Impaired acetylcholine-induced cutaneous vasodilation in young smokers: roles of nitric oxide and prostanoids

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Fujii N, Reinke MC, Brunt VE, Minson CT. Impaired acetylcholine-induced cutaneous vasodilation in young smokers: roles of nitric oxide and prostanoids. Am J Physiol Heart Circ Physiol 304: H667–H673, 2013. First published January 11, 2013; doi:10.1152/ajpheart.00731.2012.—Cigarette smoking attenuates acetylcholine (ACH)-induced cutaneous vasodilation in humans, but the underlying mechanisms are unknown. We tested the hypothesis that smokers have impaired nitric oxide (NO)- and cyclooxygenase (COX)-dependent cutaneous vasodilation to ACH infusion. Twelve young smokers, who have smoked more than 5.2 ± 0.7 yr with an average daily consumption of 11.4 ± 2.6 cigarettes, and 12 nonsmokers were tested. Age, body mass index, and resting mean arterial pressure were similar between the groups. Cutaneous vascular conductance (CVC) was evaluated as laser-Doppler flux divided by mean arterial pressure, normalized to maximal CVC (local heating to 43.0°C plus sodium nitroprusside administration). We evaluated the increase in CVC from baseline to peak (CVCΔpeak) and area under the curve of CVC (CVCΔAUC) during a bolus infusion (1 min) of 137.5 μM ACH at four intradermal microdialysis sites: 1) Ringer (control), 2) 10 mM Nω-nitro-L-arginine methyl ester (L-NAME; NO synthase inhibitor), 3) 10 mM ketorolac (COX inhibitor), and 4) combination of L-NAME + ketorolac. CVCΔpeak and CVCΔAUC at the Ringer site in nonsmokers were greater than in smokers (CVCΔpeak, 42.9 ± 5.1 vs. 22.3 ± 3.5%max·s, P < 0.05; and CVCΔAUC, 8.085 ± 1.055 vs. 3.145 ± 359%max·s, P < 0.05). In nonsmokers, CVCΔpeak and CVCΔAUC at the L-NAME site were lower than the Ringer site (CVCΔpeak, 29.5 ± 6.2%max·s, P < 0.05; and CVCΔAUC, 5.377 ± 1.109%max·s, P < 0.05), but in smokers, there were no differences between the Ringer and L-NAME sites (CVCΔpeak, 16.8 ± 4.3%max, P = 0.11; and CVCΔAUC, 2.679 ± 785%max·s, P = 0.30). CVCΔpeak and CVCΔAUC were reduced with ketorolac in nonsmokers (CVCΔpeak, 13.3 ± 3.6%max·s, P < 0.05; and CVCΔAUC, 1.967 ± 527%max·s, P < 0.05) and smokers (CVCΔpeak, 7.8 ± 1.8%max·s, P < 0.05; and CVCΔAUC, 1.246 ± 305%max·s, P < 0.05) and at the combination site in nonsmokers (CVCΔpeak, 15.9 ± 3.1%max·s, P < 0.05; and CVCΔAUC, 2.660 ± 512%max·s, P < 0.05) and smokers (CVCΔpeak, 11.5 ± 2.6%max·s, P < 0.05; and CVCΔAUC, 1.693 ± 409%max·s, P < 0.05), but the magnitudes were greater in nonsmokers (P < 0.05). These results suggest that impaired ACH-induced skin vasodilation in young smokers is related to diminished NO- and COX-dependent vasodilation.

Acetylcholine; acetylcholine; cigarette; skin; microvascular

Almost 6 million people die from tobacco use and exposure each year (50). Given that smoking is a major independent risk factor for hypertension, myocardial infarction, and atherosclerosis (2, 48) and that chronic exposure to cigarette smoking changes the structure and function of conduit arteries (39), it is not surprising that the majority of tobacco-related deaths are due to cardiovascular disease (8). In addition, chronic smoking has been reported to attenuate the function of the microcirculation, as acetylcholine (ACH)-induced vasodilation, which is an index of endothelial function and has been used in clinical studies (47), is impaired in human skin in smokers compared with controls when ACH is administered using the iontophoresis method (6, 7, 18, 35). Given that microvascular dysfunction is a crucial step in the complications that lead to cardiovascular disease (27), advancing our understanding of the mechanisms behind how smoking affects skin microvascular function would provide strategies to ameliorate dysfunction of the microcirculation and thus tobacco-related deaths. Currently, the mechanisms behind the impaired ACH-induced skin vasodilation in smokers are unknown.

ACH stimulates the endothelium, producing several substances that can induce a direct vasodilation of the smooth muscle: nitric oxide (NO), formed via NO synthase (NOS); prostacyclin (PGI2), produced via the cyclooxygenase (COX) pathway; and endothelial-dependent hyperpolarizing factors (EDHF) that stimulate calcium-activated potassium channels and thus hyperpolarize vascular smooth muscle. Importantly, the substance(s) responsible for vasodilation to ACH varies across vascular beds (41). In healthy human skin, ACH-induced cutaneous vasodilation is attenuated by administration of NOS and/or COX inhibitors (17, 21, 26), suggesting that both NO and COX pathways contribute to ACH-induced cutaneous vasodilation. As such, the impaired ACH-induced cutaneous vasodilation in smokers may be due to decreased contribution(s) of NO and/or the COX pathway to vasodilation. Supporting this notion, previous studies in vivo have shown that acute or chronic exposure to cigarette smoke extract or compounds found in cigarette smoke, including nicotine, carbon monoxide, and free radicals, lowers the bioavailability of NO (14, 36) and PGE2 (1, 5, 19, 32, 37).

Using the above information as background, we hypothesized that impaired ACH-mediated cutaneous vasodilation in smokers is due to diminished NO- and COX-dependent cutaneous vasodilation.

MATERIALS AND METHODS

Subjects. Twelve nonsmokers and twelve smokers participated in this study, which was approved by the Institutional Review Board at The University of Oregon and conformed to the guidelines set forth by the Declaration of Helsinki. The characteristics of the subjects are shown in Table 1. Verbal and written informed consent was obtained from all subjects before their participation in the study. Smokers were defined as having smoked for at least 1 yr with an average daily cigarette consumption of > 6. Subjects were excluded if they had a history of hypertension, heart disease, diabetes, or autonomic disorders. All subjects were not currently taking prescription medications with the exception of oral contraceptives. All subjects abstained from the use of all medications, including nonsteroidal anti-inflammatory agents, and alcohol, caffeine, and exercise for at least 24 h before the study. The smokers abstained from smoking for at least 12 h before...
Baseline was recorded for at least 10 min, followed by ACh infusion procedure. The dose of 137.5 μM ACh (Sigma-Aldrich) was first administered at a rate of 2.0 μL/min over one of four skin sites for 1 min. We previously confirmed that this dose of ACh induces similar cutaneous vasodilation as observed during whole body heating at rest (17). Others have demonstrated that a similar dose of ACh (100 μM) induced a similar amount of cutaneous vasodilation (26). At least 2 min after the first ACh infusion, the same ACh concentration was applied to another of the four skin sites, and this procedure continued until all four sites received ACh. Thereafter, this four-site ACh infusion was duplicated as a second trial. The first and second ACh infusions within the same skin sites were separated by at least 20 min. Liquid switch stopcocks were used to obtain precise volumes of infusions. Arterial blood pressure measurements were taken at baseline, peak, and return to baseline during each ACh infusion. After completion of both ACh infusions, infusion of 56 mM sodium nitroprusside (Nitropress, Ciba Pharmaceuticals, East Hanover, NJ) at a rate of 2.0 μL/min and local heating of the skin to 43.0°C were applied to all skin sites to induce maximal cutaneous vasodilation (CVCmax). This normalization is necessary to minimize the effect of site-to-site heterogeneity in the level of skin blood flow (27).

Data acquisition and analyses. Data were recorded and stored on a computer using Windaq data acquisition software (Dataq Instruments, Akron, OH). All CVC data were expressed as percentages of maximal CVC (%CVCmax). Initial baseline CVC (CVCbaseline) at each site was obtained during the 10-min baseline measurement. The increase in CVC during ACh infusion from the baseline to 30-s averaged peak value (CVCpeak) was evaluated. We also evaluated the area under the curve of the ACh response (CVCauc; expressed as %CVCmax). The beginning and ending points for the CVCauc analysis are the time the infusion began and the 10th min of infusion. Five minutes of baseline was taken before the start of the infusion, which was used to calculate CVCauc and CVCpeak, thus excluding the influence of differencing baselines of CVC across skin sites or groups. There was approximately a 4-min delay from the start of infusion to the initiation of vasodilation. Since CVC values were stable from preinfusion to the initiation of vasodilation, we did not consider the delay for the calculation of CVCauc and CVCpeak. Because CVC responses to ACh infusion were highly reproducible between the first and second trials for CVCpeak (r = 0.97) and CVCauc (r = 0.92), averaged values were used for data analyses.

Statistical analyses. A two-way repeated-measures analysis of variance was conducted in each group with factors of drug: Ringer, L-NAME, Keto, and combination (Combo) and time (Fig. 1). A two-way, mixed-model, repeated-measures analysis of variance was conducted with factors of smoking habit (nonsmoker and smoker) and time (Fig. 2). When a significant main effect was detected, significant differences of paired variables between groups or drug sites were determined by t-test with correction of Holm method so that α-level was kept at 0.05. The Holm statistical model, which is a modified Bonferroni method, has been developed to increase statistical power since the original Bonferroni method is too conservative. The optimality of the Holm method for these analyses has been previously validated (12). t-tests were also used to determine significant differences in other comparisons including physical characteristics (Table 1) and whether CVCpeak or CVCauc is different from baseline values before ACh infusion. If CVCauc is not significantly different from baseline, it suggests no vasodilation to ACh administration, whereas if CVCauc value is positive and significantly different from baseline, it is suggested that there is ACh-induced vasodilation. Pearson’s product moment correlation coefficients were used to relate CVCauc or CVCpeak with smoking years, daily numbers of cigarettes consumed, age, body weight, body mass index (BMI), and arterial blood pressures. The level of significance was set at α = 0.05. Values are presented as mean ± standard error (SE).
RESULTS

Physical characteristics. The physical characteristics of the subjects are presented in Table 1. No significant differences were observed between the groups for age, height, body weight, BMI, and blood pressure variables.

ACh-induced cutaneous vasodilation. Figure 1 shows the ACh-induced changes in CVC in nonsmokers and smokers. After administration of ACh, CVC increased, after which it went back to baseline values in all sites. CVC at the Ringer site during minutes 4–7 was significantly higher compared with the other three sites, both in nonsmokers and smokers. Figure 2A indicates averaged data for CVCAUC in both groups. As predicted, CVCAUC at the Ringer site was significantly attenuated in smokers compared with nonsmokers. L-NAME administration significantly reduced CVCAUC relative to that at the Ringer site in nonsmokers. By contrast, L-NAME administration did not affect CVCAUC compared with the Ringer site in smokers. Administrations of Keto and/or combination of L-NAME and Keto significantly lowered CVCAUC compared with the Ringer site in both groups; however, these reductions were significantly less in the smokers. Irrespective of skin sites or groups, CVCAUC was always significantly higher than baseline, suggesting that ACh-induced vasodilation was observed in all cases and that the double blockade did not abolish the response to ACh. Results of CVC/H9004 peak were similar to those of CVCAUC (Fig. 2B).

Baseline and maximal cutaneous vascular tone. Table 2 displays results of CVCBaseline and CVCMAX. No differences in
Table 2. CVC\textsubscript{baseline} and CVC\textsubscript{max}

<table>
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<tr>
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<th>CVC\textsubscript{baseline}, mV/MAP\textsuperscript{-100}</th>
<th>CVC\textsubscript{baseline}, % maximal</th>
<th>CVC\textsubscript{max}, mV/MAP\textsuperscript{-100}</th>
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<tr>
<td></td>
<td>Non-smokers</td>
<td>Smokers</td>
<td>(P) value</td>
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<td>Ringer</td>
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<tr>
<td>l-NAME</td>
<td>23 ± 4</td>
<td>25 ± 4</td>
<td>0.41</td>
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<tr>
<td>Keto</td>
<td>22 ± 4</td>
<td>17 ± 3</td>
<td>0.16</td>
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<td>Combo</td>
<td>28 ± 6</td>
<td>28 ± 6</td>
<td>0.48</td>
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<td>Pooled across the 4 sites</td>
<td>31 ± 5</td>
<td>27 ± 5</td>
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<td>26 ± 7</td>
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Values are means ± SE; CVC\textsubscript{baseline}, baseline cutaneous vascular conductance; CVC\textsubscript{max}, maximal cutaneous vascular conductance; l-NAME, nitric oxide synthase inhibitor \(N\textsuperscript{O}\)-nitro-l-arginine methyl ester; Keto, non-specific cyclooxygenase inhibitor ketorolac; Combo, combination of l-NAME and ketorolac.

CVC\textsubscript{baseline} were observed between the groups at all sites, as well as between the groups for the pooled data across the four sites, though we observed a near significant difference in CVC\textsubscript{baseline} between the groups at the Ringer site when expressed as % maximal between the groups (\(P = 0.05\)). Although a trend was observed in each site, CVC\textsubscript{max} in smokers was not significantly different from that in non-smokers in any individual site due to the high degree of variability. However, when the data were pooled across all four sites, pooled CVC\textsubscript{max} in smokers was significantly lower than that in non-smokers.

Correlation analyses. Although CVC\textsubscript{AUC} at the l-NAME, Keto, and Combo sites displayed large individual variations irrespective of group (non-smoker or smoker), they were not significantly correlated with age, body weight, BMI, or arterial blood pressures across subjects (\(n = 24\)). Thus the observed individual variation cannot be attributed to any single factor. In smokers, CVC\textsubscript{AUC} at the l-NAME, Keto, and Combo sites were not significantly correlated with years of smoking or daily number of cigarettes consumed. Similar correlation results were also observed for CVC\textsubscript{Δpeak}.

DISCUSSION

We are the first to investigate the mechanisms by which chronic cigarette smoking attenuates ACh-induced cutaneous vasodilation in humans using the microdialysis technique. The main findings of the present study are that 1) ACh-induced cutaneous vasodilation in smokers is attenuated compared with non-smokers, 2) inhibition of NOS attenuates the ACh-induced cutaneous vasodilation in non-smokers but not in smokers, and 3) inhibition of COX reduces the ACh-induced cutaneous vasodilation in both non-smokers and smokers with a smaller reduction being observed in smokers.

The mechanisms of ACh-mediated cutaneous vasodilation. The present study showed that inhibiting NO production significantly lowered CVC\textsubscript{AUC} and CVC\textsubscript{Δpeak} in non-smokers (Fig. 2, A and B). Additionally, CVC\textsubscript{AUC} and CVC\textsubscript{Δpeak} were attenuated by administering Keto in non-smokers (Fig. 2, A and B). These results suggest that both NO and COX pathways contribute to ACh-mediated cutaneous vasodilation. This observation is consistent with some (21, 26), but not all (17), previous studies. The reason for the discrepancy is currently unclear, but large individual variation in the response when NO production is blocked with l-NAME, as observed in the present study, might be the reason. On the other hand, even when both NO and COX pathways were blocked, a significant ACh-induced cutaneous vasodilation remained in the present study in both groups (Fig. 2, A and B), similar to previous studies (17, 21–23, 26). The involvement of EDHFs in ACh-induced vasodilation is suggested in rat hepatic (3) and retinal (30) arteries. Also, the involvement of EDHFs in the cutaneous vasculature in humans is suggested for the hyperemic response to external pressure (9), cutaneous reactive hyperemia following arterial occlusion (24), and thermal hyperemia induced by local skin heating (4). Therefore, we speculate that EDHFs may also be involved in ACh-induced cutaneous vasodilation in humans. However, given that ACh binds to muscarinic receptors in the smooth muscle cells causing vasodilation, the residual vasodilation with l-NAME and Keto is not necessarily attributable to EDHFs. Further studies specifically investigating calcium-activated potassium channels (EDHFs activate the channels and cause hyperpolarization and vasodilation) are required to determine a role for EDHFs in ACh-induced cutaneous vasodilation.

The response to combined l-NAME and Keto infusions on CVC\textsubscript{Δpeak} and CVC\textsubscript{AUC} (Fig. 2, A and B) accounts for a large portion of the vasodilation to ACh at the Ringer site; however, the summed effects of l-NAME and Keto were greater than that of the Combo site. This finding is consistent with the concept of cross talk between the NO, COX, and EDHF pathways. In support of this suggestion, the contribution of EDHFs to ACh-induced vasodilation is increased when both the NO and COX pathways are inhibited (43). Since this EDHF-induced vasodilation is blocked by sulfaphenazole, an inhibitor of cytochrome P-450 (43), it is speculated that blocking the COX pathway leaves more available arachidonic acid to be metabolized by P-450, causing EDHF-dependent vasodilation. Also, our results may potentially be due to the dependence of the COX system on the NO system as NO activates COX enzymes (42).

Chronic smoking-induced attenuation of ACh induced cutaneous vasodilation. CVC\textsubscript{AUC} and CVC\textsubscript{Δpeak} values at the Ringer site were significantly lower in smokers than in non-smokers (Fig. 2, A and B), suggesting that chronic smoking attenuates ACh-induced cutaneous vasodilation, which is consistent with the results from previous studies using the iontophoresis method (6, 7, 18, 35). More importantly, the present study is the first to explore the underlying mechanisms of the impaired cutaneous vasodilation response to ACh in smokers.

l-NAME reduced CVC\textsubscript{AUC} and CVC\textsubscript{Δpeak} in non-smokers relative to the Ringer site, whereas no effect was observed in smokers (Fig. 2, A and B), suggesting that chronic smoking impairs NO-mediated vasodilation, contributing to the impairment of ACh-induced vasodilation in the cutaneous vessels. Cigarette smoke is a rich source of reactive oxygen species (ROS), such as superoxide (38). Superoxide significantly lowers NO availability in forearm circulation (14). Also, a linkage...
between ROS and the NO pathway is suggested in human skin since ascorbate (an antioxidant) augments cutaneous active vasodilation during whole body heating in hypertensive humans, and this effect can be decreased by L-NAME (16). Therefore, a chronic increase in ROS by cigarette smoking seems to be a major factor for the impaired NO-mediated cutaneous vasodilation in smokers. Another possibility is that chronic smoking induces an increase in plasma endothelin-1 (34), which may have attenuated the NO-dependent vasodilation in the skin vessels through direct interaction between cyclic guanosine monophosphate and the intracellular pathway activated by endothelin receptor type A, as suggested in rabbit cerebral arteries (11).

Keto administration lowered CVC_{AUC} and CVC_{peak} in both groups, but smaller reductions were observed in smokers (Fig. 2, A and B). It is therefore suggested that smokers have impaired COX-dependent cutaneous vasodilation, contributing to the attenuated ACh-induced cutaneous vasodilation. Consistent with this notion, acute or chronic exposure to cigarette smoke extract or nicotine reduced the formation of PGL_2 in animal arteries (1, 19, 37), humans umbilical vessels (5, 19), and human urine (32). On the other hand, superoxide production from chronic smoking might decrease COX-dependent vasoconstricting prostanooids (e.g., thromboxane A_2), as demonstrated in rat aortic rings (40), counteracting the effect of PGL_2-induced vasodilation and contributing to the impaired COX-mediated cutaneous vasodilation.

When we blocked both NO and COX, there were no significant differences in CVC_{AUC} and CVC_{peak} between the groups (Fig. 2, A and B). With the assumption that the remaining vasodilation is mediated by EDHFs, our results may indicate that EDHF-dependent cutaneous vasodilation is not impaired in smokers. Inconsistent with this notion, however, a recent cross-sectional study by Thelen et al. (49) showed that compared with nonsmokers, smokers have lower plasma level of epoxyeicosatrienoic acids, which is a type of EDHF that has been shown to be involved in human skin in thermal hyperemia induced by local skin heating (4). Direct further studies are clearly needed to elucidate the effects of chronic smoking on EDHF-mediated cutaneous vasodilation in humans. Since aging (15, 17, 29), hypertension (44), and disease status (45, 46) influence skin endothelial function, we recruited healthy young smokers and matched controls to avoid these confounding factors. Also, earlier studies have investigated the acute (10, 35, 36) and chronic (6, 7, 18, 35) influences of cigarette smoking and the compounds found in cigarette smoke on skin vessel function, but importantly, the effect of smoking is different depending on whether one smokes cigarettes acutely or chronically (6). To exclude the acute effect, we had the subjects not smoke for more than 12 h before the study. Therefore, the observed attenuation of ACh-induced cutaneous vasodilation in smokers of the present study is not likely caused by the above factors.

Chronic cigarette smoking and premature skin aging. Aging causes skin microvascular dysfunction as NO-mediated cutaneous vasodilation during local heating (29), mild whole body heating (15), and COX-induced cutaneous vasodilation to ACh administration (17) were attenuated in aged humans compared with young ones. Similarly, the present study showed that NO- and COX-dependent cutaneous vasodilation was impaired in smokers compared with nonsmokers. Therefore, the present study extends the notion that cigarette smoking causes premature skin aging; i.e., it causes not only wrinkle formation (31) but also impairment of skin endothelial dysfunction via a similar mechanism to aging.

The effect of chronic smoking on maximal cutaneous vascular tone. Our results showed that CVC_{max} induced by sodium nitroprusside administration combined with local heating was significantly attenuated in smokers (Table 2). We speculate that this attenuation is due to diminished smooth muscle function or structural limitations to vasodilation as suggested in previous studies (6, 7, 35). Chronic smoking has been reported to induce 1) an increased Rho-associated protein kinase, known to play a major role in smooth muscle contraction (33); 2) an upregulation of voltage-gated Ca^{2+} channels in vascular smooth muscle cells (10); 3) an elevation in endothelin-1, which activates voltage-gated Ca^{2+} channels in smooth muscle cell (13); and 4) smooth muscle cell proliferation (51). Furthermore, it has been reported that cigarette smoking lowers peripheral capillary density in guinea pigs (52), capillary density of the skin in smokers may have been lower than nonsmokers, resulting in lower red blood cell concentration at the laser-Doppler sites and a consequently lower CVC_{max} in smokers.

Whatever the mechanism, the lower CVC_{max} in smokers is important in terms of the interpretation of the ACh responses. To minimize the site-to-site variations in skin blood flow (27), we expressed CVC_{peak} and CVC_{AUC} as %CVC_{max} if anything, representing the data as %CVC_{max} would have resulted in an overestimation of the ACh-mediated cutaneous vasodilation in smokers. Had we presented absolute values, the difference between groups would have been even greater. We observed a near significant difference in CVC_{baseline} at the Ringer site expressed as %maximal CVC between groups (P = 0.05). It might be that impaired NO- and COX-dependent pathways in smokers upregulates EDHF-mediated vasodilation, given the existence of complex cross talk between the NO, COX, and EDHFs pathways (as discussed above). However, this difference could also be attributed to the lower CVC_{max} at the Ringer site in smokers.

Individual variation in drug effects. The effects of the drugs on ACh-induced cutaneous vasodilation were largely different depending on the subjects (e.g., Keto administration almost abolished ACh-mediated cutaneous vasodilation in some subjects, but barely attenuated the vasodilation in others). This individual variation was seen in both nonsmokers and smokers. We attempted to elucidate the factor(s) underlying the individual variation with correlation analyses to relate CVC_{AUC} and CVC_{peak} at each site with smoking years, daily numbers of cigarettes consumed, age, body weight, BMI, and arterial blood pressure. However, no significant relationships were found. Although we did not have sufficient statistical power to make a comparison between the sexes, there were no observed trends suggesting a sex difference in CVC_{AUC} and CVC_{peak}. Perhaps the above factors and/or fitness level, smoking habit, sex, etc., interact in a complex manner, relating to the individual variation.

Limitations. We found that the effects of Keto on CVC_{AUC} and CVC_{peak} were significantly less in smokers than non-smokers when expressed as %CVC_{max} (Fig. 2, A and B). However, the results can be interpreted differently when the data are expressed relative to the Ringer site (%change from Ringer site), CVC_{AUC} and CVC_{peak} were similarly reduced by L-NAME in both groups when expressed as a relative value.
ameliorate dysfunction of the microvasculature, such as in the ing medicines that improve NO and COX pathways may the complications leading to cardiovascular disease (27), it is needed NO- and COX-dependent vasodilation, which resemblesduced skin vasodilation in young smokers is related to dimin-
ished NO- and COX-dependent vasodilation, which resembles the effect of aging on skin endothelial function (15, 17, 29).
Given that microvascular dysfunction may be a crucial step in the complications leading to cardiovascular disease (27), it is speculated that in smokers, countermeasures such as prescribing medicines that improve NO and COX pathways may ameliorate dysfunction of the microvasculature, such as in the skin, potentially reducing smoking-related cardiovascular disease and death, though this point needs to be tested in future studies.

Perspectives. Our results indicated that impaired ACh-in-
duced skin vasodilation in young smokers is related to dimin-
ished NO- and COX-dependent vasodilation, which resembles the effect of aging on skin endothelial function (15, 17, 29).
Given that microvascular dysfunction may be a crucial step in the complications leading to cardiovascular disease (27), it is speculated that in smokers, countermeasures such as prescribing medicines that improve NO and COX pathways may ameliorate dysfunction of the microvasculature, such as in the skin, potentially reducing smoking-related cardiovascular disease and death, though this point needs to be tested in future studies.

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

AUTHOR CONTRIBUTIONS
N.F., M.C.R., and V.E.B. performed experiments; N.F. and M.C.R. analyzed data; N.F., M.C.R., V.E.B., and C.T.M. interpreted results of experiments; N.F. and M.C.R. prepared figures; N.F. and M.C.R. drafted manuscript; N.F., M.C.R., and V.E.B. analyzed data; N.F., M.C.R., V.E.B., and C.T.M. conceived and designed research.

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