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ORIGINAL ARTICLE

Longitudinal analysis of cytokines, chemokines, and inflammatory markers in a middle-aged cohort

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Abstract Systemic chronic low-grade inflammation increases with aging and contributes to the risk or progression of a myriad of chronic diseases. Greater midlife inflammation has detrimental effects on future health outcomes. However, few studies have quantified inflammatory markers from midlife longitudinal measurements. Here, we measured cytokines, chemokines, and clinical biomarkers of inflammation at three different time points in a cohort of middle-aged (mean age 48) African American and White men and women. We analyzed longitudinal data for these inflammatory markers examining possible interactions across time, age, race, and sex. We report sex differences in the levels of CXCL10/IP-10, CXCL11/ITAC, and uric acid. Ferritin levels differed by sex and race; the highest levels were in White men and lowest in White women. MCP-1 and WBC count were higher in White participants than African American participants, and uric acid levels were lower for older White participants, but higher for older African American participants. In this longitudinal study, we found that IL-22 levels decreased over time while ferritin levels increased over time. For high sensitivity C-reactive protein (hsCRP), values changed differently over time across sex where men's values increased and women's values decreased over time. In addition, IL-10, CXCL11/ITAC, and hsCRP levels decreased over time in White participants, but not for African American participants. These data indicate that cytokine, chemokine, and clinical inflammatory biomarkers vary across time, age, sex, and race in a middle-aged cohort. Understanding inflammation at midlife may provide keys to reducing negative health outcomes later in life.

Keywords Inflammation · Longitudinal · Chemokines · Cytokines · Aging

Introduction

Aging is a complex process in which the gradual accumulation of cellular and molecular damage progressively impairs tissue and organ function, ultimately heightening vulnerability to disease and increasing the risk of death. Chronic inflammatory conditions are now recognized as major drivers of global mortality [1], with more than half of all deaths believed to be linked to inflammation-related diseases [1, 2]. Evidence suggests that suppressing these inflammatory processes in early or mid-life can curb later morbidity and mortality [3]. This chronic

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inflammatory milieu underpins many age-associated disorders and heightens vulnerability to non-communicable diseases (reviewed in [2]).

With advancing age, multiple factors contribute to a state of low-grade chronic inflammation, termed "inflamm-aging" [4]. These factors include the accumulation of senescent cells, immune system dysregulation, gut microbiome changes, oxidative stress, and persistent activation of innate immunity [5–7]. In particular, senescent cells secrete a myriad of factors into the extracellular space, what is known as the senescence-associated secretory phenotype (SASP) [8]. These SASP components include pro-inflammatory cytokines (such as IL-6, IFN-γ, IL-1β, TNFα), chemokines (such as MCP-1, IL-8), growth factors, proteases, soluble receptors, and various non-protein factors [7–9]. SASP contributes to inflammaging, accelerated aging, and the development of age-related disease.

Evidence suggests that a higher inflammatory state in midlife can contribute to later life morbidity and mortality. For example, systemic inflammation measured using a composite of fibrinogen, white blood cell count, von Willebrand factor, and factor VIII levels, during midlife was associated with cognitive decline later in life [10]. Several cytokines, IL-1β, IL-6, and IL-18, have been reported to be associated with a faster decline in cognitive performance in a middle-aged cohort of African American and White adults [11]. The cytokine IL-6 has been associated with mortality, although the majority of these studies were in older populations and in some cases the data are not consistent [12–15]. The most well-studied clinical biomarker of inflammation is the acute phase protein, high-sensitivity C-reactive protein (hsCRP). hsCRP is widely utilized clinically to assess cardiovascular risk and is also associated with non-vascular and vascular mortality as well as coronary heart disease [16, 17]. Therefore, although there is compelling evidence that some inflammatory markers are associated with future risk for morbidity and mortality, there is limited information about biomarkers that mechanistically are driven by different inflammatory pathways. To bridge this gap in knowledge, we recently assayed a subset of circulating inflammatory proteins in middle-aged African American and White men from the HANDLS cohort [18]. Elevated levels of E-selectin, MCP-1, and P-selectin were linked to an increased risk of mortality. Additionally, a significant interaction was observed between sex and IL-6 in relation to mortality; higher IL-6 levels were associated with greater mortality risk, with this effect being notably stronger in women than in men [18]. Moreover, among White participants, those with high sRAGE levels exhibited significantly better survival compared to those with low sRAGE levels, whereas African American participants showed similar survival rates regardless of sRAGE concentration [18].

Few studies have repeated measures of cytokines and chemokines in longitudinal studies. Recent data from the Bambuí Project, a longitudinal community-based prospective cohort study of aging in Brazil, examined cytokines and chemokines at study enrollment and at 10-year follow-up [19]. In this older cohort (≥ 60 years at enrollment), levels of several chemokines and cytokines increased over the 10-year time period, with TNFα having the most notable differences [19]. In the Doetinchem Cohort Study in the Netherlands, cytokines and chemokines were measured every 5 years over a 20-year time period (baseline age 42–53 years) and several were associated with clinical outcomes at the study endpoint [20]. In a recent longitudinal study in China, cytokines were examined in participants who were cognitively intact at baseline but who developed Alzheimer's disease at the 10-year follow-up (mean baseline age 61.1 years) compared to age- and sex-matched controls (mean baseline age 62.1 years) [21]. Measurements every 2 years for cytokines including IFN-γ, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-22, and TNF-α showed significantly increased values over the 10-year follow-up. Interestingly, for IL-1β and IL-6, the longitudinal changes were more pronounced in the Alzheimer's disease group than in the controls [21]. In a subsample of older adults (≥ 60 years, mean age 76.5 years for men and 78.1 years for women) in the Swedish National Study on Aging and Care in Kungsholmen (SNAC-K), a panel of potential aging biomarkers were assayed at baseline and a 6-year follow-up. Levels of several inflammatory and metabolic markers were altered with age and time [22]. In the Stanford-Ellison Cohort, levels of a broad range of cytokines rose preceding a cancer diagnosis, but only in those aged > 80 years [23]. In the InCHIANTI cohort from Italy, high baseline levels of IL-6 and faster increase overtime in IL-6 were associated with a faster increase in the number of chronic diseases over time in adults aged 60 and older [24]. These data

indicate the importance of examining cytokines and chemokines longitudinally and their relationship with aging and age-related disease. However, the majority of these studies were performed in older adults. Therefore, it is important to address whether there are changes in cytokine and chemokine levels in populations at middle-age that may be at risk for accelerated aging.

In this longitudinal study, we measured cytokines, chemokines, and clinical biomarkers of inflammation at three different time points in a cohort of middle-aged African American and White men and women. We analyzed longitudinal data for these inflammatory markers and examined how age, race, and sex may interact to explain changes in levels over time.

Methods

Study sample

We selected participants from the HANDLS study of the National Institute on Aging Intramural Research Program, National Institutes of Health. HANDLS was initiated in 2004 and is an ongoing, longitudinal study consisting of White and African American adult women and men aged 30–64 years at baseline living in Baltimore, Maryland, USA [25]. Race was self-identified as African American or White. Sex is sex assigned at birth. Participants had household incomes either above or below poverty defined by 125% of the 2004 U.S. Health and Human Services Poverty Guidelines at enrollment [26]. Baseline data (visit 1) were collected between 2004 and 2009 through home visits and physical examinations on medical research vehicles (MRV). Participants visited the MRV for follow-up in-person examinations at visit 2 (2009–2013), visit 3 (2013–2017), and visit 4 (2017–2020). Participants provided written informed consent. The HANDLS study is approved by the Institutional Review Board of the National Institutes of Health.

We selected 68 people who were alive in 2022 and had three fasting serum samples across visits 1–4 (Fig. 1). These samples will be referred to as time 1, 2, and 3. Participants who had HIV were excluded. The cohort was selected so there were not significant differences in the numbers of people across categories of race and sex.

A fasting blood sample was collected in the morning into BD Vacutainer® serum separator tubes. Samples were centrifuged at room temperature at 1142 *g* for 15 min with the brake on. Serum was then aliquoted into cryotubes and stored at − 80 °C until use.

Laboratory clinical assays for blood levels of hsCRP, ferritin, uric acid, and white blood cell (WBC) count were measured by Quest Diagnostics (Nichols Institute, Chantilly, VA, USA).

Cytokine and chemokine assays

Cytokines and chemokines were assayed from serum using the Simoa™ CorPlex ™ Human Cytokine Panel 1 10-Plex Array (IFN-γ, IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-22, TNFα) and Human Chemokine 4-Plex Array (CXCL10/IP-10, ITAC, MCP-1, MIP-3b) on the SP-X platform by Quanterix (Billerica, MA, USA). The cytokine array had a mean intra-assay coefficient of variation (CV) of 4.4% and inter-assay CV of 7.5% (Supplementary Table 1). The chemokine array had a mean intra-assay CV of 2.8% and inter-assay CV of 8.1% (Supplementary Table 1). Values below the limit of quantitation were excluded. Cytokines/chemokines with more than 25% of values below the limit of quantification were excluded from analyses: IFNγ (26%), IL-12p70 (86%), IL-1β (79%), IL-4 (76%), and IL-5 (75%).

Statistics

Test of differences for race used the Pearson Chi-squared test. Test of differences for age used Student's *t*-test. Mixed effects linear regression was employed to account for the three repeated measurements with a random intercept for each participant. Age at time 1 and time elapsed were included in the models as decade units centered at age 50 ((age – 50)/10) and years elapsed since time 1. The following cytokines, chemokines, and lab values were evaluated as dependent variables in the regressions: IL-6, IL-8, IL-10, IL-22, TNFα, CXCL10/IP-10, ITAC, MCP-1, MIP-3b, hsCRP, ferritin, uric acid, and WBC. Backward elimination was used for model selections starting with all possible two-way interactions across race, sex, age at time 1 (decade units), and elapsed time (years since time 1). Interactions that were not significant at a cutoff of $p = 0.05$ were removed but all main

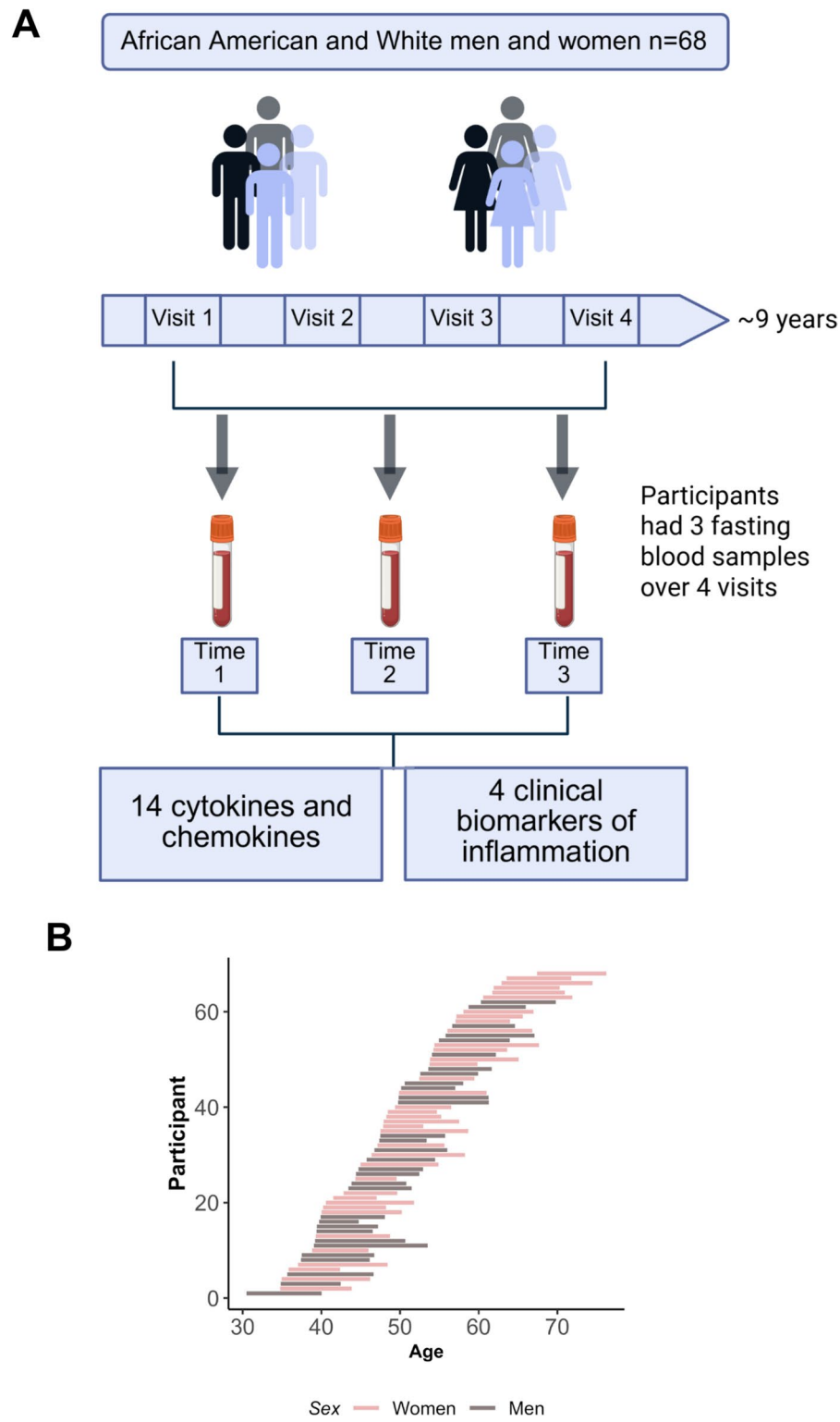


Fig. 1 Schematic of longitudinal study design and cohort demographics. **A** Participants ($n = 68$) chosen for this subcohort had three fasting blood samples at three time points. Chemokines, cytokines, and inflammatory markers were assayed. The figure was created in BioRender. Noren Hooten, N. (2026) https://BioRender.com/259pxj0. **B** Graph indicates the sex and age of each participant included in this study. The start of the line indicates age at time 1, end of the line indicates age at time 3, and length of the line represents time in study

effects were retained. All dependent variables were positively skewed and thus log base 2 transformed for use in the regression models. In keeping with other similar publications [19, 21], statistical significance was considered a p-value < 0.05 and correction for multiple testing was not applied. Analyses were performed in R (version 4.5.1) using packages lme4 and lmerTest [27, 28].

Results

As few studies have examined serum cytokines and chemokines in a longitudinal study, we assayed chemokines, cytokines, and inflammatory markers in 68 people over three time points (Fig. 1A). The cohort did not significantly differ in proportions across race and sex, and mean age at time 1 was similar among men and women in the sample (Table 1). Participants were aged between 30 and 67 years at their first blood sample with time 3 an average of 8.8 years after time 1 (maximum 14.5 years) (Fig. 1B). Of the 14 chemokines and cytokines that we assayed, 5 (IFNγ, IL-12p70, IL-1β, IL-4, IL-5) were excluded for having more than 25% of values below the limit of quantitation. We also included clinical laboratory markers of inflammation: hsCRP, ferritin, uric acid, and WBC. We used mixed effects linear regression accounting for the three timepoints of data to assess the relationship of these cytokines, chemokines, and inflammatory markers with race, sex, age at time 1, and elapsed time (Table 2, Supplementary Table 2). Results from all mixed effects linear regression analyses are reported in Table 2 and Supplementary Table 2. Only significant findings are visualized and described in detail and reported in Figs. 2, 3, 4, and 5.

There were overall differences related to sex across all time points for four inflammatory markers. Women had an overall higher level than the men in the sample for CXCL10/IP-10 ($p = 0.040$, Fig. 2A) and CXCL11/ITAC ($p = 0.002$, Fig. 2B). This pattern was in the opposite direction for uric acid where men had a higher overall level than women ($p < 0.001$, Fig. 2C). For ferritin, values differed based on an interaction between race and sex. Among White participants, men had higher ferritin values than women but values did not differ significantly among African American participants ($p = 0.015$, Fig. 2D).

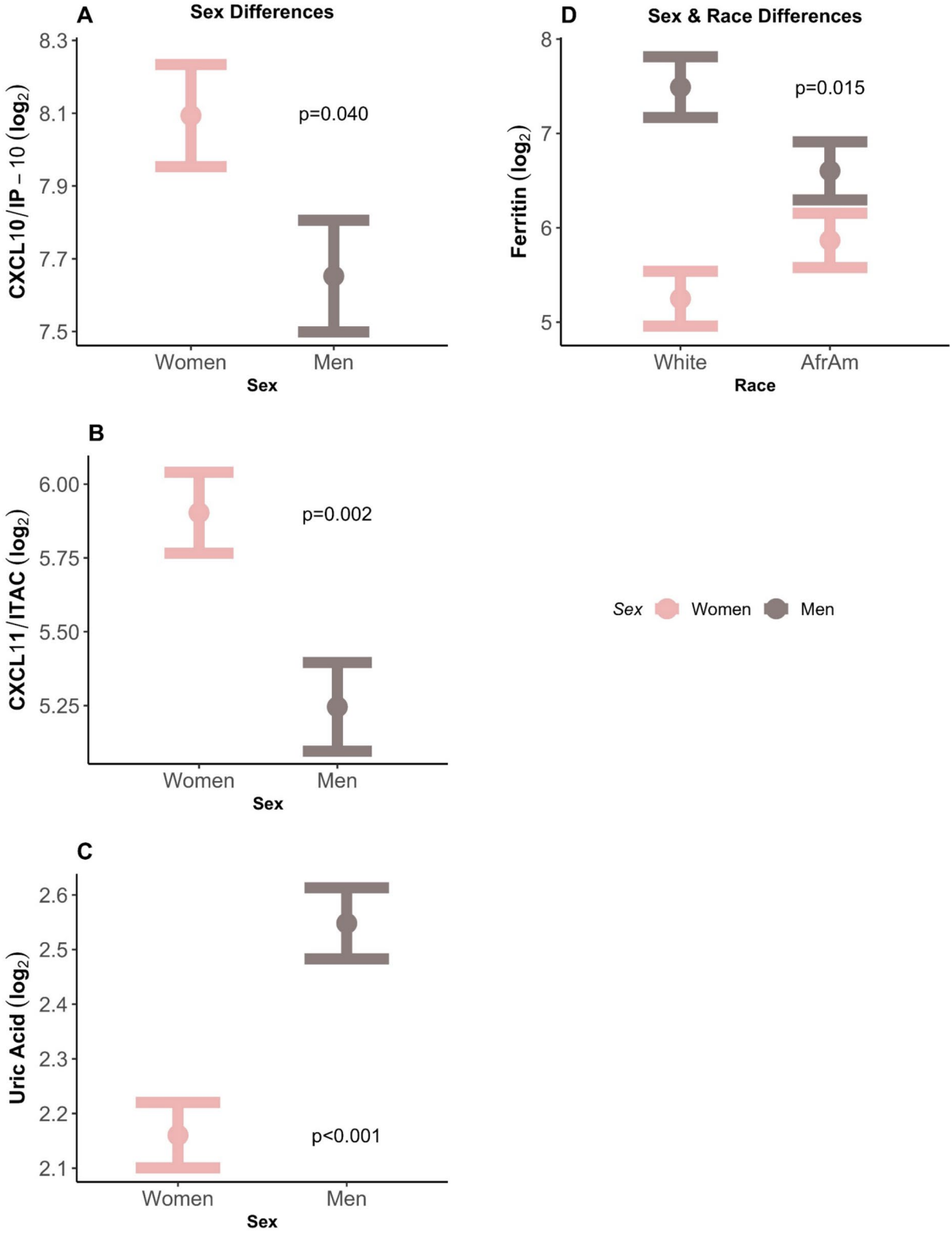
There were also overall differences related to self-identified race across all time points for three markers.

Table 1 HANDLS longitudinal subcohort

Variable	**Total (N=68)**	**Women (N=37)**	**Men (N=31)**	***p*-value**[a]
African American, N (%)	35 (51)	19 (51)	16 (52)	1.000
Age at time 1, years, mean (SD)	47.74 (8.50)	49.27 (8.93)	45.91 (7.71)	0.105
Variable, mean (SD) [missing[b]**]**	**Time 1**	**Time 2**	**Time 3**	
Elapsed time (years)	0	4.82 (1.66)	8.80 (2.02)	
IL-6 (pg/ml)	3.69 (10.7) [1]	3.99 (10.8) [1]	2.93 (3.54)	
IL-8 (pg/ml)	26.34 (19.12)	26.66 (20.76)	27.43 (25.23)	
IL-10 (pg/ml)	1.35 (2.01)	1.16 (1.54)	1.13 (1.73)	
IL-22 (pg/ml)	0.74 (0.44)	0.60 (0.39)	0.65 (0.48) [1]	
TNFα (pg/ml)	2.87 (1.42)	2.63 (1.01)	2.85 (1.53)	
CXCL10/IP-10 (pg/ml)	350.52 (407.81)	327.14 (339.00)	284.32 (227.09)	
CXCL11/ITAC (pg/ml)	68.31 (56.92)	65.83 (56.26)	63.14 (55.32)	
MCP-1 (pg/ml)	1141.06 (472.04)	1131.97 (431.30)	1119.61 (406.97)	
MIP-3b (pg/ml)	130.80 (95.21)	109.45 (63.95)	119.16 (82.92)	
High sensitivity C-reactive protein (mg/l)	6.83 (11.17) [2]	6.05 (7.33)	5.12 (5.41)	
Ferritin (ng/ml)	127.21 (226.96) [1]	146.93 (272.03)	150.34 (230.92)	
Uric acid (mg/dl)	5.26 (1.62) [1]	5.41 (1.47)	5.42 (1.67)	
White blood cell count (count x 10^9/l)	5.95 (2.00) [1]	6.10 (1.98) [1]	5.91 (1.81) [2]	

[a]Test of differences used the Pearson Chi-squared test for race and Student's t-test for age

[b]Brackets are not present if there are no missing observations


A
Sex Differences
CXCL10/IP – 10 (log2)
8.3
8.1
7.9
7.7
7.5
p=0.040
Women
Men
Sex
D
Sex & Race Differences
Ferritin (log2)
8
7
6
5
p=0.015
White
AfrAm
Race
B
CXCL11/ITAC (log2)
6.00
5.75
5.50
5.25
p=0.002
Women
Men
Sex
Sex
Women
Men
C
Uric Acid (log2)
2.6
2.5
2.4
2.3
2.2
2.1
p<0.001
Women
Men
Sex

◂**Fig. 2** Chemokine and inflammatory marker levels differ by sex in longitudinal analysis. Mixed effects linear regression accounting for the three timepoints of data was used to assess the relationship of cytokines, chemokines, and inflammatory markers with age, race, sex, and elapsed time. **A**–**C** Sex differences in chemokine and inflammatory marker levels. **D** Sex and race differences for ferritin levels. The plots show the linear regression values ± standard error of the estimated values. *p*-values for the coefficient are included on the plots. AfrAm = African American

White participants had higher levels of MCP-1 ($p < 0.001$, Fig. 3A) and WBC count ($p = 0.002$, Fig. 3B) than African American participants. For uric acid, values differed by race depending on age at time 1 representing differences in an overall age cohort ($p = 0.002$, Fig. 3C). Among participants who entered the study after age 50, African American participants had higher uric acid values than White participants while this relationship was the opposite for those under 40.

The multiple measurements for all participants provided the opportunity to identify trends over time. IL-22 showed an overall decrease over time for all participants ($p = 0.001$, Fig. 4A) while ferritin showed an overall increase over time ($p < 0.001$, Fig. 4B). For hsCRP, men and women had different trends over time with men showing an increase and women showing a slight decrease ($p = 0.048$, Fig. 4C).

Some markers indicated change over time differentially by race. For IL-10 ($p = 0.007$, Fig. 5A) and CXCL11/ITAC ($p = 0.029$, Fig. 5B), White participants had values decrease over time while those for African American participants did not change. It is noteworthy that for CXCL11/ITAC, African American participants had higher values than White participants at time 1. For hsCRP, African American participants' values increased over time while those of White participants decreased ($p = 0.015$, Fig. 5C).

Discussion

In this longitudinal study, we measured circulating cytokines, chemokines, and clinical biomarkers of inflammation at three different time points in African American and White men and women. We conducted a longitudinal analysis of inflammatory markers, evaluating their changes over time and interactions with demographic variables including age at time 1, race, and sex. We report sex differences in the levels of CXCL10/IP-10, CXCL11/ITAC, and uric acid. Ferritin levels differed by sex and race, where the highest levels were in White men compared to White women and African American men and women. MCP-1 and WBC count were higher in White participants and uric acid levels were lower with age in White participants, but higher with age in African American participants. In our longitudinal study, we were able to analyze trends over time and found that IL-22 levels decreased over time while ferritin levels increased over time. There was an interaction with sex and time where hsCRP values increased over time in men but decreased over time in women. In addition, IL-10, CXCL11/ITAC, and hsCRP levels decreased over time in White participants, but this was not the case for African American participants.

As there are few longitudinal studies of inflammatory markers including cytokines, chemokines, and clinical inflammatory markers, this study sheds light on the changes in these markers in a middle-aged cohort of African American and White men and women. Few aging studies incorporate both African American and White participants in longitudinal studies [14]. Furthermore, as middle-age is critical for future health outcomes, it is important to characterize changes in levels of inflammatory markers during this time period. Other measures of systemic inflammation at midlife, for example, using a composite of fibrinogen, WBC count, von Willebrand factor, and factor VIII levels, have been associated with cognitive decline later in life [10]. Another study showed that cytokines levels of IL-1β, IL-6, and IL-18 at midlife have been reported to be associated with a faster decline in cognitive performance [11]. The midlife time period is critical and may be associated with silent subclinical inflammation prior to the onset of disease. Adult middle-age constitutes a vulnerable window in which subclinical inflammation may develop insidiously, preceding the manifestation of clinical disease. Identification of at-risk individuals along with targeted preventive interventions during middle-age offers a unique opportunity to mitigate inflammatory progression and subsequent health outcomes, including cardiovascular disease, metabolic syndrome, and dementia [29–33]. In addition, higher levels of inflammation are associated with frailty [34], and frailty at midlife is associated with risk of mortality [35]. Therefore, assessment of

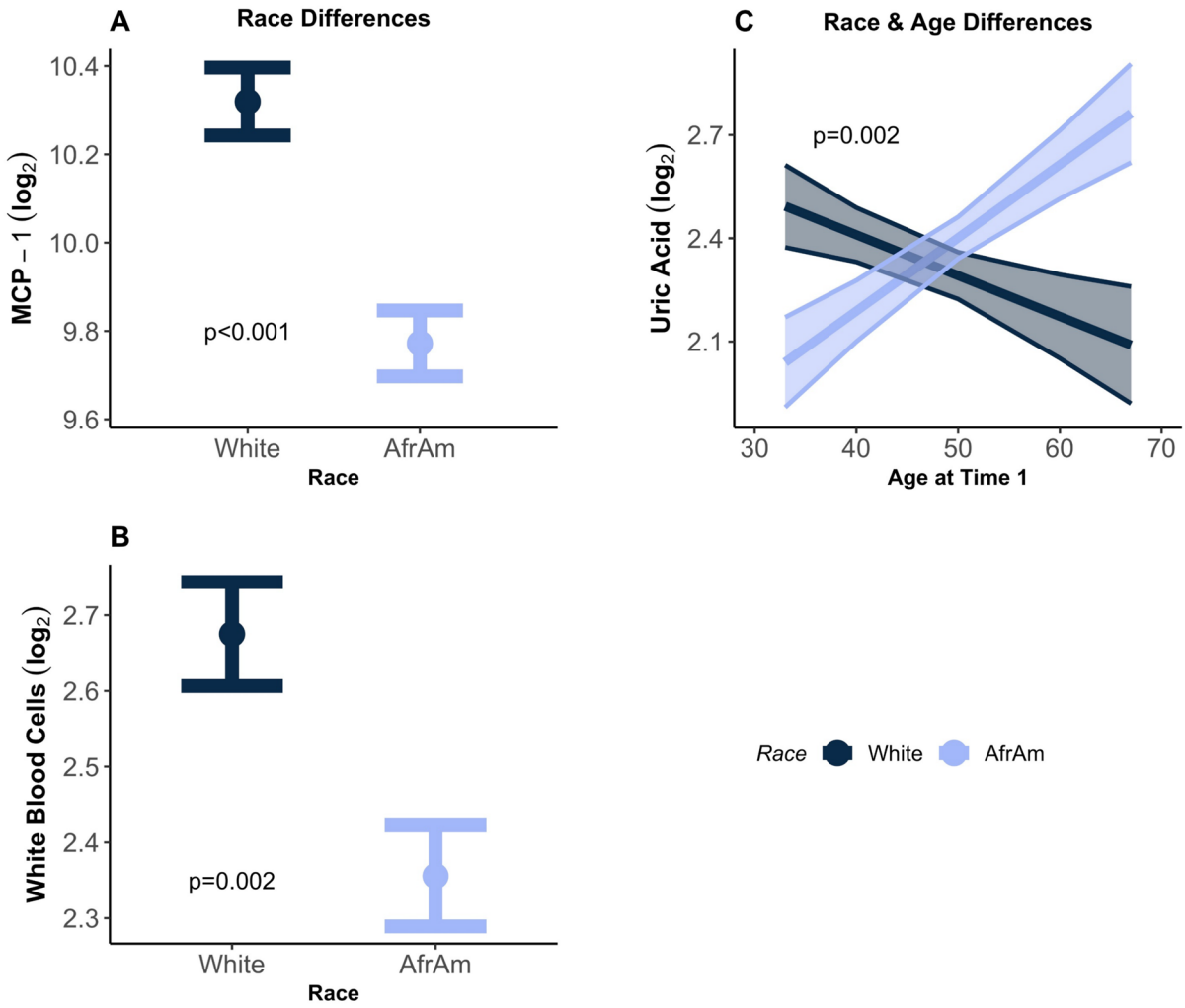


Fig. 3 Chemokine and inflammatory marker levels differ by race in longitudinal analysis. Mixed effects linear regression accounting for the three timepoints of data was used to assess the relationship of cytokines, chemokines, and inflammatory markers with age, race, sex, and elapsed time. **A**, **B** Race differences in MCP-1 and white blood cell count. **C** Race and age differences for uric acid. *p*-values for the coefficient are included on the plots. The plots show the linear regression values ± standard error of the estimated values. AfrAm = African American

inflammatory biomarkers at midlife is important to determine the various pathways that may be altered during this time period that may put individuals at risk for chronic disease or mortality.

We found that there were sex differences in chemokine levels. CXCL10/IP-10 and CXCL11/ITAC levels were higher in women. Our data are consistent with two recent reports showing significantly higher levels of CXCL10/IP-10 in women from two different Brazilian cohorts [36, 37]. In an older cohort of men (mean age = 76.5) and women (78.1) from the Swedish National Study on Aging and Care in Kungsholmen, no sex differences were found in CXCL10/IP-10 [22]. There were no sex differences in CXCL10/IP-10 in a longitudinal middle-aged cohort from the Netherlands [20]. CXCL10/IP-10 is an IFN-γ induced proinflammatory chemokine that plays an important role in inflammatory and immune responses and is associated with infectious diseases, chronic inflammatory and autoimmune diseases, and cancer [38]. CXCL11/ITAC also plays similar roles in immune

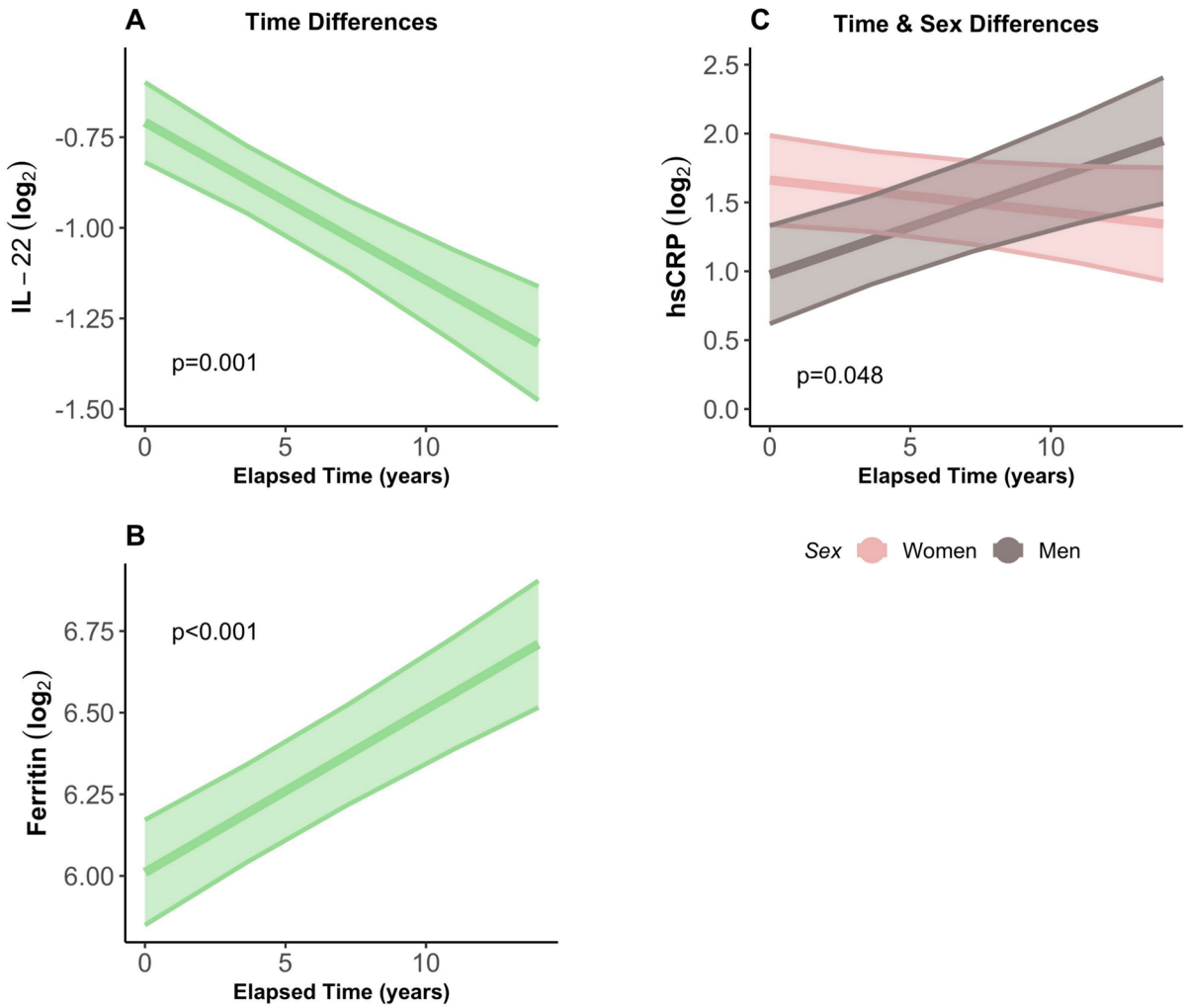


Fig. 4 Cytokine and inflammatory marker levels differ over time in our longitudinal analysis. Mixed effects linear regression accounting for the three timepoints of data was used to assess the relationship of cytokines, chemokines, and inflammatory markers with age, race, sex, and elapsed time. **A**, **B** Changes over time for IL-22 and ferritin levels. **C** Changes over time differ by sex for high sensitivity C-reactive protein (hsCRP). *p*-values for the coefficient are included on the plots. The plots show the linear regression values ± standard error of the estimated values

responses and disease, and is an IFN-γ induced ligand for CXCR3 [39]. However, few studies measure CXCL11/ITAC compared to other chemokines and little is known about sex differences in CXCL11/ITAC. Higher levels have been observed with age, but no differences were observed with sex in a cohort from the Netherlands [20]. In general, sex differences in chemokine levels may be driven by hormone levels and/or physiological and pathological drivers of these factors. Future longitudinal studies should be aimed at deciphering sex differences in chemokine levels.

Although ferritin is typically a cytosolic multimeric protein, it can also be secreted into the circulation. Ferritin stores iron and is an important regulator of iron homeostasis. Serum ferritin levels can be used as a measure of iron content and to diagnosis iron deficiency [40]. Serum ferritin is also an acute phase protein and an indicator of both acute and chronic inflammation, with elevated levels reported across numerous inflammatory conditions, including rheumatoid arthritis, systemic lupus erythematosus, chronic kidney disease, COVID-19, acute infections, thyroiditis, and others (reviewed in [40]). Our

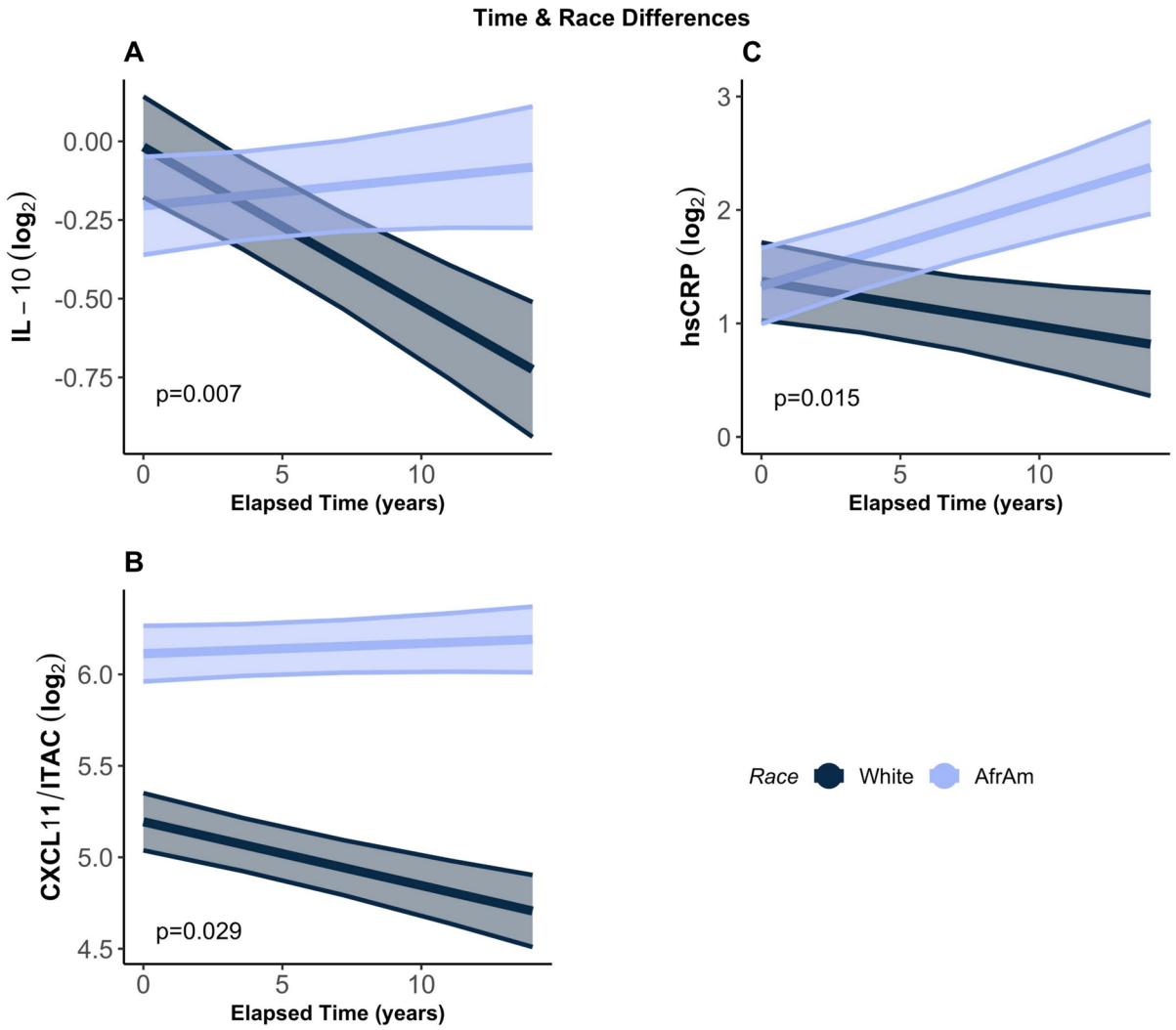


Fig. 5 Cytokine and inflammatory marker levels differ with race over time in our longitudinal analysis. Mixed effects linear regression accounting for the three timepoints of data was used to assess the relationship of cytokines, chemokines, and inflammatory markers with age, race, sex, and elapsed time. **A**–**C** IL-10, high sensitivity C-reactive protein (hsCRP) and CXCL11/ITAC levels decrease over time in White participants. *p*-values for the coefficient are included on the plots. The plots show the linear regression values ± standard error of the estimated values. AfrAm = African American

findings indicate that ferritin concentrations vary by both sex and race, with White men exhibiting the highest levels compared to White women and African American men and women. Several mechanisms may contribute to these differences. Sex-related variation in iron status has been well documented, with women typically exhibiting lower ferritin levels due in part to menstrual blood loss and increased iron requirements during reproductive years [41]. Race differences in ferritin levels and iron deficiency have also been described, which may be linked to differences in dietary iron intake, lifestyle factors, neighborhood factors, and other social determinants of health [41]. These factors also may contribute to the higher ferritin levels we observed with time along with menopause in women, which is consistent with previous literature [42]. Higher ferritin with time may also be reflective of a higher inflammatory state in older individuals. Our finding mirrors earlier work by Cankurtaran and colleagues who found that serum ferritin

Table 2 Linear mixed model regression results for the longitudinal inflammatory marker analyses

Marker	Sex	Race	Time	Age
Cytokines				
IL-10			White: lower over time	
IL-22			Lower over time	
Chemokines				
CXCL10/IP-10	Higher in women			
CXCL11/ITAC	Higher in women	White lower, and decrease over time	White lower, and decrease over time	
MCP-1		Higher in White		
MIP-3b				
Clinical inflammatory				
hsCRP	Men: higher over time	AfrAm: higher over time	Men: higher over time AfrAm: higher over time	
Ferritin	Highest in White men, Lowest in White women	Highest in White men, Lowest in White women	Higher over time	
Uric acid	Higher in men	Over 50: higher in AfAm		Over 50: higher in AfAm
WBC count		Higher in White		

Only significant results ($p < 0.05$) are summarized from linear mixed model regression models for each marker examining all possible two-way interactions across sex, race, elapsed time (years since time 1), and age at time 1 (decade units). Significant results are plotted in Figs. 2, 3, 4, 5

levels increase with age and may reflect a subclinical inflammatory state [43]. However, our findings should be interpreted with caution given the observational nature of our study. Taken together, these findings underscore the importance of considering both sex, race, age, and the inflammatory state when interpreting variability in ferritin levels.

We reported race differences in MCP-1, WBC count, and uric acid levels. Racial differences in WBC count is well known [44]. Our data reporting higher circulating levels of MCP-1 in White individuals compared to African American individuals is consistent with previous findings [18, 45]. MCP-1 (also known as CCL-2) is a chemokine that stimulates the chemotaxis of monocytes. Circulating levels of MCP-1 are associated with mortality [18, 46] and evidence from mouse models and from frail older adults with aortic stenosis has led to MCP-1 being proposed as a biomarker of biological aging [47].

We report that uric acid levels were lower with age in White participants, but higher with age in African American participants. Elevated serum uric acids levels (also known as hyperuricemia) can cause gout, which is the most common inflammatory arthritis, and is associated with various other conditions including cardiovascular and kidney disease [48–50]. Emerging trends indicate higher incidence and prevalence of gout and hyperuricemia in African American adults compared to White adults [49]. These differences have been attributed to social determinants of health [49] and point to possible opportunities for intervention which may attenuate this rise in goat and hyperuricemia in African American adults. In addition, the higher levels of uric acid we observed in men compared to women is also consistent with recent trends in the literature [49].

Here, we found IL-22 levels decreased over time. Little is known about IL-22 levels with age and time. IL-22 is a pleiotropic cytokine with important roles in preventing intestinal inflammation, maintaining gut homeostasis and systemic metabolic control [51]. As this cytokine has key functions in maintaining gut barrier integrity and mucosal immunity [51], a decline over time may contribute to higher intestinal permeability in older individuals, termed the "leaky gut of aging," which may lead to microbial leaking

into the bloodstream and contribute to chronic inflammation and inflamm-aging. Consistent with this potential role in "healthy aging," IL-22 levels were reported to be higher in centenarians [52]. However, given that this is an observational study we can only speculate as to the mechanism that may drive IL-22 levels decreasing over time.

hsCRP values increased over time in men but decreased over time in women. Few longitudinal studies have examined repeated time points of hsCRP measurements. There were no differences in the trajectories of hsCRP between men and women in the Doetinchem Cohort Study [20]. Given that hsCRP values are widely used clinically [53], it is important in future studies to examine hsCRP in longitudinal studies by sex. In addition, IL-10, CXCL11/ITAC, and hsCRP levels decreased over time in White participants, but this was not the case for African American participants. There are limited studies examining CXCL11/ITAC in longitudinal studies or across time and race. CXCL11/ITAC was higher with age in the Doetinchem Cohort Study, which is in contrast to our findings [20]. However, these variations in CXCL11/ITAC with age/time may be due to population differences. Little is known about race differences in CXCL11/ITAC. In a recent meta-analysis, CRP levels were found to be higher in Black individuals compared to White individuals, whereas IL-10 was lower in Black individuals [54]. For IL-10, there were only 4 studies examining race differences and the findings were not robust [54]. Consistent with our findings for White participants, there was a trend in lower IL-10 with age in a longitudinal cohort from the Netherlands [20]. Differences in IL-10 levels with race may be due to genetic polymorphisms that have been reported in ethnic groups [55]. Therefore, it will be important in future studies to analyze cytokine levels in the context of ancestry and also to include longitudinal analyses [55].

Our study has several notable strengths. The cohort comprises middle-aged African American and White men and women, enabling us to examine sex, race, and age in our studies. Another strength is the quantification of cytokines, chemokines and clinical biomarkers of inflammation at three different time points in this cohort, as few studies have multiple measurements over time in African American and White individuals at midlife. Our results should be taken in the context of several limitations. The study is observational and descriptive. The size of our cohort may limit the detectability of some associations or addition of other covariates. It also limits the inclusion of potential cofounding factors, such as lifestyle and metabolic factors or the presence of chronic diseases, which may affect the inflammatory state. The small cohort size also limits extensive sensitivity analyses. This cohort is an urban, middle-aged cohort, which may limit the generalizability of the findings. It is also a community-dwelling cohort and thus the participants may have baseline comorbidities that may influence inflammation. Both a strength and limitation of our cohort is that the cohort is middle-aged. This is a critical time period in which inflammation may precede the onset of disease and at-risk populations may experience accelerated aging, which may potentiate the occurrence of age-related chronic disease and conditions [14]. However, the age of the cohort may preclude the comparison to other aging studies that use older cohorts. As the nature of this study was to examine the longitudinal change in markers of inflammation, this does necessitate that participants survived over time and therefore there is a bias against those participants that succumbed to early mortality during this time period.

Our findings in our longitudinal study show that cytokines, chemokines and clinical markers of inflammation vary with time, age, sex, and race in a middle-aged cohort. We found that there were time differences in a cytokine, IL-22; sex differences in chemokines (CXCL10/IP-10 and CXCL11/ITAC); race and time differences in CXCL11/ITAC; race and time differences in IL-10; race differences in MCP-1 and WBC count; sex, race, and time differences in hsCRP and ferritin; and lastly, sex, race, and age differences in uric acid. This study builds upon current literature examining some of these inflammatory markers in older cohorts and describes changes that occur at midlife. This time period is critical as higher inflammation at midlife has been shown to have detrimental effects on health outcomes later in life. Additional longitudinal studies are warranted to further characterize changes in inflammatory markers at midlife in African American and White adults.

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Author contribution NNH, NAM, ABZ, and MKE conceived and designed the study. NAM performed statistical analyses. NE performed medical evaluations on all participants. ABZ and MKE are co-principal investigators for HANDLS. NNH and NAM wrote the manuscript with input from all the authors.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request through the HANDLS website https://handls.nih.gov/.

Declarations

Conflict of interest The authors declare no competing interests.

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Supplementary Tables

Noren Hooten et al., Longitudinal analysis of cytokines, chemokines and inflammatory markers in a middle-aged cohort

Supplementary Table 1. Cytokine and chemokine array inter- and intra-assay coefficients of variation.

	Inter-assay CV (%)	Intra-assay CV (%)
Cytokine Array		
IFNγ	6.9	3.3
IL-10	8.6	4.1
IL-12p70	6.7	5.8
IL-1β	5.5	3.7
IL-22	6.6	2.6
IL-4	8.3	7.9
IL-5	10.6	4.3
IL-6	6.7	3.3
IL-8	7.1	5.5
TNFα	7.7	3.6
Chemokine Array		
CXCL10/IP-10	10.5	2.0
CXCL11/ITAC	8.6	2.9
MCP-1	6.1	2.5
MIP-3β	7.2	3.9

Mean coefficient of variation (CV) for precision for these arrays.

Supplement Table 2. Summary of significant results for mixed model linear regressions across three time points.

	Sex	Race	Time	Age [1]	Sex*Race	Time*Sex	Time*Race [2]	Age*Race
IL-6	0.297	0.376	0.380	0.566	-	-	-	-
IL-8	0.868	0.273	0.724	0.475	-	-	-	-
IL-10	0.464	0.406	**0.002**	0.563	-	-	**0.007**	-
IL-22	0.121	0.999	**0.001**	0.596	-	-	-	-
TNFα	0.169	0.696	0.619	0.337	-	-	-	-
CXCL10/ IP-10	**0.040**	0.998	0.824	0.081	-	-	-	-
CXCL11/ ITAC	**0.002**	**<0.001**	**0.012**	0.735	-	-	**0.029**	-
MCP-1	0.969	**<0.001**	0.979	0.131	-	-	-	-
MIP-3b	0.213	0.296	0.259	0.549	-	-	-	-
hsCRP	0.160	0.928	**0.048**	0.552	-	**0.048**	**0.015**	-
Ferritin	**<0.001**	0.137	**<0.001**	0.099	**0.015**	-	-	-
Uric acid	**<0.001**	0.233	0.178	0.126	-	-	-	**0.002**
WBC	0.291	**0.002**	0.477	0.249	-	-	-	-

1. Age is in decade units centered at 50 ((Age – 50)/10)

2. All possible two-way interactions were included in each regression and non-significant interactions were removed while main effects were retained. P-values <0.05 are in bold