Menopause Is Associated With Immune Activation in Women With HIV

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Background. Persistent immune activation due to gut barrier dysfunction is a suspected cause of morbidity in HIV, but the impact of menopause on this pathway is unknown.

Methods. In 350 women with HIV from the Women's Interagency HIV Study, plasma biomarkers of gut barrier dysfunction (intestinal fatty acid binding protein; IFAB), innate immune activation (soluble CD14 and CD163; sCD14, sCD163), and systemic inflammation (interleukin-6 and tumor necrosis factor receptor 1; IL-6, TNFR1) were measured at 674 person-visits spanning ≤2 years.

Results. Menopause (post- vs premenopausal status) was associated with higher plasma sCD14 and sCD163 in linear mixed-effects regression adjusting for age and other covariates (β = 161.89 ng/mL; 95% confidence interval [CI], 18.37–305.41 and 65.48 ng/mL, 95% CI, 6.64–124.33, respectively); but not with plasma IFAB, IL-6, or TNFR1. In piece-wise linear mixed-effects regression of biomarkers on years before/after the final menstrual period, sCD14 increased during the menopausal transition by 250.71 ng/mL per year (95% CI, 16.63–484.79; $P = .04$), but not in premenopausal or postmenopausal periods.

Conclusions. In women with HIV, menopause may increase innate immune activation, but data did not support an influence on the gut barrier or inflammation. Clinical implications of immune activation during menopausal transition warrant further investigation.

Keywords. HIV; immune activation; inflammation; menopause; soluble CD14; soluble CD163.

Human immunodeficiency virus (HIV) infection leads to persistent immune activation and inflammation even with antiretroviral therapy (ART), which may contribute to higher risk of non-AIDS–related conditions (eg, cardiovascular disease [CVD], cancer) [1]. There are several hypothesized causes of immune activation in HIV, including low-level viral replication, coinfections, lymphoid fibrosis, and microbial translocation [2]. Microbial translocation is a process in which damage to the gut epithelial barrier, caused by HIV infection, allows microbial products to translocate from gut to circulation, activating

an innate immune response [3]. Gut barrier dysfunction and innate immune activation have been associated with CVD and mortality in treated HIV infection [4–10]. Understanding drivers of immune activation and inflammation in HIV is important for prevention of non-AIDS–related diseases [11].

A recent report of 65 women without HIV found that biomarkers of gut barrier dysfunction, microbial translocation, and immune activation increased during the menopausal transition [12]. Menopause marks the end of the reproductive phase of a woman's life, when complete depletion of ovarian follicles leads to loss of ovarian production of sex hormones estradiol and progesterone [13]. Both hormones appear important for gut barrier integrity [14–18], and estradiol also has well known cardioprotective properties [19–21]. In the context of HIV infection, estradiol and progesterone can inhibit HIV replication and are thought to maintain HIV latency [22, 23]. Women with HIV have lower estradiol and ovarian reserve [24, 25], higher levels of microbial translocation and immune activation [26], and higher cardiovascular risks [27–29] than women without HIV, suggesting that menopause-related decline in sex

hormones may pose unique morbidity risks. However, the effect of the menopausal transition on microbial translocation, immune activation, and inflammation is unknown in women with HIV.

Here, we examined the association of menopause status with plasma biomarkers of gut barrier dysfunction, innate immune activation, and systemic inflammation in women with HIV from the Women's Interagency HIV Study (WIHS). Additionally, we explored changes in these biomarkers over time relative to the final menstrual period, to understand effects of the menopausal transition above those of chronological aging alone [30].

METHODS

Study Population

The WIHS was a multicenter cohort of women with and without HIV in the United States that collected clinical, demographic, and behavioral data semiannually through interviews, physical examinations, and laboratory tests [31]. The present study is nested within a WIHS substudy [32, 33] that measured plasma biomarkers in women with HIV on ART from 10 sites (Bronx, NY; Brooklyn, NY; Washington, DC; San Francisco, CA; Chicago, IL; Chapel Hill, NC; Atlanta, GA; Miami, FL; Birmingham, AL; and Jackson, MI), without hepatitis B surface antigenemia or active hepatitis C virus infection at the time of enrollment into WIHS, and without self-reported cancer or autoimmune disease [33]. Biomarkers were measured on annual (longitudinal) samples collected from 2013 to 2015 for women from the northern sites (Bronx, Brooklyn, DC, San Francisco, Chicago; up to 3 samples per woman); or on a single sample collected in 2015 for women from the southern sites (Chapel Hill, Atlanta, Miami, Birmingham, Jackson). Thus, the number of samples per participant ranged from 1 to 3. Exclusions were performed at the level of the person-visit: we excluded person-visits with pregnancy, hormone replacement therapy in the past 6 months, hormonal contraceptive use during postmenopause, uncertain menopause status, and person-visits following a hysterectomy or oophorectomy that precluded a natural menopause (further description below). A total of 674 person-visits from 350 women contributed to the analysis (171, 34, and 145 participants provided 1, 2, and 3 person-visits, respectively). This research was approved by the Institutional Review Boards of all WIHS sites, and was conducted in accordance with the Declaration of Helsinki. Participants provided written informed consent.

Definition of Menopause Status

We used each participant's longitudinal survey history since their enrollment in the WIHS to identify if/when they reached their natural final menstrual period, and accordingly classify women as premenopausal or postmenopausal at each WIHS visit. We also used the longitudinal history to identify if/when a participant had a surgical menopause, to exclude visits after surgical menopause from analysis. Menopause status was

assessed semiannually with: "Have you been through menopause (the change of life)?" defined to participants as not having menstruated for 12 or more months. For participants who were postmenopausal at their most current visit, we obtained the date of the final menstrual period from the "first day of most recent period" at their last premenopausal study visit; or, if the date was missing, from the midpoint date of the time interval in which they had their last period (ie, within last month, 1–3 months, 3–6 months, etc.); or if that was also missing, we used the date of the last premenopausal visit. All visits prior to the final menstrual period were considered premenopausal, while all visits after the final menstrual period (ie, including the final premenopausal visit) were considered postmenopausal. For participants who were premenopausal at their most current visit, final menstrual period was not captured and all prior visits were considered premenopausal. If a woman reported surgery (hysterectomy or oophorectomy), all visits after the surgery date or the visit when surgery was first reported were coded as missing menopause status, unless the surgery occurred at least 2 years after a natural final menstrual period was captured. Lastly, if a woman reported menopause and later reported being premenopausal, visits between the first report of menopause and the last reported premenopausal visit were coded as missing due to uncertainty. Person-visits missing menopause status, as defined here, were excluded from analysis.

Covariate Data

Variables considered for inclusion in statistical models are shown in Table 1. Covariates were allowed to vary by person-visit within participants, excepting static variables (eg, race).

Biomarker Measurement

Enzyme-linked immunosorbent assays (R&D Systems) were used to assess plasma biomarkers of gut barrier dysfunction (intestinal fatty acid binding protein; IFAB), innate immune activation (soluble CD14 and CD163; sCD14, sCD163), and systemic inflammation (interleukin-6 and tumor necrosis factor receptor 1; IL-6, TNFR1) at Columbia University's Irving Institute for Clinical and Translational Research, Biomarkers Core lab. All samples were stored at −80°C prior to analysis. Assays were performed in duplicate with positive and negative controls on each plate. Six samples below lower limit of detection (LOD) for sCD14 were assigned a value of LOD/2. Five samples above upper limit of detection for sCD163 were assigned the upper limit value. Two samples missing IL-6 and TNFR1 were excluded from analysis of these outcomes.

Statistical Analysis

General Principles

Longitudinal stability of biomarkers was assessed using intraclass correlation coefficients [34]. Biomarkers with right skew (IFAB, IL-6, TNFR1) were log transformed for regression analyses. Characteristics were compared between pre- and postmenopausal women at the first biomarker visit (ie, baseline

Table 1. Characteristics of the Study Participants by Menopause Status[a]

Characteristic	Premenopause (n = 257)	Postmenopause (n = 93)	Standardized Mean Difference
Age, y, mean (SD)	40.4 (6.4)	53.0 (4.5)	2.27
Race, %			0.22
Black/African-American	70.0	60.2	
White	9.3	15.1	
Other or multiracial	20.6	24.7	
Hispanic, %	9.7	16.1	0.19
Country of birth, %			0.30
United States	77.4	82.8	
Puerto Rico or other US territories	0.4	3.2	
Other countries	22.2	14.0	
Education, %			0.21
Less than high school	27.2	33.3	
Completed high school	28.0	32.3	
Some college	33.9	25.8	
Completed college or any graduate school	10.9	8.6	
Employed, %	44.7	38.7	0.12
Alcohol use, %			0.35
Abstainer	47.1	63.4	
>0–7 drinks/wk	44.4	29.0	
>7–12 drinks/wk	3.9	4.3	
>12 drinks/wk	4.7	3.2	
Cigarette smoking history, %			0.35
Never smoker	49.0	32.3	
Current smoker	28.0	36.6	
Former smoker	23.0	31.2	
Drug use, %[b]	16.3	20.4	0.11
Hormonal contraceptive use, %	13.2	0	0.55
HIV load, copies/mL, mean (SD)	4394.3 (26 608.4)	211.0 (1313.8)	0.22
Undetectable HIV load, %	76.7	83.9	0.18
CD4 count, cells/mm^3, mean (SD)	648.9 (307.7)	663.8 (338.2)	0.05
CD4 count ≥500 cells/mm^3, %	68.9	62.4	0.14
BMI, kg/m^2, mean (SD)	33.2 (9.7)	30.9 (8.0)	0.25
BMI category, %			0.25
Underweight or healthy weight, <25 kg/m^2	16.0	23.7	
Overweight, ≥25 and <30 kg/m^2	26.1	30.1	
Obese, ≥30 kg/m^2	58.0	46.2	
Diabetes, %[c]	10.9	19.4	0.24
Hypertension, %[d]	31.1	53.8	0.47

Abbreviations: BMI, body mass index; HIV, human immunodeficiency virus.

[a]Data are presented for the first visit with biomarker data. Missing data for covariates were imputed based on the immediately prior study visit with the data, if available (missingness was <1% for all covariates).

[b]Injected or noninjected drug use including marijuana.

[c]Ever any fasting glucose ≥126 mg/dL, hemoglobin A1C ≥6.5%, self-reported diabetes, or diabetes medication.

[d]Systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, self-report, or use of antihypertensive medication.

visit) using standardized mean differences. All analyses were conducted using R 4.0.3.

Association of Menopause Status With Biomarker Outcomes

We used linear mixed effects models (lme4 package, R) to examine the association of menopause status (post- vs premenopause) with the continuous biomarker outcomes: log(IFAB), sCD14, sCD163, log(IL-6), and log(TNFR1). A random intercept was included to account for within-subject correlation. We developed nested models to serially adjust for potential confounders: model 1, unadjusted; model 2, age only; model 3, age, race, ethnicity, hormonal contraceptive use, HIV load, and CD4 count; model 4–model 3, variables plus any variables associated with menopause status (standardized mean difference >0.1). Because pre- and postmenopausal women differ in age, we also ran these models in a sensitivity analysis, in a subgroup of pre- and postmenopausal person-visits matched on age. We used nearest neighbor matching of the propensity score, with a caliper of 1 standard deviation (MatchIt package, R).

Biomarker Outcomes Relative to Time of Final Menstrual Period

The purpose of this analysis was to differentiate whether biomarkers increase at a constant rate over time, or accelerate in increase during the menopausal transition, suggesting an effect of ovarian versus chronological aging. We used a previously described [30] 2-stage approach in women with an observed final menstrual period, involving: (1) selection of knots from nonparametric locally weighted scatter plot smoothing curves of each biomarker trajectory over time since final menstrual period, and (2) piece-wise mixed effects linear regression with fixed knots to estimate biomarker change during each trajectory phase. In more detail, inflection points associated with increase or decrease of biomarkers around the final menstrual period were chosen visually from smoothing curves; or, if no obvious inflections were observed, default knots of 1 year before/after the final menstrual period were used based on previous literature [30]. Using these knots, time was divided into 3 segments: premenopausal, menopausal transition, and postmenopausal segments. Next, we fit 2 models for each biomarker as a function of time since final menstrual period: a linear mixed effects model, which assumes a constant slope across the 3 time segments; and a piece-wise mixed effects linear model, which allows slope to differ across the segments. The model does not force the 3 segments to connect, allowing more flexibility to accommodate distinct a priori stages of women's reproductive life (although goodness of fit was similar in connected models). A random intercept was included. We considered models unadjusted for covariates, as well as adjusted for age at final menstrual period, race, ethnicity, hormonal contraceptive use, HIV load, CD4 count, and other variables associated with menopause status (standardized mean difference >0.1). The Akaike information criterion (AIC) was used to assess model fit, and the likelihood ratio test (LRT) was used to compare nested models (linear vs piece-wise). For interpretation, a similar biomarker change (slope) across all 3 time segments was considered consistent with chronological aging, but a difference in slope across segments, steeper during the menopausal transition compared to pre- or postmenopausal segments, was considered consistent with changes driven by ovarian aging [30]. For visualization, we plotted annual biomarker means over time before/after the final menstrual period, alongside estimates from piece-wise linear models.

RESULTS

Participant Characteristics

Of 350 women included in this analysis, 257 were premenopausal and 93 were postmenopausal at the baseline biomarker visit, with 8 switching from pre- to postmenopausal status over the 2 year follow-up of the biomarker study. Average age was 40 and 53 years for pre- and postmenopausal women, respectively. The majority of women identified as black (67%), were born in the United States (79%), and were obese (55%). All were on ART, with 79% virally suppressed and 67% with normal CD4 cell count ($\geq$500 cells/mm^3) at the baseline biomarker visit (Table 1). Women with 2–3 biomarker samples differed from women with only 1 sample on demographic characteristics, as expected given differences between northern and southern study sites; however, the proportion of postmenopausal women was the same (Supplementary Table 1). Within women providing longitudinal samples over up to 3 visits spanning up to 2 years, consistency was good for sCD163, moderate for IFAB, and poor for sCD14, IL-6, and TNFR1 (Table 2).

Association of Menopause Status and Biomarker Outcomes

For this analysis, we included all women providing at least 1 (but up to 3) person-visits with menopause and biomarker data (up to 350 women; up to 674 person-visits). Menopause status was associated with plasma sCD14 and sCD163 in linear mixed effects models adjusted for age, race, ethnicity, hormonal contraceptive use, HIV load, CD4 cell count, country of birth, educational attainment, employment status, alcohol use, smoking status, drug use, body mass index (BMI), diabetes, and hypertension (Table 3). Postmenopausal women had an estimated 161.89 ng/mL (95% CI, 18.37–305.41) and 65.48 ng/mL (95% CI, 6.64–124.33) higher plasma sCD14 and sCD163, respectively, than premenopausal women (P = .03 for both). A significant association of menopause status with plasma IFAB was attenuated upon adjustment for age, while menopause status was not associated with plasma IL-6 or TNFR1 in unadjusted or adjusted models (Table 3). Results were similar in a sensitivity analysis matching pre- and postmenopausal person-visits on age (Supplementary Table 2), despite reduced sample size due to exclusion of person-visits that could not be matched on age. Results were also similar in sensitivity analyses excluding premenopausal person-visits for women that later had surgical menopause, or excluding premenopausal person-visits for women who did not have a menstrual period within the last 3 months (Supplementary Tables 3 and 4). Interestingly, the association of menopause with sCD14 was only observed among women with controlled HIV (undetectable viral load and CD4 count $\geq$ 500 cells/mm^3; P interaction = .03; Supplementary Table 5).

Table 2. Intraclass Correlation Coefficients for Biomarkers of Gut Barrier Dysfunction, Innate Immune Activation, and Inflammation, Across up to 2 Years With up to 3 Visits per Participant

Biomarker	n Women (n Person-Visits)	Intraclass Correlation Coefficient (95% CI)
log(IFAB, pg/mL)	350 (674)	0.57 (.49–.64)
sCD14, ng/mL	350 (674)	0.22 (.11–.32)
sCD163, ng/mL	350 (674)	0.81 (.77–.84)
log(IL-6, pg/mL)	349 (672)	0.20 (.10–.31)
log(TNFR1, pg/mL)	350 (672)	0.35 (.25–.44)

Abbreviations: CI, confidence interval; IFAB, intestinal fatty acid binding protein; IL-6, interleukin 6; TNFR1, tumor necrosis factor receptor 1.

Table 3. Association of Menopause Status (Post- vs Premenopause) With Biomarkers of Gut Barrier Dysfunction, Innate Immune Activation, and Inflammation in Linear Mixed Effects Models

Model	log(IFAB, pg/mL)		sCD14, ng/mL		sCD163, ng/mL		log(IL-6, pg/mL)		log(TNFR1, pg/mL)	
	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P	β (95% CI)	P
1	0.29 (.15 to .42)	<.01	175.08 (64.86 to 285.3)	<.01	36.46 (−12.99 to 85.9)	.15	0.09 (−.05 to .24)	.20	0.06 (−.02 to .13)	.12
2	0.09 (−.08 to .27)	.30	146.45 (−2.19 to 295.1)	.05	50.69 (−10.85 to 112.23)	.11	−0.04 (−.23 to .15)	.71	0.04 (−.06 to .14)	.40
3	0.10 (−.08 to .28)	.27	175.25 (30.21 to 320.28)	.02	55.94 (−3.07 to 114.95)	.06	−0.03 (−.22 to .16)	.75	0.05 (−.04 to .15)	.28
4	0.07 (−.10 to .25)	.41	161.89 (18.37 to 305.41)	.03	65.48 (6.64 to 124.33)	.03	−0.01 (−.18 to .17)	.93	0.06 (−.04 to .15)	.24

Abbreviations: BMI, body mass index; CI, confidence interval; HIV, human immunodeficiency virus; IFAB, intestinal fatty acid binding protein; IL-6, interleukin 6; TNFR1, tumor necrosis factor receptor 1.

All models include a random intercept. Model 1 is unadjusted for covariates; model 2 adjusts for age only; model 3 adjusts for age, race, ethnicity, hormonal contraceptive use, HIV load, and CD4 cell count; model 4 adjusts for all variables in model 3 plus country of birth, alcohol use, smoking status, drug use, educational attainment, employment status, BMI, diabetes, and hypertension. Number of unique IDs and person-visits, respectively, included in each model are: IFAB, sCD14, sCD163 (350, 674), IL-6 (349, 672), TNFR1 (350, 672). β represents difference in each outcome for post- compared to pre-menopausal women.

### Association of Time Before/After the Final Menstrual Period With Biomarker Outcomes

For this analysis, we included a subset of 126 women who had a prospectively observed final menstrual period (ie, gone through the menopausal transition) during their participation in the WIHS, which ranged from 3 to 25 (median 17) years; these women provided 286 biomarker person-visits. Premenopausal women in this subset tended to be older than those in the full dataset (Supplementary Table 6), and there was a strong correlation between age and time before/after the final menstrual period ($r = 0.66$, $P < .0001$). Nonparametric locally weighted scatter plot smoothing curves suggested that the change (slope) of plasma sCD14 and sCD163 over time relative to the final menstrual period, may differ during the menopausal transition compared with pre- or postmenopausal periods (Figure 1). Inflection points (knots) for piece-wise linear mixed effects models were chosen visually for sCD14 (0.5 years before/1.7 years after final menstrual period) and sCD163 (0.5 years before/1.5 years after final menstrual period). Default points of 1 year before/after the final menstrual period [30] were used for plasma IFAB, IL-6, and TNFR1 due to lack of obvious change in slope around the final menstrual period.

In piece-wise linear mixed effects models, we observed a significant increase in sCD14 during the menopausal transition (within 0.5 years before and 1.7 years after the final menstrual period); for each year in this period, sCD14 increased by 250.71 ng/mL (95% CI, 16.63–484.79; $P = .04$). The slope of sCD14 in the menopausal transition period differed from the premenopausal (>0.5 years before final menstrual period) and postmenopausal (>1.7 years after final menstrual period) periods (Table 4 and Figure 2). Additionally, the piece-wise linear model for sCD14 provided a marginally better fit than a linear model (piece-wise AIC = 4420, linear AIC = 4443, LRT $P = .06$). Results were similar adjusting for covariates (Table 4). For other outcomes (plasma IFAB, sCD163, IL-6, and TNFR1), we did not observe associations with time before/after the final menstrual period in piece-wise models.

DISCUSSION

In this study of women with HIV, we observed higher levels of innate immune activation, measured by plasma sCD14 and sCD163, in postmenopausal compared to premenopausal women. Additionally, our results suggested that plasma sCD14 increases specifically during the menopausal transition, consistent with changes driven by ovarian rather than chronological aging. However, menopause was not associated with IFAB, a biomarker of gut barrier dysfunction, or IL-6 and TNFR1, biomarkers of systemic inflammation. Due to the short follow-up in this study of up to 2 years, we did not have sufficient sample size to explore within-woman change in biomarkers as women transition from pre- to postmenopause—only 8 women transitioned during follow-up. Nevertheless, our results suggest a risk of increased immune activation during the menopausal transition in women with HIV.

Plasma sCD14 and sCD163 are nonspecific markers of monocyte and macrophage activation [35, 36]. Functioning as cell surface receptors, CD14 and CD163 are released upon ligand binding and cell activation, resulting in their soluble forms. CD14 binds lipopolysaccharide (LPS), a cell wall component of gram-negative bacteria, as well as other bacterial and endogenous compounds [35]. Additionally, inflammatory cytokines IL-6 and IL-1β induce release of sCD14 [35], and sCD14 has been recognized as an acute phase protein [37]. CD163 is a receptor for hemoglobin-haptoglobin complexes, and also binds gram-positive and gram-negative bacteria [36]. Thus, sCD14 and sCD163 may indicate microbial translocation, but could also relate to other homeostatic or inflammatory processes. sCD14 and sCD163 have been associated with CVD risk [7–10] and mortality [4–6] in HIV, suggesting that menopause-related increases in sCD14 and sCD163 may have clinical consequences. For example, the magnitude of increase in sCD14 we observed per year during the menopausal transition is comparable to the difference in sCD14 observed between HIV patients who died and controls in a mortality study [5]. However, the observed increase in sCD14 during the menopausal transition did not appear to be maintained postmenopause—in our piece-wise
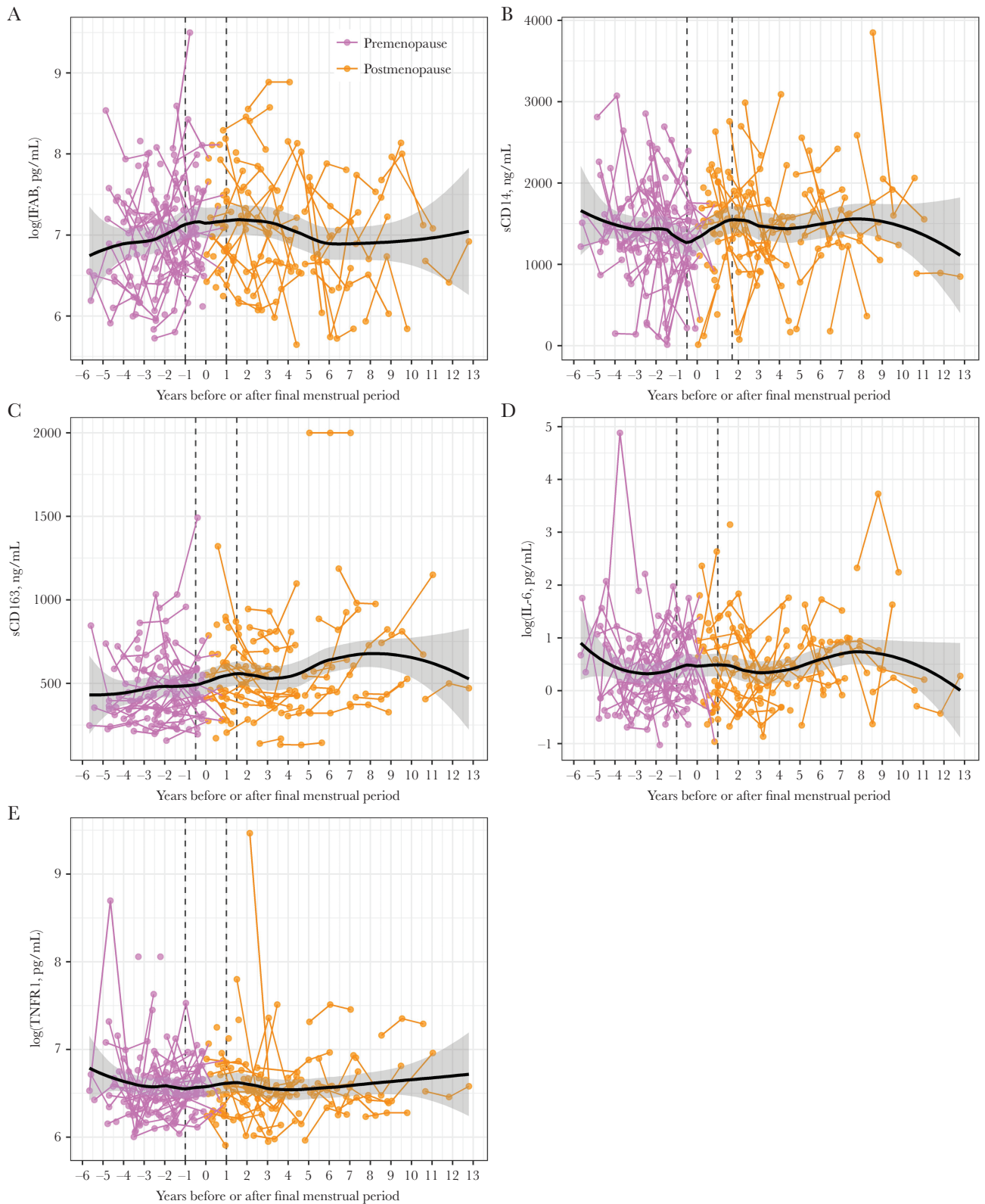
Figure 1. Scatter plots of gut barrier dysfunction, innate immune activation, and inflammation biomarkers over years before/after the final menstrual period. In scatter plots of (*A*) log(IFAB), (*B*) sCD14, (*C*) sCD163, (*D*) log(IL-6), and (*E*) log(TNFR1), lines connect points from the same woman, and points/lines are colored according to menopause status. Plots are overlaid with nonparametric locally weighted smoothing curves, shown with black curves and 95% confidence intervals. Inflection points used in piece-wise linear mixed effects models are shown with dashed vertical lines; points were visually chosen for sCD14 and sCD163, while default points of 1 year before and 1 year after the final menstrual period were used for IFAB, IL-6, and TNFR1 due to lack of obvious change in slope around the final menstrual period. Number of unique identities and person-visits, respectively, included in each plot are: IFAB, sCD14, and sCD163, 350, 674; IL-6, 349, 672; and TNFR1, 350, 672. Abbreviations: IFAB, intestinal fatty acid binding protein; IL-6, interleukin 6; sCD14, soluble CD14; sCD163, soluble CD163; TNFR1, tumor necrosis factor receptor 1.

Table 4. Association of Years Before/After Final Menstrual Period With sCD14 (ng/mL) in Linear and Piece-Wise Linear Mixed Effects Models,[a] to Distinguish Effects of Chronological and Ovarian Aging

	Unadjusted			Multivariate Adjusted		
	β (95% CI)	P Value	AIC	β (95% CI)	P Value	AIC
Linear model	4.92 (−37.48 to 47.31)	.82	4443.21	0.58 (−44.04 to 45.20)	.98	4241.58
Piece-wise model[b]	…		4420.31	…		4216.18
Slope in segment 2, menopausal transition	250.71 (16.63 to 484.79)	.04	…	300.13 (56.75 to 543.51)	.02	…
Slope difference, segment 1 vs 2	−303.80 (−557.27 to −50.34)	.02	…	−375.54 (−640.18 to −110.90)	.01	…
Slope difference, segment 3 vs 2	−242.19 (−483.18 to −1.20)	.05	…	−294.04 (−545.14 to −42.93)	.02	…
LRT[c]	…	.06	…	…		0.01

Abbreviations: AIC, Akaike information criterion; LRT, likelihood ratio test.

[a]All models include a random intercept. Linear model includes terms for time since final menstrual period and period; piece-wise model includes terms for time since final menstrual period, period, and time*period. β represents change in sCD14 (ng/mL) per year. Adjusted model adjusts for age at final menstrual period, race, ethnicity, hormonal contraceptive use, HIV load, CD4 cell count, country of birth, alcohol use, smoking status, drug use, educational attainment, employment status, BMI, diabetes, and hypertension. Included in each model were 126 unique IDs and 286 person-visits.

[b]The piece-wise model allows for different slopes in 3 time segments: segment 1 (over 0.5 years before final menstrual period); segment 2, ie, menopausal transition (within 0.5 years before and 1.7 years after final menstrual period); and segment 3 (over 1.7 years after final menstrual period).

[c]LRT comparing the piece-wise and linear model.

linear regression, the trajectory in the postmenopausal period did not align with the accelerated trajectory during the menopausal transition (Figure 2); and, although sCD163 was elevated in post- compared to premenopausal women, the increase during the menopausal transition was not statistically significant in our piece-wise models. The menopausal transition itself is increasingly recognized as a pivotal time for CVD prevention [21]. Whether the sharp but fleeting observed increase in

sCD14 during the menopausal transition in women with HIV is important for disease risk will need to be evaluated in future research.

A plausible mechanism for the observed menopause-related increase in innate immune activation may be preventive effects of sex hormones on gut permeability and microbial translocation. Experimental evidence indicates that estrogen and progesterone maintain the gut barrier [14–18], and in human studies
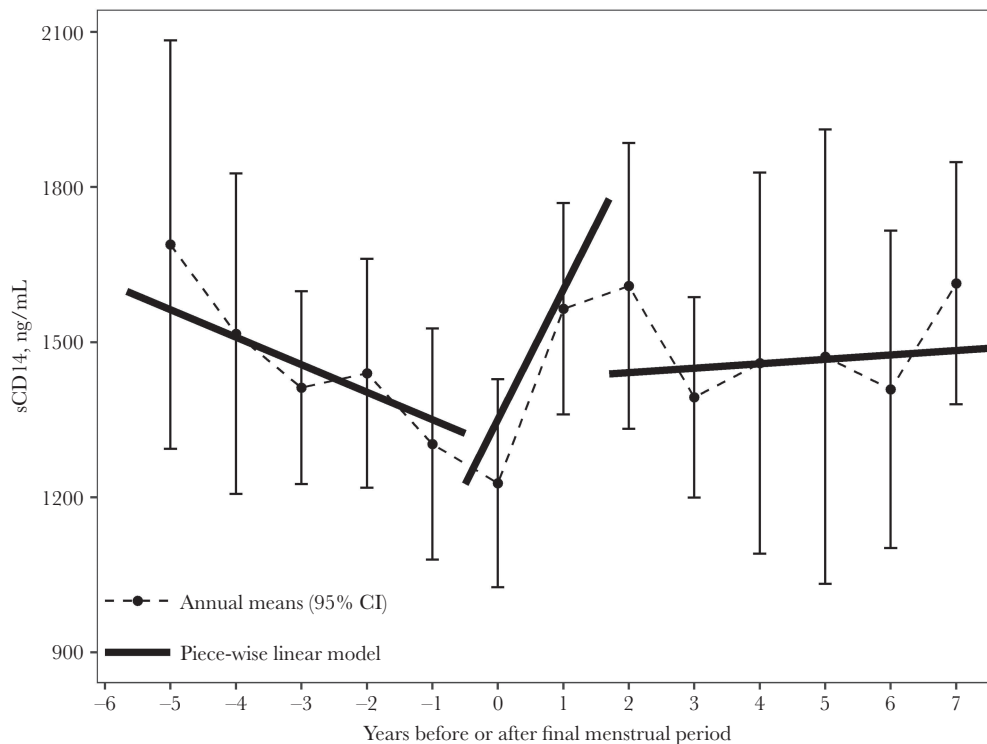
Figure 2. Means of sCD14 (ng/mL) in years before/after the final menstrual period. Estimates from unadjusted piece-wise linear mixed effects model (described in Table 4) are overlaid on the plot. Data include 126 unique identities and 286 person-visits. Abbreviations: CI, confidence interval; sCD14, soluble CD14.

progesterone has been inversely correlated with microbial translocation [38, 39]. Thus, declines in estrogen and progesterone during the menopausal transition could impair the gut barrier and increase microbial translocation and immune activation. Only 1 study has examined the effect of menopause on the gut barrier, microbial translocation, and immune activation: in 65 women without HIV from the Study of Women's Health Across the Nation (SWAN), plasma IFAB, lipopolysaccharide binding protein (LBP), and sCD14 increased significantly within woman from pre- to postmenopause, while lower plasma estradiol was associated with higher IFAB and sCD14 [12]. Although that analysis did not adjust for concurrent age, it suggests that microbial translocation increases over the menopausal transition, related to sex hormone changes. In our analysis, we did not observe an association of menopause with plasma IFAB after adjustment for age. IFAB is a cytosolic protein in enteroctyes, found to a greater extent in the small intestine than the colon, that is released upon gut epithelial damage [40]. Sex hormones may modulate the gut barrier via other mechanisms not captured by the IFAB biomarker, such as through maintenance of tight junctions [41]. Thus, an effect of menopause on gut barrier dysfunction cannot be ruled out here.

Aside from effects of menopause on the gut barrier, there may be other mechanisms for menopausal effects on immune activation in HIV. Estradiol and progesterone can inhibit HIV replication and regulate HIV latency [22, 23], suggesting that HIV reactivation may occur during menopause and cause immune activation. The effect of menopause on sCD14 in our study was only observed in women with controlled HIV, suggesting that HIV control may be an important effect modifier although the mechanism is unclear. Estrogens also interact with the gut microbiota [42], which in turn may modulate host immunity in HIV [43, 44]. In a previous study in WIHS we observed that postmenopausal status in women with HIV was associated with increased Enterobacteriales [45], which may preferentially translocate from the gut to the circulation in HIV infection [46], and lead to immune activation [44]. Research incorporating the gut microbiome and biomarkers of microbial translocation will assist with connecting these factors during menopause.

We did not find an association of menopause with biomarkers of systemic inflammation: IL-6, an inflammatory cytokine, and TNFR1, receptor for the cytokine tumor necrosis factor-α (TNF-α). Prior studies in women without HIV have observed increases in inflammatory cytokines, including IL-6 and TNF-α, in post- compared to premenopausal women, although not always [47–49]. Our observation of menopause-related increases in immune activation, but not inflammation, obscures the consequences of our findings, as inflammation is typically a direct consequence of immune activation. Alternatively, it is possible that IL-6 and TNFR1 are not ideal biomarkers to capture an effect of menopause on inflammation in the context of women with HIV.

This study was strengthened by the large sample size, longitudinal data collection, and the well-characterized WIHS cohort providing detailed information on prospectively ascertained menopause and other covariates. We also utilized best statistical practices from the leading menopausal transition cohort study (SWAN) [30] to assess biomarker changes during the menopausal transition. We were limited by the short follow-up period (up to 2 years) for this biomarker study, which prevented us from having sufficient person-visits to determine within-woman changes as women shift from pre- to postmenopause. We also did not have available data on other biomarkers of gut barrier dysfunction and microbial translocation (eg, tight junction biomarkers, LPS, or LBP), which have been studied. Lastly, we did not have hormonal measures of menopause and were limited by self-report data of menopause and last menstrual period, which could result in misclassification.

In summary, postmenopausal women with HIV had evidence of increased innate immune activation relative to premenopausal women, with this increase occurring during the menopausal transition. However, menopause was not associated with increases in key markers of systemic inflammation, making the consequences of the menopause-related immune activation unclear. Additional longitudinal research following women with HIV through the pre- and postmenopausal stages is necessary to explore whether women who experience an increase in immune activation during menopause also experience increased inflammation and other disease risks. Compounded effects of HIV, aging, and menopause on immune activation could put aging women with HIV at particularly high risk of non-AIDS–related disease and mortality. Thus, research on the health of women with HIV during and after the menopausal transition is of paramount importance, including clinical research on the effectiveness of interventions (eg, health monitoring, lifestyle modifications, hormone therapy) to reduce disease risk.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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