promoting access to White Rose research papers

Universities of Leeds, Sheffield and York
http://eprints.whiterose.ac.uk/

This is an author produced version of a paper published in **British Journal of Nutrition.**

White Rose Research Online URL for this paper:

http://eprints.whiterose.ac.uk/78310/

Paper:
Rhodes, LE, Darby, G, Farrar, MD, Bennett, S, Watson, REB, Massey, KA, Nicolaou, A, Clarke, KA, Dew, TP and Williamson, G (2013) *Oral green tea catechin metabolites are incorporated into human skin and protect against UV radiation-induced cutaneous inflammation in association with reduced production of pro-inflammatory eicosanoid 12-hydroxyeicosatetraenoic acid.* British Journal of Nutrition, 110 (5). 891 - 900.

http://dx.doi.org/10.1017/S0007114512006071

White Rose Research Online
eprints@whiterose.ac.uk

Oral green tea catechin metabolites are incorporated into human skin and protect against UVR-induced cutaneous inflammation in association with reduced production of pro-inflammatory eicosanoid 12-HETE

Lesley E Rhodes[1], Gemma Darby[1], Karen A Massey[2], Kayleigh A Clarke[3], Tristan P Dew[3], Mark D Farrar[1], Susan Bennett[1], Rachel EB Watson[1], Gary Williamson[3], Anna Nicolaou[2]

[1]Dermatology Centre, Institute of Inflammation and Repair, University of Manchester, Manchester Academic Health Science Centre, Salford Royal NHS Foundation Hospital, Manchester, UK, [2]School of Pharmacy and Centre for Skin Sciences, School of Life Sciences, University of Bradford, Bradford, UK, [3]School of Food Science and Nutrition, University of Leeds, Leeds.

Address correspondence to: L. E. Rhodes, Photobiology Unit, Dermatology Centre, University of Manchester, Salford Royal NHS Foundation Hospital, Manchester, M6 8HD, UK. Tel: +44 161 2061150. Fax: +44 161 2061156. Email: lesley.e.rhodes@manchester.ac.uk

Running head: GTC, skin uptake and UVR-induced 12-HETE

Key words: green tea catechins, bioavailability, skin, 12-HETE

Abbreviations used: COX, cyclooxygenase; CYP, cytochrome P450; EC, (-)-epicatechin; ECG, (-)-epicatechin-3-*O*-gallate; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin-3-*O*-gallate; GTC, green tea catechins; HETE, hydroxyeicosatetraenoic acid; LOX, lipoxygenase; MED, minimal erythema dose; MRM, multiple reaction monitoring; PL, phospholipase; PG, prostaglandin; TPA, 12-*O*-tetradecanpylphorbol-13-acetone; UVR, ultraviolet radiation.

ABSTRACT

Green tea catechins (GTC) reduce ultraviolet radiation (UVR)-induced inflammation in experimental models but human studies are scarce, and their cutaneous bioavailability and mechanism of photoprotection are unknown. We aimed to examine oral GTC cutaneous uptake, ability to protect human skin against erythema induced by a UVR dose-range, and impact on potent cyclooxygenase and lipoxygenase-produced mediators of UVR-inflammation, prostaglandin (PG)E_2 and 12-hydroxyeicosatetraenoic acid (HETE), respectively. In an open oral intervention study, 16 healthy humans (phototype I/II) were given low-dose GTC (540 mg) with vitamin C (50 mg) daily for 12 weeks. Pre- and post-supplementation, buttock skin was exposed to UVR and resultant erythema quantified. Skin blister fluid and biopsies were taken from unexposed and UVR-exposed skin 24h-post a pro-inflammatory UVR challenge (3 minimal erythema doses). Urine, skin tissue and fluid were analysed for catechin content, and skin fluid for PGE_2 and 12-HETE, by liquid chromatography coupled to tandem mass spectrometry. Fourteen completing subjects were supplement-compliant (12F, median 42.5y, range 29-59y). Benzoic acid levels were increased in skin fluid post-supplementation (P=0.03), and methylated gallic acid and several intact catechins and hydroxyphenyl-valerolactones were detected in skin tissue and fluid. Area-under-curve analysis for UVR-erythema revealed reduced response post-GTC (P=0.037). Pre-supplementation, PGE_2 and 12-HETE were UVR-induced (P=0.003, P=0.0001). After GTC, UVR-induced 12-HETE reduced from mean±SD 64±42 to 41±32 pg/μL (P=0.01) while PGE_2 was unaltered. Thus GTC intake results in incorporation of catechin metabolites in human skin associated with abrogated UVR-induced 12-HETE; this may contribute to protection against sunburn inflammation and potentially longer-term UVR-mediated damage.

INTRODUCTION

Ultraviolet radiation (UVR) in sunlight is a key environmental stressor impacting on skin health. Acute effects include sunburn (an inflammatory response), immune-suppression and photosensitivity, while repeated exposures lead to photoageing and photocarcinogenesis [1]. Sunburn is characterised clinically by erythema due to vasodilatation, and histologically a dermal infiltrate of neutrophils and mononuclear cells is observed [2, 3]. Activation of cutaneous phospholipase (PL) A_2 by UVR is a key part of the inflammatory response, releasing membrane esterified fatty acids, including arachidonic acid that is subsequently metabolised by cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP) isozymes to produce eicosanoids with vasodilatory and chemoattractant properties ([4]). Potent pro-inflammatory mediators prostaglandin (PG) E_2 and 12- hydroxyeicosatetraenoic acid (HETE) are the most abundant eicosanoids at the peak of the sunburn response, correlating with UVR-upregulated expression of COX-2 and 12-LOX in human skin [4].

The polyphenols are plant-derived molecules, many exhibiting anti-inflammatory properties [5, 6]. Their oral intake is associated with health benefits including reduced risk of cancer and cardiovascular disease [7, 8]. Studies performed largely in experimental models suggest polyphenols from various sources may protect skin against adverse effects of UVR, including carcinogenesis [1, 9, 10]. Green tea is widely consumed worldwide and contains several polyphenols of the catechin family, i.e. green tea catechins (GTC), principally (-)-epicatechin (EC), (-)-epicatechin-3-*O*-gallate (ECG), (-)-epigallocatechin (EGC) and (-)-epigallocatechin-3-*O*-gallate (EGCG; [11]). Emerging evidence suggests GTC can protect against cutaneous damage. Specifically, oral GTC protected against UVR-induced skin inflammation and carcinogenesis in hairless mice [12], whilst in humans, topically applied GTC reduced UVR-induced DNA damage, erythema and leucocytic infiltrate [13, 14], and oral green tea extract reduced skin erythema following a UVR challenge near the sunburn threshold [15]. Some of these effects may be mediated via effects on COX and LOX isozymes, as EGCG, EGC, ECG and EC have been reported to reduce the production of PGE_2 and/or 12-HETE in experimental systems [16-18] and oral GTC to reduce UVR-induced COX-2 protein expression and PGE_2 production in mouse epidermis [10]. However, it is unknown whether these findings have relevance to human skin.

Despite increasing evidence of their photoprotective potential, there is a dearth of information on cutaneous bioavailability of oral GTC in humans, reflecting the challenges of their tissue assessment. Moreover, the molecular mechanism(s) underlying protection from UVR-induced inflammation is unexplored in humans. Potentially this may be conveyed

59 through impact on key COX and LOX-derived pro-inflammatory eicosanoids mediating the
60 sunburn response, which additionally exhibit promoting properties in skin carcinogenesis [4,
61 19, 20]. Thus, the aims of our novel study were to examine directly in humans *in vivo* for
62 evidence of cutaneous uptake of orally administered GTC, to evaluate for impact of GTC on
63 sunburn over a range of pro-inflammatory UVR doses, and explore whether the underlying
64 mechanism of protection could be GTC modulation of PGE_2 and/or 12-HETE formation.

METHODS

Subjects and study design

This was an open oral intervention study conducted in the Photobiology Unit, Dermatology Centre, Salford Royal NHS Foundation Hospital, Manchester, UK. Subjects (n=16) were white Caucasian males and females, sun-reactive skin type I-II (easy sunburn, minimal tanning). The exclusion criteria were: history of skin cancer or a photosensitivity disorder; use of a sunbed or sunbathing in the 3 months prior to the study, taking photoactive medication or nutritional supplements, consuming more than 2 cups of tea per day, and currently pregnant or breastfeeding. Ethical approval was obtained from the North Manchester Research Ethics Committee (reference 08/H1006/79). Written informed consent was obtained from the participants and the study adhered to Declaration of Helsinki principles.

Dietary supplements

Subjects took oral supplements comprising 540 mg GTC with 50 mg vitamin C, daily for 12 weeks. These were in the form of 3 capsules each containing 450 mg green tea extract (total 1350 mg tea, 540 mg GTC; Table 1) and 2 capsules each containing 25 mg vitamin C (total 50 mg vitamin C), and were taken with breakfast each morning. The low dose vitamin C was added to stabilise the green tea extract in the gut lumen [(21)]; oral vitamin C supplementation alone has been shown to have no impact on UVR-erythema [(22)]. Supplements were provided by Nestec Ltd (Lausanne, Switzerland) and packaged by Laboratoire LPH (St Bonnet de Rochefort, France). Compliance was assessed by counting the residual capsules in the dispensed containers that volunteers were asked to return, and through analysis of 24 h urine samples collected from all volunteers pre and after 1 day, 6 weeks and 12 weeks supplementation.

UVR exposure

UVR exposures were performed using a solar simulator with emission of UVB and UVA mimicking that of sunlight (emission 290-400 nm; Newport Spectra-Physics Ltd). Irradiance of the light source was measured 10 cm from the source prior to each irradiation, using a radiometer (model IL 730A; International Light, USA) calibrated for use with the light source, to ensure consistency of doses applied. The minimal erythema dose (MED) of UVR of each subject was assessed at baseline and post-supplementation, following application of a geometric series of 10 doses of solar simulated UVR (erythemally weighted doses 6.6-68

mJ/cm^2) to upper buttock skin (1 cm diameter circular sites). Irradiated sites were examined visually after 24 h, with the MED defined as the lowest dose producing visually discernible erythema. Erythema at each site was quantified as described in the following section. At 24 h prior to skin tissue and blister fluid sampling, doses of UVR of 3 x the individual's pre-supplementation MED were given to sites on one buttock; this does was selected in order to provoke an inflammatory response sufficient to significantly elevate cutaneous eicosanoid levels [4].

Quantification of the UVR-induced erythemal responses

The intensity of erythema (erythema index) was quantified using a reflectance instrument (Diastron) in n=10 subjects. Readings were taken in triplicate from each exposed site and from adjacent unexposed skin, and erythema expressed as the difference between these readings (ΔE). Dose-response modelling was performed using a dedicated data analysis package (Regional Medical Physics Department, Gateshead & Tyneside Health Authority, UK) to calculate each subject's D_{30}, the UVR dose producing a ΔE of 30 arbitrary units, a threshold value that approximates an individual's visual MED.

Skin biopsy and suction blister fluid sampling

UVR-exposed (3 x MED) and -protected areas of upper buttock skin were sampled at baseline and post-supplementation; UVR exposures were limited to 1 buttock and the other buttock provided the unexposed skin and blister fluid samples. Skin punch biopsies (5 mm diameter) were taken after intradermal injection of lignocaine, as described [4], snap frozen and stored at -80°C. Suction blisters were raised using suctions cups with a central aperture diameter of 1 cm and vacuum of 250 mm Hg as described previously [4]. Skin blister fluid was aspirated with a 23-gauge needle, snap frozen in liquid nitrogen and stored at -80^{o}C until analysis. Samples destined for polyphenol analysis were combined with 25 µL NaH_2PO_4 (0.4 mol/L, pH 3.6) containing 200 g/L ascorbic acid and 1 g/L EDTA, prior to freezing.

Eicosanoid analysis

Eicosanoids in skin blister fluid were analysed by liquid chromatography coupled to electrospray ionisation tandem mass spectrometry (LC/ESI-MS/MS) as described previously [23, 24]. In summary, skin fluid samples (typically 50-200 µL) were diluted with methanol-water (15% w/w) up to 3 mL. Internal standards (40 ng PGB_2-*d*4 and 80 ng 12-HETE-*d*8;

Cayman Chemicals) were then added and resultant solutions acidified to pH 3.0, followed by solid-phase extraction (C18-E cartridges; Phenomenex) to reduce matrix effects and semi-purify the lipid mediators. Eicosanoids were analysed on a C18 column (Luna 5 µm; Phenomenex) using a Waters Alliance 2695 HPLC pump coupled to a triple-quadrupole mass spectrometer equipped with an electrospray ionisation probe (Quattro Ultima, Waters). Instrument control and data acquisition were performed using MassLynx 4.0 software (Waters). The following multiple reaction monitoring (MRM) transitions were used for the assay: PGE_2 *m/z* 351 > 271; 12-HETE *m/z* 319 >179.

Polyphenol analysis of urine, skin tissue and blister fluid

Urine was collected in HCl-washed flasks containing ascorbate (approx 1 g/L), and stored in aliquots at -80°C. Blister fluid and urine samples were enzymatically hydrolysed in line with previous literature [25], with adjustments. Following thawing at 5°C, urine was adjusted to pH 5.0 with NaOH (0.1 mol/L). A 40 µL aliquot of urine or blister fluid was combined with 4 µL NaH_2PO_4 solution (0.4 mol/L, pH 5.0) containing 200 g/L ascorbic acid and 1 g/L EDTA, 20 µL sodium acetate buffer (0.2 mol/L, pH 5.0) containing 0.012 µg taxifolin internal standard (Extrasynthese) and 5 U sulfatase (Type VIII, Sigma). Based on previous optimization work, 100 U and 200 U β-glucuronidase (Type X, Sigma) in NaH_2PO_4 (75 mmol/L, pH 6.8) were added to blister and urine samples, respectively, and incubated at 37°C for 45 and 60 min respectively. Samples were extracted with 3 x 250 µL ethyl acetate, with vortexing and centrifugal separation at each step. The combined extracts were dried under nitrogen and frozen at -80°C. Samples and reagents were handled on ice throughout extraction. Dried samples were reconstituted with 12 µL 20% (v/v) acetonitrile containing 1 g/L ascorbic acid, and sealed in a micro-well plate before analysis. With the exception of hippuric acids (which were poorly partitioned into ethyl acetate), the average extraction efficiency for catechins and phenolic acids reported (Table 2) was 84.7 ± 13.0%, whilst internal standard extraction efficiency was consistently at 100%.

Polyphenol-conjugates required extraction from biopsy tissue before enzyme hydrolysis. Additionally, Chu et al [26] highlighted problems using traditional ascorbate/EDTA solutions to stabilise catechins when handling tissue, owing to intrinsic iron content, and proposed the use of sodium dithionite, a reducing agent that does not take part in Fenton reactions. Biopsies were thawed at room temperature immediately before extraction then kept on ice throughout the procedure. Biopsies were washed in hexane to remove blood residue. A section of dermis was separated with a scalpel and weighed. To this, 250 µL

nitrogen-flushed chloroform containing 0.1 g/L butylated hydroxytoluene, and 250 µL sodium dithionite (0.3 mol/L) in sodium acetate buffer (0.2 mol/L, pH 5.0) were added. Samples were homogenized (Turrax micro homogenizer, IKA), with the sample being returned to ice at regular intervals, then vortexed and separated by centrifugation. The aqueous layer was removed and a second 250 µL aliquot of sodium dithionate in sodium acetate buffer added for a repeat extraction. Excess chloroform was removed via nitrogen drying, and the combined extracts mixed with 50 µL sodium acetate buffer (0.2 mol/L, pH 5.0) containing 0.012 µg taxifolin internal standard, 10 U sulfatase and 200 U β-glucuronidase. After 60 min incubation at 37^{o}C the extraction proceeded as for blisters/urine, using 3 x 400 µL ethyl acetate.

Samples were analysed using an Agilent 1200 SL HPLC system, which comprised a binary pump, degasser, well plate autosampler (5^{o}C), and column oven (35^{o}C) connected to a 6410 triple quadrupole LC-MS/MS. A 5 µL aliquot was injected onto a Kinetex C18 microbore column (2.6 µm, 150 x 2.1 mm; Phenomenex) running a binary gradient of LC-MS grade water (Millipore) vs. acetonitrile (Fisher) both with 0.2% (v/v) formic acid, at 0.3 mL/min. The gradient started at 5% acetonitrile for first 5.8 min, rose to 30% over 29.2 min, then increased to 95% acetonitrile over 2.4 min. This was held for a further 3.6 min to wash the column then returned to 5% acetonitrile over 3.6 min, re-equilibrating over a further 10.9 min. The flow was passed into an electrospray source, with gas temperature 350^{o}C, flowing at 11L/min, with a 30 psi nebulizer pressure. Analytes were detected in negative mode, using Dynamic MRM acquisition. Where available, analyte transmission and MS^2 transition parameters were individually optimized using standards. Internal standards for EC, (+)-catechin, EGC, ECG, EGCG and taxifolin were obtained from Extrasynthese. The retention times of gallocatechin, catechin gallate and gallocatechin gallate were determined by placing aqueous solutions of the relevant epi-isomers into a boiling water bath for 1 h. The chromatographic method did not distinguish between (+)- and (-)- enantiomers. The 3' and 4' mono-methylated forms of EC and EGC were obtained from Nacalai Tesque. Benzoic acid, 3-hydoxy benzoic acid, hippuric acid, 3,4-dihydroxyphenylacetic acid, and 3-(2′,4′-dihydroxyphenyl)propionic acid were obtained from Fluka and 4-hydroxy benzoic acid from Aldrich. Vanillic acid, 3,5 dihydroxy benzoic acid, gallic acid, syringic acid, 3- and 4-hydroxyphenyl acetic acids and 3-(3′-hydroxyphenyl)-propionic acid were obtained from Alfa Aesar. 3- and 4- methyl gallic acids were obtained from Apin Chemicals, and 2,4-dihydroxy benzoic acid, 2,4,6-trihydroxy benzoic acid, 2-hydroxyphenyl acetic acid, and 2-hydroxy hippuric acid from Acros Organics. All standards were of HPLC quality (>95%

purity). As commercial standards for hydroxyphenyl-valerolactones were not available, these were tentatively identified using previously reported MS^2 fragment patterns [27]. Analyte transmission and quantifying/qualifying MS^2 transition parameters were individually optimized using repeat injections of extracted urine. A total of 3 hydroxyphenyl-valerolactones were followed, namely 5-(3',4',5'-trihydroxyphenyl)-γ-valerolactone (M4; *m/z* 223 > 179+138), 5-(3',4'-dihydroxyphenyl)-valerolactone (M6; *m/z* 207> 163+122) and 5-(3',5'-dihydroxyphenyl)-valerolactone (M6': *m/z* 207> 163+123). M6 vs M6' retention time was differentiated using a synthetic M6 standard [28], which was used to quantify all hydroxyphenyl-valerolactones. Following peak integration, peak areas were normalised to internal standard. Whilst response factors for hippuric and benzoic acids were low (on column limit of quantitation of 3.45 pmol and 50 pmol respectively) the universally high levels of these compounds in urine, skin fluid and tissue meant quantification was achievable. The average on column limit of quantitation for all other compounds was 380 fmol ± 365 fmol.

Statistical analysis

Parametric data were tested using the paired t-test. The Wilcoxon signed rank test was used for data not satisfying assumptions of normality. Analyses were performed using StatsDirect (v2.7.7, StatsDirect Ltd.). Statistical significance was accepted at $P < 0.05$. Data are shown as mean ±SD and presented graphically as mean ±SEM.

RESULTS

Study subjects and compliance

Of the 16 subjects recruited to the study, 1 withdrew before completion for reasons unrelated to the study. The supplement was well tolerated; 4 subjects reported mild nausea following its ingestion. Post-supplementation, all 4 major epicatechins and their metabolites were present in urine at day 1, week 6 and week 12, from 14 of the 15 subjects completing the study (Table 2). Thus, 1 subject was non-compliant and 14 subjects (12 female) with a median age of 42.5 years (range 29-59) were included in study analyses.

Urinary metabolites

Of 35 tea phenolics and metabolites investigated, t-test analysis showed 20 components were significantly higher in week 12 urine samples compared to baseline ($P < 0.05$; n=13 due to absent record of one sample volume; Table 2), whilst 8 of these were consistently higher in

all participants. As well as several intact catechins, gallic acid and methylated metabolites, hydroxyphenyl-valerolactones, benzoic acid and its glycine conjugate, hippuric acid were all increased in urine following GTC consumption. Based on a daily intake of 129.2 μmol of EC and 482.9 μmol of EGC respectively, average urine excretion of all intact EC and EGC metabolites (including methylated forms) represented 6.1 and 7.1% of the dose, respectively.

Skin uptake

Skin fluid and biopsy (dermal) samples were taken from a subgroup of 10 participants at baseline and week 12, and subjected to qualitative analysis (Table 3). A total of 20 different phenolic compounds were observed in both sample types following supplementation. In blister fluid, hippuric, benzoic and 4-hydroxybenzoic acids were consistently present in all 10 participants. Interestingly, methylated gallic acid and several intact catechins and catechin ring-fission products were also observed, with 4-*O*-methyl gallic acid present in half of the subjects, and EGC, M4 and M6 hydroxyphenyl-valerolactones observed in fluid from 2 participants (Figure 1). Change from baseline was only statistically significant for benzoic acid ($P = 0.03$). Benzoic acid and its 4-hydroxylated form were also detected in all biopsy samples, whilst hippuric acid was only observed in 6 volunteers. Following supplementation, 4'-*O*-methylated EGC (n=4), EGC (n=1), EC (n=2), EGCG (n=1) and 4-*O*-methyl gallic acid (n=2), were observed in the dermis of certain volunteers.

UVR erythema dose-response

The median MED was 35 mJ/cm^2 at baseline and this was unchanged post-supplementation. Dose-response analysis showed a small increase in D_{30} from a mean ±SD of 28.0 ±7.7 mJ/cm^2 at baseline to 32.9 ±11.0 mJ/cm^2 post supplementation although this did not reach statistical significance ($P = 0.17$). However, GTC supplementation resulted in a significant decrease in erythema at the maximum UVR dose given (68 mJ/cm^2 erythemally weighted UVR) with ΔE falling from 100.2 ±21.4 at baseline to 81.2 ±23.2 post-supplementation ($P = 0.006$; Figure 2a). Area under curve analysis of the UVR-erythema dose-response showed a significant reduction in the erythema response post-supplementation ($P = 0.037$; Figure 2b).

Production of PGE_2

Pre-supplementation, mean ±SD concentration of PGE_2 in blister fluid from unexposed skin was 49.1 ±34.9 pg/μL. Production of PGE_2 significantly increased by ~2.3-fold following

exposure to 3 x MED UVR (P = 0.003; Figure 3a). Post-supplementation, PGE_2 in unexposed skin was similar to baseline (47.5 ±30.5 pg/µL). Exposure to the same UVR dose as at baseline produced a significant rise in PGE_2 (~2.4-fold; P = 0.001), with no significant difference in PGE_2 concentration between exposed skin at baseline and post-supplementation.

Production of 12-HETE

Pre-supplementation, the concentration of 12-HETE was significantly ~5-fold higher in UVR-exposed skin compared to unexposed skin (P = 0.0001). Following supplementation, the UVR-induced rise in 12-HETE was ~2.7-fold (P = 0.004; Figure 3b), with significantly lower concentration of 12-HETE in UVR-exposed skin compared to baseline (P = 0.01), and no significance difference in unexposed skin.

DISCUSSION

This human oral intervention study is novel in several respects: it evaluates cutaneous uptake of catechins and catechin metabolites, measures the impact of low dose green tea supplementation on pro-inflammatory UVR challenges to the skin, and examines the potential for protection through reduction of pro-inflammatory eicosanoid production. Our data provide the first evidence that GTC can be taken up into the skin following oral intake in humans, and indicate their complex skin incorporation pattern. Significant reduction was found in the cutaneous UVR-erythema dose-response, with greatest effect at higher doses, and this reduced inflammation may be attributable to the associated significant abrogation of UVR-upregulation of the potent pro-inflammatory 12-LOX metabolite, 12-HETE. In contrast, no evidence was found for mediation of the protection conferred by GTC through an impact on the COX-2 metabolite PGE_2.

The finding that GTC protect against UVR-induced erythema in humans is supported by previous studies of its topical application [13, 14] and a recent oral study [15]. In the latter, volunteers consumed a green tea beverage providing a much higher dose of 1402 mg catechins/day for 12 weeks and this protected against the threshold erythema induced by the single UVR dose tested. We found a small (non-statistically significant) effect at the threshold value D_{30} and demonstrated how oral supplementation with GTC can protect against the inflammation produced over a range of higher UVR doses, such as can be achieved when individuals over-expose themselves to sunlight. Since one large cup of green tea (250 ml) contains approximately 300 mg of catechins (EC, ECG, EGC and EGCG) then

the modest level of GTC intake in our study, i.e. approximately 540 mg, is seen to be readily achievable in daily life, and this is already consumed in many parts of the world.

Compliance with supplement ingestion was confirmed by demonstration of the urinary content of all four major categories of catechins in GTC, in all but one completing volunteer who was then excluded. As expected, the predominant intact catechins found in urine were not gallate esters, and the bioavailability of EC and EGC was in-line with reported studies [29, 30]. GTC intervention resulted in a significant increase in the excretion of the majority of intact catechins from baseline at day 1, and throughout the 12-week study, with no apparent accumulation or adaptive response during this time. However, the excretion of several general polyphenol breakdown products, including hippuric, benzoic and syringic acids, were only significantly elevated from baseline after 12 weeks intervention. Hippuric acid has previously been reported as the primary urinary metabolite following both green and black tea intervention, with participants excreting 3.8 ±0.3 and 4.2 ±0.3 mmol/24 h respectively, following a 6g/day intervention with tea solids [31]. Whilst hippuric acid was indeed the major urinary metabolite detected in our study (5.3 ±1.7 mmol/24 h post supplementation), its significant increase from baseline (at week 12) was only in the order of ~30%. Hippuric acid is a terminal metabolite of benzoic acid, which itself is a colonic breakdown product common to various phenolic substances. Hippuric acid excretion is therefore not unique to GTC *per se*, and its use as a biomarker of catechin consumption in free-living populations is limited. Hydroxyphenyl-valerolactones are catechin metabolites produced by colonic ring fission: M4 and M6' are predominantly derived from EGC, and M6 from EC [27]. Previously, Lee et al [30] reported M6 as accounting for 11.2% of EC dose in 8 human subjects, although considerable variability was observed in M6 plasma levels. Urinary M4 was reported to account for just 1.4% of the EGC dose. In our study, M6 accounted for ~24% of EC dose on average at week 12, with M4 and M6' accounting for ~4% and ~3% of the EGC dose. Levels of hydroxyphenyl-valerolactone excretion were significantly increased compared to baseline at day 1 and throughout the 12 week intervention, without a significant change in the level of excretion between acute and chronic GTC consumption. Therefore, we propose that these compounds may therefore serve as a useful biomarker of EC and EGC intake, over both the short and long term.

Detecting polyphenols and metabolites in tissues is a challenge since they bind to proteins, are at low levels and extraction methods are in development. We discovered that benzoic acid, its 4-hydroxyl form and its glycine-conjugate hippuric acid were typically present in both skin blister fluid and dermis. Wide inter-individual differences in oral

bioavailability and metabolism of polyphenols in foods are commonly reported [30, 32]. Consistent with this, intact catechins, gallic acids and catechin ring-fission products were observed in the skin fluid and dermal samples of some, but not all volunteers following GTC supplementation. However, significant post-supplement increases in blister fluid benzoic acid content indicates that volunteers experienced an increase in polyphenol metabolites in the target area as a consequence of GTC intervention, at least partially derived from metabolism by colonic microflora.

The reduced inflammatory response to UVR on GTC was associated with significant reduction in UVR-induction of the hydroxy fatty acid 12-HETE, the most abundant pro-inflammatory eicosanoid induced in human skin by UVR exposure. As well as being a leucocyte chemoattractant, this potent keratinocyte-derived mediator has been shown to cause a dose-related erythema when applied to human skin *in vivo* [33]. While more attention has focused on the role of PGE_2 in mediating erythema, COX-2 inhibitors only partially suppress UVR-erythema whilst completely suppressing UVR-induced PGE_2 [34], and LOX-derived mediators could also contribute [35]. Promotion of neutrophil and mononuclear cell migration into the dermis by 12-HETE may further augment the dermal vasodilatation and leucocytic infiltration through neutrophil release of vasodilatory nitric oxide, reactive oxygen species and chemokines [36]. Other antioxidant and cell signalling activities of GTC may also contribute to reduction of UVR-inflammation [1, 9], including through modulation of transcription factor NF-κB [37], nitric oxide [19, 38] and reduced formation/enhanced repair of UVR-induced DNA damage [10, 14, 39].

Our data indicate a direct effect of oral GTC on 12-LOX and/or possibly CYP isoforms producing 12-HETE following UVR, but not on COX-2 (Figure 4). This contrasts with studies in prostate and colon cancer cell lines, where the most abundant polyphenolic compound in tea, EGCG, inhibited protein and/or mRNA expression of COX-2 [40, 41]. However, EGCG, EGC and ECG are reported to inhibit LOX activity in colonic mucosa [16] and EC to inhibit activity of human platelet 12-LOX [17]. Topical green tea polyphenols (1-24 mg in 200 µL acetone) in mice reduced the activity of both LOX and COX enzymes after 12-*O*-tetradecanoylphorbol-13-acetate-induced tumour production, resulting in decreased PGE_2 and 12-HETE production [42]. Differences in findings are not unexpected between experimental models and human skin *in vivo*, and the catechin dose applied might also influence outcomes [43, 44].

Ultraviolet radiation is the principal aetiological factor in the majority of skin cancers, through its actions as a tumour-promoter, as well as an initiator of DNA damage that can lead

368 to mutagenesis, and repeated acute UVR insults to the skin are a risk factor for skin cancer
369 development. Interestingly, 12-HETE is over-expressed in a variety of human tumours,
370 including skin cancer, and it has tumour promoting ability which is thought to be conveyed
371 by its anti-apoptotic and angiogenic properties [45, 46]. Moreover, inhibitors of 12-HETE are
372 successful in protecting against tumorigenesis in cancer cell lines [47]. This adds to other
373 evidence suggesting GTC may have potential for development as an effective and safe
374 chemopreventive agent in humans, as in murine UVR-induced skin tumours [9].
375 In summary, this work indicates that following oral ingestion, green tea catechin
376 metabolites reach the skin target organ in humans, and that they suppress the biosynthesis of
377 eicosanoid 12-HETE and sunburn erythema induced by pro-inflammatory UVR challenges.
378 Manipulation of pro-inflammatory signalling pathways through supplementation with
379 nutritional bioactives is an attractive strategy for photoprotection in humans, and may
380 represent a complementary approach to topical sunscreens which are infrequently and
381 generally poorly applied [48]. Further studies are indicated to assess 12-LOX as a molecular
382 target of oral GTC in human skin, alongside scrutiny for their potential longer-term
383 photoprotective benefit.

ACKNOWLEDGEMENTS

Supported by grant BB/G005575/1 from the Biotechnology and Biological Sciences Research Council (BBSRC) Diet and Health Research Industry Club (DRINC). We thank Begonia Batolomé (Instituto de Investigación en Ciencias de la Alimentación CSIC-UAM, Madrid) for the kind provision of M6 hydroxyphenyl-valerolactone standard, and Andrew Healey (Analytical Centre, University of Bradford) for excellent technical assistance.

LER, GW, AN and REBW designed the research; GD, KAM, KAC, TPD and SB conducted the research; GD, KAM, KAC, TPD, MDF and AN analyzed data; LER, GD and MDF wrote the paper and all authors contributed to later drafts; LER, GW and AN had primary responsibility for final content. All authors read and approved the final manuscript. None of the authors declared a conflict of interest.

REFERENCES

1. Swindells K & Rhodes LE (2004) Influence of oral antioxidants on ultraviolet radiation-induced skin damage in humans. *Photodermatol Photoimmunol Photomed* **20,** 297-304.
2. Hawk JL, Murphy GM & Holden CA (1988) The presence of neutrophils in human cutaneous ultraviolet-B inflammation. *Br J Dermatol*, **118**, 27-30.
3. Strickland I, Rhodes LE, Flanagan BF *et al*. (1997) TNF-α and IL-8 are upregulated in the epidermis of normal human skin after UVB exposure: correlation with neutrophil accumulation and E-selectin expression. *J Invest Dermatol* **108**, 763-8.
4. Rhodes LE, Gledhill K, Masoodi M *et al.* (2009) The sunburn response in human skin is characterized by sequential eicosanoid profiles that may mediate its early and late phases. *FASEB J* **23**, 3947-56.
5. Navarro-Peran E, Cabezas-Herrera J, Sanchez-Del-Campo L *et al*. (2008) The anti-inflammatory and anti-cancer properties of epigallocatechin-3-gallate are mediated by folate cycle disruption, adenosine release and NF-kappaB suppression. *Inflamm Res* **57**, 472-8.
6. Rahman I, Biswas SK & Kirkham PA (2006) Regulation of inflammation and redox signaling by dietary polyphenols. *Biochem Pharmacol* **72,** 1439-52.
7. Lambert JD & Elias RJ (2010) The antioxidant and pro-oxidant activities of green tea polyphenols: A role in cancer prevention. *Arch Biochem Biophys* **501,** 65-72.
8. Hooper L, Kroon PA, Rimm EB (2008) Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* **88**, 38-50.
9. Afaq F & Mukhtar H (2006) Botanical antioxidants in the prevention of photocarcinogenesis and photoaging. *Exp Dermatol* **15**, 678-84.
10. Meeran SM, Akhtar S & Katiyar SK (2009) Inhibition of UVB-induced skin tumor development by drinking green tea polyphenols is mediated through DNA repair and subsequent inhibition of inflammation. *J Invest Dermatol* **129**, 1258-70.
11. Neveu V, Perez-Jimenez J, Vos F *et al*. (2010) Phenol-Explorer: an online comprehensive database on polyphenol contents in foods. *Database (Oxford).* **2010**, bap024..
12. Afaq F, Ahmad N & Mukhtar H (2003) Suppression of UVB-induced phosphorylation of mitogen-activated protein kinases and nuclear factor kappa B by green tea polyphenol in SKH-1 hairless mice. *Oncogene* **22**, 9254-64.

13. Katiyar SK, Matsui MS, Elmets CA *et al*. (1999) Polyphenolic antioxidant (-)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin. *Photochem Photobiol* **69,** 148-53.
14. Katiyar SK, Perez A & Mukhtar H (2000) Green tea polyphenol treatment to human skin prevents formation of ultraviolet light B-induced pyrimidine dimers in DNA. *Clin Cancer Res* **6**, 3864-9.
15. Heinrich U, Moore CE, De Spirt S *et al*. (2011) Green tea polyphenols provide photoprotection, increase microcirculation, and modulate skin properties of women. *J Nutr* **141**, 1202-8.
16. Hong J, Smith TJ, Ho CT *et al*. (2001) Effects of purified green and black tea polyphenols on cyclooxygenase and lipoxygenase-dependent metabolism of arachidonic acid in human colon mucosa and colon tumour tissues. *Biochem Pharmacol* **62**, 1175-83.
17. Schewe T, Sadik C, Klotz LO *et al*. (2001) Polyphenols of cocoa: inhibition of mammalian 15-lipoxygenase. *Biol Chem* **382**, 1687-96.
18. Singh T & Katiyar SK (2011) Green tea catechins reduce invasive potential of human melanoma cells by targeting COX-2, PGE_2 receptors and epithelial-to-mesenchymal transition. *PLoS One* **6,** e25224.
19. Rhodes LE, Belgi G, Parslew R *et al*. (2001) Ultraviolet-B-induced erythema is mediated by nitric oxide and prostaglandin E_2 in combination. *J Invest Dermatol* **117**, 880-5.
20. Pilkington SM, Watson RE, Nicolaou A *et al*. (2011) Omega-3 polyunsaturated fatty acids: photoprotective macronutrients. *Exp Dermatol* **20,** 537-43.
21. Chen ZY, Zhu QY, Wong YF *et al*. (1998) Stabilizing effect of ascorbic acid on green tea catechins. *J Agr Food Chem* **46**, 2512-6.
22. McArdle F, Rhodes LE, Parslew R *et al*. (2002) UVR-induced oxidative stress in human skin in vivo: effects of vitamin C supplementation. *Free Rad Biol Med* **33**, 1355-62.
23. Masoodi M & Nicolaou A (2006) Lipidomic analysis of twenty-seven prostanoids and isoprostanes by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* **20,** 3023-9.
24. Masoodi M, Mir AA, Petasis NA *et al*. (2008) Simultaneous lipidomic analysis of three families of bioactive lipid mediators leukotrienes, resolvins, protectins and related

hydroxy-fatty acids by liquid chromatography/electrospray tandem mass spectrometry. *Rapid Commun Mass Spectrom* **22**, 75-83.

25. Li C, Lee MJ, Sheng S *et al*. (2000) Structural identification of two metabolites of catechins and their kinetics in human urine and blood after tea ingestion. *Chem Res Toxicol* **13**, 177-84.
26. Chu KO, Wang CC, Chu CY *et al*. (2004) Determination of catechins and catechin gallates in tissues by liquid chromatography with coulometric array detection and selective solid phase extraction. *J Chrom B* **810**, 187-95.
27. Sang S, Lee MJ, Yang I *et al*. (2008) Human urinary metabolite profile of tea polyphenols analyzed by liquid chromatography/electrospray ionization tandem mass spectrometry with data-dependent acquisition. *Rapid Commun Mass Spectrom* **22,** 1567-78.
28. Sanchez-Patan F, Chioua M, Garrido I *et al*. (2011) Synthesis, analytical features, and biological relevance of 5-(3',4'-dihydroxyphenyl)-gamma-valerolactone, a microbial metabolite derived from the catabolism of dietary flavan-3-ols. *J Agric Food Chem* **59**, 7083-91.
29. Van Amelsvoort JJM, Van Het Hof KH, Mathot JNJJ *et al*. (2001) Plasma concentrations of individual tea catechins after a single oral dose in humans. *Xenobiotica* **31**, 891-901.
30. Lee MJ, Maliakal P, Chen L *et al*. (2002) Pharmacokinetics of tea catechins after ingestion of green tea and (⁻)-epigallocatechin-3-gallate by humans: formation of different metabolites and individual variability. *Cancer Epidem Biomar* **11**, 1025-32.
31. Mulder TP, Rietveld AG & van Amelsvoort JM (2005) Consumption of both black tea and green tea results in an increase in the excretion of hippuric acid into urine. *Am J Clin Nutr* **81**, 256S-60S.
32. Hong YJ & Mitchell AE (2004) Metabolic profiling of flavonol metabolites in human urine by liquid chromatography and tandem mass spectrometry. *J Agric Food Chem* **52**, 6794-801.
33. Wollard PM, Cunnigham FM, Murphy GM *et al*. (1989) A comparison of the proinflammatory effects of 12(R)- and 12(S)-hydroxy-5,8,10,14-eicosatetraenoic acid in human skin. *Prostaglandins* **38,** 465-71.
34. Kozuka T, Francis DM & Greaves MW (1983) Arachidonic acid metabolites and the skin. *Ann Acad Med Singapore* **12**, 87-91.

35. Ruzicka T (1992) The role of the epidermal 12-hydroxyeicosatetraenoic acid receptor in the skin. *Eicosanoids* **5**, S63-5.
36. Dowd PM, Kobza Black A *et al*. (1985) Cutaneous responses to 12-hydroxy-5,8,10,14-eicosatetraenoic acid (12-HETE). *J Invest Dermatol* **84**, 537-41.
37. Luo D, Min W, Lin XF *et al*. (2006) Effect of epigallocatechingallate on ultraviolet B-induced photodamage in a keratinocyte cell line. *Am J Chin Med* **34**, 911-22.
38. Galleano M, Pechanova O & Fraga CG (2010) Hypertension, nitric oxide, oxidants, and dietary plant polyphenols. *Curr Pharm Biotechnol* **11**, 837-48.
39. Nichols JA & Katiyar SK (2010) Skin photoprotection by natural polyphenols: anti-inflammatory, antioxidant and DNA repair mechanisms. *Arch Dermatol Res* **302**, 71-83.
40. Hussain T, Gupta S, Adhami VM *et al*. (2005) Green tea constituent epigallocatechin-3-gallate selectively inhibits COX-2 without affecting COX-1 expression in human prostate carcinoma cells. *Int J Cancer* **113**, 660-9.
41. Peng G, Dixon DA, Muga SJ *et al*. (2006) Green tea polyphenol (-)-epigallocatechin-3-gallate inhibits cyclooxygenase-2 expression in colon carcinogenesis. *Mol Carcinogenesis* **45**, 309-19.
42. Katiyar SK, Agarwal R, Wood GS *et al*. (1992) Inhibition of 12-O-tetradecanoylphorbol-13-acetate-caused tumor promotion in 7,12-dimethylbenz[a]anthracene-initiated SENCAR mouse skin by a polyphenolic fraction isolated from green tea. *Cancer Res* **52**, 6890-7.
43. Ahmad N, Feyes DK, Nieminen AL *et al*. (1997) Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *J Natl Cancer Inst* **89**, 1881-6.
44. Ahmad N, Gupta S & Mukhtar H (2000) Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor κB in cancer cells versus normal cells. *Arch Biochem Biophys* **376,** 338-46.
45. Tang DG, Chen YQ & Honn KV (1996) Arachidonate lipoxygenases as essential regulators of cell survival and apoptosis. *Proc Natl Acad Sci USA* **93**, 5241-6.
46. Winer I, Normolle DP, Shureiqi I *et al*. (2002) Expression of 12-lipoxygenase as a biomarker for melanoma carcinogenesis. *Melanoma Res* **12**, 429-34.
47. Greene ER, Huang S, Serhan CN *et al*. (2011) Regulation of inflammation in cancer by eicosanoids. *Prostaglandins Other Lipid Mediat* **96**, 27-36.

48. Rhodes LE & Diffey BL (1996) Quantitative assessment of sunscreen application technique by in vivo fluorescence spectroscopy. *J Soc Cosmet Chem* **47,** 109-15.

TABLE 1

Catechin and gallic acid content of green tea extract[1]

GTC	mg/450 mg capsule
Gallic acid	0.4 ± 0.0
Catechin	2.1 ± 0.0
Epicatechin	12.5 ± 0.2
Gallocatechin	12.4 ± 0.6
Epigallocatechin	49.3 ± 3.9
Catechin gallate	0.3 ± 0.0
Epicatechin gallate	26.0 ± 0.2
Gallocatechin gallate	4.5 ± 0.4
Epigallocatechin gallate	72.6 ± 3.1
Total	180.0 ± 8.3

[1] Values are mean ±SD. Contents of 3 capsules were homogenized and extracted in triplicate.

TABLE 2

Green tea catechins and their metabolites significantly increased in urine post-supplementation (n=13)

Compound	Amount excreted in urine (µmol)							
	Baseline		Day One		Week 6		Week 12	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
EC[1]	0.3	0.4	7[5]	4	5[5]	4	7[5]	5
3'-*O*-methyl EC[1]	0.06	0.08	0.6[5]	0.3	0.5[5]	0.4	0.6[5]	0.3
4'-*O*-methyl EC[1]	0.04	0.05	0.2[4]	0.2	0.2[4]	0.2	0.3[5]	0.2
ECG	0.000	0.002	0.01[5]	0.01	0.02[5]	0.01	0.01[4]	0.01
EGC[1]	0.2	0.4	22[5]	13	20[5]	16	25[5]	20
3'-*O*-methyl EGC[1]	0.01	0.04	0.2[5]	0.1	0.2[5]	0.2	0.2[5]	0.2
4'-*O*-methyl EGC[1]	0	0	8[4]	8	8[4]	9	9[4]	8
EGCG	0.00	0.01	0.06[5]	0.05	0.06[5]	0.04	0.08[4]	0.09
Catechin	0.01	0.02	0.2[5]	0.1	0.1[5]	0.1	0.2[5]	0.2
Gallocatechin	0	0	0.4[4]	0.5	0.3[3]	0.5	0.6[4]	0.6
Gallocatechin gallate	0	0	0.003	0.00	0	0	0.01[5]	0.02
Gallic acid	0.6	0.7	1	1	0.7[3]	0.5	1[4]	1
3-*O*-methyl gallic acid	0.6	0.6	1	1	0.9	0.8	1[3]	1
3-hydroxybenzoic acid	1	1	2	2	2	3	4[3]	4
M4 valerolactone[1,2]	0.3	0.4	30[4]	27	18[3]	25	21[4]	21
M6' valerolactone[2]	0.5	0.7	18[4]	16	12[4]	13	15[4]	15
M6 valerolactone	10	12	33[4]	25	27[3]	28	31[3]	24
Syringic acid	2	1	4	5	3	2	4[3]	4
Benzoic acid	81	83	95	60	101	132	140[3]	120
Hippuric acid	4000	2200	5100	2500	4300	1900	5300[3]	1700

[1] Increased excretion of metabolite from baseline to week 12 in 100% of subjects

[2] M4 and M6' hydroxyphenyl-valerolactone calculated as M6 equivalents

[3] $P < 0.001$ (2-tailed paired t-test), from baseline

[4] $P < 0.01$ (2-tailed paired t-test), from baseline

[5] $P < 0.05$ (2-tailed paired t-test), from baseline

TABLE 3

Presence of green tea catechins and their metabolites in skin blister fluid and tissue samples post-supplementation (week 12; n=10)[1]

	Skin blister fluid		Skin biopsy	
Compound	Change from average baseline value	Detected in n participants	Change from average baseline value	Detected in n participants
Benzoic acid	+36%[2]	10	ND	10
4-OH-benzoic acid	ND	10	ND	10
Hippuric acid	ND	10	ND	6
4-*O*-Me-gallic acid	ND	5	ND	2
EC	-	-	PPS	2
EGC	PPS	2	PPS	1
EGC-4-Me	-	-	PPS	4
EGCG	-	-	PPS	1
M4 valerolactone	PPS	2	-	-
M6 valerolactone	PPS	2	-	-

[1] Paired t-test performed only for compounds present in all subjects. EC, epicatechin; ECG, epicatechin-3-*O*-gallate; EGC, epigallocatechin; EGCG, epigallocatechin-3-*O*-gallate; ND, no significant difference; PPS, only present post-supplementation.

[2] $P = 0.03$ (2-tailed paired t-test) compared with baseline.

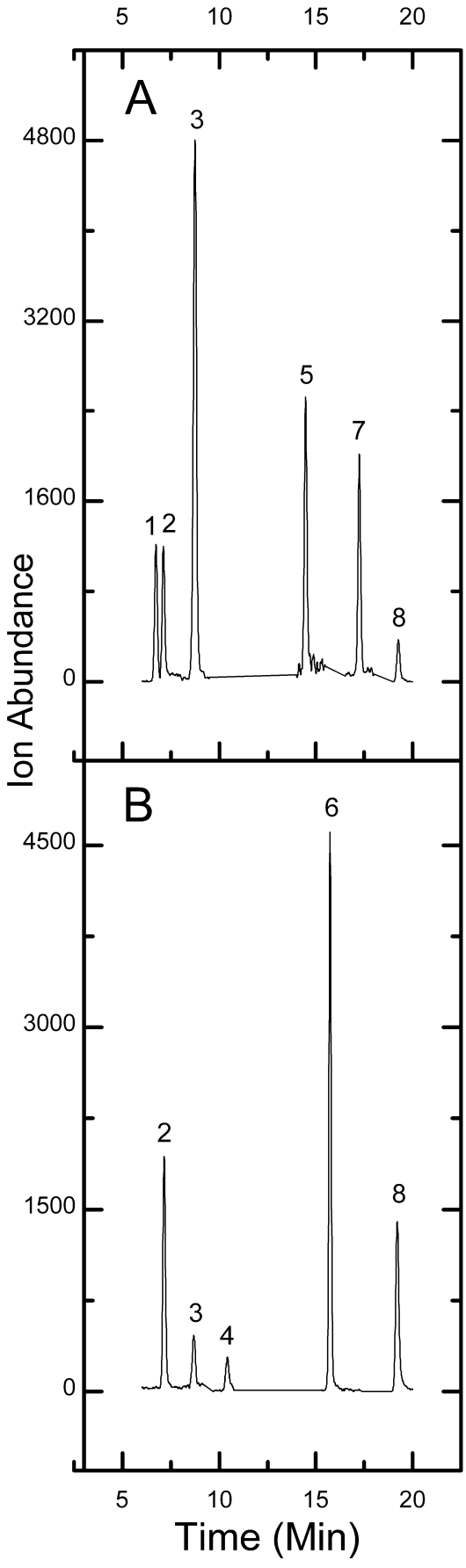
FIGURE LEGENDS

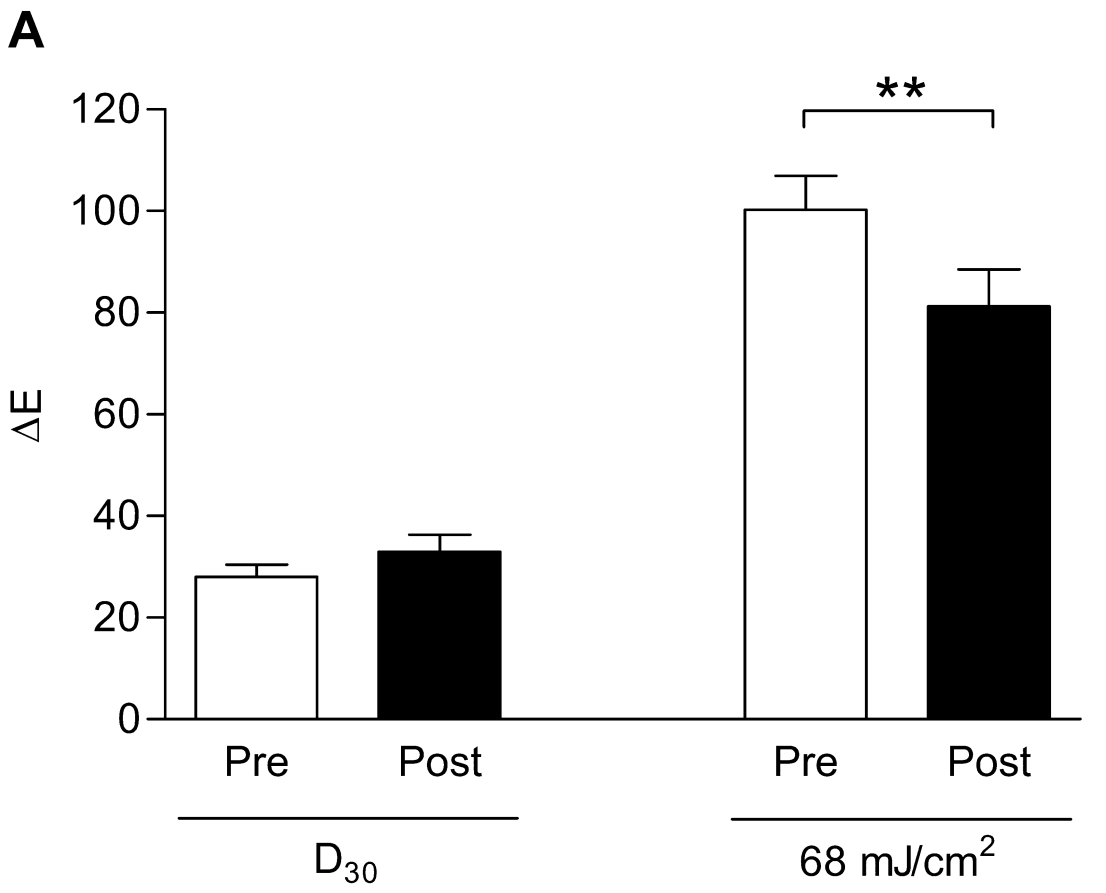
FIGURE 1. LC-MS/MS total ion current chromatogram of major compounds in skin fluid (A) and dermal skin tissue extract (B) post green tea catechin supplementation (week 12). Peak identities and multiple reaction monitoring m/z transitions are 1. M4 hydroxyphenyl-valerolactone (223>179); 2. 4-hydroxybenzoic acid (137>93); 3. Hippuric acid (178>134); 4. 2,4-dihydroxybenzoic acid (153>109); 5. M6 hydroxyphenyl-valerolactone (207>163); 6. Epicatechin (289>245); 7. 3-(3′-hydroxyphenyl)-propionic acid (165>121); 8. Benzoic acid (121>77).

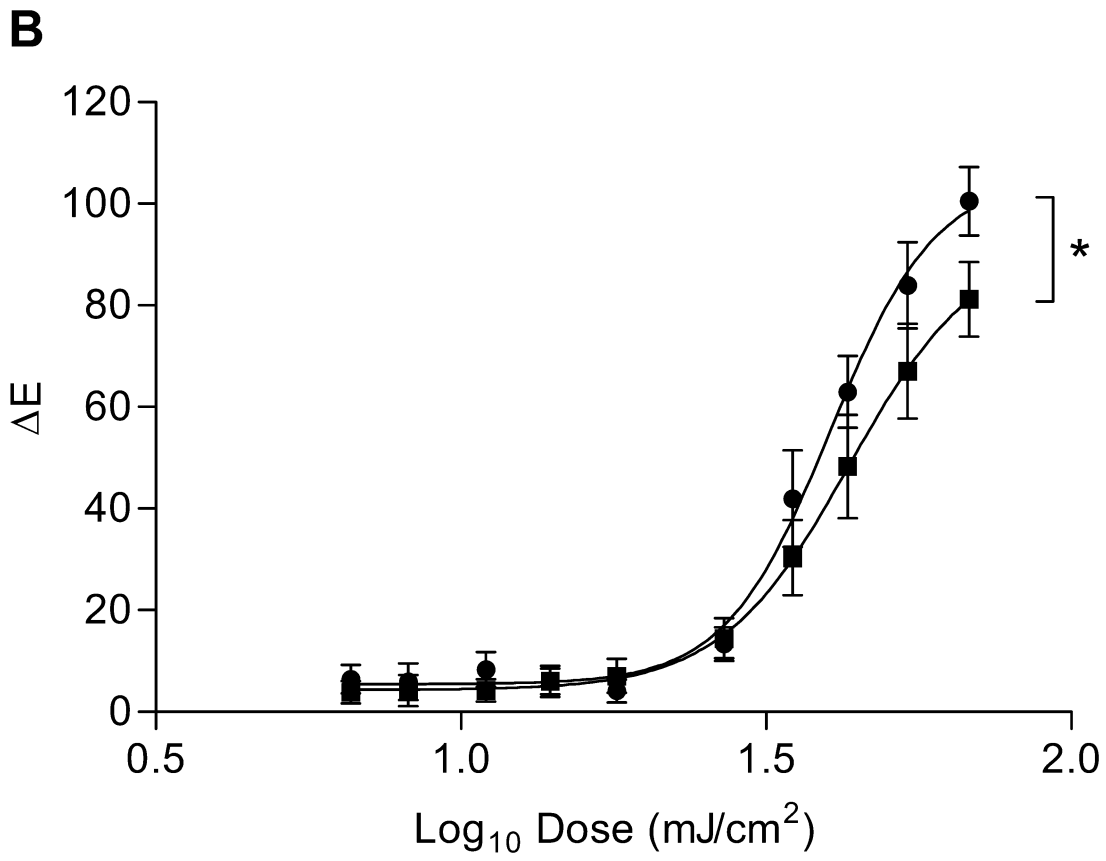
FIGURE 2. Impact of oral green tea catechins on UV radiation-induced erythema. Erythema response to solar simulated UV radiation at the D_{30} and the highest dose (68 mJ/cm^2), pre and post 12 weeks supplementation (A). UV radiation-erythema dose-response curves pre (circles) and post (squares) 12 weeks supplementation (B). Data are mean ± SEM, n=10. *P < 0.05, **P < 0.01 (2-tailed paired t-test).

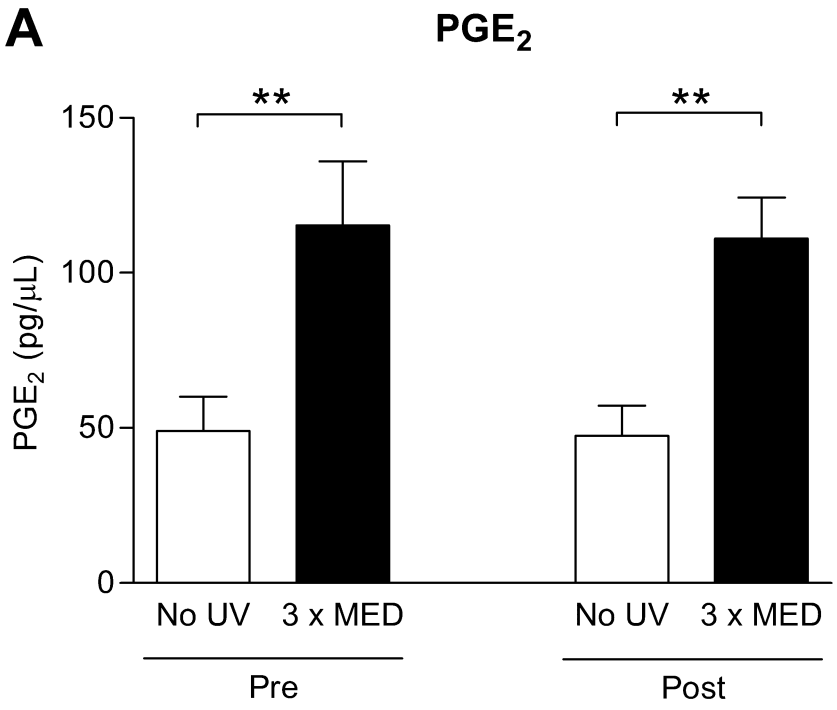
FIGURE 3. Concentration of PGE_2 (A; n=10) and 12-HETE (B; n=14) in skin fluid from unexposed skin and skin exposed to 3 x MED solar simulated UVR both pre- and post-supplementation for 12 weeks with green tea catechins. Data are mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 (2-tailed paired t-test for PGE_2, Wilcoxon signed rank test for 12-HETE). HETE, hydroxyeicosatetraenoic acid; MED, minimal erythema dose; PG, prostaglandin.

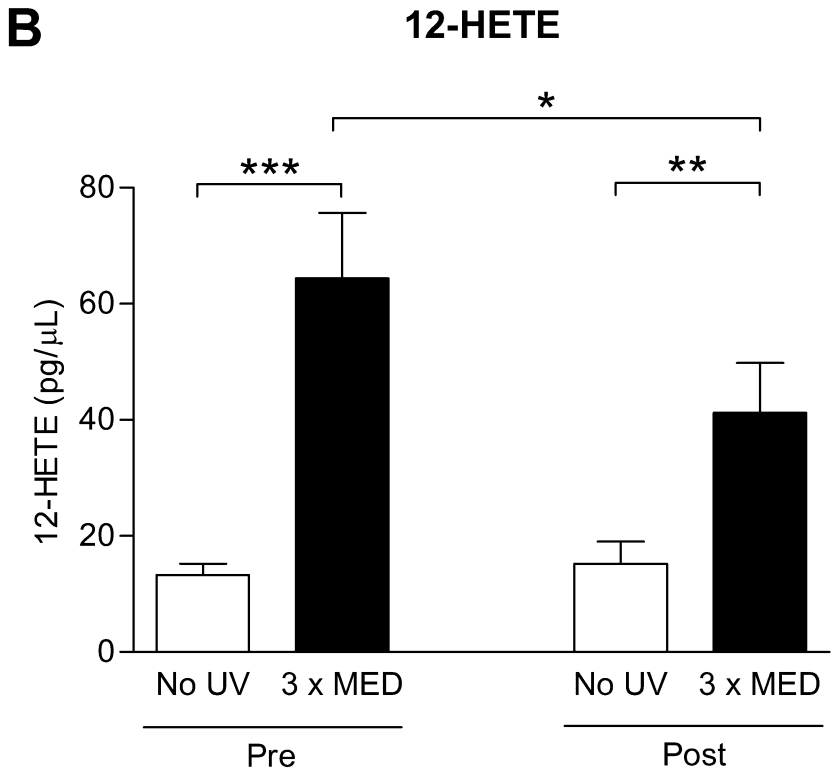
FIGURE 4. Schematic to illustrate proposed mechanism of the impact of GTC and metabolites on UV radiation-induced 12-HETE production. COX, cyclooxygenase; $cPLA_2$, cutaneous phospholipase A_2; GTC, green tea catechins; HETE, hydroxyeicosatetraenoic acid; LOX, lipoxygenase.

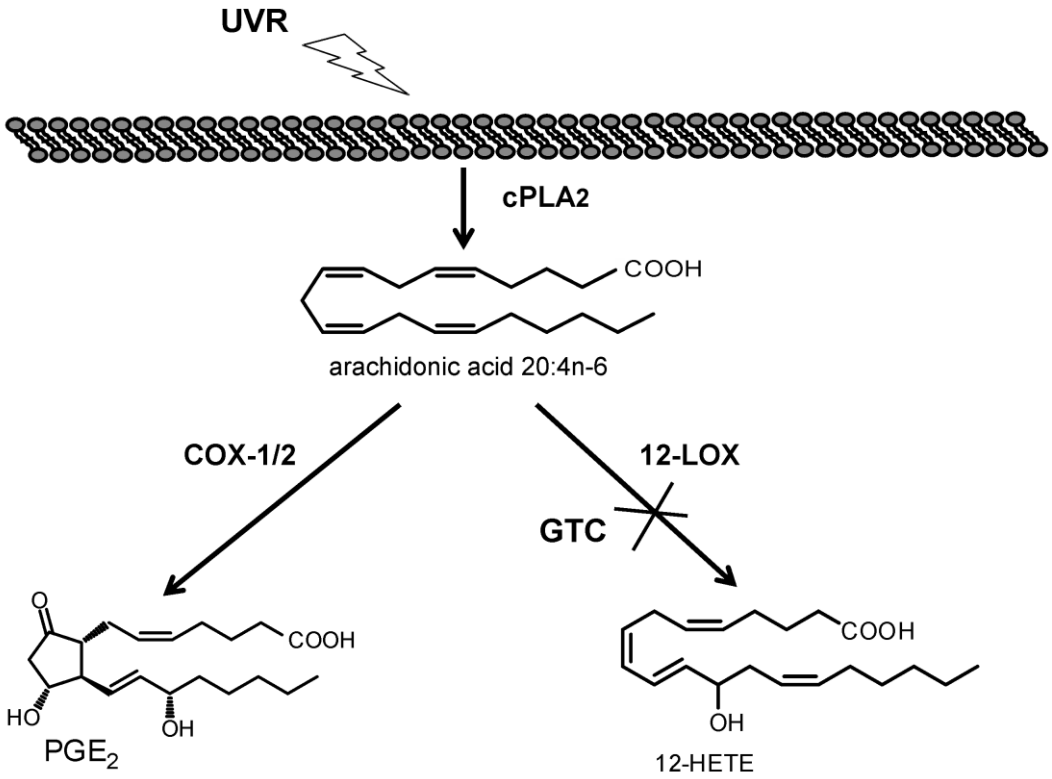

A
B
1
2
3
4
5
6
7
8
Ion Abundance
Time (Min)
0
1600
3200
4800
1500
3000
4500
5
10
15
20


A
**
120
100
80
60
40
20
0
ΔE
Pre
Post
Pre
Post
D30
68 mJ/cm2


B
120
100
80
60
40
20
0
ΔE
*
0.5
1.0
1.5
2.0
Log10 Dose (mJ/cm2)


A
PGE2
**
**
150
100
50
0
PGE2 (pg/μL)
No UV
3 x MED
No UV
3 x MED
Pre
Post


B
12-HETE
*
***
**
80
60
40
20
0
12-HETE (pg/μL)
No UV
3 x MED
No UV
3 x MED
Pre
Post


UVR
cPLA2
COOH
arachidonic acid 20:4n-6
COX-1/2
12-LOX
GTC
O
COOH
HO
OH
PGE2
COOH
OH
12-HETE