; C: Chi Squared Test; E: Fisher's Exact Test			

Table 1: Characteristics of individuals with or without insulin resistance

Covariates	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Continuous variables				
Age (years)	0.98 (0.96, 1.00)	0.053	0.67 (0.86, 1.12)	0.262
Waist circumference (cm)	1.05 (1.03, 1.07)	<0.0001	1.11 (0.72, 1.70)	0.639
Hip circumference (cm)	0.97 (0.95, 0.98)	<0.0001	0.81 (0.50, 1.32)	0.401
Waist-to-Hip circumference ratio	246.4 (54.7, 1108.9)	<0.0001	NB	NB
Visceral Fat Levels (IU)	1.14 (1.09, 1.19)	<0.0001	0.88 (0.59, 1.31)	0.536
CD4 cell count (cells/ μ L)	1.00 (0.99, 1.00)	0.096	1.00 (0.99, 1.00)	0.312
Haemoglobin (g/dl)	0.94 (0.83, 1.05)	0.273	1.39 (0.68, 2.82)	0.365
Fasting Blood Glucose (mmol/l)	1.32 (1.11, 1.59)	0.002	2.76 (1.15, 6.59)	0.022
Viral load (copies/mL)	1.00 (0.99, 1.00)	0.074	1.00 (0.99, 1.00)	0.600
Body Mass Index (kg/ m ²)	1.04 (0.99, 1.09)	0.087	1.02 (0.54, 1.93)	0.945
Total Cholesterol (mmol/l)	1.01 (0.94, 1.09)	0.729	0.96 (0.32, 2.87)	0.940
High Density Lipoprotein (mmol/l)	0.33 (0.25, 0.44)	<0.0001	2.42 (0.40, 14.7)	0.336
Low Density Lipoprotein (mmol/l)	1.29 (1.07, 1.56)	0.008	1.92 (0.26, 14.1)	0.520
Triglycerides (mmol/l)	1.15 (1.06, 1.25)	0.002	1.17 (0.74, 1.86)	0.493

C-reactive protein (hsCRP)(mg/L)	1.55 (1.36, 1.77)	<0.0001	0.95 (0.49, 1.84)	0.875
Tumor necrosis factor alpha (TNF-α)(ng/mL)	1.00 (0.99, 1.00)	0.193	1.03 (0.91, 1.17)	0.635
Type of facility	ref	0.896	ref	0.611
Health center	0.97 (0.65, 1.45)		0.41(0.01, 12.9)	
Hospital				
WHO clinical stage	ref	0.666	ref	NB
1	0.81 (0.31, 2.10)	0.376	NB	NB
2	0.38 (0.05, 3.21)	0.558	NB	NB
3	2.30 (0.14, 37.0)		NB	
4				
ART Regimen	ref	0.490	ref	NB
Others	1.73 (0.36, 0.8.27)		NB	
TDF/XTC/EFV				
Hepatitis B surface antigen	ref	0.465	ref	NB
Negative	0.66 (0.21, 2.03)		NB	
Positive				
Rapid Plasma Reagin (Syphilis)	ref	0.459	ref	NB
Negative	1.27 (0.67, 2.42)		NB	
Positive				
Non-Viral Suppression (NVS) (VL ≥20 copies/mL)	ref	0.048		
No	1.44 (0.95, 2.17)	<0.0001		
Yes	ref			
Treatment Success (VL<1,000 copies/mL)	0.26 (0.14, 0.48)			
No				
Yes				
W: Wilcoxon/Mann-Whitney Test Rank-Sum Test; C: Chi Squared Test; E: Fisher's Exact Test; NB: Values not estimated due to perfect prediction				

Table 2: Covariates and their association/risk for development of insulin resistance

Sex was not associated with IR, 55 (38.7%) males had IR versus 87 (61.3%) females; p value=0.104. A significantly lower proportion of patients with IR were virologically suppressed at one-year compared to individuals without IR, 82 (58%) of 142 patients [95% CI: 0.54-0.70] versus 232 (70%) of 331 patients [95% CI: 0.65-0.75], respectively; p value=0.042.

When analyzed by VL threshold, specifically treatment success versus treatment failure, a significantly higher (3.3-fold) proportion of individuals with IR (19%) had treatment failure compared to patients without IR (5.7%); p value<0.0001. Of note, 427 (90.3%) participants had treatment success, and this was associated with a significantly lower (74% lower risk) likelihood of developing IR (odds ratio (OR)=0.26 [0.14, 0.48], p value<0.0001.

Among those without evidence of viral suppression, there was an increased odds of having IR; specifically, OR=1.44 (0.95, 2.17), p value 0.048). Similarly, the waist-to-hip circumference ratio was also associated with increased odds of IR.

A number of models were run including

1) Age, Waist circumference, Visceral fat, CD4, Hgb, Viral Load (VL), Triglycerides/HDL ratio, CRP, TNF alpha, Sex, Non-Viral Suppression (NVS) (VL ≥ 20 c/mL)

2) Age, Waist to Hip circumference ratio, Visceral fat, CD4, Hgb, VL, Triglycerides/HDL ratio, CRP, TNF alpha, NVS

3) Age, BMI, Visceral fat, CD4, Hb, VL, LDL, CRP, TNF alpha, NVS and

4) Age, BMI, Visceral fat, CD4, Hb, VL, Triglycerides/HDL ratio, CRP, TNF alpha, NVS

Using the best adjusted model (Table 3), NVS, TNF-α, CRP, waist-to-hip ratio and visceral fat were associated with increased odds of IR.

Odds Ratio of Insulin Resistance				
Characteristics	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Continuous variables				
Age (years)	0.98 (0.96, 1.00)	0.019	0.94 (0.89, 0.99)	0.014
Waist circumference (cm)	1.05 (1.03, 1.07)	<0.0001	1.05 (1.00, 1.11)	0.073 (F/M)
Hip circumference (cm)	0.97 (0.95, 0.98)	<0.0001	0.96 (0.92, 1.01)	0.087
Waist to Hip circumference ratio	246.4 (54.7, 1108.9)	<0.0001		
Visceral Fat Levels (IU)	1.14 (1.09, 1.19)	<0.0001		
CD4 cell count (cells per μ L)	1.00 (0.99, 1.00)	0.096		
Hemoglobin (g/dl)	0.94 (0.83, 1.05)	0.273	1.22 (0.93, 1.59)	0.149
Viral load (copies /mL)	1.00 (0.99, 1.00)	0.074		
Body Mass Index (m/kg ²)	1.04 (0.99, 1.09)	0.087		
Total Cholesterol (mmol/l)	1.01 (0.94, 1.09)	0.729		
High Density Lipoprotein (mmol/l)	0.33 (0.25, 0.44)	<0.0001		
Low Density Lipoprotein (mmol/l)	1.29 (1.07, 1.56)	0.008	1.74 (1.02, 2.94)	0.041
Triglycerides (mmol/l)	1.15 (1.06, 1.25)	0.002	1.21 (1.05, 1.40)	0.010
C-reactive protein (hsCRP)(mg/L)	1.55 (1.36, 1.77)	<0.0001		
Tumor necrosis factor alpha (TNF- α)(ng/mL)	1.00 (0.99, 1.00)	0.193	1.04 (1.01, 1.07)	0.022
Categorical variables				
Type of facility	ref	0.896		
Health center	0.97 (0.65, 1.45)			
Hospital				
Sex	ref	0.105		
Male	1.39 (0.93, 2.08)			
Female				
WHO clinical stage	ref	0.666		
1	0.81 (0.31, 2.10)	0.376		
2	0.38 (0.05, 3.21)	0.558		
3	2.30 (0.14, 37.0)			
4				
ART Regimen	ref	0.490		
Others	1.73 (0.36, 0.8.27)			
TDF/XTC/EFV				
Test for hepatitis B	ref	0.465		
Negative	0.66 (0.21, 2.03)			
Positive				
Test for syphilis	ref	0.459		
Negative	1.27 (0.67, 2.42)			
Positive				
Non-Viral Suppression (NVS) (VL \geq 20 copies/mL)	ref	0.048		

No	1.44 (0.95, 2.17)	<0.0001		
Yes	ref			
Treatment Success (VL<1,000 copies/mL)	0.26 (0.14, 0.48)			
No				
Yes				

W: Wilcoxon/Mann-Whitney Test rank-sum test; C: Chi Squared Test; E: Fisher's Exact Test; NB: Values not estimated due to perfect prediction

Table 3: Best adjusted model for odds of insulin resistance

Discussion

In this HIV-positive population of Zambian adults who have been receiving combination antiretroviral therapy (ART) for a mean time of 12.2 months, 30% of them had insulin resistance (IR) as ascertained using the Homeostatic Model Assessment (HOMA). This is the first time IR has been studied in Zambia and also in the region among individuals who are HIV-positive and currently receiving ART. When analyzed by VL threshold, specifically, treatment success and treatment failure; 19% of individuals with IR had treatment failure compared to 5.7% without (p value<0.0001). The vast majority (90.3%) of study participants had treatment success and this was associated with a significantly lower (74% lower risk) likelihood of developing IR (OR=0.26 [0.14, 0.48], p value<0.0001).

Using the VL threshold of <20 copies/mL to define viral suppression (Murray, Vos et al.), this study found that a significantly lower proportion of patients with IR were virologically suppressed at one-year compared to individuals without IR, 58% [0.54-0.70] versus 70% [0.65-0.75], p value=0.042. Individuals with viremia or NVS had increased odds (OR 1.44, 95%CI: 0.95-2.17, p=0.048) and those with treatment success (showed a lower likelihood of IR; Odds ratio (OR)=0.26 [0.14, 0.48], p value<0.0001).

These findings are interesting in that they provide some insight into the possibility that the risk for the development of IR may be diminished in patients with both viral suppression and treatment success. Since individuals in this study were on ARV medications that have not been implicated in the development of IR, we could not explicitly explain these findings based on viral suppression and treatment success.

The percentage of individuals with IR is comparable to studies performed among different populations and regions of the world [24,28,29]. This study showed similar results to a study conducted in Spain where 265 HIV-positive adults on ART without DM at baseline had an IR prevalence of 21% [30]. A similar study in Cameroon, Central Africa showed a much higher prevalence of 47.3 among HIV-positive adults (48.5% among HIV-positive, ART-treated adults versus 38.5% among ART-naïve adults) compared to our study. This particular study was hospital-based and even though the majority (93.3%) of patients on ART were receiving 1st line regimens, their ARV medications were different and the total duration on ART of their study population was also longer (i.e. 72 months) [31]. These discrepancies in the duration of treatment and ART regimens would also explain the difference in results, as exposure to certain ARV medications has also been implicated in the development of IR [18,19,22]. The ARV medications this Cameroonian population was primarily exposed to; namely protease inhibitors and thymidine analogues are the ARV medications which have largely been implicated in IR and DM [22,32].

The trend of increased inflammatory markers (highly sensitive C-reactive protein (hsCRP) and tumor necrosis factor-alpha (TNF-α) for those patients with IR may imply that inflammation may be associated with the development of IR in patients with HIV. In our study, the median hsCRP levels were 2.38 mg/L (95%CI: 1.40, 3.49) for those with IR compared to 0.79 mg/L (95%CI: 0.47, 1.58) for those without IR; p value<0.0001. Similarly, TNF-α levels were 33.9 pg/mL (95%CI: 27.5, 42.2) Vs 21.7 pg/mL (95%CI: 14.9, 29.9) among those study participants with and without IR, respectively. Using quantile regression, the risk for the development of IR appeared to be positively correlated with increasing hsCRP and TNF-α levels when evaluating these biomarker values as continuous covariates. Using logistic regression coefficients estimates of log odds of IR association with hsCRP and TNF-α, CRP was significantly associated with IR (0.431; p value<0.0001).

Though our study does not prove whether or not inflammation is the cause of IR, it does highlight the importance of the interdependence between inflammation and IR. As reported by Banks et al when evaluating the relationship between TNF-α and hsCRP with risk for IR, our study showed a similar positive association [33,34]. Other authors have suggested that in HIV, an infectious stimuli may result in persistent cytokine activation leading to prolonged release of TNF- and other inflammatory cytokines that lead to and affect the risk for the development of IR [24]. This phenomenon may be present even in the absence of exposure to ARV medications typically associated with IR risk [24]. It is therefore possible that our findings may be explained by an active infectious stimuli (uncontrolled HIV) in viremic individuals, both lower levels (VL>20 copies/mL) and higher levels (VL>1,000 copies/mL) of viremia leading to varying levels of cytokine activation with the extensive and prolonged release of the TNF- and other inflammatory cytokines with subsequent reduction in insulin sensitivity. Insulin resistance may also be due to the HIV-1 accessory proteins of Tat and Vpr with Vpr obstructing the transcriptional activity of insulin through inhibition of the PPAR-c activity and Tat activating the nuclear factor-κβ with induction of TNF- and blocking of the uptake of free fatty acids by adipocytes [24,34,35]. The fact that markers of inflammation are decreased on ART may explain the observed lower levels in patients with viral suppression and also reduced odds for risk of developing IR [36-38]. It is thus plausible that controlling HIV infection with ART may be protective towards IR and this may be a result of reduced inflammation in virally suppressed individuals.

Besides inflammatory markers and NVS, we observed that individuals with increasing waist-to-hip circumference ratio, body mass indices, visceral fat levels, and levels of LDL cholesterol and triglycerides had an increased odd of developing IR. We observed increased odds of developing IR among individuals with increased visceral fat levels and increased Waist-to-Hip circumference ratios

suggesting that visceral fat in HIV-positive individuals may be a predictor of IR. Hadigan et al described IR in HIV-positive adults and demonstrated significant hyperinsulinemia and truncal adiposity in HIV-positive women independent of PI exposure [38-40]. IR and dyslipidemia are common metabolic disorders among PLWH either directly (i.e. for dyslipidemia) and indirectly (i.e., HIV-associated inflammation) and/or ART (i.e. especially older generation ART regimens) [41]. There are still ongoing studies evaluating the specific link between visceral adipose tissue accumulation and IR. Suggested mechanisms are largely centered upon insulin signaling and may be related to: a) the accumulation of excess lipid in liver resulting in cell autonomous impairment in insulin signaling and b) visceral adipose tissue inflammation and inflammatory cytokine production contributing to impairment in insulin signaling [42-44].

These data demonstrate significant rates of IR in HIV-positive, ART-treated adults having both increased visceral fat levels and waist-to-hip circumference ratios, and based on this a simpler and certainly less invasive means to monitor for IR; may be to regularly perform WHRs among at-risk individuals. Performing WHRs at some frequency may also be a way to diagnose additional adverse health events such as diabetes mellitus and cardiovascular disease. This monitoring may be especially critical in HIV-positive adults where a variety of mechanisms such inflammation, ART regimen exposure, and HIV are simultaneously interacting and have been linked with the development of IR, DM and cardiovascular disease.

We believe this is the first study to highlight that early ART leading to virologic suppression may reduce one's risk for the development of IR. Our study is in contrast to the Rwanda study which was done among HIV-positive women where ART use did not predict change in log₁₀-HOMA and use or duration of AZT, d4T and EFV was not associated with HOMA change [45]. This could be due to differences in ARV medications used, and was primarily done in a female population, and the investigative team did not use viral load to determine treatment response.

Both bacterial and viral infections have been associated with reduced insulin sensitivity and similar to our findings, risk for development of IR may be independent of exposure to certain ARV medications or classes of medications (e.g. PIs) [24,46]. Assessing the prevalence of IR in a Spanish cohort of HIV-positive adults, Araujo et al. attributed the lower prevalence of IR to participants in their cohort receiving newer antiretroviral medications [30]. It is therefore plausible to have different IR levels and risk depending on the type of ARV medications used. Previously used ARV medications (i.e. d4T, AZT, ddI, etc.) were associated with IR and persons receiving them also had more medication-related side effects such as lipodystrophy, both of which have been implicated in IR emergence. Additionally, HIV-positive adults receiving these older ARV regimens may be more likely to be non-adherent due to unfavorable side effects and/or significant pill burden that would lead to suboptimal virologic suppression that could directly influence insulin sensitivity. Our study, however, suggested that ART itself did not have any direct influence on the development of IR in contrast to what has been reported elsewhere [30].

Since ARV medications themselves may be linked to IR, it is therefore critical that the selection of ART regimens is taken into account when deciding upon what ART regimens to initiate (or switch to) at the individual patient-level. Recent reports of the newly introduced integrase strand inhibitor dolutegravir (DTG), that is being rolled out to majority of individuals on ART being linked to increasing

weight, are concerning and warrant further study. Recently, Venter and group reported increased weight on DTG based therapy in south African HIV positive adults much more in female [47,48]. These recent data that Menard et al describe as "an unexpected bothering side effect" are worrisome as DM rates may increase independently of the metabolic syndrome and as a result there may be more intermediate- and longer-term risks associated with DTG exposure than previously elucidated [48-50]. Our study participants were not on DTG-based ART, thus we could not study these potential causal relationships and certainly additional studies are needed.

Our study has several limitations. 1) This was a cross sectional study and thus we could not establish pre-treatment/baseline levels of IR in our population and other pre-treatment parameters such as viral load, HIVDR, visceral fat levels and anthropometric indices. 2) Our team did not have an HIV-negative control group or an HIV-positive, ART-naive group which would have helped to disentangle the role of HIV and ART exposure in the development of and progression of IR.

Conclusion

In summary, we have shown that insulin resistance rates in HIV-positive adults receiving ART for 1 year are high and associated with both non-viral suppression (viral load >20 copies/mL) and treatment failure (viral load >1,000 copies/mL). Insulin resistance was further associated with high inflammatory markers (hsCRP and TNF- α), visceral fat and obesity. These findings may have wider implications for a number of reasons:

Individuals with HIV are now living longer and are at high risk for NCDs including diabetes mellitus.

People Living with HIV (PLHIV) have systemic inflammation (as evidenced in this study), another factor associated with type 2 diabetes.

PLHIV may have lip dystrophic features with increasing obesity related to both HIV infection and ART exposure, both of which are related to incident type2 diabetes.

The wide scale introduction of DTG, with improved potency (i.e. higher viral suppression rates) and tolerability, may be associated with obesity which has been linked to IR and incident DM.

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