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ORIGINAL CONTRIBUTION

A preliminary investigation of the impact of catechol-O-methyltransferase genotype on the absorption and metabolism of green tea catechins

Rosalind J. Miller · Kim G. Jackson · Tony Dadd · Beate Nicol · Joanne L. Dick · Andrew E. Mayes · A. Louise Brown · Anne M. Minihane

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Abstract
Purpose Green tea is thought to possess many beneficial effects on human health. However, the extent of green tea polyphenol biotransformation may affect its proposed therapeutic effects. Catechol-O-methyltransferase (COMT), the enzyme responsible for polyphenolic methylation, has a common polymorphism in the genetic code at position 158 reported to result in a 40% reduction in enzyme activity in in vitro studies. The current preliminary study was designed to investigate the impact of COMT genotype on green tea catechin absorption and metabolism in humans.
Methods Twenty participants (10 of each homozygous COMT genotype) were recruited, and plasma concentration profiles were produced for epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), epicatechin (EC) and 4′-O-methyl EGCG after 1.1 g of Sunphenon decaffeinated green tea extract (836 mg green tea catechins), with a meal given after 60 min.
Results For the entire group, EGCG, EGC, EC, ECG and 4′-O-methyl EGCG reached maximum concentrations of 1.09, 0.41, 0.33, 0.16 and 0.08 μM at 81.5, 98.5, 99.0, 85.5 and 96.5 min, respectively. Bimodal curves were observed for the non-gallated green tea catechins EGC and EC as opposed to single-peaked curves for the gallated green tea catechins EGCG and ECG. No significant parametric differences between COMT genotype groups were found.
Conclusions In conclusion, the COMT Val(158/108)Met does not appear to have a dramatic influence on EGCG absorption and elimination. However, further pharmacokinetic research is needed to substantiate these findings.

Keywords COMT · Flavan-3-ol · Green tea · Pharmacokinetics

R. J. Miller (✉) · K. G. Jackson · A. M. Minihane
Department of Food and Nutritional Sciences, University of Reading, PO Box 226, Whiteknights, Reading, Berkshire RG6 6AP, UK
e-mail: r.j.moore@reading.ac.uk

T. Dadd · B. Nicol · J. L. Dick · A. E. Mayes · A. L. Brown
Unilever Discover, Colworth Science Park, Sharnbrook MK44 1LQ, UK

Introduction

Numerous potential health benefits of green tea (*Camellia sinensis)* have been recently reported, including anti-carcinogenic [1–3], anti-diabetic [4–6], anti-atherosclerotic [7, 8], hypolipidaemic [9, 10], anti-inflammatory [11] and anti-obesogenic [12] effects. The biopotency of green tea has been attributed to its polyphenol content, with epigallocatechin gallate (EGCG) being the most abundant green tea polyphenol followed by epigallocatechin (EGC), epicatechin (EC) and epicatechin gallate (ECG). All compounds belong to the flavan-3-ol subgroup of polyphenols and are commonly known as the green tea catechins. In addition to being the most abundant green tea catechin, EGCG is considered by many to be the most bioactive compound [13–16].

Flavan-3-ols have a low bioavailability with only a small percentage being absorbed from the small intestine. In the intestinal epithelial cells, flavan-3-ols are sulphated, glucuronidated or methylated by phase II enzymes before entering the systemic circulation with further biotransformation occurring in the liver, kidneys and blood [17]. This biotransformation affects the chemico-physical properties of the enzymatic substrates, promoting an increased rate of

urinary excretion. The extent and type of this biotransformation is dependent on the flavan-3-ol. For example, gallated flavan-3-ols, such as EGCG, mainly occur in the non-conjugated form in the plasma (77–90%); however, a small percentage is methylated by the enzyme catechol-O-methyltransferase (COMT) (an enzyme also important in the metabolism of catecholamine neurotransmitters, catecholestrogens and catechol-containing drugs) to produce the major products 4′-O-methyl EGCG and 4″-O-methyl EGCG, which can be further methylated to 4′4″-O-dimethyl EGCG and ultimately the tentatively assigned 3′4′4″-O-trimethyl EGCG [17]. Non-gallated flavan-3-ols, such as EC and EGC, are found to be mostly in the conjugated form (79 and 69%), the major products being 3′ and 4′-O-methyl EC, 4′-O-methyl EGC, 4′-O-methyl EGC-glucuronide and 4′-O-methyl EGC-sulphate [18]. The majority of research to date indicates a reduced biological activity with biotransformed green tea catechins [19–24]. However, a small selection of studies claim indifferent or even enhanced metabolite activity compared to the parent compound [25–30].

A single-nucleotide polymorphism in the gene encoding for COMT, found at position 158 of the membrane-bound form and 108 of the soluble form resulting in a valine (G) to methionine (A) substitution (Val(158/108)Met), has been shown to reduce the enzymatic activity by ~40% due to increased thermo instability [31–33]. The work to date has been carried out in vitro, using human brain tissue and human blood lymphocyte cells [31], human liver cells and red blood cells [33] and transfected human embryonic and animal kidney cells [32], with catechol as a substrate. It has been hypothesized that individuals with at least one copy of the low-activity co-dominant COMT A allele would not metabolize green tea catechins as quickly and would thus have a greater benefit from green tea consumption. Indeed, tea drinkers with the low-activity COMT enzyme (AA or AG genotype) have been shown to have a lower risk (OR 0.48) of developing breast cancer compared to those who do not drink tea. This inverse relationship was not evident in individuals homozygous for the high-activity COMT enzyme (GG) [34].

However, the impact of COMT genotype on flavan-3-ol metabolism in humans is unknown. In the current study, using a prospective recruitment approach, the extent of EGCG, EGC and EC methylation after green tea catechin supplementation is investigated in individuals with a COMT GG compared to a COMT AA genotype.

Materials and methods

Materials

Sunphenon 90LB decaffeinated green tea extract was purchased from Taiyo International (Mie, Japan) and encapsulated by DHP (Wales, UK). The HPLC standards EGCG, EC, ECG, EGC, GCG, GC, CG and catechin were purchased from Sigma–Aldrich (St Louis, MO, USA). 4′-O-Methyl EGCG was purchased from Timtec LLC (Newark, DE, USA). 4″-O-Methyl EGCG, 4′4″-O-dimethyl EGCG and 4′,3″,4″-O-trimethyl EGCG were synthesized in-house as described by Meng et al. [35]. β-Glucuronidase type H-5 enzyme (*Helix pomatia*) with an activity of ≥400,000 units/g and secondary sulphatase activity of ≥40,000 units/g in the commercial enzyme preparation was purchased from Sigma–Aldrich.

Study design

The study protocol was approved by the University of Reading Research and Ethics Committee and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Due to the lack of human studies in the literature examining the methylation rates of green tea catechins in individuals with different COMT genotypes, there was insufficient data to conduct an a priori power calculation. This study should therefore be regarded as a pilot study for future larger-scale studies. Twenty (10 (7:3 males/females) of each homozygous COMT GG and AA genotype) healthy, non-smoking participants (mean (SE) age 54.6 (3.2) years and BMI 27.4 (0.4) kg/m^2) were recruited, and all participants provided written informed consent prior to commencing the study. Acceptance onto the study was dependent on having blood pressure and biochemical screening parameters within a specified range (blood pressure < 160/100 mmHg, total cholesterol < 8.0 mmol/L, ALT < 45 U/L, γGT < 55 U/L, bilirubin < 20.5 μmol/L and haemoglobin > 12.5 g/dl), homozygous COMT genotype and age between 18 and 70 years. In total, 55 subjects were screened, of which 25 met the suitable criteria. Five subjects subsequently withdrew before completing the study visit. Participants followed a low-catechol diet for 24 h before the study by avoiding fruit juices and smoothies, alcoholic beverages, cocoa, tea, coffee and high-catechol-containing fruits and vegetables (apples, onions, grapes and berries). After an overnight 12-h fast, participants attended the human investigation unit, and an indwelling cannula was inserted into the antecubital vein in the forearm. Two decaffeinated green tea capsules providing a total of 836 mg green tea catechins (Sunphenon 90 LB) were consumed in the fasted state. The polyphenol composition of the green tea extract is shown in Table 1. The capsules were consumed in a fasted state to take account of the accumulating data, showing that the oral bioavailability of green tea catechins is greater in the fasted compared with the fed state [36, 37]. However, high doses of green tea catechins (≥800 mg) on an empty stomach have been reported to induce

Table 1 Composition of study capsules Sunphenon 90LB

Chemical	Percentage of extract	Content per capsule[a] (mg)	Study dose[b] (mg)
Epigallocatechin gallate (EGCG)	40.71	224	448
Epigallocatechin (EGC)	16.27	89	178
Epicatechin (EC)	8.74	48	96
Epicatechin gallate (ECG)	6.02	33	66
Gallocatechin (GC)	2.02	11	22
Gallocatechin gallate (GCG)	1.27	7	14
Catechin	1.16	6	12
Gallic acid (GA)	0.75	4	8
Catechin gallate (CG)	0.03	<1	<1
Caffeine	0.6	3	6

[a] Capsule total weight ~550 mg

[b] Study dose consisted of two capsules taken with water

gastrointestinal complaints [36, 38]. To prevent any gastrointestinal discomfort from the catechin supplementation in a fasted state, a standardized carbohydrate-rich breakfast meal (567 kcals, 107 g carbohydrate, 18.1 g protein and 7.4 g fat) consisting of 100 g Kelloggs Frosties and 150 mL semi-skimmed milk was given 60 min after the green tea capsules. A second standardized low-flavonoid lunch meal (661 kcals, 74.4 g carbohydrate, 15.8 g protein and 33.3 g fat) consisting of a soft cheese and cucumber sandwich, ready salted crisps and two shortbread biscuits was given at 240 min. Blood samples were taken at baseline (0 min) and 30, 60, 90, 120, 150, 180, 240, 360 and 480 min after capsule consumption and collected in K_2E EDTA tubes. Samples were centrifuged at 1,700×*g* for 10 min at 4 °C, and 10 µL 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 plasma aliquot for the analysis of green tea catechins. Plasma was stored at −80 °C until further analysis.

DNA isolation and COMT genotyping

The buffy coat layer was isolated from the K_2E EDTA screening blood sample by centrifugation at 1,700 g for 10 min at 4 °C. The DNA from the buffy coat layer was extracted using the QIAmp DNA Mini Kit (Qiagen Ltd, UK). Allelic discrimination of the COMT rs4680 gene variant was conducted using a TaqMan Drug Metabolism Genotyping Assay (Applied Biosystems, Warrington, UK). The prevalence of the COMT (rs4680) GG, GA and AA genotypes in European populations is 22, 53 and 25%, respectively (HapMap).

Sample preparation and HPLC/UPLC green tea catechin analysis

Defrosted plasma samples (200 µL) were stabilized by the addition of 20 µL of 1% ascorbic acid containing 0.1% EDTA. Glucuronidase buffer (1.5 M sodium acetate), glucuronidase and internal standard (ethyl gallate) were added and samples incubated at 37 °C for 45 min. These optimal conditions for complete hydrolysis have been previously investigated [39]. In order to isolate the catechins from plasma, 300 µL of 0.2% ascorbic acid and 10 µL of 2 N hydrogen chloride were added, samples vortexed and incubated at 4 °C for 10 min followed by the addition of 1 mL ethyl acetate (EtOAc). Samples were then vortexed again, centrifuged (3,000×*g*) for 10 min, and the EtOAc fraction (containing the catechins) was collected. Ascorbic acid (0.2%) was added and solvent evaporated under nitrogen gas. Samples were then dissolved in stabilizer solution (2% hydrogen acetate, 10% methyl cyanide, 250 mg/L EDTA and 250 mg/L ascorbic acid) and sonicated for 10 min.

Quantification of plasma total EGCG, 4′-O-methyl EGCG, 4″-O-methyl EGCG, 4′4″-O-dimethyl EGCG and 3′4′4″-O-trimethyl EGCG was performed by HPLC with coulometric array electrochemical detection (HPLC ESA CoulArray 5,600 was equipped with ESA 580 HPLC-pumps, ESA column oven 82,105, Waters autosampler 717 plus and Phenomenex Synergi Polar 250 × 4.6 mm column). Cell potentials 0, 50, 100, 150, 200, 300, 400 and 500 mV were used with 50 mM phosphate buffer (pH 3.4) with the addition of 10% acetonitrile (mobile phase A) and 50 mM phosphate buffer (pH 3.4) with the addition of 50% acetonitrile (mobile phase B). Column temperature was 35 °C, and the flow rate was 1 mL/min. Calibration standards from 0 to 500 ng/mL were used.

Plasma EGC, ECG, EC, GCG, GC, CG and catechin were quantified by UPLC-Q-TOF Mass Spectrometry (Micromass Q-Tof Premier Mass Spectrometer, Waters Acquity UPLC system and Waters Reagent Manager Pump) with an Acquity UPLC BEH Phenyl 2.1 × 100 mm, 1.7-µm column at 40 °C, flow rate 0.4 mL using a solvent system of 0.1% formic acid in water and acetonitrile as eluents.

Statistical analysis

Mean catechin concentrations were calculated for each time point and COMT genotype group and modelled by cubic spline interpolation with the exclusion of baseline values (0 min). Pharmacokinetic parameters for maximum concentration (C_{max}), time to reach C_{max} (T_{max}), area under the time–concentration curve (AUC), elimination half-life ($t^1/_2$e) and absorption half-life ($t^1/_2$a) were estimated for both COMT genotypes and for the entire study population. In the analysis of each of the pharmacokinetic parameters, the test statistic is the difference in parameter estimates between the two genotypes. For data in which cubic spline interpolation was not suitable (for data with a high frequency of concentrations below the limits of detection, for example 4″-O-methyl EGCG), an AUC corresponding to the sum of the trapeziums under the polygons defined by the horizontal line at the minimum detection limit and the medians of the data at each time point were used. The null hypothesis is that there is no difference between the genotypes. The test statistic is compared with the null distribution, which was created by resampling (drawing repeated samples from the data, but randomizing the genotypes to the individuals), to see whether the result is unusual when compared to those that could occur by chance. As the curve estimates are calculated using the mean values, there are no estimates of variation. A second resampling method was applied to enable the estimates of variability to be calculated for future prospective power calculations. The statistical package Statistical Analysis Software (SAS) version 9.2 (SAS Institute Inc, USA) was used. Differences were considered statistically significant at $p < 0.05$.

Results

The green tea extract dose was well tolerated, and no adverse events were reported during the study. Pharmacokinetic analysis for the entire group ($n = 20$) was performed on EC, EGC, ECG, EGCG and 4′-O-methyl EGCG plasma concentrations (Table 3). EGCG was found in the highest concentration in the capsules (Table 1), and this was reflected in the plasma concentrations with a C_{max} of 1.09 μM, followed by EGC, EC and ECG with a C_{max} of 405, 331 and 160 nM, respectively. The methylated form of EGCG, 4′-O-methyl EGCG, was found to have a C_{max} of 77.1 nM. EGCG was also found to be at the maximum concentration within the shortest time, with a T_{max} of 81.5 min. EGC, ECG, EC and 4′-O-methyl EGCG peaked at 98.5, 85.5, 99.0 and 96.5 min, respectively. The concentration profiles (Fig. 1) for the non-gallated catechins (EGC and EC) are suggestive of a biphasic response.

The study group was divided according to COMT genotype with ten participants for each homozygous COMT group (GG and AA). There were no significant differences in clinical and anthropometric measures between genotype groups (Table 2). Pharmacokinetic analysis was also performed for the two genotype groups (Fig. 1). Significant differences were not found for C_{max}, T_{max}, AUC, EGCG half-life absorption ($t^1/_2$a) and EGCG half-life elimination ($t^1/_2$e) between the two homozygous COMT groups (Table 3). However, there was a tendency for a difference in the concentration curves for 4′-O-methyl EGCG between the genotype groups, with a greater C_{max} and AUC response in the AA individuals (Fig. 1d; Table 3). There was also a tendency towards a higher C_{max} for the AA genotype for EC, EGC and ECG, compared with the GG genotype (Table 3). Figure 1 shows the plasma concentrations of 4′-O-methyl EGCG and 4″-O-methyl EGCG for both COMT genotypes. Single-observation standard deviation estimates of 4′-O-methyl EGCG for prospective power calculations are 32.2 min 0.065 and 8.57 μM for T_{max}, C_{max} and AUC, respectively. Due to the large number of non-detectable and trace values, cubic spline interpolation modelling curves were not produced for 4″-O-methyl EGCG, GCG, GC, CG, catechin, 4′4″-O-dimethyl EGCG and 3′4′4″-O-trimethyl EGCG. 4″-O-Methyl EGCG AUC analysis was performed for the median values above the detection limit (21 nM) giving an AUC of 432 and 76 nM for the AA and GG groups, respectively ($p = 0.214$).

Discussion

This study investigated the absorption and metabolism of green tea catechins in healthy individuals and is the first to examine the effect of the COMT Val(158/108)Met polymorphism on this process.

Metabolism of green tea catechins

Green tea catechins have low bioavailability due to limited systemic absorption and first-pass phase I and II metabolism, which results in plasma levels in the micromolar range. In the current study, C_{max} and T_{max} values in relation to quantities ingested are similar or slightly lower to those found in other studies. This study observed a C_{max} of 1.09 μM (from a 448 mg EGCG dose) and T_{max} of 81.5 min for EGCG. Ullmann et al. [40] administered a single 400-mg EGCG dose and found an average C_{max} of 1.36 μM and T_{max} of 86.4 min. A second study by this research group showed a C_{max} of 1.15 μM and T_{max} of 84 min with a 400-mg EGCG dose [41]. Yang et al. administered 4.5 g green tea containing 328.5 mg EGCG,

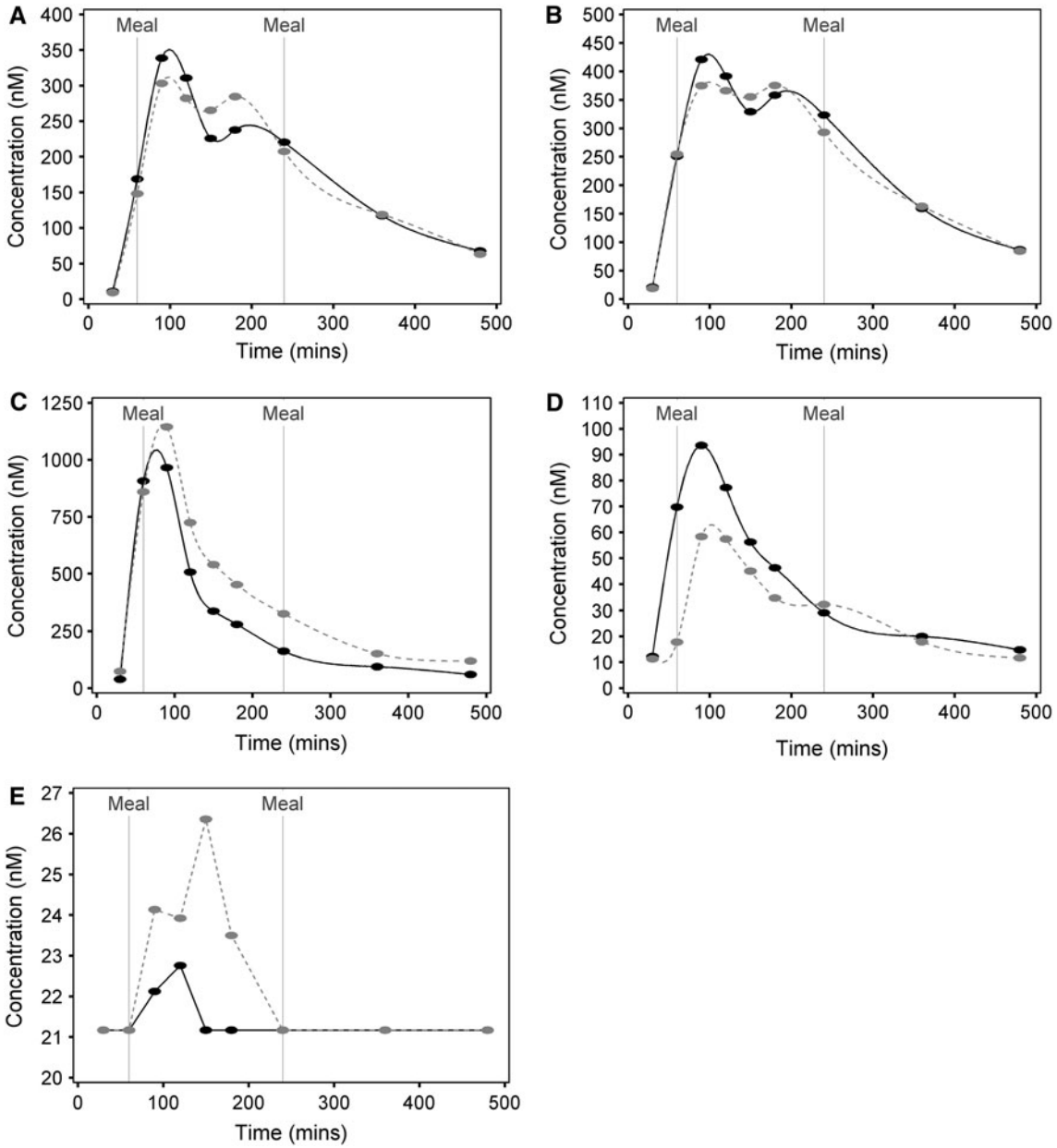

Fig. 1 Mean plasma concentration curves modelled by cubic spline interpolation for **a** epicatechin (EC), **b** epigallocatechin (EGC), **c** epigallocatechin gallate (EGCG) and **d** 4′-O-methyl EGCG and median concentration plots for **e** 4″-O-methyl EGCG for COMT GG (*grey circles* (*filled circle*)) and COMT AA (*black circles* (*filled circle*)) genotype groups. Note the scale of the *y*-axis is different for each figure

306 mg EGC and 112.5 mg EC, resulting in C_{max} levels of 0.70, 1.80 and 0.65 μM, respectively. However, the T_{max} for EGCG, EGC and EC in the aforementioned study was rather different from the current study at 162, 78 and 108 min, respectively, compared with 81.5, 98.5 and 99 min in the current trial. This could be due to the form of green tea (tea beverage as opposed to capsule containing extract) or the additional coffee whitener, sucrose and vanilla flavour the researchers added to the test beverage, which may have affected absorption [42]. Although studies have been conducted to determine the effect of the food matrix on flavan-3-ol absorption [43, 44], the outcomes have been inconsistent and further research is required.

It is interesting to note the bimodal curve produced from the non-gallated catechins, which is also evident when the data is viewed at an individual level (data not shown). This finding may be indicative of a meal effect from the high-carbohydrate breakfast given 1 h after capsule consumption. This study was different in design to previous pharmacokinetic studies, in which subjects remained fasted

Table 2 Screening characteristics according to COMT genotype*

Parameter	COMT AA	COMT GG	*p*-value
Sex (male/female)	7:3	7:3	0.99
Age (years)	58 (12)	51 (16)	0.33
Weight (kg)	83.7 (12)	83.9 (7)	0.98
BMI (kg/m^2)	27.0 (2)	27.7 (2)	0.48
Systolic BP (mmHg)	130 (13)	122 (10)	0.13
Diastolic BP (mmHg)	80 (7)	75 (7)	0.10
Cholesterol (mmol/L)	5.7 (0.8)	5.2 (1.0)	0.23
Triglycerides (mmol/L)	1.4 (0.5)	1.4 (0.8)	0.85

* Values represent mean (SD)

for a few hours after green tea catechin supplementation or consumed a meal at the same time as supplementation. The bimodal curve observed may be the result of the meal delaying absorption and thus causing a second smaller peak once meal digestion has progressed. For example, gallated species may form complexes with the food components, preventing further absorption and resulting in a single peak. Previous studies have shown substantial diminished green tea catechin absorption when supplements are consumed with a meal compared to without a meal [36, 37]. Indeed, Chow et al. found a significantly higher plasma EGCG ($C_{\max}$ 1.74 μM, $T_{\max}$ 93.9 min) in fasted participants compared to when EGCG was administered with food ($C_{\max}$ 0.31 μM, $T_{\max}$ 122.9 min), with a 400-mg EGCG dose, in the form of Polyphenon E (green tea catechin mixture) [36]. However, $C_{\max}$ levels are substantially larger in the fasted group than the current study ($C_{\max}$ 1.09 μM), which indicates a possible reduction/restriction in absorption when the food was given 1 h after supplementation.

Another possible explanation for EGC and EC bimodal curves is a depletion of precursors for conjugation reactions (uridine-5′-diphosphoglucuronic acid) for the non-gallated flavan-3-ols. Non-gallated flavanols such as EGC and EC are mainly found in plasma in the conjugated form, unlike gallated flavanols such as EGCG which are mainly in the non-conjugated form. These conjugation precursors may be replenished after meal consumption [45]. Indeed, total non-gallated flavanols in plasma have been shown to be decreased when consumed in the fasting state compared with the fed state, which may be attributable to a depletion in glucuronidation precursors when fasted [46]. Interestingly, Chow et al. [36] also found a reduction in plasma total (non-conjugated and conjugated) non-gallated catechins in the fasted compared to the fed state. Finally, enterohepatic circulation of absorbed EGC and EC cannot be excluded as a possibility to explain the bimodal nature of the appearance of these compounds in the plasma.

Impact of COMT genotype

One of the aims of the present study was to examine the plasma concentration profiles of the green tea catechins according to the COMT rs4680 genotype. Differences were not found in the profiles of the plasma catechins between COMT groups after acute consumption of the green tea

Table 3 Plasma green tea catechin pharmacokinetic parameters for entire study population and COMT AA and GG genotype groups after mixed green tea catechin supplementation

Catechin	Parameter	Entire study population	COMT rs4680 AA	COMT rs4680 GG	*p*-value (Diff AA-GG)
EC	$T_{\max}$	99.0	99.5	98.0	0.832
	$C_{\max}$	0.331	0.351	0.312	0.583
	AUC	79.7	80.2	79.1	0.943
EGC	$T_{\max}$	98.5	98.5	99.5	0.968
	$C_{\max}$	0.405	0.430	0.381	0.536
	AUC	109.9	111.6	108.4	0.877
EGCG	$T_{\max}$	81.5	76.5	85.5	0.370
	$C_{\max}$	1.09	1.04	1.16	0.731
	*t*1/2e	–	42.5	53.0	0.461
	*t*1/2a	–	44.5	48.5	0.508
	AUC	150.4	125.4	174.7	0.270
ECG*	$T_{\max}$	85.5	90.0	78.0	0.270
	$C_{\max}$	0.160	0.187	0.142	0.329
	AUC	28.5	34.8	22.8	0.141
4′-O-methyl EGCG	$T_{\max}$	96.5	90.0	101.5	0.999
	$C_{\max}$	0.077	0.093	0.064	0.315
	AUC	14.7	17.4	12.7	0.226

EC epicatechin; *EGC* epigallocatechin; *EGCG* epigallocaetchin gallate; *ECG* epicatechin gallate; T_{max} (*min*) time to maximum concentration; C_{max} (*μM*), concentration maximum; $t^1/_2e$ (*min*) half-life of elimination; $t^1/_2a$ (*min*) half-life of absorption and *AUC* (*μM·min*) area under the curve.
* High frequency of trace and non-detectable levels found

extract. Albeit non-significant, 4′-O-methyl EGCG was found to have a higher C_{max} and AUC in the AA COMT genotype group, which was initially proposed to have a slower methylation enzymatic function, based on previous in vitro work that has examined the impact of COMT genotype on catechol metabolism [31–33]. Although pharmacokinetic parametrics and statistical analysis were not performed due to non-detectable and trace levels in several study participants, 4″-O-methyl EGCG was perceived as being slightly higher in the COMT GG genotype group. The COMT polymorphism is thought to inflict small differences in the intramolecular packing around residue 108/158, resulting in an amino acid displacement towards the SAM binding site. As a result, there may be partial unfolding of the protein structure and aggregation at physiological temperatures instigating lower enzyme stability and activity [47]. It is possible that the structural change results from the valine to methionine substitution, consequent on a differential preference for methylation position. It is also possible that the 4′-O-methyl EGCG produced in the GG COMT group has been methylated more rapidly and transformed into the secondary and tertiary methylation products, 4′4″-O-dimethyl EGCG and 3′4′4″-O-trimethyl EGCG. In a study using rat liver cytosol, the 4″-position on EGCG was found to be strongly favoured for COMT methylation compared with the 4′-position. With longer incubation times (20 min), 4′4″-DiMeEGCG was the major product found [17]. This may provide an explanation for the seemingly evident lag in COMT GG 4′-O-methyl EGCG plasma appearance, which appears to peak at the time of highest EGCG concentration, suggesting that the COMT enzyme may have become saturated at this time point inhibiting the production of secondary and tertiary methylation products.

COMT phenotypic differences with respect to the methylation of the endogenous substrate, dopamine, have been observed [48–52]. However, the current study found a lack of effect on the metabolism of green tea catechin, EGCG. The low bioavailability of the green tea catechins may help to explain the findings. Green tea catechins are poorly absorbed and quickly eliminated. Dietary catechins are also thought to have better enzyme–substrate interactions and binding affinity [53], which may help to counteract the physiological effects of the polymorphism. Catecholamines, such as dopamine, have much higher affinity for the membrane-bound COMT enzyme which is predominantly found in the brain tissue, whilst soluble COMT, mainly found in the peripheral organs is thought to be mainly responsible for the methylation of dietary catechins. These differences in COMT-mediated catechin and catecholamine methylation may explain the lack of effect of genotype on catechin metabolism, despite the relatively consistently observed effect on dopamine metabolism.

Future recommendations

Due to the novel nature of this study, no human data were available to derive a meaningful power calculation. Therefore, a relatively small study population group was used. The pharmacokinetic parameters were calculated from blood sampling at 30-min intervals; more frequent sampling would improve upon the derived estimates for the metabolism of green tea catechins. This study provides a basis for the design of future studies examining COMT genotype.

In conclusion, this study demonstrated a possible difference in non-gallated and gallated catechin absorption patterns following a green tea extract in healthy men and women. The main finding of the study is that, relative to dopamine, the COMT Val(158/108)Met appears to have a less dramatic influence on EGCG absorption and elimination. However, further large-scale pharmacokinetic studies are needed to fill the knowledge gap in this subject area and to substantiate these findings.

Conflict of interest Unilever Discover, Colworth, UK, sponsored the PhD studies of RJM. AMM and KGJ have a number of ongoing collaborative projects with Unilever Discover, Colworth, UK.

References

1. Nakachi K, Matsuyama S, Miyake S, Suganuma M, Imai K (2000) Preventive effects of drinking green tea on cancer and cardiovascular disease: epidemiological evidence for multiple targeting prevention. Biofactors 13:49–54
2. Kuroda Y, Hara Y (1999) Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutat Res 436:69–97
3. Clark J, You M (2006) Chemoprevention of lung cancer by tea. Mol Nutr Food Res 50:144–151
4. Anderson RA, Polansky MM (2002) Tea enhances insulin activity. J Agric Food Chem 50:7182–7186
5. Tsuneki H, Ishizuka M, Terasawa M, Wu JB, Sasaoka T, Kimura I (2004) Effect of green tea on blood glucose levels and serum proteomic patterns in diabetic (db/db) mice and on glucose metabolism in healthy humans. BMC Pharmacol 4:18
6. Wolfram S, Raederstorff D, Preller M, Wang Y, Teixeira SR, Riegger C, Weber P (2006) Epigallocatechin gallate supplementation alleviates diabetes in rodents. J Nutr 136:2512–2518
7. Kavantzas N, Chatziioannou A, Yanni AE, Tsakayannis D, Balafoutas D, Agrogiannis G, Perrea D (2006) Effect of green tea on angiogenesis and severity of atherosclerosis in cholesterol-fed rabbit. Vascul Pharmacol 44:461–463
8. Sasazuki S, Kodama H, Yoshimasu K, Liu Y, Washio M, Tanaka K, Tokunaga S, Kono S, Arai H, Doi Y, Kawano T, Nakagaki O, Takada K, Koyanagi S, Hiyamuta K, Nii T, Shirai K, Ideishi M, Arakawa K, Mohri M, Takeshita A (2000) Relation between green tea consumption and the severity of coronary atherosclerosis among Japanese men and women. Ann Epidemiol 10:401–408
9. Unno T, Tago M, Suzuki Y, Nozawa A, Sagesaka YM, Kakuda T, Egawa K, Kondo K (2005) Effect of tea catechins on postprandial plasma lipid responses in human subjects. Br J Nutr 93:543–547

10. Anandh Babu PV, Sabitha KE, Shyamaladevi CS (2006) Green tea extract impedes dyslipidaemia and development of cardiac dysfunction in streptozotocin-diabetic rats. Clin Exp Pharmacol Physiol 33:1184–1189
11. Tedeschi E, Suzuki H, Menegazzi M (2002) Antiinflammatory action of EGCG, the main component of green tea, through STAT-1 inhibition. Ann N Y Acad Sci 973:435–437
12. Lin JK, Lin-Shiau SY (2006) Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols. Mol Nutr Food Res 50:211–217
13. Tachibana H (2009) Molecular basis for cancer chemoprevention by green tea polyphenol EGCG. Forum Nutr 61:156–169
14. Tipoe GL, Leung TM, Hung MW, Fung ML (2007) Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection. Cardiovasc Hematol Disord Drug Targets 7:135–144
15. Wolfram S, Wang Y, Thielecke F (2006) Anti-obesity effects of green tea: from bedside to bench. Mol Nutr Food Res 50:176–187
16. Ju J, Lu G, Lambert JD, Yang CS (2007) Inhibition of carcinogenesis by tea constituents. Semin Cancer Biol 17:395–402
17. Lu H, Meng XF, Yang CS (2003) Enzymology of methylation of tea catechins and inhibition of catechol-O-methyltransferase by (−)-epigallocatechin gallate. Drug Metab Dispos 31:572–579
18. Moore RJ, Jackson KG, Minihane AM (2009) Green tea (*Camellia sinensis*) catechins and vascular function. Br J Nutr 102:1790–1802
19. Huo C, Yang H, Cui QC, Dou QP, Chan TH (2010) Proteasome inhibition in human breast cancer cells with high catechol-O-methyltransferase activity by green tea polyphenol EGCG analogs. Bioorg Med Chem 18:1252–1258
20. Dou QP (2009) Molecular mechanisms of green tea polyphenols. Nutr Cancer 61:827–835
21. Harada M, Kan Y, Naoki H, Fukui Y, Kageyama N, Nakai M, Miki W, Kiso Y (1999) Identification of the major antioxidative metabolites in biological fluids of the rat with ingested (+)-catechin and (−)-epicatechin. Biosci Biotechnol Biochem 63:973–977
22. Landis-Piwowar KR, Milacic V, Dou QP (2008) Relationship between the methylation status of dietary flavonoids and their growth-inhibitory and apoptosis-inducing activities in human cancer cells. J Cell Biochem 105:514–523
23. Yano S, Fujimura Y, Umeda D, Miyase T, Yamada K, Tachibana H (2007) Relationship between the biological activities of methylated derivatives of (−)-epigallocatechin-3-O-gallate (EGCG) and their cell surface binding activities. J Agric Food Chem 55:7144–7148
24. Duenas M, Gonzalez-Manzano S, Gonzalez-Paramas A, Santos-Buelga C (2010) Antioxidant evaluation of O-methylated metabolites of catechin, epicatechin and quercetin. J Pharm Biomed Anal 51:443–449
25. Koga T, Meydani M (2001) Effect of plasma metabolites of (+)-catechin and quercetin on monocyte adhesion to human aortic endothelial cells. Am J Clin Nutr 73:941–948
26. Lu H, Meng XF, Li C, Sang SM, Patten C, Sheng SQ, Hong JG, Bai NS, Winnik B, Ho CT, Yang CS (2003) Glucuronides of tea catechins: enzymology of biosynthesis and biological activities. Drug Metab Dispos 31:452–461
27. Lambert JD, Rice JE, Hong J, Hou Z, Yang CS (2005) Synthesis and biological activity of the tea catechin metabolites, M4 and M6 and their methoxy-derivatives. Bioorg Med Chem Lett 15:873–876
28. Nanjo F, Mori M, Goto K, Hara Y (1999) Radical scavenging activity of tea catechins and their related compounds. Biosci Biotechnol Biochem 63:1621–1623
29. Sano M, Suzuki M, Miyase T, Yoshino K, Maeda-Yamamoto M (1999) Novel antiallergic catechin derivatives isolated from oolong tea. J Agric Food Chem 47:1906–1910
30. Chiu FL, Lin JK (2005) HPLC analysis of naturally occurring methylated catechins, 3' '- and 4' '-methyl-epigallocatechin gallate, in various fresh tea leaves and commercial teas and their potent inhibitory effects on inducible nitric oxide synthase in macrophages. J Agric Food Chem 53:7035–7042
31. Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM, Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75:807–821
32. Shield AJ, Thomae BA, Eckloff BW, Wieben ED, Weinshilboum RM (2004) Human catechol O-methyltransferase genetic variation: gene resequencing and functional characterization of variant allozymes. Mol Psychiatry 9:151–160
33. Boudikova B, Szumlanski C, Maidak B, Weinshilboum R (1990) Human liver catechol-O-methyltransferase pharmacogenetics. Clin Pharmacol Ther 48:381–389
34. Wu AH, Tseng CC, Van den Berg D, Yu MC (2003) Tea intake, COMT genotype, and breast cancer in Asian-American women. Cancer Res 63:7526–7529
35. Meng X, Sang S, Zhu N, Lu H, Sheng S, Lee MJ, Ho CT, Yang CS (2002) Identification and characterization of methylated and ring-fission metabolites of tea catechins formed in humans, mice, and rats. Chem Res Toxicol 15:1042–1050
36. Chow HHS, Hakim IA, Vining DR, Crowel JA, Ranger-Moore J, Chew WM, Celaya CA, Rodney SR, Hara Y, Alberts DS (2005) Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. Clin Cancer Res 11:4627–4633
37. Foster DR, Sowinski KM, Chow HH, Overholser BR (2007) Limited sampling strategies to estimate exposure to the green tea polyphenol, epigallocatechin gallate, in fasting and fed conditions. Ther Drug Monit 29:835–842
38. Chow HHS, Cai Y, Hakim IA, Crowell JA, Shahi F, Brooks CA, Dorr RT, Hara Y, Alberts DS (2003) Pharmacokinetics and safety of green tea polyphenols after multiple-dose administration of epigallocatechin gallate and Polyphenon E in healthy individuals. Clin Cancer Res 9:3312–3319
39. Grun CH, van Dorsten FA, Jacobs DM, Le Belleguic M, van Velzen EJ, Bingham MO, Janssen HG, van Duynhoven JP (2008) GC-MS methods for metabolic profiling of microbial fermentation products of dietary polyphenols in human and in vitro intervention studies. J Chromatogr B Analyt Technol Biomed Life Sci 871:212–219
40. Ullmann U, Haller J, Decourt JP, Girault N, Girault J, Richard-Caubron AS, Pineau B, Weber P (2003) A single ascending dose study of epigallocatechin gallate in healthy volunteers. J Int Med Res 31:88–101
41. Ullmann U, Haller J, Decourt JD, Girault J, Spitzer V, Weber P (2004) Plasma-kinetic characteristics of purified and isolated green tea catechin epigallocatechin gallate (EGCG) after 10 days repeated dosing in healthy volunteers. Int J Vit Nutr Res 74:269–278
42. Yang CS, Chen LS, Lee MJ, Balentine D, Kuo MC, Schantz SP (1998) Blood and urine levels of tea catechins after ingestion of different amounts of green tea by human volunteers. Cancer Epidemiol Biomark Prev 7:351–354
43. Neilson AP, Sapper TN, Janle EM, Rudolph R, Matusheski NV, Ferruzzi MG (2010) Chocolate matrix factors modulate the pharmacokinetic behavior of cocoa flavan-3-ol phase II metabolites following oral consumption by Sprague-Dawley rats. J Agric Food Chem 58:6685–6691
44. Scholz S, Williamson G (2007) Interactions affecting the bioavailability of dietary polyphenols in vivo. Int J Vitam Nutr Res 77:224–235

45. Henning SM, Choo JJ, Heber D (2008) Nongallated compared with gallated flavan-3-ols in green and black tea are more bioavailable. J Nutr 138:1529S–1534S
46. Price VF, Jollow DJ (1989) Effect of glucose and gluconeogenic substrates on fasting-induced suppression of acetaminophen glucuronidation in the rat. Biochem Pharmacol 38:289–297
47. Rutherford K, Alphandery E, McMillan A, Daggett V, Parson WW (2008) The V108 M mutation decreases the structural stability of catechol O-methyltransferase. Biochim Biophys Acta 1784:1098–1105
48. Barnett JH, Heron J, Goldman D, Jones PB, Xu K (2009) Effects of catechol-O-methyltransferase on normal variation in the cognitive function of children. Am J Psychiatry 166:909–916
49. Dickinson D, Elvevag B (2009) Genes, cognition and brain through a COMT lens. Neurosci 164:72–87
50. Zhang Z, Lindpaintner K, Che R, He Z, Wang P, Yang P, Feng G, He L, Shi Y (2009) The Val/Met functional polymorphism in COMT confers susceptibility to bipolar disorder: evidence from an association study and a meta-analysis. J Neural Transm 116:1193–1200
51. Ehrlich S, Morrow EM, Roffman JL, Wallace SR, Naylor M, Bockholt HJ, Lundquist A, Yendiki A, Ho BC, White T, Manoach DS, Clark VP, Calhoun VD, Gollub RL, Holt DJ (2009) The COMT Val108/158Met polymorphism and medial temporal lobe volumetry in patients with schizophrenia and healthy adults. Neuroimage 53:992–1000
52. Harrison PJ, Weinberger DR (2005) Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence. Mol Psychiatry 10:40–68; image 45
53. Bai HW, Shim JY, Yu J, Zhu BT (2007) Biochemical and molecular modeling studies of the O-methylation of various endogenous and exogenous catechol substrates catalyzed by recombinant human soluble and membrane-bound catechol-O-methyltransferases. Chem Res Toxicol 20:1409–1425