Aging and aerobic fitness affect the contribution of noradrenergic sympathetic nerves to the rapid cutaneous vasodilator response to local heating

GARRY A. TEW[1], JOHN M. SAXTON[2], MARKOS KLONIZAKIS[1], JAMES MOSS[1], ALAN D. RUDDOCK[1], GARY J. HODGES[3]

[1]Centre for Sport and Exercise Science, Sheffield Hallam University, UK
[2]School of Allied Health Professions, University of East Anglia, UK
[3]Department of Kinesiology, The University of Alabama, USA

Running head: Sympathetic nerves, thermal hyperemia and aging

KEYWORDS: Skin blood flow, age, heat

Word Count: 5,327

Corresponding author:
Gary J. Hodges, PhD
Department of Kinesiology
The University of Alabama
112 Moore Hall
Tuscaloosa, AL 35487, USA
Phone: (205) 348-2151
Fax: (205) 348-0867
Email: ghodges1@bamaed.ua.edu

ABSTRACT

Sedentary aging results in a diminished rapid cutaneous vasodilator response to local heating. We investigated whether this diminished response was due to altered contributions of noradrenergic sympathetic nerves; assessing 1) the age-related decline and, 2) the effect of aerobic fitness. We measured skin blood flow (SkBF) (laser-Doppler flowmetry) in young (24±1 yr) and older (64±1 yr) endurance-trained and sedentary men (n=7 per group) at baseline and during 35 min of local skin heating to 42 °C at three forearm sites: 1) untreated; 2) bretylium tosylate (BT), preventing neurotransmitter release from noradrenergic sympathetic nerves; and 3) yohimbine and propranolol (YP), antagonising α- and β-adrenergic receptors. SkBF was converted to cutaneous vascular conductance (CVC) (SkBF/mean arterial pressure) and normalized to maximal CVC (%CVCmax) achieved by skin heating to 44 °C. Pharmacological agents were administered using microdialysis. In the young trained, the rapid vasodilator response was reduced at the BT and YP sites (P<0.05); by contrast, in the young sedentary and older trained, YP had no effect (P>0.05) but treatment with BT did (P>0.05). Neither BT nor YP treatments affected the rapid vasodilator response in the older sedentary group (P>0.05). These data suggest that the age-related reduction in the rapid vasodilator response is due to an impairment of sympathetic-dependent mechanisms, which can be partly attenuated with habitual aerobic exercise. Rapid vasodilation involves noradrenergic neurotransmitters in young trained men, and non-adrenergic sympathetic cotransmitters (e.g., neuropeptide Y) in young sedentary and older trained men, possibly as a compensatory mechanism. Finally, in older sedentary men, the rapid vasodilation appears not to involve the sympathetic system.

INTRODUCTION

In humans, the cutaneous circulation performs a major role in the control of body temperature through the level of its perfusion. Under conditions of heat stress, skin blood flow (SkBF) can increase to greater than 6 L/min (30). In contrast, during exposure to extreme cold, SkBF can fall to almost zero (17). In non-glabrous (hairy) skin, the SkBF response to thermal stimuli local to the site of measurement appears to be achieved via a sympathetic noradrenergic system releasing norepinephrine (NE) and the cotransmitter neuropeptide Y (NPY) and a non-adrenergic system that is heavily dependent on nitric oxide (NO) (12-13, 15).

The skin hyperemic response to a non-painful, rapid heat stimulus is commonly used as a test of microvascular and endothelial function (6, 22) and involves at least two independent phases: an initial, rapid transitory rise, followed by a nadir, ultimately succeeded by a secondary rise and prolonged plateau (20, 23). The specific mechanisms underpinning these phases are complex and not completely understood. The rapid initial peak of the vasodilator response is thought to be primarily mediated by an axon reflex via activation of transient receptor potential vanilloid-1 (TRPV-1) receptors in C-fibre afferent nociceptive neurones (41). These sensory neurones might increase skin blood flow (SkBF) through the release of neuropeptides such as calcitonin gene-related peptide and/or substance P (4); however, these theories have yet to be directly tested. Additionally, NO has been shown to contribute modestly to the initial peak of the vasodilator response to rapid local heating (20, 23). The secondary rise and plateau in SkBF, in contrast, is heavily dependent on NO synthesis as inhibition of NO synthase (NOS) reduces this phase by approximately 70% (20, 23).

Recent work (12-13, 15) supports the somewhat counterintuitive concept that cutaneous noradrenergic sympathetic nerves are also involved in the cutaneous vasodilator response to local heating. Indeed, pre-synaptic blockade of neurotransmitter release from these nerves with bretylium tosylate (BT) abolishes the rapid vasodilator (initial) phase and greatly reduces the overall vasodilator response to slow local heating (+0.1°C·min^{-1}) (13, 15). By performing separate post-synaptic antagonism of α- and β-adrenergic receptors and of Y_1 receptors, Hodges et al. (13) found evidence of roles for both NE and the cotransmitter NPY. Adrenergic

involvement in thermal hyperemia also has a rate dependency: the initial peak evoked by slow local heating (+0.1°C·min^{-1}) is completely abolished by pre-treatment with BT; by contrast, the initial peak evoked by rapid local heating (+2°C·min^{-1}) is only halved under conditions of sympathetic nerve blockade (12).

The initial rapid peak and secondary plateau are diminished with sedentary aging (24, 34). Whereas the diminished secondary plateau of older adults is largely explained by attenuated NO-mediated vasodilation (2, 24), the mechanisms underpinning the decline in the initial rapid vasodilation are less clear. Diminished functioning of local sensory nerves might be implicated, because sensory nerve function blockade using a topical local anesthetic cream abolishes the difference between young adults and older sedentary adults (33). In that study, the size of the initial peak and the contribution of sensory nerves to the initial peak were similar between older endurance-trained adults and younger adults, suggesting that regular aerobic exercise can preserve sensory nerve-mediated vasodilator function in older adults. Previous studies have demonstrated that aging is associated with decreases in skin sympathetic efferent outflow in response to heat exposure (10) and vasoconstrictor responsiveness to NE (36, 40). Therefore, the diminished initial peak of sedentary older adults might also involve a decreased contribution of noradrenergic sympathetic nerves.

Hence, the primary aim of this study was to investigate the role of cutaneous noradrenergic sympathetic nerves in the age-related decline in the initial rapid vasodilator response to local heating. A secondary aim was to further investigate the effect of regular aerobic exercise (as reflected by a higher aerobic fitness) on the initial peak in both young and older adults, assessing whether the effects of habitual exercise on cutaneous vasodilation can be explained by altered contributions of sympathetic neurotransmitters. We hypothesized that the contribution of noradrenergic sympathetic nerves to the initial vasodilator response would be greater in the young and well-trained individuals compared to the older sedentary individuals.

MATERIALS AND METHODS

Ethics approval

This study was approved by the Ethics Committee of Sheffield Hallam University and conducted according to the principles of the Declaration of Helsinki. Written, informed consent was obtained before participants entered the study.

Participants

We recruited 28 men who were equally divided among four groups: young endurance-trained (24 ± 1 yr), young sedentary (25 ± 1 yr), older endurance-trained (64 ± 1 yr) and older sedentary (64 ± 1 yr). The trained participants were recruited from running and cycling clubs in and around Sheffield, UK. They had all performed vigorous endurance exercise for ≥3 times·week^{-1}, ≥30 min·session^{-1} and ≥5 years. The sedentary participants reported undertaking no regular exercise. All participants were healthy, non-smokers, free from cardiovascular disease and diabetes, and were not taking any medications. The participants attended the testing facility on two separate occasions. For both sessions, they were asked to refrain from caffeine, alcohol, and exercise for 24 h prior to their attendance. The participants are the same as those described in a recently published article by our group (33).

Visit 1: Assessment of cardiopulmonary fitness

Participants completed a continuous, incremental cycling test to volitional exhaustion on an electronically-braked cycle ergometer (Excalibur Sport, Lode, The Netherlands). Pedalling frequency was self-selected within the range of 60 to 90 rev·min^{-1}. After a 2-min warm-up against no resistance (0 W), the intensity of exercise was increased by 20 to 30 W·min^{-1}. Participants were encouraged to continue cycling to volitional exhaustion or until a plateau in oxygen consumption was observed. Heart rate was recorded continuously by electrocardiogram (Cardioperfect, Welch Allyn, USA). The volume of oxygen consumed during exercise was calculated from minute ventilation, measured using a pneumotach, and simultaneous breath-by-breath analysis of expired gas fractions (Ultima CardiO$_2$, MedGraphics, USA). Gas analysers and flow probes were calibrated before each test. Oxygen consumption was expressed relative to body mass (mL·kg^{-1}·min^{-1}). Maximal oxygen consumption ($\dot{V}O_{2max}$) was calculated as the highest consecutive

20-second period of gas exchange data in the last minute before volitional exhaustion, which generally occurred due to leg fatigue and/or breathlessness.

Visit 2: Assessment of SkBF responses to local heating

Instrumentation

The microvascular assessments were performed in a temperature-controlled room (22 to 24°C) with participants resting supine and the experimental (left) arm positioned at heart level for the entire protocol. Blood pressure was measured automatically on the right arm every 2 min (Dinamap Dash 2500, GE Healthcare, USA).

Two microdialysis fibers (Linear 30, CMA Microdialysis Ltd, Stockholm, Sweden) with a membrane length of 10 mm and a 6-kDa molecular mass cut-off were placed ~5 cm apart in the dermal layer of skin on the ventral aspect of the left forearm. Before implantation, the skin was temporarily anesthetised by applying an ice pack for 5 min (11). A 21-gauge needle was introduced aseptically into the dermis along a length of ~2.5 cm before exiting. A microdialysis fiber was threaded through the lumen of the needle, before removing the needle to leave the fiber in place. All microdialysis fibers were placed in this manner. To allow for the effects of the insertion trauma to subside, we waited 1.5-2 h before beginning the protocol (13).

To obtain an index of SkBF, cutaneous red blood cell flux was measured using laser Doppler flowmetry (Periflux 5000 System, Perimed AB, Järfälla, Sweden) at the two microdialysis sites, and at a third "no fiber" control site. Local heater discs (Model 455, Perimed AB) were used to control local skin temperature and integrating laser Doppler probes (Model 413, Perimed AB) were placed in the centre of each local heating disc.

Drugs

Blockade of neurotransmitter release from sympathetic adrenergic nerves was achieved at one of the microdialysis sites by administering a 20 mM solution of BT (US Pharmacopeia, Rockville, MD, USA). Administration of BT causes a selective and localised blockade of neurotransmitter release from cutaneous sympathetic adrenergic nerves lasting several hours (19).

Blockade of the α- and β-adrenergic receptors was achieved by administering a combination of 5 mM yohimbine (Sigma Aldrich, St. Louis, MO, US) and 1 mM propranolol (Sigma Aldrich) to antagonise those receptors. Herein, these skin sites will be termed the YP sites. Yohimbine is traditionally regarded as an α_2-adrenergic antagonist; however, this combination and concentration of adrenergic antagonists has previously been shown to be effective in inhibiting the cutaneous vascular responses to exogenous NE (18, 31), suggesting that all α- and β-adrenergic receptors are blocked.

As for previous studies investigating the role of sympathetic-dependent mechanisms in cutaneous vasodilation, all drugs were infused at a rate of 4 $\mu L \cdot min^{-1}$ (12-13).

Protocol

Data collection began after the trauma resolution period. Baseline data were recorded for 5 min with the local heating disc temperature at 33 °C. The temperature of the discs was then increased at a rate of 1 °C every 10 s to 42 °C (34) and held constant at this temperature for 35 min (32). After this, local heating temperature was increased to 44 °C for 10 min to induce maximal SkBF (37). No participants experienced any pain or discomfort during the local heating protocol.

Data collection and analysis

SkBF data were divided by mean arterial pressure to calculate cutaneous vascular conductance (CVC). CVC data were expressed as raw values (au/mmHg) and as a percentage of maximal vasodilation recorded during local heating to 44 °C (%CVCmax). Because of the rapid and transient nature of the initial peak responses, stable 30-s periods of SkBF were used for analysis. For the secondary plateau and maximal SkBF phases, stable 2-min periods of SkBF were used for analysis.

To assess the contribution of sympathetic adrenergic nerves to the initial peak in each group, we compared the SkBF responses between the control and drug sites. For example, similar responses between all three sites would suggest that NE and NPY do not contribute to the initial peak. If the initial peak is equally depressed at the BT and YP sites, this would indicate that NE contributes to the initial peak, whereas

NPY does not. Finally, if the initial peak is depressed at the BT site, but not the YP site, this would indicate that NPY contributes to the initial peak, whereas NE does not.

Participant characteristics were compared among groups using a one-way independent ANOVA (SAS v9.1, SAS Institute, Cary, NC). The effects of age, training status, and pharmacological manipulations on hemodynamic measures were assessed using a three-way ANOVA. Where significant interaction effects were observed, Tukey's *post hoc* analyses were used to identify significant differences in the pairwise comparisons. Statistical significance was set at $P<0.05$ and all data are presented as means ± S.E.M.

RESULTS

Participant characteristics

The characteristics of the participants have been reported previously (33). Briefly, the groups did not differ in body mass, stature, or resting systolic or diastolic blood pressure (P>0.05). All participants were normotensive and achieved $\dot{V}O_{2max}$ according to standard criteria (16). The $\dot{V}O_{2max}$ of the young trained (58 ± 3 mL·kg^{-1}·min^{-1}) was higher (P<0.05) than that of the young sedentary (40 ± 2 mL·kg^{-1}·min^{-1}), older trained (44 ± 2 mL·kg^{-1}·min^{-1}), and older sedentary (28 ± 2 mL·kg^{-1}·min^{-1}). The $\dot{V}O_{2max}$ of the older sedentary was lower than that of all other groups (P<0.05), and there was no difference between the young sedentary and older trained (P>0.05).

CVC responses

Local heating resulted in the characteristic biphasic SkBF response previously described (23), i.e., an initial rapid increase and peak at the onset of heating, a brief nadir, and then a slower rise and plateau. This pattern was seen in all four groups and at all skin sites.

Normalized baseline

Baseline CVC did not differ among groups at each skin site (P>0.05). For example, control-site baseline CVC for the young trained, young sedentary, older trained and older sedentary was 7 ± 1, 8 ± 1, 7 ± 1, and 8 ± 1 %CVCmax, respectively (P>0.05). Furthermore, baseline CVC did not differ among skin sites within any of the groups (P>0.05) (e.g. young sedentary control site: 8 ± 1, BT site: 9 ± 1, and YP site: 9 ± 1 %CVCmax), indicating no effect of pharmacological treatment.

Normalized initial peak

Figure 1 shows the normalized initial peak data for all groups at the control, BT, and YP sites. At the control site, the initial peak of the young trained and older trained (82 ± 3 and 79 ± 3 %CVCmax, respectively) was higher (P<0.05) than that of the young sedentary and older sedentary (74 ± 3 and 66 ± 5 %CVCmax, respectively). The initial peak of the older sedentary was also lower than that of the young sedentary (P<0.05), and there was no difference between the young trained and older trained (P>0.05). The difference in the initial peak between trained and sedentary groups

was more pronounced in the older men compared to the young men (19 ± 4 vs. 11 ± 2 %, respectively; $P<0.05$).

The initial peak at the BT site was lower ($P<0.05$) than that at the control site in all groups except the older sedentary (control minus BT: young trained 10 ± 3, young sedentary 7 ± 2, older trained 9 ± 2, older sedentary 2 ± 1 %CVCmax) (Fig. 1). In addition, the initial peak at the BT site did not differ between the young trained, young sedentary, and older trained ($P>0.05$), whereas the responses of the older sedentary were lower than those of the young trained and older trained ($P<0.05$).

In the young trained, the initial peak at the YP site was lower than that at the control site (control minus YP: 11 ± 3 %CVCmax; $P<0.05$); however, the initial peak was similar between the BT and YP sites (72 ± 6 and 72 ± 4 %CVCmax, respectively; $P>0.05$), suggesting that NE contributes to the initial peak in young trained adults, whereas NPY does not (Fig. 1). In the young sedentary and older trained, the initial peak at the YP site (75 ± 2 and 77 ± 3 %CVCmax, respectively) did not differ ($P>0.05$) to that at the control site (74 ± 3 and 79 ± 4 %CVCmax, respectively). Considering the reduced vasodilator response under conditions of BT but not YP in these groups, this suggests a role for NPY, but not NE, in the initial peak of these groups. As with BT treatment, YP did not effect the initial peak in the older sedentary (control minus YP: 0 ± 1 %CVCmax; $P>0.05$).

Normalized plateau

Figure 2 shows the normalized plateau data for all groups at the control, BT, and YP sites. The plateau at the control site did not differ among groups ($P>0.05$). The plateau at the BT site was lower ($P<0.05$) than that at the control site for the young trained (71 ± 1 vs. 91 ± 2 %CVCmax, respectively) and older trained (85 ± 3 vs. 93 ± 2 %CVCmax, respectively). In contrast, the plateau was similar ($P>0.05$) between BT and control sites in the young sedentary (85 ± 5 vs. 90 ± 3 %CVCmax, respectively) and older sedentary (91 ± 3 vs. 94 ± 1 %CVCmax, respectively). The plateau at the YP site in the young trained and older trained (90 ± 2 and 92 ± 3 %CVCmax, respectively) did not differ to that at the control site ($P>0.05$). In these groups, BT but not YP reduced vasodilatation; this suggests a role for NPY, but not NE, in the plateau of these groups. As with BT treatment, YP did not affect ($P>0.05$) the plateau

in the young sedentary and older sedentary (88 ± 2 and 91 ± 2 %CVCmax, respectively).

Raw CVC responses

The findings for the raw (non-normalized) data (Table 1) are similar to those for the normalized data. There was no effect of group or treatment on baseline responses ($P>0.05$). The control-site initial peak was higher in the young trained compared to all other groups ($P<0.05$), whereas the response of the older sedentary was lower than all other groups ($P<0.05$). There was no difference between the young sedentary and older trained ($P>0.05$). The control-site plateau was higher in the young trained compared to all other groups ($P<0.05$), and there were no differences among the remaining three groups ($P>0.05$). Treatment with BT and YP reduced the initial peak and plateau in the young trained only ($P<0.05$), and these phases did not differ between groups at the BT and YP sites ($P>0.05$). Finally, in the young trained, the initial peak did not differ between BT and YP sites ($P>0.05$), whereas the plateau was lower at the BT site compared to the YP site ($P<0.05$).

DISCUSSION

The main novel findings from this study are: i) the age-related decline in the rapid skin hyperemic response to localized heating is partially explained by a diminished contribution of noradrenergic sympathetic nerves; ii) the contribution of noradrenergic sympathetic nerves to the initial peak is greater in individuals who have a higher aerobic fitness; and, iii) the sympathetic neurotransmitters contributing to the initial peak vary between young trained, young sedentary and older trained adults. Our data suggest that NE contributes to the initial peak of the young trained, whereas NPY does not. Conversely, NPY seems to play a role in the initial peak of the young sedentary and older trained, whereas NE does not. In the older sedentary, there is a significant reduction in the initial peak and no involvement of noradrenergic sympathetic nerves.

Effects of aging on the initial peak and potential mechanisms

The observation that the rapid skin hyperemic response to local heating was higher in both young groups compared to the older sedentary group is consistent with previous findings (24, 33-34). It has been suggested that a smaller initial peak might be associated with a greater risk of local tissue damage in response to directly applied heat (24, 41). This seems logical since a rapid increase in SkBF will minimize the heat transferred to the underlying tissues; however, further research is needed to substantiate this suggestion, especially since the initial peak normally does not usually occur until 3 to 4 min after the initiation of skin heating. In addition, the age-related decline in the initial peak might be associated with impaired wound healing. Indeed, the magnitude of the initial peak reflects sensory nerve function (e.g., (33)), which is known to be an important contributory factor to wound healing capacity (29).

Our current findings suggest that the age-related decline is partly due to a diminished contribution of noradrenergic sympathetic nerves. Indeed, sympathetic nerve blockade decreased the initial peak in the young, but not the older sedentary, such that the between-group difference in the initial peak was smaller at the BT site than that at the control site (Figure 1). This finding may be due to the generalized decline in skin sympathetic efferent activity that occurs with primary aging (10), although other factors such as a reduced release and post-junctional binding of

sympathetic transmitters cannot be excluded. To improve our understanding of this matter, further research is needed to clarify the mechanisms by which noradrenergic sympathetic nerves contribute to local heating-induced cutaneous vasodilation. Previous research suggests that noradrenergic sympathetic nerves may sensitize the vascular responsiveness to local skin heating, which would affect the initial rise (axon reflex) in blood flow. Indeed, Houghton et al. (15) reported that low-dose NE infusion decreased the temperature threshold of the axon reflex response to slow local heating, and Drummond and Lipnicki (7) observed that iontophoresis of NE caused an axon reflex response in immediately adjacent skin that was blocked by pre-treatment with a local anesthetic cream. There might also be an important interaction between NE and/or NPY and the production of NO via endothelial NOS (1, 5, 38), which would probably contribute more to the plateau phase of the local heating response; however, this is yet to be directly tested.

Importantly, the age-related decrement in the initial peak was not completely abolished by sympathetic nerve blockade, indicating that other factors are involved. One such factor might be a diminished functioning of heat-sensitive nociceptors, since sensory nerve function blockade has been shown to abolish the difference in the initial peak between young adults and older sedentary adults (33). Nitric oxide might also be implicated given that it contributes modestly to the initial peak (20, 23); however, a previous study showed that NOS inhibition did not abolish the difference in the initial peak between young and older adults (24). Nevertheless, NO might be involved via potential interactions with sensory nerves (41) and/or noradrenergic sympathetic nerves (13). Further research is needed to understand how noradrenergic sympathetic nerves, sensory nerves, and NO interact in the rapid vasodilator response to local heating and what their respective roles are in the age-related decline in the initial peak. The current data would suggest that there is a considerable degree of redundancy among these systems, similar to what is known with reflex vasodilation in response to increases in core temperature (3).

Effects of aerobic fitness on the initial peak and potential mechanisms

The age-related decline in the initial peak was not present in the older trained, which is consistent with our previous findings (33-34) and indicates that participating in regular aerobic exercise preserves the capacity to rapidly increase SkBF in response

to skin heating into advanced age. Exercise training also appears to be associated with a higher initial peak in young adults; however, the impact of exercise training seems greater in older adults (Figure 1), perhaps because these individuals have greater potential for improvement relative to their younger counterparts. This is consistent with what is known about conduit artery and resistance vessel function; exercise training seems to enhance vascular function to a greater extent in those with depressed function at baseline (35).

The difference in SkBF between the control and BT sites was lower in the sedentary groups, indicating that regular aerobic exercise can also increase the contribution of noradrenergic sympathetic nerves to the initial peak in both young and older adults. As for the aging data, the underpinning mechanisms of this finding are unclear. Nevertheless, the current study provides novel and important data on the effects of aging and aerobic fitness on local heating-induced cutaneous vasodilation and the contribution of sympathetic neurotransmitters to this response. Further research is needed to identify the acute and chronic effects of exercise on cutaneous neurovascular function, including noradrenergic sympathetic nerve function.

Group-specific roles of NE and NPY in the initial peak

Our findings also indicate that the sympathetic neurotransmitters contributing to the initial peak vary between young trained, young sedentary, and older trained adults. Indeed, NE seemed to play a role in the initial peak of the young trained and NPY did not. By contrast, NPY and not NE appeared to play a role in the initial peak of the young sedentary and older trained groups. Neither NE nor NPY appear to be involved in the relatively diminished initial peak of the older sedentary. Aging and sedentary behavior lead to an increase in sympathetic outflow and it is under stressful conditions that NPY appears to play a role in sympathetic function (26, 42). We propose our data indicate that a role for NPY only occurs as a compensatory mechanism. NE is the neurotransmitter usually used (young trained), but with a sedentary lifestyle (young untrained) or primary aging (older trained) it would appear that NPY is required to compensate for a loss of adrenergic function. Currently, we are unable to speculate as to whether this is due to pre- or post-synaptic alterations, i.e. whether these changes are due to alterations in transmitter synthesis and release or in receptor density or affinity. The combination of a sedentary lifestyle and

aging appears to result in a complete loss of sympathetic involvement, such that the initial peak responses under control conditions for the older sedentary group were somewhat similar to the responses achieved at the BT-treated sites for the other three groups. Also note the absence of any change following treatment with BT or YP.

Effects of age and aerobic fitness on the plateau and maximum CVC responses and the role of noradrenergic sympathetic nerves

The secondary plateau of the SkBF response to local heating, did not differ between groups when the data were normalized to maximal CVC (Figure 2), which is in contrast to some of the previous studies that have investigated the impact of age and exercise training on local heating-induced SkBF responses (8-9, 14, 21, 24, 27-28, 39), but not all (2, 25). However, this finding might simply reflect our approach of normalizing data to CVC values recorded during local heating at 44 °C. Although this method, which is used to account for the wide heterogeneity in capillary density across the forearm, is acceptable in healthy young adults and in mechanistically-driven, carefully controlled studies (22), it might be inappropriate for comparing data between young and older adults (24-25), because of the age-associated decline in the maximal SkBF response to local heating (21). For example, the CVC responses during the plateau phase were similar between groups when expressed as %CVCmax, but lower in the older groups compared to the young trained when expressed as raw CVC (Table 1). Therefore, although participants from all groups reached a similar %CVCmax, lower maximal CVC values in aged skin would probably translate to a lower absolute SkBF for a given %CVCmax. Because of this issue, we chose to present data both as raw CVC and %CVCmax. Reassuringly, the interpretation of the initial peak data did not change greatly between these different methods.

Our findings indicate that the contribution of cutaneous sympathetic nerves to the plateau phase of heat-induced vasodilation is dependent on the individual's aerobic fitness. Indeed, sympathetic nerve blockade using BT decreased the plateau in the young and older trained, but not in the young and older sedentary groups (Figure 2). Three other studies have demonstrated that cutaneous noradrenergic sympathetic nerves contribute to the plateau in young healthy adults (12-13, 15). The data of

these studies are not directly comparable with our own because two of the three studies used a slow heating protocol (+0.1 °C·min^{-1}) (13, 15), and the fitness/training status of the participants was unclear throughout. Whereas both NE and NPY contribute to the plateau response to slow local heating (13, 15), it seems that the sympathetic-related contribution to rapid local heating (in the trained groups) involves NPY only. Further research is needed to help understand this difference and how exercise training alters the contribution of sympathetic neurotransmitters.

A final curious observation that warrants discussion is that maximal raw CVC in the young trained group was significantly greater at the control sites compared with the BT and YP treated sites (Table 1). No differences were observed among the sites in the other three groups. This may be due to the fact that the BT and YP treated sites had microdialysis fibers present, while the control site did not; however, as this scenario did not occur in any of the other groups we feel this is unlikely. What this might indicate, is that, in young trained adults, either noradrenergic sympathetic nerves contribute to the maximal CVC response to local heating at 44 °C (similar to what we have observed for initial peak and plateau phase data) or that local heating to 44 °C was an insufficient stimulus to elicit a maximal vasodilator response. As we are unable to definitively state whether or not "true" maximum CVC was obtained at every skin site in the different groups, inspection of the raw CVC responses (Table 1) is particularly important in the interpretation of our results. Reassuringly, both methods of data presentation support our interpretation of the initial peak results.

Experimental considerations

It might be argued that our assessment of drug effects was clouded by the fact that we did not have a microdialysis fiber at the control site. Indeed, it has previously been demonstrated that fiber placement alone decreases the peak reflex cutaneous vasodilator response to whole body heating (11). However, that study also showed that this attenuation did not occur if ice was applied for 5 min before fiber placement. Since we used ice in this manner, any reported between-site differences are probably due to the action of the drugs and not the absence of a microdialysis fiber at the control site. Furthermore, our pilot tests (*n*=5) investigating this issue indicated that the SkBF response to rapid local heating is not affected by ice treatment + fiber placement (e.g., normalized initial peak: fiber 71 ± 7 %CVCmax vs. no-fiber 67 ±

542 7 %CVCmax; raw maximum: fiber 2.56 ± 0.22 au/mmHg vs. no-fiber 2.36 ± 0.12
543 au/mmHg; P = 0.13 and P = 0.40, respectively).
544
545 Another potential limitation is that we did not use a NPY-specific antagonist such as
546 BIBP-3226 to assess the contribution of NPY to the thermal hyperemic response.
547 Hodges et al. (13) previously assessed the contribution of NE and NPY to the
548 cutaneous vasodilator response to slow local heating using a 4-site "Latin-square"
549 design: (i) control, (ii) α- and β-adrenoceptor antagonism, (iii) Y_1-receptor
550 antagonism, and (iv) a combination of (ii) and (iii). We could not use this approach
551 because we currently only have a 3-channel laser Doppler flowmeter. Nevertheless,
552 with only 3 sites (control, neurotransmitter block, and α- and β-adrenoceptor
553 antagonism), we were essentially able to obtain the effects of NE and, indirectly NPY.
554
555 In summary, we present a comparison of cutaneous microvascular responses to
556 localised heating between young and older endurance-trained and sedentary
557 individuals, with specific focus on the initial vasodilator response and the contribution
558 of noradrenergic sympathetic nerves to this phase. At untreated control sites, the
559 initial vasodilator response to local heating was lower in the older sedentary
560 compared to both young groups. The lower responses of the older sedentary
561 appeared to be partly explained by diminished contribution of noradrenergic
562 sympathetic nerves. Our findings also indicate that the sympathetic contribution to
563 the initial peak can be preserved into advanced age by maintaining a high level of
564 aerobic fitness and/or participating in regular aerobic exercise. Finally, the
565 sympathetic neurotransmitters contributing to the initial peak vary between young
566 trained, young sedentary and older trained men. Specifically, NE seems to play a
567 role in the initial peak of young trained men, whereas NPY does not. Conversely,
568 NPY seems to play a role in the initial peak of young sedentary and older trained
569 men, whereas NE does not. Finally, in older sedentary men, the rapid vasodilation is
570 greatly reduced with no involvement of the sympathetic system.
571
572 **ACKNOWLEDGEMENTS**
573 We would like to thank the participants for their time and commitment to this study,
574 which was funded by Sheffield Hallam University.
575

DISCLOSURES

The authors report no potential conflicts of interest, financial or otherwise.

REFERENCES

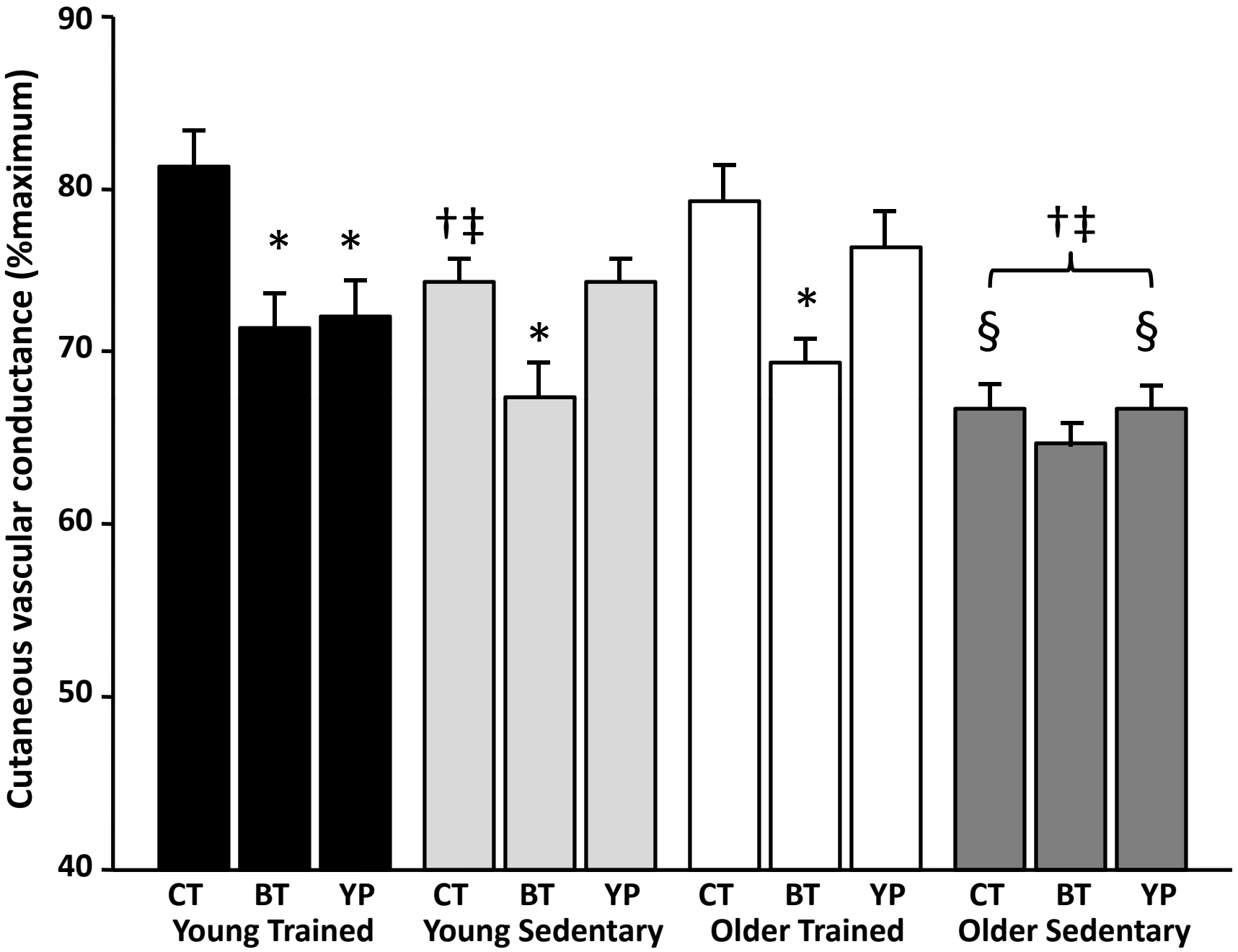
1. **Angus JA, Cocks TM, and Satoh K**. The alpha adrenoceptors on endothelial cells. *Fed Proc* 45: 2355-2359, 1986.
2. **Black MA, Green DJ, and Cable NT**. Exercise prevents age-related decline in nitric-oxide-mediated vasodilator function in cutaneous microvessels. *J Physiol* 586: 3511-3524, 2008.
3. **Charkoudian N**. Mechanisms and modifiers of reflex induced cutaneous vasodilation and vasoconstriction in humans. *J Appl Physiol* 109: 1221-1228, 2010.
4. **Charkoudian N**. Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clin Proc* 78: 603-612, 2003.
5. **Cocks TM, and Angus JA**. Endothelium-dependent relaxation of coronary arteries by noradrenaline and serotonin. *Nature* 305: 627-630, 1983.
6. **Cracowski JL, Minson CT, Salvat-Melis M, and Halliwill JR**. Methodological issues in the assessment of skin microvascular endothelial function in humans. *Trends Pharmacol Sci* 27: 503-508, 2006.
7. **Drummond PD, and Lipnicki DM**. Noradrenaline provokes axon reflex hyperaemia in the skin of the human forearm. *J Auton Nerv Syst* 77: 39-44, 1999.
8. **Evans E, Rendell M, Bartek J, Connor S, Bamisedun O, Dovgan D, and Giitter M**. Thermally-induced cutaneous vasodilatation in aging. *J Gerontol* 48: M53-57, 1993.
9. **Franzoni F, Galetta F, Morizzo C, Lubrano V, Palombo C, Santoro G, Ferrannini E, and Quinones-Galvan A**. Effects of age and physical fitness on microcirculatory function. *Clin Sci (Lond)* 106: 329-335, 2004.
10. **Grassi G, Seravalle G, Turri C, Bertinieri G, Dell'Oro R, and Mancia G**. Impairment of thermoregulatory control of skin sympathetic nerve traffic in the elderly. *Circulation* 108: 729-735, 2003.
11. **Hodges GJ, Chiu C, Kosiba WA, Zhao K, and Johnson JM**. The effect of microdialysis needle trauma on cutaneous vascular responses in humans. *J Appl Physiol* 106: 1112-1118, 2009.
12. **Hodges GJ, Kosiba WA, Zhao K, and Johnson JM**. The involvement of heating rate and vasoconstrictor nerves in the cutaneous vasodilator response to skin warming. *Am J Physiol Heart Circ Physiol* 296: H51-56, 2009.
13. **Hodges GJ, Kosiba WA, Zhao K, and Johnson JM**. The involvement of norepinephrine, neuropeptide Y, and nitric oxide in the cutaneous vasodilator response to local heating in humans. *J Appl Physiol* 105: 233-240, 2008.
14. **Hodges GJ, Sharp L, Stephenson C, Patwala AY, George KP, Goldspink DF, and Tim Cable N**. The effect of 48 weeks of aerobic exercise training on cutaneous vasodilator function in post-menopausal females. *Eur J Appl Physiol* 108: 1259-1267, 2010.
15. **Houghton BL, Meendering JR, Wong BJ, and Minson CT**. Nitric oxide and noradrenaline contribute to the temperature threshold of the axon reflex response to gradual local heating in human skin. *J Physiol* 572: 811-820, 2006.
16. **Howley ET, Bassett DR, Jr., and Welch HG**. Criteria for maximal oxygen uptake: review and commentary. *Med Sci Sports Exerc* 27: 1292-1301, 1995.

623 17. **Johnson JM, and Kellogg DL, Jr.** Local thermal control of the human
624 cutaneous circulation. *J Appl Physiol* 109: 1229-1238, 2010.
625 18. **Johnson JM, Yen TC, Zhao K, and Kosiba WA**. Sympathetic, sensory, and
626 nonneuronal contributions to the cutaneous vasoconstrictor response to local cooling.
627 *Am J Physiol Heart Circ Physiol* 288: H1573-1579, 2005.
628 19. **Kellogg DL, Jr., Johnson JM, and Kosiba WA**. Selective abolition of
629 adrenergic vasoconstrictor responses in skin by local iontophoresis of bretylium. *Am*
630 *J Physiol* 257: H1599-1606, 1989.
631 20. **Kellogg DL, Jr., Liu Y, Kosiba IF, and O'Donnell D**. Role of nitric oxide in
632 the vascular effects of local warming of the skin in humans. *J Appl Physiol* 86: 1185-
633 1190, 1999.
634 21. **Martin HL, Loomis JL, and Kenney WL**. Maximal skin vascular conductance
635 in subjects aged 5-85 yr. *J Appl Physiol* 79: 297-301, 1995.
636 22. **Minson CT**. Thermal Provocation to Evaluate Microvascular Reactivity in
637 Human Skin. *Journal of applied physiology (Bethesda, Md: 1985)* 2010.
638 23. **Minson CT, Berry LT, and Joyner MJ**. Nitric oxide and neurally mediated
639 regulation of skin blood flow during local heating. *J Appl Physiol* 91: 1619-1626,
640 2001.
641 24. **Minson CT, Holowatz LA, Wong BJ, Kenney WL, and Wilkins BW**.
642 Decreased nitric oxide- and axon reflex-mediated cutaneous vasodilation with age
643 during local heating. *J Appl Physiol* 93: 1644-1649, 2002.
644 25. **Munce TA, and Kenney WL**. Age-specific modification of local cutaneous
645 vasodilation by capsaicin-sensitive primary afferents. *Journal of applied physiology*
646 *(Bethesda, Md: 1985)* 95: 1016-1024, 2003.
647 26. **Qureshi NU, Dayao EK, Shirali S, Zukowska-Grojec Z, and Hauser GJ**.
648 Endogenous neuropeptide Y mediates vasoconstriction during endotoxic and
649 hemorrhagic shock. *Regul Pept* 75-76: 215-220, 1998.
650 27. **Richardson D**. Effects of age on cutaneous circulatory response to direct
651 heat on the forearm. *J Gerontol* 44: M189-194, 1989.
652 28. **Rooke GA, Savage MV, and Brengelmann GL**. Maximal skin blood flow is
653 decreased in elderly men. *J Appl Physiol* 77: 11-14, 1994.
654 29. **Roosterman D, Goerge T, Schneider SW, Bunnett NW, and Steinhoff M**.
655 Neuronal control of skin function: the skin as a neuroimmunoendocrine organ.
656 *Physiol Rev* 86: 1309-1379, 2006.
657 30. **Rowell LB**. Cardiovascular aspects of human thermoregulation. *Circ Res* 52:
658 367-379, 1983.
659 31. **Stephens DP, Saad AR, Bennett LA, Kosiba WA, and Johnson JM**.
660 Neuropeptide Y antagonism reduces reflex cutaneous vasoconstriction in humans.
661 *Am J Physiol Heart Circ Physiol* 287: H1404-1409, 2004.
662 32. **Strom NA, Sawyer JR, Roberts SK, Kingsley-Berg SM, and Charkoudian**
663 **N**. Local sensory nerve control of skin blood flow during local warming in type 2
664 diabetes mellitus. *J Appl Physiol* 108: 293-297, 2010.
665 33. **Tew GA, Klonizakis M, Moss J, Ruddock AD, Saxton JM, and Hodges GJ**.
666 Role of sensory nerves in the rapid cutaneous vasodilator response to local heating
667 in young and older endurance-trained and untrained men. *Exp Physiol* 2010.
668 34. **Tew GA, Klonizakis M, and Saxton JM**. Effects of ageing and fitness on
669 skin-microvessel vasodilator function in humans. *Eur J Appl Physiol* 109: 173-181,
670 2010.

35. **Thijssen DH, Maiorana AJ, O'Driscoll G, Cable NT, Hopman MT, and Green DJ**. Impact of inactivity and exercise on the vasculature in humans. *Eur J Appl Physiol* 108: 845-875, 2010.
36. **Thompson CS, Holowatz LA, and Kenney WL**. Cutaneous vasoconstrictor responses to norepinephrine are attenuated in older humans. *Am J Physiol Regul Integr Comp Physiol* 288: R1108-1113, 2005.
37. **Van Duijnhoven NT, Janssen TW, Green DJ, Minson CT, Hopman MT, and Thijssen DH**. Effect of functional electrostimulation on impaired skin vasodilator responses to local heating in spinal cord injury. *J Appl Physiol* 106: 1065-1071, 2009.
38. **Vanhoutte PM, and Miller VM**. Alpha 2-adrenoceptors and endothelium-derived relaxing factor. *Am J Med* 87: 1S-5S, 1989.
39. **Weiss M, Milman B, Rosen B, Eisenstein Z, and Zimlichman R**. Analysis of the diminished skin perfusion in elderly people by laser Doppler flowmetry. *Age Ageing* 21: 237-241, 1992.
40. **Wilson TE, Monahan KD, Short DS, and Ray CA**. Effect of age on cutaneous vasoconstrictor responses to norepinephrine in humans. *Am J Physiol Regul Integr Comp Physiol* 287: R1230-1234, 2004.
41. **Wong BJ, and Fieger SM**. Transient receptor potential vanilloid type-1 (TRPV-1) channels contribute to cutaneous thermal hyperaemia in humans. *J Physiol* 588: 4317-4326, 2010.
42. **Zukowska-Grojec Z, and Vaz AC**. Role of neuropeptide Y (NPY) in cardiovascular responses to stress. *Synapse* 2: 293-298, 1988.

712 **FIGURE LEGENDS**

713 Fig. 1: Initial vasodilation to local skin heating. Data are means + S.E.M. for each
714 group and skin site. CT, control sites; BT, bretylium tosylate treated sites; YP,
715 yohimbine and propranolol treated sites. Symbols indicate $P<0.05$ as follows: * *vs.*
716 control site within group; † *vs.* young trained; ‡ *vs*. older trained; § *vs.* young untrained.
717
718 Fig. 2: Normalized secondary plateau CVC responses to local heating. Data are
719 means + S.E.M. for each group and skin site. CT, control sites; BT, bretylium tosylate
720 treated sites; YP, yohimbine and propranolol treated sites. Symbols indicate $P<0.05$
721 as follows: * *vs.* control site within group.


Cutaneous vascular conductance (%maximum)
90
80
70
60
50
40
*
*
†‡
*
*
†‡
§
§
CT
BT
YP
CT
BT
YP
CT
BT
YP
CT
BT
YP
Young Trained
Young Sedentary
Older Trained
Older Sedentary

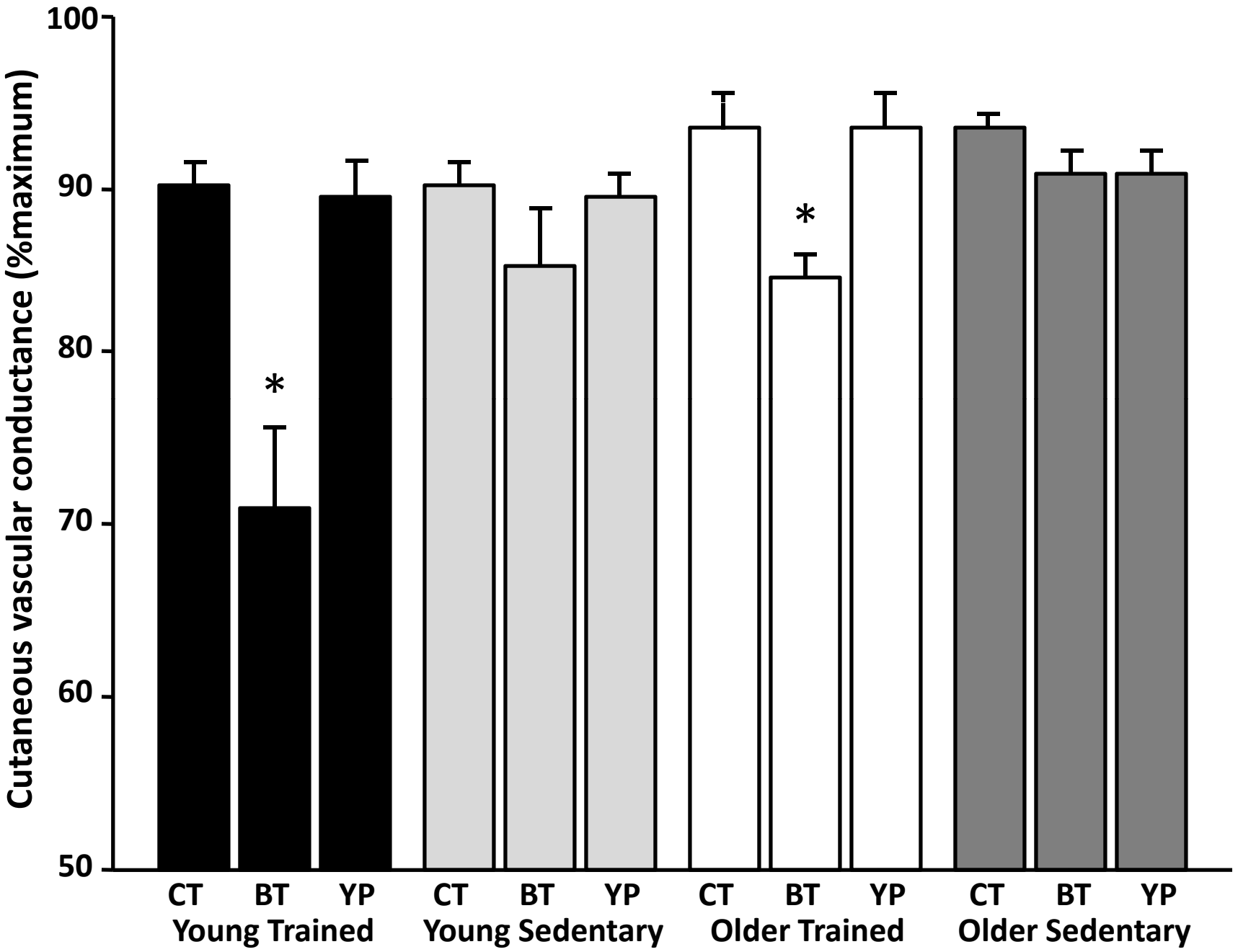

Cutaneous vascular conductance (%maximum)
100
90
80
70
60
50
*
*
CT
BT
YP
CT
BT
YP
CT
BT
YP
CT
BT
YP
Young Trained
Young Sedentary
Older Trained
Older Sedentary

Table 1. Raw CVC data for each phase of the local heating protocol

	Young trained	Young sedentary	Older trained	Older sedentary
Baseline				
CT	0.23 ± 0.03	0.22 ± 0.02	0.25 ± 0.03	0.20 ± 0.03
BT	0.19 ± 0.03	0.23 ± 0.03	0.20 ± 0.03	0.25 ± 0.02
YP	0.24 ± 0.02	0.26 ± 0.02	0.27 ± 0.04	0.26 ± 0.04
Initial peak				
CT	2.66 ± 0.13	2.05 ± 0.13†	2.02 ± 0.23†	1.75 ± 0.12†‡§
BT	1.70 ± 0.28*	2.01 ± 0.22	1.95 ± 0.34	1.82 ± 0.19
YP	1.77 ± 0.20*	1.89 ± 0.13	2.05 ± 0.25	1.67 ± 0.07
Plateau				
CT	2.95 ± 0.16	2.48 ± 0.15†	2.38 ± 0.25†	2.50 ± 0.11†
BT	1.77 ± 0.36*	2.39 ± 0.36†	2.35 ± 0.36†	2.63 ± 0.32†
YP	2.18 ± 0.15$^{*\$}$	2.48 ± 0.22†	2.42 ± 0.24†	2.46 ± 0.36†
Maximum				
CT	3.27 ± 0.18	2.74 ± 0.23†	2.55 ± 0.20†	2.64 ± 0.25†
BT	2.42 ± 0.24*	2.73 ± 0.26	2.73 ± 0.28	2.87 ± 0.27
YP	2.44 ± 0.17*	2.80 ± 0.22	2.64 ± 0.24	2.65 ± 0.36

Data are expressed as means ± S.E.M. CVC, cutaneous vascular conductance. *Different to the corresponding control (CT) site (P<0.05); $^{\$}$Different to the corresponding bretylium (BT) site (P<0.05); †Different to the young trained (P<0.05); ‡Different to the young sedentary (P<0.05); §Different to the older sedentary (P<0.05).