Sex and Obesity are Associated with Residual Viremia in ART-Suppressed Individuals

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Introduction

The sex of an individual influences HIV levels prior to antiretroviral therapy (ART) (*Napravnik, JAIDS, 2002*) and adipose tissue has been proposed to harbor part of the HIV reservoir (*Koethe, Compr Physiol, 2017*).

The effect of host characteristics, including sex and body-mass index (BMI), on HIV persistence during ART remains incompletely understood.

We evaluated factors associated with HIV persistence in a large cohort of people with well-documented, long-term virologic suppression (**ACTG A5321**).

Methods

Participants who initiated ART during chronic infection with sustained virologic suppression had virologic measurements of **plasma HIV RNA by single copy assay (SCA), PBMC-associated HIV DNA and RNA (CA-DNA, CA-RNA).**

Immunologic measurements included **soluble markers IL-6, IP-10, neopterin, sCD163, sCD14, and TNF;** and cellular markers **CD38, HLA-DR, Ki-67, and PD-1.**

We assessed the effect of **age, sex (reported at birth), BMI, waist circumference (WC), years on ART, pre-ART HIV RNA, pre-ART CD4 count, and initial ART regimen (PI, NNRTI or INSTI)** on HIV persistence.

All measurements of HIV persistence, inflammation, and T cell activation were performed on blood samples while on ART. A5321 study entry occurred at a median of 7 years after initiation of ART (**Table 1**). Participant characteristics were assessed at the timepoint that virologic and inflammatory/activation markers were measured. WC was assessed just before this time point.

Results

295 participants (53 females) evaluated after a median of 7 years on ART (**Table 1**).

1. **Males were more likely than females to have detectable plasma SCA** ≥0.4 copies/mL (52% vs 29%, p=0.003) **(Figure 1A),** even after adjusting for age, pre-ART HIV RNA and CD4 count, years on ART and BMI (p=0.004).
2. **Higher BMI and higher WC were each associated with higher SCA levels** (r=0.12 and 0.13, p<0.04) even after adjustment for age, sex, pre-ART HIV RNA and CD4 count, and years on ART. The proportion of participants with detectable viremia increased in a step-wise fashion by BMI category: normal/underweight 38%; overweight 50%; obese 55% (**Figure 1B**). WC data not shown.
3. **Sex and BMI remained significantly associated with SCA** after controlling for differences in pre-ART CD4 count, pre-ART viral load, age, sex, duration of ART, and the soluble markers: IL-6, IP-10, neopterin, sCD163, sCD14, or TNF. (**Table 2**).
4. **IL-6, sCD163, and sCD14 in blood were significantly higher in females** than males (p=0.002, 0.05, 0.02, respectively). **IL-6 was positively associated with BMI** (p<0.001) (**Table 3**) in unadjusted analyses.
5. **Sex, BMI and WC were not associated with CA-DNA or CA-RNA** during ART suppression**.**
6. Confirming previous results, CA-DNA, CA-RNA and plasma SCA were **positively** correlated with pre-ART HIV RNA (r=0.35, 0.29, 0.20; respectively, p-values <0.001), and **negatively** with pre-ART CD4 count (-0.35, -0.21, -0.12, respectively, all p<0.05).
7. ART regimen type **was not associated** with HIV persistence measures (SCA, CA-DNA, CA-RNA) after controlling for duration of ART suppression.

Table 1 Participant Characteristics

		Total (N=295)
Age at A5321 entry (years)	Median	48
	Q1, Q3	41, 54
	Min, Max	23, 74
Sex	Male	242 (82%)
	Female	53 (18%)
Race/ethnicity	White Non-Hispanic	165 (56%)
	Black Non-Hispanic	58 (20%)
	Hispanic (Regardless of Race)	64 (22%)
	Asian, Pacific Islander	3 (1%)
	American Indian, Alaskan Native	3 (1%)
	More than one race	2 (1%)
Years on ART at A5321 entry	Median	7
	Q1, Q3	6, 10
	Min, Max	4, 16
Height (cm)	Median	174
	Q1, Q3	167.6, 180.3
	Min, Max	152.0, 200.2
Weight (kg)	Median	81.6
	Q1, Q3	70.8, 94.1
	Min, Max	46.3, 149.0
BMI (kg/m²)	Median	27
	Q1, Q3	24.3, 31.0
	Min, Max	16.7, 56.5
	Underweight (< 18.5)	2 (1%)
	Normal (18.5 - <25)	89 (30%)
	Overweight (25 - <30)	124 (42%)
	Obese (≥30)	80 (27%)
Waist Circumference (cm)	Median	94.4
	Q1, Q3	87.1, 102.0
	Min, Max	68.0, 143.5
Hip Circumference (cm)	Median	99.5
	Q1, Q3	93.5, 107.3
	Min, Max	72.0, 155.9
Waist Hip Ratio	Median	0.9
	Q1, Q3	0.9, 1.0
	Min, Max	0.7, 1.2
Initial ART Regimen	NNRTI + NRTI	138 (47%)
	PI + NRTI	104 (35%)
	InSTI + NRTI	41 (14%)
	Other	12 (4%)

Table 1 *contains participant characteristics at pre-ART and A5321 study entry for participants with at least one HIV persistence measure. This population had 18% females and 14% starting an integrase inhibitor-based initial regimen.*

Figure 1 Male Sex and Higher BMI are Associated with Low-level Viremia on ART

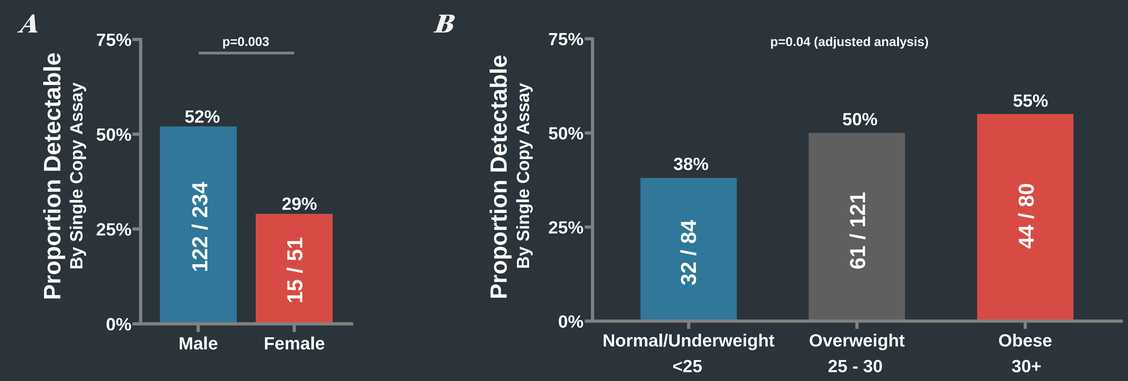

Figure 1 *Plasma single copy assay values from **285 of 295 total participants** were grouped according to participant sex (**A**) or BMI (**B**). Illustrated are the proportions of HIV RNA above 0.4 cp per mL by integrase single copy assay of approximately 5mL of plasma. Significant adjusted correlations of **sex (p=0.003)** or **BMI (p=0.04)** with plasma SCA detectability. The normal/underweight group in Panel B contains 2 underweight (<18.5) and 82 normal weight (18.5-25).*

Conclusions

1. **Females have lower residual viremia on ART than males** even after adjusting for age, pre-ART HIV RNA and CD4 count, years on ART and BMI. This may reflect the effect of estrogen on HIV expression or other biologic, immunologic, or sex differences.
2. **Higher BMI and obesity are associated with higher levels of residual viremia** in persons on long-term ART. This finding suggests that adipose tissue may be an important site of HIV production due to its proinflammatory milieu or altered ARV penetration.
3. **ART regimen was not associated with HIV persistence** after adjustment for ART duration**.**
4. Studies of the **mechanism** by which obesity and sex affect HIV persistence are needed to inform cure strategies.

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Table 2 Sex and BMI Remain Correlated with SCA After Adjusting for Soluble Inflammatory Biomarkers

	BMI		Sex
	Spearman	*P-value*	*P-value*
Unadjusted	0.09	0.13	**0.005**
Adjusted for pre-ART HIV-RNA	0.11	0.07	**0.018**
Adjusted for Age, Sex*, pre-ART CD4, pre-ART HIV RNA and Years on ART	0.12	**0.038**	**0.009**
Adjusted for Age, Sex*, pre-ART CD4, pre-ART HIV RNA, Years on ART and IL-6	0.1	0.08	**0.008**
Adjusted for Age, Sex*, pre-ART CD4, pre-ART HIV RNA, Years on ART and IP-10	0.12	**0.048**	**0.007**
Adjusted for Age, Sex*, pre-ART CD4, pre-ART HIV RNA, Years on ART and Neopterin	0.12	**0.039**	**0.008**
Adjusted for Age, Sex*, pre-ART CD4, pre-ART HIV RNA, Years on ART and sCD14	0.13	**0.029**	**0.005**
Adjusted for Age, Sex*, pre-ART CD4, pre-ART HIV RNA, Years on ART and sCD163	0.12	**0.047**	**0.009**
Adjusted for Age, Sex*, pre-ART CD4, pre-ART HIV RNA, Years on ART and TNF	0.13	**0.032**	**0.008**

Table 2 *presents Spearman correlations of BMI with SCA at A5321 entry, individually adjusting for each soluble biomarker of inflammation in addition to age at A5321 entry, sex of the participant, pre-ART CD4⁺ T cell count, pre-ART HIV RNA and years on ART at A5321 entry. Spearman correlations of BMI vs. SCA remain significant after adjusting for each soluble biomarker, except only marginally significant for IL-6 (p = 0.08).*

Using regression models, females continued to have significantly lower levels of SCA compared to males even after adjusting for each soluble biomarker of inflammation in addition to age, pre-ART CD4⁺ T cell count, pre-ART HIV RNA and years on ART at A5321 entry.

*N=285 for each. * Analysis of sex-based differences were not adjusted for sex.*

Table 3 Female Sex and BMI are Associated with Markers of Inflammation

		Male (N=242)	Female (N=53)	p-value	BMI Spearman	BMI p-value
IL-6 (pg/mL)	*Median*	1.38	2.03	**0.002**	0.3	**<0.001**
	Q1 - Q3	0.88 - 1.96	1.13 - 2.98			
IP-10 (pg/mL)	*Median*	120	128	0.7	0.09	0.13
	Q1 - Q3	84 - 164	93 - 157			
neopterin (nM)	*Median*	9.27	8.7	0.49	0.01	0.84
	Q1 - Q3	7.32 - 11.07	6.95 - 11.45			
sCD163 (ng/mL)	*Median*	522	580	**0.05**	0.11	0.07
	Q1 - Q3	381 - 732	437 - 916			
sCD14 (ng/mL)	*Median*	1893	2175	**0.023**	-0.02	0.74
	Q1 - Q3	1,431 - 2,406	1,663 - 2,530			
TNF (pg/mL)	*Median*	1.97	1.72	0.32	0.05	0.4
	Q1 - Q3	1.16 - 3.38	1.08 - 2.91			
%CD4⁺CD38⁺HLA-DR⁺	*Median*	3.9	3.3	0.09	-0.18	0.08
	Q1, Q3	3.00, 5.50	2.20, 4.50			
	N	77	21			
%CD4⁺Ki-67⁺	*Median*	0.6	0.5	0.26	-0.12	0.26
	Q1, Q3	0.50, 1.00	0.40, 0.70			
	N	77	21			
%CD4⁺PD-1^hi	*Median*	39.05	49.8	0.22	-0.01	0.93
	Q1, Q3	18.20, 57.25	22.10, 69.35			
	N	72	20			

Table 3 *compares males to females, or BMI, to cell-surface markers and soluble markers of inflammation. IL-6, sCD163, and sCD14 were higher in females (p=0.002, 0.05, and 0.02, respectively) and BMI (N=285) was associated with IL-6 (p<0.001).*