**INTRODUCTION**

The link between chronic systemic inflammation and cardiovascular disease (CVD) progression has been well-established.\(^1,2\) High sensitivity C-reactive protein (CRP) levels have long since become the primary marker for systemic inflammation and a powerful predictor of CVD risk.\(^3,4\) Concordantly, CRP levels have been linked to other markers of CVD risk such as lipid profile, body mass index, blood pressure and more.\(^5,6\) Several intervention studies have shown that aerobic exercise training (AEXT) can improve systemic inflammation and CRP levels.\(^7,8\) AEXT has been well-studied in its ability to improve numerous CVD risk factors as well as reducing actual occurrence of CVD.\(^9,10\) However, to the best of our knowledge no study has yet investigated whether systemic inflammation itself limits CVD risk factor modification by means of AEXT. As such, the primary aim of our study was to investigate whether a moderate-intensity AEXT intervention would elicit the same adaptations in a group with low-to-average vs a group with high levels of systemic inflammation as evidenced by CRP levels. For the study population, we chose African Americans who present the highest incidence of CVD as well as higher levels of inflammation when compared with other racial groups.\(^11,12\) Measures included several well-established markers of CVD, as well as endothelial microparticles (EMPs), a biomarker of endothelial health, which is becoming a powerful predictor and mediator of CVD.\(^13–16\) We hypothesized that the high inflammation group would show a blunted exercise response when compared with the low-to-average inflammation group in regard to predictors of CVD.

**Keywords:** Inflammation; Exercise; Cardiovascular Disease

**Methods**

Participants

Each participant gave written informed consent and all study protocols were approved by the Temple University Institution-
Thirty-seven African American participants, aged 40-75 years, from the Fit4Life study were included in the study and stratified by CRP levels into low (LI) and high (HI) inflammation groups. Using American Heart Association guidelines, participants with levels in the low or average risk range (0-1 and 1-3 mg/L, respectively) comprised the LI group (n=23); participants with levels in the high risk range (≥3 mg/L) comprised the HI group (n=14). All participants were sedentary (aerobic exercise ≤ two times per week), non-diabetic (fasting blood glucose ≤ 125 mg/dL), non-smoking (≥ 2 years), had a clinical blood pressure <160/100 mm Hg, were non-hyperlipidemic (total cholesterol ≤ 240mg/dL), had no signs of cardiovascular/renal/pulmonary disease or acute infection and were not on any medication.

Screening and Testing

After initial phone interviews, participants were invited to three consecutive screening visits to determine eligibility according to the study criteria. The first visit followed a 12-hour overnight fast and included collection of blood and urine samples for analysis of blood chemistry and urinalysis by Quest Diagnostics. Systemic inflammation levels were determined by the high-sensitivity C-reactive protein (CRP) test. Glomerular filtration rate was determined using the four-variable CKD-EPI equation that uses race, age, sex and serum creatinine levels. The second visit was a cardiologist-administered physical examination and review of health history. The third visit required the participants to undergo a cycle ergometer echocardiogram stress test to further rule out cardio-pulmonary abnormalities and disease among the study sample. Additionally, participants’ clinical blood pressures were assessed by measuring seated blood pressure using an aneroid sphygmomanometer (Omron, Model 11-675D, Vernon Hills, IL) and averaging three measures each from three separate visits. During each visit, participants had to rest for five minutes prior to the first measurement. Each consecutive measure was five minutes apart. Measures of internal consistency and reliability were .9 for Cronbach’s alpha and a mean of .75 for inter-items correlation for both systolic and diastolic blood pressure measurements. Additional entry examination was performed by study personnel to determine bodyweight and body fat levels via bioelectrical impedance analysis at 50 kHz using the single frequency impedance instrument, ImpediMed DF50 (San Diego, CA). Measures for blood pressure, body composition, exercise performance, as well as measures involving collection of blood and urine were repeated after the exercise intervention. Discrepancies in the number of data points for individual measurements were the result of sample loss or assay failure in rare cases.

Endothelial Microparticle Analysis

EMPs were analyzed as previously reported. Briefly, plasma samples were centrifuged twice at 1500g for 20 minutes at 24°C to obtain cell free plasma. 100 µL of sample was then incubated with fluorochrome-labeled antibodies and fixed with 93 µL of 10% formaldehyde followed by gentle agitation (500 RPM) for 20 minutes at 24°C. Samples were then diluted in 500 mL of .22 µm double-filtered phosphate-buffered saline and analyzed using a BDLS-RII flow cytometer and BD FACSDIVA software (BD Biosciences, San Jose, CA). Labeled events <1.0 µm were defined as EMPs and identified through the use of a logarithmic scale for forward scatter signal, side scatter signal and each fluorescent channel. The flow rate was set to medium and all samples were run for 180 seconds yielding a mean sample volume of 101 µL per 180 seconds. EMPs were expressed as events per µL plasma. EMPs labeled with CD31+/CD42b- were used to...
identify EMPs released as a result of endothelial cell apoptosis, whereas EMPs labeled with CD62E+ were used to identify EMPs released as a result of endothelial cell activation. Inter-Assay and Intra-Assay CVs for CD62E+ EMPs were 15% and 8%, respectively, and 6% and 8% for CD31+/CD42b- EMPs, respectively.

**Exercise Training Intervention**

Each participant performed a sub-maximal graded exercise test using the modified Bruce Protocol to determine aerobic fitness levels through continuous measurement of oxygen consumption via a metabolic cart (Vmax Encore, SensorMedics, Yorba Linda, CA) for estimation of maximal aerobic fitness capacity (VO2max). Each test was preceded by flow volume and gas mixture calibration to ensure validity and reproducibility. Participants then underwent six months of closely-supervised AEXT. Initially, participants exercised for 20 minutes at an intensity of 50% of VO2max three times per week. Frequency was maintained and duration of exercise was increased weekly in five minute increments until a total exercise time of 40 minutes was achieved. At this point exercise intensity was increased from 50% of VO2max to 65% of VO2max in weekly increments of 5% of VO2max. For the remainder of the intervention, participants exercised at this moderate intensity for 40 minutes three times per week. Exercise modes included treadmill walking/jogging, stair stepping, stationary cycling, rowing ergometry, arm ergometry and elliptical cross-training. Exercise duration and intensity were supervised by study personnel through the use of heart rate monitors and recorded every 10 minutes for the duration of exercise.

**Dietary Normalization**

Prior to baseline testing, all participants underwent a six-week dietary stabilization period. Participants met with a registered dietitian once a week and were instructed to follow the guidelines of the Dietary Approaches to Stop Hypertension (DASH) diet. Three-day dietary food logs were collected at baseline and every six weeks to ensure dietary compliance. Additionally, participants were instructed to not change dietary habits to lose weight since this was an exercise intervention study, not a weight-loss study.

### Table 1. Participant characteristics and baseline and final biomarkers for low inflammatory and high inflammatory groups

<table>
<thead>
<tr>
<th></th>
<th>Low inflammatory group (CRP &lt; 3)</th>
<th>High inflammatory group (CRP ≥ 3)</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Final</td>
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<tr>
<td>N mean</td>
<td></td>
<td>mean</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>23</td>
<td>53 ± 1.4</td>
</tr>
<tr>
<td>Height, cm</td>
<td>22</td>
<td>167 ± 1.3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>22</td>
<td>82.4 ± 3.8</td>
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<tr>
<td>BMI, kg/m²°</td>
<td>23</td>
<td>29.4 ± 1.0</td>
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<tr>
<td>Body fat, %</td>
<td>21</td>
<td>39.9 ± 2.1</td>
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<tr>
<td>Cholesterol, mg/mL</td>
<td>20</td>
<td>187 ± 5.4</td>
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<tr>
<td>Triglycerides, mg/mL</td>
<td>20</td>
<td>87 ± 4.5</td>
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<tr>
<td>HDL, mg/mL</td>
<td>20</td>
<td>66 ± 4.1</td>
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<tr>
<td>LDL, mg/mL</td>
<td>20</td>
<td>104 ± 4.5</td>
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<tr>
<td>Glucose, mg/mL</td>
<td>20</td>
<td>92 ± 2.7</td>
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<tr>
<td>CRP, mg/L</td>
<td>23</td>
<td>13 ± 2.3</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>22</td>
<td>121 ± 2.3</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>22</td>
<td>78 ± 1.4</td>
</tr>
<tr>
<td>VO₂max, mL/kg/min</td>
<td>23</td>
<td>27.8 ± 3.1</td>
</tr>
<tr>
<td>GFR, mL/1.73m²/min</td>
<td>18</td>
<td>93.3 ± 3.6</td>
</tr>
<tr>
<td>CD31+/CD42b EMPs, events/µL</td>
<td>17</td>
<td>3.7 ± .5</td>
</tr>
<tr>
<td>CD62E+ EMPs, events/µL</td>
<td>18</td>
<td>39.2 ± 6.4</td>
</tr>
</tbody>
</table>

a. Significantly different (P ≤ .05) after AEXT vs before AEXT within the same group.
b. Significantly different (P ≤ .001) after AEXT vs before AEXT within the same group.
c. Significantly different (P ≤ .05) between groups at the same time point (before or after AEXT).
d. Significantly different (P ≤ .001) between groups at the same time point (before or after AEXT).

AEXT, aerobic exercise training.
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Statistical Analysis

Data are expressed as mean ± standard error of the mean. The distribution of each variable was examined using the Shapiro-Wilk test of normality. Non-parametric tests were used when appropriate. To conduct before and after AEXT comparisons within groups, paired-sample tests were used (T-test or Wilcoxin signed-rank test). To assess between group comparisons at the same time points (before or after AEXT), independent-samples tests were performed (T-test or Mann-Whitney U test). For correlation analysis, Pearson product-moment correlation coefficient or Spearman’s rank correlation coefficient were determined. Statistical significance was defined as P≤.05. Statistical analyses were performed using PASW version 17.0 and Sample Power version 3 (SPSS Inc., Chicago, IL). Post-hoc power analysis and effect size determination was carried out with G*Power version 3.1 (Universität Düsseldorf). Differences in the number of participants between variables are a result of issues with participant scheduling, acquisition of blood samples, or assay procedure.

RESULTS

The study group consisted of 37 participants, with 23 in the LI group (CRP<3 mg/L) and 14 in the HI group (CRP≥3 mg/L). Participant characteristics and findings are presented in Table 1.

C-reactive Protein

By study design, the LI group had significantly lower CRP levels than the HI group before AEXT (1.3±0.2 vs 6.4±0.6 mg/L, P≤.001). However, only the HI group lowered CRP levels after AEXT and did so by 31% (6.4±0.6 vs 4.4±0.7 mg/L, P≤.001). Correlation analysis failed to reveal a correlation between body mass index or body fat percentage with CRP.

Aerobic Fitness

Aerobic fitness expressed as VO2max was significantly higher in the LI group when compared with the HI group before AEXT (27.8 ±1.3 vs 23.4±1.0 mL/kg/min, P≤.02). The LI group increased VO2max by 10% (27.8±1.3 vs 30.5±1.4, P≤.003), whereas the HI demonstrated no change after AEXT.

Body Mass Index

Body mass index, although not different between groups before AEXT (29.4±1.0 vs 32.7±1.6 kg/m2) was lowered in the LI group by 3% (29.4±1.0 vs 28.6±0.9 kg/m2, P≤0.01) with no change occurring in the HI group.

Plasma Triglycerides

Both groups had similar triglyceride levels before AEXT (87±9.4 vs 2.2±0.3 events/µL). Both the LI (3.7±0.5 vs 48.5±9.2 events/µL) and HI (3.6±0.7 vs 19.7±4.0 events/µL, P≤.002) lowered their levels after AEXT by 39% and 59% respectively.

Plasma Glucose

Glucose levels, although not significantly different between groups before AEXT (97±1.9 mg/dL), improved in the HI group by 10% after AEXT (97±1.9 vs 87±2.7 mg/dL, P≤.14) and no change occurred in the LI group.

DISCUSSION

Our pilot study revealed that mild-intensity AEXT improved several CVD risk factors in a sample of African Americans with lower levels
of inflammation. Conversely, in the HI group, our intervention failed to improve body mass index, triglyceride levels, VO₂ max, glomerular filtration rate, and EMP levels, all established markers of CVD risk that are well known to respond to AEXT.9,10,20 Nonetheless, these results need to be interpreted cautiously due to the small sample size of our study, which should only be regarded as a pilot project.

Our specific finding that CRP levels improved in response to AEXT only in the HI group is well-aligned with previous research.7,8,21 The lack of improvement in the LI group was expected since optimal levels of CRP had already been reached in this group. Interestingly, BMI only improved in the LI group. Previous studies have sought to demonstrate a link between obesity, specifically amounts of adipose tissue and its direct effects on systemic inflammation. It has been hypothesized that local cytokine production in adipose tissue increases the hepatic inflammatory response leading to systemic inflammation.22–25

In our sample of African Americans, CRP levels were not correlated with BMI or amounts of body fat. This was true when looking at both the HI and LI groups separately and the sample as a whole. Also, BMI and body fat levels were not different before AEXT between groups, suggesting that the difference in CRP between groups was not driven by differences in body fat. The fact that CRP levels improved in the HI group in the absence of improvements in body composition further suggests that, in our sample, obesity was not related to systemic inflammation. Although purely speculative, this may indicate a population-specific phenomenon unique to African Americans that may warrant further exploration.

Another surprising finding was the fact that aerobic fitness only improved in the LI group that incidentally also had higher initial levels of aerobic fitness. Previous studies have reported that higher levels of aerobic fitness and/or higher levels of physical activity were related to lower levels of systemic inflammation.26,27 This could explain the discrepancy of aerobic fitness between our groups prior to exercise, although we controlled for physical activity levels in our sample prior to the inclusion into our study.

The lack of improvement of aerobic fitness in the HI group, which is not something to be expected in light of a six-month aerobic exercise intervention, could possibly be explained by other diminished cardiovascular components influenced by chronic and systemic inflammation, which were not assessed in our study. It should also be noted that although the HI group improved CRP levels, on average, they still had what would clinically be considered high levels of systemic inflammation. This in itself may hinder positive cardiovascular adaptations to exercise until normalization of inflammation occurs.

Triglyceride levels have been strongly associated with CRP levels in numerous studies.6,25,28,29 Statin therapy has been used successfully to lower CRP levels.30,31 It may, therefore, seem surprising that triglyceride levels were similar in both the LI and HI groups prior to AEXT. However, in our study, triglyceride levels only improved in the LI group. One could expect that the HI group would show similar responses especially given the fact that CRP levels improved markedly in this group. But as mentioned before, the observed improvements in systemic inflammation did not lead to a normalization of inflammation. This might suggest that inflammation itself negatively impacts exercise mediated pathways that influence lipid profile markers.

Similar to triglycerides, increased glucose levels have been linked to CRP levels.6,32–34 This could explain the finding that the HI group improved glucose levels after AEXT while at the same time improving systemic inflammation.

Another interesting finding is the improvement of glomerular filtration rate in the LI but not the HI group. Chronic kidney disease has previously been linked to increased mortality from CVD. Additionally, patients with chronic kidney disease have an increased prevalence of inflammatory markers.35,36 Again, the lack of improvement in the HI group might support the hypothesis that even though CRP levels
were reduced, inflammation may still have been too high to elicit positive AEXT mediated changes in terms of improving kidney function.

One of the most defined targets of systemic inflammation and CRP is vascular function especially in relation to the development of atherosclerosis and hypertension.37–39 Recently, EMPs have been identified as novel markers of vascular disease stage.13–16,19 Increases in CD62+ labeled EMPs are usually associated with activation of endothelial cells, which is a sign of early stage endothelial dysfunction, a predecessor for atherosclerosis and hypertension. Conversely, CD31+/CD42b- labeled EMPs are considered a measure of late stage endothelial dysfunction and endothelial cell apoptosis.19 We found that the LI group improved both of these markers, whereas the HI group only improved the marker of early stage endothelial dysfunction. While this might merely be a function of our underpowered sample, it may also implicate inflammation as an impediment to AEXT mediated changes in endothelial health status at least in terms of CRP levels. A standard mild-dose AEXT intervention failed to improve numerous well-established CVD risk factors in the HI group, while improvements were observed in the LI group. Further studies with a larger sample are needed to confirm these findings. If indeed replicable, this would indicate that standard mild-intensity AEXT may not be enough to overcome inflammation as a mediator of certain CVD risk factors. Additional inflammation-lowering strategies, such as an increased or perhaps more frequent exercise stimulus, or even supplementary pharmacological interventions in combination with AEXT, may be necessary to improve CVD risk profile in African Americans with high levels of systemic inflammation.

CONCLUSION

Prior to AEXT, the HI group demonstrated higher CVD risk only in terms of CRP levels. A standard mild-dose AEXT intervention failed to improve numerous well-established CVD risk factors in the HI group, while improvements were observed in the LI group. Further studies with a larger sample are needed to confirm these findings. If indeed replicable, this would indicate that standard mild-intensity AEXT may not be enough to overcome inflammation as a mediator of certain CVD risk factors. Additional inflammation-lowering strategies, such as an increased or perhaps more frequent exercise stimulus, or even supplementary pharmacological interventions in combination with AEXT, may be necessary to improve CVD risk profile in African Americans with high levels of systemic inflammation.

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Conflict of Interest

No conflicts of interest to report.

Author Contributions

Research concept and design: Kretzschmar, Feairheller, Sturgeon, Brown; Acquisition of data: Kretzschmar, Babbit, Diaz, Sturgeon, Perkins-Ball, Williamson, Ling, Grimm; Data analysis and interpretation: Kretzschmar, Diaz, Sturgeon, Perkins-Ball, Williamson, Grimm, Brown; Manuscript draft: Kretzschmar, Feairheller, Grimm; Statistical expertise: Kretzschmar, Diaz, Williamson, Grimm, Brown; Acquisition of funding: Brown; Administrative: Babbit, Feairheller, Sturgeon, Perkins-Ball, Ling; Supervision: Brown

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