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Letter to Editor

## Dietary supplementation of omega-3 fatty acid eicosapentaenoic acid does not ameliorate pruritus in murine models of atopic dermatitis and psoriasis

Pruritus, or itch, is an uncomfortable sensation that elicits a desire to scratch. Several dermatologic disorders such as atopic dermatitis (AD) and psoriasis cause chronic pruritus that may greatly reduce the quality of life of those affected. Since scratching damages the skin barrier of the affected area and exacerbates the symptoms, mitigation of pruritus is extremely important for the treatment of these diseases. However, this type of chronic pruritus is generally resistant to antihistamines, the most widely used antipruritic drugs. Therefore, there is a great demand for the development of new treatments for antihistamine-resistant pruritus.

Arachidonic acid (AA), one of the most abundant omega-6 fatty acids, is a precursor of proinflammatory eicosanoids. The role and effects of eicosanoids in pruritus have been extensively studied [1]. The pruritogenicity of AA-derived mediators such as prostaglandin $E_2$ ($PGE_2$), thromboxane $A_2$ ($TXA_2$), and leukotriene $B_4$ ($LTB_4$) has been demonstrated, with intradermal injection of these lipids eliciting pruritus. $LTB_4$ is also implicated in pruritus in a number of dermatologic disorders including AD, passive cutaneous anaphylaxis, and contact dermatitis. Thus, dietary manipulation of fatty acid composition to reduce pruritogenic lipid mediators might be useful to treat antihistamine-resistant pruritus in various dermatologic conditions.

In the present study using well-established mouse models of AD and psoriasis, we investigated the effect on pruritus of dietary supplementation of eicosapentaenoic acid (EPA), an omega-3 PUFA that counteracts the production and function of AA-derived mediators. Four-week-old NC/Nga mice were fed a control diet (AIN-93 M) or a diet containing 5% (w/w) EPA for a month. AD-like dermatitis was then induced by topical application of *Dermatophagoides farinae* body extract (Dfb) ointment twice a week for 3 weeks. AD-like dermatitis as evaluated by the clinical score progressed at a comparable rate in both groups (Fig. 1a). The TEWL, SC hydration, and pH of the skin surface did not differ significantly between the two groups (Fig. 1b–d). EPA-fed mice with AD had no less frequent scratching than did control mice (Fig. 1e).

EPA supplementation of mice in the psoriasis model was performed similarly to that in the AD model. Psoriasis-like dermatitis was induced by topical application of 5% imiquimod cream once daily for 5 days. The clinical scores of the psoriasis-like dermatitis in the EPA-fed mice were comparable to those in the control group (Fig. 2a). The increase of epidermal thickness was also comparable in both groups (data not shown). As with the AD model, EPA-fed mice had no less frequent scratching than did the control mice (Fig. 2b).

Lipidomic analysis of the skin tissues revealed that EPA supplementation markedly reduced the levels of AA and AA-derived lipid mediators including $PGE_2$, $TXB_2$ (the stable metabolite of $TXA_2$), and $LTB_4$ (Figs. 1f and 2 c). At the same time, EPA and EPA-derived metabolites were remarkably higher in the skin tissues from EPA-fed mice (Figs. 1g and 2 d). These substances included resolvin $E_1$ ($RvE_1$), an EPA metabolite that blocks $LTB_4$ signaling by binding to the $LTB_4$ receptor BLT1 [2,3]. The levels of docosahexaenoic acid (DHA) and DHA metabolites did not differ significantly or were even slightly lower in the EPA-fed mice than in controls (Figs. 1h and 2 e).

In this study, we performed extensive lipidomic analysis of the skin of mice treated to induce AD- and psoriasis-like conditions. EPA supplementation led to a marked decrease in skin levels of potentially pruritogenic lipid mediators including $PGE_2$ and $LTB_4$, as well as a drastic increase in EPA-derived metabolites, including $RvE_1$, which blocks the $LTB_4$–BLT1 axis. Nevertheless, the frequency of scratching was not significantly different in EPA-fed versus control mice. Given that pruritus is a secondary symptom arising from skin diseases, investigating the net effect of dietary omega-3 fatty acids solely on pruritus is challenging because these fatty acids often ameliorate the primary signs of the disease. Indeed, in most of the previous studies demonstrating the efficacy of omega-3 fatty acids to reduce pruritus in various dermatitis models, the signs of the dermatitis were also ameliorated [4–6]. In contrast, in the present study, the signs of the AD or psoriasis that developed did not differ between EPA-fed and control mice. This discrepancy in our findings with those from previous studies may be attributable to the relatively short time we fed the mice EPA, only 1 month prior to the pathogenic challenge. Several other studies fed the omega-3 diet for a longer period [4–6]. Even so, as revealed by our lipidomic analysis, EPA supplementation had a great impact on the lipid mediator profiles in the skin of the mice. We had hypothesized that such changes would be sufficient to mitigate pruritus. However, our results lead us to conclude that, at least in these models, the lipid

*Abbreviations:* PUFA, polyunsaturated fatty acid; AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LA, linoleic acid; ALA, α-linolenic acid; $PGE_2$, prostaglandin $E_2$; $TXA_2$, thromboxane $A_2$; $TXB_2$, thromboxane $B_2$; $LTB_4$, leukotriene $B_4$; $RvE_1$, resolvin $E_1$; AD, atopic dermatitis; Dfb, *Dermatophagoides farinae* body extract; TEWL, transepidermal water loss; SC, stratum corneum; SDS, sodium dodecyl sulfate.

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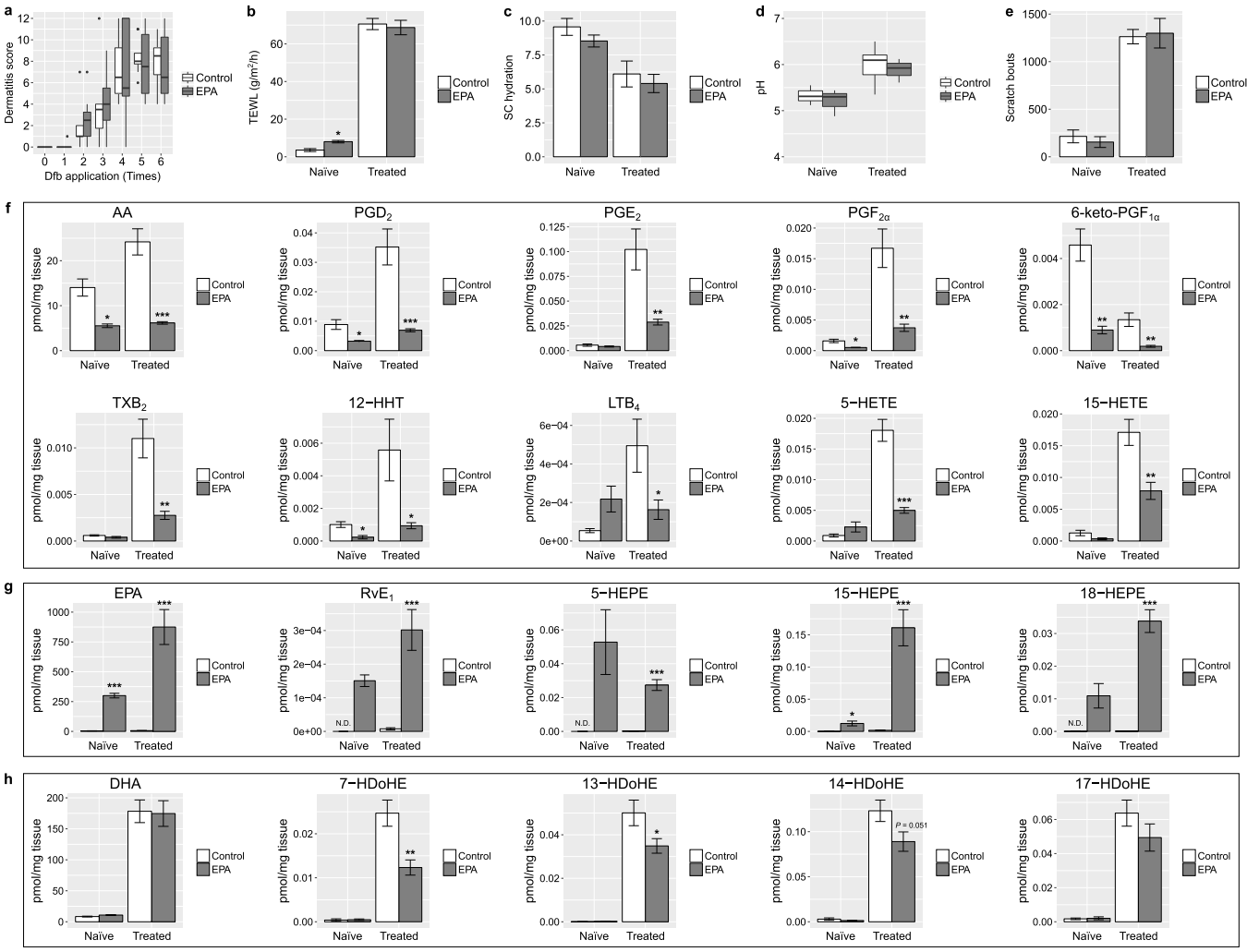


**Fig. 1.** Effects of EPA supplementation on a Dfb-induced AD model.
Male 4-week-old NC/Nga mice were fed a control diet or an EPA-rich diet for one month. AD was induced by topical applications of Dfb ointment twice a week for 3 weeks. (a) Dermatitis scores of Dfb-treated mice. The scores of naïve mice were 0. There was no significant difference between control and EPA-fed mice (Mann-Whitney *U*-test). (b–c) TEWL (b) and SC hydration (c). Data represent mean values ± SEM (n = 3 for naïve mice, n = 8 for treated mice). $^{*}P < 0.05$ (EPA-supplemented vs. control). Unpaired two-tailed *t*-test was used. There was no significant difference between Dfb-treated control and EPA-fed mice. (d) The skin surface pH did not differ significantly between control and EPA-fed mice (Mann-Whitney *U*-test). (e) Number of scratching bouts over 12 h. Data represent mean values ± SEM (n = 3 for naïve mice, n = 8 for treated mice). There was no significant difference between control and EPA-fed mice (unpaired, two-tailed *t*-test). (f–h) Lipidomic analysis of the skin of mice with Dfb-induced AD. The levels of free AA and AA metabolites (f), free EPA and EPA metabolites (g), and free DHA and DHA metabolites (h) were quantified by high performance liquid chromatography-tandem mass spectrometry. Data represent mean values ± SEM (n = 3 for naïve mice, n = 8 for treated mice). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (EPA-supplemented vs. control). Unpaired two-tailed *t*-test was used. N.D., not detected or below limit of quantitation.

mediators described above are not critically involved in the evocation of pruritus.

A caveat of this study is that we fed mice only EPA, but not other omega-3 PUFAs such as DHA. Metabolites of omega-3 PUFAs have varying therapeutic properties such as anti-inflammatory and anti-allergic activity [3]. EPA supplementation did not significantly increase the levels of DHA and DHA-derived metabolites in mouse tissues in our study, consistent with the results of previous studies [7–9]. This is likely due to the slow enzymatic conversion of EPA to DHA. Thus, it is possible that the pruritus of the AD and psoriasis models would be ameliorated by supplementation with DHA or a DHA-rich diet (e.g., fish oil) through production of as yet unknown DHA-derived antipruritogenic mediators. Ramsden et al. reported that metabolites derived from linoleic acid (LA; 18:2n-6), another very common omega-6 PUFA, are abundant in human psoriatic skin. Intradermal injection of some of these metabolites in mice induced pruritus [10]. Thus, it is possible that supplementation with ingredients such as linseed oil and perilla seed oil, which are rich in α-linolenic acid (ALA; 18:3n-3), the omega-3 counterpart of LA, may counteract LA-derived metabolites and have a therapeutic effect on pruritus. Further studies using various PUFA-manipulated diets will, it is hoped, lead to truly effective dietary treatment for antihistamine-resistant pruritus.

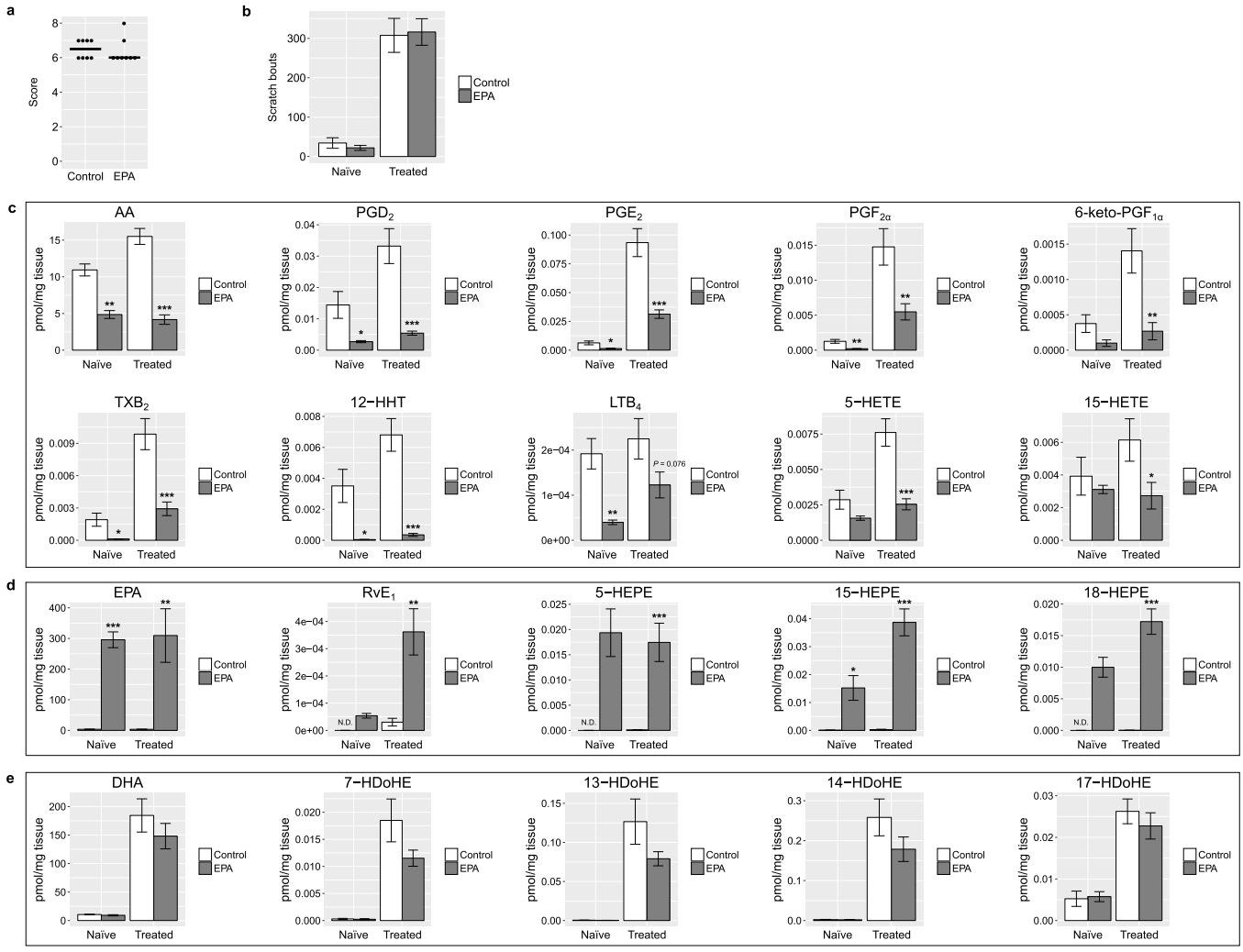


**Fig. 2.** Effects of EPA supplementation on an imiquimod-induced psoriasis model.
Male 4-week-old C57BL/6 J mice were fed a control diet or an EPA-rich diet for one month. Psoriasis was induced by topical applications of imiquimod cream once daily for 5 days. (a) Dermatitis scores of imiquimod-treated mice. The scores of naïve mice were 0. The line represents the median. No significant difference was found between control and EPA-fed mice (Mann-Whitney *U*-test). (b) Number of scratching bouts over 3 h. Data represent mean values ± SEM (n = 3–4 for naïve mice, n = 8 for treated mice). There was no significant difference between control and EPA-fed mice (unpaired two-tailed *t*-test). (c–e) Lipidomic analysis of the skin of mice with imiquimod-induced psoriasis. The levels of free AA and AA metabolites (c), free EPA and EPA metabolites (e), and free DHA and DHA metabolites (f) were quantified by high performance liquid chromatography-tandem mass spectrometry. Data represent mean values ± SEM (n = 3–4 for naïve mice, n = 8 for treated mice). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ (EPA-supplemented vs. control). Unpaired two-tailed *t*-test was used. N.D., not detected or below limit of quantitation.

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## Declaration of Competing Interest

The authors have no conflict of interest to declare.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.07.010.

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