ABSTRACT

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

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

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

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

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

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

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

Participants

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

Experimental Design

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

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

Experimental Measures

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

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

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

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

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

response.[20] The *rapid 42°C* and *rapid 39°C* protocols were examined simultaneously on the dominant arm using LDF.

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

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

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

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

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

Data Analysis

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

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

Statistical Methods

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

RESULTS

One participant was removed from the *gradual 42°C*$_{LDF}$ analysis for both experimental trials (due to probe failure) and five participants were removed from the *gradual 42°C*$_{FLPI}$ analysis for both trials (linked to excessive movement artefacts), giving a population of n=19 and n=15, respectively. No participants were removed from the *rapid 39°C* and *rapid 42°C* analysis (both n=20). Baseline MAP was not different between conditions (108±11, 108±11, P=0.73) and showed no change across time

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

Rapid local heating: impact of tea

Rapid 39°C. Local heating induced a typical pattern of an initial peak, nadir and plateau in cutaneous blood flow. Therefore, a main effect of time was demonstrated for absolute flux, CVC and %CVC_{max} (Table 1). However, we found no effect of the intervention or a timeXintervention-interaction for absolute flux, CVC or %CVC_{max} (Table 1).

Rapid 42°C. Local heating induced a typical pattern of an initial peak, nadir and plateau in cutaneous blood flow. Consequently, a main effect of time was demonstrated for absolute flux, CVC and %CVC_{max} (Table 1), whilst no main effect of intervention or timeXintervention-interaction was found for absolute flux, CVC or %CVC_{max} (Table 1).

Gradual local heating: impact of tea

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

found when CVC was normalised for maximum perfusion (%CVC_{max}, P=0.82, Table 2). No timeXintervention-interaction was found for absolute flux (P=0.93), CVC (P=0.95) or %CVC_{max} (P=0.98, Table 2).

Gradual 42°C$_{FLPI}$. Local heating induced a gradual, slow heating response with no detectable initial axon reflex-induced peak or nadir (Table 2). Tea ingestion was associated with a significantly higher absolute flux (P=0.00) and CVC (P=0.00), but not when CVC was normalised to maximum CVC (%CVC_{max}, P=0.35, Table 2). No timeXintervention-interaction was present for absolute flux (P=0.50), CVC (P=0.66) or %CVC_{max} (P=1.00, Table 2).

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

DISCUSSION

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

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

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

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

The higher vasodilatory responses that we observed following gradual heating of the skin were demonstrated for arbitrary flux and CVC values, for both LDF and FLPI techniques. However, the difference in responses between the tea and placebo trials was not significant when data were expressed as $\%CVC_{max}$. The skin is commonly heated to 44°C to reach maximal vasodilation and expressing CVC as a percentage of maximal perfusion is often considered the preferred method of data expression,[16]

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

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

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

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

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

STATEMENT OF AUTHORSHIP

K. A. W., D. A. L., D. H. J. T., N. D. H., R. D. and Y. d. G. designed research; K. A. W. conducted research; K. A. W., D. A. L. and Y. d. G. analysed data; K. A. W., D. A. L., D. H. J. T., R. D. and N. D. H. wrote the paper; R. D. and D. H. J. T. had primary responsibility for final content. All authors read and approved the final manuscript.

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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

	Intervention (mean ± SD)		LMM		
Rapid 39°C	**Placebo**	**Tea**	**time**	**tea**	**time*tea**
Absolute flux(PU)					
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			
Absolute CVC (PU/mmHg)					
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			
Maximal CVC (%CVC_{max})					
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			
Rapid 42°C					
Absolute flux(PU)					
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			
Absolute CVC (PU/mmHg)					
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			
Maximal CVC (%CVC_{max})					
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$_{LDF}$ (42°C)*

488 and *gradual$_{FLPI}$ (42°C)* protocols for placebo and tea interventions.

489

	Intervention (mean ± SD)		LMM		
Gradual$_{LDF}$ (42°C)	**Placebo**	**Tea**	**time**	**tea**	**time*tea**
Absolute flux(PU)					
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			
Absolute CVC (PU/mmHg)					
Baseline	0.25 ± 0.11	0.23 ± 0.09			
Plateau 42°*C*	2.51 ± 0.76	2.61 ± 0.64	<0.001*	0.04^	0.95
Plateau 44°*C*	2.80 ± 0.82	2.93 ± 0.51			
Maximal CVC (%CVC$_{max}$)					
Baseline	9 ± 5	8 ± 3			
Plateau 42°*C*	90 ± 7	89 ± 14	<0.001*	0.82	0.98
Gradual$_{FLPI}$ (42°C)					
Absolute flux(PU)					
Baseline	30 ± 9	36 ± 8			
Plateau 42°*C*	197 ± 51	222 ± 50	<0.001*	<0.001^	0.50
Plateau 44°*C*	216 ± 65	253 ± 68			
Absolute CVC (PU/mmHg)					
Baseline	0.29 ± 0.09	0.36 ± 0.07			
Plateau 42°*C*	1.85 ± 0.55	2.10 ± 0.57	<0.001*	<0.001^	0.66
Plateau 44°*C*	2.01 ± 0.64	2.34 ± 0.72			
Maximal CVC (%CVC$_{max}$)					
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. ^Main effect of intervention; placebo

491 vs. tea *P*<0.05.

FIGURE LEGENDS

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

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

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

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