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INFLUÊNCIA DA SUPLEMENTAÇÃO DE ÁCIDOS GRAXOS DURANTE DUAS GERAÇÕES DE RATOS: PARÂMETROS COMPORTAMENTAIS E OXIDATIVOS APÓS ESTRESSE AGUDO

DISSERTAÇÃO DE MESTRADO

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Santa Maria, RS, Brasil

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INFLUÊNCIA DA SUPLEMENTAÇÃO DE ÁCIDOS GRAXOS DURANTE DUAS GERAÇÕES DE RATOS: PARÂMETROS COMPORTAMENTAIS E OXIDATIVOS APÓS ESTRESSE AGUDO

Camila Simonetti Pase

Dissertação apresentada ao Curso de Mestrado do Programa de Pós-Graduação em Farmacologia, Área de Concentração em Neuropsicofarmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Mestre em Farmacologia**.

Orientadora: Profª. Dra. Marilise Escobar Bürger

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elaborada por
Camila Simonetti Pase

como requisito parcial para obtenção do grau de **Mestre em Farmacologia**

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Santa Maria, 30 de agosto de 2013.

“No momento em que uma célula masculina microscópica e serpenteante encaminha-se para a célula-ovo muito maior e liga-se a ela, um ser humano começa a existir, e a Nutrição tem início. Este período de desenvolvimento, quando as coisas podem ser definitivamente ‘certas’ ou ‘erradas’, é de vital importância, e a nutrição pode exercer uma profunda influência que se expande por toda a vida.”

(Roger Willians)

Dedico este trabalho à Inês S. Pase ,
admirável lutadora e incansável mãe que me mostrou que
nada é impossível pra quem tem amor à vida e à família.
Mãe, te amo pra sempre!

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As minhas amadas irmãs Duda e Amandinha, que de maneiras diferentes me ensinaram diversas coisas.

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À Universidade Federal de Santa Maria, ao Programa de Pós-Graduação em Farmacologia e seus professores e funcionários, pela possibilidade de realização deste curso.

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“Eu poderia viver recluso numa casca de noz
e me considerar rei do espaço infinito...”

Shakespeare (Hamlet, ato 2, cena 2)

RESUMO

Dissertação de Mestrado
Programa de Pós-Graduação em Farmacologia
Universidade Federal de Santa Maria

INFLUÊNCIA DE DIFERENTES ÁCIDOS GRAXOS SOBRE PARÂMETROS COMPORTAMENTAIS E OXIDATIVOS EM RATOS SUBMETIDOS AO ESTRESSE AGUDO

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ORIENTADORA: MARILISE ESCOBAR BÜRGER
Data e Local da Defesa: Santa Maria, 30 de agosto de 2013.

Os ácidos graxos (AG) são constituintes importantes das membranas fosfolipídicas cerebrais e desempenham importantes funções no sistema nervoso central (SNC) podendo modificar a plasticidade e fluidez, além de atuar de forma decisiva no desenvolvimento de patologias cognitivas e neuropsiquiátricas. Durante as últimas décadas, foram observadas mudanças nos hábitos alimentares, as quais possibilitaram o aumento do consumo de ácidos graxos *trans,* em detrimento do consumo de ácidos graxos poliinsaturados (AGPI), principalmente o ômega-3. Além disso, situações frequentes de estresse devido a pressão do mundo exterior também podem estar associadas com o desenvolvimento de doenças que envolvem o SNC e alterações na função metabólica, particularmente no metabolismo dos ácidos graxos. Neste estudo, duas gerações sequenciais de ratas foram suplementadas com óleo de soja (C-OS), óleo de peixe (OP) e gordura vegetal hidrogenada (GVH) durante a gestação, lactação e após o desmame. Aos 41 dias de idade, parte dos animais machos, da segunda geração, foram expostos ao estresse agudo (EA-2h), enquanto a outra metade foi utilizada como controles, e avaliados em campo aberto e labirinto em cruz elevado, seguido por eutanásia para análises bioquímicas. O grupo suplementado com GVH, não expostos ao estresse, apresentou maiores sintomas de ansiedade, enquanto os grupos C-OS e OP não mostraram esses comportamentos. Entre os grupos expostos ao EA, o GVH mostrou maior locomoção e sintomas semelhantes à ansiedade, mas isso não foi observado no grupo OP. As análises bioquímicas mostraram níveis mais elevados de peroxidação lipídica e menor viabilidade celular no córtex do grupo GVH. Além disso, os ratos tratados com GVH mostraram reduzida atividade da catalase no estriado e hipocampo, bem como aumento da geração de espécies reativas no estriado, ao passo que OP foi associado com aumento da viabilidade celular no hipocampo. Entre os grupos expostos ao EA, o grupo GVH mostrou aumento da geração de espécies reativas no cérebro, diminuição da viabilidade celular no córtex e estriado, e diminuição da atividade da catalase no estriado e hipocampo. Os resultados mostram que a presença de AG durante o desenvolvimento e crescimento ao longo de duas gerações é capaz de modificar parâmetros de comportamento e status oxidativo do cérebro. Tomados em conjunto, nossos dados suportam a ideia de que o consumo regular de uma dieta rica em ácidos graxos monoinsaturados (AGM) e AGPI, e particularmente pobre em alimentos processados, pode ajudar a prevenir o desenvolvimento de distúrbios emocionais, além de sugerir a influência nociva do consumo de gordura *trans* ao longo de gerações, a qual é capaz de aumentar parâmetros de emocionalidade e ansiedade após situações estressantes da vida

cotidiana podendo desencadear condições neurológicas e neuropsiquiátricas mais graves.

Palavras-chave: Sistema nervoso central. Ácidos graxos *trans*. Ácidos graxos n-3. Ansiedade. Estresse oxidativo. Estresse agudo.

ABSTRACT

Master Dissertation
Graduate Program in Pharmacology
Federal University of Santa Maria

INFLUENCE OF DIFFERENT FATTY ACID ON BEHAVIORAL PARAMETERS AND OXIDATIVE STRESS IN RATS AFTER ACUTE STRESS

AUTHOR: CAMILA SIMONETTI PASE
ADVISOR: MARILISE ESCOBAR BÜRGER
Date and Place of defense: August 30, 2013, Santa Maria.

The fatty acids (FA) are important constituents of brain phospholipid membranes and play important roles in the central nervous system (CNS) may modify the plasticity and fluidity, and act decisively in the development of cognitive and neuropsychiatric disorders. During the last decades, we observed changes in eating habits, which enabled increased consumption of *trans* fatty acids at the expense of consumption of polyunsaturated fatty acids (PUFA), especially omega-3. Furthermore, frequent situations of stress due to pressure of the outside world may also be associated with the development of diseases involving CNS and changes in metabolic function, particularly in the metabolism of fatty acids. In this study, two sequential generations of rats were supplemented with soybean oil (C-SO), fish oil (FO) and hydrogenated vegetable fat (HVF) during pregnancy and lactation. At 41 days of age, half of the male animals of each group were exposed to acute stress (AE-2h) and evaluated in the open field and elevated plus maze, followed by euthanasia for biochemical analysis. The HVF supplemented group had higher anxiety symptoms, while groups C-SO and FO did not show these behaviors. Among the groups exposed to AE, the HVF showed greater locomotion and symptoms similar to anxiety, but this was not observed in the FO. Biochemical analyzes showed higher levels of lipid peroxidation and reduced cell viability in the cortex of HVF group. Furthermore, the HVF treated rats showed reduced catalase activity in the striatum and hippocampus, and increased generation of reactive species in the striatum, while FO was associated with increased cell viability in the hippocampus. Among the groups exposed to AE, the HVF group showed increased generation of reactive species in the brain, decreased cell viability in the cortex and striatum, and decreased catalase activity in the striatum and hippocampus. The results show that the presence of FA for the development and growth over two generations is capable of modifying parameters of oxidative status and behavior of the brain. Taken together, our data support the idea that regular consumption of a diet rich in monounsaturated fatty acids (MUFA) and PUFA, and particularly low in processed foods, can help prevent the development of emotional disorders, and suggest the influence harmful consumption of *trans* fat over generations, which is able to increase parameters of emotion and nervousness after stressful situations of everyday life can trigger neurological and neuropsychiatric conditions more severe.

Keywords: Central nervous system. *Trans* fatty acids. n-3 fatty acids. Anxiety. Oxidative stress. Acute stress.

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LISTA DE ABREVIATURAS E SIGLAS

AGT - ácidos graxos *trans*

SNC - sistema nervoso central

AGPI - ácidos graxos poliinsaturados

AG - ácido graxo

AGS – ácidos graxos saturados

AGMI - ácidos graxos monoinsaturados

AGE - ácidos graxos essenciais

LA - ácido linoleico

AA - ácido araquidônico

ALA - ácido α-linolênico

DHA - ácido docosahexaenóico

EPA - ácido eicosapentaenoico

EO – estresse oxidativo

GVH – gordura vegetal hidrogenada

OP – óleo de peixe

OS – óleo de soja

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APRESENTAÇÃO

Esta dissertação está estruturada em seções dispostas da seguinte forma: Introdução, Objetivos, Artigo Científico, Conclusões, Perspectivas e Referências Bibliográficas.

No item **INTRODUÇÃO** encontram-se considerações iniciais sobre o tema desenvolvido nesta dissertação. Os resultados estão apresentados sob a forma de artigo no item **ARTIGO CIENTÍFICO**, onde se encontram os itens Introdução, Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas, representando a íntegra deste estudo.

Ao fim desta dissertação encontra-se o item **CONCLUSÕES**, no qual há interpretações e comentários gerais sobre o artigo científico contido neste estudo.

As **REFERÊNCIAS BIBLIOGRÁFICAS** referem-se somente às citações apresentadas no item **INTRODUÇÃO** desta dissertação.

1 INTRODUÇÃO

Inúmeras evidências atualmente indicam que a etiologia de várias doenças neurológicas é multifatorial e envolve uma ampla variedade de fatores como inflamação, metabolismo e também genética. Característicos da sociedade moderna, a alteração nos hábitos alimentares com o elevado consumo de alimentos industrializados, principalmente os *fast-food,* ricos em ácidos graxos *trans* (AGT) e saturados (CRAIG-SCHMIDT, 2006) e situações frequentes de estresse, devido a pressão do mundo exterior, (FERRAZ et al., 2011) podem ser considerados fatores de risco para o desenvolvimento de doenças que envolvem o sistema nervoso central (SNC).

Nas últimas décadas, foi observado um aumento na utilização de gordura vegetal hidrogenada e gordura saturada nos alimentos industrializados (BAGGIO e BRAGAGNOLO, 2006) acompanhado por uma significativa redução do consumo de alimentos ricos em ômega-3 (n-3) e um aumento na ingestão de AGT (PFEUFFER e SCHREZENMEIER, 2006). Essa mudança nos hábitos alimentares promoveu um aumento da relação n-6/n-3 de ácidos graxos poliinsaturados (AGPI), principalmente pela ingestão reduzida de ácidos graxos ômega-3 (n-3) (AILHAUD et al., 2006). Desta forma, o consumo de AGT pode culminar em uma perda de ingestão de ácidos graxos essenciais representando uma perda no valor nutricional dos alimentos, e consequentemente, ter um impacto perigoso e imprevizível para a saúde humana, uma vez que estes desempenham um papel funcional importante sobre as membranas biológicas (SARSILMAZ et al., 2003).

Além disso, o estresse também está ligado ao desenvolvimento de doenças do SNC (CHROUSOS, 2009; McEWEN, 2008; SCHMIDT, STERLEMANN e MÜLLER, 2008). Atualmente, é considerado uma adaptação funcional orgânica, que tem como causa principal a pressão do mundo exterior. Embora a base subjacente para a mudança funcional endócrina que ocorre durante o estresse ainda seja desconhecida, existem trabalhos sugerindo que as mudanças na função metabólica, particularmente no metabolismo de ácidos graxos, influenciam mecanismos centrais que regulam as respostas individuais ao estresse (LAUGERO et al., 2002). Além disso, a influência da dieta sobre a manifestação de diferentes tipos de

comportamentos induzidos por agentes estressores (FERRAZ et al., 2008) tem sido observada.

A seguir, um breve referencial teórico sobre os principais ácidos graxos constituintes da dieta e sua possível ação sobre as membranas cerebrais. Serão também considerados os efeitos do estresse sobre o SNC, além da interação entre ácidos graxos da dieta e estresse sobre a proteção contra danos cerebrais.

1.1 Ácidos Graxos: Química, classificação geral e principais representantes

Os ácidos graxos (AG) são substâncias encontradas em uma ampla variedade de alimentos e possuem funções estruturais, protetoras e de fornecimento e armazenamento de energia (COSTA e SILVA, 2002). São formados por uma cadeia hidrocarbonada (2 a 20 ou mais átomos), contendo uma carboxila (COOH) em um extremo da cadeia e uma metila (CH3) na extremidade oposta (MARSZALEK e LODISH, 2005). A nomenclatura dos ácidos graxos refere-se ao número de átomos de carbono, quantidade e posição das duplas ligações em relação ao grupo metila. Existe inúmeros comprimentos de cadeia, variando de ácidos graxos de 4 carbonos em lipídeos de produtos lácteos a ácidos graxos contendo 30 carbonos em alguns lipídios marinhos (LEHNINGER, NELSON e COX, 2002; RATNAYAKE e GALLI, 2009) (Figura 1). 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. Os AG são classificados também de acordo com o número de insaturações presentes em sua cadeia carbonada (LEHNINGER, NELSON e COX, 2002). Assim, os AG saturados (AGS) não possuem duplas ligações e os insaturados contêm em sua cadeia uma – monoinsaturados (AGMI) - ou mais duplas ligações – poliinsaturados (AGPI) (KIM et al., 2005). As múltiplas duplas ligações de carbono presentes na cadeia dificultam a interação molecular, conferindo característica líquida a esses ácidos graxos, a temperatura ambiente, importante propriedade física requerida para manutenção do alto grau de flexibilidade da bicamada lipídica das membranas celulares (CHALON, 2006; HULBERT et al., 2005; INNIS, 2007; MARZZOCO e TORRES, 1999).

Em mamíferos, incluindo seres humanos, ácidos graxos saturados e monoinsaturados podem ser obtidos pela dieta ou, podem ser sintetizados pela síntese “de novo” de ácido graxo. Pela dessaturação dos ácidos graxos saturados podem ser sintetizados os ácidos graxos monoinsaturados n-9. Essa conversão é realizada pela Δ9 desaturase, que é uma enzima muito ativa em tecidos de mamíferos, que introduz uma dupla ligação entre a posição n-9 – n-10 da cadeia de ácidos graxos (RATNAYAKE e GALLI, 2009). Assim, estes ácidos graxos

sintetizados pelo organismo são considerados não essenciais. Os AGS são encontrados em alimentos de origem animal como carne, leite, manteiga, queijo (ácidos palmítico - C16:0 e esteárico - C18:0), certos vegetais como coco, palma e dendê (ácidos caprílico - C8:0 e cáprico - C10:0) (CARVALHO et al., 2003; SALEM, 1999), assim como os AGMI, os quais também se encontram no azeite de oliva, óleo de canola e de soja e em nozes, sendo o ácido oléico (C18:1) o principal representante da classe (DUNCAN, SCHMIDT e GIUGLIANI, 2004).

Porém, os mamíferos não conseguem sintetizar ácidos graxos com a dupla ligação no carbono n-6 ou n-3, por esta razão, os AGPI classificados principalmente nas séries ômega 6 e ômega 3, diferenciando-se pela posição da primeira dupla ligação a partir do grupo metílico terminal da cadeia são considerados ácidos graxos essenciais (AGE) (HARDMAN, 2004; SIMOPOULOS, 2006; YAQOOB, 2004; YAQOOB e CALDER, 2007). Dentre os representantes da série n-6, destaca-se o ácido linoléico (C18:2, LA) abundante nos óleos vegetais como de girassol, milho, soja, etc. (McCUSKER e GRANT-KELS, 2010; SANGIOVANNI e CHEW, 2005). O ácido α-linolênico (C18:3, ALA), principal representante da série n-3, é encontrado em 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, HEIDMANN e OETTERER, 2003; WAINWRIGHT, 1992). Os AG com maior número de carbonos e maior quantidade de duplas ligações como o ácido eicosapentaenóico (EPA, C20:5 n-3) e o ácido docosahexaenóico (DHA, C22:6 n-3), são encontrados tanto nos vegetais (algas, fitoplâncton), quanto nos animais de origem marinha (peixes, crustáceos) (GIBSON, 2004; HULBERT et al., 2005; WAINWRIGHT, 1992).

Os AGT são isômeros geométricos e de posição dos AG insaturados naturais e também fazem parte da dieta humana. Produtos de origem animal como carne e leite de animais ruminantes são as principais fontes de AGT, porém, o avanço da industrialização e mudanças na dieta ocidental vem promovendo um considerável aumento no consumo deste lipídio (PADOVESE e MANCINI-FILHO, 2002).

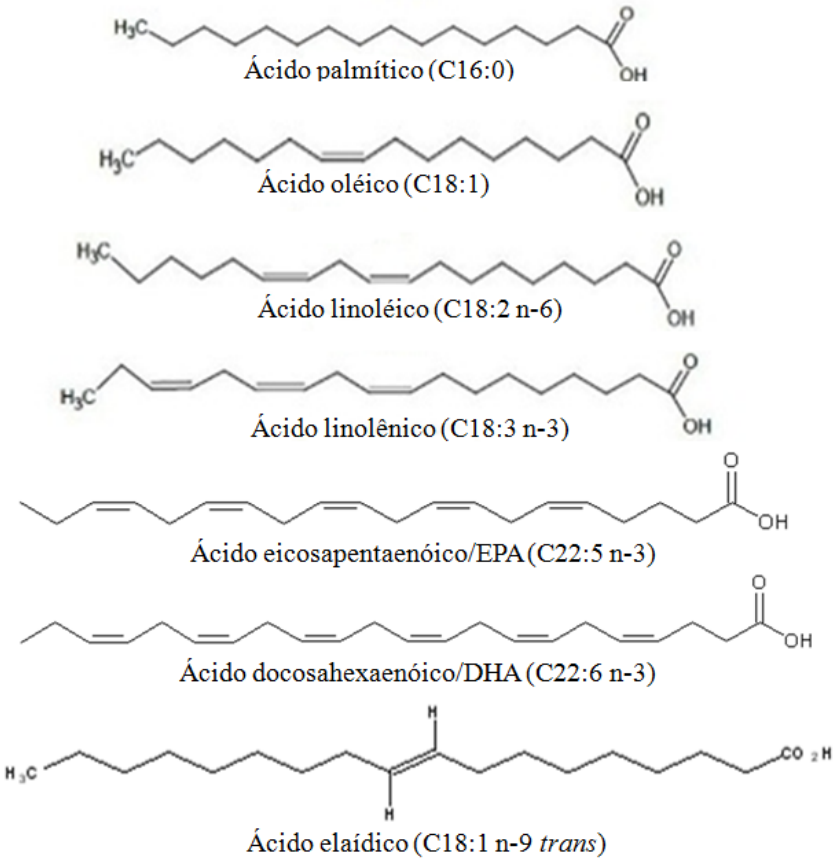

Figura 1. Principais representantes dos AG.

1.2 Ácidos Graxos Poliinsaturados

No fim da década de 20, ao observar alguns sinais e sintomas em pessoas que tinham restrição de gorduras em suas dietas, pesquisadores constataram que havia compostos que eram essenciais para a saúde do organismo (BURR e BURR, 1929) e desde então os ácidos graxos vem sendo estudados. Os AGPI são considerados AG essenciais, pois as células animais não possuem as enzimas dessaturases capazes de especificamente colocar as duplas ligações nas posições n-3 e n-6, de forma que seu suprimento depende unicamente da dieta alimentar (BOURRE, 2004; CARRIE et al., 2000; OKEN e BELFORT, 2010).

Os ácidos linoléico e α-linolênico, quando consumidos, podem ser alongados em cadeias de, pelo menos, 20 ou 22 carbonos (Figura 2). O LA pode ser metabolizado em outros AG da série n-6, incluindo o ácido araquidônico (AA, 20:4 n-

6), enquanto o ALA é metabolizado em outros AG n-3, entre eles o EPA e o DHA (MANTZIORIS, CLELAND e GIBSON, 2000). Este processo metabólico é mediado por enzimas conhecidas como alongases (adição de duas unidades de carbono) e dessaturases (adição da dupla ligação), as quais participam na formação dos AGPI de cadeia longa, resultando em uma competição metabólica entre os dois grupos (SALEM, 1999). Como resultado dessa competição, Emken et al. (1994) demostraram uma redução em aproximadamente 50% de AGPI de cadeia longa formados a partir de ALA quando o consumo de LA foi duplicado. Neste sentido, um excesso de LA poderá dificultar a transformação de ALA nos derivados EPA e DHA e vice-versa. O equilíbrio do consumo desses dois subtipos de ácidos graxos é necessário para a manutenção adequada de diferentes funções fisiológicas (SALEM, 1999).

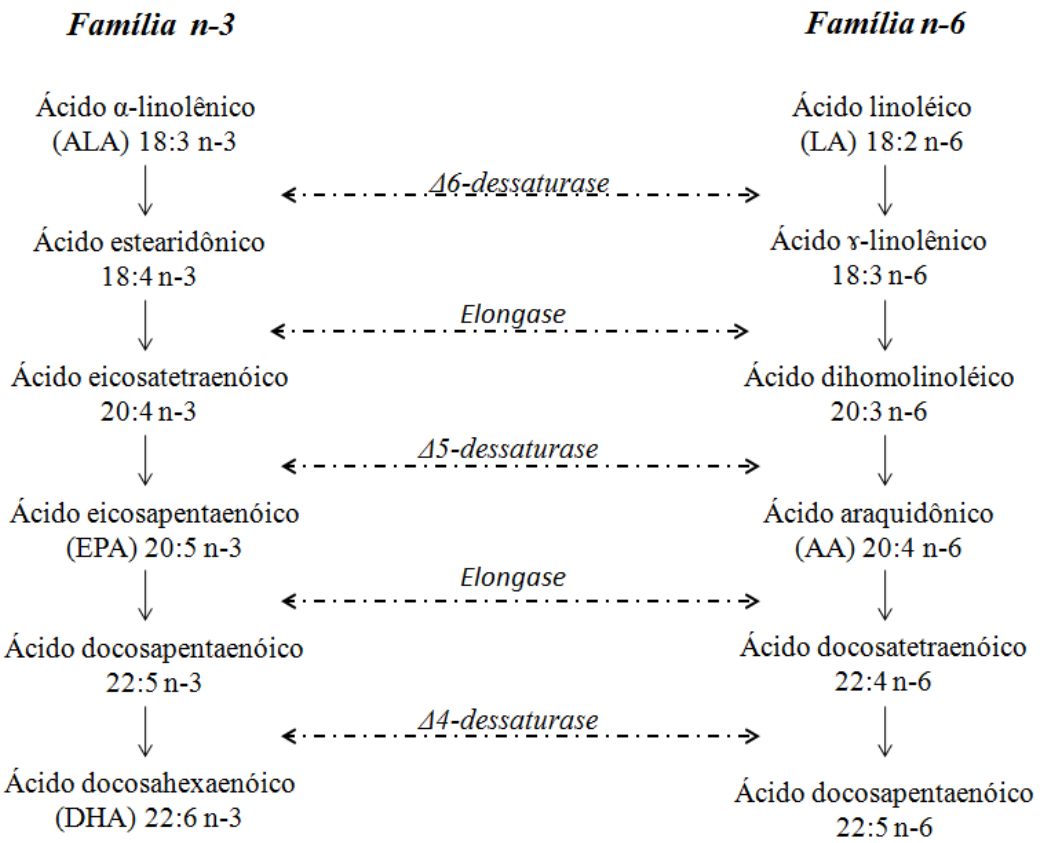

Figura 2. Via metabólica dos ácidos graxos essenciais de cadeia longa (adaptado de LAURITZEN et al., 2001).

Os efeitos benéficos dos AGPI sobre as funções cerebrais parece estar relacionado à sua influência sobre a fluidez da membrana, atividade de enzimas ligadas a membrana, número e afinidade de receptores, produção e atividade de neurotransmissores, além da transdução de sinais que controlam a atividade de neurotransmissores e fatores de crescimento neuronal (CLARKE et al., 2005; DAS, 2003; LEVANT, RADEL e CARLSON, 2004; McNAMARA e CARLSON, 2006; SUMIYOSHI et al., 2008; YEHUDA, RABINOVITZ e MOSTOFSKY, 2005).

1.3 Ácidos graxos essenciais e SNC

A literatura atual possui resultados consistentes que indicam que tanto a parte química, quanto funcional do desenvolvimento e maturação do cérebro pode ser influenciada pela dieta (WAINWRIGHT, 2002). O cérebro necessita de um aporte adequado de ácidos graxos para manter sua integridade estrutural e, consequentemente suas funções normais, principalmente por apresentar a segunda maior concentração de lipídios do corpo, depois do tecido adiposo, representando 36-60% do tecido nervoso (MARTEINSDOTTIR et al., 1998; SINCLAIR et al., 2007; UAUY e DANGOUR, 2006).

O DHA é o principal componente dos fosfolipídios das membranas neuronais, abrangendo cerca de 17% do total dos ácidos graxos nesse tecido (HORROCKS e FAROOQUI, 2004; LEHNINGER et al., 1998; MORIGUCHI, GEINER e SALEM, 2000; SALEM et al., 2001), sendo um dos grandes responsáveis por aumentar a fluidez da membrana e a plasticidade sináptica, contribuindo para as funções cerebrais via processos de transdução de sinal (MITCHELL et al., 2003; MURPHY, 1990). A presença do DHA nessas estruturas favorece a flexibilidade e a mobilidade das proteínas na bicamada de fosfolipídios, características essenciais para as respectivas funções (BERTRAND, O´KUSKY e INNIS, 2006; INNIS, 2007; PONGRAC, SLACK e INNIS, 2007; SALEM et al., 2001; SHAIKH et al., 2003; SINCLAIR et al., 2007; SU et al., 2003; STILLWELL et al., 2005).

Dados experimentais demonstraram que o DHA e o EPA são antioxidantes nutricionais e reduzem a formação de peróxidos de lipídeos no cérebro de ratos (CHOI-KWON et al., 2004; HOSSAIN et al., 1999) além de proteger ratos jovens

contra eventos de excitotoxicidade , como convulsão e isquemia (BAS et al., 2007; HÖGYES et al., 2003; STROKIN, SERGEEVA e REISER, 2007). Em humanos, o DHA parece exercer um efeito neuroprotetor, uma vez que baixos níveis deste ácido graxo foram associados com doenças neurodegenerativas, como a doença de Alzheimer (SCHAEFER et al., 2006; SODERBERG et al., 1991). A atividade neuroprotetora do DHA também foi evidenciada através de suas propriedades antioxidantes *in vivo* (CALON et al., 2004; HASHIMOTO et al., 2002; YAVIN, BRAND e GREEN, 2002) e *in vitro*, como no aumento da atividade da glutationa redutase (HASHIMOTO et al., 2002), diminuição da oxidação de proteínas (CALON et al., 2004) e dos níveis de peróxidos de lipídios e espécies reativas de oxigênio (HASHIMOTO et al., 2002; 2006). Estudos demonstraram ainda que o DHA participa diretamente da modulação da expressão gênica, de processos que envolvem estresse oxidativo, sinalização e divisão celular, crescimento e apoptose (SIMOPOULOS, 2006; YAVIN, 2006). Além disso, DHA e EPA podem modificar a produção e a função de neurotransmissores, tais como a serotonina e a dopamina (DUBOIS et al., 2006; FENTON, HIBBLEN e KNABLE, 2000). Sendo assim, DHA e EPA participam de numerosas funções celulares, incluindo a fluidez e a atividade enzimática de membrana e síntese de eicosanóides, os quais são essenciais para o desenvolvimento e a manutenção das funções cerebrais (MAZZA et al., 2007).

De particular importância para as funções do SNC, a deficiência dietética e baixos níveis endógenos de ácidos graxos n-3 tem sido associados a um pior prognóstico de doenças psiquiátricas, depressão (FERRAZ et al., 2008), desordens de hiperatividade (BURGESS et al., 2000), processo de envelhecimento e déficits de aprendizado e memória (BOURRE, 2004). Por outro lado, a suplementação destes ácidos graxos foi benéfica em pacientes com depressão, doença bipolar e esquizofrenia (STOKES e PEET, 2004), capaz de reduzir o estresse oxidativo (EO) em regiões cerebrais críticas, como o corpo estriado (SARSILMAZ et al., 2003) e o hipotálamo (SONGUR et al., 2004), podendo exibir proteção contra parâmetros oxidantes presentes em doenças neurológicas e neuropsiquiátricas (BLACK et al., 1984).

1.4 Ácidos graxos *trans* na dieta

Os ácidos graxos na dieta humana são encontrados naturalmente na configuração *cis*, a qual os átomos de hidrogênio ligados aos carbonos insaturados encontram-se no mesmo plano. Um notável papel desempenhado pela ligação *cis* ocorre nas membranas biológicas constituídas por lipídios, onde o número total de ligações *cis* vai influenciar a sua fluidez. Os ácidos graxos com uma ou mais instaurações na configuração *trans*, ou seja, com os átomos de hidrogênio ligados aos carbonos insaturados em planos opostos, são denominados ácidos graxos *trans* (AGT) (MARTIN et al., 2007). A configuração *trans* resulta em uma conformação molecular linear, similar aos ácidos graxos saturados. Essa conformação mais rígida resulta em diferentes propriedades físicas, tal como ponto de fusão mais alto e melhor estabilidade termodinâmica, associadas às modificações das características químicas sensoriais (REMIG et al., 2010; STENDER, ASTRUP e DYERBERG, 2008) (Figura 3).

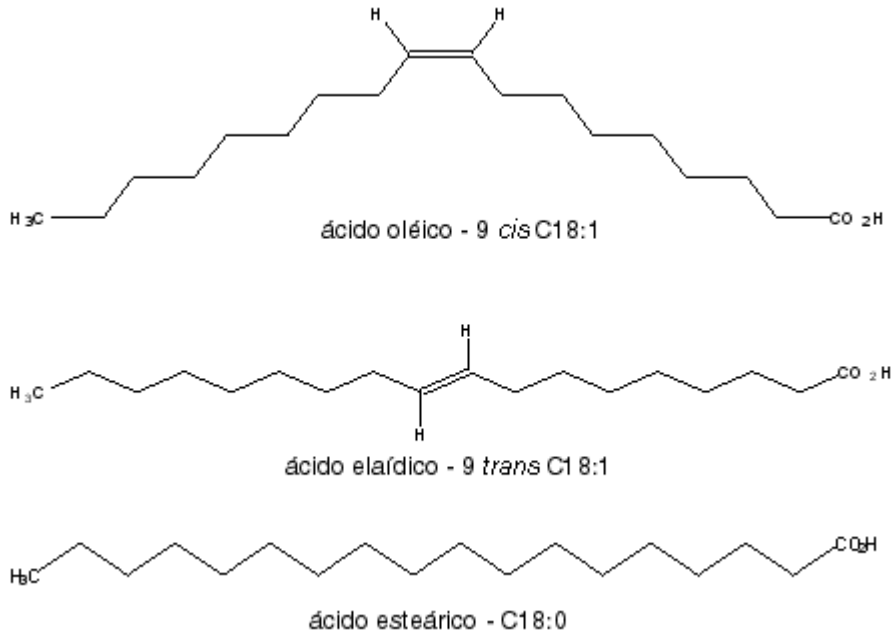

Figura 3. Estrutura química dos ácidos graxos insaturados oléico (*cis*) e elaídico (*trans*), com 18 carbonos, e do ácido graxo saturado correspondente, ácido esteárico (COSTA, BRESSAN e SABARENSE, 2006).

A hidrogenação industrial de óleos vegetais é responsável pela maior parte da

ingestão de AGT na dieta humana. Entretanto, AGT também são encontrados naturalmente em baixas concentrações em derivados do leite e gorduras animais, através da hidrogenação biológica no estômago de ruminantes (CRAIG-SCHMIDT, 2006; LARQUE, ZAMOR e GIL, 2001).

No Brasil, a hidrogenação comercial de óleos vegetais data da década de 50, visando à produção de margarinas e gorduras para frituras. Com o desenvolvimento de técnicas de hidrogenação seletiva, os óleos vegetais processados, rapidamente substituíram as gorduras animais na dieta dos brasileiros. Estas gorduras têm sido largamente empregadas na produção de diversos alimentos, como margarinas, coberturas de chocolate, biscoitos, produtos de panificação, sorvetes, massas e batatas “chips”, entre outros (MARTIN et al., 2005; SEMMA, 2002) com o objetivo de dar mais sabor e crocância, e principalmente menor rancificação, alongando a vida de prateleira do produto.

Os primeiros estudos relacionando modificações na estrutura molecular dos lipídios com alterações nos seus efeitos biológicos e consequentemente sobre a saúde dos indivíduos foram realizados no ano de 1969. Apesar da sua utilidade tecnológica, os efeitos do consumo desses ácidos graxos nos alimentos têm apresentado grande controvérsia, no que diz respeito ao efeito dos AGT na fisiologia e metabolismo humano.

1.5 Efeitos dos ácidos graxos *trans* sobre a saúde

As primeiras evidências sobre os efeitos do consumo de AGT levaram em consideração os níveis séricos de lipídeos. Estudos sugerem que o consumo elevado desses ácidos graxos pode induzir o aumento nos níveis de LDL-colesterol e triglicerídeos, acompanhado de uma diminuição em HDL-colesterol e consequente aumento da razão colesterol total/HDL-colesterol, os quais são considerados fatores de risco para o desenvolvimento de doenças coronarianas (LICHTENSTEIN et al., 2001; MEIJER, 2001; MENSINK e KATAN, 1993).

As preocupações com os efeitos dos AGT na saúde têm aumentado, uma vez que estes isômeros são estruturalmente similares às gorduras saturadas, modificando as funções metabólicas das gorduras poliinsaturadas e podem competir

com os ácidos graxos essenciais em vias metabólicas complexas (MARTIN et al., 2004).

De modo particular, os efeitos de uma deficiência em ácidos graxos essenciais provocados pela incorporação de AGT são importantes principalmente sobre o SNC, no qual os AGPI são constituintes fundamentais. Tem sido observado que animais suplementados com AGT apresentaram proporções elevadas de AG da série n-6 e diminuição de DHA (PETTERSEN e OPSTVEDT, 1992), sugerindo que a composição de AGPI no cérebro é influenciada pelo consumo de AGT (KIRSTEIN, HOY e HOLMER, 1983; LARQUE et al., 2003). Além disso, os AGT podem ser incorporados em fosfolipídios de membrana, alterando a fluidez, propriedades bioquímicas, e a função das células (SALEM et al., 2001). Especificadamente, uma deficiência em ALA foi capaz de promover déficits de memória, sensoriais, motores e motivacionais em ratos (FRANCÈS, MONIER e BOURRE, 1995; WAINWRIGHT, 1992, 2002; YAMAMOTO et al., 1987). Níveis reduzidos de AGPI também foram encontrados no cérebro *post mortem* de pacientes esquizofrênicos (HORROBIN et al., 1991; YAO, LEONARD e REDDY, 2000), e prejuízos na neurotransmissão dopaminérgica podem estar relacionadas (DELION et al., 1994; ZIMMER et al., 1998; 2000).

Ainda, outros estudos mostram que os AGT inibem a reação de dessaturação dos LA e ALA para AA, DHA e EPA, favorecendo o metabolismo de isômeros *trans* incomuns que, se incorporados aos tecidos, alteram as funções das membranas ou dos eicosanóides (INNIS, 2006; INNIS e KING, 1999). Assim, esta incorporação também pode influenciar mecanismos pró-inflamatórios e pró-apoptóticos através do aumento dos níveis do fator de necrose tumoral, interleucina-6 e proteína C reativa (MOZAFFARIAN et al., 2006), sendo ainda um campo fértil para a pesquisa, considerando que muitas controvérsias ainda persistem quanto ao metabolismo e os efeitos desses ácidos graxos na fisiologia e metabolismo humano.

1.6 Ácidos graxos e nutrição materna

Evidências demonstram a importância dos ácidos graxos essenciais, principalmente o DHA para o desenvolvimento cerebral pré-natal, onde participam

ativamente na estrutura, função e plasticidade sináptica (INNIS, 2007; MARTIN e BAZAN, 1992; NEURINGER et al., 1986; SALEM et al., 2001). A presença do DHA durante a gestação e lactação é essencial para a maturação cortical, neurogênese e mielinização, podendo atenuar riscos de prejuízos cognitivos e susceptibilidade às desordens psiquiátricas (BORSONELO e GALDUROZ, 2008; EILANDER et al., 2007; McNAMARA e CARLSON, 2006;). Esses ácidos graxos passam da mãe para o feto pela barreira placentária e, após o nascimento, pelo leite materno. Durante o último trimestre intrauterino e os primeiros 18 meses da vida pós-natal humana, e nos primeiros 15 dias após o nascimento em ratos (DOBBING e SANDS, 1979), o DHA e AA são acumulados rapidamente nos fosfolipídios de membrana do sistema nervoso central. É neste período que ocorre a explosão do crescimento cerebral (CARLSON, 2001; DIJCK-BROUWER et al., 2005).

Estudos sugerem que um fornecimento insuficiente de ácidos graxos n-3 durante o desenvolvimento pré e pós-natal diminui o conteúdo de DHA nos tecidos neurais (SCHIEFERMEIER e YAVIN, 2002), o que pode afetar o cone de crescimento dos neurônios, levando a redução da densidade neuronal e arborização dendrítica, em regiões como hipocampo, hipotálamo e córtex (AHMAD et al., 2002; CALDERON e KIM, 2004; WAINWRIGHT et al., 1998), podendo assim interferir na liberação de neurotransmissores (CHALON, 2006; ZIMMER et al., 2002). Além disso, pode ocorrer uma variedade de déficits visuais, olfatórios, cognitivos e comportamentais em modelos animais (LIM et al., 2005; MORIGUCHI et al., 2001; NIU et al., 2004). Porém, o suprimento de DHA através do aleitamento materno tem mostrado melhorar o desenvolvimento mental em crianças (HIBBELN, FERGUSON e BLASBALG, 2006).

A relação entre consumo de AGT e a fase gestacional está sendo observada em alguns estudos. Já foi relatado uma correlação inversa entre o peso ao nascer com uma ingestão de isômeros *trans*, sugerindo que os AGT também podem ser transferidos ao feto através da placenta (KOLETZKO e MÜLLER, 1990). Além disso, como os AGT podem inibir as enzimas Δ6 e Δ5 dessaturase, dificultando o metabolismo dos ácidos graxos essenciais, este processo em humanos provoca um impacto na fase gestacional por alterar o desenvolvimento intra-uterino pela inibição da síntese dos AA e DHA (DECSI e KOLETZKO, 1995), prejudicando a função motora durante o desenvolvimento (BOOYENS e MERWE, 1992).

1.7 Relação entre ácidos graxos e estresse

O estresse é reconhecido em sua cronicidade e identificado como o mal do século XXI**,** segundo a Organização Mundial da Saúde (2010). Suas repercussões estão diretamente ligadas à qualidade de vida do indivíduo, da família e da sociedade. É entendido como um processo complexo e multidimensional em que atuam estressores físicos e/ou psicológicos, sendo definido como um estado de homeostase ameaçado, ou em desequilíbrio, e é controlado por uma série de respostas viscerais e comportamentais, visando à restauração da homeostase do organismo (CARRASCO e VAN de KAR, 2003; HARBUZ e LIGHTMAN, 1992; HERMAN e CULLINAN, 1997).

As respostas ao estresse incluem a ativação do sistema nervoso autônomo e do eixo hipotálamo-hipófise-adrenal, a qual acarreta, respectivamente, a secreção de catecolaminas e a liberação de glicocorticóides pelo córtex adrenal (HARBUZ e LIGHTMAN, 1992; HERMAN e CULLINAN, 1997). O aumento sérico dos glicocorticóides pode desencadear alterações biológicas importantes para o organismo, incluindo o desenvolvimento de algumas disfunções psicológicas (McEWEN, 2010).

Tem sido sugerido também que o estresse pode alterar a composição das membranas celulares e funções associadas, além de levar à significativa redução da arborização dendrítica nas regiões do giro do cíngulo, hipocampo, região da amígdala e córtex préfrontal, os quais também predispõem a comportamentos ansiosos e depressivos, demonstrando a influência direta do estresse no sistema nervoso central (LUPIEN et al., 2009).

Recentes evidências demonstram a existência de influência da dieta sobre a manifestação de diferentes tipos de comportamentos induzidos por agentes estressores (FERRAZ et al., 2011; 2008) e o envolvimento dos AGPI na reatividade e sensibilidade do indivíduo ao estresse (McNAMARA e CARLSON, 2006). Estudos têm mostrado que dieta com óleo de peixe, rica em DHA exerce efeitos anti-estresse (FEDOROVA e SALEM, 2006; ROSS, 2009; TAKEUCHI, IWANAGA e HARADA, 2003) e reduz sintomas depressivos (COLANGELO et al., 2009), possivelmente por reduzir os níveis de glicocorticóides no cérebro através da regulação do eixo do

estresse (FERRAZ et al., 2011), sugerindo que a ingestão de AG n-3 pode apresentar efeitos protetores em transtornos psiquiátricos (FREEMAN et al., 2006). Outro recente estudo avaliou a suplementação de AGPI n-3 em pacientes com lesão acidental e mostrou uma redução significativa dos sintomas do transtorno de estresse pós-traumático (MATSUOKA et al., 2010). Estudo com humanos e animais mostra também que a exposição crônica ao estresse é capaz de aumentar a geração de espécies reativas de oxigênio, provocando aumento da peroxidação lipídica no cérebro com consequente dano tecidual (LUCCA et al., 2009; MATSUMOTO et al., 1999; SAHIN e GUMUSLU, 2004; ZAFIR e BANU, 2009).

Ao fornecer uma base fisiológica para a associação entre o metabolismo dos ácidos graxos e doenças do SNC, tem sido sugerido que a desregulação ou deficiência de ácidos graxos podem prejudicar a estabilidade e a composição das membranas neuronais (HORROBIN, 1996; SU, 2009) levando ao funcionamento anormal do cérebro (HIBBELN et al., 1998, 2004). Embora fatores dietéticos sejam os principais responsáveis pelas alterações no equilíbrio e metabolismo dos ácidos graxos, outros, como o estresse pode significativamente contribuir para variação do perfil dos ácidos graxos presentes no organismo favorecendo o desenvolvimento de doenças relacionadas ao SNC.

Sendo assim, os hábitos alimentares atualmente adotados podem influenciar de maneira significativa o conteúdo de AG das membranas cerebrais, uma vez que grande parte dos alimentos ingeridos pela sociedade moderna é rico em AGT. Deve-se considerar ainda a grande incidência de doenças neuropsiquiátricas na população e a influência dos AGT da dieta e fatores estressantes na etiologia destas doenças. Como o mecanismo pelo qual os mesmos causam efeitos deletérios à saúde não está completamente entendido, além de pouco se conhecer sobre sua influência nos tecidos cerebrais, mais estudos se fazem necessários, justificando assim, o estudo apresentado nesta dissertação.

2 OBJETIVOS

2.1 Objetivo Geral

Avaliar a influência do consumo de diferentes ácidos graxos ao longo de duas gerações de ratos sobre parâmetros comportamentais e oxidativos após exposição ao estresse agudo.

2.2 Objetivos Específicos

- ✓ Avaliar a influência do consumo de óleo de soja (rico em ácidos graxos n-6), óleo de peixe (rico em ácidos graxos n-3) e gordura vegetal hidrogenada (rica em ácidos graxos *trans*) ao longo de duas gerações de ratos sobre parâmetros comportamentais após a exposição aguda ao estresse: atividade locomotora, exploratória e parâmetros de ansiedade.

- ✓ Avaliar a influência do consumo dos diferentes ácidos graxos sobre danos oxidativos e status antioxidante no cérebro, após exposição aguda ao estresse: níveis de peroxidação lipídica, geração de espécies reativas de oxigênio, viabilidade celular e atividade da catalase.

3 ARTIGO CIENTÍFICO

Os resultados inseridos nesta dissertação apresentam-se sob a forma de artigo científico, o qual se encontra aqui estruturado. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se no próprio artigo, o qual está disposto da mesma forma que foi publicado.

INFLUENCE OF PERINATAL TRANS FAT ON BEHAVIORAL RESPONSES AND BRAIN OXIDATIVE STATUS OF ADOLESCENT RATS ACUTELY EXPOSED TO STRESS

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INFLUENCE OF PERINATAL *TRANS* FAT ON BEHAVIORAL RESPONSES AND BRAIN OXIDATIVE STATUS OF ADOLESCENT RATS ACUTELY EXPOSED TO STRESS

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Abstract—Because consumption of processed foods has increased in the last decades and so far its potential influence on emotionality and susceptibility to stress is unknown, we studied the influence of different fatty acids (FA) on behavioral and biochemical parameters after acute restrain stress (AS) exposure. Two sequential generations of female rats were supplemented with soybean oil (control group; C-SO), fish oil (FO) and hydrogenated vegetable fat (HVF) from pregnancy and during lactation. At 41 days of age, half the animals of each supplemented group were exposed to AS and observed in open field and elevated plus maze task, followed by euthanasia for biochemical assessments. The HVF-supplemented group showed higher anxiety-like symptoms per se, while the C-SO and FO groups did not show these behaviors. Among groups exposed to AS, HVF showed locomotor restlessness in the open field, while both C-SO and HVF groups showed anxiety-like symptoms in the elevated plus maze, but this was not observed in the FO group. Biochemical evaluations showed higher lipoperoxidation levels and lower cell viability in cortex in the HVF group. In addition, HVF-treated rats showed reduced catalase activity in striatum and hippocampus, as well as increased generation of reactive species in striatum, while FO was associated with increased cell viability in the hippocampus. Among groups exposed to AS, HVF increased reactive species generation in the brain, decreased cell viability in the cortex and striatum, and decreased catalase activity in the striatum and hippocampus. Taken together, our findings show that the type of FA provided during development and growth over two generations is able to modify the brain oxidative status, which was particularly adversely affected by *trans* fat. In addition, the harmful influence of chronic consumption of *trans* fats as observed in this study can enhance emotionality and anxiety parameters resulting from stressful situations of everyday life, which can trigger more severe neuropsychiatric conditions.

Key words: *trans* fatty acids, polyunsaturated fatty acids, acute stress, anxiety, oxidative stress.

INTRODUCTION

The consumption of processed foods that are rich in saturated and *trans* fatty acids (TFA) (Popkin, 1998; Allison et al., 1999) has increased in Western countries, mainly due to its practicality and durability, but chronic intake has brought health concerns (Hu et al., 1997; Wandall, 2008). Even though TFA are produced by the hydrogenation of both vegetable and animal oils and fats, their prolonged consumption can bring about a loss of essential fatty acids (EFA) and thus significantly impair human health (Teixeira et al., 2011, 2012; Trevizol et al., 2011). In fact, EFA are structural and functional components of neuronal membrane phospholipids that are recognized as essential because of the mammalian inability to synthesize fatty acids (FA) with a double bond past the Δ-9 position (Songur et al., 2004; Zararsiz et al., 2006). EFA are polyunsaturated fatty acids (PUFA) of the omega-3 series and include the α-linolenic acid (18:3n-3) and its derivatives docosahexaenoic acid (22:6n-3) and eicosapentaenoic acid (20:5n-3), which are found in fish oil and in smaller amounts in soybean and canola oils. Similarly, the linoleic acid (18:2n-6) and its metabolite arachidonic acid (20:4n-6) are EFA and PUFA of the omega-6 series, respectively, and they are abundantly found in most types of vegetable oils (Simopoulos, 1999; Holub, 2002; Benatti et al., 2004). Both n-3 and n-6 FA are important for biochemical integrity of the brain, playing different roles in the modulation of both the central nervous system (Salem et al., 2001; Contreras and Rapoport, 2002) and the immune system (Song and Horrobin, 2004).

Of particular importance, docosahexaenoic, eicosapentaenoic or arachidonic acids are long-chain PUFA and are functional components of membrane phospholipids in glia, neurons (Rapoport, 2001), and

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Abbreviations: ANOVA, analysis of variance; AS, acute stress; C-SO, control group – soybean oil; EFA, essential fatty acids; FA, fatty acids; FO, fish oil; HVF, hydrogenated vegetable fat; MDA, malondialdehyde; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PUFA, polyunsaturated fatty acids; TBARS, thiobarbituric acid reactive species; TFA, *trans* fatty acids.

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immune cells (Vlaardingerbroek et al., 2006). The growing consumption of processed foods, which became a common source of hydrogenated vegetable fat (HVF, rich in TFA) along the second half of the 20th century (Pfeuffer and Schrezenmeir, 2006) compromises the incorporation of these EFA derivatives into neuronal membrane phospholipids, impairing their fluidity and synaptic plasticity (Jump, 2002). Previous studies from our research group have shown the influence of dietary FA on the development of neuronal disorders such as mania (Trevizol et al., 2011) and brain oxidative impairments (Barcelos et al., 2009), but further studies are necessary.

Different subtypes of neuroses and neuropsychiatric diseases often begin after excessive stress and anxiety symptoms, which are part of the daily life (Arnett, 1999; Sevgi et al., 2006; Romeo, 2010). It has been shown that the continuous consumption of saturated FA is related to aggressive behavior in rodents (Raygada et al., 1998), but also that a diet enriched with n-3 PUFA is able to decrease anxiety symptoms in rats (Takeuchi et al., 2003). In this sense, stress and anxiety are characterized by excessive worry, tension, fatigue, difficulty concentrating, and sleep disturbances (Narrow et al., 2002; Wittchen, 2002), which may impair productivity and daily living skills. It is generally accepted that stress increases the risk for affective and anxiety disorders, which are closely related to the development of depression (Matuszewich et al., 2007). Currently, about 18% of young Americans have anxiety disorders (Kessler et al., 2005), and globally, it is estimated that about 15% of the young adult population will be affected by recurring emotional disorders (American Psychiatric Association, 2000), whose severity can progress to profound depression of difficult treatment.

Our current understanding of the relationship between diet, everyday life stress and psychiatric disorders is limited. Considering that dietary FA are largely incorporated into neuronal membranes during the period of cerebral development (Bourre et al., 1989) and that neural functions may be affected, especially during the pre- and postnatal period (Wauben et al., 2001), we proposed to compare behavioral parameters of anxiety and brain oxidative status in a second generation of young rats, which were born and grew under the same original supplementation rich in n-6 (Control), n-3 or TFA as their mothers, after exposure to acute stress.

EXPERIMENTAL PROCEDURES

Animals

Pregnant female Wistar rats (n = 21) from the breeding facility of the Universidade Federal of Santa Maria (UFSM), RS, Brazil, were kept in plexiglas cages with free access to food and water in a room with controlled temperature (22–23 °C) and on a 12-h light/dark cycle with lights on at 7:00 a.m. Rats were randomly assigned to one of three experimental groups (n = 7 for each group): Control-soybean oil (C-SO, rich in n-6 FA), fish oil (FO, rich in n-3 FA) and HVF (rich in TFA), which were supplemented daily by oral gavage with 3.0 g/kg body weight (Ferraz et al., 2008, 2011) from conception until weaning (Table 1). SO was used as the control group, mainly because it contains adequate levels of PUFA, n-6/n-3 ratio within acceptable limits (Simopoulos, 2002; 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 in order to prevent metabolic differences between animals of different experimental groups (McDonald et al., 2011; Khalkhal et al., 2012) and cause interference on the antioxidant defense system (Diniz et al., 2004), also modifying the content and metabolism of dopamine and serotonin (Wright et al., 2011). One female pup of each litter was maintained on the same supplementation until adulthood, when they were mated. These dams were kept on the same original supplementation until weaning of the litter of the second generation, when the pups were supplemented until 40 days of age. At day 41, half the adolescent male rats of each supplemented group (n = 7) were exposed to the acute restrain stress procedure, which is described below. After 24 h of AS exposure, animals were submitted to behavioral assessments followed by the biochemical determinations described below. All procedures were in accordance with the rules of the Committee on the Care and Use of Experimental Animals of the UFSM, which follows international rules (NIH Publication No. 80-23; revised 1978).

Acute restrain stress (AS) procedure

The acute stress procedure was adapted from the restraint method reported by Billing et al. (2012). Restraint stress consisted in placing the animal for 2 h in a well-ventilated acrylic glass tube (25 cm length, 5 cm diameter, 0.1 cm wall) immobilized in a supine position. Control rats were never exposed to the restraint procedure but were simply handled for 30 s and returned to their homecage.

Behavioral evaluations

Open field test. In this test the rats were placed individually at the center of an open field arena (40 × 40 × 30 cm) with black plywood walls and a white floor divided into nine equal squares, as previously described by Kerr et al. (2005). The number of square crossings (horizontal squares crossed) and rearings (vertical movements) were recorded for 5 min under dim light (30 lx) (Kabuki et al., 2009) and used as measures of spontaneous locomotor activity and exploratory behavior, respectively. In addition, the number of fecal pellets was also counted while the animals remained in the open field, whose increase confirms anxiety-like behavior in this paradigm, together with anxiety-like symptoms observed in the elevated plus maze (Katz et al., 1981; Crawley et al., 1997).

Elevated plus maze test. This test is based on the innate fear rodents have for open and elevated spaces

Table 1. Total fatty acid composition of diets lipids

Fatty acids (∑)	Soybean oil (%) (control group)	Fish oil (%)	Hydrogenated vegetable fat (%)
SFA	10.50	17.70	15.80
MUFA	34.00	36.61	43.41
PUFA	54.50	45.29	29.45
TFA	0.40	0.30	11.82
SFA/PUFA ratio	0.19	0.39	0.53
PUFA n-6	49.00	21.40	27.78
PUFA n-3	4.95	19.0	1.84
n-6/n-3 ratio	9.89	1.12	15.09

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TFA: *trans* fatty acids.

(Montgomery, 1955). The apparatus was made of wood and consisted of a plus-shaped platform elevated 50 cm from the floor. Two opposite arms (50 cm × 10 cm) were enclosed by 40-cm high walls whereas the other two arms had no walls. The four arms had at their intersection a central platform (10 cm × 10 cm), which gave access to any of the four arms. At the beginning of each test, the rat was placed at the central platform facing an open arm. Time spent in open arms, head dipping frequency (an ethological measure which consists of the exploratory movement of head/shoulders over the sides of the open arms and downwards to the floor), and time spent in the closed arms were recorded during a five-minute test and used as measures of anxiety (Hlavacova et al., 2010), while the total number of entries in both open and closed arms was used as index of locomotor activity (Rodgers and Dalvi, 1997). The apparatus was cleaned with a 20% alcohol solution before the introduction of each animal. To prevent any possible interference with behavioral paradigms, an interval of 2 days between each behavioral test was allowed (Schmitt and Hiemke, 1998).

Tissue preparations

After the acute restrain stress (AS) procedure and 24 h after the last behavioral evaluation (Sachdeva et al., 2011), all animals were anesthetized (sodium pentobarbital, 50 mg/kg body weight i.p.) and euthanized by cervical decapitation. Their brains were removed, put on ice, and cut coronally at the caudal border of the olfactory tubercle for the removal of cortex, hippocampus and striatum. One half of each tissue was homogenized with 0.1 M Tris–HCl, pH 7.4 and centrifuged at 3000*g* (10 min), and the supernatants were used for biochemical assays. The other half of each tissue was sliced (0.4 mm) in a McIlwain chopper, and used for viability assay.

Lipid peroxidation measure. Lipoperoxidation was estimated by the quantification of thiobarbituric acid reactive species (TBARS). Lipid peroxidation was measured through the pink chromogen produced by the reaction of thiobarbituric acid with malondialdehyde (MDA) at 100 °C, measured spectrophotometrically at 535 nm, according to Ohkawa et al. (1979). Results were expressed as ηmol MDA/g tissue.

Catalase activity. Catalase activity was spectrophotometrically quantified in tissues by the method of Aebi (1984), which involves monitoring the disappearance of H_2O_2 in the presence of the cell homogenate (pH 7.0 at 25 °C) at 240 nm. The enzymatic activity was expressed in μmol H_2O_2/min/g tissue.

Evaluation of reactive species formation with DCH (dichlorofluorescein). Reactive species levels were measured using the oxidant sensing fluorescent probe, 2′,7′-dichlorofluorescein diacetate (Hempel et al., 1999). The oxidation to fluorescent dichlorofluorescein (DCF) was determined at 488 nm for excitation and 525 nm for emission. Aliquots of cortex, hippocampus and striatum (20 μL) were added to a medium containing Tris–HCl buffer (0.01 mM; pH 7.4) and 2′,7′-dichlorofluorescein diacetate (10 μM). After 2′,7′-dichlorofluorescein diacetate addition, the medium was incubated in the dark for 1 h until fluorescence measurement procedure. DCF-reactive species levels were corrected by the protein content (Lowry et al., 1951) and expressed as a percentage of values from control.

Slices viability. The viability of cortex, striatum and hippocampus slices was quantified by measuring the reduction of 3-(4,5-dimethyllthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to a dark violet formazan product by mitochondrial dehydrogenases (Mosmann, 1983). MTT reduction assays were performed in plates containing 500 μL of phosphate-buffered 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. 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.

Statistical analysis

Behavioral and biochemical measures were analyzed by two-way analysis of variance (ANOVA) (3 supplementations (C-SO/FO/HVF) × 2 treatments (AS – acute stress/NS – no stress) followed by Duncan's multiple range test, when appropriate. $P < 0.05$ was regarded as statistically significant.

RESULTS

Locomotor and exploratory performance evaluated in open field (Fig. 1)

Two-way ANOVA revealed a significant main effect of supplementation × AS interaction on number of crossings [$F(2,36) = 12.55$; $P < 0.001$], a significant main effect of AS [$F(1,36) = 5.83$; $P < 0.05$] and a significant supplementation × AS interaction on number of rearings [$F(2,36) = 11.02$; $P < 0.001$]. Post hoc comparisons showed no difference in crossing and rearing numbers among animals not exposed to AS, but after this exposure, an increase in crossings (Fig. 1A) and rearings (Fig. 1B) was observed in HVF group in comparison to FO and C-SO groups, which showed similar values between each other.

Moreover, there was a significant main effect of supplementations on the number of fecal pellets [$F(2,36) = 8.69$; $P < 0.05$]. Post hoc test showed that HVF-supplemented animals had increased the number of fecal pellets per se and after AS exposure as compared to FO and C-SO groups (Fig. 1C), which were statistically similar to each other with regard to the number of fecal pellets.

Anxiety-like symptoms evaluated in elevated plus maze (Fig. 2)

Two-way ANOVA revealed a significant main effect of supplementation and AS on time spent in the open arms [$F(2,36) = 14.63$, ($P < 0.01$) and $F(1,36) = 8.09$; $P < 0.05$] respectively. Post hoc analysis showed that HVF spent less time per se in open arms than C-SO and FO groups. Among AS-exposed groups, both HVF and C-SO spent less time in open arms of elevated plus maze than FO. In fact, AS exposure reduced this behavioral time in C-SO but not in the other groups (Fig. 2A).

Two-way ANOVA revealed a significant main effect of supplementation on head dipping frequency [$F(2,36) = 12.02$; $P < 0.001$]. Post hoc analysis showed increased head dipping in C-SO and FO as compared to HVF. After AS, head dipping was decreased in C-SO, and was similar to that of the HVF group. The FO group did not show any changes in this behavioral parameter after AS (Fig. 2B).

A significant main effect of supplementation and AS [$F(2,36) = 12.93$; $F(1,36) = 28.30$; $P < 0.001$], respectively, was observed in the time spent in closed arms of the elevated plus maze. Post hoc analysis showed that the HVF group stayed longer in closed arms than did the FO and C-SO groups. AS exposure increased time spent in closed arms of C-SO and HVF, whose values were higher than those of FO. In fact, AS exposure did not affect this behavioral parameter in FO-supplemented rats (Fig. 2C). The total number of entries, showed no differences across the experimental groups (Fig. 2D).

Biochemical assays

Generation of TBARS and reactive species levels, catalase activity and cell viability in cortex, striatum and hippocampus are shown in Fig. 3 and Table 2. In the cortex, two-way ANOVA revealed a significant main effect of AS and a significant supplementation × AS interaction on TBARS levels [$F(1,36) = 6.09$; $F(2,36) = 6.49$; $P < 0.05$] respectively, a significant

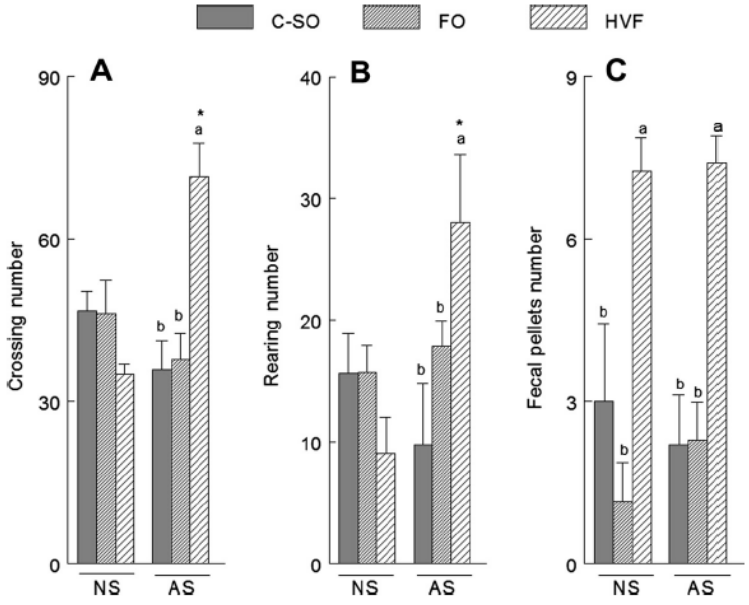

Fig. 1. Influence of supplementation with soybean oil (control group; C-SO), fish oil (FO) or hydrogenated vegetable fat (HVF) over second generation of young rats acutely exposed or not to stress on locomotor (A) and exploratory (B) activities and emotionality (C), which were observed in open field task. Abbreviations: NS – no stress, AS – acute stress. Data are expressed as mean ± S.E.M. Lowercase indicates significant difference between the supplemented groups and the same stress condition ($P < 0.05$); *indicates significant difference between stress conditions in the same oral supplementation ($P < 0.05$).

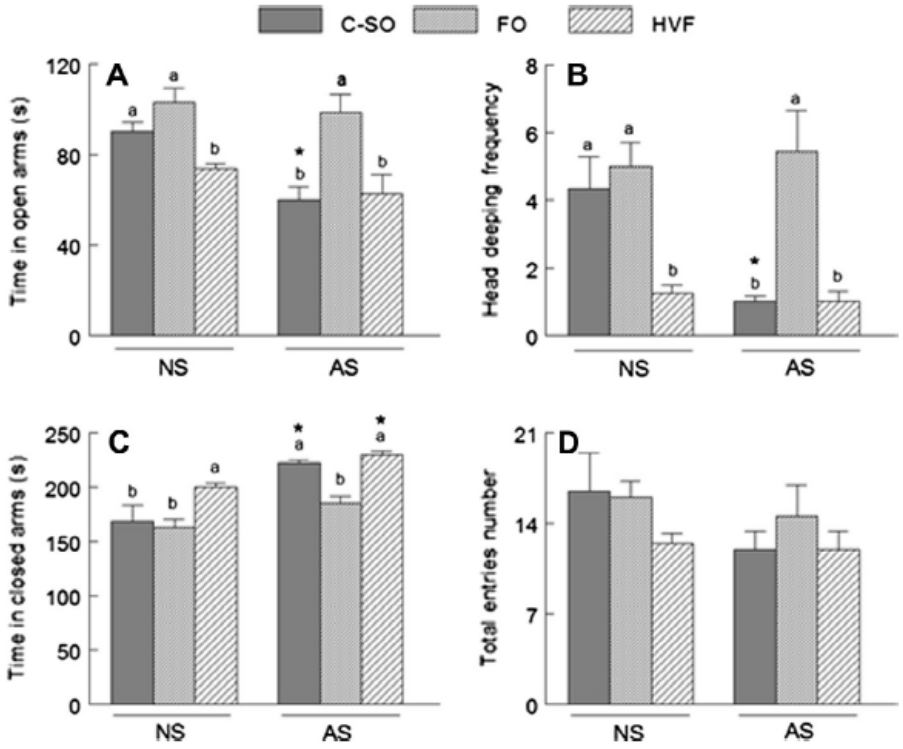

Fig. 2. Influence of supplementation with soybean oil (control group; C-SO), fish oil (FO) or hydrogenated vegetable fat (HVF) over second generation of young rats acutely exposed or not to acute stress on time spent in open arms (A), frequency of head dipping (B), time spent in closed arms (C) and entries number in both open and closed arms (D) of Elevated Plus Maze task. Abbreviations: NS – no stress, AS – acute stress. Data are expressed as mean ± S.E.M. Lowercase indicates significant difference between the supplemented groups and the same stress condition ($P < 0.05$); *indicates significant difference between stress conditions in the same oral supplementation ($P < 0.05$).

main effect of AS on catalase activity [$F(1,36) = 5.59$; $P < 0.05$], and a significant main effect of supplementation on reactive species generation and cell viability [$F(2,36) = 3.74$ and 6.80; $P < 0.05$], respectively. Post hoc test showed that TBARS levels were higher in the cortex of HVF-supplemented animals than in C-SO and FO-supplemented groups (Fig. 3A). This was not observed across AS-exposed groups, which showed similar TBARS levels between each other. Comparison within the same supplemented group showed that AS exposure was able to reduce TBARS levels and increase catalase activity, but this effect was not observed in C-SO and FO groups (Fig. 3A, D, respectively). Statistical analyses revealed a significant negative correlation between catalase activity and TBARS levels in the cortex ($r^2 = 0.23$; $P = 0.001$, Fig. 4). Supplementations did not affect reactive species generation in the cortex, but this brain area showed decreased cell viability in HVF as compared to FO (Table 2). Across AS-exposed groups, HVF showed higher reactive species generation and reduced cell viability than did FO.

In the striatum, there was a significant main effect of AS on TBARS levels [$F(1,36) = 4.06$; $P < 0.05$], a significant main effect of supplementation on catalase activity [$F(2,36) = 5.77$; $P < 0.05$] and a significant main effect of supplementation on reactive species generation and on cell viability [$F(2,36) = 15.49$ and 8.21; $P < 0.05$], respectively. Duncan's test showed no differences in TBARS levels in groups not exposed to AS (Fig. 3B), but HVF-supplemented animals had lower activity of catalase in the striatum in relation to C-SO (Fig. 3E). Within the same supplementation, AS exposure increased TBARS levels in the HVF, but did not affect it in the other groups; however, no difference in this oxidative parameter was observed between the different supplementations after AS exposure (Fig. 3B). AS exposure did not change catalase activity in the striatum, but it remained decreased in HVF in relation to both C-SO and FO groups (Fig. 3E). HVF supplementation increased reactive species production and reduced cell viability in the striatum (Table 2). HVF-supplemented animals exposed to AS showed higher reactive species levels than those treated with C-SO and FO, also showing reduced cell viability in relation to FO (Table 2).

In the hippocampus, two-way ANOVA revealed a significant main effect of AS on TBARS levels [$F(1,36) = 14.27$; $P < 0.001$], a significant main effect of supplementation and a significant supplementation × AS interaction on catalase activity [$F(2,36) = 13.58$; $P < 0.001$; 4.79; $P < 0.05$] respectively; a significant main effect of AS on reactive species generation [$F(1,36) = 7.19$; $P < 0.05$] and a significant main effect of supplementation on cell viability [$F(2,36) = 4.76$; $P < 0.05$]. Duncan's test showed no differences across supplementations on TBARS levels in the hippocampus (Fig. 3C), but catalase activity was decreased in HVF as compared to C-SO and FO groups (Fig. 3F). Within the same

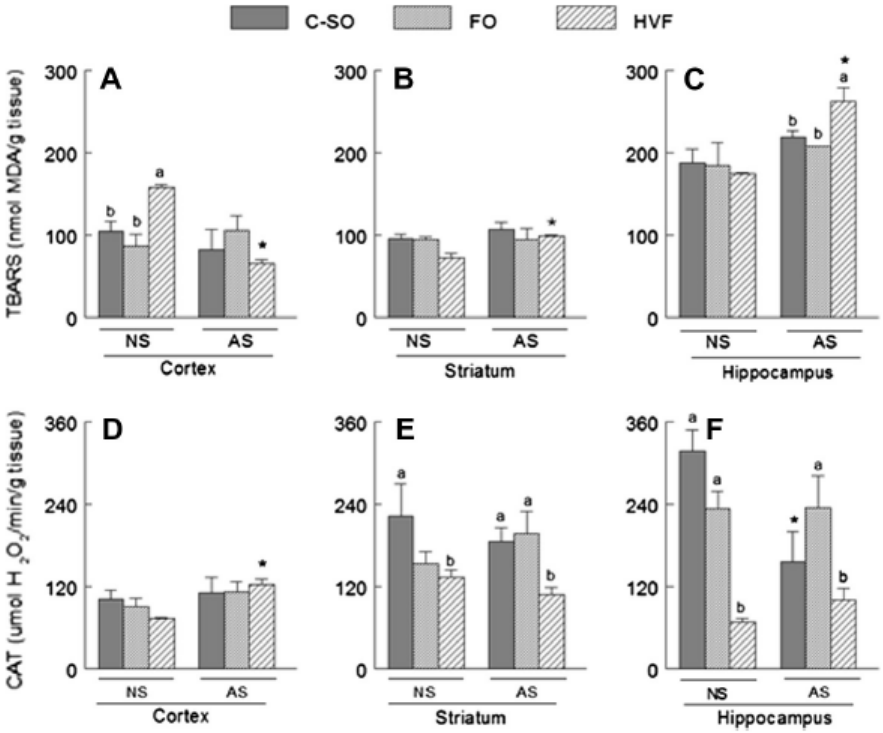

Fig. 3. Influence of supplementation with soybean oil (control group; C-SO), fish oil (FO) or hydrogenated vegetable fat (HVF) over second generation of young rats acutely exposed or not to acute stress, on TBARS levels evaluated in cortex (A), striatum (B) and hippocampus (C), as well as on catalase (CAT) activity, which was measured in the same brain areas (D, E, and F, respectively). *Abbreviations:* NS, no stress; AS, acute stress. Data are expressed as mean ± S.E.M. Lowercase indicates significant difference between the supplemented groups and the same stress condition ($P < 0.05$); *indicates significant difference between stress conditions in the same oral supplementation ($P < 0.05$).

supplementation, AS exposure increased TBARS levels in the HVF, whose values were higher than those observed in AS-exposed C-SO and FO groups (Fig. 3C). AS exposure decreased catalase activity in the C-SO group, while between the different supplementations, catalase activity was lower in HVF than in FO (Fig. 3F). Supplementations did not cause differences in reactive species generation, but the hippocampal cell viability was higher in FO than in C-SO and HVF groups (Table 2). AS exposure increased reactive species generation in HVF, whose level was higher than in C-SO and FO (Table 2). Hippocampal cell

Table 2. Influence of supplementation with soybean oil (control group; C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF) over second generation of young rats acutely exposed or not to acute stress, on reactive species (DCF-RS) generation and cell viability evaluated in different brain areas

			DCF-RS generation	MTT reduction levels
Cortex	C-SO	NS	100.00 ± 24.92	100.00 ± 6.50
		AS	102.00 ± 21.67	106.95 ± 5.66
	FO	NS	106.84 ± 21.58	121.27 ± 14.67[a]
		AS	72.58 ± 2.86[b]	121.41 ± 16.49[a]
	HVF	NS	127.45 ± 11.54	86.91 ± 0.40[b]
		AS	152.55 ± 23.74[a]	76.85 ± 2.21[b]
Striatum	C-SO	NS	100.00 ± 16.00[b]	100.00 ± 4.46[b]
		AS	102.42 ± 0.98[b]	106.55 ± 13.24
	FO	NS	126.11 ± 4.25[b]	131.60 ± 16.40[a]
		AS	118.71 ± 6.73[b]	114.63 ± 6.94[a]
	HVF	NS	247.74 ± 58.72[a]	84.41 ± 0.24[b]
		AS	217.14 ± 34.53[a]	80.49 ± 0.28[b]
Hippocampus	C-SO	NS	100.00 ± 18.60	100.00 ± 19.59[b]
		AS	102.22 ± 17.72[b]	128.46 ± 16.96
	FO	NS	71.63 ± 15.99	196.29 ± 34.81[a]
		AS	124.66 ± 36.45[b]	154.05 ± 29.65
	HVF	NS	79.33 ± 0.52	102.28 ± 4.98[b]
		AS	192.95 ± 43.76[a*]	117.78 ± 15.55

Abbreviations: NS, no stress, AS, acute stress. Data are expressed as mean ± S.E.M. Lowercase indicates significant difference between the supplemented groups and the same stress condition ($P < 0.05$); *indicates significant difference between stress conditions in the same oral supplementation ($P < 0.05$).

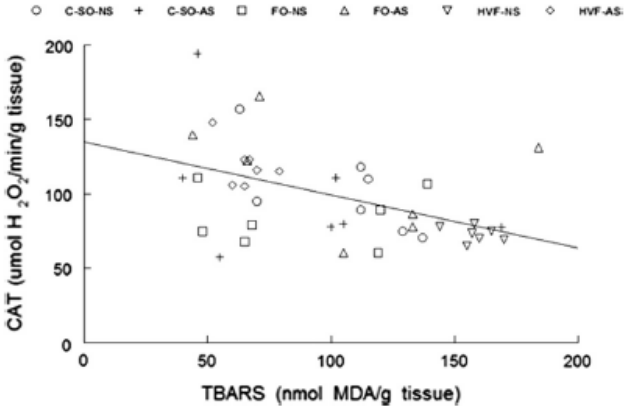

Fig. 4. Linear regression analysis between CAT activity and TBARS levels in the cortex of rats supplemented with soybean oil (control group; C-SO), fish oil (FO) or hydrogenated vegetal fat (HVF) over second generation of young rats acutely exposed or not to acute stress. Abbreviations: NS – no stress, AS – acute stress. Statistical analysis revealed *P* significance level for the r^2 value: 0.23.

viability was similar across the different experimental groups exposed to AS.

DISCUSSION

In this study, our aim was to evaluate the influence of FO and HVF on anxious behaviors and brain oxidative status of rats exposed or not to AS in young age. Dietary supplementations were initiated from pregnancy of the first generation of rats and maintained until adolescence of the second generation. Together with soybean oil, which was the control group (C-SO) here, these isocaloric fats are the most widely consumed forms of fat today. They have been shown to be primarily rich in n-3, TFA, and n-6, respectively. In addition, their n-6/n-3 FA ratios are optimum for FO, above the recommended levels for HVF (Simopoulos, 1991, 2002), and acceptable for C-SO (Table 1).

Some reports have highlighted that a more direct uptake of FA by brain membranes occurs primarily during the perinatal period (Neuringer et al., 1988), affecting membrane composition and so influencing its structure and neurotransmission (Fernstrom, 1999). Interestingly, while excessive or chronic consumption of processed foods, which are rich in TFA, can be a risk factor for neuropsychiatric conditions (Teixeira et al., 2011; Trevizol et al., 2011), stress exposure also represents a risk factor for the development of neuropsychiatric diseases (Schmidt et al., 2008), mainly during adolescence (Doremus-Fitzwater et al., 2009). In this period, the hippocampal volume, cell number and dendrite density undergo development (McCormick et al., 2008). In this sense, models of acute stress are able to induce physiological and behavioral changes (Belda et al., 2008) which may be related to depression, panic disorder and drug addiction (Spear, 2000).

Different studies have shown that locomotor changes are indicative of high reactivity to novelty as expressed by the increased number of crossings and rearings in the open field (Lapiz et al., 2003; Guo et al., 2004), which may be related to increased impulsiveness. Comparable behavior was observed in our study, since HVF supplementation was also associated with increased locomotor index in this paradigm. Because incorporation of TFA into neural membranes can affect neurotransmission (Acar et al., 2003), it could be expected that HVF intake would result in heightened locomotor response to novelty. In the present study, the locomotor status observed in the HVF-supplemented group was accompanied by increased emotionality in the open field, as indicated by the increased number of fecal pellets in animals exposed or not to AS.

Recently we reported high anxiety-like symptoms, impairment of memory (Teixeira et al., 2011), and higher susceptibility to develop movement disorders in aging rats (Teixeira et al., 2012), characteristics that were associated with the incorporation of TFA in brain membranes. From a nutritional viewpoint, the consumption of TFA can represent a significant loss of EFA, compromising physiological functions of neuronal membranes (Mahfouz et al., 1984). In contrast, a balanced intake of n-3 FA has been linked to higher fluidity and plasticity of the brain neuronal membranes (Wu et al., 2008; Bhatia et al., 2011). Literature is rich in studies on omega-3 deficiency due to its minimal dietary intake seen in the last decades, but few studies have focused on the lack of n-3 FA as a consequence of excessive consumption of processed foods, which contain plenty of saturated FA and TFA. In addition to increased locomotor status and emotionality among HVF-treated rats in the open field, in the present study, second generation rats born and grown under the influence of TFA showed spontaneous anxiety-like symptoms per se, which remained after AS exposure, as observed by fewer head dippings and shorter time spent in the open arms and longer time spent in the closed arms of the elevated plus maze. These findings cannot be considered as a locomotor artifact, because all experimental groups showed similar number of entries in both closed and open arms of the elevated plus maze (Holmes and Rodgers, 2003). Interestingly, C-SO-supplemented animals did not show anxiety-like symptoms per se as observed in the HVF group, but AS exposure was able to facilitate the development of anxiety-like symptoms in the C-SO- and not in the FO-treated group. From these findings we can assume that while the provision of n-3 FA may be related to anxiolytic properties per se, the offer of TFA can be linked to hyperactivity and an anxiogenic role, as observed in the open field and elevated plus maze, respectively.

Considering that dietary TFA compete with EFA for incorporation in membrane phospholipids (Pettersen and Opstvedt, 1992), inhibiting the activity of desaturases. and that desaturases are generators of long-chain PUFA (Wauben et al., 2001; Larque et al., 2003), whose lack modifies the fluidity and permeability of neuronal membranes (Chatgilialoglu et al., 2002; Roach et al., 2004), we can hypothesize that: (i) a diet rich in n-3 FA during development is able to contribute to the maintenance of emotional stability of adulthood in

stressful situations of everyday life; (ii) while a diet rich in n-6 FA during both the gestational and developmental periods does not precipitate emotional impairments per se, it affords no protection against stressful situations common in adulthood; and (iii) the consumption of TFA throughout life may by itself make individuals more agitated and anxious and facilitate the development of neuropsychiatric diseases after stressful situations. While high emotionality is predictive of stress development, stressful experiences during early life can alter normal developmental processes (Gilad et al., 2000) resulting in biochemical, physiological and behavioral abnormalities in adult animals.

Some authors showed that biochemical changes can reflect oxidative damage implicated in diseases (Galaris and Evangelou, 2002; Giles et al., 2003), while dietary FA composition was also reported as one of the major factors capable of modulating oxidative stress in brain tissues (Virgili et al., 1996). Here, HVF supplementation was associated with the development of oxidative damages, as observed by higher lipoperoxidation levels in cortex of animals unexposed to stress and in the hippocampus of rats exposed to AS. Although the different supplementations were not associated per se with differences in striatum, AS exposure was able to increase lipoperoxidation in the striatum of the HVF-treated group only. Besides lipoperoxidation, HVF increased per se generation of reactive species in the striatum, while AS exposure increased such species in all evaluated brain areas of this group. In addition, reduced cell viability was observed in cortex, striatum and hippocampus of HVF-supplemented rats not exposed to stress, whose damage remained after AS exposure in all evaluated areas. In contrast, FO and C-SO supplementations were not associated with development of lipoperoxidation or reactive species generation in the evaluated brain areas of animals exposed or not to AS.

Recently, Pal et al. (2006) and Boufleur et al. (2012, 2013) showed impairment of oxidative status after stress exposure confirming the high susceptibility of the central nervous system to oxidative damage. According to Van Ginkel and Sevanian (1994), lipoperoxidation can result in loss of long-chain PUFA, structural changes, and decreased membrane fluidity, which may result in the oxidation of enzymes and receptors, leading to their inactivation (Losch and Bieger, 2000). On the other hand, some studies reported neuroprotective effects of PUFA (Lonergan et al., 2002; Martin et al., 2002a,b) According to Sarsilmaz et al. (2003), the provision of n-3 PUFA may affect the oxidant/antioxidant status of the brain, so stabilizing its membranous structures possibly due to its incorporation into cell membranes. One possibility is that n-3 EFA may inhibit the phosphoslipase A_2 function, acting as an antiapoptotic (Ghule et al., 2012) and neuroprotective (Mirnikjoo et al., 2001; Barcelos et al., 2009) agent. Our findings are in agreement with these data, once FO supplementation was associated with lower oxidative damage, particularly as evidenced by lower generation of reactive species in all evaluated brain areas. Furthermore, higher neuronal viability was manifest in the FO-supplemented group, either exposed or not to stress. With regard to antioxidant defenses, Wang et al. (2012) reported that n-3 PUFA is able to enhance the activity and expression of some antioxidant enzymes. Here, decreased catalase activity was observed in striatum and hippocampus of the HVF-supplemented group, either exposed or not to AS, but C-SO- and FO-supplemented animals unexposed to AS showed higher catalase activity in striatum and hippocampus and in hippocampus, respectively. Of particular importance, our findings also showed that AS exposure was related to increased catalase activity in cortex of the HVF-supplemented group, which occurred in parallel to a decrease in lipoperoxidation levels in the same brain area. This negative correlation reinforces the coordinated action of catalase to neutralize hydrogen peroxide, thus playing a fundamental role in detoxification of these reactive species, as widely reported in the literature (Abilio et al., 2004; Teixeira et al., 2008, 2009).

So far, no studies had shown the influence of *trans* fats in a multigenerational model on behavioral and biochemical changes, more exactly on the development of anxiety-like symptoms and their relationships with brain oxidative damage, because the deleterious effects of an inadequate diet during both gestational and perinatal periods can be reflected over generations. From this study, we can propose that prolonged consumption of processed foods during early stages of life is able to increase emotionality and facilitate the development of neuropsychiatric diseases, especially after stress situations of everyday life.

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4 CONCLUSÕES

Através dos resultados obtidos podemos chegar às seguintes conclusões:

- ✓ De modo geral, o consumo de GVH associado ao estresse agudo mostrou prejuízos comportamentais e influência negativa sobre parâmetros oxidativos no cérebro dos animais;

- ✓ O consumo crônico de AGT ofertado através da suplementação com gordura vegetal hidrogenada (GVH) durante o período inicial e de crescimento dos animais, ao longo de duas gerações, foi capaz de facilitar o desenvolvimento de sintomas de ansiedade, observados nos testes do labirinto em cruz elevado e campo aberto;

- ✓ O consumo crônico de GVH associado com exposição ao estresse agudo resultou em alterações nos parâmetros locomotores, o que sugere exacerbação de sintomas de hiperatividade;

- ✓ Os animais suplementados com óleo de peixe (OP) durante duas gerações apresentaram reduzido índice de ansiedade após exposição ao estresse agudo, indicando que o aporte de AG n-3 em fases iniciais da vida pode ser refletido sobre a composição fosfolipídica das membranas neuronais, sugerindo maior estabilidade sobre a neurotransmissão;

- ✓ Os AGT provenientes da suplementação induziram maiores danos oxidativos que foram exacerbados após exposição ao estresse, em diferentes regiões cerebrais;

- ✓ A suplementação com OP mostrou potencial neuroprotetor, observado pela maior proteção antioxidante, a qual foi superior em relação aos grupos suplementados com óleo de soja (OS) e GVH.

PERSPECTIVAS

Com base nos resultados obtidos no presente trabalho, estudos mais aprofundados fazem-se necessários com a finalidade de investigar os mecanismos moleculares em nível central envolvidos nas respostas comportamentais e bioquímicas decorrentes do consumo de AG *trans* após exposição a situações de estresse, bem como os sistemas neurotransmissores envolvidos e a participação de vias de sinalização celular. Além disso, análises da incorporação de AG em regiões específicas cerebrais devem ser futuramente conduzidas.

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