Oral vitamin C enhances the adrenergic vasoconstrictor response to local cooling in human skin

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Yamazaki F. Oral vitamin C enhances the adrenergic vasoconstrictor response to local cooling in human skin. J Appl Physiol 112: 1689–1697, 2012. First published March 1, 2012; doi:10.1152/japplphysiol.00043.2012.—Local administration of ascorbic acid (Asc) at a supraphysiological concentration inhibits the cutaneous vasoconstrictor response to local cooling (LC), However, whether orally ingesting Asc inhibits the LC-induced vasoconstrictor response remains unknown. The purpose of the present study was to examine the acute influence of oral Asc on the adrenergic vasoconstrictor response to LC in human skin. In experiment 1, skin blood flow (SkBF) was measured by laser-Doppler flowmetry at three sites (forearm, calf, palm). The three skin sites were locally cooled from 34 to 24°C at −1°C/min and maintained at 24°C for 20 min before (Pre) and 1.5 h after (Post) oral Asc (2-g single dose) or placebo supplementation. Cutaneous vascular conductance (CVC) was calculated as the ratio of SkBF to blood pressure and expressed relative to the baseline value before LC. Oral Asc enhanced (P < 0.05) the reductions in CVC in the forearm (Pre, −50.3 ± 3.3%; Post, −57.8 ± 2.2%), calf (Pre, −52.6 ± 3.7%; Post, −66.1 ± 4.3%), and palm (Pre, −46.2 ± 6.2%; Post, −60.4 ± 5.6%) during LC. The placebo did not change the responses at any site. In experiment 2, to examine whether the increased vasoconstrictor response caused by oral Asc is due to the adrenergic system, the release of neurotransmitters from adrenergic nerves in forearm skin was blocked locally by iontophoresis of bretylium tosylate (BT). Oral Asc enhanced (P < 0.05) the reductions in CVC at untreated control sites but did not change the responses at BT-treated sites during LC. In experiment 3, to further examine whether adrenergically mediated vasoconstriction is enhanced by oral Asc, 0.1 mM tyramine was administered using intradermal microdialysis in the forearm skin at 34°C in the Pre and Post periods. Oral Asc increased (P < 0.05) the tyramine-induced reduction in CVC. These findings suggest that oral Asc acutely enhances the cutaneous vasoconstrictor responses to LC through the modification of adrenergic sympathetic mechanisms.

ASCORBIC ACID (Asc), a water-soluble vitamin, is essential for the synthesis of collagen, carnitine, and neurotransmitters (36, 38, 43). Humans have to take in Asc from fruits and vegetables or in tablet form because they cannot produce it themselves. The Asc present in foods is absorbed by Na+-dependent Asc transporters in the intestine (54) and influences physiological functions including peripheral vasomotion. At normal temperatures, orally or locally supplemented Asc has been shown to acutely improve endothelium-dependent vasodilator function in healthy smokers (42, 46), healthy postmenopausal women (41), and healthy older adults (8), as well as in patients with coronary heart disease (35).

Recent studies have found roles for Asc in thermoregulatory skin vasomotor control during heat and cold stress. Holowatz et al. (22, 23) showed during heat stress that local supplementation with Asc augments reflex cutaneous vasodilation in older or hypertensive subjects. With regard to the influence of Asc on skin vasomotor control during cold stress, it has been reported that local administration of Asc decreased the cutaneous vasoconstrictor response during local cooling (LC) (55). The inhibitory effect of Asc disappeared when LC was applied to skin with adrenergic receptor blockage (55). Cooling caused redistribution of β2-adrenergic receptors to the cell surface, mediated by cold-induced activation of Rho kinase (2, 25). Cold-induced constriction is initiated by mitochondrial generation of reactive oxygen species (ROS), which stimulate Rho kinase signaling in smooth muscle cells (3). Therefore, it was suggested that increased quenching of ROS by local administration of Asc decreased the efficacy of adrenergic receptors, decreasing the cutaneous vasoconstrictor response during LC (55). In addition, the results from that study (55) suggested that Asc supplementation does not alter the vasoconstrictor response during increased sympathetic adrenergic activity caused by whole body cooling. The unaltered vasoconstrictor response during whole body cooling implied that adrenergic pathways were functional despite an expected inhibition of postsynaptic adrenoceptor function by Asc supplementation during LC.

In the previous studies (22, 23, 55), Asc was given by intradermal microdialysis in a supraphysiological concentration for elucidating the contribution of oxidant stress mechanisms in skin vasomotor control. However, it is still unclear whether orally ingested Asc inhibits LC-induced vasoconstrictor response in healthy human. It is possible that differences in the Asc concentration in extracellular space result in different vasomotor responses to thermal stress. In interstitial space, Asc in pharmacological concentrations generates Asc radical and H2O2, which can act as an endothelium-derived hyperpolarizing factor (EDHF) in animals and humans (14, 48). In intravascular space, it has been proposed that Asc radicals are reduced by the erythrocyte plasma membrane redox system, and any formed H2O2 is immediately destroyed by plasma catalase and erythrocyte glutathione peroxidase (7, 40). When plasma Asc is changed within the physiological range by oral ingestion, it is speculated that cutaneous vasomotor reactivity is minimally influenced for the limited bioavailability of Asc and the limited production of H2O2.

In this study, to further understand the acute influence of Asc on cutaneous vasoconstrictor control during LC, we examined the vasoconstrictor responses to LC before and 1.5 h after the oral ingestion of Asc. On the basis of previous findings, we
hypothesized that oral Asc minimally affects the LC-induced vasoconstrictor response because of the low effectiveness for altering adrenoceptor function.

METHODS

Subjects

Twelve female and 10 male volunteers participated in the experiments. Their mean (±SE) age was 22 ± 1 yr, weight was 55 ± 2 kg, and height was 163 ± 2 cm. All subjects were healthy nonsmokers with no history of cardiovascular disease and with no use of vitamin supplements in daily life. The menstrual status of the female subjects was recorded, but their responses did not differ perceptively from those of the male subjects, and their results were combined for analysis. Written, informed consent was obtained after a thorough explanation of the present study, including its purpose and risks. The experiments were approved by the Ethics Committee of Medical Care and Research of the University of Occupational and Environmental Health.

Measurements

Skin blood flow (SkBF) was monitored continuously with laser-Doppler flow meters (ALF21, Advance, Tokyo, Japan). Mean arterial pressure (MAP) was measured by an automated oscillometric blood pressure device (BP-203i, Colin, Komaki, Japan) every 5 min. Cuta-neous vascular conductance (CVC) was calculated from the ratio of SkBF to MAP. Sublingual temperature (Tsl) was measured with a polyethylene-sealed thermocouple placed in the sublingual sulcus. Whole body skin temperature (Tsk) was recorded from six thermocouples placed on the body surface (52). Heart rate (HR) was measured from electrocardiographs. The measured variables were recorded by a data logger (DE1200 Universal, NEC Sanei, Tokyo, Japan).

Local Skin Cooling Procedures

Local temperature (Tloc) of the 6.3-cm² area surrounding the site of SkBF measurement was controlled by a custom-built metal sleeve for the flow probe that had a Peltier element. A thermocouple between the skin and the sleeve served for measurement and feedback control. Tloc can be precisely maintained within 0.1°C with this controller. Tloc at all SkBF measurement sites was maintained at 34°C for baseline measurements before LC. For LC, Tloc was decreased to 24°C at a rate of −1.0°C/min, maintained at 24°C for 10 or 20 min, and then returned to 34°C. The cooling rate was selected to activate adrenergic mechanisms with decreasing vasodilator action that occurred through tonic sympathetic adrenergic nerve endings, was applied iontophoretically to two areas (each 0.64 cm²) of skin on the forearm (26, 28). The iontophoresis was applied at 400 μA/cm² for 10 min. This method produces a selective local blockade of the cutaneous adrenergic vasoconstrictor system (28). Forearm SkBF was monitored at an untreated site and the two BT-treated sites. Subjects wore a tube-lined, water-perfused suit, and whole body Tsk was controlled by changing the water temperature of the suit. Approximately 1.5 h after the application of BT, data collection began with control period at Tloc of 34°C for 10–15 min, followed by the LC protocol. In this experiment, the Tloc at all SkBF measurement sites was maintained at 24°C for 10 min. Subjects then swallowed Asc tablets in the same way as experiment 1 and rested supine for 60 min. Again, the LC protocol was performed the same as pre-Asc ingestion. Subsequently, whole body cooling (3-min duration, aggressive cooling) was induced by perfusing the suit with cold water to lower whole body Tsk quickly from 35–36°C to 33–34°C to verify an adequate blockade of the vasoconstrictor nerves (28). The use of the suit allows sympathetic vasoconstrictor nerves to be activated without cooling of the sites where SkBF was measured. Because BT acts presynaptically to block neurotransmitter release, the period of cooling was kept brief to avoid α-adrenergic receptor activation by circulating catecholamines (28).

Experimental Protocols

The experiments consisted of three different protocols. For each protocol, the subjects arrived in the laboratory at 0900 after having abstained from caffeine and alcohol for at least 1 day and from food for at least 2 h. All experiments were conducted in an environmental chamber maintained at an ambient temperature of 27°C and 50% humidity. After the subject entered the chamber, a cuff for measuring arterial pressure, electrodes, thermocouple, and sensor probes were applied while he/she rested supine on a bed. To decrease tonic vasoconstrictor activity during the baseline control and therefore to clearly observe cutaneous vasoconstriction in response to LC, whole body Tsk except for the head and arms was kept at 35–36°C using a water-perfused suit or blanket.

Experiment 1. Four female and three male subjects participated. SkBF was measured on the forearm, calf, and palm. Data collection began with a 5-min control period, followed by the LC protocol described above. Tloc at all SkBF measurement sites was maintained at 24°C for 20 min. Following this, subjects were given tablets containing 2 g of Asc or glucose-containing placebo tablets with 100 ml of distilled water. Asc concentrations in human blood plasma were 27–89 μM (24, 34), and a single 2-g dose of Asc produces a 2.5-fold increase in the plasma Asc concentration after 1–5 h (35, 45). The 2-g dose was selected because it can produce a significant increase in plasma Asc levels within the physiological range. After the subjects rested supine for 60 min, data was again collected with another 5-min control period. The LC protocol was performed in the same way as for pre-ingestion. The order of experiments was randomized at intervals of >7 days.

Experiment 2. Contrary to our hypothesis, LC-induced change in CVC was increased by the oral ingestion of Asc in experiment 1. Therefore, we conducted experiment 2 to test whether the Asc-induced change in CVC was due to an altered adrenergic vasoconstrictor function. Four female and four male subjects participated. Bretlytim tosylate (BT; Sigma, St. Louis, MO), which blocks the release of neurotransmitters including norepinephrine (NE) from sympathetic adrenergic nerve endings, was applied iontophoretically to two areas (each 0.64 cm²) of skin on the forearm (26, 28). The iontophoresis was applied at 400 μA/cm² for 10 min. This method produces a selective local blockade of the cutaneous adrenergic vasoconstrictor system (28). Forearm SkBF was monitored at an untreated site and the two BT-treated sites. Subjects wore a tube-lined, water-perfused suit, and whole body Tsk was controlled by changing the water temperature of the suit. Approximately 1.5 h after the application of BT, data collection began with control period at Tloc of 34°C for 10–15 min, followed by the LC protocol. In this experiment, the Tloc at all SkBF measurement sites was maintained at 24°C for 10 min. Subjects then swallowed Asc tablets in the same way as experiment 1 and rested supine for 60 min. Again, the LC protocol was performed the same as pre-Asc ingestion. Subsequently, whole body cooling (3-min duration, aggressive cooling) was induced by perfusing the cloth with cool water to lower whole body Tsk quickly from 35–36°C to 33–34°C to verify an adequate blockade of the vasoconstrictor nerves (28). The use of the suit allows sympathetic vasoconstrictor nerves to be activated without cooling of the sites where SkBF was measured. Because BT acts presynaptically to block neurotransmitter release, the period of cooling was kept brief to avoid α-adrenergic receptor activation by circulating catecholamines (28).

Experiment 3. LC-induced reduction in CVC at BT-treated sites remained unchanged by the oral ingestion of Asc in experiment 2. Given the findings from experiment 2, a follow-up experiment was conducted to determine the effect of oral Asc on adrenergic vasoconstrictor function at normal Tsk. Four female and three male subjects participated. Each subject had two microdialysis probes placed intra-dermally on the ventral aspect of the forearm. These probes consisted of 1 cm of microdialysis tubing (regenerated cellulose, inner diameter of 200 μm, 18-kDa nominal molecular mass cutoff) attached at each end to polyimide tubing. Before implantation, the area of skin was temporarily anesthetized by the application of a cold pack for 5 min. Needles (25 gauge) were inserted intradermally into the arm −2.5 cm. The probes were then fed through the lumen of the needle. The probes were aligned such that the microdialysis membranes were centered within the dermis. The needles were then removed, leaving the probes in place. To allow for the effects of the insertion trauma, subjects rested supine, except if they needed to urinate, for −1.5 h before the experiment began.

The experiment began with two sites being perfused at 4 μl/min with saline for 20–30 min. After SkBF stabilized, 0.1 mM tyramine was infused at one site for 20 min at 4 μl/min to release neurotransmitters from sympathetic adrenergic nerve endings. Following this, site 1 was perfused with saline again. Subjects then swallowed Asc tablets the same as in experiment 1 and rested supine for 70–80 min. Tyramine was then infused at both sites for 20 min. Tloc at the two SkBF measurement sites was maintained at 34°C throughout the experiment.
Data processing and statistical analysis

The measured variables were sampled every 1 s and averaged over 5-min intervals. The data for the baseline control were averaged over the 5-min period before the application of LC or tyramine. CVC measurements from the two BT sites were averaged because there was no difference in the responses between sites. The changes in CVC were expressed as percent changes from the baseline and used to measure the sensitivity of vasoconstriction. CVC was also expressed as absolute values (arbitrary units) to determine changes in the baseline with Asc. To eliminate possible differences between sites in each region and between days in SkBF changes during LC, changes in CVC at a given skin site were compared before and after ingestion of Asc. Effects of Asc and cooling or tyramine on changes in CVC were evaluated using a two-way, repeated-measures ANOVA. When significant F ratios were defined, Fisher’s protected least significant difference between the means was calculated. Effects of oral Asc on absolute CVC values during the baseline control were evaluated using the Student’s paired t-test. Statistical differences in absolute CVC values at the baseline level between sites were evaluated using the Student’s unpaired t-test. P < 0.05 was considered significant. All data are expressed as means ± SE.

RESULTS

In experiment 1, oral Asc tended to increase the baseline CVC presented in absolute terms at nonglabrous sites (i.e., forearm and calf), the increase in the calf being statistically significant (Table 1). However, it did not alter the baseline CVC at glabrous sites (i.e., palm) (Table 1). By contrast, oral ingestion of the placebo did not change the absolute CVC baseline at nonglabrous sites, whereas it decreased (P < 0.05) the baseline CVC at palm sites (Table 1). Figure 1 shows changes in CVC in response to LC in the Asc-treated and placebo-administered subjects in experiment 1. LC decreased (P < 0.0001) CVC at all three sites (Fig. 1, A–C), the changes being greater (P < 0.0001) post-Asc ingestion (forearm, −57.8 ± 2.2%; calf, −66.1 ± 4.3%; palm, −60.4 ± 5.6%) than pre-Asc ingestion (forearm, −50.3 ± 3.5%; calf, −52.6 ± 3.7%; palm, −46.2 ± 6.2%). Greater reductions in the ingestion of Asc occurred in the early phase (<10 min) of cooling. The patterns of change in CVC at any sites in the later phase (10–30 min) of cooling post-Asc ingestion were analogous (P > 0.49) to those pre-Asc ingestion. The placebo did not alter LC-induced CVC reductions at any site (Fig. 1, D–F).

In experiment 2, as shown in Fig. 2, LC decreased the SkBF at BT-treated and untreated sites pre- and post-Asc ingestion. In both periods, local warming from 24 to 34°C after LC completely recovered SkBF at untreated sites but not at BT-treated sites to precooling levels. Precooling baseline CVC levels at untreated sites did not differ (P = 0.17) between pre-Asc ingestion (0.049 ± 0.008 AU) and post-Asc ingestion (0.058 ± 0.011 AU), whereas, at BT-treated sites, precooling baseline levels in absolute terms were lower (P < 0.01) post-Asc ingestion (0.042 ± 0.008 AU) than pre-Asc ingestion (0.056 ± 0.011 AU). Figure 3 shows changes in forearm CVC in response to LC in experiment 2. At untreated sites, the LC-induced changes in CVC before and after oral Asc were similar to those for the forearm in experiment 1 (Fig. 3A). LC decreased (P < 0.001) CVC at BT-treated sites, although BT treatment diminished the rapid reduction of CVC in the early phase of cooling (Fig. 3B). The LC-induced changes in CVC at BT-treated sites did not differ between the pre- and post-Asc ingestion periods.

Whole body cooling decreased (P < 0.01) CVC at untreated sites (−42 ± 5%). BT treatment completely negated (P = 0.71) the cold stress-induced decrease of CVC (−2 ± 4%), suggesting that the functional adrenergic blockade in the skin was effective throughout the experiments.

Table 1. Cutaneous vascular conductance levels during precooling baseline before and after the oral ingestion of Asc or placebo in experiment 1

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Forearm</th>
<th>Calf</th>
<th>Palm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Asc</td>
<td>0.055 ± 0.009</td>
<td>0.047 ± 0.013</td>
<td>0.271 ± 0.060</td>
</tr>
<tr>
<td>Post-Asc</td>
<td>0.062 ± 0.010</td>
<td>0.071 ± 0.014</td>
<td>0.226 ± 0.042</td>
</tr>
<tr>
<td>Preplacebo</td>
<td>0.060 ± 0.010</td>
<td>0.042 ± 0.011</td>
<td>0.197 ± 0.026</td>
</tr>
<tr>
<td>Postplacebo</td>
<td>0.056 ± 0.009</td>
<td>0.030 ± 0.004</td>
<td>0.153 ± 0.017</td>
</tr>
</tbody>
</table>

Values are means ± SE. Cutaneous vascular conductance was expressed as absolute values (arbitrary units). Asc, ascorbic acid. †Significant difference vs. pre-ingestion (P < 0.05).

DISCUSSION

There were three major findings from the present study. First, oral Asc enhanced the cutaneous vasoconstrictor response at glabrous and nonglabrous sites during LC (experiment 1). Second, blockade of the release of neurotransmitters from adrenergic nerve endings diminished the effect of oral Asc on the cutaneous vasoconstrictor response to LC (experiment 2). Finally, oral Asc augmented the vasoconstrictor re-
sponse to the presynaptic release of noradrenergic neurotransmitters in the skin (experiment 3). These findings suggest that the facilitating effect of Asc on vasoconstrictor sensitivity to direct skin cooling was mainly mediated through the modification of adrenergic mechanisms.

During exposure to a cold environment, skin vessels constrict via reflex and local mechanisms for preventing hypothermia. In nonglabrous skin, the reflex vasoconstriction that occurs during whole body cooling is known to occur with the release of NE and cotransmitters including neuropeptide Y from sympathetic adrenergic nerve terminals (50, 51). Conversely, localized cooling of the cutaneous blood vessels evokes vasoconstriction through both adrenergic and nonadrenergic mechanisms. In the present study, the facilitating effects of Asc on cutaneous vasoconstrictor responses were observed in the early phase of cooling (10 min). In the early phase of LC, the immediate vasoconstrictor response to cooling is attributable to adrenergic mechanisms (27, 44, 58). Cooling enhances vasoconstrictor response throughout the upregulation of postsynaptic mechanisms more than it compensates for cooling-induced depression in the formation and release of NE (4). That is, the direct cooling in the skin vessels induces an augmentation of α2-adrenoceptor reactivity (11–13), with a translocation of α2c-adrenoceptors from the Golgi apparatus to the vascular smooth muscle cell surface by Rho kinase (2, 25). Importantly, cold-induced constriction is initiated by mitochondrial generation of ROS, which stimulate Rho kinase signaling in smooth muscle cells (3). Involvement in the Rho kinase signaling system in the early phase cold-induced vasoconstriction also has been shown in a human study (53). The activation of Rho kinase promotes calcium sensitization of

![Fig. 1. Effects of oral ascorbate (Asc) (A–C) and placebo (D–F) on changes in cutaneous vascular conductance (CVC) in the forearm, calf, and palm during local cooling in experiment 1. ○, Data pre-ingestion; ●, data post-ingestion. *Significant difference vs. precooling baseline (P < 0.05). †Significant difference vs. pre-Asc ingestion (P < 0.05).]
the vascular smooth muscle by inactivating the myosin light chain phosphatase (49).

As direct cooling proceeds, nonadrenergic vasoconstrictor mechanisms prevail in the local component of skin cooling (27, 44, 58). As the primary nonadrenergic mechanism, longer term cooling influences the NO system (20, 58). It has been reported that the activation of Rho kinase was also involved in the nonadrenergic vasoconstriction in the later phase of cooling (53). The findings from a prior study (55), however, suggested that Asc has no effect in the role of the NO system in cutaneous vasoconstriction during LC. In the later phase of cooling (>10 min), the patterns of change in CVC after Asc ingestion were similar to those before Asc ingestion (Fig. 1). Additionally, the later vasoconstriction at BT-treated sites during 15–20 min of cooling did not differ before and after Asc ingestion (Fig. 3). These results suggest a minor role for the NO system in the enhancing action of Asc on the vasoconstrictor response.

In other words, the changes in CVC at BT-treated sites did not differ after Asc ingestion in experiment 2, suggesting that the enhancing effect of oral Asc on LC-induced vasoconstrictor responses was attributed to noradrenergic sympathetic mechanisms. Regarding the presynaptic modulation in the noradrenergic sympathetic pathway, Asc might influence the synaptic vesicle endocytosis and exocytosis of neurotransmitters. It has been indicated that Asc augments NE biosynthesis within 30 min in adrenal chromaffin cells (37). Such a facilitating action of Asc might counterbalance the inhibitory influence of cooling in NE synthesis, whereas in the catecholamine-secreting cell line PC12 cells, 0.2 mM Asc fully inhibited the enhancement of catecholamine secretion caused by hypoxia (15). Since Asc enhances the biological actions of catecholamines by preventing their oxidation (29, 39), the increased vasoconstrictor response to LC after Asc ingestion might be due to increased vasoconstrictor sensitivity to extant NE rather than a net increase in NE release.

The prior observations regarding postsynaptic modulation due to Asc are inconsistent, perhaps due to differences in the experimental methods used including Asc concentrations and tissue temperatures. Asc at >15 μM, including the entire physiological range of 40–100 μM, enhances exogenous NE-induced contractions of rabbit aortic smooth muscle independent of the oxidation of NE at 37°C (10), suggesting an increased sensitivity of existing adrenoceptors in the smooth muscle cell membrane. In human skin in vivo, local administration of Asc at a higher concentration (10 mM) in the interstitium using intradermal microdialysis inhibited the vasoconstrictor response to LC through adrenoceptor function. It
was speculated that the antioxidative effect of Asc decreased cooling-induced production of ROS and thereby inhibited ROS-mediated translocation in α2c-adrenergic receptors in vascular smooth muscle cells. Since the Asc concentration used in prior study was much higher (~50 times or more) than those in plasma after the oral ingestion of 2 g of Asc (35, 45, 46), oral supplementation might be unsuitable for increased quenching of ROS in smooth muscle cells because of its limited bioavailability. Furthermore, local administration of Asc in supraphysiological concentrations generates H2O2, which can act as an EDHF (14, 48), but the production of H2O2 at vasoactive levels would not happen with oral Asc supplementation. Thus Asc at a physiological concentration acutely enhances the cooling-induced vasoconstrictor response by preventing the oxidation of neurotransmitters in combination with the putative offsetting effect on inhibition of NE release during cooling without significant inhibition of α2c-adrenergic receptor sensitivity.

Cold-sensitive afferent in skin plays a role in initial vasoconstrictor response to LC (19, 27). Hodges et al. (19) reported that topical anesthesia blocking sensory nerve function reversed the initial vasoconstrictor response during LC to one of vasodilation regardless of adrenergically intact skin. If direct skin cooling stimulates the release of neurotransmitters from adrenergic nerve terminals by an unknown mechanism linking sensory nerves, supplemental Asc might enhance release of adrenergic neurotransmitters by the putative influence on activation in cold-sensitive, Ca2+-permeable channels (i.e., TRPM8) in sensory nerve endings, because Ca2+ channels are typical targets of ROS (5). This speculation partly explains why localized (by intradermal microdialysis) and systemic administrations of Asc induced different influences on cutaneous vasoconstrictor responses during LC, but the acute effect of Asc on cold-sensitive afferent function remains unknown.

In experiment 3, to examine the effect of oral Asc on adrenergic vasoconstrictor response at normal temperatures without increasing adrenoceptor sensitivity by direct cooling of the skin, tyramine was locally administered at the SkBF measurement sites on the forearm using intradermal microdialysis at a Tsk of 34°C. It is important to note that α1- and α2-adrenergic receptors in the human cutaneous vessels are functional at normal Tsk (59). The tyramine-induced reduction in CVC was greater post-Asc ingestion than pre-Asc ingestion, suggesting that oral Asc could acutely enhance the vasoconstrictor response to NE and the cotransmitters in nonglabrous skin. The results from prior and present studies support the finding from experiment 2 of an enhancing effect of oral Asc on the adrenergic vasoconstrictor response. The putative facilitating action of Asc on NE biosynthesis might not contribute to the altered adrenergic vasoconstrictor response. Lang et al. (32, 33) recently reported in normal Tsk condition that local supplementation of tyrosine or tetrahydrobiopterin (BH4), the essential cofactor for tyrosine hydroxylase, enhanced tyramine-induced cutaneous vasoconstriction in older subjects but not in young subjects. These findings suggest that the supplemenations of the precursors of catecholamines or reducing agents including Asc do not significantly augment NE biosynthesis in cooling, aging, or disease related-depression-free conditions. The actions of Asc on the bioavailability of adrenergic neurotransmitters and the upregulation of native α1- and α2-adrenergic receptor-mediated mechanisms may explain the prior finding that local administration of Asc did not alter the vasoconstrictor response to whole body cooling.
Despite an expected inhibition of postsynaptic $\alpha_2c$-adrenoceptor function (55).

Oral Asc could substantially increase the absolute precooling baseline levels of CVC at glabrous and nonglabrous sites. This is based on the following findings from experiment 1: 1) ingestion of the placebo did not change baseline CVC in the forearm and calf, whereas ingestion of Asc significantly increased the baseline CVC in the calf, and 2) the baseline CVC in the palm did not change after the ingestion of Asc but decreased after ingestion of the placebo, that is, oral Asc had a net vasodilator effect on the palm. For the interpretation of these findings, circadian change in sympathetic skin vasoconstrictor function must at least be considered. During the time period (0900–1300) that experiments were performed, it has been reported that Tsk in acral regions such as the foot and hand decreased, whereas that in nonacral regions such as the abdomen and thigh increased, in parallel with a changing rectal temperature (31). The findings from a prior report (31) and the placebo trials in experiment 1 suggest an increase of sympathetic skin vasoconstrictor activity throughout the experimental period, because skin vasomotor control in acral regions (especially glabrous area) is mainly mediated by the adrenergic vasoconstrictor system (26, 56). The pineal hormone melatonin has a causal role in nocturnal changes in body temperature by affecting the vasoconstrictor system (1), its secretion being decreased in the morning during a normal sleep–wake cycle (9). Interestingly, the oral administration of melatonin in the daytime reportedly increased Tsk and CVC in glabrous regions (foot, palm) but not in nonglabrous regions (forearm, abdomen) (1, 30).

Regarding the effect of Asc on baseline CVC, a previous study (55) found that inhibition of NO synthase (NOS) diminished the increase in baseline CVC caused by the local administration of Asc. Additionally, in a preliminary study ($n = 1$), the tendency to increase SkBF in the forearm with orally ingesting Asc was completely abolished by the NOS inhibitor given through intradermal microdialysis. The bioavailability of NO controlling basal vascular tone may contribute to cutaneous vasodilation after Asc ingestion, because Asc increases the bioavailability of NO by 1) quenching the ROS produced through a variety of enzymatic sources, 2) stabilizing the essential NOS cofactor BH$_4$, 3) promoting the breakdown of the endogenous NOS inhibitors, and 4) inhibiting the arginase pathway (16, 21, 47). The greater increase in baseline CVC in the calf compared with forearm after the ingestion of Asc may be due to differences in the effect of Asc on functional NO synthesis between the arms and legs. Collectively, it is thought that NO-dependent vasodilator effect of Asc abrogated the time-dependent increase in core temperature during the experimental period due to increased heat dissipation from skin.

In experiment 2, however, the significant decrease in the precooling baseline CVC at BT-treated sites post-Asc ingestion is inconsistent with the role of a functional NOS, a nonadrenergic mechanism, in the cutaneous vasodilator effect of Asc. As shown in Fig. 2, insufficient recovery of nonadrenergic skin vasodilation after LC was observed during rewarming from 24 to 34°C and also has been reported previously (58). It is thought that the attenuation of vasodilator responses during rewarming after LC following Asc ingestion was responsible for the difference in baseline CVC levels at BT-treated sites. Since an intact adrenergic function was required for NO-dependent skin vasodilation during local skin heating from 33 or 34 to 40°C (17, 18), the interaction between adrenergic and NO functions might be involved in the attenuation of vasodilator responses.

Ingestion of Asc might increase baseline CVC through the inhibition of centrally driven sympathetic vasoconstrictor activity. However, any such effect of Asc on the baseline levels was negligible, if any, in the interpretation of the results, because tonic adrenergic vasoconstrictor activity during the baseline control was sufficiently inhibited at least in forearm skin under warmer conditions (Tsk = 35–36°C). This speculation is supported by the observation that BT treatment did not increase baseline CVC above the levels at untreated sites in experiment 2. The findings that HR and blood pressure were not changed by the ingestion of Asc negate a significant role of centrally driven sympathetic activity.

In conclusion, the findings of this study suggest that an orally ingested antioxidant vitamin C acutely increases the cutaneous vasoconstrictor response to local skin cooling through adrenergic mechanisms in humans.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: F.Y. conception and design of research; F.Y. performed experiments; F.Y. analyzed data; F.Y. interpreted results of experiments; F.Y. prepared figures; F.Y. drafted the manuscript; F.Y. edited and revised the manuscript; F.Y. approved the final version of the manuscript.

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