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SKIN BLOOD FLOW AND LOCAL TEMPERATURE INDEPENDENTLY MODIFY SWEAT RATE DURING PASSIVE HEAT STRESS IN HUMANS

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Running Head: Local cooling, skin blood flow, and sweat rate

ABSTRACT

Sweat rate (SR) is reduced in locally cooled skin, which may result from decreased temperature and/or parallel reductions in skin blood flow. The purpose of this study was to test the hypotheses that decreased skin blood flow and decreased local temperature each independently attenuate sweating. In Protocols I and II, 8 subjects rested supine while wearing a water-perfused suit for the control of whole-body skin and internal temperatures. While 34 °C water perfused the suit, 4 microdialysis membranes were placed in posterior forearm skin not covered by the suit in order to manipulate skin blood flow using vasoactive agents. Each site was instrumented for control of local temperature and measurement of local sweat rate (capacitance hygrometry) and skin blood flow (laser-Doppler flowmetry). In Protocol I, 2 sites received norepinephrine to reduce skin blood flow while 2 sites received Ringer's solution (control). All sites were maintained at 34 °C. In Protocol II, all sites received 28 mM sodium nitroprusside to equalize skin blood flow between sites prior to local cooling to 20 °C (2 sites) or maintenance at 34 °C (2 sites). In both protocols individuals were then passively heated to increase core temperature ~1 °C. Both decreased skin blood flow and decreased local temperature attenuated the slope of the SR to mean body temperature relationship (2.0 ± 1.2 vs. 1.0 ± 0.7 (mg·cm^{-2}·min^{-1})/°C for the effect of decreased skin blood flow, P = 0.01; 1.2 ± 0.9 vs. 0.07 ± 0.05 (mg·cm^{-2}·min^{-1})/°C for the effect of decreased local temperature, P = 0.02). Furthermore, local cooling delayed the onset of sweating (mean body temperature of 37.5 ± 0.4 °C vs. 37.6 ± 0.4 °C, P = 0.03). These data demonstrate that local cooling attenuates sweating by independent effects of decreased skin blood flow and decreased local skin temperature.

KEY WORDS: Skin temperature, Thermoregulation, Microdialysis, Laser-Doppler flowmetry

INTRODUCTION

In humans, one of the primary thermoregulatory responses to increased core and skin temperatures is sweating from eccrine sweat glands. Several conditions, such as aging, diabetes, multiple sclerosis, skin grafting, prolonged bed rest, and some variations of heat stroke (1, 7, 9-11, 13, 18, 37) are associated with diminished sweating, and thereby have the potential to reduce heat tolerance. It is important to understand neural and non-neural factors that modulate the sweating response to more fully understand thermoregulatory abnormalities in these and other conditions.

Thermoregulatory sweating is primarily initiated by increased internal (core) and skin temperatures, with the associated afferent neural signals integrated at the hypothalamus (31, 33). Initially, sweating occurs via stimulation of sweat glands upon neurotransmitter release from sympathetic cholinergic neurons, but periglandular conditions can modulate the sweating response. For example, changes in local skin temperature can modify sweat rate (SR), as evidenced by accentuated sweating with local skin heating (20, 25-27, 29) and attenuated sweating with local skin cooling (2, 8, 22, 25, 26). The mechanism for attenuation of sweating by local skin cooling remains unclear but is likely peripheral in nature since attenuated sweating has been demonstrated at locally cooled sites despite continually increasing core body temperature and stable mean whole-body skin temperature (26). Hence, local temperature may directly affect sweat glands and/or neurotransmitter release from sudorific neurons.

In addition to decreasing regional skin temperature, local cooling also reduces skin blood flow (3, 15, 16). Occluding the arterial supply of a limb during either heat stress or administration of sudorific drugs decreases SR distal to the occlusion (4, 5, 12, 20, 29). However, it is unknown whether the magnitude of the reduction in skin blood flow associated

with local cooling, which is much less than that relative to complete ischemia, likewise impairs sweating responsiveness. Given these prior observations, the purpose of the present study was to test the hypotheses that 1) decreased skin blood flow, independent of local temperature, and 2) decreased local temperature, independent of skin blood flow, attenuate sweating during whole-body heat stress.

METHODS

Subjects

Subjects were non-smokers and were free of any known cardiovascular, metabolic, or neurological diseases. Seven men and 1 woman completed Protocol I and 6 men and 2 women completed Protocol II. Their mean (± SD) age, height, and weight were 38 ± 10 y, 175.9 ± 7.5 cm, and 72.2 ± 13.5 kg for Protocol I and 31 ± 12 y, 173.8 ± 7.0 cm, and 69.8 ± 12.1 kg for Protocol II. The phase of the menstrual cycle was not controlled. Study approval was obtained by the institutional review boards at the University of Texas Southwestern Medical Center at Dallas and at Texas Health Presbyterian Hospital Dallas, and subjects provided written informed consent prior to enrolling.

Instrumentation

For both protocols, subjects arrived to the laboratory having abstained from alcohol consumption during the previous 24 h and caffeine during the previous 12 h. Subjects swallowed a temperature-sensing pill (HQ Inc., Palmetto, FL) for the measurement of core temperature (T_c) from intestinal temperature. Heart rate was continuously obtained from an electrocardiogram (HP Patient Monitor, Agilent, Santa Clara, CA), and mean skin temperature ($\overline{T}_{sk}$) was obtained from the weighted average of regional temperatures measured from

thermocouples (Omega Engineering, Inc., Stamford, CT) taped to the lateral calf, lateral thigh, lower back, lower abdomen, upper back, and chest (35). Mean body temperature ($\overline{T}_b$) was calculated as (34):

$$\overline{T}_b = 0.8 \cdot T_c + 0.2 \cdot \overline{T}_{sk}.$$

After this initial phase of instrumentation, subjects donned a tube-lined water perfusion suit (Med-Eng, Ottawa, Canada) over shorts (or over shorts and a sports bra for the women). The suit covered the entire body except for the feet, hands, face, head, and one forearm. Changing the temperature of the water perfusing the suit permitted control of $\overline{T}_{sk}$, T_c, and thereby $\overline{T}_b$.

Once clothed in the suit, subjects rested supine while 4 microdialysis probes (Bioanalytical Systems, West Lafayette, IN) were placed in dorsal forearm skin not covered by the suit. Each probe was initially perfused with lactated Ringer's solution (Baxter, Deerfield, IL) at a rate of 2 µl/min via a perfusion pump (Harvard Apparatus, Holliston, MA) while hyperemia associated with probe insertion trauma subsided (90 – 120 min). Skin blood flow was indexed at each site using a laser-Doppler flow probe (Model DP7a, Moor Instruments, Wilmington, DE) housed in a customized Peltier thermoelectric cooling/heating plate combined with a SR capsule (covering approximately 0.64 cm^2) that permitted the control of local skin temperature while simultaneously measuring skin blood flow and local SR. The Peltier/laser-Doppler probe/SR capsule apparatus was centered over the membrane portion of each microdialysis probe. SR was measured using the ventilated-capsule method (Vaisala, Woburn, WA) with compressed nitrogen delivered at 150 ml/min. A thermocouple (Type T, Omega Engineering, Stamford, CT) was placed between the Peltier element and the skin surface for the measurement of local skin temperature (TC-1000 Thermocouple Meter, Sable Systems, Las Vegas, NV).

Arterial blood pressure was measured using electrosphygmomanometry of the brachial artery (Tango, SunTech Medical Instruments, Raleigh, NC) with the cuff placed on the arm not instrumented with the microdialysis probes. Mean arterial pressure was calculated as 1/3 pulse pressure + diastolic pressure.

Procedures for Protocol I

Throughout instrumentation, and while the hyperemic response associated with insertion trauma from placement of the microdialysis probe subsided, 34 °C water was perfused through the suit. Once the hyperemic response subsided and skin blood flow was stable, 2 of the sites received 1×10^{-3} M norepinephrine (NE; Sigma-Aldrich, A9512) to cause cutaneous vasoconstriction. The other 2 sites served as control sites and continued to receive the vehicle (lactated Ringer's solution).

Upon stable reductions in skin blood flow at the sites receiving NE (approximately 10 min from the start of NE administration), the water perfusing the suit was switched to 48 °C to elicit a whole-body heat stress. Each microdialysis probe was perfused continuously with its respective solution (i.e., NE for the experimental sites and Ringer's solution for the control sites) throughout the heat stress. The duration of whole-body heating was based on achieving an increase in T_c of ~ 1 °C. Subjects were then cooled by decreasing the temperature of water perfusing the suit while instrumentation was removed.

Procedures for Protocol II

Similar to Protocol I, throughout instrumentation and while hyperemia associated with microdialysis probe placement subsided, 34 °C water was perfused through the suit. Once hyperemia subsided and skin blood flow was stable, 28 mM sodium nitroprusside (SNP; a nitric oxide donor) was administered to all microdialysis sites to elicit maximal cutaneous vasodilation

(17, 24, 38). After 20 min, 2 microdialysis sites were cooled to 20 °C while the other 2 sites remained at 34 °C. Once skin blood flow and temperature were stable at the locally-cooled sites, the water temperature perfusing the suit was increased to 48 °C to elicit a whole-body heat stress like in Protocol I. All sites continued to receive 28 mM SNP throughout the heat stress. As in Protocol I, whole-body heating was administered until achieving an increase in T_c of ~ 1 °C. Subjects were then cooled by switching the water perfusing the suit to a lower temperature while instrumentation was removed.

Data Analysis

In Protocol I, in order to more robustly assess the effect of the difference in skin blood flow on sweat rate, data from the single NE site and the single control site with the greatest difference in skin blood flow were statistically analyzed. In Protocol II, in order to more robustly assess the effect of the difference in local temperature on sweat rate, data from the single locally-cooled site and the single control site with the most similar skin blood flows were statistically analyzed. Data for both protocols were statistically analyzed in the same manner. Data were acquired continuously at a sampling rate of 50 Hz using a data acquisition system (Biopac, Santa Barbara, CA). SR and temperature (i.e., $\overline{T}_b$, $\overline{T}_{sk}$, T_c) data were averaged every 30 s during heat stress. Changes in temperature variables before heating compared to the end of the heat stress were analyzed using one-tailed paired samples t-tests. The onset of sweating for each site was determined by an experienced investigator blinded to the sites (i.e., Protocol I: NE and control; Protocol II: cool and control) by visually inspecting SR graphed relative to time. The $\overline{T}_b$ at the indicated time for the onset of sweating was then identified and reported as the temperature threshold for the onset of sweating. This value was compared between sites using a one-tailed paired samples t-test. The $\overline{T}_b$ at the plateau in the SR response, or at the final $\overline{T}_b$ if a plateau did

not occur, was identified. The slope of the SR:$\overline{T}_b$ relationship was calculated using linear regression of all data points between the onset of sweating and end of heat stress (or plateau of SR if applicable). This slope was compared between sites using a one-tailed paired samples t-test. Additionally, sweat rate at each microdialysis site, across changes in T_c, was analyzed using a two-way (site × ΔT_c in 0.1 °C increments) repeated measures analysis of variance (ANOVA). The Greenhouse-Geisser adjustment to degrees of freedom was utilized for all ANOVA tests.

Absolute skin blood flux values from laser-Doppler flowmetry provide an index (in arbitrary units) of skin blood flow, whereas values normalized to maximum cutaneous vasodilation provide an index of neurovascular control. Since the proposed hypothesis in Protocol I was that decreased skin blood flow attenuates SR, it is appropriate to analyze absolute values obtained from laser-Doppler flowmetry, as opposed to analyzing normalized values relative to maximal cutaneous vasodilation. Furthermore, after 1+ hours of NE administration in Protocol I, maximal cutaneous vasodilation is likely unattainable by often employed techniques such as local heating and/or administration of SNP, or if it is attainable it would require an inordinate amount of time. Thus, skin blood flow data are presented as absolute perfusion units. These data were analyzed across changes in T_c using a two-way (site × ΔT_c in 0.1 °C increments) repeated measures ANOVA. Local skin temperatures also were analyzed using a two-way (site × ΔT_c in 0.1 °C increments) repeated measures ANOVA. Values are means ± SD. A P value < 0.05 was considered statistically significant.

RESULTS

Protocol I

Whole-body passive heat stress increased T_c by 1.1 ± 0.1 °C, $\overline{T}_{sk}$ by 4.3 ± 0.9 °C, and $\overline{T}_b$ by 1.7 ± 0.2 °C (all $P < 0.001$). Average local skin temperatures increased slightly during heat stress (~0.6 °C on average at Control and ~0.5 °C on average at NE; $P = 0.03$ for ΔT_c main effect) but were not different between treatment sites (Control: 34.2 ± 0.4 °C; NE: 34.2 ± 0.2 °C; $P = 0.84$ for site main effect). As intended, skin blood flow was significantly higher at the Control site versus the NE site for the duration of the heat stress (Figure 1; $P < 0.001$ for site main effect).

There was no effect of skin blood flow on the $\overline{T}_b$ threshold for the onset of sweating (Control: 37.1 ± 0.3 °C; NE: 37.1 ± 0.4 °C; $P = 0.20$). There was a significant interaction between treatment site and ΔT_c thus indicating that the increase in SR during heat stress was attenuated at the site where skin blood flow was reduced via NE administration (Figure 2; $P = 0.02$). SR at the NE site at end of heat stress was ~50% of the SR at the Control site (0.5 ± 0.4 $mg \cdot cm^{-2} \cdot min^{-1}$ vs. 1.0 ± 0.6 $mg \cdot cm^{-2} \cdot min^{-1}$, respectively, $P = 0.02$). Likewise, the slope of the SR:$\overline{T}_b$ relation was attenuated by ~50% at the NE site where skin blood flow was profoundly reduced (Figure 3; $P = 0.01$).

Protocol II

Like in Protocol I, whole-body passive heat stress increased T_c by 1.1 ± 0.0 °C, $\overline{T}_{sk}$ by 4.3 ± 0.4 °C, and $\overline{T}_b$ by 1.7 ± 0.1 °C (all $P < 0.001$). As intended, local skin temperatures were clamped at 34.3 ± 0.2 °C and 20.1 ± 0.2°C at the Control and Cool sites, respectively ($P < 0.001$ for site main effect).

Figure 4 shows skin blood flow values at both Control and Cool sites throughout passive heat stress. The effect, while statistically significant, was small (Control: 145.8 ± 11.0 perfusion units; Cool: 156.2 ± 11.9 perfusion units; P = 0.02), so despite differing local temperatures, administration of 28 mM SNP was moderately effective in matching skin blood flow between sites during whole-body heat stress. Notwithstanding slightly higher skin blood flow at the Cool site, the onset of sweating was delayed at that site ($\overline{T}_b$ for onset sweating at Control: 37.5 ± 0.4 °C; Cool: 37.6 ± 0.4 °C; P = 0.02), although the effect was small (0.07 ± 0.05 °C on average). Similar to Protocol I, there was a significant interaction between treatment site and ΔT_c thus indicating that the increase in SR during heat stress was attenuated by local cooling (Figure 5; P = 0.02). Likewise, SR sensitivity, as indicated by the slope of the SR:$\overline{T}_b$ relation, was ~50% smaller at the Cool site relative to the Control site (Figure 6; P = 0.02), which resulted in a SR at the end of heat stress that was half as high at the Cool site relative to the Control site (0.3 ± 0.2 $mg \cdot cm^{-2} \cdot min^{-1}$ vs. 0.7 ± 0.3 $mg \cdot cm^{-2} \cdot min^{-1}$, respectively, P = 0.002).

DISCUSSION

The primary finding from this study is that decreased skin blood flow and decreased local skin temperature each independently diminish the elevation of SR relative to the elevation in $\overline{T}_b$ induced by whole-body heat stress. The onset of sweating was not affected by decreased skin blood flow and was only marginally affected by decreased local temperature. For both protocols, the neural drive for sweating was the same between sites. Therefore, these findings suggest that attenuated SR during local cooling observed in previous studies (20, 26, 29) was likely due to decreased skin blood flow, decreased local temperature, or some combination of these effects.

The present findings are consistent with findings of reduced sweating during decreased skin blood flow via ischemia (4, 5, 12, 20, 29) or secondary to decreased local skin temperature (20, 26, 29). Despite these similarities, to our knowledge the present protocols are the first to independently manipulate skin blood flow and local skin temperature while simultaneously observing the effects on local sweat rate. The mechanism for the reduction in sweating associated with ischemia is likely different relative to reduced skin blood flow when local temperature is decreased. Mitigation of sweating during ischemia is proposed to result from an interruption of nerve transmission across the neuroglandular junction secondary to inadequate oxygen tension necessary for transmitter synthesis (12). In the present study it is unlikely that decreased skin blood flow at the NE site resulted in inadequate oxygen tension necessary for transmitter synthesis since, in the presence of NE, the skin still receives nutritive blood flow, just at a lower level, as opposed to ischemia in which there is a complete absence of blood flow to the skin. Moreover, decreased skin blood flow via adrenergic vasoconstriction, similar to the present study, mitigates sweating to administration of exogenous acetylcholine (thus bypassing the need for transmitter synthesis since acetylcholine can bind directly to muscarinic receptors on sweat glands) (6).

In Protocol I, even though skin blood flow averaged ~66 perfusion units lower at the NE site relative to the control site, the threshold for the onset of sweating was similar between sites when expressed relative to time (data not shown) or to increased $\overline{T}_b$ from pre-heat stress, which further refutes the idea that decreased skin blood flow at the NE site impairs neurotransmitter synthesis. In contrast, the slope of the SR:$\overline{T}_b$ relation was ~50% lower at the NE site during the remainder of heat stress (Figures 1 and 3). Although speculative, a possible explanation for the observed findings may be that substances ordinarily released as a result of increased shear stress

255 associated with high skin blood flow [e.g., nitric oxide (21, 30)] that have been shown to amplify
256 sweating (19, 36) were not present in the same proportions at the NE site relative to the control
257 site. In support of this hypothesis, inhibition of nitric oxide attenuates sweating in horses and
258 humans (19, 23, 36). We speculate that when differences in skin blood flow were smaller (such
259 as early in heat stress at the onset of sweating), shear stress-mediated release of nitric oxide (if
260 present in the skin) was low resulting in sweating not being different between sites, but as heat
261 stress progressed and differences in skin blood flow became larger, perhaps more nitric oxide
262 was released at the control site thereby sensitizing sweat glands.

263 In light of the potential effect of shear stress-induced nitric oxide release in altering SR
264 sensitivity, it is noteworthy that in Protocol II SR sensitivity was depressed when local
265 temperature was decreased but when skin blood flow, and nitric oxide availability (via
266 continuous SNP administration) were similar between sites. It may be that local cooling
267 mitigates sudorific neurotransmitter release, as has been suggested by others (25, 26, 33).
268 Alternatively, given that local warming sensitizes sweat glands (2, 27, 28), it may be that local
269 cooling results in the opposite effect, i.e., desensitization of receptors on sweat glands.
270 Regardless of the possible mechanisms, the methods employed in previous studies investigating
271 sweating responses to skin cooling do not permit the independent evaluation of blood flow and
272 local skin temperature on sweating, given decreases in skin blood flow secondary to local
273 cooling were not controlled in those studies (2, 8, 20, 22, 25, 26).

274 It might be argued that in Protocol I NE itself could attenuate sweating by an
275 unrecognized mechanism. However, NE and other adrenergic compounds stimulate sweating by
276 binding to adrenergic receptors on sweat glands (6, 14, 31, 32), so any potential direct effect of
277 NE on sweat glands would have likely been stimulatory rather than inhibitory. Furthermore,

Collins et al. (6) observed that when epinephrine was combined with a local injection of acetylcholine, SR was depressed but only in the presence of vasoconstriction, which, in light of the findings from Protocol I, suggests that such sweating depression could result from vasoconstriction, not from a direct effect of NE. Since NE per se has not been shown to attenuate sweating in the absence of vasoconstriction, it is unlikely that the attenuation of sweating at the NE site in the current study was due to a direct effect of NE, independent of the cutaneous vasculature.

A perceived limitation of the current study may be the use of $\overline{T}_b$ in the calculation of SR sensitivity (i.e., slope) instead of other measures of core body temperature such as esophageal or intestinal temperature. It is recognized that previous studies on this topic (26) utilized esophageal temperature instead of $\overline{T}_b$ as the denominator in the SR slope calculation. Since thermoregulatory sweating is an integrated response incorporating afferent signals from both skin and internal temperature receptors, we felt it more appropriate to calculate the slope as SR versus $\overline{T}_b$. Our intent was not to generalize the sweating onset threshold and slope in the present study to other studies, but rather to compare these responses between the control and experimental sites exposed to the same $\overline{T}_b$ stimulus. Any variable that demarcates sweating at each microdialysis site across time (e.g., time, esophageal temperature, $\overline{T}_b$, etc.) would therefore have been appropriate to test our hypothesis.

In summary, decreased skin blood flow and decreased local skin temperature each independently diminish SR sensitivity during passive heat stress in humans. The precise mechanisms for this modulation cannot be determined from the obtained data, but it is postulated that reduced shear-stress mediated nitric oxide release secondary to reduced skin blood flow, as well as direct effects of decreased local temperature on sweat gland receptors or neurotransmitter

301 release, may be responsible. However, it should be noted that shear-stress mediated nitric oxide
302 release in human skin is controversial and unclearly delineated (38). Accordingly, further
303 studies are warranted to evaluate the potential mechanisms responsible for the observed findings.
304

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FIGURE LEGENDS

Figure 1. Skin blood flow (mean ± SD) during whole-body passive heat stress at control and norepinephrine (NE) treated sites. Skin blood flow was reduced at the NE site relative to the control site throughout heat stress. *P < 0.001 for Control vs. NE site main effect.

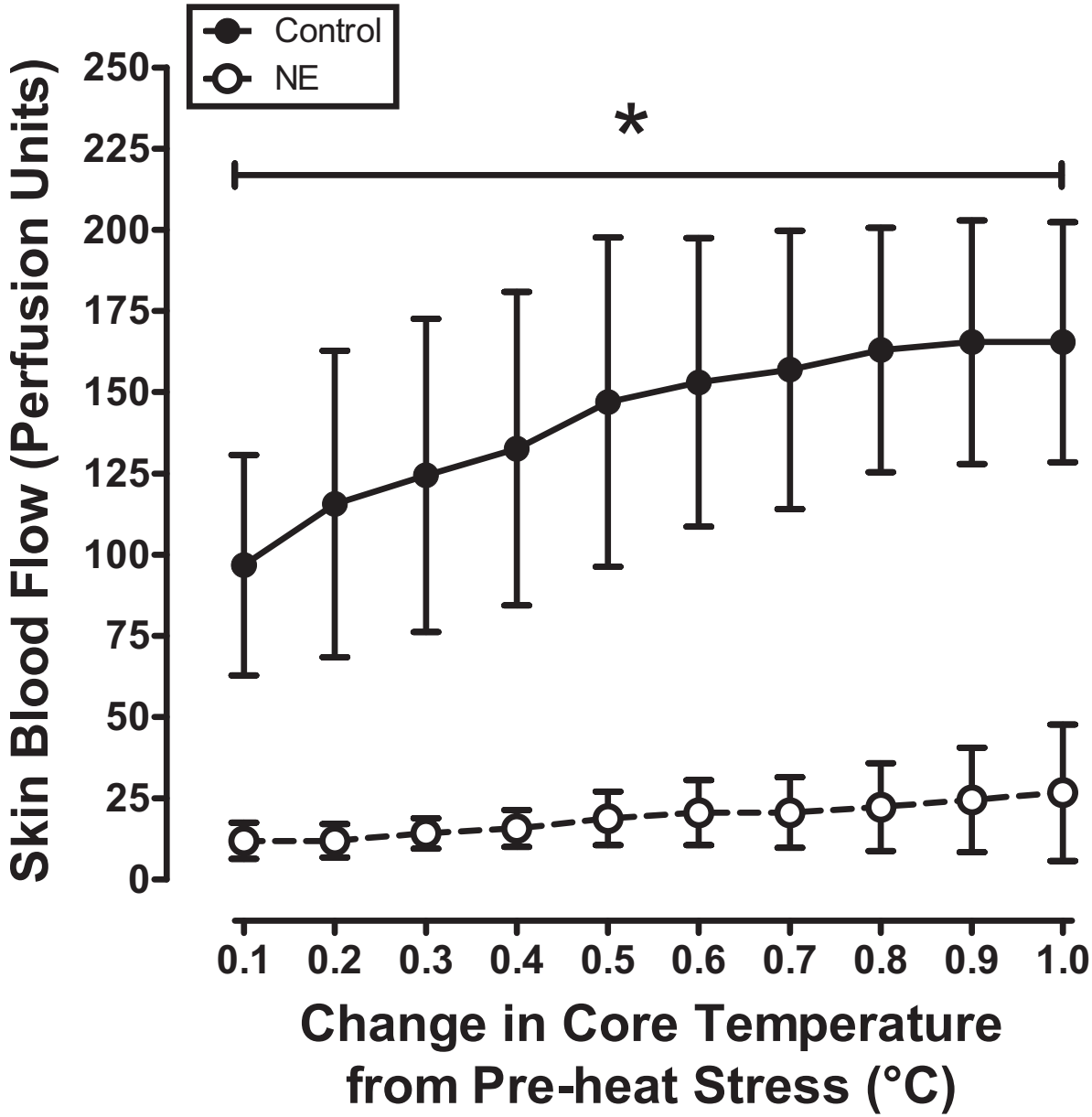
Figure 2. Sweat rate (mean ± SD) during whole-body passive heat stress at control and norepinephrine (NE) treated sites. *P = 0.02 for treatment site × ΔT_c interaction, thus indicating that the increase in SR during heat stress was attenuated by reduced skin blood flow at the NE-treated site.

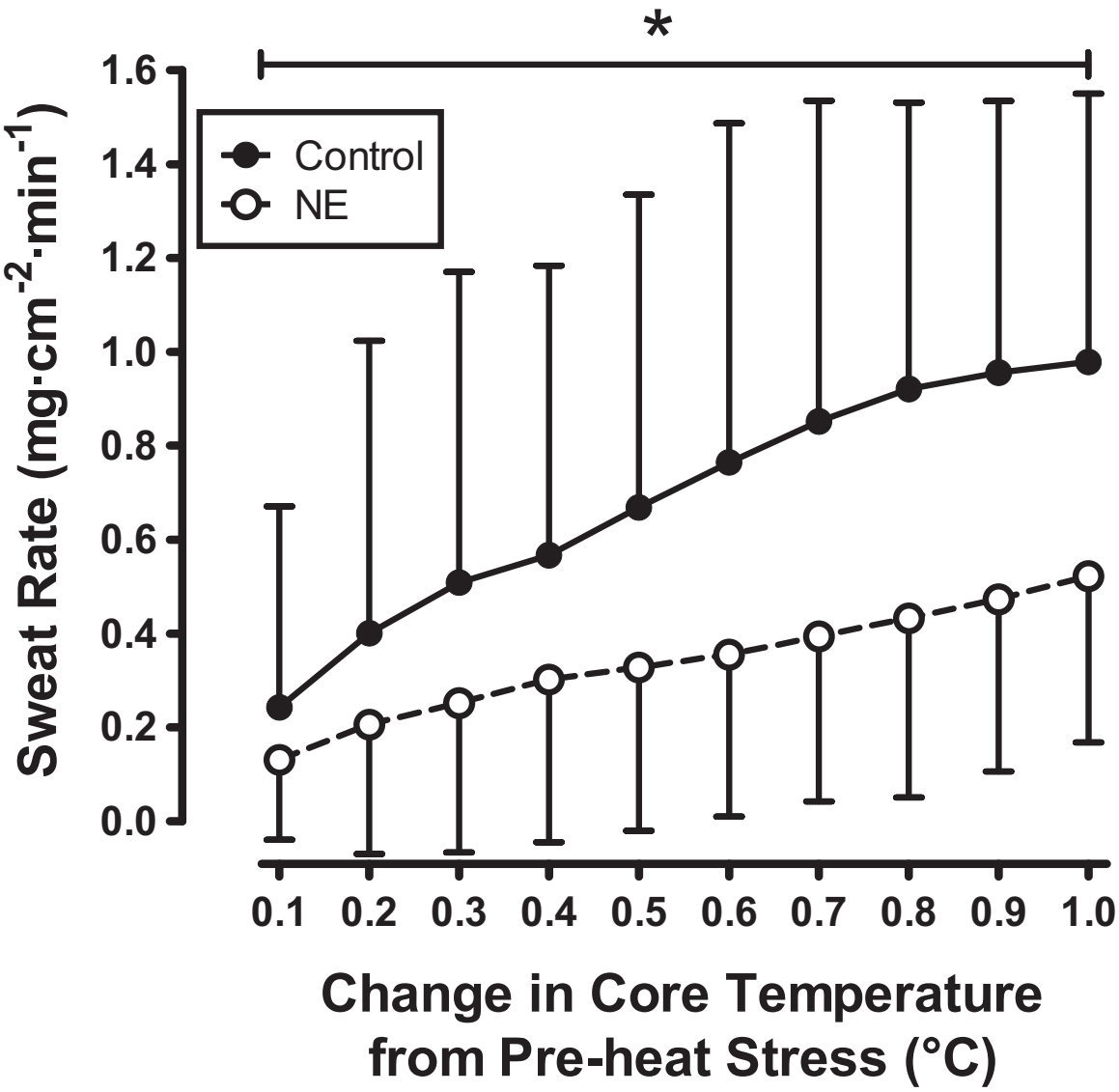
Figure 3. Slope of sweat rate (SR; mean ± SD) to mean body temperature ($\overline{T}_b$) during heat stress between control and norepinephrine (NE) treated sites. The slope of the SR:$\overline{T}_b$ relation was significantly higher at the Control relative to the NE site. *P = 0.01 versus NE site.

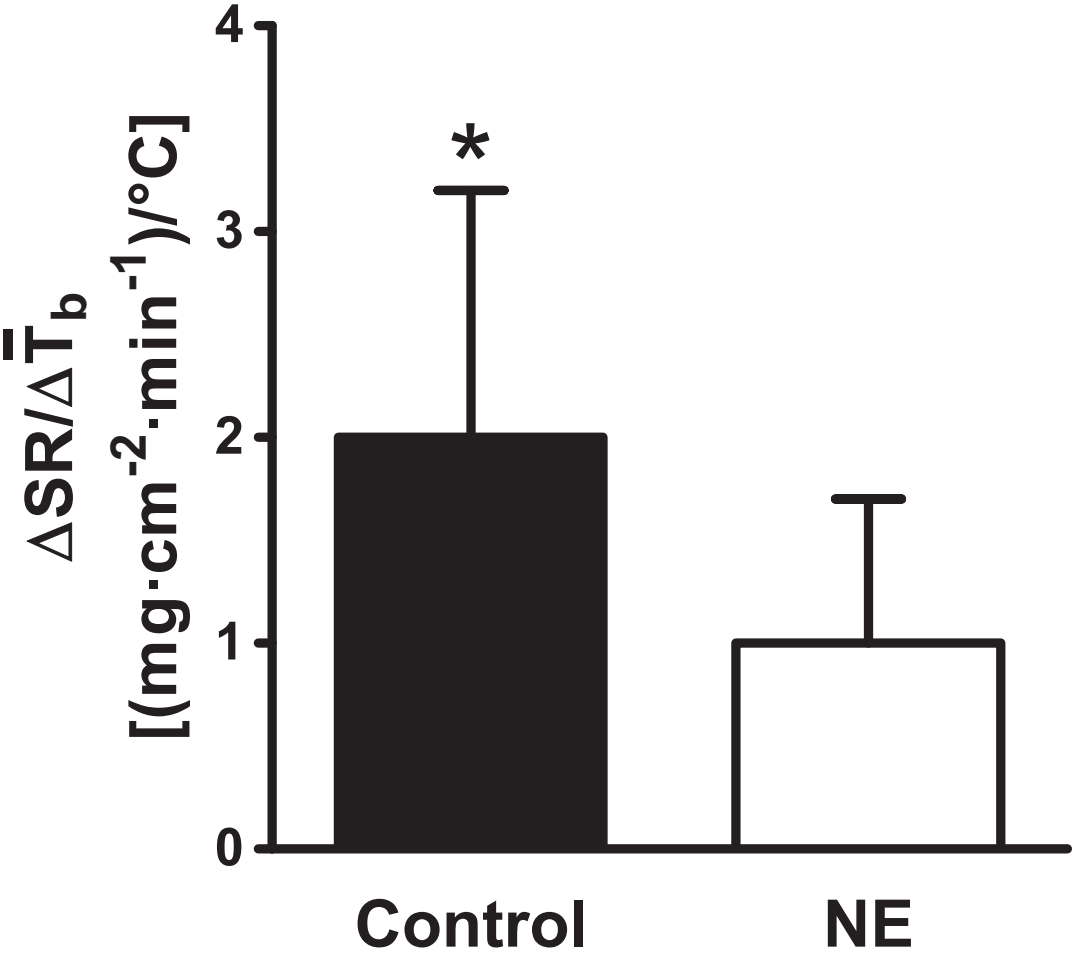
Figure 4. Skin blood flow (mean ± SD) during whole-body passive heat stress at control and locally cooled sites. Despite sodium nitroprusside being administered at all sites, skin blood flow was slightly higher (18 perfusion units) at the Cool relative to the Control site near the end of heat stress. *P = 0.02 for Control vs. Cool site main effect.

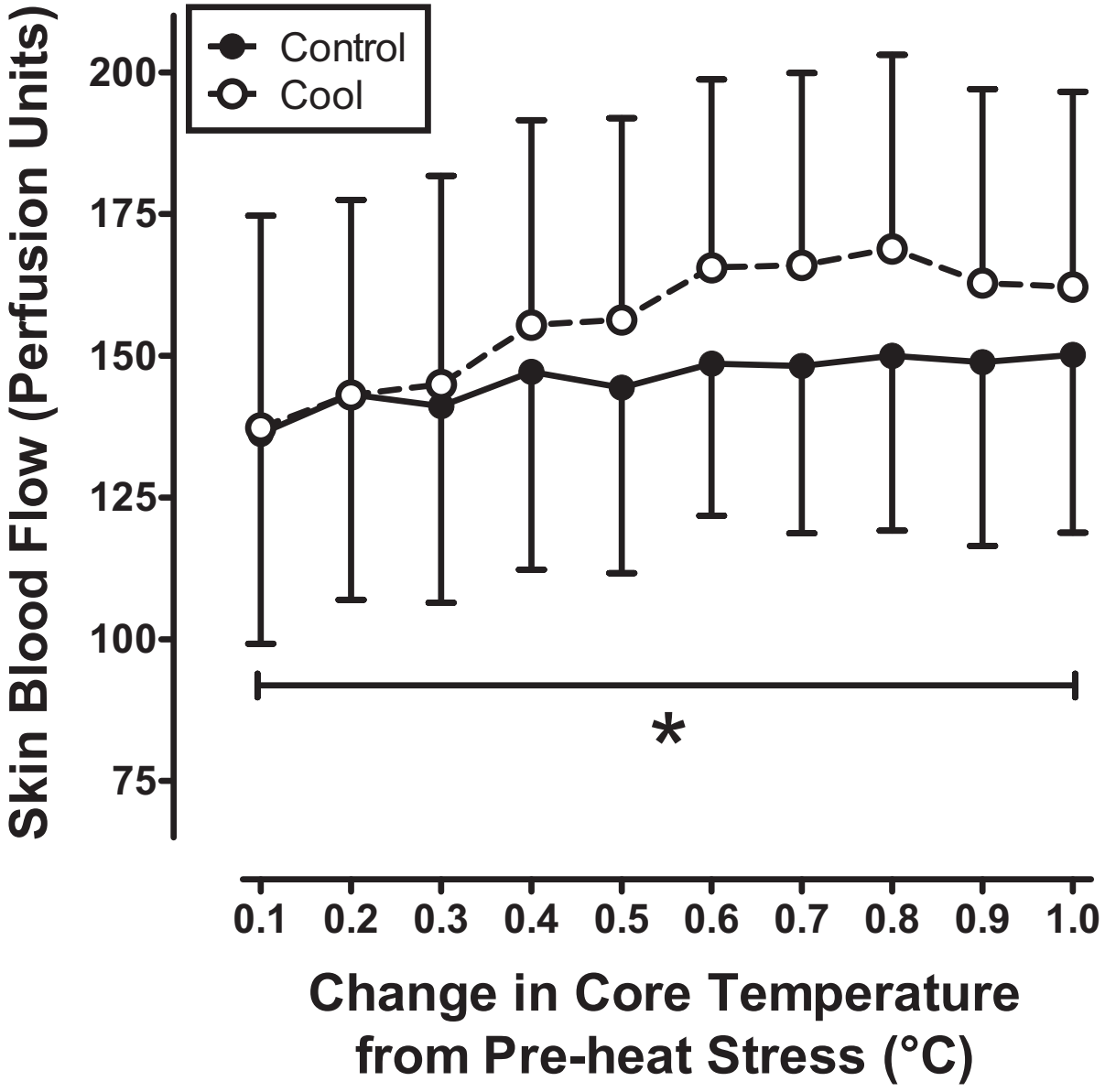
Figure 5. Sweat rate (mean ± SD) during whole-body passive heat stress at control (34 °C) and locally cooled (20 °C) sites. *P = 0.02 for treatment site × ΔT_c interaction, thus indicating that the increase in SR during heat stress was attenuated at the locally cooled site.

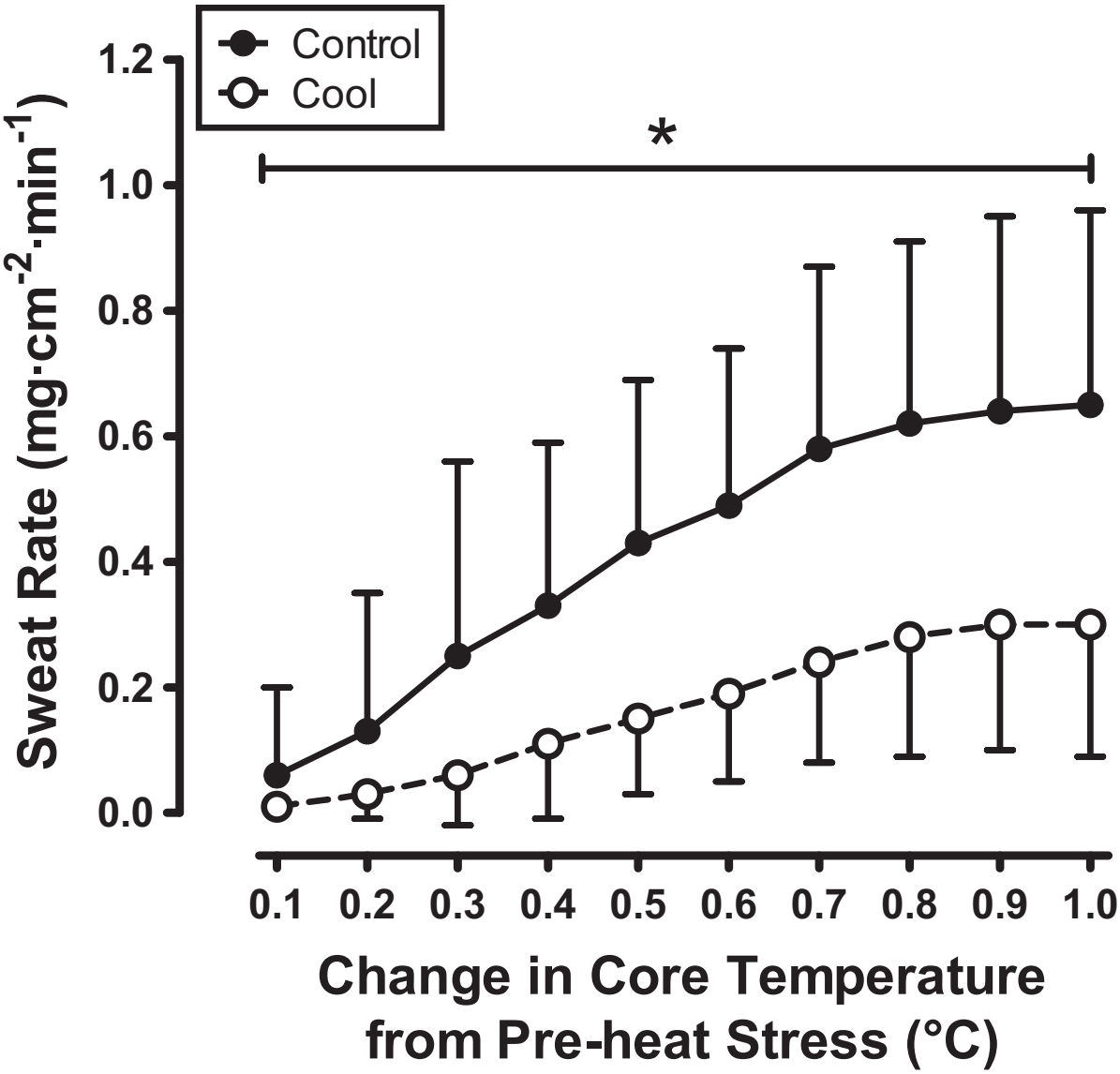
Figure 6. Slope of sweat rate (SR; mean ± SD) to mean body temperature ($\overline{T}_b$) during heat stress between control and locally cooled sites. The slope of the SR:$\overline{T}_b$ relation was significantly higher at the Control relative to the Cool site. *P = 0.02 versus Cool site.

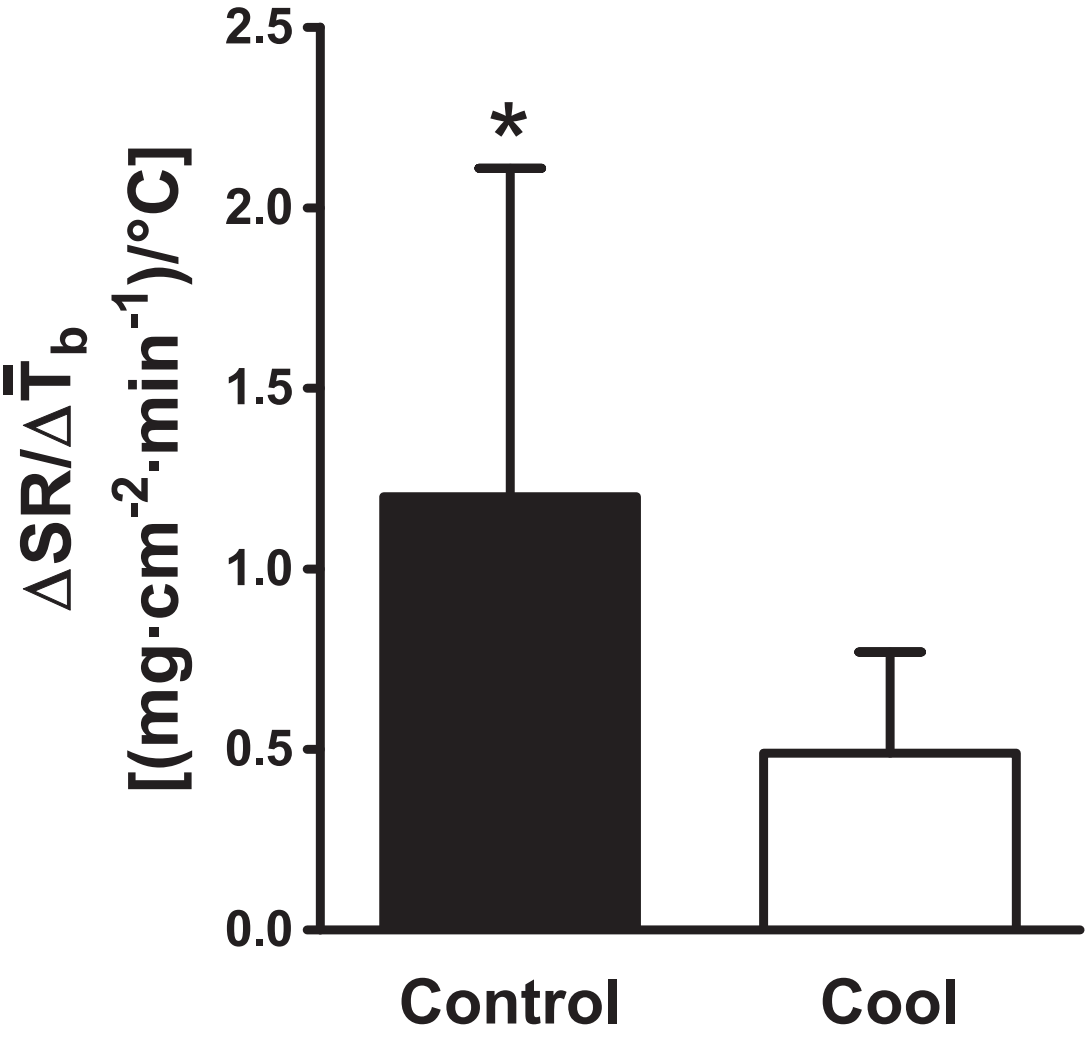

Control
NE
*
Skin Blood Flow (Perfusion Units)
250
225
200
175
150
125
100
75
50
25
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Change in Core Temperature
from Pre-heat Stress (°C)


*
Control
NE
Sweat Rate (mg·cm-2·min-1)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Change in Core Temperature
from Pre-heat Stress (°C)


4
3
2
1
0
*
$\Delta SR/\Delta \bar{T}_b$
[(mg·cm^{-2}·min^{-1})/°C]
Control
NE


Control
Cool
Skin Blood Flow (Perfusion Units)
200
175
150
125
100
75
*
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Change in Core Temperature
from Pre-heat Stress (°C)


Control
Cool
*
Sweat Rate (mg·cm-2·min-1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Change in Core Temperature
from Pre-heat Stress (°C)


2.5
2.0
1.5
1.0
0.5
0.0
*
ΔSR/ΔT̄b
[(mg·cm-2·min-1)/°C]
Control
Cool