#A-792-0129-03596

Association of markers of gut microbial translocation and inflammation with insulin resistance in HIV-infected persons

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Abstract

Background Microbial translocation has been proposed as an important driver of immune activation and inflammation in virologically suppressed HIV-infected adults. It is hypothesized that microbial translocation induces alterations in glucose metabolism that lead to insulin resistance, even after controlling for other traditional determinants of diabetes.

Methods This was a cross sectional study of 377 HIV-infected patients on highly active antiretroviral therapy (HAART) and 241 HIV-uninfected controls. Cryopreserved plasma was assessed for the gut barrier marker intestinal fatty binding protein (i-FABP), a surrogate of microbial translocation, as well as sCD14 and CD163, both markers of monocyte activation, and interleukin 6 (IL-6) an inflammatory cytokines. Association between these markers and insulin resistance, quantified by Homeostasis Model Assessment (HOMA), was evaluated using multivariable regression after controlling for traditional and HIV-related factors.

Results Mean iFABP levels were significantly higher in HIV-infected persons compared with controls (543 vs. 907, p<0.001). Mean concentrations of sCD14 (1.71 vs. 150, p<0.001), CD163 (624 vs. 478, p=0.013) and IL-6 (1.0 vs 0.81, p<0.001) were also higher in HIV-infected participants. Among HIV-infected patients, those in the highest tertile of iFABP levels had significantly lower CD4 nadirs (p=0.027) and were more likely to have a history of opportunistic infections (p=0.044) and active Hepatitis C infection (p=0.002) than others..

In unadjusted linear regression, increasing sCD163 levels were associated with increased insulin resistance . While traditional determinants, including body mass index and hip circumference were also predictive of insulin resistance, neither sCD14 levels nor i-FABP levels were associated with increasing insulin resistance in adjusted multivariate models.

Conclusions Gut epithelial barrier dysfunction, immune activation and inflammation were higher in HIV-infected patients compared to controls. While increased epithelial dysfunction was seen in HIV-infected patients, i-FABP was not predictive of insulin resistance. By contrast, sCD163 levels were independently associated with insulin resistance in multivariable models. Prospective studies are warranted to better elucidate the role of sCD163 in the pathogenesis of diabetes.

Introduction

- Aging-related comorbidities, such as diabetes mellitus, are increasingly important in the management of HIV-infected patients. The etiology of disorders of glucose metabolism is multifactorial.
- Translocation of microbial products from the gut has been postulated as one possible explanation for ongoing inflammation and immune activation that may also contribute to insulin resistance.
- We sought to determine whether soluble markers of intestinal epithelial damage, monocyte activation, and systemic inflammation were associated with alterations in glucose metabolism in HIV and HCV infected women and men, even after controlling for traditional determinants of diabetes.

Methods

Study Population

- Participants of the Women's Interagency HIV Study (WIHS) and the Study of Visceral Adiposity, HIV, and HCV: Biologic Mediators of Steatosis (VAHH) were included in cross-sectional analysis.
- **WIHS** is a multicenter prospective study established in 1994 to investigate the progression of HIV in women with and at risk for HIV. Women enrolled in a Liver Steatosis Substudy and a Fibroscan Substudy) at 3 WIHS sites (San Francisco, DC and Chicago) were included.
- **VAHH** enrolled 224 participants (98% men) with HIV monoinfection (n=64), HIV/HCV coinfection (n=27), HCV monoinfection (n=55), and neither HIV nor HCV infection (n=78) between October 2010 and June 2014 from the San Francisco Veterans Affairs Medical Center.
- After exclusion of participant with diabetes mellitus, WIHS and VAHH data were pooled in order to examine the relationship of markers of microbial translocation, monocyte activation, and inflammation with insulin resistance in HIV-infected and uninfected women and men.

Measurements

Primary Predictors

- Intestinal Fatty Acid Binding Protein (IFABP), a marker of enterocyte degradation and correlate of microbial translocation
- soluble (s) CD14 and sCD163, a marker of monocyte activation
- Interleukin (IL)-6, a marker of systemic inflammation

Secondary Predictors

Infection status (HIV infection, Hepatitis C virus (HCV) infection), **demographic** (age at index visit, race/ethnicity, gender); behavioral (cigarette use and alcohol use); **anthropometric** measures (body mass index (BMI), waist circumference, hip circumference); **metabolic** (lipid profile, estimated glomerular filtration rate);**HIV-related** measures (current CD4 cell count, CD4 cell count nadir, current HIV RNA level, history of clinical AIDS and current use of HAART) .

Outcome

Insulin resistance estimated using the Homeostasis Model Assessment (HOMA-IR) defined as fasting insulin (μU/mL) x glucose (mg/dL)/405.

Statistical Analysis

Multivariable linear mixed models were used to assess the associations of HIV status and each of the assayed inflammatory markers in unadjusted, demographic adjusted and fully adjusted multivariate models. In models with missing cases, multiple imputation using the Chained Equations method was used to impute missing covariates with ten repetitions.

Results

Table 1. Demographics and clinical characteristics stratified by HIV and hepatitis C virus infection status*

Characteristics Median (IQR) or n (%)	Control n=146	HIV monoinfected n=220	HCV monoinfected n=64	HIV/HCV-coinfection n= 89	P-value§
Demographics					
Age, yrs	49 (39, 55)	47 (41, 53)	57 (53, 60)	52 (48, 57)	<0.001
Female	74 (51%)	173 (79%)	17 (27%)	65 (73%)	<0.001
Race/ethnicity					0.002
White	37 (34%)	63 (30%)	25 (52%)	18 (23%)	
African American	43 (39%)	104 (50%)	12 (25%)	47 (60%)	
Hispanic	18 (16%)	29 (14%)	9 (19%)	13 (17%)	
Other¶	12 (4%)	12 (6%)	2 (4%)	1 (1%)	
Lifestyle					
Current Smoker	62 (43%)	77 (35%)	32 (51%)	55 (63%)	0.001
Smoking history, yrs	12 (0, 23)	10 (0, 21)	28 (18, 33)	30 (23, 37)	<0.001
Alcohol consumption					0.128
None	51 (35%)	77 (35%)	20 (32%)	40 (46%)	
>0-7 drinks/wk	57 (39%)	103 (47%)	24 (38%)	30 (34%)	
>7-12 drinks/wk	9 (6%)	17 (8%)	6 (10%)	4 (5%)	
>12 drinks/wk	29 (20%)	23 (11%)	13 (21%)	14 (16%)	
Metabolic					
BMI, kg/m^2	28.2 (24.7, 31.3)	26.1 (22.7, 29.1)	26.1 (22.3, 29.1)	24.2 (22.1, 28.4)	0.001
Waist circumference, cm	96 (86, 106)	90 (81, 98)	94 (83, 106)	88 (81, 98)	<0.001
Hip circumference, cm	103 (96, 111)	97 (92, 105)	98 (93, 109)	96 (89, 105)	<0.001
Total Cholesterol, mg/dl	182 (163, 208)	181 (155, 208)	160 (143, 184)	165 (138, 187)	<0.001
Triglycerides, mg/dl	94 (64, 143)	104 (79, 150)	82 (67, 102)	97 (75, 144)	0.010
LDL, mg/dl	103 (85, 129)	101 (81, 121)	93 (68, 105)	82 (64, 104)	<0.001
HDL, mg/dl	52 (44, 68)	53 (41, 65)	50.5 (39, 64)	52 (38, 64)	0.527
Estimated GFR, mL/min/1.73m2	99 (88, 111)	97 (85, 114)	98 (86, 109)	86 (73, 107)	<0.001
APRI score+	0.26 (0.21, 0.36)	0.29 (0.22, 0.40)	0.74 (0.36, 1.13)	0.63 (0.40, 1.10)	<0.001
I-FABP, pg/ml	520 (333, 805)	760 (519, 1426)	761 (505, 1209)	976 (463, 1564)	<0.001
sCD14, ng/ml	1105 (1008, 1360)	1514 (1225, 1837)	1351 (1083, 1585)	1628 (1354, 1924)	<0.001
sCD163, ng/ml	347 (269, 433)	402 (308, 571)	788 (515, 1146)	908 (626, 1282)	<0.001
IL-6, pg/ml	0.73 (0.44, 1.10)	0.80 (0.54, 1.25)	0.98 (0.65, 1.61)	1.39 (0.89, 2.10)	0.006
HIV specific Parameters					
Current CD4, cells/mm^3	-	588 (379, 798)	-	504 (285, 678)	<0.001
CD4 nadir, cells/mm^3	-	288 (175, 420)	-	223 (131, 290)	<0.001
History of AIDS	-	81 (37%)	-	50 (56%)	0.001
Undetectable Viral Load	-	149 (68%)	-	53 (60%)	0.134
Current HAART use	-	152 (69%)	-	75 (84%)	0.020

HCV, hepatitis C virus; HDL, high-density lipoprotein; LDL, low-density lipoprotein,
* All values are median (interquartile range) unless otherwise noted.
§ P value from Pearson χ^2 test, Wilcoxon rank-sum test or Fischer's exact test
¶ Includes Asian, Pacific Islander, Native American, Alaskan and other study participants
+ APRI = ((AST/Top normal AST)/Platelets) * 100

Figure 1. Marginal age, race, and gender-adjusted means for HOMA-IR stratified by infection status.

HIV/HCV-coinfected and HCV-monoinfected adults had greater HOMA-IR than HIV-monoinfected and controls (demographic-adjusted mean [95% CI]: 1.96 [1.51, 2.54] and 1.65 [1.22, 2.24] vs. 1.41 [1.18, 1.67] and 1.44 [1.17, 1.75], respectively).

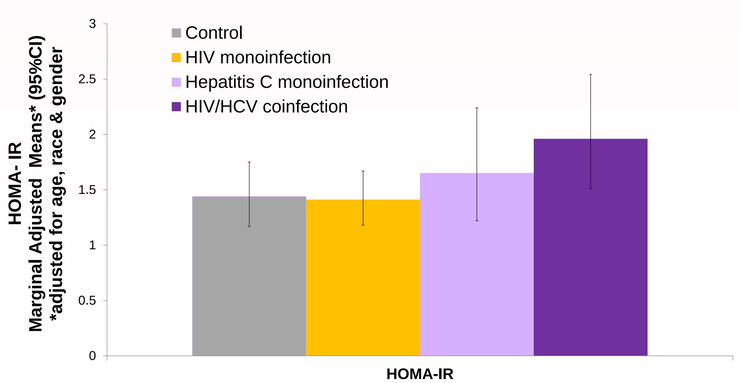

Table 2. Association of HIV, HCV, and HIV/HCV with HOMA-IR compared to controls after adjustment of demographic, lifestyle, and metabolic factors plus adjustment for I-FABP, sCD163, sCD14, and IL-6. HIV/HCV-coinfected had significantly greater HOMA-IR than controls after adjustment for demographic, lifestyle, and metabolic factors; the association weakened after additional adjustment for sCD163, but not with I-FABP, sCD14, or IL-6.

Infection Status	Adjusted* Estimate (95%CI)	Adjusted** + I-FABP (per doubling) Estimate (95%CI)	Adjusted** + sCD163 (per doubling) Estimate (95%CI)	Adjusted** + sCD14 (per doubling) Estimate (95%CI)	Adjusted** + IL-6 (per doubling) Estimate (95%CI)
HIV monoinfection	0.97 (0.79, 1.19) p=0.790	1.05 (0.84, 1.30) P=0.670	0.98 (0.79, 1.22) P=0.922	1.11 (0.88, 1.37) p=0.362	1.05 (0.85, 1.30) P=0.660
HCV monoinfection	1.12 (0.83, 1.50) p=0.460	1.15 (0.85, 1.55) p=0.360	0.89 (0.64, 1.25) p=0.489	1.19 (0.88, 1.60) p=0.267	1.14 (0.85, 1.54) p=0.380
HIV/HCV coinfection	**1.36 (1.04, 1.80) p=0.025**	1.47 (1.12, 1.94) p=0.006	1.07 (0.77, 1.48) p=0.688	1.60 (1.20, 2.13) P=0.001	1.45 (1.10, 1.92) P=0.009

*Adjusted for gender, age, race; **Adjusted for gender, age, race and serum IFABP concentration

Table 3. Association of markers of microbial translocation and inflammation by HIV and HCV status. sCD163 was independently associated with greater HOMA-IR in all four groups.

Parameter	HOMA-IR Unadjusted Estimate (95% CI)	HOMA-IR Demographic adjusted* Estimate (95% CI)	HOMA-IR Fully adjusted** Estimate (95% CI)
HIV monoinfection			
I-FABP (per doubling)	0.99 (0.91, 1.08) p=0.931	0.94 (0.86, 1.02) p=0.182	0.97 (0.90, 1.05) p=0.49
sCD163 (per doubling)§	**1.28 (1.17, 1.42) p<0.001**	**1.28 (1.15, 1.41) p<0.001**	**1.27 (1.15, 1.39) p<0.001**
sCD14 (per doubling)§	0.92 (0.85, 0.99) p=0.052	0.94 (0.88, 1.02) p=0.131	1.02 (0.95, 1.09) p=0.611
IL-6 (per doubling)§	1.02 (0.95, 1.10) p=0.471	0.94 (0.86, 1.03) p=0.320	0.97 (0.88, 1.05) p=0.374
HCV monoinfection			
I-FABP (per doubling)	0.98 (0.90, 1.06) p=0.597	0.93 (0.86, 1.01) p=0.097	0.97 (0.90, 1.04) P=0.397
sCD163 (per doubling)§	**1.31 (1.19, 1.45) p<0.001**	**1.29 (1.17, 1.44) p<0.001**	**1.27 (1.16, 1.40) p<0.001**
sCD14 (per doubling)§	0.90 (0.84, 0.98) p=0.014	0.94 (0.87, 1.01) p=0.101	1.04 (0.96, 1.12) p=-0.341
1L-6 (per doubling)§	1.03 (0.96, 1.12) p=0.392	1.05 (0.97, 1.13) p=0.270	1.03 (0.96, 1.11) P=0.351
HIV/HCV coinfection			
I-FABP (per doubling)	0.97 (0.89, 1.05) p=0.428	0.92 (0.85, 1.00) p=0.044	0.95 (0.88, 1.02) p=0.174
sCD163 (per doubling)§	**1.31 (1.18, 1.46) p<0.001**	**1.26 (1.12, 1.40) p<0.001**	**1.20 (1.08, 1.33) p=0.001**
sCD14 (per doubling)§	0.88 (0.82, 0.94) p=0.001	0.92 (0.85, 0.99) p=0.031	1.02 (0.94, 1.09) p=0.683
1L-6 (per doubling)§	1.00 (0.93, 1.09) p=0.768	1.02 (0.94, 1.11) p=0.524	1.01 (0.94, 1.09) p=0.757
Controls			
I-FABP (per doubling)	0.97 (0.89, 1.06) p=0.560	0.92 (0.84, 1.00) p=0.052	0.94 (0.86, 1.02) p=0.113
sCD163 (per doubling)§	**1.35 (1.21, 1.49) p<0.001**	**1.29 (1.16, 1.43) p<0.001**	**1.23 (1.12, 1.36) p<0.001**
sCD14 (per doubling)§	0.90 (0.84, 0.98) p=0.010	0.93 (0.86, 1.00) p=0.048	0.99 (0.92, 1.07) p=0.809
1L-6 (per doubling)§	1.03 (0.96, 1.12) p=0.412	1.04 (0.96, 1.13) p=0.318	1.03 (0.95, 1.10) p=0.501

*Adjusted for gender, age, race; **Adjusted for age, gender, race, smoking, alcohol, BMI and waist circumference
§ I-FABP added to each model. All other variables added individually not sequentially.

Conclusions

- HIV/HCV-coinfected adults have greater HOMA-IR than controls, even after controlling for traditional risk factors for diabetes mellitus
- We found that higher levels of sCD163 may partly explain the association of HIV and HCV with HOMA-IR. However, neither I-FABP, IL-6 nor sCD14 were associated with greater HOMA-IR
- Interestingly, regardless of infection status, higher sCD163 was associated with greater HOMA-IR, suggesting that the association is independent of the HIV-associated cascade of microbial translocation, immune activation, and inflammation.
- Prospective studies are warranted to elucidate the role of CD163 in the pathogenesis of disorders of glucose metabolism, and whether increasing levels of CD163 might predict DM risk.

Acknowledgements

The WIHS is funded primarily by the National Institute of Allergy and Infectious Diseases (U01-AI-103401, U01-AI-103408, UO1-AI-35004, UO1-AI-31834, UO1-AI-34994, U01-AI-103397, U01-AI-103390, UO1-AI-34989, and UO1-AI-42590), with additional co-funding from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), the National Cancer Institute (NCI), the National Institute on Drug Abuse (NIDA), and the National Institute on Mental Health (NIMH).
The VAHH Study is funded by R01 AI 087176 (PCT), which was administered by the Northern California Institute for Research and Education and with resources of the Veterans Affairs Medical Center, San Francisco, CA.
The study was also supported by the UCSF Liver Center [P30 DK026743], K24 AI 108516 (PCT), and the UCOP Global Food Initiative Fellowship Program.