

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

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34 **ABSTRACT**

35 Sedentary aging results in a diminished rapid cutaneous vasodilator response to
36 local heating. We investigated whether this diminished response was due to altered
37 contributions of noradrenergic sympathetic nerves; assessing 1) the age-related
38 decline and, 2) the effect of aerobic fitness. We measured skin blood flow (SkBF)
39 (laser-Doppler flowmetry) in young (24 ± 1 yr) and older (64 ± 1 yr) endurance-trained
40 and sedentary men ($n=7$ per group) at baseline and during 35 min of local skin
41 heating to $42\text{ }^{\circ}\text{C}$ at three forearm sites: 1) untreated; 2) bretylium tosylate (BT),
42 preventing neurotransmitter release from noradrenergic sympathetic nerves; and 3)
43 yohimbine and propranolol (YP), antagonising α - and β -adrenergic receptors. SkBF
44 was converted to cutaneous vascular conductance (CVC) (SkBF/mean arterial
45 pressure) and normalized to maximal CVC (%CVCmax) achieved by skin heating to
46 $44\text{ }^{\circ}\text{C}$. Pharmacological agents were administered using microdialysis. In the young
47 trained, the rapid vasodilator response was reduced at the BT and YP sites ($P<0.05$);
48 by contrast, in the young sedentary and older trained, YP had no effect ($P>0.05$) but
49 treatment with BT did ($P>0.05$). Neither BT nor YP treatments affected the rapid
50 vasodilator response in the older sedentary group ($P>0.05$). These data suggest that
51 the age-related reduction in the rapid vasodilator response is due to an impairment
52 of sympathetic-dependent mechanisms, which can be partly attenuated with habitual
53 aerobic exercise. Rapid vasodilation involves noradrenergic neurotransmitters in
54 young trained men, and non-adrenergic sympathetic cotransmitters (e.g.,
55 neuropeptide Y) in young sedentary and older trained men, possibly as a
56 compensatory mechanism. Finally, in older sedentary men, the rapid vasodilation
57 appears not to involve the sympathetic system.

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68 **INTRODUCTION**

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

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78 The skin hyperemic response to a non-painful, rapid heat stimulus is commonly used
79 as a test of microvascular and endothelial function (6, 22) and involves at least two
80 independent phases: an initial, rapid transitory rise, followed by a nadir, ultimately
81 succeeded by a secondary rise and prolonged plateau (20, 23). The specific
82 mechanisms underpinning these phases are complex and not completely understood.
83 The rapid initial peak of the vasodilator response is thought to be primarily mediated
84 by an axon reflex via activation of transient receptor potential vanilloid-1 (TRPV-1)
85 receptors in C-fibre afferent nociceptive neurones (41). These sensory neurones
86 might increase skin blood flow (SkBF) through the release of neuropeptides such as
87 calcitonin gene-related peptide and/or substance P (4); however, these theories
88 have yet to be directly tested. Additionally, NO has been shown to contribute
89 modestly to the initial peak of the vasodilator response to rapid local heating (20, 23).
90 The secondary rise and plateau in SkBF, in contrast, is heavily dependent on NO
91 synthesis as inhibition of NO synthase (NOS) reduces this phase by approximately
92 70% (20, 23).

93

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

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

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

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

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136 **MATERIALS AND METHODS**

137 **Ethics approval**

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

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142 **Participants**

143 We recruited 28 men who were equally divided among four groups: young
144 endurance-trained (24 ± 1 yr), young sedentary (25 ± 1 yr), older endurance-trained
145 (64 ± 1 yr) and older sedentary (64 ± 1 yr). The trained participants were recruited
146 from running and cycling clubs in and around Sheffield, UK. They had all performed
147 vigorous endurance exercise for ≥ 3 times \cdot week $^{-1}$, ≥ 30 min \cdot session $^{-1}$ and ≥ 5 years.
148 The sedentary participants reported undertaking no regular exercise. All participants
149 were healthy, non-smokers, free from cardiovascular disease and diabetes, and
150 were not taking any medications. The participants attended the testing facility on two
151 separate occasions. For both sessions, they were asked to refrain from caffeine,
152 alcohol, and exercise for 24 h prior to their attendance. The participants are the
153 same as those described in a recently published article by our group (33).

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155 **Visit 1: Assessment of cardiopulmonary fitness**

156 Participants completed a continuous, incremental cycling test to volitional exhaustion
157 on an electronically-braked cycle ergometer (Excalibur Sport, Lode, The
158 Netherlands). Pedalling frequency was self-selected within the range of 60 to 90
159 rev \cdot min $^{-1}$. After a 2-min warm-up against no resistance (0 W), the intensity of
160 exercise was increased by 20 to 30 W \cdot min $^{-1}$. Participants were encouraged to
161 continue cycling to volitional exhaustion or until a plateau in oxygen consumption
162 was observed. Heart rate was recorded continuously by electrocardiogram
163 (Cardioperfect, Welch Allyn, USA). The volume of oxygen consumed during exercise
164 was calculated from minute ventilation, measured using a pneumotach, and
165 simultaneous breath-by-breath analysis of expired gas fractions (Ultima CardIO₂,
166 MedGraphics, USA). Gas analysers and flow probes were calibrated before each
167 test. Oxygen consumption was expressed relative to body mass (mL \cdot kg $^{-1}$ \cdot min $^{-1}$).
168 Maximal oxygen consumption ($\dot{V} O_{2\max}$) was calculated as the highest consecutive

169 20-second period of gas exchange data in the last minute before volitional
170 exhaustion, which generally occurred due to leg fatigue and/or breathlessness.

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172 **Visit 2: Assessment of SkBF responses to local heating**

173 ***Instrumentation***

174 The microvascular assessments were performed in a temperature-controlled room
175 (22 to 24°C) with participants resting supine and the experimental (left) arm
176 positioned at heart level for the entire protocol. Blood pressure was measured
177 automatically on the right arm every 2 min (Dinamap Dash 2500, GE Healthcare,
178 USA).

179

180 Two microdialysis fibers (Linear 30, CMA Microdialysis Ltd, Stockholm, Sweden)
181 with a membrane length of 10 mm and a 6-kDa molecular mass cut-off were placed
182 ~5 cm apart in the dermal layer of skin on the ventral aspect of the left forearm.
183 Before implantation, the skin was temporarily anesthetised by applying an ice pack
184 for 5 min (11). A 21-gauge needle was introduced aseptically into the dermis along a
185 length of ~2.5 cm before exiting. A microdialysis fiber was threaded through the
186 lumen of the needle, before removing the needle to leave the fiber in place. All
187 microdialysis fibers were placed in this manner. To allow for the effects of the
188 insertion trauma to subside, we waited 1.5-2 h before beginning the protocol (13).

189

190 To obtain an index of SkBF, cutaneous red blood cell flux was measured using laser
191 Doppler flowmetry (Periflux 5000 System, Perimed AB, Järfälla, Sweden) at the two
192 microdialysis sites, and at a third "no fiber" control site. Local heater discs (Model
193 455, Perimed AB) were used to control local skin temperature and integrating laser
194 Doppler probes (Model 413, Perimed AB) were placed in the centre of each local
195 heating disc.

196

197 ***Drugs***

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

203

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

212

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

215

216 **Protocol**

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

223

224 **Data collection and analysis**

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

231

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

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

240

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

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271 **RESULTS**

272 **Participant characteristics**

273 The characteristics of the participants have been reported previously (33). Briefly,
274 the groups did not differ in body mass, stature, or resting systolic or diastolic blood
275 pressure ($P>0.05$). All participants were normotensive and achieved $\dot{V} O_{2\max}$
276 according to standard criteria (16). The $\dot{V} O_{2\max}$ of the young trained ($58 \pm 3 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$)
277 ($1\cdot\text{min}^{-1}$) was higher ($P<0.05$) than that of the young sedentary ($40 \pm 2 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$),
278 older trained ($44 \pm 2 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), and older sedentary ($28 \pm 2 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$). The
279 $\dot{V} O_{2\max}$ of the older sedentary was lower than that of all other groups ($P<0.05$), and
280 there was no difference between the young sedentary and older trained ($P>0.05$).

281

282 **CVC responses**

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

287

288 *Normalized baseline*

289 Baseline CVC did not differ among groups at each skin site ($P>0.05$). For example,
290 control-site baseline CVC for the young trained, young sedentary, older trained and
291 older sedentary was 7 ± 1 , 8 ± 1 , 7 ± 1 , and 8 ± 1 %CVCmax, respectively ($P>0.05$).
292 Furthermore, baseline CVC did not differ among skin sites within any of the groups
293 ($P>0.05$) (e.g. young sedentary control site: 8 ± 1 , BT site: 9 ± 1 , and YP site: $9 \pm$
294 1 %CVCmax), indicating no effect of pharmacological treatment.

295

296 *Normalized initial peak*

297 Figure 1 shows the normalized initial peak data for all groups at the control, BT, and
298 YP sites. At the control site, the initial peak of the young trained and older trained (82
299 ± 3 and 79 ± 3 %CVCmax, respectively) was higher ($P<0.05$) than that of the young
300 sedentary and older sedentary (74 ± 3 and 66 ± 5 %CVCmax, respectively). The
301 initial peak of the older sedentary was also lower than that of the young sedentary
302 ($P<0.05$), and there was no difference between the young trained and older trained
303 ($P>0.05$). The difference in the initial peak between trained and sedentary groups

304 was more pronounced in the older men compared to the young men (19 ± 4 vs. $11 \pm$
305 2 %, respectively; $P < 0.05$).

306

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

313

314 In the young trained, the initial peak at the YP site was lower than that at the control
315 site (control minus YP: 11 ± 3 %CVCmax; $P < 0.05$); however, the initial peak was
316 similar between the BT and YP sites (72 ± 6 and 72 ± 4 %CVCmax, respectively;
317 $P > 0.05$), suggesting that NE contributes to the initial peak in young trained adults,
318 whereas NPY does not (Fig. 1). In the young sedentary and older trained, the initial
319 peak at the YP site (75 ± 2 and 77 ± 3 %CVCmax, respectively) did not differ
320 ($P > 0.05$) to that at the control site (74 ± 3 and 79 ± 4 %CVCmax, respectively).
321 Considering the reduced vasodilator response under conditions of BT but not YP in
322 these groups, this suggests a role for NPY, but not NE, in the initial peak of these
323 groups. As with BT treatment, YP did not effect the initial peak in the older sedentary
324 (control minus YP: 0 ± 1 %CVCmax; $P > 0.05$).

325

326 *Normalized plateau*

327 Figure 2 shows the normalized plateau data for all groups at the control, BT, and YP
328 sites. The plateau at the control site did not differ among groups ($P > 0.05$). The
329 plateau at the BT site was lower ($P < 0.05$) than that at the control site for the young
330 trained (71 ± 1 vs. 91 ± 2 %CVCmax, respectively) and older trained (85 ± 3 vs. $93 \pm$
331 2 %CVCmax, respectively). In contrast, the plateau was similar ($P > 0.05$) between BT
332 and control sites in the young sedentary (85 ± 5 vs. 90 ± 3 %CVCmax, respectively)
333 and older sedentary (91 ± 3 vs. 94 ± 1 %CVCmax, respectively). The plateau at the
334 YP site in the young trained and older trained (90 ± 2 and 92 ± 3 %CVCmax,
335 respectively) did not differ to that at the control site ($P > 0.05$). In these groups, BT but
336 not YP reduced vasodilatation; this suggests a role for NPY, but not NE, in the
337 plateau of these groups. As with BT treatment, YP did not affect ($P > 0.05$) the plateau

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

340

341 *Raw CVC responses*

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

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372 **DISCUSSION**

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

384

385 **Effects of aging on the initial peak and potential mechanisms**

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

398

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

406 sympathetic transmitters cannot be excluded. To improve our understanding of this
407 matter, further research is needed to clarify the mechanisms by which noradrenergic
408 sympathetic nerves contribute to local heating-induced cutaneous vasodilation.
409 Previous research suggests that noradrenergic sympathetic nerves may sensitize
410 the vascular responsiveness to local skin heating, which would affect the initial rise
411 (axon reflex) in blood flow. Indeed, Houghton et al. (15) reported that low-dose NE
412 infusion decreased the temperature threshold of the axon reflex response to slow
413 local heating, and Drummond and Lipnicki (7) observed that iontophoresis of NE
414 caused an axon reflex response in immediately adjacent skin that was blocked by
415 pre-treatment with a local anesthetic cream. There might also be an important
416 interaction between NE and/or NPY and the production of NO via endothelial NOS (1,
417 5, 38), which would probably contribute more to the plateau phase of the local
418 heating response; however, this is yet to be directly tested.

419

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

435

436 **Effects of aerobic fitness on the initial peak and potential mechanisms**

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

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

447

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

457

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

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

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

479

480 **Effects of age and aerobic fitness on the plateau and maximum CVC** 481 **responses and the role of noradrenergic sympathetic nerves**

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

501

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

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

515

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

530

531 **Experimental considerations**

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

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

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575

576 **DISCLOSURES**

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

578

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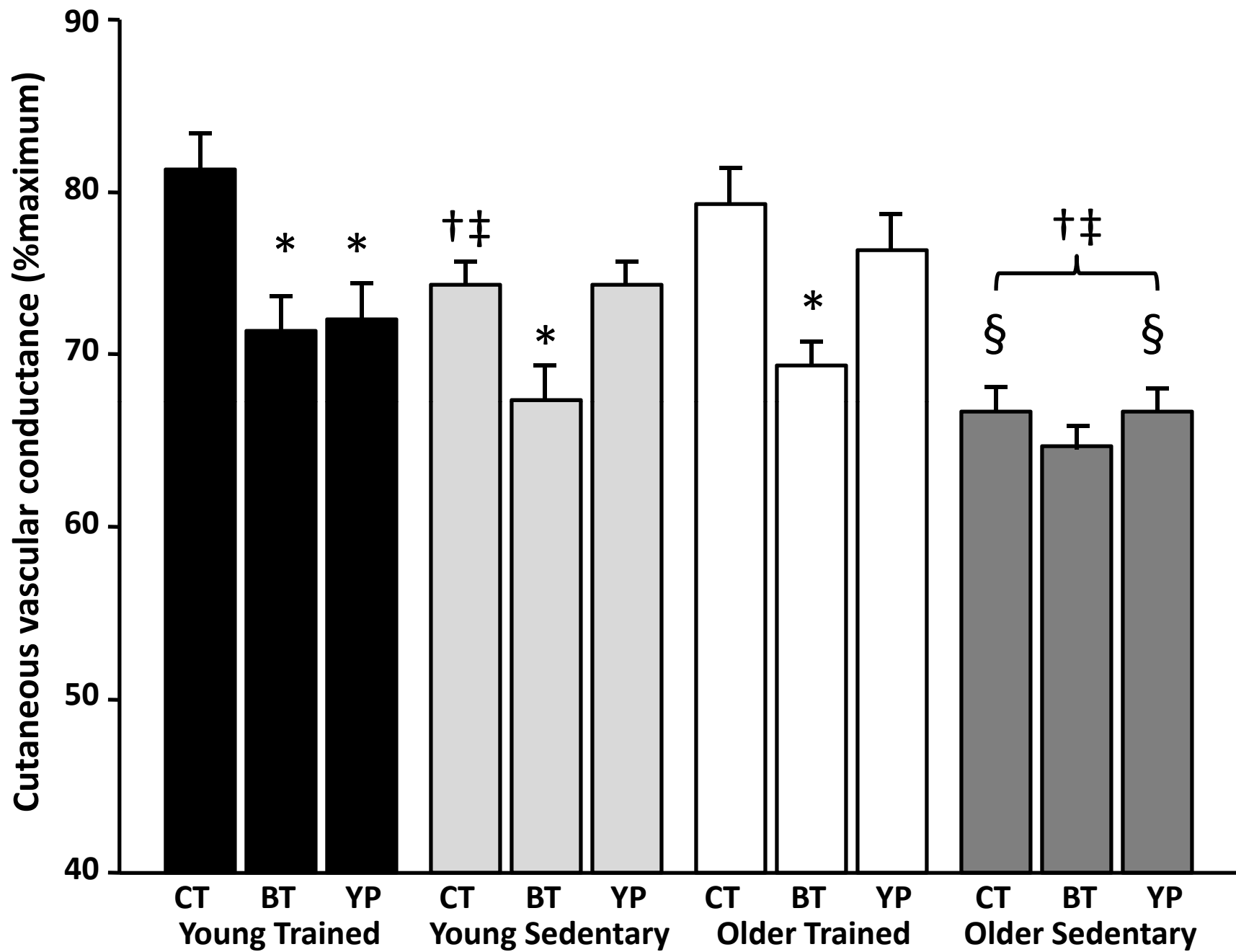
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712 **FIGURE LEGENDS**

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.



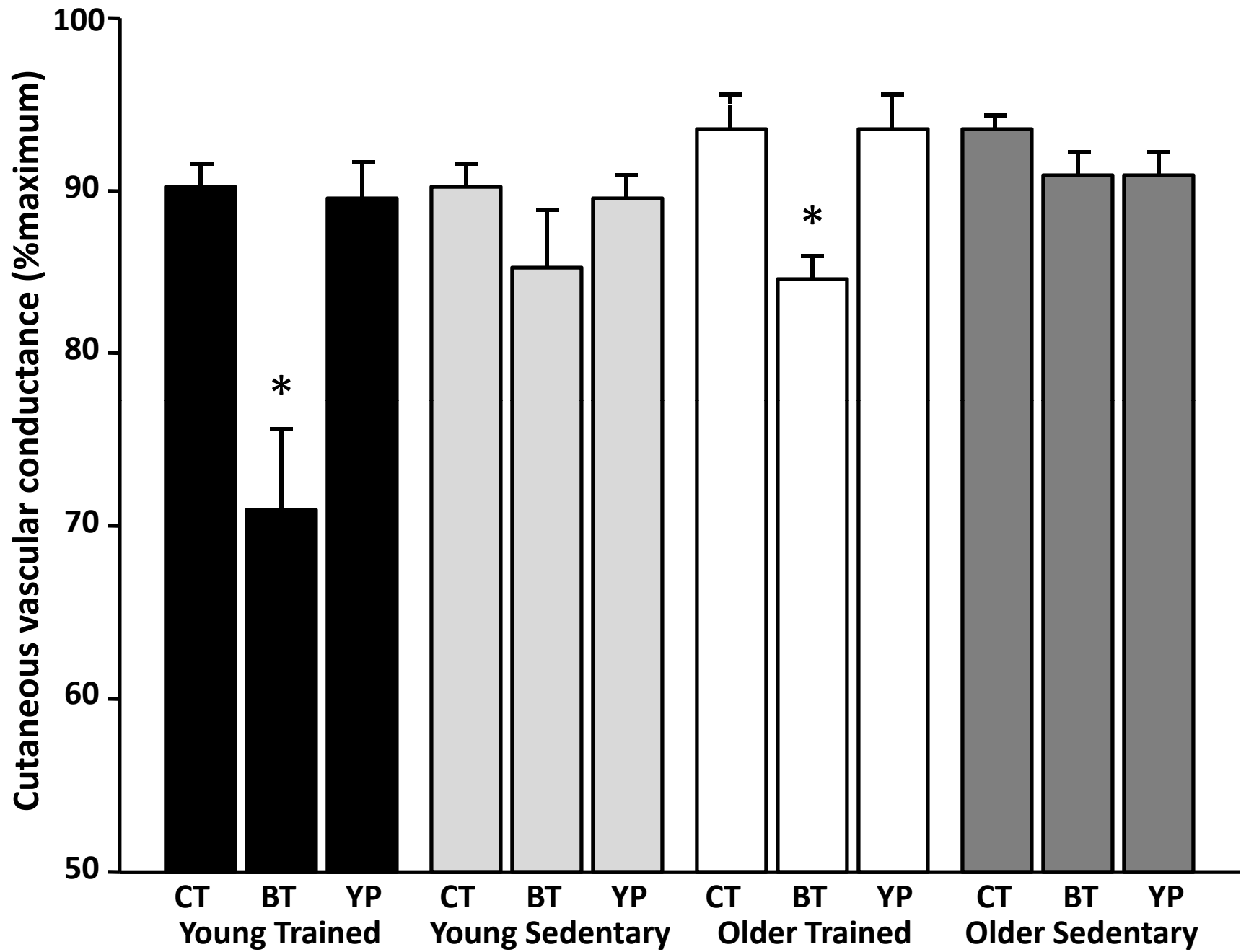


Table 1. Raw CVC data for each phase of the local heating protocol

| | 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 ($P<0.05$).