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

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**WEPEB094**

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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, have become increasingly important in the management of HIV-infected patients. The etiology of abnormalities 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 inflammation and intestinal epithelial damage 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**

- The Women's Interagency HIV Study (WIHS) is a multicenter prospective cohort study established in 1994 to investigate the progression of HIV in women with and at risk for HIV. 440 WIHS women enrolled in a prospective Metabolic Substudy between 2003 and 2005 from 3 WIHS sites (San Francisco, Bronx and Chicago) were included in the analysis.
- The VAHH Study 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 the ages of 35 and 70 from October 2010 through June 2014.
- After exclusion of men and women with diabetes mellitus, data from the WIHS and VAHH were pooled in order to examine cross-sectionally the relationship of markers of microbial translocation with insulin resistance in HIV-infected and uninfected women and men.

**Measurements**

*Primary Predictors*

Intestinal Fatty Acid Binding Protein (IFABP), soluble CD14, CD163 and IL-6 . I-FABP is a marker of enterocyte degradation and correlate of microbial translocation. Soluble CD14, CD163 and IL-6 are indicators of bacteria-associated monocyte activation and 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 quantified using the Homeostasis Model Assessment defined as 10-hour 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

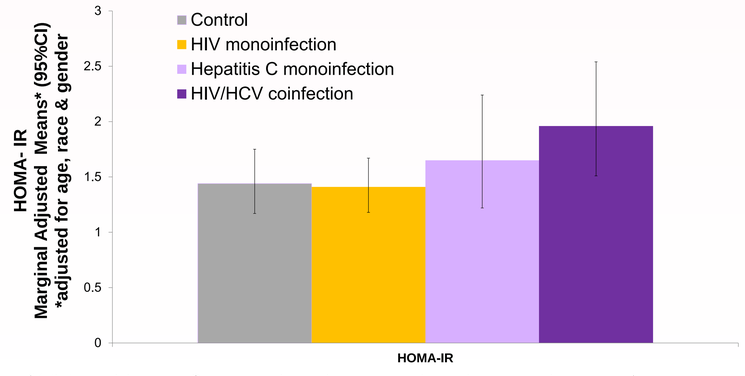
## Results

**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 |
| HOMA-IR | 1.35 (0.62, 2.17) | 1.15 (0.44, 2.15) | 1.60 (1.09, 2.57) | 1.81 (0.76, 4.32) | 0.024 |
| 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 |
| CD163, 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 $\chi^2$ 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. Comparison of marginal adjusted means for HOMA IR stratified by infection status, adjusted for age, race and gender**




(Multiple imputation using Chained Equation method was used to impute missing outcome values)

**Table 2. Association of HIV monoinfection, HCV monoinfection, HIV/HCV coinfection with IR adjusted for markers of microbial translocation, monocyte activation, and inflammation, compared to controls¶**

| 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 |

¶ Multiple imputation using Chained Equation method was used to impute missing outcome values
*Adjusted for gender, age, race
**Adjusted for gender, age, race and serum IFABP concentration

**Table 3. Associations of markers of microbial translocation and inflammation by HIV and HCV status¶**

| 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) | 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. (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<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 |

¶ Multiple imputation using Chained Equation method was used to impute missing outcome values
*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

- There is considerably more gut epithelial barrier dysfunction and immune activation in HIV and HCV monoinfected individuals and even more in HIV/HCV coinfected individuals compared to controls.
- HIV/HCV-coinfected adults have greater HOMA-IR than controls, even after controlling for established determinants of diabetes, including BMI and advanced HIV disease.
- However, neither i-FABP, IL-6 nor sCD14 were associated with increasing insulin resistance in multivariable analysis. By contrast higher levels of sCD163 were associated with increasing insulin resistance
- 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 diabetes, as well as evaluate the utility of using plasma CD163 as a simple and reliable measure of insulin resistance in HIV and HCV-infected individuals.

## Acknowledgements

The WIHS and the VAHH studies are funded primarily by the NIAID, 3U01AI034989-21S1 & 5R01AI087176-04 .
This analysis, was funded in part by a grant from the UCOP Global Food Initiative Fellowship Program