Short Communication

The impact of the catechol-*O*-methyltransferase genotype on the acute responsiveness of vascular reactivity to a green tea extract

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

[1]*Department of Food and Nutritional Sciences, University of Reading, Whiteknights, PO Box 226, Reading, Berkshire RG6 6AP, UK*
[2]*Unilever Discover, Colworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, UK*

Abstract

The beneficial effects of green tea catechins, such as the proposed improvement in endothelial function, may be influenced by phase II metabolism during and after absorption. The methylation enzyme, catechol-*O*-methyltransferase (COMT), has a missense mutation rs4680 (G to A), proposed to result in a 40 % reduction in enzyme activity. In the present pilot study, twenty subjects (ten of each homozygous COMT genotype) were recruited. Green tea extract capsules (836 mg green tea catechins) were given in a fasted state, and a high-carbohydrate breakfast was given after 60 min. Blood samples and vascular function measurements were taken at regular intervals. The change in digital volume pulse stiffness index (SI) from baseline was shown to be different between genotype groups at 120 and 240 min, with a lower SI in the GG individuals ($P \leq 0.044$). The change in blood pressure from baseline also differed between genotype groups, with a greater increase in systolic ($P = 0.023$) and diastolic ($P = 0.034$) blood pressure at 120 min in the GG group. The AA group was shown to have a greater increase in insulin concentrations at 120 min ($P = 0.019$) and 180 min ($P = 0.008$) compared with baseline, despite similar glucose profiles. No genotypic differences were found in vascular reactivity measured using laser Doppler iontophoresis, total nitrite, lipids, plasma total antioxidant capacity or inflammatory markers after ingestion of the green tea extract. In conclusion, SI and insulin response to the glucose load differed between the COMT genotype groups, and this may be suggestive of a green tea extract and genotype interaction.

Key words: Blood pressure: Endothelial function: Flavan-3-ol: Green tea: Insulin

Green tea contains high levels of polyphenols (approximately 70 % of dry weight), which are mainly green tea catechins belonging to the flavan-3-ol family of polyphenols. Of highest abundance is epigallocatechin gallate, followed by epigallocatechin, epicatechin and epicatechin gallate. Epidemiological studies have repeatedly observed a beneficial effect of green tea consumption on the risk of CVD[1–5]. In addition, acute and chronic human intervention studies have demonstrated benefits of green tea and its constituent catechins on vascular function and reactivity[6–9]. Proposed molecular mechanisms for improved vascular reactivity include increased endothelial NO synthase activity[10], reduced cytokine[11,12] and pro-oxidant production[13] and NADPH oxidase inhibition[14].

Flavan-3-ol *O*-methylation is a major pathway of flavonoid metabolism, which mainly occurs in the intestinal tract, liver and kidney. This phase II metabolism is thought to reduce the biological activity of flavan-3-ol polyphenols. Indeed, variability in flavonoid *O*-methylation has been shown to influence the effect of dietary flavonoids on endothelial function[15]. *O*-Methylation of flavonoids is catalysed by the enzyme catechol-*O*-methyltransferase (COMT). A common genetic missense mutation (G to A base change) results in a valine-to-methionine amino acid substitution at position 108 in the soluble protein and at position 158 in the membrane-bound COMT. This has been proposed to alter the function of the COMT enzyme by reducing its thermostability and resulting in a

proposed 40% reduction in enzyme activity[16–18]. In addition to flavonoids, COMT also metabolises a number of other exogenous and endogenous compounds including catecholamines, oestradiols, neurotransmitters and medicinal drugs. Although the impact of the COMT genotype on the metabolic impact of a number of these compounds has been described[19–21], the impact of the COMT Val[108/158] Met polymorphism on catechin metabolism and its physiological impact on vascular function are unknown. The primary aim of the present study was to assess the effect of the COMT genotype on vascular function after acute green tea consumption, with an assessment of the COMT genotype on metabolic responses as a secondary aim.

Methods

Study population

In the present study, twenty healthy, non-smoking, overweight (BMI $25–32 kg/m^2$) subjects (fourteen males and six females), ten of each homozygous COMT genotype (AA and GG), were recruited at the Hugh Sinclair Unit of Human Nutrition, University of Reading, UK, from Reading and the surrounding areas. Acceptance onto the study was dependent on having blood pressure and biochemical measurements in the normal range (blood pressure < 160/100 mmHg, total cholesterol < 8·0 mmol/l, alanine transaminase < 45 U/l, γ-glutamyl transferase < 55 U/l, total bilirubin < 20·5 μmol/l and Hb > 125 g/l), homozygous COMT genotype and age between 18 and 70 years. In total, fifty-five subjects were screened, of which twenty-five met the suitable criteria. Before completing the study visit, five subjects subsequently withdrew. The study procedure followed the Declaration of Helsinki and was approved by the University of Reading Research and Ethics Committee. Subjects gave written informed consent before commencing the study.

DNA isolation and catechol-O-methyltransferase genotyping

DNA was extracted from the leucocyte buffy coat layer using the QIAmp DNA Mini Kit (Qiagen Limited, Crawley, UK). Allelic discrimination of the COMT rs4680 gene variant was conducted using a TaqMan Drug Metabolism Genotyping Assay (Applied Biosystems, Warrington, UK).

Acute intervention study design

For 24 h before the study visit, subjects refrained from intensive exercise and consumed a low-catechin diet by avoiding fruit juices, fruit smoothies, alcoholic beverages, cocoa, tea, coffee and specific high catechol-containing fruits (apples, grapes and berries) and vegetables (onions). After a 12 h overnight fast, baseline vascular function and clinic blood pressure measurements (in the supine position) were taken followed by insertion of an indwelling cannula into the forearm antecubital vein. Subjects were given two Sunphenon 90LB decaffeinated green tea extract (Taiyo International, Mie, Japan) capsules, providing a total of 836 mg green tea catechins with 250 ml water. The main green tea polyphenols, epigallocatechin gallate, epigallocatechin, epicatechin and epicatechin gallate, were present in the following amounts in the dose administered: 448, 178, 96 and 66 mg, respectively. Blood samples were taken at baseline (0 min) and 120, 180, 240, 360 and 480 min after the consumption of capsules. Samples were centrifuged at 1700 g for 10 min at 4°C, and plasma aliquots were stored at − 80°C until further analysis. Vascular measurements were repeated at 120, 240, 360 and 480 min. A high-carbohydrate breakfast meal (2372·3 kJ (567 kcal), 107 g carbohydrate, 18·1 g protein and 7·4 g fat) was given 60 min after capsule administration in order to avoid any gastrointestinal complaints reported to occur when a high dose of catechin supplementation is consumed in a fasted state[22,23]. A second standardised low-flavanoid meal (2765·6 kJ (661 kcal), 74·4 g carbohydrate, 15·8 g protein and 33·3 g fat) was given at 240 min.

Vascular function measurements

Vascular function was assessed by laser Doppler iontophoresis (LDI) and digital volume pulse (DVP). All measurements were carried out in a temperature-controlled environment. The LDI (Moor Instruments Limited, Dorset, UK) methodology has been described previously by Armah et al.[24]. Briefly, using laser technology, the response of the dermal microcirculation to the iontophoretic application of the endothelium-dependent and -independent vasoactive drugs, 1% acetylcholine and 1% sodium nitroprusside (Sigma, Dorset, UK), is non-invasively observed. DVP (CareFusion, San Diego, CA, USA) measures the transmission of IR light through the finger tip which is directly proportional to the amount of blood in the finger pulp. A systolic and diastolic pulse waveform is produced from which indices are calculated. The stiffness index is a measure of large arterial stiffness and relates to the timing of the diastolic component in comparison with the systolic component. The reflection index is a measure of the tone of the small arteries and is the height of the diastolic component relative to the systolic component.

Plasma analysis

Quantification of plasma TAG, total cholesterol, NEFA, total antioxidant capacity and glucose was conducted using the ILAB 600 clinical chemistry analyser (Instrumentation Laboratory, Warrington, UK) using enzyme-based colorimetric kits supplied by Instrumentation Laboratory (TAG, total cholesterol and glucose), Alpha Laboratories (NEFA; Eastleigh, Hampshire, UK) and Medicon (total antioxidant capacity; Athens, Greece). ELISA were used for the measurement

of plasma insulin (Dako, Denmark) and soluble vascular cellular adhesive molecule 1 (R&D systems, Minneapolis, MN, USA). Plasma total nitrite was measured using a Nitric Oxide Quantitation Kit (Active Motif, Carlsbad, CA, USA).

Statistical analysis

Baseline data were analysed using a simple unpaired *t* test and by general linear regression with adjustments for age, BMI and sex. Baseline data are presented as means and standard deviations. The experimental data were modelled using a general linear mixed model in Statistical Analysis Systems, with a change from baseline as the response variable. Fixed effects were included as follows: continuous covariates for baseline value, age and BMI, and categorical covariates for sex, time, COMT genotype and the interaction between time and genotype. A random effect was also included to account for increased correlation of results within subjects. Slice effects for each time point gave the evidence for the difference between the genotypes. The null hypothesis was that there is no difference between the genotypes. Data are presented as means with their standard errors and means with 95 % CI. The Statistical Analysis Systems statistical package version 9.2 (SAS Institute, Inc., Cary, NC, USA) was used.

Results

Population characteristics at baseline

There were no detectable differences between the genotype groups with respect to mean age (AA 57·8 (SD 12) and GG 51·4 (SD 16) years; $P = 0.33$), BMI (AA 27·0 (SD 2·3) and GG 27·7 (SD 1·7) kg/m^2; $P = 0.48$) or sex ratio (male:female 7:3). A baseline difference was detected between the two genotype groups for LDI$_{Ach}$ (Ach, acetylcholine) area under the curve (AA 1547·4 (SD 723) and GG 1023·7 (SD 413) arbitrary units; $P = 0.050$). Baseline diastolic blood pressure was found to be significantly different between the genotypes (AA 79 (SD 9) and GG 72 (SD 4) mmHg; $P = 0.046$). However, when the baseline cofactors BMI, age and sex were included as covariates, the difference between the genotype subgroups was no longer significant ($P = 0.11$). No significant differences were found in baseline systolic blood pressure (AA 133·1 (SD 15·3) and GG 125·6 (SD 13·9) mmHg) with exclusion ($P = 0.27$) and inclusion ($P = 0.36$) of baseline covariates. No differences were found in baseline biochemical measurements between the genotype groups (data not shown).

Vascular and biochemical profiles post-intervention

A significant difference was found with a change from baseline for DVP stiffness index measurements at 120 min (AA −0·2 (95 % CI −1·7, 1·3), GG −2·1 (95 % CI −3·6, −0·7) m/s; $P = 0.044$) and 240 min (AA 0·6 (95 % CI

−0·7, 1·9) GG −1·5 (95 % CI −2·9, −0·1) m/s; $P = 0.026$) (Fig. 1). At the 120 min time point, the change in blood pressure was also different between the two groups, with systolic blood pressure reducing by 6·2 (95 % CI −9·6, −2·9) mmHg in the AA group but increasing by 4·3 (95 % CI −3·4, 12·1) mmHg in the GG group ($P = 0.023$). Diastolic blood pressure fell by 7·7 (95 % CI −11·3, −4·1) mmHg in the AA group and by 0·1 (95 % CI −2·4, 2·2) mmHg in the GG group ($P = 0.034$).

The most notable metabolic effect was a genotype difference in insulin response after the meal, with the GG group displaying 50 and 72 % higher plasma insulin changes from baseline values at 120 ($P = 0.019$) and 180 ($P = 0.008$) min, respectively, after the consumption of green tea extract capsules, compared with the AA group (Fig. 1) with no evidential difference in plasma glucose response. No evidence was found for any differences between the genotypes for LDI, reflection index digital volume pulse, soluble vascular cellular adhesive molecule 1, total antioxidant capacity, total nitrite, TAG and NEFA responses (Table 1).

Discussion

Green tea and its associated polyphenol content have been demonstrated to acutely improve endothelial function[6–9]. The present study investigated for the first time the effect of the COMT genotype on endothelial function and a number of its physiological determinants, following acute ingestion of the green tea extract. The proposed low-activity AA genotype is prevalent in approximately 25 % of Caucasians, a similar estimate to the prevalence of the high-activity GG genotype.

No differences were found in microvessel endothelial function as assessed by the LDI methodology. However, a difference was found between the genotypes in the DVP arterial stiffness index measurement. The reduction from baseline was greater in the GG genotype group at the 120 and 240 min time points, where the plasma green tea catechins are at their highest level (peak in the plasma approximately 1·5 h[25–27]). This finding is contradictory to our initial predictions, as COMT GG is the high-enzyme activity genotype which if previous propositions are correct[16–18] would result in a greater rate of metabolism of green tea catechins leading to higher levels of the less metabolically active methylated catechins. However, this is based on the assumption that metabolised catechins are less biologically active with respect to the vascular response, and this is not necessarily the case. Indeed, data from cocoa studies indicate that *O*-methylated metabolites may be more vasoactive than their parent compounds. Vascular function benefits of cocoa products have been postulated to be due to the NO-preserving activity of (−)-epicatechin, and this effect, which is the result of the inhibition of endothelial NADPH, requires COMT-mediated conversion to *O*-methyl esters[14,28].
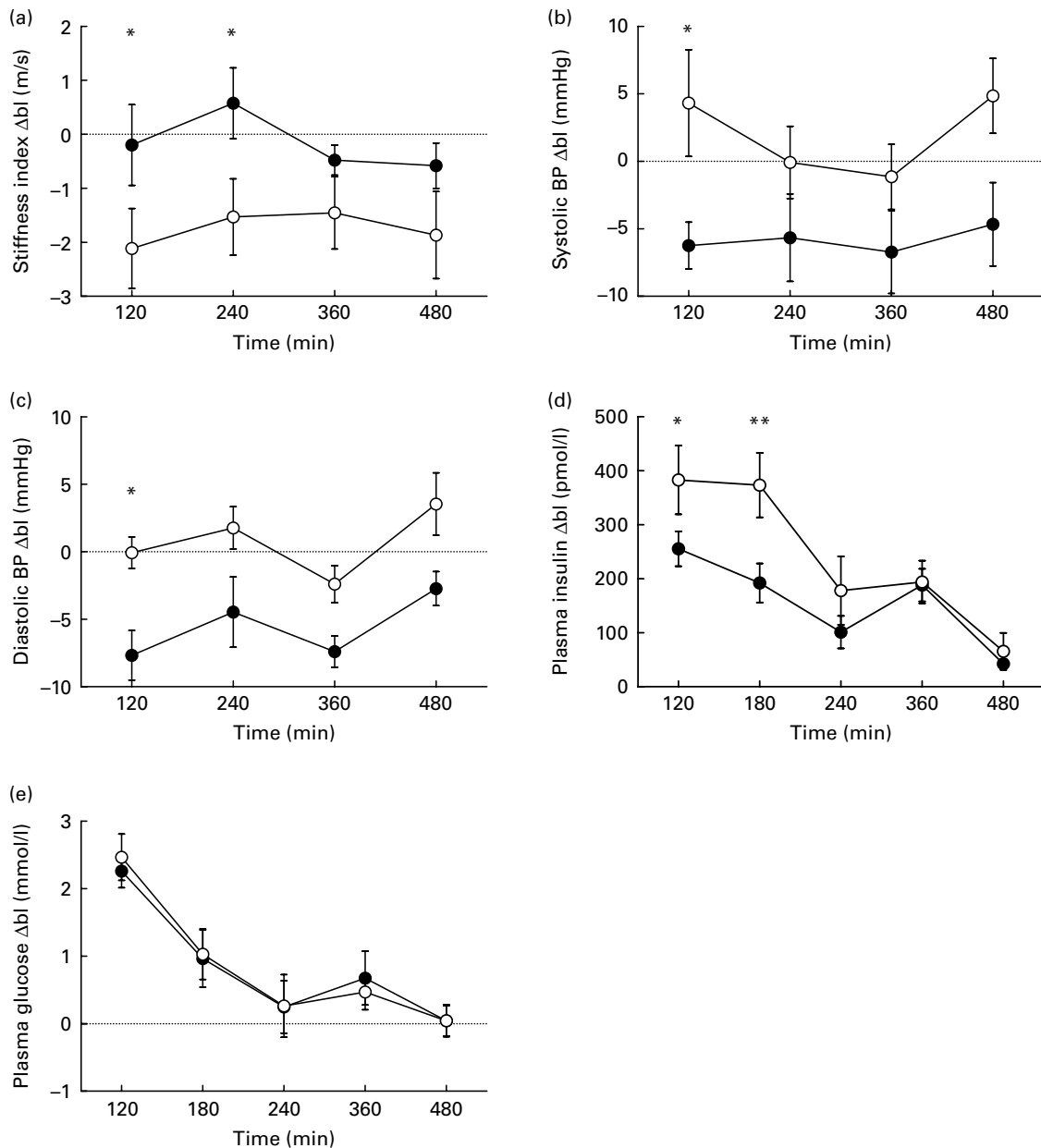
Fig. 1. Mean change from baseline (bl) (..., $t = 0$) for (a) stiffness index, (b) systolic blood pressure (BP), (c) diastolic BP, (d) insulin and (e) glucose for the catechol-O-methyltransferase (COMT) AA (●) and COMT GG (○) genotype groups after the ingestion of the green tea extract. Values are means, with standard errors represented by vertical bars. Significant differences between the genotype groups: * $P < 0.05$, ** $P < 0.01$. Meals were given at 60 and 240 min. Mean baseline values for the AA and GG genotype groups: stiffness index 7·9 (SD 1·7) and 8·2 (SD 2·5) m/s; systolic BP 133 (SD 15) and 126 (SD 14) mmHg; diastolic BP 79 (SD 9) and 72 (SD 4) mmHg; insulin 38·3 (SD 29·2) and 39·6 (SD 23·9) pmol/l; glucose 5·4 (SD 0·3) and 5·3 (SD 0·4) mmol/l.

Blood pressure measurements were found to be different between the genotype groups after the ingestion of the green tea extract, with a significant reduction in systolic and diastolic measurements at the 120 min time point in the AA genotype group relative to GG homozygotes. This is as initially predicted as the AA genotype group are thought to methylate the catechins at a slower rate and therefore have a greater beneficial effect. Surprisingly, this opposes the stiffness index findings, a measurement which has been shown to be modestly correlated with blood pressure[29]. A genotypic difference for baseline

blood pressure was also evident, specifically for diastolic blood pressure, with the AA genotype group demonstrating a higher baseline diastolic blood pressure. Similar differences in baseline blood pressure between COMT homozygous genotypes have also been shown elsewhere[30,31]. The observed reduction in blood pressure in the AA genotype group could possibly be a consequence of the genotypic differences found in insulin sensitivity when a meal was given at 60 min. Alternatively, as the AA genotype group has been proposed to have decreased catecholamine metabolism and increased sensitivity to stress and

Table 1. Change from baseline ($t = 0$) for metabolic and vascular function measurements after the ingestion of the green tea extract for the catechol-*O*-methyltransferase AA and GG genotype groups*

(Mean values and 95 % confidence intervals)

Measure	Time	AA group		GG group		AA−GG P†
		Mean	95 % CI	Mean	95 % CI	
TAG (mmol/l)	120	−0·02	−0·08, 0·05	0·11	−0·01, 0·2	0·60
	180	0·05	0·002, 0·1	0·20	0·003, 0·4	0·59
	240	0·09	−0·009, 0·2	0·22	−0·01, 0·5	0·49
	360	0·67	0·5, 0·9	0·90	0·5, 1·3	0·28
	480	1·10	0·8, 1·4	1·30	0·8, 1·8	0·42
Total antioxidant capacity (mmol/l)	120	0·02	−0·003, 0·04	−0·03	−0·2, 0·1	0·48
	180	0·02	−0·07, 0·1	0·02	−0·04, 0·08	0·56
	240	−0·02	−0·05, 0·004	−0·02	−0·1, 0·06	0·73
	360	−0·01	−0·03, 0·006	−0·03	−0·1, 0·06	0·67
	480	−0·01	−0·04, 0·02	−0·03	−0·1, 0·08	0·84
NEFA (μmol/l)	120	−64	−136, 9	−166	−313, −19	0·13
	180	−155	−305, −5	−342	−501, −183	0·56
	240	−233	−290, −176	−407	−541, −274	0·88
	360	−224	−280, −167	−323	−496, −151	0·11
	480	−115	−216, −13	−261	−426, −97	0·85
Total nitrite (μmol/l)	120	−1·42	−2·3, −0·5	−0·52	−2·4, 1·3	0·32
	180	−2·26	−3·1, −1·5	−2·74	−4·2, −1·3	0·83
	240	−2·51	−3·2, −1·8	−2·59	−4·1, −1·1	0·93
	360	−2·30	−3·2, −1·4	−1·83	−3·6, −0·1	0·49
	480	−1·60	−2·9, −0·3	−0·29	−2·5, 1·9	0·24
VCAM-1 (ng/ml)	120	−31·9	−64·5, 0·7	−14·2	−52·1, 23·7	0·90
	180	−25·4	−80·1, 29·3	−12·1	−60·3, 36·2	0·94
	240	1·6	−42·5, 45·6	20·4	−9·4, 50·1	0·86
	360	−29·6	−61·6, 1·8	−4·8	−66·5, 57·0	0·71
	480	20·4	−66·4, 25·6	30·9	−2·5, 64·3	0·28
LDI$_{Ach}$ AUC (AU)	120	−514	−879, −150	−167	−374, 41	0·84
	240	−321	−659, 18	−45	−364, 274	0·91
	360	−423	−762, −83	−72	−402, 258	0·89
	480	−214	−537, 109	56	−222, 336	0·90
LDI$_{Snp}$ AUC (AU)	120	−503	−850, −156	−64	−419, 291	0·35
	240	−294	−724, 137	95	−375, 564	0·36
	360	−222	−597, 153	153	−224, 530	0·64
	480	−104	−458, 249	342	−70, 755	0·26
RI$_{DVP}$ (%)	120	−2·5	−13·5, 8·5	1·3	−13·8, 16·3	0·97
	240	4·7	−4·3, 13·7	6·2	−8·3, 20·7	0·61
	360	−4·5	−11·9, 3·0	−0·1	−18·2, 18·0	0·93
	480	−1·9	−8·2, 4·4	2·3	−14·0, 18·7	0·59

VCAM-1, vascular cellular adhesive molecule-1; LDI, laser Doppler iontophoresis; Ach, acetylcholine; AUC, area under the curve; AU, arbitrary units; Snp, sodium nitroprusside; RI$_{DVP}$, reflection index digital volume pulse.

* Mean baseline values for the AA and GG genotype groups: TAG 1·2 (SD 0·1) and 1·1 (SD 0·2) mmol/l; NEFA 304 (SD 86) and 465 (SD 212) μmol/l; nitrite 9·9 (SD 2·2) and 12·1 (SD 4·2) μmol/l; VCAM-1 548 (SD 69) and 518 (SD 81) ng/ml); LDI$_{Ach}$ AUC 1547 (SD 723) and 1024 (SD 413) AU; LDI$_{Snp}$ AUC 1728 (SD 895) and 1344 (SD 748) AU; RI$_{DVP}$ 64·2 (SD 9) and 59·7 (SD 24) %.

† P values adjusted for age, BMI, sex and baseline.

anxiety[21,32,33], a reduction in blood pressure may be a result of a decrease in anxiety during progression of the study visit.

A novel finding was the difference in insulin response between the genotypes, with the homozygous COMT AA genotype showing greater postprandial insulin levels. A number of chronic animal studies[34−36] have shown improvements in insulin sensitivity and glucose tolerance with green tea supplementation. In humans, chronic studies with green tea have shown inconsistent results, with most showing no effect[37−39]. Acute human interventions into green tea and insulin response are lacking. However, a study by Venables *et al.*[40] found an improvement in insulin sensitivity and glucose tolerance after acute ingestion of the green tea extract compared with placebo. In addition, a study investigating the metabonomics of human urine collected between 2 and 4 h after the consumption of 300 ml green tea demonstrated an effect on metabolites involved in glucose metabolism including glucose, lactate and pyruvate[41]. Green tea aside, a recent study has shown the frequency of the COMT GG genotypes to be 11·6 % higher in insulin-resistant and type 2 diabetics compared with that of the COMT AA and GA genotype groups. The authors have suggested this finding to be a result of catecholamine involvement in energy and glucose homeostasis[42]. The potential ability of green tea to alter insulin sensitivity, potentially in a genotype-specific manner, is of wide public health relevance and worthy of further investigation.

A limitation of the present pilot study is the lack of a control arm. As a result, it is currently unknown whether the differences observed between the genotype groups are simply an effect of the genotype or due to the green tea

catechin ingestion. There is currently a dearth of studies examining the impact of genotype on the vascular response to green tea catechins. Therefore, no data were available on which to base a meaningful power calculation. As a result, a potential limitation may be the relatively small number of subjects who took part in the present exploratory study. A larger placebo-controlled study is warranted to substantiate these preliminary findings.

In conclusion, preliminary investigations have shown possible effects of the COMT genotype on acute vascular reactivity and insulin response to green tea catechins. This may be indicative of direct genotype differences or a genotype × treatment interaction. Randomised controlled trials investigating the impact of this common genotype on green tea catechin metabolism and the associated effects on vascular function are needed to confirm these initial findings.

Acknowledgements

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