Title Responses to a single dose of different polyphenols on the microcirculation and systemic circulation in rats.

Name of authors Nozomi Aruga¹, Megumi Toriigahara¹, Masahiro Shibata¹, Takeshi Ishii², Tsutomu Nakayama³, Naomi Osakabe^{1*}

Full affiliation

1. Department of Bio-science and Engineering, Shibaura Institute of Technology, 307 Fukasaku, Munumaku, Saitama, 337-8570, Japan

2. Department of Food and Nutritional Sciences, University of Shizuoka, Yada 52-1, Surugaku, Shizuoka 422-8526, Japan

3. School of Food Science and Technology, Nippon Veterinary and Life Science University, 1-7-1 Kyonanchō, Musashino-shi, Tokyo 180-8602 Japan

***Corresponding author:**

Department of Bio-science and Engineering, Shibaura Institute of Technology, 307 Fukasaku, Munumaku, Saitama, 337-8570, Japan

Tel +81-48-720-6031, Fax +81-48-720-6011, email

nao-osa@sic.shibaura-it.ac.jp

1 **Abstract**

2 Numerous studies suggest the ingestion of polyphenols can potentially
3 confer haemodynamic benefits. The association between different
4 polyphenolic chemical structures and their physiologic effects on systemic
5 haemodynamic variables and microcirculation are less well known. This
6 study investigated the effects of a single 10 mg/kg body weight dose of
7 different polyphenolic compounds on haemodynamic and
8 microcirculatory parameters. Key results from laser Doppler flowmetry
9 showed that flavan-3-ols, theaflavins, and quercetin significantly
10 increased cremaster artery blood flow. Flavan 3-ols, theaflavins,
11 (-)-epicatechin, daidzein, and cyanidin significantly elevated mean blood
12 pressure, and flavan-3-ols significantly increased the heart rate. In
13 contrast, hesperidin, epigallocatechin gallate, trans-resveratrol, gnetin C
14 or curcumin did not result in any detectable changes. Our results suggest
15 that polyphenols can contribute variably to alterations in the
16 haemodynamic and microcirculatory status in rats depending on their
17 characteristic chemical structures.

18
19 **Keywords:** *cremaster artery, cremaster muscle, microcirculation,*
20 *polyphenols, systemic circulation*

21

22

23 1. Introduction

24 Polyphenols are widely distributed in fruits, vegetables, nuts, seeds,
25 and beverages, which can impart colour and taste to edible plants or
26 processed foods derived from plant materials (Cheynier 2005). Several
27 thousand polyphenolic compounds have been identified in plants. These
28 compounds are classified into different groups on the basis of the number
29 of phenol rings they contain and the structural elements that bind these
30 rings together (Bravo 1998).

31 Numerous studies have established that some polyphenols can
32 contribute to the reduction of cardiovascular disease risk (Scalbert et al.
33 2005, Arts & Hollman 2005). The results of numerous epidemiological
34 studies have suggested that the frequency of polyphenol intake is
35 negatively correlated with the risk of coronary heart disease, myocardial
36 infarction, and stroke (Visioli & Davalos 2011, Lecour & Lamont 2011). In
37 addition, intervention trials have shown that repeated ingestion of
38 polyphenol-rich foods resulted in a significant hypotensive effect in
39 healthy and mildly-hypertensive subjects (Rodrigo et al. 2012, Michalska
40 et al. 2010, Galleano et al. 2010, Medina-Remón et al. 2013). These
41 previous data have shown that the ingestion of polyphenol-containing
42 foods supported an improvement in endothelial function, as detected by
43 flow-mediated dilation (FMD) and/or an elevation of nitric oxide (NO)
44 metabolites levels in the blood. Alteration of endothelial function was
45 observed not only after repeated ingestion of polyphenols, but also by a

46 single dose of polyphenol-rich food. Several of these studies indicated that
47 endothelial function was altered a few hours after the ingestion of
48 polyphenol-containing foods (Li et al. 2013, Wong et al. 2011, Matsushima
49 et al. 2013). In addition, Hooper et al. (2012) performed a meta-analysis
50 that showed a single dose of chocolate was significantly more effective at
51 increasing FMD than repeated ingestion. In our previous study in rats,
52 we found that a single dose of polyphenol, containing the polyphenolic
53 fraction derived from cocoa (the flavan-3-ol fraction), resulted in a rapid
54 increase in skeletal muscle blood flow, a transient rise in blood pressure
55 and heart rate, induction of phosphorylation of aortic endothelial nitric
56 oxide synthase (eNOS), and an elevation of nitrate and nitrite levels in
57 the blood (Inagawa et al. 2014). According to these perception, initial
58 haemodynamic changes were important to elucidation of the effect of
59 polyphenols on circulating system.

60 To date, polyphenols have been studied in relation to their effects on
61 circulation, but not enough is known about the correlations between
62 polyphenols and their potential to alter haemodynamic as a consequence
63 of their structure-activity relationship. In this rodent study, we compared
64 a single dose of several different polyphenols and the response of systemic
65 circulation and microcirculation by measuring blood pressure, heart rate,
66 and blood flow in the cremaster artery using laser Doppler flowmetry.

67

68 2. Materials and Methods

69 **2.1. Materials**

70 The flavan-3-ol fraction was provided by Meiji Co., Ltd (Tokyo, Japan)
71 and was prepared from cocoa using a method described in a previous
72 report (Osakabe et al. 1998). In brief, the cocoa powder was defatted with
73 n-hexane and the residue was extracted with acetone. The n-butanol
74 dissolved fraction of the extract was subsequently applied to a Diaion
75 HP2MG column (Mitsubishi Kasei Co. Ltd., Tokyo, Japan). The fraction
76 eluted with 80% ethanol was collected, freeze-dried, and used in the
77 experiments. The concentration of catechins and procyanidins in this
78 fraction was determined by high-performance liquid chromatography
79 (HPLC) (Natsume et al. 2000). Flavan-3-ol fraction contained 4.56%
80 (+)-catechin, 6.43% (-)-epicatechin, 3.93% procyanidin B2, 2.36%
81 procyanidin C1, and 1.45 % cinnamtannin A2. The theaflavin (TF)
82 fraction was prepared from green tea leaves. The crude TF mixture was
83 purchased from Yaizu Suisankagaku- Industry Co. Ltd., Shizuoka,
84 Japan., which was prepared by incubating catechins derived from green
85 tea leaves in the presence of polyphenol oxidase, The purified TF mixture
86 was prepared, as follows: the crude TFs was solubilized in water and the
87 catechins and caffeine in the solution were excluded with medium
88 pressure column chromatography with a reverse-phase preparative
89 column and with the gradient mobile phase, 10- 80% acetonitrile. These
90 TF were further purified by preparative HPLC with the Capcell Pak C18
91 (20 mm x 250 mm, Shiseido, Japan) and a mobile phase of 22%

92 acetonitrile in the presence of 0.05% phosphoric acid. The aqueous
93 solutions containing the respective TFs were treated with a Sep-Pak C18
94 cartridge, eluted with ethanol, and lyophilized. The concentration of each
95 TF in the mixture was analyzed by HPLC. The resulting mixture
96 contained 4.6 % TF (TF1), 16.36 % theaflavin-3-*O*-gallate (TF2A), 11.2%
97 theaflavin 3'-*O*-gallate (TF2B) and 26.2 % of theaflavin-3,3'-di-*O*-gallate
98 (TF3). (-)-Epicatechin (purity assessment by HPLC, >90%), hesperidin
99 (purity assessment by HPLC, >90%), quercetin (purity assessment by
100 HPLC, >95%), daidzein (purity assessment by HPLC, >95%) and
101 curcumin (purity assessment by HPLC, >97%) were purchased from
102 Tokyo Chemical Industry (Tokyo, Japan), cyanidin (purity assessment by
103 HPLC, >98%) was purchased from Santa Cruz Biotechnology (Santa Cruz,
104 CA, USA), epigallocatechin gallate (EGCG, purity assessment by HPLC,
105 >98%) was purchased from the Cayman Chemical Company (Ann Arbor,
106 MI, USA). *Trans*-resveratrol (purity assessment by HPLC, >97%) and
107 gnetin C (purity assessment by HPLC, >97%) were provided by Yamada
108 Bee Company, Inc. (Okayama, Japan). The chemical structures used in
109 this study are shown in Fig. 1. Urethane and Krebs-Ringer bicarbonate
110 buffer were purchased from Sigma Chemicals (St. Louis, MO, USA).

111

112 **2.2. Animals and Diets**

113 This study was approved by the Animal Care and Use Committee of
114 Shibaura Institute of Technology (Saitama, Japan). All animals received

115 humane care under the guidelines of this institution. Male Wistar rats
116 weighing 200-250 g (8 to 10 weeks old) were obtained from Saitama
117 Experimental Animal Supply (Tokyo, Japan). The rats were housed in a
118 temperature-regulated room (23-25 °C) with controlled lighting (12-h
119 light and dark cycles). A basal Oriental MF diet was obtained from the
120 Oriental Yeast, Co. Ltd., Tokyo, Japan.

121

122 **2.3. Experimental Procedures**

123 The animals were fed the basal diet for 4 days. Eighteen to 24 animals
124 were randomly allocated into each treatment group. The change in blood
125 flow in the cremaster artery was measured in 10 to 12 animals and the
126 changes in blood pressure and heart rate were determined in the other
127 animals in each group. Under anesthesia using urethane (1 g/kg body
128 weight, s.c.), the cremaster muscle was exteriorized and carefully spread
129 out over an optically clear viewing pedestal, and the tissue surface was
130 superfused with Krebs-Ringer bicarbonate buffer (pH 7.3-7.4) and 95%
131 N₂–5 % CO₂ at 37 °C, a tail cuff was attached, and an indwelling gastric
132 feeding tube was inserted into the stomach of each animal. After a
133 post-surgical 15 min of equilibration period, blood flow in the cremaster
134 muscle was measured by a laser Doppler blood perfusion imager
135 (Periscan PIM-2, Perimed Co. Ltd.) for 15 min. Distilled water (vehicle),
136 or polyphenols suspended in distilled water, were administered to the
137 animals via the feeding tube and blood flow in the cremaster artery was

138 measured for 60 min. In addition, as similar as above, blood pressure and
139 heart rate of the animals in each group were measured by the tail-cuff
140 method (BP-98A Softron, Tokyo Japan) for 30 min under anesthesia.

141

142 **2.4. Statistical Methods**

143 The data were reported as the mean and standard error. Statistical
144 analyses were performed by Dunnett's test. Statistical probability of $P <$
145 0.05 was considered significant.

146

147 **3. Results**

148 **3.1. Flavan-3-ols**

149 As shown in Fig.1A, blood flow in the cremaster artery was increased
150 following administration of 10 mg/kg body weight dose of flavan 3-ols,
151 and there was a significant difference versus the vehicle 35 to 55 min
152 after the treatment. There was also significant difference 45 and 50 min
153 after 1 mg/kg body weight dose of flavan-3-ols. The change in heart rate
154 and mean blood pressure (MBP) after a single dose of flavan-3-ols is
155 shown in Fig.s1B and 1C. An increase in heart rate occurred at 10 mg/ kg
156 flavan-3-ols in the groups of rats. Significant increases were observed
157 15,18, and 27 min after the treatment. MBP was also significantly
158 increased following administration of a 1- and 10 mg/kg body weight dose
159 of flavan-3-ols. The significant increase for the group of animals receiving
160 10 mg/kg body weight of flavan-3-ols versus vehicle was evident from 9 to

161 30 min after administration, and for the group of animals receiving
162 1mg/kg body weight of flavan-3-ols the difference versus vehicle occurred
163 from 15 to 30 min after dose administration. Dose-dependent
164 haemodynamic changes in the rats were observed following dosing of
165 flavan-3-ols and provided the basis for the rationale to fix the dose of each
166 polyphenol at 10 mg/kg body weight.

167

168 **3.2. Tea polyphenols**

169 The acute haemodynamic changes in rats following treatment with tea
170 polyphenols is shown in Fig. 2. The change observed in their
171 microcirculation was a result of the significantly increased blood flow in
172 the cremaster artery after ingestion of TFs. In contrast, changes were not
173 detected in rats dosed with (-)-epicatechin and EGCG (Fig. 2A). The TFs
174 increased blood flow soon after treatment, and there was a significant
175 difference from 5 min to the end of the measurement period. Heart rate
176 was not changed by the ingestion of tea polyphenols (Fig. 2B). The
177 elevation in MBP occurred in groups of rats that received (-)-epicatechin
178 and TFs (Fig. 2C). The significant difference between rats receiving the
179 vehicle versus (-)-epicatechin was evident from 18 to 30 min after
180 treatment, whereas for the vehicle versus TFs, the significant difference
181 occurred 18 min after dosing.

182

183 **3.3. Flavonoids**

184 Acute responses to treatment after administering a single dose of
185 flavonoids are shown in Fig. 3. Blood flow in the cremaster artery of rats
186 was significantly increased by a single dose of quercetin; however, this
187 change was not observed following the administration of a dose of
188 hesperidin, cyanidin, or daidzein (Fig. 3A). From 30 to 60 min after a dose
189 of quercetin, blood flow was significantly increased. No change in heart
190 rate was detected following a dose of flavonoids (Fig. 3B). MBP was
191 elevated in response to a dose of cyanidin, with a significant difference
192 being evident from 12 to 30 min after dosing. Daidzein treatment also
193 resulted in a significant elevation of MBP 6 to 30 min after treatment.

194

195 **3.4. *Trans*-resveratorol, gnetin C and curcumin**

196 The haemodynamic measurements taken in rats after they received a
197 single dose of *trans*-resveratorol, gnetin C, or curcumin is shown in Fig. 5.
198 There were no significant changes following the ingestion of these
199 polyphenols.

200

201 **4. Discussion**

202 The results of our previous study showed that the administration of a
203 single dose of flavan-3-ols increased MBP and heart rate, and the level of
204 these parameters begun to decrease 45 min after treatment, and returned
205 to baseline 60 min after treatment (Inagawa et al. 2014). We confirmed
206 not only these haemodynamic changes, but also the elevation of the blood

207 flow in artery, RBC velocity in the arteriole and recruited capillaries
208 resulting hypoxia of the skeletal muscle, in addition of antiatherogenic
209 changes (e.g. phosphorylation of eNOS) of endothelial cells in aorta by the
210 single dose of flavan-3-ols. In line with our previous report, we
211 measured blood flow in the cremaster artery for 60 min, and MBP and
212 heart rate were also measured for 30 min. In present manuscript, we
213 focused on the difference of haemodynamic alteration after
214 administration of a single dose of each under similar experimental
215 conditions. The results of this study are summarized in Table 1. A 10
216 mg/kg body weight dose of flavan-3-ols increased not only blood flow in
217 the cremaster artery, but also heart rate and MBP. TFs and quercetin
218 also demonstrated the ability to increase blood flow in the cremaster
219 artery. Cyanidin and daidzein each demonstrated a potent effect on the
220 elevation of MBP (Table 1). These results suggested that efficacy in terms
221 of haemodynamic alterations was attributable to the large difference
222 between polyphenols, possibly as a result of the differences in their
223 chemical structures. The evaluation of the RBC velocity, hypoxia or the
224 expression of the proteins after treatment of the chemicals which
225 observed remarkable haemodynamic changes in present study needs to
226 be further investigated in the future.

227 A number of studies have supported that HR and BP are dramatically
228 increased at the beginning of the exercise; however, these changes were
229 returned to the normal level after vessel dilatation induced by NO

230 production resulting endothelial eNOS phosphorylation induced by shear
231 stress and circumferential stress(Birk et al . 2013). In addition,
232 continuous exercise performed restructuring of the vessels and
233 angiogenesis resulting anti-atherogenic genes in endothelial cells, as a
234 result, it revealed hypotensive action (Katayama et al. 2013). In our
235 previous studies (Osakabe et al. 2012, Watanabe et al. 2014), repeating
236 treatment of flavan-3-ols showed significant hypotensive effect, and
237 single oral dose of flavan-3-ols transient increased HR and BP as shown
238 in present study. Therefore, the initial haemodynamic changes were
239 suggested to be important in the effect of polyphenols on circulating
240 system.

241 Numerous intervention trials support the finding that ingestion of
242 polyphenol-rich foods can have a hypotensive effect in healthy and
243 mildly-hypertensive subjects (Rodrigo et al. 2012, Michalska et al. 2010,
244 Galleano et al. 2010, Medina-Remón et al. 2013). Recent meta-analyses
245 have indicated that blood pressure was significantly decreased by the
246 ingestion of chocolate, which is one of the typical flavan-3-ol- rich foods
247 (Hooper et al. 2012, Desch et al. 2010, Grassi et al. 2010). The
248 meta-analyses also reported that blood pressure was reduced after
249 consumption of catechins contained in green tea, TFs contained in black
250 tea (Hartley et al. 2013), or isoflavones found in soy protein (Liu et al.
251 2012). Few reports have confirmed that the ingestion of quercetin
252 (Edwards et al. 2007, Larson et al. 2012) and cyanidin (Hellström et al.

253 2010, Kivimäk et al. 2013) can have a hypotensive effect, and there is
254 limited information of the hypotensive effect of *trans*-resveratrol, gnetin
255 C, and curcumin in healthy or hypertensive subjects. It was interesting
256 that the chemicals represented haemodynamic changes in this study
257 showed hypotensive effect in previous studies.

258 In addition, numerous reports have revealed that FMD was increased a
259 few hours after the administration of a single dose of polyphenols. Two
260 hours after ingestion of dark chocolate, FMD has been shown to be
261 significantly increased (Hooper et al. 2012, Monahan 2012). Furthermore,
262 a significant increase in FMD has been detected after the ingestion of a
263 single dose of green or black tea (Grassi et al. 2009, Jochmann et al. 2008,
264 Kim et al. 2006). An increase in FMD has also been observed in relation
265 to cyanidin-rich anthocyanin fractions (Rodriguez-Mateos et al. Zhu et al.
266 2011). Of particular interest is a report that notes a single dose of
267 chocolate was significantly more effective at increasing FMD than
268 repeated chocolate ingestion (Hooper et al. 2012). In the present study,
269 several polyphenols exhibited the effect of increasing blood flow in the
270 cremaster artery, heart rate, and/ or MBP as shown in Table 1. It has
271 been reported that shear stress-induced blood flow (Chien 2007, Harrison
272 et al. 2006, Shin et al. 2002) and arterial circumferential wall stress
273 increased by blood pressure (Laughlin et al. 2008, Awolesi et al. 1994) are
274 primary signals for anti-atherogenic responses such as eNOS
275 phosphorylation in arterial endothelial cells. We confirmed that eNOS

276 phosphorylation and nitrate and nitrite levels in blood were significantly
277 increased 60 min after a single 10 mg/kg body weight dose of flavan-3-ols
278 in our previous study (Inagawa et al 2014). The haemodynamic
279 alterations characterized by an increase in blood flow and blood pressure
280 following the administration of a single dose of polyphenols may have
281 induced eNOS phosphorylation and resulted in NO production in
282 endothelial cells. According to these results, an acute increase in FMD in
283 the intervention trial is suggested to be due to these anti-atherogenic
284 responses. The correlation between the acute increase in FMD and blood
285 pressure following a single dose of polyphenol remains to be fully
286 elucidated; further studies are needed to clarify the relationship between
287 them.

288 As described previously, flavan-3-ols demonstrate potent activity
289 and can influence the circulatory response (Fig. 2). On the other hand,
290 (-)-epicatechin increased blood pressure slightly, but had no effect on
291 blood flow in the cremaster artery or heart rate (Fig. 3). Flavan-3-ols
292 contain catechins and (-)-epicatechin oligomers as procyanidins, and their
293 activity seemed to be attributable to them. The alteration to
294 haemodynamic status associated with flavan-3-ols or flavan3-ol-rich food
295 has been thoroughly investigated via *in vitro* methods (Fitzpatrick et al.
296 2002, Davis et al. 2006), in experimental animals (Cui et al. 2012,
297 Balasuriya & Rupasinghe 2012) and by intervention trials (Hooper et
298 al. 2012, Desch et al. 2010, Grassi et al. 2010, Monahan, 2012). The

findings from these studies were based on using a flavan 3-ols mixture of catechins and procyanidins. There have been few *in vitro* studies that have evaluated the effects of isolated procyanidin (Byun et al. 2012a, Byun 2012b); however, at present no *in vivo* study has been performed to address this possibility. A limited amount is also known in relation to the effect of isolated TFs on endothelial function. In contrast, the effect of (-)-epicatechin on haemodynamic status has been well studied. (-)-Epicatechin has been shown to induce endothelial cell NO synthesis (Brossette et al. 2011, Huang et al. 1999), and its hypotensive effect in experimental animals has also been reported (Gabriel et al. 2014, Litterio et al. 2012). The results of the present study suggested that catechin oligomers such as procyanidins and TFs have a potent effect on haemodynamic status. Additional experiments to assess the bioactivities of catechin oligomers were needed. In conclusion, a 10 mg/kg body weight dose of flavan-3-ols increased blood flow in the cremaster artery, heart rate and MBP, TFs and quercetin demonstrated the capacity to increase blood flow in cremaster artery, and cyanidin and daidzein each had a potent effect on MBP, causing it to rise. Our results suggest polyphenols can contribute variably to alterations in the haemodynamic and microcirculatory status in rats depending on their characteristic chemical structure.

322 **ACKNOWLEDGMENTS**

323 We thank Meiji Co. Ltd. for the donation of and flavan 3-ols fraction.

324 We also thank Yamada Bee Company, Inc. for the donation of

325 *trans*-resveratrol and gnetin C.

326 This work was supported by Grant-in-Aid for Project of Shizuoka

327 Prefecture and Shizuoka City Collaboration of Regional Entities for

328 Advancement of Technological Excellence, Japan Science and Technology

329 Agency (JST) and also supported by Yamada Research Grant.

REFERENCES

- Arts, I.C., & Hollman PC. (2005) Polyphenols and disease risk in epidemiologic studies. *American Journal of Clinical Nutrition*, 81, 317S-325S.
- Awolesi, M.A., Widmann, M.D., Sessa, W.C., & Sumpio, B.E. (1994) Cyclic strain increases endothelial nitric oxide synthase activity. *Surgery*, 116, 439-444.
- Balasuriya, N., & Rupasinghe, H.P.(2012) Antihypertensive properties of flavonoid-rich apple peel extract. *Food Chemistry*, 135, 2320-2325.
- Birk, G.K., Dawson, E.A., Batterham, A.M., Atkinson, G., Cable, T., Thijssen, D.H. & Green, D.J. (2013) Effects of exercise intensity on flow mediated dilation in healthy humans. *International Journal of Sports and Medicine* 34, 409-414.
- Bravo, L. (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Review*, 56, 317-333.
- Brossette, T., Hundsdörfer, C., Kröncke, K.D., Sies, H., & Stahl, W. (2011) Direct evidence that (-)-epicatechin increases nitric oxide levels in human endothelial cells. *European Journal of Nutrition*, 50, 595-599.
- Byun, E.B., Ishikawa, T., Suyama, A., Kono, M., Nakashima, S., Kanda, T., Miyamoto, T., & Matsui, T.A. (2012) Procyanidin trimer, C1, promotes NO production in rat aortic endothelial

353 cells via both hyperpolarization and PI3K/Akt pathways.
 354 European Journal of Pharmacology, 692, 52-60.
 355 Byunm M.W. (2011) Effect of procyanidin C1 on nitric oxide
 356 production and hyperpolarization through Ca(2+)-dependent
 357 pathway in endothelial cells. Journal of Medicinal Food, 15,
 358 1032-1037.
 359 Cheynier, V. (2005) Polyphenols in foods are more complex than often
 360 thought. American Journal of Clinical Nutrition, 81,
 361 223S-229S.
 362 Chien, S. (2007) Mechanotransduction and endothelial cell
 363 homeostasis: the wisdom of the cell. American Journal of
 364 Physiology Heart and Circulatory Physiology, 292,
 365 H1209-224.
 366 Cui, X., Liu, X., Feng, H., Zhao, S., & Gao, H. (2012) Grape seed
 367 proanthocyanidin extracts enhance endothelial nitric oxide
 368 synthase expression through 5'-AMP activated protein
 369 kinase/Sirtuin 1-Krüppel like factor 2 pathway and modulate
 370 blood pressure in ouabain induced hypertensive rats. Biological
 371 & Pharmaceutical Bulletin, 35, 2192-2197.
 372 Davis, P.A., Polagruto, J.A., Valacchi, G., Phung, A., Soucek, K., Keen,
 373 C.L., & Gershwin, M.E. (2006) Effect of apple extracts on
 374 NF-kappaB activation in human umbilical vein endothelial
 375 cells. Experimental Biology and Medicine, 231, 594-598.

376 Desch, S., Schmidt, J., Kobler, D., Sonnabend, M., Eitel, I., Sareban,
 377 M., Rahimi, K., Schuler, G., & Thiele, H (2010) Effect of cocoa
 378 products on blood pressure: systematic review and
 379 meta-analysis. *American Journal of Hypertension*, 23, 97-103.
 380 Edwards, R.L., Lyon, T., Litwin, S.E., Rabovsky, A., Symons, J.D., &
 381 Jalili, T. (2007) Quercetin reduces blood pressure in
 382 hypertensive subjects. *Journal of Nutrition*, 137, 2405-2411.
 383 Fitzpatrick, D.F., Bing, B., Maggi, D.A., Fleming, R.C., & O'Malley,
 384 R.M. (2002) Vasodilating procyanidins derived from grape seeds.
 385 *New York Academy of Sciences*, 957, 78–89.
 386 Gabrielm G.S., Pilar, O.V., Claudia Maria, V., Leticia, G.S., German,
 387 C.C., Eduardo, M., Santiago, V., Francisco, V., Guillermo, C., &
 388 Israel, R.S. (2014) Effects of (-)-epicatechin on a diet-induced rat
 389 model of cardiometabolic risk factors. *European Journal of*
 390 *Pharmacology*, 728, 24-30.
 391 Galleano, M., Pechanova, O., & Fraga, C.G. (2010) Hypertension,
 392 nitric oxide, oxidants, and dietary plant polyphenols. *Current*
 393 *Pharmaceutical Biotechnology*, 11, 837-848.
 394 Grassi, D., Desideri, G., & Ferri, C. (2010) Blood pressure and
 395 cardiovascular risk: what about cocoa and chocolate? *Archives*
 396 *of Biochemistry and Biophysics*, 501, 112-115.
 397 Grassi, D., Mulder, T.P., Draijer, R., Desideri, G., Molhuizen, H.O., &
 398 Ferri, C. (2009) Black tea consumption dose-dependently

399 improves flow-mediated dilation in healthy males. *Journal of*
 400 *Hypertension*, 27, 774-781.

401 Harrison, D.G., Widder, J., Grumbach, I., Chen, W., Weber, M., &
 402 Searles, C. (2006) Endothelial mechanotransduction, nitric
 403 oxide and vascular inflammation. *Journal of Internal Medicine*,
 404 259, 351-363.

405 Hartley, L., Flowers, N., Holmes, J., Clarke, A., Stranges, S., Hooper,
 406 L., & Rees, K. (2013) Green and black tea for the primary
 407 prevention of cardiovascular disease. *Cochrane Database of*
 408 *Systematic Reviews*, 6, CD009934.

409 Hellström, J.K., Shikov, A.N., Makarova, M.N., Pihlanto, A.M.,
 410 Pozharitskaya, O.N., Ryhänen, E., Kivijärvi, P., Makarov, V.G.
 411 & Mattila, P.H. (2010) Blood pressure-lowering properties of
 412 chokeberry (*Aronia mitchurinii*, var. Viking) *Journal of*
 413 *Functional Foods*, 2, 163-169.

414 Hooper, L., Kay, C., Abdelhamid, A., Kroon, P.A., Cohn, J.S., Rimm,
 415 E.B., & Cassidy, A. (2012) Effects of chocolate, cocoa, and flavan
 416 3-ols on cardiovascular health: a systematic review and
 417 meta-analysis of randomized trials. *American Journal of*
 418 *Clinical Nutrition*, 95, 740-751.

419 Huang, Y., Chan, N.W., Lau, C.W., Yao, X.Q., Chan, F.L., & Chen,
 420 Z.Y. (1999) Involvement of endothelium/nitric oxide in
 421 vasorelaxation induced by purified green tea

422 (-)epicatechin. *Biochimica et Biophysica Acta*, 1427, 322-328.

423 Ingawa, K., Aruga, N., Matsumura, Y., Shibata, M., & Osakabe, N.

424 (2014) Alteration of the systemic and microcirculation by a

425 single oral dose of flavan-3-ols. *PLoS One*, 9, e94853.

426 Jochmann, N., Lorenz, M., Krosigk, A.V., Martus, P., Böhm, V.,

427 Baumann, G., Stangl, K., & Stangl, V. (2008) The efficacy of

428 black tea in ameliorating endothelial function is equivalent to

429 that of green tea. *Br Journal of Nutrition*, 99, 863-868.

430 Katayama, K., Fujita, O., Iemitsu, M., Kawano, H., Iwamoto, E.,

431 Saito, M. & Ishida, K. (2013) The effect of acute exercise in

432 hypoxia on flow-mediated vasodilation. *European Journal of*

433 *Applied Physiology* 113, 349-357.

434 Kim, W., Jeong, M.H., Cho, S.H., Yun, J.H., Chae, H.J., Ahn, Y.K., Lee,

435 M.C., Cheng, X., Kondo, T., Murohara, T., & Kang, J.C. (2006)

436 Effect of green tea consumption on endothelial function and

437 circulating endothelial progenitor cells in chronic

438 smokers. *Circulation Journal*, 70, 1052-1057.

439 Kivimäki, A.S., Siltari, A., Ehlers, P.I., Korpela, R. & Vapaatalo, H.

440 (2013) Lingonberry juice lowers blood pressure of

441 spontaneously hypertensive rats (SHR) *Journal of Functional*

442 *Foods*, 5, 1432-1440.

443 Larson, A., Witman, M.A., Guo, Y., Ives, S., Richardson, R.S., Bruno,

444 R.S., Jalili, T., & Symons, J.D. (2012) Acute, quercetin-induced

445 reductions in blood pressure in hypertensive individuals are
 446 not secondary to lower plasma angiotensin-converting enzyme
 447 activity or endothelin-1: nitric oxide. *Nutrition Research*, 32,
 448 557-564.

449 Laughlin, M.H., Newcomer, S.C., & Bender, S.B. (2008) Importance of
 450 haemodynamic forces as signals for exercise-induced changes
 451 in endothelial cell phenotype. *Journal of Applied Physiology*, 104,
 452 588-600.

453 Lecour, S., & Lamont, K.T. (2011) Natural polyphenols and
 454 cardioprotection. *Mini-Reviews in Medicinal Chemistry*, 11,
 455 1191-1199

456 Li, S.H., Tian, H.B., Zhao, H.J., Chen, L.H., & Cui, L.Q. (2013) The
 457 acute effects of grape polyphenols supplementation on
 458 endothelial function in adults: meta-analyses of controlled
 459 trials. *PLoS One*, 8, e69818.

460 Litterio, M.C., Jagers, G., Sagdicoglu Celep, G., Adamo, A.M., Costa,
 461 M.A., Oteiza, P.I., Fraga, C.G., & Galleano, M. (2012) Blood
 462 pressure-lowering effect of dietary (-)-epicatechin
 463 administration in L-NAME-treated rats is associated with
 464 restored nitric oxide levels. *Free Radical Biology Medicine*,
 465 15;53, 1894-1902.

466 Liu, X.X., Li, S.H., Chen, J.Z., Sun, K., Wang, X.J., Wang, X.G., & Hui,
 467 R.T. (2012) Effect of soy isoflavones on blood pressure: a

468 meta-analysis of randomized controlled trials *Nutrition,*
 469 *Metabolism & Cardiovascular Diseases*, 22, 463-470.
 470 Matsushima, A., Furuuchi, R., Sakaguchi, Y., Goto, H., Yokoyama, T.,
 471 Nishida, H., & Hirayama, M. (2013) Acute and chronic
 472 flow-mediated dilation and blood pressure responses to daily
 473 intake of boysenberry juice: a preliminary study *Journal of*
 474 *Nutrition & Food Sciences*, 6, 988-992.
 475 Medina-Remón, A., Estruch, R., Tresserra-Rimbau, A.,
 476 Vallverdú-Queralt, A., & Lamuela-Raventós, R.M. (2013) The
 477 effect of polyphenol consumption on blood
 478 pressure. *Mini-Reviews in Medicinal Chemistry*, 13, 1137-1149.
 479 Michalska, M., Gluba, A., Mikhailidis, D.P., Nowak, P.,
 480 Bielecka-Dabrowa, A., Rysz, J., & Banach, M. (2010) The role of
 481 polyphenols in cardiovascular disease. *Medical Science Monitor*,
 482 16, RA110-119.
 483 Monahan, K.D. (2012) Effect of cocoa/chocolate ingestion on brachial
 484 artery flow-mediated dilation and its relevance to
 485 cardiovascular health and disease in humans. *Archives of*
 486 *Biochemistry and Biophysics*, 527, 90-94.
 487 Natsume, M., Osakabe, N., Yamagishi, M., Takizawa, T., Nakamura,
 488 T., Miyatake, H., Hatano, T., & Yoshida, T. (2000) Analyses of
 489 polyphenols in cacao liquor, cocoa, and chocolate by
 490 normal-phase and reversed-phase HPLC. *Bioscience,*

491 Biotechnology, and Biochemistry. Bioscience, 64, 2581-2587
 492 Osakabe N. & Shibata M. (2012) Ingestion of cocoa ameliorates
 493 endothelial dysfunction in mesentery arterioles induced by
 494 high fat diet in rats: an in vivo intravital microscopy study.
 495 Life Science 9, 1196-1200.
 496 Osakabe, N., Yamagishi, M., Sanbongi, C., Natsume, M., Takizawa, T.,
 497 & Osawa, T. (1998) The antioxidative substances in cacao liquor.
 498 Journal of Nutritional Science and Vitaminology, 44, 313-321.
 499 Rodrigo, R., Gil, D., Miranda-Merchak, A., & Kalantzidis, G. (2012)
 500 Antihypertensive role of polyphenols. Advances in Clinical
 501 Chemistry, 58, 225-254.
 502 Rodriguez-Mateos, A., Rendeiro, C., Bergillos-Meca, T., Tabatabaee,
 503 S., George, T.W., Heiss, C., & Spencer, J.P. (2013) Intake and
 504 time dependence of blueberry flavonoid-induced improvements
 505 in vascular function: a randomized, controlled, double-blind,
 506 crossover intervention study with mechanistic insights into
 507 biological activity. American Journal of Clinical Nutrition,
 508 98, 1179-1191.
 509 Scalbert, A., Johnson, I.T., & Saltmarsh, M. (2005) Polyphenols:
 510 antioxidants and beyond. American Journal of Clinical
 511 Nutrition, 8, :215S-217S.
 512 Shin, H.Y., Gerritsen, M.E., & Bizios, R. (2002) Regulation of
 513 endothelial cell proliferation and apoptosis by cyclic

514 pressureAnnals of Biomedical Engineering, 30, 297-304.

515 Visioli, F., Davalos, A.(2011) Polyphenols and cardiovascular disease:

516 a critical summary of the evidence., Mini-Reviews in Medicinal

517 Chemistry, 11, 1186-1190.

518 Watanabe, N., Inagawa, K., Shibata, M.& Osakabe, N. (2014)

519 Flavan-3-ol fraction from cocoa powder promotes mitochondrial

520 biogenesis in skeletal muscle in mice. Lipids in Health and

521 Disease. 13, 64.

522 Wong, R.H., Howe, P.R., Buckley, J.D., Coates, A.M., Kunz, I., & Berry,

523 N.M. (2011) Acute resveratrol supplementation improves

524 flow-mediated dilatation in overweight/obese individuals with

525 mildly elevated blood pressure. Nutrition, Metabolism &

526 Cardiovascular Disease, 21, 851-856

527 Zhu, Y., Xia, M., Yang, Y., Liu, F., Li, Z., Hao, Y., Mi, M., Jin, T., &

528 Ling, W. (2011) Purified anthocyanin supplementation

529 improves endothelial function via NO-cGMP activation in

530 hypercholesterolemic individuals. Clinical Chemistry, 57,

531 1524-1533.

532

533

534 FIG. LEGEND

535

536 **Fig. 1.** Chemical structures of the polyphenols used in this study.

537 Substances used included the following: flavan-3-ols (a); (-)-epicatechin
538 (b); epigallocatechin gallate (EGCG) (c); theaflavins (d); quercetin (e);
539 cyanidin (f); hesperidin (g); daidzein (h); *trans*-resveratrol (i); gnetin C (j);
540 and curcumin (k).

541

542 **Fig. 2.** Haemodynamic changes after flavan-3-ol administration. Shown is
543 the change in blood flow in the cremaster artery (A).; the change in heart
544 rate (B); and the change in blood pressure (C). A black circle represents
545 the vehicle (n=10).; an orange triangle represents a 1mg/kg body weight
546 dose of flavan-3-ols (n=10).; a red square represents a 10 mg/kg body
547 weight dose of flavan-3-ols (n=10). Each value represents the mean and
548 standard error. * $P < 0.05$ and ** $P < 0.01$ compared with vehicle.

549

550 **Fig. 3.** Haemodynamic changes after administration of a single dose of
551 tea polyphenols. Shown is the change in blood flow in the cremaster
552 artery (A).; the change in heart rate (B); and the change in blood pressure
553 (C). A black circle represents vehicle (n=10).; a blue square represents a
554 10 mg/kg body weight dose of epigallocatechin gallate (EGCG) (n=12).; a
555 red triangle represents a 10mg/kg body weight dose of (-)-epicatechin
556 (n=10).; a green circle represents theaflavins (n=10). Each value
557 represents the mean and standard error. * $P < 0.05$ and ** $P < 0.01$
558 compared with vehicle.

559

560 **Fig. 4.** Haemodynamic changes after flavonoid administration. Shown is
561 the change in blood flow in the cremaster artery (A).; the change in heart
562 rate (B).; and the change in blood pressure. (C). A black circle represents
563 vehicle (n=10).; a blue square represents a 10 mg/kg body weight dose of
564 cyanidin (n=10).; a red triangle represents a 10 mg/kg body weight dose of
565 quercetin (n=10) ; an green circle represents a 10 mg/kg body weight dose
566 of hesperidin (n=10).; and a purple rhombus represents a 10mg/kg body
567 weight dose of daidzein (n=10). Each value represents the mean and
568 standard error. * $P < 0.05$ and ** $P < 0.01$ compared with vehicle.

569

570 **Fig. 5.** Haemodynamic changes after administration of *trans*-resveratrol,
571 gnetin C, and curcumin. Shown is the change in blood flow in the
572 cremaster artery (A).; the change in heart rate (B).; and the change in
573 blood pressure (C). A black circle represents vehicle (n=10).; a blue square
574 represents a 10 mg/kg body weight dose of gnetin C (n=12).; a red triangle
575 represents a 10 mg/kg body weight dose of *trans*-resveratrol (n=10).; and
576 a green circle represents a 10 mg/kg body weight dose of curcumin (n=10).
577 Each value represents the mean and standard error. * $P < 0.05$ and ** $P <$
578 0.01 compared with vehicle.

Table 1 The change of micro- and systemic circulation after single oral administration of polyphenols

	blood flow in cremaster artery	heart rate	mean blood pressure
flavan-3-ols	↑ ↑	↑	↑ ↑
theaflavins	↑ ↑	→	↑
(-)-epicatechin	→	→	↑ ↑
epigallocatechin gallate	→	→	→
quercetin	↑ ↑	→	→
hesperidin	→	→	→
cyanidin	→	→	↑ ↑
daizein	→	→	↑ ↑
<i>trans</i> -resveratorol	→	→	→
gnetin C	→	→	→
crucumine	→	→	→

Data are for an assigned as follows; → no significantly change ; ↑; occasional significant difference ,↑↑, consistent significant difference versus vehicle.

Figure

Fig.1 Chemical structures of the polyphenols used in this study. Substances used included the following: flavan-3-ols (A); (-)-epicatechin (B); epigallocatechin gallate (EGCG) (C); theaflavins (D); quercetin (E); cyanidin (F); hesperidin (G); daidzein (H); *trans*-resveratrol (I); gnetin C (J); and curcumin (K).

Fig.2. Hemodynamic changes after flavan-3-ol administration. Shown is the change in blood flow in the cremaster artery (A).; the change in heart rate (B); and the change in blood pressure (C). A closed circle represents the vehicle (n=10).; an open triangle represents a 1mg/kg body weight dose of flavan-3-ols (n=10).; an open square represents a 10 mg/kg body weight dose of flavan-3-ols (n=10). Each value represents the mean and standard error. * $P < 0.05$ and ** $P < 0.01$ compared with vehicle.

Fig.3. Hemodynamic changes after administration of a single dose of tea polyphenols. Shown is the change in blood flow in the cremaster artery (A); the change in heart rate (B); and the change in blood pressure (C). A closed circle represents vehicle (n=10); an open square represents a 10 mg/kg body weight dose of epigallocatechin gallate (EGCG) (n=12); an open triangle represents a 10mg/kg body weight dose of (-)-epicatechin (n=10); an open circle represents theaflavins (n=10). Each value represents the mean and standard error. * $P < 0.05$ and ** $P < 0.01$ compared with vehicle.

Fig.4 Hemodynamic changes after flavonoid administration. Shown is the change in blood flow in the cremaster artery (A).; the change in heart rate (B).; and the change in blood pressure.; (C). A closed circle represents vehicle (n=10).; an open triangle represents a 10 mg/kg body weight dose of quercetin (n=10).; an open square represents a 10 mg/kg body weight dose of cyanidin (n=10).; an open circle represents a 10 mg/kg body weight dose of hesperidin (n=10).; and an open rhombus represents a 10mg/kg body weight dose of daidzein (n=10). Each value represents the mean and standard error. * $P < 0.05$ and ** $P < 0.01$ compared with vehicle.

Fig.5 Hemodynamic changes after administration of *trans*-resveratrol, gnetin C, and curcumin. Shown is the change in blood flow in the cremaster artery (A).; the change in heart rate (B).; and the change in blood pressure (C). A closed circle represents vehicle (n=10).; an open triangle represents a 10 mg/kg body weight dose of *trans*-resveratrol (n=10).; an open square represents a 10 mg/kg body weight dose of gnetin C (n=12).; and an open circle represents a 10 mg/kg body weight dose of curcumin (n=10). Each value represents the mean and standard error. * $P < 0.05$ and ** $P < 0.01$ compared with vehicle.