The acute effect of black tea consumption on resistance artery endothelial function in healthy subjects. A randomized controlled trial

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ABSTRACT

Background & Aims: Black tea is a main source of flavonoids in the Western diet and has been associated with reduced risk for cardiovascular disease, possibly through lowering blood pressure. These effects may be mediated through improving endothelial function of resistance arteries. The aim of this study was therefore to examine the acute impact of black tea on forearm resistance artery endothelial function in healthy, normotensive middle-aged subjects.

Methods: Twenty middle-aged men and women (age-range 45-75 years) were recruited into a double-blind, randomized, placebo-controlled crossover intervention study. Forearm resistance artery blood flow (FBF, measured using venous occlusion plethysmography) in response to incremental doses of acetylcholine, sodium nitroprusside and L-N^G-monomethyl arginine were determined 2 hours after consumption of either black tea containing ~400 mg flavonoids (equivalent to 2-3 cups of tea) or a taste- and color-matched placebo.

Results: The mean FBF-response to acetylcholine after tea consumption was 23% higher compared to the response after placebo (95% CI: -20%, +88%), but this difference did not reach statistical significance (P=0.32). No significant differences in the FBF-responses to sodium nitroprusside and L-N^G-monomethyl arginine were found between the tea and placebo interventions (P=0.96 and 0.74, respectively). Correcting FBF for changes in blood pressure did not alter the outcomes.

Conclusions: We found no evidence that acute intake of black tea significantly altered endothelium-dependent vasodilation of forearm resistance arteries in healthy middle-aged subjects. Interventions with a longer duration of tea ingestion are required to further

explore the (long-term) impact of tea flavonoids on blood pressure regulatory mechanisms. This trial was registered at clinicaltrials.gov as NCT02328339.

Keywords: tea, flavonoids, randomized controlled trial, resistance arteries, endothelial function, blood pressure

INTRODUCTION

High blood pressure is a major risk factor for cardiovascular diseases (CVD) which are estimated to currently represent ~13% of the global mortality rate (equivalent to 7.5 million deaths annually) [1]. Changes in lifestyle, such as diet, can lower blood pressure and, as a consequence, reduce CVD risk in both symptomatic and asymptomatic subjects [2, 3]. For example, a high dietary intake of flavonoids has been associated with lower CVD risk and a better CVD risk factor profile in prospective follow-up studies [4, 5], and improvements in CVD risk factors in human-intervention studies [6]. Black tea, brewed from the leaves of *Camellia sinensis*, represents a major source of dietary flavonoids in most Western countries [7, 8]. Data from prospective observational studies have shown associations between tea consumption and lower CVD incidence and mortality [9]. These associations may, at least partly, be mediated through the blood pressure lowering effects of tea [10, 11].

Resistance vessel endothelial function plays an important role in blood pressure regulation [12]. Consequently, the blood pressure lowering effects of tea may be facilitated through improvements in endothelial function, mediating a drop in peripheral vascular resistance. It has been described previously that tea consumption results in improved endothelial function in conduit arteries [13]. Whilst these observations suggest an impact of tea on vascular health at conduit artery level, regulation of blood pressure is typically ascribed to resistance arteries. To date, only few studies have directly evaluated the effects of tea flavonoids on resistance arteries. For example, a recent study found that consumption of a flavonoid-rich fraction of black tea to improve (post-prandial) perfusion of resistance arteries in insulin-resistant men [14]. Another study found improved

resistance artery endothelial function after consumption of isolated green tea flavonoids in male smokers [15]. Whether such improvements in resistance artery endothelial function are present in the general population after ordinary black tea consumption is currently unknown.

The purpose of this study is to examine the impact of an acute dose of black tea on resistance artery endothelial function, evaluated by means of the isolated and perfused forearm technique [16], in a group of healthy middle-aged men and women. The study hypothesis was that, in agreement with earlier findings in conduit arteries, acute tea ingestion improves endothelial function in resistance arteries as indicated by an increase in the acetylcholine-mediated forearm blood flow (FBF) response compared to placebo.

MATERIALS AND METHODS

Study participants

Twenty middle-aged (median age 63 years, range 50-72 years) men (n=10) and post-menopausal women (n=10) without a history of cardiovascular diseases or diabetes mellitus were included. None of the participants used medication known to influence endothelial function. Subjects were selected from a database with volunteers who showed interest in contributing to studies as a participant. Current smokers, subjects who stopped smoking less than 6 months before study participation, subjects with a self-reported alcohol intake of ≥21 units per week and subjects who performed over 2 hours of strenuous exercise per week were excluded. Use of medication that does not influence endothelial function was allowed if medication use was stable for ≥3 months. This study

was performed according to the guidelines stated in the declaration of Helsinki 2013 and the Dutch Medical Research Involving Human Subjects Act (WMO). This study was approved by the Ethics Committee of the Radboud University Medical Center Nijmegen (CMO Arnhem-Nijmegen). All participants provided written informed consent prior to participation in the study.

Study design

This study followed a double-blind randomized cross-over design. Subjects reported twice to our laboratory. On both days, subjects ingested either black tea or a taste-, color- and temperature-matched placebo beverage in a randomized order. Subsequently, subjects were instrumented for assessment of forearm resistance artery endothelial function. Changes in FBF were measured using venous occlusion plethysmography (VOP) during intrabrachial administration of vasoactive drugs, which is the gold standard for assessment of endothelial function [16]. Increasing doses of acetylcholine (ACh; endothelium-dependent vasodilator), sodium nitroprusside (SNP; endothelium-independent vasodilator), and L-N^G-Monomethyl-arginine (L-NMMA; endothelium-dependent vasoconstrictor) were used. This allowed us to explore the impact of black tea on forearm resistance artery endothelium-dependent and -independent dilation as well as the contribution of nitric oxide (NO) to baseline vascular tone.

Tea and placebo

The intervention product was prepared using commercially available black tea (Lipton Yellow Label, Unilever B.V., The Netherlands) according to a standardized brewing protocol which produced tea infusions with a total flavonoid content of 1.28 ± 0.06 mg/ml

(as determined with the Folin Ciocalteu assay using gallic acid as a standard [17, 18]) and a caffeine content of 0.47 ± 0.02 mg/ml (as determined with reverse phase high-performance liquid chromatography [19]). Due to the duration of the FBF protocol, subjects were provided with a loading dose of 240 ml test product (307 mg flavonoids) given 2 hours before, and a maintenance dose of 120 ml (102 mg flavonoids) given 10 minutes before the start of the measurement. This amounted to a total flavonoid dose of approximately 409 mg (and ~150 mg caffeine, equivalent to ~3 cups of black tea [20]). The timing and size of the respective tea (flavonoid) doses were based on previously published plasma kinetic profiles of tea flavonoids [21, 22]. The placebo was provided as a powder which contained no flavonoids and consisted of 93.4% maltodextrin, 6% tea flavor and 0.6% silicon dioxide. For the loading and maintenance doses, 2 and 1 grams of placebo powder was respectively dissolved in 240- and 120 ml hot water. The test products were freshly prepared for each subject by an analyst not involved in the FBF measurements.

Protocol

Subjects underwent a medical screening, consisting of a medical history, physical examination (including measurement of body weight and -height) and collection of blood for assessment of fasting lipid spectrum and glucose levels. Upon approval for inclusion, 2 subsequent testing days were scheduled, with an interval of 2 to 6 weeks. During the week preceding the measurements, subjects were instructed not to consume tea (or tea-containing products) and foods high in flavonoids (e.g. cocoa, chocolate, red wine). In the 24 hours prior to the measurements, subjects were instructed to additionally avoid strenuous exercise and abstain from or vitamin C and from products containing caffeine

or alcohol. All measurements were performed after an overnight fast in a quiet, darkened, air-controlled room (22˚C).

Venous occlusion plethysmography: After the tea/placebo loading dose (and before the maintenance dose), the brachial artery of the non-dominant arm was cannulated for vasoactive drug infusion and intra-arterial blood pressure monitoring. Forearm blood flow was measured in both the experimental and contralateral forearm by ECG-triggered VOP. Mercury-in-silastic strain gauges were placed around the widest portion of the upper third of both forearms to quantify changes in FBF from changes in forearm volume. At least 20 minutes after cannulation of the brachial artery, and 10 minutes after consumption of the maintenance dose, infusion of the vasoactive drugs started. Following a fixed order, ACh was administered at 5, 10, 20 and 40 μg/ml, followed by SNP at 2, 4, and 8 μg/ml and L-NMMA at 2, 4 and 8 μmol/ml, respectively. Each dose was infused for 5 minutes at an infusion rate of 1 ml per 1000 ml of forearm volume per minute. Forearm volume was individually determined by measurement of water displacement in a glass column. Between each series of drug infusions, FBF was allowed to return to basal value during a 30 minute washout period (**Figure 1**).

This protocol was repeated at the next visit, during which subjects received the other intervention (according to a computer-generated randomized allocation sequence between subjects). The reproducibility of VOP to assess forearm blood flow shows a coefficient of variation of 8.6% (i.e. 7 days between testing) [23]. Forearm blood flow was calculated using standard formulae and expressed as ml/100 ml forearm volume/min as previously reported [23]. This data analysis was performed by two investigators

(TLCW & DMB) blinded to the subject's allocation to treatment. To account for any potential systemic hemodynamic variation, FBF data were also analyzed in terms of changes in forearm vascular resistance (FVR, calculated by dividing mean arterial pressure (MAP) by FBF) and changes in the blood flow ratio between the infusion and control arm (FBF ratio). For all measures the area under the dose-response curve (AUC), expressed in arbitrary units (AU), for each drug was calculated and analyzed as the primary outcome measure.

Statistical methods

It was estimated that to detect a 15% increase in the mean FBF AUC response to ACh with 80% power and at the 5% significance level, a sample size of 20 participants was required to complete the study (assuming a standard deviation of 20% for a within-subject difference of two forearm blood flow measurements [23]).

The statistical analysis was performed using SAS software version 9.4 (SAS Institute, Cary, NC). Data are expressed as mean ± SD, unless otherwise stated. Due to the skewed nature of the FBF data, logarithmic transformation was performed prior to analysis. Changes in FBF AUC responses to the different vasoactive drugs were analyzed using a series of Mixed ANOVA models. In each case the log of the mean recorded FBF per drug dosing level was treated as the response; Treatment, Period and Dose were treated as fixed effects; the log of the mean baseline FBF for the treatment arm in question and the average baseline value across both treatment arms were treated as covariates; subject and subject*visit were treated as random effects. Similar models were used to examine the

effect of the interventions on FVR, FBF ratio, MAP and heart rate. Conclusions were drawn by comparing treatments across all doses at a 5% level of significance.

Both an Intention-To-Treat (ITT) and a Per Protocol (PP) analysis were performed. The ITT population was defined as all subjects randomized in the study and having completed at least one intervention. The PP was the population in which data from subjects who were non-compliant, who took concomitant medication or who had an adverse event that could have influenced vascular function have been removed. Where baseline data were deemed invalid, the subject concerned was necessarily omitted from the analysis in question. Where only a subset of dosed responses were deemed invalid the remaining data were employed, the analysis approach adjusting for the estimated effects of missing data.

RESULTS

Baseline characteristics of the study participants are provided in Table 1. All 20 subjects completed the study. During blind review, all data from one subject who had an adverse event (emesis after test product consumption) and another who reported gastric illness symptoms on the day prior to a measurement visit were excluded from the PP analyses. Additionally, FBF data from 4 different subjects had to be removed from the PP and ITT analyses due to technical problems (n=2 during L-NMMA, n=1 during ACh, n=1 during SNP). An overview of the trial design and subject disposition is provided in **Figure 2**. Since the PP and ITT outcomes did not differ, the PP data are presented.

Hemodynamic effects

Baseline heart rate and MAP did not differ between the placebo and tea intervention periods. Heart rate remained stable throughout all three drug infusions (Table 2). During ACh infusion, MAP increased after the tea administration (P=0.03, Table 2), but this difference did not persist during the SNP and L-NMMA infusions.

Resistance artery endothelial function

Due to a skewed distribution, data analysis was performed on log-transformed data for FBF (and back-transformed data to present in figures). The estimated mean difference in the log FBF-AUC response to ACh for tea *vs* placebo was 0.21 AU (95% CI: -0.22, 0.63). This corresponds to a +23% (95% CI: -20%, +88%) difference in the mean FBF-response to ACh after tea consumption compared to the response after placebo, but this did not reach statistical significance (**Figure 3**, P=0.32). No significant differences in the FBF-AUC responses between tea and placebo were found during infusion of SNP and L-NMMA (Figure 3). Throughout the study, contralateral FBF remained constant (data not shown).

Analyzing data as FVR or FBF ratio, minimizing the impact of changes in blood pressure, or potential systemic effects of the study drugs, revealed no significant differences between tea and placebo during infusion of ACh or L-NMMA (all P > 0.10, Table 2). Whilst FVR did not differ between tea and placebo for SNP infusion, FBF-ratio response to SNP was different between both trials, with a larger FBF-ratio response after placebo compared to tea (Table 2, P=0.04). The difference in change over time were not

statistically significant between the two interventions however (SNP dose*treatment interaction: P = 0.65).

DISCUSSION

Epidemiological data suggest the presence of an inverse association between consumption of tea beverages prepared from the leaves of *Camellia sinensis* and the risk of stroke as well as prominent risk factors thereof such as blood pressure and arterial stiffness [24-26]. Reductions in blood pressure following continued tea consumption provide a plausible explanation for these epidemiological observations [11, 27]. This acute intervention study aimed to determine whether the consumption of a black tea beverage, providing approximately 400 mg flavonoids, would result in acute effects on forearm resistance artery endothelial function – an important blood pressure regulatory mechanism. Our hypothesis was however not confirmed as we did not find a statistically significant increase in endothelium-dependent vasodilation in forearm resistance vessels compared to placebo in healthy middle-aged subjects.

A few previous intervention studies did demonstrate some evidence for acute effects of tea consumption on resistance arteries. For example, Fuchs *et al.* found tea intake to prevent significant elevation in postprandial forearm vascular resistance [14]. Furthermore, Oyama *et al.* found significant increases in both ACh and reactive hyperemia-induced changes in FBF two hours after consumption of a large dose of isolated green tea flavonoids in smokers [15, 28]. Direct comparisons between these studies and ours, however, is difficult since important differences are present between

studies. Whilst we included healthy middle-aged subjects, previous work included subjects with increased risk for cardiovascular disease with *a priori* endothelial dysfunction (i.e. smokers and insulin resistant obese), potentially making it easier to observe an effect from a food product to improve endothelial function. Secondly, we explored the impact of ~3 cups of normal black tea to match a real-life situation, whilst previous work examined isolated tea flavonoids and compared this with low or placebo-controlled caffeine content. Lastly, since the blood pressure lowering effects of tea intake are relatively modest (approx. 2 mmHg [10, 29]), it is possible that, if changes in blood flow in resistance arteries and in vascular resistance do indeed contribute to the blood pressure lowering effects, the effects on these measures might be quite small. Nonetheless, the 23% increase in the FBF-AUC response to ACh that was observed in healthy middle-aged subjects was within the expected range based on the abovementioned studies. However, due to a larger variation than expected, this effect was not statistically significant.

Despite the blood pressure lowering effects of its longer term consumption [10, 29], tea has previously been demonstrated to have acute pressor effects, possibly due to the caffeine content [30]. In accordance with these findings, we did see a statistically significant increase in MAP during the ACh infusion protocol (Table 2). The effect was short-lived however and was not evident during infusion of SNP or L-NMMA. It is unlikely that the change in MAP affected the FBF response to ACh infusion. Indeed, analyzing the data in terms of FVR and the blood flow ratio of the infused to the control arm (both of which correct for systemic hemodynamic changes which might affect local blood flow) did not alter the conclusions of our work.

297

298 It is interesting to note that several previous human intervention studies have found
299 improvements in brachial artery endothelial function, as measured by flow mediated
300 dilation (FMD) [13]. Tea flavonoids and their metabolites may affect conduit artery FMD
301 by improving NO bioavailability through stimulation of endothelial NO synthase activity
302 and prevention of superoxide-mediated NO breakdown [31]. We hypothesized that these
303 effects would also be present at the resistance artery level. It is however important to note
304 that that the values obtained by these two techniques do not always correlate [32, 33],
305 suggesting that an intervention might elicit an effect in one vessel type but not another.
306 As such, differential effects of blood pressure lowering drugs on conduit- versus
307 resistance arteries, underlines the concept that endothelium is a paracrine organ, whose
308 function/dysfunction can vary depending on which vascular district is explored or which
309 stimulus is employed [34].

310

311 Tea ingestion acutely improves conduit artery endothelial function, with peak
312 improvements in FMD seen within ~2 hours after intake [13]. Longer duration studies
313 have also demonstrated improvements in fasting FMD following continued tea intake for
314 several days/weeks [35-37]. These findings suggest that the initial acute effects on
315 endothelial function, in conduit arteries at least, following tea ingestion may become
316 sustained with continued intake over longer time periods. Interestingly, sustained
317 improvements in FMD following longer-term tea intake have in some cases been
318 accompanied by reductions in blood pressure and measures of small vessel tone [35, 38,
319 39], suggesting that beneficial effects on resistance artery endothelial function might have
320 been observed after a more prolonged (several days/weeks) exposure. This provides

further support that acute and chronic effects of tea (on blood pressure, endothelial function) may not be interchangeable and that future studies are required to better understand the long-term effect of tea ingestion.

A potential factor which may have influenced the outcome of this study is the caffeine content (150 mg) of the test product. We did not control for caffeine in the placebo since our intent was to examine the impact of black tea on resistance artery endothelial function such as present in a real-life situation and not the effect of its individual components. It is however important to note that caffeine is a nonselective competitive antagonist of adenosine receptors, known to acutely increase peripheral vascular resistance and reduce resting blood flow in the forearm microcirculation [40]. The effects of caffeine on endothelium-dependent dilation in resistance and conduit arteries are not consistent however. Indeed, intake of a high dose of caffeine (300 mg) was found to augment the increase in FBF responses to ACh in one study [41], whereas studies on brachial artery FMD have produced conflicting results [36, 42-44]. It is therefore difficult to conclusively comment on the potential confounding effects of caffeine in this study based on currently available evidence.

Strengths of this study include the blinded within-subject crossover design and use of a robust measure of resistance artery endothelial function. Some limitations need to be taken into account. In this study FBF responses were assessed at 2 h after tea intake, a time-point based on the anticipated time of peak tea flavonoid plasma concentrations. Since we did not measure circulating or urinary levels of tea flavonoids or their metabolites, we could not confirm the time-course of the bioavailability. However,

previous studies provided sufficient evidence for the elevation of flavonoids and their metabolites several hours after consumption of tea [21, 22, 45], which makes it unlikely that we failed to assess resistance artery responses during the peak in plasma flavonoid concentrations.

In conclusion, this study does not support the hypothesis that tea consumption leads to an immediate improvement in resistance artery endothelial function in healthy middle-aged subjects. Further evidence is required, preferably from interventions with a longer duration, in order to determine whether tea consumption affects peripheral vascular resistance and if so, which mechanistic pathways are involved.

AUTHORS' CONTRIBUTIONS TO MANUSCRIPT

The authors' responsibilities were as follows—AG, TPM and DHT: conceived and designed the study; TLW, DMB, SHR, NR: conducted the human study; TLW: performed the data analysis, MJR: conducted the statistical analysis; AG, TLW and DHT: drafted the manuscript; All of the authors made significant contributions to this manuscript. All authors read and approved the final manuscript.

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DISCLOSURES

AG, TPM and MJR are employed by Unilever R&D. Unilever produces foods of which some are marketed to fit in a healthy diet and lifestyle. No other authors declare a conflict of interest.

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LEGENDS FOR FIGURES

Figure 1. Schematic presentation of measurement protocol. Concentrations presented are in µg/L for ACh and SNP and µmol/L for L-NMMA. ACh, acetylcholine; FBF, forearm blood flow; L-NMMA, N^G-Monomethyl-L-arginine; SNP, sodium nitroprusside.

Figure 2. Enrollment, randomization and trial design. *One subject experienced an adverse event (emesis after test product consumption) and was subsequently excluded from the Per Protocol analyses. ACh, acetylcholine; FBF, forearm blood flow; L-NMMA, N^G-Monomethyl-L-arginine; SNP, sodium nitroprusside.

Figure 3. Mean (±95% CI) forearm blood flow area under the curve during infusion of acetylcholine (ACh, administered at 5, 10, 20 and 40 µg/ml) sodium nitroprusside (SNP, administered at 2, 4, and 8 µg/ml) and N^G-Monomethyl-L-arginine (L-NMMA, administered at 2, 4 and 8 µmol/ml) after consumption of ~400 mg tea flavonoids (open bars) or placebo (shaded bars).

525 **TABLES**

526 **Table 1.** Characteristics of subjects included in the trial. Data are presented as mean ±
527 SD.

Characteristics		
N	20	
Gender, females/males	10/10	
Age (years)	62.2	± 6.2
Weight (kg)	74.2	± 14.6
Body Mass Index (kg/m^2)	24.6	± 4.2
Systolic blood pressure (mmHg)	130.4	± 11.1
Diastolic blood pressure (mmHg)	79.4	± 7.9
Plasma glucose (mmol/l)	4.8	± 0.3
Total cholesterol (mmol/l)	6.0	± 1.2
HDL cholesterol (mmol/l)	1.7	± 0.5
LDL cholesterol (mmol/l)	4.0	± 1.0
Triglycerides (mmol/l)	1.3	± 0.3

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531 **Table 2.** Resistance vessel, blood pressure and heart rate responses to infusion of vasoactive drugs after tea and placebo. Data are presented

532 as median (IQR).

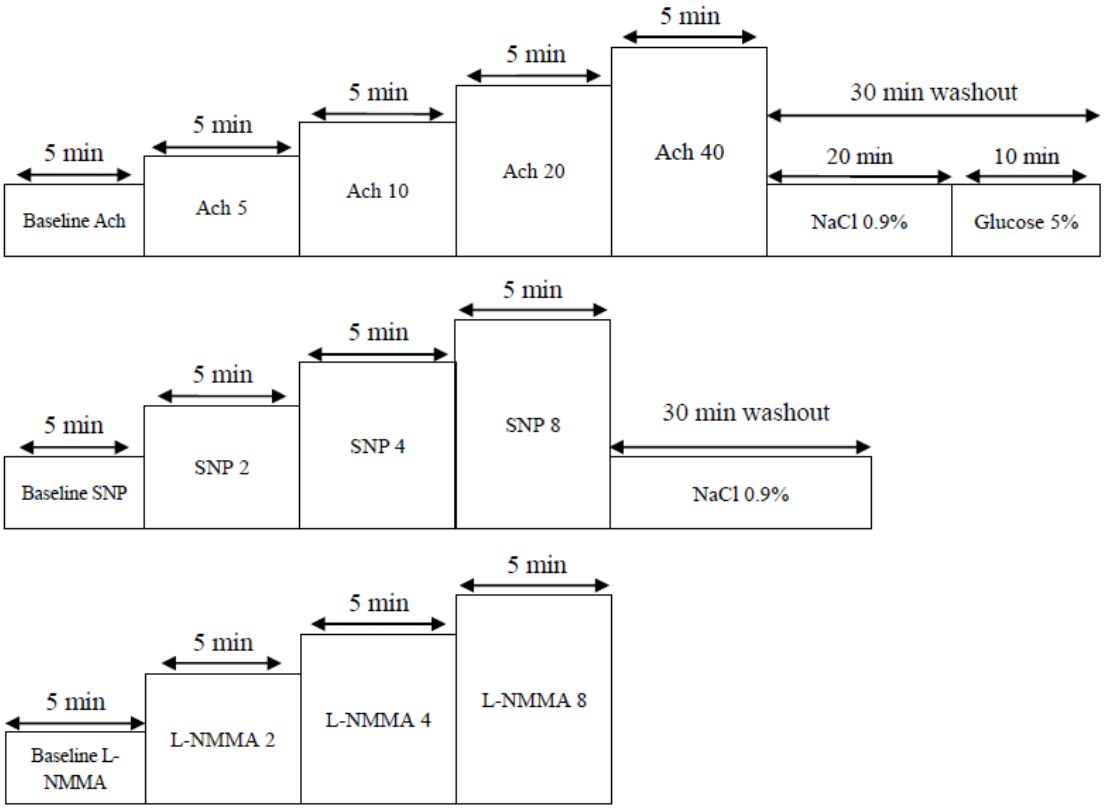
ACh						
FBF (ml/100ml/min)	**Baseline**	**5 µg/ml**	**10 µg/ml**	**20 µg/ml**	**40 µg/ml**	**P-value***
Placebo	1.1 (0.6-1.8)	1.7 (0.9-2.0)	2.2 (1.4-3.1)	2.4 (1.5-4.3)	4.1 (1.6-6.9)	0.32
Tea	1.3 (0.9-2.3)	2.3 (1.5-3.6)	2.5 (1.3-4.4)	2.8 (1.6-6.3)	5.6 (2.1-8.0)	
FVR (mmHg/100ml/min)						
Placebo	90 (52-138)	55 (49-110)	48 (30-69)	38 (21-60)	21 (13-65)	0.78
Tea	73 (48-111)	43 (27-72)	41 (24-81)	32 (17-64)	17 (12-49)	
FBF Ratio						
Placebo	1.3 (0.9-1.7)	1.3 (1.0-2.0)	2.1 (1.1-3.3)	1.8 (1.5-5.2)	3.3 (1.4-8.7)	0.77
Tea	1.2 (0.9-2.0)	1.3 (1.0-3.8)	1.8 (0.8-4.1)	2.2 (1.2-4.4)	3.5 (1.6-7.8)	
MAP (mmHg)						
Placebo	96 (88-101)	97 (88-100)	96 (88-101)	90 (86-101)	92 (86-102)	**0.03**
Tea	98 (93-105)	98 (94-104)	98 (93-107)	100 (94-106)	101 (93-108)	
HR (beats/min)						
Placebo	59 (57-62)	59 (57-63)	59 (58-60)	59 (58-64)	60 (57-62)	0.52
Tea	58 (56-66)	58 (56-65)	59 (56-63)	58 (56-64)	58 (57-65)	
SNP						
FBF (ml/100ml/min)	**Baseline**	**2 µg/ml**	**4 µg/ml**	**8 µg/ml**		**P-value***
Placebo	1.1 (0.8-1.6)	3.8 (3.0-6.3)	5.4 (3.6-7.6)	6.3 (4.4-10.5)		0.96
Tea	1.4 (0.8-1.9)	4.7 (2.9-6.2)	5.4 (3.6-9.2)	6.6 (5.2-10.0)		
FVR (mmHg/100ml/min)						
Placebo	92 (65-109)	23 (13-30)	15 (12-26)	14 (8-18)		0.18
Tea	69 (46-100)	21 (16-35)	21 (10-29)	14 (9-19)		
FBF Ratio						
Placebo	1.0 (0.7-1.7)	3.5 (2.5-5.0)	5.3 (3.6-8.1)	7.0 (4.3-9.3)		**0.04**
Tea	1.3 (0.7-1.7)	2.7 (2.1-5.0)	3.6 (2.7-6.4)	5.6 (3.6-7.3)		

MAP (mmHg)					
Placebo	95 (90-102)	94 (90-104)	94 (86-103)	91 (87-100)	0.44
Tea	99 (92-109)	99 (94-106)	100 (90-106)	98 (90-105)	
HR (beats/min)					
Placebo	58 (52-61)	61 (54-62)	59 (57-61)	61 (55-65)	0.58
Tea	59 (55-66)	61 (56-63)	60 (57-63)	62 (58-64)	
L-NMMA					
FBF (ml/100ml/min)	**Baseline**	**2 µmol/ml**	**4 µmol/ml**	**8 µmol/ml**	**P-value***
Placebo	1.5 (0.8-1.8)	1.1 (0.7-1.3)	1.0 (0.5-1.3)	1.0 (0.6-1.2)	0.74
Tea	1.5 (1.2-2.8)	1.1 (0.9-1.5)	1.2 (0.7-1.7)	1.1 (0.7-2.1)	
FVR (mmHg/100ml/min)					
Placebo	71 (51-115)	89 (71-166)	98 (77-210)	103 (88-182)	0.63
Tea	63 (42-82)	85 (63-110)	89 (72-150)	99 (48-157)	
FBF Ratio					
Placebo	0.9 (0.7-1.6)	0.8 (0.7-1.0)	0.6 (0.5-0.9)	0.7 (0.6-0.9)	0.87
Tea	1.3 (0.9-1.7)	0.8 (0.6-1.3)	0.6 (0.5-1.0)	0.9 (0.4-1.4)	
MAP (mmHg)					
Placebo	95 (92-105)	97 (94-106)	99 (93-107)	100 (92-107)	0.90
Tea	101 (91-108)	98 (92-109)	101 (93-110)	103 (94-111)	
HR (beats/min)					
Placebo	60 (57-63)	60 (57-63)	60 (59-64)	60 (58-65)	0.92
Tea	59 (56-62)	59 (58-63)	60 (58-64)	61 (58-66)	

*P-values refer to mixed ANOVA models with the log of the outcome parameter in question (FBF, FVR, Ratio, MAP or HR) per drug dosing level as the response, treatment, period and dose as fixed effects, the log of the baseline of the outcome parameter for the treatment arm in question and the average baseline value across both treatment arms as covariates and subject as well as subject*visit as random effects. FBF, forearm blood flow; FVR, forearm vascular resistance; FBF ratio, blood flow ratio between the infusion and control arm; MAP, mean arterial pressure; HR, heart rate. *P-values in bold are significantly different *vs* placebo at $P<0.05$.

FIGURES

Figure 1:



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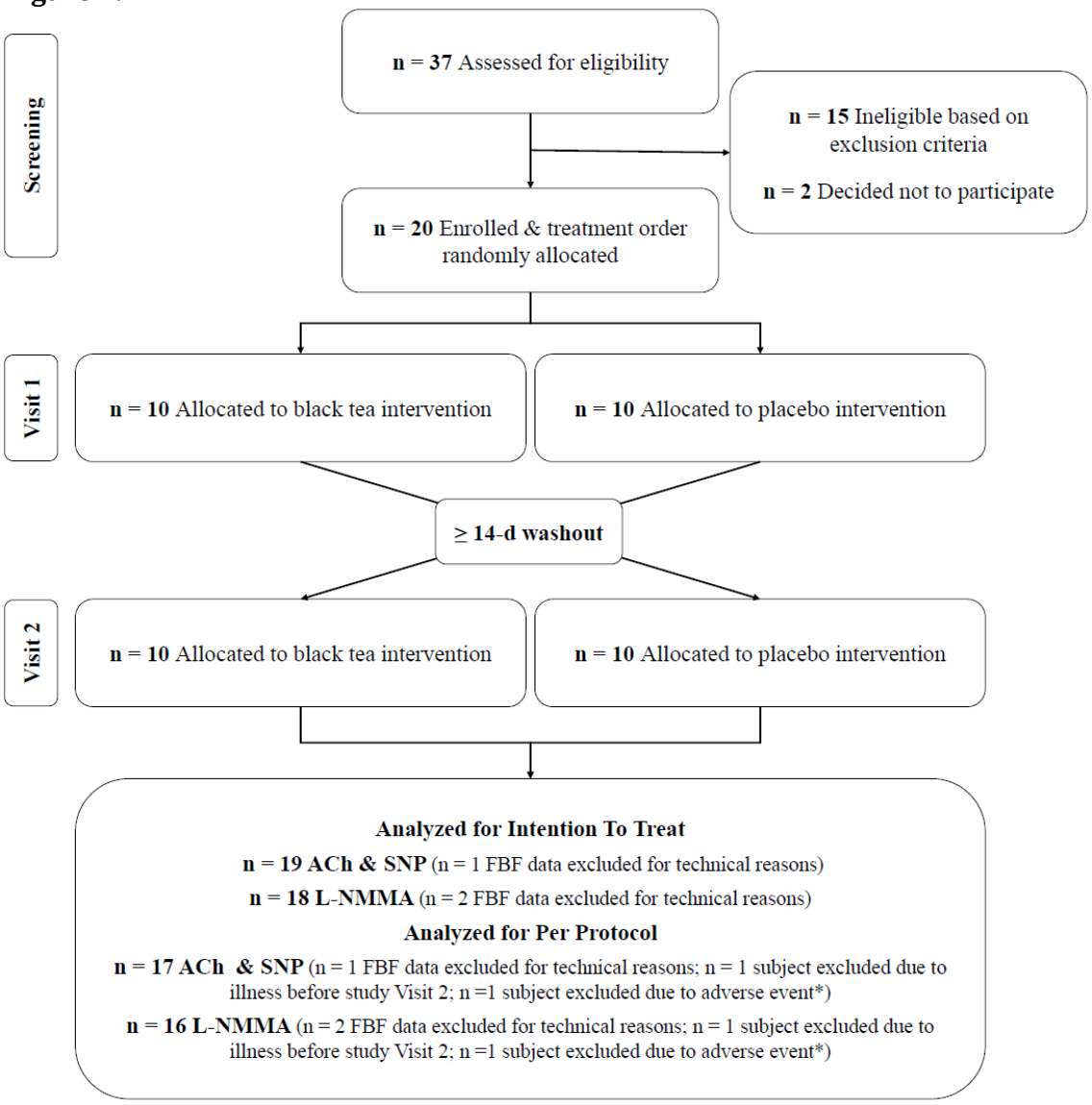

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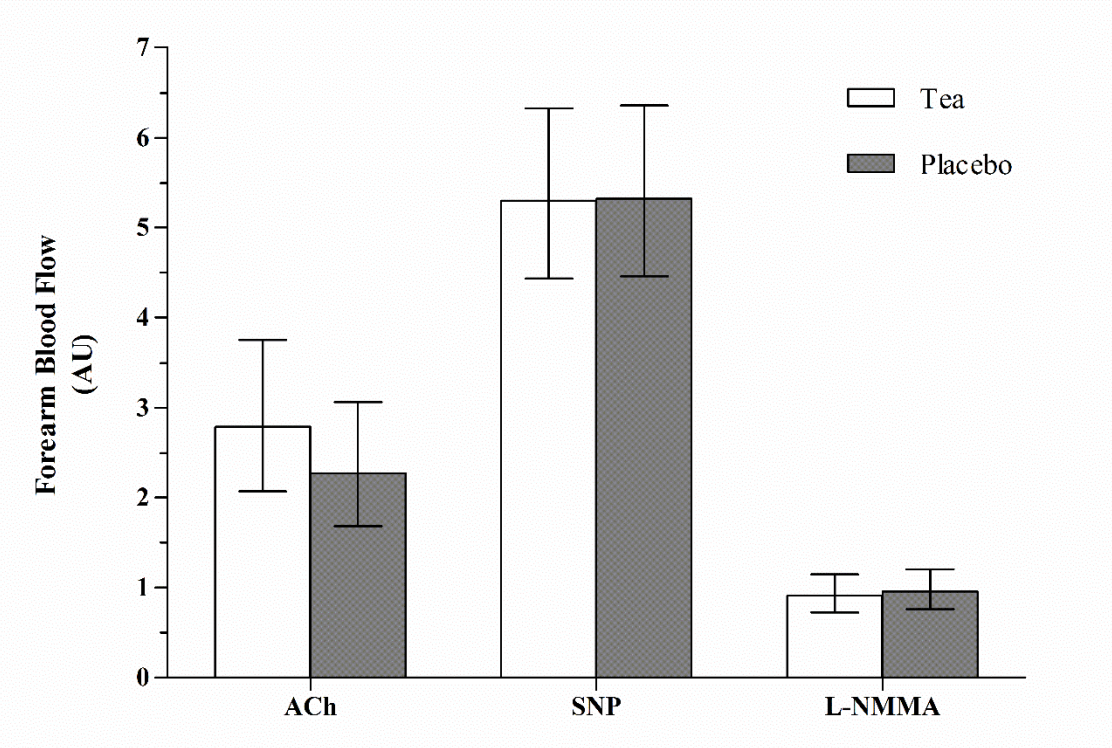
545 **Figure 3:**



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