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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 × 34 × 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