Racial Differences in Fc gamma Receptor Expression and Shear Stress Response to CRP in Endothelial Cells

BY

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THESIS
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SUMMARY

C-reactive protein (CRP) is a robust and independent marker of inflammation. It also decreases endothelial nitric oxide synthase (eNOS) expression and bioactivity, increases vasoconstrictor release, and increases adhesion molecule expression. African Americans (AA) have higher levels of CRP than Caucasians (CA), and CRP was found to cause a greater reduction in eNOS expression and bioactivity in the AA endothelial cells (ECs) compared with CA. In addition, AA ECs have shown larger improvements in EC health in response to the exercise mimetic, high levels of laminar shear stress (HiLSS).

CRP mediates the biological effects in ECs via Fcy receptors. CRP binding to Fcy receptors inhibits eNOS activation via Protein Phosphatase 2A (PP2A), a down-stream protein that dephosphorylates eNOS rendering it inactive. Preliminary data from the doctoral candidate shows racial differences in the expression of PP2A with CRP incubation.

The current study used an in vitro cell model to investigate how CRP may differentially affect ECs from AA and CA donors and whether HiLSS eliminates racial differences in CRP-induced proinflammatory and proatherogenic effects. Major findings of this study are: 1) CRP induced racial differences in eNOS expression and activity, NO bioavailability, ET-1 release, and expression of adhesion molecules; 2) CRP receptors (FcyRIIB) have higher levels of expression in AA ECs than in CA ECs under basal conditions and after CRP incubation, which partially contributed to the CRP-induced racial
SUMMARY (continued)

differences; 3) The exercise mimetic, HiLSS, counterbalanced the antagonistic effects of CRP, eliminated racial differences in suppressed eNOS expression and bioactivity, reduced NO bioavailability, and increased ET-1 release and adhesion molecules after CRP pre-incubation.

The significance of this current study provides a better understanding of the cellular mechanism of racial differences in endothelial dysfunction. This study provides evidence that HiLSS is effective in reversing the proinflammatory and proatherogenic effects induced by CRP and thereby eliminating the racial differences. Most importantly, this study is targeted to a high-risk population and provides evidence that exercise is particularly beneficial to the vascular function of AA.
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1.1 Introduction

African American (AA) adults have one of the highest rates of cardiovascular diseases (CVD) in the world [1], which contributes to greater cardiovascular mortality compared with Caucasians (CA). Endothelial dysfunction is a common feature of CVD risk factors such as hypertension, diabetes, obesity, kidney disease and hyperlipidemia [2]. Previous studies suggest racial differences in endothelial function exist in healthy and diseased populations at both clinical and cellular levels [3-6]. Exercise as an intervention is effective in improving endothelial function and CVD risk factors [7, 8].

Nitric oxide (NO) derived from endothelial nitric oxide synthase (eNOS) is one of the major blood vessel vasodilators. C-reactive protein (CRP), a risk marker for CVD, causes a reduction in eNOS expression and bioactivity in ECs [9]. Higher CRP levels in the AA population have been reported in previous studies from others and by our group [10, 11]. We showed that six months aerobic exercise significantly decreased CRP levels and increased endothelial-dependent dilation in AA [12]. In EC culture studies, the exercise mimetic, high levels of laminar shear stress (HiLSS), is effective at enhancing NO production in AA [13]. No study has combined these known facts to further investigate the mechanism of racial differences in endothelial dysfunction, and the potential role of exercise as a treatment.
Preliminary data generated by the doctoral candidate demonstrated racial differences in EC response to CRP stimulation: ECs from AA donors showed a significantly greater reduction of eNOS expression, a greater expression of interleukin-6 (IL-6), adhesion molecules intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and endothelin-converting enzyme-1 (ECE-1) than those from CA donors at the same concentration of CRP; Compared with CA, ECs from AA donors had significantly greater attenuation of eNOS activation after 1 hr CRP incubation. At the same time, eNOS recovery was detected in response to HiLSS after CRP incubation.

These findings underline the importance of studying endothelial function under different flow conditions. No study has assessed endothelial function with CRP stimulation and shear flow particularly in AA. There is a need for a study to investigate the effects of exercise training on endothelial function under CRP stimulation in AA and CA ECs, because AA tend to have high levels of CRP.

Based on the doctoral candidate’s preliminary finding and the current literature, the hypotheses of the proposed study are 1) CRP receptors (Fcγ receptors) in AA ECs will have a higher level of expression than CA ECs with/without CRP incubation; 2) An exercise mimetic, HiLSS, will attenuate the CRP-induced antagonism on endothelial function, and eliminate the racial differences after CRP pre-incubation.

**AIM 1:** Determine Fcγ receptors (FcγRIIB) expression in AA and CA ECs with and without CRP incubation. Western blot and qRT-PCR were used to
assess the expression of Fcγ receptors. In addition, the Fcγ receptor expression levels was also assessed under different concentrations of CRP.

Working hypothesis: Fcγ receptors in AA ECs will have a higher level of expression than in CA ECs with/without CRP incubation.

**AIM 2:** Measure eNOS expression and bioactivity, NO bioavailability, ET-1 release, adhesion molecules followed by application of HiLSS to both AA and CA ECs after CRP pre-incubation.

Working hypothesis: An exercise mimetic, HiLSS, will attenuate the CRP-induced antagonism in ECs, and eliminate the racial differences in suppressed eNOS expression and bioactivity, reduced NO bioavailability, increased ET-1 release and adhesion molecules after CRP pre-incubation.
2.0 Literature Review

2.1 General introduction to C-reactive protein

2.1.1 History

C-reactive protein (CRP) is a highly conserved blood plasma protein discovered by Tillet and Francis at Rockefeller University in 1930. In the first report, the level of the substance – Fraction C (later renamed as C-reactive protein or CRP) increased to high concentrations in acute pneumococcal pneumonia patients during the early stage of inflammation and reacted with the C-polysaccharide of Pneumococcus [14]. Subsequently, more studies have shown the acute phase reactant role of CRP [15].

Although CRP was discovered just less than 100 years, it actually evolved long before the adaptive immune system existed. Since its discovery, substantial research has been done to reveal CRP structure, biochemistry, and function. At the very beginning of tissue injury, infection or inflammation, CRP is secreted by the liver in response to the Interleukin-6 (IL-6) released by macrophages and adipocytes. As an acute phase reactant, CRP plays an important role in inflammation and autoimmunity. CRP started receiving increased attention relative to cardiovascular disease (CVD) after studies showed increased levels of CRP among patients with acute or chronic atherothrombotic disease [16, 17].
2.1.2 Structure

CRP is primarily produced and secreted by the liver, other sites such as kidney, local atherosclerotic tissues, neurons and alveolar macrophages also have been reported to synthesize CRP [18-21]. Circulating CRP is exclusively produced by hepatocytes and transcriptionally driven by IL-6, with synergistic enhancement by IL-1. Activation of IL-1 is mediated through the transcription factors STAT3 and NF-κB. [22]. Human aortic arterial ECs (HAECs) have been demonstrated to synthesize and secrete CRP [23].

CRP is synthesized as a monomer and assembled into pentamers in the liver endoplasmic reticulum, and retained in hepatocytes endoplasmic reticulum in the resting state [24]. In response to stimuli, the binding of CRP to carboxylesterases decreases and CRP is quickly secreted into blood [25].

CRP belongs to the pentraxin family, which is characterized by containing a pentraxin protein domain. CRP is formed as a ring by five symmetry monomers each contains 206 amino acids in 14 antiparallel β-strands, arranged in two β-sheets. The pentamer is relatively stable and resistant to heating and proteases. In contrast, the five monomers are connected by noncovalent bonds and are not able to reassemble after high pH exposure.

X-ray crystallography indicated each monomer of CRP is folded into two antiparallel beta sheets with a flattened jellyroll topology. There are two important
residues—Phe66 and Glu81 on the recognition face, B face, that mediate the binding of phosphocholine. Binding sites of C1q and Fcγ receptors are located on the opposite side, A face, of the pentamer.

The functions of two different forms of CRP are not clear. To date, there are still many controversies about the biological effects of pentameric CRP and monomeric CRP. Pentameric CRP might be proatherogenic while monomeric CRP might be antiatherogenic, but this assumption is questionable. Pentameric CRP was reported to increase adhesion molecules on ECs but then it was shown that the effects might be caused by lipopolysaccharide and sodium azide in commercial CRP preparations [26, 27]. Findings from an 8 week treatment (2.5 mg/kg subcutaneously, weekly) of pentameric CRP to 14-week-old female ApoE−/− mice resulted in a 4-fold higher mean aortic plaque area compared with the saline controls, whereas the mean plaque size was decreased by approximately 50% in monomeric CRP-treated ApoE−/− mice (2.5 mg/kg subcutaneously, weekly) [28]. It has also been reported that monomeric CRP evokes increased IL-8 and monocyte chemotactic protein 1 (MCP-1) production in HAECs, which occurs through anchoring to lipid rafts, rather than binding to the Fcγ receptors on the cell surface [29].

2.1.3 Function

A variety of proteins acutely respond to injury or inflammation with dramatic concentration changes. As a host defense protein, CRP rapidly participates in the systemic response to inflammation and reaches its peak blood
level at 24-48 hr and returns to basal levels as the inflammation resolves [30].

One of the main functions of CRP is to specifically recognize and bind to molecular configurations (ligands and effectors) and finally eliminate foreign pathogens. The protective role of CRP has been shown in human CRP transgenic mice infected with S. pneumoniae. Serum CRP rapidly increased as expected following the infection. It was shown that bacteremia in CRP-transgenic mice was 400-fold lower than control mice; the CRP-transgenic mice also had a significantly higher percent survival and survival time than their control counterparts [31].

CRP binds to ligand phosphocholine only during cell death or apoptosis to initiate the immunologic response, which cannot happen in normal cells [32]. Except for phosphocholine, CRP also binds to a number of ligands, such as phosphoethanolamine, fibronectin, chromatin and histones, to activate the C1q complement pathway [33-35]. Extensive studies have shown that CRP can act as an opsonin for bacteria to activate C1q complement system leading to phagocytosis of nuclear components [36, 37]. CRP induces expression of several cell adhesion molecules as well as tissue factors [38]. CRP mediates LDL uptake by endothelial macrophages, induces monocyte recruitment into the arterial wall, and enhances production of MCP-1 [39]. CRP could interact with a variety of cell types and the responses differ from each other with limited reproducibility. One major reason for the inconsistent results is the contamination of endotoxin, which causes a strong synergistic biological response with CRP. Later studies noticed
the potential influences of contamination and removed endotoxin and sodium azide from purchased CRP before application. The biological effects of CRP have been confirmed and are not attributable to endotoxin [40].

In humans, the plasma level of CRP can dramatically increase up to 1000 fold within the 24 hrs of initial inflammatory stimulus [41]. Under this condition, during the acute phase, CRP suppresses inflammation. Exceptions are in systemic lupus erythematos (SLE), and perhaps in scleroderma, where CRP levels have been shown to not be associated with inflammation [42].

In humans, chronic elevated levels of CRP are associated with increased cardiovascular risk in which is CRP levels have long been employed for clinical purposes [43]. Recent studies show significant evidence that the role of CRP is not only a biomarker of future cardiovascular events, but actually participates in the CVD progression [44].

The independent prognostic significance of CRP has been determined in many human trials. In the Women’s Health Study, which included 28,263 subjects, plasma CRP was the only marker that independently predicted the risk of thromboembolic stroke [45]. Levels of hs-CRP were measured in 3771 patients with stable coronary artery disease from the Prevention of Events With Angiotensin-Converting Enzyme Inhibition (PEACE) trial with a median follow-up period of 4.8 years. After adjustment for baseline characteristics and treatments, an elevated level of high sensitivity-CRP (hs-CRP) still independently predicted cardiovascular events [46]. Because of its high correlation with CVD, stable
biochemistry, ease of measurement, the Centers for Disease Control and American Heart Association recommend CRP as a reliable biomarker of hypertension and coronary heart disease [47].

### 2.1.4 Mechanism

The CRP gene is located on the first chromosome at position 1q23.2. Studies on genetic polymorphisms of CRP have been conducted to investigate the intra-individual variability in basal CRP levels, which are genetically regulated. It has been shown that polymorphisms in the 3’ untranslated region and one synonymous mutation in exon 2 might be associated with lower basal levels of serum CRP [48].

Although basal CRP level is substantially influenced by genetics, the contribution of environmental and intrinsic variables, such as lifestyle, age, gender, smoking, and blood pressure status, cannot be neglected. In addition, the CRP promoter contains both IL-1 and IL-6 responsive elements therefore IL-6, IL-1, TNF-α and other cytokines can all contribute to CRP levels [22, 49]. On the other hand, polymorphisms in CRP receptor (Fcγ receptor) genes may also be one of the factors that contribute to higher CVD risk.

The activities of CRP are largely dependent on its interaction with Fcγ receptors. The CRP Fcγ receptor signaling pathway is not well understood due to the complexity of the Fcγ receptor system. For many years, it appeared that only one CRP receptor participated in the signaling cascade, but later evidence showed that two of three families of Fcγ receptors actually bind to CRP and the expression of different families of Fcγ receptors vary among cell types [50-52].
Fcγ receptors (IgG receptors) were first found to be associated with CRP when it was found that IgG could completely block CRP binding to leukocytes and neutrophils [50]. The differences between these two types of Fcγ receptors are the immunoreceptor tyrosine-based motifs: activation motif of FcγRI located in its γ-chain subunit while the inhibitory motif of FcγRIIB is located in its cytoplasmic domain. Activating FcγR is required to initiate inhibitory FcγR function [53]. Findings from previous studies strongly support that CRP directly interacts with Fcγ receptors [54-56]. FcγRIIB (CD32) and FcγRI (CD64) have been confirmed to be the Fcγ receptors that interact with CRP through gene knock out experiments [57, 58]. For example, CRP did not attenuate carotid artery conductance induced by Ach in FcγRIIB−/− mice, which suggests that CRP mediates its effects through FcγRIIB receptors [59]. Pre-incubation with antibodies to block FcγRI and FcγRIIB in cultured ECs effectively prevented CRP-induced effects [60]. In cells not expressing Fcγ receptors, CRP does not antagonize eNOS activation. In addition, investigators were able to determine that compared with IgG, CRP has higher affinity for Fcγ receptors. CRP binding to FcγRI initiates important signaling cascades that regulate inflammation, and induces potent anti-inflammatory effects by synthesizing cytokines, especially IL-10 and IL-1 receptor antagonist (IL-1RA) [61, 62]. While high levels of CRP acutely play an anti-inflammatory role by increasing IL-10 and IL-1RA, chronic, low levels of CRP are proinflammatory and proatherosclerotic. The different effects may be due to different affinities of CRP to Fcγ receptors.
Studies have confirmed that CRP elicits responses through binding to FcγRIIa [63]. The phagocytosis of zymosan by neutrophils after CRP stimulation was blocked by FcγRIIa inhibitors. CRP does not bind to FcγRIIIB (CD16), a major inflammatory receptor expressed on inflammatory macrophages, mast cells and natural killer cells [64].

CRP binds to neutrophils and monocytes and activates phagocytosis of CRP-opsonized particles and cytokines production [65], which is similar to aggregated IgG and with production of IL-1β, IL-6, and TNF-α.

2.2 Cardiovascular disease and CRP

Large amounts of published data support a role for CRP as a strong predictor for CVD. CRP satisfies three major criteria to be a CVD biomarker; accuracy, reliability, and likelihood of beneficial intervention [66]. The AHA and the CDC categorize CRP levels into 0 to 1, 1 to 3, and ≥ 3 mg/L, which correspond to low-, moderate-, and high-risk groups for future cardiovascular events [47]. More than a dozen population-based studies have demonstrated that elevated levels of CRP in apparently healthy individuals can be an independent predictor of future onset of cardiovascular events such as myocardial infarction (MI), coronary heart disease (CHD), stroke, unstable angina, peripheral arterial disease, and sudden cardiac death [67, 68].

The prognostic relevance of CRP has been confirmed by several large cohort studies. CRP levels have been found to be positively and significantly associated with the incidence of future CHD events in initially healthy middle-
aged subjects after adjustment for all other vascular risk factors [69]. Data from the Physicians’ Health Study found that healthy male physicians who developed symptomatic peripheral arterial disease were in the highest quartile of baseline CRP (> 2.1 mg/L) had a 2-fold higher risk of stroke, a 3-fold higher risk of MI after a 60-month follow-up period, compared with the lowest quartile (< 0.55 mg/L) [70].

In another study, both males and females with high levels of CRP consistently demonstrated high vascular risk, even in the absence of hyperlipidemia [71].

In many clinical studies, hs-CRP has been shown to be the strongest predictor of cardiovascular events among numerous biomarkers, such as serum amyloid A (SAA), IL-6, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and ICAM-1. A large prospective and nested case-control study was conducted to evaluate 12 inflammation markers and the relative risk of cardiovascular events. Hs-CRP had the highest prognostic value for future cardiovascular events among the 12 inflammation markers. Predictive values in models that included CRP were significantly higher than those including traditional risk factors [45]. One study also found that individuals with the highest baseline CRP levels had a 5-fold increased risk of any vascular event and a 7-fold increase in risk of MI and stroke [67]. Together these results indicate that CRP is capable to predict future vascular events in current low-risk population without other risk factors appearance.

Cross sectional data suggest that CRP significantly correlates with calculated 10-year Framingham Coronary Heart Disease Risk in men and
women not taking hormone replacement therapy (HRT) and that CRP levels have minimal correlation with most individual risk components (age, HDL-C, LDL-C, blood pressure, diabetes and smoking) in the Framingham Coronary Heart Disease Risk Score [72]. Algorithms that add CRP to traditional risk factors significantly improved future event prediction [73, 74]. This indicates an adjunctive role of CRP for the global prediction of CVD based on Framingham risk factors.

2.3 Inflammation and CRP

Inflammation is believed to be a pivotal contributor to the initiation and progression of pathogenesis of cardiovascular events. CRP is a promising biomarker of inflammation in the clinical environment due to its long half-life and high stability in circulation. Chronic, low levels of serum CRP have been shown to correlate with systemic inflammation and atherosclerosis.

It has been suggested that CRP is not only a marker of systemic levels of inflammation but may have direct effects on the development and progression of atherothrombosis [75]. CRP binds complement C1q in activated ECs with an upregulation of chemokines and adhesion molecules and thereby participates in atherogenesis [76]. In a CRP transgenic mouse model, enhanced thrombosis has been demonstrated, but whether CRP accelerates atherogenesis progression remains unclear [77]. It has been reported that elevations in serum CRP may decrease stability of the fibrous cap tissue, trigger plaque rupture and form occlusive thrombosis [69].
Investigators have conducted studies to elucidate the roles of CRP in inflammation and its biological responses. The primary function of CRP during tissue injury is to activate the complement cascade in order to remove the dead cells and therefore CRP could potentially promote vascular damage [78]. Injection of human CRP into mice with myocardial infarction caused enhanced tissue damage, and the depletion of C1q complement eliminated the effect [79].

Animal studies have demonstrated CRP has an anti-inflammatory function in different models. In those studies, rabbit CRP transgenic mice have shown resistance to endotoxin or platelet-activating factor (PAF), but not TNF-α [80]. Several groups reported that passive administration of CRP could initiate protection from endotoxin [61, 81]. *In vitro* study results showed that CRP could inhibit neutrophil migration to chemotactic factors [82]. However, the mechanism of these protective effects has not been investigated.

The participation of Fcγ receptors in CRP induced protection has been studied by using FcγRIIB-deficient mice. CRP failed to protect FcγRIIB-deficient mice from lipopolysaccharide (LPS) shock and actually increased lethality in these mice. The increased production of IL-10 in response to LPS was found to be anti-inflammatory both *in vivo* and in bone-marrow macrophage cultures from normal mice, but not in γ-chain deficient mice and macrophage cultures from these mice. CRP binding to FcγRIIB suppresses the IL-12 production in response to LPS [61].
CRP promotes inflammatory cell adhesion via p38 mitogen-activated protein kinase (MAPK)-dependent upregulation of IL-8 [44]. CRP induces endothelial apoptosis and proinflammatory mediators by initiating production of TNF-α, IL-1β and Matrix metalloproteinase-9 (MMP-9) [83]. CRP also has been shown to induce the production of endothelial microparticles (EMPs) and circulating ECs in vivo and in vitro, which are biomarkers of endothelial dysfunction [84, 85].

2.4 Endothelial dysfunction and CRP

Endothelial dysfunction is the initial and integral component for future cardiovascular disease, which primarily manifests as nitric oxide (NO) deficiency and augmented expression of endothelin-1 (ET-1), angiotensin II (Ang II), plasminogen activator inhibitor 1 (PAI-1), adhesion molecules, and cytokines. Endothelial dysfunction shifts the endothelium toward suppressed vasodilation, proinflammatory and atherogenic states. The inverse association of CRP and endothelial function was first shown when acetylcholine (Ach)-induced brachial artery dilation was reduced in patients with elevated levels of serum CRP [86].

There is substantial evidence that CRP has direct effects on endothelial function. In both in vivo mouse models and in vitro EC cultures, CRP has been shown to attenuate survival, differentiation, and function of endothelial progenitor cells [87, 88]; activate ECs to express adhesion molecules; reduce eNOS mRNA and protein expression and eNOS bioactivity in dose- and time-dependent manner [89]. Importantly, CRP antagonism of eNOS activity has been associated
with decreased endothelium-dependent vasodilation, which increases the risk for CVD in the long-term [90]. CRP induces release of endothelin-1 (ET-1), a potent vasoconstrictor and a mediator of CRP-induced upregulation of adhesion molecules and MCP-1 from ECs [91].

Nitric Oxide (NO) is one of the most important vasodilators released by endothelium. It inhibits inflammation, platelet aggregation and smooth muscle cells proliferation. NO production is caused by an eNOS signaling complex that includes two important proteins, Hsp90 and calmodulin. CRP treatment (12.5 and 25 µg/mL) of HAECs for 24 h significantly increased eNOS binding to caveolin-1 and decreased binding to Hsp90, which resulted in decreased eNOS activation [92].

In EC experiments, incubation CRP at 5 µg/mL for 1 hr impaired diverse agonists of (insulin, VEGF, estrogen, and high-density lipoprotein) activation of eNOS, resulting in blunted L-arginine conversion to L-citruline by eNOS to generate NO. In an in vivo mouse study, Ach-induced carotid artery vascular conductance and eNOS activation were blunted by CRP treatment. Endothelium-dependent vasodilation was potently reduced in CRP-overexpression transgenic mice compared with control mice [59]. CRP treatment in porcine coronary arterioles significantly attenuated NO production and vasodilation, and increased nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) activity and superoxide production [93]. Compared with human serum albumin (HuSA) treated rats, human CRP-treated rats exhibited reduced endothelium-dependent
vasodilation by reducing eNOS cofactor tetrahydrobiopterin (BH4) availability, resulting in uncoupling eNOS and decreased eNOS dimer : monomer ratio [94]. Prolonged exposure (24 hr and longer) of ECs to CRP destabilized eNOS mRNA and decreased eNOS expression [95]. In CF1-CRP transgenic mice, CRP blunted eNOS mRNA and enzyme abundance in carotid arteries at 9 µg/mL in vivo [94].

Strong evidence from in vitro studies has suggested that CRP directly interacts with ECs, inducing pro-inflammatory cytokines and monocyte infiltration. A recent report has indicated that human CRP may directly increase thrombosis in mice with vascular injury, which suggests that CRP expression has a proatherogenic effect in vivo [96]. After CRP incubation, increased mRNA and protein expression and activity of plasminogen activator inhibitor 1 (PAI-1), a proatherogenic serine protease inhibitor and a marker of atherothrombosis, were detected in human aortic ECs [97]. CRP overexpression markedly augmented aortic endothelial staining for ICAM-1, VCAM-1, E-selectin, MCP-1, and enhanced macrophage recruitment and infiltration [98, 99]. CRP also promotes platelet adhesion to ECs through increasing P-selectin expression.

Prostacyclin (PGI2) is another important vasodilator secreted by ECs. It inhibits platelet aggregation and smooth muscle cell proliferation. CRP incubation decreases prostacyclin in both HAECs and human coronary artery ECs [100]. CRP enhances lectin-like OxLDL receptor-1 (LOX-1) mRNA and protein expression, resulting in increased human monocyte adhesion to ECs and OxLDL
uptake by these cells. Studies also have shown that blocking FcγRIIB and FcγRI inhibits CRP-induced OxLDL uptake [99].

2.5 Racial Differences in cardiovascular disease and endothelial function

It is well documented that the prevalence of CVD in AA is significantly higher than CA. Not only the prevalence but also the onset of CVD happens earlier with greater severity in AA. Essential hypertension in AA is approximately twice that of CA. Except for socioeconomic status and physical activity levels, genetic susceptibility might substantially contribute to higher cardiovascular risk factors and pathogenesis of hypertension in AA.

AA healthy adults have impaired endothelial -dependent and -independent dilation: Vasodilator response to Ach (a vasodilator that stimulates endothelial release of NO) and sodium nitroprusside (an exogenous NO donor) was lower than CA, suggesting that cGMP-mediated smooth muscle relaxation is blunted in AA [101, 102]. Isoproterenol-induced (a β-adrenergic agonist whose vasodilator effect stems from the combination of direct smooth muscle stimulation and endothelial NO release) vasoreactivity was lower in AA than in CA both before and after NG-monomethyl-L-arginine (eNOS inhibitor) implying that cAMP-dependent vasodilator response to isoproterenol is diminished in African Americans.

The kinetics of NO/O2−/ONOO− has been detected in Human Umbilical Vein Endothelial Cells (HUVECs) from both AA and CA donors. Compared to CA HUVECs, NO/O2−/ONOO− balance in AA HUVECs was closer to redox states
meaning that oxidant/antioxidant ratio was higher in AA. Due to increased $O^2-$ production mediated by upregulated NADPH oxidase activity followed by eNOS uncoupling, NO bioavailability in AA HUVECs was lower than in CA [3]. This likely contributes to the decreased endothelial function in AA.

Plasma concentrations of ET-1 are significant higher (four to eight fold) in normotensive and hypertensive AA than CA [103, 104]. ET-1 is a potent vasoconstrictor and has been proposed to regulate blood pressure through multiple pathways. It is reported that Angiotensin II (ANG II) upregulates ET-1 production in cultured ECs through angiotensin-converting enzyme (ACE)–sensitive mechanism [105]. There are two subtypes of ET-1 receptors, ETA and ETB. ETA receptors are expressed on smooth muscle cells, and promote vasoconstriction and cell proliferation. ETB receptors are found on the endothelium and smooth muscle, and stimulation of ETB receptors results in vasodilation via release of NO or prostacyclins. The ratio and relative density of ETA and ETB is important to determine the contractile response to ET-1 [106]. AA males possess two-fold higher expression of ET-1 receptors than AA females. One study found that both subtypes of ET-1 receptors are expressed on smooth muscle cells of saphenous veins of AA, but only ETA receptors were found in CA veins. Specifically, the total ETB receptor density was lower compared to AA [107]. This decrease in the ratio of endothelial to smooth muscle ETB suggests a shift in favor of vasoconstriction-promoting receptors.
Significant racial differences were found in levels of serum CRP in several large-scale studies. For example, in Dallas Heart Study, a total of 2749 African Americans and Caucasians were measured for serum CRP levels. The median CRP concentration in AA was significantly higher than CA (3.0 vs. 2.3 mg/l; p < 0.001) [108]. Genetic factors have been shown to account for 40% of the variance in basal serum CRP levels [109]. Polymorphisms within CRP gene promoter locus were demonstrated to associate with higher basal CRP levels in AA. Genome-wide association studies have shown a CRP-associated variant of triggering receptors expressed by myeloid cells 2 (TREM2) in chromosomal region 6p21 (p = 10^{-10}) that appeared to be related to higher CRP levels in AA [110]. However, whether there are racial differences in Fcγ receptor expression is unknown. Potential differences in Fcγ receptor expression in ECs of AA and CA might lead to the differential effects of CRP inhibition on eNOS expression and activity.

2.6 Sex differences in CRP

Overall, females have substantially higher median CRP levels compared with males. CRP levels are significantly influenced by the quantity and distribution of adipose tissue. In Dallas Heart Study, CRP and body fat were measured in 1166 male and 1413 female middle-aged subjects [98]. The results showed that CRP levels were strongly associated with total fat mass in both sexes, and significantly higher fat mass and CRP levels were observed in the female subjects. Data from the Multiethnic Study of Atherosclerosis (MESA)
cohort suggest that the sex discrepancies and associations between CRP and body mass remain in both high and low BMI groups even after multivariable adjustment [111].

2.7 Regulation and mechanism of CRP antagonistic effects to endothelial cells

CRP mediates its down stream signaling pathway by binding to cell plasma membranes via IgG Fcγ receptors (FcγRs). CRP and immune complex IgG have similar ability to antagonize eNOS expression and NO bioavailability, resulting in endothelial migration inhibition, adhesion molecule production, impaired vasodilation and hypertension. CRP attenuation of eNOS activity may act through multiple mechanisms.

CRP-induced inhibition effects on eNOS requires the activating Fcγ receptors, FcγRI, which is functionally coupled with the inhibitory FcγRIIB to phosphorylate its immunoreceptor tyrosine-based inhibitory motif (ITIM), followed by phosphorylation of Tyr1020 to active Src kinase dependent SH2 domain containing inositol 5'-phosphatase 1 (SHIP-1) [112]. SHIP-1 inhibits downstream signaling of phosphatidylinositol 3-kinase (PI3K) thereby blunting activation of Akt, which phosphorylates eNOS on Ser1179, resulting in impaired eNOS activity and decreased NO bioavailability. Phosphorylation of Akt is counter-regulated by the phosphatase PP2A. Inhibition by okadaic acid or knockdown of PP2A by short-interference RNA reverses CRP antagonism of eNOS [59]. This signaling pathway provides more evidence that CRP is not merely a passive CVD
biomarker but an active mediator in the pathological process. From a therapeutic perspective, prevention of Fcγ receptors activation could be considered to inhibit the antagonism of eNOS under higher levels of CRP such is often observed in AA.

2.8 Exercise and CRP

Exercise is associated with decreased risk factors of CVD, which may result, in part, from its effects on reducing low-grade systematic inflammation. Acutely, exercise produces a quick, short inflammatory response, while long-term exercise training demonstrates an “anti-inflammatory” effect [113].

Results from several studies consistently show that along with white blood cell count, IL-1 and creatinine kinase (CK), CRP concentrations acutely increased (200%-2000%) immediately after different types of exercise (marathon race, cycling, canoeing, etc.) and returned to baseline levels within 48 h. CRP, IL-1, IL-6 and TNF-α are involved in post-exercise inflammation [114-116]. During and immediately after exercise, IL-6 is the earliest and greatest responder to exercise, which stimulates hepatocytes to produce CRP. At the same time, exercise also acutely increases various anti-inflammatory mediators, such as IL-1 receptor antagonist, soluble TNF-α receptors, IL-10 and IL-8 [113].

The major cause of increased IL-6 following acute exercise is muscle damage. Recently, studies have revealed that a complex intramuscular signaling pathway stimulates IL-6 release from exercised muscle, which is independent of muscle damage [117]. Consequently, damaged muscle initiates a repair
response, inducing macrophages into the muscle and releasing more IL-6. Post-exercise CRP levels seem to be proportional to the intensity and duration of exercise and related to the type of exercise and the muscle group involved.

Cross-sectional studies demonstrate that the concentration of serum inflammation markers is negatively correlated with regular physical activity. Physically active elderly individuals have lower CRP levels than their counterparts [118]. The effects of exercise training on CRP levels vary by type of exercise. Baseline levels of CRP were significantly lower in swimmers and rowers compared to untrained control subjects, while there was no difference in soccer players [119]. The National Health and Nutrition Examination Survey (NHANES) III demonstrated that the amount of leisure-time physical activity is inversely associated with CRP levels in 13748 subjects (p < 0.001), which remained significant after adjusting for cofounders such as age, gender, race, education, body mass index (BMI), hypertension, and cholesterol etc [120].

Prospective studies have shown that exercise training reduces CRP levels. A randomized trial of 39 subjects demonstrated that CRP levels were significantly reduced after three and six months of supervised active and passive leg exercise training [121]. Similar effects were found in subjects after nine months of marathon training, which significantly reduced CRP levels by 31% compared with non-training controls [122]. It is likely that exercise training reduces CRP by reducing the up-stream cytokines released from adipose tissue, skeletal muscle, and mononuclear cells.
2.9 Mechanism of reduced CRP levels by exercise training

Upregulation of CRP production could be elicited by many stimuli. Increased IL-6 production by increased adipose tissue contributes to an elevated CRP level. Exercise training decreases IL-6 production by reducing adipose mass, which results in reduced CRP production. A ten months, 3 days/week, 45 min/day of aerobic exercise training resulted in significant reductions in CRP, TNF-α, and IL-18 in subjects aged 64 and above. Resistance training also has been reported to significantly reduce serum CRP levels in previously sedentary subjects after 10 weeks, while no changes were detected in IL-6 [123].

It has reported that exercise training with weight loss lowered CRP levels. A total of 199 subjects completed a 2-month supervised aerobic exercise training program. Significant weight reduction was observed and variables related to cardiovascular risk were improved. The changes in CRP levels were disproportionately associated with the loss of body weight [124]. Another study found a strong association between CRP and the level of physical activity after adjusting for body mass index and waist-to-hip ratio, which suggests that physical activity influences the inflammatory process may through other mechanisms [125].

The mechanism underlying the exercise training-induced reduction in inflammation and decreases in serum CRP levels is not well established. Exercise training may reduce CRP levels by reducing adiposity, which secretes IL-6 and TNF-α may contribute to the elevated CRP levels observed in obesity.
However, there are studies including ours that show independent effects of exercise training on CRP levels [126, 127]. In addition, exercise training increases anti-oxidant capacity, which is a potential mechanism to lower CRP levels. It is proposed that exercise training-induced increases in β-adrenergic receptor sensitivity leads to reduction of IL-6 from adipocytes with less IL-6 released into circulation resulting in a consequent reduction in CRP production.

2.10 Exercise training, shear stress and endothelial function

Regular exercise training improves endothelial function by preserving and augmenting NO bioavailability due to chronic increases in the expression of eNOS and eNOS activity. Exercise training is proven to mitigate inflammation partly by improving endothelial health. ECs are known to secret IL-1 and IL-6 and under inflammation conditions, ECs could increase IL production and adhesion molecules. The total anti-inflammation effect reduces oxidation of LDL, which further improves endothelial function by preventing endothelial injury and inflammation. Exercise training is well known to reduce inflammation markers associated with endothelial dysfunction, such as ICAM-1, VCAM-1, MCP-1 [128].

A well-controlled study investigated the effects of exercise training on coronary endothelial function in 19 patients with coronary endothelial dysfunction. Conditions that might be cofounders including smoking, diabetes, hypertension, hypercholesterolemia and ventricular tachyarrhythmias were excluded. After four weeks of exercise training, the mean vasoconstrictive responses to the intermediate dose of acetylcholine were significantly attenuated compared with
baseline; significantly greater increases in coronary blood-flow velocity in response to acetylcholine from baseline compared to the control group [7]. In another study, FMD and glyceryltrinitrate (GTN)-mediated dilation were measured before and after 10-week exercise training in 25 healthy military subjects. Only FMD was found to significantly increase compared with baseline, while no change in GTN-mediated dilation was observed [8].

The endothelium serves not only as a static barrier between circulating blood and vessel wall, it is a dynamic system that actively regulates vascular remodeling, modulates vasomotor tone and function by synthesizing and releasing vasodilators and vasoconstrictors. The movement of viscous fluid over ECs results in a tractive horizontal force on the cell surface. Shear stress defined by force per unit surface area is a physiologically relevant mechanical force to regulate endothelial function. LSS regulates endothelial gene expression through complex mechanoreceptors and mechanotransduction process. The mechanisms underlying how LSS mediates downstream signaling pathways are not fully understood.

Shear stress is highly associated with the location of atherosclerotic lesions, which occur preferentially in regions of low or disturbed shear stress at vessel branch points, bifurcations and regions of high curvature. Extreme shear stress, either extremely high or low, has been demonstrated to contribute to vessel wall pathology. In vitro, cultured ECs exposed to low-levels of shear stress (< 5 dyne/cm²) display an atherogenic phenotype. High levels of physiological
LSS (> 15 dyne/cm²) are considered to be atheroprotective by inducing atheroprotective gene expression profile and initiating endothelial quiescence [124]. Using the DNA microarray technique, data from cultured ECs in response to 24 hr of 12 dyne/cm² showed that long-term high levels of LSS downregulated genes related to inflammation and proliferation [124].

A large number of studies have been done that investigated the influences of steady LSS on ECs in vitro using a specially designed apparatus—cone-and-plate system. The theory and design of this cone-and-plate system has been fully defined and tested. Different shear rates, exposure intervals and durations can be precisely controlled by this experimental system to simulate different physiological conditions (e.g. sedentary and exercise).

HiLSS exposure triggers a number of events, of which the most important one may be an increases in eNOS mRNA, protein, and activity. The central role of eNOS in shear stress-mediated vasodilation has been confirmed by numerous studies [129-131]. HiLSS activates a pathway involving PI3K and phosphorylation of Akt, which phosphorylates eNOS. Acute LSS stimulation immediately increases prostacyclin and NO, both are vasodilators and also inhibit smooth muscle proliferation, and hinder platelet activation. Expression of vasoconstrictors, inflammatory mediators and adhesion molecules are suppressed by HiLSS. Morphologic changes of ECs can be observed after exposure ≥ 12 hr of steady HiLSS (> 5 dyne/cm²). The shape of ECs changes from polygonal to ellipsoidal shape and align in the same direction of the fluid
force. These atheroprotective responses to HiLSS are due to shear regulation of endothelial phenotype. On the other hand, proinflammatory mediator expression is increased at LoLSS shear region. In one study in cultured ECs, CRP, VCAM-1, and ICAM-1 expression were upregulated up to 3-fold and IL-6 up to 16-fold by LoLSS, compared with the control [132]. HiLSS has been reported to increase ICAM-1 expression and prevent the VCAM-1 upregulation induced by TNF-α [133]. Whether HiLSS prevents the antagonistic effects of CRP on eNOS and endothelial function has not been studied. If HiLSS can antagonize the effects of CRP on eNOS, then in regards to endothelial function and vascular inflammation, African Americans may benefit more from moderate aerobic exercise training compared to CA.
3.0 Methods

3.1 Cell Culture

Human umbilical vein ECs (HUVECs, Lonza) were cultured in the endothelial basal medium-2 media containing 2% fetal bovine serum (FBS) and growth supplements and will be used between passages 2-6.

The rationale for using HUVECs is based on the needs of the project, which is to study ECs response to LSS. Vein ECs are not arterial ECs and ECs in different vascular beds can have varying functions. HUVECs have been one of the most studied EC types and yield reproducible biologic responses and demonstrate most of the same patterns of gene expression as ECs from arteries. Approximately 500 studies have been published on the effects of shear stress on HUVECs. LSS gene expression studies show that responses of arterial and venous EC are very similar.

Eight cell lines were used, four from AA and four from CA with sex evenly split. All cells were maintained at 37°C in a 5% CO\textsuperscript{2} atmosphere, in 100 mm glass tissue culture dishes. Incubation of CRP was done when cells reaches log phase and at 80%–90% confluence. HUVECs were treated identically following the instructions from Lonza, and samples used for all assays were tested in duplicate.

The doctoral candidate recognizes that researchers who conduct primarily human studies summarily believe that sample sizes required for human and cell
studies are similar. Human studies require such large sample sizes because of the tremendous heterogeneity introduced by the presence of the complex biology and environmental influences. Cell culture studies inherently have less random error because complex physiology and other confounding factors are eliminated. In addition, environmental influences are negligible as comparison studies are performed in parallel. While this does limit external validity, it allows for a smaller sample size than in human in vivo studies. Samples sizes in nearly all published EC culture studies that show statistical significance typically are in the range of N=3-4. Actually, an N>5 experimental units is considered a large sample size.

3.2 Materials

Recombinant human CRP was purchased from Calbiochem. All CRP preparations were purified under sterile conditions using Endotoxin-removal columns (Pierce Biochemicals) and the CRP was used only if the concentration of endotoxin was ≤0.125 EU/mL. All cell culture media was endotoxin-free. Different Concentrations range from 0 to 100 µg/mL of purified CRP were applied to the confluent ECs for 1 hr and 24 hr. The eNOS agonists VEGF (2.4 pmol/L, 100 ng/ml), was used to test eNOS activation in preliminary experiments. Static controls and sheared HUVECs were treated with VEGF (2.4 pmol/L, 100 ng/ml, 5 min) and cell lysates were analyzed for phospho-eNOS (peNOS) and total eNOS. For inhibitory experiments, HUVECs were pretreated with piceatannol (syk kinase inhibitor) and FcγRIIB antibodies.
3.3 Experimental Procedures

**In vitro LSS:** HUVECs were grown to confluent monolayers in 100-mm glass tissue culture dishes. There are three different combinations of LSS and CRP conditions: the application of LSS before CRP incubation (LSS+ CRP+), the application of LSS with CRP incubation (LSS & CRP) and the application of LSS after CRP incubation (CRP+ LSS+). The different timing of LSS application was to simulate individuals with previous or current physical active lifestyle experiencing inflammatory stimuli, and individuals with previous sedentary lifestyle starting to exercise after experiencing inflammatory stimuli, respectively. Depending on the assigned conditions, the ECs were exposed to static culture condition and LSS (5 and 20 dyne/cm²) before, during and after 24 hr CRP pre-incubation at 10 µg/mL. Shear stress was applied via a cone-and-plate shear apparatus for 24 hr using a rotating cone (0.5° cone angle) unidirectionally in medium as previously described [134]. Each condition was run in duplicate.

**Western Blot:** Immediately following LSS application, both the static and LSS culture dishes were harvested for protein analysis as previously described [135]. Aliquots of cell lysate were separated by NuPAGE (Bis-Tris) gels and transferred to PVDF membranes, which were blocked with 5% nonfat dry milk dissolved in Tris-Buffered saline and then incubated overnight with primary antibodies at 4°C. Immunoreactive proteins were detected by chemiluminescence with Thermo Scientific SuperSignal (Pierce Biotechnology, IL). Primary antibodies included:
anti-eNOS (BD Biosciences, CA), anti-peNOS-1177 (BD Biosciences, CA), FcγRIIB, ICAM-1, VCAM-1, CD32, CD64, PP2A, P-Akt, Akt. The anti-β-actin antibody (Santa Cruz Biotechnology, CA) was used as an internal control.

**Quantitative Reverse Transcriptase Polymerase Chain Reaction (qRT-PCR):**

qRT-PCR was used to determine FcγRIIB, eNOS, ICAM-1 and VCAM-1 gene expression in EC tissue samples. EC samples were homogenized and RNA was extracted and stored at -80°C. Later, the reverse transcriptase reaction was performed using commercial first strand kits (LifeTechnology) and random hexamer primers, as described in the manufacture’s protocol. ThermoFisher primers were used: FcγRIIB, Hs01634996_s1; eNOS, Hs01574659_m1; VCAM-1, Hs01003372_m1; and ICAM-1, Hs00164932_m1. To perform the PCR reactions, Taqman® gene expression Q-PCR master mix was used and all reactions were performed in duplicate according to the manufacturer’s instructions. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA levels were measured as an internal control, and all data is represented relative to its expression (i.e., using the ΔΔ-Ct method) as fold change from SED/H2O group.

**NO, ET-1 and IL-6 measurements:** Following LSS application, cell supernatant was collected for measurements from both static and LSS treated HUVECs and immediately stored at −80°C until assay. NO, ET-1 and IL-6 Assay Kits (Abcam,
Cambridge, MA) were used to detect the NO and ET-1 production in the cell culture media.

3.4 Statistical Analysis

All variables were checked for normal distribution with the boxplot and Shapiro-Wilk test (p > .05). Descriptive statistics were performed. Statistical differences between groups were assessed by the two-tailed independent Student t-test. Within-HUVEC group analysis was performed using paired t-tests comparing control and stimulated values. One-way (within ethnicity and within experimental conditions) and Two-way ANOVA was computed to examine any race by shear stress interaction effects. Post hoc adjustments for multiple comparisons were done using the Bonferroni's test. Western Blot densitometry analyses were completed using the ImageJ software to quantify protein expression levels. β-actin was employed as an internal control expression and all protein measurements were normalized to it. Normalized values were analyzed by using SPSS version 21.0 (IBM, Chicago, IL). Data are expressed as mean ± SE and the level of significance set at p ≤ .05.
4.0 Results

As shown in Fig. 1A, basal eNOS expression did not differ between AA and CA. In Fig. 1 B-D, CRP (25, 50, and 100 µg/mL) reduced eNOS expression as the dose increased (data not shown) and elicited the differential eNOS expression between racial groups. At each CRP concentration, eNOS expression in AA HUVECs is significantly lower than in CA (p < .01). peNOS was assessed in order to detect the activation form of eNOS. Dose response experiments using different doses of CRP (10, 25 and 50 µg/mL) revealed that 1 hr of CRP incubation induced attenuation of peNOS (Fig. 2). At 25 and 50 µg/mL dose conditions, compared with their control, a greater attenuation of peNOS was found in AA (Fig. 2B1 and 2B2). Fig. 3B shows the racial differences in eNOS activation after 1 hr CRP incubation and that peNOS was significantly lower in AA HUVECs than in CA (p < .01). A dose of 10 µg/mL is a physiological level of CRP in individuals with inflammation and a high-risk category of hypertension. Therefore, this dose was used to study the CRP-induced effects in subsequent experiments.

IgG (100 µg/mL) was used as an FcγRIIB blocker (IgG) to test if the racial differences were possibly mediated through FcγRIIB receptors. eNOS mRNA levels were lower in AA than in CA under basal conditions, CRP, and IgG + CRP conditions although no statistical significance was found (Fig. 4A). Western Blot data showed that the racial differences only occurred under CRP (10 µg/mL, 24
hr) incubation (Fig. 4B3). 1 hr pre-incubation with 100 µg/mL IgG attenuated the differences between racial groups (Fig. 4B4). Fig. 5 shows CRP upregulated VCAM-1 and ICAM-1 protein expression in both racial groups. In addition, it is important to note that AA HUVECs show greater fold changes of VCAM-1 and ICAM-1, compared with CA. IgG and CRP upregulated VCAM-1 and ICAM-1 expression as evidenced by PCR and Western blot analysis (Fig. 6A and Fig. 7A), which suggests the activation of FcγRIIB receptors is involved in adhesion molecule augmentation. Similar to eNOS protein expression, the racial differences in VCAM-1 protein expression can only be found under CRP incubation at 10 µg/mL. Strong racial differences of ICAM-1 existed under basal and all the other conditions (Fig. 7B1, 7B2, and 7B3), and IgG actually decreased the differences between AA and CA HUVECs (Fig. 7B4). These results suggest that the activation of FcγRIIB is not the only cause for CRP-induced racial differences in HUVECs.

Because CRP-induced biological effects are mediated by FcγRIIB receptors and we have previously shown that the racial differences are elicited through activation of FcγRIIB, the effect of CRP on FcγRIIB receptor expression was tested. Increased FcγRIIB expression levels were detected by PCR and Western blot analysis (Fig. 8A). Under basal and CRP incubation conditions, AA HUVECs expressed significantly higher levels of FcγRIIB receptors than CA (Fig. 8B1 and 8B2). To further explore the effects of CRP on FcγRIIB expression, we examined FcγRIIB expression under different CRP concentrations (10, 25, and
50 µg/mL) and found a dose-dependent relationship. Larger fold changes were observed in AA HUVECs than in CA (Fig. 9A and 9B).

Not only the increased CRP production in acute and chronic inflammation is largely regulated by IL-6, and vise versa, CRP also activate IL-6 production from ECs [91]. The effect of CRP (25, 50, and 100 µg/mL) on IL-6 production was also tested. A dose-dependent relationship with consistently higher levels of IL-6 was observed in AA HUVECs media than in CA but no statistical significance was achieved (Fig. 10).

After identifying the racial differences in CRP-induced effects on ECs, the role of shear stress in CRP-induced effects on ECs was tested. CRP-induced attenuation of eNOS expression was not reversed by the static condition but was reversed when HUVECs were exposed to HiLSS (20 dyne/cm²) after CRP incubation in both racial groups (Fig. 11).

Blocking FcyRIIB with either antibodies or piceatannol resulted in a modest inhibition of CRP effects. CRP-induced eNOS expression attenuation was inhibited in all conditions involved HiLSS (Fig. 12A1 and 12A2). Greater but not significant inhibition to the attenuation was observed in both racial groups in the application of HiLSS before CRP incubation, compared with the application of HiLSS after CRP incubation. As expected, LoLSS exposure did not reverse eNOS expression attenuation and even exacerbated it in CA HUVECs (Fig. 12B1 and 12B2).
HiLSS eliminated the racial differences in basal FcyRIIB protein expression between CA and AA (Fig. 13A), whereas LoLSS failed to have an effect on the racial differences of CRP-induced FcyRIIB expression, which remained the same after 24 hr LoLSS (Fig. 13B).

FcyRIIB protein expression was examined after 24 hr of HiLSS and LoLSS exposure (Fig. 14). HiLSS reduced FcyRIIB expression after 6 hr and both CA and AA HUVECs in a time-dependent fashion up to 24 hr under HiLSS condition (Fig. 14A1 and 14A2). LoLSS slightly upregulated FcyRIIB expression after 24 hr exposure (Fig. 14B1 and 14B2).

HiLSS with CRP incubation simultaneously, CRP incubation followed by HiLSS, HiLSS followed by CRP incubation and HiLSS only conditions strongly inhibited FcyRIIB upregulation in both racial groups (Fig. 15A and 15B). The application of HiLSS before CRP incubation showed greater inhibition to FcyRIIB expression than the application of HiLSS following CRP incubation. FcyRIIB expression was also tested under multiple LoLSS conditions (Fig. 16A and 16B). LoLSS failed to change FcyRIIB expression. VCAM-1 expression was inhibited by all conditions with HiLSS (Fig. 17A1 and 17A2). HiLSS has been reported to upregulate whereas LoLSS downregulates ICAM-1 expression on ECs. ICAM-1 was enhanced after CRP incubation and HiLSS had an add-on effect on increased expression (Fig. 17B1 and 17B2). LoLSS exposure upregulated VCAM-1 with and without CRP incubation (Fig. 18A1 and 18A2). CRP-induced ICAM-1 upregulation was inhibited by LoLSS exposure in AA HUVECs (Fig.
18B2). CA HUVECs responded differently under CRP incubation followed by LoLSS application and LoLSS application followed by CRP incubation conditions by increasing ICAM-1 expression (Fig. 18B1).

NOx was measured in cell culture media under multiple conditions (Fig. 19). NOx production exhibited a similar pattern to eNOS expression. It is worth noting that a higher NOx production was detected in AA compared to CA media under HiLSS only condition (Fig. 19A). HiLSS but not LoLSS exposure significantly inhibited to the release of ET-1, with or without CRP incubation (Fig. 20A and 20B). Again, the application of HiLSS before CRP incubation had greater inhibitory effects on ET-1 than the application of HiLSS after CRP incubation.
Fig. 1. eNOS protein expression with different concentrations of CRP incubation in AA and CA HUVECs.

HUVECs from Caucasian (CA, N = 4) and African American (AA, N = 4) donors were incubated with different concentrations of CRP (0, 25, 50, 100 µg/mL) for 24 hr (mean ± SE). Independent-samples t-tests were run to determine if there were differences in eNOS expression between CA and AA HUVECs.

** p < .01.
This figure suggests:
a. No differences were detected in basal eNOS expression between AA and CA;
b. significant racial differences were detected in the reduction of eNOS expression response to CRP. AA HUVECs have significant greater reduction than CA ECs in all CRP conditions.
Fig. 2

A1

peNOS  

eNOS

CRP (µg/mL)

+ VEGF (100 ng/mL)

A2

CA: eNOS bioactivity after 1 hr CRP pre-incubation

B1

peNOS  

eNOS

CRP (µg/mL)

+ VEGF (100 ng/mL)

B2

AA: eNOS bioactivity after 1 hr CRP pre-incubation
Fig. 2. CRP inhibits eNOS phosphorylation.

HUVECs were pre-incubated with CRP (10, 25, 50 µg/mL) for 1 hr, treated with vascular endothelial growth factor (VEGF, 100 ng/mL) for 5 min and lysates were analyzed for phospho-eNOS (peNOS, ser1177) and total eNOS (mean ± SE).

One-way repeated measures ANOVA tests were conducted to determine whether there were statistically significant differences in peNOS at different CRP concentrations.

* p < .05, ** p < .01, *** p < .005, **** p < .001.

Both figures suggest that CRP inhibits eNOS bioactivity in a dose-dependent manner. Compared with CA, peNOS in AA HUVECs showed a significantly greater reduction as the CRP dose increased.
**Fig. 3.** eNOS phosphorylation at basal and CRP pre-incubation conditions between CA and AA HUVECs.

All 8 cell lines from CA and AA incubated with and without CRP (10 µg/mL) for 1 hr followed by 5 min VEGF (100 ng/mL) stimulation (mean ± SE). Independent-samples t-tests were run to determine if there were differences in eNOS bioactivity between CA and AA HUVECs at baseline and after 1 hr CRP incubation.

**p < .01.**

A. No significant differences were found in peNOS expression without CRP pre-incubation.

B. CRP incubation caused significantly greater peNOS expression attenuation in AA than in CA HUVECs.
Fig. 4

A  eNOS

Relative mRNA expression

Control  IgG  CRP  IgG + CRP

B1  Control

B2  100 µg/mL IgG

B3  10 µg/mL CRP

B4  100 µg/mL IgG + 10 µg/mL CRP

B1-B4: Western blot images of eNOS and β-Actin expression under different conditions.
Fig. 4. Blocking effects of IgG (FcyRIIB inhibitor) on eNOS.

HUVECs from CA and AA were exposed to 100 µg/mL IgG, 10 µg/mL CRP for 24 hr and 1 hr 100 µg/mL IgG pre-incubation followed by 10 µg/mL CRP for 24 hr, respectively (mean ± SE).

** p < .01.

A. mRNA expression of eNOS.

A two-way ANOVA was conducted to examine the effects of ethnicity and multiple incubation conditions on eNOS expression. Residual analysis was performed to test for the assumptions of the two-way ANOVA. Outliers were excluded and the data was normally distributed at each condition, as assessed by boxplot and Shapiro-Wilk test (p > .05), respectively.

No interaction effect and statistical significances were found between ethnicity in all conditions. Lower levels of eNOS expression were detected in the CRP only condition but it did not reach statistical significance.

B. Independent-samples t-tests were run to determine if there were differences in eNOS expression between CA and AA HUVECs under multiple incubation conditions.

1. No significant differences were detected in basal eNOS expression between CA and AA HUVECs.
2. IgG failed to cause racial differences in eNOS expression.
3. eNOS protein expression in AA is significantly lower than in CA after CRP incubation.
4. With IgG pre-incubation, CRP failed to cause racial differences in eNOS expression.

This result suggests that the mechanism of CRP-induced racial differences in eNOS expression may involve the participation of FcyRIIB receptors.
**Fig. 5**

VCAM-1 and ICAM-1 protein expression with and without CRP incubation (10 µg/mL, 24 hr) in AA and CA HUVECs (mean ± SE).

**p < .01, *** p <.005.

This figure shows that CRP incubation significantly increases VCAM-1 and ICAM-1 protein expression in both AA and CA HUVECs.
Fig. 6

A

VCAM

HUVEC Relative mRNA

Control IgG CRP IgG + CRP

Referent Control CA AA

B1

Control

VCAM-1/β-Actin

VCAM-1

β-Actin

CA AA

B2

100 µg/mL IgG

VCAM-1/β-Actin

VCAM-1

β-Actin

CA AA

B3

10 µg/mL CRP

VCAM-1/β-Actin

VCAM-1

β-Actin

CA AA

B4

100 µg/mL IgG + 10 µg/mL CRP

VCAM-1/β-Actin

VCAM-1

β-Actin

CA AA
Fig. 6. Blocking effects of IgG (FcyRIIB inhibitor) on VCAM-1.

HUVECs from CA and AA were exposed to 100 µg/mL IgG, 10 µg/mL CRP for 24 hr and 1 hr 100 µg/mL IgG pre-incubation followed by 10 µg/mL CRP for 24 hr, respectively (mean ± SE).

*** p < .005.

A. mRNA expression of VCAM-1.

VCAM-1 is higher in AA under all conditions, although statistical significance was only found under CRP (10 µg/mL) incubation.

B.
1. No significant differences were detected in basal VCAM-1 expression between CA and AA HUVECs.
2. IgG failed to cause racial differences in VCAM-1 expression.
3. VCAM-1 protein expression in AA is significantly higher than in CA after CRP incubation.
4. With IgG pre-incubation, CRP failed to cause racial differences in VCAM-1 expression.

This result suggests that the mechanism of CRP-induced racial differences in VCAM-1 expression may involve the participation of FcyRIIB receptors.
Fig. 7

A) HUVEC Relative mRNA

Control  IgG  CRP  IgG + CRP

ICAM-1/β-Actin

B1) Control

ICAM-1

β-Actin

B2) 100 µg/mL IgG

ICAM-1

β-Actin

B3) 10 µg/mL CRP

ICAM-1

β-Actin

B4) 100 µg/mL IgG + 10 µg/mL CRP

ICAM-1

β-Actin
**Fig. 7. Blocking effects of IgG (FcyRIIB inhibitor) on ICAM-1.**

HUVECs from CA and AA were exposed to 100 µg/mL IgG, 10 µg/mL CRP for 24 hr and 1 hr 100 µg/mL IgG pre-incubation followed by 10 µg/mL CRP for 24 hr, respectively (mean ± SE).

* p < .05, ** p < .01, *** p < .005, **** p < .001.

A. mRNA expression of ICAM-1.
ICAM-1 mRNA expression is higher in AA in all conditions, although statistical significance only found under CRP incubation.

B.
1. Basal ICAM-1 expression is significantly higher in AA than in CA HUVECs.
2. Significant racial differences in ICAM-1 expression between AA and CA HUVECs remain in IgG incubation condition.
3. ICAM-1 protein expression in AA is significantly higher than in CA after CRP incubation.
4. With IgG pre-incubation, racial differences in ICAM-1 expression remain between AA and CA HUVECs.

This result suggests that the mechanism of CRP-induced racial differences in ICAM-1 expression may involve the participation of FcyRIIB receptors, and there might be pathways other than Fcy that contribute to the disparities.
**Fig. 8.** Effect of CRP incubation (10 µg/mL, 24 hr) on FcγR1IB receptor expression (mean ± SE).

* p < .05, ** p < .01.

A. mRNA expression of FcγR1IB receptors. This figure shows that
a. CRP incubation increases FcγR1IB receptor mRNA expression;
b. FcγR1IB receptor mRNA expression in AA are higher than in CA HUVECs under both basal and CRP incubation conditions.
B
1. At basal condition, FcγRIIB receptor protein expression is significantly higher in AA than in CA HUVECs.
2. After 24 hr CRP incubation, racial differences in FcγRIIB receptor protein expression remain between AA and CA HUVECs. Compared with CA, AA HUVECs expressed significantly higher levels of FcγRIIB receptors.
Fig. 9

**Fig. 9.** Dose-dependent FcγRIIB receptors protein expression with CRP (10, 25, 50 µg/mL, 24 hr) incubation in CA and AA HUVECs (mean ± SE).

One-way repeated measures ANOVA tests were conducted to determine whether there were statistically significant differences in FcγRIIB receptor expression at different CRP concentrations.

**p < .01, *** p < .005, **** p < .001.**

This figure suggests:
a. CRP increases FcγRIIB receptor expression in a dose-dependent manner in both racial groups.
b. The augment folds of FcγRIIB receptors in AA are higher than in CA at each CRP concentration.
Fig. 10. Effects of CRP incubation (10, 25, 50 µg/mL) for 24 hr on IL-6 (mean ± SE).

Two-way ANOVA tests were conducted to examine the effects of ethnicity and multiple incubation conditions on IL-6 production. Residual analysis was performed to test for the assumptions of the two-way ANOVA.

There was no statistically significant interaction between ethnicity and incubation conditions on IL-6 production, $F(7, 48) = 2.306, p = .706$, partial $\eta^2 = .625$.

The IL-6 production tended to be higher in the AA group under 25, 50, and 100 µg/mL CRP conditions, though the differences were not significant.
Fig. 11. HiLSS reverses the reduction of eNOS protein expression after CRP pre-incubation.

HUVECs were pre-incubation with CRP (10 µg/mL) for 24 hr followed by static control or HiLSS (20 dyne/cm²) for another 24 hr (mean ± SE).

**** p < .001, between CRP + HiLSS and CRP + Static within subjects.
## p < .005, between CA and AA.

This figure suggests:
a. HiLSS is effective in the recovery of eNOS from CRP incubation;
b. AA HUVECs may have similar or greater recovery responses to HiLSS after CRP incubation.
Fig. 12. eNOS protein expression under different HiLSS and LoLSS conditions (mean ± SE).

HiLSS & CRP: Simultaneous application of HiLSS with CRP incubation for 24 hr; HiLSS+: HiLSS only for 24 hr; CRP+ HiLSS+: CRP only for the first 24 hr and followed by HiLSS only for another 24 hr;
HiLSS+ CRP+: HiLSS only for the first 24 hr and followed by CRP only for another 24 hr;

LoLSS & CRP: Simultaneous application of LoLSS with CRP incubation for 24 hr;
LoLSS+: LoLSS only for 24 hr;
CRP+ LoLSS+: CRP only for the first 24 hr and followed by LoLSS only for another 24 hr;
LoLSS+ CRP+: LoLSS only for the first 24 hr and followed by CRP only for another 24 hr.

One-way repeated measures ANOVA tests were conducted to determine whether there were statistically significant differences in eNOS expression under different incubation conditions. Outliers were excluded and the data was normally distributed at each condition, as assessed by boxplot and Shapiro-Wilk test (p > .05), respectively.

* p < .05, ** p < .01, *** p < .005, **** p < .001, compared with control.
# p < .05, ## p < .01, ### p < .005, #### p < .001, compared with CRP+.

A.
1. The eNOS expression in CA HUVECs statistically significant changes under different incubation conditions, F (7, 35) = 14.699, p < .0005, partial $\eta^2 = .75$.
Post hoc analysis with a Bonferroni adjustment revealed that eNOS expression was statistically significantly increased from CRP+ condition to HiLSS+ CRP+ condition (0.539 (95% CI, -.962 to -.116, p = .017), and from CRP+ condition to CRP & HiLSS condition, (0.401(95% CI, -.784 to -.019, p = .040), but not from CRP+ condition to CRP+ HiLSS+ condition (0.356 (95% CI, -0.843 to 0.130), p = .191).

2. The eNOS expression in AA HUVECs statistically significant changes under different incubation conditions, F (7, 35) = 29.067, p < .0005, partial $\eta^2 = .95$.
Post hoc analysis with a Bonferroni adjustment revealed that eNOS expression was statistically significantly increased from CRP+ condition to CRP & HiLSS condition, (0.507 (95% CI, -.828 to -.187, p = .020), from CRP+ condition to CRP+ HiLSS+ condition (0.285 (95% CI, -.824 to .306, p = .045), and from CRP+ condition to HiLSS+ CRP+ condition (0.352 (95% CI, -0.145 to 0.441), p = .034).

These figures suggests:
a. HiLSS & CRP significantly increases eNOS expression in both CA and AA HUVECs, compared with CRP+.
b. HiLSS+ condition increases eNOS expression as expected. HiLSS+ CRP+ and CRP+ HiLSS+ have similar effects to eNOS expression.
B. 

1. The eNOS expression in CA HUVECs statistically significant changes under different incubation conditions, $F (7, 35) = 35.320$, $p < .0005$, partial $\eta^2 = .88$. Post hoc analysis with a Bonferroni adjustment revealed that eNOS expression was statistically significantly decreased from CRP+ condition to CRP+ LoLSS condition, $(-.366$ (95% CI, .103 to .629, $p = .011)$, and from CRP+ condition to LoLSS+ CRP+ condition $(-0.518$ (95% CI, .295 to .741), $p = .001$).

2. The eNOS expression in AA HUVECs statistically significant changes under different incubation conditions, $F (7, 35) = 12.717$, $p < .0005$, partial $\eta^2 = .72$. Post hoc analysis with a Bonferroni adjustment revealed that eNOS expression was statistically significantly increased from CRP+ condition to CRP+ LoLSS+ condition, $(0.381$ (95% CI, .004 to .758, $p = .047)$.

This figure suggests:

a. LoLSS & CRP significantly decreases eNOS expression in CA HUVECs, compared with CRP+.

b. No significant change was found between LoLSS & CRP condition and CRP+ condition in AA HUVECs. Compared with the control condition, LoLSS+ condition decreases eNOS expression as expected.

c. In CA HUVECs, eNOS expression attenuation is higher in LoLSS+ CRP+, compared with CRP+ LoLSS+. In AA HUVECs, no significant differences were found between LoLSS+ CRP+ condition and CRP+ LoLSS+ condition.
**Fig. 13**

A. FcγRIIB receptors protein after 24 H HiLSS

B. FcγRIIB receptors protein after 24 H LoLSS

**Fig. 13.** FcγRIIB receptor protein expression after 24 hr HiLSS and LoLSS between CA and AA HUVECs (mean ± SE).

** p < .01.

A. No statistical significances of FcγRIIB receptor expression were found between CA and AA HUVECs after 24 hr HiLSS exposure.

B. FcγRIIB receptor expression is significantly higher in AA HUVECs than in CA after 24 hr LoLSS.
Fig. 14

Fig. 14. Time-dependent FcγRIIB receptor expression with HiLSS and LoLSS in CA and AA HUVECs (mean ± SE).

One-way repeated measures ANOVA tests were conducted to determine whether there were statistically significant differences in FcγRIIB receptor expression at different time point under HiLSS or LoLSS conditions.

* p < .05, ** p < .01, **** p < .001, compared with control.

A.
  a. HiLSS inhibits FcγRIIB receptor expression in a time-dependent manner.
b. At the 24 hr time point, inhibition of FcyRIIB receptors by HiLSS is greater in AA than in CA HUVECs.

B.  
  a. LoLSS slightly increases FcyRIIB receptor expression. 
  b. At the 24 hr time point, FcyRIIB receptor expression increased in CA but not in AA HUVECs.
**Fig. 15**

**A:** FcγRIIB Receptors protein expression under different HiLSS conditions.

**B:** FcγRIIB Receptors protein expression under different HiLSS conditions.

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**Fig. 15.** FcγRIIB receptor protein expression under different HiLSS conditions.

Effects of different combinations of HiLSS application and CRP incubation were evaluated (mean ± SE). CRP antibody (AT10-IgG1, (20 µg/mL) and the Syk kinase inhibitor piceatannol (50 µmol/L) were applied 1 hr before CRP incubation (10 µg/mL, 24 hr).

One-way repeated measures ANOVA tests were conducted to determine whether there were statistically significant differences in FcγRIIB receptor protein expression under different incubation conditions. Outliers were excluded and the data was normally distributed at each condition, as assessed by boxplot and Shapiro-Wilk test (p > .05), respectively.

* p < .05, ** p < .01, *** p < .005, **** p < .001, compared with control.
# p < .05, #### p < .001, compared with CRP+.

**A.**

The FcγRIIB receptor protein expression in CA HUVECs statistically significant changes under different incubation conditions, F (7, 35) = 26.670, p < .0005, partial η² = .84. Post hoc analysis with a Bonferroni adjustment revealed that FcγRIIB receptor protein expression was statistically significantly decreased from CRP+ condition to HiLSS+ CRP+ condition, (1.651 (95% CI, 0.188 to 3.114, p = .029). No changes was statistically significant from CRP+ condition to HiLSS&
CRP condition (0.846 (95% CI, -0.812 to 2.503 p = .776), and from CRP+ condition to CRP+ HiLSS+ condition (0.608 (95% CI, -0.867 to 2.082, p = 1.000).

B. The FcγRIIB receptor protein expression in AA HUVECs statistically significant changes under different incubation conditions, F (7, 35) = 182.923, p < .0005, partial $\eta^2$ = .97. Post hoc analysis with a Bonferroni adjustment revealed that FcγRIIB receptor protein expression was statistically significantly decreased from CRP+ condition to HiLSS & CRP condition, (0.958 (95% CI, 0.056 to 1.860, p = .038), from CRP+ condition to CRP+ HiLSS+ condition, (1.523 (95% CI, 0.418 to 2.628, p = 0.011), and from CRP+ condition to HiLSS+ CRP+ condition, (2.954 (95% CI, 1.782 to 4.126, p = 0.001).

This figure suggests:

a. CRP induced FcγRIIB receptor expression augmentation partially blunted by FcγRIIB antibody and piceatannol.

b. HiLSS & CRP significantly inhibited FcγRIIB receptor expression, compared with CRP only incubation condition. HiLSS+ condition inhibits FcγRIIB receptor expression at the greatest level, compared with other conditions.

c. HiLSS+ CRP+ condition has higher inhibitive effects on FcγRIIB receptor expression than CRP+ HiLSS+ condition.
Fig. 16. FcγRIIB receptor protein expression under different LoLSS conditions.

CRP antibody (AT10-IgG1, (20 µg/mL) and the Syk kinase inhibitor piceatannol (50 µmol/L) were applied 1 hr before CRP incubation (10 µg/mL, 24 hr). Effects of different combinations of LoLSS application and CRP incubation were evaluated (mean ± SE).

One-way repeated measures ANOVA tests were conducted to determine whether there were statistically significant differences in FcγRIIB receptor protein expression under different incubation conditions. Outliers were excluded and the data was normally distributed at each condition, as assessed by boxplot and Shapiro-Wilk test (p > .05), respectively.

* p < .05, ** p < .01, *** p < .005, **** p < .001, compared with control.
# p < .05, ## p < .01, ### p < .005, #### p < .001, compared with CRP+.

A.
The FcγRIIB receptor protein expression in CA HUVECs statistically significant changes under different incubation conditions, F (7, 35) = 51.217, p < .0005, partial η² = .91. No changes was statistically significant from CRP+ condition to LoLSS& CRP condition (0.331 (95% CI, -1.611 to 0.949 p = 1.000), from CRP+ condition to CRP+ LoLSS+ condition (0.668 (95% CI, -2.116 to 0.780, p = 1.000) and from CRP+ condition to LoLSS+ CRP+ condition (0.124 (95% CI, -1.537 to 1.290, p = 1.000).
B. The FcγRIIB receptor protein expression in AA HUVECs statistically significant changes under different incubation conditions, $F(7, 35) = 129.484$, $p < .0005$, partial $\eta^2 = .96$. Post hoc analysis with a Bonferroni adjustment revealed that FcγRIIB receptor protein expression was statistically significantly decreased from CRP+ condition to LoLSS & CRP condition ($2.900$ (95% CI, 0.268 to 5.531, $p = .032$). No changes was statistically significant from CRP+ condition to CRP+ LoLSS+ condition (0.650 (95% CI, -3.388 to 2.089, $p = 1.000$) and from CRP+ condition to LoLSS+ CRP+ condition (0.066 (95% CI, -2.270 to 2.402, $p = 1.000$).

This figure suggests:

a. CRP induced FcγRIIB receptor expression augmentation partially blunted by FcγRIIB antibody and piceatannol.

b. In AA HUVECs, LoLSS & CRP significantly inhibited FcγRIIB receptor expression, compared with CRP only incubation condition, but this reaction did not happen in CA HUVECs. LoLSS+ condition slightly increased FcγRIIB receptor expression in CA HUVECs but not in AA.

c. LoLSS+ CRP+ condition has similar effects to FcγRIIB receptor expression with CRP+ LoLSS+ condition in both CA and AA HUVECs.
Fig. 17. VCAM-1 and ICAM-1 protein expression under different HiLSS and LoLSS conditions (mean ± SE).

One-way repeated measures ANOVA tests were conducted to determine whether there were statistically significant differences in VCAM-1 and ICAM-1 expression under different incubation conditions. Outliers were excluded and the
data was normally distributed at each condition, as assessed by boxplot and
Shapiro-Wilk test (p > .05), respectively.

* p < .05, ** p < .01, *** p < .005, **** p < .001, compared with control.
# p < .05, ## p < .01, ### p < .005, #### p < .001, compared with CRP+.

A.
1. The VCAM-1 expression in CA HUVECs statistically significant changes under
different incubation conditions, F (7, 35) = 65.343, p < .0005, partial $\eta^2 = .93$.
   Post hoc analysis with a Bonferroni adjustment revealed that ICAM-1 expression
   was statistically significantly decreased from CRP+ condition to HiLSS & CRP
   condition, (1.787 (95% CI, 0.557 to 3.016, p = .009), from CRP+ condition to
   CRP+ HiLSS+ condition (2.176 (95% CI, 0.925 to 3.427 p = .004), and from
   CRP+ condition to HiLSS+ CRP+ condition (2.034 (95% CI, 1.000 to 3.069, p
   = .002).

2. The VCAM-1 expression in AA HUVECs statistically significant changes under
different incubation conditions, F (7, 35) = 165.947, p < .0005, partial $\eta^2 = .97$.
   Post hoc analysis with a Bonferroni adjustment revealed that ICAM-1 expression
   was statistically significantly decreased from CRP+ condition to HiLSS & CRP
   condition, (2.892 (95% CI, 2.239 to 3.545, p < .0005), from CRP+ condition to
   CRP+ HiLSS+ condition (2.796 (95% CI, 1.537 to 4.055, p = .001), and from
   CRP+ condition to HiLSS+ CRP+ condition (3.265 (95% CI, 2.432 to 4.098, p
   < .0005).

This figure suggests:
   a. HiLSS & CRP significantly decreases VCAM-1 expression in both CA and AA
   HUVECs.
   b. HiLSS+ condition decreases VCAM-1 expression in both CA and AA HUVECs,
   which is in line with previous study results.
   c. HiLSS+ CRP+ and CRP+ HiLSS+ have similar effects to VCAM-1 expression
   in both CA and AA HUVECs.

B.
1. The ICAM-1 expression in CA HUVECs statistically significant changes under
different incubation conditions, F (7, 35) = 477.375, p < .0005, partial $\eta^2 = .99$.
   Post hoc analysis with a Bonferroni adjustment revealed that ICAM-1 expression
   was statistically significantly increased from CRP+ condition to HiLSS & CRP
   condition, (2.228 (95% CI, -3.490 to -0.965, p = .004), from CRP+ condition to
   CRP+ HiLSS+ condition (4.743 (95% CI, -5.825 to -3.662 p < .0005), and from
   CRP+ condition to HiLSS+ CRP+ condition (5.398 (95% CI, -6.066 to -4.731, p
   < .0005).
2. The ICAM-1 expression in AA HUVECs statistically significant changes under different incubation conditions, $F (7, 35) = 39.007, p < .0005$, partial $\eta^2 = .89$. Post hoc analysis with a Bonferroni adjustment revealed that ICAM-1 expression was statistically significantly increased from CRP+ condition to HiLSS & CRP condition, (1.526 (95% CI, -2.435 to -0.618, $p = .004$). No changes was statistically significant from CRP+ condition to CRP+ HiLSS+ condition (0.767 (95% CI, -2.204 to 0.669 $p = .650$), and from CRP+ condition to HiLSS+ CRP+ condition (0.220 (95% CI, -1.246 to 0.805, $p = 1.000$).

This figure suggests:

a. HiLSS & CRP significantly increases ICAM-1 expression.
b. HiLSS+ condition increases ICAM-1 expression, which is consistent with previous study results.
c. HiLSS+ CRP+ and CRP+ HiLSS+ have similar effects to ICAM-1 expression.
Fig. 18

Fig. 18. VCAM-1 and ICAM-1 protein expression under different LoLSS conditions (mean ± SE).

One-way repeated measures ANOVA tests were conducted to determine whether there were statistically significant differences in VCAM-1 and ICAM-1 expression under different incubation conditions. Outliers were excluded and the data was normally distributed at each condition, as assessed by boxplot and Shapiro-Wilk test (p > .05), respectively.

* p < .05, ** p < .01, *** p < .005, **** p < .001, compared with control.
# p < .05, ## p < .01, ### p < .005, #### p < .001, compared with CRP+.
A. The VCAM-1 expression in CA HUVECs statistically significant changes under different incubation conditions, $F(7, 35) = 15.700, p < .0005$, partial $\eta^2 = .76$. No changes were statistically significantly from CRP+ condition to other LoLSS involved conditions. From CRP+ condition to LoLSS & CRP condition (0.648 (95% CI, -2.556 to 1.260, $p = 1$), from CRP+ condition to CRP+ LoLSS+ condition, (0.983 (95% CI, -4.993 to 3.026, $p = 1$), and from CRP+ condition to LoLSS+ CRP+ condition (0.828 (95% CI, -5.007 to 3.350), $p = 1.000$).

2. The VCAM-1 expression in AA HUVECs statistically significant changes under different incubation conditions, $F(7, 35) = 40.568, p < .0005$, partial $\eta^2 = .89$. No changes were statistically significantly from CRP+ condition to other LoLSS involved conditions. From CRP+ condition to LoLSS & CRP condition (1.013 (95% CI, -2.467 to 0.442, $p = .236$), from CRP+ condition to CRP+ LoLSS+ condition, (0.331 (95% CI, -1.401 to 0.738, $p = 1.000$), and from CRP+ condition to LoLSS+ CRP+ condition (0.194 (95% CI, -1.364 to 0.976), $p = 1.000$).

This figure suggests:

a. LoLSS failed to cause any changes to VCAM-1 expression in both CA and AA HUVECs, compared with CRP+ condition.

b. LoLSS+ condition increases VCAM-1 expression, compared with control, which is in line with previous study results.

c. LoLSS+ CRP+ and CRP+ LoLSS+ have similar effects to VCAM-1 expression in both CA and AA HUVECs.
B.

1. The ICAM-1 expression in CA HUVECs statistically significant changes under different incubation conditions, $F (7, 35) = 19.201$, $p < .0005$, partial $\eta^2 = .79$. No changes were statistically significantly from CRP+ condition to other LoLSS involved conditions except LoLSS+. From CRP+ condition to LoLSS & CRP condition ($0.116$ (95% CI, -.595 to 0.828, $p = 1.000$), from CRP+ condition to CRP+ LoLSS+ condition, (0.310 (95% CI, -1.316 to 0.696, $p = 1.000$), and from CRP+ condition to LoLSS+ CRP+ condition (0.550 (95% CI, -1.330 to 0.231), $p = .225$).

2. The ICAM-1 expression in AA HUVECs statistically significant changes under different incubation conditions, $F (7, 35) = 32.779$, $p < .0005$, partial $\eta^2 = .87$. Post hoc analysis with a Bonferroni adjustment revealed that ICAM-1 expression was statistically significantly decreased from CRP+ condition to CRP+ LoLSS+ condition, (2.778 (95% CI, .611 to 4.945, $p = .016$), and from CRP+ condition to LoLSS+ CRP+ condition (3.198 (95% CI, 1.011 to 5.385), $p = .009$). No changes were statistically significantly from CRP+ condition to LoLSS & CRP condition (1.210 (95% CI, -1.817 to 4.236, $p = 1$).

This figure suggests:

a. LoLSS & CRP significantly decreases ICAM-1 expression from CRP+ in AA but not in CA HUVECs.
b. LoLSS+ condition decreases ICAM-1 expression, which is in line with previous study results.
c. LoLSS+ CRP+ and CRP+ LoLSS+ have similar effects to ICAM-1 expression. However, LoLSS exposure pre- and post- to CRP incubation have opposite effects between AA and CA. CRP+ LoLSS+ and LoLSS+ CRP+ conditions in CA HUVECs significantly increase ICAM-1 expression, compared with CRP+. 
Fig. 19. NO production under different HiLSS and LoLSS conditions between CA and AA HUVECs (mean ± SE).

Two-way ANOVA tests were conducted to examine the effects of ethnicity and multiple incubation conditions on NO production. Residual analysis was performed to test for the assumptions of the two-way ANOVA.

A. The interaction effect between ethnicity and incubation conditions on NO production was statistically significant, F (7, 48) = 2.475, P = 0.0298, partial η² = .265. Therefore, the effect of each incubation conditions is not the same for each ethnicity.

There was a statistically significant difference in mean NO production between CA and AA under HiLSS+ condition, For CA and AA under HiLSS+ condition, mean NOx production was 5.348 (95% CI, 2.19 to 8.50) higher for AA than CA, F (1, 48) = 11.604, p = .001, partial η² = .195.

There were statistically significant differences in mean NO production for AA HUVECs under either CRP+, HiLSS&CRP, HiLSS+, CRP+ HiLSS+, and HiLSS+ CRP+ conditons, F (7, 48) = 50.353, p < .0005, partial η² = .880, as for CA, significant differences in mean NO production were found under either CRP+, HiLSS+, and HiLSS+ CRP+ conditons. F (7, 48) = 29.559, p < .0005, partial η² = .812. NO production was significantly higher under HiLSS+ CRP+ than CRP+ HiLSS+ condition in both CA (p = 0.004) and AA (p = 0.011)
This figure suggests:
a. Compared with CRP+ condition, NO production significantly increases in HiLSS+ CRP+ condition in both CA and AA HUVECs. AA HUVECs responded to more HiLSS involved conditions.
b. NO production reaches peak levels in HiLSS+ condition for both CA and AA HUVECs. AA HUVECs display a greater response under HiLSS+ than CA.
c. Pre HiLSS exposure might have greater protection than post HiLSS exposure to CRP incubation in terms of NO production.

B. There was no statistically significant interaction between ethnicity and incubation conditions on NO production, $F(7, 48) = 0.1192, p = 0.9967$, partial $\eta^2 = .017$.

There were statistically significant differences in mean NO production for AA HUVECs under either CRP+, LoLSS&CRP, and LoLSS+ CRP+ conditions, $F(7, 48) = 8.238$, $p < .0005$, partial $\eta^2 = .546$, as for CA, significant differences in mean NO production were found under either CRP+, LoLSS&CRP, CRP+ LoLSS+ and LoLSS+ CRP+ conditions. $F(7, 48) = 9.108$, $p < .0005$, partial $\eta^2 = .570$. The simple main effect of ethnicity on mean NO production for different incubation conditions was not statistically significant.

This figure suggests:
a. Compared with CRP+ condition, NO production remains at a similar level in LoLSS & CRP condition in both CA and AA HUVECs.
b. NO production is at a similar level in control and LoLSS+ conditions in both CA and AA HUVECs.
c. Compared with CRP+ condition, no significant differences in NO production were detected in both CRP+ LoLSS+ and LoLSS+ CRP+ conditions in both CA and AA HUVECs.
Fig. 20. ET-1 production under different HiLSS and LoLSS conditions between CA and AA HUVECs (mean ± SE).

Two-way ANOVA tests were conducted to examine the effects of ethnicity and multiple incubation conditions on ET-1 production. Residual analysis was performed to test for the assumptions of the two-way ANOVA.

A. There was no statistically significant interaction between ethnicity and incubation conditions on ET-1 production, F (7, 48) = 1.989, p = .076, partial $\eta^2 = .225$.

There were statistically significant differences in mean ET-1 production between CA and CA under CRP+ and CRP+ HiLSS+ conditions. For CA and AA under CRP+ condition, mean ET-1 production was 3.364 (95% CI, 0.92 to 5.80) higher for AA than CA, F (1, 48) = 7.687, p = .008, partial $\eta^2 = .138$. Under CRP+ HiLSS+ condition, mean ET-1 production was 3.722 (95% CI, 1.28 to 6.16) higher for AA than CA, F (1, 48) = 9.408, p = .004, partial $\eta^2 = .164$.

This figure suggests:

a. Compared with CRP+ condition, ET-1 production significantly decreases in any HiLSS with CRP condition in both CA and AA HUVECs.

b. ET-1 production reaches lowest levels in HiLSS+ condition for both CA and AA HUVECs.

c. ET-1 production in HiLSS+ CRP+ condition is significantly lower than in CRP+ HiLSS+ condition in both CA and AA HUVECs, which indicated a greater protection under pre HiLSS exposure than post HiLSS.
B.
There was no statistically significant interaction between ethnicity and incubation conditions on ET-1 production, F (7, 48) = 1.781, p = .113, partial η² = .206.

There were statistically significant differences in mean ET-1 production between CA and CA under CRP+, LoLSS&CRP, CRP+ LoLSS+ and LoLSS+ CRP+ conditions. For CA and AA under CRP+ condition, mean ET-1 production was 3.696 (95% CI, 0.94 to 6.45) higher for AA than CA, F (1, 48) = 6.267, p = .010, partial η² = .131. Under LoLSS&CRP condition, mean ET-1 production was 3.524 (95% CI, 0.77 to 6.28) higher for AA than CA, F (1, 48) = 6.607, p = .013, partial η² = .121. Under CRP+ LoLSS+ condition, mean ET-1 production was 3.023 (95% CI, 0.27 to 5.78) higher for AA than CA, F (1, 48) = 4.860, p = .032, partial η² = .092. Under LoLSS+ CRP+ condition, mean ET-1 production was 3.737 (95% CI, 0.98 to 6.49) higher for AA than CA, F (1, 48) = 7.429, p = .009, partial η² = .134.

This figure suggests:

a. Compared with CRP+ condition, ET-1 production remains at similar levels in LoLSS & CRP, CRP+ LoLSS+ and LoLSS+ CRP+ conditions in CA and AA HUVECs.

b. ET-1 production is at similar levels between control and LoLSS+ conditions.
5.0 Discussion

The principal findings of this study were that: 1) Caucasian (CA) and African American (AA) HUVECs responded differently to CRP incubation, with AA HUVECs showing greater impairment of eNOS expression and activity, NO bioavailability reduction, ET-1 release, and adhesion molecule upregulation; 2) AA HUVECs had higher levels of FcγRIIB receptor expression under both basal and CRP incubation conditions, which may partially contribute to the racial differences of CRP effects on endothelium; 3) HiLSS inhibited whereas LoLSS slightly increased FcγRIIB receptor protein expression, with or without CRP incubation; 4) HiLSS reversed the detrimental effects of CRP and AA HUVECs were more responsive to HiLSS than CA, with or without CRP incubation; and 5) Compared with the application of HiLSS simultaneously with CRP incubation and the application of HiLSS following CRP incubation, the application of HiLSS before CRP incubation had larger effects in protecting NO bioavailability and inhibiting FcγRIIB receptor expression. These results support the hypothesis that FcγRIIB receptors have higher expression levels in AA than CA HUVECs with/without CRP incubation and HiLSS is able to attenuate the CRP-induced proatherogenic effects and eliminate the racial differences in suppressed eNOS expression and bioactivity after CRP pre-incubation.

To our knowledge, the current study is the first to evaluate racial differences in the CRP-induced biological effects on ECs and the possible
mechanisms and the role of LSS on this process. Data on racial differences are mostly only available in humans and they are largely descriptive in nature. While the majority of the studies focus on circulating levels of biomarkers such as CRP, NO, ET-1 or adhesion molecules, few studies reported the racial differences in cell models and even fewer have data on the disparities in response of endothelium to a stimuli. In the current study, eNOS expression and activity, NO bioavailability, ET-1 release, and adhesion molecules were evaluated and compared between CA and AA HUVECs under multiple stimulative and inhibitive conditions. Overall the results from this study suggest that the differential expression of FcγRIIB on HUVECs in the two racial groups may partially contribute to CRP-induced racial disparities and that AA HUVECs may be more responsive to HiLSS, as evidenced by greater inhibition of FcγRIIB receptor expression.

It has been demonstrated that compared with CA, there is a reduction of NO bioavailability accompanied with an increased level of ONOO⁻ and O₂⁻ in ECs from AA, which may contribute to the higher prevalence and severity of hypertension in AA [3]. Extensive epidemiological studies provide solid evidence that serum CRP levels in AA are higher than in CA. With similar levels of CVD risks, AA have higher levels of CRP than CA [108, 136, 137]. Elevated levels of serum CRP are associated with reduced NO levels. In a comparative study, AA diabetic patients with microalbuminuria, a population typically associated with higher levels of CRP, showed a blunted response to vasodilators due to
decreased levels of NO production [138]. The current hypothesis about reduced NO bioavailability is that higher oxidative stress levels keep the oxidant/antioxidant balance closer to the redox state in AA. However, there are studies that have shown that reduced NO-dependent vasodilation is not associated with higher oxidative stress levels. Moreover, inflammation largely causes endothelial dysfunction and reduces NO bioavailability in healthy subjects; the reduction is only partially reversed by local-antioxidants, which suggests a direct role of inflammation in endothelial dysfunction. CRP is a well-acknowledged inflammation biomarker and has been shown by numerous studies to play an important role in mediating the process of endothelial dysfunction. Little is known about the racial differences of the interaction of endothelium with CRP and the potential underlying mechanisms. Whether or not CRP causes racial differences in NO bioavailability in ECs and why is unknown.

Studies showed that HUVECs obtained from AA were significantly more likely than those obtained from CA to have higher oxidative stress levels, higher adhesive molecular levels, higher inflammation, and a greater degree of fibrinolytic potential [139, 140]. In addition, AA HUVECs haven been shown to produce more EMPs with the TNF-α stimulation than their CA counterparts [4]. It is worth noting that the EMP level was lower in AA HUVECs under superoxide dismutase (SOD) and TNF-α + SOD conditions, which suggests that AA HUVECs are more responsive to both proatherogenic and antiatherogenic stimuli. These results suggest that racial differences in response to inflammation
mediators on endothelium could exist. The results from the current study are consistent with these previous findings: under the same dose of CRP incubation, AA HUVECs exhibited significantly greater reductions on eNOS expression and activity, and lower NOx and higher ET-1 release without statistical significance. The racial differences in CRP-induced NO bioavailability may converge with other mechanisms, such as oxidative stress, leading to eventual endothelial dysfunction and vascular diseases in AA.

There is a scarcity of knowledge about the FcγRIIB receptors on ECs and even less knowledge on racial differences. Several groups have examined the binding of CRP to Fcγ receptors by knocking out all 3 Fcγ receptors (FcγRI, FcγRII, FcγRIII) on leukocytes. Stimulatory receptors, FcγRI and FcγRIIA, and the inhibitory receptor FcγRIIB all bind to CRP. But only FcγRIIB, which blocks activating signals after binding, is expressed on HUVECs. It has been reported that by binding to Fcγ receptors on ECs, FcγRI and FcγRIIB specifically, CRP is able to decrease eNOS and prostacyclin, and increase IL-8 and adhesion molecules [44]. Blocking FcγRI and FcγRIIB by antibodies or transfection with small interference RNA effectively attenuated NF-κB activity and inhibited VCAM-1 and ICAM-1 upregulation [141]. Similar effects were found in other studies by inhibiting the decrease in eNOS expression and the increase in IL-8 after CRP incubation [60, 112]. Thus, Fcγ receptors serve as a link between CRP and CRP-induced biological effects.
Sustained elevated levels of CRP are associated with low-grade systemic inflammation, obesity, or hereditary tendencies. Genetic polymorphisms could be one of the contributors to basal CRP levels and CRP receptor expression so there is possibility that these polymorphisms affect the risk for CVD. CRP and Fcγ receptor polymorphisms in different populations have been extensively studied to analyze the risk of developing SLE. Individuals with the 409G/390T haplotype had the highest basal CRP levels [142] while FcγRIIB$^{232}$ genotypes have been shown to clearly associate with SLE susceptibility. FcγR genetic polymorphisms introduce further variations between populations. There is a higher FcγRIIB $^{T/T232}$ genotype frequency of 11% and an allele frequency of 29% in African Americans, but only 0.7-1% of populations of European origin [143]. Li et al. [144] found that the active FcγRIIB allele at residue 187 is associated with its enhanced function, which was observed more frequently in AA. These results suggest a possibility of higher levels of FcγRIIB receptors on HUVECs, which might be the key to CRP-induced racial differences on ECs.

The current study provides evidence that in cultured ECs, higher levels of FcγRIIB receptor expression contribute to, at least partially, the greater CRP-induced biological effects in AA. The synergistic effects of increased CRP receptors and greater response to CRP stimulation may explain the racial differences in endothelial function, particularly in studies involving inflammatory or hypertensive populations. In the current study, incubation with IgG (an FcγR inhibitor) was able to cause Fcγ signaling pathway activation and subsequent
biological effects, but failed in eliciting racial differences as CRP did. In addition, the pre IgG incubation eliminated or decreased the inhibition from CRP. Although CRP exhibits functional similarities with IgG, the results from this study suggest that the inhibition of eNOS by CRP is partially, but not completely mediated through the Fcγ receptor pathways.

One of the most important roles of the endothelium of the vasculature is sensing blood flow to regulate vascular function. HiLSS has been known to inhibit EC proliferation and apoptosis induced by hydrogen peroxide and TNF-α exposure. HiLSS has been demonstrated to have an antiapoptotic effect by upregulating SOD and activating eNOS, which eventually increases NO production [145]. In the current study, NOx levels were similar between CA and AA under CRP incubation followed by HiLSS application and HiLSS application followed by CRP incubation conditions, and a significantly higher level of NOx in AA HUVECs was observed only in HiLSS only condition; while NOx remained lower in AA HUVECs under LoLSS only, CRP incubation followed by LoLSS application and LoLSS application followed by CRP incubation conditions. Considering the relatively lower levels of NO under CRP only condition, AA HUVECs were more responsive to HiLSS than CA. Another evidence is that the application of HiLSS after CRP incubation rescued eNOS expression in both racial groups, however, compared to CA, AA ECs exhibited a greater level of rescued eNOS. Potential mechanisms remain to be investigated. These data also suggest that HiLSS maintains FcγRIIB receptor expression on the HUVECs.
at lower levels than in HUVECs subjected to static flow or LoLSS. HiLSS-induced inhibition of FcγRIIB receptor expression and function may contribute to the antiatherogenic effect of laminar flow by affecting NO bioavailability, vasoconstrictor and proatherogenic adhesion molecules production. This may help regulate endothelial function and cell survival and integrity under inflammatory conditions to protect endothelium from moving toward the proatherogenic status. It is well documented that atherosclerotic lesions are preferentially found in areas with low or turbulent shear stress. When AA HUVECs were exposed to LoLSS, it did not inhibit the CRP-induced effects, and also, did not achieve the same extent of response as CA HUVECs. Lower levels of NO and higher levels of ET-1 were detected in LoLSS only condition in AA compared with CA, although the differences did not reach statistical significance.

Very few studies about CRP and ECs were conducted under shear stress conditions to create a flow environment similar to physiological condition. In a cell study performed by Devaraj et al. [141], CRP-induced monocyte-EC adhesion on HAECs was examined under static and low shear flow (2 dyne/cm²) conditions. No study to date has been done to investigate the role of high/low levels of LSS on CRP-induced biological effects and the potential racial differences. Recently, it has been shown that AA HUVECs respond differently to high levels of LSS, which was evidenced by a significantly larger reduction in NADPH subunit expression and higher increased levels of antioxidant production [13]. Based on these previous findings combined with the results from the current study, it
appears that in terms of endothelial function, AA may benefit more from high levels of blood flow generated by aerobic exercise compared with CA. Interestingly, LoLSS significantly increased FcγRIIB receptor expression after 24 hr in CA ECs but not in AA ECs.

It has been determined that CRP elicits increased ICAM-1 and VCAM-1 expression on ECs, which are important markers and contributors to the development and progression of atherosclerosis. Previous cell studies by others have shown that 24 hr CRP incubation (10 mg/L) induced an up to 10-fold increase ICAM-1 expression and a significant upregulation of VCAM-1 expression [76]. The results from the current study are consistent with these findings that showed racial differences in response to CRP in terms of adhesion molecules. Because elevated levels of adhesion molecules result in heightened CVD risk, and considering the higher circulating CRP levels and the greater response to CRP in AA as well as how HiLSS appears to be effective in reversing the detrimental effects specifically to the AA population, it is recommended to promote aerobic exercise as a vascular risk prevention and treatment for AA.

Numerous studies reported that AA exhibit greater blood pressure increases in response to vasoconstrictors than CA. ET-1, a potent vasoconstrictor, is released by ECs and elicits smooth muscle contraction. Data from human studies have found that AA have higher basal ET-1 levels than CA regardless of blood pressure status [103, 146]. Higher absolute levels of ET-1
were detected in AA after acute stress in normotensive youth [104]. Data from the current study are in agreement with the results from other studies that higher levels of CRP were detected in the cell media from AA. There are both in vivo and in vitro data on the regulatory effects of exercise or shear stress on ET-1. It is reported that ET-1 secretion is regulated by shear stress is in a dose-dependent manner and is independent of protein kinase C and cAMP [147]. In the current study, only HiLSS was able to inhibit CRP-induced ET-1 production and the application of HiLSS before CRP incubation had greater inhibition than the application of HiLSS after CRP exposure.

The biological significance and consequences of the greater response to CRP in cultured HUVECs in AA may contribute to the reduced endothelial function and higher prevalence of atherogenesis in AA with chronic low grade systemic inflammation. For example, decreased levels of NO are closely associated with inflammation and endothelial activation as well as smooth muscle cell activation. This chronic proinflammatory predisposition may eventually result in early vascular injury and endothelial dysfunction and hence contribute to the increased prevalence of CVD in AA.

Ideally, a study should use ECs directly obtained from sedentary CA and AA adults with different levels of CRP, followed by the completion of aerobic exercise training. However, since obtaining the large number of adult-derived EC samples is not feasible and aerobic exercise training may decrease the serum CRP level, the in vitro HUVECs model was chosen as an alternative. Advantages
of the well-established, controlled cell culture model are the relative stable environment and controllable flow stress. Since efforts were made to conduct the comparison analysis under identical conditions, it is reasonable to assume that the significant racial differences in the measured levels of mRNAs and proteins reflect inherent differences in the expression of each protein by cell line donors. The results from this study are consistent with previously reported findings in other human and cell studies and contribute to the insight and overall understanding of racial differences in inflammation and endothelial function. In fact, results from our group have consistently supported the concept that AA ECs are generally more responsive to proinflammatory or proatherogenic stimulus, and at the same time, are more responsive to the exercise mimetic--HiLSS.

Although the results from this cell model may not necessarily reflect the inflammation status and exercise effects under normal physiological conditions in the human body, this study may provide new insight into the mechanism of racial differences in inflammation and the potential larger exercise benefits to AA in terms of endothelial health.

Although the observation is novel, the results and conclusion drawn from the in vitro HUVEC culture study should be interpreted cautiously for several reasons. Firstly, long-term influences of risk factors and other physiological changes cannot be included and accurately measured in this cell model. Further study will need to examine the FcγR expression in ECs from human arteries under both steady flow and turbulent flow conditions. Second, we used only
HUVECs as the cell model but there are different EC types throughout the human body. The expression of FcγR might not differ between racial groups in other ECs. Third, it is unclear about the ratio of pentameric and monomeric CRP in the commercial CRP and the roles of them are still controversial.

A better understanding of the racial differences in endothelial function will help to improve hypertension prevention and treatment. To conclude, the results of the current study suggest that the racial differences in CRP-induced effects on ECs are through the differential expression of FcγRIIB, and that HiLSS may affect the expression of FcγRIIB and protect EC from atherogenesis.
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