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RESEARCH ARTICLE

The impact of the catechol-*O*-methyltransferase genotype on vascular function and blood pressure after acute green tea ingestion

*Rosalind J. Miller[1,2], Kim G. Jackson[1,2], Tony Dadd[3], Andrew E. Mayes[3], A. Louise Brown[3], Julie A. Lovegrove[1,2] and Anne M. Minihane[1]**

[1] Department of Food and Nutritional Sciences, University of Reading, Reading, UK
[2] Institute for Cardiovascular and Metabolic Research (ICMR), University of Reading, Reading, UK
[3] Unilever Discover, Colworth Science Park, Sharnbrook, Bedford, UK

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Scope: Evidence for the benefits of green tea catechins on vascular function is inconsistent, with genotype potentially contributing to the heterogeneity in response. Here, the impact of the catechol-*O*-methyltransferase (COMT) genotype on vascular function and blood pressure (BP) after green tea extract ingestion are reported.

Methods and results: Fifty subjects ($n = 25$ of the proposed low-activity [AA] and of the high-activity [GG] COMT rs4680 genotype), completed a randomized, double-blind, crossover study. Peripheral arterial tonometry, digital volume pulse (DVP), and BP were assessed at baseline and 90 min after 1.06 g of green tea extract or placebo. A 5.5 h and subsequent 18.5 h urine collection was performed to assess green tea catechin excretion. A genotype × treatment interaction was observed for DVP reflection index ($p = 0.014$), with green tea extract in the AA COMT group attenuating the increase observed with placebo. A tendency for a greater increase in diastolic BP was evident at 90 min after the green tea extract compared to placebo ($p = 0.07$). A genotypic effect was observed for urinary methylated epigallocatechin during the first 5.5 h, with the GG COMT group demonstrating a greater concentration ($p = 0.049$).

Conclusion: Differences in small vessel tone according to COMT genotype were evident after acute green tea extract.

Keywords:
Blood pressure / Catechol-*O*-methyltransferase / Endothelial function / Flavonoids / Green tea

1 Introduction

Cardiovascular disease (CVD) is the number one cause of death across the globe with an estimated 80% of cases believed to be preventable through lifestyle modifications such as diet and exercise [1]. Endothelial dysfunction and an associated loss of vascular reactivity and decreased vascular tone have been repeatedly recognized as a critical early step in the process of atherogenesis, and as such is considered to represent an intermediate phenotype for vascular disease [2]. Thus, substances with the ability to prevent damage or restore endothelial function have important clinical applications. A number of acute and chronic human studies have shown favorable effects of green tea and associated green tea polyphenols on noninvasively assessed endothelial function [3–7] but findings are not entirely consistent [3, 8, 9]. Possible reasons for differences in study results include heterogeneities in study design, dose and form of green tea catechin administration, the gender, age or health status of participants or genetic variation between study populations.

Correspondence: Dr. Kim G. Jackson, Department of Food and Nutritional Sciences, University of Reading, P.O. Box 226, Whiteknights, Reading, Berkshire, RG6 6AP, UK
E-mail: k.g.jackson@reading.ac.uk
Fax: +44-118-378-7708

Abbreviations: AI, augmentation index; **COMT**, catechol-*O*-methyltransferase; **CVD**, cardiovascular disease; **DVP**, digital volume pulse; **EC**, epicatechin; **ECG**, epicatechin gallate; **EGC**, epigallocatechin; **EGCG**, epigallocatechin gallate; **NEFA**, nonesterified fatty acid; **RHI**, reactive hyperemia index; **RI**, reflection index; **SI**, stiffness index; **TNF-α**, tumur necrosis factor alpha; **VCAM-1**, vascular cellular adhesion molecule-1

*Current address: Diet and Health Group, School of Medicine, University of East Anglia, Norwich, UK.

Catechol-*O*-methyltransferase (COMT), a phase II enzyme, plays an important role in the metabolism and rapid inactivation/detoxification of various catechol-containing endobiotics and xenobiotics, such as catecholamines (dopamine and epinephrine), catechol estrogens, and medicinal and dietary catecholic polyphenols. The COMT gene has been found to have a common nonsynonymous G to A polymorphism (rs4680) resulting in a valine to methionine amino acid substitution at position 108/158 of the membrane-bound/soluble form of the protein. The polymorphism is thought to produce a less-stable protein, which in vitro studies have proposed to result in a 40% decrease in enzyme activity with respect to catechol metabolism [10–12]. The prevalence of the homozygous low-activity COMT genotype (AA) in Caucasian populations is approximately 25%, a figure which is substantially lower in African and Asian populations. The effect of COMT genotype on green tea catechin metabolism in vivo is unknown. However, low-activity COMT has been shown to metabolize estrogens (another substrate for COMT) at a different rate to the high-activity form [13]. The main objective of the current study was to investigate, using a placebo-controlled design, the acute impact of a green tea extract on vascular reactivity and the potential modifying effect of the COMT Val108/158Met polymorphism. Green tea extract is hypothesized to have a beneficial impact on vascular function, with the low-activity COMT genotype demonstrating the greatest beneficial effect.

2 Materials and methods

2.1 Subject selection

Fifty healthy male subjects (25 GG and 25 AA COMT genotype) were prospectively recruited from Reading and surrounding areas. Subjects were not suffering from any known form of gastrointestinal disorder, CVD, kidney or liver disease, or diabetes and were not taking any blood pressure (BP) or cholesterol-lowering medications. All screening parameters were below or above a specified level (BP $<$ 140/90 mmHg, total cholesterol $<$ 8.0 mmol/L, alanine aminotransferase $<$ 50 U/L, γ-glutamyl transferase $<$ 55 U/L, bilirubin $<$ 25 μmol/L, fasting triacylglycerol $<$ 4.0 mmol/L, fasting glucose $<$ 7.0 mmol/L, and hemaglobin $>$12.5 g/dL). The study was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. Informed consent was gained from all subjects and the study was given a favorable ethical opinion to proceed by the University of Reading's Research Ethics Committee (permission number 09/22). Treatment order was randomized by a study-independent statistician using a permuted block method: multiple codes corresponding to the green tea extract/placebo treatments were employed to allow rolling recruitment to take place without undue risks of imbalance or unblinding.

2.2 DNA extraction and COMT genotyping

COMT genotype of each subject was determined following DNA isolation using TaqMan PCR Technology (Applied Biosystems 7300 Instrument, Applied Biosystems, Warrington, UK). The buffy coat layer was isolated from the K_2E EDTA screening blood sample by centrifugation at 1700 × *g* for 10 min at 4°C and DNA was extracted using the QIAmp DNA Mini Kit (Qiagen Ltd., Crawley, UK). Allelic discrimination of the COMT rs4680 gene variant was conducted using a TaqMan Drug Metabolism Genotyping Assay (Applied Biosystems).

2.3 Study design

This was a double-blinded, placebo-controlled, randomized, crossover study. For 48 h before each study visit, subjects consumed a low-flavonoid diet by avoiding all fruit and vegetables (except those of very low flavonoid content), fruit juice, jams and preserves, chocolate and chocolate-containing products, tea, coffee, soy products, and alcohol. To avoid caffeine withdrawal, regular coffee/tea drinkers were asked to replace usual caffeine intake with other caffeine-containing drinks. Subjects were also asked to avoid intensive exercise for 24 h before each study visit. A standardized low-flavonoid ready meal was consumed by the subjects on the evening prior to the study visits. Subjects visited the Hugh Sinclair Unit of Human Nutrition on two separate occasions at least 10 days apart after a 12 h overnight fast. An investigator-led 24 h food recall was completed at the beginning of each visit to assess compliance to the low-flavonoid diet. Weight measurements were performed followed by insertion of an indwelling cannula into the antecubital vein of the forearm. Subjects were rested for 30 min in a quiet, temperature-controlled room before assessment of vascular function by peripheral arterial tonometry (Endo-PAT, Itamar, Israel), pulse contour analysis (digital volume pulse [DVP], CareFusion, UK), and static BP assessment. A baseline blood sample was collected followed by ingestion of two decaffeinated green tea extract capsules (Sunphenon 90LB, Taiyo International, Japan) containing a total of 828 mg green tea catechins (equivalent to ∼8 cups of green tea; Table 1, Fig. 1) or visually identical lactose placebo capsules taken with 250 mL water. A decaffeinated green tea extract was chosen to eliminate the potential confounding effects of caffeine, a proposed modulator of vascular function. Vascular function assessment was repeated at 90 min (previously derived time of peak catechin appearance in plasma [14]). Blood samples were then taken after the vascular measurements at 105 min and then at hourly intervals at 165 and 225 min. Blood samples were used for assessment of plasma green tea catechin concentrations, vascular function modulators, and inflammatory biomarkers. A 24 h urine collection was performed (start $t = 0$ min) for assessment of EGC and 4′-O-methyl EGC concentration, split into two collection periods (5.5 h (duration of the study day) and 18.5 h) to help capture potential genotypic differences in excretion rates.

Table 1. Composition of study capsules Sunphenon 90LB

Chemical	Percentage of extract	Content per capsule[a)] (mg)	Study dose[b)] (mg)
Epigallocatechin gallate (EGCG)	43.0	230	460
Epigallocatechin (EGC)	10.9	58	116
Epicatechin gallate (ECG)	8.9	47	94
Epicatechin (EC)	8.3	44	88
Gallocatechin (GC)	2.0	11	22
Gallocatechin gallate (GCG)	1.7	9	18
Catechin	1.2	6	12
Gallic acid (GA)	1.0	5	10
Catechin gallate (CG)	0.2	1	2
Caffeine	0.6	3	6

a) Capsule contents total weight ~530 mg.
b) Study dose consisted of two capsules taken with water.

2.4 Vascular function assessment

Vascular function was assessed noninvasively using peripheral arterial tonometry, a method used in an increasing number of studies including the Framingham Heart [15], Heart SCORE study [16], and short-term dietary intervention studies [17, 18]. The Endo-PAT measures endothelium-mediated changes in vascular tone using biosensors placed on the index finger. Using a standard BP cuff on the upper arm, the brachial artery is occluded for 5 min and when released quickly, the surge in blood flow causes shear stress endothelium-dependent flow-mediated dilatation. The other index finger acts as a control and is used to correct for nonendothelial-dependent changes in vascular tone. A postocclusion to preocclusion ratio is calculated by the software to produce an Endo-PAT reactive hyperemia index (RHI). In addition, automated analysis of the pulse waveform, specifically the height of the reflected wave compared to the direct wave, is used to produce the augmentation index (AI).

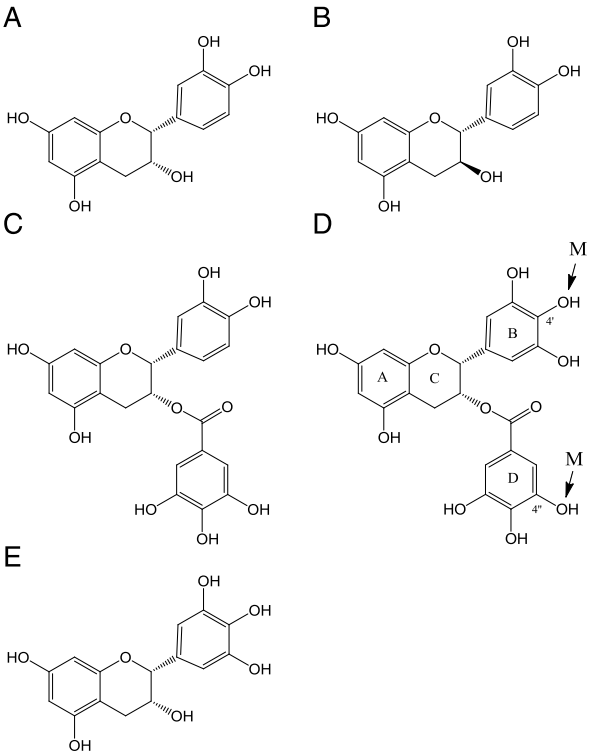

Figure 1. Green tea catechins (A) (−)-epicatechin; (B) (+)-catechin; (C) (−)-epicatechin gallate; (D) (−)-epigallocatechin gallate (M = methylation sites of 4′ and 4″-*O*-methyl epigallocatechin gallate); (E) (−)-epigallocatechin.

The DVP is recorded by measuring the transmission of infrared light absorbed through the finger via a photoplethysmograph finger probe. The amount of light is directly proportional to the volume of blood in the finger pulp. A pulse waveform is produced which is used to derive the reflection index (RI) and stiffness index (SI). The RI is the height of the diastolic peak as a percentage of the systolic peak and relates to the tone of the small arteries. The SI is the time period between the systolic and diastolic peaks (corrected for subject height) and is strongly influenced by large arterial stiffness [19].

2.5 Blood and urine sample analysis

Blood samples were collected into K_2E EDTA and lithium heparin tubes, centrifuged at 1700 × *g* for 10 min at 4°C before storage at −80°C until analysis. To prevent green tea catechin degradation, 10 μL of ascorbic acid buffer (0.4 M sodium dihydrogen phosphate, 0.1% EDTA, and 20% ascorbic acid, pH 3.6) was added to each 500 μL aliquot of sample intended for green tea catechin analysis before storage at −80°C. Fasting plasma triglycerides, nonesterified fatty acid (NEFA), and cholesterol were measured using the ILAB 600 clinical chemistry analyzer (Instrumentation Laboratory, Warrington, UK) using enzyme-based colorimetric kits supplied by Instrumentation Laboratory (triglycerides and cholesterol) and Alpha Laboratories (Eastleigh, UK) (NEFA). Interassay coefficients of variance were 2.4, 2.0, and 2.4%, for cholesterol, triglycerides, and NEFA, respectively. Plasma total nitrite (includes nitrate), which serves as a biomarker for nitric oxide (a potent endothelial derived vasodilator) was measured using a nitric oxide quantitation kit (Active Motif, CA). Commercially available ELISA kits were used to measure endothelin-1

(endothelial-derived vasoconstrictor), vascular cellular adhesion molecule-1 (VCAM-1) and tumor necrosis factor alpha (TNF-α) (RnD Systems, MN). Interassay coefficients of variance were 11.8, 8.5 and 6.9% for endothelin-1, VCAM-1 and TNF-α, respectively.

Urine collection was split into two periods, baseline to 5.5h and 5.5h to 24h. Urine was collected in containers containing 200 mg of the preservative ascorbic acid. Total volume by weight was recorded and samples were mixed thoroughly before aliquots were removed and acidified with 1M HCl. Urine samples were stored at −80°C prior to analysis.

2.6 Green tea catechin analysis using mass spectrometry

Extraction of catechins from plasma and urine samples was performed as previously described [14]. Total (parent and conjugated forms) plasma catechin, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) and 4′-*O*-methyl EGCG, 4′′-*O*-methyl EGCG and 4′4′′-*O*-methyl EGCG were measured by UPLC-MS/MS (Chemical structures; Fig. 1). Reference standards were obtained from Sigma-Aldrich, Missouri (EC, EGC, ECG, and EGCG), Timtec, LLC (4′-*O*-methyl EGCG) or synthesized in-house as described by Meng et al. [20] (4′′-*O*-methyl EGCG and 4′4′′-*O*-methyl EGCG). Samples were analyzed using UPLC (Waters Acquity LC system equipped with autosampler and BEH Sheild RP18 1.7 μm 2.1 × 100 mm column) with mass spectrometry using a column temperature of 60°C, flow rate of 0.44 mL/min with 0.1% acetic acid in methanol as mobile phase A and 0.1% acetic acid (aq) as mobile phase B. The elution started with 5% phase A, increased to 15% after 0.7 min, 42% at 3.2 min, 100% at 3.3 min and decreased back to 5% at 9 min. The tandem MS analyses were carried out on a TQD mass spectrometer (Waters Quattro Ultima) equipped with an electrospray interface. The analyses were done in positive mode and the ionization source working conditions were as follows: capillary voltage, 3.83 kV; source temperature, 120°C; cone gas flow rate, 200 L/h; desolvation gas flow rate, 1000 L/h; desolvation temperature, 450°C; and dwell time 20 ms.

Total EGC and 4′-*O*-methyl EGC were measured in the urine samples by HPLC MS. Reference standards were obtained from Sigma-Aldrich (EGC) and Timtec TLL (4′-*O*-methyl EGC). A mixed working standard was prepared from stock solutions (2 mg/mL EGC and 1 mg/mL 4′-*O*-methyl EGC in methanol) and stabilizer solution (250 mg/L ascorbic acid, 250 mg/L EDTA in 2% acetic acid, 10% acetonitrile) with a final EGC and 4′-*O*-methyl EGC concentration of 10 μg/mL. A Micromass ZMD Single Quadrupol Mass Spectrometer with Waters Alliance 2795 HPLC system was used with a Phenomenex Synergi 4u Polar-RP 80A, 150 × 2 mm column. A flow rate of 0.2 mL/min, column temp of 30°C and injection volume of 50 μL was used. The solvent system was composed of 0.1% formic acid in water and 0.1% of formic acid in acetonitrile as eluents. EGC and 4′-*O*-methyl EGC were detected in positive electrospray mode with optimized tune parameters of 307.0 m/z for EGC and 321.0 m/z for 4′*O*-methyl EGC. Data acquisition was carried out using MassLynx v 4.1 software.

2.7 Statistical analysis

Taking a 5% significance level, the study was calculated to have 80% power to detect an Endo-PAT RHI response of 0.2 between treatments, with a within-subject variance of 0.13 previously derived from an internal study (data unpublished). Upon study completion, a blind review of the data was performed using a randomized pseudo-treatment allocation. TNF-α was subsequently log transformed to improve the model fit. The statistical analyses for the primary endpoints were adjusted for the fixed effect covariates of both subject baseline and subject-corrected period baseline (the difference between the baseline on each visit and the subject mean baseline for the two study days) [21], period of treatment, COMT genotype, age in completed years, and BMI on study day. Subject number was included as a random effect. The modifying effect of the COMT genotype on the treatment effect (the genotype × treatment interaction) was tested independently of the main treatment effect. Values of endothelin-1, total nitrite, VCAM, and TNF-α taken at 105, 165, and 225 min were compared to values taken at 0 min using repeated measures time-dependent analysis. The fixed effect covariates of subject baseline, subject-corrected period baseline, BMI and age on study day, COMT genotype, and treatment were included as covariate × time interactions. The potential modifying effect of COMT with treatment was investigated using the three-way interaction of these two factors with time. Baseline data are presented as mean and SD values. Change from baseline data in tables and figures is presented as estimated mean and SE values. The statistical package SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) was used.

3 Results

3.1 Baseline characteristics

Of the 50 subjects who completed the study (25 of each homozygous COMT genotype), three were subsequently removed from the per protocol analysis dataset before treatment unblinding, due to protocol nonadherence. Tables 2 and 3 show the baseline characteristics of the participants according to COMT genotype. Groups were well matched with respect to age and BMI. A difference in diastolic BP between genotype groups was evident with the AA group presenting with a higher mean baseline BP than the GG group ($p = 0.014$).

3.2 Vascular function

A significant genotype × treatment interaction in the vascular function measure DVP RI was observed ($p = 0.014$)

Table 2. Baseline parameters of the study participants in the COMT (rs4680) genotype groups

	COMT AA ($n = 25$)		COMT GG ($n = 22$)		
	Mean	SD	Mean	SD	p-value[a)]
Subject characteristics					
Age (y)	37.9	18.2	34.2	17.2	ns
BMI (kg/m^2)	24.6	2.9	23.7	2.8	ns
Systolic BP (mmHg)	121	10	119	11	ns
Diastolic BP (mmHg)	69	6	65	8	0.014
Heart rate (bpm)	61.1	11.4	62.2	9.0	ns
Biochemical measures					
Total cholesterol (mmol/L)	4.64	0.90	4.18	1.0	ns
Triglycerides (mmol/L)	1.18	0.55	1.04	0.51	ns
NEFA (μmol/L)	456	143	430	195	ns
Vascular function					
Endo-PAT RHI	1.89	0.64	1.95	0.60	ns
Endo-PAT AI	−5.3	15.2	−7.1	13.0	ns
DVP SI (m/s)	7.0	1.8	6.5	1.5	ns
DVP RI (%)	62.0	16.4	57.8	14.6	ns

a) Differences between genotype groups assessed by general linear regression adjusted for age, BMI, and visit, ns = $p > 0.05$. Endo-PAT = peripheral arterial tonometry, RHI = reactive hyperemia index, AI = augmentation index, DVP = digital volume pulse, SI = stiffness index, RI = reflection index, BP = blood pressure.

(Fig. 2). Although a significant treatment effect was not found for the group as a whole, a treatment effect was found in the AA genotype group with the ingestion of the green tea extract showing a more beneficial change in RI from baseline compared to placebo ($p = 0.029$). The GG genotype demonstrated no evidence for a treatment effect in RI. A possible treatment effect in diastolic BP was also found, with the green tea extract showing a greater increase in diastolic BP (difference from (Δ) baseline (and SE) 1.04 (0.64) and 2.66 (0.75) mmHg for placebo and green tea, respectively, ($p = 0.07$)). A similar tendency was found with systolic BP (Δ baseline 0.70 (1.15) mmHg and 2.17 (1.18) mmHg for placebo and green tea, respectively, ($p = 0.17$)). No treatment effects or genotype × treatment interactions were found in the Endo-PAT, DVP SI, total nitrite, endothelin-1, TNF-α, and VCAM measures (Fig. 2 and Table 3).

3.3 HPLC plasma and urine analysis

Evidence for a difference in urinary 4′-*O*-methyl EGC between genotype groups was found during the 5.5 h collection period, with the AA genotype demonstrating a lower concentration compared to the GG genotype ($p = 0.049$). No evidence was found for a difference between genotypes for urinary 4′-*O*-methyl EGC for the remaining 18.5 h collection or for total EGC during the 5.5 h and 18.5 h collections (Table 4). No evidence for a difference in plasma green tea catechin concentrations was observed between genotype groups at the time points measured (Fig. 3).

For the group as a whole, total ECG, 4′-*O*-methyl EGCG, 4″-*O*-methyl EGCG, and 4′4″-*O*-methyl EGCG plasma concentrations (at 105 min) were found to be negatively correlated with DVP SI at 90 min ($r = -0.55, -0.56, -0.54, -0.58$ ($p = 0.035$, 0.030, 0.037, and 0.023), respectively). No significant associations were found with any of the other green tea catechins, however, there was a tendency toward a negative association with total EGCG and DVP SI ($r = -0.46$; $p = 0.085$).

4 Discussion

There are inconsistencies in the literature regarding the acute impact of green tea consumption on vascular reactivity and tone. However, a recent meta-analysis, including both green and black tea, has suggested a substantial improvement in endothelial function with moderate consumption [22]. In the current study, for the group as a whole, no significant treatment effect on vascular function was observed, which is in agreement with a number of studies [9, 14] but not others [3, 6, 7, 23]. Interestingly, a COMT genotype × treatment interaction emerged for a number of the outcomes. As far as the authors are aware, the current study is the first placebo-controlled investigation of the effects of green tea on vascular function in different COMT genotype groups. A genotype × treatment interaction was evident in the DVP RI, a measure of small vessel tone. In the AA genotype group, ingestion of the green tea extract was found to attenuate the increase in DVP RI following placebo treatment. However, as more than one measure of vascular function has been assessed in the current study, and genotype-mediated differences in response were only evident for one measure, the clinical significance of the observed interaction is likely to be modest and needs to be considered with caution. No differences between genotype groups were evident in the Endo-PAT measurements, although a similar trend was found with the AI

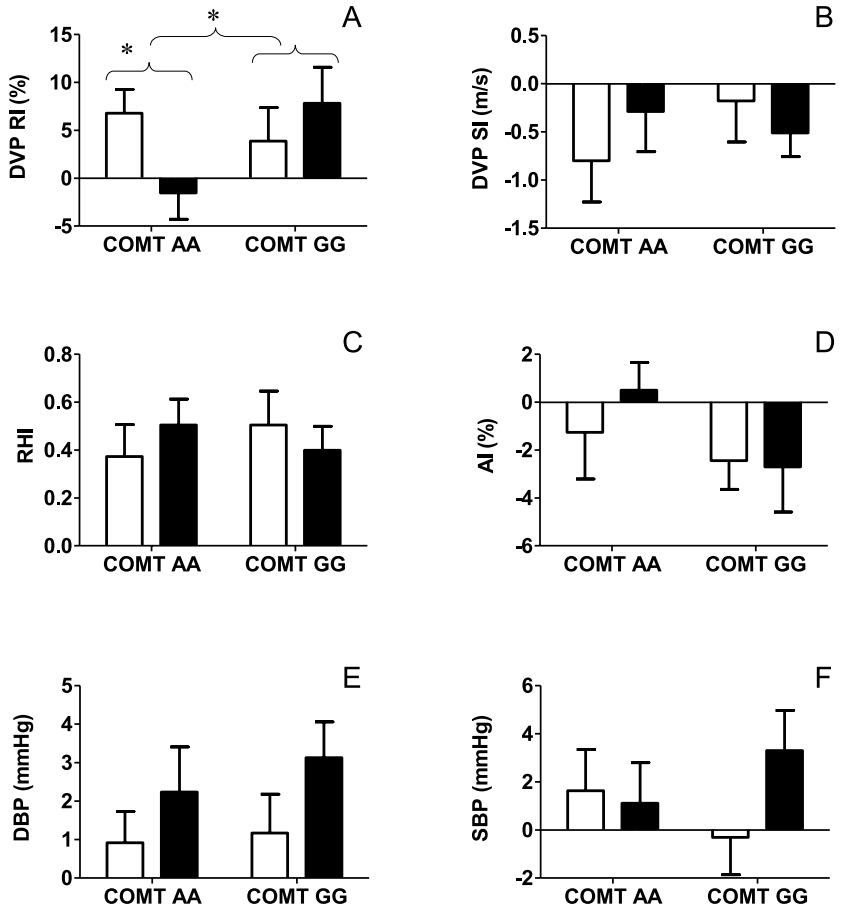

Figure 2. Absolute mean change at 90 min from baseline (and SEM) for (A) digital volume pulse (DVP) reflection index (RI), (B) DVP stiffness index (SI), (C) Endo-PAT reactive hyperemia index (RHI), (D) Endo-PAT augmentation index (AI), (E) diastolic blood pressure (DBP), and (F) systolic blood pressure (SBP) for COMT AA and COMT GG genotype groups after placebo (white bars) and green tea extract (black bars). A COMT genotype × treatment interaction was demonstrated for DVP RI ($p = 0.014$). Within the COMT AA genotype group, a treatment effect was also evident ($p = 0.029$).

measurement as with the comparable RI measurement. The advantages of the Endo-PAT and DVP in comparison to other more commonly used methods such as flow-mediated dilation and forearm plethysmography include user-independence via automated data processing and analysis, ease of use, noninvasive methodology, and reproducibility. Unlike ultrasound flow-mediated vasodilation methodology, the Endo-PAT and DVP measure vascular function across several different types of vascular beds.

The COMT AA group has been proposed to methylate the green tea catechins at a slower rate. Indeed, a difference in mono-methylated EGC between genotype groups was evident in the urine samples collected during the first 5.5 h after green tea catechin consumption, with the COMT AA group demonstrating a lower concentration compared to the COMT GG group. Thus, the current finding demonstrating a greater beneficial effect of the green tea on small vessel tone in the AA genotype group could be speculated to be due to differing vasoactivities of the parent and methylated green tea catechin forms. A number of in vitro and human studies indicate a decreased activity of methylated compounds compared to the parent compounds [24–28]. In vitro investigations have indicated enhancement in the production of the potent vasodilator, nitric oxide, with parent green tea catechins. Potential mechanisms include the activation of the phosphatidylinositol-3-OH-kinase and Akt-dependent cellular pathway [29]. Alternative mechanisms include the reversal of noradrenaline and KCl inhibition of cAMP and cGMP production [30], the stimulation of the potent vasodilator, prostacyclin [31], and the inhibition of BP mediator, angiotensin-converting enzyme [32]. The effects of methylated green tea catechins remain under-investigated. However, in vitro studies have shown mono-O-methylated flavanols to protect nitric oxide from peroxynitrate formation by inhibiting NADPH oxidase and the production of superoxide free radicals [33].

No evidence for a difference in plasma green tea catechin concentrations between genotype groups was evident in the current study. However, a potential limitation of this study was the limited number of time points used to measure the levels of circulating catechins (0, 105, 165, 225 min). Thus, a more detailed pharmacokinetic profile in each COMT genotype group may have highlighted potential differences. Alternatively, differences in catecholamine metabolism may also have contributed to the differential genotypic effect on DVP RI since green tea catechins are competitive and

Table 3. Mean subject baseline and change from baseline for vascular function and inflammatory biochemical markers, according to COMT (rs4680) genotype

	Baseline 0 min	Genotype baseline differences (p)	Placebo change from baseline			Green tea change from baseline			Treatment effect (p)	Treatment × genotype interaction (p =)
			105 min	165 min	225 min	105 min	165 min	225 min		
VCAM-1 (ng/mL)										
AA	751 (187)	ns	1 (16)	−9 (18)	−4 (23)	−27 (18)	−51 (23)	−23 (18)	ns	ns
GG	780 (221)		−25 (22)	−72 (22)	−47 (31)	0.7 (18)	−2 (15)	1 (19)		
Endothelin-1 (pg/mL)										
AA	0.90 (0.26)	ns	0.2 (0.04)	−0.03 (0.03)	0.01 (0.04)	0.2 (0.05)	−0.03 (0.06)	0.02(0.05)	ns	ns
GG	0.86 (0.34)		0.2 (0.07)	−0.07 (0.05)	−0.01 (0.05)	0.3 (0.05)	0.05 (0.06)	0.1 (0.07)		
Total nitrite (μmol/L)										
AA	18.1 (2.9)	ns	−0.5 (0.4)	−0.3 (0.4)	−1.4 (0.4)	−0.03 (0.4)	−0.3 (0.4)	−0.2 (0.5)	ns	ns
GG	18.2 (3.8)		−1.0 (0.6)	−2.0 (0.8)	−1.3 (0.7)	−0.4 (0.6)	−0.7 (0.6)	−0.6 (0.7)		
TNF-α (pg/mL)										
AA	1.1 (0.5)	ns	−0.06 (0.05)	−0.08 (0.05)	−0.13 (0.06)	−0.05 (0.02)	−0.10 (0.05)	−0.09 (0.04)	ns	ns
GG	1.2 (0.8)		−0.03 (0.03)	−0.02 (0.06)	−0.03 (0.07)	−0.04 (0.03)	−0.07 (0.05)	−0.12 (0.05)		

Values represent mean (SEM). Differences between treatments and treatment × genotype interactions assessed by mixed model analysis adjusted for age, BMI, visit, subject, subject baseline, and subject-corrected period baseline. Differences between genotype baselines assessed by general linear regression adjusted for age, BMI, and visit. ns = $p > 0.05$.
VCAM-1 = vascular cellular adhesive molecule-1, TNF-α = tumor necrosis factor alpha.

noncompetitive inhibitors of COMT [34,35]. Therefore, it may be possible that green tea catechins inhibited catecholamine metabolism to a greater extent in the low-activity COMT genotype group, therefore maintaining catecholamines in their active form. Adrenaline (a key catecholamine) is a potent modulator of vascular tone and differences in adrenaline metabolism between genotype groups may help to explain the observed improvement in the RI in the COMT AA group, although this requires further investigation.

In the current study for the group as a whole, plasma total (conjugated and nonconjugated) ECG, 4′-*O*-methyl EGCG, 4″-*O*-methyl EGCG and 4′4″-*O*-methyl EGCG were found to be negatively correlated with DVP SI, a measure of large arterial stiffness. Interestingly, our previous work suggested a reduction in DVP SI after green tea consumption in the proposed faster methylating GG genotype group only [14]. This indicates that participants who methylate flavonoids at a faster rate have a greater improvement in large artery stiffness. Indeed as aforementioned, methylated EC has been shown to inhibit NADPH oxidase in vitro potentially elevating nitric oxide bioavailability, an effect not found with the parent compound [33]. Combining the aforementioned results, it is possible the genotype groups derive differential localized benefits from green tea, resulting in improvements in either large or small vessel function. Thus, methylated catechins may instigate a beneficial effect in the large vasculature while the unmethylated parent forms may have greatest activity in the small vasculature. The possible opposing activities of methylated and parent compounds on the different vasculatures may help to explain the nil effects found with the Endo-PAT RHI measure that encompasses vascular function of the entire limb. Unlike our previous findings, in the current study, we did not observe any significant differences in DVP SI between COMT genotype groups. It is possible that the population group of overweight sedentary males and females, the timings of vascular measurements, and the high carbohydrate breakfast 1 h after the GTE in the pilot study may have exaggerated some of the differences between the genotype groups. It is also worth noting our previous study was a pilot study designed for power-calculation purposes and was not placebo-controlled.

As found in our previous study [14], a difference in the baseline diastolic BP between genotype groups was evident, with the AA (low activity) genotype group having a higher diastolic BP. It is possible this difference is also related to COMT methylation of endogenous catecholamines. Indeed a study investigating COMT activity in rats found a positive association between COMT activity in the cerebral cortex and BP regulation [36]. An interesting finding for the entire study population was the greater increase in diastolic BP 90 min after the green tea extract compared to placebo. A number of studies have also shown increases in BP after acute green tea [9,23,37], while others have found no demonstrable effect [3,7,38]. As a variety of population and chronic studies have shown beneficial effects of green tea on BP, acute green tea consumption may be accountable for a rapid-onset,

Table 4. Urinary total EGC and 4′-*O*-methyl EGC after green tea ingestion according to COMT (rs4680) genotype

	COMT AA ($n = 25$)		COMT GG ($n = 22$)		
	Mean	SEM	Mean	SEM	*p*-value
5.5 h					
EGC (ng/mL)	4428	607	5865	792	ns
4′-*O*-methyl EGC (ng/mL)	1271	192	1975	265	0.049
18.5 h					
EGC (ng/mL)	3854	704	3032	594	ns
4′-*O*-methyl EGC (ng/mL)	1542	205	1534	223	ns
24 h (5.5 h + 18.5 h)					
EGC (ng/mL)	8105	884	8509	1039	ns
4′-*O*-methyl EGC (ng/mL)	2763	268	3357	363	ns

Differences between genotype groups assessed by mixed model analysis adjusted for age, BMI, and visit. ns = $p > 0.05$. EGC = epigallocatechin.

short-lived increase in BP [39–42] as a result of green tea catechin inhibition of catecholamine methylation. Alternatively, the increase in BP may indicate the presence of pressor substances, other than caffeine, which have not yet been identified in green tea.

In conclusion, in the AA genotype group, ingestion of the green tea extract led to an attenuation of the decrease in small arterial tone observed with the placebo while no differences were found in the GG genotype group. It is tentatively speculated that an inhibition of COMT-mediated endogenous

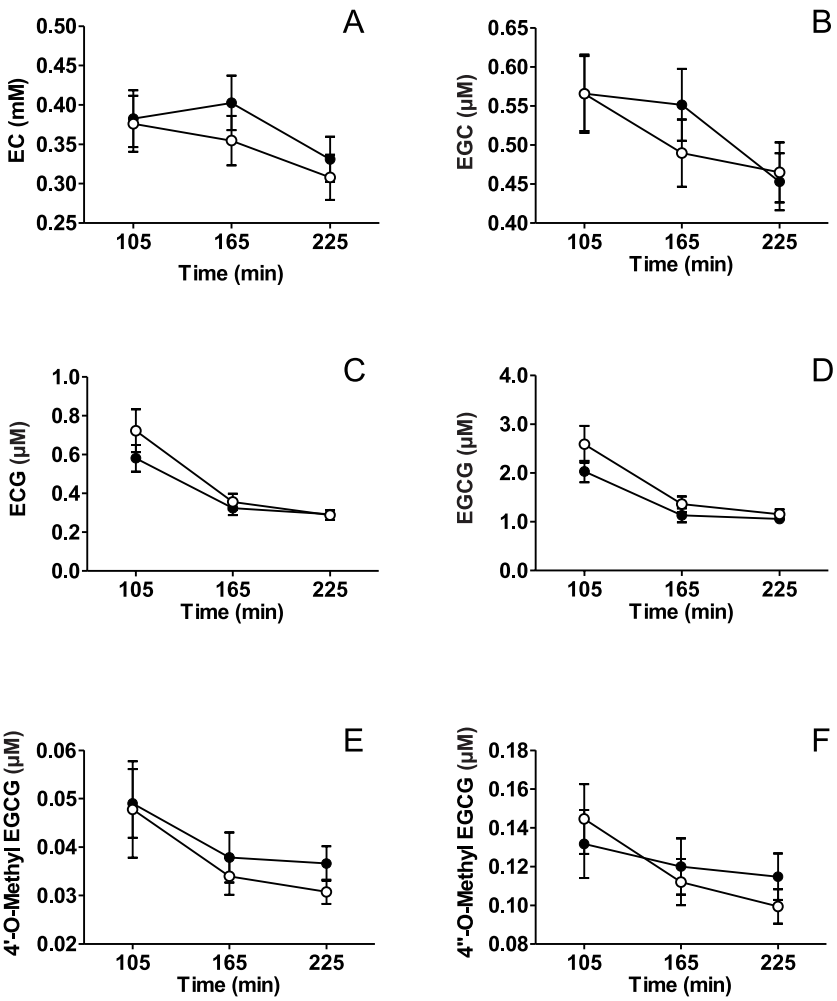

Figure 3. Mean plasma green tea catechin concentrations (and SEM) (A) epicatechin (EC), (B) epigallocatechin (EGC), (C) epicatechin gallate (ECG), (D) epigallocatechin gallate (EGCG), (E) 4′-*O*-methyl EGCG, and (F) 4′′-*O*-methyl EGCG for COMT AA (●) and COMT GG (○) genotype groups after consumption of green tea extract.

catecholamine methylation by the green tea catechins or differing vasoactivities of the parent and methylated green tea catechin forms in the genotype groups may have contributed to the findings. Additional research is required in order to gain a full understanding of the effects of green tea catechins on micro- and macrovascular function and to elucidate the impact of the COMT genotype on the response. Such studies should include a simultaneous investigation of the impact of catechins and COMT genotype on catecholamine metabolism in order to provide a more comprehensive mechanistic insight into the findings.

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