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**Acute black tea consumption improves cutaneous vascular function in healthy middle-aged humans.**

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### Article

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## 1 ABSTRACT

2 **Background & Aims.** Dietary flavonoids, such as those present in black tea, are  
3 associated with reduced risk of cardiovascular disease (CVD), possibly through  
4 improving nitric oxide (NO) mediated vascular function. The aim of this study was to  
5 examine the effect of acute black tea ingestion on cutaneous microvascular function.

6 **Methods.** Twenty healthy participants ( $58 \pm 5$  yr, 9 men) attended two experimental  
7 trials (tea, placebo), 7-days apart in a randomised, controlled, double-blind, cross-over  
8 design. Participants ingested a single dose of 200ml black tea or placebo, followed by  
9 assessment of forearm cutaneous perfusion using laser-Doppler flowmetry (LDF)  
10 using three distinct heating protocols, enabling us to distinguish between axon- and  
11 endothelium-dependent vasodilation: 1. *rapid 42°C*, 2. *rapid 39°C* and 3. *gradual 42°C*.  
12 On the contralateral arm, full-field laser perfusion imaging (FLPI) was used to assess  
13 forearm perfusion during *gradual 42°C*. Data were presented as cutaneous vascular  
14 conductance (CVC; flux/mean arterial pressure, MAP) and CVC expressed as a  
15 percentage of maximal CVC (%CVC<sub>max</sub>).

16 **Results.** Rapid local heating to 39°C or 42°C demonstrated no effect of tea for flux,  
17 CVC or %CVC<sub>max</sub> (all  $P > 0.05$ ). Gradual local heating to 42°C, however, produced a  
18 higher skin blood flow following black tea ingestion for absolute CVC ( $P = 0.04$ ) when  
19 measured by LDF, and higher absolute flux ( $P < 0.001$ ) and CVC ( $P < 0.001$ ) measured  
20 with FLPI. No effect of tea was found for %CVC<sub>max</sub> when assessed by either LDF or  
21 FLPI.

22 **Conclusions.** Acute tea ingestion enhanced cutaneous vascular responses to  
23 gradual local heating to 42°C in healthy, middle-aged participants, possibly through a  
24 mechanism related to activation of endothelium-derived chemical mediators, such as

25 NO. These improvements may contribute to the cardiovascular health benefits of  
26 regular tea ingestion.

27

28 **Key words: tea; flavonoids; cardiovascular health; vascular function;**  
29 **microcirculation.**

## 30 INTRODUCTION

31 Cardiovascular disease (CVD) remains the leading cause of global mortality,  
32 representing ~30% of all deaths.<sup>1</sup> The role of dietary factors on CVD risk has been  
33 frequently explored in recent years, with a high dietary flavonoid intake being  
34 associated with a reduction in CVD risk.<sup>2</sup> Tea, produced from the plant *Camillia sinesis*,  
35 is the major dietary source of flavonoids in many countries globally<sup>3</sup> and can be found  
36 as catechins and flavonols in green tea and theaflavins, thearubigins and flavonols in  
37 black tea.<sup>4</sup> Accordingly, several studies have revealed a strong, inverse relation  
38 between regular intake of tea and cardiovascular risk.<sup>5, 6</sup>

39

40 A frequently cited explanation for the cardioprotective effects of black and green tea  
41 ingestion relates to the reduction in blood pressure following chronic consumption.<sup>5-7</sup>  
42 Further research found that acute and regular tea ingestion improves nitric oxide-  
43 mediated, endothelium-dependent dilation of conduit arteries.<sup>6, 8-11</sup> Both conduit and  
44 resistance vessels have demonstrated improved endothelial function following tea  
45 ingestion in both healthy individuals<sup>6, 8</sup> and in those with CVD.<sup>10</sup> Thus, the general  
46 consensus is that regular tea ingestion improves blood pressure by virtue of a  
47 generalised improvement of endothelial function and lowering of peripheral vascular  
48 resistance.<sup>6, 8, 10-12</sup>

49

50 Despite encouraging data supporting a beneficial effect of tea ingestion in larger  
51 (conduit) vessels, no previous study has explored the effect of black tea on small  
52 vessels (skin microcirculation). Therefore, our aim was to examine cutaneous vascular  
53 responses to local skin heating. Given the complexity of the cutaneous vascular  
54 system and contribution of distinct mechanisms for skin dilation when gradually or

55 rapidly heating the skin, we adopted a comprehensive approach of using rapid *and*  
56 gradual local skin heating protocols simultaneously. We hypothesised that black tea  
57 ingestion would be associated with increased cutaneous microcirculation responses  
58 for both rapid and gradual heating protocols.

59

## 60 **MATERIALS AND METHODS**

### 61 **Participants**

62 Twenty middle-aged male (n=9) and post-menopausal female (n=11) participants  
63 were recruited through local advertisement. All participants were healthy and non-  
64 smokers (58±5yrs, height 1.70±0.1m, weight 75.9±16.1kg, BMI 26±4 kg/m<sup>2</sup>, baseline  
65 mean arterial pressure 104±8mmHg). Individuals with a medical history of  
66 hypercholesterolaemia (total cholesterol >6.5mmol/l),<sup>13</sup> cardiovascular disease and  
67 hypertension (systolic blood pressure ≥140mmHg, diastolic blood pressure  
68 ≥90mmHg)<sup>14</sup> were excluded. Participants were not taking any vasoactive medications  
69 or supplements. After being fully informed of the methods, written informed consent  
70 was obtained from all participants. The study conformed to the Declaration of Helsinki  
71 and was approved by the Research Ethics Committee of Liverpool John Moores  
72 University.

73

### 74 **Experimental Design**

75 All participants performed two experimental trials (tea and control), 7-days apart in a  
76 randomised, controlled, double-blind, cross-over design (figure 1). The cross-over  
77 design was chosen to eliminate between-participant variability, taking into account a  
78 6-day washout period between the two interventions to avoid any carry-over effects,  
79 which is in accordance with previous similar designed cross-over tea vascular function

80 studies.<sup>6,7</sup> Computer-generated randomisation was used to reduce potential selection  
81 bias. Upon arrival to the laboratory, and 2h prior to microvascular assessment,  
82 participants ingested a tea drink (containing 300 mg flavonoids, 75 mg caffeine and  
83 2.8 g sucrose) or a taste and appearance matched placebo drink (0 mg flavonoids, 75  
84 mg caffeine, 2.7 g sucrose, tea flavour and caramel colour), prepared by dissolving  
85 two sachets in 200 ml hot water. Participants subsequently rested for 2h prior to  
86 commencement of testing to match peak plasma concentrations of flavonoids and  
87 other metabolites such as phenolic acids, with testing of skin microcirculation. During  
88 each testing day, baseline and thermally stimulated forearm cutaneous blood flow was  
89 examined simultaneously using rapid (to 39 and 42°C) and gradual (to 42°C) local  
90 heating protocols. Since these protocols reflect different dilator mechanisms and a  
91 distinct role of the NO-pathway, they provide complementary insight into the impact of  
92 black tea on cutaneous microvasculature. Rapid local heating was performed at two  
93 different sites (i.e. two different local heating protocols) on the dominant forearm and  
94 examined using laser Doppler flowmetry (LDF). Gradual local heating to 42°C was  
95 performed on the dominant forearm using LDF and on the contralateral (non-dominant)  
96 arm using laser speckle imaging to provide whole forearm cutaneous microcirculation  
97 function.

98

### 99 **Experimental Measures**

100 All participants fasted for at least six hours and refrained from alcohol, food products  
101 high in polyphenols (dark chocolate, red wine), caffeine and exercise for 24h prior to  
102 testing.<sup>15</sup> Participants were asked to refrain from drinking all types of tea for a period  
103 of one week prior to each trial. Sips of water were permitted prior to testing to ensure

104 that participants were well hydrated. All trials were conducted in a quiet, temperature  
105 controlled environment ( $23.4\pm 0.6^{\circ}\text{C}$ )<sup>15, 16</sup> and at the same time of day to reduce any  
106 circadian influences on vascular function.<sup>15</sup> Following a 20-minute stabilisation period,  
107 the LDF equipment was calibrated using two generic points, 0 and 250 PU, a zeroing  
108 disk and motility standard, according to manufacturer's guidelines (Perimed AB,  
109 Järfälla, Stockholm, Sweden). Two hours following tea ingestion, participants  
110 assumed a comfortable, supine position on a bed, with the head slightly elevated and  
111 the hand of each testing arm relaxed, supinated and supported by a vacuum cushion  
112 to minimise microcirculatory fluctuations resulting from motion artefact.<sup>15, 16</sup> If  
113 necessary, forearm measurement sites were shaved 24h prior to testing to avoid any  
114 inflammatory response that may affect cutaneous blood flow; we inspected the  
115 forearms prior to each trial to ensure that no skin damage was present that may  
116 adversely influence cutaneous blood flow responses. Participants were instrumented  
117 for LDF measurements on the dominant forearm; three heating discs (Perimed 355,  
118 Perimed AB, Järfälla, Stockholm, Sweden) were placed ~5cm apart on the dominant  
119 forearm, with a 7-laser array probe (PF 413, Perimed AB, Järfälla, Stockholm, Sweden)  
120 placed into each heater and firmly attached to the skin using adhesive stickers and  
121 medical tape. Following sterilisation of the non-dominant arm measurement site,  
122 participants were instrumented for laser speckle imaging using the technique of full-  
123 field laser perfusion imaging (FLPI); a water-filled clear heating probe (Moor VHP3,  
124 Moor Instruments, Axminster, UK) was placed on the skin and attached using an  
125 adhesive sticker to obtain a good seal. Measurement sites were randomly chosen,  
126 avoiding visible veins, hair follicles and dermatological lesions.<sup>16</sup> Upon completion of  
127 the first experimental trial, the location of the LDF and FLPI assessment sites was  
128 marked on the skin, with digital photographs and measurements taken to the nearest

129 millimetre using anatomical and skin-surface landmarks for reference, to ensure  
130 accurate re-selection of probe sites for the second trial. Stature (seca 217 stadiometer,  
131 seca UK, Birmingham, UK) and body mass (seca 767 calibrated electronic scales,  
132 Germany) were recorded using standardised protocols. Body mass index was  
133 calculated (BMI) as the body weight (kg) divided by the height squared ( $m^2$ ).

134

135 Both LDF and FLPI provide non-invasive, continuous measures of cutaneous blood  
136 flow.<sup>17</sup> By using a combination of these techniques, it is possible to simultaneously  
137 evaluate superficial (<300 micron) and deeper (1-1.5mm) skin blood flow via FLPI and  
138 LDF, respectively. Rather than assessing overall microvascular function, using local  
139 thermal hyperaemia provides a more comprehensive assessment of microvascular  
140 reactivity to acute tea ingestion and the complex neural and chemically-mediated  
141 pathways underlying microvascular function. Distinct rapid and gradual local heating  
142 protocols all provide a different type of vasodilation that likely relates to different  
143 vasodilator pathways.

144

145 *Rapid local heating.* First, we adopted the classic local heating protocol *rapid 42°C*  
146 (0.5°C per 5s, 30-min at 42°C, 20-min at 44°C), which induces a rapid, transient axon-  
147 reflex, followed by a more gradual, but sustained, heating response. The plateau  
148 phase represents 80-90% of the maximal response, and is partly (60-70%) NO-  
149 mediated.<sup>18, 19</sup> Secondly, we examined a more recently introduced protocol; *rapid 39°C*  
150 (0.5°C per 5s, 30-min at 39°C, 20-min at 44°C), that also induces an axon-reflex and  
151 gradual plateau during the heating response. By stopping the heating protocol at 39°C,  
152 the plateau phase is largely NO-mediated and only represents 50% of the maximal



153 response.<sup>20</sup> The *rapid 42°C* and *rapid 39°C* protocols were examined simultaneously  
154 on the dominant arm using LDF.

155

156 *Gradual local heating.* We examined an adapted version of the *gradual* local heating  
157 protocol that increases to 42°C (0.5°C per 2min30s, 30-min at 42°C, 20-min at 44°C),  
158 and induces a slow heating response that is largely NO-mediated and reflects 80-90%  
159 of the maximal response.<sup>21</sup> This protocol was examined on the dominant arm using  
160 LDF, covering 6mm<sup>2</sup> of skin at a penetration depth of ~1-1.5mm, and on the  
161 contralateral arm using FLPI, which covers an area of skin up to 30,000mm<sup>2</sup> at a depth  
162 of ~0.3mm.<sup>22</sup>

163

164 *Laser Doppler Flowmetry (LDF).* Laser Doppler flowmetry is a non-invasive technique  
165 that is routinely used to study microvascular function,<sup>16, 18, 22</sup> and is sensitive in  
166 detecting changes in skin perfusion over a period of time and in response to a stimulus,  
167 such as local thermal hyperaemia.<sup>16</sup> LDF is concerned with the reflection of a laser  
168 beam that undergoes a change in wavelength, or Doppler shift, when it detects moving  
169 red blood cells, the magnitude and frequency of which is related to the concentration  
170 and velocity of blood cells and is recorded as a signal of red blood cell flux (RBCF).<sup>16,</sup>  
171 <sup>22</sup> Following a 20-minute acclimation period, cutaneous blood flow was measured as  
172 RBCF at the chosen probe sites using a laser Doppler flowmeter (Periflux system 5000,  
173 Perimed AB, Järfälla, Stockholm, Sweden). The local heating discs were connected  
174 to a heating unit (Peritemp 4005 heater, Perimed AB, Järfälla, Stockholm, Sweden)  
175 which was manually controlled to perform the temperature stages of the local heating  
176 protocols. Baseline skin RBCF was recorded with the local heating disc temperature

177 set at 33°C for 10-minutes for each measurement site. Subsequently, local skin  
178 temperature was heated using the three distinct protocols.

179

180 *Full-field Laser Perfusion Imaging (FLPI)*. The FLPI technique, also known as laser  
181 speckle contrast imaging, exploits the fact that the random speckle pattern that is  
182 generated when tissue is illuminated by laser light, changes when blood cells move  
183 within the region of interest.<sup>23</sup> High levels of movement (fast flow) produce a more  
184 blurred pattern, associated with a reduction in contrast in that region. Low contrast  
185 corresponds with high flow and high contrast corresponds with low flow. The strengths  
186 of this technique are that video frame rate blood flow images (up to 25 per second)  
187 enable the tracking of fast transient blood flow changes and provides high spatial and  
188 temporal resolution. This device works with a near infra-red laser diode (785nm) and  
189 is able to scan skin surfaces from 5mm x 7mm to 15cm x 20cm, to a depth of  
190 approximately 150-300 micron and is safe for human use. Following a 20-minute  
191 acclimation period, FLPI recordings of the non-dominant forearm were performed  
192 using a blood flow imaging system (moorFLPI-1, Moor Instruments, Axminster, UK)  
193 with a laser wavelength of 785nm and sampling frequency of 25Hz. The distance  
194 between the laser head and skin surface was fixed at 15cm.<sup>24</sup> A skin heater module  
195 (moorVMS-HEAT, Moor Instruments, Axminster, UK) was used to manually set the  
196 baseline temperature at 33°C for 10-minutes and to perform the incremental local  
197 heating protocol; *gradual 42°C<sub>FLPI</sub>* (0.5°C per 2min30s, 30-min at 42°C, 20-min at  
198 44°C).<sup>21</sup>

199

200 *Haemodynamics*. Heart rate (HR) and blood pressure were recorded at the beginning  
201 and at the end of the 20-minute acclimation period using an automated  
202 sphygmomanometer (Dinamap V100, GE Healthcare, UK) positioned on the ankle,  
203 corresponding to the same laterality as their dominant arm. Thereafter, mean arterial  
204 pressure (MAP, mV/mmHg) and HR were recorded at 5-minute intervals throughout  
205 the local heating protocols. MAP was used to calculate cutaneous vascular  
206 conductance ( $CVC=RBCF/MAP$ ), thereby accounting for changes in skin blood flow  
207 resulting from variations in blood pressure.<sup>16, 18, 25</sup>

208

## 209 **Data Analysis**

210 Data analysis was performed blind. Cutaneous RBCF (PU) was expressed as  
211 cutaneous vascular conductance (CVC), as described previously.<sup>16</sup> Artefact in the  
212 data, due to unwanted subject movement, was identified and removed prior to  
213 analysis. Baseline laser Doppler RBCF was averaged over a stable 10-minute  
214 baseline period. For the *rapid 42°C* and *rapid 39°C* protocols, following initiation of  
215 heating, initial peak and nadir CVC values were calculated over a stable 60-second  
216 period,<sup>18</sup> with the initial peak identified as the highest value and the nadir as the lowest  
217 value during the first 5-10 minutes of local heating.<sup>26</sup> A clear nadir was not detected in  
218 all measurement traces, which is typical of this type of thermal provocation test. In  
219 those traces (~5%), data was included from a 60-second period, 1-minute after the  
220 initial peak. This value was always lower than the initial peak. CVC was calculated  
221 over a stable 60-second period for the final minute of each temperature increment (34-  
222 41°C) of the *gradual 42°C* local heating protocol. For each of the three protocols, *rapid*  
223 *42°C*, *rapid 39°C* and *gradual 42°C*, plateau phases during heating (42°C, 39°C and  
224 maximal 44°C) were averaged over the last 5-minutes of each phase. Data at baseline

225 and at the various plateau phases were also normalised to the maximal CVC achieved  
226 at 44°C ( $\%CVC_{max}=[CVC/CVC_{max}] \times 100$ ).<sup>25</sup> All data were collected in LabChart 7.0  
227 (ADInstruments, Dunedin, New Zealand).

228

## 229 **Statistical Methods**

230 Data were expressed as mean $\pm$ SD and statistical significance was set at  $P<0.05$ . For  
231 all protocols, linear mixed models (main effects of condition and time) were used to  
232 examine the impact of acute tea ingestion on blood pressure and forearm skin  
233 microcirculation. The repeated covariance type was Unstructured and Condition, Time  
234 and Condition\*Time was specified as Fixed Effects (intercept was included) and as  
235 Estimated Marginal Means. We interpreted the Test of Fixed Effects Condition\*Time  
236 interaction. Significant main effects of Time or Condition or a Time\*Condition  
237 interaction were followed up with a simple main effects analysis and the least  
238 significant difference (LSD) approach to multiple comparisons.<sup>27</sup> Data were stored and  
239 transformed within Microsoft Excel (Microsoft Office 2010, Microsoft Corporation), and  
240 statistical analyses were performed using SPSS 22.0 (SPSS, Chicago, IL, USA).

241

242

## 243 **RESULTS**

244 One participant was removed from the *gradual 42°C<sub>LDF</sub>* analysis for both experimental  
245 trials (due to probe failure) and five participants were removed from the *gradual*  
246 *42°C<sub>FLPI</sub>* analysis for both trials (linked to excessive movement artefacts), giving a  
247 population of  $n=19$  and  $n=15$ , respectively. No participants were removed from the  
248 *rapid 39°C* and *rapid 42°C* analysis (both  $n=20$ ). Baseline MAP was not different  
249 between conditions ( $108\pm 11$ ,  $108\pm 11$ ,  $P=0.73$ ) and showed no change across time

250 (P=0.52). There were no differences in baseline cutaneous perfusion between trials  
251 for measurement sites that underwent *rapid 39°C* or *rapid 42°C* local heating for  
252 absolute flux, CVC or %CVC<sub>max</sub> (Table 1). Also the site that underwent *gradual 42°C*  
253 local heating using LDF showed no difference in baseline cutaneous blood flow  
254 between trials for absolute flux, CVC or %CVC<sub>max</sub> (Table 2). However, using FLPI, a  
255 significantly higher baseline perfusion was found after tea ingestion for cutaneous flux  
256 and CVC, but not for %CVC<sub>max</sub> (Table 2).

257

#### 258 *Rapid local heating: impact of tea*

259 *Rapid 39°C*. Local heating induced a typical pattern of an initial peak, nadir and plateau  
260 in cutaneous blood flow. Therefore, a main effect of time was demonstrated for  
261 absolute flux, CVC and %CVC<sub>max</sub> (Table 1). However, we found no effect of the  
262 intervention or a timeXintervention-interaction for absolute flux, CVC or %CVC<sub>max</sub>  
263 (Table 1).

264 *Rapid 42°C*. Local heating induced a typical pattern of an initial peak, nadir and plateau  
265 in cutaneous blood flow. Consequently, a main effect of time was demonstrated for  
266 absolute flux, CVC and %CVC<sub>max</sub> (Table 1), whilst no main effect of intervention or  
267 timeXintervention-interaction was found for absolute flux, CVC or %CVC<sub>max</sub> (Table 1).

268

#### 269 *Gradual local heating: impact of tea*

270 *Gradual 42°C<sub>LDF</sub>*. Local heating induced a gradual, slow heating response with no  
271 detectable initial axon reflex-induced peak or nadir with a main effect of time (Table 2).  
272 A higher skin blood flow throughout the heating protocol was observed during the trial  
273 preceded by black tea for absolute CVC (P=0.04), with a trend towards significance  
274 when data were presented as absolute flux (P=0.06, Table 2). No effect of tea was

275 found when CVC was normalised for maximum perfusion (%CVC<sub>max</sub>, P=0.82, Table  
276 2). No timeXintervention-interaction was found for absolute flux (P=0.93), CVC  
277 (P=0.95) or %CVC<sub>max</sub> (P=0.98, Table 2).

278 *Gradual 42°C<sub>FLPI</sub>*. Local heating induced a gradual, slow heating response with no  
279 detectable initial axon reflex-induced peak or nadir (Table 2). Tea ingestion was  
280 associated with a significantly higher absolute flux (P=0.00) and CVC (P=0.00), but  
281 not when CVC was normalised to maximum CVC (%CVC<sub>max</sub>, P=0.35, Table 2). No  
282 timeXintervention-interaction was present for absolute flux (P=0.50), CVC (P=0.66)  
283 or %CVC<sub>max</sub> (P=1.00, Table 2).

284

285 Our statistical analysis revealed no presence of a carry-over effect.

286

## 287 **DISCUSSION**

288 The aim of this study was to test the hypothesis that a single dose of black tea  
289 ingestion improves cutaneous microcirculation following both rapid and gradual local  
290 skin heating. We found that gradual local heating of the skin to 42°C induced a greater  
291 vasodilatory response following tea ingestion compared to placebo when expressed  
292 as absolute flux and CVC. The ability of tea to improve local gradual heating responses  
293 in the skin was reinforced by the observation that both LDF and FLPI, two distinct but  
294 accepted techniques to assess skin perfusion, detected this effect. Conversely, rapid  
295 local heating did not demonstrate a significant increase in cutaneous microcirculation  
296 with tea ingestion, either for the *rapid 39°C* or *rapid 42°C* protocols. Taken together,  
297 our study provides some further evidence that regular tea ingestion may mediate its  
298 potential cardiovascular benefits via improvements in (cutaneous) microvascular  
299 function.

300

301 Our study is the first to explore the acute effects of tea ingestion on the cutaneous  
302 microcirculation whilst adopting a rigorous protocol involving blind analysis of rapid  
303 and gradual heating protocols as well as two distinct, accepted techniques. This  
304 observation fits with the general observation of tea being able to enhance endothelial  
305 function in conduit vessels when assessed by flow-mediated dilation (FMD).<sup>6, 9</sup> Taken  
306 together, these findings suggest that acute tea ingestion improves vascular function  
307 across the vascular tree, including skin microvessels, possibly via upregulation of  
308 vasodilator mechanisms.

309

310 In contrast to gradual local heating, rapid heating of the skin did not alter cutaneous  
311 vascular function following tea ingestion when compared to placebo. Our findings were  
312 similar for both rapid heating protocols (*rapid 39°C* and *rapid 42°C*). Interestingly, a  
313 recent observational study<sup>28</sup> found improved microvascular function following regular  
314 consumption of green tea (14 days) using rapid heating (whilst no measure of gradual  
315 heating was included). Important differences were present between studies, especially  
316 since this previous study did not include a placebo control, did not fully adhere to  
317 guidelines for vascular assessment (e.g. control of menstrual cycle),<sup>15</sup> and was limited  
318 by a lack of control of dietary habits.<sup>6</sup> Furthermore, whilst our study investigated the  
319 acute (2h) effects of tea, they examined a protocol of 14 days of green tea. Despite  
320 the *rapid 39°C* and *gradual 42°C* protocols both being linked to the release of NO,  
321 distinct responses are clearly evident between the gradual and rapid heating protocols  
322 in our study. Different vasodilator pathways directly influence the cutaneous  
323 microcirculation, including neurogenic reflexes and local chemical mediators.<sup>18, 21, 25</sup>  
324 The rate at which the skin is heated, alters the contribution of these vasodilator

325 pathways, with rapid (0.5°C per 5s) local heating inducing a transient axon-reflex  
326 mediated vasodilation that is produced via activation of heat sensitive  
327 sensory/nociceptive nerves releasing calcitonin gene-related peptide (CGRP) and  
328 substance P and adrenergic nerves releasing norepinephrine and neuropeptide Y.<sup>18</sup>  
329 <sup>29</sup> This initial neurogenic response is followed by a more gradual, sustained  
330 vasodilation. In both phases, vasodilation occurs through complex pathways that lead  
331 to the production of NO and smooth muscle relaxation via hyperpolarization from  
332 endothelial derived hyperpolarization factors (EDHFs),<sup>29</sup> with a greater (but not  
333 exclusive) contribution of NO during the plateau phase.<sup>18, 20</sup> Furthermore, the relative  
334 contribution of NO to the vasodilation during the plateau phase of the rapid heating  
335 protocols depends upon the target heating temperature, as the heating response to  
336 39°C seems to depend more on NO than the response to 42°C.<sup>18, 20</sup> These studies,  
337 therefore, demonstrate that the underlying mechanism for cutaneous vasodilation  
338 differ based on the rate and maximum level of heating. The different vasodilator  
339 pathways for these heating protocols may contribute to the distinct findings in our study.  
340 From a methodological perspective, the differences between rapid and gradual local  
341 heating highlight the importance of using multiple heating protocols simultaneously  
342 when exploring the impact of an intervention on skin perfusion.

343

344 The higher vasodilatory responses that we observed following gradual heating of the  
345 skin were demonstrated for arbitrary flux and CVC values, for both LDF and FLPI  
346 techniques. However, the difference in responses between the tea and placebo trials  
347 was not significant when data were expressed as %CVC<sub>max</sub>. The skin is commonly  
348 heated to 44°C to reach maximal vasodilation and expressing CVC as a percentage  
349 of maximal perfusion is often considered the preferred method of data expression,<sup>16</sup>



350 with improved reproducibility compared to flux or CVC.<sup>25</sup> Despite a main effect of tea  
351 on flux and CVC, post-hoc analyses revealed no differences between trials at 44°C  
352 (LFD: flux=0.17 and CVC=0.19; FLPI: flux=0.09 and CVC=0.08). However, the  
353 magnitude of differences in flux and CVC between tea and placebo are larger than  
354 one may expect based on day-to-day variation.<sup>25</sup> This provides some indication that  
355 the tea intervention may have altered cutaneous perfusion at 44°C local heating.

356

357 *Clinical Relevance.* Tea consumption is known to have cardiovascular benefits,  
358 including a reduction in blood pressure after short- to long-term intervention, possibly  
359 mediated (in part) by improved endothelial function of conduit vessels.<sup>8, 9, 12</sup> In our  
360 study, cutaneous microcirculation responses to gradual heating improved following tea  
361 ingestion. We speculate that these findings may have implications for individuals with  
362 microvascular complications and skin endothelial dysfunction, such as type 2 diabetes  
363 mellitus. Interestingly, consumption of tea has been associated with a reduced risk for  
364 type 2 diabetes mellitus.<sup>30</sup> Our findings thus support the hypothesis that regular tea  
365 consumption may have potential benefit in such patient groups. Future studies are  
366 warranted to explore this hypothesis.

367

368 *Limitations.* Due to our modest sample size, we are unable to generalise our findings  
369 towards the wider populace. Furthermore, although we included a middle-aged  
370 population who likely are at an increased risk of CVD, we cannot simply extrapolate  
371 our findings to clinical groups. Moreover, our population may have impaired  
372 endothelial function as blunted cutaneous NO-mediated vasodilation has been  
373 demonstrated in older individuals,<sup>21</sup> suggesting that young healthy volunteers may  
374 exhibit different results than our older population. Therefore, future work is required to

375 explore the potential impact of acute as well as chronic tea ingestion on cutaneous  
376 vascular function in both individuals with compromised endothelial function and in  
377 young, healthy individuals. A further limitation is that we did not obtain plasma  
378 measures of flavonoids or NO compounds and, therefore, our study does not provide  
379 any biochemical or biomolecular insight into the mechanisms underlying the  
380 improvement in cutaneous microvascular function. However, it is important to  
381 emphasise that this was not the purpose of our study, particularly given that we are  
382 the first to explore the effects of acute tea ingestion on the cutaneous microcirculation.

383

384 In conclusion, our findings suggest that acute tea ingestion enhances cutaneous  
385 vascular function in a healthy, middle-aged population, when measured following  
386 gradual local heating to 42°C. Therefore, these data suggest that acute tea ingestion  
387 has a beneficial impact on vascular function at the microcirculatory level, which is likely  
388 achieved through a mechanism related to activation of endothelium-derived  
389 vasodilators. These improvements in cutaneous microvascular function may  
390 contribute to the potential cardiovascular health benefits of regular tea ingestion.  
391 Future studies are required to explore the acute and chronic effects of tea on  
392 individuals with increased CVD risk and in clinical populations with *a priori* endothelial  
393 dysfunction.

394

#### 395 **STATEMENT OF AUTHORSHIP**

396 K. A. W., D. A. L., D. H. J. T., N. D. H., R. D. and Y. d. G. designed research; K. A.  
397 W. conducted research; K. A. W., D. A. L. and Y. d. G. analysed data; K. A. W., D. A.  
398 L., D. H. J. T., R. D. and N. D. H. wrote the paper; R. D. and D. H. J. T. had primary  
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400

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406

407 **References**

- 408 1. WHO. Cardiovascular Diseases (CVDs). 2016. Available from:  
409 <http://www.who.int/mediacentre/factsheets/fs317/en/>.
- 410 2. Peterson JJ, Dwyer JT, Jacques PF, McCullough ML. Associations between flavonoids and  
411 cardiovascular disease incidence or mortality in European and US populations. *Nutrition Reviews*.  
412 2012;70(9):491-508.
- 413 3. Yahya HM, Day A, Lawton C, Myrissa K, Croden F, Dye L, et al. Dietary intake of 20 polyphenol  
414 subclasses in a cohort of UK women. *European Journal of Nutrition*. 2016;55(5):1839-47.
- 415 4. Hodgson JM, Croft KD. Tea flavonoids and cardiovascular health. *Molecular aspects of*  
416 *medicine*. 2010;31(6):495-502.
- 417 5. Greyling A, Ras RT, Zock PL, Lorenz M, Hopman MT, Thijssen DHJ, et al. The effect of black tea  
418 on blood pressure: A systematic review with meta-analysis of randomized controlled trials. *PLoS ONE*.  
419 2014;9(7):1-9.
- 420 6. Grassi D, Mulder TPJ, Draijer R, Desideri G, Molhuizen HOF, Ferri C. Black tea consumption  
421 dose-dependently improves flow-mediated dilation in healthy males. *Journal of Hypertension*.  
422 2009;27(4):774-81.
- 423 7. Grassi D, Draijer R, Desideri G, Mulder T, Ferri C. Black Tea Lowers Blood Pressure and Wave  
424 Reflections in Fasted and Postprandial Conditions in Hypertensive Patients: A Randomised Study.  
425 *Nutrients*. 2015;7(2):1037.
- 426 8. Hodgson JM, Puddey IB, Burke V, Watts GF, Beilin LJ. Regular ingestion of black tea improves  
427 brachial artery vasodilator function. *Clinical Science*. 2002;102(2):195-201.
- 428 9. Schreuder THA, Eijsvogels TMH, Greyling A, Draijer R, Hopman MTE, Thijssen DHJ. Effect of  
429 black tea consumption on brachial artery flow-mediated dilation and ischaemia-reperfusion in  
430 humans. *Applied Physiology, Nutrition & Metabolism* 2014;39(2):145-51.
- 431 10. Duffy SJ, Keaney Jr JF, Holbrook M, Gokce N, Swerdloff PL, Frei B, et al. Short- and long-term  
432 black tea consumption reverses endothelial dysfunction in patients with coronary artery disease.  
433 *Circulation*. 2001;104(2):151-6.
- 434 11. Hodgson JM, Burke V, Puddey IB. Acute effects of tea on fasting and postprandial vascular  
435 function and blood pressure in humans. *Journal of Hypertension*. 2005;23(1):47-54.
- 436 12. Ras RT, Zock PL, Draijer R. Tea Consumption Enhances Endothelial-Dependent Vasodilation; a  
437 Meta-Analysis. *PLoS ONE*. 2011;6(3):e16974.
- 438 13. Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. Clinical Practice  
439 Guidelines Committee of the Spanish Society of Cardiology [ESC/EAS Guidelines for the management  
440 of dyslipidaemias]. *Revista espanola de cardiologia*. 2011;64:1168 e1- e0.
- 441 14. NICE. Hypertension: quick reference guide: clinical management of primary hypertension in  
442 adults. 2011 ed. London: National Institute for Health and Clinical Excellence.2011.
- 443 15. Thijssen DHJ, Black MA, Pyke KE, Padilla J, Atkinson G, Harris RA, et al. Assessment of flow-  
444 mediated dilation in humans: a methodological and physiological guideline. *American Journal of*  
445 *Physiology - Heart and Circulatory Physiology*. 2011;300(1):H2-H12.
- 446 16. Cracowski JL, Minson CT, Salvat-Melis M, Halliwill JR. Methodological issues in the assessment  
447 of skin microvascular endothelial function in humans. *Trends in Pharmacological Sciences*.  
448 2006;27(9):503-8.
- 449 17. Roustit M, Cracowski JL. Non-invasive assessment of skin microvascular function in humans:  
450 an insight into methods. *Microcirculation*. 2012;19(1):47-64.
- 451 18. Minson CT, Berry LT, Joyner MJ. Nitric oxide and neurally mediated regulation of skin blood  
452 flow during local heating. *Journal of Applied Physiology*. 2001;91:1619-26.
- 453 19. Kellogg DL, Liu Y, Kosiba IF, O'Donnell D. Role of nitric oxide in the vascular effects of local  
454 warming of the skin in humans. *Journal of Applied Physiology*. 1999;86(4):1185-90.

- 455 20. Choi PJ, Brunt VE, Fujii N, Minson CT. New approach to measure cutaneous microvascular  
456 function: an improved test of NO-mediated vasodilation by thermal hyperemia. *Journal of Applied*  
457 *Physiology*. 2014;117(3):277-83.
- 458 21. Black MA, Green DJ, Cable NT. Exercise prevents age-related decline in nitric-oxide-mediated  
459 vasodilator function in cutaneous microvessels. *Journal of Physiology*. 2008;586(14):3511-24.
- 460 22. Cracowski J-L, Roustit M. Current methods to assess human cutaneous blood flow. An updated  
461 focus on laser based-techniques. *Microcirculation*. 2015:n/a-n/a.
- 462 23. Briers D, Duncan DD, Hirst E, Kirkpatrick SJ, Larsson M, Steenbergen W, et al. Laser speckle  
463 contrast imaging: theoretical and practical limitations. *BIOMEDO*. 2013;18(6):066018-.
- 464 24. Mahé G, Haj-Yassin F, Rousseau P, Humeau A, Durand S, Leftheriotis G, et al. Distance between  
465 laser head and skin does not influence skin blood flow values recorded by laser speckle imaging.  
466 *Microvascular Research*. 2011;82(3):439-42.
- 467 25. Dawson EA, Low DA, Meeuwis IH, Kerstens FG, Atkinson CL, Cable NT, et al. Reproducibility of  
468 cutaneous vascular conductance responses to slow local heating assessed using seven-laser array  
469 probes. *Microcirculation*. 2015;22(4):276-84.
- 470 26. Van Duijnhoven NT, Janssen TW, Green DJ, Minson CT, Hopman MT, Thijssen DH. Effect of  
471 functional electrostimulation on impaired skin vasodilator responses to local heating in spinal cord  
472 injury. *Journal of Applied Physiology*. 2009;106(4):1065-71.
- 473 27. Perneger TV. What's wrong with Bonferroni adjustments? *British Medical Journal*.  
474 1998;316:1236.
- 475 28. Wasilewski R, Ubara EO, Klonizakis M. Assessing the effects of a short-term green tea  
476 intervention in skin microvascular and oxygen tension in older and younger adults. *Microvascular*  
477 *Research*. 2016;107:65-71.
- 478 29. Johnson JM, Minson CT, Kellogg DL, Jr. Cutaneous vasodilator and vasoconstrictor  
479 mechanisms in temperature regulation. *Comprehensive Physiology*. 2014;4(1):33-89.
- 480 30. Yang W-S, Wang W-Y, Fan W-Y, Deng Q, Wang X. Tea consumption and risk of type 2 diabetes:  
481 a dose-response meta-analysis of cohort studies. *British Journal of Nutrition*. 2014;111(08):1329-39.

482

483 **Table 1.** Laser Doppler flowmetry cutaneous blood flow responses to local heating  
 484 for the *rapid 39°C* and *rapid 42°C* protocols for placebo and tea interventions.

485

<b>Rapid 39°C</b>	<b>Intervention (mean ± SD)</b>		<b>LMM</b>		
	<b>Placebo</b>	<b>Tea</b>	<b>time</b>	<b>tea</b>	<b>time*tea</b>
<i>Absolute flux(PU)</i>					
Baseline	22 ± 11	21 ± 8			
Axon-reflex	108 ± 38	103 ± 50			
Nadir	57 ± 25	52 ± 26	<0.001*	0.14	0.76
Plateau 39°C	136 ± 53	123 ± 70			
Plateau 44°C	288 ± 61	263 ± 61			
<i>Absolute CVC (PU/mmHg)</i>					
Baseline	0.21 ± 0.12	0.21 ± 0.10			
Axon-reflex	1.03 ± 0.39	0.99 ± 0.47			
Nadir	0.54 ± 0.25	0.50 ± 0.26	<0.001*	0.27	0.91
Plateau 39°C	1.29 ± 0.52	1.17 ± 0.65			
Plateau 44°C	2.70 ± 0.67	2.52 ± 0.59			
<i>Maximal CVC (%CVC<sub>max</sub>)</i>					
Baseline	8 ± 4	8 ± 3			
Axon-reflex	39 ± 15	39 ± 15			
Nadir	20 ± 10	20 ± 10	<0.001*	0.76	0.99
Plateau 39°C	48 ± 15	46 ± 21			
<b>Rapid 42°C</b>					
<i>Absolute flux(PU)</i>					
Baseline	22 ± 9	25 ± 16			
Axon-reflex	199 ± 60	208 ± 60			
Nadir	165 ± 64	177 ± 74	<0.001*	0.51	0.99
Plateau 42°C	252 ± 72	253 ± 67			
Plateau 44°C	300 ± 79	302 ± 63			
<i>Absolute CVC (PU/mmHg)</i>					
Baseline	0.21 ± 0.10	0.25 ± 0.16			
Axon-reflex	1.90 ± 0.61	2.00 ± 0.61			
Nadir	1.57 ± 0.64	1.71 ± 0.74	<0.001*	0.29	1.00
Plateau 42°C	2.39 ± 0.74	2.43 ± 0.66			
Plateau 44°C	2.81 ± 0.81	2.91 ± 0.74			
<i>Maximal CVC (%CVC<sub>max</sub>)</i>					
Baseline	8 ± 3	8 ± 4			
Axon-reflex	67 ± 11	68 ± 11			
Nadir	55 ± 16	57 ± 19	<0.001*	0.65	0.95
Plateau 42°C	85 ± 8	83 ± 12			

486 Data are mean ± SD. \*Main effect of time  $P < 0.001$  vs baseline.

487 **Table 2.** Cutaneous blood flow responses to local heating for the *gradual<sub>LDF</sub>* (42°C)  
 488 and *gradual<sub>FLPI</sub>* (42°C) protocols for placebo and tea interventions.

489

<b><i>Gradual<sub>LDF</sub></i> (42°C)</b>	<b>Intervention (mean ± SD)</b>		<b>LMM</b>		
	<b>Placebo</b>	<b>Tea</b>	<b>time</b>	<b>tea</b>	<b>time*tea</b>
<b><i>Absolute flux(PU)</i></b>					
Baseline	26 ± 11	24 ± 9			
Plateau 42°C	268 ± 79	278 ± 61	<0.001*	0.06	0.93
Plateau 44°C	302 ± 84	319 ± 45			
<b><i>Absolute CVC (PU/mmHg)</i></b>					
Baseline	0.25 ± 0.11	0.23 ± 0.09			
Plateau 42°C	2.51 ± 0.76	2.61 ± 0.64	<0.001*	0.04 <sup>^</sup>	0.95
Plateau 44°C	2.80 ± 0.82	2.93 ± 0.51			
<b><i>Maximal CVC (%CVC<sub>max</sub>)</i></b>					
Baseline	9 ± 5	8 ± 3			
Plateau 42°C	90 ± 7	89 ± 14	<0.001*	0.82	0.98
<b><i>Gradual<sub>FLPI</sub></i> (42°C)</b>					
<b><i>Absolute flux(PU)</i></b>					
Baseline	30 ± 9	36 ± 8			
Plateau 42°C	197 ± 51	222 ± 50	<0.001*	<0.001 <sup>^</sup>	0.50
Plateau 44°C	216 ± 65	253 ± 68			
<b><i>Absolute CVC (PU/mmHg)</i></b>					
Baseline	0.29 ± 0.09	0.36 ± 0.07			
Plateau 42°C	1.85 ± 0.55	2.10 ± 0.57	<0.001*	<0.001 <sup>^</sup>	0.66
Plateau 44°C	2.01 ± 0.64	2.34 ± 0.72			
<b><i>Maximal CVC (%CVC<sub>max</sub>)</i></b>					
Baseline	17 ± 11	17 ± 8			
Plateau 42°C	94 ± 10	91 ± 6	<0.001*	0.35	1.00

490 Data are mean ± SD. \*Main effect of time  $P < 0.001$  vs baseline. <sup>^</sup>Main effect of intervention; placebo

491 vs. tea  $P < 0.05$ .

492 **FIGURE LEGENDS**

493 **Figure 1.** CONSORT diagram showing the flow of participants through each stage of  
494 the randomised trial.

495

496 **Figure 2.** Study overview and schematic depicting the stages of the local heating  
497 protocols. Light grey shading denotes local heating, mid grey shading  
498 represents the plateau and dark grey shading represents the maximal  
499 plateau.

500

501 **Figure 3.** Cutaneous vascular conductance (CVC) responses across time points  
502 (baseline at 33 °C, axon peak, axon nadir, plateau at 39/42 °C and maximal  
503 plateau at 44 °C) following rapid local heating for **A.** *rapid 39°C* and **B.**  
504 *rapid 42°C* in 20 healthy volunteers when heating was preceded by  
505 ingestion of placebo (open squares) or tea (solid triangles). Data are  
506 presented as means, with error bars representing SE.

507

508 **Figure 4.** Cutaneous vascular conductance (CVC) responses across time points  
509 (from baseline at 33 °C to maximal plateau at 44 °C) following gradual local  
510 heating using **A.** laser-Doppler flowmetry (LDF) and **B.** full-field laser  
511 perfusion imaging (FLPI) in 20 healthy volunteers when heating was  
512 preceded by ingestion of placebo (open squares) or tea (solid triangles).  
513 Data are presented as means, with error bars representing SE. \*Main effect  
514 of condition  $P < 0.05$  placebo vs tea.