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INFLUÊNCIA DA SUPLEMENTAÇÃO DE DIFERENTES ÁCIDOS GRAXOS SOBRE O FOTODANO DA PELE INDUZIDO PELA EXPOSIÇÃO DE ROEDORES À RADIAÇÃO ULTRAVIOLETA

TESE DE DOUTORADO

Raquel Cristine Silva Barcelos

Santa Maria, RS, Brasil

2014

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Farmacologia, Área de Concentração em Neuropsicofarmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito para obtenção do grau de **Doutora em Farmacologia.**

Orientadora: Profª. Dra. Marilise Escobar Bürger

Santa Maria, RS, Brasil

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Centro de Ciências da Saúde
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aprova a Tese de Doutorado

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elaborada por
Raquel Cristine Silva Barcelos

como requisito parcial para obtenção do grau de
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Dedico esta tese ao Cristian

e à minha família.

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mas renunciar ao entusiasmo enruga a alma.

Albert Schweitzer

RESUMO

Tese de doutorado
Programa de Pós-Graduação em Farmacologia
Universidade Federal de Santa Maria, RS, Brasil

INFLUÊNCIA DA SUPLEMENTAÇÃO DE DIFERENTES ÁCIDOS GRAXOS SOBRE O FOTODANO DA PELE INDUZIDO PELA EXPOSIÇÃO DE ROEDORES À RADIAÇÃO ULTRAVIOLETA

Autora: Raquel Cristine Silva Barcelos
Orientadora: Profª. Drª. Marilise Escobar Bürger
Local e Data da Defesa: Santa Maria, 18 de fevereiro de 2014.

Os ácidos graxos (AG) provenientes da dieta são fundamentais para a estrutura e função dos fosfolipídeos das membranas celulares, nas quais os AG poliinsaturados (AGPI) de cadeia longa aumentam a sua fluidez, enquanto os AG *trans* (AGT) a tornam mais rígida. Nos últimos anos, a diminuição da camada de ozônio tem aumentado a exposição humana à radiação ultravioleta (RUV), causando consequências deletérias sobre a homeostase cutânea. Por outro lado, os hábitos de vida e os padrões alimentares, especialmente em países ocidentais, tem apresentado um consumo crescente de alimentos processados ricos em AGT, cujas consequências cutâneas ainda não apresentam validação científica. Considerando que a saúde da pele está parcialmente relacionada aos lipídios que a compõem, este estudo foi desenvolvido para avaliar a influência da suplementação de diferentes óleos ou gordura em diferentes períodos da vida de roedores sobre os danos oxidativos induzidos pela exposição aguda e crônica à RUV. Camundongos Swiss machos recém desmamados foram diariamente suplementados (3g/kg; p.o.) com óleo de soja (rico em AGPI n-6) (grupo controle), óleo de peixe (rico em AGPI n-3) ou gordura vegetal hidrogenada (GVH; rica em AGT) até 90 dias de idade, quando a pele da região dorsal foi agudamente exposta à RUV. A suplementação com óleo de peixe foi relacionada à incorporação de AGPI n-3 no tecido cutâneo dos camundongos, enquanto os grupos suplementados com óleo de soja e GVH apresentaram incorporação de AGPI n-6 e AGT, respectivamente. Tais incorporações exerceram influências sobre o desenvolvimento de danos oxidativos induzidos pela RUV na pele dos camundongos, de modo que o grupo GVH mostrou maiores níveis de peroxidação lipídica e carbonilação protéica, acompanhados de maior espessamento da pele (edema), menor atividade da catalase (CAT) e viabilidade celular. Enquanto o óleo de soja foi associado a uma prevenção parcial dos danos observados no grupo GVH, a suplementação com óleo de peixe preveniu os danos oxidativos cutâneos. Sequencialmente, o segundo e terceiro protocolos experimentais foram desenvolvidos com a 1ª e a 2ª gerações de ratas adultas nascidas sob a suplementação diária dos mesmos óleos utilizados no experimento 1 (óleo de soja, óleo de peixe e GVH) e, aos 90 dias de idade, parte de cada grupo experimental foi exposto à RUV 3x/ semana, durante 12 semanas. Animais de 1ª geração (experimento 2) tratados com óleo de peixe apresentaram maior incorporação de n-3 FA e menor razão n-6/n-3 na pele dorsal, enquanto o grupo GVH mostrou maior incorporação de AGT. Análises bioquímicas mostraram um aumento dos níveis de proteína carbonil (PC), *per se*, menor funcionalidade das enzimas mitocondriais e diminuição de algumas defesas antioxidantes (glutationa reduzida (GSH) e vitamina C (VIT C)) na pele dorsal do grupo suplementado com GVH. Após exposição à RUV, este mesmo grupo experimental apresentou maior escore de rugas, maior geração de espécies reativas (ER) e níveis de PC, os quais foram acompanhados de uma diminuição dos níveis de GSH e de VIT C na pele dorsal. Contrariamente, o grupo óleo de peixe mostrou menor escore de

rugas e espessamento da pele após exposição à RUV, além de apresentar menores níveis de PC e maior funcionalidade das enzimas mitocondriais. Adicionalmente, observou-se uma correlação positiva entre a geração de ER induzida pela RUV e a espessura da pele, rugas e PC, enquanto uma correlação negativa entre a geração de ER induzidas pela RUV e a funcionalidade das enzimas mitocondriais, e entre os níveis de PC e GSH, SOD e VIT C. Animais de 2ª geração (experimento 3) tratados com óleo de peixe apresentaram maior incorporação AG n-3 e menor razão n-6/n-3 na pele dorsal, enquanto que os AGT foram incorporados apenas no grupo GVH. Este último grupo experimental apresentou alterações bioquímicas *per se*: maior geração de ER, menor funcionalidade das enzimas mitocondriais e maior atividade da Na^+K^+ATPase. A exposição do grupo GVH à RUV aumentou a rugosidade da pele, aumentou a geração de ER e reduziu a funcionalidade das enzimas mitocondriais, além de diminuir os níveis de GSH. No grupo óleo de peixe, a exposição à RUV foi associada à menor espessura da pele e à redução dos níveis de PC, além do aumento da atividade da CAT e da preservação da atividade da Na^+K^+ATPase. Os AGPI n-3 competem com AGPI n-6 pela atividade das elongases e dessaturases, as quais originam AGPI de cadeia longa n-3 ou n-6, respectivamente, que são incorporados aos fosfolipídeos das membranas celulares. Tal incorporação permite a atividade da ciclooxigenase-2 (COX-2) sobre os mesmos, originando metabólitos ativos da série 3 (prostaglandinas (PG) e tromboxanos (TX) da série 3) ou da série 6 (PG e TX da série 2), respectivamente. Os metabólitos da série 3 são menos pró-inflamatórios que aqueles da série 2, o que pode em parte explicar nossos achados. Além disto, até o momento, nenhum estudo mostrou a geração de metabólitos de AGT, nem mesmo sua influência sobre o processo inflamatório e pró-oxidante nas membranas celulares. Como os AGT têm sido descritos por inibir a atividade das dessaturases, nós sugerimos que a presença de AGT nas membranas pode estar inibindo a incorporação de AGPI n-3 e, dessa maneira, reduzir a geração de seus metabólitos, os quais são reconhecidamente benéficos. Tomados em conjunto, os dados apresentados nesta tese sugerem que hábitos alimentares saudáveis, que inclui uma ingesta reduzida de alimentos ricos em AGT e a inclusão de AGPI n-3, acompanhado de cuidados frente à exposição solar, podem contribuir para a prevenção de afecções cutâneas e doenças de pele associadas à exposição UV.

Palavras-chave: ácidos graxos; *trans* fatty acids; pele; radiação ultravioleta; estresse oxidativo

ABSTRACT

Doctoral Thesis
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INFLUENCE OF DIFFERENT FATTY ACID SUPPLEMENTATION ON SKIN PHOTODAMAGE ULTRAVIOLET RADIATION-INDUCED IN RODENTS

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Diet fatty acids (FA) are fundamental to the phospholipids structure and function of cell membranes, in which long chain polyunsaturated FA (PUFA) increase its fluidity, while the *trans* FA (TFA) to make it more rigid. Also, the barrier function and hydration are highly dependent on the skin composition and structure, as well as the organization of lipids in the cell matrix. In recent years, the ozone layer depletion has increased human exposure to ultraviolet radiation (UVR), inducing deleterious effects on skin homeostasis. Moreover, lifestyle habits and eating patterns, especially in Western countries, has shown an increasing consumption of processed foods rich in AGT, whose cutaneous consequences do not present scientific validation. Whereas the skin health is partially related to the lipids that compose it, this study was designed to evaluate the effect of supplementation of different oils or fat in distinctlife periods of rodents on oxidative damage acute and chronic exposure to UVR-induced. Male Swiss mice weanling were supplemented daily (3g/kg, po) with soybean oil (C-SO; rich in n-6 PUFA), fish oil (FO; rich in n-3 PUFA) or hydrogenated vegetable fat (HVF; rich in TFA) until 90 days old and the dorsal skin was acutely exposed to UVR. The FO supplementation showed n-3 PUFA incorporation in mice skin, while the groups supplemented with soybean oil and HVF showed incorporation of n-6 PUFA and TFA, respectively. Such skin incorporations exerted influences on the development of UVR - induced oxidative damage in the mice skin and HVF group showed the highest protein carbonylation (PC) levels and lipid peroxidation, accompanied by larger skin thickening (edema), lower catalase (CAT) activity and cell survival. While soybean oil was associated with a partial prevention of damage observed in HVF group, FO supplementation prevented cutaneous oxidative damage UVR-induced. Sequentially, second and third experimental protocols were developed with the first and second generations offsprings born adult rats under daily supplementation of the same oils used in experiment 1 (SO, FO and HVF) and at 90 days old, each experimental group were exposed to UVR 3x/ week for 12 weeks. Animals first generation offspring (experiment 2) FO supplemented treated showed higher incorporation of n-3 FA and lower n-6/n-3 ratio in the dorsal skin, while the HVF group showed greater incorporation of TFA. Biochemical analyzes showed higher PC levels, *per se*, and smaller functionality of mitochondrial enzymes and decrease of some antioxidant defenses ((reduced glutathione (GSH) and vitamin C (VIT C)) in the dorsal skin HVF supplemented group. After UVR exposure, the same experimental group showed higher wrinkles scores, increased reactive species (RS) generation and PC levels, which were accompanied by decrease in GSH and VIT C skin levels. In contrast, FO group showed lower wrinkles scores and skin thickening after UVR exposure, besides lower PC levels and increased of the functionality of mitochondrial enzymes. Additionally, we observed a positive correlation between the RS generation-UVR induced and skin thickness, wrinkles and PC

levels, while a negative correlation between the RS generation-UVR induced and functionality of mitochondrial enzymes, and between PC levels and GSH, SOD and VIT C.Animals of the second generation offspring (experiment 3) supplemented with FO showed higher n-3 FA incorporation and lower n-6/n-3 ratio in the dorsal skin, while TFA were incorporated only in HVF group. The latter experimental group showed biochemical changes *per se*: high RS generation, lower functionality of mitochondrial enzymes and increased Na^+K^+-ATPase activity. UVR exposure increased skin wrinkling andRS generation, besides reduced functionality of mitochondrial enzymes and GSH levels in HFV group. FO groupUVR exposure showed reduced skin thickness and PC levels, besides increase CAT activity and the preservation of Na^+K^+-ATPase activity. Whereas the n-3 PUFA compete with n-6 PUFA for desaturases and elongases activities, which originate from long chain n-3 or n-6 PUFA, respectively, which are incorporated into the cell membranesphospholipids.Such incorporation allows the cyclooxygenase-2 (COX-2) activity over them, originating active metabolites of the series 3 (prostaglandins (PG) and thromboxanes (TX)) or series 2 (PG and TX series 2), respectively. Series 3 metabolites are less pro-inflammatory than those of series 2, which may partly explain our findings. Moreover, to date, no study has shown the metabolites generation of AGT, even their influence on inflammation and pro-oxidant in cell membranes. How TFA have been reported to inhibit the desaturases activity, we suggest that the presence of AGT in the membranes may be inhibiting the n-3 PUFA incorporation and, thus, reduce the metabolites generation, which are known to be beneficial. Taken together, the data presented in this thesis suggest that healthy eating habits that include reduced intake of foods rich in AGT and the inclusion of n-3 PUFA, accompanied by care front sun exposure can contribute to the prevention of skin diseases and skin diseases associated with UV exposure.

Keywords: fatty acids, *trans* fatty acids, ultraviolet radiation, oxidative stress

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LISTA DE ABREVIATURAS

AA – ácido araquidônico
AG – ácidos graxos
AGE – ácidos graxos essenciais
AGPI – ácidos graxos poliinsaturados
AGT – ácidos graxos *trans*
AGI – ácidos graxos insaturados
AGS – ácidos graxos saturados
AGMI – ácidos graxos monoinsaturados
ALA – ácido α-linoléico
CAT – catalase
COX – ciclooxigenase
DA – dermatite atópica
DGLA – ácido dihomo-γ-linoléico
DHA – ácido docosahexaenóico
EO – estresse oxidativo
EPA – ácido eicosapentaenóico
EROs – espécies reativas de oxigênio
GLA – ácido γ-linoléico
GPx – glutationa peroxidase
GSH – glutationa reduzida
GVH – gordura vegetal hidrogenada
IL-8 – interleucina 8
LA – ácido linoléico
LOX – lipooxigenase
LPO – peroxidação lipídica ou lipoperoxidação
LT – leucotrienos
MED – dose eritematosa mínima
PG – prostaglandinas
PGI_3 – prostaglandina I_3
PLA_2 – fosfolipase A_2
RL – radicais livres
RUV – radiação ultravioleta
RUVA – radiação ultravioleta A
RUVB – radiação ultravioleta B
RUVC – radiação ultravioleta C
SC – stratum corneum
SOD – superóxido dismutase
TBARS – substâncias reativas ao ácido tiobarbitúrico
TX – tramboxanos
TXA_2 – tramboxano A_2
VIT C – vitamina C
5-LOX – lipooxigenase 5

LISTA DE APÊNDICES

SUMÁRIO

APRESENTAÇÃO

Esta tese está estruturada em seções dispostas da seguinte forma: Introdução, Objetivos, Desenvolvimento (Revisão bibliográfica, Artigo 1 e 2, Manuscrito Científico 1, Discussão e Referências, Apêndice 1 (Artigo 3 e resultados adicionais) e Conclusão.

Os itens Materiais e Métodos, Resultados, Discussão e Referências encontram-se inseridos nos próprios artigos e manuscritosna seção **PRODUÇÃO CIENTÍFICA** e representam a íntegra deste estudo.

Ao fimencontram-se os ítens **DISCUSSÃO** e **CONCLUSÃO**, nos quais há interpretações e comentários gerais dosmanuscritos científicos contidos neste estudo.

As **REFERÊNCIAS** referem-se somente às citações que aparecem nos itens **INTRODUÇÃO** e **DISCUSSÃO**.

O **APÊNDICE 1** refere-se aos experimentos executados durante realização de Doutorado Sanduíche.

1. INTRODUÇÃO

Em países em que a exposição à radiação solar é alta, como no Brasil e outros países situados em proximidade à linha do Equador, os quais apresentam uma média anual de 280 dias de sol (RODRIGUES; MATAJS, 2005), o risco de alteração da homeostase da pele é maior (SÁNCHEZ-CAMPILLO et al., 2009). Da mesma forma, a evolução recente do estilo de vida tem impulsionado o desenvolvimento de atividades ao ar livre e, consequentemente, o aumento da exposição da pele aos raios ultravioleta (UV) solares (BOISNIC et al., 2005), fatores determinantes para a elevação de alterações na pele relacionadas à exposição àquela radiação (MILLER; WEINSTOCK, 1994; PEUS et al., 1999).

A radiação UV (RUV) representa uma parte do espectro solar com efeitos biológicos potentes sobre a pele e é o principal agente envolvido no fotodano cutâneo, através da indução da formação de radicais livres (RL) e subsequente estresse oxidativo (EO) (IDSON, 1988; LAVKET; KAIDBEY, 1997; RANGARAJAN; ZATZ, 1999). O fotoenvelhecimento e o fotodano têm sido objeto de vários estudos e pesquisas que buscam minimizar ou reverter desordens de pele (BANDO et al., 2004; BARG et al., 2014; BOLFA et al., 2013; CAMPANINI et al., 2013; CASAGRANDE et al., 2006; HWANG et al., 2006; KAWADA et al., 2013; KITAZAWA et al., 2005; LEE et al., 2013; MARTIN et al., 2008; PARK et al., 2014; RAJNOCHOVÁ SVOBODOVÁ et al., 2013; REEVE et al., 2005; SÁNCHEZ-CAMPILLO et al., 2009; SONG et al., 2006; SVOBODOVA et al., 2003; VAID et al., 2014; VICENTINI et al., 2008). Os agentes que podem reduzir a intensidade da ação da exposição à RUV, atuando tanto como uma barreira de proteção (AL MAHROOS et al., 2002; MOYAL, 1998; SEITE et al., 2000), quanto como antioxidante (BARG et al., 2014; CASAGRANDE et al., 2006; FONSECA et al., 2010; HWANG et al., 2006; MITANI et al., 2001; SHARMA; KAUR, 2006; XIONG et al. 2014), podem servir como um lenitivo para os fotodanos cutâneos (BANDO et al., 2004; BARG et al., 2014; CAMPANINI et al., 2013;CASAGRANDE et al., 2006; FONSECA et al., 2010; 2011a; b; HWANG et al., 2006; KITAZAWA et al., 2005; MARTIN et al., 2008; REEVE et al., 2005; SÁNCHEZ-CAMPILLO et al., 2009; SONG et al., 2006; SVOBODOVA et al., 2003; VICENTINI et al., 2008; 2010; 2011).

A preservação da saúde da pele contra os danos associados a exposição à RUV também está relacionada comos lipídios que a compõem. Os lipídios estão presentes no

stratum corneum da epiderme, que é a camada mais externa da pele, bem como em membranas celulares (DENDA et al., 1993; IMOKAWA et al., 1991; RAWLINGS et al., 1994a; ROGERS et al., 1996), e consistem em ácidos graxos (AG), fosfolípidos, e espingolipídios, colesterol, triglicerídios, esqualeno e ésteres de cera (GRAY; YARDLEY, 1975). Esses lípidos desempenham um papel importante nas funções fisiológicas da pele, cuja composição afeta os mecanismos de sinalização celular (JUMP, 2004) e determinam a sua morfologia e histologia (SCHNEIDER; WOHLRAB; NEUBERT, 1997). Nesse contexto, os ácidos graxos essenciais (AGE) e seus derivados desempenham um papel crucial na fluidez e flexibilidade da membrana celular e afetam a atividade de proteínas, como receptores, transportadores e enzimas (FOSTER; HARDY; ALANY, 2010). Nesse sentido, os AGE são capazes de manter a integridade estrutural da pele e, consequentemente, a sua permeabilidade (HORROBIN, 2000; MANKU et al., 1982; WRIGHT, 1991; ZIBOH; MILLER; CHO, 2000).

Nas últimas décadas, nos países ocidentais foi observado um aumento do consumo de alimentos industrializados que contêm quantidades significativas de ácidos graxos saturados (AGS), ácidos graxos monoinsaturados (AGM) e ácidos graxos poliinsaturados (AGPI) n-6, bem comoconcentrações consideráveis de ácidos graxos *trans* (AGT) (ALLISON et al., 1999; BAGGIO; BRAGAGNOLO, 2006; HULSHOF et al., 1999; SAGUY; DANA, 2003). Essa mudança nos hábitos alimentares promoveu um aumento da relação entre AGPI n-6/n-3, principalmente pela ingestão reduzida de AGPI n-3 (AILHAUD et al., 2006). O aumento do consumo de AGT representa uma perda no valor nutricional dos alimentos e o impacto desta condição na saúde humana precisa ser monitorado.

Há um interesse crescente no papel da nutrição materna sobre a saúde das gerações (AGALE et al., 2010; LEVANT; OZIAS; CARLSON, 2007; MARSZALEK; LODISH, 2005; RAO et al., 2007), no entanto, o impacto da suplementação com diferentes AG sobre o fotodano da pele induzido pela RUV não tem sido explorado. Nos seres humanose animais, a quantidade e a natureza dos AG presentes na dieta, assim como a mobilização dos seus estoques maternos durante a gravidez (AL et al., 1995; HORNSTRA et al., 1995; OTTO et al., 2001) são refletidas nos tecidos fetais (AMUSQUIVAR; HERRERA, 2003; FRIESEN; INNIS, 2006; INNIS, 2005; KRAUSS-ETSCHMANN et al., 2007; LEVANT et al., 2006) etêm sido identificadas como determinantes do risco de desenvolvimento de doenças crônicas na prole (ARMITAGE et al., 2005;BUCKLEY et al., 2005; GHOSH et al., 2001), possivelmente com efeitos a longo prazo (OZANNE et al., 1998).

A perspectiva de validação dos resultados esperados dessa pesquisa poderá contribuir na compreensão da influência do consumo de diferentes AG nas desordens da pele. E a

ampliação das possibilidades de produção de novos elementos de qualidade de vida pode funcionar como um novo motivador de consumo de uma alimentação com qualidade capaz de favorecer a saúde física e mental e, também, acionar o surgimento de novas tecnologias de aproveitamento e industrialização de alimentos para a humanidade. Já está comprovado que os AGPI n-3 possuem atividade antiinflamatória e antioxidante, demonstrada através de experimentação clínica e animal (BARCELOS et al., 2010; CHOI-KWON et al., 2004; YILMAZ et al., 2004). No entanto, a influência da suplementação com GVH, fonte de AGT, em comparação com o óleo de peixe, fonte de AGPI n-3, óleo de soja, fonte de AGPI n-6 e, sobre os danos oxidativos da pele dorsal de roedores induzidos pela RUV ainda não possuem validação científica.

2. OBJETIVOS

2.1 Objetivo geral

Avaliar a influência da suplementação de diferentes AG sobre os danos oxidativos na pele de roedores expostos à RUV.

2.2 Objetivos específicos

- Avaliar a incorporação de AG n-3, n-6 e *trans* na pele de roedores suplementados com diferentes óleos/gordura como fonte destes AG em diferentes épocas de vida;
- Avaliar a influência da incorporação dos diferentes AG sobre a espessura da pele, parâmetros de EO, defesas antioxidantes e viabilidade celular na pele de roedores expostos aguda e cronicamente à RUV;
- Avaliar a influência da suplementação dos diferentes óleos e gordura (óleo de peixe, óleo de soja e gordura vegetal hidrogenada (GVH)) sobre o processo de fotoenvelhecimento da pele de ratas de 1ª e 2ª geração e repetidamente expostas à RUV;
- Avaliar a influência da suplementação de diferentes AG sobre a espessura da pele, parâmetros de EO, defesas antioxidantes e sobrevivência mitocondrial cutânea.

3. DESENVOLVIMENTO

3.1. Revisão bibliográfica

3.1.1 A pele

A pele é o maior órgão do corpo humano, ocupando uma superfície de cerca de 1,5 a 2,0 m^2 (PROKSCH et al., 1993; SOUZA, 2009). A sua principal função éservir como um sistema de defesa primário (LEE et al., 2006), protegendo os órgãos internos por agir como uma barreira eficaz contra os efeitos nocivos do ambiente e de agentes xenobióticos (HAKOZAKI et al., 2008; NICHOLS; KATIYAR, 2010).

A principal barreirada pelesitua-se na sua camadamais externa, o *stratum corneum*, que é constituído por corneócitos rodeados por regiões lipídicas, dos quais a organização é considerada muito importante para a função de barreira da pele (BOUWSTRA;PONEC, 2006; TFAYLI et al., 2013). Devido à excepcional composição lipídica do *stratum corneum*, com ceramidas de cadeia longa, AGlivres e colesterol, a organização de lípidos é diferente das outras membranas biológicas (PONEC et al., 1988; TFAYLI et al., 2013; WERTZ; DOWNING, 1991). Nesse sentido, desordens cutâneas são acompanhadas por uma organização lipídica alterada e por uma redução na função de barreira da pele (DOERING; PROIA; SANDHOFF; 1999; HOLLERAN et al., 1994; SIDRANSKY et al., 1996).

O *stratum corneum* é a camada mais externa da pele e a sua função de barreira depende, em grande parte, da composição e a estrutura, bem como da organização dos lipídios na sua matriz extracelular (TFAYLIet al., 2013). As ceramidas, os AG livres e colesterol representam as principais classes de lipídios presentes nessa matriz (TFAYLIet al., 2013).

Recentemente, o reconhecimentodos cuidados com a pelehumana aumentou marcadamente. Pesquisas emmuitos campos, incluindo cosméticos, alimentação e suplementosforam ativamenteconduzidos parareduzir oupara melhoraras afecções cutâneas causadas por fatoresinternos ou externos, como a RUV (KIM et al., 2012a; LEI; LI; WANG, 2010).

3.1.2 A radiação ultravioleta

A importância da RUV e o seu impacto sobre a saúde humana têm sido amplamente discutidos durante as últimas décadas (UNEP,2005). Desde meados dos anos 1980, o nível mundial dessa radiação solar como consequência da diminuição da camada de ozônio (DAMERIS, 2009; LI; STOLARSKI; NEWMANet al., 2009; McKENZIE et al., 2007) tem gerado preocupação, devido as suas implicações para a saúde (DE GRUIJL et al., 2003; GALLAGHER; LEE, 2006). A camada de ozônio age como um escudo fino na atmosfera estratosférica, protegendo a vidana Terra dos raios UV solares, absorvendo toda a radiação UVC, a maioria da UVB e muito pouco a UVA (LAUTENSCHLAGER; WULF; PITTELKOWet al., 2007). Os cientistas começaram a se preocupar com a redução da camada de ozônio (ARMSTRONG; KRICKER, 2001; DAMERIS, 2009; KRIPKE, 1988; LI et al., 2009; McKENZIE et al., 2007), resultante da ação de produtos químicos (clorofluorcarbonos) e outras substâncias que destroem o ozônio liberados por indústrias e escape dos automóveis na atmosfera (ARMSTRONG; KRICKER, 2001), já que uma diminuição aproximada de1% nos níveis de ozônio corresponde a um aumento de 1% a 2% na mortalidade causada por melanoma (LAUTENSCHLAGER; WULF; PITTELKOW et al., 2007). Da mesma forma, uma redução de 10% nos níveis de ozônio causará 300.000 novos casos de câncer de pele não melanomae 4.500 novos casos de melanoma (WHO, 2009).

O risco de câncer continua a crescer com o aumento constante da esperança de vida e de alterações prejudiciais nos hábitos alimentares, estilo de vida e condições ambientais (SINGH; KATIYAR, 2013). Os cânceres da pele representam os tumores malignos mais comuns em humanos (SINGH; KATIYAR, 2013) e têm predominância em adultos brancos (INCA). Segundo o Instituto Nacional de Câncer (INCA), no Brasil o câncer de pele é o mais freqüente e corresponde a 25% de todos os tumores malignos registrados no país. Em 2012, a estimativa para o Brasil foi de 62.680 novos casos de câncer de pele não melanoma entre homens e 71.490 entre mulheres. Esses valores correspondem a um risco estimado de 65 novos casos a cada 100 mil homens e 71 para cada 100 mil mulheres. O câncer de pele não melanoma é o mais incidente em homens nas regiões Centro-oeste (124/100mil), Sul (38/100mil), enquanto nas regiões Sudeste (73/100mil) e Nordeste (39/100mil) é o segundo mais frequente. Nas mulheres é o mais freqüente em todas as regiões, com um risco estimado de 109/100mil nas regiões Centro-oeste, 91/100mil na região Sudeste, 68/100 mil na região

Sul, 43/100mil na região Norte e 42/100mil na região Nordeste. Quanto ao melanoma da pele, sua letalidade é elevada, porém, sua incidência é baixa (3.170 novos casos em homens e 3.060 novos casos em mulheres). As maiores taxas estimadas em homens e mulheres encontram-se na região Sul (INCA).

A luz solar é um espectro contínuo de radiação eletromagnética que está dividido em três espectros principais de comprimento de onda: UV, visível e infravermelho (SOEHNGE et al., 1997). A faixa UV pode ser segmentada em três grupos com base nos comprimentos de onda: radiação de onda curta (UVC; 100-280nm), onda média (UVB; 280-320nm) e onda longa (UVA; 320-400nm) (AFAQ; MUKHTAR, 2006; CIE, 1987; FREDERICK et al., 1994; GODAR, 2005). Aproximadamente90-99% da energia solar UV que atinge a superfície da terra é UVA e somente 1-10% é UVB (MILLER et al., 1998; PASTILA; LESZCZYNSKI, 2007) (figura 1).

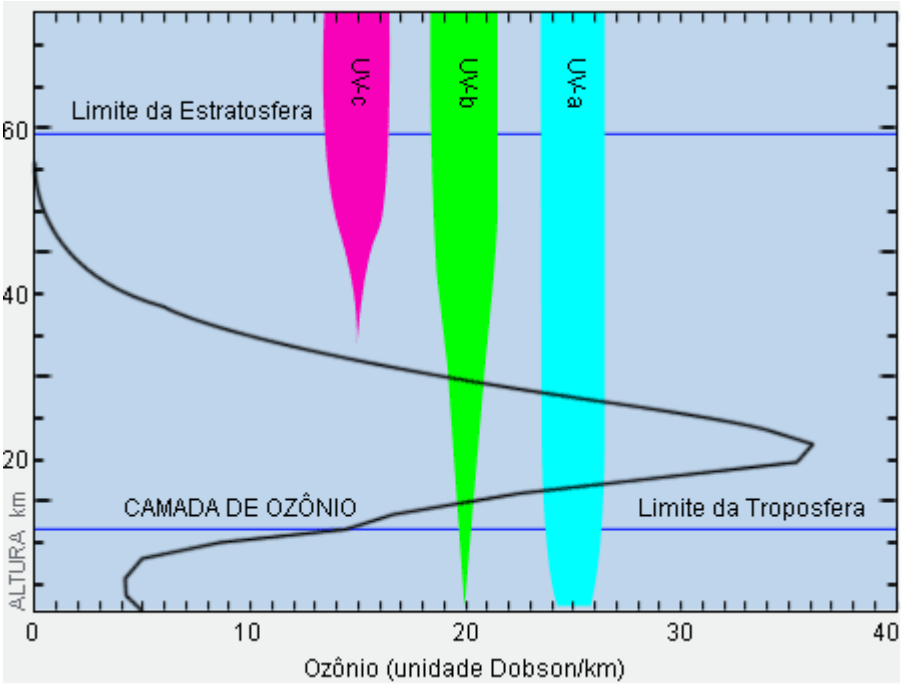


Figura 1. Absorção da RUV pela camada de ozônio.
Fonte: figura adaptada de www.nasa.gov

Cada espectro da RUV tem um limite característico de eficiência na penetração das camadas epidérmicas e dérmicas da pele humana e de roedores (NICHOLS; KATIYAR, 2010). A radiação solar UVC é bloqueada pela camada de ozônio e normalmente não alcança a superfície terrestre e, desta forma, seu papel patogênico torna-se mínimo. Por outro lado, esse espectro possui uma enorme energia e é mutagênico na natureza, podendo penetrar na

pele numa profundidade de, aproximadamente, 60 a 80 μm, causando danos ao DNA (NICHOLS; KATIYAR, 2010). A RUVB constitui cerca de 5% da RUV solar total e é a principal responsável por uma variedade de desordens da pele. Essa radiação pode penetrar na pele a uma profundidade de cerca de 160 a 180 μm, atravessando toda a epiderme e derme. Tal espectro pode produzir efeitos biológicos diretos e indiretos em muitos sistemas biológicos como a indução de EO e de danos ao DNA, queimaduras cutâneas, envelhecimento prematuro e câncer de pele (BACHELOR; BOWDEN, 2004; BOWDEN, 2004; DE GRUIJL; VAN DER LEUN, 1994; ICHIHASHI et al., 2003; MUKHTAR; ELMETS, 1996; NICHOLS; KATIYAR, 2010; PARISIetal, 2007; SETLOW, 1974; WHO, 2002). A RUVA compreende o maior espectro da RUV solar (90-95%), é a porção menosabsorvida pelacamada de ozônio, podendo penetrar na pele até a profundidade de, aproximadamente, 1000 μm. A exposição à RUVA causa lesões oculares (HEISLER; GRANT, 2000; KIMLIN et al., 2002) e induz a geração de RL, que podem causar danos a macromoléculas celulares, como proteínas, lipídios e DNA (DIGIOVANI, 1992), culminando no fotoenvelhecimento (KRUTMANN, 2001). Presume-se que grande parte da ação mutagênica e cancerígena da RUVA seja mediada pelas espécieds reativas de oxigênio (EROs) (DE GRUIJL et al., 2000; RUNGER, 1999) (figura 2).

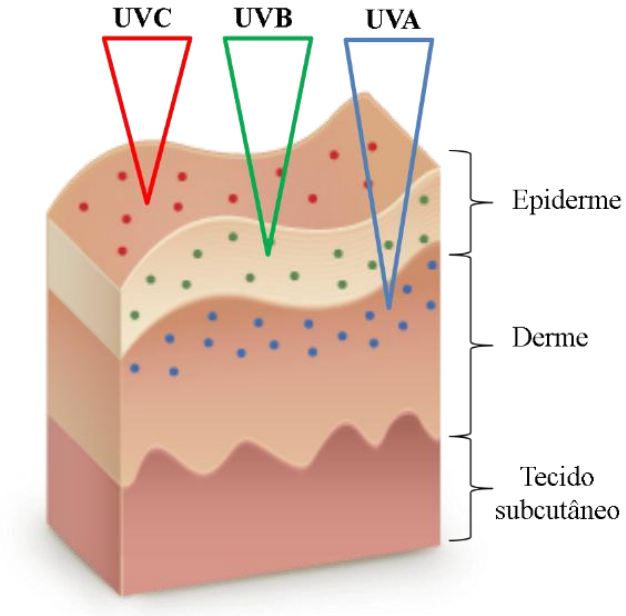


Figura 2. Eficiência de penetração das radiações UVA, UVB e UVC na pele
Fonte: figura adaptada de www.skinlaser.com.br

O envelhecimento cutâneo ocorre por dois mecanismos distintos, nomeadamente o envelhecimento intrínseco e o envelhecimento extrínseco ou fotoenvelhecimento (MALERICH; BERSON, 2014). O envelhecimento intrínseco depende tempo e, portanto, éinevitável (RANGARAJAN; DREHER, 2010) e as alterações ocorrem parcialmente como resultado do dano endógeno cumulativo devido à formação contínua de EROs geradas pelo metabolismo oxidativo celular (PUIZINA-IVIC, 2008). Apesar de um forte sistema de defesa antioxidante, o dano gerado pelas EROs afeta os constituintes celulares como proteínas, lipídios e DNA(KATIYAR et al., 2001; LARSSON et al., 2005; SHINDO et al., 1993). Também possui um componente genético devido à diminuição dos níveis dos hormônios sexuais. O envelhecimento extrínseco ocorre devido a vários fatores como a radiação ionizante, o estresse físico e psicológico grave, a ingestão de álcool, a má nutrição e o excesso de comida, a poluição ambiental e a exposição à RUV (MALERICH; BERSON, 2014; PUIZINA-IVIC, 2008). Entre todos esses fatores ambientais, a RUV contribui até 80% para a indução do envelhecimento extrínseco (MALERICH; BERSON, 2014; PUIZINA-IVIC, 2008).

A RUVé um importante estressante ambiental, responsável por queimaduras cutâneas, envelhecimento precocee câncer de pele (BODE; DONG, 2009; BOUKAMP, 2005; GRUBER et al., 2007; SPRECHER, 2007). Além disso, causa danos diretos e indiretos ao DNA e RNA, proteínas e lipídios, mutações de genes, imunossupressão, respostas inflamatórias, alterações nas vias de sinalização intracelular. Também ativa a fosfolipase A_2 (PLA_2, do inglês *phospholipase* A_2) citosólica e a cicloxigenase-2 (COX-2) a aumentarem a produção de prostaglandina E_2 (PGE_2) (BUCKMAN et al., 1998; CHEN et al., 1996; LIU, MIZU; YAMAUCHI, 2010) através da elevada produção de EROs e RL (IMLAY; LINN, 1988). O subsequente EO parece contribuir para aquelesprocessos patológicos (BENDER et al., 1997; BODE et al., 2003; 2005; HERRLICH et al., 2008; MEERAN; PUNATHIL; KATIYAR et al., 2008; MUTHUSAMY; PIVA, 2010;RASS; REICHRATH, 2008).

A exposição à RUV é o fator chave para o início de várias desordens de pele, como rugas, descamação, ressecamento, alterações do pigmento, incluindo hipopigmentação, hiperpigmentação, fotoenvelhecimento e câncer de pele (DE GRUIJL; VAN DER LEUN, 1994; ICHIHASHI et al., 2003; MUKHTAR; ELMETS, 1996; NICHOLS; KATIYAR, 2010). Embora muitos fatores ambientais e genéticos contribuam para o desenvolvimento das desordens de pele, o mais importante fator é a exposição da pele à RUV (NICHOLS; KATIYAR, 2010).

3.1.3 Estresse oxidativo e sistema de defesa antioxidante da pele

Para lidar com os efeitos nocivos do EO induzido pela RUV, a pele possui mecanismos de defesa antioxidante (BRIGANTI; PICARDO, 2003; NICHOLS; KATIYAR, 2010). Esses mecanismos evitam a lesão oxidativa dos lipídios estruturais e proteínas (KATIYAR et al., 2001; LARSSON et al., 2005; SHINDO et al., 1993), contribuindo para a integridade da membrana celular, a qual é condição essencial para uma pele saudável (BRIGANTI; PICARDO, 2003). Neste sentido, o sistema redox celular desempenha um papel fundamental na homeostase da pele e as doenças nesse órgão podem resultar de um desequilíbrio entre estímulos pró-oxidantes e antioxidantes (BRIGANTI; PICARDO, 2003).

O sistema antioxidante da pele é constituído por antioxidantes enzimáticos e não enzimáticos (BRIGANTI; PICARDO, 2003; LOPEZ-TORRES et al., 1998). Entre os antioxidantes enzimáticos, a glutationa peroxidase (GPx), a catalase (CAT) e a superóxido dismutase (SOD) desempenham um papel central. Os antioxidantes não enzimáticos presentes nas células da epiderme são o α-tocoferol, a ubiquinona, o β-caroteno, o ácido ascórbico e a glutationa reduzida (GSH) (BRIGANTI; PICARDO, 2003; BURTON; INGOLD, 1984; DI MASCIO et al., 1990; MEISTER, 1988; KENNEDY; LIEBLER, 1992; SCHAFER; BUETTNER, 2001; SIES, 1999). Os peróxidos lipídicos e seus produtos de metabolismo, como o malondialdeído, podem afetar direta ou indiretamente muitas funções essenciais para a homeostase das células e dos tecidos. Como conseqüência, o aumento da peroxidação da membrana lipídica pode evocar uma resposta imune e inflamatória, ativar a expressão gênica, a proliferação celular ou iniciar a apoptose. Consequentemente, há uma estreita relação entre a produção de EROs, o prejuízo da defesa antioxidante, o dano peroxidativo da membrana celular e os processos inflamatórios ou patológicos degenerativos (BRIGANTI; PICARDO, 2003).Vários estudos conduzidos com base na teoria dos RL para o envelhecimento demonstraram níveis basais reduzidos de enzimas antioxidantes (SOD, CAT e GPx) e de antioxidantes não enzimáticos (GSH, vitamina C (VIT C) e α-tocoferol) e aumento de peróxidos lipídicos na pele (LIPPMAN, 1985; LOPEZ-TORRES et al., 1994; NIWA et al., 1987; 1988; SHINDO et al., 1991; UEDA; SUGIURA, 1989).

Embora a pele possua um sistema antioxidante elaborado para lidar com o EO induzido pela RUV (BRIGANTI; PICARDO, 2003; NICHOLS; KATIYAR, 2010), a exposição aguda e crônica a essa radiação e o consenquente aumento na geração de EROs (ZHANG et al., 1997), que excede a capacidade antioxidante cutânea, leva ao dano oxidativo,

podendo resultar em desordens e doenças de pele (BRIGANTI; PICARDO, 2003). Neste sentido, agentes quimioprotetores e quimioterapêuticos estão sendo pesquisados e o uso de suplementações e/ou intervenções dietéticas estão tornando-se cada vez mais populares como um meio para a proteção contra as desordens e doenças de pele induzidas pela RUV (BARG et al., 2014; CHO et al., 2007; FONSECA et al., 2010; HAMA et al., 2012; ISHII et al., 2014; JEON et al., 2003; KANG et al., 2009; KAWADA et al., 2013; KIM et al., 2004; KIMURA; SUMIYOSHI; KOBAYASHI, 2014; LEE et al., 2013; LOU et al., 2011; MINAMI et al., 2009; RAJNOCHOVÁ SVOBODOVÁ et al, 2013; SÁNCHEZ-CAMPILLO et al., 2009; SHAHBAKHTI et al., 2004; SHARMA; KATIYAR, 2010; TAKEMURA et al., 2002; VAYALIL et al., 2004).

3.1.4 Enzima Na^+K^+ATPase

Além as enzimas citadas anteriormente, uma importante enzima presente nas membranas celulares e muito sensível a agentes oxidantes é a Na^+K^+ATPase (CARFAGNA; PONSLER; MUHOBERAC, 1996; FOLMER et al., 2004). A bomba Na^+K^+ATPase é responsável pelo transporte ativo de íons sódio e potássio através da membrana plasmática para manter a sua excitabilidade (RIBEIRO et al., 2007). Alguns estudos demonstram que a Na^+K^+ATPase é altamente vulnerável ao ataque dos RL (LEES, 1993; KURELLAet al., 1997; YOUSEFet al., 2002), relacionando o EO e a função da enzima através de alterações observadas em sua atividade decorrente da peroxidação lipídica (KAUR;SHARMA; SINGH, 2001; PIERRE et al., 1999; TEIXEIRA et al., 2011; 2012).

A atividade aumentada da Na^+K^+ATPase altera a transmissão celular e pode reduzir a entrada de cálcio na célula. Da mesma forma, as modificações nos fosfolipídios de membrana também alteram os sistemas de transporte de cálcio (GOLDMAN; ALBERS, 1973).Em condições basais, a epiderme de mamíferos normalmente exibe um distinto gradiente de cálcio, com baixos níveis na camada basal e níveis elevados no estrato granuloso(MENON et al, 1985; FORSLIND et al, 1995; MAURO et al, 1998). Mudanças no cálcio presente no estrato granuloso regulam a resposta secretora dos corpos lamelares frente às alterações da permeabilidade da barreiracutânea e as perturbações na permeabilidade da função de barreira da pelepode transitoriamente alterar este gradiente de cálcio epidérmico (MAO-QIANG et al, 1997). A perda de cálcio epidérmico após a interrupção da barreira da pele estimula a

secreção de corpos lamelares, configurando uma resposta que facilita a recuperação normal barreiracutânea após pertubações na sua integridade induzidas por agentes xenobióticos, como a RUV (ELIAS et al.,2002).

3.1.5Modelo animal para estudo das desordens cutâneas

Camundongos *hairless* têm sido utilizados como modelo animal para o estudo das desordens cutâneas induzidas pela RUV por muitos anos. Tais animais possuem a pele desprovida de pelos e a depilaçãonão é necessáriaantes do início dos estudos de desordens e doenças cutâneas induzidas pela RUV, bem caracterizadaseprontamenteobservadas nesses animais (BENAVIDES et al., 2009). Da mesma forma, são empregados para avaliação da atividade fotoprotetora de substâncias pela determinação dos teores dos componentes da matriz extracelular e de marcadores de EO. Várias pesquisas utilizando esses animais têm relatado mudanças qualitativas e quantitativas que se assemelham aos danosinduzidos pela RUV que ocorrem na pele humana (BING-RONG et al., 2008; CAMPANINI et al., 2013; CHEN et al., 2008; DAI et al., 2007; FONSECA et al., 2010; HACHIYA et al., 2009; ITO et al., 2010; KATIYAR; MEERAN, 2007; KAWADA et al., 2013; KIM et al., 2012b;KIMURA; SUMIYOSHI; KOBAYASHI, 2014; LEE et al., 2013; MEERAN et al., 2009; MINAMI et al., 2009; OBA; EDWARDS, 2006; PARK et al., 2014; RODRÍGUES-YANES et al., 2012; SHARMA; KAUR, 2006; TSOYI et al., 2008; VAID et al., 2014; YIN et al., 2013).

No entanto, camundongos Swiss foram muito pouco utilizados, após depilação, em estudos distintos que confirmaram claramentequeo própolis e o sesamol são eficazes na prevenção dos danos cutâneos induzidos pela exposição à RUV (BOLFA et al., 2013; SHARMA; KAUR, 2006). Da mesma forma, poucos estudos utilizaram ratos na pesquisa dos efeitos cutâneos deletérios relacionados à exposição à RUV (BARG et al., 2014; LAM et al., 2011; SEVIN et al., 2007; SHI; RUAN, 2013), porém estudos relacionados à influência da idade (HUI et al., 2010; LIANG et al., 2010), hormônios (ESREFOGLU et al., 2005; TRESGUERRES et al., 2008) ou a pele plantar das patas traseiras (TSUKAHARA et al., 1999; 2001a; b; c; 2006) com o fotoenvelhecimento induzido pela RUV têm empregados esses animais.

3.1.6 Ácidos graxos essenciais

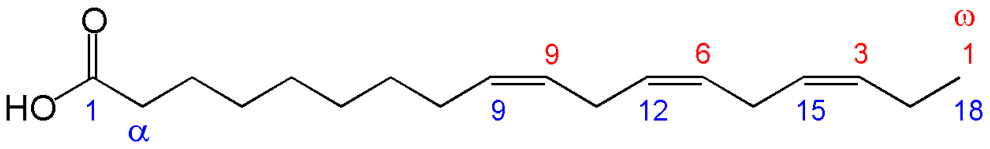
3.1.6.1 Química, classificação e principais representantes

Os AG são substâncias encontradas em uma ampla variedade de alimentos, os quais, na forma de lipídios, possuem funções estruturais, protetoras e de fornecimento e armazenamento de energia (COSTA; SILVA, 2002). São constituídos por cadeias de hidrocarbonetos (2 a 20 ou mais átomos)comumgrupo carboxila (COOH) em uma extremidade da cadeia e um grupo metila (CH_3) na extremidade oposta (n ou ω final) (MARSZALEK; LODISH, 2005). Na nomenclatura dos AG, o primeiro número indica o comprimento da cadeia de carbono; o segundo número, após os dois pontos, refere-se ao número de duplas ligações e o terceiro número, depois do n, representa o número de carbonos a partir da metila terminal da molécula para a primeira dupla ligação (WAINWRIGHT, 1992).

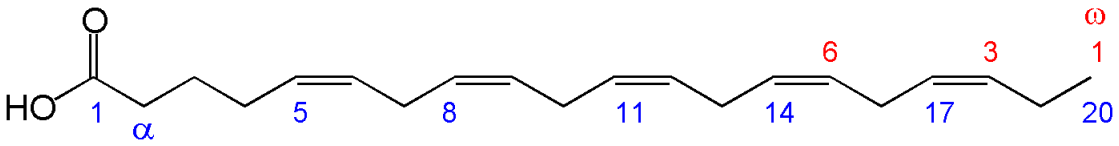
Quanto à extensão da cadeia, os AG classificam-se em AG de cadeia curta com cauda alifática de menos de 6 átomos de carbono; de cadeia média, com cauda alifática de 6 a 12 carbonos; de cadeia longa,com cauda alifática de mais de 12 carbonos; e de cadeia muito longa, com cauda alifática contendo mais de 22 átomos de carbono. Quando se trata de AGE costuma-se usar uma terminologia ligeiramente diferente: os de cadeia curta possuem 18 carbonos e os de cadeia longa têm 20 ou mais átomos de carbon (LEHNINGER; NELSON; COX, 2002).

Os AG também podem ser classificados de acordo com o número de insaturações presentes em sua cadeia carbonada (LEHNINGER; NELSON; COX, 2002). Assim, os AGS não possuem duplas ligações e os insaturados (AGI) contêm em sua cadeia uma – monoinsaturados (AGMI) – ou mais duplas ligações – poliinsaturados (AGPI), não saturadas com hidrogênio (KIM et al., 2010). Os AG com a designação n-3 e n-6 são AGPI, possuem a primeira dupla ligação da cadeia hidrocarbonada localizada no terceiro e sexto carbono a partir do n terminal, respectivamente (KNUTZON et al., 1998) e são componentes da dieta humana (MARSZALEK; LODISH, 2005). Dentre os alimentos que constituem as principais fontes de AG n-3 figuram a carne de peixes marinhos de águas geladas e profundas (sardinha, salmão, cavala, truta, arenque), óleos e produtos derivados de pescados, nozes e óleos vegetais (chia, canola e linhaça) (LARSSON et al., 2004; SOCCOL et al., 2003; WAINWRIGHT, 1992). Os AGPI n-6 são encontrados nos óleos vegetais de soja, girassol,

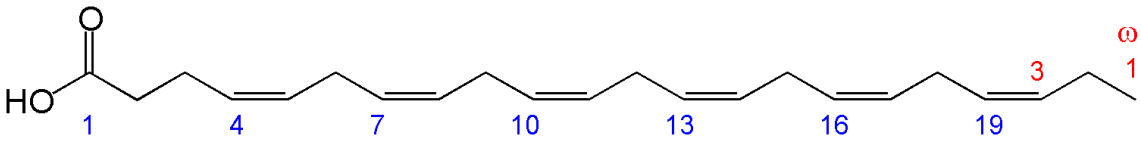
milho, cártamo, semente de uva, papoula, gérmen de trigo e semente de algodão (McCUSKER; GRANT-KELS, 2010; SANGIOVANNI; CHEW, 2005). Entre os componentes dessas duas classes de AGPI de cadeia longa, o ácido α-linolénico (ALA, 18:3 n-3) e o ácido linoléico (LA, 18:2 n-6) (figura 3) são considerados AGE porque as células animais não possuem as enzimas dessaturases capazes de especificamente colocar as duplas ligações nas posições n-3 en-6 (KNUTZON et al., 1998). Dessa forma, devem ser obtidos através da dieta e/ou suplementação (YEHUDA; RABINOVITZ; MOSTOFSKY, 2005).



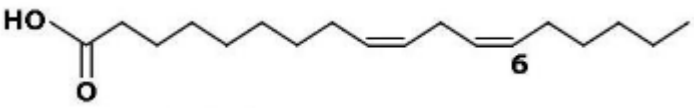
Ácido Alfa-linolênico (ALA; 18:3 n-3)



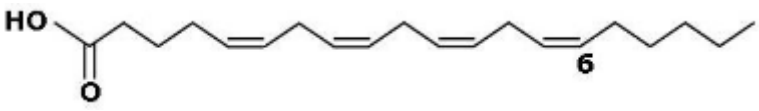
Ácido Eicosapentaenóico (EPA; 20:5 n-3)



Ácido Docosahexaenóico (DHA; 22:6 n-3)



Ácido Linoléico (LA; 18:2 n-6)



Ácido Araquidônico (AA; 20:4 n-6)

Figura 3. Principais representantes dos AGPI n-3 e n-6

3.1.6.2 Síntese dos AGPI n-3 e n-6 de cadeia longa

As séries n-3 e n-6 dependem das mesmas enzimaspara a produção dos seus derivados de cadeia longa (McCUSKER; GRANT-KELS, 2010; SALEM, 1999). Duas vias sintéticas completamente distintas são responsáveis pela produção dos AGPI n-3 e n-6 de cadeia longa (figura 4) a partir de AG de cadeia curta e os componentes dessas duas classes não são interconversíveis. Assim, através de vários passos de síntese – dessaturação (adição de dupla ligação), elongação (adição de duas unidades de carbono), dessaturação e parcial peroxissomal β-oxidação - o ácido araquidônico (AA, 20:4 n-6), um AGPI n-6 de cadeia longa, é produzido a partir do LA, um AGPI n-6 de cadeia curta. Da mesma forma, o ácido docosahexaenóico (DHA, 22:6 n-3) e o ácido eicosapentaenóico (EPA, 20:5 n-3), AGPI n-3 de cadeia longa, são produzidos pelas mesmas enzimas de síntese dos AGPI n-6, a partir do AG n-3 de cadeia curta ALA. O ALA não é produzido pelo organismo de mamíferos, o que requer sua ingestão a partir da dieta (SALEM, 1999). Cerca de 5% a 10% desse AG podem ser convertidos em EPA e 1% a DHA (ANDERSON; MA, 2009; JUMP, 2002; SARSILMAZ et al., 2003ª; b), mas também ambos podem vir através dos alimentos (BURR, 2000). Dessa forma, embora o ALA seja precursor para a síntese de EPA e DHA, a conversão EPA a DHA é altamente ineficiente, como demonstrado pela falta de aumento de DHA no plasma e tecidos após suplementação com EPA (GRIMSGAARD et al., 1997). Uma complexa via sintética tem sido descrita para a síntese de DHA a partir do EPA (SPRECHER, 1986; SPRECHER et al., 1995). Inversamente, a retroconversão de DHA a EPA é possível e. após uma ingestão de DHA, um pequeno aumento de EPA no plasma e nos tecidos é observado (BROSSARD et al., 1996). No todo, entretanto, a taxa de conversão de EPA e DHA a partir de ALA é baixa (ANDERSON; MA, 2009; EMKEN; ADLOF; GULLEY, 1994; GERSTER, 1998; GHAFOORUNISSA, 1998), varia entre indivíduos (JUMP, 2002) e parece ser mais limitada em homens jovens, que em mulheres jovens (BURDGE, 2004; BURDGE; JONES; WOOTTON, 2002; SMIT et al., 2003). Para atingir níveis teciduais adequados de EPA e DHA, eles devem ser obtidos a partir de suplementação e/ou da dieta, especialmente a partir de subprodutos de peixes marinhos, nos quais há o acúmulo de EPA e DHA ao longo da cadeia alimentar, a partir do fitoplâncton (YEHUDA; RABINOVITZ; MOSTOFSKY, 2005).

A competição da Δ6-dessaturase na conversão de ALA a ácido estearidônico (18:4 n-3) e LA a ácido γ-linoléico (GLA,18:3 n-6) significa que as dietas com elevado teor de AG n-6 pode reduzir a conversão de AG n-3 em 40% (EMKEN; ADLOF; GULLEY, 1994). A dieta

ocidental tipicamente têm uma elevada relação n-6/n-3(DAS, 2006) e a conversão de ALA a DHA é susceptível de ser comprometida. Então, a necessidade de uma ingestão adequada de ALA, com um equilíbrio adequado de n-6/n-3, é bem reconhecida, e a inclusão de DHA pré-formado, em circunstâncias onde a conversão de ALA em DHA pode ser insuficiente é reconhecida (SALEM, 1999).

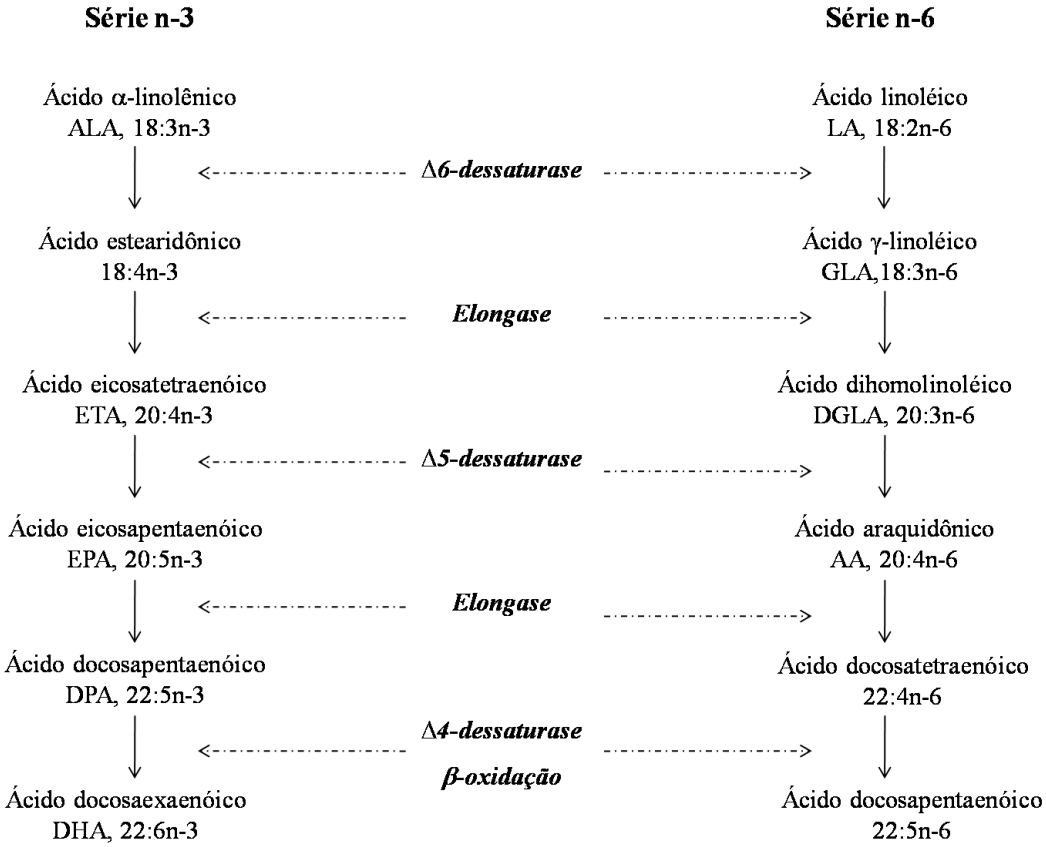


Figura 4. Síntese de AGPI de cadeia longa n-3 e n-6

3.1.6.3 Síntese de AGE na pele

A síntese dos AGE na pele é diferente que na maioria dos outros tecidos (HORROBIN, 1989). A epiderme não possui as enzimas Δ5-dessaturase e Δ6-dessaturase (CHAPKIN et al., 1986; CHAPKIN; ZIBOH, 1984) e a conversão do LA a GLA e dihomo-γ-linolênico (DGLA, 20:3 n-6) a AA não é possível, embora a conversão GLA a DGLA ocorra (figura 5). A epiderme deve, portanto, obte ro LA, o GLA, o DGLA e o AA do sangue, AG n-6, que devem

ser sintetizados por outros tecidos, principalmente pelo fígado (CHAPKIN et al., 1986; CHAPKIN; ZIBOH, 1984; ZIBOH; CHAPKIN, 1987). Como a epiderme renova-se rapidamente, não há estoques substanciais de GLA, DGLA, ou AA, tornando-a depende da formação contínua desses AG pelo fígado e seu transporte pelo sangue (CHAPKIN et al., 1986; CHAPKIN; ZIBOH, 1984; ZIBOH; CHAPKIN, 1987).

Tem sido demonstrado que a síntese de AG de cadeia curta a AG de cadeia longa é vulnerável a uma variedade de fatores que podem afetar o estado da pele, como o envelhecimento, o diabetes mellitus, a alta ingestão de álcool, a liberação de catecolaminas durante o estresse e as dietas ricas em açúcares simples e em AGT ou em AGS (BRENNER, 1982). Algumas das conhecidas consequências cutâneas desses fatores pode se relacionar coma deficiência da oferta de AGE para a pele, tornando-a escamosa, áspera, seca e causando hiperproliferação epidérmica, hipertrofia das glândulas sebáceas, fragilização dos capilares cutâneos e cicatrização deficiente possivelmente como resultado da deficiente formação de colágeno (IMOKAWA et al., 1989; SHERERTZ, 1986; SINCLAIR, 1958; ZIBOH; CHAPKIN, 1987).

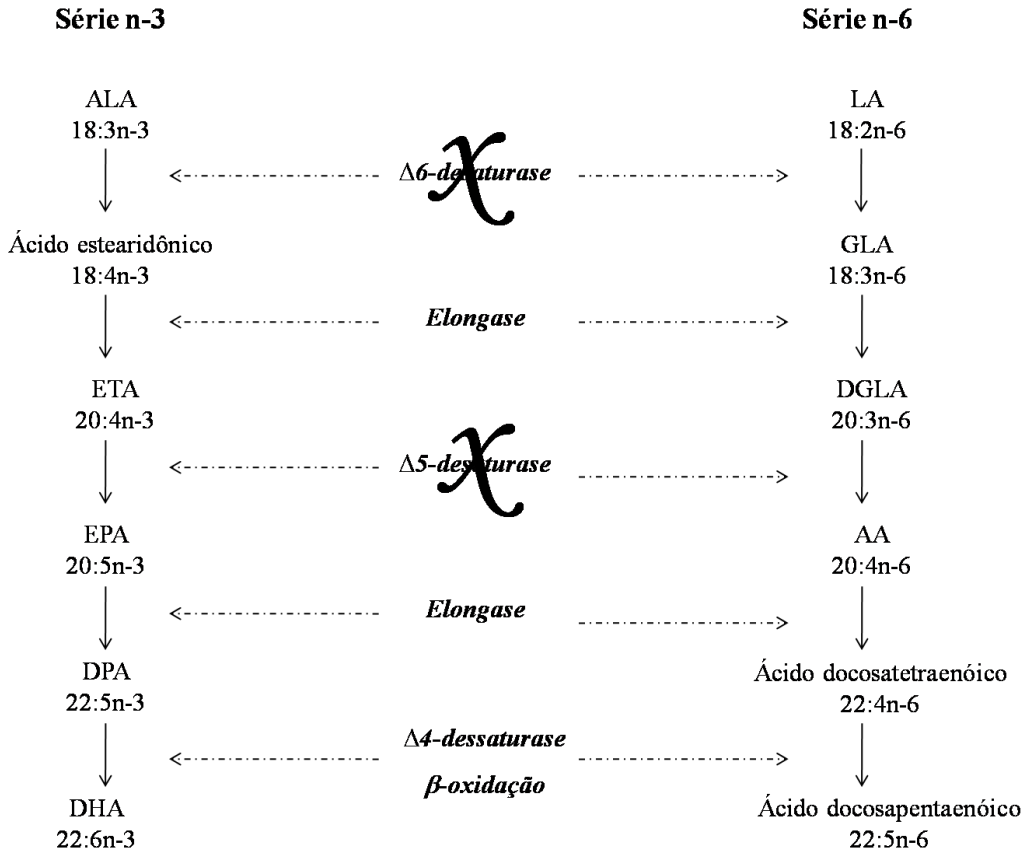


Figura 5. Síntese dos AGE n-3 e n-6 na pele: ausência das enzimas Δ5-dessaturase e Δ6-dessaturase.

3.1.6.4 Incorporação dos AGE n-3 e n-6 às membranas plasmáticas

O mecanismo proposto para os benefícios dos AGPI n-3 parece estar relacionado à sua incorporação nas membranas celulares (CLANDININ et al., 1994), promovendo mudanças na sua fluidez e função, bem como alterações na expressão gênica e produção de eicosanóides (RIEDIGER et al., 2009). O aumento do conteúdo dos AGPI n-3 na dieta causa a substituição parcial dos AGPI n-6, especificamente o AA, das membranas fosfolipídicas (MICKLEBOROUGH et al., 2003), pelos AGPI n-3 EPA e DHA. Nesse sentido, as proporções de AGPI n-3e n-6 presentes nos lípidios teciduais podem ser influenciadas pelo seu aporte dietético (HWANG; BOUDREAU; CHANMUGAM, 1988; HWANG; CARROLL, 1980; MOHRHAUER; HOLMAN, 1963a; b; PRASAD; CULP; LANDS, 1987) e pelas interações metabólicas competitivas entre esses AGPI (LANDS, 1991; LANDS; CRAWFORD, 1977; LANDS; MORRIS; LIBELT, 1991).

3.1.6.5 Metabolismo dos AGE n-3 e n-6

Além do seu papel estrutural e funcional sobre a membrana (WAINWRIGHT, 1992), os AGPI também exercem uma função reguladora através da produção de eicosanóides, que incluem prostaglandinas (PG), tromboxanos (TX) e leucotrienos (LT) (SPRECHER, 1986;WAINWRIGHT, 1992; WEBER *In* KARNOVSKY et al., 1988) (figura 6). Essa família de compostos fisiologicamente ativos é derivada de AG de 20 carbonos após a sua liberação da membrana plasmática pela ação específica da enzima PLA_2, diferem entre as séries n-3 e n-6 e são metabolizados através de duas vias (MASSARO et al., 2008; WAINWRIGHT, 1992).

A primeira via envolve reações catalisadas pelas enzimas COX e lipoxigenase (LOX). Quando o precursor é um AGPI n-6, como o AA, os prostanóides originados são da série 2, incluindo prostaciclina (PGI_2) e TXA_2; quando o precursor é um AGPI n-3, como o EPA e o DHA, os prostanóides originados são da série 3 (PGI_3 e TXA_3)(figura 4) (MASSARO et al., 2008). O aumento do consumo de AGPI n-3 provoca uma substituição parcial dos AGPI n-6, diminuindo as proporções relativas de AA nos fosfolipídios da membrana celular (LANDS et

al., 1992). Isso favorece a síntese de prostanóides biologicamente menos ativos, ou seja, com menores atividades de agregação plaquetária e vasoconstritora (MASSARO et al., 2008).

A segunda via envolve compostos tipicamente produzidos durante a resolução da inflamação e com potente atividade inflamatória (MASSARO et al., 2008). Esses compostos foram primeiro identificadas por Serhan et al. com o nome de resolvinas, para enfatizar o seu isolamento e produção durante a fase de resolução da inflamação aguda e para demonstrar a contribuição frequente da biossíntese transcelular desses novos mediadores (SERHAN; CHIANG, 2008). A produção das resolvinas é mediada por uma série de atividades da COX-2 e da lipooxigenase 5 (5-LOX) sobre o EPA, produzindo resolvinas das séries E_1 e E_2 e sobre o DHA, produzindo resolvinas da série D, com potente atividade antiinflamatória (SERHAN; CHIANG, 2008).

Diferentes mecanismos têm sido propostos para explicar os efeitos protetores dos AGPIn-3 em condições oxidativas. Alguns estudos têm enfatizado a propriedade antioxidante dos AGPI n-3 (OKUYAMA; ORIKASA; NISHIDA, 2008; TARDIVEL et al., 2009; SAW et al., 2010) sobre as EROs. Da mesma forma, pesquisas com animais de laboratório demonstraram que o DHA e EPA são antioxidantes nutricionais indiretos e reduzem peróxidos de lipídios no cérebro (BARCELOS et al., 2010; CHOI-KWON et al., 2004; HOSSAIN et al., 1999) e no fígado de ratos (YILMAZ et al., 2004). A atividade neuroprotetora do DHA também foi evidenciada através das propriedades antioxidantes indiretas *in vivo* (BAZAN, 2005; CALON et al., 2004; HASHIMOTO et al., 2002; WU; YING; GOMEZ-PINILLA, 2004; YAVIN et al., 2002), através do aumento da atividade da glutationa redutase (HASHIMOTO, 2002), diminuição da oxidação de proteínas (CALON et al., 2004; WU; YING; GOMEZ-PINILLA, 2004) e dos níveis de peróxidos de lipídios e EROs (HASHIMOTO et al., 2002; 2006).

Já foi demonstrado que a suplementação com AGPI n-3, assim como o tratamento tópico, possuem propriedades antiinflamatórias na pele exposta à RUV (JIN et al., 2010;PUPE e al., 2002; SHAHBAKHTI et al., 2004). Da mesma fora, estudos clínicos demonstraram que uma dieta rica em AGPI n-3 reduziu o eritema solar e a produção de PGE_2 na pele (ORENGO; BLACK; WOLF, 1992; RHODES et al., 1994; 1995; 2003) e seu precursor, o ALA, foi benéfico na prevenção de lesões cutâneas induzidas pela RUVB (TAKEMURA et al., 2002). Os AGPI n-3 também estão relacionados com uma menor sensibilidade cutânea (pós-irritação com nicotinato), aspereza e escamação e maior hidratação à pele (NEUKAM et al., 2011). Recentemente foi demonstrado que o EPA reduziu a secreção de interleucina-8 (IL-8) induzida pela RUV em queratinócitos (STOREY et al., 2005),

podendo ter efeito fotoprotetor e antiinflamatório em queratinócitos expostos à RUV (CHÊNEet al., 2007). Os AGPI n-3 podem ser úteis para a prevenção da carcinogênese da pele (LOU et al., 2011; MILLER; GAUDETTE, 1996) e podem aliviar erupções cutâneas de fotossensibilidade, aumentando o limiar de queimaduras solares e reduzindo os danos causados ao DNA (RHODES et al., 2003). Além disso, foi demonstrado que o EPA é uma agente potencial para a prevenção e tratamento do fotoenvelhecimento da pele induzido pela RUV (KIM et al., 2005).

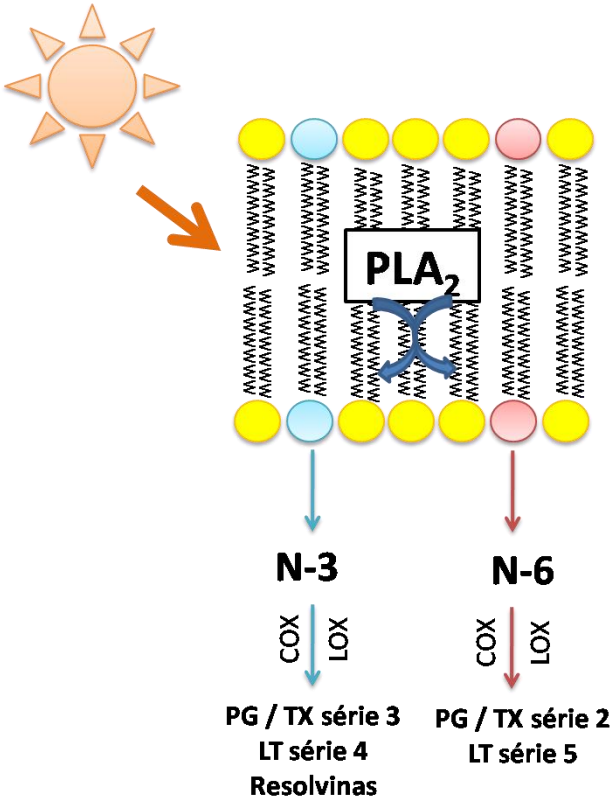


Figura 6. Esquema do metabolismo dos AGPI n-3 e n-6

Adaptado de Massaro et al., 2008

3.1.7 Razão n-6/n-3

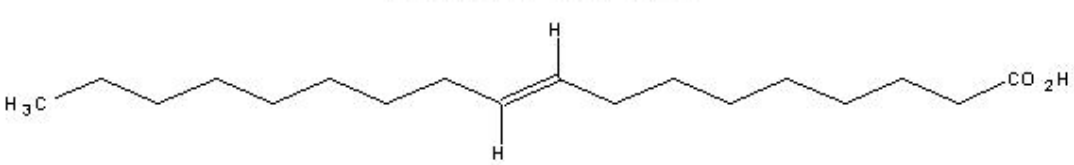
Em virtude da prevalência de AGPI n-6 na dieta ocidental (DAS, 2006), os eicosanóides derivados do AA são aqueles que geralmente predominam, sendo o EPA e DHA minimamente produzidos (SIMOPOULOS, 1989). Tal fato é consequência da competição enzimática, uma vez que ambas as classes de AG dependem das mesmas enzimas para a

produção dos seus derivados de cadeia longa (BRENNER; PELUFFO, 1969; BUDOWSKI; CRAWFORF, 1985). Além disso, durante o cozimento e o processamento dos alimentos, os AGPI n-3 são perdidos ou oxidados (DAS, 2006). Essa consequente deficiência em AGPI n-3 na dieta ocidental e abundância em AGPI n-6, resulta em uma razão n-6/n-3 muito elevada. Tal fato pode estar associado à moderna prevalência de doenças cardiovasculares, câncer, diabetes e desordens e doenças neurodegenerativas e cutâneas, os quais afetam milhões de pessoas em todo o mundo (SIMOPOULOS,2003; VIOLA; VIOLA, 2009). A implicação dessa condição em situações patológicas resultou na preocupação com a reparação adequada de uma razão n-6/n-3 (SIMOPOULOS, 1989). Portanto, uma proporção equilibrada desses AGPI é dependente da ingestão nutricional (VIOLA; VIOLA, 2009). Nesse sentido, parece ser importante que os AGPI n-3 e n-6 estejam presentes numa proporção correta na dieta, pois o excesso de LA pode inibir a síntese endógena de EPA e DHA, com consequentes danos para o organismo (BRENNER; PELUFFO, 1969; BUDOWSKI; CRAWFORF, 1985).

Não há um consenso a respeito da proporção homeostática celular ideal. Há autores que afirmam que a razão de 3:1 (DAS, 2002; OLLIS; MEYER; HOWE, 1999) é a ideal, outros que defendem a 6:1 (WIJENDRAN; HAYES, 2004). Parece importante que não seja inferior a 10:1, já que os AGPI n-3 de cadeia longa são fundamentais para a manutenção da homeostase da pele, mantendo-a bem hidratadae evitando várias desordens, como dermatite atópica, psoríase, acne e eczema (VIOLA; VIOLA, 2009).

3.1.8 Ácidos graxos *trans*

Segundo a Comissão do Codex Alimentarius (2004), os AGT são definidos como todos os isômeros geométricos de AGM e AGPI com duplas ligações carbono-carbono não conjugadas na configuração *trans* (interrompidas por, no mínimo, um grupo metil), incluídos os monômeros e os isômeros *trans* de AGPI com ligações duplas não conjugadas, produzidos através da hidrogenação de óleos e gorduras de origem vegetal e animal em presença de um catalisador químico. Essa definição, contudo, exclui os AGT conjugados presentes naturalmente nas gorduras animal e derivados. Por sua vez, o Food and Drug Administration (FDA) (EUA, 2003) definiu AGT como AGI que contêm uma ou mais duplas ligações isoladas (não conjugadas) na configuração *trans*.



Ácido Elaídico 9 *trans* (18:1*t*)

Figura 7. Representante de AGT

Há duas fontes alimentares principais de gorduras *trans*. Em primeiro lugar, essas gorduras são formadas naturalmente por bactérias presentes no rúmen de animais ruminantes (KHANAL; DHIMAN, 2004). Dessa forma, carnes e seus derivados, assim como produtos lácteos provenientes desses animais contêm pequenas quantidades de gorduras *trans*, razão pela qual é muito difícil eliminar tais gorduras de uma dieta equilibrada (JAKOBSEN et al., 2006; KHANAL; DHIMAN, 2004). Em segundo lugar, as gorduras *trans* são geradas a partir da hidrogenação de óleos vegetais (MOZAFFARIAN et al., 2006), produzindo uma configuração mais rígida e um ponto de fusão intermediário altamente desejável na indústria alimentícia. Essa hidrogenação é utilizada, principalmente, para converter óleos líquidos a gorduras sólidas, fornecendo características favoráveis aos alimentos, como textura e sensação na boca, e melhor estabilidade oxidativa dessas gorduras, aumentando a vida de prateleira do produto (KHANAL; DHIMAN, 2004; REMIG et al., 2010; STENDER; ASTRUP; DYERBERG,2008). Traços de gorduras *trans* são também produzidos durante o processo de desinfecção ou refino de óleos vegetais (TASAN; DEMIRCI, 2003). Uma vez que pequenas quantidades de gorduras *trans* estão presentes em gorduras vegetais que não são objeto de hidrogenação e também são encontradas em fontes naturais, é impossível eliminá-las completamente da dieta, mesmo que a hidrogenação comercial cesse (REMIG et al., 2010). A primeira fonte pode conter até 60% de AG na forma *trans* em relação ao teor de gordura em ruminantes, que geralmente não ultrapassa 6% (STENDER; ASTRUP; DYERBERG, 2008).

Os AGT estão presentes em quantidades variadas em diferentes produtos, mas principalmente nos alimentos processados com óleos parcialmente hidrogenados, como recheios de biscoitos, formulações de bases para sopas e cremes, sorvetes, batata frita, salgadinhos de pacotes, pipoca para microondas, margarinas, empanados de frango, pães,

lanches de preparação rápida (“fast-foods”), bolos e tortas industrializados (SEMMA, 2002).

A ingestão de gorduras aumentou mais de 60% durante os últimos 35 anos (WELLS; BUZBY, 2008) e representa, atualmente, entre 1.7 a 8% do total de ingestão mundial de lipídios (OSSO et al., 2008). A ingestão de AGT tem sido associada a um risco aumentando para doenças cardiovasculares (MOZAFFARIAN et al., 2004; OOMEN et al., 2001; SUN et al., 2007), diabetes tipo 2 (HU et al., 1997; OOMEN et al., 2001; SUN et al., 2007), disfunção cognitiva (FILLIT et al., 2008; KODL; SEAQUIST, 2008; MORGAN et al., 2007; RAZAY et al., 2006; YAFFE, 2007) e doenças inflamatórias (MOZAFFARIAN et al., 2004). As gorduras alteradas e parcialmente hidrogenadas bloqueiam a utilização de AGE, causando efeitos nocivos principalmente por interferir na produção de eicosanóides e pela alteração de parâmetros de EO (CASSAGNO et al., 2005; KINSELLA etal., 1981; SÁNCHEZ-MORENO et al., 2004; SUGANOetal.,1989).

Os AGT da dieta são absorvidos e transportados até as células, onde são utilizados como fonte de energia ou depositados nos tecidos para utilização futura (CURI, 2002; EMKEN, 1979; KUMMEROW, 1974; 1975; 1979). Nos tecidos humanos, a absorção, o transporte, a incorporação e a excreção dos AGT ocorrem de forma similar a outros AG da dieta, competindo inclusive pelos mesmos sistemas enzimáticos envolvidos na síntese de AGPI (MAHFOUZ; KUMMEROW, 1999), porém, esses não apresentam a mesma atividade dos AGE (KHOSLA; HAYES, 1996). Aparentemente, as concentrações de AGT incorporados aos tecidos refletem seu consumo (EMKEN, 1979). Estudos relacionados à incorporação dos AGT em tecidos de ratos mostraram que os teores de isômeros *trans* adicionados nas dietas foram suficientemente incorporados e metabolizados, alterando o perfil de AG nos tecidos desses animais (JOHNSTON; KUMMEROW; WALTON, 1958; LOÏ et al., 2000; SABARENSE; MANCINI-FILHO, 2003; TEIXEIRA et al., 2011; 2012; TREVIZOL et al., 2013).

Os AGT são capazes de modular a função celular, alterando a fluidez de membrana (menos fluída) e as respostas dos receptores de membrana, quando incorporados aos fosfolipídios de membranas celulares (CHAPMAN; OWENS; WALKER, 1966; GURR; HARWOOD, 1996; ROACH et al., 2004). Em uma revisão sobre possíveis mecanismos moleculares dos AGT, foi sugerido que poderiam afetar as funções e repostas celulares devido à sua capacidade de ligação e modulação dos receptores nucleares, os quais regulam a transcrição de genes (MOZAFFARIAN et al., 2006). Estudos mostram também que os AGT inibem a reação de dessaturação do LA a AA, e ALA a DHA e EPA, favorecendo o metabolismo de AGT monoméricos e de AGPI n-3 ou n-6 em isômeros incomuns que, se

incorporados aos tecidos, alteram as funções das membranas, de enzimas ligadas às membranas (elongases, dessaturases and PG sintetase) ou dos eicosanóides (CHAPMAN; OWENS; WALKER, 1966; INNIS, 2006).

Alguns estudos demonstraram que os AGT não possuem efeitos adversos em ratos, quando ingeridos com adequadas quantidades de AGE (MOORE; ALFIN-SLATER; ALFTERGOOD, 1980; INNIS, 2005; INNIS, 2003; LOÏ et al., 2000; MORGADO et al., 1999). Nesse sentido, a presença de AGT na dieta tende a aumentar a necessidade de AGE (KINSELLA et al., 1981). Na deficiência de AGE, os AGT acentuam os sintomas dérmicos e reduzem a atividade das dessaturases (HANSEN et al., 1958; KINSELLA et al., 1981; KUMMEROW et al., 2004; PRIVETT et al., 1977; ROSENTHAL; DOLORESCO, 1984; WERTZ; CHO; DOWNING, 1983).

3.1.9 Ácidos graxos e a nutrição materna

Uma área de pesquisa que requer mais atenção é o impacto da alimentação materna sobre a prole (BUCKLEY, 2005). A mãe é aprincipal fontede AGE parao feto e para o lactente (RAO et al., 2007) e a gravidez aumenta a necessidade materna de AGE, da qual os estoques são mobilizadospara fornecê-los à prole (MONIQUE, 1995). Nesse contexto, oambiente uterino é susceptível à influência nutricional materna, como o excesso no consumo de AGE, que tem sido associado à diminuição da duração da gestação e/ou crescimento fetal retardado (GRANDJEAN et al., 2001; GRANDJEAN; WEIHE, 1993; OKEN et al., 2004; OLSEN et al., 1992; 1993; RUMPet al., 2001; THORSDOTTIR et al., 2004) e aumento da morbidade infantil (THORSDOTTIR et al., 2004). Por outro lado, reduzidos níveis de consumo de AGE estão associados a várias anormalidades não neuronais, como crescimento reduzido, dificuldades reprodutivas, lesões da pele, polidipsia e neuronais, que incluem a redução da habilidade visual e de aprendizagem (BOURRE et al., 1989a; b; CONNOR; NEURINGER; REISBICK,1992; HOLMAN; JOHNSON; HATCH,1982).Não apenas a quantidade, mas também o tipo de AG influencia o desenvolvimento dessas doenças (STORLIEN et al., 1991), ou seja, quanto mais AGI presentes na dieta, menos prejudicial será tal dieta (BUCKLEY et al., 2005). Além disso, a classe do AGI consumido parece ser outro fator no desenvolvimento de doenças (BUCKLEY et al., 2005), como na diabetes, sobre a

qual os AGPI n-3 são particularmente benéficos à ação da insulina (STORLIEN et al., 1987), contrário ao consumo de uma dieta rica em AGPI n-6 (STORLIEN et al., 1996).

Durante o desenvolvimento embrionário e pós-natal, o maior acréscimo de AGPI ocorre durante o último trimestre e os primeiros 6-10 meses após o nascimento nos seres humanos (CLANDININ et al., 1980) e nos primeiros 15 dias após o nascimento em ratos (DOBBING; SANDS, 1979). O feto recebe os AGPI através da placenta e o recém-nascido mamífero, através do leite materno. A ingestão de AGPI n-3 é acompanhada por níveis elevados de DHA nos fosfolipídios de todos os tecidos (ALSTED; HEY, 1992), sugerindo que esses AG de cadeia longasão mais prontamente incorporados. Durante a gestação, os AGT são transferidos para o feto e são secretados no leite, demonstrando uma exposição do feto aos AGT presentes na dieta materna (MOORE; DHOPESHWARKAR, 1980). Desde que a dieta materna influencia o perfil lipídico tecidual da prole, torna-se importante investigar a influência da suplementação materna com GVH, rica em AGT, em comparação com a suplemetação com óleo de soja e óleo de peixe, fontes de AG n-6 e n-3, respectivamente, sobre o fotodano da pele induzido pela RUV em roedores em distintos estágios sobre a primeira e segunda geração em ratos.

3.2 PRODUÇÃO CIENTÍFICA

Os resultados inseridos nesta tese apresentam-se sob a forma de artigos científicos 1 e 2 e manuscritos 1, os quais se encontram aqui estruturados. Os ítens Materiais e Métodos, Resultados, Discussão e Referências encontram-se no próprio artigo e nos manuscritos, os quais estão dispostos da mesma forma que foram publicados (2) e submetido (1).

3.2.1 ARTIGO 1

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ORIGINAL ARTICLE

Trans Fat Supplementation Increases UV-Radiation-Induced Oxidative Damage on Skin of Mice

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Abstract We evaluated the influence of fish oil (FO, rich in n-3 FA), soybean oil (SO, rich in n-6 FA) and hydrogenated vegetable fat (HVF, rich in *trans* FA) on the oxidative status and viability of skin cells of mice exposed to ultraviolet radiation (UVR). Mice were supplemented with FO, SO or HVF for three months and exposed to UVR (2.72 mJ/cm^2) for 2 days. One day after the last UVR session, the FO group showed higher levels of n-3 fatty acids (FA), while the HVF showed higher incorporation of *trans* FA (TFA) in dorsal skin. UVR increased lipid peroxidation and protein carbonyl levels of the HVF and to a lesser extent of the control and SO groups. Although all irradiated groups showed increased skin thickness, this increase was slighter in FO mice. UVR exposure reduced skin cell viability of the control, SO and HVF groups, while FO prevented this. Catalase activity was reduced independently of the supplementation and SOD level was increased in C and FO groups after UVR exposure; FO prevented the UVR-induced increase in glutathione levels, which was observed in skin of the control, SO and HVF mice. Our results showed the beneficial effects of FO supplementation, as well as the harmful effects of *trans* FA, whose intensity can increase vulnerability to skin diseases.

Keywords Oxidative stress · Skin · Fatty acids · PUFA · *Trans* fatty acids · Ultraviolet radiation

Abbreviations

ARA	Arachidonic acid
ALA	α-Linolenic acid
CAT	Catalase
COX-2	Cyclooxygenase-2
DHA	Docosahexaenoic acid
DMSO	Dimethylsulfoxide
EFA	Essential fatty acid(s)
EPA	Eicosapentaenoic acid
FA	Fatty acid(s)
FO	Fish oil
GPx	Glutathione peroxidase
GSH	Gluthatione
HVF	Hydrogenated vegetable fat
LA	Linoleic acid
LOX	Lipoxygenase
LP	Lipid peroxidation
MDA	Malondialdehyde
MED	Minimal erythemal dose
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MUFA	Monounsaturated fatty acid(s)
PC	Protein carbonyl
PG	Prostaglandins
PGE_2	Prostaglandins of series 2
PGE_3	Prostaglandins of series 3

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PUFA	Polyunsaturated fatty acid(s)
ROS	Reactive oxygen species
SFA	Saturated fatty acid(s)
SO	Soybean oil
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
TBA	Thiobarbituric acid
TFA	*Trans* FA
UVR	Ultraviolet radiation

Introduction

Repeated exposure to solar ultraviolet radiation (UVR) is known to cause inflammatory processes, gene mutation, photoaging and development of skin cancer, which is the most common of all human cancers [1]. While the skin is the first physical, biochemical and immunological barrier against UVR [2], this radiation constitute a primary cause of oxidative stress consequent to reactive oxygen species (ROS) generation [3]. These species are free radicals (FR), whose bioaccumulation is related to damages in proteins, lipids and DNA [4]. Besides glutathione as an important nonenzymatic antioxidant [5], enzymatic antioxidant defenses are also present in skin and are represented by glutathione peroxidase (GPx), glutathione reductase, superoxide dismutase (SOD) and catalase (CAT). These two last have been considered the major antioxidant enzymes protecting the epidermis [6].

Due to the emission of environmental pollutants [7, 8], the thinning of the ozone layer has increased the incidence of diseases related to UVR exposure [9], whose gravity will tend to increase in coming years. The preservation of skin health against sun exposure damage is also related to the lipids that compose it. Lipids are present in the stratum corneum of the epidermis, which is the outermost layer of the skin, as well as in cell lipid membranes [10–13], and consist of fatty acids (FA), phospholipids, sphingolipids, cholesterols, triglycerides, squalene and wax esters [14]. These lipids play an important role in physiological functions of the skin, whose composition affects cellular signaling mechanisms [15]. Of particular importance, essential fatty acids (EFA) and their derivatives play a crucial role in the fluidity and flexibility of cell membranes and affect protein activity such as receptors, transporters and enzymes [16]. Notably, EFA are able to maintain the structural integrity of the skin and consequently its permeability [17–20]. In this sense, FA can be classified as saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) [21]. However, another basis of classification for EFA is the position of unsaturation (n-3 or n-6). In fact, these EFA are referred to as essential because they cannot be synthesized by mammals and must be acquired from the diet [22]. While n-3 FA, which include α-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) [23] are widely found in marine fishes and oil seeds [24], members of the n-6 series consist of linoleic acid (LA, 18:2n-6) and arachidonic acid (ARA, 20:4n-6), which are plentiful in nature and are present in the seeds of most plants such as soybean, safflower, sunflower, and corn, among others [25].

EFA are metabolized to highly active eicosanoid products such as prostaglandins (PG) and leukotrienes, which modulate inflammatory, immunologic, and proliferative responses including those of the skin cells [20, 26]. Beneficial effects of n-3 FA consumption have been observed in inflammatory diseases [27, 28], as well in central nervous system diseases related to oxidative stress [29, 30] and to apoptosis [31]. In fact, EPA and DHA compete with ARA for its incorporation into the lipid cell membranes [32] and serve as a substrate for cyclooxygenase-2 (COX-2) [33]. In addition, this enzyme acts on n-6 PUFA and originates PG of series 2 (PGE_2), while n-3 PUFA generates PGE of series 3 (PGE_3) [33], which are less mutagenic and pro-inflammatory than the former. Thus, n-3 PUFA can be useful in the prevention of some skin diseases, as they are able to minimize inflammatory processes, while n-6 FA enhances them [34]. Furthermore, the need to ensure a sufficient intake of n-3 EFA in order to maintain skin homeostasis has long been advised [22]. In this sense, n-3 FA keep the skin hydrated and could potentially contribute to reduction of sunburn response, an acute inflammatory reaction of the skin against UV irradiation [27, 35].

In addition to n-3 and n-6 PUFA, *trans* FA (TFA) are defined as geometrical isomers of MUFA and PUFA, which are produced by hydrogenation of both vegetable and animal oils and fats. In recent years, the influence of TFA on the susceptibility to developing of different disorders has been discussed [36–38]. Considering that FA exert a fundamental role in cell membrane phospholipids and that their influence on the development of skin photodamage is poorly understood, we decided to investigate the influence of TFA supplementation, in comparison to n-3 and n-6 FA, on UVR-induced skin oxidative injury in mice.

Materials and Methods

Male *Swiss* mice from the breeding facility of Universidade Federal de Santa Maria (UFSM), RS, Brazil, were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (23 ± 1 °C) and a 12-h

light/dark cycle. After 1 week of acclimatization, the mice were assigned to the experimental groups. This study was approved by the Animal Ethical Committee of the Universidade Federal de Santa Maria (027132-UFSM), which is affiliated to the Council for Control of Animal Experiments (CONCEA), following international norms of animal care and maintenance.

Weaned male *Swiss* mice (56) were randomly assigned to 4 experimental groups according to oral supplementation: control, (water, C); fish oil (FO, rich in n-3 FA; Herbarium®, Colombo, PR, Brazil); soybean oil (SO, rich in n-6 FA) or hydrogenated vegetable fat (HVF, rich in *trans* and saturated FA) (Table 1).

Due to the presence of vitamin E (0.037UI/100 g) in the soybean oil, this vitamin was added to other supplementations and the control, in order to maintain the same intake of this antioxidant agent in all experimental groups. Animals received these supplements once a day by gavage (3 g/kg; p.o.) [39] for 90 days and thereafter, one half of the mice in all experimental groups were exposed to UVR, totaling eight experimental groups ($n = 7$). The length of time chosen for supplementation (90 days) was based on previous studies by other laboratories evaluating the influence of TFA [40] and an oil mixture containing vitamin E [41] on the skin of mice. The body weight gain of the animals, as well as the daily food consumption was monitored throughout the experimental protocol. Twenty-four hours after the last oral supplementation, animals were anesthetized with ketamine plus xylazine (60 and 15 mg/kg, intramuscular, respectively) and placed in a mirrored box where they were exposed to UVR (irradiated groups). The irradiation source was a Philips TL/12RS 40 W lamp (São Paulo, SP, Brazil) placed 30 cm above the mice's dorsal skin, which emits in the range of 280–400 nm with an output peak at 315 nm. Ultraviolet radiation B (UVB) was 73 % of the total UVR under the present experimental conditions.

In order to select which UVR dose to use, oxidative parameters were assessed by determination of thiobarbituric acid reactive substances (TBARS) [42], which estimates LP levels. Preliminary assays of LP were performed on the skin of mice exposed to different doses of UVR in comparison to non-irradiated animals. By monitoring the lamp's output using a radiometer (Digital Meter Ultraviolet Light MRU-201, Instrutherm, São Paulo, SP, Brazil), it was found that UVR doses ranged from 1.36 to 5.44 mJ/cm^2, and the resulting LP in the skin of animals varied, respectively, from 193.37 to 870.80 nmol MDA/g tissue. While the lowest dose tested did not attain a significant statistical difference (1.36 mJ/cm^2), the highest dose increased LP levels in relation to the control group (5.44 mJ/cm^2), and therefore this dose was chosen to evaluate the influence of FA supplementation on UVR-induced skin oxidative damage.

Firstly, the MED of mouse dorsal skin was determined, which is defined as the minimum amount of radiation exposure required to produce erythema with sharp margins after 24 h [43]. To determine the MED, male Swiss mice (3 weeks-old) ($n = 5$) were exposed to UVR for different times. The time to produce MED was chosen and converted into UVR dose (J/cm^2) using a radiometer (Digital Meter Ultraviolet Light MRU-201, Instrutherm, São Paulo, SP, Brazil).

Twenty-four hours after the last UVR exposure, animals were anesthetized (ketamine/xylazine, 60 and 15 mg/kg, intramuscular, respectively), euthanized by cervical dislocation and their dorsal skin was removed immediately. For biochemical assays, dorsal skin was homogenized in Tris HCl 10 mM pH 7.4 and centrifuged at 3,640*g* for 15 min. The supernatants were used for analysis. For GSH levels, the dorsal skin was homogenized in potassium phosphate buffer (PPB) 1 M pH 7.4 and centrifuged at 3,640*g* for 15 min. All tissues were stored at 20 °C until the assays.

The fat was extracted from the dorsal skin samples using chloroform and methanol as described by Bligh and Dyer [44] and used for FA profile determination. To prevent lipid oxidation during and after extraction, 0.02 % butyl hydroxyl-toluene was added to the chloroform used [45]. Fatty acid composition was determined by gas chromatography. Fat was saponified in a methanolic KOH solution and then esterified in a methanolic H_2SO_4 solution [46]. Methylated FA were analyzed using a gas chromatograph (Agilent Technologies - HP 6890 N) equipped with a capillary column DB-23 (60 m × 0.25 mm × 0.25μm) and a 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 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 FA methyl esters (37-component FAME Mix, C 22:5n-3 and PUFA no. 2 from Sigma, Saint Louis, MO, USA and C 22:5n-6 from Nu-Chek Prep. Inc., Elysian, MN, USA) were performed under the same conditions and the subsequent retention times were used to identify the FA. Fatty acids were expressed as percentages of the total FA content.

LP was estimated through the pink chromogen produced by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) formed during lipid oxidation of skin homogenates, and measured spectrophotometrically at 535 nm [42]. Results were expressed as nmol MDA/g tissue. The protein carbonyl (PC) level was measured according to Yan et al. [47], with some modifications. Aliquots of skin tissue homogenized in Tris HCl buffer (10 mM; pH 7.4) were mixed with 0.2 mL of 2,4-dinitrophenylhydrazine (10 mM DNPH). After 1 h of incubation at room temperature in the dark, 0.5 mL of denaturing

buffer (150 mM sodium phosphate buffer, pH 6.8, 3 % SDS), 2 mL of heptane (99.5 %) and 2 mL of ethanol (99.8 %) were added sequentially and mixed for 40 s and centrifuged for 15 min. After that, the protein isolated from the interface was washed twice with ethyl acetate/ethanol 1:1 (v/v) and suspended in denaturing buffer. Each sample was measured at 370 nm against the corresponding HCl sample (blank), and total carbonylation calculated using a molar extinction coefficient of 22,000 $M^{-1}cm^{-1}$ according to Levine et al. [48].

Catalase (CAT) activity was spectrophotometrically quantified in skin tissue according to Aebi [49], which involves monitoring the disappearance of H_2O_2 in the presence of cell homogenate (pH 7 at 25 °C) at 240 nm. The enzymatic activity was expressed in 1 µmol H_2O_2/min/g tissue.

Superoxide dismutase (SOD) activity was assayed spectrophotometrically as described by Misra and Fridovich [50]. Briefly, epinephrine rapidly auto-oxidizes at pH 10.2 producing adrenochrome, a pink colored product that can be detected at 480 nm. The addition of samples containing SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored for 120 s at intervals of 15 s. The amount of enzyme required to produce 50 % inhibition at 40 °C was defined as one unit of enzyme activity. SOD activity was expressed as U/mg protein. Protein content was determined by the method of Lowry et al. [51].

Gluthatione (GSH) levels were determined after reaction of skin tissue homogenates with 5,5′-dithiobis-(2-nitrobenzoic acid). The yellow color formed was read at 412 nm, in accordance with Boyne and Ellman [52]. A standard curve using GSH was plotted in order to calculate the content of GSH, expressed as µmol GSH/g tissue.

The viability of dorsal skin slices was quantified by measuring the reduction of [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]-MTT to a dark violet formazan product by mitochondrial dehydrogenases [53]. Slices (0.4 mm) of the dorsal skin of mice were prepared with a McIlwain chopper. MTT reduction assays were performed in plates containing 500 µL of phosphate buffer saline, and the reaction was started by adding MTT to a final concentration of 0.1 mg/mL. After 1 h of incubation at 37 °C, the medium was removed and the slices dissolved in dimethylsulfoxide (DMSO). The MTT reduction was measured spectrophotometrically by the difference in absorbance between 570 and 630 nm. Data were calculated as a percentage of values from control.

To assess the extent of UVR-induced edema, briefly, the mice's dorsal skin was lifted up by pinching gently and the skin bi-fold thickness was measured using a dial caliper 24 h after the last UV irradiation. Changes in skin bi-fold thickness were determined by measurements of the same site within the area of exposure per mouse [54]. Edema formation was expressed as net increase in skin thickness between experimental (UVR) and control (non-UVR) groups. The grader was blinded to the group.

All the data were analyzed by two-way ANOVA [4 (C, SO, FO and HVF] X [2 (non irradiated/irradiated)], followed by Tukey's test when appropriate (Software package Statistica for Windows version 8.0 was used). Value of $P < 0.05$ was considered significant for all comparisons made.

Results

No differences in body weight gain or food consumption were observed between the different experimental groups (data not shown). LP was developed after two exposures (27MED) to UVR, and therefore was used to develop the experimental protocol of the present study, i.e. UVR exposure (one dose of 2.72 mJ/cm^2) every other day for 3 days, yielding a total dose of 5.44 mJ/cm^2.

Exposure of mice to 0.20 mJ/cm^2 UVR produced a minimally perceptible erythema that was taken as the MED (data not shown).

UVR exposure caused no changes in FA composition of the control, FO, SO and HVF groups. Between irradiated groups, incorporation of TFA in skin was higher in HVF-supplemented mice (0.41 %) than in the C, SO and FO groups. On the other hand, FO supplementation was related to the lower n-6/n-3 (17.35 %) ratio than were the other experimental groups, while incorporation of MUFA in the skin was higher in both FO and HVF groups (10.7 and 6 %, respectively) than in the C group. FO and SO supplementations increased the skin n-3 FA incorporation (72 and 56 %, respectively) in relation to the C group (Table 2).

Two-way ANOVA of lipid peroxidation (LP) revealed a significant effect of supplementation and UVR [$F(3,48) = 48.33$; $F(1,48) = 175.97$; $P < 0.001$, respectively] and a significant supplementation x UVR interaction [$F(3,48) = 44.92$; $P < 0.001$]. A post-hoc test showed that while no differences of LP were observed in the groups not exposed to UVR, LP levels were higher in HVF and C than in SO and FO groups in irradiated animals, which showed similar values between each other. In fact, UVR exposure increased skin LP levels in the C and HVF, but not in SO and FO groups (Fig. 1A).

Two-way ANOVA of protein carbonyl (PC) levels revealed a significant effect of supplementation and UVR [$F(3,48) = 125.45$; $F(1,48) = 201.61$; $P < 0.001$, respectively] and a significant supplementation x UVR interaction [$F(3,48) = 8.03$; $P < 0.001$]. Similar to LP, the post hoc test showed that between groups not exposed to UVR, HVF increased skin PC levels in relation to other groups. In

Table 1 Fatty acids composition (% of total identified FA) of the food and oils/fat supplemented to different experimental groups

Fatty acids	Chow	FO	SO	HVF
C 14:0	0.51	8.44	0.10	0.18
C 16:0	17.54	19.01	11.09	13.09
C 18:0	5.86	3.93	5.35	11.10
C 20:0	0.54	0.00	0.57	0.53
Σ SFA	24.45	31.38	17.11	24.90
C 16:1n-7	0.65	9.38	0.00	0.00
C 18:1n-7	0.00	0.00	0.77	2.23
C 18:1n-9*c*	32.61	12.07	24.82	33.19
C 18:1n-9*t*	0.51	0.00	0.15	19.45
C 20:1n-9	0.57	1.25	0.30	0.34
C 22:1n-9	0.00	0.00	0.00	0.00
Σ MUFA	34.34	22.70	26.04	55.21
C 18:3n-3	2.88	1.35	4.48	0.48
C 20:5 n-3	0.00	21.29	0.00	0.00
C 22:5 n-3	0.00	2.58	0.00	0.00
C 22:6 n-3	0.00	13.71	0.00	0.00
Σ n-3	2.88	38.93	4.48	0.48
C 18:2n-6	37.33	1.40	49.86	10.25
C 20:2n-6	0.00	0.00	0.00	0.00
C 20:3n-6	0.00	0.00	0.00	0.00
C 20:4n-6	0.00	1.48	0.06	0.03
C 22:4n-6	0.00	0.00	0.00	0.00
Σ n-6	37.33	2.88	49.92	10.28
C 18:1n-9*t*	0.51	0.00	0.15	19.45
Σ TFA	0.51	0.00	0.15	19.45
n-6/n-3 ratio	12.96	0.07	11.14	21.42

SFA saturated fatty acids, *MUFA* Monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids, *TFA trans* fatty acids

Values represent means ± SEM ($P < 0.05$)

addition, the PC level was lower in the FO group than in both C and SO groups, whose values were comparable to each other. UVR exposure was able to increase the skin PC levels in all supplemented groups, and these levels were higher in C and HVF than in SO and FO, but in turn lower in FO than in SO group (Fig. 1B).

Two-way ANOVA of skin thickness revealed a significant effect of supplementation and UVR [$F(3,48) = 5.60$, $P < 0.05$; $F(1,48) = 297.14$; $P < 0.001$, respectively] and a significant supplementation FA × UVR interaction [$F(3,48) = 8.33$; $P < 0.001$]. Tukey's test showed that skin thickness was similar among animals not exposed to irradiation; however, UVR exposure was able to increase this parameter in all experimental groups. Across irradiated animals, skin thickness was higher in SO and HVF than in C and FO groups, which showed similar values to each other (Fig. 2A).

Two-way ANOVA of skin cell viability revealed a significant effect of supplementation and UVR [$F(3,48) = 9.37$; $F(1,48) = 16.06$; $P < 0.001$, respectively]. UVR exposure reduced the percentage of cell viability in the skin of the C group. Across irradiated animals, the FO group showed higher cell viability, whose percentage was higher than in the HVF and C groups. The SO group showed a partial protective effect on the skin, since its percentage of cell viability was not different from those of the other UVR-exposed groups (Fig. 2B).

Two-way ANOVA of glutathione (GSH) levels revealed a significant effect of supplementation and UVR [$F(3,48) = 6.75$; $F(1,48) = 21.31$; $P < 0.001$, respectively] and a significant supplementation × UVR interaction [$F(3,48) = 2.95$; $P < 0.05$]. Tukey's test showed no differences of GSH levels in skin of animals not exposed to irradiation. UVR exposure increased GSH levels in skin of both C and HVF groups.

Across irradiated animals, GSH levels were similar in C, SO and HVF groups, but not in FO, whose value was lower than in all other groups (Table 3).

Two-way ANOVA of catalase (CAT) activity revealed a significant effect of UVR [$F(1,48) = 54.95$; $P < 0.001$ and a significant supplementation × UVR interaction [$F(3,48) = 15.91$; $P < 0.001$]. Post-hoc test showed that FO supplementation increased per se the skin CAT activity when compared to the C group. UVR exposure was able to decrease the CAT activity in the skin of SO, FO and HVF groups, whose values were similar to each other but smaller than in the C group (Table 3).

Two-way ANOVA of superoxide dismutase (SOD) levels revealed a significant effect of UVR [$F(1,48) = 40,65$; $P < 0.001$]. Tukey's test showed no differences in the SOD levels among experimental groups no exposed to irradiation. Even though UVR exposure increased SOD levels in the skin of C and FO mice, these values were statistically similar across all irradiated groups (Table 3).

Discussion

In this study we evaluated the influence of SO, FO and HVF supplementation, which are rich in n-6, n-3 and *trans* FA, respectively, on the skin photodamage of mice exposed to UVR. Our objective was to explore isocaloric diets (the most consumed currently) and show that even in similar levels, SO is less deleterious than HVF, and both SO and HVF are more harmful than FO. In our findings, the higher incorporation of TFA observed in HVF-supplemented mice showed that FA can be easily renovated in the skin, suggesting two possibilities: (1) TFA can be substrates for COX and lipoxygenase (LOX), whose activities

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

Fatty acids	N-UVR				UVR			
	C	FO	SO	HVF	C	FO	SO	HVF
C 14:0	0.80 ± 0.02^{a}	1.02 ± 0.06^{a}	0.79 ± 0.04^{a}	1.00 ± 0.08^{a}	1.28 ± 0.21^{a,*}	0.97 ± 0.03^{a}	0.87 ± 0.07^{b}	0.96 ± 0.06^{a}
C 16:0	21.05 ± 0.40^{a}	20.48 ± 0.80^{a}	20.32 ± 0.75^{a}	22.06 ± 1.04^{a}	20.26 ± 0.47^{a}	18.91 ± 0.43^{a}	20.10 ± 0.94^{a}	20.98 ± 0.66^{a}
C 18:0	5.16 ± 0.30^{a}	3.32 ± 0.20^{b}	3.98 ± 0.20^{ab}	3.39 ± 0.25^{b}	7.68 ± 0.73^{a,*}	4.55 ± 0.77^{b}	5.62 ± 0.54^{b,*}	4.29 ± 0.12^{b}
C 20:0	0.45 ± 0.06^{a}	0.62 ± 0.11^{a}	0.72 ± 0.15^{a}	0.66 ± 0.13^{a}	0.43 ± 0.18^{b}	0.57 ± 0.11^{ab}	0.88 ± 0.13^{a}	0.70 ± 0.05^{ab}
Σ SFA	27.46 ± 0.55^{a}	25.44 ± 1.19^{a}	25.81 ± 0.97^{a}	27.11 ± 1.47^{a}	29.65 ± 0.98^{a}	25.00 ± 1.12^{b}	27.47 ± 1.52^{ab}	26.93 ± 0.76^{ab}
C 16:1n-7	1.79 ± 0.17^{c}	4.21 ± 0.18^{a}	2.83 ± 0.12^{b}	4.49 ± 0.18^{a}	1.63 ± 0.27^{c}	3.17 ± 0.31^{b,*}	2.93 ± 0.19^{b}	4.02 ± 0.40^{a}
C 18:1n-7	1.52 ± 0.06^{b}	1.73 ± 0.06^{a}	1.75 ± 0.04^{a}	1.73 ± 0.07^{a}	1.57 ± 0.03^{b}	1.93 ± 0.03^{a,*}	1.79 ± 0.06^{a}	1.57 ± 0.07^{b}
C 18:1n-9c	35.44 ± 0.32^{a}	35.29 ± 1.09^{a}	34.13 ± 0.86^{a}	34.04 ± 1.23^{a}	33.85 ± 0.94^{a}	35.72 ± 1.28^{a}	32.14 ± 1.56^{a}	33.23 ± 0.65^{a}
C 18:1n-9t	0.00^{b}	0.06 ± 0.05^{b}	0.00^{b}	0.34 ± 0.09^{a}	0.03 ± 0.03^{b}	0.00^{b}	0.00^{b}	0.41 ± 0.11^{a}
C 20:1n-9	0.91 ± 0.08^{a}	0.88 ± 0.11^{a}	0.99 ± 0.14^{a}	0.95 ± 0.07^{a}	0.68 ± 0.28^{a}	1.06 ± 0.19^{a}	1.23 ± 0.12^{a}	1.00 ± 0.06^{a}
C 22:1n-9	0.08 ± 0.05^{b}	0.52 ± 0.12^{ab}	0.65 ± 0.19^{a}	0.54 ± 0.13^{ab}	0.56 ± 0.13^{a}	0.52 ± 0.10^{a}	0.75 ± 0.17^{a}	0.41 ± 0.12^{a}
Σ MUFA	39.74 ± 0.45^{a}	42.69 ± 1.15^{a}	40.35 ± 0.80^{a}	42.09 ± 1.11^{a}	38.32 ± 1.33^{b}	42.40 ± 1.25^{a}	38.84 ± 1.38^{ab}	40.64 ± 0.53^{a}
C 18:3n-3	1.08 ± 0.05^{a}	1.21 ± 0.07^{a}	1.33 ± 0.03^{a}	0.99 ± 0.22^{a}	0.71 ± 0.27^{a}	0.96 ± 0.05^{a}	1.06 ± 0.06^{a}	0.95 ± 0.19^{a}
C 22:6n-3	0.11 ± 0.05^{b}	0.64 ± 0.11^{a}	0.19 ± 0.07^{b}	0.27 ± 0.04^{ab}	0.26 ± 0.11^{b}	0.71 ± 0.21^{a}	0.46 ± 0.08^{ab}	0.28 ± 0.10^{b}
Σ n-3	1.19 ± 0.09^{b}	1.85 ± 0.13^{a}	1.52 ± 0.11^{ab}	1.26 ± 0.19^{ab}	0.97 ± 0.31^{b}	1.67 ± 0.14^{a}	1.52 ± 0.11^{a}	1.23 ± 0.15^{ab}
C 18:2n-6	28.94 ± 0.51^{a}	27.10 ± 0.70^{ab}	28.73 ± 0.61^{a}	26.05 ± 1.00^{b}	26.91 ± 1.50^{a}	27.46 ± 0.50^{a}	26.77 ± 0.70^{a}	26.87 ± 0.80^{a}
C 20:2n-6	0.25 ± 0.05^{a}	0.10 ± 0.03^{a}	0.18 ± 0.04^{a}	0.13 ± 0.03^{a}	0.19 ± 0.09^{a}	0.16 ± 0.04^{a}	0.21 ± 0.05^{a}	0.35 ± 0.27^{a}
C 20:3n-6	0.05 ± 0.03^{a}	0.09 ± 0.03^{a}	0.02 ± 0.02^{a}	0.12 ± 0.03^{a}	0.11 ± 0.05^{a}	0.08 ± 0.02^{a}	0.12 ± 0.04^{a}	0.50 ± 0.03^{a}
C 20:4n-6	1.03 ± 0.13^{a}	0.69 ± 0.07^{a}	1.10 ± 0.18^{a}	1.04 ± 0.16^{a}	1.78 ± 0.56^{a}	1.28 ± 0.40^{a}	2.05 ± 0.28^{a}	1.39 ± 0.25^{a}
C 22:4n-6	0.13 ± 0.04^{a}	0.00^{a}	0.13 ± 0.04^{a}	0.12 ± 0.05^{a}	0.23 ± 0.11^{a}	0.00^{b}	0.29 ± 0.07^{a}	0.12 ± 0.06^{ab}
Σ n-6	30.40 ± 0.09^{a}	27.98 ± 0.13^{b}	30.16 ± 0.11^{a}	27.46 ± 0.19^{b}	29.22 ± 0.31^{a}	28.98 ± 0.14^{a}	29.44 ± 0.11^{a}	29.23 ± 0.15^{a}
C 18:1n-9t	0.00^{b}	0.06 ± 0.05^{b}	0.00^{b}	0.34 ± 0.09^{a}	0.03 ± 0.03^{b}	0.00^{b}	0.00^{b}	0.41 ± 0.11^{a}
Σ TFA	0.00^{b}	0.06 ± 0.05^{b}	0.00^{b}	0.34 ± 0.09^{a}	0.03 ± 0.03^{b}	0.00^{b}	0.00^{b}	0.41 ± 0.11^{a}
n-6/n-3 ratio	25.54 ± 1.81^{a}	15.12 ± 1.28^{b}	19.84 ± 1.28^{ab}	21.79 ± 1.76^{a}	30.12 ± 1.91^{a}	17.35 ± 1.01^{c}	19.36 ± 1.16^{bc}	23.76 ± 4.40^{ab}

SFA saturated fatty acids, *UFA* unsaturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

Considering the number of unsaturations, TFA were added to the MUFA content

Data expressed as means ± SEM ($n = 7$)

Different lower case letters (a–c) indicate significant difference between supplementations in the same irradiation condition ($P < 0.05$)

* Indicates a significant difference from the non-irradiated group in the same supplementation ($P < 0.05$)

are able to generate *trans*-prostanoids endowed with hitherto unknown properties; (2) TFA have been described as potent inhibitors of Δ-5 [55] and Δ-6 desaturases [56], suppressing the synthesis of ARA, EPA and DHA and exacerbating symptoms of EFA deficiency [57, 58] in the skin. Other reports have shown the importance of the intake of n-3 PUFA and/or their precursors, which play an important role in the physiology and homeostasis of skin [24, 59]. In this sense, n-3 PUFA derivatives constitute a skin health strategy and are substrates for COX-2, whose activity results in synthesis of the PGE_3 [33], which are less pro-inflammatory than PGE_2 originated from n-6 FA [33]. In addition, there is evidence in the literature of the modulatory effects of n-3 PUFA on COX-2 and PGE_2 levels [60], indicating a possible protective role of these dietary FA against UVR-induced photodamage [59, 61]. The nutritional supply of n-3 PUFA has been associated with reduced erythema and low levels of PGE_2 following UVR exposure [62], especially because n-3 FA are recognized as modulating immune responses of the skin. Human studies showed reduced sensibility to sunburn and a longer time for the skin to redden in subjects treated with n-3 FA, showing that consumption of these FA is related to a lower susceptibility to UVR [63]. On the other hand, n-6 PUFA present in SO produces more potent pro-inflammatory eicosanoids [64], and are related to increased UVR-induced carcinogenesis in mice [65].

Besides FA incorporation in the skin, we also evaluated the influence of UVR on oxidative damages, antioxidant defenses, epidermal thickness, and cell survival in skin of FO, SO or HVF supplemented mice. Of particular importance, HVF is rich in TFA, which has been associated with development of erythema, inflammation and cancer due to pro-oxidative and apoptotic properties of ROS generation [66]. Such reactive species oxidize lipids and proteins, resulting in photooxidative damage of cells and the extracellular matrix [67]. In this context, the continuous LP may lead to degeneration and loss of fluidity of the cell membrane, which is considered an important event involved in cell death. Together with protein oxidation, this oxidative

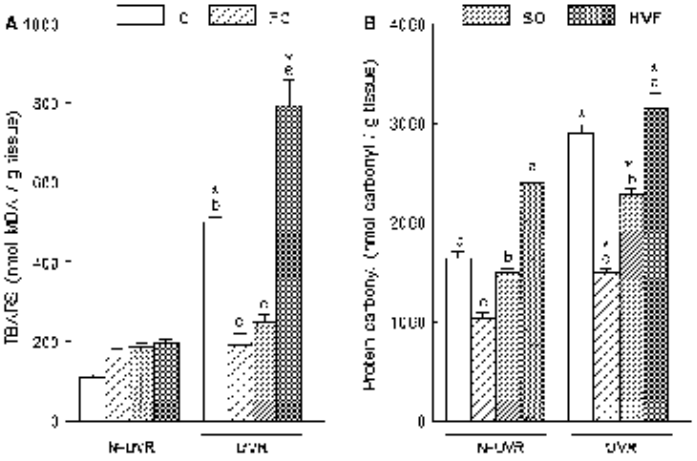

Fig. 1 Influence of n-3, n-6 and *trans* fatty acids provided from 90 days of supplementation with fish oil (FO), soybean oil (SO) and hydrogenated vegetal fat (HVF), respectively, on lipid peroxidation (**A**) and protein oxidation (**B**) levels in the dorsal skin of mice exposed or not to UV irradiation. Data expressed as means ± SEM ($n = 7$). *Different lowercase letters* (*a*–*c*) indicate significant differences between supplementations under the same irradiation condition ($P < 0.05$); *asterisks* indicate a significant difference from the non-irradiated group with the same supplementation ($P < 0.05$)

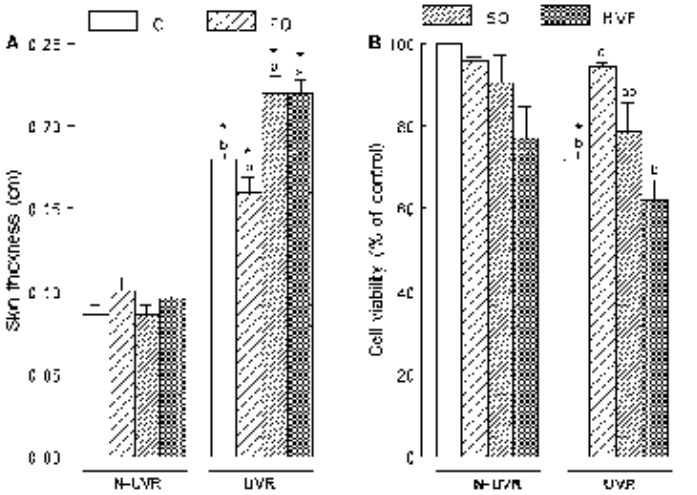

Fig. 2 Influence of n-3, n-6 and *trans* fatty acids provided from 90 days of supplementation with fish oil (FO), soybean oil (SO) and hydrogenated vegetal fat (HVF), respectively, on the thickness (**A**) and cellular survival (**B**) of dorsal skin of mice exposed or not to UV irradiation. Data expressed as means ± SEM ($n = 7$). *Different lowercase letters* (*a*, *b*) indicate significant difference between supplementations in the same irradiation condition ($P < 0.05$); *asterisks* indicate a significant difference from the non-irradiated group with the same supplementation ($P < 0.05$)

stress marker is able to inactivate membrane enzymes and increase permeability of ions, which may lead to disruption of cell membrane potential [68–70] and also DNA damage [71]. Considering that elevated levels of LP and PC may represent the initial and final stage of cell destruction, our findings showed that UVR exposure increased LP levels in skin of the control and HVF-supplemented mice, while increased PC levels were observed in the control, SO and HVF groups, but not in the FO group. These findings indicate that TFA supplementation was associated with the highest UVR-induced damage, while n-3 FA (predominantly present in FO), and in minor proportion n-6 (found in higher proportion in SO), were able to prevent this. While the influence of TFA derivatives incorporated in the skin is poorly understood, LC n-3 PUFA are precursors of eicosanoids and docosanoids [72], and already described as having anti-apoptotic properties [31]. While MUFA present in FO, SO and HVF groups did not alter the skin's sensitivity to sunburn or its time to redden [73], few studies on the influence of TFA on skin health have been carried out so far [40, 55].

Table 3 Influence of n-3, n-6 and *trans* fatty acids provided from 90 days of supplementation with fish oil (FO), soybean oil (SO) and hydrogenated vegetal fat (HVF), respectively, on the antioxidant status of dorsal skin of mice exposed (UVR) or not (N-UVR) to irradiation

Groups	GSH µmol GSH/g skin	CAT 1 µmol H_2O_2/min/g skin	SOD U/mg protein
N-UVR			
C	1.55 ± 0.13	314.53 ± 18.05^{b}	17.49 ± 1.19
FO	1.30 ± 0.60	448.79 ± 34.69^{a}	15.25 ± 0.72
SO	2.35 ± 0.33	419.95 ± 9.55^{ab}	18.36 ± 1.22
HVF	1.07 ± 0.24	393.39 ± 19.71^{ab}	18.28 ± 1.09
UVR			
C	3.59 ± 0.41^{a*}	398.16 ± 46.90^{a}	29.67 ± 3.77*
FO	1.19 ± 0.07^{b}	205.04 ± 13.38^{b*}	24.84 ± 1.91*
SO	3.77 ± 0.54^{a}	202.51 ± 23.26^{b*}	26.56 ± 3.92
HVF	3.26 ± 0.44^{a*}	231.49 ± 14.66^{b*}	26.81 ± 1.18

GSH glutathione, *CAT* Catalase, *SOD* superoxide dismutase

Data expressed as means ± SEM ($n = 7$)

Different lower case letters (a–b) indicate significant difference between supplementations in the same irradiation condition ($P < 0.05$)

* Indicates a significant difference from the non-irradiated group with the same supplementation ($P < 0.05$)

In our study, greater UVR-induced skin thickening was observed in both TFA and SO supplemented animals, whose mechanism may be due to generation of proinflammatory cytokines [74], increased blood flow and higher vascular permeability, which together lead to inflammation and edema development. Thus, the deleterious effects of TFA have been related to their greater impact on n-3 EFA metabolite generation, and consequently reducing the fluidity and stability [75] of the cell membrane, making it more susceptible to oxidative damage. Such reports are especially relevant to the last two decades, since consumption of manufactured foods has increased in Western countries. This change in eating habits has contributed to increases in the n-6/n-3 PUFA ratio, mainly due to reduced intake of n-3 FA [76]. Although the increased consumption of TFA may represent a loss of the nutritional value of foods, the impact of this condition on the antioxidant defense system of the skin and thus on UVR-induced photodamage has been poorly understood. UVR acts as a pro-oxidant agent [77], and is recognized as increasing ROS generation, which is closely related to oxidative stress development [77, 78]. Our findings showed as well that proinflammatory and prooxidative effects of UVR exposure were reflected on skin cell viability of control, SO and HVF, but not of FO-supplemented mice, which did not show a reduction in skin cell viability. So it is possible to hypothesize that the deleterious effects of UVR exposure can be aggravated by a lower generation of eicosanoids and docosanoids and lower fluidity of the cell membrane, which can interfere with the antioxidant activity of membrane-bound enzymes [79]. In this sense, Sander et al. [6] reported that acute and chronic photodamage in human skin is related to depletion of antioxidant enzymes' expression and increased oxidative protein modifications. Enzymes such as CAT and SOD [6, 80] and non-enzymatic compounds such as GSH [77, 81] give important protection against UVR-induced skin oxidative processes. In this study, UVR exposure decreased CAT activity in the FO, SO and HVF groups, indicating that the type of FA do not interfere with the skin CAT activity. Different to CAT activity, SOD levels were increased by UVR exposure, especially in C and FO groups. Other studies have shown contradictory effects of UVR exposure on the antioxidant defense system of the skin: while an increase in SOD levels in response to UVR-induced, oxidative stress was shown by some authors [82, 83], others showed that the enzymatic antioxidant defense system can be impaired by UVR [84–86], making skin damage from sun exposure even more serious. Although the exact influence of FA on the antioxidant defenses of UVR-exposed skin is not known yet, glutathione is a reduced (GSH) or oxidized (GSSG) tripeptide, which is considered one of the most important endogenous antioxidant defenses due to its nucleophilic properties [87]. Considering that UVR-induced oxidative stress strongly challenges the glutathione system [88], in the present study UVR exposure was related to higher GSH levels of control, SO and HVF groups, but this effect was not observed in FO supplemented mice. We believe that this findings are consistent with the fewer pro-inflammatory properties of the metabolites generated by n-3 FA [31], which are different from those derived from n-6 [34, 72] and *trans* FA [89, 90]. In this sense, eicosanoids resulting from the metabolism of n-3 FA contribute to a decrease in the

production of pro-inflammatory cytokines, reducing UVR-related photodamage [91]. Such findings can be closely related to lower production of ROS, and consequently a reduced generation of oxidative damage, leading us to suggest that a higher generation of pro-inflammatory cytokines, as observed in *trans*-FA and less in n-6 FA, can be related to increased generation of ROS and oxidative damages in the skin exposed to sunlight.

In conclusion, the results of this study indicate that consumption of HVF during the developmental period leads to incorporation of TFA in skin and facilitates UVR-induced damage observed by increased levels of LP and PC, as well as to decreased skin cell survival and increased skin thickness. These pro-oxidative and pro-inflammatory events were observed to be less in SO-supplemented mice, which showed higher n-6 FA levels, but were not observed in the FO group at all, which showed a higher incorporation of LC n-3 PUFA and a lower n-6/n-3 ratio in the skin. Furthermore, n-3 FA exerted photo-protective effects, mainly because this supplementation was able to prevent the development of oxidative damage, maintaining both normal skin thickness and cell survival. This study shows the critical role of a diet rich in TFA and poor in n-3 EFA on the development of inflammatory and oxidative processes in skin, which can culminate in death of the cells exposed to UVR. Taken together, these events may lead to the development of skin cancer, which constitutes a preventable disease.

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3.2.2 Manuscrito 1: Submetido para periódico científico e por isso será apresentado conforme normas da revista, aguardando orientação dos revisores.

Influence of *trans* fat on skin Damage and Photoaging in first Generation rats Exposed to UV Radiation

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Abstract

The influence of dietary fatty acids (FA) on photoaging, oxidative damage and mitochondrial viability in skin of rats exposed to ultraviolet radiation (UVR) was evaluated. Wistar rats of first generation were supplemented with soybean oil (SO, rich in n-6 FA), fish oil (FO, rich in n-3 FA) and hydrogenated vegetable fat (HVF, rich in *trans* FA- TFA) until adulthood, when half of each group was exposed to UVR. The FO group presented higher skin n-3 FA incorporation and lower n-6/n-3 ratio; the HVF group showed higher TFA incorporation, increased protein carbonyl (PC) levels *per se*, and decreased mitochondrial integrity and antioxidant defenses. After UVR, the HVF group showed increased skin wrinkles, reactive species (RS) generation and PC levels, with decreased skin antioxidant defenses. The FO group showed lesser wrinkling and skin thickness, lower PC levels, and increased mitochondrial integrity. RS generation was positively correlated with skin thickness, wrinkles and PC levels, while negative correlations between RS generation and mitochondrial integrity, as well as between PC levels and antioxidant defenses (glutathione reduzed (GSH), superoxide dismutase (SOD) and vitamin C (VIT C)) were observed. While n-3 polyunsaturated FA (PUFA) play a protective role in skin, once incorporated, TFA make it more susceptible to developing UVR-induced disorders.

Keywords: Oxidative stress; skin; fatty acids; PUFA; *trans*fat; ultraviolet radiation

Introduction

Ultraviolet radiation (UVR) is a major environmental factor related to harmful effects in human skin (1), whose pathophysiology are closely related to oxidative, inflammatory and degenerative processes, consequent of an overproduction of reactive species (RS), which may affect the antioxidant defense system (2-4). RS-sensitive signaling pathways activation (5-6) may participate in short- and long-term UVR-induced skin pathologies, such as erythema, inflammation, photoaging and tumors (7-8). Dietary fat is an additional but modifiable known environmental factor, that increases the susceptibility to skin disease (10).

Theessential fatty acids (EFA)can be classified intotwo main families according totheir unsaturation state, namely, omega-3 (n-3) and omega-6 (n-6) polyunsaturated FA (PUFA)(11,12). The n-3 PUFA include eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) (11), which are mainly found in flesh of marine fish (tuna, sardines, salmon), nuts and vegetable oils (flaxseed and *Salvia hispanica*) (13,14). The n-6 PUFA include linoleic acid (LA, 18:2 n-6) and arachidonic acid (AA, 20:4 n-6) (11), which are abundantly obtained from most types of vegetable oils including soybean and sunflower oil (15). Omega-3 PUFA are involved in resolution of inflammatory processes, as their regular consumption has been found to reduce erythema and prevent UVR-induced wrinkles, mainly due to their indirectantioxidant property (10,16-18). On the other hand, n-6 FA tend to promote increased inflammatory processes (23), thus facilitating development of edema and photoaging.

Trans fatty acids (TFA) are sourced from the hydrogenation of vegetable oils, which have been significantly added to processed foods, setting an increasein TFA consumption and currently accounting for 0.5-3% of total calories consumed by most Western people (24,25). This change in eating habits promoted an increase in the n-6/n-3 ratio, mainly due to reduced n-3 PUFA intake (37). Considering that the influence of dietary FA on photoaging and UVR-

induced cutaneous oxidative damage still lacks scientific validation, this study aimed to evaluate the influence TFA on photoaging and oxidative status in rat skin associated with repeated UVR exposure.

Materials and methods

Animals

The animals from the breeding facility of Universidade Federal de Santa Maria (UFSM), RS, Brazil, were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (23±1°C) and 12 h light/dark cycle. After one week of acclimatization, the rats were assigned to the experimental groups. This study was approved by the Animal Ethical Committee of the Universidade Federal de Santa Maria (027132-UFSM), which is affiliated to the Council for Control of Animal Experiments (CONCEA), following international norms of animal care and maintenance.

Virgin female Wistar rats were randomly assigned to two experimental groups according to oral supplementation: soybean oil (C-SO, rich in n-6 PUFA)orhydrogenated vegetable fat (HVF, rich in TFA and SFA). SO was used as a control group, mainly because it contains adequate levels of PUFA, n-6/n-3 ratio within acceptable limits (38-40) and because of its elevated consumption worldwide (41,42). Control (C-SO) and experimental group (HVF) were isocaloric in order to prevent metabolic differences between animals of different experimental groups (43,44), which could cause interferences in the antioxidant defense system (45). Animals received these supplementations once a day by gavage (3g/kg; p.o.) (32-34) from pregnancy and lactation. Immediately after weaning, the offspring were kept in the same maternal supplementation for 90 days. 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 four experimental groups (n=7). FA supplementation was carried

out from prenatal period until adulthood of the first generation to study its influence on skin incorporation as related to UVR-induced cutaneous alterations. The duration of supplementation (90 days) was chosen based on previous studies that evaluated the influence of TFA (10) on the skin of rodents. Food intake and body weight gain were monitored throughout the study period in all of the animals.

Radiation exposure

The source of UVR used was a Philips TL/12RS 40W 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. Initially, we determined the Minimal Erythemal Dose (MED) of rat dorsal skin, which is defined as the minimum amount of radiation exposure required to produce erythema with sharp margins after 24h (48). Therefore, female Wistar rats (8 weeks-old) (n=5) wereexposedto UVRfor differenttimes. The timeto produce MED was chosenand converted intoUVR dose (J/cm^2).

After 90 days of supplementation, animals were anesthetized with ketamine plus xylazine (60 and 15 mg/kg, intramuscular, respectively) and placed in a mirrored box where they were exposed to UVR (irradiated groups) three times a week for twelve weeks. During this period, UVR sessions were conductedthree times a week with an UVR dose of 0.25 J/cm^2/3x/week, yielding a total dose of 0.75 J/cm^2/week for total 12 weeks. Supplementations were maintained throughout the irradiation period.

Evaluation of wrinkle formation

After 12 weeks of UVR irradiation, animals were anesthetized (ketamine/xylazine, 60/15 mg/kg, intramuscular, respectively) for evaluation of dorsal skin wrinkle formation. Skin wrinkles were analyzed according to the scale ofBisset et al. (49) modified by Inomata et al. (50). Three investigators individually determined wrinkle formation scores in blind fashion for each animal, as described in Table 1.

Table 1. Grading of rat dorsal skin wrinkles according to the scale of Bisset et al. (49) modified by Inomata et al. (50).

	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

Determination of skin thickness as an inflammatory biomarker

Briefly, the rat's dorsal skin was lifted up by pinching gently and the skin bi-fold thickness was measured using a caliper at week 12 of UVR irradiation to assess the extent of edema. Changes in skin bi-fold thickness were determined by measurements of the same site within the area of exposure per rat (51). Edema formation was expressed as net increase in skin thickness between experimental (UVR) and control (non-UVR) groups. The grader was blind to the groups.

Preparation of skin sample

At week 12 of UVR irradiation, animals were euthanized and dorsal skin fragments were excised from each rat. A part of the dorsal skin was dissected and stored at -80°C degree for lipid profile determination of. For gluthatione (GSH) levels, the dorsal skin was homogenized in potassium phosphate buffer 1M pH 7.4 and centrifuged at 3640g for 15 min. For all other biochemical assays, the dorsal skin was homogenized in Tris HCl 10mM pH 7.4 and centrifuged at 3640g for 15 min. The supernatants were used for analysis. All tissues were stored at -20°C until assayed.

Lipid profile analysis

The fat was extracted from dorsal skin samples of non irradiated groups using chloroform and methanol as described by Bligh and Dyer (52) and used for FA profile determination. To prevent lipid oxidation during and after extraction, 0.02% butyl hydroxyl-toluene was added to the chloroform used (53). Fatty acid composition was determined by gas chromatography. Fat was saponified in methanolic KOH solution and then esterified in methanolic H_2SO_4 solution (54). Methylated FA were analyzed using a gas chromatograph (Agilent Technologies - HP 6890N) equipped with a capillary column DB-23 (60m x 0.25mm x 0.25μm) and flame ionization detector. The temperature of the injector port was set at 280°C and the carrier gas was nitrogen (0.9mL/min). After injection (1μL, 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 FA methyl esters (37-component FAME Mix, C 22:5n-3 and PUFA no. 2 from Sigma, Saint Louis, MO, USA and C 22:5n-6 from NuChek Prep. Inc., Elysian, MN, USA) were performed under the same conditions and the subsequent retention times were used to identify the FA. Fatty acids were expressed as percentage of the total FA content.

Estimation of skin oxidative damage

Protein carbonylated (PC) level was measured according to Yan et al. (56), with some modifications. Aliquots of skin tissue homogenized in Tris HCl buffer (10mM; pH 7.4) were mixed with 0.2 mL of 2,4-dinitrophenylhydrazine (10mM DNPH). After 1h of incubation at room temperature in the dark, 0.5mL of denaturing buffer (150mM sodium phosphate buffer, pH 6.8, 3% SDS), 2mL of heptane (99.5%) and 2mL of ethanol (99.8%) were added sequentially and mixed for 40s and centrifuged for 15min. After that, protein isolated from the interface was washed twice with ethyl acetate/ethanol 1:1 (v/v) and suspended in denaturing buffer. Each sample was measured at 370nm against the corresponding HCl sample (blank), and total carbonylation calculated using a molar extinction coefficient of 22,000$M^{-1}cm^{-1}$ according to Levine et al. (1990).

RS levels were quantified using the oxidant sensing fluorescent probe, 2,7-dichlorofluorescein diacetate (DCHF–DA) (58). The oxidation (DCHF–DA) to fluorescent dichlorofluorescein (DCF) was determined at 488nm for excitation and 525nm for emission. Skin samples (20 µL) were added to a medium containing TrisHCl buffer (0.01 mM; pH 7.4) and DCFH-DA (10 µM). After DCFH-DA addition, the medium was incubated in the dark for 1 h until fluorescence measurement procedure. DCF-RS levels were corrected by the protein content (59) and expressed as a percentage of values from control.

Estimation of skin antioxidant defenses

Superoxide dismutase (SOD) activity was assayed spectrophotometrically as described by Misra and Fridovich (61). Briefly, epinephrine rapidly auto-oxidizes at pH 10.2 producing adrenochrome, a pink colored product that can be detected at 480nm. The addition of samples containing SOD inhibits the auto-oxidation of epinephrine. The rate of inhibition was monitored for 120s at intervals of 15s. The amount of enzyme required to produce 50%

inhibition at 40°C was defined as one unit of enzyme activity. SOD activity was expressed as U/mg protein. Protein content was determined by the method of Lowry et al. (59).

GSH levels were determined after reaction of skin tissue homogenates with 5,5'-dithiobis-(2-nitrobenzoic acid). The yellow color formed was read at 412nm, in accordance with Boyne and Ellman (62). A standard curve using GSH was plotted in order to calculate GSH content, expressed as µmol GSH/g tissue.

Estimation of mitochondrial integrity

Mitochondrial integrity was quantified by measuring reduction of [3-(4,5-dimethyllthiazol-2-yl)-2,5-diphenyltetrazolium bromide]-MTT to a dark violet formazan product by mitochondrial dehydrogenases (63). Slices (0.4mm) of dorsal skin were prepared with a Mcllwain chopper. MTT reduction assays were performed in plates containing 500µL of phosphate buffer saline, and the reaction was started by adding MTT to a final concentration of 0.1mg/mL. After 1h of incubation at 37°C, the medium was removed and the slices dissolved in dimethylsulfoxide (DMSO). MTT reduction was measured spectrophotometrically by the difference in absorbance between 570 and 630nm. Data were calculated as a percentage of values from control.

Statistical analysis

All the data were analyzed by two-way ANOVA [3 (SO, FO and HVF) X 2 (non irradiated/ irradiated)], except skin FA quantification, which was analyzed by one-way ANOVA followed by Duncan's test for all comparisons when appropriate. $P<0.05$ was considered significant for all comparisons made (Statistica for Windows version 8.0 was used).

Results

Food intake and body weight

Rats supplemented with diets rich in either C-SO or HFV had no significant differences in food intake and body weight over the 12 weeks of UVR irradiation (data not shown).

Fatty acids composition of the skin (Table 2)

The type of FA provided in the diet modified the lipid profile of the rats' dorsal skin: compared to the C-SO group, the HVF group showed higher MUFA (10.50%; P=0.0000) and TFA (535%; P=0.02) incorporation and the FO group higher n-3 PUFA (20.52%; P=0.0000) and lower n-6/n-3 (35.68%; P=0.0000) ratio.

UVR-induced wrinkles and thickening of dorsal skin

Two-way ANOVA of wrinkle score revealed a significant main effect of supplementation and UVR exposure [$F(2,36)=5.39$, $P<0.01$; $F(1,36)=255.66$; $P<0.001$, respectively] and a significant supplementation x UVR interaction [$F(2,36)=5.39$; $P<0.01$]. Two-way ANOVA of skin thickness revealed a significant main effect of supplementation and UVR exposure [$F(2,36)=5.99$, $P<0.01$; $F(1,36)=286.64$; $P<0.001$, respectively] and a significant supplementation x UVR interaction [$F(2,36)=5.35$; $P<0.01$].

Duncan's test showed that wrinkle scores and skin thickness were similar among animals not exposed to UVR. UVR was able to modify these parameters in all experimental groups, but FO-supplemented rats showed lower skin thickening than both C-SO and HVF did, whose values were comparable to each other (Fig. 1).

Biochemical measurements

Influence of fat supplementations on RS generation, PC levels and mitochondrial integrity in dorsal skin (Fig. 2).

Two-way ANOVA of RS generation, PC levels and mitochondrial integrity revealed a significant main effect of supplementation [$F(2,36)=10.70$, $P<0.001$; 14.01 $P<0.001$ and 66.53, $P<0.001$, respectively] and UVR exposure [$F(1,36)=8.80$, $P<0.01$;26.54, $P<0.001$ and 85.11,$P<0.001$, respectively] in dorsal skin tissue. Additionally, two-way ANOVA of RS generation and mitochondrial integrity revealed a significant supplementation x UVR interaction [$F(2,36)=3.22$, $P<0.051$ and 7.40, $P<0.01$, respectively].

Post hoc test showed that FO supplementation decreased *per se* the RS generation in relation to control (C-SO) group. UVR exposure increased RS generation in skin of rats supplemented with HVF when compared to non-irradiated animals, but this effect was not observed in C-SO and FO. In fact, between irradiated groups, RS generation was higher in HVF than in FO and C-SO groups (Fig. 2).

Duncan's test showed that HFV-supplementation increased PC levels *per se* in non-irradiated animals, but UVR exposure increased significantly this oxidative marker in all experimental groups (C-SO, FO and HVF). Between irradiated animals, PC levels were higher in HVF than in C-SO, and the lowest in FO (Fig. 2).

The mitochondrial integrity was reduced *per se* in skin of HVF and increased in FO in comparison to C-SO. UVR exposure was able to reduce significantly the mitochondrial integrity of all experimental groups. In fact, between irradiated groups, FO supplementation was related to increased mitochondrial integrity in relation to both HVF and C-SO supplementations (Fig. 2).

Influence of fat supplementations on antioxidant defenses in dorsal skin (Fig. 3)

Two-way ANOVA of SOD and GSH levels revealed a significant main effect of UVR exposure [F(1,36)=46.61 and 4.93, $P<0.001$, respectively]. Two way ANOVA of VIT C levels revealed a significant main effect of supplementation and UVR exposure [F(2,36)=34.74, $P<0.001$ and F(1,36)=7.08, $P<0.05$; respectively].

Post hoc test showed no difference of SOD levels in dorsal skin of experimental groups; however, after UVR exposure, the level of this antioxidant was similarly decreased in all groups (Fig. 3).

Duncan´s test showed that HFV supplementation was able to decrease GSH levels *per se* in comparison to C-SO and FO. UVR exposure decreased significantly the GSH levels in all groups. In irradiated animals, HVF showed the lowest level of GSH, which was significantly different from both C-SO and FO (Fig 3). *Post-hoc* test showed that HFV was related to lower levels of VIT C in dorsal skin, which was higher in FO than in C-SO. Although UVR exposure was able to decrease the level of this antioxidant compound in HVF, it was not in C-SO and FO (Fig. 3).

Interestingly, positive correlations were observed between RS generation and skin thickness ($P<0.001$), wrinkles score ($P<0.05$) and PC levels ($P<0.001$). Additionally, negative relationships were observed between RS generation and mitochondrial integrity ($P<0.05$), and between PC levels and GSH ($P<0.001$), SOD ($P<0.001$) and VIT C ($P<0.05$) levels (Table 3).

Discussion

Here we evaluated the influence of n-3 and n-6 FA and TFA on oxidative status and photoaging in the skin of first generation rats which had been supplemented with these different fats from intrauterine life to adulthood, and then chronically exposed to UVR. While FO, and to a lesser extent, SO supplementation attenuated photoaging, prevented RS

generation and reduced PC levels, thus partially preserving the antioxidant system and mitochondrial integrity of skin cells exposed to UVR, HFV supplementation failed to do so. Different studies have reported that UVR exposure induces skin oxidative damage, while natural product supplementation from different sources may be protective (64-73).

We also found that there were no differences in food intake and body weight gain between the different supplemented groups from the beginning to the end of the experimental protocol, confirming recent literature data, when dietary n-3 and n-6 PUFA were related to no differences in these parameters (41,74,75). In addition, FA composition of the different oils/ fat used in this study showed that FO was rich in n-3 PUFA, while HVF had a predominance of SFA and the lowest n-6/n-3 ratio. Additionally, C-SO presented the highest n-6 PUFA level, while HVF offered the highest TFA and MUFA levels. In this sense, skin FA incorporation is reflected by type and proportion of FA present in the supplementation offered to animals. In other words, higher n-3 FA incorporation was found in the skin of FO-, n-6 FA in SO-, and TFA in HFV-supplemented groups. Moreover, these supplementations across one generation showed a 3.23% incorporation of n-3 FA, 35% of n-6 FA, and 1.27% of TFA in skin of C-SO, FO and HVF groups, respectively. Such phenomenon also occurs in other tissues, such as brain, retina and erythrocytes (32,42,76,77). Our resultsare consistent with those reported by Rosenthal and Doloresco (78), who demonstrated that human skin fibroblasts readily incorporate exogenous free FA into cellular phospholipids and triacylglycerol, facilitating the nutritional modification of cellular composition. Indeed, a recent study of our group showed that 90-day supplementation with SO, FO and HFV was sufficient to change the skin FA incorporation in mice, thus affecting the UVR exposure-induced damage (10). Therefore, the present findings indicate that the type of FA (n-3, n-6 or TFA) is crucial for the prevention of UVR-induced skin oxidative damage, as skin FA are modified by supplementation and/or dietary manipulations.

Skin incorporation of TFA can induce changes in cutaneous oxidative status, as observed here by increased RS generation, PC levels and decreased mitochondrial integrity. Teixeira et al. (41,42) showed significant brain incorporation of TFA in rats after long-term intake of *trans* fat, which was associated with movement disorders and memory loss. Similarly, Tsai et al. (79) related TFA brain incorporation with apoptotic and oxidative effects in PC12 cells. Phivilay et al. (80) also showed changes in the brain FA profile after dietary TFA intake, however no changes in an Alzheimer's animal model were observed. On the other hand, the incorporation of n-3 PUFA prevented RS generation and was related to lower protein oxidation levels after UVR.

The cutaneous content of n-3 PUFA may be increased through dietary intake in humans (16,81), mainly because these FA compete with AA for incorporation into cellular phospholipids and for cyclooxigenase (COX) and lipoxygenase (LOX) metabolism (82). Therefore, FO supplementation, which is rich in n-3 PUFA, may afford further protection against UVR-mediated oxidative damage in the skin. In this study, chronic UVR exposure increased skin thickness in all supplemented groups; however, FO supplementation was related to development of slight edema, while HVF was associated with more severe edema after 12 weeks of UVR. UVR exposure was reported to increase synthesis and release of pro-inflammatory cytokines, such as PGE_2, IL-8, TNF-α, IL-1α, and IL-6 in skin, and keratinocytes and fibroblasts (17,83), which may be related to edema formation (18). In fact, n-6 PUFA metabolism is competitively inhibited by n-3 PUFA for incorporation into cell membrane phospholipids (84,85), as well as for COX-2, serving as competitive inhibitors (86), which together could modify the UVR-induced skin damage (18,83). In this sense, the augmented skin thickness observed in C-SO and HVF supplemented groups can be related to generation of inflammatory cytokines by n-6 PUFA and TFA metabolism, leading to edema development. On the other hand, n-3 PUFA have shown inhibition of the vascular endothelial

growth factor (VEGF) expression in colon cancer cells, suggesting one possible mechanism, such as the negative regulation of the COX-2/PGE_2 pathway, which can be involved inprevention of UVR-induced skin edema, as observed in this study. Moreover, the lesser skin thickness observed in the FO group may be also related to generation of n-3 PUFA metabolites, which are known for their less proinflammatory properties (23), thus attenuating UVR-induced skin inflammation.

Chronic UVR exposure is able to induce skin photoaging by increasing cutaneous oxidative damage due to increased RS generation (87). In this sense, RS generation plays an important role in the skin photoaging process, which eventually leads to increased collagen breakdown (88). Also, wrinkle formation occurs by matrix destruction and skin inflammation (89). In this study, we were interested in the influence of different dietary FA on skin aging, particularly on the development of skin wrinkles in animals chronically exposed to UVR. We found that chronic UVR was able to induce wrinkling in dorsal skin of rats, whose gravity was dependent on the type of supplemented FA. Although all our experimental groups developed skin wrinkles, fewer wrinkles were found in the FO than in the C-SO and HVF groups. Indeed, this photoaging phenomenon was milder in FO-supplemented animals, allowing us to hypothesize a mitigation of wrinkle formation by regulating RS generation, which can be related to an interference in the inflammation signaling pathway. Thus, the attenuated skin wrinkling following FO supplementation might be related to a combination of both antioxidant and less pro-inflammatory properties previously attributed to n-3 PUFA (10,21,23,90-92). On the other hand, HVF supplementation was related to higher UVR-induced RS generation and, consequently, increased dorsal wrinkles, which can be associated with the inflammatory property of *trans* fat (10,26).

Our current findings also showed that chronic UVR exposure was able to cause a marked decrease of SOD levels in all irradiated groups. Some laboratories reported a

reduction of SOD level after UVR exposure in *in vivo* studies (93-95). If SOD converts superoxide radicals into hydrogen peroxides (93,96,97), then we can infer that SOD levels were decreased as an attempt to neutralize RS, which were generated by UVR exposure, whose process was successful in C-SO and FO, but not in the HVF group. Moreover, ascorbate and GSH are thiols that have been shown to be major contributors to the antioxidant action via reduction of phenoxyl radicals and generation of the ascorbyl radicals (98,99). In fact, GSH is involved in the regulation of OS signaling pathways (100,101), besides monitoring the intracellular redox balance by both H_2O_2 content and peroxidase-catalyzed reactions (102). In addition, GSH plays an important role in maintaining intracellular protein integrity via disulfide linkages reduction, regulating their biosynthesis and cellular sulfhydryl balance (103). Thiols are highly reductive in physiological conditions, and GSH detoxifies highly reactive peroxides, acting as a critical antioxidant (104,105). Reduced levels of GSH favors free radicals generation, therefore, GSH is a scavenger of RS preventing UVR-induced damage. Oxidative conditions like repeated UVR exposure play a major role in modulating redox states by altering the dynamic equilibrium of GSH homeostasis (65,66,71). Inflammatory reaction via increased cytokine release can be potentially enhanced by GSH depletion, besides being able to activate OS through increased RS production (106,107). In this study, GSH depletion resulted in an overabundance of RS, which was observed in the HFV group, subsequently leading to a higher production of cytokines, causing oxidative and inflammatory processes in the skin. So, we found that the HFV supplementation favored RS generation, which was exacerbated by the reduced GSH level in skin as a result of UVR exposure. Moreover, SO and FO supplementations were able to prevent UVR-induced depletion of VIT C levels in skin, whereas HVF supplementation failed to do so. Vitamins A, E and C have been reported to exhibit photoprotective effects on the skin due to their antioxidant activities (108-110). In addition, polyphenolic compounds such as vitamin C,

lutein, and lycopene have been reported in several animals studies as protection agents against UVR-induced skin damage via antioxidant, antiinflammatory or cell cycle regulatory pathways (111-115).

In this study, SO and FO supplementations showed RS scavenging effects, differently from HVF, which was further evaluated with regard to its protective effects against UVR-induced cell damage. In this sense, the influence of n-3 and n-6 PUFA and TFA on UVR-induced skin mitochondrial damage were measured by the MTT assay: the conversion of MTT could serve as an indirect indicator of cell metabolism, since its reduction to formazan in viable cell happens through reactions catalyzed by mitochondrial dehydrogenases, coupled to oxidative phosphorylation (116). Our results demonstrated that UVR exposure significantly reduced formazan in the HVF group, but a significant prevention of this was observed in the C-SO and FO groups. The n-3 PUFA have been reported to have protective properties against oxidative stress (10,19-21,118-222). Our results here show for the first time that TFA intake may potentiate the effects of UVR-induced oxidative damage associated with decreased mitochondrial integrity, as demonstrated here and elsewhere (10-31).

Interestingly, positive correlations between RS generation and skin thickness, wrinkle formation and PC levels reinforce a coordinated action of UVR-induced RS generation in a causal relationship with damage. In addition, the negative correlations observed between RS generation and mitochondrial integrity, as well as between PC levels and GSH, SOD and VIT C, indicate a crucial protective function of these antioxidants, as widely described in the literature (123-125). These negative correlations also point to a possible impairment to neutralize UVR-induced RS generation. In fact, such endogenous antioxidants exert a fundamental role in RS detoxification (126-127).

So far, no studies had shown the influence of *trans* fat intake on the skin in a multigenerational model together with biochemical changes, focusing on photoaging and its

relationships with skin oxidative damage. The deleterious influence of inadequate dietary habits during gestational and perinatal periods until adulthood can be reflected over generations. From these findings, we suggest that long lasting consumption of processed foods, which are rich in TFA, is able to increase skin oxidative damage, thus facilitating the development of photoagingand ultimately leading to skin cancer. Nutritional strategies with the potential to mitigate UVR-induced cutaneous damage may improve the safety and health of the human skin.

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Figure Captions

Figure 1. Influence of n-3, n-6 and *trans* fatty acids provided during one generation 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±S.E.M. (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 ($P<0.05$).

Figure 2. Influence of n-3, n-6 and *trans* fatty acids provided during one generation of rats supplemented with control-soybean oil (C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF), respectively, on reactive species (RS) generation (A), protein carbonyl levels (B) and mitochondrial integrity (C) in dorsal skin of rats exposed or not to UV irradiation. Data expressed as means±S.E.M. (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 ($P<0.05$).

Figure 3. Influence of n-3, n-6 and *trans* fatty acids provided during one generation of rats supplemented with control-soybean oil (C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF), respectively, on superoxide dismutase (SOD) (A), glutathione (GSH) (B), and vitamin C (VIT C) (C) levels in dorsal skin of rats exposed or not to UV irradiation. Data expressed as means±S.E.M. (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 ($P<0.05$).

Table 2. Fatty acids composition (% of total identified FA) of the chow and oils/fat supplemented to different experimental groups.

Fatty acids	Chow	SO	FO	HVF
Σ SFA	24.45	17.11	31.38	24.90
Σ MUFA	34.34	26.04	22.70	55.21
C 18:3n-3	2.88	4.48	1.35	0.48
C 20:5 n-3	nd	nd	21.29	nd
C 22:5 n-3	nd	nd	2.58	nd
C 22:6 n-3	nd	nd	13.71	nd
Σ n-3	2.88	4.48	38.93	0.48
C 18:2n-6	37.33	49.86	1.40	10.25
C 20:2n-6	nd	nd	nd	nd
C 20:3n-6	nd	nd	nd	nd
C 20:4n-6	nd	0.06	1.48	0.03
C 22:4n-6	nd	nd	nd	nd
Σ n-6	37.33	49.92	2.88	10.28
Σ TFA	0.51	0.15	nd	19.45
n-6/n-3 ratio	12.96	11.14	0.07	21.42

SFA: saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA: *trans* fatty acids; nd: fatty acids no detected.Values represent the mean±S.E.M. (P<0.05).

Table 3. 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**
Σ SFA	27.00±0.29[a]	28.86±0.19[b]	26.09±0.18[c]
Σ MUFA	35.31±0.20[b]	35.44±0.2[b]	39.02±0.49[a]
C 18:3n-3	1.37±0.04[a]	1.24±0.04[b]	1.21±0.02[b]
C 20:3n-3	0.01±0.01[a]	0.03±0.01[a]	0.01±0.01[a]
C 20:5n-3	0.13±0.04[a]	0.22±0.03[a]	nd
C 22:6n-3	0.82±0.14[b]	1.29±0.04[a]	0.43±0.02[c]
Σ n-3	2.68±0.16[b]	3.23±0.15[a]	1.86±0.17[c]
C 18:2n-6	32.62±0.55[a]	30.40±0.22[b]	30.58±0.32[b]
C 18:3n-6	0.14±0.02[a]	0.11±0.02[a]	0.15±0.02[a]
C 20:3n-6	0.18±0.02[a]	0.19±0.02[a]	0.14±0.02[a]
C 20:4n-6	1.72±0.07[a]	1.57±0.07[a]	1.68±0.07[a]
Σ n-6	35.00±0.43[a]	32.46±0.42[b]	33.01±0.46[b]
C 18:1n-9*t*	0.20±0.08[a]	0.06±0.08[b]	1.18±0.08[b]
C 18:2n-6*t*	nd	nd	0.09±0.03
Σ TFA	0.20±0.03[b]	0.06±0.02[b]	1.27±0.017[a]
n-6/n-3 ratio	14.46±0.78[b]	9.30±0.82[c]	17.81±0.82[a]

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Considering the number of unsaturations, TFA were added to the MUFA content. nd: fatty acids no detected. Different lower case letters (a-c) indicate significant difference among supplementations (P<0.05).

Table 4. Linear regression analysis of the RS generation and PC levels in the skin of rats supplemented with soybean oil (control group; C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF) over one generation of rats chronically exposed to UVR. Linear regression was evidenced by Person´s correlation coefficients (n=7).

Reactive species generation		
Parameter	r^2	***P***
Skin thickness	0.27	0.000
Wrinkles	0.21	0.002
Mitochondrial integrity	-0.10	0.047
PC	0.26	0.000
Protein carbonyl levels		
GSH	-0.37	0.000
SOD	-0.34	0.000
VIT C	-0.25	0.003

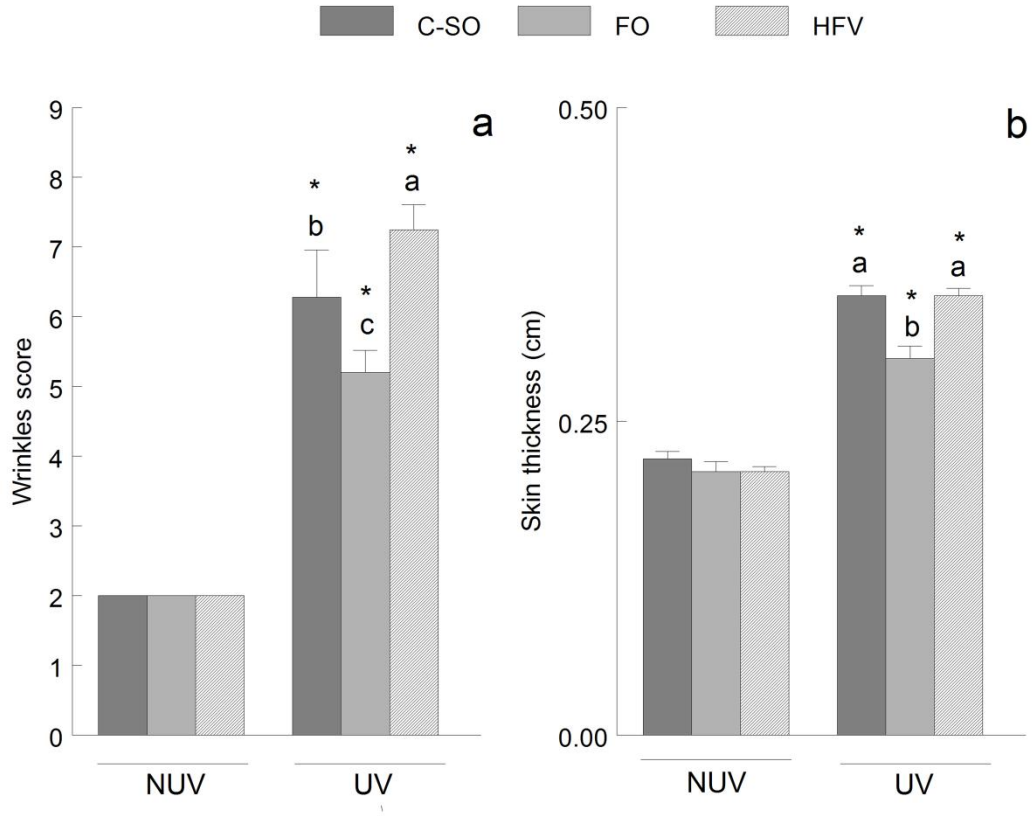
C-SO
FO
HFV
a
b
Wrinkles score
Skin thickness (cm)
0
1
2
3
4
5
6
7
8
9
0.00
0.25
0.50
NUV
UV
NUV
UV

Figure 1

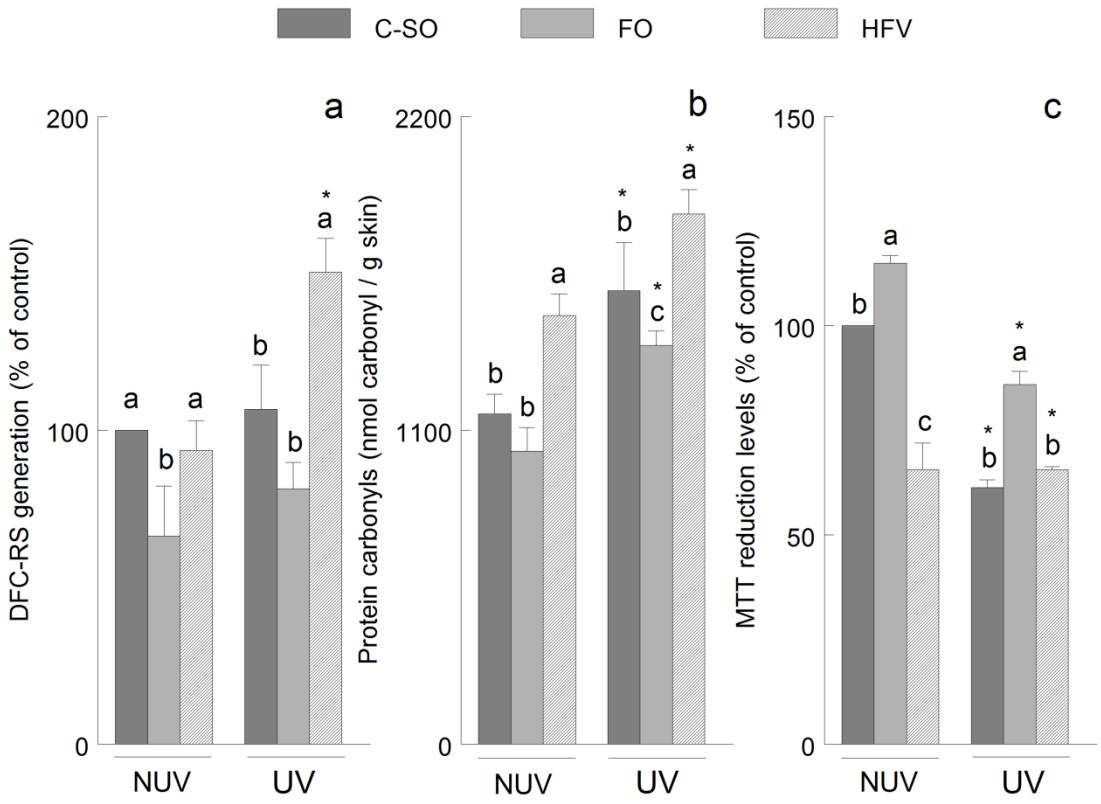
C-SO
FO
HFV
a
b
c
DFC-RS generation (% of control)
Protein carbonyls (nmol carbonyl / g skin)
MTT reduction levels (% of control)
200
100
0
2200
1100
150
50
NUV
UV

Figure 2

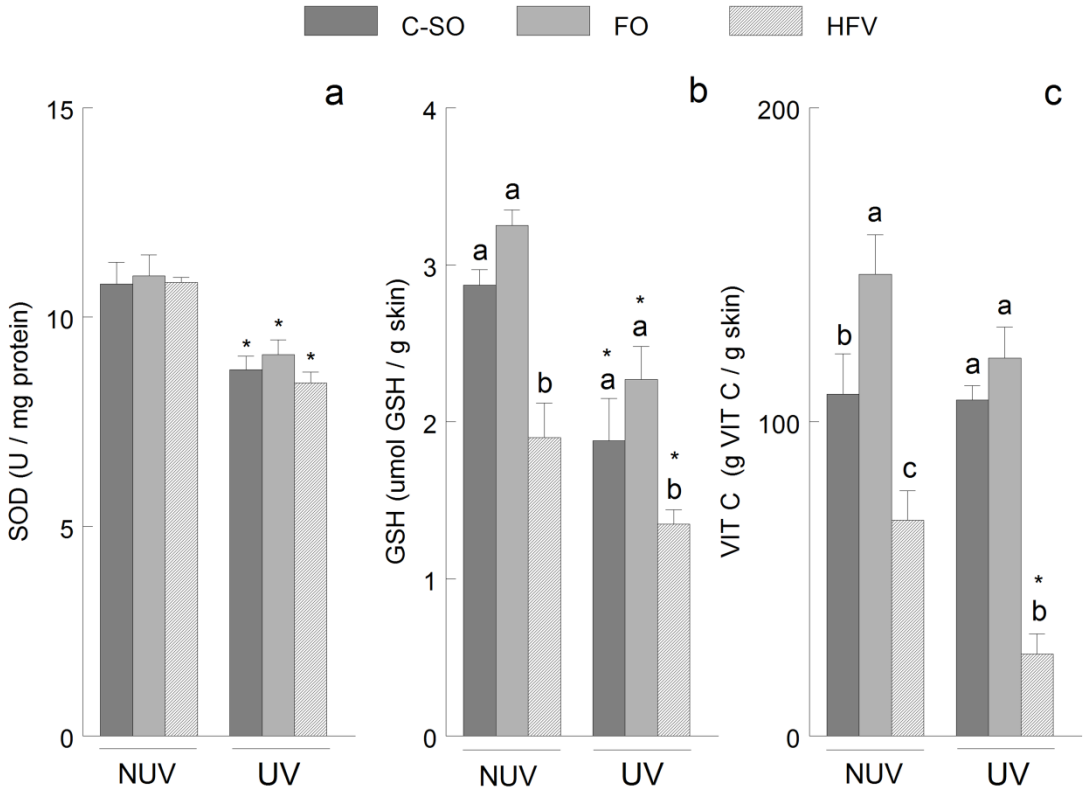
C-SO
FO
HFV
a
b
c
SOD (U / mg protein)
GSH (umol GSH / g skin)
VIT C (g VIT C / g skin)
NUV
UV

Figure 3

3.2.3 ARTIGO 2

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

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Ultraviolet radiation

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; Paze 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; Saikai 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 Química Final®, Rio de Janeiro-Brazil) was added sequentially. Protein isolated was washed twice with ethyl acetate/ethanol (Vetec Química 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-9t	0.00	0.00	0.84 ± 0.05
C 18:2n-6t	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 ± 0.05[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 (Proquimios®, 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, Proquimios®, 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].

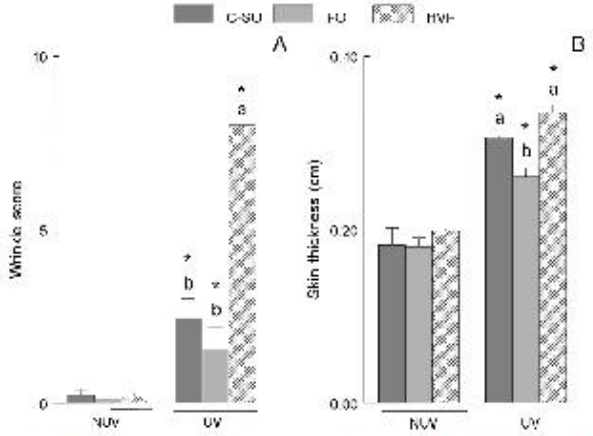

Fig. 1. Influence of n-3, n-6 and *trans* fatty acids provided across two generations of rats supplemented with control-soybean oil (C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF), respectively, on wrinkle score (A) and skin thickness (B) in dorsal skin of rats exposed or not to UV irradiation. 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 ($P < 0.05$).

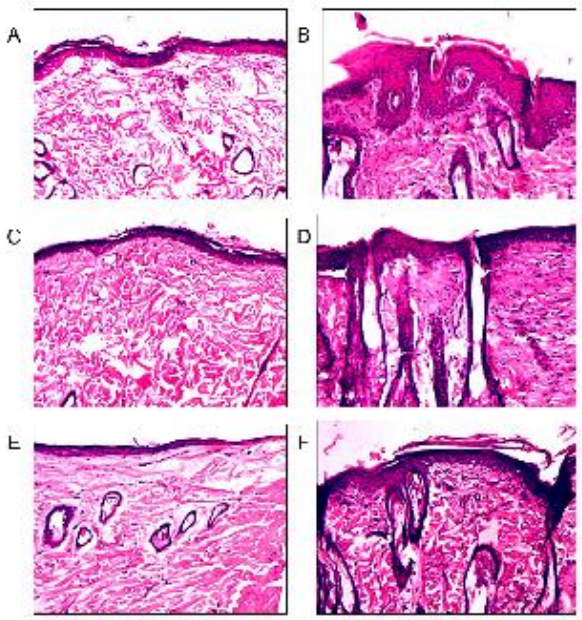

Fig. 2. Effects of oral supplementation of different fatty acids on UVR-induced epidermal thickness on the back of rat skin at the end of week 12. Each photograph is representative of seven animals. Photomicrograph of histologic sections of rat skin in control and supplemented groups after haematoxylin 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.

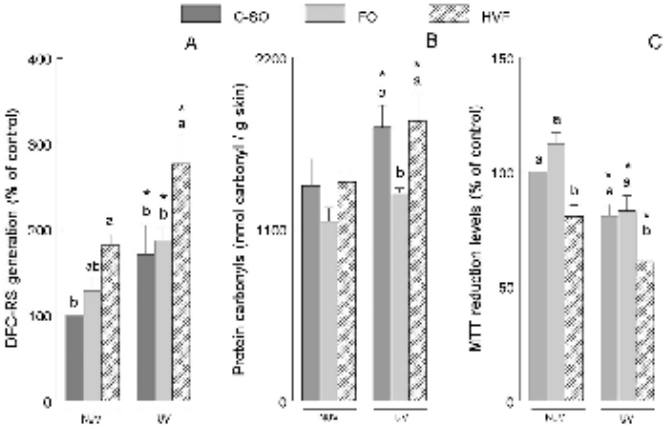

Fig. 3. Influence of n-3, n-6 and *trans* fatty acids provided across two generations of rats supplemented with control-soybean oil (C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF), respectively, on the reactive oxygen species (ROS) generation (A), protein carbonyl levels (B) and mitochondrial 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

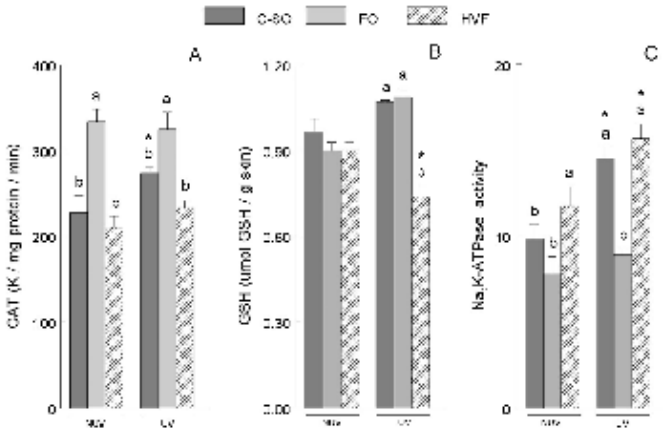

Fig. 4. Influence of n-3, n-6 and *trans* fatty acids provided across two generations of rats supplemented with control-soybean oil (C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF), respectively, on catalase (CAT) activity (A), glutathione (GSH) level (B) and Na^+K^+-ATPase activity (C) of dorsal 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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4. DISCUSSÃO

Nos últimos anos, nosso grupo de pesquisa tem estudado a influência da suplementação de diferentes óleos ou gordura, os quais são fontes de AGS, AGPI das séries n-3 e n-6, e *trans*, sobre o desenvolvimento de distúrbios do movimento (BARCELOS et al., 2010;TEIXEIRA et al., 2012), prejuízos de memória (TEIXEIRA et al., 2011), maior suscetibilidade para desenvolver mania (TREVIZOL et al., 2011), estresse (PASE et al., 2013) e maior preferência por drogas psicoestimulantes (KUHN et al., 2013). Tais transtornos têm sua fisiopatologia direta ou indiretamente relacionada ao desenvolvimento de EO, o qual também pode ser influenciado pela natureza dos AG presentes nos fosfolipídios das membranas celulares que revestem os neurônios.

No presente estudo, a influência da suplementação destes mesmos óleos e gordura foi avaliada em animais expostos de forma aguda e crônica à RUV em diferentes estágios da vida. Nesse contexto, a quantificação do perfil lipídico cutâneo após a suplementação oral em diferentes períodos de vida e sua relação com (i) a oxidação de lipídios e proteínas; (ii) a geraçãode espécies reativas; (iii) a atividade e/ou nível de antioxidantes enzimáticos e não enzimáticos tais como CAT, SOD, GSH e VIT C); (iv) a atividade das enzimas mitocondriais; (v) espessura da pele (vi) formação de rugas cutâneas e (vii) atividade da enzima Na^+K^+ATPase foram avaliados. As avaliações foram precedidas pela suplementação dos óleos e gordura imediatamente após o desmame até a vida adulta, e durante os períodos de gestação, amamentação e crescimento dos animais no decorrer da primeira e segunda gerações, correspondendo ao artigo 1 e 2, e manuscritos 1 apresentados aqui, respectivamente. Na verdade, tal abordagem sobre a saúde da pele de animais foi consideradaum grande desafio, já que até o momento, os estudos do grupo de pesquisa estavam voltados para as patologias do sistema nervo central, como descrito anteriormente. Assim, três novos desafios foram criados: a padronizaçãode um modelo animal de dano oxidativo cutâneo induzido pela RUV em pelo menos duas espécies de roedores (camundongos Swiss e ratos Wistar); a suplementação adequada dos animais antes e durante a gestação, bem como durante o crescimento das ninhadas por duas gerações e a padronização de técnicas de determinação de danos oxidativos e defesas antioxidantes em tecido cutâneo.

Diferentes metodologias que avaliam os efeitos deletérios da RUV sobre a pele têm sido descritas na literatura e estas incluem a pele humana (PORTUGAL-COHEN et al., 2009;

SHAHBAKHTI et al., 2004), cultura de células (CHÊNE et al., 2007; KIM et al., 2005; PARK et al., 2011; PUPE et al., 2002), peixe-zebra (ANCHELIN et al., 2013; GERHARD et al., 2002; HENRIQUES et al., 2013; KELLER; MURTHA, 2004; KISHI et al., 2003; TSAI et al., 2007; YU et al., 2006; ZHDANOVA et al., 2008), camundongos *hairless* (AHN, HWANG; LEE, 2007; CASAGRANDE et al., 2006; PERES et al., 2011; RODRÍGUES-YANES et al., 2012; SHARMA; KATIYAR, 2010; SUMIYOSHO; KIMURA, 2009;VICENTINI et al., 2008), camundongos Swiss e C57BL/6 (DAI et al., 2007; MEERAN et al., 2009; SHARMA; KAUR, 2006), ratos *hairless* (ROMEU et al., 2002), ratos Wistar e Sprague-Dawley (BARG et al., 2014; HE et al., 2004; LAM et al., 2011; LIANG et al., 2010; SEVIN et al., 2007; SHI; RUAN, 2013) e pele plantar de ratos (TSUKAHARA et al., 1999, 2001a; b; c; 2006). A partir destas considerações, o artigo 1 foi desenvolvido em camundongos Swiss e avaliou os danos oxidativos da pele exposta agudamente à RUV, enquanto que no artigo 2 e no manuscrito 1 foram desenvolvidos em ratos Wistar, já que tais espécies apresentavam metodologias validadas e descritas na literatura.

Neste sentido, diferentes estudos desenvolvidos com roedores têm escolhido espécies portadoras de pêlos na superfície corporal ao invés de espécies geneticamente modificadas ou *hairless*, principalmente porque suas respostas experimentais qualitativas e quantitativas são mais próximas daquelas observadas na pele humana (BING-RONG et al., 2008; DAI et al., 2007; ITO et al., 2010; KATIYAR; MEERAN, 2007; MEERAN et al., 2009; SHARMA; KAUR, 2006), facilitando assim, sua interpretação. Então, a partir das espécies eleitas (Swiss para camundongos e Wistar para ratos) para o nosso estudo, o método de depilação mais adequado foi também padronizado, cuja escolha recaiu para uma associação entre a redução do comprimento dos pelos com auxílio de uma tesoura, seguida de um creme depilatório, como forma de expor a pele dos animais às RUV. A etapa seguinte foi determinar a dose eritematosa mínima (MED, do inglês *minimal erythemal dose*) para ambas as espécies. A MED é definida como o tempo necessário para que haja formação de um eritema discreto, com bordas bem definidas e sem formação de bolhas, após exposição única à RUV (PARK et al., 2006) e tem por finalidade avaliar a sensibilidade da pele à RUV (SÁ et al., 2002). E, tanto para camundongos Swiss, como para ratos Wistar, obteve-se a dose de 0.25 J/cm^2 como sendo a MED.

Na sequência, seguiu-se à padronização das doses de RUV a serem utilizadas tanto nos experimentos de exposição aguda para camundongos Swiss, como nos experimentos de exposição crônica (repetida) à RUV para ratos Wistar. Para o cálculo das doses de RUV utilizou-se a fórmula proposta por Diffey (2002). Após a observação de diferentes curvas, a

dose de exposição aguda para os camundongos Swiss foi de 2.42 J/cm^2 em dois dias alternados, totalizando uma dose de 5.44 J/cm^2. Considerando-seque a exposiçãoda pele à RUV induz à formação de RL e subsequente EO (AHN; HWANG; LEE, 2007; HE et al., 2004; TERRA et al., 2012), esta dose de exposição à RUV foi suficiente para induzir peroxidação lipídica máxima (SHARMA; KAUR, 2006) no protocolo de exposição aguda. Para o protocolo de exposição crônica à RUV, a dose escolhida foi baseada na MED encontrada para ratos Wistar, a qual foi 0.25 J/cm^2, equivalente a 1 MED, a ser empregada 3x/semana, durante 12 semanas, correspondendo a 0.25 J/cm^2/3x/semana e totalizando 0.75 J/cm^2/semana durante 12 semanas.

A partir dos experimentos descritos no artigo 1 foi possível observar que uma suplementação oral de óleos e gordura a partir do desmame de camundongos Swiss e mantida por 90 dias permitiu uma incorporação significativa dos diferentes AG na pele dorsal dos animais. Deste modo, mesmo sem diferença significativa, a maior incorporação de AGPI n-3 foi observada na pele dorsal dos animais suplementados com óleo de peixe, enquanto AGPI n-6 foram mais incorporados nos animais suplementados com óleo de soja. Por outro lado, incorporação de AGT foi significativamente maior nos animais suplementados com GVH. Tomados em conjunto, estes dados também mostram que o óleo de peixe foi relacionado à menor razão n-6/n-3 cutânea, quando comparado com óleo de soja e GVH. Dados da literatura têm apontado uma razão máxima de 10 como sendo o limite aceitável para manutenção da fisiologia e funcionalidade das células (VIOLA; VIOLA, 2009), porém até o momento não existe um consenso acerca da razão ideal. De fato, essa razão mostrada em nosso estudo ainda não foi documentada na literatura, tornando difícil a compreensão do que seria o aceitável para a pele. Uma vez que os n-3 PUFA estão em constante competição metabólica com os n-6 PUFA, a razão n-6/n-3 desempenha um papel fundamental na determinação do efeito global sobre a fotocarcinogênese da pele (BLACK et al., 1992). Assim, é possível confirmar que o perfil lipídico da pele pode ser alterado pelos AG presentes na dieta, especialmente quando a suplementação é iniciada no período pós-desmame, o qual corresponde à fase de crescimento corporal. Alguns estudos têm enfatizado que as membranas celulares são mais vulneráveis aos insultos nutricionais durante o período de crescimento, o qual ocorre no último trimestre de gravidez em humanos e no período pós-natal em roedores (OLSEN et al., 1992; DOBBING; SANDS, 1979). Neste sentido, a maior oferta de AG de uma série pode inibir as reações de síntese (dessaturação e elongação) de outros AG presentes em menor proporção (PAWLOSKY et al., 2003). Deste modo, o maior aporte de LA, um AG pertencente à série n-6, pode inibir ou impedir a conversão de ALA em derivados de cadeia

longa (EPA e DHA) pertencentes à série n-3, por competir pela enzima Δ6-dessaturase (VANCASSEL et al., 2007). Da mesma forma, os AGT são capazes de inibir os sistemas enzimáticos responsáveis pela síntese de AG de cadeia longa, interferindo na biossíntese de AGPI n-3 e n-6 (BOURRE et al., 1990; KUMMEROW et al., 2004; ROSENTHAL; DOLORESCO, 1984) e sua consequente incorporação na pele.

A incorporação de AGT nas membranas celulares cutâneas foi associada ao aumento dos danos oxidativos da pele exposta à RUV, expresso pelos níveis aumentados de marcadores de oxidação lipídica e protéica cutânea, os quais foram capazes de causar alterações significativas sobre as enzimas mitocondriais, cuja menor atividade reflete a extensão do dano observado na pele dos animais. Dados da literatura têm mostrado que a oxidação de lipídeos e proteínas pode levar a degeneração da membrana celular, alterando sua permeabilidade e inativando enzimas ligadas às membranas, culminando na morte celular (BUS; GIBSON, 1979; GIROTTI, 1990; SCHARFFETTER-KOCHANEK et al., 1997; SUN, 1990). Assim, acreditamosque aincorporação de AGT nasmembranas celulares da pele foi suficiente para causar alterações funcionais nesse tecido, que estão fortementeenvolvidas nos danos oxidativos induzidos pela RUV. Além disso, o grupo suplementado com GVH apresentou maior espessamento da pele, o qual é uma resposta inflamatória característica da exposição à RUV. Esse achado está de acordo com Han et al. (2002), que mostrou uma relação entre o consumodeuma dieta rica em GVH com o aumento da produção decitocinas pró-inflamatóriase e EO. Por outro lado, a incorporação cutânea de AGPI n-3 observada no presente estudo foi capaz de prevenir os danos oxidativos da pele exposta à RUV, demonstrado pela menor peroxidação lipídica e protéica junto com a função normal das enzimas mitocondriais. Nesse contexto, é possível atribuir tais resultados à geração de metabólitos dos AGPI n-3, os quais apresentam menor atividade pró-inflamatória e pró-apoptótica na pele dos animais suplementados com óleo de peixe, que são diferentes daqueles gerados pelo metabolismo dos AGPI n-6 e AGT. Assim, a redução dos danos cutâneos induzido pela exposição à RUV observados no presente estudo, poderia ser atribuída aos eicosanoides e docosanóides resultantes do metabolismo dos AGPI n-3.

Sequencialmente, os dados mostrados nos artigo 2 e manuscrito 1 mostram que a suplementação mais prolongada de GVH e, em menor proporção, de óleo de soja, facilita o desenvolvimento de fotoenvelhecimento induzido pela RUV em ratas Wistar de 1ª e 2ª geração. A exposição crônica (repetida) à RUV aumentou os danos oxidativos cutâneos no grupo suplementado com GVH, observados pela maior geração de espécies reativas e desenvolvimento de peroxidação lipídica, menor atividade das enzimas mitocondriais e

redução das defesas antioxidantes. Em contrapartida, a suplementação de óleo de peixe, rico em AGPI n-3, mostrou proteção sobre os efeitos deletérios cutâneos da RUV. Os AG podem modular condições fisiológicas e patológicas através de mecanismos que envolvem a cascata inflamatória (KANG; WEYLANDT, 2008). Desta forma, dependendo do AG precursor, haverá a formação de mediadores com características antagônicas e com diferentes atividades biológicas (BLACK et al., 2006). Enquanto as consequências da incorporação cutânea dos AGT são pouco descritas e pobremente compreendidas, os AGPI n-3 apresentam propriedades antiinflamatórias e anti-apoptóticas (BARCELOS et al., 2010; BAZAN, 2005; 2007; CALON et al., 2004; HASHIMOTO et al., 2002; 2006; YAVIN et al., 2002; MASSARO et al., 2008; WU et al., 2004), demonstrando um potencial preventivo para desenvolver danos oxidativos. Por sua vez, os AGPI n-6 são precursores de mediadores pró-inflamatórios, conferindo um fator positivo para a geração de espécies reativas, as quais, em conjunto, podem culminar em danos oxidativos cutâneos pós-RUV (OKUYAMA; KOBAYASHI; WATANABE, 1996). Deste modo, enquanto os AGPI n-3 estão envolvidos na redução da inflamação, os AGPI n-6 tendem a promover a inflamação: após serem liberados da membrana fosfolipídica pela ação da enzima PLA_2, os AGPI n-3 são metabolizados pela COX e LOX, gerando mediadores com menor atividade inflamatória (PGs da série 3), ao passo que o metabolismo dos AGPI n-6 geram metabólitos com potente atividade inflamatória (PGs da série 2). Diferentes estudos *in vivo* e *in vitro* mostraram que os AGPI n-3 são capazes de atuar através da redução da produção de PGs derivadas do AA e de outros mediadores inflamatórios, exercendo seus efeitos antiinflamatórios por antagonizar a síntese ou o metabolismo do AA. Da mesma forma, os AGT podem inibir as enzimas responsáveis pela síntese dos AGE e, dessa maneira, podem reduzir a geração dos seus metabólitos, porém tal evento é apenas teórico, não tendo sido ainda demonstrado experimentalmente.

A atividade da Na^+K^+ATPase é outro fator importante para os processos de sinalização celular através da manutenção do gradiente eletroquímico transmembrana (MOSELEY et al., 2007; STAHL; HARRIS, 1986). Alterações na atividade dessa enzima podem representar um importante marcador de toxicidade (CHAUDHARY; PARVEZ, 2012; LEES, 1993), uma vez que a sua atividade é sensivelmente modificada pela geração OS (TEIXEIRA et al., 2011; 2012). Da mesma forma, a atividade alterada da Ca,MgAPTase é utilizada como um marcador de dano oxidativo e um importante parâmetro em estudos dos efeitos de manipulações dietéticas (BARTOLI, 1995). Neste contexto, a avaliação da atividade da Na^+K^+ATPase cutânea constituiu uma ferramenta para estimar os efeitos nocivos da RUV. Neste estudo, foi

observado um aumento significativo da atividade dessa enzima nos animais suplementados com óleo de soja e GVH, através dos grupos experimentais C-SO e HVF, respectivamente, após exposição crônica à RUV. Por outro lado, no grupo suplementado com óleo de peixe, a atividade da Na^+K^+ATPase manteve-se inalterada. A bomba de Na^+K^+ATPase é responsável pelo transporte ativo de íons de sódio e potássio através da membrana plasmática para manter a sua excitabilidade (RIBEIRO et al., 2007) e o marcado aumento da sua atividade pode alterar a transmissão celular e diminuir a entrada de cálcio da célula. A redução de cálcio pela epiderme está relacionada com perturbações da integridade da barreira cutânea induzidas pela RUV e estimula a secreção de corpos lamelares do estrato granuloso, o que facilita a recuperação de barreira (ELIAS et al., 2002).

O envolvimento ativo dos AG na saúde cutânea e na função de barreira cutânea justifica a escolha da suplementação com AGPI n-3 como uma estratégia eficaz para a melhoria de condições inflamatórias (CALDER et al., 2009) cutâneas. Os efeitos dos AGPI n-3 de cadeia longa sobre a produção de eicosanóides através da sua habilidade de oferecer substratos alternativos para as enzimas metabolizadoras de lipídios, exercem seus efeitos através de seu papel na sinalização celular, na expressão gênica e, consequentemente, nos níveis enzimáticos (CALDER, 2006). Geralmente, os mediadores produzidos a partir da metabolização dos AGPI n-3 são menos inflamatórios e moléculas quimio-atratoras menos potentes que os seus homólogos derivados do AA, um AGPI n-6 (NICOLAO, 2013). Nesse sentido, é possível afirmar que a formação desses mediadores reduz a concentração dos eicosanóides derivados do AA, resultando em um ambiente menos inflamatório.

A pele humana responde as intervenções nutricionais e estudos com óleo de peixe têm mostrado a redução do eritema e dos níveis de PGE_2 após exposição à RUV (RHODES et al., 1995), configurando uma possível estratégia quimio-preventiva para resolver os efeitos nocivos da radiação solar. Já foi demostrado o papel dos mediadores derivados dos AGPI n-3 na cicatrização de feridas, processo no qual o perfil de tais moléculas lipídicas quimio-atrativas podem ter influência sobre o infiltrado leucocitário e o grau de epitelização (LU; TIAN; HONG, 2010; McDANIEL; MASSEY; NICOLAOU, 2011; MARTIN; LEIBOVICH, 2005). Além disso, intervenções dietéticas com AGPI n-3 demonstraram uma melhoria na psoríase e na dermatite atópica, elucidando um importante papel na restauração das propriedades da barreira cutânea (FLUHR; CAVALLOTTI; BERARDESCA, 2008), fortalecendo a evidência do seu papel benéfico na saúde da pele (MAYSER; GRIMM; GRIMMINGER, 2002; GUECK et al., 2004).

Com base em todos os experimentos que abrangem essa tese e devido as propriedades antiinflamatórias dos AGPI n-3, os estudos realizados sugerem que a ingesta continuada de alimentos processados, ricos em GVH, em detrimento de óleos vegetais e/ou animais ricos em AGPI n-3, podem modificar os padrões de dano e os mecanismos de defesa da pele frente a exposição à RUV, contribuindo para o desenvolvimento de eritema, inflamação, envelhecimento e até mesmo melanomas e câncer de pele, cuja incidência, e por analogia ou coincidência, têm aumentado nas últimas décadas.

Dados adicionais contidos no item apêndice 1 desta tese foram desenvolvidos em estudos de doutorado sanduíche, cujos resultados refletem a escassês de estudos sobre a secura da pele, a qual pode configurar um fator agravante para os danos cutâneos decorrentes da exposição à RUV, cujo tema ainda carece de estudos. Neste sentido, a identificação da secura dapele easquestões clínicas envolvidas requer abordagensque possam funcionar como ferramentas na busca pela melhora dos pacientes, além de envolver a pesquisa acerca da sua etiologia, prevenção e tratamento (GUENTHER etal., 2012).A perspectiva de publicação dos achados resultantes dos estudos apresentados aqui deverá contribuir na produção de novos elementos de pesquisa que busquem uma melhor qualidade de vida, atuando como um fator motivacional para uma alimentação mais saudável, e também acionando o desenvolvimento de novas estratégias de aproveitamento de alimentos para a humanidade.

5. CONCLUSÕES

- ✓ A suplementação com óleo de peixe, óleo de soja e GVH desde o período perinatal até a idade adulta, bem como no decorrer da primeira e segunda gerações permitiu uma incorporação aumentada de AG n-3, n-6 e *trans* na pele, respectivamente, mostrando que o perfil lipídico cutâneo reflete rapidamente a oferta exógena proveniente de diversas fontes como a ingestão direta, via placenta e leite materno;
- ✓ A incorporação de AG n-3, n-6 e *trans* provenientes das respectivas suplementações apresentam diferentes respostas sobre o status oxidativo da pele exposta à RUV, modificando também sua espessura, atividade do sistema de defesa antioxidante, atividade de enzimas mitocondriais e transmembrana;
- ✓ A suplementação de GVH aumentou os danos cutâneos induzidos pela RUV, facilitando o desenvolvimento de rugosidades e hipertrofia cutânea;
- ✓ Os AGT foram capazes de favorecer o desenvolvimento de alterações relacionadas à exposição UV, as quais incluem envelhecimento, edema inflamatório e danos oxidativos, os quais podem facilitar o desenvolvimento de doenças mais severas como melanomas e câncer de pele.

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7. APÊNDICE 1

A revisão bibliográfica a seguir refere-se aos estudos realizados durante a realização de Doutorado Sanduíche no Research Institute for Medicines and Pharmaceutical Sciences (iMED.UL) da Faculdade de Farmácia da Universidade de Lisboa, na cidade de Lisboa/Portugal.

Os resultados a seguir apresentam-se estruturados nos ítens Materiais e Métodos, Resultados, Discussão e Referências, os quais estão dispostos da mesma forma que foi publicado (artigo 3) e em fase de redação (manuscrito 2), apresentado como um resumo estendido.

7.1REVISÃO BIBLIOGRÁFICA

7.1.1 A pele

O *stratum corneum* é a camada mais externa da pele e desempenha um importante papel de barreira contra a perda de água e as agressões químicas externas (LODÉN, 2003; TFAYLI et al., 2013). Apesar dos avanços na compreensão da estrutura, fisiologia e função da pele, a hidratação e seus meandros continuam a ser uma área ativa de pesquisa. Um nível mais elevado de hidratação que 10% do peso total do *stratum corneum* tem sido considerado característica de pele humana normal "não-seca" (MADISON, 2003; YADAV et al., 2009).

A hidratação da pele depende, entre outros, de dois grandes fatores: da presença de fatores de hidratação natural (do inglês *natural moisturizing factors* (NMF)) (MADISON, 2003) e da estrutura e composição dos lípidos no SC (GUILLARD; TFAYLI, 2011; TFAYLI et al., 2010). Os NMF consistem principalmente de aminoácidos e seus derivados, ácido láctico, uréia e açúcares, formando uma mistura altamente higroscópica e que representa de 5 a 30% do peso seco total do *stratum corneum* (MADISON, 2003; YADAV; WICKETT, 2009). Contrariamente ao NMF, a matriz de lipídios tem um papel direto na hidratação da pele pela prevenção da perda de água através da sua função de barreira, principalmente devido à combinação única de ceramidas (45-50% de peso), colesterol (25%) e AG livres (10-15%) no *stratum corneum* (TFAYLI et al., 2013). Além da composição lipídica, está bem estabelecido que a organização de ceramidas, AG de cadeia longa, na matriz intercelular do *stratum corneum* humano desempenha um papel chave na criação da barreira cutânea (BINIEK; LEVI; DAUSKARDT, 2012; BOUWSTRA et al., 2003; 2006; PILGRAM et al., 2001; TFAYLI et al., 2013).

7.1.2 A pele seca

A pele seca é um problema global e extremamente comum (MATTS et al., 2005). Pesquisas nos Estados Unidos, França e Japão mostraram que aproximadamente 40% da população apresenta problemas relacionados à pele seca (KITAMURA, 2002). A pele seca ou eczema xerótico pode ser diagnosticado como xerose, eczema asteatótico, eczema

disidróticoou eczema craquele (NORMAN, 2003). A superfície da pele seca é menos flexível que a pele normal, o que contribui para a sensação irregular sensível ao toque (FLYNN et al., 2001). Também apresenta-se escamosa, áspera, fissurada e rachada e, geralmente, tal condição dermatológica está intimamente associada com a desagradável sensação somatossensorial de coceira ou prurido (NOJIMA et al., 2003;. NORMAN, 2003; TOMINAGA et al., 2007). Algumas doenças sistêmicas como a insuficiência renal, a coletasis, a uremia e a síndrome da imunodeficiência adquirida (SIDA), assim como a pele envelhecida e quase todas as doenças dermatológicas que se manifestam comouma pele xerótica são acompanhadas pelo prurido (OKAWA et al., 2012;. KAMO et al., 2011; MIYAMOTO et al., 2002; TOMINAGA et al., 2007; WAHLGREN, 1999; YOSIPOVITCH, 2004b). A secura da pele ocasionavárias doenças cutâneas pruriginosas, como a dermatite atópica (DA) e a xerose (LODEN; MAIBACH, 2006). O contato da pele com agentes ou alérgenos pruriginosos provoca coceira aguda; enquanto a coceira crônica se manifesta nas condições patológicas da pele e nas doenças sistêmicas (DI NARDO et al., 1998; KRAJNIK; ZYLICZ, 2001; LONG; MARKS, 1992).

A condição normal da pele também pode ser afetada por variações sazonais que podem desencadear várias doenças cutâneas (LODEN; MAIBACH, 2006;. YOSIPOVITCH et al., 2004a), como as dermatoses comuns (psoríase, xerose e DA) (LODEN; MAIBACH, 2006; YOSIPOVITCH et al., 2004b; PINNAGODA et al., 1989). Da mesma forma, o uso freqüente de detergentes, sabonetes e irritantes tópicos têm a capacidade de induzir o prurido xerótico (YOSIPOVITCH, 2004b). Algumas evidências sugerem que o inverno (LODEN; MAIBACH, 2006; YOSIPOVITCH et al., 2004a) e a diminuição da humidade ambiental precipita aquelas desordens cutâneas (RYCROFT; SMITH, 1980), enquanto que a hidratação da pele parece diminuí-las (CHERNOSKY, 1976; RAWLINGS et al., 1994b).

A incidência da pele seca pruriginosa aumenta com a idade e é um dos problemas dermatológicos mais comuns que acometem os idosos (NORMAN, 2003). A condição ocorre mais freqüentemente nas pernas, mas as mãos e o tronco também podem ser afetados (NORMAN, 2003). As fissuras ou rachaduras decorrentes da perda de água pela epiderme pode romper capilares dérmicos e induzir a formação defissuras e sangramento cutâneo (ANDERSON; MILLER,2000). Além disso, o prurido e as lesões cutâneas resultantes podem levar às abrasões secundárias que produzem, por sua vez, resposta inflamatória, líquen simples crônico e até mesmo manchas edemaciadas (ANDERSON; MILLER,2000). Consequentemente, os patógenos e alérgenos ambientais podem facilmente penetrar na pele, aumentando o risco de alergias e dermatite irritante, o que pode levar a uma dermatite

persistentee, possivelmente, mais extensa, apesar da terapia (ANDERSON; MILLER, 2000). Desse modo, uma infecção secundária, risco inerente de qualquer interrupçã ona barreira cutânea, também pode ocorrer (NORMAN, 2003).

A pele seca possui uma menor capacidade de retenção de água, característica regulada pelafunção de barreirada pele no *stratum corneum* (CHANDAR et al., 2009; LEVEQUE e tal., 1987; MIYAMOTO et al., 2002). A securada pele tem sido relacionada com o rompimento da barreira cutânea e ao prurido (HARDING et al., 2000; LONG; MARKS, 1992; MORTON et al., 1996). Nesse contexto, a secura da pele é caracterizada por redução da hidratação do *stratum corneum* e a ruptura da barreira cutânea é caracterizada por um aumento da perda de água transepidérmica (TEWL, do inglês *transepidermal water loss*) (MIYAMOTO et al., 2002). Alterações nahidratação do *stratum corneum*e a TEWL não estão relacionadas com o prurido em alguns estudos (DELEIXHE-MAUHIN et al., 1993; OSTLERE et al., 1994; KATO et al., 2000). Assim, os mecanismos do prurido relacionados à pele seca são pouco estabelecidos e tratamentos para tal desordem ainda não foram estabelecidos (MIYAMOTO et al., 2002).

6.1.3 Modelos experimentais e patofisiologia da pele seca associada ao prurido

A patofisiologia da pele seca envolve fatores que podem contribuir para a sensação de prurido, como a alteração da estrutura do *stratum corneum*, anormalidades da queratinização, proliferação, metabolismo da água, níveis de citocinas, pH e lípidos de superfície (ELIAS; GHADIALLY, 2002; YOSIPOVITCH, 2004). O modelo animal de pele seca utilizando camundongos proporcionou novo entendimento sobre o prurido associado com a pele seca (YOSIPOVITCH, 2004).

O modelo experimental de prurido associado a pele seca foi demonstrado através da exposição tópica de camundongos à acetona (KAMO et al., 2011; OKAWA et al., 2012; TOMINAGA et al., 2007). Miyamoto et al. (2002) recentemente desenvolveu um modelo animal utilizando camundongos no qual a pele foi exposta a uma mistura de acetona e éter dietílico, seguida da exposição à água. Tal protocolo induziu uma desidratação do*stratum corneum* e aumentou a TWEL, além de aumentar os movimentos espontâneos de coçar a pele seca com a pata traseira (MIYAMOTO et al., 2002). Outros protocolos experimentais de modelo animal para rompimento da barreira cutânea foram demonstrados por uma dieta sem

AGE, pela exposição tópicaà uma solução orgânica ou surfactante e indução de interrupção física da integridade cutânea através da utilização de uma fita autocolante (GRUBAUER et al., 1987; DENDA et al., 1998). Tais modelos experimentais removem os componentes lipídicos (AG livres, colesterol e ceramidas) do *stratum corneum*, os quais desempenham um papel importante na função de barreira cutânea (ELIAS, 1983; GRUBAUER et al., 1989). Por sua vez, a exposição dos animais a um ambiente seco ou a exposição cutânea à água ou aos solventes orgânicos podem perturbar a barreira cutânea, causando perda de componentes aquosos, como aminoácidos, hidroxila, uréia e pirrolidona carboxilato (JOKURA et al., 1995; SATO et al., 2001).Apesar de a investigação e a compreensão sobre o prurido crônico associado à pele seca ter aumentado nos últimos anos, sua patogênese ainda permanece obscura e controversa (BACK et al., 2012).

A histamina é um mediador pruriginoso secretada pelos grânulos dos mastócitos (AKASAKA et al., 2011). Nesse sentido, a terapia anti-histamínica têm sido utilizada para o tratamento das condições agudas de prurido (KENNARD; ELLIS, 1991) e o antagonismo dos receptores de histamina H_1 é o mecanismo dessa inibição (WAHLGREN, 1992). No entanto, na prática clínica é comum a ocorrência de prurido resistente a ação dos anti-histamínicos (WAHLGREN, 1992). Novos insights sobre o potencial envolvimento de outros mediadores químicos, além da histamina, no prurido presente em desordens cutâneas crônicas foram demosntrados, incluindo a substância P (HÄGERMARK et al., 1978) e a triptase (STEINHOFF et al., 2003). A substância P, um neuropeptídio membro da família da taquiquinina, é conhecido por atuar como um neurotransmissor ou neuromodulador no sistema nervoso periférico e central de mamíferos (PERNOW, 1983) e tem sido associada aos processos inflamatórios e de dor (OTSUKA; YOSHIOKA, 1993). O comportamento de coçar em camundongos que mimetizam o prurido em humanos (ANDOH; KURAISHI, 1998; KURAISHI et al., 1995) e o aumento da produção de leucotrienos (LT) B_4 (ANDOH et al., 2001), um potente que pruritogênio que induz uma resposta associada ao prurido, foram demonstrados pela administração de substância P em ratos (ANDOH; KURAISHI, 1998).

6.1.4 Dispositivos de medição

O conteúdo de água da derme e da epiderme é definido como a hidratação da pele (MANFREDINI et al., 2013). Funcionalmente, a quantidade de água na pele pode ser dividida

em água ligada e água livre: a primeira está associada à diferentes moléculas como a filagrina e outros componentes do NMF na epiderme, enquanto a última se difunde a partir da pele para o ambiente externo (MANFREDINI et al., 2013). A hidratação anormal da pele resulta numa pele seca e escamosa e é um dos sintomas mais comuns presentes em muitos distúrbios dermatológicos (BERARDESCA et al., 1990, BYRNE, 2010; SERUP; BLICHMANN, 1987).

A avaliação do teor de água da pele *in vivo* é de grande interesse para muitas pesquisas e possui implicações positivas para o tratamento clínico (MANFREDINI et al., 2013). Embora nem sempre seja possível quantificar a hidratação exata da pele, existem diversas abordagens para a determinação não invasiva do conteúdo de água da pele (MANFREDINI et al., 2013). Os avanços tecnológicos em dispositivos de medição tornaram possíveis as análises não invasivas de parâmetros cutâneos, facilitando e ampliando o conhecimento da pele humana saudável e de suas patologias (BENEVENUTO et al., 2010; EGAWA et al., 2002; FANG et al., 2003; FUKADA et al., 2012; HORRI et al., 2011; JEON et al., 2010; LODEN et al,, 2013). Abaixo estão listadas algumas das abordagens para a medição do teor de umidade cutâneo, que são métodos complementares, sendo recomendado seu uso combinado (BLICHMANN; SERUP, 1988):

- SKICON: o dispositivo é baseado na medição da condutância e mede o teor de umidade do *stratum corneum*. Possui maior sensibilidade na medição do aumento da hidratação (BLICHMANN; SERUP, 1988).
- CORNEOMETER (Courage Khazaka-Electrônico): este dispositivo baseia-se na medida da capacitância da pele. A profundidade de penetração do campo eléctrico é muito pequena, de modo que apenas a umidade do *stratum corneum* é medido. Pode ser mais sensível para a medição da diminuição da hidratação cutânea (BLICHMANN; SERUP, 1988).
- TEWAMETER (Courage-Khazaka Eletrônico): este dispositivo mede a TEWL.
- PADRÃO DE SUPERFÍCIE DA PELE: medida pela observação da réplica negativa usando material de borracha. As réplicas de superfície da pele podem ser analisadas com um analisador de imagem.

A película lipídica externa configura-se como um marcador de segurança das patologias da pele, facilmente controlável por meio de técnicas de monitorização não invasivas (DOWNING; STEWART, 1985, PASSI et al., 1991). Os lipídios da superfície da pele são os primeiros alvos das agressões ambientais, e também representam uma ferramenta

adequada para a pesquisa da penetração de drogas através da pele e medição das reações químicas aos medicamentos, produtos químicos industriais, têxteis, jóias ou partículas em contato prolongado e direto com pele (STEFANIAK; HARVEY, 2006).

6.1.5 Tratamentos para a pele seca

Há registros de tratamentos para a pele seca tão cedo quanto 3000aC (MATTS et al., 2005). Apesar dos avanços atuais da ciência cosmética, ainda não existe um tratamento definitivo para essa condição dermatológica (KITAMURA, 2002). Os cremes e loções hidratantes são benéficos na prevenção e tratamento da pele seca, nos distúrbios de barreira cutânea e envelhecimento da pele (MANFREDINI et al., 2013; RODRIGUES et al., 1997). Sua eficácia é influenciada por fatores como a formulação, patogênese da pele seca e adesão do paciente ao tratamento (LODEN, 2012). Além disso, há o envolvimento de razões fisiológicas, raciais e culturais que envolvem o uso de hidratantes (FOTOH et al., 2008). Fotoh et al. (2008) relataram o uso generalizado de hidratantes por caucasianos, enquanto os indivíduos com pele mais escura o fazem apenas pela necessidade de combater o ressecamento da pele.

A adição de água à pele envolve a hidratação (MANFREDINI et al., 2013). Assim, os agentes umectantes são adicionados às formulações hidratantes para melhorar a capacidade de ligação da água no *stratum corneum* (MANFREDINI et al., 2013). Diferentes formulações de hidratantes podem ter diferentes efeitos sobre a epiderme e afetar a sua integridade, o conteúdo total de água, as propriedades físicas, a descamação e a espessura do *stratum corneum* (LODEN et al., 2004).

O interesse no emprego de gorduras dietéticas no tratamento de doenças de pele émarcado pelo estudo histórico de Burr e Burr em 1929 (BURR; BURR, 1929; 1930), no qual ratos alimentados com uma dieta desprovida de gordura apresentaram retardo no crescimento, falhas reprodutivas e erupções cutâneas caracterizadas por descamação e eritema, com aumento da perda transepidérmica de água. Tais manifestações regrediram quando a dieta foi suplementada com LA e ALA. Originalmente conhecidas como vitamina F, essas gorduras logo vieram a ser conhecidas como AGE (McCUSKER; GRANT-KELS, 2010). Este estudo, bem como outros estudos iniciais, têm sido criticados porque não foi feita uma distinção entre a suplementação com o LA, AGPI n-6, ou ALA, AGPI n-3 (CUNNANE, 2003). A

diferenciação entre os dois é importante, porque seus papéis são distintos: LA e seus produtos servem como precursores estruturais para as ceramidas do *stratum corneum*, enquanto que o ALA e seus derivados servem como moduladores da resposta imune (McCUSKER; GRANT-KELS, 2010).

Recentemente, houve um interesse crescente no uso de suplementos nutricionais para beneficiar a pele humana (BUONOCORE et al., 2012; DAYAN et al., 2011; GUILLOU et al., 2011; MARINI et al., 2012; NEUKAM et al., 2010). O controle da composição lipídica da pele através de uma intervenção nutracêutica ou dietética surge como uma possibilidade e uma perspectiva promissora (NEUKAM et al., 2010; PUPE et al., 2002; TAKEMURA et al., 2002; VIOLA; VIOLA, 2009), uma vez que os fatores nutricionais podem beneficiar a fisiologia da pele (BUONOCORE et al., 2012; DAYAN et al., 2011; GUILLOU et al., 2011; MARINI et al., 2012; NEUKAM et al., 2010; PUPE et al., 2002; TAKEMURA et al., 2002; VIOLA; VIOLA, 2009). Entretanto, a literatura é carente acerca dos efeitos dos nutrientes dietéticos consumidos/suplementados por longo prazo sobre as propriedades da pele, incluindo TEWL, hidratação, elasticidade e microcirculação epidérmica. A composição lipídica cutânea é fisiologicamente relevante para um desempenho ideal da pele e pode representar uma abordagem poderosa para a prevenção e/ou tratamento da secura e envelhecimento da pele, como forma de aumentar a fotoproteção da pele e e as consequentes doenças cutâneas.

2. OBJETIVOS

2.1 Objetivo geral

Desenvolver modelo animal de pele seca e DA em ratos para avaliar a influência da suplementação com óleo de peixe, rico em AGPI n-3, sobre os parâmetros fisiológicos da pele como perda transepidérmica de água, hidratação, circulação e pruridocutâneo.

2.2 Objetivos específicos

- Desenvolver modelo animal de pele seca e DA em ratos e avaliar parâmetros fisiológicos da pele como perda transepidérmica de água, hidratação e prurido cutâneo nesses animais;
- Avaliar a influência dos AG n-3 sobre a perda transepidérmica de água, hidratação, microcirculação cutânea em modelo animal de pele seca em ratos suplementados com óleo de peixe.

3. PRODUÇÃO CIENTÍFICA

Os resultados inseridos nesta tese apresentam-se sob a forma de artigo 3 e manuscrito 2, os quais se encontram aqui estruturados. Os ítens Materiais e Métodos, Resultados, Discussão e Referências encontram-se no próprio artigo e no manuscrito, os quais estão dispostos da mesma forma que foi publicado (artigo 3) e submetido (manuscrito 2).

3.1 Artigo 3:

BARCELOS, R. C. S. et al. Atopic dermatitis-like disease in a rat model. **Biomed. Biopharm. Res.**, v. 2, p. 217-224, 2013

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Atopic dermatitis-like disease in a rat model

Modelo tipo de dermatite atópica em rato

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Abstract

The Atopic dermatitis (AD) is a most important topic in clinical dermatology. In fact, the pathogenesis of this chronic inflammatory skin disease, primarily characterized by pruritus and dry skin, is still far from being fully understood. To look further into this complex disease, male adult Wistar rats ($n = 10$) were used as a model where acetone (AA) acted as the active challenger in a 3-day treatment setting, and compared with water (AW). On day 3, one hour after the last treatment, the AA area exhibited higher transepidermal water loss (*TEWL*), capillary blood flow, and reduced hydration when compared to AW. The scratching behavior was markedly higher in the AA treated group ($n = 5$) than in the AW group ($n = 5$). These interesting results justify the application of this model as a clinical experimental tool for AD research.

Keywords: atopic dermatitis; pathophysiology; animal model; inflammation, pruritus

Resumo

A dermatite atópica (DA) é um tema importante na dermatologia clínica. Na verdade, a patogénese dessa doença inflamatória crónica da pele, caracterizada principalmente por pele seca e prurido, ainda está longe de ser totalmente compreendida. A fim de saber mais acerca desta complexa doença, ratos Wistar machos e adultos ($n = 10$) foram utilizados como modelo animal, nos quais o tratamento com acetona (AA) foi comparado com o tratamento com água por 3 dias (AW). No dia 3, uma hora após o último tratamento, a AA mostrou maior perda transepidérmica de água (*TEWL*), fluxo sanguíneo capilar e reduzida hidratação quando comparada com AW. A análise comportamental mostrou que a acção de coçar foi marcadamente mais frequente no grupo AA ($n = 5$) quando comparado ao grupo AW ($n = 5$). Estes resultados justificam a implementação deste modelo animal como uma ferramenta experimental para investigação da AD.

Palavras-chave: dermatite atópica, fisiopatologia, modelo animal, inflamação, prurido

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Introduction

Dry skin is a common global problem [1]. Recent studies from the USA, France and Japan report that 40 % of the population apparently suffers from a dry skin related problem [2]. Dry skin, or xerotic eczema, can be diagnosed as xerosis, asteatotic eczema, deshydrotic eczema or eczema craquelé [3]. Scaly, rough, fissured / cracked surface are typical in dry skin and such dermatological conditions are closely related to the unpleasant somatosensory sensation of itching or pruritus [3-5]. Skin dryness is present in many pruritic cutaneous diseases, such as xerosis and atopic dermatitis (AD), which is actually the most prevalent hallmark of these conditions involving dry skin and pruritus [6]. AD is the most common higher occurrence inflammatory skin condition during infancy and childhood [7] so that in the last two decades, its prevalence has increased between 18% and 25 % in some developed countries [8]. AD is non-contagious and characterized in acute phases by intense itching, erythematous rash, vesicles and oedema, and lichenification in chronic stages [7,9,10]. Patients with AD-induced visible dermatologic signs and disruptive symptoms have their lives and social relationships negatively affected, with reduced sense of well-being and self-esteem, anxiety, psychosocial stress, sleep deprivation, and poor professional performance [11]. AD complex pathogenesis still remains unclear and only partially understood [7] despite improved knowledge of the topic due to growing research, especially in recent years. It is thought to result from a complex interaction between the skin barrier disruption, immune abnormalities, and environmental and infectious agents [12].
The present study aims to develop an animal model, where a controlled AD-like condition is evoked, in order to look deeper into the pathophysiological processes involved in this skin disease.

Introdução

A pele seca é um problema global comum [1]. Estudos recentes dos EUA, França e Japão relataram que 40 % da população sofre aparentemente de um problema relacionado com a pele seca [2]. A pele seca ou eczema xerótico pode ser diagnosticado como xerose, eczema asteatótico, eczema desidrótico ou eczema *craquelé* [3]. A superfície seca, escamosa e fissurada/rachada são típicas na pele seca e tais condições dermatológicas estão intimamente relacionadas com a desagradável sensação somatossensorial de coceira ou prurido [3-5]. O ressecamento da pele está presente em muitas doenças cutâneas pruriginosas, como a xerose e a dermatite atópica (DA), a qual é, atualmente, a característica mais prevalente das condições que envolvem a pele seca e o prurido [6]. A DA é a condição inflamatória da pele de maior ocorrência durante a infância [7], e nas duas últimas décadas, a sua prevalência aumentou entre 18 % e 25 % em alguns países desenvolvidos [8]. A DA não é contagiosa e caracteriza-se , na sua fase aguda, por prurido intenso, exantema eritematoso, vesículas e edema, e liquenificação na fase crônica [7,9,10]. Pacientes com sinais dermatológicos visíveis induzidos pela DA têm suas vidas e relações sociais negativamente afetadas, com redução da sensação de bem-estar e auto-estima, ansiedade, estresse psicossocial, privação de sono e baixo desempenho profissional [11]. Apesar do conhecimento aprimorado proporcionado pela investigação crescente, especialmente nos últimos anos, a complexa patogênese da DA permanece obscura e ainda parcialmente compreendida [7], e pode ser resultado de uma complexa interação entre o comprometimento da barreira da pele, anormalias imunológicas e a acção de agentes ambientais e infecciosos [12].
O presente estudo teve como objetivo desenvolver um modelo animal de DA sob condições controladas, a fim de saber mais acerca dos processos fisiopatológicos envolvidos nessa doença de pele.

Materials and methods

Animals

Male Wistar rats weighing (447±14) g (n = 10) were obtained from Harlan Laboratories Inc. (Barcelona, Spain). The animals were housed under controlled temperature (between 20 ºC and 24 ºC) and 12 h light/dark cycle (light between 07:00 and 19:00). Food and water were freely available. Animals were fed with standard laboratory chow (4RF21 GLP; Mucedolasrl, Milan, Italy). Procedures for animal experiments were conducted in accordance with the relevant European Community

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Animais

Ratos Wistar machos pesando (447±14) g (n = 10) foram obtidos ao Laboratório Harlan Inc. (Barcelona, Espanha). Os animais foram mantidos sob temperatura controlada (entre 20 ºC e 24 ºC), ciclo claro/escuro de 12h (luz acesas entre 7:00 e 19:00) e comida e água *ad libitum* e alimentados com ração padrão (BPL 4RF21; Mucedolasrl, Milão, Itália). Os procedimentos de experimentação animal foram conduzidos em harmonia com as regras aplicadas na União Europeia, consideran-

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and National rules on animals protection for experimental and other scientific purposes [13-15].

Chemicals

Acetone and ether were acquired from Sigma Chemicals Co. (St. Louis, MO, USA).

Cutaneous barrier alteration

The rats' dorsal body hair (6 cm x 6 cm) was removed 24 h before the experiment by an electric razor followed by a soft hair-removing cream. The animal dorsum was divided by the median line into two areas. To modify the animal's epidermal barrier, the left side of the area was exposed to acetone, applied with soaked cotton wool (3 cm x 3 cm) for 5 min (acetone area; *AA*), while the right side was exposed to sterile water (water area; *WA*) and served as the control (Figure 1). This procedure took place for 3 consecutive days (at 9:00AM) under deep sedation induced by inhalation of ether to prevent additional stress stimulus. On day 3, approximately 1 h after the last application, the skin physiology and the scratching behavior were assessed.

do ainda as regras nacionais aplicáveis à proteção para experimentação animal e outros fins científicos [13-15].

Químicos

A acetona e o éter foram adquiridos da Sigma Chemical Co. (St. Louis, MO, EUA).

Alteração da barreira cutânea

O pêlo dorsal dos ratos foi removida (6 cm x 6 cm) 24h antes das experiências com auxílio de um barbeador elétrico, seguido da aplicação tópica de um creme depilatório. O dorso dos animais foi divididos pela linha mediana em duas áreas. Para modificar barreira epidérmica do animal, a área do lado esquerdo foi exposta à acetona, aplicada com um algodão embebido (3cm x 3 cm) durante 5 min (área da acetona, AA), enquanto a área do lado direito foi exposta à água esterilizada (área da água, WA) e serviu como controlo (Figura 1). Este procedimento foi realizado durante três dias consecutivos (9:00AM), sob sedação profunda induzida por inalação de éter para prevenir qualquer estímulo estressante ao animal. No dia 3, cerca de 1 h após a última aplicação, a fisiologia da pele e a acção de coçar foram avaliados.

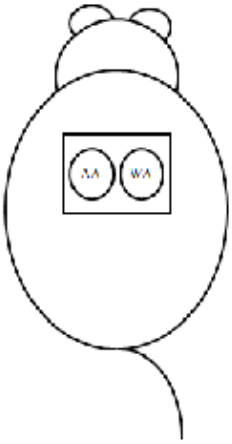

Figure 1
Graphic scheme illustrating the preparation of the model for the experimental procedure. The marked dorsal area on the left was exposed to acetone (AA) and the right area exposed to sterile water (WA) served as the control (see text).

Figura 1
Esquema gráfico ilustrando o modelo para o procedimento experimental. A região dorsal da esquerda foi exposta à acetona (AA), a área dorsal à direita foi exposta à água estéril (WA) e serviu como controlo (ver texto).

Skin physiology

The rats skin physiology was assessed by non-invasive techniques providing the quantitative measurement of the epidermal barrier, the epidermal water content, and local microcirculation, all obtained in both dorsum (WA and AA)treated areas during sedation. The epidermal barrier, expressed by the Transepidermal Water Loss (*TEWL*) was measured by evaporimetry using the Tewameter TM300 (Courage+Khazaka GmbH, Cologne, RFA) system and its unit has the symbol (g h^{-1}

Fisiologia da pele

A fisiologia da pele dos ratos foram avaliadas através de métodos não-invasivos, proporcionando a medição quantitativa da barreira epidérmica, do teor de água da epiderme, e da microcirculação local, todos obtidos em ambas as áreas dorsais (WA e AA) tratadas sob sedação. A barreira epidérmica, medida pela perda de água transepidérmica (*TEWL*) foi obtida através de evaporimetria usando o sistema Tewameter TM300 (Courage + Khazaka GmbH, Colônia, Alemanha), tendo como símbolo de

m^{-2}). Epidermal water was obtained by an electrometric system, a Corneometer CM825 (Courage+Khazaka Electronic GmbH, Cologne, Germany) expressed in arbitrary units (UA□s). Local capillary blood flow was determined by laser Doppler flowmetry (LDF) (PeriFlux PF5000, PF5010 System, Perimed, Sweden) expressed in arbitrary units (BPU□s). Measurements took place 1h after the last exposure (day 3) under controlled temperature and humidity conditions ((22±4) °C, (45±5) % *rh*), and were expressed as mean values (obtained from 3 sequential assessments).

Scratching behavior

Evaluation of the scratching behavior was performed according to the protocol described by Okawa et al. [16]. Male Wistar rats (10-week-old), randomly assigned to 2 experimental groups (n=5) according to the previously described procedure were exposed to the acetone challenge test. For the control group, sterile water was used instead of acetone. After 1h of the last cutaneous exposure to acetone or sterile water, each rat was individually placed into an acrylic box (60 cm x 34 cm x 18cm) and acclimatised to the experimental environment for 5 min. The number of scratching actions was counted for 30 minutes by trained observers unaware of the treatments. One scratching action corresponds to a series of scratch movements on the dorsal skin using the hind paws, as previously reported [16,17] and is expressed as a scratching behavior number for each animal for a 30 min period.

Statistical analysis

All data are presented as mean ± SEM. The skin TEWL, hydration, capillary blood flow and scratching behavior data were analyzed using the Wilcoxon test. Differences were considered statistically significant when $p \leq 0,05$. All statistical analyses were carried out using Prisma software.

Results

Transepidermal Water Loss (TEWL), skin hydration and capillary blood flow of the skin

Following the acetone exposure the *TEWL* markedly increased, by nearly 4 times (p = 0,0059), when compared with the control (Table 1 and Figure 2A). On the contrary, the skin hydration decreased in the area (p = 0,0020) to half of the control value (Table 1 and Figure 2B). The acetone exposure also significantly increased (p = 0,0020) the capillary blood flow when compared to the control (Table 1 and Figure 2C).

unidade g h^{-1} m^{-2}). A água da epiderme foi medida por um sistema eletrométrico, o Corneometer CM825 (Courage + Khazaka Electronic GmbH, Colônia, Alemanha), e expressa em unidades arbitrárias (UA'S). O fluxo sanguíneo capilar local foi determinado por Laser Doppler (LDF) (PeriFlux PF5000, PF5010 System, Perimed, Suécia) expresso em unidades arbitrárias (BPU's). As medições foram realizadas 1h após a última exposição (dia 3) sob condições de temperatura e umidade controladas ((22±4) °C, 45±5) % *hr*), e foram expressas como valores médios (obtidos a partir de três avaliações sequenciais).

Análise comportamental

A avaliação da acção de coçar foi realizada de acordo com o protocolo descrito por Okawa et al. [16]. Ratos machos Wistar (10 semanas de idade), divididos aleatoriamente em dois grupos experimentais (n=5) foram expostos à acetona de acordo com o procedimento descrito anteriormente. Para o grupo controlo, a água estéril foi usada em vez de acetona. Uma hora após a última exposição cutânea à acetona ou à água estéril, cada rato foi colocado individualmente em uma caixa de acrílico (60 cm x 34 cm x18cm) e aclimatados ao ambiente experimental durante 5min. O número de ações de coçar foi mensurado durante 30min por observadores treinados desconhecendo os tratamentos. Uma ação de coçar corresponde a uma série de movimentos em direção a pele dorsal usando as patas traseiras, como relatado anteriormente [16,17] e expresso como o número de atos para cada animal durante 30 min.

Análise estatística

Todos os dados são apresentados como média ± EPM. A TEWL, a hidratação, o fluxo sanguíneo capilar e os dados da análise comportamental foram analisados utilizando o teste de Wilcoxon. Diferenças estatisticamente significativas foram consideradas quando $p \leq 0,05$. Todas as análises estatísticas foram realizadas utilizando o software Prisma□.

Resultados

Perda de água transepidérmica (TEWL), hidratação e fluxo sanguíneo capilar da pele

Após a exposição à acetona, a *TWEL* aumentou acentuadamente, aproximadamente 4 vezes (p = 0,0059), quando comparados com o grupo controlo (Tabela 1 e Figura 2A). Contrariamente, a hidratação da pele diminuiu na AA (p = 0,0020) para metade do valor do grupo controlo (WA) (Tabela 1 e Figura 2B). A exposição à acetona aumentou significativamente (p = 0,0020) o fluxo sanguíneo capilar quando comparado ao grupo controlo (Tabela 1 e Figura 2C).

Table 1 - Biometric and behavioral results of the acetone (AA) and water (WA) exposed areas of rats' pre-shaved dorsum skin. For Transepidermal Water Loss (*TEWL*), hydration and capillary blood flow $n = 10$ and $n = 5$ for scratching behavioral. Data expressed as means ± S.E.M.

Tabela 1 - Resultados biométricos e comportamentais das áreas expostas à acetona (AA) e à água (WA) na pele dorsal pré-depilada de ratos da perda de água transepidérmica (*TEWL*), hidratação e fluxo sanguíneo capilar $n = 10$ e $n = 5$ para a análise comportamental. Dados expressos como média ± E.P.M.

	WA	AA	*p*
***TEWL* / (g h^{-1} m^{-2})**	7.68 ± 0.47	28.39 ± 1.28	0.0059
Hydration / AU Hidratação / UA	23.70 ± 1.98	12.53 ± 0.71	0.0020
Capillary blood flow / BPU Fluxo sanguíneo capilar / BPU	16.56 ± 0.74	29.09 ± 1.87	0.0020
Scratching behavior / (number/30 min) / Ação de coçar / (número / 30 min)	0.66 ± 0.50	6.44 ± 0.16	0.0090

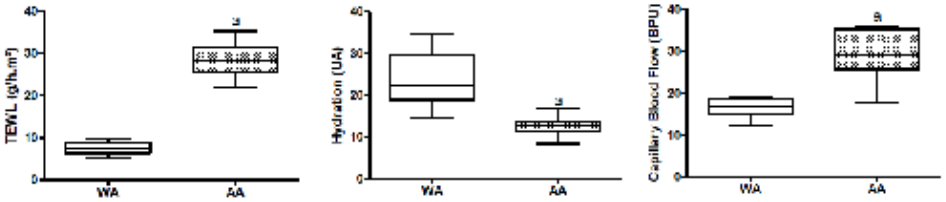

Figure 2 - Effect of acetone exposure of rats' pre-shaved dorsum skin on Transepidermal Water Loss (*TEWL*) (A), hydration (B) and capillary blood flow (C). Data expressed as means±S.E.M ($n = 10$). *Indicates significant difference between acetone exposed area (AA) and water exposed area (WA) ($p < 0,01$).

Figura 2 - Efeitos da exposição à acetona da pele dorsal pré-depilada de ratos sobre a perda transepidérmica de água (*TEWL*) (A), hidratação (B) e fluxo sanguíneo capilar (C). Dados expressos como média ± E.P.M. ($n = 10$). *Indica a existência de diferença significativa entre a área exposta à acetona (AA) e a exposta à água (WA) ($p < 0,01$).

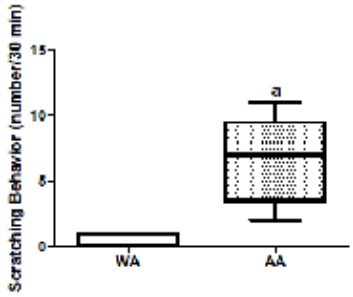

Figure 3 - Rats' scratching behavior (number of scratchings/30 min) 1 h after the last cutaneous acetone ($n = 5$) or water exposure ($n = 5$). *Indicates significant difference between acetone exposed area (AA) and water exposed area (WA) ($p<0,01$). Data are expressed as mean ± S.E.M.

Figura 3 - Análise do comportamento dos ratos (número de atos de coçar/30 min) 1 h após a última exposição cutânea à acetona ($n = 5$) ou à água estéril ($n = 5$). *Indica a existência de diferença significativa entre a área exposta à acetona (AA) e a área exposta à água (WA) ($p <0,01$). Os dados são expressos como média ± E.P.M.

The scratching behavior measured by the number of scratching actions registered for 30 min, also changed after the 3 days acetone exposure treatment, significantly increasing ($p = 0,0090$) when compared to the control (Table 1 and Figure 3).

Discussion

The cellular and molecular mechanisms causing atopic dermatitis (AD) are still unclear, partly due to the limitations of current in vitro research methodologies and the paucity of suitable models.

The main goal for this animal model is to replicate the most relevant features identified in the human AD pathophysiology in order to develop a useful experimental instrument for research, namely to evaluate new therapeutic approaches in AD. Ideally, the animal model used for this kind of study should be reliable, affordable and allow researchers to quantify the symptoms that are usually induced by AD. Existing animal models of AD are generally explored in mice dorsum [5,16,18,19], abdomen and ears [20] and also in Wistar rats' dorsal skin [21]. This last animal model has been established through subcutaneous injection of capsaicin into newborn rat pups within 48h of birth [21,22]. As a consequence, this AD model in rat authentically represents the chronically relapsing pruritic dermatitis, similar to human AD, but it does not represent the characteristic cutaneous injuries of the disease.

In our protocol, an AD-like disease was induced in Wistar rats through repeated exposure to acetone of previously prepared dorsal skin. The obtained skin lesions were visually very similar to those observed in human AD, showing various degrees of signs and symptoms such as deep excoriation and increased microcirculation in addition to those signs related to skin dryness such as scaly, rough, fissured and cracked surface. These were also biometrically quantified by elevated local blood flow, *TWEL* and lower hydration.

Skin dryness results from the reduction in the water-holding capacity of the stratum corneum (SC), which is also related to the cutaneous barrier function [5,19,23,24]. The cutaneous barrier impairment is normally revealed by a consistent increase in TEWL [19]. In the present study, acetone exposure of the rat's dorsum modified the epidermal barrier function, as demonstrated by the increase in TEWL and reduced the skin hydration (Fig. 2), resulting in dry skin. Previous studies have shown that acetone exposure on hairless mice removes SC lipid components, resulting in an acute barrier obliteration [25,26] and TEWL increase, in addition to a SC hydration reduction in the first hour after exposure [5]. Thus, the acetone exposed area re-

A análise comportamental de coçar, mensurado pelo número de ações de coçar durante 30min, também aumentou significativamente após 3 dias de exposição à acetona, aumentando significativamente ($p = 0,0090$), quando comparado ao grupo controlo (Tabela 1 e Figura 3).

Discussão

Os mecanismos celulares e moleculares que causam a DA são ainda pouco entendidos, em parte devido às limitações das atuais metodologias de pesquisa *in vitro* e da escassez de modelos adequados.

O principal objetivo para este modelo animal é a replicação da mais relevante característica identificada na fisiopatologia da DA humana, a fim de desenvolver uma ferramenta experimental útil para a pesquisa na avaliação de novas abordagens terapêuticas na DA. Idealmente, o modelo animal utilizado para este tipo de estudo deve ser confiável, acessível e permitir quantificar os sintomas usualmente induzidos pela DA. Os modelos animais de DA existentes são geralmente explorados utilizando o dorso [5,16,18,19], abdômen e orelhas [20] de camundongos, assim somo o dorso de ratos Wistar [21]. Este último modelo animal tem sido estabelecido através de injeção subcutânea de capsaicina em ratos recém-nascidos dentro de 48h após o nascimento [21,22]. Como resultado, este modelo de DA em ratos representa autenticamente a dermatite pruriginosa crônica recidivante, semelhante à DA humana, porém não representa as lesões cutâneas características da doença.

Em nosso protocolo, a DA foi induzida em ratos Wistar através da exposição repetida da pele dorsal previamente depilada à acetona. As lesões cutâneas obtidas foram visualmente muito semelhantes às observadas na DA humana, mostrando vários graus de sinais e sintomas, como escoriações profundas e aumento da microcirculação, além dos sinais relacionados com a secura da pele, como a presença da superfície escamosa, áspera, e fissurada. Tais sinais foram biometricamente caracterizados pelo elevado fluxo sanguíneo local, *TEWL* e menor hidratação.

A secura da pele resulta da diminuição da capacidade de retenção de água pelo *stratum corneum* (SC) e está relacionada com a função de barreira cutânea [5,19,23,24]. O comprometimento da barreira cutânea é normalmente demonstrado pelo aumento na TEWL [19]. No presente estudo, a exposição da pele dorsal dos ratos à acetona modificou a função de barreira da epiderme, como demonstrado pelo aumento da TEWL e redução da hidratação superficial (Figura 2), resultando em pele seca. Estudos anteriores têm demonstrado que a exposição da pele dorsal de camundongos *hairless* à acetona remove componentes lipídicos do SC, resultando no compro-

produced the most prevalent condition manifested in AD, that is, dry skin.

After acetone exposure a significant increase in the local capillary blood flow was also registered. Such an event is related to the barrier's integrity [27] and suggests a reaction to the acetone-induced skin dryness. Barrier dysfunction might amplify the response to acetone-induced irritating stimuli and promote its inflammatory potential via a modulation of signal molecules [28]. A recent paper demonstrated that female volunteers with sensitive skin had capillary blood flow decreased after flaxseed oil supplementation, suggesting an improved epidermal function and a modulated inflammation dependent of signaling molecules [27].

The pruritus or itching accompanies several skin disorders, such as AD which promotes scratching and is responsible for additional related lesions [29]. One important finding in the present experiment is that repeated cutaneous exposure to acetone increased action of scratching in rats. The skin dryness might be a more important cause of scratching than the barrier impairment [19]. Thus, in this sense, the scratching behavior may be considered as an expression of the itch response and can be used as an indicator for the screening test of anti-pruritic drugs [19].

metimento agudo da barreira cutânea [25,26] aumentando a TEWL, além de reduzira hidratação do SC na primeira hora após a exposição [5]. Nesse contexto, a área dorsal exposta à acetona (AA) reproduziu a condição mais prevalente manifestada na DA, a pele seca.

Após a exposição à acetona, foi também registrado um aumento significativo no fluxo sanguíneo capilar local. Tal evento está relacionado com a integridade da barreira cutânea [27] e sugere uma reação para a secura da pele induzida pela acetona. A disfunção da barreira cutânea pode amplificar a resposta a estímulos irritantes induzidos pela acetona e promover uma reação inflamatória através de uma modulação das moléculas sinalizadoras [28]. Um estudo recente demonstrou que mulheres voluntárias com pele sensível tiveram o fluxo sanguíneo capilar diminuído após a suplementação com óleo de linhaça, sugerindo uma melhoria na função epidérmica, além da modulação da inflamação dependente de moléculas sinalizadoras [27].

O prurido acompanha várias doenças de pele como a DA, promove a acção de coçar e é responsável por lesões adicionais relacionadas [29]. Um importante achado da presente experiência é que a exposição cutânea repetida à acetona aumentou a frequência do coçar nos ratos. O ressecamento da pele pode ser a principal causa para o coçar, mais que o comprometimento da barreira cutânea [19]. Neste sentido, o coçar pode ser considerado como uma expressão da resposta pruriginosa e pode ser usado como um indicador para a pesquisa de drogas antipruriginosas [19].

Conclusion

These results suggest the obvious usefulness of acetone to induce a human AD-like condition in Wistar rats. With this model, skin injuries are easy to evoke and promptly reproducible, animal stress is reduced and the cost is very acceptable. So, this model might be especially interesting for AD pathophysiology basic research to assess the efficacy of new therapeutic approaches in human AD.

Conclusão

Estes resultados sugerem a utilidade da acetona para induzir uma condição dermatológica que mimetiza a DA humana em ratos Wistar. Com este modelo, as lesões de pele são facilmente induzíveis e prontamente reprodutíveis, e o estresse animal é reduzido, além do custo muito aceitável. Assim, este modelo pode ser especialmente interessante para a pesquisa básica da fisiopatologia da DA, bem como para a avaliação da eficácia de novas abordagens terapêuticas da DA humana.

Conflict of Interest

The authors declare that there is no financial or personal relationship that can be understood as representing a potential conflict of interest.

Conflito de interesses

Os autores declaram que não há nenhuma relação financeira ou pessoal, que possa ser entendida como um potencial conflito de interesses.

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3.2 Manuscrito 2: Este manuscrito encontra-se em fase de redação, e por isso será apresentado na forma de um resumo expandido, composto de materiais e métodos, e resultados.

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

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Abstract

Dry skin causes pruritus and discomfort in patients with xerosis and atopic dermatitis.Several experimental and epidemiological studies strongly suggest that diet plays a important role in the prevention of many chronic diseases. Skin properties can be modulated by dietary fatty acids (FA), especially polyunsaturated (PUFA). The present study was performed to evaluate the effect of daily supplementation with fish oil (FO), source n-3 PUFA, on dry skin model rat and itch-related. FO was given to male Wistar adult rats by daily supplementation by gavage (3g/kg) for 90 day and submitted to dry skin model acetone-induced every 30 days. Transepidermal water loss (TEWL), hydration, cutaneous microcirculation andscratching behavior were evaluated. The control group supplemented with n-3 PUFA in an amount equal to that contained in a Western diet developed dry skin and itch. The FO supplementation alleviated skin barrier defects, evidenced by reduction of the TWEL, and by hydration and cutaneous microcirculation increasing, as well as, completely prevented to the itch-related scratching acetone-induced. In conclusion, the consumption of foods rich in n-3 PUFA in a long term is able to influence the skin barrier integrality and functionality against to skin disorders.

Keywords: dry skin, itch, fish oil, PUFA, skin barrier function

Materials and methods

Animals

Male Wistar rats (447±14g) under were kept under conventional conditions in cages with free access to food and water in a room with controlled temperature (23±1°C) and 12 h light/dark cycle. This study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals approved by The Portuguese Pharmacological Society.

Drug

Acetone was purchased from Sigma Chemicals Co. (St. Louis, MO, USA).

Experimental procedure

Rats were randomly assigned to 2 experimental groups according to oral supplementation (n=10): control, (water, C) or fish oil (FO, rich in n-3 PUFA; Herbarium®, Colombo, PR, Brazil). Animals received these supplementations once a day by gavage (3g/kg; p.o.) (BARCELOS et al., 2014; KUHN et al., 2013; PASE et al., 2013; TREVIZOL et al., 2013) for 90 days and every 30 days all animals were submitted to dry skin model. The supplementation time was based on previous studies by our group (BARCELOS et al., 2013; 2014) and other studies evaluating the influence of *trans* fatty acids (SAKAI et al., 2009) and an oil mixture containing vitamin E (CHO et al., 2007) on skin of rodents.

Treatment of cutaneous barrier disruption

The hair of rats was shaved using an electric shaver and after depilatory cream over the dorsal part (6x6cm) at least 24 h before the start of the experiment and divided into two areas according treatment cutaneous. To disrupt the cutaneous barrier, on the right side of the shaved back was applied the treatment with cotton (3x3cm) soaked with acetone for 5 min (acetone area; AA). The left side the shaved area was treated with sterile water (water area; WA) instead of acetone and served as the control for the experiment (Figure 1). The above treatment was performed daily (9:00AM) under anesthesia (ketamine/xylazine, 60/15 mg/kg, IM, respectively) for 3 consecutive days. After 1h of the last cutaneous acetone or steril water treatment, measurements involved non-invasive techniques allowed to quantify the skin characteristics.

Acetone-induced dry skin model rat

The hair of rats was shaved under anesthesia (ketamine/xylazine, 60/15 mg/kg, IM, respectively) using an electric shaver and after a soft hair-removing cream over the dorsal part (6x6cm) at least 24 h before the start of the experiment and divided into two areas according treatment cutaneous. Acetone treatment was used as described previously with some modifications (TOMINAGA et al., 2007) for skin barrier disruption. The right side of the shaved back was applied the treatment with cotton (3x3cm) soaked with acetone for 5 min (acetone area; AA). The left side the shaved area was treated with sterile water (water area; WA) instead of acetone and served as the control for the experiment (Figure 1). The above treatment was performed daily (9:00) under ether anesthesia for 3 consecutive days. After 1h of the last cutaneous acetone or steril water treatment on 30^{th}, 60^{th} and 90^{th} of the FO supplementation, the scratching behavior was quantified and measurements involved non-invasive techniques allowed to quantify the skin characteristics.

Transepidermal water loss (TEWL), skin hydration and cutaneous microcirculation

Animals were slightly anesthetized by inhalation of ether, in a temperature (22-24±8ºC) and humidity (40-50%) controlled room, and TEWL was then measured at both dorsal areas (WA and AA) using Tewameter TM 210 (Courage + Khazaka GmbH, Cologne, Germany). TEWL is given in grams per hour per square meter ($g/h.m^2$). Skin hydration was evaluated by measuring electrical capacitance with a Corneometer CM825 (Courage + Khazaka Electronic GmbH, Cologne, Germany) and is given in arbitrary units (CM unit). Capillary blood flow was determined by laser Doppler flowmetry (O_2C System, Lea Instruments, Giessen, Germany) and is given in arbitrary units (AU). Measurement was repeated 3 times to give rise to an average value for each area in each rat.

Measurement of scratching behavior

The scratching behavior of rats was quantified for 30 min at 1 h after the acetone treatment on day 3. The number of scratching actions was counted. It was considered one scratching action to be a series of scratch movements on the dorsal skin using the hind paws, as reported previously (AKASAKA et al., 2011).

Table 1. Fatty acids composition (% of total identified FA) of the chow and fish oil supplemented to different experimental groups.

Fatty acids	Chow	FO
Σ SFA	24.45	31.38
Σ MUFA	34.34	22.70
C 18:3n-3	2.88	1.35
C 20:5 n-3	0.00	21.29
C 22:5 n-3	0.00	2.58
C 22:6 n-3	0.00	13.71
Σ n-3	2.88	38.93
Σ n-6	37.33	2.88
n-6/n-3 ratio	12.96	0.07

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids. Values representthe mean±S.E.M. (P<0.05).

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

Fatty acids (mean±SEM)	**C**	**FO**
Σ SFA	34.04±0.95	34.82±0.77
Σ MUFA	34.46±0.97	33.43±1.02
C 18:3n-3	1.23±0.04	1.24±0.04
C 20:5n-3	0.56±0.18	1.24±0.14*
C22:5n-3	0.71±0.12	1.19±0.06*
C 22:6n-3	1.64±0.29	2.97±0.12*
Σ n-3	4.13±0.59	6.57±0.39*
Σ n-6	26.80±0.87	25.81±0.25
n-6/n-3 ratio	7.84±0.96	4.06±0.29*

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.*Indicate significant difference of the control (C) group (P<0.05).

Legends

Figure 1. Influence of n-3 FA provided during 90 days to rats supplemented with FO on transpidermal water loss (TWEL) in dorsal skin of rats exposed or not to acetone. Data expressed as means±S.E.M. (n=10). (P<0.05) [&]Indicates significant difference between groups in the same evaluation time. *Indicates significant difference from basal in the same group. [+]Indicates significant difference from 1h in the same group. [#]Indicates significant difference from 24h in the same group.

Figure 2. Influence of n-3 FA provided during 90 days to rats supplemented with FO on dorsal skin hydration of rats exposed or not to acetone. Data expressed as means±S.E.M. (n=10). (P<0.05)[&]Indicates significant difference between groups in the same evaluation time. *Indicates significant difference from basal in the same group. [+]Indicates significant difference from 1h in the same group. [#]Indicates significant difference from 24h in the same group.

Figure 3. Influence of n-3 FA provided during 90 days to rats supplemented with FO on dorsal skin capillary blood flow of rats exposed or not to acetone. Data expressed as means±S.E.M. (n=10). (P<0.05) [&]Indicates significant difference between groups in the same evaluation time; *Indicates significant difference from basal in the same group. [+]Indicates significant difference from 1h in the same group. [#]Indicates significant difference from 24h in the same group.

Figure 4. Rats' scratching behavior (number of scratchings/30 min) 1h after the last cutaneous acetone (n=5) or water exposure (n=5). *Indicates significant difference from control (C) group. [+]Indicates significant difference from FO group. Data are expressed as mean ± S.E.M. (P<0.05)

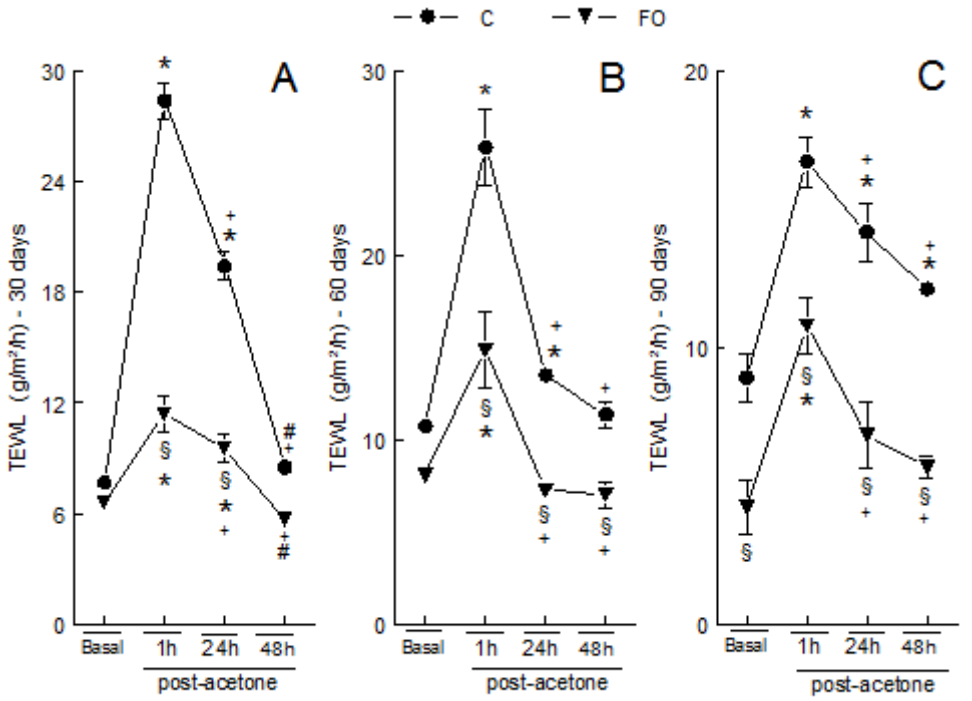

Figure 1

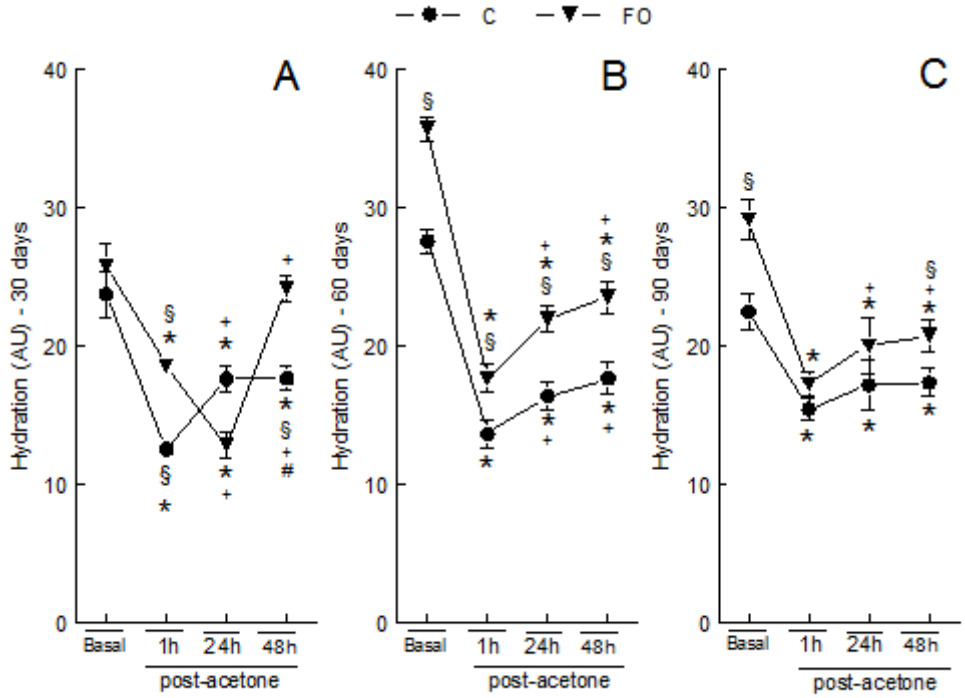

Figure 2

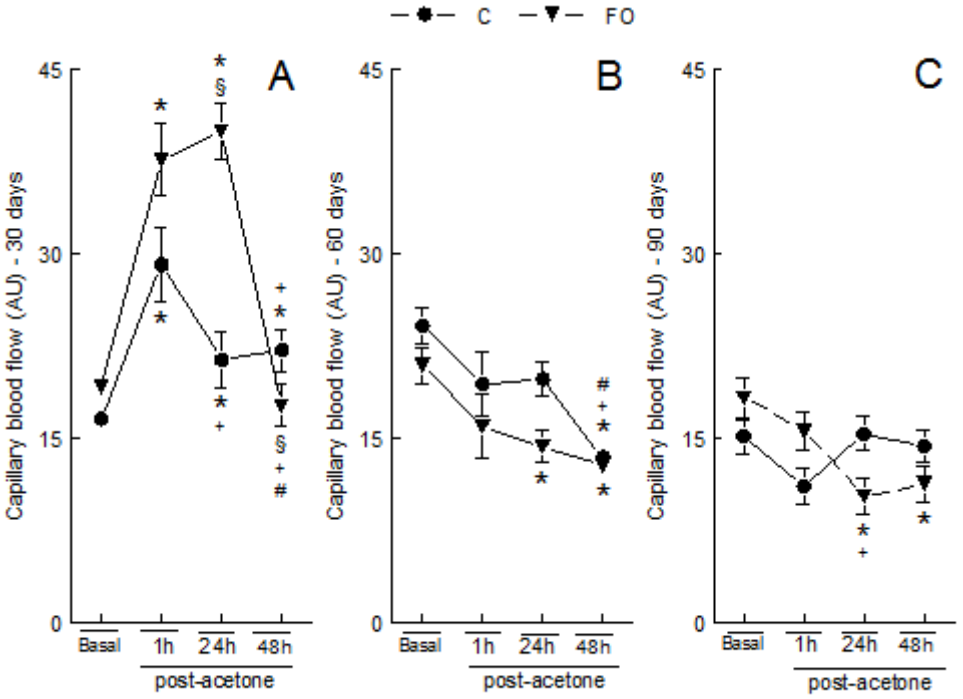

Figure 3

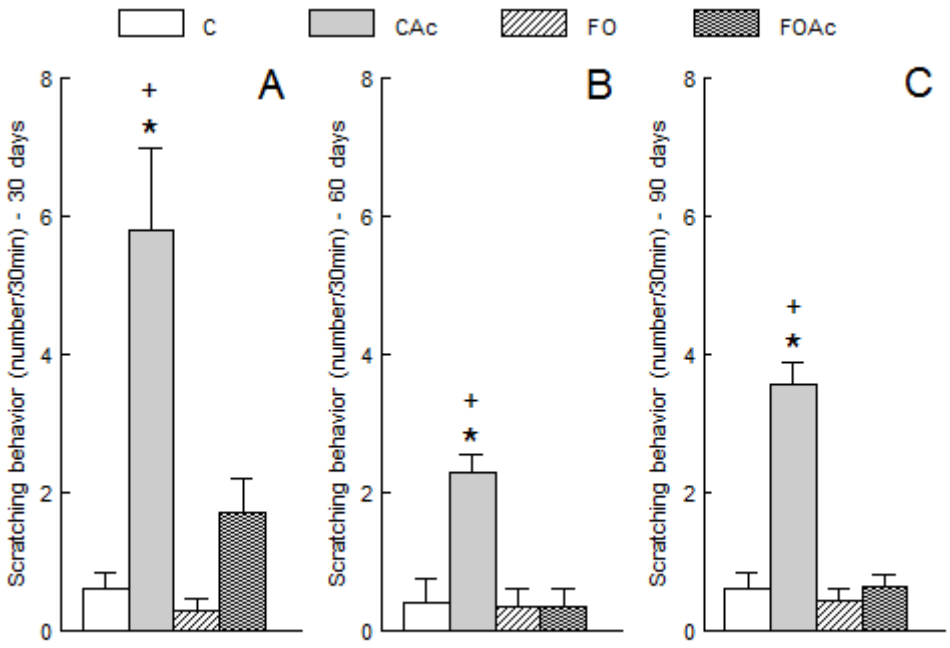

Figure 4

DISCUSSÃO

Na continuação dos estudos, realizou-se Doutorado Sanduíche no Research Institute for Medicines and Pharmaceutical Sciences (iMED.UL) da Faculdade de Farmácia da Universidade de Lisboa, na cidade de Lisboa/Portugal. O estudo conduzido foi centralizado na padronização de um modelo experimental de dermatite atópica/pele seca em ratos Wistar e na investigação da possível influência da suplementação diária de óleo de peixe, fonte de AG n-3, sobre parâmetros de perda transepidérmica de água, hidratação, microcirculação e prurido da pele no modelo animal anteriormente citado.

O estudo realizado configurou-se pelo propósito de complementação do estudo da influência dos AGPI n-3 sobre a pele iniciado no Programa de Pós-graduação em Farmacologia na Universidade Federal de Santa Maria (UFSM). Configurou-se também pela possibilidade de oportunizar intercâmbio de estudos, processos e resultados já obtidos nas experiências em desenvolvimento nesta pesquisa e nas desenvolvidas no iMED da Faculdade de Farmácia da Universidade de Lisboa (Portugal). Os AG podem modular as condições fisiológicas e patológicas através de vários mecanismos, tais como a resposta inflamatória (KANG; WEYLANDT, 2008) e têm recebido grande atenção nos últimos anos por seu papel fundamental na prevenção e tratamento de doenças (GEBAUER et al., 2006).

Os resultados obtidos em Portugal e publicados noartigo 3 demonstraram a possibilidade de desenvolvimento de modelo animal de dermatite atópica através da exposição da pele dorsal previamente depilada de ratos Wistar à acetona. Nesse modelo, as lesões cutâneas mimetizaram àquelas presentes na dermatite atópica, com alterações fisiológicas da pele características dessa desordem cutânea, nomeadamente, maior TEWL, menor hidratação e maior circulação sanguínea cutânea. Ademais, as lesões cutâneas foram facilmente induzíveis, além de tal modelo animal possuir um baixo custo de execução. Os animais também desenvolveram prurido na pele dorsal exposta à acetona, quantificado através dos movimentos de coçar, sintoma associado às afecções cutâneas que se apresentam como uma pele seca. Assim, tal modelo pode servir como uma ferramenta para o estudo da fisiopatologia da dermatite atópica humana e ser útil para a avaliação da eficácia de novas abordagens terapêuticas para seu tratamento.

Na continuidade dos estudos, prosseguimos com a avaliação da influência da suplementação diária com óleo de peixe no modelo animal de pele seca em ratos

anteriormente desenvolvido, correspondente ao manuscrito 2. Nesse momento, podemos comprovar que as lesões induzidas pela exposição tópica à acetona são facilmente reproduzíveis e induzíveis. Assim, o fenótipo e a fisiologia cutânea da dermatite atópica, com maior TEWL, menor hidratação e maior circulação sanguínea cutânea, associados ao prurido, foram observadas nos animais expostos topicamente à acetona, de ambos os grupos controle e óleo de peixe. A suplementação com óleo da peixe *per se* melhorou a hidratação da pele dorsal dos ratos de maneira tempo-dependente e, pós-acetona, esse grupo apresentou as mesmas alterações observadas no grupo controle exposto à acetona, porém em menor grau. Além disso, o prurido foi totalmente prevenido pela suplementação com óleo de peixe. Esses resultados demonstram as propriedades de recuperação da barreira cutânea, hidratante a antiinflamatória do óleo de peixe, relacionadas e demonstradas através da menor perda transepidérmica de água e ausência de pruriro, maior hidratação e menor circulação cutânea pós-acetona, respectivamente.

CONCLUSÃO

- ✓ A exposição da pele de ratos à acetona induz danos à integridade da barreira cutânea, em parte, pela remoção de lipídios e pode servir como modelo de DA humana;
- ✓ A suplementação com óleo de peixe altera a resposta cutânea frente ao modelo animal de pele seca;
- ✓ A suplementação ou dieta rica em óleo de peixe torna a pele menos vulnerável aos danos oxidativos causados pela por agentes externos como a RUV ou indutores de pele seca.

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CONCLUSÃO GERAL

Tomados em conjunto, os resultados apresentados nesta tese demonstram a influência deletéria do consumo de AGT sobre a pele, tornando-a mais suscetível aos danos causados pela exposição aguda e crônica à RUV. Além disso, tais estudos demonstram que os diferentes AG podem ser incorporados na pele em diferentes períodos de vida dos animais, o que configurou nosso maior objetivo e o maior achado dessa tese. Tal incorporação cutânea dos AG se relaciona diretamente com os diferentes agressores cutâneos internos (envelhecimento, doenças sistêmicas) e externos (RUV, inverno, detergentes), alterando a resposta da pele frente a esses danos, conferindo proteção ou facilitando a ocorrência das desordens cutâneas.

É possível estimar que a dieta ocidental, com abundância de AGT, especialmente devido à industrialização dos alimentos, em detrimento dos AGPI n-3, pode ser relacionada ao aumento dos danos cutâneos da exposição à RUV. Por outro lado, a partir dos resultados obtidos nos estudos da suplementação com óleo de peixe acerca de diversos parâmetros da pele (perda transepidérmica de água, hidratação, fluxo sanguíneo, prurido), é possível sugerir que o seu consumo regular e continuado é fundamental as condições de saúde da pele(MAURETTE, 2008). Felizmente, nossos estudos também apontam para a idéia que a substituição dos AG na pele é possível em curto intervalo de tempo, indicando que uma mudança dos hábitos alimentares podem reduzir os danos oxidativos e o desenvolvimento de doenças de pele de diferentes etiologias.