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1 **Impact of green tea on the deleterious cardiometabolic effects of 7-days**
2 **unhealthy lifestyle in young healthy males**

3
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13 Experiments were conducted in the Cardiovascular laboratories of the Research Institute
14 for Sport and Exercise Sciences at Liverpool John Moores University. KAR, RD, NDH,
15 DHJT and DAL designed the work; KAR, SMH, SEC, YdG and DAL were responsible
16 for the acquisition, analysis and/or interpretation of the data for the work. KAR, RD,
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18 important intellectual content. All authors approved the final version of the manuscript.

19
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25 **Running title:** Green tea and metabolic and vascular function

26 **ABSTRACT**

27 **PURPOSE:** The aim of this study was to examine if catechin-rich green tea abrogates the
28 negative effects of 7-days of physical inactivity and excessive calorie-intake on insulin
29 homeostasis and peripheral vascular function. **METHODS:** Using a randomised, double-
30 blind, crossover design, twelve healthy men (29 ± 6 yrs) underwent 7-days unhealthy
31 lifestyle (UL), including physical inactivity (-50% steps/day) and overfeeding (+50%
32 kcal/day). This was combined with green tea consumption (UL-tea; 3 doses/day) or
33 placebo (UL-placebo). Before and after each intervention, we examined post-prandial
34 blood glucose and insulin (3-hours after a 1,202 kcal meal) and upper and lower limb
35 vascular function (flow-mediated dilation (FMD%) and carotid artery reactivity
36 (CAR%)). **RESULTS:** UL-placebo increased post-prandial glucose and insulin, whilst
37 UL-tea decreased post-prandial glucose and insulin (interaction-effects: both $P<0.05$).
38 UL-placebo decreased CAR% and femoral FMD%, whilst UL-tea prevented these effects
39 (Time*Intervention interaction effects of $P<0.04$ and $P<0.001$, respectively). There was
40 no main effect of Time or Time*Intervention interaction (both $P>0.05$) for brachial
41 FMD%. **CONCLUSION:** Seven days physical inactivity and overfeeding impairs insulin
42 homeostasis and vascular function. These effects were mitigated by daily intake of
43 catechin-rich green tea.

44 **Key words:** cardiovascular disease; cardiometabolic health; flavonoids; overfeeding;
45 physical inactivity.

46 INTRODUCTION

47 Physical inactivity and poor dietary habits are major modifiable risk factors linked to
48 detrimental changes in cardiometabolic health (61). Large cohort studies revealed that a
49 physically inactive lifestyle, either classified as the lack of exercise or engagement in
50 sedentary behaviour, are strongly associated with increased cardiovascular disease (CVD)
51 risk (63). Similarly, habitual high (trans) fat and high calorie dietary intake is associated
52 with increased cardiovascular risk and development of CVD (12). Whilst the long-term
53 effects of these behaviours are well-established, relatively less work has examined
54 whether short periods of an unhealthy (high calories, low physical activity) lifestyle affect
55 cardiometabolic risk. Intermittent periods of unhealthy nutritional and physical activity
56 behaviour are frequently experienced, such as during holidays, religious festivals or
57 forced physical inactivity (e.g. hospitalisation, injury). Previous work has found that 3-
58 14 days exposure to physical inactivity and/or overfeeding impairs metabolic and
59 vascular health (5, 22, 28). Exposure to such periods of unhealthy behaviour may
60 ultimately contribute to accelerated development of cardiometabolic disorders, therefore,
61 effective strategies are needed to offset these deleterious effects of a short-term unhealthy
62 lifestyle.

63

64 Dietary interventions are inexpensive tools to combat the ever-increasing burden of CVD.
65 Bioactive compounds known as polyphenols are found in plant-derived products, such as
66 olive oil, fruits and vegetables and are suggested to be cardioprotective and exert a
67 positive influence upon cardiovascular health (33). Polyphenols are the most abundant
68 antioxidant in the human diet and can be broadly categorised into four subclasses:
69 flavonoids, phenolic acids, lignans and stilbenes. Flavonoids account for the greatest
70 proportion of polyphenols (60%) and have been linked to a reduction in CVD risk (27,

71 46). Tea is the major source of dietary flavonoids in many countries globally (65) and is
72 classified according to the fermentation process, where flavonoids present in the tea leaf
73 are oxidised following the release of intracellular polyphenol oxidase. The four major
74 types of tea are white tea, green tea (non-fermented), oolong tea (semi-fermented) and
75 black tea (fully fermented). The associated health benefits of green tea are attributed to
76 its richness in flavan-3-ols (catechins) (24). The main catechins present in green tea are
77 epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG) and
78 epigallocatechin-3-gallate (EGCG), the most abundant of which is EGCG (~59%)
79 followed by EGC (~19%), ECG (~14%) and EC (~6%) (9).

80 Several biological actions of green tea support the association with a cardioprotective
81 effect, with a direct impact of tea on the vasculature, including its effects on the vascular
82 endothelium (17), the inner lining of all blood vessels which plays a central role in
83 vascular homeostasis, and improving the bioactivity of NO (18). Furthermore, higher
84 green tea consumption is associated with lower blood pressure (43) and superior
85 endothelial function (1, 26), particularly in those with CVD or in the postprandial state
86 (11, 39, 47). Clinically, green tea ingestion is also linked to lower risk for CVD events
87 and cerebrovascular complications (e.g. stroke, dementia) (10, 59). In addition, regular
88 intake of tea, a key dietary source of flavonoids, is associated with lower risk for type 2
89 diabetes mellitus (25, 40). In support of this, some laboratory-based studies have found
90 tea to acutely improve glucose homeostasis in both healthy (64), diabetic and obese
91 individuals (4, 30, 37). The consumption of catechin-rich green tea against a background
92 of forced physical inactivity and overfeeding could mitigate the negative metabolic and
93 vascular effects of physical inactivity and overfeeding, at least in the short-term.
94 Therefore, in this study, we tested the hypothesis that daily consumption of green tea
95 abrogates the effects of 7-days unhealthy lifestyle (UL: 50% less physical activity and

96 50% more calories) on glucose-insulin homeostasis and vascular function in healthy
97 participants.

98 PARTICIPANTS AND METHODS

99 *Participants*

100 Fourteen healthy, non-smoking, habitually active male participants were recruited
101 through local advertisement (29±6 yrs, BMI 25 ± 2 kg/m² and mean arterial pressure 84±8
102 mmHg). This sample size (effect size of 0.9, beta=0.90, alpha=0.05) was based on
103 previously reported green tea-induced increases in macrovascular function (1, 26, 39) and
104 amelioration of fat loading-induced decrements in macrovascular function (11). We
105 excluded individuals with vasoactive medications, a history of hypercholesterolemia
106 (cholesterol >6.5 mmol/l), CVD and/or hypertension (systolic: ≥140 mmHg, diastolic:
107 ≥90 mmHg). We also excluded individuals with food allergies, special dietary
108 requirements, currently following a diet and/or those using dietary/vitamin supplements.
109 Nine participants were habitual users of tea (and coffee). We included physically active
110 individuals [i.e. >8,000 steps/day; (56)]. Prior to testing, fully informed written consent
111 was obtained. The study conformed to the *Declaration of Helsinki*, was approved by
112 Liverpool John Moores University's Research Ethics Committee (15/SPS/065) and was
113 registered online (clinicaltrials.gov: NCT02777853).

114

115 *Experimental Design*

116 Firstly, participants underwent a 4-day monitoring period to record physical activity level
117 and dietary intake. Subsequently, participants underwent a randomised double-blind,
118 placebo controlled, crossover trial design of 5 weeks duration; lead in period (1 week);
119 intervention period one (1 week); washout (2 weeks); and finally intervention period two
120 (1 week). A 2 week washout period was used to allow the systemic elimination of the tea
121 and unhealthy lifestyle before initiation of the subsequent 1 week intervention which was
122 based on previous short-term studies that demonstrated detrimental effects of forced

123 physical inactivity and/or overfeeding interventions on insulin sensitivity and
124 macrovascular function (5, 21, 28, 41, 60). Participants adopted an unhealthy lifestyle in
125 both intervention periods but were randomly assigned (computer-generated, simple
126 randomisation), to tea (UL-Tea) in intervention period one, followed by placebo (UL-
127 Placebo) in intervention period two, or placebo in intervention period one followed by
128 tea in intervention period two. A crossover design was chosen for this study instead of
129 the more traditional randomized, parallel-group design because within-participant
130 variation is less than between participant variation allowing for examination of possible
131 causal relationships between the interventions (green tea vs. placebo) and the outcomes.

132 *Interventions*

133 *Unhealthy Lifestyle (UL)*. Based on the 4-day control period, participants reduced daily
134 steps by 50%. Real-time feedback on step count was provided using a pedometer (Digi-
135 walker SW-701, Yamax, Japan) and verified post-hoc via a hip mounted accelerometer
136 (GT3X BT+ model, Actigraphy, Pensacola, Florida, USA). During the interventions daily
137 caloric intake was increased by 50% (overfeeding) through the provision of daily “snack
138 boxes” in addition to participants maintaining their normal diet. The snack boxes were
139 made up of 60% and 20% of fats and carbohydrates, respectively, and typically contained
140 foods such as cheddar cheese, whole milk, salami, eggs, white chocolate and croissants.
141 The participants’ baseline dietary ratios of macronutrients were 49% carbohydrates, 31%
142 fat and 20% protein. Participants also refrained from foods and beverages high in
143 flavonoids (e.g. berries, red wine, dark chocolate) and caffeine during both interventions.
144 Dietary patterns were monitored and analysed (MyFitnessPal, Baltimore, Maryland,
145 USA) through self-reported food diaries. Step count verification was performed using
146 accelerometry data (ActiLife 6, Pensacola, Florida, USA).

147 *Tea versus placebo.* Participants drank three doses of green tea (UL-Tea, Unilever,
148 Vlaardingen, The Netherlands) or placebo (UL-Placebo) per day >15-minutes before
149 breakfast, lunch and dinner. In a double-blind manner, tea was provided as a brewed
150 spray-dried tea powder form, supplied in identical, coded, laminated aluminium foil
151 sachets. Two sachets were dissolved in 300 ml boiled water. No additives were permitted
152 and tea was consumed whilst hot. This dose of green tea is estimated to contain ~300 mg
153 of flavonoids (2). Due to a difference in energy intake between green tea and placebo
154 because of maltodextrin in the green tea (19 kcal/day), daily energy intake was adjusted
155 for in the daily food intake. Placebo tea had similar colour and taste as green tea, but did
156 not contain flavonoids or caffeine (Supplemental Table S1;
157 <https://figshare.com/s/8831f983188aba13d264>). Participants were instructed to avoid all
158 other types of tea.

159

160 *Experimental Measures*

161 Participants reported to the laboratory before and after each 7-day intervention. In the
162 week preceding the pre-intervention visits, participants refrained from tea and avoided
163 food sources high in flavonoids (44). Prior to testing, participants fasted for >6-hours and
164 refrained from alcohol and strenuous physical activity for 24-hours. Measurements were
165 conducted in a quiet, temperature-controlled laboratory (22-24°C) at the same time of
166 day. Upon arrival, anthropometric measurements were recorded, including height (Seca
167 stadiometer, model 217, Birmingham, UK) and body mass (Seca, model 767, Germany).
168 Before and after each intervention, we examined vascular function and glucose
169 homeostasis/insulin sensitivity responses to a mixed meal tolerance test Assessments of
170 vascular function were always conducted first followed by the mixed meal tolerance test.

171

172 *Mixed meal tolerance test*

173 A 20G cannula (Venflon Pro, BD, NJ, USA) was inserted into the antecubital vein of one
174 arm and a three-way stopcock (BD Connecta, NJ, USA) was subsequently attached to
175 enable multiple venous blood sampling and flushing of the cannula. Baseline samples
176 were collected for glucose (5 ml) and insulin (6 ml), in silica and EDTA vacutainers,
177 respectively. After baseline assessment, participants consumed a mixed meal (1201 kcal,
178 comprising 60% carbohydrates, 33% fat and 7% protein; Supplemental Table S2;
179 <https://figshare.com/s/8831f983188aba13d264>;
180 <https://doi.org/10.6084/m9.figshare.12246035>) in ~15 min (34). Postprandial blood
181 samples were collected after 30, 60, 90, 120 and 180-min. The rationale for using a 180
182 min postprandial period was in order to ensure peak responses and subsequent declines
183 in glucose and insulin were detected as well as previous work that has demonstrated black
184 tea-induced beneficial vascular and insulin effects for 180 min after a mixed-meal
185 challenge (15). Following each blood sample, isotonic saline (3 ml; B Braun, UK) was
186 used to keep the cannula patent. All blood samples were centrifuged (1000 g for 10-min
187 at 4°C) to obtain plasma samples, which were subsequently stored in aliquots at -80°C
188 for later analysis using commercially available assays for glucose (Randox, London, UK)
189 and insulin (ELISA-kit, Invitrogen, UK). Plasma glucose was determined using an ILab-
190 600 semi-automatic spectrophotometric analyser and glucose hexokinase assay (Randox,
191 London, UK). Plasma insulin concentrations were determined using a direct insulin
192 ELISA kit (Invitrogen, UK) and insulin levels determined using a monochromator
193 microplate reader (Clariostar, BMG LABTECH, Ortenberg, Germany). Area-under-the-
194 curve (AUCs) for postprandial glucose and insulin were calculated above baseline using
195 the trapezoidal rule.

196 Insulin sensitivity was estimated using homeostasis model assessment (HOMA-IR)
197 (23) and insulin secretion from insulin and glucose levels obtained following the standard
198 meal challenge using the Matsuda index (35). β -Cell function was assessed with the oral
199 disposition index (DI_o) (57).

200 *Vascular Function.*

201 Peripheral conduit artery, largely NO-mediated, endothelial function was examined at the
202 right brachial and superficial femoral arteries using flow-mediated dilation (FMD) (53).

203 A 10 MHz multi-frequency linear array probe, attached to a high-resolution 2D duplex
204 ultrasound machine (Terason u-Smart 3300, Teratech, Burlington, MA, USA) was used.

205 Pneumatic cuffs (D.E. Hokanson, Bellevue, WA, USA), connected to a rapid inflator
206 (D.E. Hokanson, Bellevue, WA, USA), were positioned on the interrogated upper
207 forearm and thigh, distal to the imaged site. In addition to a stable B-mode image,
208 continuous Doppler velocity and diameter data were collected. Baseline images were
209 recorded for 1-minute, following which the occlusion cuffs were inflated (>220 mmHg)
210 for 5-minutes. Diameter and velocity recordings resumed 30-seconds prior to cuff
211 deflation and continued for 3-minutes after cuff deflation, according to methodological
212 guidelines (53).

213

214 Central conduit artery endothelial function was measured using the carotid artery
215 reactivity test (CAR). The CAR induces carotid artery dilation during sympathetic
216 stimulation using the cold pressor test (CPT) and is a surrogate for coronary artery
217 vasomotor function and is inversely associated with the presence of cardiovascular risk
218 factors (49, 58). Duplex ultrasound was used to examine the common carotid artery
219 (CCA) before (1-minute) and during the CPT when participants were instructed to
220 immerse their left hand (up to the wrist) in iced slush (1-5°C) for 3-minutes. Participants

221 were instructed to breathe normally throughout the CPT and to avoid breath
222 holding/hyperventilation. Beat-to-beat arterial BP (Finapres Medical Systems, The
223 Netherlands) and 5-lead ECG were recorded online throughout the CPT (LabChart 8.0,
224 AD Instruments, Dunedin, New Zealand). Baseline diameter, velocity, shear rate, and
225 blood flow were calculated as the mean of data acquired across the 1 minute preceding
226 the CPT and during the CPT, data were calculated as the mean value for 10-second
227 intervals for the 3-minutes (58).

228

229 FMD and CAR analysis was performed using custom-designed edge detection software
230 by a single trained researcher who was blinded to the treatment allocation (53). From the
231 synchronised diameter and velocity data, blood flow (the product of cross-sectional area
232 and Doppler velocity) and shear rate (four times the velocity divided by the diameter)
233 were calculated. Total shear rate area under the curve between cuff deflation and peak
234 diameter (SRAUC) was calculated and FMD and CAR were automatically calculated and
235 presented as the peak diameter change from baseline (in %). The area-under-the curve for
236 changes in diameter during the CPT (CARAUC) was calculated as the percent change of
237 the average carotid diameter during the 3-minute CPT from baseline. As part of the
238 complete study (clinicaltrials.gov: NCT02777853), we also examined microvascular
239 function via assessment of forearm skin blood flow responses to local skin heating.
240 However, due to space restrictions and this variable being a secondary outcome, these
241 data are only presented as supplements (<https://figshare.com/s/ee9578ba1100e868861f>;
242 <https://doi.org/10.6084/m9.figshare.12659987>).

243

244 *Statistical Analysis*

245 Data were expressed as mean \pm SD and statistical significance was set at $P<0.05$. Linear
246 mixed models were used to examine the effect of the 7-day intervention (“Time”: pre *vs*
247 post), and whether this effect was altered by the type of intervention (“Intervention”:
248 Placebo *vs* Tea). The repeated covariance type was Unstructured, whilst we specified
249 “Time”, “Intervention” and “Time*Intervention” as Fixed Effects (intercept was
250 included) and as Estimated Marginal Means. Significant main or interaction effects were
251 followed up with the least significant difference (LSD) approach to multiple comparisons
252 (45). Data were analysed using SPSS 22.0 (SPSS, Chicago, IL, USA).

253 **RESULTS**

254 Two participants withdrew prior to completion due to personal circumstances (n=1) and
255 being unable to tolerate the lifestyle change (n=1), whilst technical issues caused
256 incomplete data sets for some parameters. One participant was unable to complete the
257 cold pressor test due to discomfort (n=11). Due to problems with venous cannulation, one
258 participant did not complete measures of glucose handling and insulin homeostasis
259 (n=11). Self-reported compliance to tea and food boxes was 100%. Compared to baseline
260 (11,103±3,385 steps/day), a significant reduction in steps was found after UL-Placebo
261 (5,880±1,462 steps/day, $P<0.001$) and UL-Tea (5,710±1,390 steps/day, $P<0.001$) with no
262 difference between UL-Placebo and UL-Tea ($P=0.75$). Energy intake increased during
263 both UL-Placebo (3,519±1,279 kcal/day) and UL-Tea (3,516±1,210 kcal/day) compared
264 to baseline (2,373±864 kcal/day, both $P<0.001$) with no difference between UL-Placebo
265 and UL-Tea ($P=0.95$). A non-significant increase in body mass was found in UL-Placebo
266 (77.4±10.0 to 78.1±11.0 kg) and UL-Tea (76.9±9.0 to 77.6±10.6 kg, $P=0.07$), which did
267 not differ between interventions (“Time*Intervention”-interaction: $P=0.92$). A trend for
268 a “Time*Intervention” interaction was found for MAP ($P=0.06$), with small, non-
269 significant changes in opposite direction after UL-Placebo (83±5 vs 85±5 mmHg) and
270 UL-tea (84±7 vs 82±6 mmHg).

271

272 *Mixed-Meal Tolerance Test (MTT).*

273 The 3-hour mixed-meal tolerance (MTT) induced a typical initial increase and subsequent
274 decrease in glucose and insulin (Figure 1). A significant “Time*Intervention” interaction
275 effect was found for glucose and insulin ($P=0.03$ and 0.01, respectively, Figure 1). Post-
276 hoc analysis revealed that postprandial AUC for glucose (226±138 vs 261±162 mmol/L)
277 and insulin (12,562±4,498 vs 16,254±6,803 μ iu/L) were increased in UL-Placebo (both

278 $P<0.05$), whilst postprandial AUC for glucose (261 ± 120 vs 164 ± 113 mmol/L) and insulin
279 ($15,225\pm5,501$ vs $10,533\pm3,825$ miu/L) were significantly decreased in UL-Tea (both
280 $P<0.05$; Figure 2). There was a significant “Time*Intervention” interaction ($P=0.01$) for
281 the Matsuda Index responses with a reduction after UL-Placebo (3.7 ± 2.0 vs. 3.0 ± 1.3 ,
282 $P<0.05$) but no change after UL-Tea (3.3 ± 1.7 vs. 4.2 ± 2.2 , $P>0.05$). There was no
283 significant “Time*Intervention” interaction ($P=0.53$) for the HOMA-IR responses with
284 no change after either UL-Placebo (2.4 ± 1.2 vs. 2.6 ± 0.5) or UL-Tea (2.8 ± 2.1 vs. 2.5 ± 1.8).
285 There was no significant “Time*Intervention” interaction ($P=0.11$) for the β -Cell
286 function responses with no change after either UL-Placebo (9.2 ± 10.2 vs. 6.0 ± 4.7) or UL-
287 Tea (6.4 ± 5.2 vs. 8.5 ± 6.4).

288

289 *Peripheral vascular function*

290 For the brachial artery, there was no main effect of “Time”, “Intervention” or
291 “Time*Intervention” interaction for FMD%, baseline diameter or SRAUC (all $P>0.05$,
292 Table 1; Figure 3). For femoral artery FMD, there was a significant interaction of
293 “Time*Intervention” ($P<0.001$). Post-hoc analysis revealed that femoral artery FMD
294 decreased after UL-Placebo (e.g., peripheral vascular function was worse), but was
295 maintained during UL-Tea (e.g., peripheral vascular function did not change; Table 1
296 Figure 3). No effects were observed for baseline diameter or SRAUC (all $P>0.05$, Table
297 1). No significant main effects of time nor time*interaction effects were found for skin
298 microvascular function (<https://figshare.com/s/ee9578ba1100e868861f>;
299 <https://doi.org/10.6084/m9.figshare.12659987>; Table S3 and Figures S1 and S2).

300

301

302

303 *Central vascular function.*

304 For CAR (peak diameter change from baseline), there was no main effect of “Time”
305 ($P=0.85$), but there was a main effect of “Intervention” ($P=0.05$) and
306 “Time*Intervention” ($P=0.04$). Post-hoc analysis showed that CAR decreased following
307 UL-Placebo (e.g., central vascular function was worse), but was maintained during UL-
308 Tea (e.g., central vascular function did not change; Table 1, Figure 4). Similar results
309 were evident for CARAUC (the percent change of the average carotid diameter during
310 the 3-minute CPT); there was no main effect of “Time” ($P=0.88$), but there was a main
311 effect of “Intervention” ($P=0.04$) and a borderline “Time*Intervention” interaction
312 ($P=0.08$). Post-hoc analysis showed that CARAUC decreased following UL-Placebo, but
313 was maintained during UL-Tea (Figure 4). Elevations in systolic and diastolic BP during
314 CAR were not different across “Time”, “Intervention” or “Time*Intervention” (all
315 $P>0.05$, Table 1). Baseline common carotid artery diameter did not change after either
316 intervention ($P=0.59$) nor differed between conditions ($P=0.97$).

317

318 DISCUSSION

319 Our study has the following novel observations. Impairments in postprandial glucose-
320 insulin homeostasis, and also peripheral and central vascular function, in young, healthy
321 men as a result of a 7-day unhealthy lifestyle, were ameliorated with daily consumption
322 of green tea. These results highlight the rapid, detrimental impact of a short-term exposure
323 to an unhealthy lifestyle on metabolic and vascular function, and that green tea
324 consumption may (in part) alleviate these effects. This work highlights the immediate
325 impact of lifestyle-related factors for metabolic and cardiovascular health.

326

327 In the present study we found higher blood glucose and insulin levels after a mixed-meal
328 challenge (as well as a lower Matsuda Index) after 7-days following an unhealthy
329 lifestyle. This supports previous findings, in that 3-14 days exposure to excessive calorie
330 intake, physical inactivity or both can alter glucose and insulin homeostasis (5, 21, 28,
331 41, 60). These findings are clinically relevant since higher postprandial levels of blood
332 glucose and insulin fit with the presence of insulin resistance. More importantly, when
333 meals were consistently preceded with green tea, we found that these metabolic
334 derangements did not occur. Previous studies found that green tea acutely, i.e., within
335 hours, improves glucose homeostasis in healthy and pre-diabetic participants (4, 30, 37,
336 64). In addition, long-term ingestion of green tea has been linked to better metabolic
337 health through a range of mechanisms, including, a slowing of carbohydrate digestion
338 and glucose absorption, stimulation of insulin secretion, a decreased β -cell oxidative
339 damage, a modulation of liver glucose release and activation of glucose uptake receptors
340 in insulin-sensitive tissue (20). Our study extends these findings by revealing that green
341 tea is causally linked to the prevention of impairments in metabolic function in response
342 to short-term exposure to an unhealthy lifestyle. We found significant impairments in

343 peripheral and central vascular function, specifically, conduit artery vasodilator capacity
344 to increases in flow, i.e., femoral FMD, largely NO-mediated (29), was reduced by ~2%
345 points and sympathetic stimulation, i.e., CAR, likely related to NO (42), was reduced by
346 ~1.8% points after 7-days of unhealthy lifestyle, which did not occur with concomitant
347 consumption of green tea. Meta-analyses indicate a 8–13% lower risk of CV events per
348 percent point increase in FMD (54) and a 2% lower CAR is associated with the presence
349 of 2 CVD risk factors (58). Several previous studies found that a prolonged and/or
350 extreme unhealthy lifestyle, e.g., diets high in fat (particularly trans-fat) and/or
351 carbohydrate and/or physical inactivity, is associated with increased CVD risk (12, 36,
352 51), and impaired macrovascular function (13, 38) largely attributed to endothelial
353 dysfunction from increased oxidative stress and reduced NO bioavailability (3). Our
354 study, reflecting a real-world situation, i.e., holidays, further highlights that only a short
355 timeframe, e.g., 7 days, is sufficient to induce clinically meaningful detrimental vascular
356 effects, which were abrogated by regular daily consumption of green tea, likely via
357 improved activation of eNOS (31) and NO-mediated endothelial function (47), reduced
358 oxidative stress (4) and/or an improved antioxidant and anti-inflammatory capacity (52).
359 The exact constituent of green tea that causes these beneficial vascular and metabolic
360 effects in vivo is not clear. Equivocal evidence exists for the role of EGCG (32, 62) and
361 EC (14, 50) and caffeine (8, 55) in green tea-induced elevations in macrovascular
362 function. Further research is needed to identify the mechanism(s) that underlie
363 cardiovascular and metabolic benefits of green tea.

364

365 We found distinct effects in upper and lower limb FMD responses whereby divergent
366 changes were evident in femoral FMD (decreases in Placebo but maintenance in Tea) but
367 not in brachial FMD. This between-limb discrepancy may relate to differences in activity

368 level across the intervention period, in that our intervention reduced activity of the lower
369 limbs, but not necessarily upper limbs. This may underlie the decline in femoral artery
370 FMD, with preserved brachial FMD. In agreement, previous studies adopting models of
371 physical inactivity affecting lower limbs (e.g. bed rest, lower limb suspension, step
372 reduction) also report a decline in lower limb FMD, with preserved brachial artery FMD
373 (6, 22) as shear stress is reduced in the lower limb but likely preserved in the upper limb
374 where movement is not restricted. Furthermore, the lower limb vessels appear more
375 vulnerable to dysfunction and disease than upper limb vascular beds (16, 48). Similarly,
376 the lack of a time*condition interaction for forearm microvascular function is consistent
377 with the aforementioned regional FMD differences and/or differences in susceptibility for
378 dysfunction in the micro- vs. macrovasculature.

379

380 *Limitations.* Although a relatively modest sample size was included, our study was
381 sufficiently powered to demonstrate a significant impact of an unhealthy lifestyle and tea
382 via a strong methodological design (i.e. double-blind, within-subjects cross-over) across
383 a variety of outcomes from a comprehensive test protocol. It was not possible to ascribe
384 the detriments in vascular and metabolic function specifically to low physical activity or
385 overfeeding per se; this was beyond the scope of the study. Another limitation is that we
386 adopted self-reported diaries to assess participants' compliance to the caloric intervention
387 which may be subject to reporting bias. Moreover, we did not determine if the dose and
388 frequency of green tea were sufficient to raise the plasma NO bioavailability and whether
389 alternative mechanisms were evident (e.g., interaction with the gut microbiome). Only
390 young, healthy men were studied, which limits the findings to this cohort. Clearly, female
391 reproductive hormones in pre-menopausal women, as well as postmenopausal status, can
392 alter vascular function. The interaction of an unhealthy diet and physically inactive

393 lifestyle and the reproductive cycle is an important area that requires further investigation.
394 Similarly, the beneficial effects of flavonoids are more evident in diseased or at risk
395 populations; therefore, it is possible that green tea would have a greater effect in groups
396 with impaired vascular and/or metabolic function. Finally, green tea was used as the
397 intervention when various other types of tea are available, e.g., black tea, which shows
398 similar beneficial effects to green tea on vascular and metabolic function (7, 19).

399

400 *Conclusion.* In conclusion, our study reveals that only 7-days of an unhealthy lifestyle,
401 including 50% fewer steps and 50% more calories, leads to impaired postprandial
402 metabolic, as well as peripheral and central vascular, function in young, healthy men.
403 These short-term detrimental metabolic and vascular effects were prevented when green
404 tea was consumed daily. This suggests that simple dietary adjustments, such as the
405 consumption of green tea , may help to avoid short-term detrimental effects when healthy
406 participants are transiently exposed to an unhealthy lifestyle.

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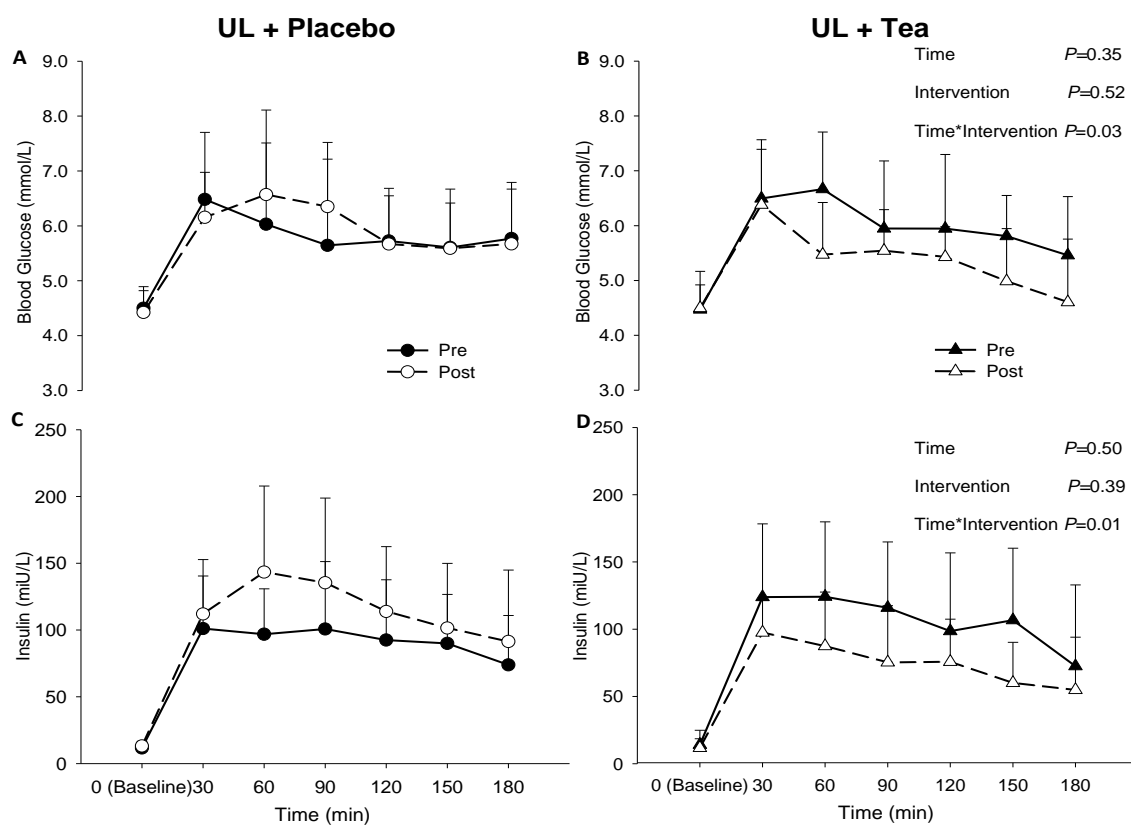


Figure 1. Presentation of glucose (A-B) and insulin (C-D) levels at baseline (0 min) and after a mixed meal tolerance test (MTT; 30, 60, 90, 120, 150 and 180-min) before (closed symbols) and after (open symbols) a 7-day unhealthy lifestyle (UL) combined with placebo (A, C) or green tea (B, D) in healthy male volunteers. Data are presented as means, with error bars representing SD. P -values refer to a 2-way linear mixed model (LMM) of time and intervention. $N=11$.

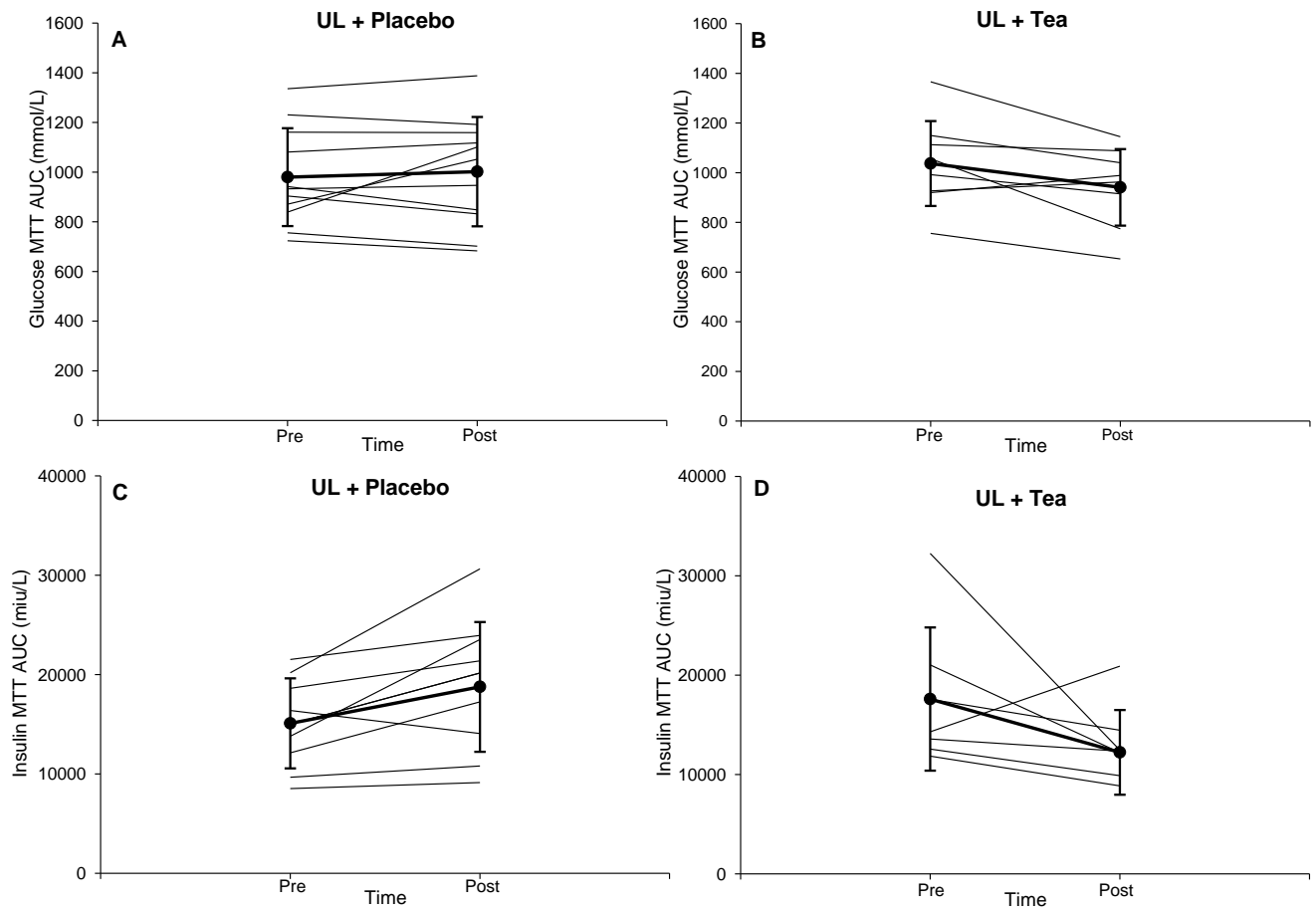


Figure 2. Presentation of individual and mean glucose (A-B) and insulin (C-D)AUC responses to a mixed meal tolerance test (MTT) before and after a 7-day unhealthy lifestyle combined with placebo (A, C) or green tea (B, D) in healthy male volunteers. Error bars represent SD. N=11.

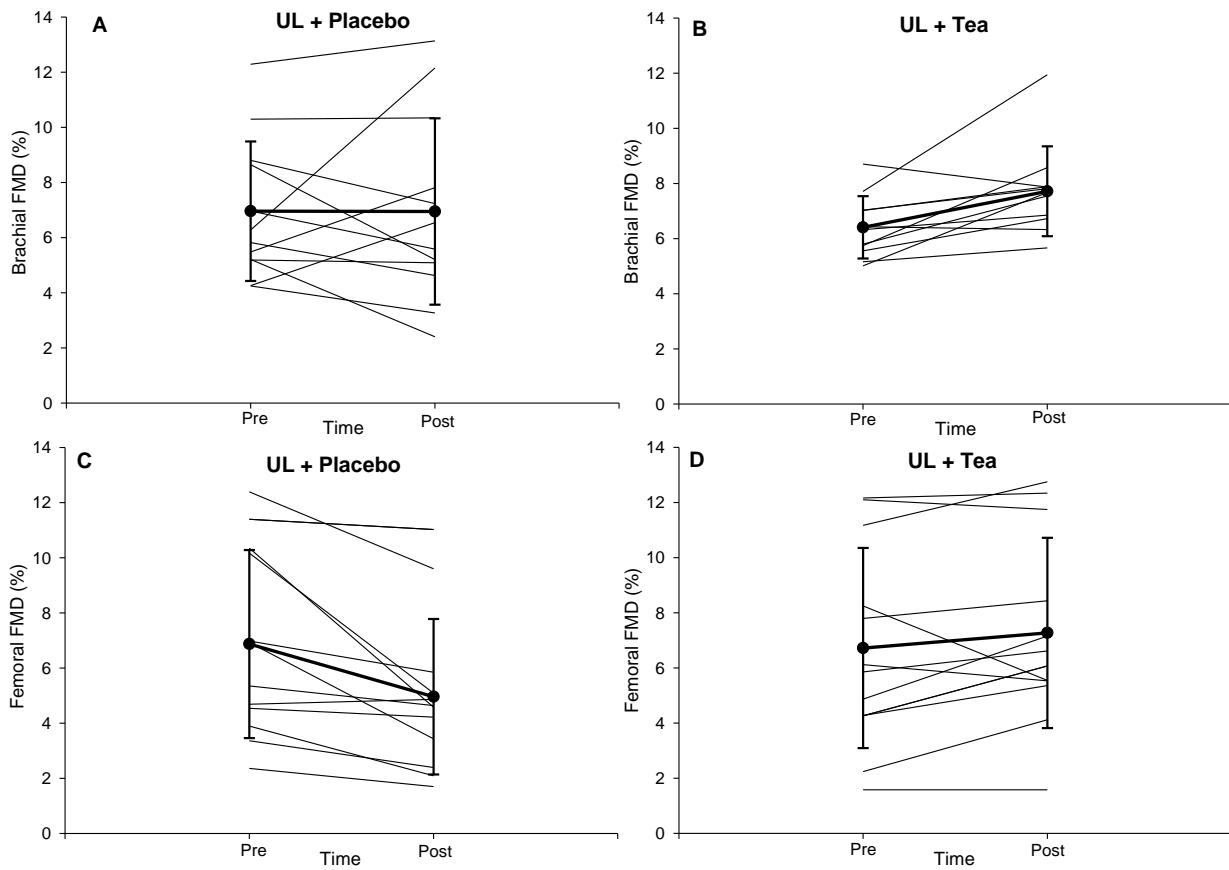


Figure 3. Presentation of individual and mean brachial (A-B) and femoral (C-D) FMD responses before and after a 7-day unhealthy lifestyle combined with placebo (A, C) or green tea (B, D) in healthy male volunteers. Error bars represent SD. N=11.

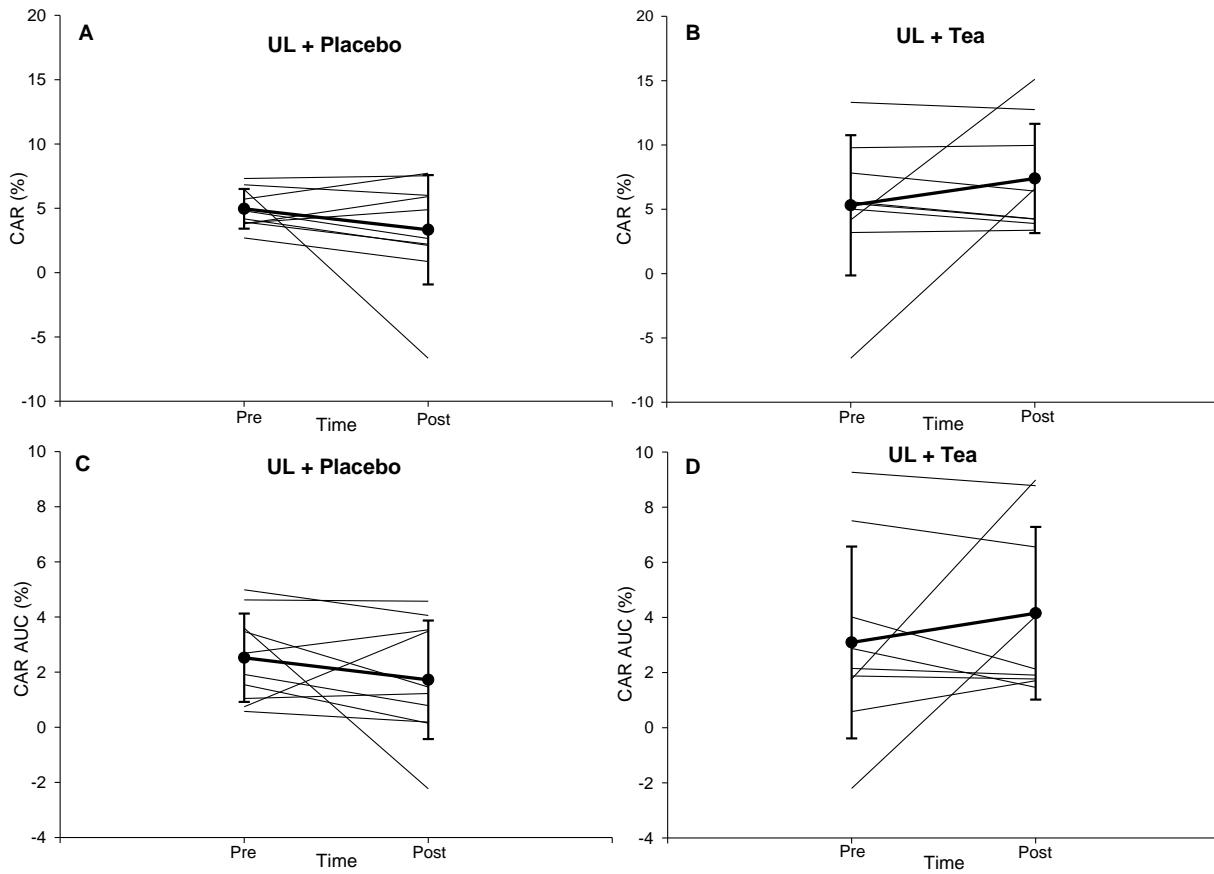


Figure 4. Presentation of individual and mean CAR (A-B) and CARAUC (C-D) responses before and after a 7-day unhealthy lifestyle combined with placebo (A, C) or green tea (B, D) in healthy male volunteers. Error bars represent SD. N=11.

Table 1. Brachial and femoral artery FMD%, baseline diameter, time-to-peak and shear rate, and carotid artery reactivity variables before and after UL-Placebo and UL-Tea interventions. N=12 for Brachial and Femoral Artery data. N=11 for Carotid Artery Reactivity data.

	Intervention (mean±SD)				LMM P Values		
	UL-Placebo		UL-Tea		Time	Intervention	T*I
	Pre	Post	Pre	Post			
Brachial Artery							
FMD (%)	7.0±2.5	7.0±3.38	7.0±1.2	7.7±1.6	0.20	0.97	0.11
Baseline diameter (cm)	0.4±0.0	0.4±0.04	0.4±0.0	0.4±0.0	0.40	0.45	0.21
Time-to-peak (s)	40±17	48±22	47±19	43±11	0.65	0.87	0.06
Shear rate (SRAUC)	17456±8205	19407±9026	21046±7317	21411±12650	0.64	0.46	0.16
Femoral Artery							
FMD (%)	7.0±3.4	5.0±2.8	6.7±3.6	7.3±3.5	0.10	0.21	0.001
Baseline diameter (cm)	0.6±0.1	0.6±0.1	0.6±0.1	0.7±0.1	0.52	0.29	0.32
Time-to-peak (s)	74±49	79±43	54±28	41±22	0.77	0.02	0.30
Shear rate (SRAUC)	17882±8353	18187±13450	15904±8525	13659±7965	0.68	0.15	0.16
Carotid Artery Reactivity							
CAR (%)	5.1±1.5	3.3±4.3	5.7±5.3	7.5±4.0	0.87	0.05	0.04
CARAUC (%)	2.6±1.5	1.7±2.2	3.4±3.4	4.2±3.0	0.88	0.04	0.08
Change in SBP (mmHg)	28±13	34±15	25±12	26±15	0.27	0.10	0.38
Change in DBP (mmHg)	18±6	22±8	18±5	14±5	0.61	0.07	0.05

Data are mean±SD. AUC, area-under-the-curve; CAR, carotid artery reactivity; DBP; diastolic blood pressure; FMD, flow-mediated dilation; SBP, systolic blood pressure; SRAUC, shear rate area-under-the-curve; T*I, Time*Intervention-interaction.