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

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

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ABSTRACT

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

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

INTRODUCTION

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

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

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

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

PARTICIPANTS AND METHODS

Participants

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

Experimental Design

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

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

Interventions

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

Mixed meal tolerance test

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

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

Vascular Function.

Peripheral conduit artery, largely NO-mediated, endothelial function was examined at the right brachial and superficial femoral arteries using flow-mediated dilation (FMD) (53). A 10 MHz multi-frequency linear array probe, attached to a high-resolution 2D duplex ultrasound machine (Terason u-Smart 3300, Teratech, Burlington, MA, USA) was used. Pneumatic cuffs (D.E. Hokanson, Bellevue, WA, USA), connected to a rapid inflator (D.E. Hokanson, Bellevue, WA, USA), were positioned on the interrogated upper forearm and thigh, distal to the imaged site. In addition to a stable B-mode image, continuous Doppler velocity and diameter data were collected. Baseline images were recorded for 1-minute, following which the occlusion cuffs were inflated (>220 mmHg) for 5-minutes. Diameter and velocity recordings resumed 30-seconds prior to cuff deflation and continued for 3-minutes after cuff deflation, according to methodological guidelines (53).

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

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

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

243

244 *Statistical Analysis*

245 Data were expressed as mean±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).

RESULTS

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

Mixed-Meal Tolerance Test (MTT).

The 3-hour mixed-meal tolerance (MTT) induced a typical initial increase and subsequent decrease in glucose and insulin (Figure 1). A significant "Time*Intervention" interaction effect was found for glucose and insulin ($P=0.03$ and 0.01, respectively, Figure 1). Post-hoc analysis revealed that postprandial AUC for glucose (226±138 *vs* 261±162 mmol/L) and insulin (12,562±4,498 *vs* 16,254±6,803 miu/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±5,501 *vs* 10,533±3,825 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

DISCUSSION

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

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

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

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

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

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

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

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

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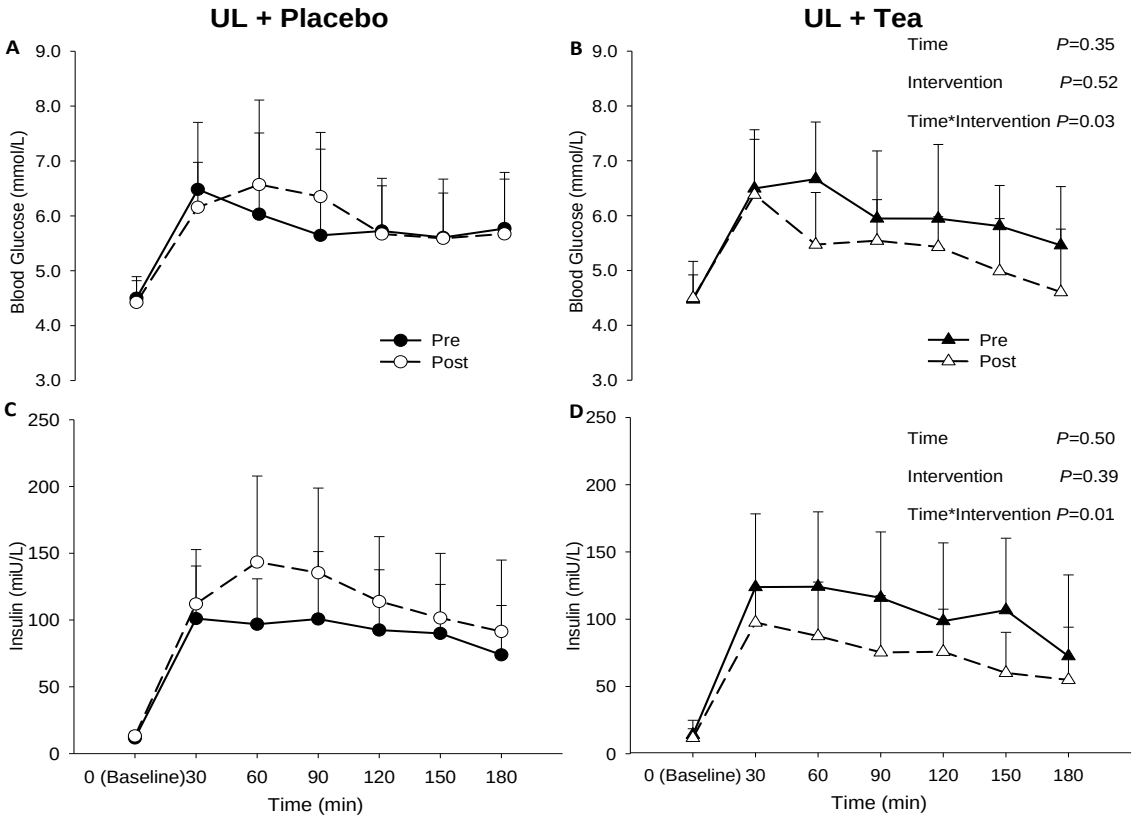

Figure 1. Presentation of glucose (A-B) and insulin (C-D) levels at baseline (0 min) and after a mixed meal tolerance test (MTT; 30, 60, 90, 120, 150 and 180-min) before (closed symbols) and after (open symbols) a 7-day unhealthy lifestyle (UL) combined with placebo (A, C) or green tea (B, D) in healthy 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.

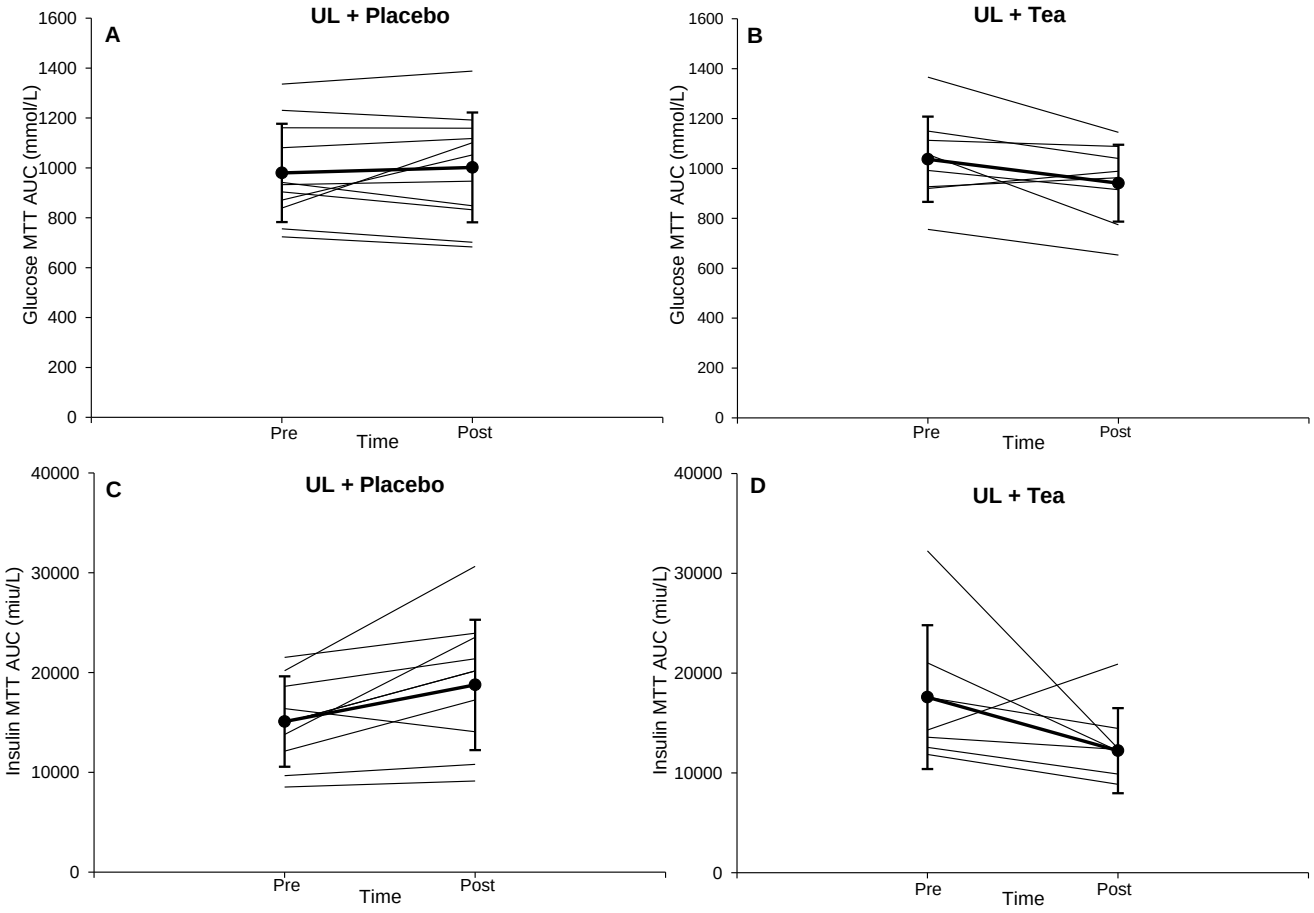

Figure 2. Presentation of individual and mean glucose (A-B) and insulin (C-D)AUC responses to a mixed meal tolerance test (MTT) before and after a 7-day unhealthy lifestyle combined with placebo (A, C) or green tea (B, D) in healthy male volunteers. Error bars represent SD. N=11.

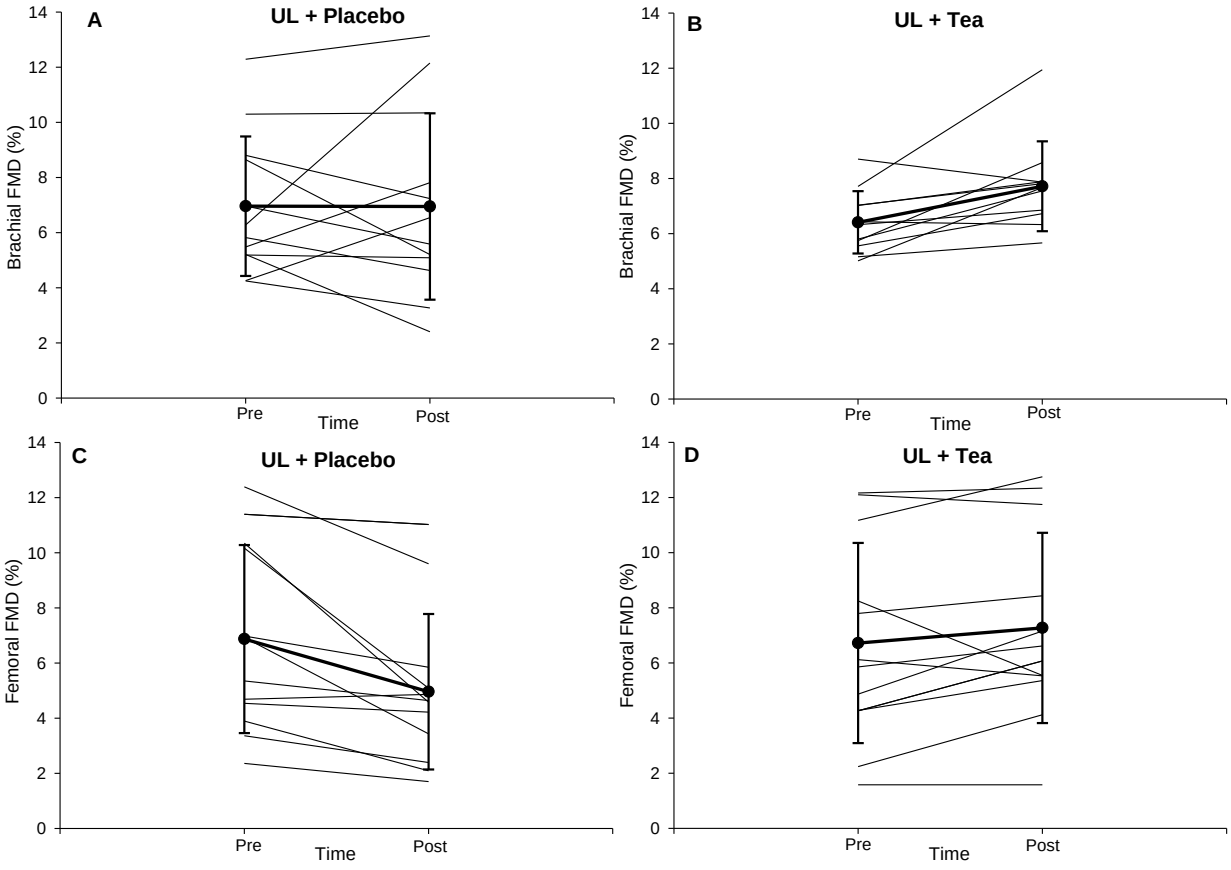

Figure 3. Presentation of individual and mean brachial (A-B) and femoral (C-D) FMD responses before and after a 7-day unhealthy lifestyle combined with placebo (A, C) or green tea (B, D) in healthy male volunteers. Error bars represent SD. N=11.

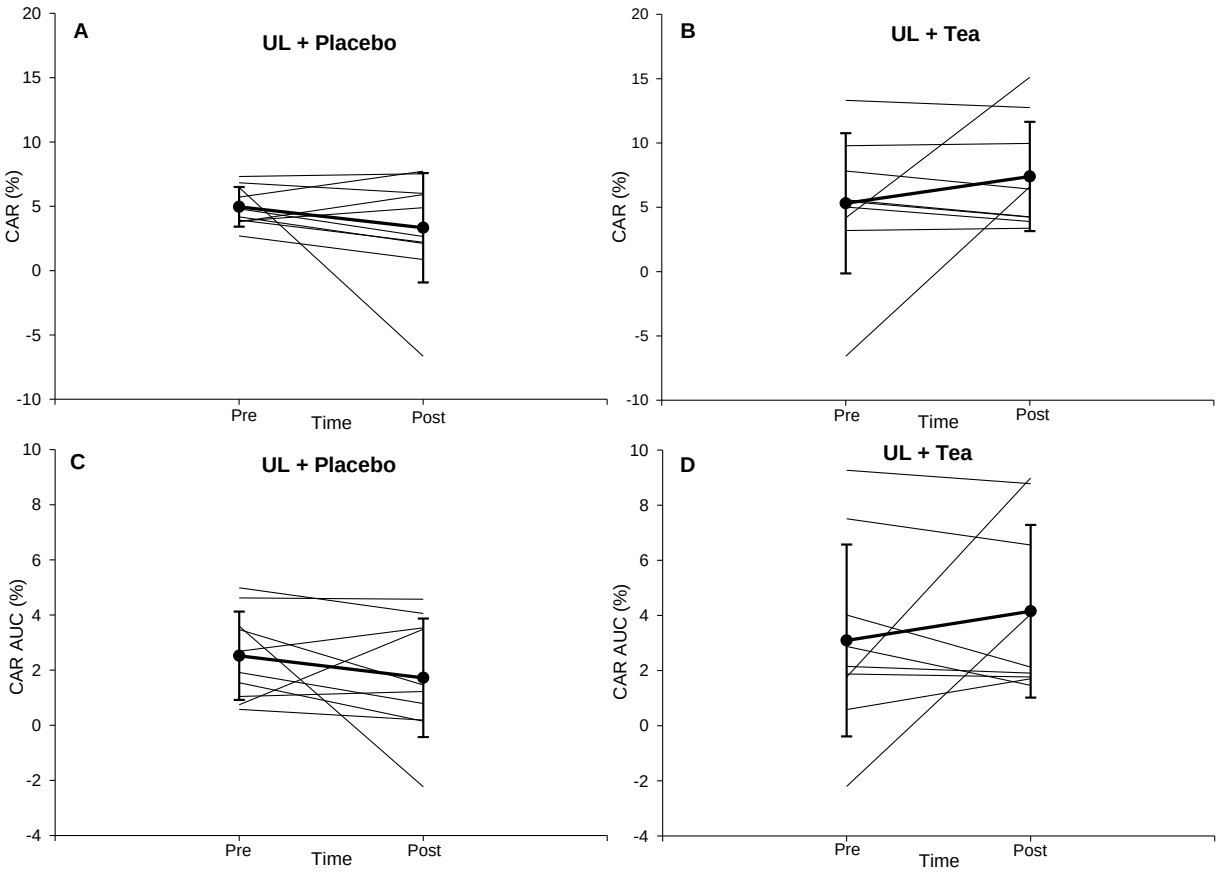

Figure 4. Presentation of individual and mean CAR (A-B) and CARAUC (C-D) responses before and after a 7-day unhealthy lifestyle combined with placebo (A, C) or green tea (B, D) in healthy male volunteers. Error bars represent SD. N=11.

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**
Brachial Artery	*Pre*	*Post*	*Pre*	*Post*			
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.