Am J Physiol Heart Circ Physiol 293: H1090–H1096, 2007.
First published May 4, 2007; doi:10.1152/ajpheart.00295.2007.

Local ascorbate administration augments NO- and non-NO-dependent reflex cutaneous vasodilation in hypertensive humans

Lacy A. Holowatz[1] and W. Larry Kenney[1,2]

[1]*Noll Laboratory,* [2]*Department of Kinesiology, Graduate Physiology Program, The Pennsylvania State University, University Park, Pennsylvania*

Submitted 8 March 2007; accepted in final form 30 April 2007

Holowatz LA, Kenney WL. Local ascorbate administration augments no- and non-no-dependent reflex cutaneous vasodilation in hypertensive humans. *Am J Physiol Heart Circ Physiol* 293: H1090–H1096, 2007. First published May 4, 2007; doi:10.1152/ajpheart.00295.2007.—Full expression of reflex cutaneous vasodilation (VD) is dependent on nitric oxide (NO) and is attenuated with essential hypertension. Decreased NO-dependent VD may be due to *1*) increased oxidant stress and/or *2*) decreased L-arginine availability through upregulated arginase activity, potentially leading to increased superoxide production through uncoupled NO synthase (NOS). The purpose of this study was to determine the effect of antioxidant supplementation (alone and combined with arginase inhibition) on attenuated NO-dependent reflex cutaneous VD in hypertensive subjects. Nine unmedicated hypertensive [HT; mean arterial pressure (MAP) = 112 ± 1 mmHg] and nine age-matched normotensive (NT; MAP = 81 ± 10 mmHg) men and women were instrumented with four intradermal microdialysis (MD) fibers: control (Ringer), NOS inhibited (NOS-I; 10 mM N^G-nitro-L-arginine), L-ascorbate supplemented (Asc; 10 mM L-ascorbate), and Asc + arginase inhibited [Asc+A-I; 10 mM L-ascorbate + 5 mM (*S*)-(2-boronoethyl)-L-cysteine-HCl + 5 mM N^{ω}-hydroxy-*nor*-L-arginine]. Oral temperature was increased by 0.8°C via a water-perfused suit. N^G-nitro-L-arginine was then ultimately perfused through all MD sites to quantify the change in VD due to NO. Red blood cell flux was measured by laser-Doppler flowmetry over each skin MD site, and cutaneous vascular conductance (CVC) was calculated (CVC = flux/MAP) and normalized to maximal CVC (%CVC_{max}; 28 mM sodium nitroprusside + local heating to 43°C). During the plateau in skin blood flow (ΔT_{or} = 0.8°C), cutaneous VD was attenuated in HT skin (NT: 42 ± 4, HT: 35 ± 3 %CVC_{max}; $P < 0.05$). Asc and Asc+A-I augmented cutaneous VD in HT (Asc: 57 ± 5, Asc+A-I: 53 ± 6 %CVC_{max}; $P < 0.05$ vs. control) but not in NT. %CVC_{max} after NOS-I in the Asc- and Asc+A-I-treated sites was increased in HT (Asc: 41 ± 4, Asc+A-I: 40 ± 4, control: 29 ± 4; $P < 0.05$). Compared with the control site, the change in %CVC_{max} within each site after NOS-I was greater in HT (Asc: −19 ± 4, Asc+A-I: −17 ± 4, control: −9 ± 2; $P < 0.05$) than in NT. Antioxidant supplementation alone or combined with arginase inhibition augments attenuated reflex cutaneous VD in hypertensive skin through NO- and non-NO-dependent mechanisms.

skin blood flow; essential hypertension; oxidative stress; temperature regulation; vitamin C

SKIN BLOOD FLOW IS reflexively controlled by dual sympathetic innervation consisting of an adrenergic vasoconstrictor system and an active vasodilator system (11). With rising body core temperature, skin blood flow is initially increased by a withdrawal of adrenergic vasoconstrictor tone and is further increased by the active vasodilator system (29). Active vasodilation is mediated by sympathetic cotransmission of ACh and an unknown neurotransmitter that mediates sweating and cutaneous vasodilation (21). Putative vasodilator pathways involved in active vasodilation include vasoactive intestinal peptide (3), histamine 1 receptor activation (38), cyclooxygenase-produced vasodilators (27), and substance P (37). Furthermore, activation of these pathways may contribute to active vasodilation in part through nitric oxide (NO)-dependent pathways, which are required for full expression of active vasodilation, contributing ~30–40% to the total vasodilatory response (20, 30).

Essential hypertension is associated with attenuated cutaneous vasodilation during local (6) and systemic thermal stress (16, 22). We (16) have recently shown that NO-dependent reflex cutaneous vasodilation is attenuated in hypertensive skin and that increasing L-arginine availability through acute arginase inhibition augments reflex vasodilation. Arginase catalyzes the conversion of L-arginine to L-ornithine and urea in the final step of the urea cycle and is capable of limiting NO production by competing for the common NO synthase substrate L-arginine (13). In addition to upregulated arginase contributing to decreased NO production, there are likely additional mechanisms impairing NO bioavailability in hypertensive cutaneous vasculature.

One potential mechanism contributing to the reduced NO bioavailability implicated in the pathogenesis of hypertensive vascular pathology is increased oxidative stress (35). In essential hypertensive vasculature, there is increased production of reactive oxygen species (ROS) coupled with decreased degradation of ROS through dysfunctional enzymatic antioxidant defense mechanisms (25). Furthermore, augmented ROS (superoxide) production reduces NO bioavailability by readily reacting with newly synthesized NO-forming peroxynitrite, which further potentiates superoxide production by uncoupling endothelial NO synthase (eNOS) (10). eNOS uncoupling can result from peroxynitrite-mediated oxidization of the essential NOS cofactor tetrahydrobiopterin (BH_4) and/or inadequate L-arginine availability (28). Together, these data suggest that there may be a mechanistic link between hypertension-associated increased arginase activity decreasing L-arginine availability and potentially contributing to increased oxidant stress through uncoupled eNOS.

In vivo human studies examining vascular oxidant stress mechanisms commonly utilize high-dose antioxidant supplementation for measurement of functional NO-dependent vasodilation. To this end, acute L-ascorbate (Asc) supplementation

Address for reprint requests and other correspondence: L. A. Holowatz, 123 Noll Laboratory, Univ. Park, PA 16802 (e-mail: lma191@psu.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

in the forearm muscle vasculature in humans with essential hypertension increases endothelium-dependent vasodilation through NO-dependent mechanisms (31). In these studies, Asc may increase NO bioavailability directly through antioxidant capabilities and/or indirectly by stabilization of the essential NOS cofactor BH_4, enabling NOS to "recouple" and produce functional NO (15, 32). The purpose of the present study was to determine the relative role of oxidant stress and augmented arginase activity on attenuated NO-dependent vasodilation in human hypertensive cutaneous vasculature. We sought to control for age-related increases in oxidant stress and arginase activity (17) by matching subjects with unmedicated essential hypertension to age- and sex-matched healthy normotensive control subjects. We hypothesized that *1*) local acute antioxidant (Asc) supplementation would modestly augment NO-dependent reflex cutaneous vasodilation and *2*) arginase inhibition (to increase L-arginine availability, enabling NOS to recouple and produce functional NO) combined with Asc supplementation would further enhance NO-dependent reflex vasodilation over Asc supplementation alone.

METHODS

Subjects. Experimental protocols were approved by the Institutional Review Board at The Pennsylvania State University. Verbal and written consents were voluntarily obtained from all subjects before their participation. Studies were performed on nine unmedicated subjects with essential hypertension (57 ± 4 yr, 6 men and 3 women) and nine normotensive age- and sex-matched control subjects (57 ± 3 yr, 6 men and 3 women). Each subject reported to the laboratory on three separate occasions to determine their blood pressure status in accordance with the guidelines set forth by the American Heart Association (1). Subjects then underwent a complete medical screening, including blood chemistry, lipid profile evaluation (Quest Diagnostics Nichol Institute, Chantilly, VA), resting ECG, and physical examination. All subjects were screened for the presence of cardiovascular disease other than hypertension, dermatological, and neurological diseases. Subjects were normally active, nondiabetic, healthy nonsmokers who were not taking medications, including aspirin therapy, hormone replacement therapy, or oral contraceptives. All premenopausal women (4 women) were studied on *days 2–7* of the follicular phase of their menstrual cycle. Seventeen of the eighteen subjects also participated in a previous study in our laboratory on a separate day (16).

Instrumentation and measurements. All protocols were performed in a thermoneutral laboratory with the subject in the semisupine position with the experimental arm at heart level. After arrival to the laboratory, subjects were instrumented with four intradermal microdialysis fibers as previously described (16–19) (MD 2000, Bioanalytical Systems) (10-mm, 30-kDa cutoff membrane) in the skin on the right ventral forearm. The microdialysis fibers were taped in place and perfused with lactated Ringer solution during the insertion trauma resolution period at a rate of 2.0 μl/min (Bee Hive controller and Baby Bee microinfusion pumps; Bioanalytical Systems) for 60–90 min.

To obtain an index of skin blood flow, cutaneous red blood cell flux was measured with an integrated laser-Doppler flowmeter probe placed in a local heater (MoorLAB, Temperature Monitor SH02; Moor Instruments, Devon, UK) on the skin directly above each microdialysis membrane. Cutaneous vascular conductance (CVC) was calculated as red blood cell flux divided by mean arterial pressure (MAP). MAP was calculated as diastolic pressure plus one-third pulse pressure.

Whole body temperature was controlled and whole body heating was conducted with a water-perfused suit as previously described (16–19). The subject's ECG was monitored throughout the protocol, and blood pressure was measured via brachial auscultation every 5 min. Oral temperature (T_{or}) was continuously monitored during baseline and throughout whole body heating with a thermistor placed in the sublingual sulcus as an index of body core temperature. The subjects were instructed to keep the thermistor in the same location in the sublingual sulcus and not to open their mouths or speak during the protocol. After a 0.8°C rise in T_{or} was reached, skin temperature and T_{or} were clamped by perfusing the suit with 46°C for the remainder of the heating protocol. Local skin temperature over each microdialysis site was maintained at 33°C during baseline and whole body heating (Moor Instruments SHO2).

Experimental protocol. After the insertion trauma resolution period (60–90 min), microdialysis sites were randomly assigned to receive *1*) lactated Ringer solution to serve as control, *2*) 10.0 mM N^G-nitro-L-arginine (L-NAME; Calbiochem, San Diego, CA) to competitively inhibit NO production by NOS, *3*) 10 mM ASC (Sigma, St. Louis, MO) to supplement antioxidants (17), or *4*) the combination of 10 mM ASC and 5.0 mM (*S*)-(2-boronoethyl)-L-cysteine-HCl and 5.0 mM N^{ω}-hydroxy-*nor*-L-arginine to supplement antioxidants and to inhibit arginase (Asc+A-I: Calbiochem) (16, 18). Our group (16–18) has previously used these doses of Asc and arginase inhibitors in conjunction with intradermal microdialysis in an aged population with known endothelial dysfunction. All pharmacological solutions were mixed just before usage, dissolved in lactated Ringer solution, sterilized with the use of syringe microfilters (Acrodisc, Pall, Ann Arbor, MI), and wrapped in foil.

All microdialysis sites were perfused continuously with assigned pharmacological agents for at least 60 min before the start of the baseline and throughout baseline (20 min) and whole body heating at a rate of 2.0 μl/min. After the baseline data collection period, whole body heating was conducted to raise T_{or} by 0.8°C. After a 0.8°C rise in T_{or}, body temperature was clamped for 30–40 min. After 10 min of stable laser-Doppler flux values, 10 mM L-NAME was perfused through all microdialysis fibers at a rate of 2.0 μl/min to inhibit NOS within each treatment site. L-NAME infusions were discontinued after laser-Doppler flux decreased to a stable plateau (Fig. 1). At the end of the whole body heating protocol, each microdialysis site was perfused with 28.0 mM sodium nitroprusside (SNP; Nitropress, Abbott Laboratories, Chicago, IL) at a rate of 4.0 μl/min to achieve maximal CVC. Local heating of the skin to 43°C was conducted simultaneously with SNP infusion to ensure that maximal CVC had been obtained.

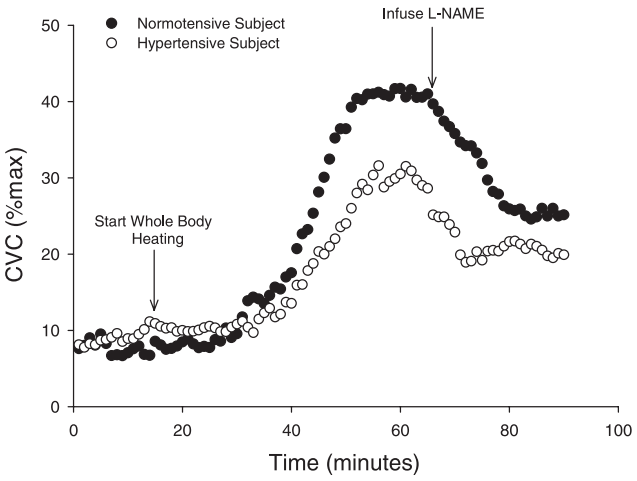

Fig. 1. Representative tracings of cutaneous vascular conductance (CVC) as a percentage of maximal vs. time from the control site in a normotensive (●) and a hypertensive (○) subject. Whole body heating started at *minute 10*, and N^G-nitro-L-arginine (L-NAME) was perfused through the microdialysis sites after oral temperature increased 0.8°C (*minute 65* in this example).

Data acquisition and analysis. Data were acquired at 40 Hz using Labview software and a National Instruments data-acquisition system (Austin, TX). Data were digitized, recorded, and stored on a personal computer for further analysis. CVC data were averaged over 3-min periods for baseline and every 0.1°C rise in T_{or} and are presented as a percentage of maximal CVC (%CVC_{max}). Δ%CVC_{max} in the control, Asc, and Asc+A-I sites is the difference between the plateau values in %CVC_{max} before and after NOS inhibition. Maximal CVC was calculated as the average of the stable 10-min plateau in laser-Doppler flux values divided by MAP during SNP and local heating.

Student's unpaired *t*-tests were used to determine significant differences between the groups for physical characteristics. Two-way repeated-measures ANOVA was conducted to detect differences from blood pressure and pharmacological treatment on maximal CVC and on the within-site Δ%CVC_{max}. A mixed model, three-way repeated-measures ANOVA was conducted to detect differences between subject groups at the pharmacological treatment sites over the rise in T_{or} (SAS, version 8.01). Tukey's post hoc tests were performed when appropriate to determine where differences between groups and drug treatments occurred. The level of significance was set at $\alpha = 0.05$. Values are presented as means ± SE.

RESULTS

Subject characteristics are presented in Table 1. The subjects were well matched for age, sex, body-mass index, total cholesterol, and high-density and low-density lipoproteins. The hypertensive subjects had significantly higher resting systolic and diastolic pressures and MAP (all $P < 0.001$).

Figure 1 illustrates the timeline of L-NAME administration with representative tracings in control microdialysis sites from a normotensive subject and a subject with essential hypertension.

Figure 2 shows the %CVC_{max} responses for all sites to the rise in body core temperature in both subject groups. %CVC_{max} in the control site was attenuated in the hypertensive subjects starting with change in T_{or} (ΔT_{or}) ≥0.6°C compared with the normotensive group ($P < 0.05$). NOS inhibition throughout whole body heating attenuated %CVC_{max} in both subject groups starting at ΔT_{or} ≥0.3°C in the hypertensive group and ΔT_{or} ≥0.2°C in the normotensive group (both $P < 0.05$). Asc and Asc+A-I significantly increased %CVC_{max} in the hypertensive group at ΔT_{or} ≥0.4°C ($P < 0.05$ vs. control) but did not alter %CVC_{max} in the normotensive group ($P > 0.05$ vs. control). There was not a significant difference between the Asc- and Asc+A-I-treated sites in either subject group ($P > 0.05$).

Table 1. *Subject characteristics*

Subject Characteristics	Normotensive	Hypertensive
Sex (no. of men, no. of women)	6, 3	6, 3
Age, yr	57±3	57±4
Body-mass index, kg/m^2	24.0±1.0	26.3±1.0
Total cholesterol, mg/dl	183±9	189±16
HDL, mg/dl	58±6	53±5
LDL, mg/dl	110±9	114±11
SBP, mmHg	116±3	159±3*
DBP, mmHg	76±1	94±1*
MAP, mmHg	81±10	112±1*

Values are means ± SE. HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure. *Significant difference vs. normotensive subject group ($P < 0.05$).

Figure 2 also illustrates the mean %CVC_{max} after NOS inhibition within the control, Asc, and Asc+A-I sites at ΔT_{or} = 0.8°C. NOS inhibition within the control site decreased %CVC_{max} in both subject groups ($P < 0.05$). Furthermore, there was not a significant difference between the groups in %CVC_{max} in the control site after NOS inhibition (%CVC_{max}: 29 ± 4 for hypertensive vs. 34 ± 4 for normotensive; $P > 0.05$). Asc and Asc+A-I increased %CVC_{max} after NOS inhibition in the hypertensive group (%CVC_{max}: 41 ± 4 Asc and 40 ± 4 for Asc+A-I; $P < 0.05$ vs. control) but not in the normotensive group (%CVC_{max}: 29 ± 3 for Asc, 30 ± 4 for Asc+A-I; $P > 0.05$ vs. control).

Figure 3 summarizes the %CVC_{max} responses in all of the treatment sites during the plateau in skin blood flow with a 0.8°C rise in body core temperature before and after within-site NOS inhibition. Compared with the normotensive group, vasodilation at the control site was attenuated in the hypertensive group ($P < 0.001$), and there was a blunted decrease with NOS inhibition (hypertensive: −8 ± 1 vs. normotensive: −14 ± 2 Δ%CVC_{max}; $P < 0.001$). Compared with their respective control sites, NOS inhibition in the Asc and combined Asc+A-I sites caused a greater Δ%CVC_{max} in the hypertensive group (Asc: −19 ± 4, Asc+A-I: −17 ± 4; $P < 0.05$ vs. control) than in the normotensive subject group (Asc: −11 ± 3, Asc+A-I: −15 ± 3; $P > 0.05$ vs. control) (Fig. 3, *inset*).

Maximal absolute CVC was attenuated in the hypertensive group compared with the normotensive group (hypertensive: 1.32 ± 0.11 vs. normotensive: 1.78 ± 0.20 flux/mmHg; $P = 0.001$). Within each subject group, there was no effect of pharmacological treatment on maximal absolute CVC ($P > 0.05$).

DISCUSSION

The primary findings of this study were that humans with essential hypertension have *1*) attenuated reflex cutaneous vasodilation that can be augmented with acute Asc supplementation alone or combined with arginase inhibition and *2*) the increase in cutaneous vasodilation with these treatments is mediated by both NO- and non-NO-dependent mechanisms (Figs. 2 and 3). These data suggest that increased ROS associated with hypertension directly decrease NO-dependent vasodilation. Contrary to our hypothesis, concurrent Asc supplementation and arginase inhibition did not result in a greater increase in vasodilation than Asc alone, suggesting that *1*) there is redundancy in the underlying cellular mechanisms that these treatments affect (ROS and uncoupled NOS) and/or *2*) that Asc supplementation alone maximized the capacity of the cutaneous vessels to vasodilate at this level of hyperthermia.

Our data confirm earlier reports (22) that reflex cutaneous vasodilation is attenuated in subjects with essential hypertension. In the present study, cutaneous vasodilation at the control site was only modestly reduced when expressed as a percentage of maximal vasodilation; however, absolute maximal CVC was significantly attenuated. We chose to represent the data as a %CVC_{max} to account for variability in capillary density under each laser-Doppler flowmeter, allowing for comparisons between microdialysis treatment sites (4). Others (6) have consistently observed a reduction in maximal CVC using both venous occlusion plethysmography to measure absolute and laser-Doppler flowmetry to measure relative changes in skin

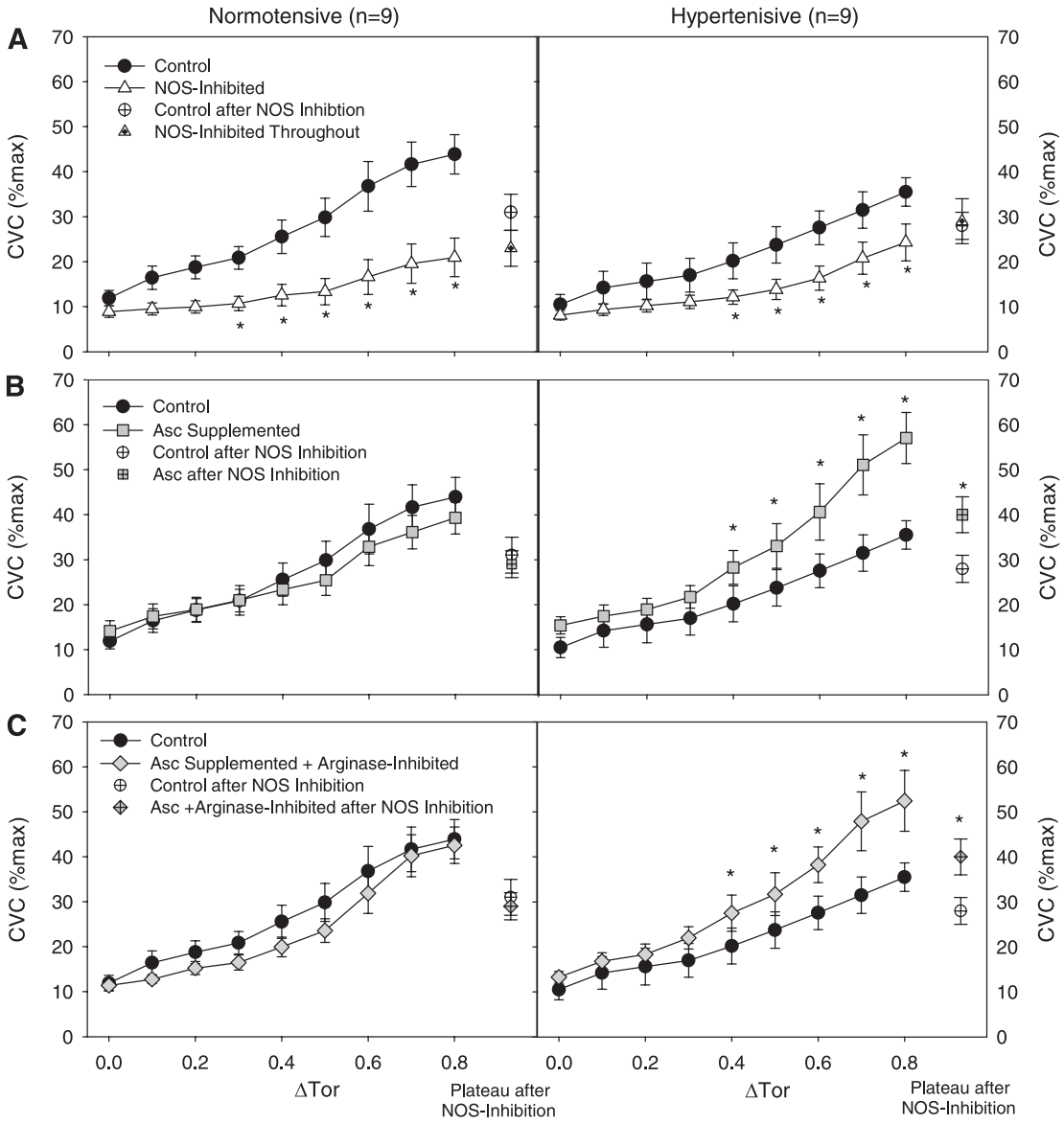

Fig. 2. Group means ± SE of CVC as a percentage of maximal response during passive whole body heating. Age-matched normotensive subject responses (*left*) and hypertensive subject responses (*right*) are shown. *A*: nitric oxide synthase (NOS)-inhibited site (○) throughout heating and during the plateau with change in oral temperature (ΔT_{or}) = 0.8°C. *B*: ascorbate (Asc)-supplemented supplemented site (gray squares) throughout heating and the mean for the stable plateau after NOS inhibition. *C*: combined Asc + arginase-inhibited site (gray diamonds) throughout heating and the mean for the stable plateau after NOS inhibition. The control site (●) results throughout heating and means for the stable plateau after NOS inhibition are also illustrated in *A–C* for comparison. Asc and Asc + arginase inhibition augmented cutaneous vasodilation during heating and after within-site NOS inhibition in the hypertensive subject group. *$P < 0.05$ significant difference vs. the control site within subject groups.

blood flow during whole arm local heating. Together, these data suggest that reflex cutaneous vasodilation is significantly attenuated with hypertension with the reduction in maximal CVC, suggesting structural maladaptations of the cutaneous vessels that limit absolute vasodilator capacity. However, we also observed a clear reduction in NO-dependent vasodilation and alterations in vascular signaling that contribute to attenuated reflex cutaneous vasodilation in humans with essential hypertension.

We found that localized Asc administration in humans with essential hypertension augmented reflex cutaneous vasodilation through NO- and non-NO-dependent mechanisms, independent of arginase inhibition. In sites treated with Asc, there was a greater decrease in skin blood flow ($\Delta\%CVC_{max}$) with NOS inhibition during established hyperthermia, and the resulting post-L-NAME plateau ($\%CVC_{max}$) was increased compared with control sites (Figs. 2 and 3), demonstrating an increase in NO- and non-NO-dependent cutaneous vasodilation, respectively.

A prooxidant vascular environment and resulting decreased NO bioavailability are implicated in the pathogenesis of hypertensive vascular disease (35). NO bioavailability is dictated

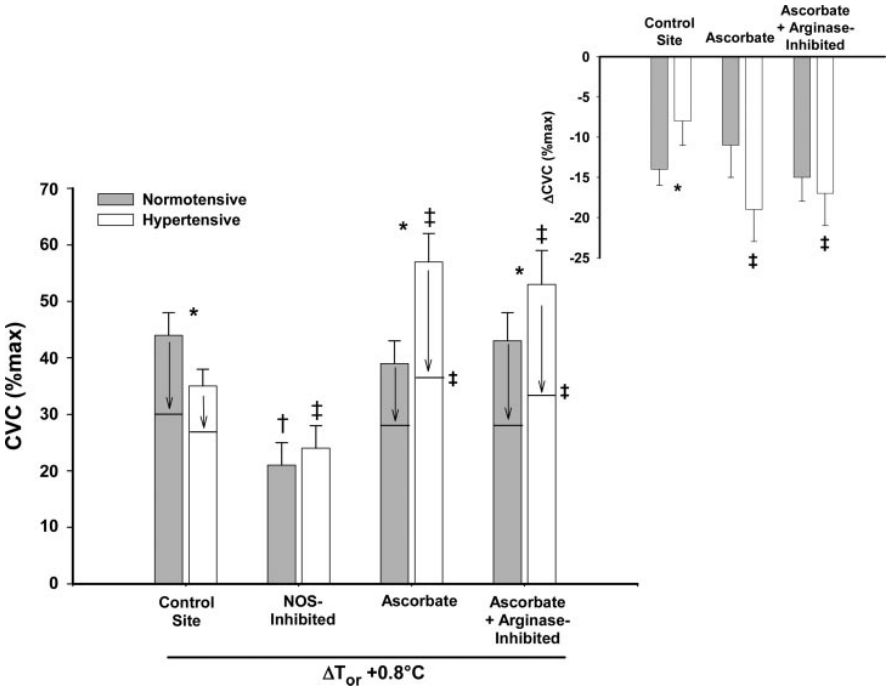

Fig. 3. Group means ± SE of CVC as a percent of maximal vasodilation with a 0.8°C ΔT_{or} in all drug treatment sites. Gray bars represent the age-matched normotensive subject group, and the open bars represent the hypertensive subject group. The decrease in CVC (ΔCVC) after NOS inhibition is illustrated with arrows and in the *inset*. Asc supplementation and combined Asc + arginase inhibition augmented CVC before NOS inhibition, the change in CVC due to NOS inhibition, and the CVC after NOS inhibition in hypertensive but not in normotensive subjects. *$P < 0.05$, significant difference between blood pressure groups; †$P < 0.001$, significant vs. control site age-matched normotensive subject group; ‡$P < 0.001$, significant vs. control site hypertensive subject group.

by the balance of NO and oxidant production and clearance. With essential hypertension oxidant production, particularly superoxide, in the vasculature increases predominantly through NAD(P)H oxidase, xanthine oxidase, and uncoupled eNOS (35). In this deleterious cycle, superoxide decreases functional NO by reacting to form peroxinitrite, which then oxidizes the critical NOS cofactor BH_4. BH_4 deficiency can then ultimately decrease functional NO production through uncoupled NOS (14, 23, 24, 33). In the present study, we chose to directly supplement antioxidants to the cutaneous vasculature to examine a functional link between hypertension-associated increased oxidant stress and reduced NO-dependent vasodilation. However, Asc is a nonspecific antioxidant and may have increased functional NO bioavailability through a variety of mechanisms, including *1*) direct scavenging of superoxide and *2*) stabilization of the essential NOS cofactor BH_4, enabling recoupling of NOS and resulting in functional NO production (15, 32). However, with the present data, we are unable to delineate which of these mechanisms led to the increase in NO-dependent vasodilation.

Other mechanisms, including inadequate L-arginine availability, can also lead to eNOS uncoupling (34). We have recently shown that acute inhibition of arginase augments vasodilation in hypertensive skin (16), suggesting that arginase activity is upregulated in hypertensive cutaneous vasculature. Together, these data suggest that hypertension-associated upregulated arginase activity may cause decreased NO production and may contribute to increased superoxide synthesis through uncoupled eNOS. We hypothesized that concurrent inhibition of arginase and Asc supplementation would result in a greater increase in NO-dependent vasodilation than Asc alone; however, our data indicate that the resulting cutaneous vasodilation from the combined treatments was not additive. One possible explanation for this finding is that there is redundancy in the underlying cellular mechanisms of action of these treatments, where both treatments likely facilitated recoupling of eNOS, thereby increasing functional NO synthesis and decreasing superoxide production. Alternatively, the individual treatments (Asc or Asc+A-I) may have maximized the capacity of the hypertensive cutaneous vessels to vasodilate reaching a "ceiling" effect with this given hyperthermic stimulus ($\Delta T_{or} = 0.8$°C). It is possible, but not likely, that a further increase in cutaneous vasodilation with the combined treatments could be unmasked with a more significant hyperthermic stimulus.

An unexpected finding from the present study was that Asc supplementation alone and combined with arginase inhibition increased the resulting $\%CVC_{max}$ after NOS inhibition in hypertensive skin (Fig. 3). These data suggest that Asc supplementation augmented cutaneous vasodilation in hypertensive vasculature by non-NO-dependent as well as NO-dependent mechanisms. The results may be due to *1*) hypertension-associated increased ROS augmenting vasoconstrictor tone or *2*) possible nonspecific effects of Asc on the putative neurotransmitter pathways and/or *3*) potential synergistic interactions between NO and these pathways involved in reflex cutaneous vasodilation.

In human cutaneous vascular smooth muscle cells, ROS act as signaling molecules to mediate cold-induced vasoconstriction through Rho kinase-dependent mechanisms (2). Because upregulated Rho kinase signaling is implicated in hypertensive vascular pathology (26), it is possible that ROS mediate enhanced vasoconstriction in the absence of a cold stimulus through Rho kinase-dependent mechanisms. Thus providing antioxidants would inhibit the ROS-mediated enhanced vasoconstriction unmasking augmented non-NO-dependent vasodilation.

Asc may also have mediated the increase in non-NO-dependent vasodilation through nonspecific effects on the putative neurotransmitter pathways implicated in reflex vasodilation. In vitro studies suggest that Asc enhances histamine 1 receptor sensitivity (7) and presynaptically increase VIP synthesis and release (5). Alternatively, increased NO itself may have augmented non-NO-dependent vasodilation through potential synergistic effects. In human skin, NO is capable of mediating cutaneous vasodilation synergistically with sympathetic cotransmitters, resulting in a combined vasodilation that is greater than the sum of the individual contributions (36). The synergistic effect may occur downstream through cross-talk between NO-mediated cGMP-dependent and sympathetic neurotransmitter(s)-mediated cAMP-dependent mechanisms (8, 9), or NO may prejunctionally enhance the release of sympathetic neurotransmitter(s) (12).

The precise effects of Asc on the mechanisms and potential interactions between NO and sympathetic neurotransmitter(s) involved in active cutaneous vasodilation remains elusive. However, our data suggest that augmenting NO bioavailability in a clinical population with compromised endothelial function can also increase non-NO-dependent vasodilation.

In conclusion, NO-dependent reflex cutaneous vasodilation is attenuated in humans with essential hypertension, and antioxidant supplementation alone or combined with arginase inhibition augments cutaneous vasodilation during hyperthermia through NO- and non-NO dependent mechanisms. These data suggest that oxidant stress is increased in hypertensive cutaneous vasculature, which decreases functional NO bioavailability. Finally, in subjects with hypertension, the resulting vasodilation with concurrent Asc supplementation and arginase inhibition was not greater than the vasodilation with Asc alone, suggesting redundancy in underlying cellular mechanisms through uncoupled eNOS and/or that Asc maximized the cutaneous vasodilatory capacity as this level of hyperthermia.

ACKNOWLEDGMENTS

We are grateful for the intellectual, technical, and data collection assistance of Caitlin Thompson-Torgerson, Jane Pierzga, James Lang, and David DeGroot.

GRANTS

This research was supported by National Institute on Aging Grant R01 AG-07004-17 (W. L. Kenney), American Heart Association predoctoral fellowship 0515392U (L. A. Holowatz), an American College of Sports Medicine Carl V. Gisolfi memorial student research grant (L. A. Holowatz), a Penn State Department of Kinesiology Dissertation Award (L. A. Holowatz), and National Center for Research Resources Grant M01 RR-10732 (General Clinical Research Center).

REFERENCES

1. **American Heart Association.** *Heart Disease and Stroke Statistics: 2006 Update*. Dallas, TX: American Heart Association, 2006.
2. **Bailey SR, Mitra S, Flavahan S, Flavahan NA.** Reactive oxygen species from smooth muscle mitochondria initiate cold-induced constriction of cutaneous arteries. *Am J Physiol Heart Circ Physiol* 289: H243–H250, 2005.
3. **Bennett LA, Johnson JM, Stephens DP, Saad AR, Kellogg DL Jr.** Evidence for a role for vasoactive intestinal peptide in active vasodilatation in the cutaneous vasculature of humans. *J Physiol* 552: 223–232, 2003.
4. **Braverman IM, Keh A, Goldminz D.** Correlation of laser Doppler wave patterns with underlying microvascular anatomy. *J Invest Dermatol* 95: 283–286, 1990.
5. **Brick PL, Howlett AC, Beinfeld MC.** Synthesis and release of vasoactive intestinal polypeptide (VIP) by mouse neuroblastoma cells: modulation by cyclic nucleotides and ascorbic acid. *Peptides* 6: 1075–1078, 1985.
6. **Carberry PA, Shepherd AM, Johnson JM.** Resting and maximal forearm skin blood flows are reduced in hypertension. *Hypertension* 20: 349–355, 1992.
7. **Dillon PF, Root-Bernstein RS, Lieder CM.** Ascorbate enhancement of H1 histamine receptor sensitivity coincides with ascorbate oxidation inhibition by histamine receptors. *Am J Physiol Cell Physiol* 291: C977–C984, 2006.
8. **Farrell DM, Bishop VS.** Permissive role for nitric oxide in active thermoregulatory vasodilation in rabbit ear. *Am J Physiol Heart Circ Physiol* 269: H1613–H1618, 1995.
9. **Farrell DM, Bishop VS.** The roles of cGMP and cAMP in active thermoregulatory vasodilation. *Am J Physiol Regul Integr Comp Physiol* 272: R975–R981, 1997.
10. **Forstermann U, Munzel T.** Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 113: 1708–1714, 2006.
11. **Grant R, Holling H.** Further observations on the vascular responses of the human limb to body warming: evidence for sympathetic vasodilator nerves in the normal subject. *Clin Sci (Lond)* 3: 273–285, 1938.
12. **Grider JR, Murthy KS, Jin JG, Makhlouf GM.** Stimulation of nitric oxide from muscle cells by VIP: prejunctional enhancement of VIP release. *Am J Physiol Gastrointest Liver Physiol* 262: G774–G778, 1992.
13. **Hecker M, Nematollahi H, Hey C, Busse R, Racke K.** Inhibition of arginase by N^G-hydroxy-L-arginine in alveolar macrophages: implications for the utilization of L-arginine for nitric oxide synthesis. *FEBS Lett* 359: 251–254, 1995.
14. **Heinzel B, John M, Klatt P, Bohme E, Mayer B.** Ca^{2+}/calmodulin-dependent formation of hydrogen peroxide by brain nitric oxide synthase. *Biochem J* 281: 627–630, 1992.
15. **Heller R, Unbehaun A, Schellenberg B, Mayer B, Werner-Felmayer G, Werner ER.** L-ascorbic acid potentiates endothelial nitric oxide synthesis via a chemical stabilization of tetrahydrobiopterin. *J Biol Chem* 276: 40–47, 2001.
16. **Holowatz LA, Kenney WL.** Upregulation of arginase activity contributes to attenuated reflex cutaneous vasodilatation in hypertensive humans. *J Physiol* 581:863–72, 2007.
17. **Holowatz LA, Thompson CS, Kenney WL.** Acute ascorbate supplementation alone or combined with arginase inhibition augments reflex cutaneous vasodilation in aged human skin. *Am J Physiol Heart Circ Physiol* 291: H2965–H2970, 2006.
18. **Holowatz LA, Thompson CS, Kenney WL.** L-Arginine supplementation or arginase inhibition augments reflex cutaneous vasodilatation in aged human skin. *J Physiol* 574: 573–581, 2006.
19. **Holowatz LA, Thompson CS, Minson CT, Kenney WL.** Mechanisms of acetylcholine-mediated vasodilatation in young and aged human skin. *J Physiol* 563: 965–973, 2005.
20. **Kellogg DL Jr, Crandall CG, Liu Y, Charkoudian N, Johnson JM.** Nitric oxide and cutaneous active vasodilation during heat stress in humans. *J Appl Physiol* 85: 824–829, 1998.
21. **Kellogg DL Jr, Pergola PE, Piest KL, Kosiba WA, Crandall CG, Grossmann M, Johnson JM.** Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res* 77: 1222–1228, 1995.
22. **Kenney WL, Kamon E, Buskirk ER.** Effect of mild essential hypertension on control of forearm blood flow during exercise in the heat. *J Appl Physiol* 56: 930–935, 1984.
23. **Kuzkaya N, Weissmann N, Harrison DG, Dikalov S.** Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase. *J Biol Chem* 278: 22546–22554, 2003.
24. **Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG.** Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest* 111: 1201–1209, 2003.
25. **Lassegue B, Griendling KK.** Reactive oxygen species in hypertension: an update. *Am J Hypertens* 17: 852–860, 2004.
26. **Masumoto A, Hirooka Y, Shimokawa H, Hironaga K, Setoguchi S, Takeshita A.** Possible involvement of Rho-kinase in the pathogenesis of hypertension in humans. *Hypertension* 38: 1307–1310, 2001.
27. **McCord GR, Cracowski JL, Minson CT.** Prostanoids contribute to cutaneous active vasodilation in humans. *Am J Physiol Regul Integr Comp Physiol* 291: R596–R602, 2006.
28. **Munzel T, Daiber A, Ullrich V, Mulsch A.** Vascular consequences of endothelial nitric oxide synthase uncoupling for the activity and expres-

sion of the soluble guanylyl cyclase and the cGMP-dependent protein kinase. *Arterioscler Thromb Vasc Biol* 25: 1551–1557, 2005.
29. **Roddie IC, Shepherd JT, Whelan RF.** The contribution of constrictor and dilator nerves to the skin vasodilatation during body heating. *J Physiol* 136: 489–497, 1957.
30. **Shastry S, Dietz NM, Halliwill JR, Reed AS, Joyner MJ.** Effects of nitric oxide synthase inhibition on cutaneous vasodilation during body heating in humans. *J Appl Physiol* 85: 830–834, 1998.
31. **Taddei S, Virdis A, Ghiadoni L, Magagna A, Salvetti A.** Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. *Circulation* 97: 2222–2229, 1998.
32. **Taddei S, Virdis A, Ghiadoni L, Salvetti G, Bernini G, Magagna A, Salvetti A.** Age-related reduction of NO availability and oxidative stress in humans. *Hypertension* 38: 274–279, 2001.
33. **Toth M, Kukor Z, Valent S.** Chemical stabilization of tetrahydrobiopterin by L-ascorbic acid: contribution to placental endothelial nitric oxide synthase activity. *Mol Hum Reprod* 8: 271–280, 2002.
34. **Vasquez-Vivar J, Kalyanaraman B, Martasek P, Hogg N, Masters BS, Karoui H, Tordo P, Pritchard KA Jr.** Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. *Proc Natl Acad Sci USA* 95: 9220–9225, 1998.
35. **Warnholtz A, Wendt M, August M, Munzel T.** Clinical aspects of reactive oxygen and nitrogen species. *Biochem Soc Symp* 71: 121–133, 2004.
36. **Wilkins BW, Holowatz LA, Wong BJ, Minson CT.** Nitric oxide is not permissive for cutaneous active vasodilatation in humans. *J Physiol* 548: 963–969, 2003.
37. **Wong BJ, Minson CT.** Neurokinin-1 receptor desensitisation attenuates cutaneous active vasodilatation in humans. *J Physiol* 577: 1043–1051, 2006.
38. **Wong BJ, Wilkins BW, Minson CT.** H1 but not H2 histamine receptor activation contributes to the rise in skin blood flow during whole body heating in humans. *J Physiol* 560: 941–948, 2004.