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

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28 **Abstract**

29 Langerhans cells (LC) are sentinels of skin's immune system, their loss from epidermis
 30 contributing to UVR-suppression of cell mediated immunity (CMI). Omega-3 polyunsaturated
 31 fatty acids ~~can~~ show potential to abrogate UVR-suppression of CMI in mice and humans,
 32 potentially through modulation of LC migration. Our objectives were to examine if
 33 eicosapentaenoic acid (EPA) ingestion influences UV-mediated effects on epidermal LC
 34 numbers and levels of immunomodulatory mediators including prostaglandin (PG)D₂, which
 35 is expressed by LC.

36 In a double-blind randomised controlled study, healthy individuals took 5g EPA-rich
 37 (n=40) or control (n=33) lipid for 12-weeks; UVR exposed and unexposed skin samples were
 38 taken pre- and post-supplementation. Epidermal LC numbers were assessed by
 39 immunofluorescence for CD1a, and skin blister fluid PG and cytokines quantified by LC-
 40 MS/MS and Luminex assay, respectively. Pre-supplementation, UVR reduced mean (SEM)
 41 LC number/mm² from 913 (28) to 322 (40) ($p<0.001$), and mean PGD₂ level by 37% from 8.1
 42 (11.6) to 5.1 (5.6) pg/ μ l; $p<0.001$), while IL-8 level increased ($p<0.001$). Despite confirmation
 43 of EPA bioavailability in red blood cells and skin in the active group, no between-group effect
 44 of EPA was found on UVR-modulation of LC numbers, PGD₂ or cytokine levels post-
 45 supplementation.

46 Thus no evidence was found for EPA abrogation of photoimmunosuppression
 47 through an impact on epidermal LC numbers. Intriguingly, UVR-exposure substantially
 48 reduced cutaneous PGD₂ levels in humans, starkly contrasting with reported effects of UVR
 49 on other skin PG. Lowered PGD₂ levels could reflect LC loss from the epidermis and/or
 50 altered dendritic cell activity, and may be relevant for phototherapy of skin disease.

51

52

53 **Introduction**

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

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

75 The response of LC and other dendritic cells to antigen are strongly influenced by
 76 signals in the skin microenvironment. Cytokines TNF- α and IL-1 β stimulate LC migration
 77 from the epidermis after exposure to antigen [13, 14], and both are upregulated in the skin in
 78 response to UVR-exposure. UVR also upregulates further cytokines possessing pro-
 79 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. PGE₂ 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 PGD₂ in the skin, alongside mast cells and keratinocytes [19]. A role for PGD₂ in photoimmunosuppression has not been explored but in murine skin and lung epithelia PGD₂ 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.

108 **Materials and Methods**

109 **Participants**

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

121

122 **Study Design and Intervention**

123 The double-blind randomised (1:1) controlled parallel-group study took place in the
124 Photobiology Unit, Dermatology Centre, Salford Royal Hospital (Manchester, UK).
125 Treatment allocation sequence was permuted block design (mixed blocks of 4 to 6) and
126 produced by the study biostatistician using statistical software (v2.7.7; StatsDirect Ltd,
127 Altrincham, UK). Encapsulated active and control lipid supplements, identical in
128 appearance, were packaged and labeled according to the allocation sequence by GP
129 solutions Ltd (Manchester, UK), and the code held by the study biostatistician until study
130 completion. All volunteers and researchers were blinded and volunteers were assigned the
131 intervention on study enrolment and concurrently randomised to have either suction blister
132 fluid sampled for analysis of eicosanoids and cytokines or skin punch biopsies taken for
133 assessment of epidermal LC. Skin sampling was performed on both unexposed and UVR-
134 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 erythral 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°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 PGB₂-d₄ (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 PGD₂ (*m/z* 351 >271) and its metabolites PGJ₂, Δ¹²-PGJ₂ (*m/z* 333 >271) and 15-deoxy-Δ^{12,14} PGJ₂ (*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-1 β , IL-4, IL-10, IL-23 and IL-17) was simultaneously quantified in suction blister fluid using the Bio-PlexTM 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 PGD₂ and its metabolites PGJ₂, Δ^{12} -PGJ₂ and 15-deoxy- $\Delta^{12,14}$ PGJ₂ were measured in skin
 249 blister fluid to explore impact of UVR and EPA; Δ^{12} -PGJ₂ was detected but below the limit of
 250 quantitation and 15-deoxy- $\Delta^{12,14}$ PGJ₂ was below limit of detection. At baseline, data from
 251 both supplement groups was combined to examine effect of UVR exposure.

252

253 *PGD₂*: At baseline, median (IQR) PGD₂ 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 PGD₂ 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 PGD₂ occurred post-UVR.
 258 Comparison of groups post-supplementation revealed that in unexposed skin PGD₂ 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 *PGJ₂*: At baseline, PGJ₂ 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 PGJ₂ were seen in UVR-exposed skin in control and EPA groups (non-significant). There
 266 were no significant differences in PGJ₂ 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.

TNF α : At baseline, median (IQR) TNF α 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 TNF α 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 TNF α 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 PGD₂. This significant UVR impact on PGD₂ production (Fig 3A) is in stark contrast to the well-described increase in skin PGE₂ 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-1 β 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 TNF α 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 PGE₂ level in this group of individuals was
 augmented at 24 hours post UVR challenge [30]. PGE₂ 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 PGE₂, we found skin PGD₂ levels in
 the same volunteers were significantly reduced by 37% after UVR-exposure (Fig 3C). PGD₂
 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 PGD₂, including hair-
 loss [57]. PGD₂ 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 PGD₂
 and PGE₂ may contribute to the therapeutic effects of phototherapy. Increased levels of the
 PGD₂ dehydration product, PGJ₂, in UVR-exposed skin is also interestingly, as J ring

metabolites, in particular 15-deoxy- $\Delta^{12,14}$ -PGJ₂, 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 PGD₂ [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 PGD₂ could partially reflect loss of LC from the epidermis. This is supported by our observation of no UVR-induced PGD₂ 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 PGD₂ correlate with impaired migration of respiratory DC, and antagonism of the DP1 receptor restores migration [16]. Constitutive levels of PGD₂ may provide an inhibitory signal to migration which can be downregulated by UVR. While we did not find an effect of EPA on PGD₂ 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 PGD₂ 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 PGD₂ level

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

385

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395

396 Conflict of Interests

397 The authors have no conflicts of interest.

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Table 1. Baseline characteristics of participants.

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>I</i>	2/33 (6)	6/40 (15)
<i>II</i>	31/33 (94)	34/40 (85)
HRT/OCP ³ (no./total (%))	2/33 (6)	6/40 (15)

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

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

³ Hormone replacement therapy/ oral contraceptive pill

Figure legends

Fig 1. Flow diagram of study design and participants.

Fig 2. UVR induces LC loss from epidermis but EPA supplementation has no impact on epidermal LC numbers. (A) LC count (mean) per mm² of epidermis and (B) images of CD1a positive LC in epidermal sheets in unexposed (open circles) and UVR-exposed skin (closed circles) at baseline (n=30) and post-supplementation (control n=12, EPA n=18); *** $p < 0.001$ (scale bar 50µm).

Fig 3. UVR reduces PGD₂ and increases IL-8 level in skin blister fluid.

Concentration (median) of (A) PGD₂, and (B) its metabolite PGJ₂ in skin blister fluid taken from unexposed (open circles) and UVR-exposed (closed circles) skin at baseline (n=36) and post-supplementation (control n=19, EPA n=17); * $p < 0.05$, ** $p < 0.01$. (C) % change in PGD₂ in UVR-exposed skin in comparison with UVR-induced % change in PGE₂ [24], in skin blister fluid at baseline (n=36). Concentration of (D) IL-8, (E) IL-10 and (F) TNF-α in skin blister fluid taken from unexposed (open circles) and UVR-exposed (closed circles) skin at baseline (n=31) and post-supplementation (control n=16 (IL-10 n=14), EPA n=15); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig 1.

Fig 2.

Fig 3.