Distinct Effects of Blood Flow and Temperature on Cutaneous Microvascular Adaptation

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ABSTRACT

CARTER, H. H., A. L. SPENCE, C. L. ATKINSON, C. J. A. PUGH, N. T. CABLE, D. H. J. THIJSSEN, L. H. NAYLOR, and D. J. GREEN. Distinct Effects of Blood Flow and Temperature on Cutaneous Microvascular Adaptation. *Med. Sci. Sports Exerc.*, Vol. 46, No. 11, pp. 2113–2121, 2014. **Purpose**: We performed two experiments to determine whether cutaneous microvascular adaptations in response to repeated core temperature (Tc) elevation are mediated by increases in skin blood flow (SkBF) and/or skin temperature. **Methods**: Healthy subjects participated for 8 wk in thrice-weekly bouts of 30-min lower limb heating (40°C). In study 1, both forearms were "clamped" at basal skin temperature throughout each heating bout (n = 9). Study 2 involved identical lower limb heating, with the forearms under ambient conditions (unclamped, n = 10). In both studies, a cuff was inflated around one forearm during the heating bouts to assess the contribution of SkBF and temperature responses. We assessed forearm SkBF responses to both lower limb (systemic reflex) heating and to local heating of the forearm skin, pre- and postintervention. **Results**: Acutely, lower limb heating increased Tc (study 1, 0.63°C ± 0.15°C; study 2, 0.69°C ± 0.19°C; $P < 0.001$) and forearm SkBF (study 1, 0.13 ± 0.03 vs 1.52 ± 0.51; study 2, 0.14 ± 0.01 vs 1.17 ± 0.38 cutaneous vascular conductance (CVC); $P < 0.001$), with skin responses significantly attenuated in the cuffed forearm ($P < 0.01$). SkBF responses to local heating decreased in study 1 (clamped forearms; week 0 vs week 8, 1.46 ± 0.52 vs 0.99 ± 0.44 CVC; $P < 0.05$), whereas increases occurred in study 2 (unclamped; week 0 vs week 8, 1.89 ± 0.57 vs 2.27 ± 0.52 CVC; $P < 0.05$). Cuff placement abolished local adaptations in both studies. **Conclusions**: Our results indicate that repeated increases in SkBF *and* skin temperature result in increased skin flux responses to local heating, whereas repeated increases in SkBF in the absence of change in skin temperature induced the opposite response. Repeated increases in Tc induce intrinsic microvascular changes, the nature of which are dependent upon both SkBF and skin temperature. **Key Words:** CORE TEMPERATURE, LOCAL HEATING, SKIN BLOOD FLOW, THERMOREGULATION

BASIC SCIENCES

In a recent review (21), Kenney et al. expertly summarized research pertaining to skin blood flow (SkBF) changes after exercise training and/or heat acclimation, including studies that indicate that higher active vasodilator activity and SkBF occur for a given core temperature (Tc) *during* exercise (23). Much of this adaptation can be attributed to the effect of repeated Tc elevation on central hemodynamic adaptation, particularly expanded blood volume (11,17,21). Most (10,17,22,23), but not all (8), previous studies suggest that the effect of local cutaneous microvascular adaptations after exercise training and/or heat acclimation may be limited in terms of exercise performance.

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Nonetheless, several recent studies indicate that local microvascular adaptations are apparent after training/acclimation (1,14), consistent with changes observed in conduit and resistance vessels (5). This raises the important issue of potential microvascular health benefit from repeated exposure to passive heating in humans, given that enhanced nitric oxide (NO)–mediated microvascular function should be associated with decreased progression or risk of microvascular disease (9). However, the relative effect of episodic increases in SkBF, versus skin temperature, in cutaneous microvascular adaptation to repeated body heating has not previously been addressed *in vivo*.

In the present study, we therefore used two novel experimental models to dissect the independent effects of repeated increases in SkBF, versus increases in skin temperature, on cutaneous microvascular adaptation in humans. In the first study, subjects underwent three times weekly bouts of 30 min of lower limb heating (40°C) in a custom-designed immersion bath, which isolated heating to the lower limbs. Throughout each of these heating bouts, a pneumatic cuff was positioned and inflated around one forearm to unilaterally manipulate SkBF in the upper limbs, which allowed for a within-subject bilateral assessment of the role of blood

flow in microvascular adaptation to repeated increases in Tc. To further isolate the effect of changes in SkBF from those associated with blood flow–mediated increases in skin temperature, both forearms were "clamped" at 30°C via immersion in thermostatically controlled baths throughout each bout of lower limb heating in study 1. A subsequent experiment (study 2) was identical in design (8 wk, three times weekly, lower limb heating, unilateral cuff inflation), with the important difference that both forearms were in ambient conditions during the heating bouts; that is, skin temperatures were "unclamped" and therefore allowed to increase with the rise in SkBF that accompanied Tc elevation. Collectively, these experiments, involving repeated Tc elevation, allowed us to isolate the effect of increases in SkBF *per se* from the effects of repeated increases in skin temperature. We hypothesized that repeated increases in SkBF would enhance the intrinsic capacity of the skin to vasodilate and that the combination of increased SkBF and elevated local skin temperature would further enhance this localized vascular adaptation.

MATERIALS AND METHODS

Ethical Approval

This study complied with the Declaration of Helsinki, and the human research ethics committee of the University of Western Australia approved the experimental protocol. All subjects provided a written informed consent before participating in the study.

Subject Characteristics

Nine young, healthy, recreationally active (≤2 h of physical activity per week) males were recruited for study 1, and 10 were recruited for study 2 (Table 1). Subjects had no history of cardiovascular, musculoskeletal, or metabolic disease and did not smoke or take medication. Women were excluded from this study because of the well-established effects of estrogen on hemodynamic and vascular responses. All subjects commenced the intervention between the months of May and July and were therefore exposed to similar natural environments before and throughout the interventions.

Study Designs

Study 1: repeated lower limb heating, unilateral cuff inflation to manipulate forearm SkBF, forearm temperatures clamped (30°C). All subjects underwent baseline assessments and were then required to attend the laboratory three times per week for 8 wk. During each of these sessions, subjects were seated in a custom-designed inflatable recovery bath (iC-iBody; iCoolsport, Queensland, Australia) and immersed up to their waist in warm water (40°C) for a period of 30 min. The water bath temperature was maintained and continuously circulated via a thermostatic heating pump (IC-Heat; iCoolsport, Queensland, Australia). A thick plastic sheet was placed over this bath to isolate heating to the legs, leaving the upper body unaffected by the heating stimulus.

TABLE 1. Subject characteristics.

Characteristics	Study 1 (n = 9)	Study 2 (n = 10)
Age (yr)	24.3 ± 2.9	25.8 ± 3.1
Height (m)	1.76 ± 0.07	1.78 ± 0.09
Weight (kg)	76.6 ± 8.2	78.8 ± 8.2
Body mass index (kg·m^{-2})	24.6 ± 2.6	25.0 ± 1.8
Mean arterial pressure (mm Hg)	88 ± 6	93 ± 5
HR (bpm)	61 ± 9	62 ± 5

Values are mean ± SD.

To eliminate the effect of lower limb heating and Tc-induced reflex increases in SkBF on forearm temperature, each forearm was immersed up to the elbows in thermostatically controlled euthermic water throughout each of the 30-min lower limb heating bouts. Pilot studies indicated that resting forearm skin temperatures averaged approximately 30°C in our laboratory; therefore, these water baths were set and maintained at this basal temperature. Finally, a pneumatic cuff was positioned and inflated to 80 mm Hg around one forearm throughout each of the 30-min heating periods to attenuate the increase in SkBF induced by elevation in Tc in this limb. In this way, we controlled for the effect of skin temperature during core heating while manipulating SkBF bilaterally.

Study 2: repeated lower limb heating, unilateral cuff inflation to manipulate forearm SkBF, forearm temperatures unclamped. This experiment was identical to the one previously discussed, with the exception that the forearms were not placed in temperature baths during the repeated episodes of lower limb heating. By comparing the results of this experiment with those of the previously discussed one, we were able to address the effect of repeated skin temperature elevation on cutaneous microvascular adaptation in response to core heating.

Experimental Outcomes

Acute effect of leg heating on forearm SkBF responses at study entry. At study entry, to assess the efficacy of manipulation of our independent variable, SkBF values were determined using seven Doppler array laser probes (model 413, PeriFlux 5001 System; Perimed AB, Sweden) in *both* experimental studies previously described. That is, skin perfusion was assessed across a 30-min period during isolated lower limb heating (40°C) under circumstances where both forearms were immersed at 30°C (study 1) (Fig. 1A–B) and both forearms were under ambient conditions (study 2) (Fig. 1C–D). The effect of forearm cuff placement during both studies was also assessed. SkBF measures were collected simultaneously in both arms, effectively eliminating any effect of systemic hemodynamics. The data are presented in cutaneous vascular conductance (CVC).

Effect of repeated lower limb heating on the relation between Tc and forearm skin perfusion responses. To assess the effect of repeated lower limb

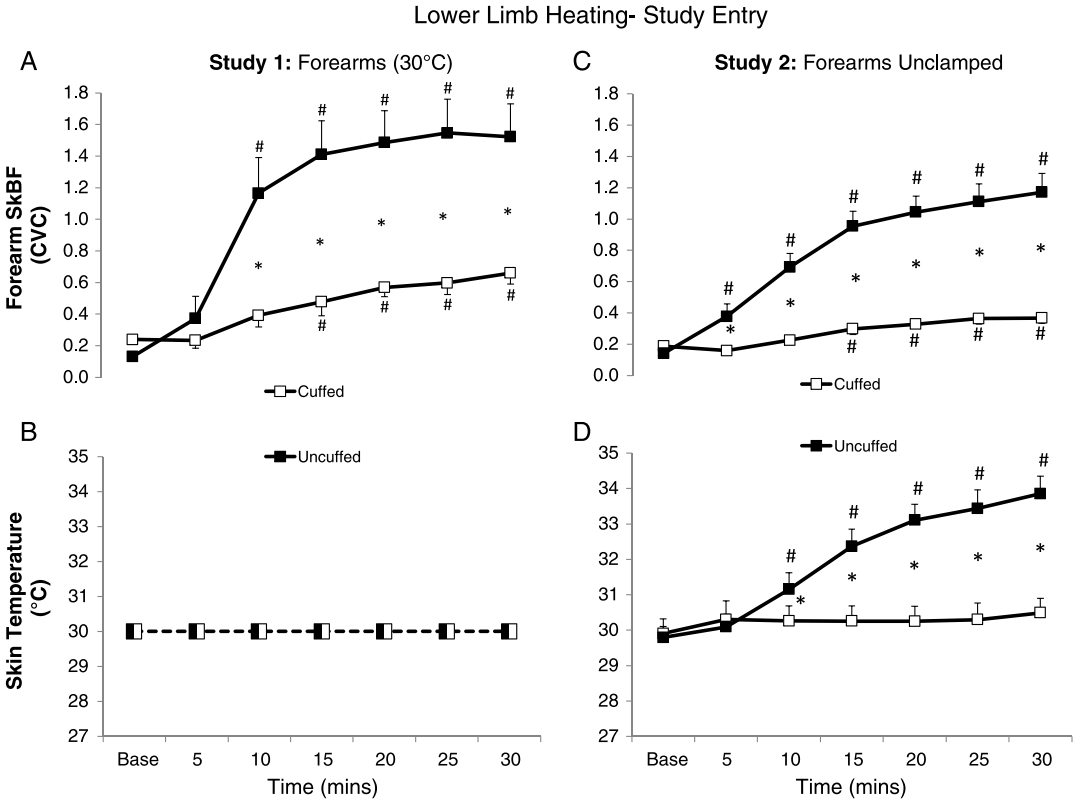

FIGURE 1—Forearm SkBF (in CVC) in study 1 (A) and study 2 (C) and temperature in study 1 (B) and study 2 (D) in the cuffed (*open squares*) and uncuffed (*closed squares*) forearms at baseline and during 30 min of lower limb heating (5-min intervals). Data are mean ± SE. *Significantly different between the cuffed and uncuffed forearms at $P < 0.05$. #Significantly different at $P < 0.05$ from baseline.

heating on adaptations in the relation between Tc elevation and skin perfusion, a subgroup of six subjects (23.3 ± 1.8 yr) in study 1 and eight subjects (25.8 ± 3.1 yr) in study 2 had measures collected throughout the heating protocols previously described. Laser Doppler (model 413, PeriFlux 5001 System; Perimed AB, Sweden) was used to record changes in SkBF throughout the 30-min leg immersions. Tc was measured from rectal temperature probes throughout immersion (RET-1; Physitemp Instruments, Inc., Clifton, NJ). In this way, it was possible to assess the effect of repeated episodic elevation in Tc on adaptations in forearm skin perfusion during an acute bout of lower limb heating undertaken pre- and postintervention.

Effect of repeated lower limb heating on SkBF responses to localized heating. At weeks 0 and 8, all subjects attended an experimental laboratory session, having fasted for a minimum of 8 h and abstained from alcohol, caffeine, and vigorous exercise for at least 24 h. These studies were conducted in a quiet euthermic environment, with subjects at rest and seated comfortably. The assessments were performed at the same time of the day. They did not involve lower limb heating or cuffing or any intervention other than localized heating of the skin using heater discs, as will be described later.

Laser Doppler probe sites on each forearm were shaved and cleaned 24 h before the laboratory attendance. Photographs of the sites were taken and measurements of bony anthropometric landmarks were made at baseline so that similar placement sites were selected on each forearm for repeated measures at 8 wk. Local heater discs (Perimed 455, Stockholm, Sweden) were attached to the forearms using double-sided adhesive rings. The seven laser Doppler array probes (model 413, Periflux 5001 System; Perimed AB, Sweden) were then fitted into the middle of the localized heating discs. Room temperature was controlled and recorded throughout all assessments.

Once instrumented, the heater discs were increased to 33°C and remained at this temperature for a 20-min baseline period. The heater discs were then increased in increments of 0.5°C every 5 min until 42°C was reached, so as to minimize any effect of axon reflexes (15), which are less NO dependent than the protocol adopted in this study (1). Finally, the heater discs remained at 42°C for a further 30 min. Previous articles have established that SkBF in response to this gradual heating protocol is largely NO mediated (1,16). Blood pressure was recorded every 5 min at the ankle using a Dinamap automated monitor and later corrected for the hydrostatic column (6) and used to calculate CVC. All laser Doppler, room temperature, and Tc measurements were relayed and recorded in real time onto a laptop using the software program LabChart 7 (ADInstruments, Sydney, Australia).

Data Analysis

Laser Doppler data. Skin perfusion unit (PU) data from the cuffed and uncuffed arms were averaged over a stable 30-s period at the end of every 5-min interval to assess SkBF. Calibration of the probes were undertaken before the experiments using two generic points, 0 and 250 PU, in accordance with calibration guidelines using a zeroing disk and motility standard (Periflux System; Perimed AB, Sweden). Measurements in PU were converted to CVC, which was calculated as PU/Dinamap mean arterial pressure. All core and room temperature readings were averaged at the end of each 5-min interval in degrees Celsius.

Statistics. SkBF CVC outcome data were compared within subjects across two study time points (0 and 8 wk) using two-factor ANOVA, with planned comparisons performed on four temperature points: 34°C, 40°C–42°C, and 42°C + 30 min. *Post hoc t*-tests were performed where significance was detected at $P < 0.05$ using least significant difference tests to correct for multiple comparisons.

RESULTS

Acute Effect of Lower Limb Heating on Forearm SkBF Responses at Study Entry

Study 1: repeated lower limb heating, unilateral cuff inflation to manipulate forearm SkBF, forearm temperatures clamped (30°C). Despite being bathed at a constant skin temperature of 30°C, forearm SkBF increased significantly in both the uncuffed and cuffed forearms during 30 min of lower limb heating at 40°C ($P < 0.001$ and $P < 0.001$, respectively) (Fig. 1A). However, inflation of the cuff around one forearm significantly attenuated the increase in SkBF compared with that in the uncuffed arm ($P < 0.01$). Skin temperatures were maintained in both arms at 30°C throughout the heating session (Fig. 1B).

Study 2: repeated lower limb heating, unilateral cuff inflation to manipulate forearm SkBF, forearm temperatures unclamped. Resting SkBF was similar between forearms ($P = 0.29$). SkBF increased in both the uncuffed and cuffed arms during 30 min of lower limb heating ($P < 0.001$ and $P < 0.001$, respectively) (Fig. 1C). Inflation of the cuff significantly reduced the increase in SkBF in the cuffed arm compared with that in the uncuffed arm ($P < 0.001$), with differences at all time points ($P < 0.05$) (Fig. 1C). Similarly, skin temperatures in the uncuffed arm significantly increased across the 30-min lower limb heating period ($P < 0.001$). However, in the contralateral limb, cuff inflation abolished increases in skin temperature throughout the heating bout (Fig. 1D).

Comparison of SkBF and temperature between the studies. No difference was evident between SkBF responses in the uncuffed arms throughout the lower limb heating bouts between the studies ($P = 0.14$) (Fig. 1A and C, *closed symbols*). A significant difference in uncuffed limb skin temperature responses was evident between the studies

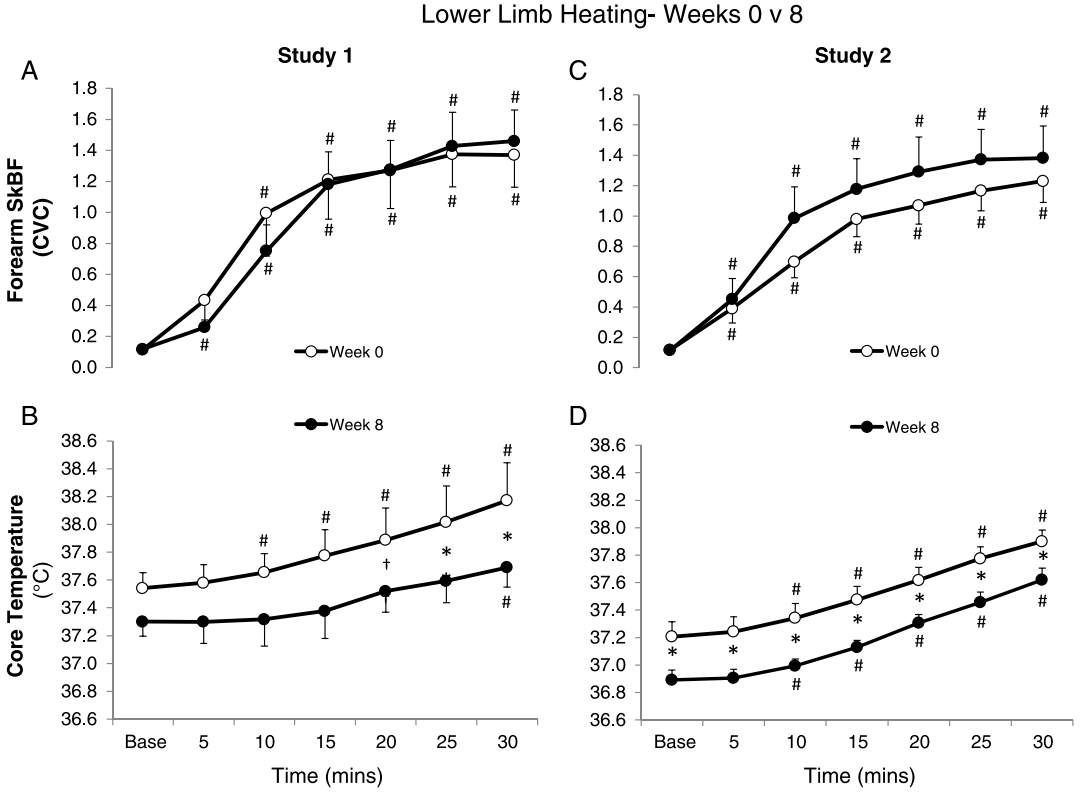

FIGURE 2—Forearm SkBF (in CVC) in study 1 (A) and study 2 (C) and Tc in study 1 (B) and study 2 (D) in response to 30 min of lower limb heating at weeks 0 (*open circles*) and 8 (*closed circles*). Data are mean ± SE. *Significantly different between weeks at $P < 0.05$. †Significantly different between weeks at $P = 0.05$. #Significantly different at $P < 0.05$ from baseline.

($P < 0.01$), indicative of the effect of forearm water immersion at 30°C.

The reported data therefore indicate that, although SkBF increased in the uncuffed arm in both experiments, skin temperature was effectively controlled in study 1. Similarly, cuff placement effectively attenuated SkBF responses between the limbs. These experimental manipulations therefore allow us to distinguish between the effects of repeated elevations in SkBF in the presence and absence of affiliated increases in skin temperature.

Effect of Repeated Lower Limb Heating on the Relation between Tc and Forearm Skin Perfusion Responses

Study 1: repeated lower limb heating, forearm temperatures clamped (30°C). During the first and last bout of 30-min heating (i.e., weeks 0 and 8), the forearms were in ambient air for both experiments so that the effect of repeated core heating on the relation between Tc and SkBF could be assessed.

Forearm perfusion did not change across the 8 wk in response to lower limb heating ($P = 0.75$) (Fig. 2A), whereas Tc responses to lower limb heating decreased after 8 wk ($P < 0.05$) (Fig. 2B). When change in SkBF was plotted against change in Tc across the 30-min session at weeks 0 and 8, a higher SkBF was apparent for a given change in Tc (Fig. 3A).

Study 2: repeated lower limb heating, forearm temperatures unclamped. In keeping with the data previously given, forearm perfusion did not significantly change across the 8 wk in response to lower limb heating in this experiment ($P = 0.31$) (Fig. 2C), whereas resting Tc at week 8 was significantly lower compared with that in week 0 (37.2 ± 0.3 vs 36.9 ± 0.2, $P < 0.001$). Similarly, Tc was significantly lower during lower limb heating at week 8 compared with that at week 0 ($P < 0.001$), with differences at every 5-min interval ($P < 0.05$) across the 30 min (Fig. 2D). When change in SkBF was plotted against change in Tc across the 30-min session at weeks 0 and 8, a higher SkBF was evident for a given change in Tc (Fig. 3B). No differences were evident between mean arterial pressure responses during the lower limb heating bouts at weeks 0 and 8 (weeks 0 vs 8, 97 ± 8 vs 96 ± 6, 87 ± 7 vs 89 ± 6, 86 ± 7 vs 87 ± 5, and 86 ± 9 vs 87 ± 7; $P = 0.88$).

Effect of Repeated Lower Limb Heating on SkBF Responses to a Standardized Local Heating Stimulus

Study 1: repeated lower limb heating, unilateral cuff inflation to manipulate forearm SkBF, forearm temperatures clamped (30°C). Mean CVC values at 33°C were similar between weeks 0 and 8 in the cuffed arm (0.29 ± 0.10 vs 0.29 ± 0.11, $P = 0.87$). In addition, there was no significant difference between weeks 0 and 8 in responses to incremental heating in the cuffed arm ($P = 0.72$) (Fig. 4A).

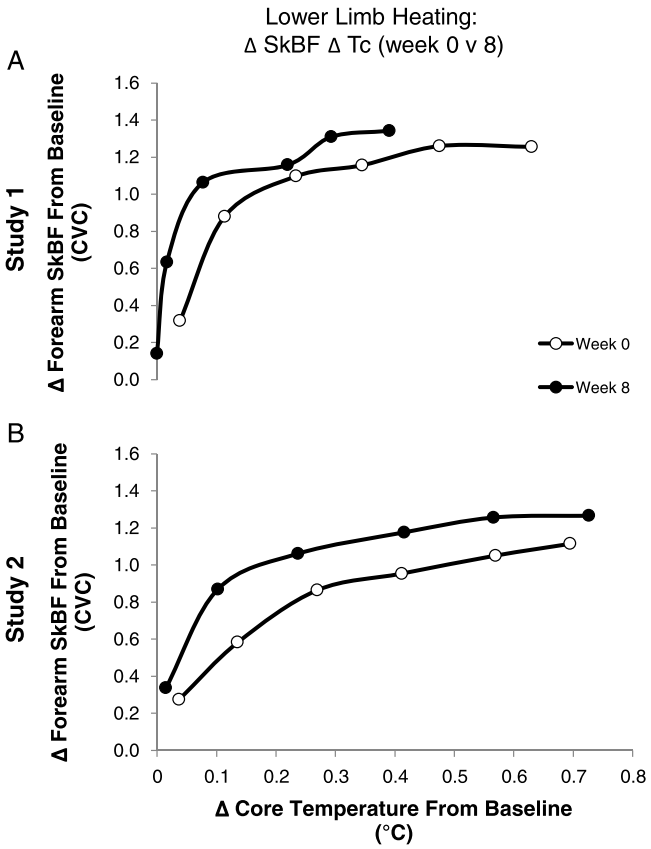

FIGURE 3—Change in forearm SkBF (in CVC) (*y*-axis) versus change in Tc (*x*-axis) during 30 min of lower limb heating (5-min intervals) at weeks 0 (*open circles*) and 8 (*closed circles*) in study 1 (A) and study 2 (B). Data are derived from Figure 2.

In the uncuffed arm, mean CVC values at 33°C remained similar between weeks 0 and 8 (0.21 ± 0.09 vs 0.17 ± 0.08, $P = 0.16$). However, a *decrease* in SkBF responsiveness to incremental heating throughout the local heating protocol was evident at week 8 compared with that at baseline ($P = 0.05$) (Fig. 4B).

Study 2: repeated lower limb heating, unilateral cuff inflation to manipulate forearm SkBF, forearm temperatures unclamped. Mean CVC values at 33°C were similar between weeks 0 and 8 in the cuffed arm (0.22 ± 0.06 vs 0.29 ± 0.09), and there was no significant difference in the response to incremental heating between weeks 0 and 8 in the cuffed arm ($P = 0.69$) (Fig. 4C).

In the uncuffed arm, mean CVC values at 33°C remained similar across weeks 0 and 8 (0.20 ± 0.07 vs 0.23 ± 0.11). In contrast to the previously mentioned results from study 1, SkBF responses to incremental heating between weeks 0 and 8 *increased* ($P < 0.05$) (Fig. 4D).

Change in response to local heating: direct comparison of the effect of skin temperature manipulation. A significant difference was apparent between change in CVC (baseline to week 8) at temperature points 40°C,

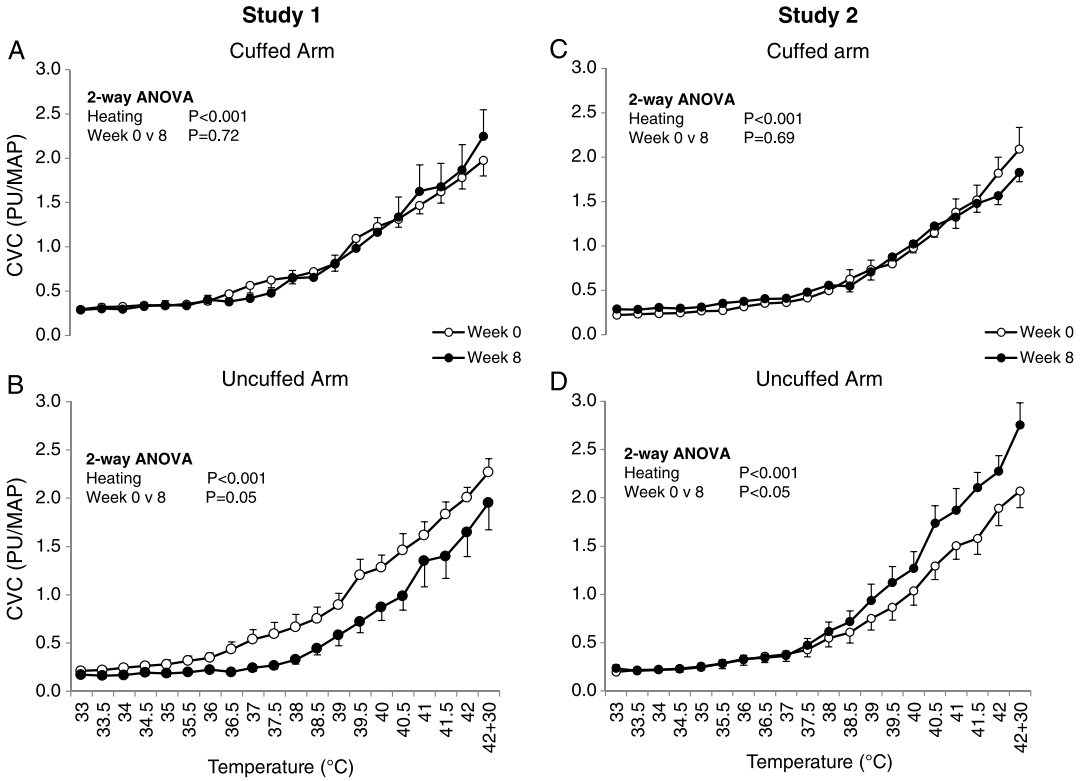

FIGURE 4—Forearm SkBF (in CVC) during the entire gradual heating protocol in the cuffed arms in study 1 (A) and study 2 (C) and uncuffed arms in study 1 (B) and study 2 (D) arms at weeks 0 (*open circles*) and 8 (*closed circles*). Data are mean ± SE. MAP, mean arterial pressure.

41.5°C, and 42°C − 30 min between studies 1 and 2 (two-way between-subjects ANOVA, all *post hoc* $P < 0.05$) (Fig. 5).

DISCUSSION

In the present study, 8 wk of repeated thermoregulatory reflex-mediated increases in forearm SkBF induced divergent cutaneous microvascular adaptations to a local heating stimulus, dependent upon whether skin temperatures were elevated or not during the lower limb heating bouts. When skin temperatures were prevented from rising across the 8-wk intervention period (study 1), postintervention localized heating elicited decreases in SkBF responsiveness, whereas repeated elevation in both SkBF and temperature (study 2) elicited increased responsiveness to our standardized local heating test. Finally, when both SkBF and temperature were attenuated using cuff placement, no adaptations were apparent in either study. Taken together, these data suggest that intrinsic cutaneous microvascular adaptation is dependent upon repetitive episodic increases in blood flow and that skin temperature modifies the direction of this response.

A recent study involving thrice-weekly bouts of bilateral forearm heating (42°C) for 8 wk elicited an increase in SkBF responses to local heating in the uncuffed, but not in the cuffed, forearm (4). This study suggested that SkBF and/or skin temperature were key stimuli for cutaneous microvascular adaptation, but it did not allow us to distinguish between these stimuli. This previous study involved localized forearm heating and did not induce Tc elevation or reflex thermoregulatory changes. We therefore devised the current experiments to assess the relative effect of repeated increases in SkBF and/or skin temperature on cutaneous microvascular adaptations in response to thermoregulatory-reflex mediated increases in forearm SkBF. In these studies, we heated the legs of subjects while clamping skin temperatures (study 1) in the presence of rising SkBF, whereas in study 2, forearm skin temperatures were not clamped and were allowed to rise with blood flow (study 2). Interestingly, and in contrast to our expectations, we observed distinct

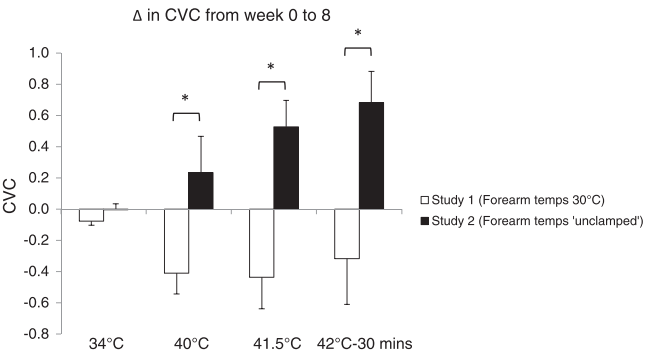

FIGURE 5—Change in CVC from week 0 to week 8 in the uncuffed arm in study 1 (*open columns*) and study 2 (*closed columns*). Data are mean ± SE. *Significantly different between studies at $P < 0.05$.

cutaneous microvascular adaptations in these experiments. Microvascular perfusion in response to a standardized local heating test increased after the latter intervention but decreased in the former.

The decrease in cutaneous microvascular responsiveness during localized heating that we observed in study 1 may conceivably relate to functional or structural microvascular adaptation. It is well established that SkBF is regulated by both neural and local mechanisms (12). For example, rapid local heating of the skin induces a transient peak in SkBF mediated by axon reflexes, a response followed by prolonged vasodilation mediated by the local release of NO (15). The gradual local heating test we used in the present study is considered to be largely NO mediated (1). In contrast, whole-body heating elicits central thermoregulatory reflexes, which increase cutaneous vasodilator outflow via sympathetic cholinergic nerves (12). There are several proposed mechanisms by which vasodilation is induced by this neural drive, including the cotransmitter concept whereby sweating and vasodilation are stimulated by the release of acetylcholine (ACh) and a vasodilator substance yet to be determined (12,24). Recently, Wong (24) reported that inhibition of sensory nerves and NO production reduced the reflex vasodilator response to whole-body heating by approximately 80% and that NO is obligatory for full expression of reflex-mediated vasodilation in the skin. It was furthermore suggested that skin temperature–mediated activation of sensory nerves may play a distinct role in reflex vasodilation compared with that associated with endothelium-dependent mechanisms. The present study adds to these important observations relating to the acute effects of body heating by addressing the chronic effects of repeated Tc elevation mediated by skin perfusion versus hyperemia associated with increased skin temperature. Future studies, similar to those previously described (24), involving blockade of specific transmitters, will provide further mechanistic insight into the distinct effects of repeated episodic changes in skin perfusion and temperature *in vivo*.

It is possible that, in the absence of changes in localized forearm skin temperature during our lower limb bath exposures, one or more of the many intrinsic vasodilators known to contribute to SkBF such as NO, endothelium-derived hyperpolarizing factor, or prostacyclin may have been downregulated. We believe that this explanation is somewhat unlikely because repeated increases in blood flow have not previously been associated with decreased expression of vasodilator substances in other arteries (7). Nonetheless, our study suggests that repeated Tc elevation, in the absence of changes in skin temperature or extrinsic skin heating, does not up-regulate reflex-mediated vasodilation. An alternate speculative explanation for our finding in study 1 invokes microvascular structural change. Theoretically, an increase in skin capillarization after repeated Tc heating and blood flow stimulation might account for the decreases in skin flux that we observed in response to local heating. Consequential prolongation in red cell transit time would enhance heat dissipation in the face of elevated Tc. By analogy, a similar prolongation in transit time due to increased capillarity has been proposed in skeletal muscle after exercise training (13). Unfortunately, we did not directly assess structural microvascular changes in the present study. Our proposal regarding adaptive skin capillarization is therefore hypothetical at this point and included here to provoke future studies, which should elicit maximal CVC or adopt novel methodologies to assess capillarization. Although we are not aware of any previous direct evidence that *skin* capillarization increases in response to either exercise training or repeated passive elevation in Tc, this possibility is consistent with our findings and previously reported changes in skeletal muscle (13) and is worthy of future study.

A possible explanation for the increase in SkBF response to local heating that we observed in study 2 and also in our previous forearm heating experiment (4) may relate to the bioavailability of vasodilators in response to the significant increases in skin temperature. It is known that increases in temperature enhance the expression of heat shock proteins (HSP), and there is a known association between HSP90 and endothelial NO synthase (eNOS) (3,19). Indeed, Shastry and Joyner (20) reported that inhibition of HSP90 (by geldanamycin) reduced SkBF in response to local heating and ACh, highlighting the essential role of HSP90 in full eNOS activation. In this context, it is possible to rationalize the current findings as reflective of heating-induced expression of HSP90 (or other HSP) and subsequent activation of eNOS, thereby enhancing functional vasodilator capacity. Such a cascade may not be apparent in study 1 because of the lack of increase in skin temperature during the lower limb heating bouts.

Apart from the adaptations previously referred to, which pertain to vasodilation in response to localized heating, we also measured SkBF and Tc changes during lower limb heating bouts before and after the intervention period. It is generally accepted that acclimation results in increases in SkBF at an earlier Tc and to a higher plateau level (21). Classic human integrative physiology studies furthermore suggest that whole-body heat exposure during brief periods of exercise training (called "acclimation" by some) exaggerates this response (17). A recent review (21) and article (11) attributed these effects on the Tc–SkBF relation to changes in blood volume, although the latter study occurred across a brief time frame and there is some evidence that blood volume changes are time dependent (18). The relative increase in SkBF for a given Tc that we observed in both current experiments (Figs. 2 and 3) is consistent with that previously observed (2,21) and strongly implies central blood volume expansion. However, a recent and carefully performed study by Lorenzo and Minson (14) demonstrated that heat acclimation involving 10 d of combined exercise and 40°C whole-body heat exposure induces localized SkBF adaptations in response to ACh administration. Our study design was different from that of Lorenzo and Minson (14) and sought to answer distinct questions, but if the mechanism

for our observed change in Tc–SkBF relation during lower limb heating was peripheral rather than central (e.g., blood volume related), then it must relate to episodic increases in skin perfusion and not to changes in skin temperature because the Tc–SkBF change was observed in both of our experiments.

One potential limitation of the present study relates to the placement of the laser Doppler probes before and after the intervention. It is possible that differential placements may have affected the changes we observed. However, we believe that our findings are robust and internally consistent for several reasons. Each subject's initial baseline probe placement was recorded using distances from anatomical landmarks, and a photograph was taken to ensure that follow-up placement was as close as possible to the baseline site. Furthermore, the study design was within subjects and between arms and truly random effects should have similarly affected both limbs. Finally, the findings of our study were very consistent within conditions: of the nine subjects in study 1, eight demonstrated decreases in response to local heating, whereas eight of 10 subjects increased in study 2. A further limitation of this study was that maximal SkBF after the local heating protocol was not induced. Elicitation of maximal SkBF by either local heating to 44°C or infusion of vasoactive substances via microdialysis, such as sodium nitroprusside, may have provided supporting evidence for structural adaptation in the cutaneous vasculature.

In summary, this experiment suggests that repetitive increases in skin microvascular hyperemia, when associated with increased skin temperature, induces distinct adaptations to those associated with repeated increases in blood flow *per se*. Although episodic increases in SkBF *may* induce microvascular changes consistent with prolonged red blood cell transit time, increases in skin temperature may be required to manifest functional adaptation and enhanced red cell flux. Interventions that repeatedly increase Tc and, consequently, both SkBF and skin temperature, likely induce central adaptations in blood volume and intrinsic microvascular changes, which combine to enhance thermoregulation.

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D. J. G., H. H. C., and D. H. J. T. conceived and designed the experiments. H. H. C., A. L. S., C. L. A., C. J. A. P., and L. H. N. performed data collection. All authors were involved in data analysis, interpretation of the results, and writing of the manuscript. All authors helped revise the manuscript critically and have approved the final submitted version.

None of the authors have declared any conflict of interest.

The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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