Effect of oral eicosapentaenoic acid on epidermal Langerhans cell numbers and PGD2 production in UVR-exposed human skin: a randomised controlled study

DOI:
10.1111/exd.13177

Document Version
Accepted author manuscript

Effect of oral eicosapentaenoic acid on epidermal Langerhans cell numbers and PGD_2 production in UVR-exposed human skin: a randomised controlled study

Suzanne M Pilkington[1], Neil K Gibbs[1], Patrick Costello[1], Susan P Bennett[3], Karen A Massey[2], Peter S Friedmann[4], Anna Nicolaou[2], Lesley E Rhodes[1,3]

[1]Centre for Dermatology, Institute of Inflammation and Repair and [2]School of Pharmacy, Faculty of Medical and Human Sciences, University of Manchester, Manchester, UK; [3]Centre for Dermatology, Salford Royal Hospital, Manchester Academic Health Science Centre, Manchester, UK; and [4]Faculty of Medicine, University of Southampton, Southampton, UK.

Correspondence: Prof Lesley Rhodes, Photobiology Unit, Centre for Dermatology, University of Manchester, Salford Royal Hospital, Manchester, UK.
Tel 00 44 161 206 1150; Email lesley.e.rhodes@manchester.ac.uk

Keywords: Photoimmunosuppression, dendritic cells, prostaglandin D_2, omega-3 fatty acids; systemic photoprotection

This study was registered at http://www.clinicaltrials.gov as NCT01032343.

Abstract

Langerhans cells (LC) are sentinels of skin's immune system, their loss from epidermis contributing to UVR-suppression of cell mediated immunity (CMI). Omega-3 polyunsaturated fatty acids ~~can~~ show potential to abrogate UVR-suppression of CMI in mice and humans, potentially through modulation of LC migration. Our objectives were to examine if eicosapentaenoic acid (EPA) ingestion influences UV-mediated effects on epidermal LC numbers and levels of immunomodulatory mediators including prostaglandin (PG)D_2, which is expressed by LC.

In a double-blind randomised controlled study, healthy individuals took 5g EPA-rich (n=40) or control (n=33) lipid for 12-weeks; UVR exposed and unexposed skin samples were taken pre- and post-supplementation. Epidermal LC numbers were assessed by immunofluorescence for CD1a, and skin blister fluid PG and cytokines quantified by LC-MS/MS and Luminex assay, respectively. Pre-supplementation, UVR reduced mean (SEM) LC number/mm^2 from 913 (28) to 322 (40) ($p<0.001$), and mean PGD_2 level by 37% from 8.1 (11.6) to 5.1 (5.6) pg/µl; $p<0.001$), while IL-8 level increased ($p<0.001$). Despite confirmation of EPA bioavailability in red blood cells and skin in the active group, no between-group effect of EPA was found on UVR-modulation of LC numbers, PGD_2 or cytokine levels post-supplementation.

Thus no evidence was found for EPA abrogation of photoimmunosuppression through an impact on epidermal LC numbers. Intriguingly, UVR-exposure substantially reduced cutaneous PGD_2 levels in humans, starkly contrasting with reported effects of UVR on other skin PG. Lowered PGD_2 levels could reflect LC loss from the epidermis and/or altered dendritic cell activity, and may be relevant for phototherapy of skin disease.

Introduction

Ultraviolet radiation (UVR) suppresses cutaneous immunity (photoimmunosuppression) and this is believed to be an important contributor to the development of skin cancers [1]. In addition to the mutagenic effects of UVR on DNA which initiate carcinogenesis, inhibition of cell mediated immunity (CMI) can allow cancerous cells to escape destruction by cytotoxic lymphocytes, facilitating tumour progression. This has been elegantly demonstrated in mouse models where antigenic tumour cells were transplanted into UVR-exposed mice where they were able to progress [2]. Moreover, immunosuppressed patients have a higher incidence of skin malignancies [3].

Dendritic cells, including epidermal Langerhans cells (LC) and dermal dendritic cells (DC), are antigen presenting cells (APC) and are amongst the first line of defence in the skin where they facilitate innate and adaptive immunity and promote antigenic tolerance [4, 5]. The LC reside above the basal layer of the epidermis and monitor the skin microenvironment for danger signals including pathogens, chemicals and tumour peptides. On capturing antigenic material they travel along the afferent lymphatics to the skin-draining lymph nodes (DLN) and activate differentiation of naïve T cells (Th-0) into T helper (Th)-1, Th-2, Th17, Th22 or Treg cells [5]. Following UVR-exposure LC migrate away from the epidermis [6], and their behaviour is altered, favouring activation of Th-2 immune responses and Treg cells over Th-1 driven CMI [7-10]; these changes are believed to contribute to UVR-induced suppression of skin immunity [11]. This can be observed clinically by diminished skin contact hypersensitivity (CHS) and delayed type hypersensitivity responses to allergens following UVR-exposure [12].

The response of LC and other dendritic cells to antigen are strongly influenced by signals in the skin microenvironment. Cytokines TNF-α and IL-1β stimulate LC migration from the epidermis after exposure to antigen [13, 14], and both are upregulated in the skin in response to UVR-exposure. UVR also upregulates further cytokines possessing pro-inflammatory (including IL-8, IL-6 and IFN–γ) and immunosuppressive (including IL-4 and IL-

10) properties [15, 16]. Moreover, prostaglandins (PG) produced in the skin are reported to regulate dendritic cell activity. PGE_2 can modulate LC migration and maturation in mice [17] and reduces the ability of bone marrow derived dendritic cells to stimulate a CMI responses following UVR-exposure [18], indicating a potential influence on antigen presenting activity during photoimmunosuppression. Interestingly, it has also been reported that human LC and dermal dendritic cells express hematopoietic PGD synthase (hPGDS) supporting these cells as a source of PGD_2 in the skin, alongside mast cells and keratinocytes [19]. A role for PGD_2 in photoimmunosuppression has not been explored but in murine skin and lung epithelia PGD_2 inhibits dendritic cell migration and stimulation of T cell responses [20, 21].

The omega-3 polyunsaturated fatty acid (n-3 PUFA) eicosapentaenoic acid (EPA) reduces UVR-suppression of CMI *in* vivo; in mice, both topical and systemic EPA-rich lipids reduced UVR-suppression of chemically induced CHS responses by up to 90% [22, 23]. Further, we recently observed in a randomised controlled trial (RCT) in humans that oral EPA supplementation showed potential to reduce ~~UVR-~~ UVR-suppression of nickel CHS [24]. While the mean group difference for the 3 solar simulated radiation (SSR) doses we employed showed no statistically significant protection by EPA, ~50% reduction of photoimmunosuppression was noted with UVR dosing equivalent to brief exposure to summer sunlight (post-hoc analysis $p<0.05$) [24]. EPA exhibits a range of activities that may contribute to protective profile, including transcriptional activation of cytokine genes and modulation of PG synthesis [25]. EPA competes with the n-6 PUFA arachidonic acid (AA) for metabolism by cyclooxygenase (COX) enzymes, and this can reduce the levels of AA-derived PG [26].

In a double-blind RCT in 79 females, the objective of the current study was to explore the impact of dietary EPA on epidermal LC numbers as a potential mechanism of abrogation of photoimmunosuppression, and to examine for influence on levels of immunomodulatory mediators. Cutaneous samples were taken from UVR-exposed and unexposed skin pre- and post- a 12-week course of supplementation, with immunofluorescence assessment of CD1a+ cells in epidermal sheets and quantification of PG and cytokines in blister fluid.

Materials and Methods

Participants

Seventy-nine healthy female volunteers were recruited from the contact dermatitis investigation unit at Salford Royal Hospital, Manchester, UK and by open advertisement between 2008 and 2010. Inclusion criteria: age 18-60 years, female, Fitzpatrick sun-reactive skin type I or II, allergic to nickel (required for the clinical photo-immunosuppression study, reported elsewhere [24]). Exclusion criteria: taking n-3 PUFA supplements or photoactive medication, pregnancy or breast feeding, sunbathing or sun bed use in the prior 3 months, history of photosensitivity, skin cancer or atopy. They did not have active contact dermatitis at the time of the study. Written informed consent was provided by all volunteers before study inclusion. Ethical approval was granted by North Manchester local research ethics committee (08/H1006/30) and the study was performed in accordance with the Declaration of Helsinki principles (revised Seoul 2008).

Study Design and Intervention

The double-blind randomised (1:1) controlled parallel-group study took place in the Photobiology Unit, Dermatology Centre, Salford Royal Hospital (Manchester, UK). Treatment allocation sequence was permuted block design (mixed blocks of 4 to 6) and produced by the study biostatistician using statistical software (v2.7.7; StatsDirect Ltd, Altrincham, UK). Encapsulated active and control lipid supplements, identical in appearance, were packaged and labeled according to the allocation sequence by GP solutions Ltd (Manchester, UK), and the code held by the study biostatistician until study completion. All volunteers and researchers were blinded and volunteers were assigned the intervention on study enrolment and concurrently randomised to have either suction blister fluid sampled for analysis of eicosanoids and cytokines or skin punch biopsies taken for assessment of epidermal LC. Skin sampling was performed on both unexposed and UVR-exposed skin. All volunteers provided blood samples pre- and post-supplementation and

compliance with supplementation was confirmed through measurement of red blood cell (RBC) EPA levels (reported in [24]). The parameters assessed here were secondary outcome measures in a larger clinical trial of oral EPA supplementation that primarily assessed impact on clinical photoimmunosuppression (nickel CHS; reported in [24]). Procedures in the different studies involved UVR-exposure to small skin areas only, at separate body sites and times, with the CHS study performed post-supplementation after completion of the current study. The n-3 PUFA supplements were 1g gelatine capsules containing Incromega E7010 SR ethyl ester (~70% EPA and 10% DHA; Croda Chemicals Leek Ltd, Staffordshire, UK). Control supplements comprised 1g gelatine capsules of identical appearance containing glyceryl tricoprylate coprate (GTCC; Croda Chemicals Leek Ltd), a medium chain triglyceride found in coconut oil, and previously used as control oil in human supplement studies [27-29]. Both supplements were taken 5 capsules daily with breakfast for 12 weeks.

UVR-exposure and Skin Sampling

All volunteers were exposed to broadband UVR (270-400nm, peak 310nm; 44% UVB, 56% UVA, 1% UVC; TL12, Philips GmbH, Hamburg, Germany or UV21, Waldmann Co., VS-Schwenningen, Germany). Lamp irradiance was monitored during each exposure using radiometers (Medical Physics Department, Dryburn Hospital and Waldmann IL730A, International Light, Newburyport, USA) traceable to the UK National Physical Laboratory. The individual's minimal erythemal dose (MED) was determined on study enrolment. Pre and post-supplementation, upper buttock sites were exposed to 4x the individual's MED. After 24h, skin suction blistering and skin punch biopsy were performed from UVR-exposed and unexposed sites (methods as described in [30]). The 4x MED dose was chosen to provide a sufficient challenge to produce quantifiable increases in cytokine and eicosanoid expression in human skin *in vivo* [31, 32].

Epidermal Langerhans Cell Counting

Skin punch biopsies (5mm) from unexposed and UVR-exposed sites were immediately placed in 0.02M ethylene diamine tetra acetic acid (EDTA) in phosphate-buffered saline (PBS). After 2h incubation at 37^{o}C, epidermis was carefully peeled from dermis using forceps. Epidermal sheets were washed in PBS, fixed in ice-cold acetone (20 minutes) and re-washed in PBS, prior to incubation with mouse CD1a monoclonal primary antibody (clone NA1/34; IgG2a (Dako, Stockport, UK)) diluted to 10µg/ml in PBS (0.1 % bovine serum albumin (BSA; Sigma-Aldrich, MO, USA) and with fluorescein isothiocyanate (FITC) conjugated goat anti-mouse secondary antibody (Dako; 1/100 in PBS (0.1% BSA)), before mounting in Citifluor media (Citifluor, London, UK). LCs were counted using an Olympus Bx50 fluorescence microscope fitted with an eyepiece graticule at 40x magnification. Fifty fields per graticule were counted for each epidermal sheet.

Suction Blister Fluid Prostaglandin Measurement

Lipidomic analysis by mass spectrometry was performed as described previously [33, 34]. In summary, blister fluid eicosanoids (50-200µl) were extracted in methanol-water (15% wt/wt) and internal standard PGB_2-*d*4 (40ng) (Cayman Chemicals, Ann Arbor, MI, USA) was added. The extract was acidified to pH3.0 and applied to preconditioned solid-phase extraction (SPE) cartridge (C18-E 500 mg, 6 mL) (Phenomenex, Macclesfield, UK) and eluted with methyl formate. Chromatographic analysis was performed on a C18 column (Luna, 5µm, 2.0mm, Phenomenex, Macclesfield, UK) using HPLC (Alliance 2695, Waters, Elstree, Hertfordshire, UK) coupled to a triple quadrupole mass spectrometer with electrospray ionisation (ESI) (Quattro Ultima, Waters). Multiple reaction monitoring transitions were used to assay for the presence of PGD_2 (*m/z* 351 >271) and its metabolites PGJ_2, Δ^{12}-PGJ_2 (m/z 333 >271) and 15-deoxy-$^{\Delta12,14}$ PGJ_2 (m/z 315 >271). Results are expressed as pg/µl of blister fluid, based on calibration lines constructed from commercially available standards (Cayman Chemicals).

Suction Blister Fluid Cytokine Measurement

A panel of cytokines (IL-8, IFN-γ, TNF-α, IL-1β, IL-4, IL-10, IL-23 and IL-17) was simultaneously quantified in suction blister fluid using the Bio-Plex™ cytokine array system (Bio-Rad Laboratories, Hercules, CA, USA) in accordance with manufacturer's instructions, as described previously [35].

Statistical analysis

The study was powered to detect a difference in clinical photoimmunosuppression responses between EPA and control supplemented groups, as previously detailed [24]. Statistical analysis was performed in SPSS 20.0. Non-normally distributed data was transformed using natural log. ANCOVA analyses compared EPA and control groups post-supplementation with baseline (pre-supplementation) data as the covariate. Paired t-tests were performed to make within-group comparisons between unexposed and UVR-exposed skin. A p value of <0.05 was considered statistically significant.

Results

Volunteers and compliance

Seventy-nine volunteers were recruited and randomised to the oral intervention: 6 did not proceed to take supplements and discontinued the study for personal reasons; no data was collected from them. Of the 73 who took supplements, 33 were randomised to control and 40 to EPA; baseline characteristics are shown (Table 1). Baseline dietary intake assessed by food frequency questionnaire was below current UK recommendations of 450mg/day total long chain n-3 PUFA [36, 37]. The EPA supplement was bioavailable in both RBC and skin ($p<0.001$) as previously reported [30]. Three volunteers in the EPA group (all suction blister subgroup) who showed no increase in RBC EPA levels post-supplementation were excluded from analyses for poor compliance (Fig 1). One individual in the EPA group declined biopsies post-supplementation and data was excluded from analyses. Of the remaining 69 volunteers, 33 were in the control and 36 in the EPA group (Fig 1). No adverse effects were reported for either supplement.

Langerhans cells

To assess the effect of UVR exposure on epidermal LC density pre-supplementation, baseline data of the two supplement groups was combined. UVR challenge produced a reduction of ~65% in mean (SEM) LC number in the epidermis at 24 hours post-exposure, from 920 (28) to 318 (39) per mm^2 ($p<0.001$) (Fig 2A). Following supplementation, the UVR-induced reduction in LC number was similar to baseline for both control (881 (46) to 218 (44) cells per mm^2; $p<0.001$) and EPA group (856 (55) to 191 (26) cells per mm^2; $p<0.001$), decreases of 75% and 78%, respectively (Fig 2A). There was no significant difference in epidermal LC numbers between control and EPA groups post-supplementation, in unexposed or UVR-exposed skin. Visualisation of LC in epidermal sheets revealed that following UVR the majority of LC lost their dendritic projections and appeared in a more rounded, migratory form. There was no apparent effect of EPA on LC morphology (Fig 2B) in unexposed or UVR-exposed skin.

246

247 **Prostaglandin production**

248 PGD_2 and its metabolites PGJ_2, Δ^{12}-PGJ_2 and 15-deoxy-$^{\Delta12,14}$ PGJ_2 were measured in skin
249 blister fluid to explore impact of UVR and EPA; Δ^{12}-PGJ_2 was detected but below the limit of
250 quantitation and 15-deoxy-$^{\Delta12,14}$ PGJ_2 was below limit of detection. At baseline, data from
251 both supplement groups was combined to examine effect of UVR exposure.

252

253 *PGD_2:* At baseline, median (IQR) PGD_2 was decreased in UVR-exposed versus unexposed
254 skin (from 8.1 (11.6) pg/µl to 5.1 (5.6) pg/µl; $p<0.001$) (Fig 3A). Post-supplementation,
255 control group PGD_2 level was similarly decreased in UVR-exposed versus unexposed skin
256 (from 8.6 (6.3) to 4.1 (4.7) pg/µl; $p<0.01$). In contrast in the EPA group post-
257 supplementation, no statistically significant reduction in PGD_2 occurred post-UVR.
258 Comparison of groups post-supplementation revealed that in unexposed skin PGD_2 was
259 ~40% lower in the EPA-unexposed versus control group (5.2 (4.8) vs 8.6 (6.3) non-
260 significant), while in UVR-exposed skin, levels were similar (4.0 (5.3) vs 4.1 (4.7) pg/µl in
261 control group).

262

263 PGJ_2: At baseline, PGJ_2 was significantly increased in UVR-exposed versus unexposed skin
264 (from 1.2 (1.3) to 2.1 (2.0); $p<0.05$) (Fig 3B). Post-supplementation small apparent increases
265 in PGJ_2 were seen in UVR-exposed skin in control and EPA groups (non-significant). There
266 were no significant differences in PGJ_2 levels between control and EPA groups post-
267 supplementation.

268

269 **Cytokine expression**

270 Of the panel of cytokines assessed, IL-10, TNFα and IL-8 were quantifiable. Whilst IFN-γ
271 was detected, levels were below the limit of quantitation, and IL-1β, IL-4, IL-17 and IL-23
272 were not detected. Due to low blister fluid volumes, five individuals (two in EPA group and
273 three in control group) were excluded from cytokine analyses, resulting in n=16 for the

control and n=15 for the EPA group. IL-10 levels for two individuals in the control group were out of range and excluded, resulting in n=14 in the control group. Baseline data for EPA and control groups were combined to assess effect of UVR on cytokine levels pre-supplementation.

IL-8: At baseline, median (IQR) IL-8 increased in UVR-exposed versus unexposed skin (791.9 (798.9) vs 238.1 (314) pg/ml; $p<0.001$; Fig 3D). Similarly, post-supplementation, a statistically significant UVR-induced rise in IL-8 was seen in the control group (from 162.3 (304.2) to 827.1 (443) pg/ml; $p<0.001$) and EPA group (from 244.5 (277.3) to 591.7 (970.9) pg/ml; $p<0.01$). There was no significant difference in IL-8 concentration in unexposed or UVR-exposed skin in control versus treatment groups post-supplementation.

IL-10: At baseline, median (IQR) IL-10 concentration apparently increased following UVR exposure, but this was not statistically significant (82 (153) vs 68.3 (142) pg/ml; Fig 3E). Similarly post-supplementation, there was an apparent increase in IL-10 concentration post-UVR in the control (90.3 (142) vs 79.6 (98) pg/ml) and EPA groups (95.8 (148) vs 70 (115) pg/ml). There was no significant difference in IL-10 concentration in unexposed or UVR-exposed skin when comparing control and EPA groups post-supplementation.

TNFα: At baseline, median (IQR) TNFα concentration was not significantly altered in UVR-exposed versus unexposed skin (67.2 (98.5) pg/ml vs 57.7 (101.8) pg/ml) at baseline (Fig 3F). Post-supplementation, there were apparent rises in TNFα in UVR-exposed versus unexposed skin, in control (84.8 (107.2) vs 54.7 (139.7)) and EPA (88.1 (149.9) vs 36.6 (66.2)) groups (both non-significant). There was no significant difference in TNFα concentration in unexposed or UVR-exposed skin when comparing control and EPA groups post-supplementation.

Discussion

In this study UVR exposure of human skin *in vivo* at baseline (pre-supplementation) significantly reduced epidermal LC density and altered the morphology of remaining LC, in association with a notable reduction in PGD_2. This significant UVR impact on PGD_2 production (Fig 3A) is in stark contrast to the well-described increase in skin PGE_2 and other eicosanoids examined following UVR-exposure to humans *in vivo* [30, 31], and this could have implications for health, including during sun-exposure and for the phototherapy of skin disorders. The subsequent investigation of the impact of 12 weeks EPA supplementation employed a robust study design and adequate sample size, and importantly, oral EPA compliance and skin bioavailability was demonstrated in these volunteers [24, 30]. No impact of EPA supplementation versus control was found on epidermal LC numbers, either in unexposed or UVR-exposed skin, and hence we found no evidence that EPA abrogates UVR-suppression of skin immunity through this mechanism in humans.

UVR induced loss of LC from the epidermis contributes to local UVR-induced immunosuppression of the skin, which is partially mediated through induction of T-reg [11]. Langerhans cell loss from the epidermis can be stimulated by a range of UVR doses, with LC cell density and size reduction occurring in a UVR-dose dependent manner [38, 39]. We observed a notable reduction in epidermal LC number of ~65% following a pro-inflammatory (4xMED) UVR exposure. This magnitude of response is in line with a previous report in human skin, where LC apoptosis in the epidermis was barely detectable after a very high (6xMED) UVR challenge, while migration was observed [6], supporting that the UVR-induced epidermal loss of LC observed in our study could be due to migration. The current study provides substantially the largest dataset to-date examining UVR-induced reduction in LC number in human epidermis. Consistent with previous observations [40, 41], notable inter-subject variation was seen in LC numbers under all treatment conditions.

While most skin blister fluid cytokines assessed in our study were below the assay detection limit, the chemokine IL-8 showed a large induction in response to UVR-exposure, in keeping with previous studies [16, 32]. TNF-α and IL-β are key cytokines involved in LC

mobilisation following exposure to UVR [13, 42], however, IL-1β was not detected and while TNF-α was present, no significant UVR-induced increase was found in blister fluid. A major source of UVR-induced TNF-α is purported to be basal keratinocytes [43], where UVR-induced nuclear DNA damage may stimulate its release [44]. The dermal neutrophil infiltrate, which is reported to peak from 14 hours post-UVR may also contribute to TNFα increase [45]. The immunosuppressive cytokine IL-10 inhibits dendritic cell IFN-γ production and initiation of CMI responses and induces tolerance [46-48]. In UVR-irradiated human skin, IL-10 is reported to be preferentially induced in infiltrating CD11b+ macrophages which peak in the dermis during the first 24 hours and in epidermis at 72 hours [49]; as blister fluid is primarily of epidermal origin [34] this might contribute to lack of significant IL-10 increase observed in this study. No effect of EPA on cytokine levels was observed. A 24 hour post-UVR time point was selected as the most appropriate for assessing cytokines and prostaglandins simultaneously [16, 31], however, other time points might reveal differences.

We previously reported the skin PGE_2 level in this group of individuals was augmented at 24 hours post UVR challenge [30]. PGE_2 stimulates IL-10 production in mouse and human model systems, favouring a Th2 response, Treg activation and immune-suppression [15, 50, 51]. In contrast to the 127% rise in PGE_2, we found skin PGD_2 levels in the same volunteers were significantly reduced by 37% after UVR-exposure (Fig 3C). PGD_2 is associated with allergic inflammatory disorders in the respiratory tract [52] and skin [53], including mast cell disorders [54] and atopic dermatitis [55, 56], and has potential relevance to the novel treatment of other conditions featuring raised cutaneous PGD_2, including hair-loss [57]. PGD_2 differentially regulates T cell responses via two receptors; the DP1 receptor mediates inhibition of Th1 functions, while the DP2 (CRTH2) receptor promotes Th2 activity [58]. In inflammatory skin disorders the contrasting effect of acute UVR exposure on PGD_2 and PGE_2 may contribute to the therapeutic effects of phototherapy. Increased levels of the PGD_2 dehydration product, PGJ_2, in UVR-exposed skin is also interestingly, as J ring

metabolites, in particular 15-deoxy-$\Delta^{12,14}$-PGJ_2, reportedly exert anti-inflammatory effects [59]. Further assessment of these metabolites in cutaneous inflammation could be valuable.

In human skin, LC, mast cells and dermal dendritic cells are primary sources of PGD_2 [19]. Post UVR-exposure of human skin, mast cell infiltration and degranulation occurs as early as 4 hours post-challenge, but by 24 hours mast cell numbers and activity have returned to normal [60], while epidermal LC are depleted. We propose that the UVR-reduction in cutaneous PGD_2 could partially reflect loss of LC from the epidermis. This is supported by our observation of no UVR-induced PGD_2 reduction in human primary keratinocytes and fibroblasts (unpublished data), or in the dermal fraction of human skin [61]. In mice ageing-associated increases in local PGD_2 correlate with impaired migration of respiratory DC, and antagonism of the DP1 receptor restores migration [16]. Constitutive levels of PGD_2 may provide an inhibitory signal to migration which can be downregulated by UVR. While we did not find an effect of EPA on PGD_2 levels in UVR-exposed skin, an apparent fall in unexposed skin compared to control (Fig 3A) was consistent with *in vitro* findings of EPA reduction of PGD_2 production in niacin- stimulated human LC [62].

Our recently reported assessment of a clinical CHS end-point in the same volunteers suggested that 12 weeks oral EPA supplementation has the potential to reduce UVR-suppression of nickel CHS [24]. However, we have found EPA to have no impact on LC number in unexposed and UVR-exposed skin when compared to the control group, when using a high UVR-dose sufficient to produce a measurable increase in PG [30, 31] Thus, our study did not support the mediation of immune-protective effects of EPA by changes in the numbers of epidermal LC, although it is conceivable there may still have been changes in LC activity. EPA may potentially exhibit greater protection with UVR at lower doses or different spectra. This could be addressed in future studies, alongside examination of impact on other DC subsets as understanding of their significance in human skin immunity becomes better understood.

In conclusion, our double blind RCT did not find evidence for an impact of oral EPA on UVR-induced reduction of epidermal LC. The significant UVR-induced fall in PGD_2 level

may have an immunomodulatory effect of relevance to the phototherapy of skin disease, and warrants further investigation.

Acknowledgements

LER, NKG and PSF designed the study, SMP, PC, SPB and KAM performed the study and data analysis, AN contributed essential equipment and reagents, SMP and LER wrote the paper and PSF and AN critically revised the paper. We acknowledge the Association of International Cancer Research for funding this study. We thank Rebecca Dearman and Ian Kimber in the Faculty of Life Sciences at the University of Manchester for use of their Luminex analyser, Donald Allan for technical support, Croda Chemicals Ltd for freely supplying the active and control lipid supplements and GP solutions Ltd for packaging the supplements. We also thank all the volunteers who took part in the study.

Conflict of Interests

The authors have no conflicts of interest.

References

1. Schwarz, T., Photoimmunosuppression. Photodermatol Photoimmunol Photomed 2002: 18: 141-145.

2. Kripke, M. L., R. M. Thorn, P. H. Lill, C. I. Civin, N. H. PazmiñoM. S. Fisher, Further characterization of immunological unresponsiveness induced in mice by ultraviolet radiation. Growth and induction of nonultraviolet-induced tumors in ultraviolet-irradiated mice. Transplantation 1979: 28: 212-217.

3. Oberyszyn, T. M., Non-melanoma skin cancer: importance of gender, immunosuppressive status and vitamin D. Cancer Lett 2008: 261: 127-136.

4. van der Aar, A. M., D. I. Picavet, F. J. Muller et al., Langerhans cells favor skin flora tolerance through limited presentation of bacterial antigens and induction of regulatory T cells. J Invest Dermatol 2013: 133: 1240-1249.

5. Seneschal, J., Rachael A. Clark, A. Gehad, Clare M. Baecher-AllanThomas S. Kupper, Human Epidermal Langerhans Cells Maintain Immune Homeostasis in Skin by Activating Skin Resident Regulatory T Cells. Immunity 2012: 36: 873-884.

6. Kolgen, W., H. Both, H. van Weelden et al., Epidermal Langerhans Cell Depletion After Artificial Ultraviolet B Irradiation of Human Skin In Vivo: Apoptosis Versus Migration. J Invest Dermatol 2002: 118: 812-817.

7. Simon, J. C., P. D. Cruz, Jr., P. R. BergstresserR. E. Tigelaar, Low dose ultraviolet B-irradiated Langerhans cells preferentially activate CD4+ cells of the T helper 2 subset. J Immunol 1990: 145: 2087-2091.

8. Simon, J. C., R. E. Tigelaar, P. R. Bergstresser, D. EdelbaumP. D. Cruz, Jr., Ultraviolet B radiation converts Langerhans cells from immunogenic to tolerogenic antigen-presenting cells. Induction of specific clonal anergy in CD4+ T helper 1 cells. J Immunol 1991: 146: 485-491.

9. Loser, K.S. Beissert, Regulation of cutaneous immunity by the environment: an important role for UV irradiation and vitamin D. Int Immunopharmacol 2009: 9: 587-589.

425 10. Denfeld, R. W., H. Hara, J. P. Tesmann, S. MartinJ. C. Simon, UVB-irradiated
426 dendritic cells are impaired in their APC function and tolerize primed Th1 cells but not naive
427 CD4+ T cells. J Leukoc Biol 2001: 69: 548-554.
428 11. Schwarz, A., M. Noordegraaf, A. Maeda, K. Torii, B. E. ClausenT. Schwarz,
429 Langerhans cells are required for UVR-induced immunosuppression. J Invest Dermatol
430 2010: 130: 1419-1427.
431 12. Damian, D. L.G. M. Halliday, Measurement of ultraviolet radiation-induced
432 suppression of recall contact and delayed-type hypersensitivity in humans. Methods 2002:
433 28: 34-45.
434 13. Cumberbatch, M., R. J. DearmanI. Kimber, Interleukin 1 beta and the stimulation of
435 Langerhans cell migration: comparisons with tumour necrosis factor alpha. Arch Dermatol
436 Res 1997: 289: 277-284.
437 14. Cumberbatch, M., I. FieldingI. Kimber, Modulation of epidermal Langerhans' cell
438 frequency by tumour necrosis factor-alpha. Immunology 1994: 81: 395-401.
439 15. Shreedhar, V., T. Giese, V. W. SungS. E. Ullrich, A cytokine cascade including
440 prostaglandin E2, IL-4, and IL-10 is responsible for UV-induced systemic immune
441 suppression. J Immunol 1998: 160: 3783-3789.
442 16. Strickland, I., L. E. Rhodes, B. F. FlanaganP. S. Friedmann, TNF-alpha and IL-8 are
443 upregulated in the epidermis of normal human skin after UVB exposure: correlation with
444 neutrophil accumulation and E-selectin expression. J Invest Dermatol 1997: 108: 763-768.
445 17. Kabashima, K., D. Sakata, M. Nagamachi, Y. Miyachi, K. InabaS. Narumiya,
446 Prostaglandin E2-EP4 signaling initiates skin immune responses by promoting migration and
447 maturation of Langerhans cells. Nat Med 2003: 9: 744-749.
448 18. Ng, R. L., J. L. Bisley, S. Gorman, M. NorvalP. H. Hart, Ultraviolet irradiation of mice
449 reduces the competency of bone marrow-derived CD11c+ cells via an indomethacin-
450 inhibitable pathway. J Immunol 2010: 185: 7207-7215.

19. Shimura, C., T. Satoh, K. Igawa et al., Dendritic Cells Express Hematopoietic Prostaglandin D Synthase and Function as a Source of Prostaglandin D2 in the Skin. Am J Pathol 2010: 176: 227-237.

20. Zhao, J., J. Zhao, K. LeggeS. Perlman, Age-related increases in PGD(2) expression impair respiratory DC migration, resulting in diminished T cell responses upon respiratory virus infection in mice. J Clin Invest 2011: 121: 4921-4930.

21. Angeli, V., C. Faveeuw, O. Roye et al., Role of the parasite-derived prostaglandin D2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. J Exp Med 2001: 193: 1135-1147.

22. Moison, R. M.G. M. Beijersbergen Van Henegouwen, Dietary eicosapentaenoic acid prevents systemic immunosuppression in mice induced by UVB radiation. Radiat Res 2001: 156: 36-44.

23. Moison, R. M., D. P. SteenvoordenG. M. Beijersbergen van Henegouwen, Topically applied eicosapentaenoic acid protects against local immunosuppression induced by UVB irradiation, cis-urocanic acid and thymidine dinucleotides. Photochem Photobiol 2001: 73: 64-70.

24. Pilkington, S. M., K. A. Massey, S. P. Bennett et al., Randomized controlled trial of oral omega-3 PUFA in solar-simulated radiation-induced suppression of human cutaneous immune responses. Am J Clin Nutr 2013: 97: 646-652.

25. Pilkington, S. M., R. E. Watson, A. NicolaouL. E. Rhodes, Omega-3 polyunsaturated fatty acids: photoprotective macronutrients. Exp Derm 2011: 20: 537-543.

26. Wada, M., C. J. DeLong, Y. H. Hong et al., Enzymes and Receptors of Prostaglandin Pathways with Arachidonic Acid-derived Versus Eicosapentaenoic Acid-derived Substrates and Products. J Biol Chem 2007: 282: 22254-22266.

27. West, N. J., S. K. Clark, R. K. Phillips et al., Eicosapentaenoic acid reduces rectal polyp number and size in familial adenomatous polyposis. Gut 2010: 59: 918-925.

28. Belluzzi, A., C. Brignola, M. Campieri, A. Pera, S. BoschiM. Miglioli, Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. N Engl J Med 1996: 334: 1557-1560.

29. Henz, B. M., S. Jablonska, P. C. van de Kerkhof et al., Double-blind, multicentre analysis of the efficacy of borage oil in patients with atopic eczema. Br J Dermatol 1999: 140: 685-688.

30. Pilkington, S. M., L. E. Rhodes, N. M. Al-Aasswad, K. A. MasseyA. Nicolaou, Impact of EPA ingestion on COX- and LOX-mediated eicosanoid synthesis in skin with and without a pro-inflammatory UVR challenge--report of a randomised controlled study in humans. Mol Nutr Food Res 2014: 58: 580-590.

31. Rhodes, L. E., K. Gledhill, M. Masoodi et al., The sunburn response in human skin is characterized by sequential eicosanoid profiles that may mediate its early and late phases. FASEB J. 2009: 23: 3947-3956.

32. Shahbakhti, H., R. E. Watson, R. M. Azurdia, C. Z. Ferreira, M. GarmynL. E. Rhodes, Influence of eicosapentaenoic acid, an omega-3 fatty acid, on ultraviolet-B generation of prostaglandin-E2 and proinflammatory cytokines interleukin-1 beta, tumor necrosis factor-alpha, interleukin-6 and interleukin-8 in human skin in vivo. Photochem Photobiol 2004: 80: 231-235.

33. Masoodi, M.A. Nicolaou, Lipidomic analysis of twenty-seven prostanoids and isoprostanes by liquid chromatography/electrospray tandem mass spectrometry. Rapid Commun Mass Spectrom 2006: 20: 3023-3029.

34. Kendall, A. C., S. M. Pilkington, K. A. Massey, G. Sassano, L. E. RhodesA. Nicolaou, Distribution of bioactive lipid mediators in human skin. J Invest Dermatol 2015: 135: 1510-1520.

35. Dearman, R. J., C. J. Betts, H. T. CaddickI. Kimber, Cytokine profiling of chemical allergens in mice: impact of mitogen on selectivity of response. J Appl Toxicol 2009: 29: 233-241.

504 36. Scientific Advisory Committee on Nutrition (SACN), *Advice on fish consumption:*
505 *benefits and risks*. 2004, London: The Stationary Office.
506 37. Wallingford, S. C., S. M. Pilkington, K. A. Massey et al., Three-way assessment of
507 long chain omega-3 polyunsaturated fatty acid nutrition: by questionnaire and matched blood
508 and skin samples. Br J Nutr 2012: 23: 1-8.
509 38. Blackburn, A., T. C. Ling, M. Brownrigg, L. E. RhodesN. K. Gibbs, UVB-induced
510 Langerhans cell trafficking in polymorphic light eruption. Br J Dermatol 2004: 150: 796.
511 39. Seite, S., H. Zucchi, D. Moyal et al., Alterations in human epidermal Langerhans cells
512 by ultraviolet radiation: quantitative and morphological study. Br J Dermatol 2003: 148: 291-
513 299.
514 40. Cumberbatch, M., M. Singh, R. J. Dearman, H. S. Young, I. KimberC. E. Griffiths,
515 Impaired Langerhans cell migration in psoriasis. J Exp Med 2006: 203: 953-960.
516 41. Cumberbatch, M., M. Bhushan, R. J. Dearman, I. KimberC. E. Griffiths, IL-1beta-
517 induced Langerhans' cell migration and TNF-alpha production in human skin: regulation by
518 lactoferrin. Clin Exp Immunol 2003: 132: 352-359.
519 42. Cumberbatch, M., R. J. DearmanI. Kimber, Langerhans cells require signals from
520 both tumour necrosis factor-alpha and interleukin-1 beta for migration. Immunology 1997:
521 92: 388-395.
522 43. Human keratinocytes are a source for tumor necrosis factor alpha: evidence for
523 synthesis and release upon stimulation with endotoxin or ultraviolet light. J Exp Med 1990:
524 172: 1609-1614.
525 44. Walker, S. L.A. R. Young, An action spectrum (290-320 nm) for TNFalpha protein in
526 human skin in vivo suggests that basal-layer epidermal DNA is the chromophore. Proc Natl
527 Acad Sci U S A 2007: 104: 19051-19054.
528 45. Hawk, J. L., G. M. MurphyC. A. Holden, The presence of neutrophils in human
529 cutaneous ultraviolet-B inflammation. Br J Dermatol 1988: 118: 27-30.

530 46. Ullrich, S. E., Mechanism involved in the systemic suppression of antigen-presenting
531 cell function by UV irradiation. Keratinocyte-derived IL-10 modulates antigen-presenting cell
532 function of splenic adherent cells. J Immunol 1994: 152: 3410-3416.
533 47. Schwarz, A., S. Grabbe, H. Riemann et al., In vivo effects of interleukin-10 on contact
534 hypersensitivity and delayed-type hypersensitivity reactions. J Invest Dermatol 1994: 103:
535 211-216.
536 48. Enk, A. H., J. Saloga, D. Becker, M. MohamadzadehJ. Knop, Induction of hapten-
537 specific tolerance by interleukin 10 in vivo. J Exp Med 1994: 179: 1397-1402.
538 49. Kang, K., A. C. Gilliam, G. Chen, E. TootellK. D. Cooper, In human skin, UVB
539 initiates early induction of IL-10 over IL-12 preferentially in the expanding dermal
540 monocytic/macrophagic population. J Invest Dermatol 1998: 111: 31-38.
541 50. Kalinski, P., C. M. Hilkens, A. Snijders, F. G. SnijdewintM. L. Kapsenberg, Dendritic
542 cells, obtained from peripheral blood precursors in the presence of PGE2, promote Th2
543 responses. Adv Exp Med Biol 1997: 417: 363-367.
544 51. Harizi, H., M. Juzan, V. Pitard, J. F. MoreauN. Gualde, Cyclooxygenase-2-issued
545 prostaglandin e(2) enhances the production of endogenous IL-10, which down-regulates
546 dendritic cell functions. J Immunol 2002: 168: 2255-2263.
547 52. García-Solaesa, V., C. Sanz-Lozano, J. Padrón-Morales et al., The prostaglandin D2
548 receptor (PTGDR) gene in asthma and allergic diseases. Allergol Immunopathol 2014: 42:
549 64-68.
550 53. Yahara, H., T. Satoh, C. MiyagishiH. Yokozeki, Increased expression of CRTH2 on
551 eosinophils in allergic skin diseases. J Eur Acad Dermatol Venereol 2010: 24: 75-76.
552 54. Butterfield, J. H.C. R. Weiler, Prevention of mast cell activation disorder-associated
553 clinical sequelae of excessive prostaglandin D(2) production. Int Archives Allergy Immunol
554 2008: 147: 338-343.
555 55. Matsushima, Y., T. Satoh, Y. Yamamoto, M. NakamuraH. Yokozeki, Distinct roles of
556 prostaglandin D2 receptors in chronic skin inflammation. Mol Immunol 2011: 49: 304-310.

557 56. Iwasaki, M., K. Nagata, S. Takano, K. Takahashi, N. IshiiZ. Ikezawa, Association of a
558 new-type prostaglandin D2 receptor CRTH2 with circulating T helper 2 cells in patients with
559 atopic dermatitis. J Invest Dermatol 2002: 119: 609-616.
560 57. Nieves, A.L. A. Garza, Does Prostaglandin D(2) hold the cure to male pattern
561 baldness? Exp Dermatol 2014: 23: 224-227.
562 58. Tanaka, K., H. Hirai, S. Takano, M. NakamuraK. Nagata, Effects of prostaglandin D2
563 on helper T cell functions. Biochem Biophys Res Commun 2004: 316: 1009-1014.
564 59. Harris, S. G., J. Padilla, L. Koumas, D. RayR. P. Phipps, Prostaglandins as
565 modulators of immunity. Trends Immunol 2002: 23: 144-150.
566 60. Gilchrest, B. A., N. A. Soter, J. S. StoffM. C. Mihm, Jr., The human sunburn reaction:
567 histologic and biochemical studies. J Am Acad Dermatol 1981: 5: 411-422.
568 61. Kendall, A. C., S. M. Pilkington, G. Sassano, L. E. RhodesA. Nicolaou, N-acyl
569 ethanolamide and eicosanoid involvement in irritant dermatitis. Br J Dermatol 2016.
570 62. VanHorn, J., J. D. Altenburg, K. A. Harvey, Z. Xu, R. J. KovacsR. A. Siddiqui,
571 Attenuation of niacin-induced prostaglandin D(2) generation by omega-3 fatty acids in THP-1
572 macrophages and Langerhans dendritic cells. J Inflamm Res 2012: 5: 37-50.
573 63. Judson, B. L., A. Miyaki, V. D. Kekatpure et al., UV Radiation Inhibits 15-
574 Hydroxyprostaglandin Dehydrogenase Levels in Human Skin: Evidence of Transcriptional
575 Suppression. Cancer Prev Res 2010: 3: 1104-1111.
576 64. Kalinski, P., J. H. Schuitemaker, C. M. HilkensM. L. Kapsenberg, Prostaglandin E2
577 induces the final maturation of IL-12-deficient CD1a+CD83+ dendritic cells: the levels of IL-
578 12 are determined during the final dendritic cell maturation and are resistant to further
579 modulation. J Immunol 1998: 161: 2804-2809.

580

581

582

583 **Table 1. Baseline characteristics of participants.**

584

Characteristic	Control	EPA
Age (yrs) (median (range))	45 (22-60)	43 (21-60)
BMI[1] (Kg/m^2) (mean (SD))	25.9 (4.4)	27.8 (5.3)
Skin type[2] (no./total (%))		
I	2/33 (6)	6/40 (15)
II	31/33 (94)	34/40 (85)
HRT/OCP[3] (no./total (%))	2/33 (6)	6/40 (15)

585 [1] BMI data from n=31 in the EPA group and n=31 in the control group

586 [2] Fitzpatrick skin type classification: *I*- always burns, never tans, *II*- usually burns, tans with

587 difficulty

588 [3] Hormone replacement therapy/ oral contraceptive pill

589

590

591

592 **Figure legends**

593

594 **Fig 1. Flow diagram of study design and participants.**

595

596 **Fig 2. UVR induces LC loss from epidermis but EPA supplementation has no impact**
597 **on epidermal LC numbers.** (A) LC count (mean) per mm^2 of epidermis and (B) images of
598 CD1a positive LC in epidermal sheets in unexposed (open circles) and UVR-exposed skin
599 (closed circles) at baseline (n=30) and post-supplementation (control n=12, EPA n=18);
600 ***p<0.001 (scale bar 50μm).

601

602 **Fig 3. UVR reduces PGD_2 and increases IL-8 level in skin blister fluid.**
603 Concentration (median) of (A) PGD_2, and (B) its metabolite PGJ_2 in skin blister fluid taken
604 from unexposed (open circles) and UVR-exposed (closed circles) skin at baseline (n=36)
605 and post-supplementation (control n=19, EPA n=17); *p<0.05, ** p<0.01. (C) % change in
606 PGD_2 in UVR-exposed skin in comparison with UVR–induced % change in PGE_2 [24], in skin
607 blister fluid at baseline (n=36). Concentration of (D) IL-8, (E) IL-10 and (F) TNF-α in skin
608 blister fluid taken from unexposed (open circles) and UVR-exposed (closed circles) skin at
609 baseline (n=31) and post-supplementation (control n=16 (IL-10 n=14), EPA n=15); *p<0.05,
610 ** p<0.01, *** p<0.001.

611

612

613

614

615

616

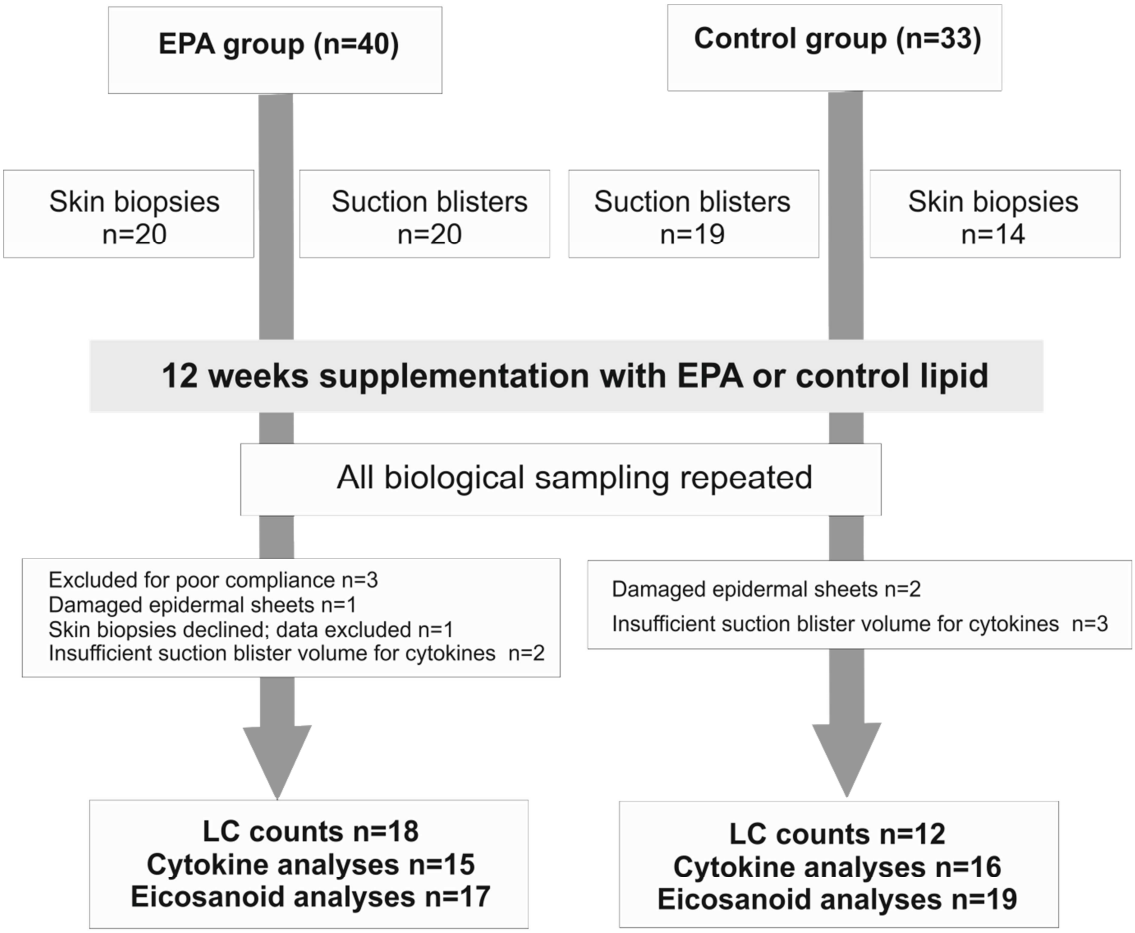
617

618

619

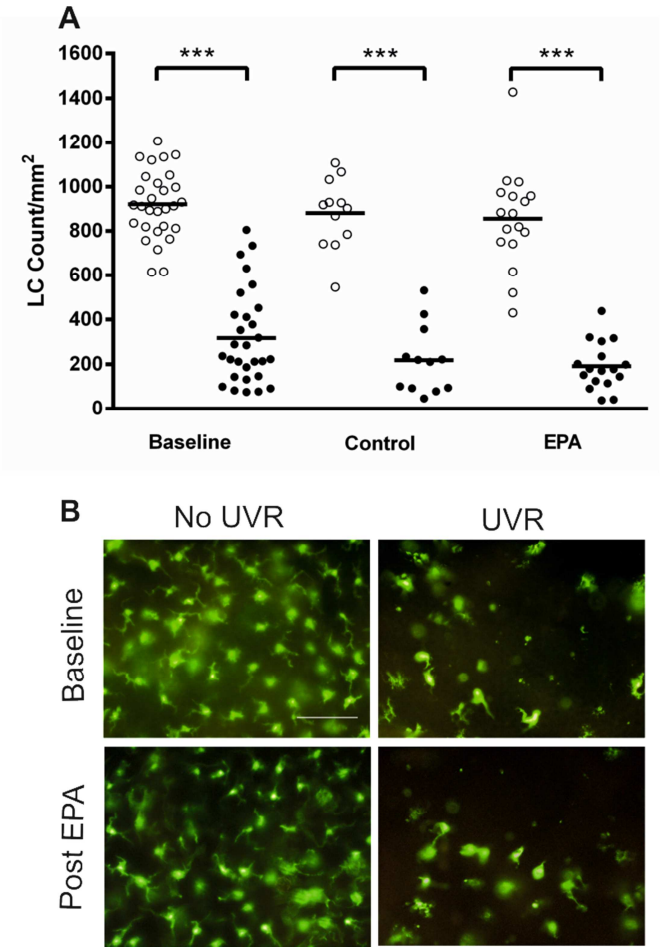
620 **Fig 1.**

621



622
623
624
625
626
627
628
629
630
631
632
633
634
635
636
637
638
639
640

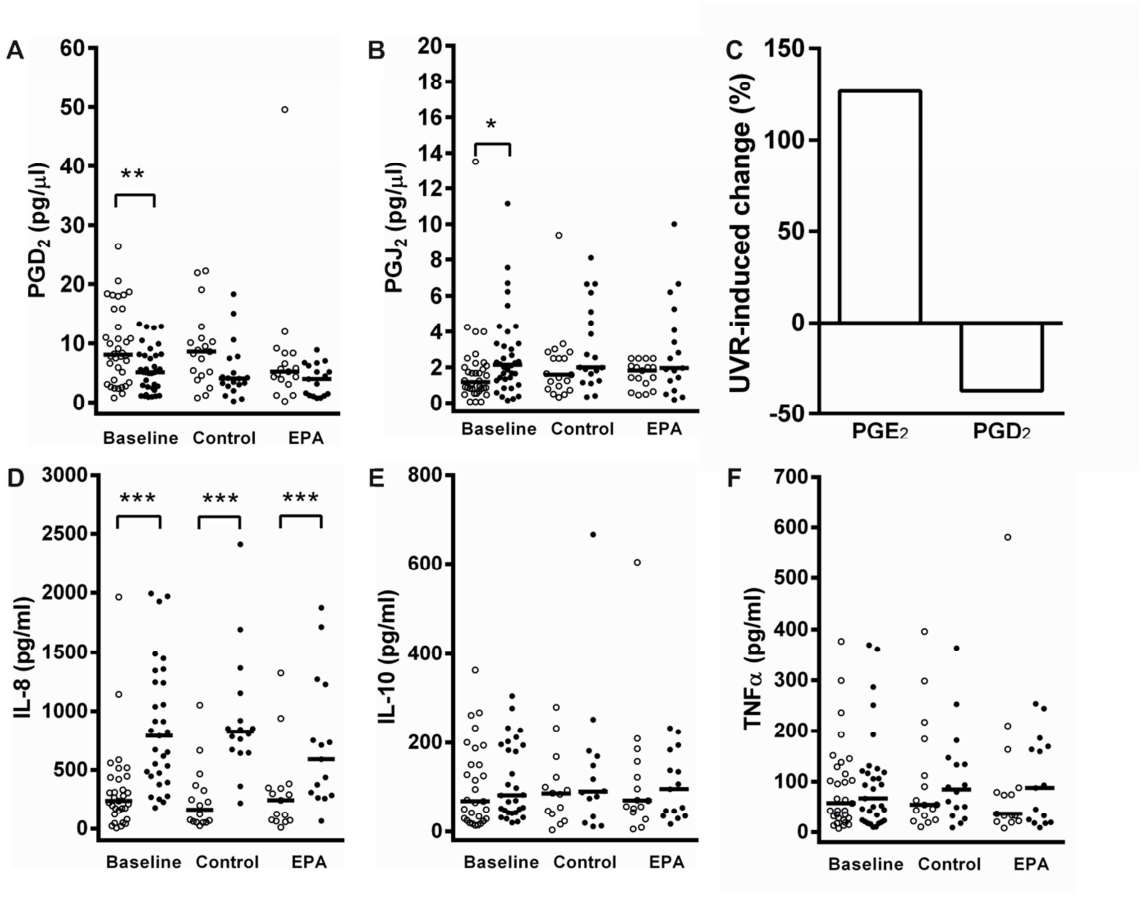
Fig 2.


A
1600
1400
1200
1000
800
600
400
200
0
LC Count/mm2
***
***
***
Baseline
Control
EPA
B
No UVR
UVR
Baseline
Post EPA

664 **Fig 3.**

665

666



667