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Attenuated cutaneous microvascular function in healthy young African Americans: Role of intradermal L-arginine supplementation

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ABSTRACT

It has been established that endothelial function in conduit vessels is reduced in young African Americans (AA) relative to Caucasian Americans (CA). However, less is known regarding endothelial function in microvasculature of young AA. We hypothesized that microvascular function in response to local heating of skin is attenuated in young AA relative to age-matched CA due largely to the lack of NO bioavailability, which is in turn improved by intradermal L-arginine supplementation and/or inhibition of arginase. Nine AA and nine CA adults participated in this study. Participants were instrumented with four microdialysis membranes in the cutaneous vasculature of one forearm and were randomly assigned to receive 1) lactated Ringer's solution as a control site; 2) 20 mM NG-nitro-L-arginine (L-NAME) to inhibit NO synthase activity; 3) 10 mM L-arginine to local supplement L-arginine; or 4) a combination of 5.0 mM (S)-(2-boronoethyl)-L-cysteine-HCL (BEC) and 5.0 mM Nω-hydroxy-nor-L-arginine (nor-NOHA) at a rate of 2.0 μl/min to locally inhibit arginase activity. Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by mean arterial pressure. All CVC data were presented as a percentage of maximal CVC (%CVCmax) that was determined by maximal cutaneous vasodilation induced by 44 °C heating plus sodium nitroprusside administration. The response during the 42 °C local heating plateau was blunted in the AA at the control site (CA: 84 ± 12 vs. AA: 62 ± 6 vs. %CVCmax; $P < 0.001$). This response was improved in AA at the L-arginine site (Control: 62 ± 6 vs. L-arginine: 70 ± 18%CVCmax; $P < 0.05$) but not in the arginase inhibited site (Control: 62 ± 6 vs. Arginase inhibited: 62 ± 13%CVCmax; $P = 0.91$). In addition, the AA group had an attenuated NO contribution to the plateau phase during 42 °C local heating relative to the CA group (CA: 56 ± 14 vs. AA: 44 ± 6 Δ %CVCmax; $P < 0.001$). These findings suggest that 1) cutaneous microvascular function in response to local heating is blunted in young AA when compared to age-matched young CA; 2) this attenuated response is partly related to decrease in NO bioavailability in young AA; and 3) a local infusion of L-arginine, but not arginase inhibition, improves cutaneous microvascular responses to local heating in young AA relative to CA.

1. Introduction

African Americans (AA) are at increased risk for developing a wide variety of cardiovascular and metabolic diseases including coronary artery disease, stroke, hypertension, metabolic syndrome, and type II diabetes relative to other populations including Caucasian Americans (CA) (Fields et al., 2004; Harris et al., 2011; Melikian et al., 2007; Mensah et al., 2005). The underlying impairments resulting in these disease states manifest during early adulthood prior to any overt signs of risk in this population (Cushman et al., 2008; Forouhi and Sattar, 2006; Kerr et al., 2008). The mechanisms resulting in increased risk in this population are multifactorial; however, it is likely that endothelial dysfunction, as indexed by a blunted vasodilatory response to a variety of stimuli, is a major contributing factor (Bassett Jr. et al., 1992; Harris et al., 2011; Kotanko and Skrabal, 1995; Melikian et al., 2007; Perregaux et al., 2000; Stein et al., 1997).

One potential mechanism that has been implicated in attenuated endothelial function in young AA is decreased nitric oxide (NO) bioavailability. NO is formed in the endothelial cells by endothelial NO synthase (eNOS), which breaks down the substrate L-arginine into NO and L-citrulline, and acts as a primary vasoactive substance that plays a critical role in vasodilation (Holowatz et al., 2006; Palmer et al., 1988).

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The exact mechanisms of a decreased NO bioavailability have not been fully elucidated. However, several potential signaling pathways, including eNOS uncoupling or dysregulated utilization of the substrate L-arginine, have been suggested. It has recently shown that a low concentration of L-arginine can negatively affect the production of NO in animals (Cooke et al., 1992) and human cells (Creager et al., 1992), indicating that a decreased NO bioavailability due to eNOS uncoupling, may be related to a decreased concentration of available L-arginine (Holowatz et al., 2006). Interestingly, in a previous study AA had an improved coronary endothelial function in response to the endothelial dependent vasodilator acetylcholine when it was co-infused with L-arginine supporting a beneficial role of L-arginine supplementation on vascular function in this population (Houghton et al., 2002). Another possible mechanism leading to eNOS uncoupling and thus reduced NO bioavailability in AA could be related to an elevated arginase activity. Arginase competes with L-arginine and therefore, with elevated Arginase activity eNOS is uncoupled leading to the production of urea and L-ornithine as opposed to NO and L-citrulline (Bachetti et al., 2004; Berkowitz et al., 2003; Elms et al., 2013; Holowatz and Kenney, 2007b; Holowatz et al., 2006; Ming et al., 2004; White et al., 2006). In this regard, a number of research studies utilizing populations with elevated cardiovascular disease risk and/or overt cardiovascular disease have implicated that decreased L-arginine availability and/or increased arginase activity could impair microvascular function secondary to decreased NO bioavailability. Furthermore, this attenuated microvascular function could be restored with both acute and chronic L-arginine supplementation and/or inhibition of arginase activity (Berkowitz et al., 2003; John and Schmieder, 2003; White et al., 2006).

To our knowledge, racial differences in the functional roles of L-arginine and arginase and its potential contribution to a reduced NO-dependent vasodilation are unknown. Thus, we hypothesized that microvascular function in response to local heating of skin is attenuated in young AA relative to age-matched CA due largely to a reduction in NO bioavailability. Furthermore, we hypothesized that this blunted response to local heating would be restored at a measurement site that is pretreated with L-arginine and another site that is pre-treated with an arginase inhibitor.

2. Methods

2.1. Ethical approval

The Institutional Review Board at The University of Texas at Austin approved all study procedures and the consent process used in the present study. Subjects were given a verbal description of all procedures and informed of the purpose and risks involved in the study before providing their informed, written consent.

2.2. Subjects

Eighteen young, healthy subjects (9 CA and 9 AA) volunteered for this study. All subjects self-identified as CA or AA and were only accepted into the study if both parents were CA or AA, respectively. Subjects were normally active, normotensive, nonsmokers, not taking any medications and had no history of cardiovascular, metabolic, or neurological disease. All female subjects were studied during the early follicular phase (within 1–3 days of the start of menstruation) to minimize the effects of female sex hormones (Charkoudian et al., 1999). Subjects were fasted at least 12 h, and having refrained from strenuous exercise, alcohol, and caffeine for 24 h before the test.

2.3. Instrumentation

Throughout the experiment the subjects sat in the semi-recumbent position on a patient bed in a temperature controlled laboratory (23–24 °C). Four intradermal microdialysis membranes (CMA 31 Linear Microdialysis Membrane, 55 kDa cut-off membrane; CMA Microdialysis AB, Holliston, MA) were instrumented on the dorsal surface of the non-dominant forearm. A 25-gauge needle was inserted into the dermal layer of the skin using aseptic techniques. Each site was placed at least 4 cm apart with entry and exit points on each site ~2.5 cm apart. The microdialysis membranes were threaded through the lumen of the needle, and the needle was withdrawn from the skin while the semi-permeable membrane portion of the membrane was advanced into the dermis. Membranes then were taped in place to secure its position and perfused with lactated Ringer's solution at a rate of 2.0 μl/min via a perfusion pump (11 PLUS; Harvard Apparatus, Holliston, MA) until the beginning of drug infusions (see below). Cutaneous red blood cell (RBC) flux, an index of skin blood flow (SkBF), was measured over each microdialysis membrane site using a laser-Doppler flowmetry probe (VP7 A/T with moorVMS-LDF2; Moor Instruments, Wilmington, DE). Each laser-Doppler probe was housed in the center of a local heating element (PeriFlux System 5000; Perimed, Sweden). Intermittingly throughout the protocol arterial blood pressure was obtained on the right arm by auscultation of the brachial artery via electrosphygmomanometry (Tango; SunTech, Raleigh, NC). Mean arterial pressure (MAP) was calculated as one-third pulse pressure plus diastolic blood pressure (DBP). Heart rate (HR) and cardiac rhythms were continuously monitored and recorded from an electrocardiogram on a patient monitor (GE DASH 4000; General Healthcare).

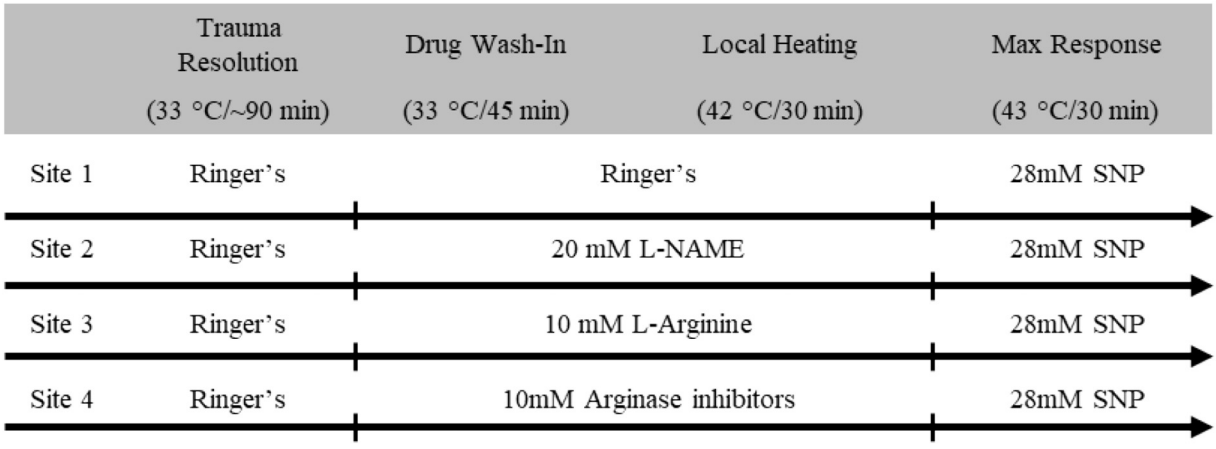

Fig. 1. An illustration of the experimental timeline for the study protocol. L-NAME, NG-nitro-L-arginine; arginase inhibitors, 5 mM BEC, (S)-(2-boronoethyl)-L-cysteine-HCL + 5 mM nor-NOHA, Nω-hydroxy-nor-L-arginine; SNP, sodium nitroprusside.