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Distinct inflammatory mediator patterns in young black and white adults: The African-PREDICT study

Running title: Inflammatory mediator profile of bi-ethnic adults.

Key Words: Inflammatory mediators, inflammation, ethnicity, blood pressure.

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Abstract

Objective: Inflammatory mediators have been implicated in the early stages of cardiovascular disease development, including hypertension. Since global reports reflect a higher hypertension prevalence in black than white populations, we hypothesise the involvement of specific inflammatory mediators. We therefore compared a detailed range of 22 inflammatory mediators between young black and white adults, and determined the relationship with blood pressure. **Approach and Results:** We included 1197 adults (20-30 years; 50% black; 52% female) with detailed ambulatory blood pressures. Blood samples were analysed for 22 inflammatory mediators. For pro-inflammatory mediators, the black adults had higher C-reactive protein, interferon-inducible T-cell alpha chemoattractant, macrophage inflammatory protein 3 alpha (all p≤0.008), but lower interferon-gamma, interleukin (IL)-1β, IL-8, IL-12, IL-17A, and tumour necrosis factor alpha (all p≤0.048). For anti-inflammatory mediators the black group consistently had lower levels (IL-5, IL-10 and IL-13 (all p≤0.012)), resulting in generally higher pro-to-anti-inflammatory ratios in black than white adults (p≤0.001). In mediators with pro- and anti-inflammatory functions, the black group had lower granulocyte-macrophage colony-stimulating factor and IL-6 (both p≤0.010). These patterns were confirmed after adjustment for age, sex and waist circumference, or when stratifying by hypertensive status, sex and socio-economic status. Multi-variable adjusted regression analyses and factor analysis yielded no relationship between inflammatory mediators and blood pressure in this young healthy population. **Conclusions:** Black and white ethnic groups each consistently presented with unique inflammatory mediator patterns regardless of blood pressure, sex or social class. No association with blood pressure was seen in either of the groups.

Abbreviations

CVD	Cardiovascular disease
SBP	Systolic blood pressure
DBP	Diastolic blood pressure
GM-CSF	Granulocyte-macrophage colony-stimulating factor
IFN-γ	Interferon gamma
IL-1 β	Interleukin 1 beta
IL-2	Interleukin 2
IL-4	Interleukin 4
IL-5	Interleukin 5
IL-6	Interleukin 6
IL-8	Interleukin 8
IL-10	Interleukin 10
IL-12	Interleukin 12
IL-13	Interleukin 13
IL-17A	Interleukin 17A
IL-23	Interleukin 23
ITAC	Interferon-inducible T-cell alpha chemoattractant
MIP-1α	Macrophage inflammatory protein 1-alpha
MIP-1β	Macrophage inflammatory protein 1-beta
MIP-3α	Macrophage inflammatory protein 3-alpha
TNFα	Tumour Necrosis Factor Alpha

Introduction

Inflammatory mediators have been implicated in the development of chronic diseases,[1-3] including hypertension and cardiovascular disease (CVD).[4] However, the physiological mechanisms through which this occur, are not all completely understood.[5-9] A number of pro-inflammatory mediators, such as CRP,[10] IL-6,[10-12] IL-17A,[12, 13] and TNF-α[12] contribute to an increase in blood pressure. In contrast, others such as IL-10, GM-CSF and IFN-γ show inverse associations with blood pressure.[12, 14] The functions of numerous other pro- and anti-inflammatory mediators such as IL-21,[15] and ITAC[16] are not yet clearly known, and neither is their association with blood pressure and CVD. Pro- and anti-inflammatory mediators are mainly produced by helper T cells and macrophages but also by other cell populations such as monocytes and certain nonimmune cells.[17]

Globally it has been reported that black populations have higher blood pressure than white populations.[18, 19] Many mechanisms have been proposed to explain this, such as a suppressed renin-angiotensin-aldosterone-system and salt sensitivity in black populations.[20-22] Based on our previous work in South Africa,[23, 24] another possible mechanism could be related to inflammation. Differences in pro- and anti-inflammatory mediator concentrations between ethnic groups have indeed been reported, e.g. in populations of African, European and South American descent.[24-30] Findings remain contradictory;[29] for example Mwantembe *et al.*[25] found that IL-1β levels were higher in black than in white adults, whereas Albandar *et al.*[27] found levels to be highest in Hispanics, followed by white individuals, while black participants exhibited the lowest levels. With regards to IL-6, Hong *et al.*[31] reported no ethnic differences between black and white participants, while Elkind *et al.*[26] found black individuals to have higher levels than white individuals. In South Africa, it was consistently shown that black individuals display higher levels of pro-inflammatory markers than whites.[23, 24, 32]

In this study we therefore compared a detailed range of 22 pro- and anti-inflammatory mediators and numerous blood pressure measurements between young black and white adults. We also investigated the relationships between blood pressure and the pro- and anti-

inflammatory mediator profile. This unique young disease-free sample allowed us to examine ethnic differences without interference from overt cardiovascular disease.

Methodology

Study population

This study forms part of the African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT).[33] We recruited young black and white, men and women, between the ages of 20-30 years. African-PREDICT included apparently healthy individuals who were HIV uninfected; had a screening office brachial blood pressure of <140 mmHg systolic and <90 mmHg diastolic; had no self-reported previous diagnosis or used any medication for a chronic disease; and, if female, were not currently pregnant or lactating. Although individuals with office brachial BP of ≥140 and/or ≥90 were excluded during screening, there was an average two-week period between the screening and research phases. Some participants were classified as being hypertensive based on 24hr ambulatory blood pressure during the research phase and were included in this study. This study analysed data for participants included in the baseline phase of the study (n=1202). Participants on anti-inflammatory medication or with missing biochemical analyses data were additionally excluded, resulting in a total population size of n=1189. The study was approved by the Health Research Ethics Committee (HREC) of the North-West University (NWU-00058-18-A1), adheres to the Declaration of Helsinki and all participants in the study provided written informed consent prior to participation.

General Measurements

Self-reported data with regards to demographic and lifestyle information were collected using a questionnaire. Socio-economic status was calculated using a point system that was adapted from Kuppuswamy's Socio-economic Status Scale[34] for a South African environment. Height, weight and waist circumference were measured using standard methods.[33] Body mass index (BMI) was calculated using weight (kg) / height(m)^2. A

compact, chest-worn accelerometric device (Actiheart4 CamNtech Ltd and CamNtech Inc, UK) was used to objectively measure physical activity over a maximum period of 7 days.

Blood pressure

Brachial blood pressure

Duplicate brachial blood pressure measurements were done on the left and right arms, with a 5-minute interval in-between, in a seated and resting state. We used the Dinamap® Procare 200 Blood Pressure Monitor (GE Medical Systems, Milwaukee, WI, USA).

Central blood pressure

cSBP was shown to provide a better estimate of cardiovascular mortality than other measures of blood pressure.[35] We therefore performed pulse wave analysis using the SphygmoCor XCEL (AtCor Medical, Sydney, Australia) device. This technique records the peripheral pressure waveforms and generates a corresponding central waveform.[36]

Ambulatory blood pressure

Participants were fitted with a validated 24-hour brachial ambulatory blood pressure (ABPM) monitor (Card(X)plore® CE120, Meditech, Budapest, Hungary). The apparatus was programmed to record every 30 minutes during the day (06h00 to 22h00) and every hour during the night (22h00 to 06h00).[37] Participants had a mean successful recording rate of 88%.

Biological sampling and biochemical analyses

Participants fasted overnight for at least eight hours prior to attending the day of research measurements. Blood samples were collected from the median cubital vein. The samples were prepared according to standardised protocols and stored at -80°C until the time of analysis.

Serum samples were analysed for high-sensitivity C-reactive protein (CRP), total cholesterol, low- and high-density lipoprotein cholesterol, glucose and γ-glutamyltransferase (GGT) (Cobas Integra® 400plus, Roche, Basel, Switzerland). Serum creatinine concentrations were

measured using the Creatinine Jaffé Gen.2 reagent (Roche, Basel, Switzerland). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology (CKD-EPI) formula, without race in the equation as this is not appropriate for a South African population.[38, 39] Serum cotinine was analysed using a chemiluminescence method on the Immulite (Siemens, Erlangen, Germany) apparatus. EDTA whole blood samples were analysed for white blood cell, neutrophil, basophil, eosinophil, lymphocyte and monocyte count (Coulter AcT5 diff OV Hematology analyzer, Beckman Coulter, Brea, CA, US).

A MILLIPEX Map Human High Sensitivity T Cell Magnetic Bead Panel (EMD Millipore, Merck, Missouri, USA) was used to analyse 21 cytokines. This multiplex panel was analysed using Luminex xMAP technology on the Luminex 200™ analyser. This technology performed immunoassays on the surface of fluorescent-coded magnetic beads known as MagPlex-C microspheres. Microspheres were colour-coded with two fluorescent dyes. Through precise concentrations of these dyes, distinctly coloured bead sets of 500 5.6 μm polystyrene microspheres or 80 6.45 μm magnetic microspheres were created, each of which was coated with a specific capture antibody. A biotinylated detection antibody was introduced, and the mixture was then incubated in a streptavidin-phycoerythrin (PE) conjugate reporter. The fluorescent intensity of the reporter was determined which correlates with the concentration of a given analyte in solution. A standard curve was generated to calculate final analyte concentration.

Statistical analyses

IBM®, SPSS® version 24 (IBM Corporation, Armonk, New York) was used for data analysis. GraphPad Prism 5.03 (GraphPad Software, San Diego) was used all for graphics. Continuous variables were inspected for normality using QQ plots as well as inspection of skewness and kurtosis. Variables with non-Gaussian distributions were logarithmically transformed. To evaluate if data should be presented and analysed independently by sex or ethnicity, we investigated the interactions of these variables on the relationship between

cSBP, 24hr systolic blood pressure (SBP) and the full range of pro- and anti-inflammatory mediators. We divided our groups by ethnicity based on the interactions found (**Table S1**). Pro- to anti-inflammatory ratios were calculated based on literature,[40, 41] and new ratios were suggested based on instances where pro-inflammatory mediators were higher and anti-inflammatory mediators were lower in the black and white groups. T-tests and Chi-square tests were used to compare the profiles of black and white participants. Analyses of covariance, adjusting for age; sex; and waist circumference, were used for ethnic comparisons of pro- and anti-inflammatory mediator concentrations. This was also done in normotensive and hypertensive groups, and groups categorised according to socio-economic status. The relationships between measures of blood pressure as the dependent variables and pro- and anti-inflammatory mediators as the main independent variables were explored using Pearson, partial and multiple regression analyses. Factor analyses were performed using the factor function of SPSS. Principal component analyses were used and factors with an eigenvalue >1 were retained. Varimax rotation was used to obtain independent interpretable factors. A factor loading of ≥0.3 was used to interpret the factor patterns. Double loading was handled by placing the variable in the factor with the strongest loading factor. Factor scores with a cumulative percentage of >50 were subsequently used for multiple regression analyses to determine the relationship between measures of blood pressure and factor scores.

Results

The general characteristics of the participants (n=1202) are shown in **Table 1.** The overall mean blood pressures of all participants were in the optimal blood pressure ranges.[37] Compared to the white group, black individuals had lower 24hr and night SBP readings (all p≤0.027), but higher office diastolic blood pressure (DBP) (p<0.001) and central SBP (p<0.001). With regards to body composition, the black group had a lower BMI and waist circumference.

Black participants showed higher levels of the pro-inflammatory mediators CRP, ITAC, MIP3-α (all p≤0.008), but lower levels of IFN-γ, IL-1β, IL-8, IL-12, IL-17A, and TNF-α (all p≤0.048). Regarding anti-inflammatory mediators, black individuals had lower levels of IL-5, IL-10 and IL-13 (all p≤0.012). In terms of mediators that display both pro- and anti-inflammatory functions, we observed lower levels of both GM-CSF and IL-6 (all p≤0.010) in the black group. Black participants generally had higher pro-to-anti-inflammatory ratios than their white counterparts (p≤0.001). The black group exhibited lower white blood cell, neutrophil, monocyte, eosinophil and basophil counts (all p≤0.013).

We also performed analyses of covariance comparing black and white individuals (**Table 2**), adjusting for age, sex and waist circumference. Findings were largely similar (**Figure 1**).

Additionally, we compared ethnic groups, stratified by hypertensive status, sex and the middle socio-economic status group to account for the role these factors may play in determining inflammatory mediator status **(Table II-VI).** Similar to the ethnic comparisons in the total group, nearly identical ethnic profiles were seen, independent of hypertension status, sex and socio-economic status. **Figure 1** summarises the pro- and anti-inflammatory mediator profiles of black and white groups based on Table 3 and Tables I-VI.

Multiple regression analyses were performed to determine whether blood pressure (24hr SBP, 24hr DBP, cSBP and nighttime SBP) is related to pro- and anti-inflammatory mediator concentrations within each ethnic group. In our young, healthy populations, the multiple regression analyses yielded no consistent statistically significant results **(Figures 2-3 and Figures I-II).**

Factor analyses were performed with the pro- and anti-inflammatory mediator data to determine factor scores (**Table VII-IX**). Factor scores were subsequently used for multiple regressions analyses to determine whether blood pressure (24hr SBP, 24hr DBP, cSBP and nighttime SBP) is related to the pro- and anti-inflammatory mediator factors; this yielded no statistically significant results (**Table X**).

Table 1. Characteristics of young black and white adults.

	Black (n=599)	White (n=590)	*P*
Age, years	24.5± 3.17	24.6 ± 3.06	0.55
Male, n (%)	296 (49.0)	281 (47.4)	0.58
Socio-economic Status			
Low, n (%)	356 (58.9)	118 (19.9)	**<0.001**
Middle, n (%)	163 (27.0)	182 (30.7)	
High, n (%)	85 (14.1)	293 (49.4)	
Office BP (mmHg)			
Brachial SBP	117 ± 12.0	116 ± 12.6	0.16
Brachial DBP	79.4 ± 8.37	77.7 ± 8.12	**<0.001**
Central SBP	110 ± 9.58	105 ± 9.69	**<0.001**
Ambulatory BP (mmHg)			
24h SBP	116 ± 8.99	118 ± 9.82	**<0.001**
24h DBP	68.8 ± 5.93	68.5 ± 5.86	0.45
Night SBP	107 ± 10.1	109 ± 10.9	**0.027**
Night DBP	59.6 ± 6.77	59.1 ± 6.70	0.14
Hypertensive status, n (%)	50 (8.40)	78 (13.2)	**0.009**
Body Composition			
Body mass index (kg/m^2)	24.0 (17.6; 35.3)	25.0 (18.8; 35.1)	**<0.001**
Waist circumference (cm)	77.0 (63.0; 97.0)	81.5 (64.9; 107)	**<0.001**
Male	75.9 (64.3; 92.3)	88.5 (74.0; 110)	**0.007**
Female	78.2 (62.0; 102)	75.7 (63.5; 99.5)	**<0.001**
Inflammatory Markers			
Pro-Inflammatory			
CRP (mg/L)	1.01 (0.09; 11.1)	0.78 (0.08; 7.89)	**0.002**
Fractalkine (pg/mL)	28.0 (9.02; 71.7)	29.3 (9.82; 74.3)	0.24
IFN-γ (pg/mL)	6.75 (1.65; 21.5)	7.82 (163; 22.0)	**0.002**
IL-1β (pg/mL)	0.98 (0.20; 3.74)	1.08 (0.27; 3.66)	**0.033**
IL-2 (pg/mL)	0.78 (0.13; 4.11)	0.85 (0.16; 3.91)	0.21
IL-7 (pg/mL)	5.63 (1.34; 18.7)	5.65 (1.12; 18.8)	0.95
IL-8 (pg/mL)	1.74 (0.45; 5.97)	1.91 (0.47; 7.88)	**0.048**
IL-12 (pg/mL)	1.75 (0.35; 6.40)	1.97 (0.45; 6.72)	**0.024**
IL-17 A (pg/mL)	3.19 (0.64; 13.5)	3.57 (0.67; 14.1)	**0.044**
IL-23 (pg/mL)	119 (13.4; 600)	133 (12.9; 668)	0.10
ITAC (pg/mL)	4.70 (1.45; 19.9)	3.67 (1.40; 11.6)	**<0.001**
MIP-1α (pg/mL)	9.57 (2.74; 26.3)	10.3 (2.85; 27.0)	0.089
MIP-1β (pg/mL)	7.06 (2.70; 15.6)	7.28 (2.88; 16.4)	0.33
MIP-3α (pg/mL)	2.17 (0.54; 8.12)	1.90 (0.48; 6.61)	**0.008**
TNF-α (pg/mL)	1.61 (0.41; 5.29)	1.79 (0.49; 5.73)	**0.016**
Anti-Inflammatory			
IL-4 (pg/mL)	42.62 (7.33; 155)	43.85 (7.92; 154)	0.61
IL-5 (pg/mL)	0.89 (0.20; 3.88)	1.02 (0.26; 3.99)	**0.012**
IL-10 (pg/mL)	4.38 (0.89; 21.1)	5.42 (1.14; 21.2)	**<0.001**
IL-13 (pg/mL)	3.88 (0.58; 23.2)	5.04 (0.69; 30.3)	**<0.001**
Pro- and Anti-Inflammatory			
IL-6 (pg/mL)	1.81 (0.25; 9.84)	2.35 (0.30; 12.8)	**<0.001**

IL-21 (pg/mL)	1.34 (0.21; 5.53)	1.49 (0.26; 6.53)	0.074
GM-CSF (pg/mL)	7.23 (1.22; 32.5)	8.47 (1.22; 38.1)	**0.010**
Pro-to-Anti Inflammatory Ratios			
IL-6 to IL-10	0.41 (0.10; 2.03)	0.43 (0.12; 2.02)	0.39
IL-1β to IL-10	0.22 (0.07;0.73)	0.19 (0.07; 0.52)	**0.001**
TNF-α to IL-10	0.37 (0.17; 0.98)	0.33 (0.15; 1.01)	**<0.001**
CRP to IL-10	0.23 (0.01; 3.80)	0.14 (0.01; 2.40)	**<0.001**
MIP-*1α* to IL-10	2.13 (0.71; 7.72)	1.82 (0.59; 5.76)	**<0.001**
ITAC to IL-4	0.11 (0.02; 0.90)	0.08 (0.02; 0.67)	**<0.001**
ITAC to IL- 5	5.33 (1.10; 32.5)	3.60 (0.79; 17.7)	**<0.001**
ITAC to IL-10	1.08 (0.27; 6.65)	0.68 (0.21; 3.12)	**<0.001**
ITAC to IL-13	1.22 (0.17; 9.92)	0.73 (0.10; 5.66)	**<0.001**
Biochemical Markers and White Blood Cell Counts			
Total Cholesterol (mmol/L)	3.47 ± 0.97	4.04 ± 1.33	**<0.001**
HDL-C (mmol/L)	1.14 ± 0.38	1.17 ± 0.46	0.28
LDL-C (mmol/L)	2.07 (0.99; 3.71)	2.45 (1.20; 4.42)	**<0.001**
Triglycerides (mmol/L)	0.64 (0.31; 1.37)	0.80 (0.33; 2.11)	**<0.001**
Glucose (mmol/L)	3.94 ± 1.03	4.25 ± 1.09	**<0.001**
eGFR (ml/min/1.73m^2)	118 ± 14.5	106 ± 16.1	**<0.001**
White blood cell counts (x10^9/L)	5.21 (3.10; 8.40)	5.79 (3.80; 9.36)	**<0.001**
Neutrophils (x10^9/L)	2.43 (1.03; 5.49)	2.93 (1.35; 5.95)	**<0.001**
Lymphocytes (x10^9/L)	2.02 (1.26; 3.24)	1.98 (1.29; 3.01)	0.26
Monocytes (x10^9/L)	0.30 (0.14; 0.57)	0.42 (0.20; 0.79)	**<0.001**
Eosinophils (x10^9/L)	0.14 (0.05; 0.41)	0.15 (0.06; 0.39)	**0.008**
Basophils (x10^9/L)	0.04 (0.01; 0.11)	0.04 (0.02; 0.10)	**0.013**
Health Behaviours			
Serum cotinine (ng/ml)	4.04 (1.00; 341)	3.22 (1.00; 308)	0.081
Self-Reported Tobacco use n (%)	154 (25.5)	132 (22.3)	0.18
γ-glutamyltransferase (U/L)	22.2 (8.40; 66.2)	14.8 (5.40; 46.6)	**<0.001**
Self-Reported Alcohol use, n (%)	333 (55.8)	330 (55.7)	0.99
Hormonal Contraceptive Use, n (% of women)	143 (47.2)	130 (41.9)	0.19

Abbreviations: SBP, Systolic blood pressure; DBP, Diastolic blood pressure; BMI, Body mass index; HDL-C High density lipoprotein cholesterol; LDL-C, Low density lipoprotein cholesterol. Bold values indicate P<0.05. Data presented as mean ± SD; or geometric mean 95 C.I. Granulocyte-macrophage colony-stimulating factor (GM-CSF), Interferon gamma (IFN-γ), Interleukin 1 beta (IL-1β), Interleukin 2 (IL-2), Interleukin 4 (IL-4), Interleukin 5 (IL-5), Interleukin 6 (IL-6), Interleukin 7 (IL-7), Interleukin 8 (IL-8), Interleukin 10 (IL-10), Interleukin 12 (IL-12), Interleukin 13 (IL-13), Interleukin 17A (IL-17A), Interleukin 21 (IL-21), Interleukin 23 (IL-23), Interferon-inducible T-cell alpha chemoattractant (ITAC), Macrophage inflammatory protein *1-alpha* (MIP-1α), Macrophage inflammatory protein *1-beta* (MIP-1β), Macrophage inflammatory protein 3-*alpha* (MIP-3α) and Tumour Necrosis Factor Alpha (TNFα).

Table 2. A comparison of cytokine concentrations between black and white individuals, adjusted for age, sex and waist circumference.

	Black (n=599)	White (n=590)	*p*	Difference: Black minus White
Inflammatory Markers				
Pro-Inflammatory				
CRP (mg/L)	1.16 (1.05; 1.29)	0.67 (0.61; 0.75)	**<0.001**	0.49
Fractalkine (pg/mL)	27.9 (26.4; 29.3)	29.5 (28.1; 31.1)	0.12	-1.60
IFN-γ (pg/mL)	6.68 (6.25; 7.14)	7.93 (7.40; 8.47)	**0.001**	-1.25
IL-1β (pg/mL)	0.97 (0.91; 1.04)	1.09 (1.02; 1.17)	**0.021**	-0.12
IL-2 (pg/mL)	0.77 (0.71; 0.85)	0.86 (0.78; 0.93)	0.12	-0.09
IL-7 (pg/mL)	5.62 (5.25; 6.01)	5.66 (5.28; 6.07)	0.86	-0.04
IL-8 (pg/mL)	1.72 (1.61; 1.84)	1.93 (1.81; 2.07)	**0.015**	-0.21
IL-12 (pg/mL)	1.74 (1.62; 1.87)	1.98 (1.85; 2.13)	**0.013**	-0.24
IL-17 A (pg/mL)	3.16 (2.92; 3.40)	3.61 (3.34; 3.90)	**0.016**	-0.45
IL-23 (pg/mL)	118 (107; 130)	135 (122; 149)	0.059	-17.0
ITAC (pg/mL)	4.70 (4.42; 5.00)	3.67 (3.45; 3.90)	**<0.001**	1.03
MIP-1α (pg/mL)	9.51 (8.95; 10.1)	10.4 (9.75; 11.0)	0.051	-0.89
MIP-1β (pg/mL)	7.24 (6.71; 7.35)	7.31 (7.00; 7.66)	0.20	-0.07
MIP-3α (pg/mL)	2.15 (2.01; 2.31)	1.91 (1.79; 2.06)	**0.022**	0.24
TNF-α (pg/mL)	1.59 (1.50; 1.70)	1.80 (1.69; 1.92)	**0.008**	-0.21
Anti-Inflammatory				
IL-4 (pg/mL)	42.5 (39.4; 45.9)	44.2 (40.1; 47.8)	0.49	-1.70
IL-5 (pg/mL)	0.88 (0.82; 0.95)	1.03 (0.96; 1.11)	**0.004**	-0.15
IL-10 (pg/mL)	4.32 (4.00; 4.66)	5.48 (5.08; 5.92)	**<0.001**	-1.16
IL-13 (pg/mL)	3.83 (3.48; 4.20)	5.11 (4.65; 5.61)	**<0.001**	-1.28
Pro- and Anti-Inflammatory				
IL-6 (pg/mL)	1.79 (1.63; 1.96)	2.38 (2.17; 2.62)	**<0.001**	-1.37
IL-21 (pg/mL)	1.32 (1.21; 1.43)	1.51 (1.39; 1.64)	**0.024**	-0.59
GM-CSF (pg/mL)	7.18 (6.58; 7.82)	8.55 (7.83; 9.33)	**0.006**	-0.19
Pro-to-Anti Inflammatory Ratios				
IL-6 to IL-10	0.41 (0.38; 0.45)	0.43 (0.40;0.47)	0.38	-0.02
IL-1β to IL-10	0.22 (0.21; 0.23)	0.19 (0.18; 0.20)	**0.001**	0.03
TNF-α to IL-10	0.37 (0.36; 0.39)	0.33 (0.32; 0.35)	**<0.001**	0.04
CRP to IL-10	0.27 (0.24; 0.31)	0.12 (0.11; 0.14)	**<0.001**	0.15
MIP-1α to IL-10	2.14 (2.01; 2.29)	1.81 (1.70; 1.93)	**<0.001**	0.33
ITAC to IL-4	0.11 (0.10; 0.12)	0.08 (0.08; 0.09)	**<0.001**	0.03
ITAC to IL- 5	5.38 (4.98; 5.82)	3.57 (3.30; 3.86)	**<0.001**	1.81
ITAC to IL-10	1.10 (1.02; 1.18)	0.67 (0.62; 0.72)	**<0.001**	0.43
ITAC to IL-13	1.24 (1.12; 1.36)	0.77 (0.65; 0.79)	**<0.001**	0.47

Bold values indicate P<0.05.

Normotensive status determined using 24-hour ambulatory blood pressure.

|	Total		NT		HT		M		F		Mid SES	
	B	**W**	**B**	**W**	**B**	**W**	**B**	**W**	**B**	**W**	**B**	**W**
Black, n	599		536		50		307		307		162	
White, n	590		507		78		311		311		180	
Pro-Inflammatory												
CRP (mg/L)												
Fractalkine (pg/mL)												
IFN-γ (pg/mL)												
IL-1β (pg/mL)												
IL-2 (pg/mL)												
IL-7 (pg/mL)												
IL-8 (pg/mL)												
IL-12 (pg/mL)												
IL-17 A (pg/mL)												
IL-23 (pg/mL)												
ITAC (pg/mL)												
MIP-1α (pg/mL)												
MIP-1β (pg/mL)												
MIP-3α (pg/mL)												
TNF-α (pg/mL)												
Anti-Inflammatory												
IL-4 (pg/mL)												
IL-5 (pg/mL)												
IL-10 (pg/mL)												
IL-13 (pg/mL)												
Pro- and Anti-Inflammatory												
IL-6 (pg/mL)												
IL-21 (pg/mL)												
GM-CSF (pg/mL)												
Pro-to-Anti Inflammatory Ratios												
IL-6 to IL-10												
IL-1β to IL-10												
TNF-α toIL-10												
CRP to IL-10												
MIP-1α to IL-10												
ITAC to IL-4												
ITAC to IL-5												
ITAC to IL-10												
ITAC to IL-13												

Figure 1. Comparison of cytokines between black and white individuals.

Cytokine concentrations that differ between black and white participants are indicated in colour (P<0.05). A darker shade indicates a higher value. Adjusted for: age, sex, waist circumference. Based on Tables 3 and II-VI. Black (B), White (W), Men (M), Women (F), Normotensive (NT), Hypertensive (HT), Middle Socio-economic Status (Mid SES)
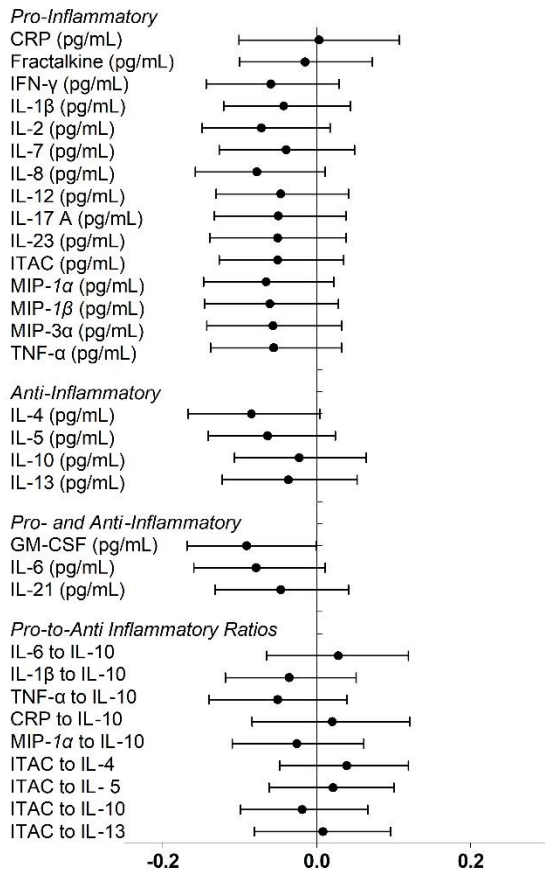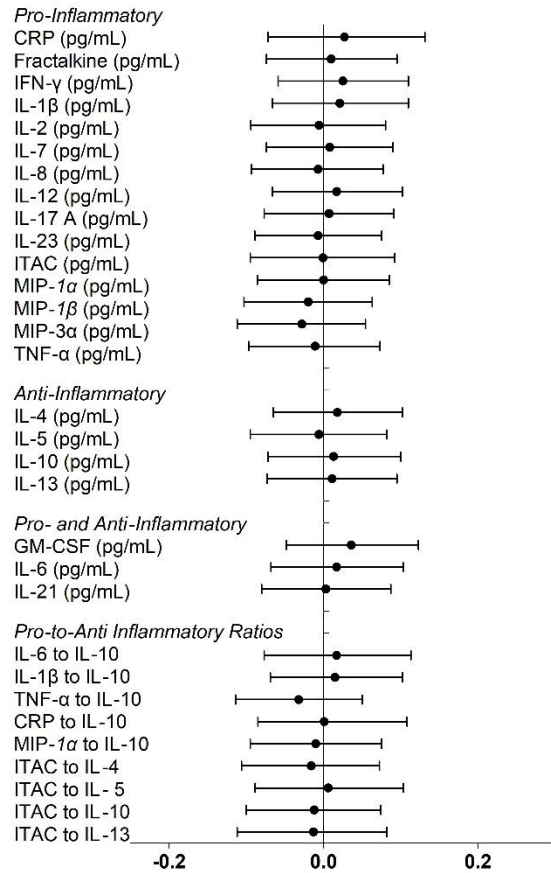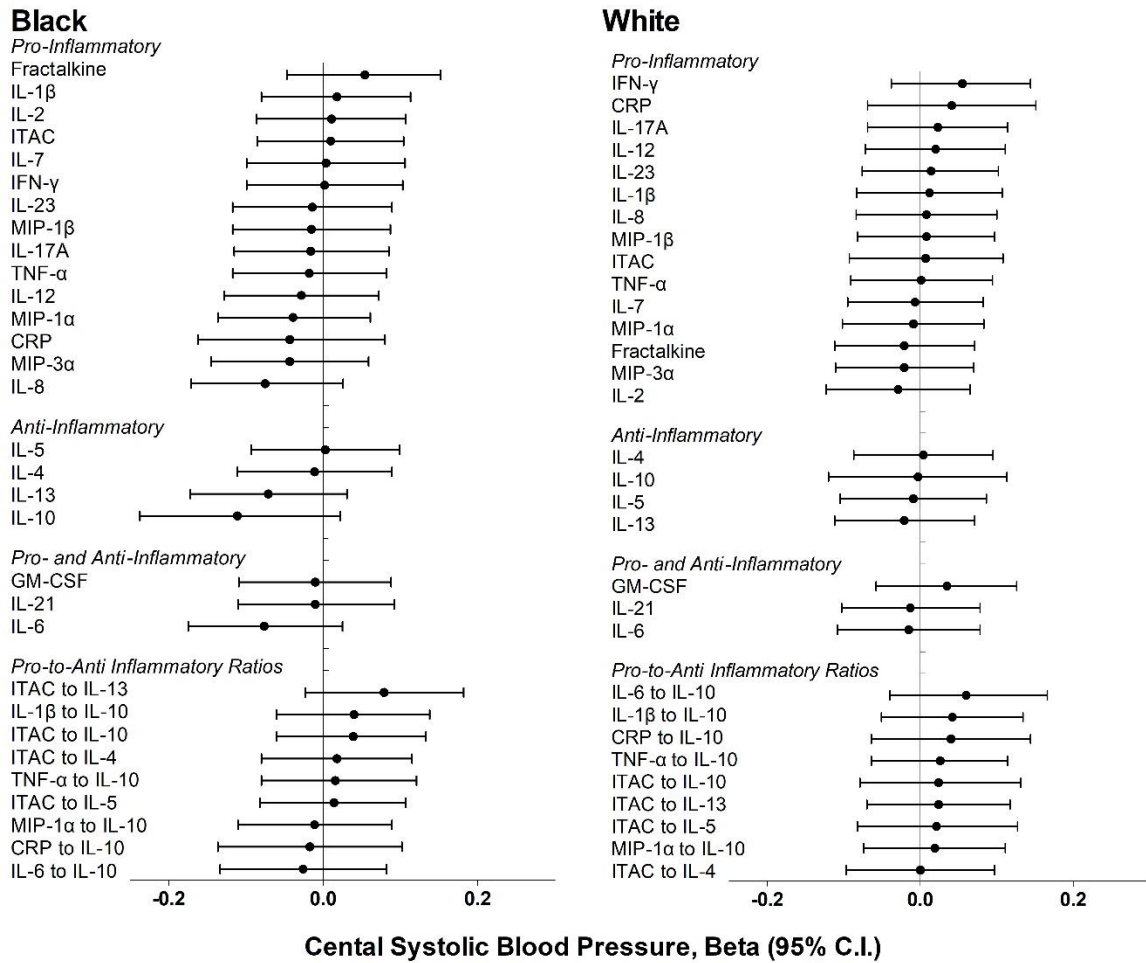
Figure 2. Multiple regression analyses showing the relationship between cytokine concentrations and 24-hour Systolic Blood Pressure in black and white adults, respectively.

Adjusted for: sex, socio-economic status, waist circumference, total cholesterol, glucose, gamma glutamyltransferase, cotinine, estimated glomerular filtration rate, activity energy expenditure.

Figure 3. Multiple regression analyses showing the relationship between cytokine concentrations and central systolic blood pressure in black and white adults, respectively.

Adjusted for: sex, socio-economic status, waist circumference, total cholesterol, glucose, gamma glutamyltransferase, cotinine, estimated glomerular filtration rate, activity energy expenditure.

Discussion

Here we report distinct pro- and anti-inflammatory mediator patterns in young and healthy white and black adults, independent of sex, hypertension or socio-economic status. Black individuals consistently showed higher pro-to-anti-inflammatory ratios when compared to their white counterparts. This suggests that the pro- and anti-inflammatory mediator profiles in this young healthy cohort are likely to be ethnic-specific and independent of blood pressure, sex or socio-economic status. With respect to blood pressure profiles, we found no independent relationships between individual inflammatory mediators or inflammatory mediator patterns with blood pressure in either of the young ethnic groups.

Ethnic Profiling

When examining the pro-to-anti-inflammatory mediator ratios, black adults consistently displayed higher ratios, even though they were leaner, had better lipid and glycaemic profiles, as well as lower immune cell counts. These results for mediator ratios were found, despite the black group having lower levels for six of the fifteen pro-inflammatory mediators compared to whites, and higher levels of only three of the fifteen mediators. It is the anti-inflammatory mediators where the black group displayed lower levels for three of the four mediators, which resulted in overall higher pro-to-anti-inflammatory ratios.

Literature regarding ethnic differences in cytokine concentrations remains inconsistent. Elkand *et al.*[26] and Schutte *et al.*[23,24] found CRP levels to be higher in black than in white populations, which is in line with our findings. However, Ford *et al.*[42] found no difference in CRP levels between African-American and white children and young adults.

Many studies and systematic reviews focussed on only a few inflammatory mediators at a time, and usually the same mediators, such as CRP, IL-6, TNF-α and IL-1β.[23, 24, 29, 43-46] This simplifies comparison between studies. In our study, we made use of a large range of mediators which, while complex, provides a better overall inflammatory profile.

One possible explanation for the ethnic-specific mediator profiles may be the differences in gene polymorphisms distribution across different ethnic groups.[47] It has been shown that differences in the inheritance of IL-6 and IL-10 genotypes in black populations result in higher expression when compared to white populations.[47] Differences in genotypes between black and white populations have also been seen where IL-2 is concerned.[48]

Fifty-nine percent of the black population (versus 20% in whites) falls within a low socio-economic category. It has recently been shown that childhood environment may play a role in the development of inflammatory phenotypes suggesting that the overall low socio-economic status of this black population may play a role.[49]

The effect of potential confounders on the difference in mediator concentrations should be taken into consideration. In our comparisons (Figure 1) we adjusted for age, sex and abdominal obesity. Yet tobacco use,[50] alcohol consumption,[51] and obesity,[52] which are often linked to socio-economic status,[53-55] may play a role in increasing pro-inflammatory mediator levels, as studies have found that those with a low socio-economic status show elevated systemic inflammation.[56, 57] However, when counteracting this by comparing black and white groups with similar mid-level socio-economic status, the original profiles remained robust, suggesting heritability.

Relationship with blood pressure

Our motivation for this ethnic comparative study was to identify the involvement of specific inflammatory mediators as a possible explanation for the high prevalence of hypertension in black populations.[58] Our analyses yielded no clear findings, with no individual mediator or pattern associating with a range of clinic, central and ambulatory blood pressure measures. Since at this early stage in life there are multiple factors that may dominate physiological variances in blood pressure – such as differences in renin-angiotensin-aldosterone profiles,[58] arterial stiffness,[59] and physical activity[60] – blood pressure may not be a measure sensitive enough to show small scale, early inflammatory changes in young otherwise healthy

individuals. It may thus be useful to focus on more sensitive markers of early cardiovascular changes such as arterial stiffness, the microvasculature,[61] and endothelial function.[62]

In addition to the relationship seen with blood pressure, inflammatory mediators are also associated with other disease states. It has been shown that IL-1β, IL-6, IL-17A and TNF-α are implicated in the development of atherosclerosis,[63, 64] while IL-10 is inversely associated.[65] More research is required regarding detailed inflammatory mediators in the development of CVD in black and white populations.

Our study results should be interpreted within the framework of its strengths and limitations. Participants were screened prior to participation using strict exclusion criteria for conditions that may affect the results. The absence of pre-existing chronic diseases afforded us the opportunity to investigate the underlying physiology in adults without influence from pathology. A further strength of our study is the inclusion of a wide range of pro- and anti-inflammatory mediators as well as our large sample size. Mediator concentrations were established using a high-sensitivity kit which allowed for detection even at low levels. In terms of limitations, the black group had a greater proportion of individuals with low socio-economic status, allowing for potential socio-economic bias. However, sensitivity analyses were performed to address this and yielded no changes in our main results. Additionally, this study uses cross-sectional data.

In conclusion, the black and white ethnic groups each consistently presented with unique inflammatory mediator patterns regardless of blood pressure, sex or socio-economic status. Despite a higher pro-to-anti-inflammatory status of the young black adults, there was no association with blood pressure in the black or white groups. Whether these ethnic specific patterns will relate to future disease development, needs to be established.

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