Assessing the effects of a short-term green tea intervention in skin microvascular and oxygen tension in older and younger adults

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Title: Assessing the effect of a short-term, green tea intervention in skin microvascular function and oxygen tension in older and younger adults.

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Abstract

Green tea consumption has been associated with a reduction in cardiovascular disease risk factors. However, there is little evidence examining its potential differing effect between younger and older populations, while little is known on its effect on the circulatory system when oxygen demand is higher.

Therefore the aim of this study was to evaluate the short-term effects of green tea consumption on microvascular functioning in both an older and younger population.

Fifteen young [24 (4.0)] and fifteen older [61 (4.0)] participants, consumed two cups of green tea daily for 14 days. We used Laser Doppler Flowmetry to assess cutaneous microvascular function and Transcutaneous Oxygen Pressure to assess skin oxygen tension. Systolic and diastolic blood pressure were also assessed on both visits.

We observed significant improvements in axon-mediated microvascular vasodilation for the younger group [1.6 (0.59) vs 2.05 (0.72), $p<0.05$] and the older group [1.25 (0.58) vs 1.65 (0.5) $p<0.05$]. Improvements in skin oxygen tension were also noted for both groups in both noted TcPO2 measures (i.e. 1.25 (0.58) vs 1.65 (0.5) ($p<0.05$), for ΔTcPO2max for the older group, between visits) respectively. Improvements were also observed for systolic blood pressure in both the younger [120 (10) vs 112 (10), $p<0.05$] and older group [129 (12) v 124 (11), $p<0.001$].

In conclusion, we observed statistically-significant improvements in microvascular function and skin oxygen tension. Our results suggest that green tea may prove beneficial as a dietary element in lifestyle interventions aiming to lower cardiovascular disease risk, in both older and younger populations.

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide (Nichols *et al.* 2014), and responsible for 1 in 4 deaths in the United Kingdom (Townsend *et al.* 2015). There are a number of factors which contribute to its development with The British Heart Foundation categorising high saturated fat and high sodium intake as known risk factors (Townsend *et al.* 2015).

Green tea (GT) and particularly its high quantities of catechins have been the subject of extensive research and have subsequently been associated with a reduction in CVD risk makers by improving endothelial functioning (Alexopoulos *et al.* 2008). The endothelium is the inner lining of all blood vessels and serves as a selectively permeable barrier between blood and tissue. It plays an essential role in the homeostasis of vascular tone and it requires an intricate balance of endothelium dependant relaxing and contracting factors (Félétou 2011).

Nitric oxide (NO) is a particularly important factor and is an endothelium dependant relaxer, generated during the conversion of L-arginine to L-citrulline by endothelial NO-synthase (Vasan *et al.* 2002). It works through counteracting the actions of contracting factors, including angiotensin-11 and endothelin-1. Various studies have isolated specific components of GT and observed an augmentation of NO status (Loke *et al.* 2008). Similarly, oral administration of catechins increased NO levels and also decreased enodthelin-1 concentrations (a vasoconstrictor) (Persson *et al.* 2006). However, the precise constituents within GT, responsible for health benefits and indeed their exact mechanisms of action, are not clear. In vivo, mice studies have revealed that consumption of GT catechins may decrease the risk of atherosclerosis by improving the bioavailability of NO and by causing vasorelaxation, however, data appeasing this in humans is presently insufficient (Velayutham *et al.* 2008). One study postulated that catechins could be the constituent responsible for their observed improvements in cell viability by reducing the intracellular Reactive Oxygen Species accumulation (Horáková 2011).

Endothelial dysfunction is a term used to describe diminished production of the supply of NO and a subsequent lack of vasodilation in the artery and is considered to be an indicator of future cardiovascular events (Deanfield *et al.* 2007). Short-term GT consumption induced a rapid improvement in endothelial dysfunction in 'healthy' smokers in a study by Kim and colleagues (2006). The study found that after 2 weeks of GT consumption, there was a significant positive amelioration in endothelial function.

All major CVD risk factors, including high blood cholesterol, hypertension, diabetes and smoking have been shown to be accompanied by endothelial dysfunction (Hadi *et al.* 2005). The assessment of endothelial function and dysfunction is therefore of great interest.

There is mounting epidemiological evidence to suggest GT may lower the risk of CVD (Kuriyama *et al.* 2008), however epidemiological studies may prove difficult in isolating cause and effect. Further, whilst positive results have been observed when participants were given GT extracts (Nagao *et al.*, 2007) the particularly high catechin content contained within these may not accurately represent those ingested through GT beverages. A standard cup of beverage GT can contain 100mg of catechins (dependent on origin and preparation) in comparison to extract formulas which can contain almost four times this amount (Astil *et al.*2001; Dullo *et al.* 1999).

We therefore conducted a study aiming to observe the short-term effects of beverage GT on the micro-circulation at rest and during sub-maximal exercise testing, making a comparison between two healthy, sedentary populations (e.g. "younger" vs an 'older"). We sought to examine whether GT exerts a physiological effect when oxygen demand is higher as well, as the circulatory system is known to react differently during rest and provocation (Abraham *et al.* 2003).
We hypothesized that GT consumption would have an overall positive effect on the endothelial function in both the older and younger healthy, sedentary groups, with the effects being greater in

the older population.

For the purposes of this study, we used Laser Doppler Flowmetry (LDF) and Transcutaneous Oxygen Pressure (TcPO2). LDF combined with local heating of the skin (usually at the forearm) provides a simple method for examining microvascular and endothelial function (Tew *et al.* 2011). TcPO2 is a non-invasive monitoring of the oxygen tension in the skin, providing us with a direct indication of the microvascular function.

Methods

Ethical Approval

The study was approved by Sheffield Hallam University Health and Wellbeing Ethics Committee (Institutional Review Board; HWB-S&E-24) and conformed to the standards set by The Declaration of Helsinki.

Participants

Fifteen healthy young participants aged 18-35 and fifteen older participants aged 55-75 participated in the study. Recruitment took place through The University of Sheffield Volunteers email, local community groups (University of the Third Age Group) and through word of mouth. Posters were also placed around the University campus.

All participants were medically screened beforehand and for each of them, height and weight were recorded and a body mass index was subsequently calculated. Body mass index was calculated using the following standard equation BMI (kgm^2) = (weight kg / (height m) ^{2}). The participants resting systolic and diastolic blood pressure were also taken. Exclusion criteria included any overt chronic disease that would affect micro- or macro- vascular functioning (e.g. diabetes, systemic sclerosis, hypertension and cardiovascular disease), pregnancy, smoking as well as regular tea-drinking (e.g. more than 4 cups per week).

Each participant provided written informed consent prior to participating in the study.

Protocol

Eligible participants were required to visit the Centre of Health and Wellbeing at Sheffield Hallam University on two occasions. On each visit, participants were required to undergo a microvascular assessment using LDF and a sub-maximal exercise test in which skin oxygen tension was assessed. Participants were given 100% green tea, of Indian origin in the form of 2.5g tea-bags (Tesco, Welwyn Garden City, UK). Participants were instructed to consume two standard cup sizes (250ml) a day for 14 days. We stipulated two cups a day as this can be considered achievable to implement for the majority of the population, with findings suggesting the average British individual already consumes 17 cups throughout the week (Contact the Elderly 2015).

In addition, all participants were instructed to avoid adding milk to the beverage and to steep the teabag for 3-4 minutes (Ryan & Petite, 2010). Participants were instructed to consume the first cup upon waking and the final cup in the afternoon, independent of food consumption. Participants were instructed to abstain from caffeine 2-3 hours prior to the assessments, so as to eliminate the confounding effect caffeine may have produced on vasorelaxation (Echerverri *et al.* 2010). Finally, participants were asked not to change their usual activities and dietary habits (aside from consuming the GT) over the next 14 days so as to best decrease the susceptibility of confounding factors. Participants were also given a 14-day log and were asked to note the times of GT consumption.

Laser Doppler Flowmetry (LDF) Measurements

A site on the upper right forearm was selected (care was taken to avoid visible veins, body hair and damaged or irritated skin). The site location was marked and measured from the wrist so as to improve reproducibility of the location for the follow up visit. The site was first cleaned using an

alcohol sterilization wipe to remove the superficial dead layer of skin, as this has been proposed to improve the reproducibility of the technique (Puissant *et al.* 2013).

Regions of the response were then recorded (Table 1). Cutaneous vascular conductance (CVC) calculations were made by dividing LDF values by the participants mean arterial pressure. These values are expressed as percentages of the peak response to heating: %CVC MAX = (CVC/maximum CVC) x 100.

Response	***Time***
Baseline	The arithmetic mean of the last 2 minutes of the initial 5 minute period.
Initial peak	The arithmetic mean of the highest consecutive 30 second period within the distinct initial hyperaemic response.
Plateau	The arithmetic mean of the last 2 minutes of heating at 42°C.
Maximum	The arithmetic mean of the last 2 minutes of heating at 44°C.

Table 1: Laser Doppler Flowmetry regions of recorded responses

All tests were conducted in a temperature controlled room with a constant ambient temperature of 24°C. Throughout LDF assessments heart rate and diastolic and systolic blood pressure were recorded from the left arm at 5 minute intervals throughout the protocol (Dinamap Dash 2500, GE Healthcare, USA). Baseline skin blood flow data was recorded for 5 minutes with the local heating disc temperature set at 30 degrees. Following this, rapid local heating was initiated to obtain maximal vasodilation and the temperature was increased by 1 degree every 10 seconds until 42°C were reached. This was then maintained for 30 minutes. After this, the temperature of the probe was quickly increased to 44°C for a final 10 min to obtain maximal vasodilatation (Tew *et al.* 2011).

Transcutaneous Oxygen Pressure (TcPO2) Measurements and Sub-Maximal Exercise Tests

At the end of each visit, a sub-maximal test was also undertaken using a standard ergometer cycle while measuring the oxygen tension of participants, which consisted of four rounds. (Table 2).

Each participant was allowed to stop the sub-maximal test when they felt that they had reached their exertion limit.

Minute Rounds	***Resistance***	***Speed RPM (Rotations per minute)***
Round 1: 5 mins	0.5kg	80RPM
2 minute rest	-Rest-	-Rest-
Round 2: 5 mins	0.75kg	80 RPM
2 minute rest	-Rest	-Rest-
Round 3: 5 mins	1kg	80 RPM
2 minute rest	-Rest-	-Rest-
Round 4: 5 mins	1.25kg	80 RPM
2 minute rest	-Rest-	-Rest-
	Power (watt) = weight of load (Kg) * revolutions per minute (rpm).	

Table 2: Sub-maximal testing protocol

During the sub-maximal test, oxygen tension was measured using TcPO2 in which sensors were non-invasively attached onto the skin and allowed to heat. The heat produced by the sensor causes a dilatation of the skin blood capillaries, which increases blood flow and causes a subsequent diffusion of oxygen through the skin to the sensor. The sensor is able to measure TcPO2 value(s) inwardly through an electrochemical process.

Measurements were performed using the TINA TCM400 TcPO2 device (Radiometer, Copenhagen, Denmark). The temperature of the probe was set to 44.5°C to allow maximal skin vasodilatation, thereby decreasing the arterial to skin surface oxygen pressure gradient. Afterwards, the TcPO2 measurements were automatically temperature-corrected to 37°C by the TINA device. The electrode was placed slightly below the right scapula on the back away from any bone.

Fixation rings were used to hold the probe in-place and this was filled with two small drops of contact fluid before attachment to the sensor. The fluid is then heated causing the skin to subsequently dilate. Heart rate was also recorded throughout the duration of the sub-maximal test.

For the purposes of this study we defined TcPO2 as raw values of the participant's oxygen perfusion, obtained directly from Transcutaneous Oxygen Pressure machine recordings (Table 3).

TcPO2 Quantity	**Definition**
Baseline	The arithmetic mean of maximum TcPO2 at rest.
TcPO2max	The highest TcPO2 value recorded every minute of exercise or at rest.
Maximum change from Baseline (ΔTcPO2max)	The outcome of the subtraction of Baseline from TcPO2max: e.g. TcPO2max – Baseline
Change in Transcutaneous Oxygen Pressure (ΔTcPO2)	The average sum of the change from Baseline at rest and exercise period: e.g. (Σ (ΔY1…n)/n) = ΔTcPO2

Table 3: TCpO2 quantities used in our study and their definitions

Self-perceived exertion and pain

During sub-maximal exercise testing, we also recorded the participants' perceived pain and exertion was measured every minute, using the CR10 (rate of perceived pain) and the rate of perceived exertion (RPE) (Borg, 1970). The CR10 scale contains 20 different perceived pain intensity levels, with 0 corresponding to no perceived pain through to 20 corresponding to maximum pain. The RPE scale contains 14 numerical values beginning at the number 6 which corresponds with no exertion and 20 corresponding to maximum exertion.

TcPO2 profiles

As suggested by Ouedraogo and colleagues (2011) we categorised study participants as "healthy" and "diseased", according to their TcPO2 profiles. Type 1 corresponding to healthy; characterised by an initial increase during exercise. Type 2 corresponding to unhealthy; characterised by initial decline during exercise.

Statistical analysis

An Analysis of variance (ANOVA) was carried out to compare the effectiveness of supplements of GT for two weeks period on BMI, Blood Pressure, perceived pain and exertion and TCPO2 and microvascular LDF values (version 18.0 for windows, SPSS Inc., Chicago Illinois). The dependent variables consisted of the variables measurements before and after intervention period. Participants' measurement before the intervention period was used as base line values for analysis. Preliminary checks were conducted using the Kolmogorov-Smirnov goodness of fit test to ensure that there were no violations of assumptions of normality, linearity, homogeneity of variances, homogeneity of regression slopes, and are reliable measurements of covariates. Significant level for all tests was set at p=0.05. The number of days between sessions and number of doses missed were used to calculate the participants' overall compliance. Differences for compliance between groups were calculated using independent t-tests. Data was stored and transformed within Microsoft Excel (Microsoft Office 2013) and statistical analysis was performed using Predictive Analytics SoftWare Statistics v18.0 (SPSS: An IBM Company, New York, USA).

Results

Participant characteristics

Fifteen younger participants were deemed eligible to participate and completed all measures of the study. The fifteen younger participants had a mean age of 24 (4.0), with a mean BMI of 24.5 (4.2). Mean systolic blood pressure was 120 (10) and there was a diastolic mean of 67 (7.0).

Similarly, fifteen older participants aged 55-75 were recruited and completed all assessments. Seven participants in the older group were female, eight were male. The fifteen older participants had a mean age of 61 (4.0) with a mean BMI of 23.8 (4.6). The mean systolic blood pressure was 129 (12) and the mean diastolic blood pressure was 73 (5.0).

	Group A (younger group)		Group B (older group)	
	Visit 1	**Visit 2**	**Visit 1**	**Visit 2**
Gender	7 male, 8 female		8 male, 7 male	
Age (years)	24 (4)†		61 (4)	
Resting BP (systolic)	120 (10)	112 (10)**	129 (12)	124 (11)*
Resting BP (diastolic)	67 (7)†	65 (6)	73 (5)	72 (8)
Height (cm)	170 (8)		169 (7)	
Weight (kg)	71.4 (15.5)	71.2 (15.7)	66.7(11.1)	66(11.2)
BMI (kg/m^2)	24.6 (4.2)	24.6 (4.2)	23.8 (4.6)	23.5(4.4)
GT Consumption Compliance (units)	26 (2)		27 (1)	

*p<0.05 between visits (within groups) ** p<0.001 between visits (within groups)
† p<0.05 between groups (at the same time-point)

Table 4: Characteristics of the experimental groups

Participant characteristics are presented in Table 4. There were no statistically significant differences between groups at baseline for body mass index (BMI), stature, resting heart rate, or systolic blood pressure. There was however a significant difference between groups for resting diastolic blood pressure with a lower value noted in the younger groups.

Side effects

No participants withdrew from the study because of adverse events associated with the treatment. One participant noted mild gastrointestinal discomfort upon commencing the GT however this resolved naturally after the first day. There were no major adverse events reported during the study.

Compliance

GT consumption compliance was 26 (2) units for the younger group and 27 (1) units in the older group. No significant difference was found when comparing groups.

Blood Pressure, BMI and Weight

There were no statistically-significant differences between groups for BMI or diastolic blood pressure at baseline or at the end of the intervention. However, there was a significant difference for systolic blood pressure measurements, at the end of the intervention for both younger ($P < 0.05$) and older participants ($p<0.001$). Whilst both groups also demonstrated a pre- to post- GT consumption decrease in diastolic blood pressure, this was not statistically-significant in any of the groups. No significant changes were noted in either BMI or weight.

Raw Cutaneous Vascular Conductance (CVC)

Baseline

Raw CVC

There were no statistically significant differences between groups for Raw CVC baseline readings at any visit. Raw CVC baseline recordings within groups improved however this too was not significant in either of the two groups (Table 5).

% CVC MAX

There were no statistical differences between groups for % CVC MAX baseline readings at either the beginning or at the end of the intervention, although post-GT consumption % CVC MAX readings were elevated for both groups (Table 5).

Initial peak

Raw CVC

There was a statistically-significant difference between groups at the beginning of the intervention for Raw CVC at initial peak with the older group demonstrating a lower value. There was not a significant difference at the end of the intervention between groups; however, there were statistically-significant improvements within both groups (Table 5).

	Group A (younger group)		Group B (older group)	
	Raw CVC	**%CVCMAX**	**Raw CVC**	**%CVCMAX**
		Baseline		
Visit 1 (Pre-intervention)	0.25 (0.14)	10.7 (5.9)	0.31 (0.13)	10.8 (7.9)
Visit 2 (Post-intervention)	0.5(0.41)	12.6(7.6)	0.39(0.15)	13.6(4.3)
		Initial Peak		
Visit 1 (Pre-intervention)	1.6 (0.59)	76.4 (23.1)	1.25 (0.58)†	65.6 (16.5)
Visit 2 (Post-intervention)	2.05(0.72)*	87.1(17.6)	1.65 (0.5)*	77.8 (17.6)
		Plateau		
Visit 1 (Pre-intervention)	1.87 (0.79)	88.1 (19.9)†	1.45 (0.45)†	72.6 (22.5)
Visit 2 (Post-intervention)	2.19 (0.87)	95.2 (19.5)	1.67 (0.47)†	86.2 (13.1)*

*p<0.05 between visits (within groups)
† p<0.05 between groups (at the same time-point)

Table 5: Cutaneous Vascular Conductance of the Experimental Groups

% CVC MAX

There were no statistical significant differences between CVC MAX at both visits, although post-GT consumption % CVC MAX values were increased for both groups (Table 5).

Plateau

Raw CVC

There was a statistically-significant difference between groups pre-green, tea at the plateau stage for Raw CVC, with the older group having a lower reading than the younger group (e.g. 1.45 (0.45) vs 1.87 (0.79) respectively; $p<0.05$). Despite the increase in both groups, statistical significance remained at the end of the intervention (1.67 (0.47) vs 2.19 (0.87) for the older and younger group respectively; $p<0.05$).

% CVC MAX

Similarly to Raw CVC, pre-GT consumption %CVC MAX was significantly different between groups, with the older group having lower values (72.6 (22.5) vs 88.1 (19.9) respectively, $p<0.05$). The differences were diminished at the end of the intervention (95.2 (19.5) vs 86.2 (13.1), younger group vs older group respectively, $p>0.05$), with the older group achieving a statistically-significant improvement (72.6 (22.5) pre- vs 86.2(13.1) post-intervention, $p<0.05$) as well.

Oxygen Tension

All participants were categorised according to their sub-maximal exercise TcPO2 profile as previously described. At baseline, we recorded a total of 12 healthy profiles and 3 disease profiles in each of the two groups. The intervention had no effect on their categorisation (Table 6). Baseline ΔTcPO2 did not differ significantly between groups and this continued in a similar trend after the intervention; but a statistical-significant difference was observed within groups at the end of the intervention, for both the younger and older groups (i.e. 7 (5.5) vs 9.7 (5.4), $p<0.05$ for the older group). A similar trend was also observed with ΔTcPO2max, with statistical significance being reached for both groups at the end of the intervention (Table 6, Figure 1).

Heart Rate, Exercise Perceived pain (CR-10) and Exertion (RPE) Parameters

There was no significant variation at peak heart rate (beats/min) for participants during the sub-maximal test, between visits, in both groups (e.g. 128 (17) vs 132 (25) for the older and younger group respectively, $p>0.05$). Similarly, no significant differences were observed between groups, pre- and post- intervention visits (Table 6). The average perception of pain and exertion were lower in the younger group when compared to the older group both prior and after GT consumption, with statistical significance being reached at both occasions (Table 6). At the end of the intervention statistical significance was observed for the exertion parameter in the younger group, but not for the pain element (Table 6). At the same time, statistical significance was observed in pain perception but not in felt exertion in the older group (Table 6).

	Group A (younger group)	p value (between visits)	Group B (older group)	p value (between visits)	p value (between groups)
ΔTcPO2 (mmHg)					
Visit 1 (Pre-intervention)	6.8 (7)	0.04*	7 (5.5)	0.04*	0.86
Visit 2 (Post-intervention)	11.5 (6.5)		9.7 (5.4)		0.62
ΔTcPO2max (mmHg)					
Visit 1 (Pre-intervention)	14.3 (9.7)	0.04*	12.1 (6)	0.02*	0.50
Visit 2 (Post-intervention)	19.5 (8.2)		16.7 (6)		0.27
In-Exercise Peak HR (beats/min)					
Visit 1 (Pre-intervention)	132 (25)	0.9	128 (17)	0.08	0.50
Visit 2 (Post-intervention)	133 (23)		124 (19)		0.29
CR-10 (pain)					
Visit 1 (Pre-intervention)	2 (1.5)	0.3	5 (3)	0.01*	0.002
Visit 2 (Post-intervention)	1.5 (1.5)		4 (2)		0.01
RPE (exertion)					
Visit 1 (Pre-intervention)	10 (3)	0.04*	15 (3)	0.08	<0.001
Visit 2 (Post-intervention)	9 (2)		14 (2)		<0.001
TcPO2 Profile Categorization					
Visit 1 (Pre-intervention)	12 healthy, 3 disease	1.0	12 healthy, 3 disease	1.0	1.0
Visit 2 (Post-intervention)	12 healthy, 3 disease		12 healthy, 3 disease		1.0

*p<0.05 between visits

Table 6: Comparison of Physiological and Exercise Data (*obtained during Sub-maximal exercise testing*) between Groups and Visits.

Discussion

Blood pressure

In our study, we noted a significant decrease in systolic blood pressure in both the younger and older group following 2 weeks of GT consumption. The inverse association between GT consumption and systolic and diastolic blood pressure is well-established: for example, a 2013 Cochrane review on tea and blood pressure reported that in all 6 studies involving GT, a

statistically-significant blood pressure reduction was observed (Hartley *et al.* 2013). Whilst significance was not reached in diastolic blood pressure post-consumption in our study, improvements were nonetheless evident in both groups. Our findings are therefore consistent with previous literature, in suggesting GT may have a beneficial effect on hypertension which in turn could reduce an individual's risk of CVD (Sowers *et al.*, 2001).

Cutaneous Microvascular Function

Whilst there is at present, a lack of research on the effects of GT on microcirculation, a number of studies exploring vascular function in general have been published in the past. Fuchs and colleagues (2014), for example, examined the effect of catechins contained within GT on vascular functioning in 24 healthy men and women aged 45-75. The study found that 500 mg of GT catechins did result in a small but significant increase in readings, suggesting GT may have had a positive effect on vascular functioning. Similar results were observed by Jochmann and colleagues (2008) on the effect of catechins on endothelial functioning using Flow Mediated Dilatation (FMD). During our study, we attempted to expand those findings and explore the effects of GT consumption with regard to specific elements of vascular function, focusing on cutaneous microcirculation.

We noted improvements in the initial peak stage in the older participants in our study following the GT intervention. The initial peak stage is a neurogenic phase, which is initiated by the activity of sensory nerves and is mediated by an axon-reflex (Minson *et al.* 2001). A decrease in an individual's nerve axon-mediated vasodilation has been associated with abnormalities in the microcirculation (Nouri *et al.* 2012). Such abnormalities may then lead to an increased risk of neuropathic changes and subsequently CVD (Kannel & McGee, 1979). It is important to note that as an individual ages there is a definable loss of organisation in the vessels of the microcirculation (Montagena and colleagues 1975). This subsequently impacts the skins ability to respond to local heating. Our finding is in-line with previous work on lifestyle interventions (exercise; Tew *et al.*

2010, high-sodium diet; Greaney *et al.* 2012), which have been shown to reverse the ageing-invoked reduction of axon-mediated vasodilation.

In our study, we observed improvements in the plateau stage for the older [for example % CVC MAX 72.6 (22.5) increasing to 86.2 (13.1)] and younger group [% CVC MAX 88.1 (19.9) increasing to 95.2 (19.5)]. As the plateau stage is largely mediated by NO, this suggests the NO levels in our participants has increased, without however, this increase reaching levels of statistical significance. As reduced bio-availabilty of NO is thought to lead to a loss of cardio-protective actions and may even increase the progression of CVD (Naseem 2005), maintaining NO at acceptable levels can reduce CVD risk. This is consistent with previous literature in which an increase in NO vasodilation was observed following the administration of specific phenols contained within GT (epicatechin and epigallocatechin) (Huang *et al.* 1999; Lorenz *et al.* 2004). The observed trend makes further research – based on an intervention that would be of greater duration and potentially include a more complex diet – the next logical step in this area.

Skin Oxygen Tension

In an attempt to explore the effects of GT on microvascular function during every day mild physical activity (such as walking, lifting and climbing stairs), we assessed TcPO2 changes during sub-maximal exercise.

It is well accepted that older people are susceptible to reduced oxygen perfusion due to degradation of endothelium dependent NO by free radicals. Their source varies and can be obtained both from the diet and environmental assaults accumulating over time (Gerhard *et al.* 1996). Such assaults may eventually cause the arteries to narrow enough to reduce blood flow/cause total occlusion resulting in high BP/ cardiac arrest (Stary *et al.* 1995). Our study is the first to report a significant improvement in oxygen tension under provocation (e.g. exercise testing) at the end of a GT intervention in both younger and older groups. This may provide an indication that such subtle

arterial defect could be reversed or supressed with GT supplementation in the short-term, in healthy but sedentary populations. This expands on earlier findings by Fuchs *et al.* (2014) who reported significant improvements in hyperaemia following a 12-day intervention.

We observed significant improvements in both the younger and older group in ΔTcPO2max (oxygen tension) when comparing groups between visits. However a greater improvement was observed in the older group, suggesting that older people may derive more benefits from a GT-based intervention. On the other hand, GT consumption appeared to have no effect on the TcPO2 profiles of the participants in our study, with no changes observed between visits. More importantly no improvement was detected in participants classified as having a "disease" profile, as described by Ouedraogo and colleagues (2011). However, it must be noted that such profiles have been reported not only in people with cardiovascular impairments but also in healthy individuals (Kubota & Zoladz, 2005). Therefore assumptions made about the wellbeing of individuals classified as "diseased" in reference to TcPO2 categorisation, should be treated with caution.

Nevertheless, it appears that although GT can improve skin oxygen tension under provocation, this improvement is related to quantity and not quality, as the latter remains unaffected. Evidently however, further research would be needed to substantiate our findings and explore the mechanisms, which are motivated by GT consumption and affect skin oxygen tension when oxygen demand is higher.

Experimental concerns

All participants in our study were healthy, with no overt chronic disease effecting their microvascular functioning. It is therefore difficult to ascertain whether similar results would have been achieved had the population had an alternative health status, and indeed how well our results could therefore be extrapolated to the wider population. Future studies need to explore this.

Conclusions

Our results are consistent with the existing literature base purporting GT's positive effect on cardiovascular risk markers (Peng *et al.* 2014; Velayutham *et al.* 2008). It supports the notion that GT may protect against cardiovascular disease and that there may be merit therefore in introducing it into the diet of those most at risk of developing the disease. Exercise and various other dietary elements of an individual's lifestyle however have been linked to reducing the risk of CVD and therefore must also be taken into consideration (Kromhout *et al.*, 2002). There is a need therefore for further research to substantiate the significance of our observations.

Conflicts of interest

There are no conflicts of interest to declare.

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Figure Legend

Figure 1: ΔTCPo2max comparison between groups, prior and after 14 days of green tea consumption (*$p<0.05$).

Figure 1

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