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

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30

31 **ABSTRACT**

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

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

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

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

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

56

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

59 **INTRODUCTION**

60 High blood pressure is a major risk factor for cardiovascular diseases (CVD) which are
61 estimated to currently represent ~13% of the global mortality rate (equivalent to 7.5
62 million deaths annually) [1]. Changes in lifestyle, such as diet, can lower blood pressure
63 and, as a consequence, reduce CVD risk in both symptomatic and asymptomatic subjects
64 [2, 3]. For example, a high dietary intake of flavonoids has been associated with lower
65 CVD risk and a better CVD risk factor profile in prospective follow-up studies [4, 5], and
66 improvements in CVD risk factors in human-intervention studies [6]. Black tea, brewed
67 from the leaves of *Camellia sinensis*, represents a major source of dietary flavonoids in
68 most Western countries [7, 8]. Data from prospective observational studies have shown
69 associations between tea consumption and lower CVD incidence and mortality [9]. These
70 associations may, at least partly, be mediated through the blood pressure lowering effects
71 of tea [10, 11].

72

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

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

87

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

94

95

96 **MATERIALS AND METHODS**

97 **Study participants**

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

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

112

113 **Study design**

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

126

127 **Tea and placebo**

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

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

145

146 **Protocol**

147 Subjects underwent a medical screening, consisting of a medical history, physical
148 examination (including measurement of body weight and -height) and collection of blood
149 for assessment of fasting lipid spectrum and glucose levels. Upon approval for inclusion,
150 2 subsequent testing days were scheduled, with an interval of 2 to 6 weeks. During the
151 week preceding the measurements, subjects were instructed not to consume tea (or tea-
152 containing products) and foods high in flavonoids (e.g. cocoa, chocolate, red wine). In
153 the 24 hours prior to the measurements, subjects were instructed to additionally avoid
154 strenuous exercise and abstain from or vitamin C and from products containing caffeine

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

157

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

172

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

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

186

187 **Statistical methods**

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

192

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

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

204

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

213

214

215 **RESULTS**

216 Baseline characteristics of the study participants are provided in Table 1. All 20 subjects
217 completed the study. During blind review, all data from one subject who had an adverse
218 event (emesis after test product consumption) and another who reported gastric illness
219 symptoms on the day prior to a measurement visit were excluded from the PP analyses.

220 Additionally, FBF data from 4 different subjects had to be removed from the PP and ITT
221 analyses due to technical problems (n=2 during L-NMMA, n=1 during ACh, n=1 during
222 SNP). An overview of the trial design and subject disposition is provided in **Figure 2**.

223 Since the PP and ITT outcomes did not differ, the PP data are presented.

224

225

226 *Hemodynamic effects*

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

231

232 *Resistance artery endothelial function*

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

242

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

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

251

252

253 **DISCUSSION**

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

265

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

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

287

288 Despite the blood pressure lowering effects of its longer term consumption [10, 29], tea
289 has previously been demonstrated to have acute pressor effects, possibly due to the
290 caffeine content [30]. In accordance with these findings, we did see a statistically
291 significant increase in MAP during the ACh infusion protocol (Table 2). The effect was
292 short-lived however and was not evident during infusion of SNP or L-NMMA. It is
293 unlikely that the change in MAP affected the FBF response to ACh infusion. Indeed,
294 analyzing the data in terms of FVR and the blood flow ratio of the infused to the control
295 arm (both of which correct for systemic hemodynamic changes which might affect local
296 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

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

324

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

338

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

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

349

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

355

356 **AUTHORS' CONTRIBUTIONS TO MANUSCRIPT**

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

362

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366

367 **DISCLOSURES**

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

371

372

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508 **LEGENDS FOR FIGURES**

509 **Figure 1.** Schematic presentation of measurement protocol. Concentrations presented
510 are in $\mu\text{g/L}$ for ACh and SNP and $\mu\text{mol/L}$ for L-NMMA. ACh, acetylcholine; FBF,
511 forearm blood flow; L-NMMA, N^G -Monomethyl-L-arginine; SNP, sodium
512 nitroprusside.

513

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

518

519 **Figure 3.** Mean ($\pm 95\%$ CI) forearm blood flow area under the curve during infusion of
520 acetylcholine (ACh, administered at 5, 10, 20 and 40 $\mu\text{g/ml}$) sodium nitroprusside (SNP,
521 administered at 2, 4, and 8 $\mu\text{g/ml}$) and N^G -Monomethyl-L-arginine (L-NMMA,
522 administered at 2, 4 and 8 $\mu\text{mol/ml}$) after consumption of ~ 400 mg tea flavonoids (open
523 bars) or placebo (shaded bars).

524

525 **TABLES**526 **Table 1.** Characteristics of subjects included in the trial. Data are presented as mean \pm

527 SD.

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

528

529

530

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)						0.78
Placebo	90 (52-138)	55 (49-110)	48 (30-69)	38 (21-60)	21 (13-65)	
Tea	73 (48-111)	43 (27-72)	41 (24-81)	32 (17-64)	17 (12-49)	
FBF Ratio						0.77
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)	
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)						0.03
Placebo	96 (88-101)	97 (88-100)	96 (88-101)	90 (86-101)	92 (86-102)	
Tea	98 (93-105)	98 (94-104)	98 (93-107)	100 (94-106)	101 (93-108)	
HR (beats/min)						0.52
Placebo	59 (57-62)	59 (57-63)	59 (58-60)	59 (58-64)	60 (57-62)	
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)						0.18
Placebo	92 (65-109)	23 (13-30)	15 (12-26)	14 (8-18)		
Tea	69 (46-100)	21 (16-35)	21 (10-29)	14 (9-19)		
FBF Ratio						0.04
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)		
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)		

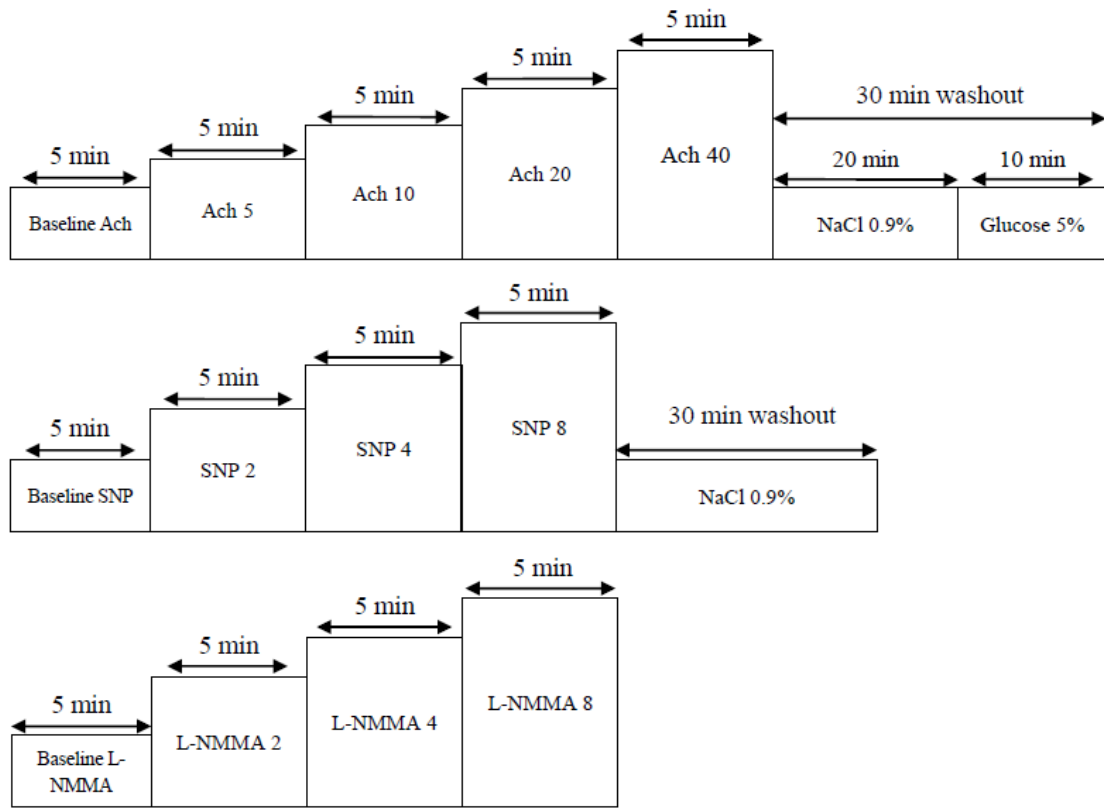
MAP (mmHg)					
Placebo	95 (90-102)	94 (90-104)	94 (86-103)	91 (87-100)	0.44
Tea	99 (92-109)	99 (94-106)	100 (90-106)	98 (90-105)	
HR (beats/min)					
Placebo	58 (52-61)	61 (54-62)	59 (57-61)	61 (55-65)	0.58
Tea	59 (55-66)	61 (56-63)	60 (57-63)	62 (58-64)	
L-NMMA					
FBF (ml/100ml/min)	Baseline	2 µmol/ml	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)	

533 *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,
534 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
535 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
536 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.

537 **FIGURES**

538

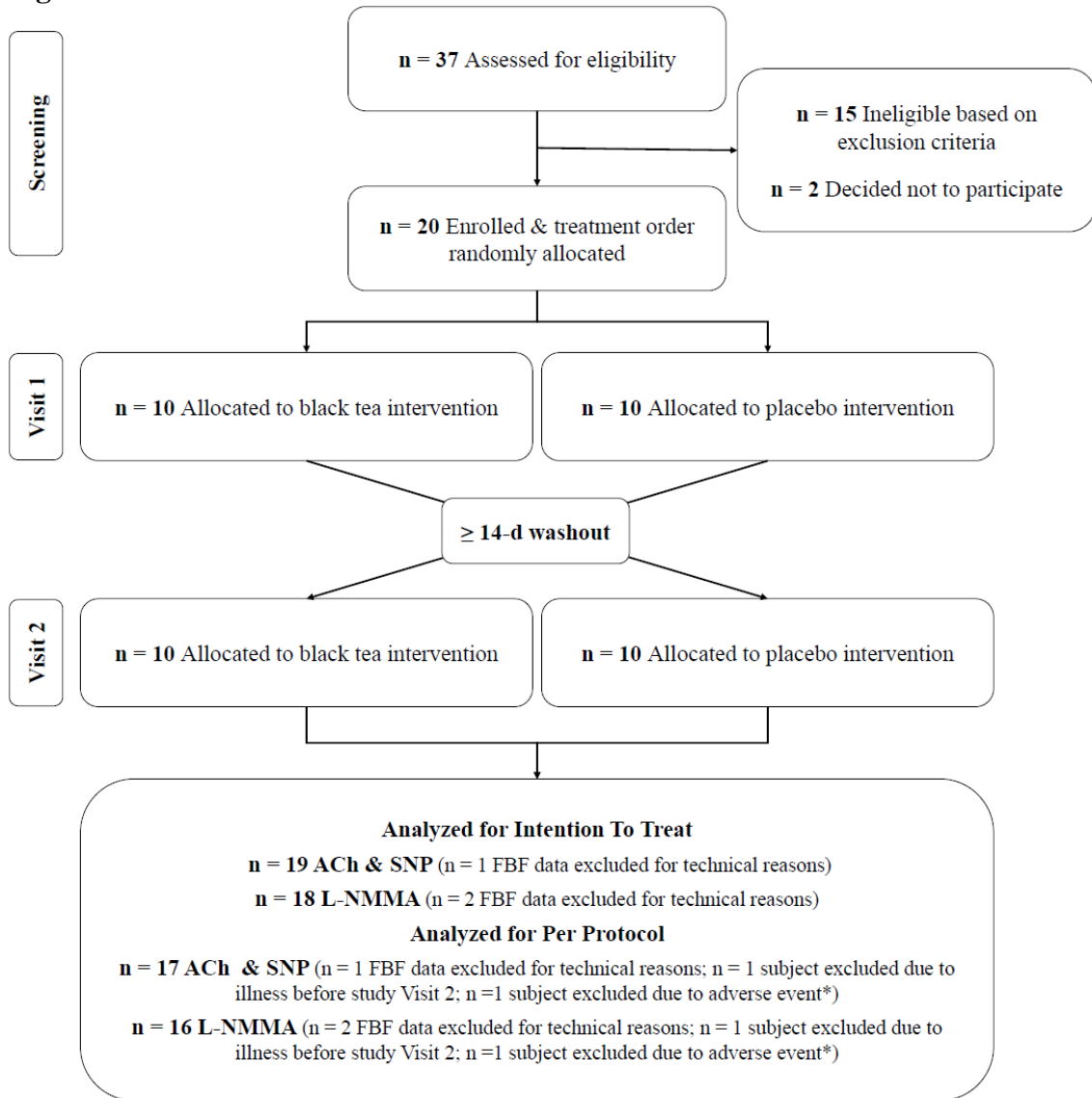
539 **Figure 1:**



540

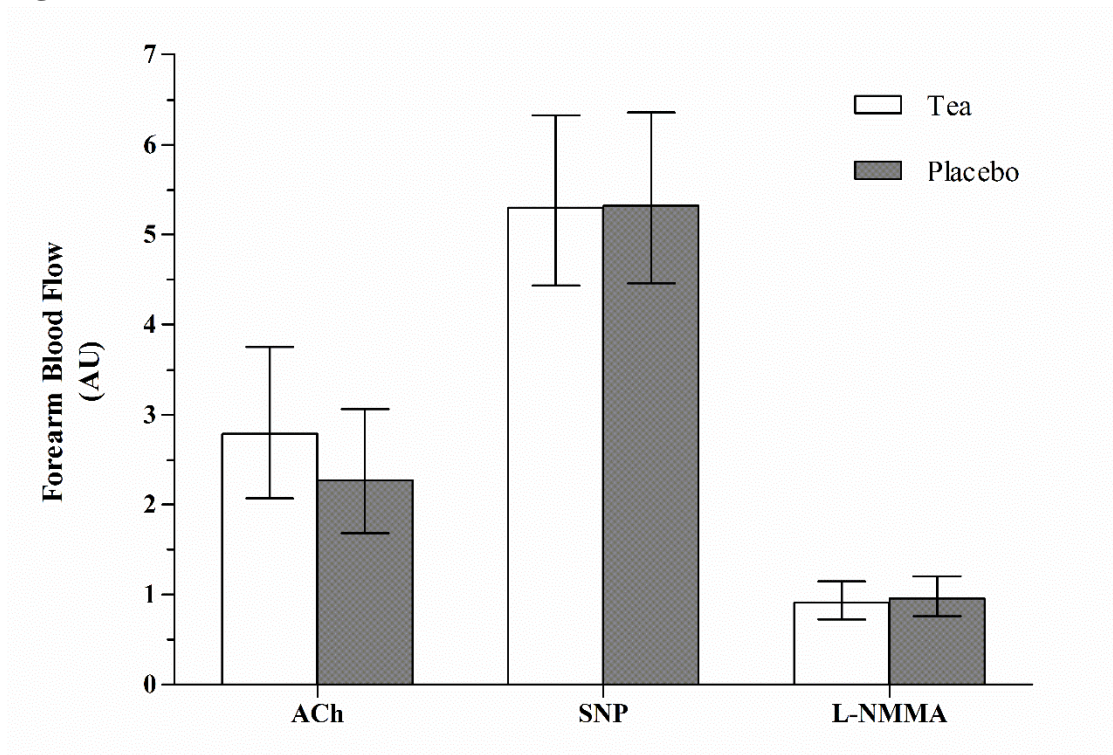
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542 **Figure 2:**



543
544

545 **Figure 3:**



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