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Are there Race-Dependent Endothelial Cell Responses to Exercise?

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Abstract

African Americans have endothelial dysfunction which likely contributes to their high prevalence of hypertension. Endothelial cell responses to stimuli could play a role in the development of endothelial dysfunction and hypertension. High physiological levels of vascular laminar shear stress can profoundly alter endothelial cell phenotype. It is not known whether there are race-dependent endothelial cell responses to laminar shear stress.

Keywords

Endothelial cell; shear stress; exercise; African American; oxidative stress; inflammation

INTRODUCTION

Nearly all published articles on hypertension and race state that African Americans have the highest prevalence in the world. 2006–2008 NHANES data show that African American men and women 20 years of age and older have a hypertension prevalence of 40 and 42% while Caucasian men and women have a hypertension prevalence of 30 and 28%. The mechanisms underlying racial disparities in cardiovascular disease (CVD) are multifactorial. Differences in endothelial cell (EC) responses to stimuli could play an important role in the development of endothelial dysfunction, hypertension, and therefore CVD. Though only a handful of studies have been conducted, they consistently show that African Americans have reduced endothelial function compared to Caucasians.

A major problem with research on the racial disparity in endothelial dysfunction is that it is conducted at the organ or physiological system level but not at the cellular level. Due to this large gap between *in vivo* physiology and *in vitro* mechanistic research relative to racial disparities in endothelial dysfunction, there continues to be only speculation about

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SUMMARY

Vascular endothelial cells from African Americans demonstrate basal oxidative stress and inflammation and may respond more robustly to laminar shear stress.

mechanisms of endothelial dysfunction in African Americans. This lack of knowledge about the EC biology of African Americans severely impedes advances in finding optimal preventive and treatment strategies that target African Americans. Our research focuses on understanding racial disparities in endothelial function at the cellular level, if they exist.

The vascular endothelium is the monolayer of ECs lining the blood vessels. Current thought about endothelial dysfunction includes a pro-oxidant, pro-inflammatory, pro-thrombotic, and apoptotic EC state which creates an arterial wall environment favoring the initiation and maintenance of high blood pressure and atherosclerosis. A quote by Le Brocq et al. from a comprehensive article on endothelial dysfunction highlights the fact that there can be different types of endothelial dysfunction, and the best therapeutic plan may vary by type: *"It is essential for researchers first to identify the type of endothelial dysfunction that applies to the disease of interest before deciding what therapeutic approach might be beneficial."* (20).

Intra-vascular laminar shear stress (LSS) is a major physiological stimulus that can profoundly affect the phenotype of the endothelium (24). Our laboratory uses various levels of *in vitro* LSS to simulate different physiological blood flow conditions. For example, we use high physiological arterial levels of LSS as an exercise mimetic EC model to better understand the endothelial cellular and molecular events related to endothelial adaptations to exercise.

Figure 1 shows our conceptual model of proposed differences in EC status and responses to LSS in African Americans and Caucasians. Our working hypothesis is that ECs obtained from African Americans exhibit greater levels of oxidative stress and inflammation compared to ECs obtained from Caucasians. Further, we hypothesize that African American ECs demonstrate greater reductions in oxidative stress and inflammation after exposure to an *in vitro* exercise mimetic, LSS, compared to Caucasian ECs. The data we present herein are, for the most part, the first of their kind. Thus, much more research is required to fully test these hypotheses.

ENDOTHELIAL DYSFUNCTION AND AFRICAN AMERICANS

Endothelial activation is an early step in endothelial dysfunction characterized by a disturbance of the regulation of vessel tone and the maintenance of a vascular environment that favors coagulation, oxidative stress, and inflammation. Endothelial dysfunction precedes and predicts the development of atherosclerotic disease in normal coronary arteries post-cardiac transplant (11) and is a prognostic indicator of cardiovascular events after statistical adjustment for other CVD risk factors (38).

Approximately ten studies have been conducted that assessed endothelial function in different races and they consistently show reduced function in African-Americans. This apparent predisposition for endothelial dysfunction likely contributes to their high prevalence of hypertension and hypertension co-morbidities.

ENDOTHELIAL SHEAR STRESS

The endothelium senses both chemical and mechanical stimuli, integrates the signals, and transduces the signals across the membrane and into the EC (2). Intra-vascular shear stress is the tangential friction force exerted by the flowing blood on the vessel wall. The endothelium responds to a sudden increase in shear stress within milliseconds. This immediate response is followed within a few hours by changes in the regulation of a subset of genes that are expressed in ECs. Thus, a critical feature of vascular endothelium is its phenotypic plasticity induced by hemodynamic cues. Mechanotransduction describes the

interaction between biomechanical forces and EC function. Therefore, the mechanical forces acting on the luminal side of ECs cause deformation of the EC which is transmitted through the cytoskeleton to the nucleus.

The type and magnitude of shear stress has an important role in long-term preservation of the structure and function of the blood vessel. Malek et al. delineated the normal magnitudes of shear stress in veins, arteries, and in low-shear and high-shear pathologic states (24). Normal arterial shear stress levels range from 10–70 dyne/cm². Lower levels (<4 dyne/cm²) stimulate an atherogenic phenotype, while levels > 15 dyne/cm² induce an atheroprotective phenotype. Studies have shown that a prolonged (24 hrs) high physiological level of LSS profoundly alter EC phenotype by modifying the gene profile creating an environment that is anti-inflammatory, anti-oxidant, and anti-apoptotic (24).

It is thought that the elevated vascular shear stress caused by aerobic exercise underlies many of the fundamental beneficial cardiovascular adaptations to aerobic exercise training. A substantial portion of the CVD risk reduction associated with exercise training cannot be entirely explained by changes in conventional CVD risk factors (29). It has been suggested that direct effects of exercise on the vessel wall may account for some of the remaining risk factor reduction gap (13).

A large body of work has shown that moderate intensity aerobic exercise training improves endothelial function in animals and humans with and without CVD risk factors. It appears that endothelial function changes occur early (2–4 weeks) in the exercise training program and may be transient phenomenon as some long-term (> 16 weeks) aerobic training studies show a return to initial levels of endothelial-dependent dilation with vascular wall structural remodeling of vessel caliber (31–30). Furthermore, resistance and conduit vessels have a different time-course of adaptation. Nonetheless, in individuals with diminished endothelial-dependent dilation, there is consistency with respect to the beneficial adaptations of the endothelium with aerobic exercise training. One question we had was since African Americans tend to have reduced endothelial function compared to Caucasians, then would the two groups' ECs respond differently to an *in vitro* application of LSS that causes adaptations of ECs that are consistent with *in vivo* endothelial function adaptations with exercise training.

Do Endothelial Nitric Oxide Synthase and Nitric Oxide Respond Differently to LSS in African American and Caucasian ECs?

The relative function of endothelial nitric oxide synthase (eNOS), the bioactivity of nitric oxide (NO), and the consequent NO-dependent dilation are considered synonymous with the degree of endothelial function. A possible reason for reduced endothelial function in African Americans compared to Caucasian could be reduced eNOS protein and NO production. In addition, given the tendency for reduced endothelial function in African Americans, it would be important to understand the capacity for adaptability of their ECs to exercise training. Therefore, we recently conducted a parallel EC culture experiment using African American and Caucasian human umbilical vein endothelial cells (HUVECs) to address this issue (9).

The HUVECs were exposed to 24 hours of 5 dyne/cm² LSS to simulate an atherogenic type of LSS, 24 hours of 20 dyne/cm² LSS to simulate a moderate exercise intensity level of LSS, and 0 dyne/cm² (static control conditions) using a custom cone and plate viscometer (9). For details about the methodology, please refer to the article by Fearheller et al (9). The Table summarizes directional changes in all endothelial factors in response to LSS in African American and Caucasian HUVECs.

Under static basal conditions, African American HUVECs exhibited significantly greater eNOS protein expression levels compared to Caucasian HUVECs. Greater eNOS protein in the basal condition in African Americans would suggest the potential for greater NO production although eNOS activation was not assessed in our study. This does not support the evidence for reduced endothelial function in African Americans. It is possible that NO production can be high but the NO bioavailability can be compromised as is the case when oxidative stress levels are also elevated within the EC.

Interestingly, we also found that the African American HUVECs had significantly greater expression levels of inducible NOS (iNOS) compared to Caucasian HUVECs. Our results were similar to those of Kalinowski et al. whose study also used African American and Caucasian HUVECs and found greater eNOS protein expression and NO levels in African American HUVECs (17). However, our study extended the results of Kalinowski et al. by showing that basal expression of iNOS was also greater in African American HUVECs.

Greater iNOS expression may be very important in revealing the African American EC phenotype. This inducible NO synthase isoform is involved in immune response and produces large amounts of NO as a defense mechanism. iNOS is up-regulated by different stimuli, especially inflammatory cytokines (16). Thus, greater iNOS expression may indicate an unhealthy or activated EC.

In response to LSS, we showed that eNOS protein expression was significantly increased in both African American and Caucasian HUVECs at 5 dyne/cm² and at 20 dyne/cm². Also, relative to 5 dyne/cm², eNOS protein expression significantly increased in both African American and Caucasian HUVECs at 20 dyne/cm² (Figure 2). Relative to static controls, HUVEC from both African American and Caucasians increased total NO production when exposed to LSS at both 5 dyne/cm² and at 20 dyne/cm² (Figure 2). Where there was a significant difference between HUVEC race was in NO production between 5 dyne/cm² and 20 dyne/cm² of LSS where NO production increased significantly only in African American HUVECs. This finding has relevant physiological implications because 5 dyne/cm² of LSS can be considered a resting arterial level, whereas 20 dyne/cm² of LSS can be considered a level achieved during “moderate” intensity aerobic exercise. The implications of these findings are that the adaptability of ECs relative to eNOS and NO appears to be similar in the two racial groups.

Different magnitudes of LSS (i.e., 5 and 20 dyne/cm²) could be thought of as different exercise intensities. A human study previously showed the exercise intensity-dependent nature of endothelial adaptations to exercise training. Goto et al. assessed forearm endothelium-dependent in healthy men after 12 weeks of cycle ergometry training at 25% (mild), 50% (moderate), and 75% (high) of maximal oxygen consumption (12). The study found that only moderate-intensity exercise training significantly increased endothelial-dependent dilation. Neither low nor high intensity training significantly increased endothelial-dependent dilation. Our findings also show the LSS magnitude-dependent nature of changes in eNOS and NO.

OXIDATIVE STRESS AND ENDOTHELIAL CELLS

Oxidative stress plays a critical role in the pathology and progression of CVD and hypertension. Reactive superoxide (O₂⁻) is primarily produced by the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX). Within the vascular wall, increased oxidative stress, as evidenced by higher O₂⁻ levels, alters blood flow regulation, membrane lipid oxidation, protein oxidation, platelet aggregation, leukocyte adhesion, and controls cellular growth. Many extensive review articles have highlighted the complex

association between reactive oxygen species (ROS) and their contributions to altered physiology and subsequent pathologies.

Within the body, the three primary antioxidant defense enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX). The SOD enzyme serves as the main protective antioxidant scavenging enzyme, where under normal physiological conditions, the dismutation of O_2^- by SOD yields hydrogen peroxide (H_2O_2). Three main isoforms of the SOD enzyme exist, manganese SOD (MnSOD/SOD2) located in the mitochondria, copper/zinc SOD (CuZnSOD/SOD1) located in the cytoplasm, and extracellular SOD (EcSOD/SOD3) located in the extracellular space. In the case of heightened NOX activity and O_2^- overproduction, O_2^- reacts with NO to produce peroxynitrite ($ONOO^-$) thus reducing NO bioavailability. When concentrations of O_2^- and NO are high, formation of $ONOO^-$ is favored because the rate of this diffusion-limited reaction is three times faster than the dismutation of O_2^- by SOD (18).

The NOX enzyme complex is composed of a membrane-bound cytochrome, which includes subunits gp91phox and p22phox, and a cytosolic component composed of subunits p47phox, p67phox, and the G protein, Rac. NOX-mediated free radical production has been associated with the pathophysiology of endothelial dysfunction, inflammation, CVD, and angiogenesis. It has been reported that in primary endothelial cultures, NOX2 and NOX4 serve as primary sources of NOX-mediated oxidative stress (32).

It has been shown that exercise training modifies the expression of NOX. In an *in vivo* study by Adams et al., left internal mammary artery rings sampled during bypass surgery from 45 patients randomized to either an exercise training or an inactive control group, were assessed for messenger RNA (mRNA) expression of NOX subunits, NOX activity, and ROS production (1). Exercise training significantly lowered the expression of gp91phox, p22phox, and NOX4. NOX enzymatic activity and ROS generation were significantly lower in the exercise training compared to the control group. Exercise training also resulted in improved endothelial function.

Very little *in vitro* research has been conducted examining racial differences between cell lines of different races. Wei et al. conducted a microarray study to assess basal gene expression in an attempt to explain racial health disparities (37). Wei et al. concluded that genetic influences stemming from ancestral continent-of-origin could impact upon endothelial cell biology and thereby contribute to disparity of vascular-related disease burden among African Americans. Kalinowski et al. found that African American HUVECs exhibited upregulated eNOS and NOX subunit expression of p67phox, p47phox, and p22phox relative to Caucasian HUVECs.

Duerrschmidt et al. used cultured HUVECs of unknown racial origin to quantify the expression of specific NOX subunits in response to LSS (8). They reported reduced mRNA expression of gp91phox and p47phox after 24 hours of LSS at 30 dyne/cm². Since *in vitro* LSS is hypothesized to simulate the shear stress conditions elicited by aerobic exercise *in vivo*, this reported *in vitro* downregulation of NOX activity may be an important contributor to the lower oxidative stress levels often observed following exercise training in human subjects. However, no study has ever investigated the possibility that there may be an influence of race on NOX subunit response to LSS.

In order for us to pursue potential race differences in EC oxidative stress, we first sought to examine whether basal oxidative-stress markers that are typically measured in humans also differ by race in cell culture (10). For detailed methodology, please refer to the article by Fearheller et al (10). For the EC experiments, African American and Caucasian HUVECs were cultured in parallel. The supernatant from confluent HUVECs was removed and

immediately stored at -80°C until assay. Cell lysate was collected by fractionation and cell culture media samples were collected.

We found heightened oxidative stress in the African Americans both *in vitro* and *in vivo*. We found no difference in plasma NO levels between the African American and Caucasian young adults. African American adults had significantly greater plasma SOD activity compared to Caucasian adults. Total antioxidant capacity (TAC) provides a relative cumulative measure of all the antioxidants present in the plasma and body fluid and is inherently a measure of the ability to scavenge H_2O_2 . African American adults also had significantly greater plasma TAC levels compared to Caucasian adults. Confirming this heightened oxidative stress, we found significantly greater plasma protein carbonyl levels in African American adults compared to Caucasian adults. Relative to the HUVECs, we found significantly lower SOD activity in African American compared to Caucasian HUVECs. To further clarify the antioxidant potential of the HUVECs, we found that African American HUVECs tended to have lower SOD1 activity. Further analysis of protein expression of SOD2 showed that African American HUVECs exhibited significantly lower basal SOD2 protein expression.

In most tissues, SOD3 exists in very small amounts, but in the circulation it can represent up to half the total SOD activity. Therefore in controlled cell-culture conditions, EC lysate would not have the same inherent percentages of SOD3. In HUVECs, we found African Americans had significantly lower levels of total SOD activity, while in human plasma we found African American adults had significantly higher SOD activity when compared to Caucasians. This difference could be attributed to the large contributions from SOD3 to the total SOD measure in plasma from the African American adults.

In summary, we found evidence of a heightened oxidative stress environment both *in vivo* and *in vitro* in African Americans. Given the often observed relationship of oxidative stress with various chronic diseases, the potential implication is that interventions aimed at reducing oxidative stress and/or increasing antioxidant capacity within the vascular wall may be especially important in African Americans. Furthermore, these observational results suggest that the race of the cell model may be important to consider in the context of *in vivo* clinical research.

Do Oxidative Stress Mechanisms in African American and Caucasian ECs Respond Differently to LSS?

In our parallel EC culture experiments using African American and Caucasian ECs (9, 10), we found greater expression levels of oxidative stress proteins during basal conditions (static) in African American HUVECs. African American HUVECs exhibited significantly greater p47phox, p22phox, NOX2, and NOX4 protein expression levels compared to Caucasian HUVECs (Figure 3).

We hypothesized that a high level of LSS would be beneficial because it would decrease NOX subunit protein expression. In our study, relative to static controls, p47phox protein expression levels significantly decreased with 20 dyne/cm² LSS only in African Americans HUVECs (9). Relative to static controls, NOX4 protein expression decreased significantly only in the African American HUVECs with 5 dyne/cm² LSS (Figure 4), but with 20 dyne/cm² LSS, NOX4 expression significantly decreased in both African American and Caucasian HUVECs (Figure 4). LSS had no effect on NOX2 protein expression in HUVECs from either race. In response to LSS, SOD2 protein expression increased significantly in African American HUVECs under the 5 dyne/cm² and 20 dyne/cm² conditions. Again, only the African American HUVECs had a significant increase in total SOD activity under the 5 dyne/cm² and 20 dyne/cm² conditions compared with static conditions. LSS had no effect

on either SOD2 protein expression or total SOD activity in the Caucasian HUVECs. Compared with static conditions, the African American HUVECs had greater TAC responses to 5 dyne/cm² and 20 dyne/cm² and of LSS. The Caucasian HUVECs did not respond to 5 dyne/cm² but did achieve significantly greater TAC levels at 20 dyne/cm² compared to static conditions.

Taken together, p47phox and NOX4 were more responsive to LSS in African American ECs and SOD2 protein expression increased significantly only in African American EC under the 5 and 20 dyne/cm² conditions. Studies have found that p47phox is required for NADPH oxidase activity and ROS production in ECs (22). It has been shown that in mice lacking p47phox, the blood pressure response to angiotensin II was blunted compared with wild-type mice, suggesting a role for p47phox and NOX activation in the blood pressure increase caused by angiotensin II (19). It has been reported that in primary EC cultures, NOX4 serves as primary source of oxidative stress (32). This NOX4 isoform has also been suggested to directly produce large amounts of the oxidant H₂O₂ (21). This information has important implications. Given that African Americans may have an EC phenotype characterized by heightened oxidative stress, then it is important to show that a nonpharmacologic intervention could be effective in reducing this vascular oxidative stress burden which could improve endothelial function and reduce the risk of hypertension in this high risk group.

INFLAMMATION AND ENDOTHELIAL CELLS

Inflammation directly damages the endothelium by activating the EC and indirectly by inducing oxidative stress. Epidemiologic data support the existence of an association between different inflammatory markers and high blood pressure. One such inflammatory marker is C-reactive protein (CRP). CRP is considered to be an important marker of both subclinical chronic vascular inflammation and increased cardiovascular risk (28). CRP is an acute phase protein that is produced by hepatocytes and is regulated by cytokines, mostly interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α)³. CRP also stimulates monocytes to increase the release of other cytokines as well as the expression of the adhesion molecules by ECs (26). In general, studies have shown a graded risk across the measured ranges of high-sensitivity CRP (hs-CRP). Increased levels of pro-inflammatory markers such as hs-CRP have been found in hypertensive individuals, even after statistical adjustment for potential confounding factors. Elevated hs-CRP levels have also been shown to be predictive of the development of hypertension in Pre-hypertensive and normotensive patients (7). One weakness of the studies mentioned above is that they are limited to Caucasian North American or European populations except for one study that included Japanese-American men in the Honolulu Heart Study.

African Americans tend to have higher levels of inflammatory markers compared to Caucasians which may be related to their greater risk of CVD-related morbidity and mortality. We have recently shown in a cohort of African American Pre-hypertensives (systolic blood pressure, 120–139 mmHg and diastolic blood pressure, 80–89 mmHg) that there is low-grade systemic inflammation as indicated by hs-CRP levels (34). In fact, their hs-CRP levels put them in the highest CVD risk category (27).

Non-pharmacological and pharmacological approaches tend to reduce vascular inflammation in patients with diabetes and hypertension. A number of studies have assessed the effects of exercise training on CRP in coronary artery disease patients. In general, these studies indicate that exercise training reduces inflammation independent of changes in obesity. Since the endothelium is a source of inflammatory cytokines, it is likely that an improvement in EC health could be a primary reason for the improvement in chronic inflammation with exercise training.

In response to pro-inflammatory cytokine stimulation, ECs undergo functional changes that include an increase in expression of adhesion molecules and induction of pro-coagulant activity. Pro-inflammatory cytokines, such as TNF- α , activate NF κ B transcription factors which regulate vascular inflammation. In addition, CRP is not simply a marker of chronic inflammation but also has direct effects on ECs by down-regulating eNOS transcription, destabilizing eNOS mRNA, and decreasing basal and stimulated NO release (35). TNF- α decreases eNOS mRNA expression level by shortening its half-life (39). Secondly, increased EC production of O₂⁻ and other free radicals during inflammation can reduce NO bioavailability. Evidence shows that chronic high physiological levels of LSS, which are consistent with atheroprotection, suppress inflammation in ECs (25).

ARE THERE RACIAL DIFFERENCES IN ENDOTHELIAL MICROPARTICLES (EMPS) AS MARKERS OF EC DAMAGE?

The physiological measurement of endothelial function does not provide specific information about the biological status of ECs. Cytokine-mediated endothelial dysfunction appears to be a predominant mechanism underlying hypertension in African Americans. EMPs are sub-microscopic membranous particles that reflect the functional state of the parental EC (14). EMPs are released from activated or apoptotic parent ECs when stimulated by pro-inflammatory cytokines, oxidative stress, or infectious agents (33). They carry a subset of membrane proteins and phospholipids, or markers, of their parent ECs including those induced by activation, apoptosis, or oxidative stress. EMPs expressing constitutive surface markers such as CD31 (platelet endothelial cell adhesion molecule; PECAM) are increased in injury and/or apoptosis (23). EMP levels are increased in pathological conditions such as diabetes, hypertension, metabolic syndrome, hypercholesterolemia, and thrombosis. Jenkins et al. recently published an article reporting that prior exercise prevents post-prandial-induced oxidative stress in circulating angiogenic cells (15). They found that the attenuated increases in oxidative stress by prior exercise was associated with increased antioxidant gene expression and reduced EMPs compared to the control trial. Thus, the level of EMPs is emerging as a novel direct marker of EC impairment mediated by activation and apoptosis.

We recently published a study that determined EMP responses to the inflammatory cytokine, TNF- α , and the antioxidant, SOD, in HUVECs obtained from African American and Caucasians (5). TNF- α is a primary inflammatory cytokine that is synthesized in several cell types involved in vascular inflammation including ECs. Intra-arterial TNF- α has been shown to cause acute local vascular inflammation that is associated with impaired endothelium-dependent relaxation (6).

In our study, EMPs were measured under four conditions: control (basal), TNF- α , SOD, and TNF- α + SOD. Culture supernatant was collected for EMP analysis by flow cytometry and IL-6 assay by ELISA. IL-6 protein expression was assessed by Western blot. We measured the cytokine IL-6 because it is produced by ECs in response to TNF- α and stimulates the synthesis of several acute-phase reaction proteins, including CRP. IL-6 prolongs endothelial dysfunction, which may in turn lead to hypertension.

We showed that the EMP level significantly increased by 89% from basal levels in the African American HUVECs under the TNF- α condition compared to a non-significant increase of just 8% in the Caucasian HUVECs (Figure 5). Compared to the EMP level under the TNF- α condition, the EMP level in the African American HUVECs was significantly lower under the SOD only condition and under the TNF- α + SOD condition. Basal IL-6 concentrations were nearly two-fold greater in the African American compared to the Caucasian HUVECs while basal IL-6 protein expression was significantly greater in the

African American cells (Figure 6). These observational results suggest that African American HUVECs may be more susceptible to the injurious effects of the pro-inflammatory cytokine, TNF- α . In addition, the results suggest that TNF- α induced oxidative stress may play a greater role in EMP generation in African American compared to Caucasian HUVECs. Since we found that the EMP response to TNF- α differed by HUVEC race, we next wondered if high physiological levels of LSS would differentially affect EMP levels in African American and Caucasian ECs.

Do African American and Caucasian ECs Show Differential EMP Generation Responses to LSS?

We conducted a similar LSS experiment and measured the generation of EMPs in African American and Caucasian HUVECs. We hypothesized that since EMPs are markers of EC damage, then low (5 dyne/cm²) physiological levels of LSS, those that are atherogenic prone, would increase EMP levels and that high (20 dyne/cm²) physiological levels of LSS, those that are atheroprotective, would decrease EMP levels. We found a tendency for a HUVEC race by LSS condition interaction with the Caucasian HUVECs showing a substantial reduction in EMP generation going from 5 to 20 dyne/cm² of LSS whereas EMP generation was not altered in the African American HUVECs (Figure 7). There was a significant main effect of LSS. There was no main effect of race. These results suggest that EMPs are responsive to LSS, though only the Caucasian HUVECs showed significantly lower levels of EMPs at 20 dyne/cm² compare to the 5 dyne/cm² condition. Indeed, much more research is required to elucidate the underlying mechanisms responsible for LSS-induced changes in EMPs.

In summary, we have shown that African American HUVECs had a much greater EMP response to TNF- α and SOD suggesting that there was greater EC damage and that it was likely that TNF- α -induced oxidation stress played a prominent role. High physiological levels of LSS are known to improve EC phenotype such that the EC is anti-inflammatory, anti-oxidative, and anti-apoptotic. We have shown that there was a significant main effect of 20 dyne/cm² of LSS to reduce EMP levels, however this effect was due primarily to the Caucasian HUVECs. This resulted in a tendency for an interactive effect of HUVEC race and LSS.

CONCLUSIONS

Our working hypothesis is that ECs obtained from African Americans exhibit greater levels of oxidative stress and inflammation compared to ECs obtained from Caucasians, and that that African American ECs demonstrate greater reductions in oxidative stress and inflammation after exposure to an *in vitro* exercise mimetic, LSS, compared to Caucasian ECs. Nearly three decades of research by numerous independent labs have clearly established that the shear effects, determined *in vitro* using aortic or venous ECs of humans and animals well reflect the *in vivo* responses in both human and animals. Even after an EC demonstrates a particular phenotype during embryonic development, transdifferentiation may occur (36) because ECs adapt to hemodynamic environmental cues (4). HUVECs yield reproducible biologic responses and demonstrate most of the same patterns of gene expression as ECs from coronary artery and aorta.

The most important findings of our research relative to responses to LSS are related to oxidative stress. Relative to static controls, p47phox protein expression levels significantly decreased with 20 dynes/cm² LSS only in African Americans. Relative to static controls, NOX4 protein expression decreased significantly only in the African American HUVECs with 5 dynes/cm² LSS. Relative to NO production, only the African American HUVECs increased NO production at 20 dynes/cm² of LSS compared to 5 dynes/cm². We are

currently conducting studies to elucidate the reasons for possible race-dependent responses to LSS. To this point, our data should be viewed as preliminary.

We recognize that human exercise training consists of intermittent bouts of exercise that occur over time and that our application of LSS occurs over 24 hours. The reason we selected 24 hours is because it is more than enough time for gene expression to occur and for protein levels to change. The adaptations in protein expression that occur after 24 hours of LSS are consistent with the changes in endothelial function in humans and animals following exercise training. The findings we report here are from our initial sets of experiments aimed at addressing our hypotheses. Of course, subsequent experimental designs will use many different types of LSS perturbations, including intermittent application.

Taken together, our data suggest that African Americans appear to have an EC phenotype characterized by heightened oxidative stress with the African American HUVECs exhibiting greater basal p47phox, NOX2, and NOX4 protein expression levels compared to Caucasian HUVECs. To compound this presumed heightened oxidative stress, the African American HUVECs had reduced antioxidant capacity exhibiting lower SOD activity and SOD2 protein expression levels. We also showed that African American HUVECs exhibit potential for a heightened inflammatory phenotype. In addition, African American HUVECs exhibited greater basal iNOS protein expression levels which could be due to the greater oxidative stress and inflammation.

It may be that the fundamental mechanism underlying endothelial dysfunction in African Americans is EC inflammation which can lead to increased oxidative stress. Interestingly, our African American subjects demonstrate an average hs-CRP concentration that puts them in the highest risk category (>3.0 mg/L) and also exhibit endothelial dysfunction, even when we analyzed data from only the Pre-hypertensive African American group.

There is a very large group of literature describing observations of racial disparities in various diseases and conditions even when genetic admixture is present. Due to the observational nature of these studies, gaining insights into specific mechanisms that can be targeted for therapy cannot be determined. Even the studies that are conducted at the organ or physiological system levels are not able to identify the fundamental cellular mechanisms underlying the potential biologic causes of racial health disparities. Thus, progress in racial health disparities research is stymied.

Based on the few EC proteins that we have investigated to date, some appear to have race-dependency in terms of their responses to LSS. Our future work will be directed at exploring specific inflammatory and oxidative stress pathways and their modifiers more deeply. Of course, EC genes that are shear stress responsive will be primary targets.

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Figure 1.

Conceptual Overview of Hypothesized Differences in Endothelial Cell Status and Responses to Laminar Shear Stress between African Americans and Caucasians.

African American and Caucasian endothelial cells shown in their basal state. We hypothesized that there would be less nitric oxide (NO), greater oxidative stress (O₂⁻), and less antioxidant capacity (Antiox) compared to the Caucasian endothelial cells in the basal condition. We also hypothesized that greater endothelial cell responsiveness to laminar shear stress in African Americans would lead to greater changes in these endothelial cell factors compared to Caucasians.

Figure 2.

Panel A - eNOS protein expression under static, 5 and 20 dyne/cm² conditions in African American (AA) and Caucasian (C) HUVECs. Panel B – nitric oxide concentrations in cell culture media under static, 5 and 20 dyne/cm² conditions in African American (AA) and Caucasian (C) HUVECs. Bars are mean $\pm$ SE. * P < 0.05 from static; # P < 0.05 from 5 dyne/cm². (Reprinted from (9). Copyright © 2011 Dove Medical Press Ltd. Used with permission.)

Figure 3. Basal NADPH oxidase subunit protein expression in African American (AA, solid bars) and Caucasian (C, open bars) HUVECs. Bars are mean $\pm$ SE. *, $p < .05$ AA vs. C. [Adapted from (9). Copyright © 2011 Dove Medical Press Ltd. Used with permission.]

Figure 4.

NADPH oxidase subunit protein expression under static, 5 and 20 dyne/cm² conditions in African American (AA) and Caucasian (C) HUVECs. Bars are mean $\pm$ SE. * P < 0.05 from static; # P < 0.05 from 5 dyne/cm²; † P < 0.05 between racial groups. (Reprinted from (9). Copyright © 2011 Dove Medical Press Ltd. Used with permission.)

*, $p < .05$ between HUVEC racial groups within condition

†, $p < .05$ compared to TNF- α condition within racial HUVEC groups

Figure 5.

EMP levels under each of the experimental conditions in African American and Caucasian HUVECs. Bars are mean $\pm$ SE. * $P < 0.05$ between HUVEC racial groups within condition. † $P < 0.05$ compared to the TNF- α condition within HUVEC racial groups. (Reprinted from (5). Copyright © 2011 Dove Medical Press Ltd. Used with permission.)

Figure 6.

IL-6 concentrations under each of the experimental conditions in African American and Caucasian HUVECs. At the top are representative Western blots of basal IL-6 protein expression levels in African American and Caucasian basal HUVECs. Bars are mean $\pm$ SE. *, $p < .05$ between HUVEC racial groups within condition. †, $p < .05$ compared to TNF- α condition within racial HUVEC groups. (Reprinted from (5). Copyright © 2011 Dove Medical Press Ltd. Used with permission.)

Figure 7.
 EMP levels under static 5 and 20 dyne/cm² conditions in African American and Caucasian HUVECs. Bars are mean $\pm$ SE.

Directional changes in endothelial factors in response to 5 and 20 dynes/cm² in African American and Caucasian HUVECS.

Table 1

Endothelial Factor	Change with 5 dyne/cm ² in African Americans	Change with 5 dyne/cm ² in Caucasians	Change with 20 dyne/cm ² in African Americans	Change with 20 dyne/cm ² in Caucasians	Change from 5 dyne/cm ² to 20 dyne/cm ² in African Americans	Change from 5 dyne/cm ² to 20 dyne/cm ² in Caucasians
eNOS protein	↑	↑	↑	↑	↑	↑
NOx	↑	↑	↑	↑	↑	↑
p47phox protein	↔	↔	↓	↔	↔	↔
NOX4 protein	↓	↔	↓	↓	↓	↔
NOX2 protein	↔	↔	↔	↔	↔	↔
SOD2 protein	↓	↔	↓	↔	↔	↔
SOD activity	↓	↔	↓	↔	↔	↔
TAC	↔	↔	↔	↔	↔	↔
EMPs	↔	↑	↔	↔	↔	↓

eNOS, endothelial nitric oxide synthase; NOx, nitric oxide end-products; p47phox, NADPH oxidase subunit; NOX2, NADPH oxidase gp-91phox subunit; NOX, homolog of NADPH oxidase gp-91phox subunit; SOD2, superoxide dismutase (mitochondrial); SOD, superoxide dismutase, TAC, total antioxidant activity; EMPs, endothelial microparticles.