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

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(Submitted 20 September 2012 – Final revision received 26 November 2012 – Accepted 26 November 2012 – First published online 28 January 2013)

Abstract
Green tea catechins (GTC) reduce UV 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 cyclo-oxygenase- and lipoxygenase-produced mediators of UVR inflammation, PGE2 and 12-hydroxyeicosatetraenoic acid (12-HETE), respectively. In an open oral intervention study, sixteen healthy human subjects (phototype I/II) were given low-dose GTC (540 mg) with vitamin C (50 mg) daily for 12 weeks. Pre- and post-supplementation, the buttock skin was exposed to UVR and the resultant erythema quantified. Skin blister fluid and biopsies were taken from the unexposed and the UVR-exposed skin 24 h after a pro-inflammatory UVR challenge (three minimal erythema doses). Urine, skin tissue and fluid were analysed for catechin content and skin fluid for PGE2 and 12-HETE by liquid chromatography coupled to tandem MS. A total of fourteen completing subjects were supplement compliant (twelve female, median 42·5 years, range 29–59 years). 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 the skin tissue and fluid. AUC analysis for UVR erythema revealed reduced response post-GTC (P=0·037). Pre-supplementation, PGE2 and 12-HETE were UVR induced (P=0·003, 0·0001). After GTC, UVR-induced 12-HETE reduced from mean 64 (SD 42) to 41 (SD 32) pg/µl (P=0·01), while PGE2 was unaltered. Thus, GTC intake results in the incorporation of catechin metabolites into human skin associated with abrogated UVR-induced 12-HETE; this may contribute to protection against sunburn inflammation and potentially longer-term UVR-mediated damage.

Key words: Green tea catechins: Bioavailability: Skin: 12-Hydroxyeicosatetraenoic acid

UV radiation (UVR) in sunlight is a key environmental stressor that makes an impact on skin health. Acute effects include sunburn (an inflammatory response), immune suppression and photosensitivity, while repeated exposures lead to photoaging and photocarcinogenesis11. Sunburn is characterised clinically by erythema due to vasodilatation and, histologically, a dermal infiltrate of neutrophils and mononuclear cells is observed2,3. Activation of cutaneous phospholipase A2 by UVR is a key part of the inflammatory response, releasing membrane-esterified fatty acids, including arachidonic acid, which is subsequently metabolised by cyclo-oxygenase (COX), lipoxygenase (LOX) and cytochrome P450 isozymes to produce eicosanoids with vasodilatory and chemottractant properties4. Potent pro-inflammatory mediators, PGE2 and 12-hydroxyeicosatetraenoic acid (12-HETE), are the most abundant eicosanoids at the peak of the sunburn response, correlating with UVR up-regulated expression of COX-2 and 12-LOX in human skin4.

Polyphenols are plant-derived molecules, many exhibiting anti-inflammatory properties5,6. Their oral intake is associated with health benefits, including reduced risk of cancer

Abbreviations: 12-HETE, 12-hydroxyeicosatetraenoic acid; COX, cyclo-oxygenase; EC, (−)-epicatechin; ECG, (−)-EC-3-O-gallate; EGC, (−)-epigallocatechin; EGCg, (−)-EGC-3-O-gallate; GTC, green tea catechins; LOX, lipoxygenase; M4, 5(3′,4′,5′)-trihydroxyphenyl)-γ-valerolactone; M6, 5(3′,4′,6′-trihydroxyphenyl)-valerolactone; MED, minimal erythema dose; UVR, UV radiation.

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and CVD\textsuperscript{7,8}. Studies performed largely in experimental models suggest that polyphenols from various sources may protect the skin against the adverse effects of UVR, including carcinogenesis\textsuperscript{13,14}. Green tea is widely consumed worldwide and contains several polyphenols of the catechin family, i.e. green tea catechins (GTC), principally (\textsuperscript{-})-epicatechin (EC), (\textsuperscript{-})-EC-3-O-gallate (EGC), (\textsuperscript{-})-epigallocatechin (EGC) and (\textsuperscript{-})-EGC-3-O-gallate (EGCG\textsuperscript{111}). Emerging evidence suggests that GTC can protect against cutaneous damage. Specifically, oral GTC protected against UVR-induced skin inflammation and carcinogenesis in hairless mice\textsuperscript{122}, whilst in human subjects, topically applied GTC reduced UVR-induced DNA damage, erythema and leucocytic infiltrate\textsuperscript{13,14}, and oral green tea extract reduced skin erythema following a UVR challenge near the sunburn threshold\textsuperscript{155}. Some of these effects may be mediated via effects on COX and LOX isozymes, as EGC, ECG, EC and EG have been reported to reduce the production of PGE\textsubscript{2} and/or 12-HETE in experimental systems\textsuperscript{16–18} and oral GTC to reduce UVR-induced COX-2 protein expression and PGE\textsubscript{2} production in mouse epidermis\textsuperscript{140}. 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 human subjects, reflecting the challenges of their tissue assessment. Moreover, the molecular mechanism(s) underlying protection from UVR-induced inflammation are unexplored in human subjects. Potentially, this may be conveyed through an impact on key COX- and LOX-derived pro-inflammatory eicosanoids mediating the sunburn response, which additionally exhibit promoting properties in skin carcinogenesis\textsuperscript{4,19,20}. Thus, the aims of the present novel study were to examine directly in human subjects in vivo for evidence of cutaneous uptake of orally administered GTC, to evaluate for impact of GTC on sunburn over a range of pro-inflammatory UVR doses and to explore whether the underlying mechanism of protection could be GTC modulation of PGE\textsubscript{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 of 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 two cups of tea per d and currently pregnant or breastfeeding. Ethical approval was obtained from the North Manchester Research Ethics Committee (reference 98/H1006/79). Written informed consent was obtained from the participants, and the study adhered to the 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 three capsules, each containing 450 mg green tea extract (total 1350 mg tea, 540 mg GTC; Table 1) and two 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\textsuperscript{21}, oral vitamin C supplementation alone has been shown to have no impact on UVR erythema\textsuperscript{22}. Supplements were provided by Nestec Limited and packaged by Laboratoire LPH. Compliance was assessed by counting the residual capsules in the dispensed containers that the volunteers were asked to return and through analysis of 24 h urine samples collected from all volunteers before and after 1 d, 6 weeks and 12 weeks supplementation.

**UV radiation 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 Limited). Irradiance of the light source was measured 10 cm from the source prior to each irradiation, using a radiometer (model IL 730A; International Light) calibrated for use with the light source, to ensure consistency of the 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 ten doses of solar-simulated UVR (erythemally weighted doses 6·6–68 mJ/cm\textsuperscript{2}) to the 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\times the individual’s pre-supplementation MED were given to sites on one buttock; this dose was selected in order to provoke an inflammatory response sufficient to significantly elevate cutaneous eicosanoid levels\textsuperscript{4}.

**Table 1. Catechin and gallic acid content of green tea extract**\textsuperscript{*} (Mean values and standard deviations)

<table>
<thead>
<tr>
<th>Component</th>
<th>Content (mg/450 mg capsule)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>0·4</td>
</tr>
<tr>
<td>Catechin</td>
<td>2·1</td>
</tr>
<tr>
<td>Epicatechin</td>
<td>12·5</td>
</tr>
<tr>
<td>Gallocatechin</td>
<td>12·4</td>
</tr>
<tr>
<td>Epigallocatechin</td>
<td>49·3</td>
</tr>
<tr>
<td>Catechin gallate</td>
<td>0·3</td>
</tr>
<tr>
<td>Epicatechin gallate</td>
<td>26·0</td>
</tr>
<tr>
<td>Gallocatechin gallate</td>
<td>4·5</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>72·6</td>
</tr>
<tr>
<td>Total</td>
<td>180·0</td>
</tr>
</tbody>
</table>

GTC, green tea catechins. *Contents of three capsules were homogenised and extracted in triplicate.
**Quantification of the UV radiation-induced erythemic responses**

The intensity of erythema (erythema index) was quantified using a reflectance instrument (Diastron) in ten 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_{0.0}, 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 × MED) and -protected areas of upper buttock skin were sampled at baseline and post-supplementation; UVR exposures were limited to one buttock and the other buttock provided the unexposed skin and blister fluid samples. Skin punch biopsies (5 mm diameter) were taken after intra-dermal injection of lignocaine, as described, snap-frozen and stored at −80°C. Suction blisters were raised using suction cups with a central aperture diameter of 1 cm and vacuum of 250 mmHg, as described previously. Skin blister fluid was aspirated with a twenty-three-gauge needle, snap-frozen in liquid N2 and stored at −80°C until analysis. Samples destined for polyphenol analysis were combined with 25 μl NaH2PO4 (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 MS, as described previously. 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-glucuronide and 80 ng 12-HETE-d_4; Cayman Chemicals) were then added and the 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 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 (approximately 1 g/l) and stored in aliquots at −80°C. Blister fluid and urine samples were enzymatically hydrolysed in line with previous literature, adjusting with 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 NaH2PO4 solution (0·4 mol/l, pH 5·0) containing 200 g/l ascorbic acid and 1 g/l EDTA, and 20 μl sodium acetate buffer (0·2 mol/l, pH 5·0) containing 0·012 μg taxifolin internal standard (Extrasynthese) and 5 U (1·39 nkat) sulphotase (Type VIII; Sigma). Based on previous optimisation work, 100 and 200 U (0·087 and 0·175 nkat) β-glucuronidase (Type X; Sigma) in NaH2PO4 (75 mmol/l, pH 6·8) were added to the blister and urine samples, respectively, and incubated at 37°C for 45 and 60 min, respectively. Samples were extracted with 3 × 250 μl ethyl acetate, with vortexing and centrifugal separation at each step. The combined extracts were dried under N2 and frozen at −80°C. Samples and reagents were handled on ice throughout extraction. Dried samples were reconstituted with 12 μl of 20% (v/v) acetonitrile containing 1 g/l ascorbic acid, and sealed in a microwell 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 (SD 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. highlighted problems using traditional ascorbate/EDTA solutions to stabilise catechins when handling tissue, owing to intrinsic Fe 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 and 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 N2-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 homogenised (Turax 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 N2 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 (2·78 nkat) sulphatase and 200 U (0·175 nkat) β-glucuronidase. After 60 min incubation at 37°C, the extraction proceeded as for blisters/urine using 3 × 400 μl ethyl acetate.

Samples were analysed using an Agilent 1200 SL HPLC system (Agilent Technologies), which comprised a binary pump, degasser, well plate autosampler (5°C) and column oven (35°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 × 2·1 mm; Phenomenex) running a binary gradient of LC-MS-grade water (Millipore) v. 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 and then increased to 95% acetonitrile over 2·4 min. This was held for a further 3·6 min to wash the column and 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°C, flowing at 11 litres/min and with a 30 pounds per square inch (psi) nebuliser pressure. Analytes were detected in negative mode using dynamic multiple reaction monitoring acquisition. Where available, analyte transmission and MS<sup>2</sup> transition parameters were individually optimised using standards. Internal standards for EC, (±)-catechin, EGC, ECG, EGCG and taxifolin were obtained from Extrasynthese. The retention times of gallocatechin, catechin, EGC, ECG, EGCG and taxifolin were obtained from Aldrich. Vanillic acid, 3,5-dihydroxybenzoic acid, 3- and 4-hydroxy 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).

A total of three 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<sub>2</sub>; m/z 207 > 163 + 123). M6 <em>v</em>. M6<sub>2</sub> 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 the internal standard. Whilst response factors for hippuric and benzoic acids were low (on column limit of quantification of 3-45 and 50 pmol, respectively), the universally high levels of these compounds in urine, skin fluid and tissue meant that quantification was achievable. The average on column limit of quantification for all other compounds was 380 (SD 365) fmol.

### Statistical analysis

Parametric data were tested using the paired <em>t</em> test. The Wilcoxon signed-rank test was used for data not satisfying assumptions of normality. Analyses were performed using StatsDirect (version 2.7.7, StatsDirect Limited). Statistical significance was accepted at <em>P</em> < 0.05. Data are shown as means and standard deviations and presented graphically as means with their standard errors.

### Results

#### Study subjects and compliance

Of the sixteen subjects recruited to the study, one withdrew before completion for reasons unrelated to the study. Of the remaining fifteen, one subject was excluded (due to a medication change) and one subject was unable to complete the urinary collection. As a result, the study was terminated after collection of baseline urine samples from fourteen subjects. Additional information about the study sample is given in Table 1.
The supplement was well-tolerated; four subjects reported mild nausea following its ingestion. Post-supplementation, all four major EC and their metabolites were present in the urine at day 1 and weeks 6 and 12 from fourteen of the fifteen subjects completing the study (Table 2). Thus, one subject was non-compliant and fourteen subjects (twelve female), with a median age of 42·5 years (range 29–59 years), were included in the study analyses.

**Urinary metabolites**

Of the thirty-five tea phenolics and metabolites investigated, $t$ test analysis showed that twenty components were significantly higher in week 12 urine samples compared with baseline ($P<0.05$; $n = 13$ due to absent record of one sample volume; Table 2), whilst eight 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 the urine following GTC consumption. Based on a daily intake of 129·2 mol of EC and 482·9 mol of EGC, 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 ten participants at baseline and week 12, and subjected to qualitative analysis (Table 3). A total of twenty different phenolic compounds were observed in both sample types following supplementation. In blister fluid, hippuric, benzoic and 4-hydroxybenzonic acids were consistently present in all ten participants. Interestingly, methylated gallic

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**Table 3. Presence of green tea catechins and their metabolites in skin blister fluid and tissue samples post-supplementation (week 12; $n = 10$)**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Skin blister fluid</th>
<th>Skin biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Change from average baseline value</td>
<td>Detected in $n$ participants</td>
</tr>
<tr>
<td>Benzoic acid</td>
<td>+36%*</td>
<td>10</td>
</tr>
<tr>
<td>4-OH-Benzoic acid</td>
<td>ND</td>
<td>10</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>ND</td>
<td>10</td>
</tr>
<tr>
<td>4-O-Me-gallic acid</td>
<td>ND</td>
<td>5</td>
</tr>
<tr>
<td>EC</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EGC</td>
<td>PPS</td>
<td>2</td>
</tr>
<tr>
<td>EGC-4-Me</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>EGCg</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>M4 valerolactone</td>
<td>PPS</td>
<td>2</td>
</tr>
<tr>
<td>M6 valerolactone</td>
<td>PPS</td>
<td>2</td>
</tr>
</tbody>
</table>

ND, no significant difference; EC, epicatechin; PPS, only present post-supplementation; EGC, epigallocatechin; EGCG, epigallocatechin-3-O-gallate; M4, 5-(3,4,5-trihydroxyphenyl)-γ-valerolactone; M6, 5-(3,4-dihydroxyphenyl)-valerolactone.

* Value was significantly different compared with baseline ($P<0.03$; two-tailed paired $t$ test).

† Paired $t$ test performed only for compounds present in all subjects.

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**Fig. 1.** Liquid chromatography–MS/MS total ion current chromatogram of (a) major compounds in skin fluid and (b) dermal skin tissue extract 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-hydroxybenzonic acid (137 > 93); 3, hippuric acid (178 > 134); 4, 2,4-dihydroxybenzonic acid (153 > 109); 5, M6 hydroxyphenyl-valerolactone (207 > 163); 6, epicatechin (289 > 245); 7, 3-(3-hydroxyphenyl)-propionic acid (165 > 121); and 8, benzoic acid (121 > 77).
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 two participants (Fig. 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 hip puric acid was only observed in six 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.

**UV radiation erythema dose–response**

The median MED was 35 mJ/cm² at baseline and this was unchanged post-supplementation. Dose–response analysis showed a small increase in D30 from a mean of 28·0 (sd 7·7) mJ/cm² at baseline to 32·9 (sd 11·0) mJ/cm² 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/cm² erythemally weighted UVR), with ∆E falling from 100·2 (sd 21·4) at baseline to 81·2 (sd 23·2) post-supplementation (P=0·006; Fig. 2(a)). AUC analysis of the UVR erythema dose–response showed a significant reduction in the erythema response post-supplementation (P=0·037; Fig. 2(b)).

**Production of PGE2**

Pre-supplementation, mean concentration of PGE2 in blister fluid from unexposed skin was 49·1 (sd 34·9) pg/µl. Production of PGE2 significantly increased by approximately 2·3-fold following exposure to 3× MED UVR (P=0·003; Fig. 3(a)). Post-supplementation, PGE2 in unexposed skin was similar to baseline (47·5 (sd 30·5) pg/µl). Exposure to the same UVR dose as at baseline produced a significant rise in PGE2 (approximately 2·4-fold; P=0·001), with no significant difference in PGE2 concentration between exposed skin at baseline and post-supplementation.

**Production of 12-hydroxyeicosatetraenoic acid**

Pre-supplementation, the concentration of 12-HETE was significantly approximately five-fold higher in UVR-exposed skin compared with unexposed skin (P=0·0001). Following supplementation, the UVR-induced rise in 12-HETE was approximately 2·7-fold (P=0·004; Fig. 3(b)), with significantly lower concentration of 12-HETE in UVR-exposed skin compared with the baseline (P=0·01), and no significant difference in unexposed skin.

**Discussion**

The present 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 human subjects 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 up-regulation 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 PGE2.

The finding that GTC protects against UVR-induced erythema in human subjects 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/d 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 D30 and demonstrated
Effects of green tea catechins on human skin

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...
thought to be conveyed by its anti-apoptotic and angiogenic properties\(^{45,46}\). Moreover, inhibitors of 12-HETE are successful in protecting against tumorigenesis in cancer cell lines\(^{47}\). This adds to the other evidence, suggesting that GTC may have potential for development as an effective and safe chemopreventive agent in human subjects, as in murine UVR-induced skin tumours\(^9\).

In summary, the present study indicates that following oral ingestion, GTC metabolites reach the target organ skin in human subjects, and that they suppress the biosynthesis of eicosanoid 12-HETE and sunburn erythema induced by pro-inflammatory UVR challenges. Manipulation of pro-inflammatory signalling pathways through supplementation with nutritional bioactives is an attractive strategy for photoprotection in human subjects and may represent a complementary approach to topical sunscreens, which are infrequently and generally poorly applied\(^{48}\). Further studies are indicated to assess 12-LOX as a molecular target of oral GTC in human skin, alongside scrutiny for their potential longer-term photoprotective benefit.

**Acknowledgements**

This study was supported by grant BB/G005575/1 from the Biotechnology and Biological Sciences Research Council Diet and Health Research Industry Club. 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. L. E. R., G. W., A. N. and R. E. B. W. designed the research; G. D., K. A. M., K. A. C., T. P. D. and S. B. conducted the research; G. D., K. A. M., K. A. C., T. P. D., M. D. F. and A. N. analysed data; L. E. R., G. D. and M. D. F. wrote the paper and all authors contributed to later drafts; L. E. R., G. W. and A. N. had primary responsibility for final content. All authors read and approved the final manuscript. None of the authors declared a conflict of interest.

**References**


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**Fig. 4.** Schematic to illustrate proposed mechanism of the impact of green tea catechins (GTC) and metabolites on UV radiation (UVR)-induced 12-hydroxyeicosatetraenoic acid (12-HETE) production, COX, cyclo-oxygenase; cPLA\(_2\), cutaneous phospholipase A\(_2\); 12-LOX, 12-lipoxygenase.

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**Diagram**

- **Arachidonic acid 20 : 4n-6**
- **COX-1/2**
- **12-LOX**
- **GTC**
- **PGE\(_2\)**
- **12-HETE**
- **COOH**
- **HO**
- **OH**
- **cPLA\(_2\)**

- **Schematic**

- **Reactive oxygen species**
- **Chemo-dilatation and leucocytic infiltration through neutrophil release**
- **Promotion of neutrophil and mononuclear cell migration into human platelet 12-LOX**

- **Topical green tea polyphenols**

- **EGCG, EGC and ECG are reported to inhibit LOX**

- **While more attention has been focused on the role of PGE\(_2\) in repair of UVR-induced DNA damage**

- **UVR erythema, whilst completely suppressing UVR-induced inflammation, COX-2 inhibitors only partially suppress**

- **Differences in bol-13-acetate-induced tumour production, resulting in both LOX and COX enzymes after 12-**

- **(1–24 mg in 200**

- **A new therapy for the prevention of skin cancer, and it has tumour-promoting ability, which is**

- **Eight potential preventive agents, as in murine UVR-induced skin tumours**

- **A new agent for photoprotection and the catechin dose applied**

- **In 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**

- **However, EGCG, EGC and ECG are reported to inhibit LOX activity in colonic mucosa**

- **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**

- **Differences in findings are not unexpected between experimental models and human skin in vivo, and the catechin dose applied might also influence outcomes**

- **UVR 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 to mutagenesis, and repeated acute UVR insults to the skin are a risk factor for skin cancer development. Interestingly, 12-HETE is over-expressed in a variety of human tumours, including skin cancer, and it has tumour-promoting ability, which is**

- **In summary, the present study indicates that following oral ingestion, GTC metabolites reach the target organ skin in human subjects, and that they suppress the biosynthesis of eicosanoid 12-HETE and sunburn erythema induced by pro-inflammatory UVR challenges. Manipulation of pro-inflammatory signalling pathways through supplementation with nutritional bioactives is an attractive strategy for photoprotection in human subjects and may represent a complementary approach to topical sunscreens, which are infrequently and generally poorly applied**

- **Further studies are indicated to assess 12-LOX as a molecular target of oral GTC in human skin, alongside scrutiny for their potential longer-term photoprotective benefit.**

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**References**


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31. Mulder TP, Rietveld AG & van Amelvoort 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, 2606S–2608S.


