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Cross-generational *trans* fat intake exacerbates UV radiation-induced damage in rat skin

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ABSTRACT

We evaluated the influence of dietary fats on ultraviolet radiation (UVR)-induced oxidative damage in skin of rats. Animals from two consecutive generations born of dams supplemented with fats during pregnancy and breastfeeding were maintained in the same supplementation: soybean-oil (SO, rich in n-6 FA, control group), fish-oil (FO, rich in n-3 FA) or hydrogenated-vegetable-fat (HVF, rich in TFA). At 90 days of age, half the animals from the 2nd generation were exposed to UVR (0.25 J/cm^2) 3×/week for 12 weeks. The FO group presented higher incorporation of n-3 FA in dorsal skin, while the HVF group incorporated TFA. Biochemical changes *per se* were observed in skin of the HVF group: greater generation of reactive oxygen species (ROS), lower mitochondrial integrity and increased Na^+K^+-ATPase activity. UVR exposure increased skin wrinkles scores and ROS generation and decreased mitochondrial integrity and reduced-glutathione levels in the HVF group. In FO, UVR exposure was associated with smaller skin thickness and reduced levels of protein–carbonyl, together with increased catalase activity and preserved Na^+K^+-ATPase function. In conclusion, while FO may be protective, *trans* fat may be harmful to skin health by making it more vulnerable to UVR injury and thus more prone to develop photoaging and skin cancer.

1. Introduction

Ultraviolet radiation (UVR) is an important environmental factor associated with human skin disorders (Nichols and Katiyar, 2010), which have been linked to oxidative damage. Such events involve inflammatory processes and damage to proteins, lipids and DNA/RNA, besides changes in cell signaling pathways (Liu et al., 2010). Additional consequences from UVR exposure are photoaging (Jin et al., 2010), which is clinically characterized by wrinkles, pigmented spots and decreased skin elasticity (Cho et al., 2007).

Fatty acids (FA) are an integral part of the mammalian skin in the form of unsaturated FA, phospholipids, sphingolipids, among others (Black, 1987), thus affecting physiological functions and cellular signaling mechanisms (Jump, 2004). Dietary FA are modifiable environmental factors known for their influence on the susceptibility to offspring diseases (Friesen and Innis, 2006), configuring the mother as primary source of FA for the pups during pregnancy and breastfeeding (Rao et al., 2007). Different studies have shown the importance of an adequate transference of essential fatty acids (EFA) from mother to the fetus, which is reflected by the type of dietary FA (Innis, 2005; Lima et al., 2004). Thus, EFA may be n-3 or n-6 polyunsaturated FA (n-3 and n-6 PUFA), which are considered essential because animals are devoid of desaturases, making the natural occurrence of double bonds in n-3 and n-6 positions impossible (Knutzon et al., 1998). In this sense, EFA should be obtained from the diet or supplementation (Yehuda et al., 2005), especially during the perinatal period, when the maternal diet should provide adequate levels of PUFA for the unborn (Jensen et al., 1996).

Abbreviations: CAT, catalase; C-SO, control-soybean oil; EFA, essential fatty acids; FA, fatty acids; FO, fish oil; GSH, reduced glutathione; HVF, hydrogenated fatty acids; Na^+K^+-ATPase, sodium and potassium ATPase; PC, protein carbonyl; PUFA, polyunsaturated fatty acids; RS, reactive species; TFA, *trans* fatty acids; UVR, ultraviolet radiation.

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Functionally, n-3 PUFA are present in membrane phospholipids, competitively inhibiting the metabolism of n-6 PUFA, thus reducing formation of n-6 long chain PUFA (LC-PUFA), such as arachidonic acid (AA). This influence is able to attenuate the inflammatory cascade by decreasing eicosanoid production (Massaro et al., 2008). In addition, n-3 FA are recognized to prevent such skin diseases as psoriasis, dermatitis, and eczema, so reducing UVR-induced inflammatory processes (Jin et al., 2010). Notably, n-3 EFA are able to maintain skin's structural integrity affecting its permeability (Horrobin, 2000) and hydration (Pupe et al., 2002), thus confirming that an adequate n-3 FA intake favors cutaneous homeostasis, which is crucial for a healthy skin (Viola and Viola, 2009). Consistent with this, recent clinical studies showed an inverse relationship between severe photoaging and both monounsaturated fatty acids (MUFA) and n-3 PUFA dietary intake, thus contributing to the protective effects of these nutrients on skin inflammatory processes (Latreille et al., 2012, 2013).

Trans fatty acids (TFA) are present in processed foods and are primarily obtained from vegetable oils by industrial hydrogenation (Fritsche and Steinhart, 1998). TFA incorporation in infants may occur from placental transfer (Larqué et al., 2001) and/or from breast milk (Innis and King, 1999), affecting the EFA profile (Larqué et al., 2000). Experimental studies showed a relationship between TFA and impaired development (Hornstra et al., 2006), also affecting n-3/n-6 PUFA synthesis (Decsi et al., 2001) and lipid metabolism (Assumpção et al., 2002). Furthermore, TFA are able to alter inflammatory markers and, when incorporated into tissues, they interfere with *cis* FA metabolism (Loï et al., 2000). In this connection, a previous study of our group showed that TFA is able to modify the skin's oxidant/antioxidant status, thus contributing to its protection or facilitating the development of skin diseases related to UV radiation (Barcelos et al., 2013).

Our current understanding of the relationship between dietary FA and UVR-induced skin diseases is limited. Considering that dietary FA may be easily incorporated into the tissues both pre- and postnatally (Alsted and Hoy, 1992), here we proposed to evaluate the UVR-induced oxidative damage to the skin of a 2nd generation of rats born and grown under the same original dietary supplementation – rich in n-6 (control), n-3 or trans FA (TFA) – as their mothers and grandmothers.

2. Materials and methods

2.1. Animals and experimental procedure

Female Wistar rats were assigned to 3 experimental groups ($n = 14$) according to oral supplementation: Soybean oil (C-SO, rich in n-6 FA; Camera®, Santa Rosa-RS, Brazil, purchased in supermarket); FO (rich in n-3 FA; Herbarium®, Brazil, donated by Herbarium® Pharmaceutical Laboratory) and hydrogenated vegetable fat (HVF, rich in TFA; Primor®, São Paulo-SP-Brazil, purchased in supermarket), whose FA composition was previously described (Barcelos et al., 2013). SO was used as a control group, mainly because it contains n-6/n-3 ratio within acceptable limits (Yehuda et al., 2002; Viola and Viola, 2009) and by its elevated consumption worldwide (Teixeira et al., 2011, 2012). Control (C-SO) and experimental groups (FO and HVF) were isocaloric to prevent metabolic differences (Khalkhal et al., 2012), which could cause interferences in the antioxidant defense system (Diniz et al., 2004). Animals were orally supplemented (3 g/kg) (Kuhn et al., 2013; Pase et al., 2013; Trevizol et al., 2013) during pregnancy and lactation (totaling 43 days). Female rats of the 1st generation were maintained in the same maternal supplementation until adulthood (90 days old), when they were mated to obtain the 2nd generation, thus including pregnancy and lactation (43 days) periods. At weaning, one female rat from each 2nd generation litter was treated with the original supplementation until 90 days of age. FA supplementation was carried out from the prenatal period of the 1st generation until adulthood of the 2nd generation to study its influence on skin incorporation as related to UVR-induced cutaneous alterations. The duration of supplementation after weaning of both 1st and 2nd generations (90 days) was chosen based on previous studies that evaluated the influence of TFA (Barcelos et al., 2013; Sakai et al., 2009). Then one half of each group was designated to UVR exposure (UVR) or not (N-UVR), and maintained in the supplementation during the irradiation period, totaling six experimental groups ($n = 7$).

2.2. Radiation exposure

The source of UVR used was a Philips TL/12RS 40 W lamp (São Paulo, SP, Brazil) placed 20 cm above the rat's dorsal skin, which emits in the range of 280–400 nm with an output peak at 315 nm. Ultraviolet radiation B (UVB) accounted for 73% of the total UVR in these experimental conditions. The energy output of the lamp was measured with a UV radiometer (Digital Meter Ultraviolet Light MRU-201, Instrutherm, São Paulo, SP, Brazil).

All animals were shaved on their back with an electric shaver, followed by the application of hair removal cream before UVR irradiation sessions, once a week. UVR sessions were conducted three times a week for 12 weeks, with an UVR dose of 0.25 J/cm^2/3×/week, yielding a total dose of 0.75 J/cm^2/week/12 weeks. Supplementations were maintained throughout the study period.

2.3. Evaluation of wrinkles formation

After 12 weeks of UVR irradiation, animals were anesthetized (ketamine/xylazine, 60 and 15 mg/kg, im, respectively) for evaluation of dorsal skin wrinkle formation. Skin wrinkles were analyzed according to the scale of Bissett et al. (1987) modified by Inomata et al. (2003). Three investigators individually determined wrinkle formation scores in blind fashion for each animal, as described in Table 1.

2.4. Determination of skin thickness as an inflammation marker – histology and microscopy

For histological analyses, skin samples from rat central dorsum were obtained at the end of the experiments and fixed in 10% buffered formalin. The paraffin-embedded skin specimens were sectioned (5 μm), deparaffinized and stained with haematoxylin and eosin for light microscopic evaluation. The stained tissue sections were examined using an optical microscope and five images (10×) were taken *per section*.

2.5. Preparation of skin samples

At week 12 of UVR exposure, animals were anesthetized, euthanized, and a part of their dorsal skin was dissected and stored at −80 °C for lipid profile determination. Dorsal skin was homogenized in potassium phosphate buffer (Proquímios®, Rio de Janeiro-Brazil) 1 M pH7.4 or Tris–HCl (Sigma–Aldrich®, São Paulo-Brazil) 0.1 mM pH7.4, for reduced glutathione (GSH) levels and all other biochemical assays, respectively.

2.6. Histological analysis

For histological analyses, skin samples from rat central dorsum were obtained at the end of the experiments and fixed in 10% buffered formalin. The paraffin-embedded skin specimens were sectioned (5 μm), deparaffinized and stained with haematoxylin and eosin for light microscopic evaluation. The stained tissue sections were examined using an optical microscope and five images (10×) were taken *per section*.

2.7. Estimation of FA incorporation in the skin

FA profile determination was performed as described by Bligh and Dyer (1959). Fats were analyzed using a gas chromatograph equipped with a capillary column DB-23 and flame ionization detector. Standard FA methyl esters (37-component FAME Mix, C22:5n-3 and PUFA no. 2, Sigma, USA and C22:5n-6, NuChek Prep. Inc., USA) were performed under the same conditions and the subsequent retention times were used to identify the FA, which were expressed as percentage of total FA content.

2.8. Biochemical assessments

ROS levels were quantified using the oxidant sensing fluorescent probe, 2,7-dichlorofluorescein diacetate (DCHF-DA, Sigma Aldrich®, São Paulo-Brazil) (Hempel et al., 1999). The oxidation (DCHF-DA) to dichlorofluorescein (DCF) was determined at 488 nm for excitation and 525 nm for emission. Skin samples were incubated for 1 h until fluorescence measurement. DCF-ROS levels were corrected by protein content (Lowry et al., 1951) and expressed as percentage of control (C-SO group).

Protein carbonyl (PC) level was measured according to Yan et al. (1995). Skin samples were mixed with 2,4-dinitrophenylhydrazine (10 mM DNPH, Sigma Aldrich®, São Paulo-Brazil) for 1 h. Denaturing buffer (150 mM sodium phosphate buffer, pH6.8, 3% SDS), heptane (99.5%) and ethanol (99.8%) (all reagents from Vetec Quimica Final®, Rio de Janeiro-Brazil) was added sequentially. Protein isolated was washed twice with ethyl acetate/ethanol (Vetec Quimica Final®, Rio de Janeiro-Brazil) 1:1 (v/v) and suspended in buffer. Each sample was measured at 370 nm against the corresponding HCl sample (blank). Total carbonylation was calculated according to Levine et al. (1990).

Table 1
Grading of rat dorsal skin wrinkles according to the scale of Bissett et al. (1987) modified by Inomata et al. (2003).

Grade	evaluation criteria
0	No wrinkles
2	A few shallow wrinkles across the back skin are observed occasionally
4	Shallow wrinkles across the back skin are observed on the whole surface
6	Some deep, long wrinkles across the back skin are observed
8	Deep, long wrinkles across the back skin are observed on the whole surface

Table 2
Fatty acid composition of the rat dorsal skin after supplementation with different fatty acids (% of total fatty acids identified).

Fatty acids (mean ± SEM)	C-SO	FO	HVF
C 18:3n-3	0.86 ± 0.04	1.20 ± 0.04	1.15 ± 0.01
C 22:6n-3	0.87 ± 0.07	1.69 ± 0.02	0.47 ± 0.01
C 18:2n-6	32.15 ± 1.52	31.89 ± 0.11	32.16 ± 0.04
C 18:3n-6	0.00	0.01 ± 0.01	0.19 ± 0.01
C 20:3n-6	0.17 ± 0.04	0.21 ± 0.01	0.16 ± 0.01
C 20:4n-6	2.71 ± 0.38	1.46 ± 0.04	1.70 ± 0.02
C 18:1n-9*t*	0.00	0.00	0.84 ± 0.05
C 18:2n-6*t*	0.00	0.02 ± 0.01	0.03 ± 0.01
Σ SFA	29.06 ± 2.27[a]	28.03 ± 0.13[a]	25.09 ± 0.08[a]
Σ MUFA	33.00 ± 0.91[a]	33.70 ± 0.08[a]	37.85 ± 0.12[a]
Σ n-3 PUFA	1.98 ± 0.06[b]	4.40 ± 0.07[a]	1.84 ± 0.02[b]
Σ n-6 PUFA	35.49 ± 1.16[a]	33.87 ± 0.13[a]	34.61 ± 0.07[a]
Σ TFA	0.00[b]	0.02 ± 0.01[b]	0.87 ± 0.01[a]
n-6/n-3 Ratio	17.92 ± 0.37[a]	7.77 ± 0.14[b]	18.88 ± 0.18[a]

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Considering the unsaturation number, TFA were added to the MUFA content. Different lower case letters (a–b) indicate significant difference among supplementations in the same irradiation condition ($P < 0.05$).

Mitochondrial integrity estimation (MTT assay) was performed according to Brustovetsky and Dubinsky (2000). This colorimetric assessment was performed by reduction of 3-bromide-4,5-dimethylthiazol-2-yl)-2,5-difeniltetrazolio (MTT, Sigma–Aldrich®, São Paulo-Brazil) in mitochondria suspension isolated from skin according to Mossman (1983). This method is based on MTT reduction by mitochondrial dehydrogenases, whose color can be spectrophotometrically measured (λ = 570–630 nm). Mitochondrial suspension was corrected by protein content (Lowry et al., 1951) and expressed as percentage of control (C-SO group).

Na^+K^+-ATPase activity was adapted from Muszbek et al. (1977). The method is based on inorganic phosphate (Pi) released by ATP (Sigma–Aldrich®, São Paulo-Brazil) hydrolysis (Atkinson et al., 1973). The formed molibdate-Pi complexes were measured spectrophotometrically at 405 nm. Values were calculated in relation to standard curve constructed with Pi at known concentrations and corrected by protein (Lowry et al., 1951).

2.9. Estimation of skin antioxidant defenses

Catalase (CAT) activity was spectrophotometrically quantified according to Aebi (1984), monitoring the disappearance of H_2O_2 (Proquímios®, Rio de Janeiro-Brazil) in the presence of homogenate at 240 nm. The enzymatic activity was expressed in K/mg protein/min. Protein content was determined by the method of Lowry et al. (1951).

Reduced glutathione (GSH) levels were determined after reaction of homogenates with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB, Proquímios®, Rio de Janeiro-Brazil). The yellow formed was read at 412 nm, according to Boyne and Ellman (1972). A standard curve using GSH was plotted in order to calculate the GSH content, expressed as μmol GSH/g tissue.

2.10. Statistical analysis

The skin FA content was analyzed by one-way ANOVA followed by Duncan's test. Skin wrinkles and thickening data, as well as biochemical evaluations, were analyzed by two-way ANOVA [3(SO, FO and HVF)] × [2(non-irradiated/irradiated)], followed by Duncan's multiple range test, when appropriate. Value of $P < 0.05$ was considered significant for all comparisons made.

3. Results

3.1. Skin fatty acids composition (Table 2)

The type of supplementation provided across two generations of rats was able to modify the lipid profile of their dorsal skin: While HVF-supplemented animals presented higher MUFA (37.85%; $P < 0.001$) and TFA (0.87%; $P < 0.001$) incorporation, the FO group showed higher n-3 PUFA (4.40%; $P < 0.001$) and lower n-6/n-3 (7.77%; $P < 0.001$) ratio than both C-SO- and HVF-supplemented rats.

3.2. UVR-induced wrinkles and thickening in dorsal skin (Figs. 1 and 2)

Two-way ANOVA of wrinkle score revealed a significant main effect of supplementation and UVR exposure [$F(2,36) = 53.54$, $P < 0.001$; $F(1,36) = 186.22$; $P < 0.001$, respectively] and a significant supplementation x UVR interaction [$F(2,36) = 53.13$; $P < 0.0001$]. Two-way ANOVA of skin thickness revealed a significant main effect of supplementation and UVR exposure [$F(2,36) = 7.20$, $P < 0.05$; $F(1,36) = 127.10$; $P < 0.001$, respectively].

Wrinkle scores and skin thickness were similar in animals unexposed to irradiation. UVR exposure modified these parameters in all experimental groups: While wrinkle scores were higher in HVF than in both FO and C-SO groups (Fig. 1A), skin thickening was lower in FO-supplemented animals than in C-SO and HVF (Figs. 1B and 2).

3.3. Biochemical measurements

3.3.1. Influence of the fat supplementations on reactive species (ROS) generation, protein carbonyl (PC) levels and mitochondrial integrity in dorsal skin (Fig. 3)

Two-way ANOVA of RS generation, PC levels and mitochondrial integrity revealed a significant main effect of supplementation [$F(2,36) = 9.81$, $P < 0.001$; 5.38 $P < 0.05$ and 12.99, $P < 0.001$, respectively] and UVR exposure [$F(1,36) = 17.54$, $P < 0.001$; 10.25, $P < 0.05$ and 23.12, $P < 0.001$, respectively] in skin tissue.

HVF supplementation increased *per se* the ROS generation in relation to control (C-SO). UVR exposure increased ROS generation in skin of C-SO- and HVF-supplemented rats when compared to non-irradiated animals, but this effect was not observed in FO. In fact, between irradiated groups, ROS generation was higher in HVF than in FO and C-SO (Fig. 3A).

Duncan's test showed no differences in the PC levels of non-irradiated animals, but UVR exposure increased this oxidative parameter in C-SO and HVF, but not in FO group. Between irradiated animals, FO supplementation was able to prevent the increase of skin PC levels, which were increased in C-SO and HVF (Fig. 3B).

The mitochondrial integrity was reduced *per se* in skin of HVF in comparison to both C-SO and FO. UVR exposure was able to reduce significantly the mitochondrial integrity of all experimental groups. In fact, between irradiated groups, mitochondrial integrity was lower in HVF than in both C-SO and FO (Fig. 3C).

3.3.2. Influence of the fat supplementations on antioxidant defenses and Na^+-K^+-ATPase activity in dorsal skin (Fig. 4)

Two-way ANOVA of CAT activity revealed a significant main effect of supplementation [$F(2,36) = 30.30$, $P < 0.001$]. Two way ANOVA of GSH levels revealed a significant main effect of supplementation [$F(2,36) = 19.55$, $P < 0.001$] and a significant supplementation × UVR interaction [$F(2,36) = 14.07$; $P < 0.001$]. Two way ANOVA of Na^+-K^+-ATPase activity revealed a significant main effect of supplementation and UVR exposure [$F(2,36) = 21.88$ and 21.72, $P < 0.001$, respectively].

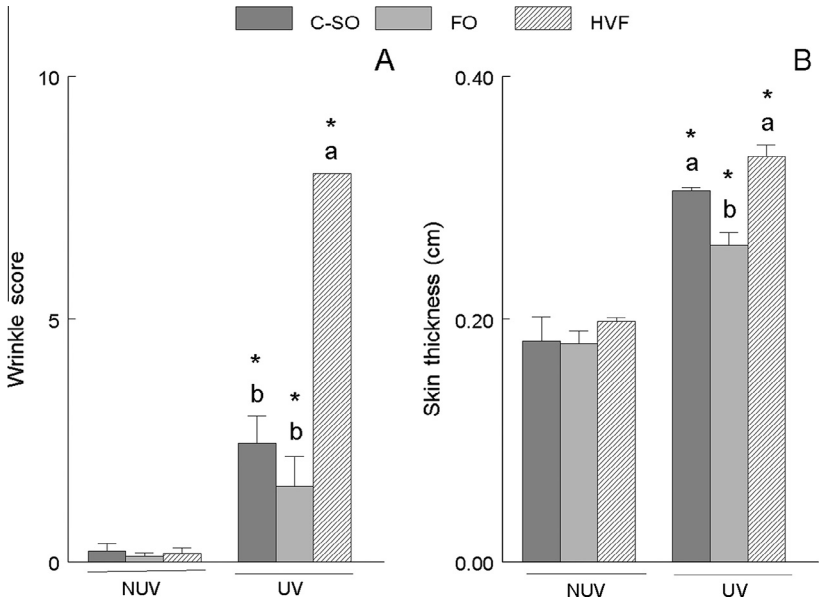

Fig. 1. Influence of n-3, n-6 and *trans* fatty acids provided across two generations of rats supplemented with control-soybean oil (C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF), respectively, on wrinkle score (A) and skin thickness (B) in dorsal skin of rats exposed or not to UV irradiation. Data expressed as means ± SEM ($n = 7$). Different lowercase letters indicate significant difference between the supplementations in the same irradiation condition ($P < 0.05$); *Indicates significant difference from non-irradiated group in the same supplementation ($P < 0.05$).

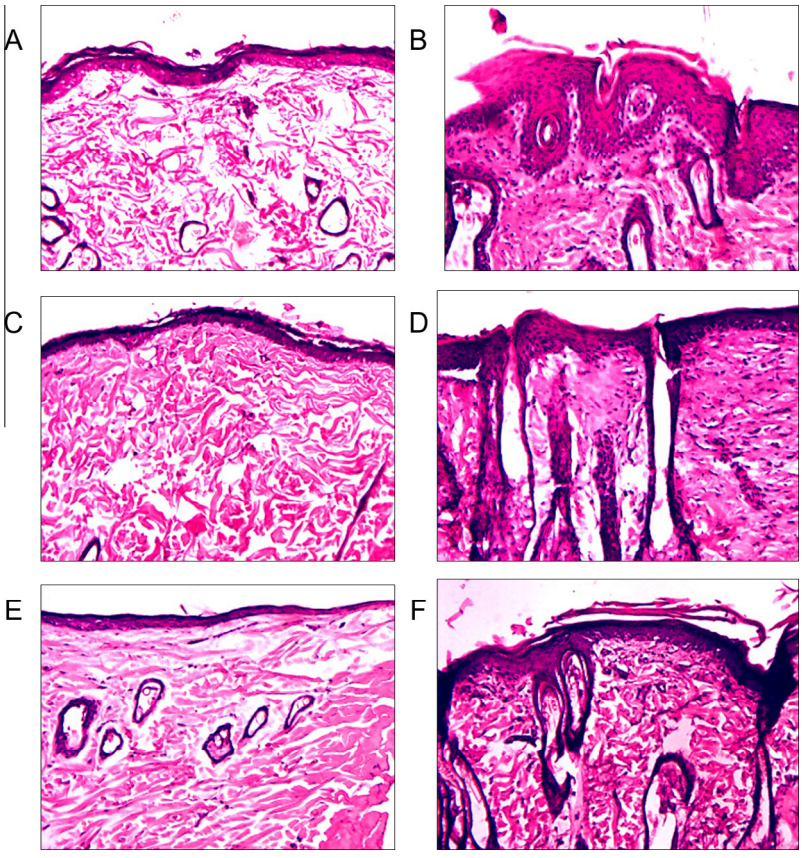

Fig. 2. Effects of oral supplementation of different fatty acids on UVR-induced epidermal thickness on the back of rat skin at the end of week 12. Each photograph is representative of seven animals. Photomicrograph of histologic sections of rat skin in control and supplemented groups after haematoxylin and eosin staining: (A) Non-irradiated control (C-SO), (B) Irradiated C-SO, (C) non-irradiated hydrogenated vegetal fat (HVF), (D) Irradiated HVF, (E) non-irradiated fish oil (FO), (F) Irradiated FO. The respective values are mean ± SEM from seven animals and the values not sharing a letter are different at $P < 0.05$. Magnification: 10-fold.

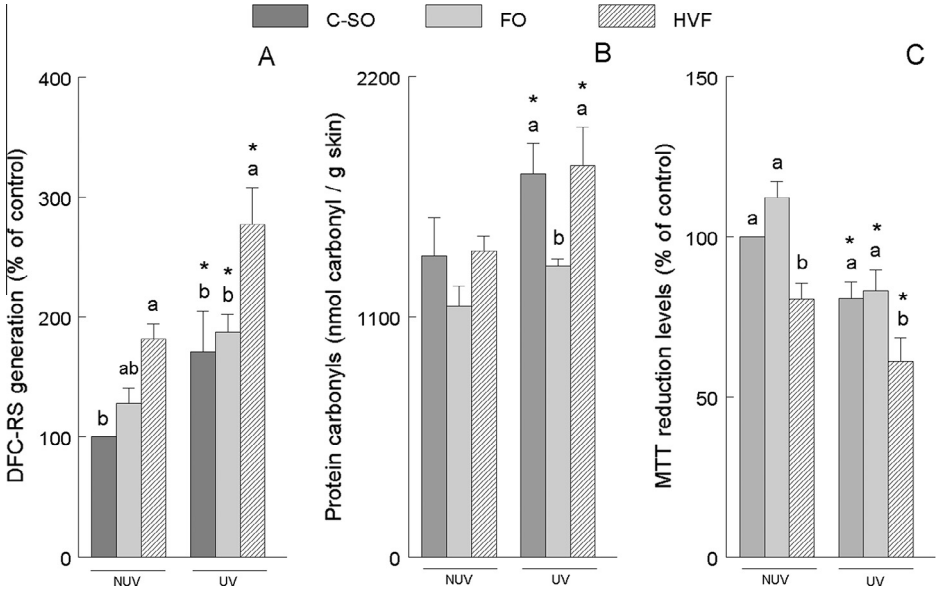

Fig. 3. Influence of n-3, n-6 and *trans* fatty acids provided across two generations of rats supplemented with control-soybean oil (C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF), respectively, on the reactive oxygen species (ROS) generation (A), protein carbonyl levels (B) and mitochondrial integrity (C) of dorsal skin of rats exposed or not to UV irradiation. Data expressed as means ± SEM ($n = 7$). Different lowercase letters indicate significant difference between the supplementations in the same irradiation condition ($P < 0.05$); *Indicates significant difference from non-irradiated group in the same supplementation ($P < 0.05$).

FO supplementation was able to increase CAT activity *per se* as compared to C-SO and HVF. UVR exposure increased significantly the CAT activity in C-SO, but not in FO and HVF. Between irradiated animals, the highest CAT activity was observed in FO, which was different from both C-SO and HVF (Fig 4A).

Duncan's test showed no differences of GSH levels in dorsal skin of animals; however, after UVR exposure, the level of this antioxidant was increased in skin of C-SO and FO, and reduced in skin of HVF group. Between animals exposed to irradiation, the GSH level was lower in HVF of than in both C-SO and FO (Fig. 4B).

The activity of Na^+K^+-ATPase was lower in skin of FO-supplemented rats than in the HVF group. UVR exposure was able to increase the activity of this enzyme in both C-SO and HVF, but not in FO group. In fact, Na^+K^+-ATPase activity was lower in the skin of FO-supplemented rats than in C-SO and HVF after UVR exposure (Fig. 4C).

4. Discussion

Our recent studies have shown impairments from TFA consumption in different animal models (Teixeira et al., 2011, 2012; Trevizol et al., 2011, 2013; Kuhn et al., 2013; Pase et al., 2013). So far, no study showed the influence of long term consumption of different fats, including *trans* fat, on UVR-induced oxidative damage in skin of rats. Our objective here was to compare isocaloric dietary supplementations, considering SO as control (C-SO group) because it is widely consumed currently (Teixeira et al., 2011, 2012). In fact, SO supplementation contains adequate levels of polyunsaturated fatty acids (PUFA) and n-6/n-3 ratio within acceptable limits (around 10) as described elsewhere (Viola and Viola, 2009; Simopoulos, 2002; Yehuda et al., 1997, 2002), which were confirmed in the current findings (Table 2). In this sense, control (C-SO) and experimental groups (FO and HVF) were isocaloric in order to prevent metabolic differences between animals of different experimental groups (McDonald et al., 2011; Khalkhal et al., 2012), which could cause interferences in the antioxidant defense system (Diniz et al., 2004). Furthermore, fats supplemented in this study were chosen mainly because these are the most used worldwide without awareness of their harm to general health and, especially, without enough research on the effects of their consumption on skin health. Considering the different oils/fats, we suggest that chronic supplementation of FO and, to a lesser extent, SO exerts protective effects, while HVF may impair the skin. Our group recently confirmed a part of this hypothesis, when 3-month HVF supplementation was sufficient to change the incorporation of FA in mice skin, thus affecting the UVR-induced damage (Barcelos et al., 2013). Here, we observed that HVF offered for two generations was critical to increase TFA and MUFA incorporation in the rats' skin, while FO increased n-3 PUFA incorporation, favoring the decreased n-6/n-3 ratio observed as well.

Skin lipids constitute a unique composition due to the high percentage of LC–FA (De Luca and Valacchi, 2010), whose packed structure (Downing, 1992; Förster, 2002) ensures an effective barrier function (Mendelsohn et al., 2006) and protection. The type of FA incorporated in this structure may variously affect the skin's protection against sun light exposure, thus inducing premature photoaging and histological changes that include increased epidermal thickness and wrinkles, besides connective tissue alterations (Rittie and Fisher, 2002). In this sense, our results showed that besides higher skin TFA and MUFA incorporation, the HVF group scored higher on UVR-induced wrinkles than the other experimental groups, allowing us to theorize that chronic HVF consumption favors photoaging. Indeed, as the skin renews itself quickly without stocking of essential fatty acids (EFA), it becomes dependent on the continuous supply of these FA from the diet. Thus, the LC-PUFA generated from hepatic elongation and desaturation are available for incorporation into skin cell membranes. (Chapkin et al., 1986, 1987; Chapkin and Ziboh, 1984; Ziboh and Chapkin, 1987). In this sense, the preservation of skin health against UV damage is also related to lipids that compose it: lipids are present in *stratum corneum* of epidermis, which is the outermost layer of skin, as well as in cell lipid membranes (Hansen et al., 1958), affecting its physiology and cellular signaling system (Jump, 2004). Taken together, these changes may facilitate skin damage, such as from UVR exposure.

In line with this, UVR-induced skin edema is a result of the activation of phospholipase-A_2 (PLA-A_2) and cyclooxygenase-2

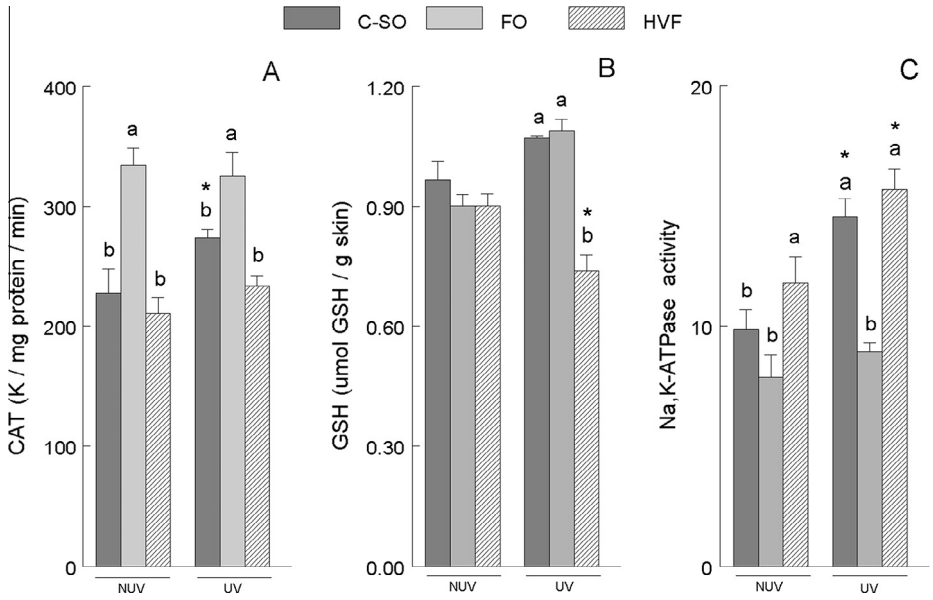

Fig. 4. Influence of n-3, n-6 and *trans* fatty acids provided across two generations of rats supplemented with control-soybean oil (C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF), respectively, on catalase (CAT) activity (A), glutathione (GSH) level (B) and Na^+K^+-ATPase activity (C) of dorsal skin of rats exposed or not to UV irradiation. Data expressed as means ± SEM ($n = 7$). Different lowercase letters indicate significant difference between the supplementations in the same irradiation condition ($P < 0.05$); *Indicates significant difference from non-irradiated group in the same supplementation ($P < 0.05$).

(COX-2), which increase prostaglandin-E_2 generation (Buckman et al., 1998) through elevated ROS production (Imlay and Linn, 1988). In this sense, skin FA are released by PLA-A_2 serving as substrates for COX-2 for eicosanoids generation (Massaro et al., 2008). While n-6 PUFA are precursors of pro-inflammatory metabolites, acting as a stimulatory factor for skin edema from ROS generation (Okuyama et al., 1996), n-3 PUFA derivatives have been shown to inhibit the linoleic acid (LA) cascade, serving as competitive inhibitors (Takemura et al., 2002), modifying the UVR-induced edema. On the other hand, little is known about the action of membrane-derived *trans*-eicosanoids, raising questions about skin health in societies with chronic consumption of foods rich in *trans* fats. In our study, greater UVR-induced skin thickening was observed in both TFA and C-SO groups, whose mechanism may be due to generation of pro-inflammatory cytokines (Meeran et al., 2009), increased blood flow and vascular permeability, which together lead to inflammation and edema development (Kajiya and Detmar, 2006). Furthermore, our experimental protocol included FO-supplemented animals, whose higher incorporation of n-3 PUFA and lower n-6/n-3 ratio was associated with decreased skin thickness. Indeed, although n-3 and n-6 PUFA are predominantly present in FO and C-SO, respectively, their functions in inflammatory processes are opposite (Lou et al., 2011): while n-3 PUFA favor the production of eicosanoids, thereby controlling the inflammation, n-6 PUFA are generators of AA, favoring physiologic inflammatory responses (Black and Rhodes, 2006). In addition, TFA have been reported to inhibit desaturase activity, so reducing the synthesis of n-3 and n-6 PUFA (Phivilay et al., 2009). Such event may impair the skin's n-3 PUFA content, which may present deficiencies consequent to processed foods consumption (Kummerow et al., 2004), common in Western diets. Moreover, metabolism of TFA is able to favor *trans*-eicosanoids generation, whose properties are currently unknown.

So far, a comparative influence of perinatal supplementation with different oils on UVR-induced photoaging and oxidative damage had not been performed. This study found that cross-generational, long term TFA supplementation caused critical damage to rat skin, as observed by increased RS generation and decreased mitochondrial integrity. Furthermore, the lower GSH level observed in the HVF group indicates an overloaded antioxidant defense system, whose intensity may partially compromise the skin's natural defenses. HVF consumption may potentially increase the risk of developing skin disorders related to UVR exposure. In addition, n-6 PUFA was less deleterious than TFA, while n-3 PUFA showed protective influences against UVR-induced skin damage. This hypothesis is supported by our findings after UVR exposure of FO-supplemented rats: (i) lesser increase of skin thickness; (ii) prevention of damage to skin proteins; (iii) increased CAT activity; and (iv) preservation of the physiologic function of Na^+K^+-ATPase. Indeed, this transmembrane enzyme may be considered a damage biomarker (Chaudhary and Parvez, 2012), since its activity is sensitively modified by OS generation (Teixeira et al., 2011, 2012). In this sense, skin Na^+K^+-ATPase activity measurements constitute a tool for estimating the harmful effects of UVR. Here, a significant increase of Na^+K^+-ATPase activity was observed in C-SO- and HVF-supplemented animals chronically exposed to UVR, while such activity remained unchanged in FO-supplemented animals. The Na^+K^+-ATPase pump is responsible for the active transport of sodium and potassium ions across the plasmatic membrane to maintain its excitability (Ribeiro et al., 2007). The marked enhance of Na^+K^+-ATPase activity alters cellular transmission and may decrease cell calcium entry. In fact, loss of calcium from epidermis, which is related to UVR-induced disruption of barrier integrity, stimulates the secretion of lamellar bodies from the outermost *stratum granulosum*, so facilitating the barrier recovery (Elias et al., 2002).

These results suggest that skin FA composition exerts an important role in UVR-induced photoaging and oxidative photodamage. In fact, ROS has been reported to play an important role in UVR-induced deleterious effects to skin (Park et al., 2006), including increased ROS generation (Jurkiewicz and Buettner, 1996), with significant consequences on DNA structure, lipids, and proteins, also affecting signal transduction pathways (Nishigori et al., 2004), which is closely linked to cell death (Cryns and Yuan, 1998). In this sense, the increased generation of ROS, as observed in the HVF group, may result from a synergistic action of both factors: TFA incorporation inhibits both elongation and desaturation of EFA, plus UVR exposure, which is promoter of pro-inflammatory events (Von Thaler et al., 2010), compromising the function of the

antioxidant defense system and affecting the skin mitochondrial integrity. In contrast, FO supplementation – and to a lesser extent SO – showed lower UVR-induced ROS generation and a minor loss of mitochondrial integrity. These events may be due to the fundamental role of n-3/n-6 PUFA in maintenance of *stratum corneum* permeability, which is required for epidermal homeostasis and skin health (McCusker and Grant-Kels, 2010). The differentiation between these two series of FA is indeed important, mainly because their roles are distinct: while n-3 PUFA are immune modulators, n-6 PUFA serve as structural precursors for the important *stratum corneum* ceramides (McCusker and Grant-Kels, 2010).

Our findings showed that pro-inflammatory and oxidative events resulting from UVR exposure were sufficient to change the antioxidant defense system, represented here by CAT activity and GSH levels. We hypothesized that skin TFA incorporation reduced cell membrane fluidity and thus affected membrane-bound enzymes (Cazzola et al., 2004). It has been suggested that photoaging might result from imperfect protection against cumulative stress from ROS generated by chronic UVR exposure (Fisher et al., 1997). A recent study of our group showed that acute UVR exposure was able to increase GSH levels in skin of HVF supplemented mice (Barcelos et al., 2013), confirming that the preservation of endogenous antioxidant defense system is an important strategy for protection against UVR-related oxidative insults o.

UVR-induced ROS and inflammatory responses have been implicated in skin diseases as well as in premature aging (Katiyar and Meeran, 2007). Our findings pointed that, upon chronic UVR exposure, rats supplemented with HVF for two generations exhibit higher wrinkles scores, related to higher levels of skin oxidative damage, than animals supplemented with SO and FO. Thus, our hypothesis is that chronic TFA provision favors an increase of ROS generation in skin and impairs the antioxidant defense system, so affecting proteins and damaging cells, possibly due to EFA deficiency, as well as *trans*-eicosanoids generation, which are involved in the exacerbation of UVR-induced injury (Cassagno et al., 2005; Sánchez-Moreno et al., 2004).

Longer sun exposure due to increased human life expectancy, along with depletion of the ozone layer, is a matter of concern. Food factors, such as *trans* fat chronic consumption, have recently emerged as a concern because they may contribute to the growing increase of photoaging and skin cancer (Sharma and Katiyar, 2010). In this context, this study supports the interpretation that the type of FA – n-3, n-6 or TFA – during development is critical for skin protection against UVR-induced damage.

In sum, TFA offered from intrauterine life until adulthood exacerbated UVR-induced cutaneous oxidative damage, while EFA may prevent it at different levels. This may bring an innovative point of view in dermatology, as the adoption of healthy dietary habits could bring an additional protection against photoaging and skin cancer.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Transparency Document

The Transparency document associated with this article can be found in the online version.

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