

The sunburn response in human skin is characterized by sequential eicosanoid profiles that may mediate its early and late phases

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ABSTRACT Sunburn is a commonly occurring acute inflammatory process, with dermal vasodilatation and leukocyte infiltration as central features. Ultraviolet (UV) B-induced hydrolysis of membrane phospholipids releases polyunsaturated fatty acids, and their subsequent metabolism by cyclooxygenases (COXs) and lipoxygenases (LOXs) may produce potent eicosanoid mediators modulating different stages of the inflammation. Our objective was to identify candidate eicosanoids formed during the sunburn reaction in relation to its clinical and histological course. We exposed skin of healthy humans ($n=32$) to UVB and, for 72 h, examined expression of proinflammatory and anti-inflammatory eicosanoids using LC/ESI-MS/MS, and examined immunohistochemical expression of COX-2, 12-LOX, 15-LOX, and leukocyte markers, while quantifying clinical erythema. We show that vasodilatory prostaglandins (PGs) PGE₂, PGF_{2 α} , and PGE₃ accompany the erythema in the first 24–48 h, associated with increased COX-2 expression at 24 h. Novel, potent leukocyte chemoattractants 11-, 12-, and 8-monohydroxy-eicosatetraenoic acid (HETE) are elevated from 4 to 72 h, in association with peak dermal neutrophil influx at 24 h, and increased dermal CD3⁺ lymphocytes and 12- and 15-LOX expression from 24 to 72 h. Anti-inflammatory metabolite 15-HETE shows later expression, peaking at 72 h. Sunburn is characterized by overlapping sequential profiles of increases in COX products followed by LOX products that may regulate subsequent events and ultimately its resolution.—Rhodes, L. E., Gledhill, K., Masoodi, M., Haylett, A. K., Brownrigg, M., Thody, A. J., Tobin, D. J., Nicolaou, A. The sunburn response in human skin is characterized by sequential eicosanoid profiles that may mediate its early and late phases. *FASEB J.* 23, 3947–3956 (2009). www.fasebj.org

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ACUTE ULTRAVIOLET (UV) B EXPOSURE of the skin produces sunburn, an inflammatory response evident

visually as erythema and characterized histologically by a mixed dermal neutrophilic and lymphocytic infiltrate. Peak vasodilatation is reached at 24 h (1) and neutrophil infiltration at 24–48 h (2–4), while dermal infiltration by T lymphocytes may exhibit a later time course (5). Whereas prostaglandin (PG) E₂ and nitric oxide have roles in the vasodilatation (1, 6, 7), and cytokines, including interleukin-8, contribute to the leukocyte infiltration (3), the mediation of induction and resolution of the sunburn response is incompletely understood and is anticipated to involve a wider range of eicosanoids.

Skin displays highly active metabolism of polyunsaturated fatty acids (PUFAs), resulting in the production of eicosanoids that modulate physiological processes at low concentrations and elicit inflammatory reactions at higher levels (8). Activation of membrane phospholipase A₂ by UVB effects release of fatty acids, notably the ω (n)-6 PUFA arachidonic acid (AA; 20:4n-6), and potentially the n-3 PUFA eicosapentaenoic acid (EPA; 20:5n-3), which has also been detected in human skin (9) (Scheme 1). Subsequently, these PUFAs may be metabolized by cyclooxygenases (COXs) and lipoxygenases (LOXs) to a wide range of eicosanoids and other lipid mediators. High COX-2, 12-LOX, and 15-LOX activity is reported in epidermal cells, and infiltrating neutrophils may possess 5-LOX activity (8, 10). Considerable species differences are reported for LOX expression (11), and additional fatty acid metabolism by cytochrome P-450 and nonenzymatic oxidation may

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contribute to production of a wide diversity of metabolites (12).

Following UVB exposure, COX-2 expression was found to be elevated in human skin when assessed at the single time point of 24 h (13). Recently, UVB was reported to modulate 12-LOX and 15-LOX expression in a keratinocyte cell line, with reciprocal regulation of these LOXs, suppressing 12-LOX, while up-regulating 15-LOX (14). However, there is scant information concerning the effects of UVB on LOX expression in human skin. Furthermore, through technical limitations, only a restricted range of UV-induced skin eicosanoids have been examined to date; consequently, detailed characterization of eicosanoids possessing properties that potentially mediate the course of human sunburn is lacking. Recently developed lipidomic assays based on electrospray ionization-liquid chromatography-tandem mass spectrometry (ESI-LC-MS-MS) now permit assessment of a wide range of metabolites (15, 16).

We examined the expression and temporal relationship of candidate eicosanoids that may mediate both the early and later phases of vasodilatation and leukocyte infiltration, and potentially also the resolution phase, of the sunburn response, in 32 healthy humans. The clinical (skin erythema) and histological (leukocyte infiltration) response was followed for 72 h, with detailed characterization of cutaneous eicosanoid expression, and direct assessment of UVB regulation of COX-2, 12-LOX, and 15-LOX expression over this period. We specifically examined for vasodilatory prostanoids derived through COX metabolism of both ω -6 and ω -3 PUFAs (*i.e.*, AA and EPA), and for a range of hydroxyeicosatetraenoic acids (HETEs) expressing chemoattractant properties for neutrophils and lymphocytes (17, 18) or anti-inflammatory properties (11).

MATERIALS AND METHODS

Subjects

The study group comprised 32 healthy white Caucasian subjects of mean age 40 yr (range 19 to 58 yr; 20 female) and sun-reactive skin types I-IV. Exclusions from the study were sunbathing or sunbed use in the prior 3 mo, photosensitizing or anti-inflammatory medication, and history of skin cancer or photosensitivity disorders. The study conformed to the Declaration of Helsinki 2000, North Manchester Research Ethics Committee approval was obtained, and all volunteers gave written informed consent.

UV irradiation

The UV radiation (UVR) source used was a fluorescent UVB lamp, (Waldmann UV6; emission 290–400 nm, peak 313 nm; Herbert Waldmann GmbH, Villingen-Schwenningen, Germany). Minimal erythema dose (MED) testing was performed by applying a geometric series of UV doses ranging from 13 to 128 mJ/cm² of erythemally weighted UVR in a horizontal row to the upper buttock skin. The MED was assessed at 24 h, and defined as the lowest dose of UVB that resulted in detectable erythema. Volunteers were given MED-related doses in order

to standardize the UVB challenge according to individual erythema sensitivity. Three sites on the buttock skin were irradiated with 4 times the individual's MED of UVB, a dose sufficient to produce a well-defined clinical and histological inflammatory response. These sites were utilized for erythema time course determination and suction blister fluid sampling ($n=22$ subjects) or skin-punch biopsy sampling for immunohistochemical investigations ($n=10$ subjects).

Assessment of erythema responses

Erythema responses were quantified using a reflectance instrument (Diastron, Andover, UK), which gives an erythema index related to the blood content of the superficial dermis. Triplicate measurements were taken from the test sites and adjacent unirradiated skin, and the means were calculated.

Suction blister sampling

Suction blister fluid was sampled from unirradiated skin and from skin at 4, 18, 24, 48, and 72 h following a dose of UVB (4×MED). Each volunteer provided a sample from an unirradiated site on one buttock, and samples from 3 irradiated sites on the contralateral buttock, each site being sampled on one occasion. Half of the volunteers were sampled at baseline, 4, 18, and 24 h, and the others at baseline, 24, 48, and 72 h, with erythema readings taken at the same time points. Suction blister cups with 1-cm central aperture were applied with a vacuum pressure of 250 mm Hg, as described previously (19). When blisters had formed (after ~90 min), the fluid was aspirated with a 23-gauge needle, snap-frozen, and stored at -80°C until analysis.

Lipidomic analysis

Lipidomic analyses were performed as described by Masoodi *et al.* (15, 16). Briefly, blister fluid samples (typical volumes 50–200 μl) were diluted with methanol-water (15% wt/wt) to a final volume of 3 ml. Internal standards (40 ng PGB2-*d4* and 80 ng 12-HETE-*d8*; Cayman Chemicals, Ann Arbor, MI, USA) were added to each sample. The resultant solutions were acidified to pH 3 and immediately applied to preconditioned solid-phase extraction cartridges (C18-E; Phenomenex, Macclesfield, UK) to extract the lipid mediators. Chromatographic analysis was performed on a C18 column (Luna 5 μm ; Phenomenex) using a Waters Alliance 2695 HPLC pump coupled to an ESI triple-quadrupole Quattro Ultima mass spectrometer (Waters, Elstree, UK). 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 of prostanoids and hydroxy fatty acids: PGE₂, m/z 351 > 271; 13,14-dihydro-15-keto PGE₂, m/z 351 > 333, 349 > 113; PGF_{2 α} , m/z 353 > 193; 13,14-dihydro-15-keto PGF_{2 α} , m/z 353 > 113; PGE₁, m/z 353 > 317; 13,14-dihydro-15-keto-PGE₁, m/z 353 > 335; PGE₃, m/z 349 > 269; LTB₄, m/z 335 > 195; 11-HETE, m/z 319 > 167; 12-HETE, m/z 319 > 179; 8-HETE, m/z 319 > 155; and 15-HETE, m/z 319 > 175. Results are expressed as picograms of metabolite per milligram of protein, based on calibration lines constructed with commercially available eicosanoid standards (Cayman Chemicals). Protein content was estimated using the Bio-Rad protein assay kit with BSA as standard (Bio-Rad, Hemel Hempstead, UK).

Immunohistochemical assessment

Skin-punch biopsies (5-mm diameter) were sampled from unirradiated skin and from skin at 4, 24, and 72 h following

exposure to $4 \times \text{MED}$ of UVB (see above). Biopsies were snap-frozen and stored at -80°C prior to immunohistochemical analysis. Frozen sections ($6 \mu\text{m}$) were air-dried before fixation in ice-cold acetone (Fisher Scientific UK, Loughborough, UK) for 10 min. Endogenous peroxidase was quenched in hydrated tissue sections with $0.5\% \text{H}_2\text{O}_2$ (Sigma-Aldrich Co., Gillingham, UK) before blocking with 10% goat serum (Sigma-Aldrich) for 1 h. Primary antibody was added to the sections at the required dilution and incubated overnight at 4°C . The primary antibodies used in this study were as follows. Neutrophil elastase: MAB 1056, dilution 1:70 (Chemicon Europe, Chandlers Ford, UK); CD3^+ : NCL-CD3, dilution 1:40 (NovoCastra Reagents, Leica Biosystems, Newcastle on Tyne, UK); 12-LOX: ab23678, dilution 1:200 (Abcam plc, Cambridge, UK); 15-LOX: 10004454, dilution 1:100 (Cayman Chemical, Boldon, UK); COX-2: ALX-210-712-1, dilution 1:100 (Alexis Biochemicals, Lausen, Switzerland). After washing in PBS, skin sections were incubated with biotinylated anti-mouse secondary antibody (DakoCytomation, Ely, UK) for 30 min, washed in PBS, and incubated with streptavidin peroxidase (DakoCytomation) for 30 min. Sections were developed using aminoethylcarbazole chromogen (Vector Laboratories, Peterborough, UK) for 5–10 min, washed in distilled water, lightly counterstained in Meyers hematoxylin (Sigma-Aldrich), and mounted in glycerol mounting medium (DakoCytomation). The sections were assessed using a Nikon Eclipse 80i microscope at $\times 200$ view and a Nikon Digital Sight DS-U1 camera (Nikon UK, Kingston on Thames, UK).

Quantification of immunohistochemical staining

Slides were masked prior to assessment. Neutrophils and CD3^+ cells were counted in 3 randomly selected epidermal and dermal high-power fields (hpf) at $\times 400$. This technique was employed for each individual at each of 4 time points. COX and LOX staining was quantified in terms of relative staining intensity, according to the following scoring system: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = intense staining. 12-LOX, 15-LOX, and COX-2 were graded in epidermal and dermal hpf at $\times 200$. Assessment was made for each individual at each of the 4 time points.

Statistical analysis

Data were analyzed with StatsDirect (Stats Direct, Altrincham, UK). Nonparametric data were analyzed using the Kruskal-Wallis test with adjustment for multiple comparisons, and parametric data were analyzed using 1-way ANOVA with Bonferroni correction. All results are compared with levels in non-UV-exposed tissue unless otherwise stated. Statistical significance was accepted at the $P < 0.05$ level.

RESULTS

UVB-erythema time course

Erythema was clinically visible at 4 h following UVB exposure, with a significant elevation of the erythema index at this time point, rising from a mean \pm SE baseline value of 39.9 ± 3.6 to 97.2 ± 13.4 at 4 h ($P < 0.001$). The erythema index peaked at 18–24 h after UVB exposure (160.8 ± 6.3 and 171.1 ± 3.6 , respectively) and remained elevated at 72 h (156.6 ± 6.4 , $P < 0.001$) (Fig. 1).

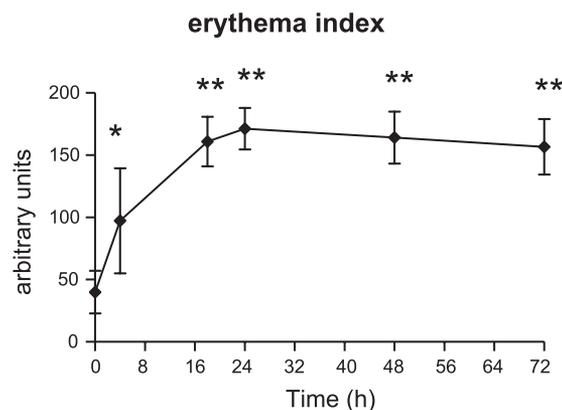


Figure 1. Time course of sunburn erythema in human skin. Buttock skin of healthy human volunteers ($n=22$) was exposed to a UVB dose equivalent to $4 \times$ each volunteer's sunburn threshold ($4 \times \text{MED}$). Erythema response was quantified using a reflectance technique, giving an erythema index (EI) proportional to the blood content of the superficial dermis. Results are expressed as mean \pm SE EI in unexposed skin and in skin at 4, 18, 24, 48, and 72 h after UVB exposure. * $P < 0.05$, ** $P < 0.001$ vs. unexposed skin.

Effect of UVB on COX-mediated eicosanoid production

PGE₂ and metabolite

Analysis of suction blister fluid from unexposed skin revealed baseline levels of PGE_2 and its inactive metabolite 13,14-dihydro-15-keto PGE_2 of 26.83 ± 3.1 and 31.7 ± 2.63 pg/mg protein, respectively. Following UVB, PGE_2 levels were above the baseline level at all time points 4–72 h, with statistically significant elevation at 18 and 24 h (32.81 ± 5.13 and 38.05 ± 3.95 pg/mg protein, respectively; $P=0.02$) (Fig. 2A), while 13,14-dihydro-5-keto PGE_2 was significantly elevated at 72 h (50.45 ± 6.86 pg/mg protein; $P=0.02$) (Fig. 2B).

PGF_{2 α} and metabolite

Baseline levels of $\text{PGF}_{2\alpha}$ and 13,14-dihydro-15-keto $\text{PGF}_{2\alpha}$ were 30.47 ± 3.53 and 0.81 ± 0.81 pg/mg protein, respectively. Prostaglandin $\text{F}_{2\alpha}$ also showed a significant rise at 18 h (44.84 ± 6.66 pg/mg protein; $P=0.03$), but in contrast to PGE_2 , it returned to baseline levels by 24 h (30.1 ± 2.57 pg/mg protein) (Fig. 2C). However, the inactive $\text{PGF}_{2\alpha}$ metabolite 13,14-dihydro-15-keto PGF_2 , was significantly elevated at 48 and 72 h (17.62 ± 4.39 pg/mg protein, $P=0.003$ and 13.65 ± 5.02 pg/mg protein, $P=0.049$, respectively) (Fig. 2D).

PGE₁ and metabolite

Baseline levels of PGE_1 and 13,14-dihydro-15-keto PGE_1 were 17.14 ± 3.58 and 7.60 ± 2.18 pg/mg protein, respectively. Following UVB, the same overall trend was noted as for the above prostanoids. Although PGE_1

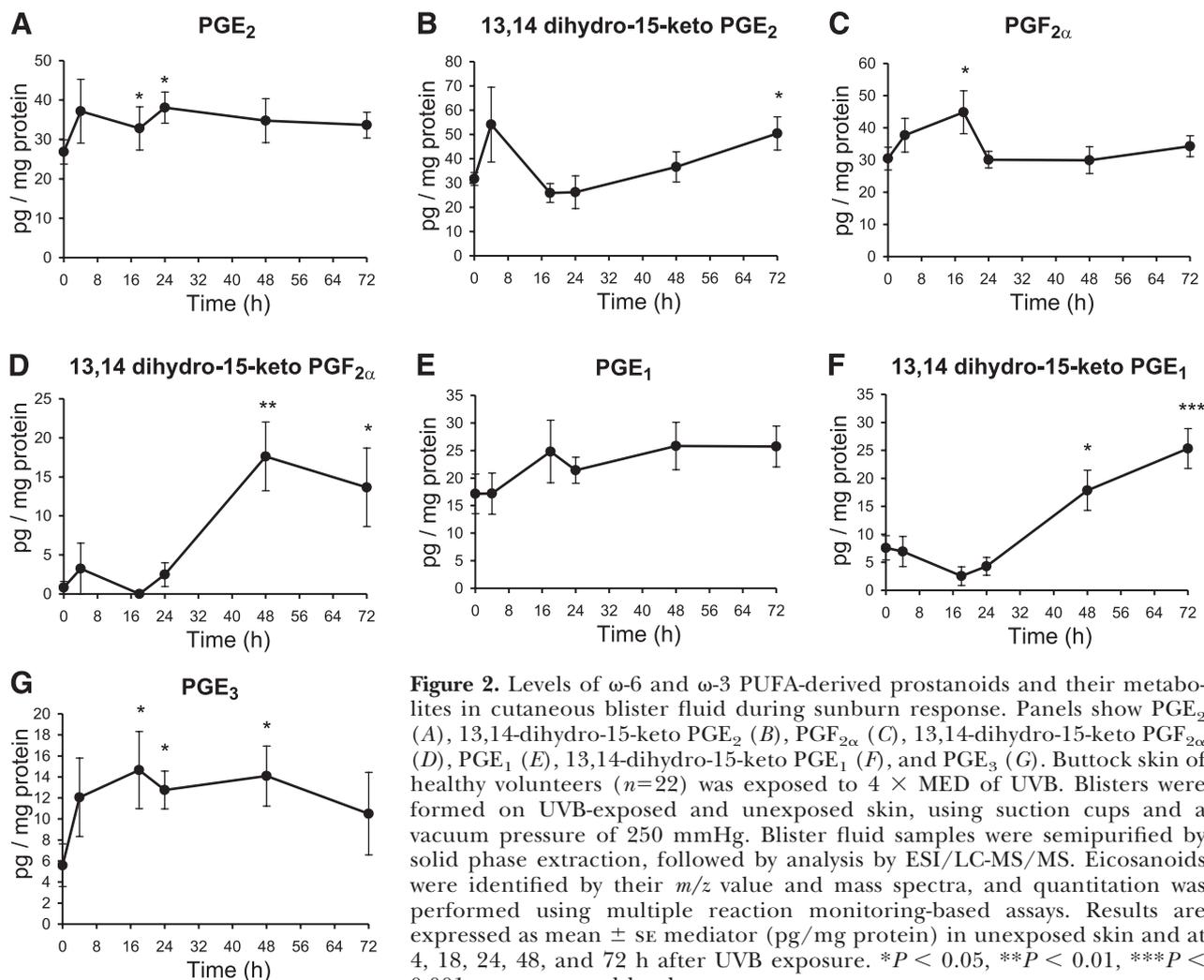


Figure 2. Levels of ω -6 and ω -3 PUFA-derived prostanooids and their metabolites in cutaneous blister fluid during sunburn response. Panels show PGE₂ (A), 13,14-dihydro-15-keto PGE₂ (B), PGF_{2 α} (C), 13,14-dihydro-15-keto PGF_{2 α} (D), PGE₁ (E), 13,14-dihydro-15-keto PGE₁ (F), and PGE₃ (G). Buttock skin of healthy volunteers ($n=22$) was exposed to $4 \times$ MED of UVB. Blisters were formed on UVB-exposed and unexposed skin, using suction cups and a vacuum pressure of 250 mmHg. Blister fluid samples were semipurified by solid phase extraction, followed by analysis by ESI/LC-MS/MS. Eicosanoids were identified by their m/z value and mass spectra, and quantitation was performed using multiple reaction monitoring-based assays. Results are expressed as mean \pm SE mediator (pg/mg protein) in unexposed skin and at 4, 18, 24, 48, and 72 h after UVB exposure. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. unexposed levels.

concentration was not significantly elevated over baseline levels (Fig. 2E), its inactive metabolite 13,14-dihydro-15 keto-PGE₁ was significantly elevated at 48 and 72 h postexposure (17.86 ± 3.58 pg/mg protein, $P=0.02$, and 25.33 ± 3.55 pg/mg protein, $P=0.0001$, respectively) (Fig. 2F).

PGE₃

Baseline level of this ω -3-PUFA-derived prostanooid was 5.60 ± 2.03 pg/mg protein. Following UVB, PGE₃ increased by 4 h (12.06 ± 3.73 pg/mg protein) and still appeared elevated above baseline at 72 h (10.5 ± 3.93 pg/mg protein), with significant elevations found at 18, 24, and 48 h (16.65 ± 3.66 pg/mg protein, $P=0.04$; 12.76 ± 1.81 pg/mg protein, $P=0.04$; and 14.08 ± 2.85 pg/mg protein, $P=0.03$, respectively) (Fig. 2G).

Effect of UVB on LOX-mediated eicosanoid production

8-HETE

Baseline level was 0.64 ± 0.64 pg/mg protein; following UVB, 8-HETE became significantly elevated at 24 h

(13.78 ± 3.69 pg/mg protein, $P=0.003$) and showed a progressive rise to 72 h (31 ± 6.11 pg/mg protein, $P<0.0001$) (Fig. 3A).

11-HETE

Baseline level was 1.17 ± 0.7 pg/mg protein; 11-HETE was significantly elevated at the first time point of assessment following UVB, *i.e.*, 4 h (5.54 ± 1.94 pg/mg protein, $P=0.02$), peaked at 18 h (10.68 ± 2.45 pg/mg protein, $P<0.0001$), and remained elevated to 72 h (10.54 ± 3.78 pg/mg protein, $P=0.0004$) (Fig. 3B).

12-HETE

Baseline level was 20.25 ± 9.82 pg/mg protein. Following UVB, 12-HETE became significantly elevated at 18 h (105.85 ± 34.64 pg/mg protein, $P=0.007$), with a progressive rise at subsequent time points and peak level at 72 h (251.51 ± 60.85 pg/mg protein, $P<0.0001$) (Fig. 3C).

15-HETE

Baseline value of 15-HETE was 2.16 ± 1.37 pg/mg protein. Following UVB, 15-HETE showed significant

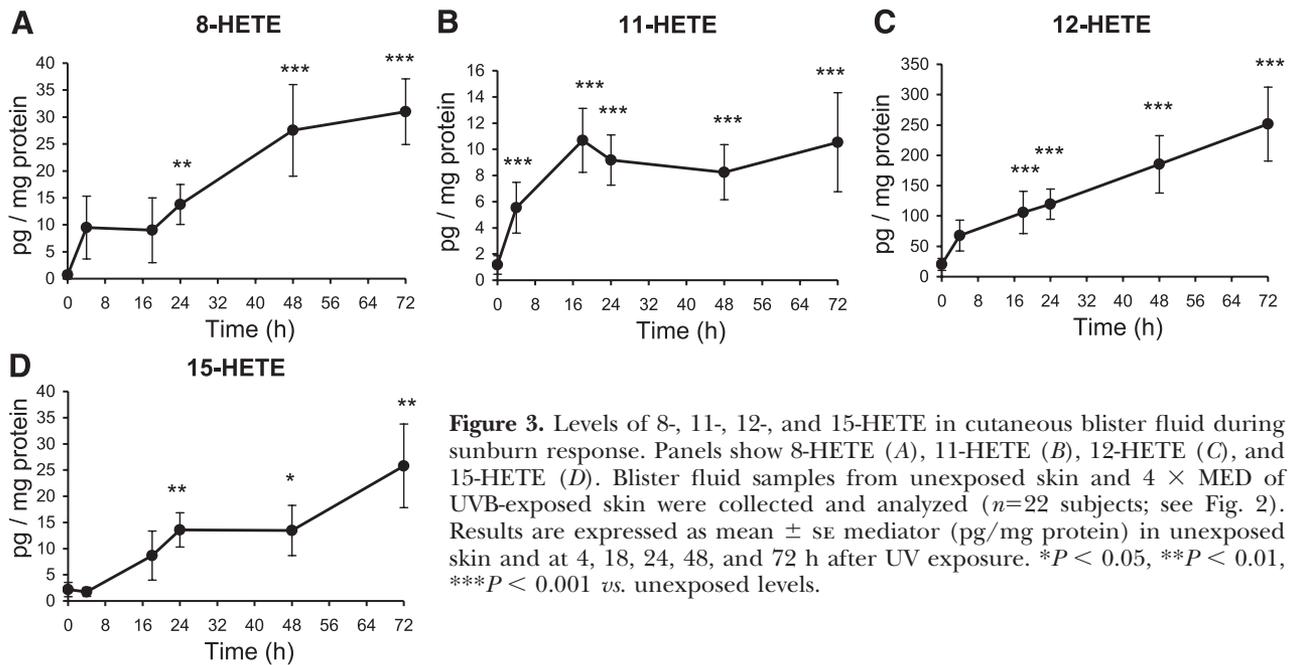


Figure 3. Levels of 8-, 11-, 12-, and 15-HETE in cutaneous blister fluid during sunburn response. Panels show 8-HETE (A), 11-HETE (B), 12-HETE (C), and 15-HETE (D). Blister fluid samples from unexposed skin and $4 \times$ MED of UVB-exposed skin were collected and analyzed ($n=22$ subjects; see Fig. 2). Results are expressed as mean \pm SE mediator (pg/mg protein) in unexposed skin and at 4, 18, 24, 48, and 72 h after UV exposure. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. unexposed levels.

elevation by 24 h (13.58 ± 3.26 pg/mg protein, $P=0.007$), and was still rising at the last (72 h) time point (25.8 ± 8 pg/mg protein, $P=0.002$) (Fig. 3D).

LTB₄

LTB₄ was undetectable at baseline and at all time points following UVB.

Cutaneous leukocytic infiltration following UVB

Neutrophils

Neutrophil polymorphs, identified by their staining with antineutrophil elastase antibody, were present in unirradiated epidermal and dermal samples (0.97 ± 0.65 and 3.07 ± 1.45 cells/hpf, respectively) (Fig. 4). Dermal neutrophil levels peaked at 24 h after UV exposure (mean 8.6 /hpf; $P=0.03$) returning to baseline levels by 72 h. The number of epidermal neutrophils was not significantly different from baseline levels.

CD3⁺ cells

Baseline levels of *CD3⁺* lymphocytes were 0.53 ± 0.3 and 10.8 ± 1.66 cells/hpf in epidermis and dermis, respectively (Fig. 5). The number of *CD3⁺*-positive cells was significantly elevated above baseline at 24 h after UVB exposure (mean 22.63 ± 5.11 cells per hpf; $P=0.02$), and remained significantly elevated at 72 h after UVB (mean 24.7 ± 2.97 cells/hpf; $P < 0.001$ vs. baseline). No significant changes were seen in epidermal levels of *CD3⁺* cells.

Cutaneous expression of COX-2, 12-LOX, and 15-LOX following UVB

COX-2

Baseline staining intensity of COX-2 was 0.14 ± 0.14 and 0.71 ± 0.29 in the epidermis and dermis, respectively (Fig. 6). A significant increase in COX-2 staining intensity was observed at 24 h after UVB exposure, for both dermis and epidermis (1.43 ± 0.37 and 1.29 ± 0.36 ; $P=0.04$ and $P=0.03$, respectively).

12-LOX

Relative baseline staining intensity of 12-LOX was 0.14 ± 0.14 and 1.43 ± 0.3 for the epidermis and dermis, respectively (Fig. 7). This was significantly elevated in the dermis at 4 h after UVB exposure (mean 2.29 ± 0.29 ; $P=0.02$), and in the epidermis at 24 and 72 h after UVB (mean 1 ± 0.22 and 1.29 ± 0.36 ; $P=0.02$ and $P=0.005$, respectively).

15-LOX

Relative baseline staining intensity for 15-LOX was 0.75 ± 0.25 and 1.13 ± 0.23 in the epidermis and dermis, respectively (Fig. 8). In the dermis a significant increase relative to baseline was seen at 24 h after UVB exposure (2.75 ± 0.16 $P=0.001$). The epidermis also showed a significant increase in staining intensity at 24 h after UVB (2 ± 0.33 ; $P=0.01$), and this remained significantly elevated at 72 h after UVB (1.63 ± 0.18 ; $P=0.02$).

DISCUSSION

This study provides for the first time a detailed characterization of the candidate eicosanoid mediators of the

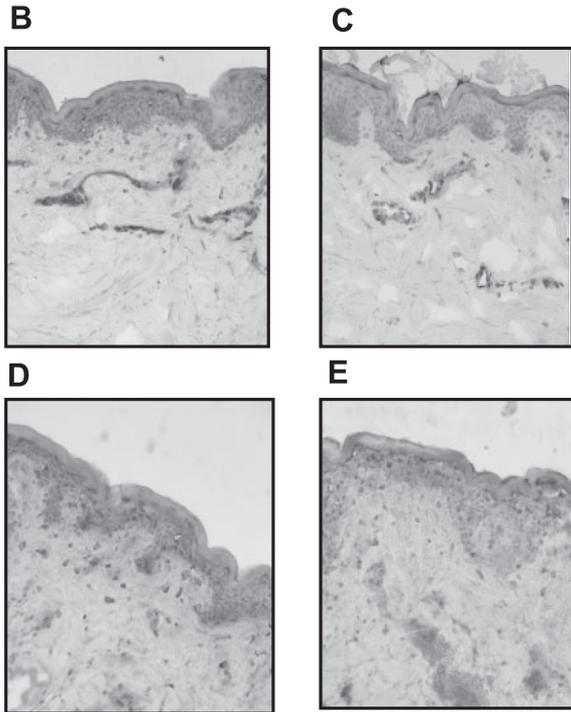
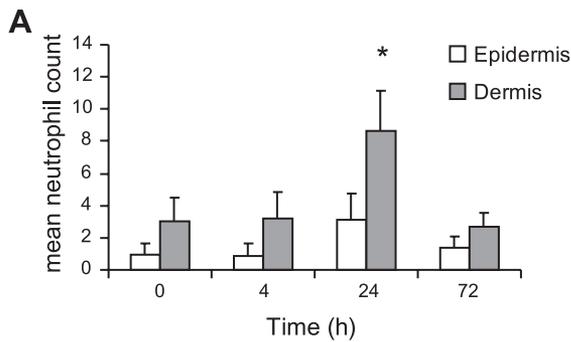


Figure 4. Neutrophil infiltrate in epidermis and dermis during sunburn response. Graphical presentation of time course (A), with representative photomicrographs of sections from unirradiated skin (B) and skin at 4 h (C), 24 h (D), and 72 h (E) after UVB exposure. Punch biopsy samples were collected from the buttock skin of healthy volunteers prior to and at intervals after exposure to $4 \times \text{MED}$ of UVB. Frozen skin sections were immunohistochemically stained using anti-neutrophil elastase monoclonal antibody. Numbers of cells staining positive per hpf ($\times 400$ original view) were counted, using 3 fields in random epidermal/dermal interface locations for each time point. Results are expressed as mean \pm SE cell count; $n = 10$. * $P < 0.05$ vs. baseline.

sunburn response up to 72 h, identifying bioactive lipid mediators that may be responsible for the principal clinical and histological components of vasodilatation and leukocyte infiltration (Scheme 1). Our data support that a range of prostanoids, including n-3 PUFA-derived PGE_3 , in addition to n-6 PUFA-derived PGE_2 , are produced during the sunburn reaction. They further suggest that the potent chemoattractants 8-HETE, 11-HETE, and 12-HETE may contribute to the leukocyte influx, while the anti-inflammatory metabolite 15-HETE may serve to limit or aid resolution of the inflammation. Eicosanoid-producing enzymes 12-LOX

and 15-LOX are both up-regulated, in contrast to recent findings in a keratinocyte cell line (14). Expression of COX and LOX does not necessarily reflect enzyme activity. However, we observe that while COX-2 shows significantly increased expression only at 24 h, 12- and 15-LOX show sustained increase in expression at 72 h, consistent with the earlier and later peaks of their respective prostanoid and HETE metabolites during the sunburn response (Scheme 2).

Our results indicate that after an early increase in n-6 PUFA-derived prostaglandins PGE_2 , $\text{PGF}_{2\alpha}$, and PGE_1 following UVB exposure, these potent mediators are enzymatically inactivated (Fig. 2), as reflected by increases in their respective 13,14-dihydro-15-keto derivatives. Although it is established that PGE_2 contributes

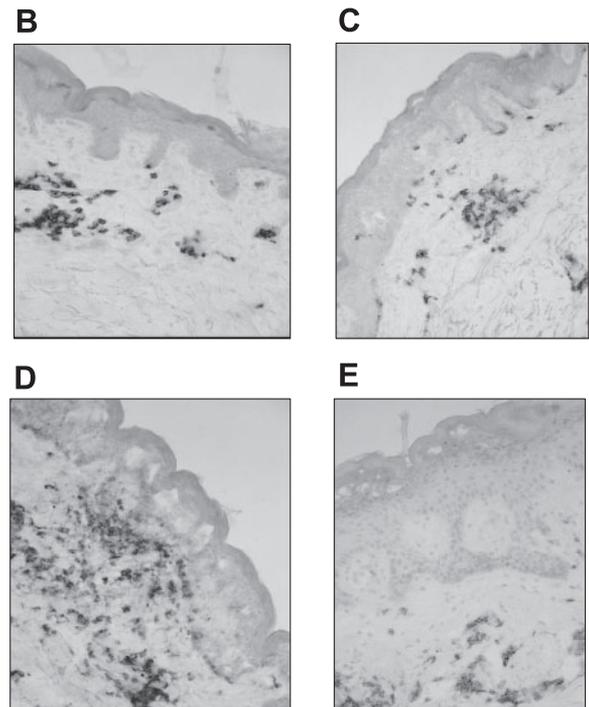
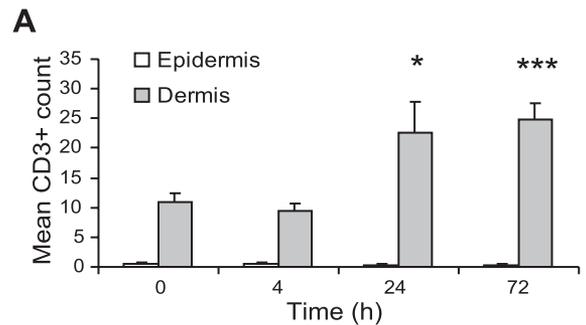


Figure 5. CD3^+ lymphocytic infiltrate in epidermis and dermis during the sunburn response. Graphical presentation of time course (A), with representative photomicrographs of sections from unexposed skin (B) and skin at 4 h (C), 24 h (D), and 72 h (E) after UVB exposure. Punch biopsy samples were collected from buttock skin of healthy volunteers, and immunohistochemical analysis was performed to determine numbers of CD3^+ cells per hpf (see Fig. 4). Results are expressed as mean \pm SE cell count; $n = 10$. * $P < 0.05$, *** $P < 0.001$ vs. baseline.

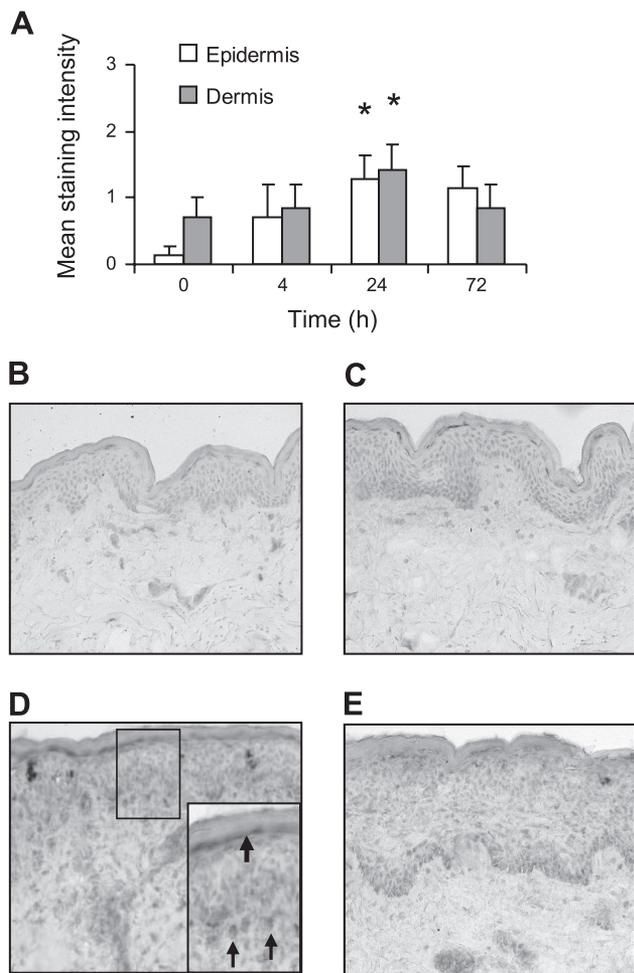


Figure 6. Expression of COX-2 in the epidermis and dermis during sunburn response. Graphical presentation of time course (A), with representative photomicrographs of sections from unexposed skin (B) and skin at 4 h (C), 24 h (D), and 72 h (E) after UVB exposure. Biopsy samples were collected from buttock skin of healthy individuals, and immunohistochemical analysis was performed to determine levels of COX-2 expression in dermis and epidermis. Frozen sections were scored for relative staining intensity: 0 = no staining; 1 = weak staining; 2 = moderate staining; 3 = intense staining. Three hpf in random epidermal/dermal interface locations were examined for each time point. Arrows indicate COX-2 staining. Results are expressed as mean \pm SE staining intensity; $n = 8$. * $P < 0.05$ vs. baseline.

to UVB-induced vasodilatation in the first 24–48 h (1, 6, 20), the contribution of $\text{PGF}_{2\alpha}$ to erythema production is uncertain. Intradermal injection of $\text{PGF}_{2\alpha}$, as with PGE_1 and PGE_2 , can produce skin erythema (21, 22), but the relevance of these findings is unclear in view of the higher than physiological doses used; moreover, $\text{PGF}_{2\alpha}$ is described as a vasoconstrictor in some systems (23). Whereas we did not directly detect a significant increase in the COX-metabolized vasodilator PGE_1 , derived from dihomogammalinolenic acid, its inactive metabolite was significantly elevated at 48–72 h after UVB exposure, indicating the earlier elevation of the mediator. This pattern of controlled prostaglandin production suggests an active response

of the tissue to UVR-induced injury. Moreover, PGE_2 has complex roles, contributing to tissue repair in addition to mediating vasodilatation and inflammation (24), and inducing COX-2, and thereby its own synthesis (25), while displaying anti-inflammatory effects, including inhibition of 5-LOX (26) and induction of 15-LOX (27–29). Thus, sunburn is a self-resolving model of injury exhibiting preparation for its resolution through the quick deactivation of proinflammatory signals and activation of repair mechanisms.

Interestingly, we found significant elevation of the n-3 PUFA-derived PGE_3 , from 18 to 48 h following UV. This mediator has not previously been reported in human skin, although its discovery is consistent with detection of the precursor n-3 PUFAs EPA and docosahexaenoic acid (DHA) in human epidermal phospho-

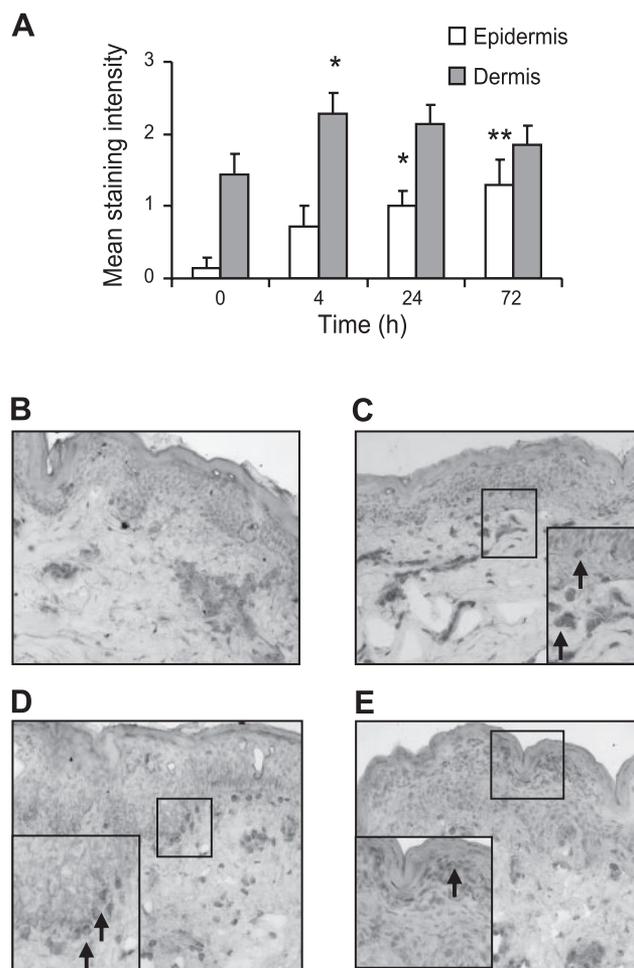


Figure 7. Expression of 12-LOX in the epidermis and dermis during the sunburn response. Graphical presentation of time course of 12-LOX (A), with representative photomicrographs of sections from unirradiated skin (B) and skin at 4 h (C), 24 h (D), and 72 h (E) after UVB exposure. Punch biopsy samples were collected from buttock skin of healthy volunteers prior to and at intervals after exposure to $4 \times \text{MED}$ of UVB. Immunohistochemical analysis was performed to determine relative levels of 12-LOX present in dermis and epidermis. Frozen sections were scored for staining intensity ($\times 200$ original view; see Fig. 6). Arrows indicate 12-LOX staining. Results are expressed as mean \pm SE staining intensity; $n = 7$. * $P < 0.05$; ** $P < 0.01$ vs. baseline.

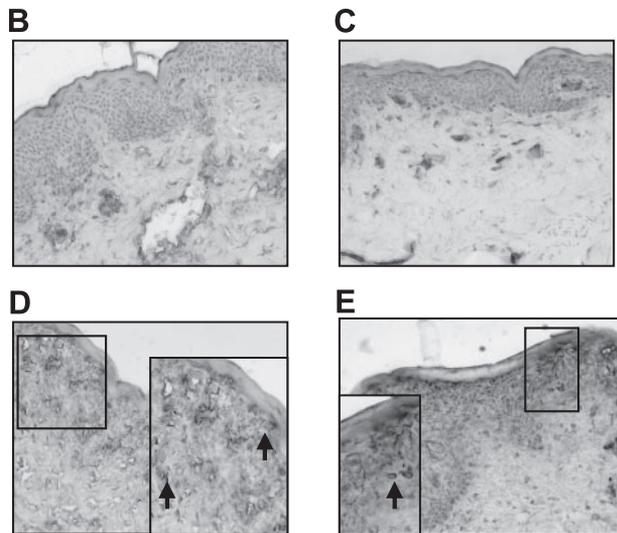
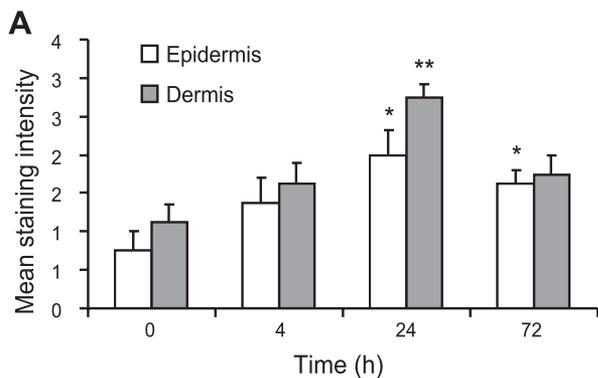
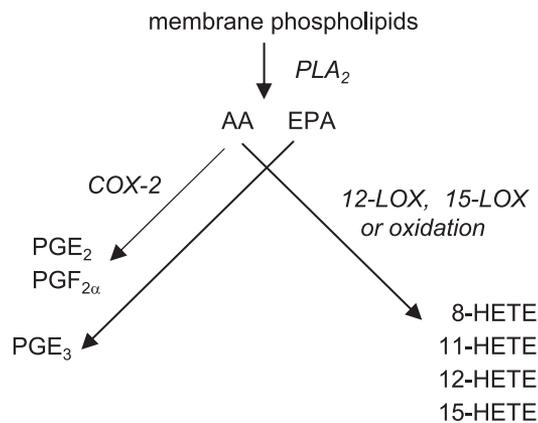


Figure 8. Expression of 15-LOX in the epidermis and dermis following UVB irradiation. Graphical presentation of time course of 15-LOX expression (A), with representative photomicrographs of sections from unirradiated skin (B) and skin at 4 h (C), 24 h (D), and 72 h (E) after UVB exposure. Punch biopsy samples were collected from buttock skin of healthy volunteers prior to and at intervals after exposure to $4 \times \text{MED}$ of UVB. Immunohistochemical analysis was performed on frozen skin sections to determine relative levels of 15-LOX in dermis and epidermis (see Fig. 6). Arrows indicate 15-LOX staining. Results are expressed as mean \pm SE staining intensity; $n = 8$. * $P < 0.05$, ** $P < 0.01$ vs. baseline.

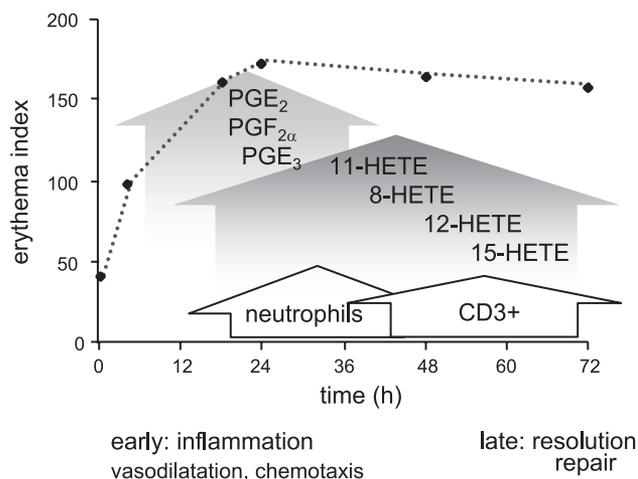
lipids (9, 30). In many respects, PGE_3 is believed to possess similar functions to PGE_2 , but with reduced potency (31), and thus PGE_3 may contribute to the erythema following UVB, although it may also reduce the efficacy of PGE_2 through acting as its partial agonist. Although neither PGE_2 nor PGE_3 was significantly elevated at 72 h, they appear not to have returned to baseline level at this time point, and the combined effects of the vasodilatory prostaglandins, together with nitric oxide (1, 7), could continue to influence sunburn erythema at the later time point. Cyclooxygenase and nitric oxide synthase inhibitors each reduce the erythema component of the sunburn response at least to 48 h after UVB exposure (1).

During sunburn, neutrophils accumulate in the dermis (2, 3) where they exert proinflammatory activities, including further release of mediators with chemoattractant properties. The infiltration occurs from 4 h



Scheme 1. COX- and LOX-generated PUFA metabolites. Simplified illustration to show COX- and LOX-generated metabolites of the principal long-chain n-6 and n-3 PUFAs, AA, and EPA, respectively, focusing on eicosanoids relevant to the present study.

after UVB onward and is consistent with our observations of the increase in a range of chemoattractant HETE. Neither 11-HETE nor 8-HETE has to our knowledge previously been reported in human skin, while 8-HETE has been detected in mouse skin (32). Their derivation is uncertain but potentially results from a combination of LOX, CYP450, and nonenzymatic oxidation pathways (11). Because both are potent neutrophil chemoattractants in *in vitro* and experimental models in other systems (18), they are anticipated to make a substantial contribution to the leukocyte chemoattraction in sunburn, particularly as there is significant elevation of 11-HETE as early as 4 h. The potent keratinocyte-derived chemoattractant 12-HETE produces a mixed neutrophilic and mononuclear infiltrate following intradermal injection (33) and has been shown *in vitro* to be a chemoattractant for lymphocytes in addition to neutrophils (17). While 12-HETE has



Scheme 2. Composite illustration of the principal events of the sunburn response. Illustration shows the skin erythema and leukocytic responses to UVB, indicating proposed key time points for contributions of COX- and LOX-generated eicosanoids.

been reported to be elevated in cutaneous inflammatory states, *i.e.*, in psoriasis (34) and contact dermatitis (35), Black *et al.* (35) found 12-HETE elevated at 24 but not 72 h after UV exposure in a study of skin blister fluid in 6 subjects, and Grundmann *et al.* (36) detected 12-HETE at 3 but not 24 h in dermal microdialysate in 3 subjects. Our findings, in a larger subject group, of higher and more sustained 12-HETE up-regulation, contrast with these reports and support a significant role for this 12-LOX-derived metabolite in the sunburn response, potentially including the prolonged (to 72 h) CD3⁺ cell infiltration.

The neutrophil chemoattractants LTB₄, believed to play a role in a range of skin inflammatory conditions (37), and 5-HETE were undetectable. It is conceivable that these mediators were present in a cellular compartment inaccessible for sampling *via* the suction blister method, since a slight although nonstatistically significant UV-associated rise in 5-HETE was detected in dermal microdialysate (36). However, LTB₄ has been detected in suction blister fluid in a range of other skin inflammatory states (38, 39), and our data correspond with earlier reports of lack of up-regulation of LTB₄ and 5-HETE following UVB exposure of mouse skin (40, 41). UVB-up-regulated PGE₂ may inhibit 5-LOX and thereby production of the LT₄ series (26), while 15-HETE may also inhibit LTB₄ synthesis (42). Thus, a specific proinflammatory profile of LOX metabolite expression is apparent in sunburn, distinct from that observed in other skin inflammatory states, providing evidence against the supposition that “LTB₄ may be a prerequisite for emigration of leukocyte into dermis” (43).

A further novel, and intriguing, finding was the significant UVB up-regulation of the anti-inflammatory eicosanoid 15-HETE, exhibiting a later response with mean levels observed to double between 48 and 72 h and potentially rising beyond 72 h. This 15-LOX metabolite has been shown to inhibit the synthesis of proinflammatory eicosanoids PGE₂ and 12-HETE in *in vitro* models (14, 44) and to inhibit the activity of 12-LOX in epidermis (45) and 5-LOX in neutrophils (44). Hence, the later elevation of this mediator could reflect a role in the resolution of sunburn, including limitation of the neutrophil influx. Other eicosanoids could potentially also possess restorative roles at later time points, including, paradoxically, 12-HETE, which is still rising at 72 h, and is described to induce fibroblast chemotaxis (46).

In summary, we have observed overlapping phases in lipid mediator profile during the sunburn response, from mainly COX-derived proinflammatory products in the early stages, to predominantly LOX products during the leukocyte infiltration phases and resolution phase, in association with UVB up-regulation of COX-2 and 12- and 15-LOX expression (Scheme 2). This study supports involvement of a specific pattern of eicosanoid products in this self-limiting inflammatory reaction. While both n-3 and n-6 PUFA-derived prostanoids are elevated and may contribute to the vasodilatation re-

sponse, 8-HETE, 11-HETE, and 12-HETE are candidate mediators of UVB-induced leukocyte chemoattraction. Our findings of an elevation of the anti-inflammatory metabolite 15-HETE in the later stages of this response warrants further examination of its potential role in resolving the inflammation. A robust resolution response may be important in protection against pathological states linked with sunburn, namely skin cancer and types of photosensitivity. FJ

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