Randomized controlled trial of oral omega-3 PUFA in solar-simulated radiation-induced suppression of human cutaneous immune responses^1–3


ABSTRACT

Background: Skin cancer is a major public health concern, and the majority of cases are caused by solar ultraviolet radiation (UVR) exposure, which suppresses skin immunity. Omega-3 (n−3) PUFAs protect against photoimmunosuppression and skin cancer in mice, but the impact in humans is unknown.

Objectives: We hypothesized that EPA-rich n−3 PUFA would abrogate photoimmunosuppression in humans. Therefore, a nutritional study was performed to assess the effect on UVR suppression of cutaneous cell-mediated immunity (CMI) reflected by nickel contact hypersensitivity (CHS).

Design: In a double-blind, randomized controlled study, 79 volunteers (nickel-allergic women, 22–60 y old, with phototype I or II) took 5 g n−3 PUFA–containing lipid (70% EPA plus 10% DHA) or a control lipid daily for 3 mo. After supplementation, nickel was applied to 3 skin sites preexposed on 3 consecutive days to 1.9, 3.8, or 7.6 J/cm^2 of solar-simulated radiation (SSR) to 3 unexposed control sites. Nickel CHS responses were quantified after 72 h and the percentage of immunosuppression by SSR was calculated. Erythrocyte [red blood cell (RBC)] EPA was measured by using gas chromatography.

Results: SSR dose-related suppression of the nickel CHS response was observed in both groups. Photoimmunosuppression appeared less in the n−3 PUFA group than in the control group (not statistically significant [mean difference (95% CI): 6.9% (−2.1%, 15.9%)]). The difference was greatest at 3.8 J/cm^2 SSR [mean difference: 11% (95% CI: 0.5%, 21.4%)]. Postsupplementation RBC EPA was 4-fold higher in the n−3 PUFA group than in the control group (mean difference: 2.69% [95% CI: 2.23%, 3.14%]), which confirmed the EPA bioavailability.

Conclusion: Oral n−3 PUFAs appear to abrogate photoimmunosuppression in human skin, providing additional support for their chemopreventive role; verification of study findings is required. This trial was registered at clinicaltrials.gov as NCT01032343. Am J Clin Nutr 2013;97:646–52.

INTRODUCTION

Solar ultraviolet radiation (UVR)^4 is the primary cause of the majority of nonmelanoma skin cancers, the incidence of which continues to rise in white-skinned populations (1, 2). UVR initiates potentially mutagenic DNA damage in keratinocytes and suppresses cell-mediated immunity (CMI), allowing tumor cells to escape immune destruction (3, 4). The role of immune suppression in skin cancer pathogenesis was discovered in mouse models, in which highly antigenic tumors that were transplanted into chronically UVR-exposed recipients were not rejected and were able to progress (5, 6). Consistent with this role, there is a greatly increased incidence of skin cancer in immunosuppressed patients, such as in patients who receive organ transplants (7). Thus, nutritional strategies with potential to abrogate UVR-induced immunosuppression (photoimmunosuppression) may assist the prevention of skin cancers in humans. Epidemiologic evidence indicates that n−3 PUFAs could present such an opportunity because high dietary levels are associated with reduced skin cancer risk (8, 9). The protective effect is believed to operate during the promotion stage of carcinogenesis. In mice, mixed n−3 PUFAs reduced the UVR suppression of CMI (10), reduced tumor multiplicity, and increased tumor latency (11–13), whereas concentrated EPA (20:5n−3) resulted in the near complete inhibition of photoimmunosuppression (14).

In humans, the cutaneous EPA content is increased with dietary intake (15, 16). The content competes with the n−6 PUFA arachidonic acid (AA; 20:4n−6) for the incorporation into cellular phospholipids and for cyclooxygenase and lipoxygenase metabolism (17). A high dietary n−6 PUFA content was

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^4Abbreviations used: AA, arachidonic acid; BHT, butylated hydroxytoluene; CHS, contact hypersensitivity; CMI, cell-mediated immunity; EI, erythema index; FAME, fatty acid methyl ester; NSAID, nonsteroidal antiinflammatory drug; PG_E2, prostaglandin E_2; RBC, red blood cell; SSR, solar-simulated radiation; UVR, ultraviolet radiation.

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reported to enhance photocarcinogenesis, potentially via the augmentation of UVR-induced immunosuppressive eicosanoids and cytokines (10, 12, 18). Thus, supplementary n−3 PUFAs, in particular EPA, may confer protection against UVR suppression of CMI in humans.

Cutaneous CMI responses involve the detection of nonself antigens by resident antigen-presenting cells, which migrate to the lymph nodes and stimulate antigen-specific T lymphocytes (19). UVR reduces CMI in human skin in a dose-dependent manner (20, 21), and this effect can be quantified by measuring the inhibition of immune responses to recall antigens applied topically or injected into the skin (22). Nickel is frequently used as the recall antigen because contact hypersensitivity (CHS) to this metal is common in the population and affects ~15% of women and ~5% of men (23). The measurement of the erythemal component of the eczematous response provides a reproducible method for the assessment of the impact of systemic agents (24, 25). We hypothesized that oral n−3 PUFA supplements would reduce photoimmunosuppression in human skin and investigated this effect in a double-blind, randomized controlled study. Seventy-nine nickel-allergic volunteers took EPA-rich n−3 PUFA or control lipid supplements for 12 wk, and the suppression of nickel CHS responses by solar-simulated radiation (SSR) was compared between groups postsupplementation. Erythrocyte [red blood cell (RBC)] EPA concentrations were analyzed to assess compliance and bioavailability.

SUBJECTS AND METHODS

Participants

Seventy-nine healthy female volunteers with nickel allergy were recruited. The selected population consisted of women in view of the high prevalence of nickel allergy in women that is attributable to skin piercing and wearing jewelry (23). Recruitment was from the contact dermatitis investigation unit at Salford Royal National Health Service Foundation Hospital and by open advertisement. Volunteers were eligible to take part if they were aged between 18–60 y and of sun reactive skin type I or II (ie, sunburns easily with no or minimal ability to tan) and were demonstrated to be positive for a nickel patch test. Volunteers were excluded from joining the study if they were taking n−3 PUFAs supplements, consumed >2 portions oily fish/wk, were pregnant or breastfeeding, had sunbathed or used a tanning bed in the 3 mo before the study, were taking photoactive or anti-inflammatory medication, or had a history of photosensitivity, skin cancer, or atopy. Ethical approval was granted by North Manchester local research ethics committee (08/H1006/30), and the study was performed in accordance with Declaration of Helsinki principles (revised Seoul 2008). Written informed consent was provided by all volunteers before inclusion in the study.

Study design and intervention

The study took place in the Photobiology Unit, Dermatological Sciences, Salford Royal National Health Service Foundation Hospital (Manchester, United Kingdom) between 2008 and 2010. It was performed as a double-blind, randomized (1:1), controlled, parallel-group study that assessed the effects of systemic long-chain n−3 PUFA compared with control lipid supplements on the UVR suppression of CMI (Figure 1). All individuals who conducted the research and sample analysis were blinded to the randomization code as were the subjects. The treatment-allocation sequence was done by using a permuted block design (mixed blocks of 4–6) and produced by the study biostatistician with
Determination of nickel patch dose

To confirm nickel allergy and determine the optimal nickel dose to use for each volunteer, patch testing was performed in each individual before recruitment. A double dilution dose series (0.15–5%) of nickel sulfate (Chemical Abstracts Service no.10101–97-0) concentrations in soft white petroleum (Chemical Abstracts Service no.8009–03-8) was used (Chemotechnique Diagnostics). Nickel sulfate ointment was applied to Finn Chamber discs (8-mm diameter; Bio-Diagnostics Ltd) in 5-mm lengths via a syringe, as described previously (27). The Finn Chamber discs were applied to the skin of the upper back and remained in place for 48 h. Contact-hypersensitivity reactions were examined 24 h after patch removal. Volunteers were recruited if they showed an eczematous response [assessed by International Contact Dermatitis Research Group scale + (weak positive reaction) or higher]. The nickel sulfate dose–producing induration with a moderate confluent erythema was chosen for the photoimmunosuppression studies of each volunteer as previously described (22).

Assessment of photoimmunosuppression

The UVR source was a solar simulator (model 91293–1000) with a 1000W xenon arc lamp, fitted with an Atmospheric Attenuation Filter (model 81017; Newport Spectra-Physics Ltd) to remove UVC and modify the spectral output to simulate the ground-level ambient solar UVR (300–400 nm). Irradiance was measured 10 cm from the source before each irradiation by using a Waldmann UVR radiometer (model IL 730A; International Light) calibrated for the source and with standards traceable to the UK National Physical Laboratory. SSR doses effective for immunosuppression were determined by previous assessment in 10 individuals (data not shown). In the randomized controlled trial, before ceasing supplementation, each volunteer was irradiated on the midback at 3 separate 2.5-cm² sites with 1.9, 3.8, and 7.6 J/cm² SSR for 3 consecutive days (Figure 2). All participants were given the same absolute doses of SSR. The highest dose of SSR applied was equivalent to ~70% of the average UVR-erythemal threshold (ie, the minimal erythema dose of the group). The predetermined dose of nickel sulfate for each individual was applied to each of the 3 SSR-exposed sites and to 3 adjacent unirradiated sites on the skin of the midback (Figure 2). The nickel remained in place for 48 h, and 24 h after patch removal, the erythema of CHS reactions was quantified by using a reflectance instrument (Diastrom Ltd) to provide erythema index (EI) values. Readings were taken in triplicate, and the mean was calculated. The mean EI of SSR-exposed sites was subtracted from the mean EI unexposed sites to determine the percentage of photoimmunosuppression of the response.

Assessment of RBC EPA content

Blood sampling

Blood samples (7 mL) were taken from the antecubital fossa of all participants and collected in 3.4-mL EDTA-containing monovette tubes (Sarstedt). Samples were centrifuged for 15 min at 1500 rpm, and RBC fractions were collected and stored at −70°C until analysis.

PUFA quantification

RBCs were defrosted on ice before extraction and incubated overnight in chloroform:methanol (4 mL; 2:1 vol:vol) with 0.01% (wt:vol) butylated hydroxytoluene (BHT) at 4°C. Samples were homogenized by using a blade homogenizer and extracted with an additional 2 vol chloroform:methanol:BHT as described previously (28). Fatty acid methyl esters (FAMEs) were prepared with boron trifluoride (14% in methanol) (Sigma...
Aldrich) and heneicosanoic acid (21:0) (ACS reagent; Sigma Aldrich) was used as the internal standard. FAME analysis was performed with an Agilent 6850 gas chromatography system (Agilent Technologies) with a BPX70 capillary column (0.25-μm film, 60 m × 0.25 mm; SGE Europe Ltd) by using helium as the carrier gas and a flame-ionization detector. Fatty acids were identified by direct comparison of individual retention times to a 37 FAME mixed standard (Supelco). Results are presented as the percentage of total fatty acids.

Sample size and statistical analysis

To detect a difference in photoimmunosuppression of 15% between treatment groups (SD: 24%) at the 5% significance level and 90% power, a sample size of ≥28 per group was estimated. The percentage of photoimmunosuppression at each test site was calculated as follows:

\[
\text{[(EI of unexposed skin} - \text{EI of SSR-exposed skin)} \div \text{EI of unexposed skin}] \times 100
\]

Statistical analyses were performed with SPSS 16.0 software (SPSS Inc). The normality of data were determined by using the Kolmogorov-Smirnov test. Photoimmunosuppression data were analyzed by using repeated-measures ANOVA. RBC EPA data were transformed by using the ln to obtain normally distributed data. ANCOVA was performed to compare RBC EPA content between n−3 PUFA and control groups postsupplementation with baseline data as the covariate. Within-group RBC EPA pre-supplementation and post-supplementation contents were assessed by using a paired t test.

RESULTS

Volunteers and compliance

Seventy-nine volunteers were recruited. Six volunteers dropped out of the study because of reasons unrelated to the study; these volunteers had been randomly assigned to the control group, but no data were collected for these individuals (Figure 1). Of the 73 individuals who remained (median age: 44 y; range: 21–60 y), 33 subjects were in the control group, and 40 subjects were in the n−3 PUFA supplementation group. An assessment of the baseline characteristics of groups revealed that the control and n−3 PUFA groups were similar in age, BMI, sun-reactive skin type, and number of individuals who were taking an oral contraceptive pill or undergoing hormone replacement therapy (Table 1). No adverse effects related to supplementation were reported. Three individuals in the n−3 PUFA group were excluded from analyses because of poor compliance (2 individuals showed no change in RBC EPA concentrations, and one individual showed decreased RBC EPA post-supplementation. The average dietary intake of EPA in the study population was 23 mg/d as assessed by using a food-frequency questionnaire, which recorded dietary information relating to the 6-mo period before the study (26). This average intake is below the current recommended intake for the United Kingdom according to recommendations made to the Food Standards Agency by the Scientific Advisory Committee on Nutrition (29).

TABLE 1
Baseline characteristics of completing participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control group</th>
<th>n−3 PUFA group</th>
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</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>45 (22–60)</td>
<td>43 (21–60)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 (19.3–34.5)</td>
<td>28.0 (19.8–44.1)</td>
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<tr>
<td>Skin type [n/total n (%)]</td>
<td>I 2/33 (6)</td>
<td>8/40 (20)</td>
</tr>
<tr>
<td></td>
<td>II 31/33 (94)</td>
<td>32/40 (80)</td>
</tr>
<tr>
<td>HRT/OCP [n/total n (%)]</td>
<td>2/33 (6)</td>
<td>7/40 (18)</td>
</tr>
</tbody>
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1 HRT, hormone replacement therapy; OCP, oral contraceptive pill.
2 Median; range in parentheses (all such values).
3 BMI data from n = 31 in the n−3 PUFA group and n = 31 in the control group.
4 Fitzpatrick skin-type classifications were as follows: I = always burns and never tans; and II = usually burns and tans with difficulty.

UVR suppression of CHS response to nickel

Data from one individual in the n−3 PUFA group was excluded because of a failure of the nickel patch test (ie, n−3 PUFA: n = 36; control: n = 33; Figure 1). In the control group the CHS response to nickel was reduced in a UVR dose–related manner as anticipated after SSR exposure; consequently, the mean (95% CI) percentage of photoimmunosuppression was 15% (3.7%, 25.2%) at 1.9 J/cm², 21% (13.9%, 28.1%) at 3.8 J/cm², and 43% (34.9%, 50.8%) at 7.6 J/cm² (Figure 3). In the n−3 PUFA group, the CHS response to nickel showed less suppression by SSR exposure in comparison with the control group, with 8% (−0.5%, 16.7%) photoimmunosuppression at 1.9 J/cm², 10% (2.3%, 17.9%) photoimmunosuppression at 3.8 J/cm², and 40% photoimmunosuppression (29.7%, 49.3%) at 7.6 J/cm². No interaction of UVR dose by treatment group interaction was observed (P = 0.58; ANOVA). The group difference in photoimmunosuppression across the 3 SSR doses was 6.9% (95% CI: −2.1%, 15.9%; P = 0.13; ANOVA). Although nonsignificant within the adjusted P limit for multiple t tests (P = 0.016), a small difference was observed between treatment groups at 3.8 J/cm² SSR [11% (0.5%, 21.4%); P = 0.04; unpaired t test]. Whether estrogen concentrations in practice influence CHS responses in women is uncertain, with conflicting
results shown in studies (30), but in the current study, the omission of individuals who were taking hormone replacement therapy or oral contraceptive pills from the analysis had no effect on the results (data not shown).

RBC EPA content

Because of technical difficulties, there was no RBC EPA data for one volunteer, and no postsupplementation EPA data for another volunteer, both subjects were in the n-3 PUFA group (ie, n-3 PUFA: n = 35; control: n = 33; Figure 1). Pre-supplementation in control and n-3 PUFA groups had a similar mean EPA content at 0.9% (95% CI: 0.7%, 1.0%) and 0.93% (0.8%, 1.1%) of total fatty acids, respectively (Figure 4). After 3 mo of supplementation, the RBC EPA content was significantly increased in the n-3 PUFA group but not in the control group compared with at baseline ($P < 0.001$ and $P = 0.23$, respectively; paired $t$ test). In addition, the EPA content in the n-3 PUFA group was 3.62% (3.2%, 4.0%) compared with 0.92% (0.8%, 1.0%) in the control group postsupplementation (mean difference: 2.69% (2.23%, 3.14%); $P < 0.001$; ANCOVA with the baseline as a covariate).

DISCUSSION

In this double-blind, randomized controlled study, we used a reproducible method for inducing cutaneous CMI responses in humans in vivo by using nickel as a recall allergen (4). This method provided an effective noninvasive system in which to assess immunosuppression by UVA and UVB components of sunlight and its potential abrogation by dietary n-3 PUFA (21, 22). We observed a UVR dose-dependent increase in the percentage of photoimmunosuppression of the nickel CHS response that reached 43% at the highest SSR dose used. Our findings were consistent with those of Damian et al (20) who reported that immunosuppression can be induced by low doses of SSR. The current study suggested that n-3 PUFAs are protective against photoimmunosuppression. Immunosuppression was 50% lower after active supplementation compared with after the control at an SSR dose of 3.8 J/cm², which was equivalent to ~15 min of summer midday sun in Manchester, United Kingdom (53.5°N). At the lowest dose of SSR used (equivalent to ~8 min of summer midday sun exposure) the percentage of immunosuppression also appeared to be abrogated by ~50% in the n-3 PUFA group, whereas immunosuppression induced by the highest SSR dose (equivalent to ~30 min of midday sun) showed little influence of n-3 PUFAs. Because the dose-response for some protective effects of EPA is unknown, higher supplemental doses remain to be explored. Thus, our data indicated that increased oral n-3 PUFA intake could provide protection from the cutaneous immunosuppression that occurs during short exposures to solar UVR.

Immunological mechanisms that underlie UVR suppression of CHS and tumor promotion are similar (4) and support the potential chemopreventive activity of these lipids in humans (8, 9). Our findings were consistent with supplementation studies in animals (14, 31) and epidemiologic surveys (8, 9) and highlighted a mechanism whereby n-3 PUFA supplementation may act to reduce UVR-induced skin cancer risk in humans (32). This study was pertinent because traditional topical sunscreens are less effective at protecting against photoimmunosuppression than against UVR-induced erythema, as indicated by the sun protection factor (33), and they are generally applied inadequately, as well as infrequently, outside of vacation periods (34, 35). In the current study, the difference in photoimmunosuppression between groups equated to an immune protection factor of ~2 for oral n-3 PUFAs. Although this change was small, a continuous low level of chemoprevention could reduce risk of skin cancer over an individual’s lifetime, which would provide a substantial effect at the population level. The dietary supplementation of 4 g n-3 PUFAs/d given in this study was equivalent to ~1.5 portions oily fish/d, but in practice, intake could be assisted by the use of n-3 PUFA–fortified foods. Omega-3 PUFAs, and in particular EPA, are widely reported to have many beneficial health effects in, eg, cardiovascular disease (36). Thus, increased dietary intake could have a range of potential benefits in addition to skin health.

The bioavailability of the n-3 PUFA supplement was confirmed by the 4-fold increase in RBC EPA; erythrocyte membranes respond more conservatively to changes in dietary lipid than plasma (37), which makes them a more suitable indicator of tissue content. Omega-3 PUFAs are readily taken up into human skin (15, 16) where they may influence UVR-induced immunosuppression through several mechanisms, including the effect on membrane fluidity, signal transduction, transcription-factor activation, and soluble mediator production (38). In addition to an effect on resident skin cells, EPA is also readily taken up into infiltrating leukocytes including T cells (39). The alteration in cyclooxygenase-2 metabolism of n-6 PUFAs is a potential mechanism for the modulation of immune responses by n-3 PUFAs. Cyclooxygenase-2 expression is upregulated in both UVR-exposed skin (40, 41) and skin tumors (42, 43) and has been identified as a pharmacologic target for the prevention of skin cancer. In mice, high dietary n-6 PUFAs increase risk of photocarcinogenesis (12, 18), the effect of which was shown to result in the development of previously latent UVR-initiated tumors (44).

The n-6 PUFA AA is metabolized by cyclooxygenase-2 to prostaglandin E2 (PGE2), which is a potent immunosuppressor (45) that also mediates UVR-induced inflammation and erythema (40, 46). In mice, UVR exposure or injection of PGE2 increases the expression of immunosuppressive cytokines IL-4 and IL-10 and, thus, shifts the balance from proinflammatory T helper 1–lymphocyte–driven CMI responses toward T helper

![FIGURE 4. RBC EPA content before and after the lipid course. Results are presented as the percentage of EPA of total fatty acids (control: n = 33; n-3 PUFA: n = 35). The mean (95% CI) treatment-group difference Post was 2.69% (2.23%, 3.14%) (***$P < 0.001$; ANCOVA with the baseline as a covariate). Post, after supplementation; Pre, before supplementation; RBC, red blood cell.](https://academic.oup.com/ajcn/article-abstract/97/3/646/4571525)
2 and T-regulatory lymphocyte responses associated with immunosuppression (47, 48). Eicosapentaenoic acid competes with AA for cyclooxygenase-2 metabolism and, thereby, reduces PGE2 concentrations in sunburnt skin (46, 49). Therefore, an increased tissue EPA content might abrogate photoimmunosuppression partially through a dampening of UVR-induced PGE2 synthesis.

Nonsteroidal antiinflammatory drugs (NSAIDs) inhibit both prostaglandin synthesis and UVR suppression of CHS (45, 50) and protect against skin cancer development (51–54). However, nonselective and selective NSAIDs are associated with gastrointestinal and cardiovascular complications, respectively (55). Omega-3 PUFA reduce UVR-induced PGE2 concentrations (46, 49) and provide a safer strategy to chemoprevention than do NSAIDs (56). Additional studies are required to verify the findings of the current study. The nickel patch was chosen as a recall antigen to assess the effect of n–3 PUFA on photoimmunosuppression; however, additional studies could use the Mantoux test, which involves the intradermal injection of purified protein derivative. Although this method is more invasive, it can be performed in anyone previously vaccinated against tuberculosis and would facilitate the investigation of a broader population. The effect of n–3 PUFA on the suppression of the initiation phase of CHS could also be assessed. However, this assessment would require the active sensitization of individuals to chemicals such as dinitrochlorobenzene (57).

In conclusion, this study suggests that supplementation with EPA-rich n–3 PUFA, which is a natural dietary agent, may protect human skin from photoimmunosuppression induced by short exposures to solar UVR. This study adds to the evidence, and indicates a potential mechanism, for protection against skin cancer by n–3 PUFA in humans.

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The authors’ responsibilities were as follows—LER, PSF, and NKG: designed the study; SMP and SPB: conducted the research; AN, KAM, and LER: wrote the manuscript; and had final responsibility for the content of the manuscript; and SMP and LER: performed statistical analyses; SMP and SPB: conducted the research; AN, KAM, and LER: wrote the manuscript; and had final responsibility for the content of the manuscript; and all authors: read and approved the final manuscript. None of the authors declared a conflict of interest.

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