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Oral supplementation with fish oil reduces dryness and pruritus in the acetone-induced dry skin rat model

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ABSTRACT

Background: Pruritus and discomfort are often present in patients with xerosis and atopic dermatitis. Several studies suggest an important role of diet in skin pathophysiology.
Objective: This study evaluated the effect of dietary fatty acids in the skin physiology via an itch-related animal model with and without supplementation with fish oil (FO), a source of polyunsaturated fatty acids (PUFA), especially omega 3 (*n*-3).
Methods: Male Wistar rats were divided into two groups—non-supplemented (control) and supplemented with FO (3 g/kg/day) by gavage for 90 days. Every 30 days, scratching and skin parameters (transepidermal water loss (TEWL), hydration, and local blood flow) were evaluated before and after dorsal skin exposure to acetone to induce the itch-related dry skin. At the end of the study, animals were sacrificed, and skin samples collected for fatty acids composition analysis by GC–FID.
Results: FO supplementation reduced the TEWL and increased the skin hydration, with significant changes from day 60 on, while skin microcirculation registered no changes. It also alleviated the acetone induced skin barrier alteration, revealed by a faster resolution of TEWL and hydration, and elimination of itch-related scratching induced by dry skin. These changes were associated with the shift in the skin fatty acids incorporation pattern (richer in *n*-3 with $n\text{-}6/n\text{-}3 < 5$) resulting from the FO supplementation.
Conclusion: Skin barrier dynamics seem to be influenced by FO *n*-3 PUFA, with suppressive effects on the scratching behaviour induced by dry skin. Hence, long-term supplementation with *n*-3 PUFA rich nutrients might reinforce and restore cutaneous integrity and function.

1. Introduction

Skin dryness and itching are present in several human diseases, such as renal failure, cholestasis, dermatoses and/or dermatitis (as seasonal winter or senile xerosis) including atopic dermatitis [1]. Dry skin, characterized clinically by a scaly, rough, cracked and fissured surface [2], is a consequence of the reduction of epidermal water-holding capacity, which is regulated by the "barrier" function mostly attributed to the *stratum corneum* (SC) [3]. The skin dryness itself and/or the cutaneous barrier disruption have been correlated to the dry skin-associated pruritus; the first shows reduced SC hydration while the last presents significantly increased transepidermal water loss (TEWL) [4,5]. These skin barrier defects, associated with the removal of the *stratum corneum* lipid components (free fatty acids, ceramides and cholesterol), can amplify the response to irritating stimuli and their inflammatory potential [6–8], predisposing the skin to pruritus. Pruritus or itching follows irritating stimuli and is an unpleasant sensation associated with the desire to scratch [9] which, in turn, directly aggravates skin barrier dysfunction [10]. The pathophysiology of itch is diverse and involves a complex network of cutaneous and

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neuronal cells, several receptors, and mediators (including leukotrienes) [11].

Eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) are representatives of omega 3 (*n*-3) polyunsaturated fatty acids (PUFA). These *n*-3 PUFA are found in fish from deep and cold marine waters (sardines, salmon, mackerel, trout, herring), fish-oils, other fish derived products and microalgae, as well as nuts and vegetable oils (chia, flaxseed and canola) [12,13]. These are regarded as essential fatty acids (EFA) because animal cells lack the desaturase enzymes capable of specifically placing the double bond at positions *n*-3 [14], hence they must be obtained through diet and/or supplementation. In addition to their structural and functional roles on the membrane [13], the *n*-3 PUFA also exert a regulatory function by modulating physiological and pathological conditions over multiple mechanisms, such as the inflammatory response through production of eicosanoids, including prostaglandins, thromboxanes and leukotrienes [13,15].

Despite the well-known differences between human and animal, the structural similarities between human and rodent cutaneous tissue [16–18] as well as several additional practicalities, prompt use of the rodent as a suitable model for skin pathophysiological processes including wound healing and drug permeation. The recently described acetone-induced dry skin model in mice [2,3,9,19] could be a useful tool to study medicines (or other products) to control or reduce pruritus associated to dry skin. Some studies strongly suggest that the *n*-3 PUFA and related monohydroxy metabolites play a crucial role in skin homoeostasis [20–22], since their content within the skin seems to modulate the skin barrier function as well as the inflammatory/immune reactions involved in several skin disorders [23], but the role of *n*-3 PUFA in dry skin and associated pruritus remains incompletely elucidated.

The present paper focuses on the cutaneous impact of the supplementation with fish oil, a well-known source of *n*-3 PUFA rich nutrients, on a acetone-induced dry skin rat model where the scratching behaviour and relevant skin physiological variables, obtained by non-invasive techniques, were regularly assessed and quantified and the effect on skin fatty acids composition assessed at the end of the study.

2. Materials and methods

2.1. Animals

Male Wistar rats (447 ± 14 g), purchased from Harlan (Barcelona, Spain), were kept under controlled room temperature (23 ± 3 °C) and 12 h light/dark cycles with free access to water and standard laboratory chow (4RF21 LPG, Mucedola Srl, Milan, Italy). All animal experiments were carried out in accordance with the relevant European Community and Portuguese National rules on animals' protection for experimental and other scientific purposes (the EEC Directive (86/609/EEC), the Portuguese laws (DL no. 129/92, Portaria no. 1005/92) and all following legislations).

2.2. Experimental procedure

Rats were randomly assigned into two experimental groups according to oral daily supplementation by gavage, for 90 days, with fish oil (3 g/kg bw, FO group, $n = 10$) rich in *n*-3 PUFA (Herbarium®, Colombo, PR, Brazil) or with water (C group for control, $n = 10$). The administered dose of FO was equivalent to a human daily dose of 486 mg/kg/day when adjusted according to the FDA converting factor [24]. The supplement amount and duration were defined based on preliminary studies on the subject [20,21,25–27] . Every 30 days all animals were submitted to the acetone-induced dry skin rat model (Section 2.3), and 1 h after the last cutaneous acetone exposure, *non-invasive measurements of skin characteristics* (Section 2.4) and *scratching behaviour* (Section 2.5) were conducted. At the end of the study animals were anaesthetized (ketamine/xylazine, 60/15 mg/kg, IM, respectively), euthanized, and dorsal skin samples immediately collected and stored at −80 °C for determination of the lipid profile.

2.3. Acetone-induced dry skin rat model

For both C and FO groups, the animals' dorsum hair (9×9 cm) was removed under anaesthesia (ketamine/xylazine, 60/15 mg/kg, IM, respectively) using an electric shaver and a soft hair-removing cream at least 24 h prior to the start of the dry skin protocol. The dry skin protocol described by Tominaga et al. [2] was used with some modifications. Each morning (by 9:00 AM) for 3 consecutive days, a 6×6 cm acetone (Sigma Chemicals Co., St. Louis, MO, USA) soaked cotton pad was applied for 5 min to the dorsum of the C and FO rats. All studied skin properties, as well as the scratching behaviour, were measured on the 3rd day, 1 h after the patch removal.

2.4. Measurements of skin properties

Relevant variables representing skin physiology namely, TEWL, epidermal hydration and skin blood flow, were all measured by non-invasive techniques on anaesthetized animals after room acclimatization (temperature 23 ± 3 °C and humidity 45 ± 4%).

TEWL was measured by a Tewameter TM300 (Courage + Khazaka GmbH, Cologne, Germany) and expressed in g/h/m^2, epidermal hydration was evaluated by electrical capacitance with a Corneometer CM825 (Courage + Khazaka Electronic GmbH, Cologne, Germany) and expressed in arbitrary units (AU), and skin blood flow determined by laser-doppler flowmetry (LDF) (PeriFlux System 5000, Perimed, Järfälla–Stockholm, Sweden) and expressed in arbitrary units (AU). Three measurements were obtained per time point and the mean value expressed for each rat.

2.5. Measurement of the scratching behaviour

The induced pruritus was quantified every 30 days by analysing the scratching behaviour of each animal. Half of the C and FO animals was randomly assigned to four experimental groups ($n = 10$). One hour after the last cutaneous exposure to acetone or sterile water, animals were acclimatized and scratching behaviour analysed according to the protocol described by Okawa et al. [19]. Briefly, each rat was individually placed into an acrylic box ($60 \times 34 \times 18$ cm) and the number of scratching actions counted by trained observers unaware of the treatments. One scratching action corresponded to a series of scratch movements on the dorsal skin using the hind paws and was expressed as a scratching behaviour number for each animal for a 30 min period [19,28].

2.6. Fatty acids profile analysis

The fat was extracted from dorsal skin samples of both C and FO groups using chloroform and methanol (as described by Bligh and Dyer [29]) and used for fatty acids profile determination. Samples of used standard chow and fish oil were also analysed. To prevent lipid oxidation during and after extraction, 0.02% butyl hydroxyltoluene was added to the chloroform used [30]. Fat was saponified in methanolic KOH solution and then esterified in methanolic H_2SO_4 solution [31]. Methylated fatty acids were analysed using a gas chromatograph (Agilent Technologies, HP 6890N) equipped with a capillary column (DB-23, 60 m × 0.25 mm × 0.25 μm) and flame ionization detector. The temperature of the injector port was set at 280 °C and the carrier gas was nitrogen (0.9 mL/min). After

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injection (1 μL, split ratio 50:1), the oven temperature was held at 160 °C for 1 min, then increased to 240 °C at 4 °C/min and held at this temperature for 9 min. Standard fatty acids methyl esters (37-component FAME Mix, C 22:5*n*-3 and PUFA no. 2 from Sigma, Saint Louis, MO, USA and C 22:5*n*-6 from NuChek Prep. Inc., Elysian, MN, USA) were analysed under the same conditions and the subsequent retention times used to identify the samples' fatty acids. Each analysed fatty acid was expressed as a percentage of the total fatty acids content.

2.7. Statistics

Means and standard errors were used for data expression. The skin fatty acids content was analysed by the one-way ANOVA followed by the Duncan's test. Whenever appropriate, skin parameters were analysed by the three-way ANOVA, followed by the Duncan's multiple range test. A 95% confidence level was adopted.

3. Results

3.1. Effect of the FO supplementation on skin's biometrical variables

A decrease on TEWL basal values compared to non-supplemented animals (control) was detected in the animals under daily supplementation with FO, not exposed to the acetone protocol (Fig. 1a). This difference was significantly different after 2 months of supplementation (Fig. 1b), and the effect was even more pronounced (50%) after 3 months of daily supplementation (Fig. 1c). In all three acetone trials, conducted for both control and FO supplemented animals, an increase in the TEWL was observed 1 h after the last acetone exposure, followed by a gradual decrease after 24 h, reaching the baseline value by 48 h (Fig. 1) or even after a longer period (control Fig 1c). Clear quantitative differences in the TEWL pattern in FO supplemented versus control animals were observed (52%, 41% and 44% reduced AUC on days (D) 30, 60 and 90 compared to control animals AUC); in FO animals, the maximum acetone-induced TEWL modification was significantly lower than in controls at all time points (1 h, 24 h and 48 h post exposure) and more rapidly returned to baseline values (24 h, $P > 0.05$) by D60 and D90 (Fig. 1b and c).

Regarding epidermal hydration, both groups exhibited similar basal values by D30 of the study (Fig. 2a). However, a 30% increase in skin hydration was observed in the supplemented animals ($P < 0.05$) by D60 (Fig. 2b), persisting at D90 (Fig. 2c). During the entire study, epidermal hydration of the skin was reduced following acetone treatment as expected (Fig. 2a–c), but FO supplemented animals demonstrated higher values when compared to control animals in almost all evaluated time points (1 h, 24 h and 48 h) and 11%, 32% and 19% increased AUC on D30, D60 and D90 compared to the control animals. By D90, the hydration pattern of the FO group, while still higher, approached the control values.

The skin blood flow (LDF) basal values obtained from the dorsal skin were similar in both groups during the full study (D30, D60 and D90). In the first dry skin trial (D30, Fig. 3a), the acetone

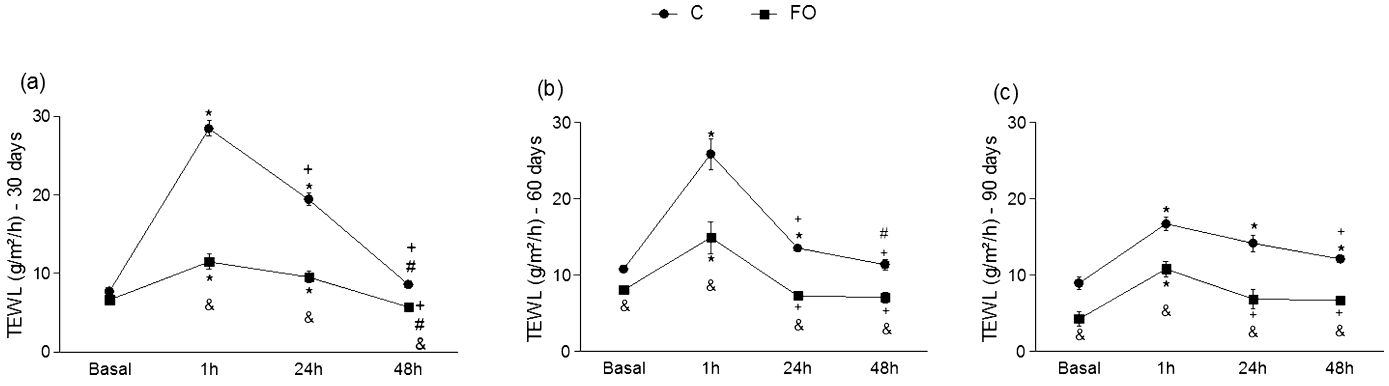

Fig. 1. Skin TEWL values, before (basal) and after (1, 24, 48 h) dorsal skin exposure to acetone, of rats fed maintenance diet alone (C, $n = 10$) or supplemented with fish oil (FO, $n = 10$) for 30 days (a), 60 days (b) and 90 days (c); data expressed as mean ± S.E.M; $^{\&}$ indicates significant difference ($P < 0.05$) between groups in the same evaluation time; * indicates significant difference ($P < 0.05$) from basal in the same group; $^{+}$ indicates significant difference ($P < 0.05$) from 1 h in the same group and $^{\#}$ indicates significant difference ($P < 0.05$) from 24 h in the same group.

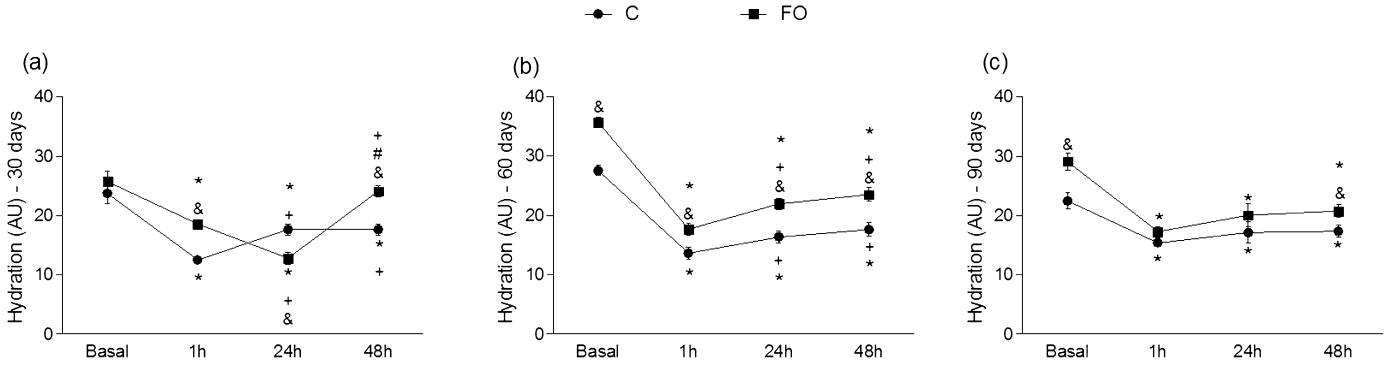

Fig. 2. Skin hydration values, before (basal) and after (1, 24, 48 h) dorsal skin exposure to acetone, of rats fed maintenance diet alone (C, $n = 10$) or supplemented with fish oil (FO, $n = 10$) for 30 days (a), 60 days (b) and 90 days (c); data expressed as mean ± S.E.M; $^{\&}$ indicates significant difference ($P < 0.05$) between groups in the same evaluation time; * indicates significant difference ($P < 0.05$) from basal in the same group; $^{+}$ indicates significant difference ($P < 0.05$) from 1 h in the same group and $^{\#}$ indicates significant difference ($P < 0.05$) from 24 h in the same group.

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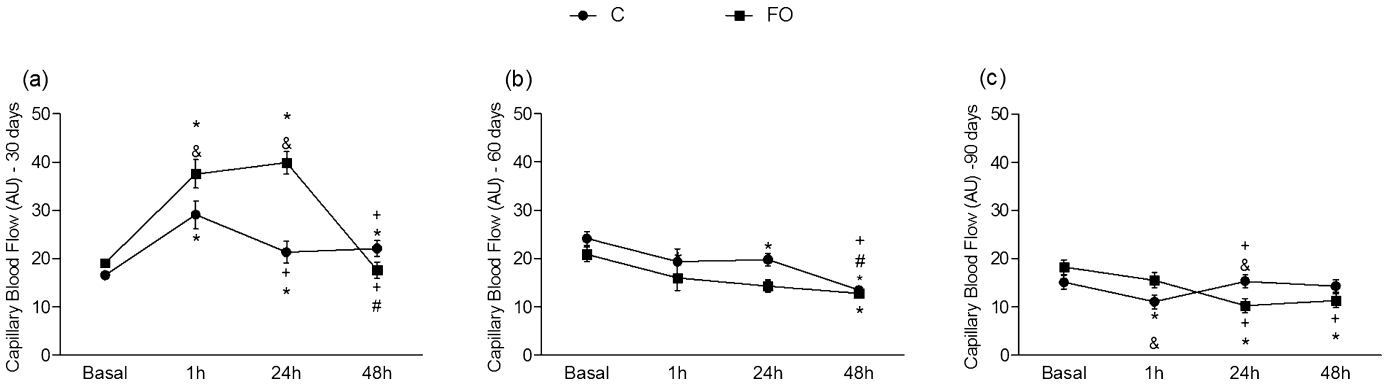

Fig. 3. Skin blood flow, before (basal) and after (1, 24, 48 h) dorsal skin exposure to acetone, of rats fed maintenance diet alone (C, $n=10$) or supplemented with fish oil (FO, $n=10$) for 30 days (a), 60 days (b) and 90 days (c); data expressed as mean ± S.E.M; $^{\&}$ indicates significant difference ($P<0.05$) between groups in the same evaluation time; * indicates significant difference ($P<0.05$) from basal in the same group; $^{+}$ indicates significant difference ($P<0.05$) from 1 h in the same group and $^{\#}$ indicates significant difference ($P<0.05$) from 24 h in the same group.

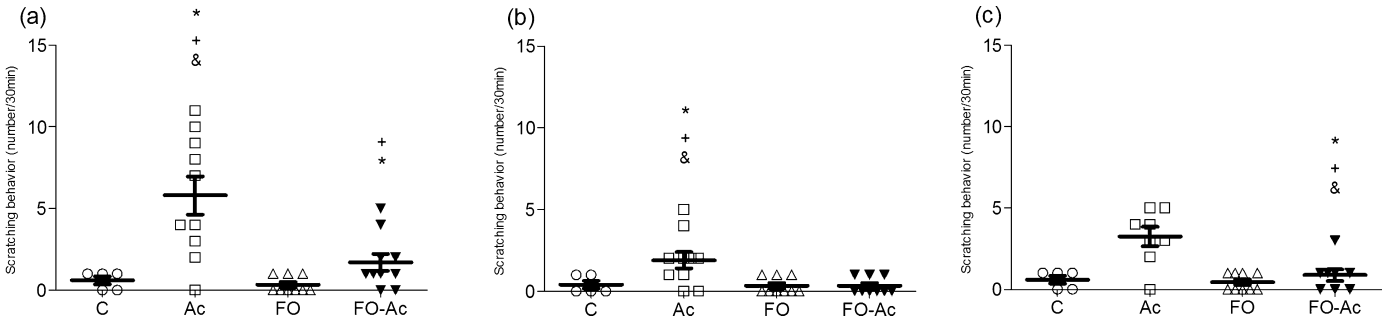

Fig. 4. Scratching behaviour, 1 h after the last cutaneous exposure to acetone (CAc, FOAc, $n=10$/group) or water (C, FO), of rats' fed maintenance diet alone (C, $n=5$) or supplemented with fish oil (FO, $n=10$) for 30 days (a), 60 days (b) and 90 days (c); data expressed as mean ± S.E.M; * indicates significant difference ($P<0.05$) from control (C) group; $^{+}$ indicates significant difference ($P<0.05$) from FO and $^{\&}$ indicates significant difference from FOAc group.

exposure increased the LDF, with 37% higher AUC values in the FO group but faster resolution (at 48 h the LDF had returned to baseline values, but not in the C group). By D60 and D90, the acetone skin exposure reduced LDF's AUC in the FO group (Fig. 3b and c) in 19% and 1%, respectively, compared to control group.

3.2. Effect of the FO supplementation on the scratching behaviour

The evaluation of the scratching behaviour confirmed that the acetone-induced dry skin is associated with pruritus, since the number of measured scratch actions increased significantly in the acetone treated animals (CAc group) (Fig. 4) when compared to the control (C) group. In the FO supplemented groups, the induced acetone-dry skin scratch actions (FOAc group) were significantly reduced when compared with the CAc group following 1 month of supplementation (Fig. 4a) and completely suppressed following 2 months or more of FO supplementation (Fig. 4b and c).

3.3. Effect of the FO supplementation on the skin fatty acids composition

The relative fatty acids composition of both diets, maintenance chow and chow supplemented with fish oil, and of rat's dorsal skin at the end of regular supplementation with fish oil (D90) is shown in Table 1. The FO supplementation improved the omega-3 PUFA (*n*-3) diet content, specially eicosapentaenoic acid (EPA, 20:5*n*-3, 3.5 times richer), docosahexaenoic acid (DHA; 22:6*n*-3, 1.7 times richer) and docosapentaenoic acid (DPA, 22:5*n*-3, absent in standard diet) without causing major changes on other fatty acid classes relative composition apart from a slight decrease in omega-6 (*n*-6) intake and consequent decrease in *n*-6/*n*-3 ratio ($P=0.010$). Overall, the relative PUFA content of both diets was similar (49.38 ± 0.11% and 49.10 ± 0.12% respectively for non- and FO supplemented diet).

The dorsal skin relative content in saturated fatty acids (∑ SFA), monounsaturated fatty acids (∑ MUFA) and *n*-6 (∑ *n*-6) was not significantly affected by the FO supplementation treatment compared to the control group. Significant increases, however, were observed in *n*-3 (∑ *n*-3, $P=0.014$) dorsal skin content of FO supplemented rats, specifically in EPA ($P=0.03$), DPA ($P=0.010$) and DHA ($P=0.0064$), with no changes in the PUFA content (30.9 ± 0.9% and 32.4 ± 0.4%, respectively, for non- and FO-supplemented animals). Therefore, the FO supplementation led to a significant ($P=0.009$) decrease of skin *n*-6/*n*-3 ratio content

Table 1
Fatty acids composition (% of total identified FAs) of the diets (fed maintenance chow alone and diet supplemented with fish oil – FO diet) and of rat's dorsal skin at the end (D90) of the repeated dose study with fish oil (FO).

Fatty acids	Diets		Skin incorporations	
	Maintenance	FO	Control group	FO group
∑ SFA	23.29 ± 0.16	23.59 ± 0.51	34.04 ± 0.95	34.82 ± 0.77
∑ MUFA	27.29 ± 0.18	27.11 ± 0.71	34.46 ± 0.97	33.43 ± 1.02
C 18:3*n*-3	1.44 ± 0.20	1.44 ± 0.11	1.23 ± 0.04	1.24 ± 0.04
C 20:5*n*-3	0.31 ± 0.08	1.10 ± 0.23	0.56 ± 0.18	**1.24 ± 0.14***
C 22:5*n*-3	0.00 ± 0.00	0.10 ± 0.05	0.71 ± 0.12	**1.19 ± 0.06***
C 22:6*n*-3	0.66 ± 0.01	1.15 ± 0.14	1.64 ± 0.29	**2.97 ± 0.12***
∑ *n*-3	3.18 ± 0.22	4.52 ± 0.28	4.13 ± 0.59	**6.57 ± 0.39***
∑ *n*-6	46.21 ± 0.23	44.58 ± 0.71	26.80 ± 0.87	25.81 ± 0.25
n-6/*n*-3 ratio	14.54 ± 0.71	**9.87 ± 0.19** *	7.84 ± 0.96	**4.06 ± 0.29***

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Values represent the mean ± S.E.M. * Indicates significant difference ($P<0.05$) compared to the control (C) group.

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when compared to control group, to levels one point below the *n*-6/*n*-3 threshold value of 5.

4. Discussion

Only a few published studies have reported the skin incorporation of fatty acids following a lipid diet or supplementation [20,21,32]. In this study, the fish oil, with a composition rich in *n*-3 PUFA, provided a daily intake *n*-6/*n*-3 ratio considered ideal [33]. The 90 day FO supplementation in the adult rats resulted in an increased incorporation of $\sum$ *n*-3 PUFA (1.6 times) into the skin, including EPA (2.2 times), DPA (1.7 times) and DHA (1.8 times), and a consequently decreased *n*-6/*n*-3 ratio for values below the threshold value of 5, much lower than that of the C group. Other tissues, such as the retina, erythrocyte and brain have also been referred for such fatty acids dietary incorporation profiles [27,34–36]. Our results agree with those obtained in human skin fibroblasts, which readily incorporate exogenous free fatty acids, facilitating nutritional modification of cellular composition [32]. Recent studies have shown that supplementation with different fatty acids (present in soybean oil, FO and hydrogenated fat vegetal) does modify the fatty acids incorporation in mouse skin as well as in rat skin (cross-generational) [20,21].

As demonstrated, skin barrier impairment occurs after topical exposure to acetone in mice [9,19]. The mechanism seems to involve the action of acetone on the skin barrier lipid complex components [37] allowing acetone to disrupt the SC and result in a defective "barrier" function [38]. In this study, TEWL was assessed as an indicator of barrier function, and the results indicated that in non-supplemented animals the topical acetone exposure increased the TEWL for 24 h after the last acetone challenge during the first two months (D30 and D60) and for 48 h on the third month (D90). The attenuation of the acetone induced increase in TEWL, observed in the FO supplemented group until 24 h after the last topical exposure to acetone during the first month and until only 1 h after the next months (D60 and D90), might reveal a protective effect against skin barrier impairment as well as a recovery improvement since TEWL returns faster to baseline values when compared to control, sustained by $46 \pm 6\%$ reduction on AUC. Effects of acetone exposure on skin dryness were also less noted ($26 \pm 6\%$ increase on AUC) and involved a faster recover in the FO supplemented animals, again suggesting the protective effect idea linked to FO. Therefore, FO seems to have an influence on the barrier dynamics. In addition, the lower basal TEWL and higher skin hydration noted at D60 and D90 in the FO group suggest a positive effect of the diet supplementation with *n*-3 PUFA on skin physiology, in particular over the barrier function and water homeostasis. Other studies have also reported additional positive influences over skin functions (including photoprotection) as a consequence of diet supplementation [20,21,36,39–41]. The observed changes on skin functions were also correlated with the shift in the skin fatty acids incorporation pattern originated by the FO supplementation—a higher incorporation of *n*-3 PUFA and lower *n*-6/*n*-3 ratio in FO supplemented animals were associated with a decrease in TEWL and an increase in skin hydration. Epidermal lipids, including EFA, play an essential role on the skin barrier function, as well as on skin's water homeostasis, while lipid disorganization seems to be involved in proinflammatory reactions and excessive water loss as happens in many different skin conditions including atopic dermatitis, psoriasis, burn or epidermolysis bullosa [7,8,23].

The skin exposure to acetone increased LDF more noticeably in the FO group after D30 of regular supplementation. In fact, such response occurred immediately after the acetone-induced cutaneous damage. We believe that the observed increase in *n*-3 PUFA skin incorporation, promoted by FO supplementation, with partial substitution of skin *n*-6 PUFA, possibly by replacing the cell membrane phospholipid–arachidonic acid (*n*-6 PUFA), decreases the production of prostanoids in favour of generally less biologically active (vasoconstriction and platelet-aggregating) molecules. Such changes in eicosanoid production cause vasodilatation and inhibition of platelet aggregation [42]. Other known effects of *n*-3 PUFA include the endothelial relaxation through up-regulation of nitric oxide synthase [43]. Such events may explain the increased blood flow observed following cutaneous acetone-exposure on D30. The observed decrease in LDF on months 2 and 3 of the FO supplementation could be related to its anti-inflammatory properties [42]. Another observed consequence of the acetone-induced barrier impairment was the itch-related scratching noted in all animals. Acetone exposure removes skin lipids, compromising function of ceramides, which are crucial for the skin's barrier role [44,45]. Skin barrier dysfunction (defects), directly resulting from lipid disorganization, can amplify the inflammatory potential and the response to irritating stimuli [6–8] in addition to the well-known associated fissured scaly skin, excessive water loss and dry skin. Our results suggest that the attenuated skin barrier defects in the acetone-exposed FO group could be related to the increased *n*-3 PUFA skin incorporation and with the improvement in scratching behaviour, meaning that the skin barrier recovery may involve EFA-dependent mechanisms. Several animal studies have associated *n*-6 PUFA, the most abundant PUFA in the epidermis, with skin permeability barrier homoeostasis [45–48] during the inflammatory response e.g. atopic dermatitis-like pruritic skin inflammation [45]. The *n*-3 PUFA, like α-linolenic acid (ALA, 18:3*n*-3) and its metabolites (DHA and EPA), are minor components of the epidermis known to produce anti-inflammatory eicosanoids, hence having immunomodulatory actions on inflammation [23,42]. In addition, DHA and EPA have been reported to reduce erythema and PGE_2 production in the skin [49–51]. In this study, a correlation between *n*-3 PUFA skin incorporation and reduced skin water loss, scaling and greater hydration was observed. Neukam et al. [8] reported similar skin effects following flaxseed oil supplementation. Of interest, we found that oral FO supplementation leading to a lower skin *n*-6/*n*-3 ratio clearly suppressed acetone-induced pruritic dry skin stimulus in rats. The observed prevention of scratching behaviour might result from the involvement of *n*-3 PUFA metabolites in the amelioration of pruritic dry skin in rats as consequence of circumventing epidermal hyperinnervation through skin barrier protection, since scratching depends on skin barrier dysfunction, and barrier defects precede skin inflammatory changes. It has been suggested [52] that in the skin barrier disruption the itch sensations are partly consequence of the hyperinnervation (increase in nerve density and alteration of epidermal innervation) a phenomenon observed in acetone-treated mice [2,9,19,53]. The same nerve growth effect may also be happening in the present study performed in acetone-treated rats, and if such event is being prevented by supplementation with a rich source of *n*-3 PUFA like is FO, as suggested by this study results, the control of epidermal innervation could be considered an antipruritic therapy target like it has been demonstrated by recent studies [9,19,53,54].

Lastly, decreased epidermal sphingomyelinase (SMase)-generating ceramides and impaired differentiation are both involved in disturbed barrier function found in atopic dermatitis [55,56]. The published effects of *n*-3 PUFA on SMase activity [57–59] are not linear and the absence of studies on both epidermal SMase and ceramidase activities calls for further research in order to elucidate the *n*-3 PUFA interaction with the most relevant lipid of skin barrier, the ceramides, and therefore the mechanisms underlying FO-induced (i) improvement of dry skin parameters and (ii) amelioration of the acetone-induced pruritic dry skin.

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In conclusion, these results suggest that, the daily oral FO supplementation in the present experimental conditions:

- improves the skin barrier function;
- promotes skin protection against dry skin stimulus and related induced pruritus;
- accelerates skin physiology recovery and
- promotes the incorporation of *n*-3 PUFA in the skin while decreasing the *n*-6/*n*-3 ratio.

Therefore, a positive influence of the FO *n*-3 PUFA on the skin barrier dynamics seems to be in place.

The tested equivalent human dose of FO, although corresponding to a dose twice the recommended human daily dose of 3–4 g/day EPA + DHA [60], has demonstrated an interesting therapeutic potential. Thus, these findings support the potential effect of *n*-3 PUFA on dry skin conditions and demonstrate that long-term supplementation with *n*-3 PUFA rich nutrients might reinforce and restore cutaneous integrity and function.

Lastly, we note that the described acetone induced dry skin rat model has proven to be easy to handle and reproducible, thus suitable to study different therapeutic tools (pharmacological or other) to control or reduce pruritus associated with dry skin.

Conflict of interest

The authors have no conflict of interest to declare.

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