UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
DEPARTAMENTO DE FISIOLOGIA E FARMACOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA

SUPLEMENTAÇÃO DE ÁCIDOS GRAXOS E ATIVIDADE FÍSICA SOBRE OS DISTÚRBIOS DO MOVIMENTO, DA MEMÓRIA E DA ANSIEDADE EM RATOS: PARÂMETROS COMPORTAMENTAIS E BIOQUÍMICOS

TESE DE DOUTORADO

Angélica Martelli Teixeira

SANTA MARIA, RS, BRASIL

2012

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Angélica Martelli Teixeira

Tese apresentada ao Curso de Doutorado 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
Doutor em Farmacologia

Orientador: Profª Drª Marilise Escobar Bürger

Santa Maria, RS, Brasil

2012

Universidade Federal de Santa Maria
Centro de Ciências da Saúde
Programa de Pós-Graduação em Farmacologia

A Comissão Examinadora, abaixo assinada, aprova a Tese de Doutorado

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elaborada por
Angélica Martelli Teixeira

Como requisito parcial para obtenção do grau de
Doutor em Farmacologia

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Santa Maria, 30 de janeiro de 2012.

Esta tese é dedicada à minha família

Agradecimentos

À Deus, por minha vida, por minha família e amigos, por Seu cuidado e proteção neste caminho. Obrigada, Pai, pelos risos, sorrisos e lágrimas, tudo é prova de Teu amor por nós.

À minha mãe Aderoci e ao meu pai Paulo, que já está nos braços de Deus, se algo de bom conquisto, é porque aprendi a amar e a lutar com vocês. Tenho muito orgulho de ser sua filha e os amo muito!

Aos meus irmãos Anelise, Adriana e Nestor, seu incentivo, força e oração são o que a Vida me deu de melhor. Amo vocês.

Ao meu noivo Paolo, principalmente pela paciência da espera...Teu amor chegou pra me dizer quem sou...

À minha orientadora Marilise, obrigada por compartilhar comigo teu conhecimento e me ajudar a descobrir novos horizontes, obrigada pelas motivações e “puxões de orelha”, pois na vida é preciso equilíbrio entre autoconfiança e humildade. Enfim, obrigada pela amizade e por todas as oportunidades e ensinamentos.

Aos queridos colegas do *Farmatox* Rachel, Dali, Fabíola, Geise, Caren, Nardeli, Flávidu, Vel, Kati, Karine e Heco, sem vocês seria impossível! Obrigada pela dedicação e pela amizade, é o que marca pra sempre.

À Camilinha, que acima de tudo se tornou uma grande amiga, obrigada por teu esforço, vontade e coragem, saiba que tens um lugar muito especial no meu coração.

Ao Pe. César, amigo, exemplo e pai espiritual, e à Karina e amigos do Oratório Mariano, que me ajudaram a buscar, entregar e amar a Deus também através deste trabalho, pois tudo vem Dele e volta para Ele.

A todos os professores e profissionais do Programa de Pós-Graduação em Farmacologia, especialmente ao coordenador professor Carlos Mello, por seu trabalho e dedicação impecáveis.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), pela bolsa de estudos e pelos recursos financeiros concedidos.

Enfim, agradeço à Universidade Federal de Santa Maria, por todo o apoio institucional, e ao Programa de Pós-Graduação em Farmacologia, pela possibilidade de realização deste curso.

“Eu vos louvarei de todo o coração, Senhor,
porque ouvistes as minhas palavras.
Na presença dos anjos eu vos cantarei.
Ante vosso santo templo prostrar-me-ei,
e louvarei o vosso nome, pela vossa bondade e fidelidade,
porque acima de todas as coisas,
exaltastes o vosso nome e a vossa promessa.
Quando vos invoquei, vós me respondestes;
fizestes crescer a força de minha alma.
Hão de vos louvar, Senhor, todos os reis da terra,
ao ouvirem as palavras de vossa boca.
E celebrarão os desígnios do Senhor:
Verdadeiramente, grande é a glória do Senhor.”

(Salmo 137, De Davi)

RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Farmacologia
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SUPLEMENTAÇÃO DE ÁCIDOS GRAXOS E ATIVIDADE FÍSICA SOBRE OS DISTÚRBIOS DO MOVIMENTO, MEMÓRIA E ANSIEDADE EM RATOS: PARÂMETROS COMPORTAMENTAIS E BIOQUÍMICOS

AUTORA: Angélica Martelli Teixeira
ORIENTADORA: Marilise Escobar Bürger
LOCAL E DATA DA DEFESA: Santa Maria, 30 de janeiro de 2012.

Ácidos graxos (AG) provenientes da alimentação são fundamentais na arquitetura das membranas fosfolipídicas cerebrais, e podem modificar plasticidade e fluidez, atuando de forma decisiva no desenvolvimento de patologias cognitivas e neuropsiquiátricas. Hábitos de vida saudável incluem alimentação balanceada e atividade física, cuja regularidade tem sido descrita como uma forma de prevenção ou reabilitação de doenças que afetam o SNC. Este estudo foi inicialmente designado para avaliar a influência do exercício regular na prevenção de danos oxidativos comumente induzidos por haloperidol. O exercício preveniu o desenvolvimento de discinesia orofacial (DO) e os prejuízos locomotores induzidos pelo antipsicótico. A atividade da catalase foi recuperada na região subcortical, prevenindo a lipoperoxidação cortical e subcortical. Ainda na região subcortical, houve uma correlação positiva entre a lipoperoxidação e a DO, concomitante à uma correlação negativa entre a atividade da catalase e a DO. Enquanto estes dados reforçam o envolvimento do estresse oxidativo (EO) no desenvolvimento dos distúrbios do movimento, o exercício físico foi capaz de aumentar a atividade do transportador de dopamina, contribuindo para a redução dos níveis do neurotransmissor no estriado, freqüentemente elevado por ação do antipsicótico.

Na seqüencia dos estudos, ratos recém desmamados foram suplementados com diferentes AG (óleo de soja-OS, rico em AG poliinsaturados; banha de porco-BP, rico em AG saturados; e gordura vegetal hidrogenada-GVH, rico em AG *trans)* e exercitados diariamente nos 3 meses finais. Após 15 meses, os animais foram designados para o experimento 1 ou 2. No primeiro, avaliou-se comportamentos de ansiedade (labirinto em cruz elevado), memória (labirinto de Barnes), bem como a atividade da Na^+K^+-ATPase no córtex e hipocampo. A suplementação GVH causou uma incorporação de cerca de 0,30% de AG *trans* no cérebro dos animais, enquanto a BP de 0,20%, não sendo observada incorporação *trans* no grupo OS. Esta incorporação não influenciou os sintomas de ansiedade nos grupos BP e GVH, mas o exercício beneficiou o grupo OS, aumentando seu comportamento exploratório e de risco. Um déficit de memória foi observado no grupo GVH, mas revertido pelo exercício físico, igualando a aquisição de memória dos três grupos experimentais. A atividade da Na^+K^+-ATPase foi menor no córtex e hipocampo dos animais tratados com GVH, não sendo modificada pelo exercício.

No 2º experimento, ratos suplementados com os diferentes AG apresentaram incorporação cerebral similar aos acima descritos. Estes animais foram submetidos a avaliações comportamentais de DO, locomoção e atividade das enzimas Na^+K^+-ATPase e catalase no estriado. A suplementação GVH foi associada ao aumento da DO, a qual foi intensificada pelo exercício nos grupos GVH e BP. A locomoção foi reduzida nestes dois grupos e não foi modificada pelo exercício. A atividade da catalase foi menor nos grupos BP e

GVH, mas elevada pelo exercício neste último. Os diferentes AG não modificaram a atividade da $Na^{+}K^{+}$-ATPase, a qual foi elevada pelo exercício nos animais suplementados com OS e BP. A incorporação de AG *trans* nas membranas cerebrais pode estar relacionada aos prejuízos motores observados, principalmente, no grupo GVH, enquanto a ausência desta incorporação no grupo OS, ao melhor desempenho motor e atividades enzimáticas.

Tomados em conjunto, os dados apresentados sugerem que hábitos de vida saudáveis, os quais incluem ingestão reduzida de AG *trans* e saturados e a prática regular de atividade física, podem ser capazes de prevenir e/ou reduzir o desenvolvimento ou conseqüências de desordens neurológicas e neuropsiquiátricas.

Palavras-chave: SNC. Exercício. Haloperidol. Ácidos Graxos *Trans*. Distúrbios Motores.

ABSTRACT

PhD Thesis
Post-graduating Course in Pharmacology
Federal University of Santa Maria

FATTY ACID SUPPLEMENTATION AND PHYSICAL ACTIVITY ON MOVEMENT DISORDERS, MEMORY AND ANXIETY OF RATS: BEHAVIORAL AND BIOCHEMICAL PARAMETERS

AUTHOR: Angélica Martelli Teixeira
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DEFENSE PLACE AND DATE: Santa Maria, january 30th, 2012.

Fatty acids (FA) from the diet play a key role in the architecture of brain membrane phospholipids, and can modify plasticity and fluidity, acting decisively in the development of cognitive and neuropsychiatric disorders. Healthy lifestyle habits include balanced diet and physical activity, which has regularly been described as a form of rehabilitation or prevention of diseases affecting the CNS. This study was initially designed to evaluate the influence of regular exercise in preventing oxidative damage commonly induced by haloperidol. Exercise prevented the development of orofacial dyskinesia (OD) and antipsychotic-induced locomotor impairments. The catalase activity was recovered in the subcortical region, preventing cortical and subcortical lipoperoxidation. Also in the subcortical region, there was a positive correlation between lipid peroxidation and the OD, concurrent with a negative correlation between catalase activity and OD. While these data reinforce the involvement of oxidative stress (OS) in the development of movement disorders, physical exercise was able to increase the activity of the dopamine transporter, thereby helping to reduce levels of the neurotransmitter in the striatum, often elevated by the antipsychotic action.

In the following studies, weaned rats were supplemented with different FA (soybean oil-SO, rich in polyunsaturated FA; lard-L, rich in saturated FA; and hydrogenated vegetable fat-HVF, rich in *trans* fatty acids) and daily exercised in the last three months. After 15 months, the animals were assigned to experiment 1 or 2. In the first one, was evaluated anxiety behavior (elevated plus-maze), memory (Barnes-maze), and the activity of Na^+K^+-ATPase in the cortex and hippocampus. The HVF supplementation caused an incorporation of about 0.30% of *trans* FA in the rat's brain, whereas L of 0.20%, with no observed *trans* incorporation in SO group. This incorporation did not influence the symptoms of anxiety in HVF and L groups, but the exercise benefited the SO group, increasing their exploratory and risk behavior. A memory deficit was observed in the HVF, but reversed by physical exercise, equaling the memory acquisition of the three experimental groups. The activity of Na^+K^+-ATPase was lower in the cortex and hippocampus of animals treated with HVF and was not modified by exercise.

In the 2nd experiment, rats supplemented with the different FA showed similar brain incorporation as described above. These animals were submitted to behavioral assessments of OD, locomotion and activity of Na^+K^+-ATPase and catalase in the striatum. The HVF supplementation was associated with increased OD, which was intensified by the exercise in the HVF and L groups. The locomotion was reduced in these two groups and was not modified by exercise. The catalase activity was lower in L and HVF groups, but elevated by exercise in the latter one. The different FA did not alter the Na^+K^+-ATPase activity, which was elevated by exercise in animals supplemented with L and SO.

The brain incorporation of *trans* FA may be related to the motor impairments mainly observed in the HVF group, while the absent incorporation in the SO group, with the best motor performance and enzymatic activities.

Taken together, the presented data suggest that healthy lifestyle habits, which include reduced intake of saturated and *trans* FA and the regular practice of physical activity, may be able to prevent and/or reduce the development or the consequences of neurological and neuropsychiatric disorders.

Keywords: CNS. Exercise. Haloperidol. *Trans* Fatty Acids. Motor Disorders.

LISTA DE ABREVIATURAS

AA – Ácido Araquidônico

AG – Ácidos Graxos

ALA – Ácido α-Linilênico

BDNF – Fator Neurotrófico Derivado do Cérebro (do inglês *Brain Derived Neurotrophic Factor*)

DA - Dopamina

DHA – Ácido Docosaexaenóico

DO – Discinesia Orofacial

EO – Estresse Oxidativo

EPA- Ácido Eicosapentaenóico

EROs – Espécies Reativas de Oxigênio

GVH – Gordura Vegetal Hidrogenada

LA – Ácido Linilêico

LC-PUFAs – Ácidos Graxos Poliinsaturados de Cadeia Longa

MMV- Movimentos de Mascar no Vazio

n-3 – Ômega-3

n-6 – Ômega-6

PUFAs – Ácidos Graxos Poliinsaturados (em inglês P*olyunsaturated Fatty Acids*)

TBARS – espécies reativas ao ácido tiobarbitúrico

TF- tremor facial

TNF – Fator de Necrose Tumoral (em inglês *Tumor Necrosis Factor*)

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APRESENTAÇÃO

No item “Introdução” encontra-se considerações iniciais sobre os temas desenvolvidos nesta tese. Os resultados estão apresentados sob a forma de artigos no item “Artigos Científicos”, onde se encontram os itens Introdução, Materiais e Métodos, Resultados, Discussão e Bibliografias, representando a íntegra deste estudo.

Os itens “Discussão Geral” e “Conclusões Finais”, encontrados no final desta tese, apresentam interpretações e comentários gerais sobre os artigos científicos aqui contidos.

As “Referências Bibliográficas” referem-se somente às citações apresentadas nos itens “Introdução” e “Discussão Geral” desta tese.

CAPÍTULO I

1. INTRODUÇÃO

Evidências das últimas décadas indicam que a etiologia de várias desordens neurológicas é multifatorial e envolve uma ampla variedade de neurônios, inflamação, metabolismo e genética. Típicos da sociedade moderna, níveis insuficientes de exercício (BÉLANGER; FOSTER, 2011) e más práticas alimentares, como o consumo elevado de alimentos *fast-food* e industrializados, ricos em ácidos graxos (AG) *trans* e saturados (CRAIG-SCHMIDT, 2006), podem ser considerados fatores de risco para o desenvolvimento de doenças envolvendo o sistema nervoso central (SNC).

A partir da segunda metade do século XX, a elevada utilização de gordura vegetal hidrogenada e gorduras saturadas pela indústria de alimentos foi acompanhada por uma significativa redução do consumo de alimentos ricos em ômega-3 (n-3) e um aumento signifcativo na ingestão de AG *trans* (PFEUFFER; SCHREZENMEIER, 2006). Alimentos como margarinas, biscoitos e hambúrgueres costumam conter grandes quantidades de ácidos graxos saturados, monoinsaturados e poliinsaturados da série ômega-6 (n-6) (BAGGIO; BRAGAGNOLO, 2006) e consideráveis quantidades de isômeros *trans* (ALLISON et al., 1999). Essa mudança nos hábitos alimentares da população aumentou também a relação n-6/n-3 de ácidos graxos poliinsaturados (do inglês, PUFAs), principalmente como consequência da redução da ingestão de ácidos graxos n-3 (AILHAUD et al., 2006). Desta forma, o consumo de AG *trans* pode culminar em uma perda de ingestão de AG essenciais e, consequentemente, ter um impacto perigoso e imprevisível para a saúde humana, uma vez que estes desempenham um papel funcional importante sobre as membranas biológicas (SARSILMAZ et al., 2003).

Por sua vez, o exercício físico proporciona mecanismos de contra-ataque, o que possibilita a reação do cérebro frente a diversos insultos. Pode-se observar benefícios na recuperação após dano cerebral decorrente de traumas e doenças (BOHANNON, 1993; GREALY; JOHNSON; RUSHTON, 1999), além de contrabalancear perdas decorrentes do envelhecimento (KRAMER et al., 1999). Estudos em um modelo animal de estresse oxidativo demonstraram efeitos benéficos da atividade física sobre a atividade enzimática e distúrbios do movimento (TEIXEIRA et al., 2008), sendo este efeito dependente da intensidade do exercício fisíco (TEIXEIRA et al., 2009). Os distúrbios de movimento estão presentes em diversas doenças neurodegenerativas de grande incidência como Parkinson, Huntington, epilepsia e discinesia tardia (ALBIN; YOUNG; PENNEY, 1989; BARTZOKIS et al., 1999; FAHN; COHEN, 1992; LOHR et al., 1990), justificando a importância destes estudos.

Além disso, segundo Gomez-Pinilla et al. (2008), dieta e exercício físico influenciam de maneira complementar as funções cerebrais através do controle homeostático e da plasticidade sináptica. A atividade física também demonstra resultados eficazes sobre hábitos alimentares prejudiciais (MOLTENI et al., 2004).

Sendo assim, os hábitos alimentares atualmente adotados podem influenciar de maneira significativa o conteúdo de AG dos tecidos cerebrais. Uma vez que grande parte dos alimentos ingeridos pela sociedade moderna é rico em AG *trans* e que pouco se conhece sobre sua incorporação no cérebro, mais estudos se fazem necessários. Deve-se considerar ainda a grande incidência de doenças neuropsiquiátricas e do movimento na população e a influência de fatores como sedentarismo e consumo de alimentos processados contendo quantidades elevadas de isômeros *trans* na etiologia destas doenças. Faz-se necessária também a pesquisa e inclusão de atividades alternativas, como a realização de exercícios físicos adequados, capazes de modular positivamente as funções cerebrais, justificando, assim, os estudos apresentados nesta tese.

1. 1 Ácidos Graxos: Classificação geral e presença nos alimentos

Ácidos graxos (AG) são substâncias encontradas em uma ampla variedade de alimentos, os quais na forma de lipídeos (formados pela esterificação de AG e alcoóis) fornecem e servem como armazenamento de energia, proteção e estrutura celular (COSTA; 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) no outro, cuja classificação varia de acordo com o número de insaturações (saturados, monoinsaturados ou poliinsaturados) e com o comprimento da cadeia (curta, média e longa) (LEHNINGER; NELSON; COX, 2002) (Figura 1).

Os AG poliinsaturados podem ser classificados principalmente nas séries ômega 6 (n-6) e ômega 3 (n-3), diferenciando-se pela posição da primeira dupla ligação a partir do grupo metílico terminal da cadeia. O ácido linoléico (C18:2, LA), representante da série n-6, é abundante nos óleos vegetais de girassol, milho, soja, etc. (SANGIOVANNI; CHEW, 2005). O ácido α-linolênico (C18:3, ALA), representante da série n-3, é encontrado em nozes e sementes oleaginosas como soja, canola e linhaça (HULBERT et al., 2004). 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; WAINWRIGHT, 1992).

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

Os AG saturados 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), além de produtos vegetais hidrogenados (CARVALHO et al., 2003; SALEM, 1999).

Os AG monoinsaturados se encontram na maioria das gorduras animais, bem como no azeite de oliva, nos óleos de canola e de soja e em nozes, sendo o ácido

oléico (C18:1) o principal representante da classe (DUNCAN; SCHMIDT; GIUGLIANI, 2004).

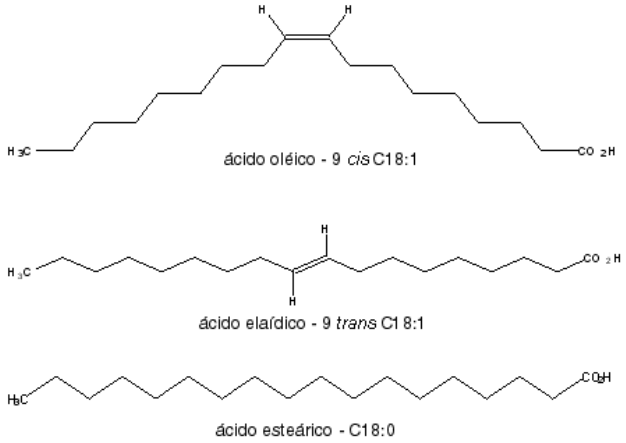

Figura 1. Representação do ácido graxo oléico (monoinsaturado cis), elaídico (monoinsaturado *trans*) e esteárico (saturado).

1.2 Ácidos Graxos Poliinsaturados (PUFAs)

Os PUFAs são considerados AG essenciais, pois não são sintetizados no organismo e devem ser obtidos através da dieta a fim de compor as membranas celulares (SOLFRIZZI et al., 2005; YEHUDA; RABINOVITZ; MOSTOFSKY, 2005). 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; 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 PUFAs de cadeia longa (LC-PUFAs), 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 LC-PUFAs 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).

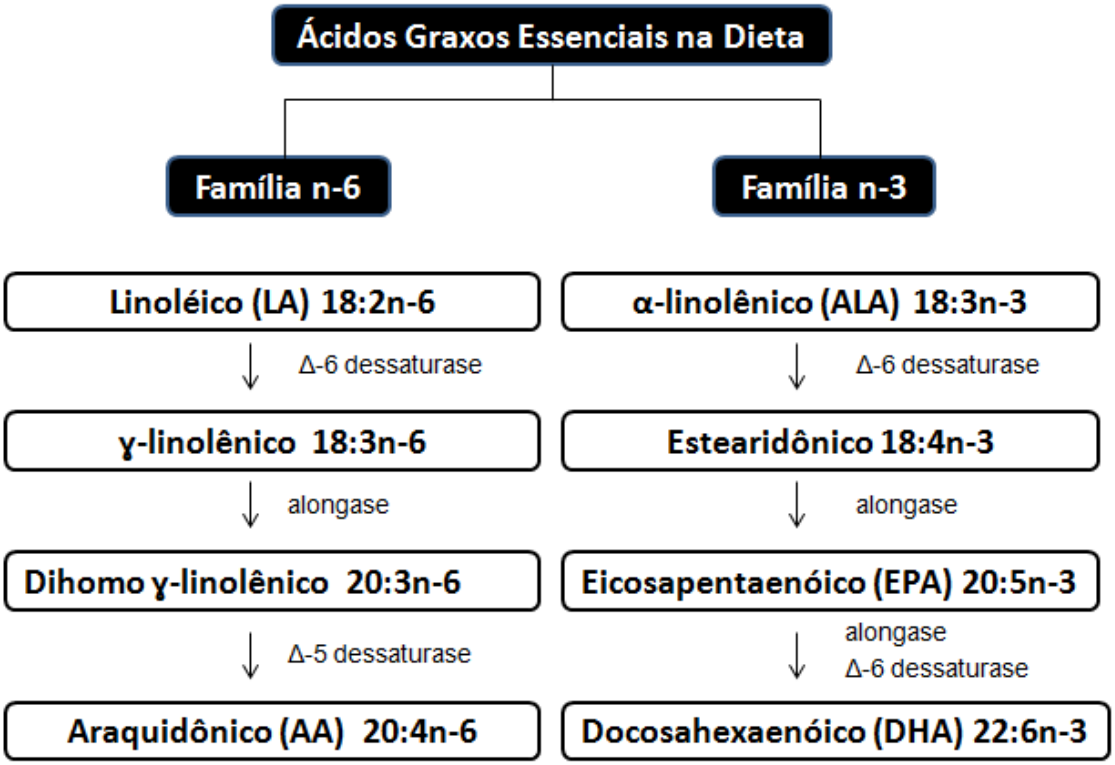

Figura 2. Via metabólica dos ácidos graxos essenciais (adaptado de LAURITZEN et al., 2001).

Os PUFAs afetam as funções cerebrais através de, pelo menos, seis tipos de modificações: (a) na fluidez da membrana; (b) na atividade de enzimas ligadas à membrana; (c) no número e afinidade de receptores; (d) na função de canais iônicos; (e) na produção e atividade de neurotransmissores; e (f) na transdução de sinais, os quais controlam a atividade de neurotransmissores e os fatores de crescimento neuronal (CLARKE et al., 2005; DAS, 2003; LEVANT; RADEL; CARLSON, 2004; McNAMARA; CARLSON, 2006; SUMIYOSHI et al., 2008; YEHUDA; RABINOVITZ; MOSTOFSKY, 2005). De particular importância para as funções do SNC, a carência de ômega-3 pode estar associada à 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 com ômega-3 é 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 desordens neurológicas e neuropsiquiátricas (BLACK; HOFF; RODIN, 1984).

1.2.1 Ácidos Graxos Essenciais e Tecido Cerebral

O cérebro necessita de um aporte adequado de ácidos graxos para manter sua integridade estrutural e, consequentemente, suas funções normais, principalmente por apresentar elevado teor de lipídeos de membranas correspondendo a 50% do seu peso seco dos quais 25% são AG essenciais (MARTEINSDOTTIR et al., 1998; UAUY; DANGOUR, 2006). Variações nos níveis de AG essenciais da dieta são capazes de alterar completamente o perfil lipídico das membranas cerebrais, modificando características como fluidez, estabilidade e suscetibilidade a danos oxidativos (GUTTERIDGE; HALLIWELL, 1994; PECK, 1994). Sabe-se que o PUFA AA (n-6) é necessário nos processos de sinalização e divisão celular, bem como um precursor de eicosanóides e leucotrienos (agentes pró-inflamatórios), os quais também desempenham funções na transmissão sináptica (ELIAS; INIS, 2001). Lukiw et al. (2005) demonstraram que o derivado endógeno do DHA (neuroprotectina D1- NPD1) exerce atividade regulatória, neuroprotetora e antiinflamatória neuronal, além de estar envolvido na programação dos genes de expressão e atividade anti-apoptótica, promovendo resistência neuronal ao EO.

O conteúdo de DHA em muitas regiões cerebrais é 30 a 50% maior que o de AA, exercendo um papel crítico no funcionamento do sistema nervoso central (particularmente membrana sináptica) e retina (BIRCH et al., 2002; CHAMPUX et al., 2002; NEURINGER, 2000; SANGIOVANNI et al., 2000). Além disso, acredita-se que deficiências de DHA durante o desenvolvimento pré e pós-natal afetam a acuidade visual, as funções cognitivas e, possivelmente, o comportamento e a suscetibilidade às desordens psiquiátricas (FUGH-BERMAN; COTT, 1999).

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). A atividade neuroprotetora do

DHA também foi evidenciada através de suas propriedades antioxidantes *in vivo* (BAZAN, 2005; CALON et al., 2004;HASHIMOTO et al., 2002; YAVIN; BRAND; GREEN, 2002) e *in vitro,* como no aumento da atividade da glutationa redutase (HASHIMOTO, 2002), diminuição da oxidação de proteínas (CALON et al., 2004) e dos níveis de peróxidos de lipídios e espécies reativas de oxigênio (EROs) (HASHIMOTO et al., 2002; 2006). Estudos demonstraram ainda que o DHA participa diretamente da modulação da expressão gênica, de processos que envolvem EO, sinalização e divisão celular, crescimento e apoptose (SIMOPOULOS, 2006; YAVIN, 2006).

A incorporação de DHA nas membranas celulares também é capaz de 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). Além disso, DHA e EPA podem modificar a produção e a função de neurotransmissores, tais como a serotonina e a dopamina (DU BOIS et al., 2006; FENTON et al., 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 e cols., 2007).

1.3 Ácidos Graxos *Trans* (AG *trans*)

Os AG *trans* não são sintetizados no organismo humano e resultam de um processo de bio-hidrogenação (ação de enzimas do rúmen, 1-8% AG *trans*) ou de processos industriais de hidrogenação parcial ou total de óleos vegetais (GEUKING, 1995). São formados a partir dos AG insaturados naturais (*cis*) através da introdução de hidrogênios nas cadeias de carbono em posição transversal (Figura 3), o que torna a molécula mais linear, uma forma raramente encontrada na natureza (HARPER, 1994). Neste sentido, os AG *trans* são praticamente inexistentes em óleos e gorduras de origem vegetal, mas pequenas quantidades podem ser formadas durante a extração, refinamento e armazenamento (GEUKING, 1995).

CIS *TRANS*

Figura 3. Configurações dos isômeros cis (forma líquida) e *trans* (forma sólida). (Fonte: WARDLAW, 2002)

O processo de hidrogenação parcial confere aos óleos vegetais algumas propriedades físicas de AG saturados, tais como consistência mais firme, elevação no ponto de fusão e maior estabilidade à oxidação lipídica (CURI et al., 2002; TARRAGO-TRANI et al., 2006). Estão presentes em uma ampla variedade de produtos manufaturados, como margarinas, biscoitos, sorvetes, salgadinhos de pacotes, pães, bolos e tortas (40-50% AG *trans*) (CRAIG-SCHMIDT; HOLZER, 2000; MOZAFFARIAN et al., 2006).

1.3.1 Ácidos Graxos *Trans* e Tecido Cerebral

Os isômeros *trans* podem ser digeridos, absorvidos e incorporados nas membranas celulares de forma semelhante aos AG com isômeros *cis* não apresentando, porém, atividade como os AG essenciais (ENIG, 2000; KHOSLA; HAYES, 1996). A incorporação dos *trans* nos tecidos depende da quantidade e do tempo de ingestão deste tipo de AG, do tipo de tecido e de isômero, além da quantidade paralelamente consumida de AG essenciais (GARDLAND et al., 1998; GEUKING, 1995;), competindo inclusive pelos mesmos sistemas enzimáticos (MAHFOUZ; KUMMEROW, 1999). De acordo com isso, Sabarense e Mancini-Filho (2003) verificaram que os isômeros *trans* são capazes de reduzir significativamente a formação de DHA no coração de ratos. Resultados semelhantes foram encontrados em ratos alimentados com óleo de palma e gordura vegetal hidrogenada (rica em *trans*), em que esta reduziu os níveis de AA e EPA no plasma

dos animais, indicando uma inibição de conversão metabólica (SILVA et al., 2005). A inibição da biossíntese de AA e DHA por isômeros *trans* é proposta ainda como responsável por afetar o crescimento intrauterino e pós-nascimento (DECSI-BROUWER; KOLETZKO, 1995). Corroborando com esta hipótese, outros modelos experimentais demonstram uma elevação do 22:5 n-6 e uma redução no conteúdo de DHA cerebral decorrentes de elevado consumo de *trans* (GRANDGIRARD et al., 1994; PETTERSON; OPSTVEDT, 1992). Este aumento na concentração de 22:5 n-6 funciona como um mecanismo compensatório, sugerindo que LC-PUFAs são influenciados por AG *trans* da dieta (GALLI; TRZECIAK; PAOLETTI, 1971).

De modo particular, os efeitos de uma deficiência em n-3 provocados pela incorporação de AG *trans* são importantes principalmente sobre o SNC, no qual PUFAs são constituintes fundamentais. Especificadamente, uma deficiência em ALA foi capaz de promover déficits de memória, sensoriais, motores e motivacionais em ratos (FRANCE`S; MONIER; BOURRE, 1995; WAINWRIGHT, 1992, 2002; YAMAMOTO et al., 1987, 1988). Níveis reduzidos de PUFAs também foram encontrados no cérebro *post mortem* de pacientes esquizofrênicos (HORROBIN et al., 1991; YAO; LEONARD; REDDY, 2000), e mudanças na neurotransmissão dopaminérgica (déficits no armazenamento de dopamina) podem estar relacionadas (DELION et al., 1994; ZIMMER et al., 1998; 2000).

De acordo, outros estudos mostram que os AG *trans* inibem a reação de dessaturação dos LA e ALA para AA, DHA e EPA, favorecendo o metabolismo de *trans* monoméricos e de AG n-6 ou n-3 em isômeros incomuns que, se incorporados aos tecidos, alteram as funções das membranas ou dos eicosanóides (INNIS, 2006; INNIS; KING, 1999). Assim, esta incorporação influenciaria mecanismos pró-inflamatórios e pró-apoptóticos podendo aumentar os níveis do fator de necrose tumoral (TNF), interleucina-6 e proteína C reativa (MOZAFFARIAN et al., 2006).

1.4 Ácidos Graxos Saturados

Um ácido graxo é saturado quando todas as ligações de carbono estão ocupadas por átomos de hidrogênio sendo, portanto, altamente estáveis. Todos os AG naturalmente existentes, como óleos de origem animal ou vegetal, apresentam

uma combinação de ácidos graxos saturados, monoinsaturados e poliinsaturados (ácidos linoléico e linolênico, principalmente) (WHITNEY; ROLFES, 2005).

Os AG saturados desempenham um papel vital na saúde dos ossos, conferindo firmeza e integridade às células (WATKINS et al., 1997), e são essenciais para o desenvolvimento do cérebro de recém-nascidos e crianças (ALFIN-SLATER; AFTERGOOD, 1980). Além disso, podem possuir propriedades antimicrobianas e estão associados ao fortalecimento do sistema imunológico (ENIG, 2000; KABARA, 1978).

Em contrapartida, considerando o SNC, a ingestão de dietas contendo um elevado teor de AG saturados está associada com déficits cognitivos mais acentuados no processo de envelhecimento (MORRIS et al., 2004), com a doença de Alzheimer (GRANT, 1997; MORRIS et al., 2003) e com um maior risco para a demência (KALMIJN et al., 1997). Um estudo avaliando os efeitos de três meses de dietas contendo elevadas quantidades de AG saturados, PUFAs ou monoinsaturados, comparadas a uma dieta com baixo teor de gordura, demonstrou maiores prejuízos cognitivos nos ratos alimentados com os AG saturados (GREENWOOD; WINOCUR, 1996). Uma grande ingestão de AG saturado compromete o desempenho cognitivo, possivelmente por reduzir os níveis do fator neurotrófico derivado do cérebro (BDNF) (MOLTENI et al., 2002), além de agravar danos causados por traumatismo sobre a neuroplasticidade e a cognição (WU et al., 2003). Em adição, os prejuízos decorrentes do consumo elevado de AG saturados podem derivar do aumento da produção de espécies reativas (BELTOWSKI et al., 2000), e uma suplementação com antioxidantes é capaz de conter seus efeitos adversos sobre a função neuronal e a cognição (WU; YING; GOMEZ-PINILLA, 2004).

Segundo alguns autores, os efeitos de uma dieta com elevado teor de AG saturados sobre a função neural podem ser o reflexo de disfunções cardiovasculares, tais como aterosclerose (KEYS, 1997; KHAN et al., 2004), desordens metabólicas (GLUCKMAN; HANSON, 2004; LUCAS, 1991), hipertensão (ROSEBOOM et al., 1999), obesidade (FARR et al., 2008) entre outros.

Modificações capazes de prejudicar a funcionalidade do SNC consequentes à incorporação de ácidos graxos saturados e, especialmente de ácidos graxos *trans*, nas membranas neurais têm sido pouco descritas na literatura.

1.5 A Influência da Atividade Física sobre o SNC

Os efeitos de uma atividade física regular se mostram promissores em diversas desordens neurológicas/neurodegenerativas, cuja fisiopatologia está relacionada ao estresse oxidativo (EO). Efeitos benéficos também são descritos quando ocorre a associação entre o exercício físico e constituintes específicos da dieta.

Wu et al. (2008) demonstram que o exercício físico tem efeitos benéficos potencializados sobre plasticidade sináptica e cognição quando associado ao ômega-3; enquanto capaz de reduzir os efeitos deletérios de uma dieta rica em AG saturados e açúcar refinado sobre os mesmos parâmetros (MOLTENI et al., 2004). Este tipo de dieta está relacionado com um déficit de aprendizagem por causar uma diminuição nos níveis do fator neurotrófico cerebral no hipocampo (MOLTENI et al., 2002), os quais são elevados pelo exercício (NEEPER et al., 1995, 1996).

Relevantemente, as membranas fosfolipídicas cerebrais, por conterem grande quantidade de AG poliinsaturados em sua composição, tornam-se susceptíveis a danos oxidativos. Estes danos oxidativos também podem ser oriundos do metabolismo energético de elementos da dieta, através da formação exacerbada de espécies reativas inicialmente na mitocôndria (CHENG; MATTSON, 1994; MOLTENI et al., 2004). Espécies reativas de oxigênio (EROs) têm sido relacionadas a doenças neurológicas e ao declínio cognitivo observado no envelhecimento, o que sugere que estas espécies desempenham um importante papel nas funções cerebrais (AHLEMEYER; KRIEGLSTEIN, 2000; CADET; BRANNOCK, 1998). A atividade física regular parece estimular o estado redox do cérebro e aumentar a atividade de enzimas antioxidantes, atenuando a formação de EROs e melhorando, assim, as funções fisiológicas e a resistência ao EO (RADAK et al., 2001; RADAK; CHUNG; GOTO, 2005), mesmo se realizado de forma intensa (OGONOVSZKY et al., 2005).

O cérebro é mais vulnerável ao EO quando comparado a outros órgãos e tecidos, pois, além de conter grande quantidade de lipídeos peroxidáveis, consome elevados níveis de oxigênio, possui baixos níveis de defesas antioxidantes e contem aminoácidos excitotóxicos (HALLIWELL; GUTTERIDGE, 1999). Muitos neurotransmissores, como a noradrenalina e a dopamina, são auto-oxidáveis e, reagindo com o oxigênio molecular para formar quinonas e semiquinonas,

consomem glutationa, o que pode gerar espécies reativas de oxigênio (EROs) durante este processo (GRAHAM, 1978). De particular importância, regiões contendo elevadas concentrações de dopamina e metais de transição, como substância negra e gânglios basais, são mais susceptíveis ao EO e ao envolvimento na patogênese de desordens degenerativas e do movimento (ELKASHEF; WYATT, 1999; LARSON et al., 1979; SACHDEV; SAHAROV; CTHCART, 1999).

Em um modelo experimental de trauma cerebral, o exercício protegeu a região do córtex da peroxidação lipídica, carbonilação de proteína e da inibição da enzima Na^+K^+-ATPse (LIMA et al., 2009). Nas membranas do SNC, a Na^+K^+-ATPse é responsável pela manutenção do gradiente eletroquímico transmembrana, podendo afetar diretamente a liberação de neurotransmissores e a atividade neural (JAMME et al., 1995; LI; STYS, 2001). Outros autores também demonstraram que o exercício facilita a recuperação após danos cerebrais (BOHANNON, 1993; GREALY; JOHNSON; RUSHTON, 1999; LINDVALL et al., 1992). Além disso, a atividade física se mostra eficaz na melhora do aprendizado e da cognição (ROGERS; MEYER; MORTEL, 1990; SUOMINEN-TROYER et al., 1986; VAN PRAAG et al., 1999), de forma especial no processo senil (KRAMER et al., 1999; LAURIN et al., 2001).

Considerando estudos clínicos, pacientes com a doença de Parkinson apresentam melhora significativa sobre a atividade motora, a cognição e o estado geral de humor, em decorrência do treinamento físico (BAATILE et al., 2000; HURWITZ, 1989; MIYAI et al., 2000; SUNVISSON et al., 1997). A atividade física está associada ainda a um menor risco para o desenvolvimento da doença de Alzheimer e demência de qualquer grau (BROE et al., 1990; LAURIN et al., 2001; SHIMAMURA et al., 1998). Benefícios semelhantes têm sido observados em pacientes portadores de esquizofrenia (CONNOLLY; KELLY, 2005; MARDER et al., 2004).

O exercício físico regular pode evocar diversas mudanças no SNC, incluindo a expressão de fatores neurotróficos (BORTZ et al., 1981; GOMEZ-PINILLA; SO; KESSLAK, 1998), neurogênese pós-natal (GOULD et al., 1999) e o aumento na densidade de vasos sanguíneos (BLACK et al., 1990; SWAIN et al., 2003). O exercício também influencia a atividade dopaminérgica central (FREED; YAMAMOTO, 1985; HATTORI; NAOI; NISHINO, 1994), sendo capaz de promover um aumento da plasticidade e funcionalidade cerebral normal e comprometida

(COTMAN; BERCHTOLD, 2002; SUTOO; AKIYAMA, 2003). Segundo Sarbadhikari e Saha (2006), esta ação sobre neurotransmissores (dopamina, serotonina e norepinefrina) ocorre através da elevação da expressão do BDNF, claramente envolvido na excitabilidade neuronal (BOLTON; PITTMAN; LO, 2000; KAFITZ et al., 1999). Existe uma estreita conexão entre BDNF, função cognitiva e homeostasia celular. Encontrando-se este fator reduzido no hipocampo de animais, são observados déficits de memória e aprendizado (MOLTENI et al., 2002), já discutidos anteriormente como sendo beneficiados pelo exercício. Esta habilidade do exercício físico em elevar a função cognitiva por ação neurotrófica pode estar relacionada com processos metabólicos e consequentes alterações nos níveis de EO (KIRKWOOD, 2002; RADAK et al., 2001).

1.6 Espécies Reativas e Estresse Oxidativo

1.6.1 Definição e Formação

Espécies reativas podem ser definidas como átomos, moléculas orgânicas ou inorgânicas capazes de existir sob formas independentes, e que contêm um ou mais elétrons desemparelhados na camada de valência (HALLIWELL, 1994; HALLIWELL; GUTTERIDGE, 1999). Esta configuração confere alta instabilidade, meia-vida curta e recombinação química quase imediata (POMPELLA, 1997). Podem ser geradas no citoplasma, na mitocôndria ou na membrana celular e, de acordo com o sítio de formação, danificar proteínas, lipídios, carboidratos e DNA (ANDERSON, 1996; YU; ANDERSON, 1997).

Uma fonte importante de espécies reativas é o sistema de transporte mitocondrial, onde a citocromo oxidase promove a redução completa de uma molécula de O_2 em duas moléculas de água (SOUTHORN; POWIS, 1988) (Figura 4).

$$O_2 + 4H^- + 4e^- \rightarrow 2H_2O$$

Figura 4. Reação de redução completa da molécula de O_2.

No entanto, nem sempre o oxigênio origina água diretamente. Por sua configuração eletrônica, o oxigênio tende a receber um elétron de cada vez formando intermediários tóxicos e reativos, tais como radical superóxido (O_2^-), peróxido de hidrogênio (H_2O_2) e radical hidroxil ($OH^\bullet$) (MENEGHINI, 1987) (Figura 5).

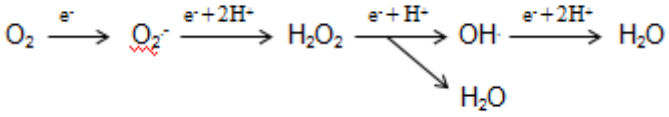

Figura 5. Reação de redução da molécula de O_2 e intermediários reativos.

O peróxido de hidrogênio (H_2O_2) é capaz de atravessar a membrana nuclear e induzir danos na molécula de DNA por meio de reações enzimáticas (ANDERSON, 1996; CHANCE; SIES; BOVERIS, 1979).

O radical hidroxil é um dos mais potentes oxidantes, pois é capaz de atravessar as membranas e reagir com moléculas, tais como lipídeos insaturados e DNA (ANDERSON, 1996).

1.6.2 Lipoperoxidação

A peroxidação lipídica (ou lipoperoxidação) é definida como a deterioração oxidativa dos lipídeos poliinsaturados presentes tanto nas membranas celulares quanto nas organelas (mitocôndrias, peroxissomas) (CHAMPE; HARVEY, 1997). O processo de lipoperoxidação consiste de reações em cadeia que podem ser

divididas em três fases: iniciação, propagação e terminação (BOVERIS, 1998) (Figura 6).

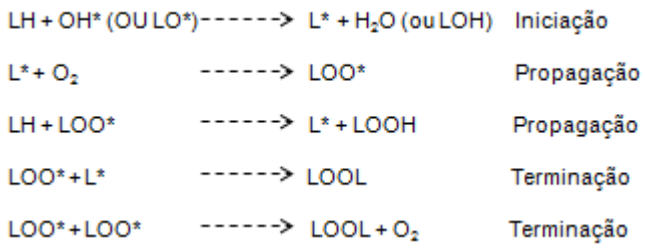

Figura 6. Etapas da lipoperoxidação.

A contínua renovação das membranas celulares promove naturalmente o processo de lipoperoxidação. No entanto, quando as defesas antioxidantes são insuficientes ou quando há uma intensa produção de espécies reativas, este processo pode se tornar tóxico. A peroxidação de membranas lipídicas pode resultar na perda de PUFAs, menor fluidez da membrana e diversas modificações estruturais resultando na perda da atividade de receptores e enzimas, além de danos diretos às proteínas de membrana (DEAN; GIESEG; DAVIES, 1993; OLIVER et al., 1987; STADTMAN, 1993). Uma medida indireta de dano celular é obtida através da quantificação do MDA (malondialdeído - produzido pela reação com o ácido tiobarbitúrico), que é um biomarcador de estresse oxidativo liberado no processo de lipoperoxidação (BROWN; KELLY, 1996; OHKAWA; OHISHI; YAGI, 1979).

1.6.3 Estresse Oxidativo e Sistema de Defesa Antioxidante

O EO ocorre quando há um desequilíbrio entre os fenômenos pró-oxidantes e as defesas antioxidantes celulares, podendo levar à degradação da membrana, disfunção celular, danos ao DNA e apoptose (FREY et al., 2006; HALLIWELL, 2006). O cérebro é extremamente sensível ao EO devido a sua grande quantidade de PUFAs e baixas defesas antioxidantes (LOHR; KUCZENSKI; NICULESCU, 2003),

além de ser responsável por consumir aproximadamente 20% do O_2 basal corporal (HALLIWELL & GUTTERIDGE, 1999).

Os antioxidantes são capazes de interceptar as espécies reativasgeradas pelo metabolismo celular ou por fontes exógenas, impedindo o ataque sobre os lipídeos, os aminoácidos das proteínas, a dupla ligação dos ácidos graxos poliinsaturados e as bases do DNA, evitando, assim, a formação de lesões e a perda da integridade celular (BIANCHI; ANTUNES, 1999; DROGE, 2002). A eficácia dos antioxidantes depende do tipo de radical gerado, do local de formação e da severidade do dano causado (HALLIWELL, 1994, 1997).

O sistema de defesa antioxidante está dividido em enzimático e não enzimático. O primeiro inclui as enzimas superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx). A SOD catalisa a remoção do radical superóxido através da conversão à H_2O_2, enquanto a enzima catalase age na eliminação do H_2O_2 promovendo sua catálise até formar água. A GPx remove H_2O_2 e forma água, convertendo a glutationa reduzida (GSH) à glutationa oxidada (GSSG) (LOHR; KUCZENSKI; NICULESCU, 2003). O sistema não enzimático inclui compostos sintetizados pelos seres vivos como bilirrubina, ceruloplasmina, hormônios sexuais, melatonina, coenzima Q e ácido úrico; além de outros compostos presentes na dieta como ácido ascórbico, α-tocoferol, β-caroteno e grupos flavonóides (SCHNEIDER; OLIVEIRA, 2004).

1.6.4 Enzima Na^+K^+-ATPase

Além das enzimas citadas, uma importante enzima presente nas membranas celulares e bastante sensível a agentes oxidantes é a Na^+K^+-ATPase (CARFAGNA; PONSLER; MUHOBERAC, 1996; FOLMER e cols., 2004). Está presente em altas concentrações no tecido cerebral, consumindo cerca de 40 a 50% do ATP gerado neste tecido (ERECINSKA; SILVER, 1994). A inativação da Na^+K^+-ATPase leva a uma despolarização parcial da membrana e sucessiva entrada de Ca^{+2} nas células neuronais, podendo culminar em excitotoxicidade (BEAL; HYMAN; KOROSHETZ, 1993). De fato, estudos relacionam o envolvimento entre o EO e a função da enzima Na^+K^+-ATPase através de alterações observadas em sua atividade decorrentes da

lipoperoxidação (KAUR; SHARMA; SINGH, 2001; PIERRE et al., 1999). Oliveira et al. (2004) mostraram que um tratamento com o antioxidante ascorbato foi capaz de prevenir a inibição desta enzima em um modelo experimental de epilepsia.

1.6.5 Estresse Oxidativo Induzido por Haloperidol

Substâncias como o haloperidol, amplamente utilizado no tratamento farmacológico de pacientes esquizofrênicos, estão associadas ao desenvolvimento de EO (BURGER et al., 2005a; CADET; LOHR, 1989). Esta medicação antipsicótica age bloqueando receptores pré e pós-sinápticos dopaminérgicos, o que pode causar um aumento secundário na síntese, liberação e metabolismo da dopamina. O catabolismo das catecolaminas e a consequente produção de espécies reativas e neurotoxicidade são bem descritos (CLOW et al., 1980; COHEN, 1984; SLIVKA; COHEN, 1985). A autooxidação da dopamina pode resultar na elevada formação de quinonas de dopamina e espécies reativas como o radical superóxido (COHEN; ZUBENKO, 1985). Ainda, a ação da enzima monoamina oxidase (MAO) sobre a dopamina pode produzir H_2O_2 (SINET; HEIKKILA; COHEN, 1980), o qual pode reagir com diferentes constituintes como aminoácidos, ácidos nucléicos, fosfolipídeos e açúcares (KONAT; WIGGINS, 1985; MELLO FILHO; MENEGHINI, 1985).

A supersensibilidade dopaminérgica é descrita por originar EO e o desenvolvimento de desordens do movimento, especialmente na região orofacial e pescoço, denominadas como discinesia tardia (ELKASHEF; WYATT, 1999; LLORCA et al., 2002; TSAI et al., 1998) e também estudadas em modelos animais (BURGER et al., 2005a b; SACHDEV; SAHAROV; CTHCART, 1999). Além da discinesia tardia, muitas doenças envolvendo desordens do movimento têm sido associadas com a geração de espécies reativas e neurodegeneração, tais como, a síndrome de Parkinson (FAHAN; COHEN, 1992) e a doença de Huntington (BEAL, 1996), principalmente.

2. OBJETIVOS

2.1 Objetivo Geral

Verificar as correlações existentes entre comportamento, estresse oxidativo e distúrbios do movimento induzidos tanto farmacologicamente quanto por ácidos graxos (AG) oriundos da dieta. Avaliar a eficácia da atividade física em ambas as situações.

2.2 Objetivos Específicos

ARTIGO 1:

1. Avaliar os danos oxidativos induzidos pela administração de haloperidol sub-crônico em ratos, através da observação dos distúrbios do movimento;

2. Avaliar os efeitos da administração do haloperidol sobre os danos oxidativos observados através da lipoperoxidação, do envolvimento da enzima catalase e do transportador de dopamina, no cérebro de ratos;

3. Avaliar os efeitos do exercício físico na prevenção dos distúrbios do movimento induzidos pela administração de haloperidol sub-crônico em ratos;

4. Verificar os efeitos do exercício físico sobre a lipoperoxidação, a atividade da enzima catalase e do transportador de dopamina, após administração sub-crônica de haloperidol;

ARTIGO 2:

5. Verificar a incorporação de AG no cérebro de animais cronicamente suplementados com diferentes AG (20% poliinsaturados, saturados e monoinsaturados, ou *trans* e saturados);

6. Verificar os efeitos da suplementação com os diferentes AG sobre aprendizado e memória, e a possível correlação com a incorporação *trans* no cérebro;

7. Verificar a influência da suplementação com os diferentes AG sobre a atividade da enzima Na^+K^+-ATPase;

8. Avaliar os efeitos do exercício físico sobre parâmetros de aprendizagem e ansiedade em animais suplementados com diferentes AG (20% poliinsaturados, saturados e monoinsaturados, ou *trans* e saturados);

9. Avaliar os efeitos do exercício em animais suplementados com os diferentes AG sobre a atividade da enzima Na^+K^+-ATPase;

ARTIGO 3:

10. Analisar a relação entre a suplementação com os diferentes AG e, principalmente, a incorporação *trans* no cérebro, com o desenvolvimento dos distúrbios do movimento;

11. Verificar se os diferentes AG e a incorporação *trans* podem modificar a atividade das enzimas catalase e Na^+K^+-ATPase;

12. Observar os efeitos da atividade física associada às diferentes suplementações e à incorporação *trans* no cérebro, sobre o desenvolvimento dos distúrbios do movimento;

13. Avaliar os efeitos da atividade física associada às diferentes suplementações e à incorporação *trans* no cérebro, sobre a atividade das enzimas catalase e Na^+K^+-ATPase.

CAPÍTULO II

3. ARTIGOS CIENTÍFICOS

3.1 ARTIGO 1:

Publicado na Revista *Neuropharmacology* 60: 432-438, 2011.

Efeitos benéficos de um novo modelo animal de atividade física sobre o desenvolvimento de desordens motoras e oxidativas induzidas por haloperidol em ratos.

Beneficial effects of an innovative exercise model on motor and oxidative disorders induced by haloperidol in rats

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ARTICLE INFO

Article history:
Received 18 August 2010
Received in revised form
27 September 2010
Accepted 20 October 2010

Keywords:
Exercise
Oral dyskinesia
Haloperidol
Oxidative stress
Excitotoxicity

ABSTRACT

Here we evaluate the influence of a new exercise protocol on movement disorders induced by neuroleptic drugs. In this animal model, involuntary movements are closely related to neuronal degeneration and oxidative stress (OS) that can be caused by pre-synaptic D2 receptor blockade increasing dopamine (DA) metabolism. The increase in vacuous chewing movements (VCM) and the reduced locomotor activity induced by haloperidol treatment (12 mg/kg-im, once a week for 4 weeks) was prevented by exercise, 5 times per week, which was initiated four weeks before the first haloperidol administration. Exercise training also prevented the increase of haloperidol-induced lipid peroxidation in the cortex and subcortical region and recovered the catalase activity in the subcortical region. There was a negative correlation between catalase activity in the subcortical region and the VCM frequency ($r = 0.50$, $p < 0.05$), as well as a positive correlation between VCM frequency and lipid peroxidation in the cortex ($r = 0.64$, $p < 0.05$) and subcortical region ($r = 0.71$, $p < 0.0001$). Both haloperidol and exercise increased DA uptake in the striatum, while the co-treatment (exercise plus haloperidol) reduced it. The striatal DA uptake correlated negatively with catalase activity ($r = 0.51$, $p < 0.05$), indicating a relationship between oxidative damage and the function of the transporter in the striatum. Our findings show that physical exercise can modulate dopamine uptake, especially when it is altered, and reveal the benefit of this new exercise protocol in the prevention of movement disorders related to oxidative damage.

1. Introduction

Extensive research demonstrates that exercise exerts neuroprotective effects mainly by reducing brain injury, and by delaying the onset of several neurodegenerative diseases (Howells et al., 2005; Kiraly and Kiraly, 2005; Smith and Zigmond, 2003; Sutoo and Akiyama, 2003). Specifically, studies with exercised animals have shown delays on the onset of the cognitive decline related to Huntington's disease (Pang et al., 2006), and improved spatial learning and memory in Alzheimer's disease (Adlard et al., 2005).

Exercise can also be associated with the generation of reactive oxygen species (ROS) depending on its different forms and intensities and the increased oxygen consumption. A single bout of physical exercise can induce the production of ROS and nitrogen reactive species and the related oxidative damage (Davies et al., 1982; Radak et al., 1999a). Oxidative stress (OS) induced by strenuous physical exercise damages various cell components, such as proteins, DNA, and membrane lipids (Hartmann et al., 1995; Ohkuwa et al., 1997).

On the other hand, regular training is known to increase the resistance against ROS-induced lipid peroxidation (Alessio and Goldfarb, 1988), and to decrease the accumulation of oxidative protein and DNA damage (Leeuwenburgh et al., 1998; Radak et al., 1999b). Furthermore, we have previously demonstrated the beneficial effects of moderate physical exercise on motor disorder and OS in rat brain (Teixeira et al., 2008) while an intense exercise program potentiated it (Teixeira et al., 2009), suggesting that physical training functions paradoxically as either an enhancer or a protector against OS.

Of particular importance, the brain is more susceptible to oxidative damage than peripheral tissues mainly because it contains high levels of membrane lipids, excitotoxic amino acids,

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doi:10.1016/j.neuropharm.2010.10.017

low levels of antioxidant defenses, and auto-oxidizable neurotransmitters (Halliwell and Gutteridge, 1999). Haloperidol has been experimentally used to induce movement disorders which are related to oxidative damage (Barcelos et al., 2009; Burger et al., 2005a, 2006; Colpo et al., 2007; Fachinetto et al., 2005; Polydoro et al., 2004), and its mechanism can be mediated by blocking pre- and post-synaptic dopamine (DA) receptors (Tsai et al., 1998). Furthermore, DA reacts with molecular oxygen to form dopamine-quinones which can deplete glutathione, generating ROS during this process (Graham, 1978). Brain areas, such as the basal ganglia, are rich in monoamines and consequently more vulnerable to free radical damage and OS (Lohr et al., 2003) associated with abnormal involuntary movements (Burger et al., 2005a, 2005b; Dawson et al., 2000; Graybiel et al., 1995; Teixeira et al., 2008, 2009).

The mechanisms which underlie the protective effects of exercise against neurodegenerative conditions are in the early stages (Radak et al., 2010; Teixeira et al., 2008; Zigmond and Smeyne, 2010) and, therefore, may be an important tool for future clinical research. Considering the close relationship between OS and neurodegenerative conditions and the findings of our previous studies, we decided to investigate the effects of an exercise protocol with resting intervals on haloperidol-induced movement disorders.

2. Material and methods

2.1. Drugs

Haloperidol decanoate (Cristalia, Brazil) was dissolved in Tween 1% and diluted with distilled water to a final concentration of 12 mg/kg/ml. The haloperidol solution and the vehicle were intramuscularly injected (i.m) at a volume of 1.0 ml/kg body weight.

2.2. Animals

Twenty-four male Wistar rats weighing about 200 g at the start of the experiments were used for the study. Groups of six animals were kept in plexiglas cages with free access to food and water in a temperature-controlled room (23 °C ± 1 °C) with a 12 h-light/dark cycle (lights on at 7:00 a.m). The number of animals used was the minimum to obtain relevant results and they were maintained and used in accordance with the guidelines of the National Council for Control of Animal Experiments (CONCEA), following international norms of animal care and maintenance. The rats were randomly assigned to four groups: sedentary-control (SC), sedentary-haloperidol (SH), exercise-control (EC), exercise-haloperidol (EH).

2.3. Training protocol and experimental procedure

All exercised rats were subjected to swimming exercise in a plastic container (depth 45 cm) under continuous supervision, with the water temperature set to 32 °C ± 1 °C, for 1 h per day (three exercise sections of 15 min interspersed with three intervals of 5 min of rest), 5 times per week. While resting, the animals were kept on a platform under water, which allowed them to rest without leaving the water (i.e., rats were partially under water).

After 12 weeks of training, half the animals of each experimental group were treated with vehicle (SC and EC groups) or haloperidol solution (SH and EH groups). Injections (im) of vehicle (vegetal oil-Tween 1%) or haloperidol (12 mg/kg/ml-Tween 1%) were done once a week for 4 weeks combined with the exercise (amounting to16 weeks of swimming).

2.4. Training assessment

The heart weight (HW), body weight (BW) and epididymal fat mass (EFM) were assessed. The HW/BW ($g \times 10^{-3}$) ratio was used as an index of cardiac hypertrophy (Tharp and Carson, 1975), and the EFM/BW ($g \times 10^{-2}$) ratio as an index of body fat (Kabir et al., 1998).

2.5. Behavioral testing

2.5.1. Quantification of vacuous chewing movements (VCMs)

Twenty-four hours after the 7th, 14th, 21st and 28th daily injection of haloperidol or vehicle solution, all the animals were observed for quantification of oral dyskinesia (OD). The animals were placed individually in cages (20 × 20 × 19 cm) containing mirrors under the floor to allow for behavioral quantification when the animal was facing away from the observer. The incidence of vacuous chewing movements (VCM) was recorded for 5 min after a 2 min adaptation period (hand operated counters were employed). The observers were blind to the drug treatments. The behavioral experiments were conducted between 09:00 and 11:00 a.m.

2.5.2. Open-field test

In order to evaluate the effects of exercise on the reduction of haloperidol-induced motor activity, the spontaneous locomotor activity of the rats was quantified just after each weekly session of oral dyskinesia. Animals were placed individually in 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 described elsewhere (Kerr et al., 2005). The number of lines crossed was recorded weekly for 5 min, for 4 weeks.

2.6. Tissue preparations

Rats were euthanatized by decapitation about 24 h after the last session of behavioral evaluation. The brains were immediately excised and put on ice. Cortical and subcortical regions were separated, weighed and homogenized in 10 volumes (w/v) of 10 mM Tris–HCl, pH 7.4, and used for thiobarbituric acid reactive substances (TBARS) and catalase activity tests. The striatum was used for DA uptake and catalase activity assays. All the biochemical assays were carried out in triplicate.

2.6.1. [^{3}H] dopamine uptake

[^{3}H] dopamine uptake was measured as described by Holz and Coyle (1974) with some modifications. The striatum was cut into 400 μm slices, which were washed with a buffered solution consisting of 127 mM NaCl, 1.2 mM Na_2HPO_4, 5.36 mM KCl, 0.44 mM KH_2PO_4, 0.95 mM $MgCl_2$, 0.70 mM $CaCl_2$, 10 mM glucose, and 1 mM Tris–HCl, pH 7.4. Slices (0.2–0.3 mg protein) were further pre-incubated in 96 well-polycarbonate plates for 15 min at 35 °C with the same buffered solution plus selegiline 1 μM [^{3}H] dopamine was added to the incubation medium and uptake was carried out for 10 min at 35 °C, after which the reaction was stopped by five washes of 30 s each with 1 mL of ice-cold buffered solution previously described, containing 1 μM selegiline and 100 μM cocaine. Immediately after washing, 0.25 mL of 0.5 M NaOH and 0.2% sodium dodecyl sulfate (SDS) were added to the slices that were digested by 10 min incubation at 60 °C. Aliquots of the lysates were taken for protein content measurement by the Lowry et al. (1951) method. For determination of the intracellular amount of dopamine, liquid scintillation counting was used. Results were expressed as [^{3}H] dopamine uptake per mg of protein.

2.6.2. Lipid peroxidation

To assess lipid peroxidation, we quantified thiobarbituric acid reactive substances (TBARS). The homogenates were centrifuged for 10 min at 1500 × g. Just after the centrifugation, an aliquot of 200 μL or of supernatant was incubated for 1 h at 37 °C and then used for lipid peroxidation quantification as described elsewhere (Ohkawa et al., 1979).

2.6.3. Catalase activity

Catalase (CAT-EC 1.11.1.6) activity was quantified by measuring the disappearance of H_2O_2 at 240 nm (Aebi et al., 1984), and expressed in mmol H_2O_2/g tissue/min.

2.7. Statistical analysis

All the data were analyzed by two-way ANOVA (2(sedentary/exercise) × 2 (control/haloperidol)) or by three-way ANOVA [2(sedentary/exercise) × 2(control/haloperidol) × 4 periods of behavioral quantifications], followed by Duncan's multiple range test, when appropriate. $P < 0.05$ was regarded as statistically significant.

3. Results

Assessment of the training program and body fat mass: Table 1 *shows the values of heart weight (mg), epididymal fat mass (mg), body weight (g), heart weight/body weight and epididymal weight/body weight ratios.* Duncan's test showed that exercise (EC and EH groups) did not increase the heart weight in relation to sedentary groups (SC and SH). However, the heart-to-body weight ratio was increased in EC and EH groups, indicating the effectiveness of the exercise protocol. Epididymal fat mass, as well as the resulting body fat mass index were decreased by training (EC and EH groups), when compared to sedentary rats (SC and SH groups, respectively). Body weight, heart weight and epididymal fat mass were not modified by haloperidol treatment.

The effects of haloperidol and exercise on oral movements (VCM) and locomotor activity are shown in Fig. 1: Duncan's multiple range test showed that haloperidol-treated rats (SH group) had increased VCMs at all times and that the exercise protocol was able to prevent

Table 1
Mean values of heart weight (HW), epididymal fat mass (EFM), final body weight (BW), heart weight/body weight and epididymal fat mass/body weight ratios of sedentary (S) and exercised (E) rats, treated with vehicle (C) or haloperidol (H).

Group	HW (g)	EFM (g)	BW (g)	HW/BW ratio ($g \times 10^{-3}$)	EFM/BW ($g \times 10^{-2}$)
SC	1.22 ± 0.16	6.91 ± 0.61	363.17 ± 20.23	3.36 ± 0.10	1.90 ± 0.10
SH	1.24 ± 0.13	7.94 ± 0.62	380.67 ± 11.14	3.26 ± 0.13	2.08 ± 0.11
EC	1.24 ± 0.16	4.84 ± 0.15*, +	309.33 ± 5.09*, +	4.00 ± 0.17*, +	1.56 ± 0.10*, +
EH	1,21 ± 0.14	5.71 ± 0.28+	329.00 ± 16.52+	3.72 ± 0.16+	1.75 ± 0.11+

Data (mean ± S.E.M.), ($n = 6$) were analyzed by two-way analysis of variance followed by Duncan's test. *($p < 0.05$) difference from sedentary-control group (SC); +($p < 0.05$) difference from sedentary-haloperidol group (SH).

it (Fig. 1A). In fact, exercised rats treated with haloperidol (EH group) showed VCM frequency similar to that of vehicle-treated rats (SC and EC groups) throughout the 4 weeks (Fig. 1A).

Haloperidol treatment decreased the locomotor activity in the four weekly evaluations (no significant variations between them), as observed by the number of crossings in the open-field, which were counteracted by exercise (EH group) at weeks 1, 2 and 4 (Fig. 1B). At week 3, the exercise failed to prevent haloperidol's effects on the number of crossings, but a non-significant tendency to do so ($p = 0.059$) was observed.

Haloperidol treatment counteracted the increase in locomotor activity caused by exercise in last week of behavioral evaluation. In fact, the activity of EH group was lower than of vehicle-treated rats (EC group) and similar to the sedentary-control group (SC). Pairwise comparisons showed that exercise (EC) caused a progressive increase in open-field locomotion from week 2 of behavioral observation onwards (Fig. 1B).

The effects of haloperidol and exercise on antioxidant defense and lipid peroxidation are shown in Fig. 2: Haloperidol treatment decreased catalase activity in all evaluated brain areas, and exercise partially prevented this effect only in the subcortical region (Fig. 2A). Except in the cortex, exercise *per se* reduced catalase activity in all evaluated brain areas. In fact, the exercised rats treated with haloperidol (E + H group) also showed lower catalase activity than the SC group (Fig. 2A) in all the evaluated brain tissues.

Haloperidol treatment induced lipid peroxidation in the cortex and subcortical region, and the exercise partially prevented this effect in the cortex and completely in subcortical region (2B). In fact, the subcortical region showed lipid peroxidation levels in control animals (SC and EC groups) similar to exercised rats treated with haloperidol (EH group). The exercise *per se* decreased the TBARS levels in cortex, and this effect was maintained after haloperidol treatment (E + H group, Fig. 2B). Of particular importance, catalase activity in the subcortical region showed a negative correlation with VCM frequency ($r = 0.50$, $p < 0.05$, Fig. 3C). The same behavioral parameter showed a positive correlation with TBARS levels in the cortex ($r = 0.64$, $p < 0.05$, Fig. 3A) and subcortical region ($r = 0.71$, $p < 0.0001$, Fig. 3B).

Effects of haloperidol and exercise on [^{3}H] dopamine uptake: Both haloperidol treatment and exercise *per se* caused a significant increase in [^{3}H] dopamine uptake in striatal slices when compared to control (SC group, Fig. 4A). Interestingly, the exercise-haloperidol co-treatment reduced [^{3}H] dopamine uptake, which was similar to control (Fig. 4A). Furthermore, striatum data showed a negative

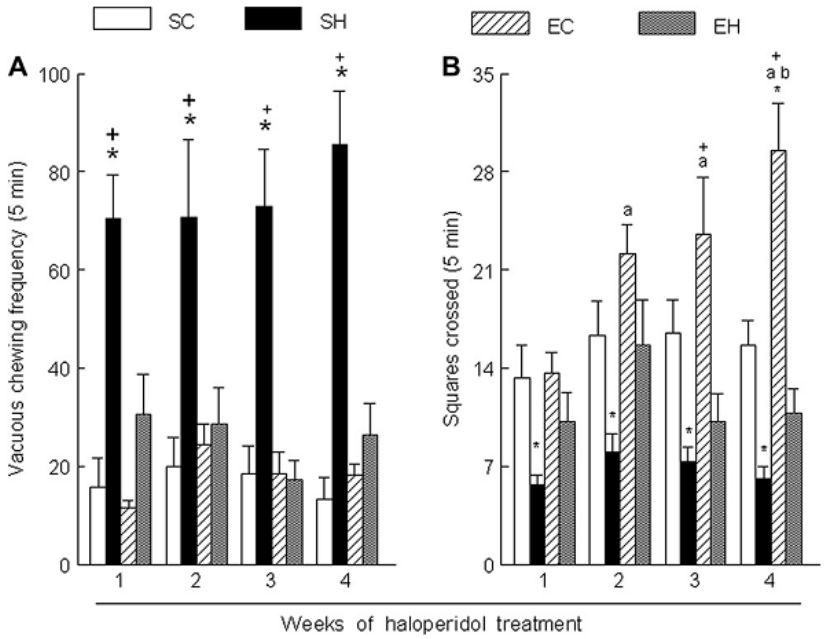

Fig. 1. Effects of haloperidol treatment (12 mg/kg-im, once a week for 4 weeks) (SH) or vehicle (SC) on the VCMs (A) and locomotor activity (B) of sedentary and exercised rats (EH or EC). Data are expressed as mean ± S.E.M. *difference from SC ($p < 0.001$); +difference from EH ($p < 0.05$) for A and B simultaneously. Lowercase "a" and "b" indicates difference from the first and third weeks, respectively ($p < 0.05$).

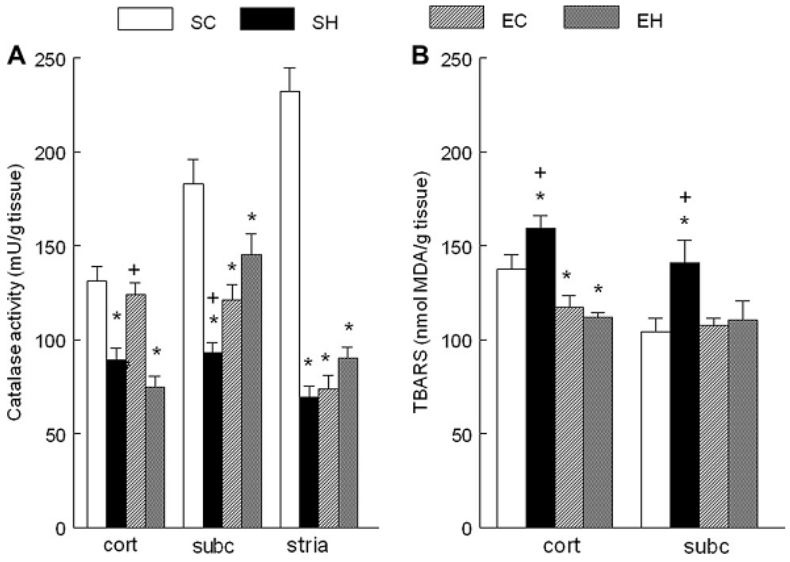

Fig. 2. Effects of haloperidol treatment (12 mg/kg-ip, once a week for 4 weeks) or vehicle on catalase activity (A), and TBARS levels in cortex, subcortical region and cerebellum of sedentary (SC or SH) and exercised rats (EH or EC). Data are expressed as mean ± S.E.M. *Difference from SC ($p < 0.05$ (A)); +difference from EH ($p < 0.05$).

correlation between [^{3}H] dopamine uptake and catalase activity ($r = 0.51$, $p < 0.05$) (Fig. 4B).

4. Discussion

In the present study, we are demonstrating the beneficial effects of a daily moderate exercise protocol interspersed with rest intervals on sub-chronic haloperidol-induced motor and oxidative damages.

4.1. Assessment of the training program and body fat mass

While moderate (Gündüz et al., 2004; Radak et al., 2001; Teixeira et al., 2008) and heavy (with overweight) continuous

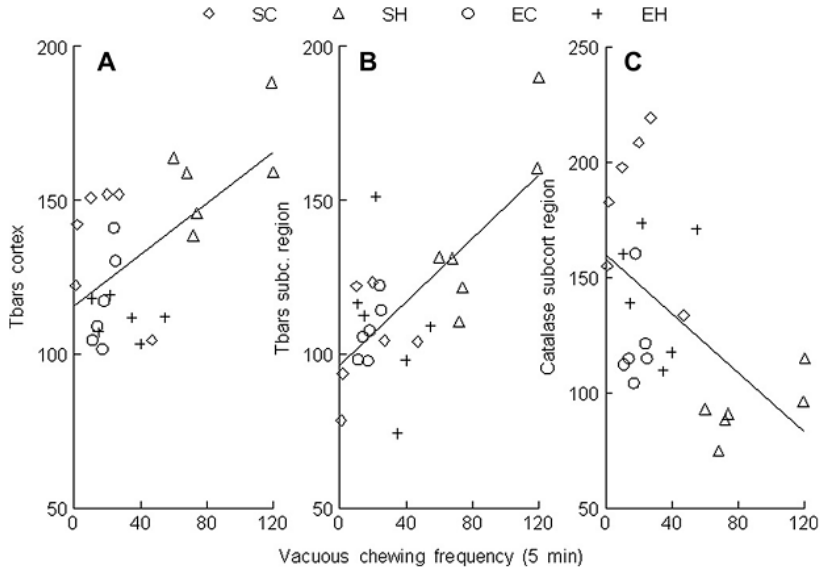

Fig. 3. Linear regression analysis between VCM frequency and TBARS levels evaluated in cortex (A) and subcortical region (B) and catalase activity in subcortical region (C) of exercised (E) and sedentary (S) rats, which were treated with haloperidol (H-12 mg/kg-im, once a week for 4 weeks) or its vehicle (C). Statistical analysis revealed the following *P* significance for the *r* values: 0.64, $p < 0.05$; 0.71, $p < 0.0001$; 0.50, $p < 0.05$, respectively.

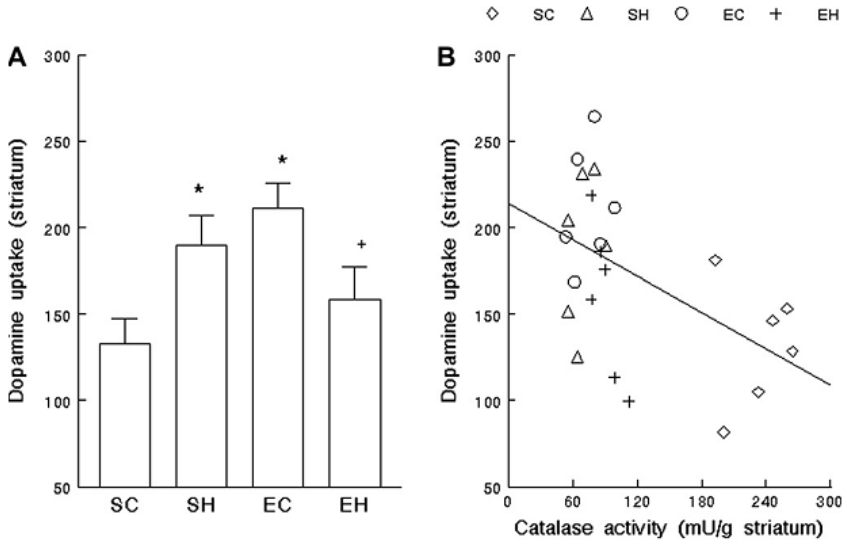

Fig. 4. A-Dopamine uptake in striatum of sedentary (S) and exercised (E) rats and treated with haloperidol (SH and EH) or vehicle (SC and EC). The haloperidol or vehicle treatment was done for 4 weeks (12 mg/kg-im, once a week), and the exercise was maintained all along, totaling 16 weeks of physical training. *Difference from SC group ($p < 0.05$); $^{+}$difference from EH group ($p < 0.05$). B-Linear regression analysis between dopamine uptake and catalase activity ($r = 0.51$, $p < 0.05$) evaluated in striatum of the rats submitted to physical activity and haloperidol.

exercise (Teixeira et al., 2009) were not associated with changes in body weight, the exercise protocol used here reduced the body weight and the fat mass index of the rats, without causing heart hypertrophy. In fact, the heart weight/body weight ratio was greater in the exercised groups, although the heart weight of these animals was unchanged. We believe that this apparent cardiac hypertrophy is a consequence of the lower body weight of exercising animals, as there was no increase in heart weight. We thus suggest that moderate exercise with rest intervals is an innovative and healthier protocol than either moderate or heavy exercise performed continuously.

4.2. Effects of haloperidol and exercise on oral movements (VCM) and locomotor activity

The exercise protocol also prevented both dyskinetic and locomotor effects induced by haloperidol. Interestingly, in the control rats (EC), exercise increased the locomotor activity in ascending order, while the other treatments did not cause this. We believe that this effect of exercise is not a consequence of emotional stress caused by swimming, mainly because the exercise program employed here was not a stress factor, as demonstrated by the reduced oral dyskinesia. In this sense, it is important to note that emotional stress has been associated with development of motor disturbances, and particularly, it causes an increase in animal models of orofacial dyskinesia (Egan et al., 1996; Glenthoj, 1993; Waddington, 1990).

4.3. Effects of haloperidol and exercise on antioxidant defense and lipid peroxidation and its relationship with the orofacial dyskinesia

Haloperidol is known to reduce motor activity and induce orofacial movements and catalepsy in experimental animals. Our findings are in accordance with previous studies from our laboratory, when continuous moderate exercise (without rest) prevented the development of orofacial movements and increased antioxidant defenses in rat striatum (Teixeira et al., 2008). In contrast, intense exercise increased the brain oxidative damage and the reserpine-induced motor disturbance (Teixeira et al., 2009). The exercise protocol used here prevented the increase of haloperidol-induced lipid peroxidation in the cortex and subcortical region, and decreased catalase activity in the subcortical region. Literature data indicates that an imbalance in production and detoxification of free radicals may contribute to the initiation of hyperkinetic movements in the orofacial regions (Cadet et al., 1986).

Of particular importance, the positive correlation observed between oral dyskinesia (VCM) and lipid peroxidation in the cortex and subcortical region reinforce the role of free radicals in the haloperidol-induced motor side-effects. Furthermore, different animal models have shown correlations between motor disturbances and oxidative damage (Fachinetto et al., 2007; Teixeira et al., 2008), confirming the involvement of OS in the development of movement disorders and neurodegeneration (Cadet and Kahler, 1994; Naidu et al., 2003; Tillerson and Miller, 2003). Concerning the antioxidant defenses, a negative correlation between oral dyskinesia development and striatal catalase activity was reported (Abilio et al., 2004). Our results also confirm these findings, reinforcing the role of this enzyme in the detoxification of free radicals in brain regions involved in motor balance. Interestingly, exercise by itself caused a significant reduction in catalase activity, which indicates that there is a complex interaction between catalase activity and other biomarkers of oxidative stress. For instance, here we have observed that exercise decreased TBARS production in different brain areas. Consequently, a reduction in catalase activity by itself cannot explain the increase in VCM; only when it occurs concomitantly with changes in other markers of oxidative stress can it predict orofacial dyskinesia.

4.4. Effects of haloperidol and exercise on [^{3}H] dopamine uptake

In addition, we also observed that haloperidol treatment and moderate exercise increased DA uptake in the striatum of rats, but

this effect did not occur when haloperidol and exercise were combined. Presently, we do not know the exact mechanism involved in these findings, but it may be useful to make speculations about some literature reports: 1) haloperidol blocks DA pre-synaptic receptors and inhibit the feedback mechanism, increasing DA release (Creese et al., 1976); 2) the expression of DA transporter (DAT) and tyrosine hydroxylase proteins (TH), as well as the level of striatal DA, are closely linked (Jaber et al., 1999); 3) the increased synaptic bioavailability of DA, through the inhibition of the feedback mechanism may lead to increased density of the DA autoreceptor, leading to the downregulation of TH (Fauchey et al., 2000); 4) Oxygen free radicals are reported to diminish the DAT function, further increasing the extracellular DA levels (Fleckenstein et al., 1977). Based on these considerations and on our findings, we can suggest that haloperidol treatment increases DA turnover, which initially stimulates uptake mechanisms, but this compensatory mechanism can be damaged after prolonged administration of a neuroleptic drug. Recently, Fachinetto et al. (2007) reported a decrease in striatal DA uptake in rats treated with a typical neuroleptic drug for 32 weeks, while our study was conducted for 4 weeks.

On the other hand, physical activity is also implicated in the increase in brain monoamines which can facilitate ROS generation and OS development in brain and other vital tissues. Indeed, *in vivo* microdialysis studies have shown that exercise increases the concentration of striatal DA in rat brain (Hattori et al., 1994). As exercise does not block pre-synaptic DA receptors, it can modulate compensatory mechanisms of DA uptake. In this sense, DA uptake by high affinity transporter (DAT) is the primary pathway for the clearance of extracellular DA and the consequent regulation of the intensity and duration of dopaminergic signaling (Kahlig and Galli, 2003). Our hypothesis supports a recent finding by Petzinger et al. (2007), where exercise changed DA neurotransmission, which was different in the injured as compared to non-injured nigrostriatal system of rats. Furthermore, literature reports suggest that physical activity can exert a positive modulation in the production of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF).

These neurotrophic factors can activate downstream pathways, which influence positively synaptic plasticity and neurotransmission (Cohen et al., 2003; Gómez-Pinilla et al., 2002). Recently, the importance of physical activity in treating neurodegenerative disorders of the basal ganglia was reported, including Parkinson's disease. In this specific case, exercise could enhance reactivity and motor behavior in patients with Parkinson's disease (Müller and Muhlack, 2009). This beneficial effect of exercise can be explained by neuroplasticity and increased dopaminergic availability (release and uptake), exerting a more critical role in maintaining normal synaptic connections than the restoration of absolute DA levels (Petzinger et al., 2007).

In this way, we may suggest that the increased levels of DA in the synaptic cleft by sub-chronic haloperidol treatment and/or moderate exercise stimulates its reuptake to maintain the balance and neuronal integrity, by different mechanisms, and may reveal differences between acute and chronic use of neuroleptic in the DA transporter affinity. However, further studies must be carried out to elucidate the exact mechanism of physical exercise on motor disorders related to oxidative damage and its influence on the pre-synaptic dopamine transporter.

In conclusion, our data suggest that moderate exercise with rest intervals exerted beneficial effects on the motor and oxidative damages induced by sub-chronic haloperidol treatment and may be useful in the prevention or amelioration of movement disorders related to neurodegenerative processes.

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3.2 ARTIGO 2:

Publicado na Revista *Neuroscience* 195: 80-88, 2011.

Efeitos do exercício físico sobre a aquisição de memória, sintomas de ansiedade e sobre a atividade de enzimas de membrana de tecido cerebral de ratos cronicamente suplementados com diferentes ácidos graxos: Prejuízos da gordura *trans*.

EXERCISE AFFECTS MEMORY ACQUISITION, ANXIETY-LIKE SYMPTOMS AND ACTIVITY OF MEMBRANE-BOUND ENZYME IN BRAIN OF RATS FED WITH DIFFERENT DIETARY FATS: IMPAIRMENTS OF *TRANS* FAT

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Abstract—Here we evaluated the influence of physical exercise on behavior parameters and enzymatic status of rats supplemented with different dietary fatty acids (FA). Male Wistar rats fed diets enriched with soybean oil (SO), lard (L), or hydrogenated vegetable fat (HVF) for 48 weeks were submitted to swimming (30 min/d, five times per week) for 90 days. Dietary FA *per se* did not cause anxiety-like symptoms in the animals, but after physical exercise, SO group showed a better behavioral performance than L and the HVF groups in elevated plus maze (EPM). In Barnes maze, HVF group showed impaired memory acquisition as compared to L group, and exercise reversed this effect. SO-fed rats showed an improvement in memory acquisition after 1 day of training, whereas lard caused an improvement of memory only from day 4. HVF-fed rats showed no improvement of memory acquisition, but this effect was reversed by exercise in all training days. A lower activity of the Na^+K^+-ATPase in brain cortex of rats fed lard and HVF was observed, and this effect was maintained after exercise. Similarly, the HVF diet was related to lower activity of hippocampal Na^+K^+-ATPase, and exercise reduced activity of this enzyme in the SO and L groups. Our findings show influences of dietary FA on memory acquisition, whereas regular exercise improved this function and was beneficial on anxiety-like symptoms. As FA are present in neuronal membrane phospholipids and play a critical role in brain function, our results suggest that low incorporation of *trans* FA in neuronal membranes may act on cortical and hippocampal Na^+K^+-ATPase activity, but this change appears to be unrelated to the behavioral parameters primarily harmed by consumption of *trans* and less so by saturated FA, which were reversed by exercise.

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Abbreviations: EFA, essential fatty acids; FA, fatty acids; HVF, hydrogenated vegetable fat; L, lard; Pi, inorganic phosphate; PUFA, polyunsaturated fatty acids; SO, soybean oil.

Key words: *trans* fatty acids, exercise, memory, anxiety, phospholipids, Na^+K^+-ATPase.

Recent studies emphasize the importance of dietary components on modulating the function of molecular systems involved with maintenance of neuronal health and activity. In particular, a diet high in saturated fat decreases synaptic plasticity and compromises learning and memory (Molteni et al., 2002), aggravating the outcome of brain insult on neuroplasticity and cognitive function (Wu et al., 2003). In this context, foods rich in *trans* fatty acids (*trans* FA) have also attracted the attention of health authorities and general public because of their growing consumption and contradictory and inconclusive data on safety (Wandall, 2008). Although *trans* FA from ruminant fats have been part of the human diet for centuries, the intake of *trans* FA increased much with the growing use of hydrogenated vegetable fat (HVF) during the second part of the 20th century (Pfeuffer and Schrezenmeir, 2006). This change in Western dietary habits is mainly related to consumption of convenience food and fast foods, often rich in saturated and monounsaturated FA (Baggio and Bragagnolo, 2006), as well as considerable amounts of *trans* FA (Allison et al., 1999). Importantly, from a dietary point of view, consumption of *trans* FA represents a loss of essential fatty acids (EFA) intake that may have a hazardous impact on human health. EFA are converted to long-chain polyunsaturated fatty acids (LC-PUFA) such as docosahexaenoic acid (DHA, 22:6-n3), eicosapentaenoic (EPA, 20:5-n3), and arachidonic acid (AA, 20:4-n6), which are integral and structural components of neural membranes. Deficiency of EFA has been related to cognitive and behavioral aberrations by changing the LC-PUFA composition of membrane phospholipids in the nervous system (Wainwright, 1997; Yehuda et al., 2005) and modulation of brain physiological functions by modifying cell permeability and synaptic membrane fluidity (Jump, 2002). The key role in normal functioning of cells is exerted by membrane-bound enzymes such as Na^+K^+-ATPase, which determine neuronal bioelectric properties by regulating the distribution of Na^+ and K^+ between the cell and the intercellular space. In addition, an increase in membrane fluidity is related to greater activation of this enzyme, whereas an increase in that rigidity inhibits its action (Srinivasarao et al., 1997).

Of particular importance, physical activity is also an important factor in maintaining a healthy brain function and has been shown to improve quality of life and decrease the

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.08.055

incidence of life-style–related diseases (Radák et al., 2004, 2005), including the consequences of inadequate diets. Furthermore, recent experimental studies by our group showed that regular exercise is able to reduce oxidative damages in brain (Teixeira et al., 2008) and peripheral tissues (Teixeira et al., 2009), showing also modulator effects on dopamine transporter (Teixeira et al., 2011). In fact, regular physical exercise has been related to reversion of harmful effects of high-fat diet on neuronal plasticity (Molteni et al., 2004), as well as to neurochemical damages caused by brain injury (Lima et al., 2009). Other studies from our laboratory have shown that dietary n-3 PUFA may change the brain oxidative status and modulate motor disorders (Barcelos et al., 2010), whereas *trans* FA facilitates neuronal disorders such as development of mania (Trevizol et al., 2011). In this sense, literature lacks studies assessing such lifestyle influences as saturated and HVF fat consumption and regular physical activity on behavior and neuronal functions, since both are closely related to changes of membrane fluidity and modulation of neurotransmitter release and uptake, respectively (Li and Stys, 2001). Of particular importance, the different FA incorporated into neuronal membranes can modify its fluidity and exert influence in the conformation and function of membrane-bound proteins such as the Na^+K^+-ATPase, which is responsible for generation and maintenance of membrane potential for neuronal excitability. Therefore, the aim of this study was to evaluate the interaction between different dietary FA (saturated, PUFA, and *trans*) and physical activity, in rats supplemented from weaning to adulthood, on behavioral parameters and its influence on Na^+K^+-ATPase activity in brain. Moreover, we are evaluating for the first time the consequences of saturated and *trans* FA incorporation on Na^+K^+-ATPase activity, which has a significant impact on neural transmission (Vajreswari et al., 1990; Kimelberg and Papahadjopoulos, 1972). The fats incorporated in the diets were chosen in the isocaloric form, mainly because these are the most consumed worldwide, with or without awareness of their benefits or harm to health.

EXPERIMENTAL PROCEDURES

Animals

Forty-eight male Wistar rats (21 days old) were used for the study. Groups of four animals were kept in plexiglas cages (Bioterium of Universidade Federal de Santa Maria, Santa Maria, RS, Brazil) with free access to food and water in a temperature-controlled room (23±1 °C) with a 12-h light/dark cycle (lights on at 7:00 AM). The number of animals used was the minimum to obtain relevant results, and they were maintained and used in accordance with the guidelines of the National Council for Control of Animal Experiments (CONCEA), following international norms of animal care and maintenance. Immediately after weaning, the rats were randomly assigned to the following three experimental groups: soybean oil (SO), lard (L), and HVF. Dietary supplementation consisted in incorporation (20%) of different FA present in soybean oil (rich in PUFA), lard (rich in monounsaturated and saturated FA), and hydrogenated vegetable fat (rich in *trans*-monounsaturated and saturated FA).

After 48 weeks of dietary supplementation, one-half of each group was designated to physical activity (exercise—E) or not (sedentary—S), and maintained in the same diet during the exercise period.

Fatty acid determination in the diets

The different supplemented diets were submitted to saponification in methanolic KOH solution and esterification in methanolic H_2SO_4 solution (Hartman and Lago, 1973). Methylated fatty acids were analyzed using an Agilent Technologies gas chromatograph (HP 6890) equipped with a Supelco SP-2560 capillary column (100 m×0.25 mm×0.20 μm) and flame ionization detector. The temperature of the injector port was set at 250 °C, and the carrier gas was nitrogen (1.1 ml/min). After injection (1 μl; split ratio, 50:1), the oven temperature was kept at 140 °C for 5 min and then raised to 240 °C at 4 °C/min and kept at this temperature for 12 min. Standard fatty acid methyl esters (Sigma, Saint Louis, MO, USA) were subjected to the same conditions, and the following retention times were used to identify the fatty acids. Results were expressed as percentage of total area of the identified fatty acids.

Exercise protocol and experimental procedure

After 48 weeks of dietary supplementation, all exercised rats were subjected to swimming exercise in a plastic container (depth, 45 cm) under continuous supervision, with water temperature set to 32±1 °C, for 30 min per day (three exercise sessions of 10 min interspersed with two 5-min rest intervals), five times per week. During the interval, the animals were kept on a platform under water, which allowed them to rest without leaving the water. After 12 weeks of training, the animals were submitted to the behavioral assessments described later in text.

Training assessment

The effectiveness of the exercise protocol was verified by lactate levels in blood, which was done at the last week of swimming. For this, blood was collected from the tail tip vein (25 μl) of sedentary and exercised rats immediately after the swimming session. Lactate concentrations of blood samples were determined in a lactate analyzer (Accutrend® Lactate) in order to determine the aerobic resistance of the animals, which indicates the effectiveness of the swimming protocol (Gobatto et al., 2001; Teixeira et al., 2009).

Behavioral tests

Elevated plus maze (EPM). In order to evaluate the effects of dietary fat type and the exercise on anxiety-like symptoms, animals were observed in the EPM, which is based on the innate fear rodents have for open and elevated spaces (Montgomery, 1955). In this paradigm, rats display a variety of behaviors related to fear, risk, and general motor activity, which can be quantified by time spent in the open arms, number of head dipping, and number of entries in both open and closed arms, respectively (Anseloni and Brandão, 1997; Martinez et al., 2007; Rodgers et al., 1997). Head dipping is an exploratory movement of head/shoulders over sides of the open arms and down toward the floor. 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×13.5 cm), which gave access to any of the four arms. At the beginning of each test, the rat was placed in the central platform facing an open arm. Time spent in the open arms and numbers of head dipping were registered during the 5-min test. The apparatus was cleaned with water using wet sponge and paper towel before the introduction of each animal (Ramos et al., 2002).

Barnes maze. To test rats' performance in a spatial learning paradigm, we chose the Barnes maze test, initially described by Barnes (1979). The apparatus was located in a 4 m×4 m test room where four visuospatial cues made of rigid black paper (different geometric forms) were affixed to the walls but not directly over one maze hole. On the first day of experiment, rats were moved to the testing room and left undisturbed for 60 min. After this habituation, the rats were trained to find the escape hole: they were placed in the escape box for 1 min, then into a cylindrical opaque chamber (start box) in the center of the maze. With lights on, the start box was removed, and the rat was allowed to explore freely and find the escape box. A maximum latency of 180 s to find it was allowed. Three trials per day for four consecutive days were given for each rat, scoring the time to reach the escape tunnel. The arena and boxes were wiped clean using distilled water both between each training session for a given rat and between each rat.

Na^+K^+-ATPase activity

The Na^+K^+-ATPase enzyme activity was determined according to method proposed by Muszbek et al. (1997), with some modifications, in cortex and hippocampus, mainly because these brain structures have been shown to influence anxiety-related cognitive–behavioral processing in rodents, human and nonhuman primates (File et al., 2000; Hollerman et al., 2000; Ribeiro et al., 1999; Wall and Messier, 2000). Briefly, the aliquots of cortex and hippocampus (20 μl) were added to a reaction medium containing NaCl (115 mM), $MgCl_2$ (2.5 mM), KCl (18 mM), and Tris–HCl buffer (45 mM and pH 7.4), with or without the Na^+K^+-ATPase enzyme inhibitor ouabaine (5 μM). The method for ATPase activity measurement was based on determination of inorganic phosphate (Pi) released to the reaction medium by hydrolysis of ATP according to the method proposed by Atkinson et al. (1973). The reaction was initiated with addition of substrate ATP (1.5 mM) to the reaction medium and was finished by addition of the color reagent (1 ml) containing ammonium molybdate (2%), Triton-100X (5%), and H_2SO_4 1.8 M (10%) after 15 min of incubation at 37 °C. The formed molybdate-Pi complexes were measured spectrophotometrically at 405 nm. Values were calculated in relation to a standard curve constructed with Pi at known concentrations and also corrected by protein content.

Brain fatty acid analyses

Fat was extracted of total brain using chloroform and methanol as described by Bligh and Dyer (1959) and used for determination of the FA acid profile. To prevent lipid oxidation during and after extraction, 0.02% butyl hydroxy toluene was added to the chloroform used. Fatty acid composition was determined by gas chromatography. Fat was saponified in methanolic KOH solution and then esterified in methanolic H_2SO_4 solution (Hartman and Lago, 1973). The fatty acid methyl esters (FAME) were analyzed using an Agilent Technologies gas chromatograph (HP 6890) fitted with a Supelco SP-2560 capillary column (100 m×0.25 mm×0.20 μm) and flame ionization detector. Temperature of the injector port was set at 250 °C, and the carrier gas was nitrogen (0.8 ml/min). After injection (1 μl; split ratio, 50:1), oven temperature was raised from 35 °C to 150 °C at 10 °C/min and held at this temperature for 2 min, then raised to 200 °C at 2 °C/min and held at this temperature for 2 min, and then raised again to 220 °C at 2 °C/min and held at this temperature for 21 min. Standard fatty acid methyl esters (37-component FAME Mix, DPA n-3 and PUFA no. 2 from Sigma, Saint Louis, MO, USA and DPA n-6 from NuChek Preparatory Inc., Elysian, MN, USA) were run under the same conditions and the subsequent retention times were used to identify fatty acids. Fatty acids were expressed as percentage of the total fatty acids content.

Table 1. Fatty acid composition of diets enriched with different fats (% of total fatty acids identified)

Fatty acids	Soybean oil	Lard	Hydrogenated vegetable fat
14:0	0.17	0.96	0.34
16:0	12.26	20.12	14.28
18:0	5.79	11.26	10.21
20:0	0.59	0.34	0.52
22:0	0.55	0.17	0.48
ΣSFA	19.36	32.85	25.83
16:1 n-7	0.20	1.31	0.36
18:1 n-9	27.45	38.19	42.53
18:1 n-9t	0.61	2.81	15.84
20:1 n-9	0.30	0.73	0.37
ΣMUFA	28.56	43.04	59.10
18:2 n-6	46.41	21.02	12.94
18:2 n-6t	nd	0.14	0.67
18:3 n-3	4.72	1.47	0.67
20:2 n-6	0.06	0.57	0.08
ΣPUFA	51.19	23.20	14.36
Σ*trans* FA	0.61	2.95	16.51
n6/n3 ratio	9.83	14.39	20.31

The following fatty acids were found at concentrations lower than 0.5% and for this reason are not shown: 12:0, 17:0, 17:1 n-7, 18:3n-6, 20:3n-3, 20:4n-6, 21:0; 23:0, 24:0. The following fatty acids were not detected in the analyzed samples: C4:0, C6:0, C8:0, C10:0, C11:0, C13:0, C14:1 n-5, C15:0, C15:1 n-5, C20:3 n-6, C22:1 n-9, C22:2 n-6, C20:5n-3, C24:1 n-9, C22:6 n-3.

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; nd, not detected.

Statistical analysis

Levels of lactate were analyzed by paired independent Student *t*-test. Diets and brain tissue FA content were analyzed by one-way ANOVA followed by Duncan test. Data of EPM and enzyme activity were analyzed by two-way ANOVA (2 (sedentary/exercise)×3 (SO/L/HVF) followed by Duncan multiple range test, when appropriate. Barnes maze test data were analyzed by three-way ANOVA (2 (sedentary/exercise)×3 (SO/L/HVF)×4 periods of behavioral quantifications in Barnes maze. This last factor was considered as a repeated measure, and pair-wise comparisons were used to compare behavior at different time points, followed by Duncan multiple range test, when appropriate. $P<0.05$ was regarded as statistically significant.

RESULTS

Total oil content of the different diets

The three dietary fats differed considerably concerning their fatty acid composition: the SO diet had a high proportion (51%) of PUFA in contrast to the L diet, which had nearly 33% of SFA, whereas the HVF diet had the highest level of *trans* FA (16%) and MUFA (57%). The n6/n3 ratio was lower in SO and was progressively higher in the L and HVF diets, respectively (Table 1).

Monitoring of body weight gain and blood lactate levels

Food intake was monitored every 2 days during all the study without significant difference (data not shown), and consequently, different diets and physical activity had no

Table 2. Effect of dietary fatty acids consumption on body weight (BW) of rats aged 21 d, 12 mon, and 15 mon (after 3 mon of physical activity); and effect of physical activity on blood lactate levels

Groups	Initial BW (21 d old)	Final BW (12 mon old)		Physical activity (15 mon old)	
				Sedentary	Exercised
SO	53.95±2.61	480.84±10.79		486.64±16.28	485.50±22.28
L	55.00±1.89	463.00±8.95		477.00±15.44	486.11±15.32
HVF	54.58±2.13	464.52±10.17		462.64±15.47	484.37±20.87
			Blood lactate level (nmol/L)	7.12±0.78	4.70±0.00*

* Difference from sedentary rats ($P<0.05$).

effect on body weight gain. As verification of the training program, exercised rats showed lower blood lactate levels than sedentary ones ($P<0.05$) (Table 2).

The effects of dietary fatty acids and physical activity on anxiety-like symptoms in EPM

Two-way ANOVA of time percentage spent in open arms of EPM revealed a significant effect of exercise [$F(1,42)=4.74$; $P<0.05$] and a significant dietary FA×exercise interaction [$F(2,42)=8.63$; $P<0.001$]. Among sedentary rats, the different dietary FA did not change the time spent in open arms. Among those trained, SO diet–fed rats spent more time in open arms than that L- ($P<0.05$) and HVF-fed ones ($P<0.001$). In fact, physical activity increased this behavioral parameter only in the SO-fed group ($P<0.001$) (Fig. 1A).

Two-way ANOVA of head-dipping frequency revealed a significant dietary FA×exercise interaction [$F(2,42)=3.80$; $P<0.05$]. Similarly to time spent in open arms, no difference of head dipping frequency was observed across sedentary rats fed with different diets. Among trained rats, this behavior was more frequent in SO-fed rats ($P<0.05$). Again, physical activity increased head-dipping frequency only in SO-fed rats ($P<0.05$) (Fig. 1B). No significant differences were observed in number of entries of both closed and open arms (data not shown).

The effects of dietary fatty acids and physical activity on rat performance in Barnes maze test

Three-way ANOVA with repeated measures (2×3×4) for number of errors in Barnes maze revealed a significant main effect of training day [$F(3,126)=20.85$; $P<0.001$], exercise [$F(1,42)=58.78$; $P<0.001$], and significant fatty acid×training day [$F(6,126)=5.34$; $P<0.001$], exercise×training day [$F(3,126)=12.79$; $P<0.001$], and FA×exercise×training day [$F(6,126)=5.19$; $P<0.001$] interactions.

Duncan test showed that the different diets were able to change memory acquisition in Barnes maze, where

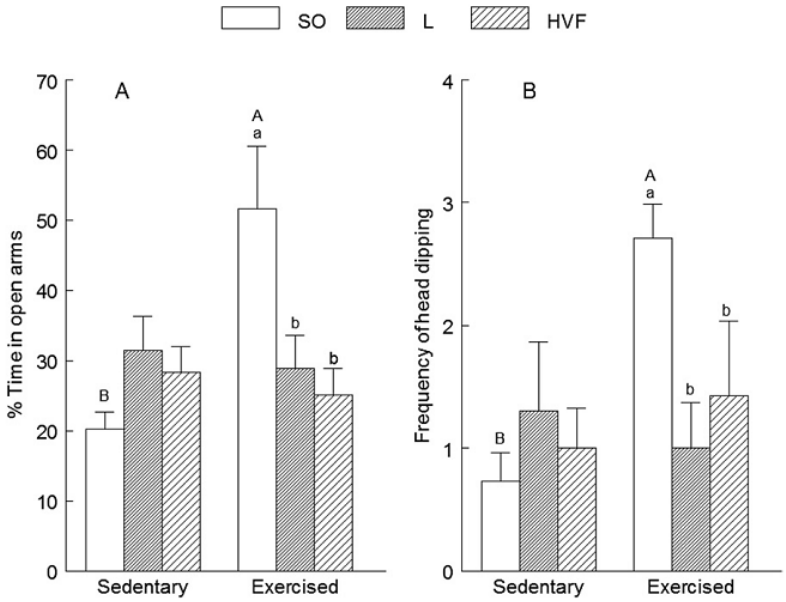

Fig. 1. Effect of diets enriched with soybean oil (SO), lard (L), or hydrogenated vegetable fat (HVF) for 15 mon and of exercise (30 min of swimming/day in the last 90 d), on time spent in open arms (A) and on head-dipping frequency (B) in EPM test. Data are expressed as means±SEM. Different lowercase letters indicate significant differences between diets and the same physical activity ($P<0.001$); different uppercase letters indicate differences between sedentary and exercised rats fed the same diet ($P<0.05$), for both (A) and (B).

SO-fed rats presented more errors on test day 1 and fewer errors on days 2 and 3 than L- and HVF-fed ones. On test day 4, both SO- and L-fed rats showed fewer errors than HVF group. On the other hand, exercise reduced the number of errors of all three experimental groups on test day 1 ($P<0.001$, 0.05, and 0.05 for SO, L, and HVF, respectively). On days 2 and 3, exercise reduced the number of errors in L and HVF groups ($P<0.05$ for both). On the last test day, physical activity reduced the number of errors of HVF-fed rats, equaling the three diet groups (Fig. 2).

Paired comparisons with repeated measure showed the influence of different diets on memory acquisition of sedentary rats. SO-fed rats showed fewer errors from training day 2 until the last session. L-fed group showed fewer errors only in the last session (day 4), whereas HVF-fed group did not show any acquisition of memory.

Among exercised animals, paired comparisons also showed a similar number of errors in the four test sessions, regardless of the diet (Fig. 2).

Fatty acid composition of total brain

Rats fed SO-, L-, and HVF-enriched diets from weaning for 15 months presented changes in FA composition of brain neuronal membranes. The highest content of PUFA was observed in the SO diet (27%), whereas the highest content of SFA and *trans* FA were observed in L (42%) and HVF (0.33%), respectively. In fact, *trans* FA were not detected in SO-fed rats, and the n6/n3 ratio showed similar values across the three different diets (Table 3).

The effects of dietary fatty acids and physical activity on Na^+K^+-ATPase in cortex and hippocampus of rats

Two-way ANOVA of Na^+K^+-ATPase activity revealed a significant main effect of dietary FA in brain cortex [$F(2,42)=16,70$; $P<0.001$] and a significant main effect of exercise in hippocampus [$F(1,42)=13.40$; $P<0.001$].

Among sedentary animals, Duncan test showed higher activity of Na^+K^+-ATPase in cortex of SO-fed rats than in L- and HVF-fed ones ($P<0.001$ and $P<0.05$, respectively), whereas hippocampal enzyme activity was higher in both SO and L groups than in HVF. Considering exercised animals, L- and HVF-fed rats showed lower Na^+K^+-ATPase activity than SO group in cortex, whereas no difference was observed in hippocampus of these animals. In addition, physical activity did not change Na^+K^+-ATPase activity in cortex of rats fed with the different diets (Fig. 3A), but significant differences were observed between sedentary and exercised rats of both SO and L diets in hippocampus, since exercise reduced enzyme activity of SO- and L-fed rats ($P<0.05$ for both), whose values were similar in the three different diets (Fig. 3B).

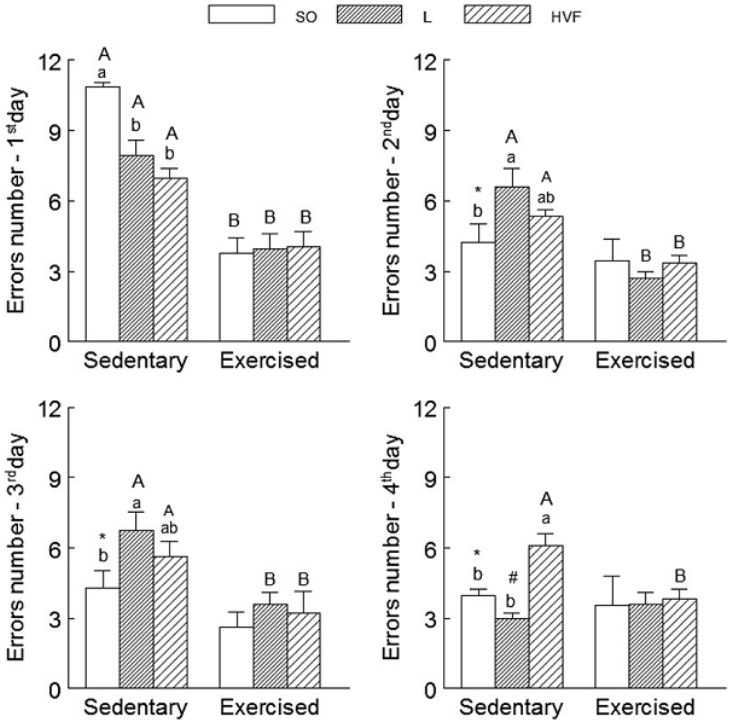

Fig. 2. Effect of diets enriched with soybean oil (SO), lard (L), or hydrogenated vegetal fat (HVF) for 15 mon and of exercise (30 min of swimming/day in the last 90 d), on number of errors in Barnes maze test (4-d observation). Data are expressed as mean±SEM. Different lowercase letters indicate significant differences between diets and the same physical activity ($P<0.001$); different uppercase letters indicate differences between sedentary and exercised rats fed the same diet ($P<0.05$); * indicates difference from the first day test for SO-fed rats ($P<0.001$); # indicates difference from the first day test for L-fed rats ($P<0.001$).

Table 3. Fatty acid composition of brain of rats after feeding with different dietary fats (% of total fatty acids identified)

Fatty acids	Mean (±SD)		
	Soybean oil	Lard	Hydrogenated vegetable fat
16:0	19.48±0.00^{c}	20.32±0.58^{a}	19.95±0.13^{a,b}
18:0	21.26±0.00^{b}	21.63±0.15^{a}	21.48±0.27^{ab}
ΣSFA	41.38	42.71	42.26
17:1 n-7	2.05±0.10	1.92±0.26	2.22±0.00
18:1 n-9	23.60±0.17^{a}	23.45±0.26^{a}	22.87±0.13^{b}
18:1 n-7	5.06±0.00^{a}	4.94±0.17^{a}	4.67±0.12^{b}
18:1 n-9t	0.00^{c}	0.20±0.00^{b}	0.33±0.00^{a}
20:1 n-9	1.12±0.00^{a}	1.02±0.00^{b}	0.92 ± 0.00^{c}
ΣMUFA	32.71	32.44	31.84
18:2 n-6	1.67±0.11^{a}	1.14±0.00^{b}	0.96±0.00^{c}
20:4 n-6	9.89±0.00	9.86±0.14	9.88±0.18
20:5 n-3	0.66±0.00^{a}	0.00^{c}	0.51±0.00^{b}
22:6 n-3	10.40±0.00^{a}	9.54±0.36^{c}	10.01±0.00^{b}
22:4 n-6	3.61±0.00	3.53±0.10	3.57±0.00
ΣPUFA	26.90	24.50	25.05
Σ*trans* FA	0.00	0.20	0.33
n6/n3 ratio	1.37	1.51	1.35

The following fatty acids were found at concentrations lower than 0.5% and for this reason are not shown: C10:0, C14:0, C16:1, C17:0, C20:3 n-6, C22:1 n-9, C22:2 n-6. The following fatty acids were not detected in the analyzed samples: C4:0, C6:0, C8:0, C11:0, C12:0, C13:0, C14:1n-5, C15:0, C15:1 n-5, C18:2n-6t, C18:3 n-3, C18:3 n-6, C20:0, C20:2 n-6, C20:3n-3, C22:0, C23:0, C24:0, C24:1 n-9.

Different lowercases indicate significant difference among S, L and HVF ($P<0.05$).

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

DISCUSSION

The supplementation in focus in this study presented different types of FA (saturated, PUFA, and *trans* FA), which were detected in diets enriched with L, SO, and HVF, respectively. Our objective was to investigate isocaloric diets (the most consumed currently) and show that, even with a great consumption, SO diet is less harmful than L and HVF diets. Immediately after the adipose tissue, the nervous system is the organ with the second greatest concentration of lipids, which participate directly in neuronal membranes functions. Reports have highlighted that the direct uptake of n-6 and n-3 PUFA by brain tissue occurs primarily in the perinatal period, but mechanisms of synthesis, transport, and metabolism of fatty acids during pregnancy vary between species and are still poorly understood (Neuringer et al., 1988). In our findings, levels of n-6 and n-3 PUFA were similar between the three different diets, reinforcing the hypothesis that these PUFA are largely incorporated into neuronal membranes during the period of cerebral development (Bourre et al., 1989). On the other hand, *trans* FA can also be incorporated in brain (Grandgirard et al., 1994), indicating that neural functions may be affected, especially during the pre- and postnatal period (Wauben et al., 2001). After 60 weeks of supplementation with different dietary FA, the consumption of HVF was found to be able to induce a slight cerebral incorporation of *trans* FA, which is sufficiently strong to modify parameters of anxiety, memory acquisition, and $Na^{+}K^{+}$-ATPase activity in brain tissues. In fact, this small incorporation (0.33%) showed that the brain is well protected against changes in its lipids composition through dietary habits. In line with our findings, Acar et al. (2003) also observed 0.3% of *trans* FA incorporation after 21 months of supplementation with diets enriched with this FA. In addition, our findings showed a subtle but significant higher level of PUFA and absence of *trans* FA in brain tissue of SO-fed rats.

In this sense, a diet rich in n-6 and saturated FA has been shown to increase aggressive behavior in rodents (Raygada et al., 1998), whereas one enriched with n-3 PUFA reduced anxiety symptoms (Ikemoto et al., 2001). In contrast, our results showed that the different dietary FA had no effect on anxiety-like symptoms of unexercised rats, but interaction with physical activity led to some interesting results. Rats fed SO-enriched diet and submitted to exercise showed less anxious behavior demonstrated by greater head-dipping frequency and longer time spent in open arms, whereas physical activity did not improve these parameters in L- and HVF-fed rats. In addition, the swimming protocol used in this study was adequate, and its efficacy was evidenced by low levels of blood lactate, which was quantified after physical training. These results are in agreement with other studies (Gobatto et al., 2001; Zhang et al., 2007) and also with previous reports of our group (Teixeira et al., 2009), when exercise was able to reverse oxidative damages related to a diet lacking in micronutrients.

At the sequence of behavioral evaluations, the diets used here also allowed us to relate brain FA incorporation to faster learning as assessed in Barnes maze. This memory paradigm was chosen to determine the hippocampus- and cortex-dependent cognitive task that requires spatial reference (Barnes, 1979; Bach et al., 1995; Oliveira et al., 2008). We observed that SO-fed rats presented a fast and sustained learning process, whereas L- and HVF-fed rats showed delayed or absent learning, respectively. Other studies reported memory impairments related to dietary FA (Bourre et al., 1989; Lamptey and Walker, 1976; Yamamoto et al., 1987; Yu et al., 2010), and their incorporation in brain membranes phospholipid were related to DNA damage and BDNF reduction in hippocampus of rats (Molteni et al., 2002).

We observed that physical activity was able to improve memory acquisition in HVF- and lard-fed rats, these effects being more subtle in the latter. Different studies have shown that exercise has a particular ability to modulate neuronal functions, such as the cognitive decline associated with aging (Friedland et al., 2001), to aid in the recovery from functional loss after CNS injury (Mattson, 2000), and to promote neurogenesis in hippocampus (Van Praag et al., 1999). Of particular importance, recent studies performed in our laboratory showed beneficial effects of exercise on motor disturbances (Teixeira et al., 2008, 2011), as well as a modulating effect on dopamine transport system when this function was damaged (Teixeira et al., 2011). While different studies have shown beneficial

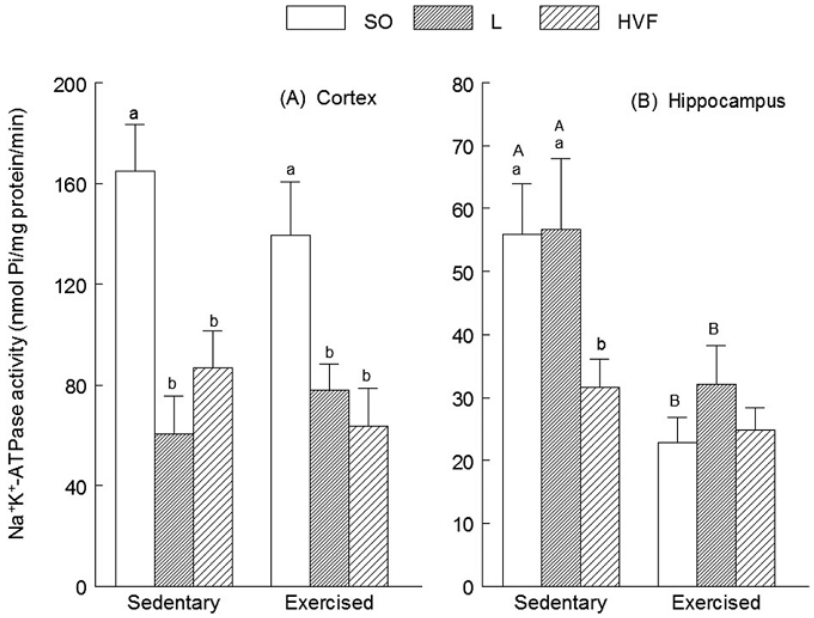

Fig. 3. Effect of diets enriched with soybean oil (SO), lard (L), or hydrogenated vegetal fat (HVF) for 15 mon and of exercise (30 min of swimming/day in the last 90 d), on Na^+K^+-ATPase activity in cortex (A) and hippocampus (B) of rats. Data are expressed as means±SEM. Different lowercase letters indicate significant differences between diets and the same physical activity ($P<0.05$); different uppercase letters indicate significant differences between sedentary and exercised rats fed the same diet ($P<0.05$).

effects of exercise on memory function, as mentioned above, our findings showed deleterious effects of diets rich in SFA and *trans* FA on the learning process, reinforcing the health effects of exercise in this situation as well. In addition, Molteni et al. (2004) showed that memory function is restored by exercise especially when its levels are reduced by saturated fat.

Concerning neuronal membrane, reports showed that membranes with high fluidity or higher PUFA to SFA ratio display an elevated activity of membrane-bound enzymes like Na^+K^+-ATPase (Srinivasarao et al., 1997; Vajreswari et al., 1990), emphasizing the importance of essential FA on this enzyme activity (Alam and Alam, 1986). Na,K-ATPase is widely diffused in brain membranes, whose activity spends about 40–50% of the ATP generated in this tissue (Erecińska and Silver, 1994). This enzyme is responsible for the generation of membrane potential through the active transport of Na^+ and K^+, and it is necessary to maintain the ionic gradient for neuronal excitability (Mobasheri et al., 2000), it and plays fundamental role in neuronal and synaptic plasticity (Scuri et al., 2007), mediating learning and memory (Zhan et al., 2004).

Our results also showed higher activity of Na^+K^+-ATPase in cortex of SO- and in hippocampus of SO- and L-fed rats. On the other hand, the consumption of HVF was not compatible with better functionality of the enzyme in both brain tissues, and this effect was not modified by exercise. Of particular importance for these findings, changes in lipid composition of the neuronal membrane may cause significant conformational modifications in membrane-bound proteins, and this could explain the changes in enzyme activity reported here. We suggest that the short incorporation of *trans* FA in brain membranes of HVF-fed rats, and to a lesser extent of lard-fed rats, may be related to lower activity of the Na^+K^+-ATPase observed in our study. Interestingly, exercise reduced the activity of this enzyme in hippocampus of rats fed with the different FA, and this result deserves further studies. Other factors not evaluated here may be involved in this effect, that is, the age of the animals (at the end of the experimental protocol animals were 16 months old). In the aging process, neuronal functions lose strength and balance, causing difficulties to recover through protocols started in maturity (12 months old). Furthermore, the aging process is known to elevate cholesterol and decrease PUFA levels changing membrane fluidity and its normal function (Kessler and Yehuda, 1985; McGahon et al., 1999a,b). Although the physiological significance of these effects could not be determined here, some hypotheses should be considered: (i) dietary saturated and *trans* FA may affect the activity of Na^+K^+-ATPase by modifying membrane fluidity, which is known to markedly affect activity of a number of membrane-bound enzymes; (ii) ionic involvement has been suggested to contribute for memory formation, and the Na^+K^+-ATPase activity is essential for maintaining ionic gradients in neurons (Conrad and Roy, 1993; Ng et al., 1992); (iii) in line with our findings, it was recently hypothesized that Na^+K^+-ATPase activity inhibition might mediate potentiation of NMDA current, which is then relevant to modulation of learning and memory acquisition (Zhang et al., 2011); (iv) Na^+K^+-ATPase activity function was related to dietary FA as well (Bourre et al., 1989; Hájek et al., 1994).

CONCLUSION

Healthy lifestyle habits that include adequate intake of PUFA and regular physical activity appear to regulate neuronal neurotransmission and may be useful to reduce anxiety-like symptoms. The data presented here showed that a predominance of dietary *trans* FA may be associated with impairments of learning, which can be protected by exercise. Moreover, as dietary FA play a critical role in brain function, a high consumption of processed foods rich in saturated and *trans* FA may promote their incorporation in neuronal membranes and alter the Na^+K^+-ATPase activity. The effects of exercise on activity of this enzyme, as well as their influence on neuronal functions, deserve further investigations.

Acknowledgments—The authors are grateful to CNPq, CAPES, and FAPERGS for fellowships and financial support.

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(Accepted 23 August 2011)
(Available online 27 August 2011)

3.3 ARTIGO 3:

Publicado na Revista *Behavioural Brain Research* 226: 504-510, 2012.

O consumo prolongado de ácidos graxos *trans* poderia facilitar o desenvolvimento dos distúrbios do movimento? Efeitos do exercício e sua influência sobre a atividade das enzimas Na^+,K^+-ATPase e catalase no estriado de ratos.

Research report

Could dietary trans fatty acids induce movement disorders? Effects of exercise and its influence on Na^+K^+-ATPase and catalase activity in rat striatum

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ARTICLE INFO

Article history:
Received 19 August 2011
Received in revised form
30 September 2011
Accepted 3 October 2011
Available online 8 October 2011

Keywords:
Orofacial dyskinesia
trans Fatty acids
Exercise
Na^+K^+-ATPase
Motor disorders

ABSTRACT

The influence of *trans* fatty acids (FA) on development of orofacial dyskinesia (OD) and locomotor activity was evaluated. Rats were fed with diets enriched with 20% soybean oil (SO; *n* – 6 FA), lard (L; saturated FA) or hydrogenated vegetable fat (HVF; *trans* FA) for 60 weeks. In the last 12 weeks each group was subdivided into sedentary and exercised (swimming). Brains of HVF and L-fed rats incorporated 0.33% and 0.20% of *trans* FA, respectively, while SO-fed group showed no incorporation of *trans* FA. HVF increased OD, while exercise exacerbated this in L and HVF-fed rats. HVF and L reduced locomotor activity, and exercise did not modify. Striatal catalase activity was reduced by L and HVF, but exercise increased its activity in the HVF-fed group. Na^+K^+-ATPase activity was not modified by dietary FA, however it was increased by exercise in striatum of SO and L-fed rats. We hypothesized that movement disorders elicited by HVF and less by L could be related to increased dopamine levels in striatum, which have been related to chronic *trans* FA intake. Exercise increased OD possibly by increase of brain dopamine levels, which generates pro-oxidant metabolites. Thus, a long-term intake of *trans* FA caused a small but significant brain incorporation of *trans* FA, which favored development of movement disorders. Exercise worsened behavioral outcomes of HVF and L-fed rats and increased Na^+K^+-ATPase activity of L and SO-fed rats, indicating its benefits. HVF blunted beneficial effects of exercise, indicating a critical role of *trans* FA in brain neurochemistry.

1. Introduction

The consumption of processed foods rich in saturated and *trans* fatty acids (FA) has increased considerably in the last century, mainly in the Western countries, and its consequences on the neuronal function are still unclear. Different studies have shown that dietary FA may change the fluidity and the physiology of the neuronal membranes. In effect, incorporation of polyunsaturated fatty acids (PUFA) increases its fluidity while saturated and *trans* FA incorporation increases its rigidity [1–4]. Fatty acids are important components in the composition of the brain and high levels of lipids are found in the neuronal membranes and myelin sheath. Consequently, changes in the composition of FA can exert important influence on the nervous system functions [5,6], as well as on development of neuronal diseases [7].

The *trans* FA content in hydrogenated vegetable fat (HVF) may account for up to 60% of the total FA [8] and as yet its consumption does not have established safety limits [9]. Literature data have indicated that intake of *trans* FA increased plasmatic marker of inflammation and decreased the cellular defenses against oxidative stress (OS) [10–12]. The presence of these *trans* FA in neuronal membranes also can favor dopaminergic neurotransmission [13], mainly due to increased turnover of dopamine, whose biotransformation is related to generation of pro-oxidant metabolites [14–16]. In fact, an increase in oxidative stress OS can increase the oxidation of fatty acids FA from neural membranes, decreasing their fluidity, which can be also involved in aging process [17–19]. Furthermore, specific aspects of lifestyle such as diet and exercise can affect synaptic plasticity and can afford neuroprotection against degenerative diseases. In this sense, regular physical activity exerts benefits in mental and physical health, increasing antioxidant defenses.

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doi:10.1016/j.bbr.2011.10.005

In addition, physical exercise can modulate dopamine levels in neostriatum and nucleus accumbens [20–22], can ameliorate impaired brain motor functions in movement disorders such as Parkinson and Huntington diseases [23–26]. On the other hand, literature data also showed that exercise could be harmful to a vulnerable nervous system [27].

Considering that little is known about the effects of elevated consumption of *trans* FA on the brain physiology, we evaluated the influence of different dietary fats (rich in $n-6$, saturated and *trans* FA) and the possible benefits of the physical activity on the movement disorders (orofacial dyskinesia – OD) and on normal spontaneous locomotor activity. The incorporation of dietary *trans* FA in the brain membranes was quantified, and its influence on the activity of catalase and Na^+K^+-ATPase in striatum was considered, mainly because this brain region is closely related to the development of movement disorders.

2. Methods

2.1. Animals

Forty-eight male Wistar rats (21 days old) were kept in Plexiglas cages with free access to food and water in a room with controlled temperature (23 ± 1 °C) and on a 12 h light/dark cycle with lights on at 7:00 a.m. The experimental protocol was approved by the Animal Ethical Committee (Universidade Federal de Santa Maria-UFSM-24/2010), which is affiliated to the Council for Control of Animal Experiments (CONCEA), following international norms of care and animal maintenance. Immediately after weaning, the rats were fed with diets enriched (20%) with either soybean oil (SO-rich in polyunsaturated fatty acids – PUFA), lard (L-rich in monounsaturated – MUFA and saturated fatty acids – SFA) or hydrogenated vegetable fat (HVF-rich in *trans*-monounsaturated and saturated fatty acids) for 60 weeks. These diets were isocaloric in order to reduce possible metabolic differences between animals of different experimental groups. In this sense, soybean oil was introduced in this study as an isocaloric control mainly by its elevated consumption worldwide.

In the last 12 weeks one half of each group was submitted to swimming (3 exercise sessions of 10 min interspersed with two 5 min rest intervals, 5 days/week). At the end of this period, all animals were submitted to the behavioral assessments described below.

2.2. Fatty acids determination in the diets

The percentage of FA present in the diets enriched with different types of fats was evaluated [28]. Methylated fatty acids were analyzed using an Agilent Technologies gas chromatograph (HP 6890) equipped with a Supelco SP-2560 capillary column (100 m × 0.25 mm × 0.20 μm) and flame ionization detector. Results were expressed as percentage of total area of the identified fatty acids.

2.3. Behavioral testing

2.3.1. Quantification of orofacial dyskinesia (OD)

The animals were placed individually in cages (20 cm × 20 cm × 19 cm) containing mirrors under the floor to allow for behavioral observation when the animal was facing away from the observer. The frequency of vacuous chewing movements (VCM) and time of facial twitching (FT) were recorded for 5 min after 2 min of adaptation (hand operated counters were employed). The observers were blind to the dietary and exercise treatments. The behavioral experiments were conducted between 09:00 and 11:00 a.m.

2.3.2. Open-field test

After the OD quantification, animals were placed individually in the center of an open-field arena (40 cm × 40 cm × 30 cm) with black plywood walls and a white floor divided into nine equal squares, as previously described by Kerr et al. [29]. The number of crossings (horizontal squares crossed) and rearings (vertical movements) were recorded for 5 min and used as measures of spontaneous locomotor activity and exploratory behavior, respectively.

2.4. Tissue preparations

Rats were euthanized by decapitation about 24 h after the last session of behavioral evaluation. The brains were removed immediately, put on ice and cut coronally at the caudal border of the olfactory tubercle. The striatum was dissected from the anterior part and homogenized in 10 volumes (w/v) of 0.1 M Tris–HCl, pH 7.4. After centrifugation (10 min at 3000 × g), the supernatant was used for Na^+K^+-ATPase and catalase activity assays, as described below follow.

Table 1
Total fatty acid composition of diets enriched with different fats.

Fatty acids	Soybean oil	Lard	Hydrogenated vegetable fat
∑SFA	19%	32%	26%
∑MUFA	29%	42%	59%
∑PUFA	51%	23%	14%
∑*trans* FA	0.6%	3%	17%
∑$n-6/n-3$ ratio	9.0	14.4	20.1

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

2.5. Catalase (CAT) enzyme activity

CAT activity was spectrophotometrically quantified in striatum by the method of Aebi [30], which involve monitoring the disappearance of H_2O_2 in the presence of brain homogenate (pH 7 at 25 °C) at 240 nm. The enzymatic activity was expressed as hydrolysis of 1 μmol H_2O_2/min/g tissue.

2.6. Na^+K^+-ATPase activity

The Na^+K^+-ATPase activity was determined in striatum sample according to the method proposed by Musbeck et al. [31], with some modifications. Briefly, the aliquots of tissue (20 μL) were added to a reaction medium containing NaCl, $MgCl_2$, KCl and Tris–HCl buffer (pH 7.4), with or without the Na^+K^+-ATPase enzyme inhibitor ouabain. The method for ATPase activity measurement was based on the determination of the inorganic phosphate (Pi) released to the reaction medium by the hydrolysis of the ATP according to the method proposed by Atkinson et al. [32]. The reaction was initiated with the addition of the substrate ATP to the reaction medium and was finished by the addition of the color reagent (1 mL) containing ammonium molybdate (2%), triton X-100 and H_2SO_4 (10%) after 15 min of incubation at 37 °C. The formed molybdate–Pi complexes were measured spectrophotometrically at 405 nm. Values were calculated in relation to a standard curve constructed with Pi at known concentrations and also corrected by the protein content. Protein was measured according to Lowry et al. [33].

2.7. Brain fatty acids analyses

Fat was extracted from diets and brain using chloroform and methanol as described by Bligh and Dyer [34] and methylated according to Hartman and Lago [28]. Fatty acid composition was determined by gas chromatography using an Agilent Technologies gas chromatograph (HP 6890) fitted with a Supelco SP-2560 capillary column (100 m × 0.25 mm × 0.20 μm) and flame ionization detector. Standard fatty acid methyl esters (37-component FAME Mix, DPA $n-3$ and PUFA no. 2 from Sigma, Saint Louis, MO, USA and DPA $n-6$ from NuChek Prep. Inc., Elysian, MN, USA) were run under the same conditions and the subsequent retention times were used to identify the fatty acids. Results were expressed as percentage of total area of the identified fatty acids.

2.8. Statistical analysis

Diets and brain tissue FA content were analyzed by one-way ANOVA followed by Duncan's test. Data of orofacial dyskinesia, spontaneous locomotor activity, catalase and Na^+K^+-ATPase activities were analyzed by two-way ANOVA (2 training conditions (sedentary/exercised) × 3 diets (soybean oil/lard/HVF) followed by Duncan's multiple range test, when appropriate. $P<0.05$ was regarded as statistically significant.

3. Results

3.1. The total oil content of the different diets is shown in Table 1

The composition of the three dietary fats differed considerably as to the prevalence of FA: while the SO diet had a high proportion (51%) of PUFA, the L diet had nearly 32% of SFA, and the HVF diet had the highest level of *trans* FA (17%) and MUFA (59%). The $n-6/n-3$ ratio was lower in SO and was progressively higher in the L and HVF diets, respectively.

Table 2
Total fatty acid composition of the brain of rats after feeding with different fats in the diet.

Fatty acids	Soybean Oil	Lard	Hydrogenated vegetable fat
∑SFA	40.19%	41.40%	40.88%
∑MUFA	31.33%	30.73%	29.89%
∑PUFA	24.18%	22.10%	22.75%
∑*trans* FA	0.00	0.20%	0.30%

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

3.2. *The effects of dietary fatty acids and physical activity on the development of orofacial dyskinesia are shown in Fig. 1*

Two-way ANOVA of VCM frequency revealed a significant effect of FA and exercise [$F(2,42) = 12.47$ and $F(1,42) = 13.79$, $P < 0.001$], respectively. Similarly, two-way ANOVA of FT time revealed a significant effect of FA and exercise [$F(2,42) = 39.73$ and $F(1,42) = 16.80$; $P < 0.001$], respectively, and also a significant diet × exercise interaction [$F(2,42) = 7.70$; $P < 0.05$].

HVF-fed rats of sedentary and exercise, showed higher VCM frequency than SO and L-fed groups (Fig. 1A). Sedentary HVF-fed rats showed greater FT time than the SO and L groups. In exercised animals, a significant sequential increase of FT time was observed in SO, L and HVF-fed rats, respectively (Fig. 1B). In fact, physical activity increased the VCM frequency and also the FT time of both L and HVF-fed rats (Fig. 1A and B).

3.3. *The effects of dietary fatty acids and physical activity on the spontaneous locomotor activity in open-field task are shown in Fig. 2*

Two-way ANOVA of crossing and rearing number revealed a significant effect of FA [$F(2,42) = 13.97$ and 9.86; $P < 0.001$, respectively]. Both sedentary and exercised groups fed L and HVF showed lower crossing (Fig. 2A) and rearing (Fig. 2B) numbers. Exercise did not modify these motor behavioral parameters.

3.4. *Fatty acid composition of total brain is shown in Table 2*

Rats fed SO, lard and HVF enriched diets from weaning for 15 months presented changes in the FA composition of brain. The highest content of PUFA was observed in the SO diet (27%), while the highest contents of SFA and *trans* FA were observed in L (42%) and HVF (0.30%), respectively. In fact, *trans* FA were not detected at all in SO-fed rats, and the $n-6/n-3$ ratio showed similar values across the three different diets (Table 2).

3.5. *The effects of dietary fatty acids and physical activity on Na^+K^+-ATPase and catalase activities in striatum of rats are shown in Fig. 3*

Two-way ANOVA of striatal Na^+K^+-ATPase activity revealed a significant main effect of FA [$F(1,42) = 4.32$; $P < 0.05$], exercise [$F(1,42) = 12.81$; $P < 0.05$] and a tendency to a significant FA × exercise interaction ($P = 0.062$). Similarly, two-way ANOVA of striatal catalase activity revealed a significant main effect of both FA and exercise [$F(2,42) = 11.70$; $P < 0.001$ and $F(1,42) = 6.78$; $P < 0.05$], respectively.

Dietary FA alone did not alter the activity of striatal Na^+K^+-ATPase, but among the exercised animals, SO fed rats showed higher Na^+K^+-ATPase activity than those fed L and HVF. Considering the same diet, the exercise increased the Na^+K^+-ATPase activity in SO and L fed rats, but not the HVF group.

In sedentary group, catalase activity was reduced in striatum of both L and HVF fed rats in relation to SO. Among exercised, the striatal catalase activity was reduced only in L fed rats. In fact, the exercise was able to increase the catalase activity of HVF fed rats.

4. Discussion

FA composition of SO enriched diet presented the highest proportion of PUFA, in contrast to L diet, with a predominance of SFA, whereas the HVF diet presented the highest level of *trans* FA and MUFA. According to Pettersen and Opstvedt [35], the brain appears to be protected from *trans* FA incorporation. Nevertheless, we showed that 60 weeks of dietary supplementation allowed an incorporation of 0.30% and 0.20% of *trans* FA in brain tissue of HVF and L-fed rats, respectively. In fact, this small FA incorporation indicates that the brain is able to select its lipid composition, which is supplied from dietary habits [13]. A similar phenomenon is also observed in other tissues, such as retina lipids [36,37]. On the other hand, this apparently slight incorporation of *trans* FA in brain membranes can induce changes in memory function and anxiety-like symptoms [38], as well as in motor control, which was observed in this study by increased OD in HVF-fed rats and decreased spontaneous locomotor activity in HVF and L-fed rats. Acar et al. [13] showed a similar incorporation of dietary *trans* FA in neuronal membranes of rats, which was related to higher levels of striatal dopamine, suggesting changes in gene expression and transcription of proteins involved in dopamine metabolism. In this study we did not measure dopamine levels in striatum, but this neurotransmitter may be involved in the increased OD observed in HVF-fed rats. Thus, we believe that a minimum incorporation of *trans* FA in brain neuronal membranes is sufficient to cause functional changes in the striatal region, which is heavily involved in movement disorders. Reinforcing our findings, recently we showed a close relation between motor disorders and oxidative damage evocated by dopamine metabolism in striatum, where movement disturbances were prevented by $n-3$ PUFA [39,40]. Furthermore, consumption of a diet high in hydrogenated fat was related to increased production of proinflammatory cytokines and OS [11]. In addition, the reduced spontaneous locomotor activity observed in L and HVF-fed rats may also be related to *trans* FA incorporation, which confers greater rigidity to neuronal membrane [41]. Our findings also showed that exercise increased OD in both L and HVF-fed rats, but not in soybean oil (SO) group, while locomotor and exploratory movements were not modified, remaining diminished in relation to SO group: we hypothesized that the presence of trans FA in neuronal membranes, which was observed in L and HVF-fed rats, may impair molecular responses of exercise, which was not efficient enough to counteract the damages evoked by this incorporation. In fact, studies have reported that incorporation of *trans* FA into membranes is related to changes to its fluidity and permeability [42] reflecting on changes in enzyme activity [38], density and function of transmembrane receptor [41], as well as modifications in neurotransmitter release such as dopamine [13]. Recently we showed the modulatory effects of exercise on striatal dopamine reuptake in situations of oxidative damage [20], and its benefits in movement disorders induced by reserpine, a pro-oxidant agent [43]. These studies are not contradictory to the findings presented here, mainly because dopaminergic activity normally increased by exercise [21] may have its response reduced due to greater membrane rigidity, which is related to *trans* FA incorporation, as mentioned above. In fact, small changes in lipid environment of receptors can lead to changes in their structures and large changes in ligand binding [44]. A minor activation of dopamine receptor especially in basal ganglia, including the striatum, can affect motor

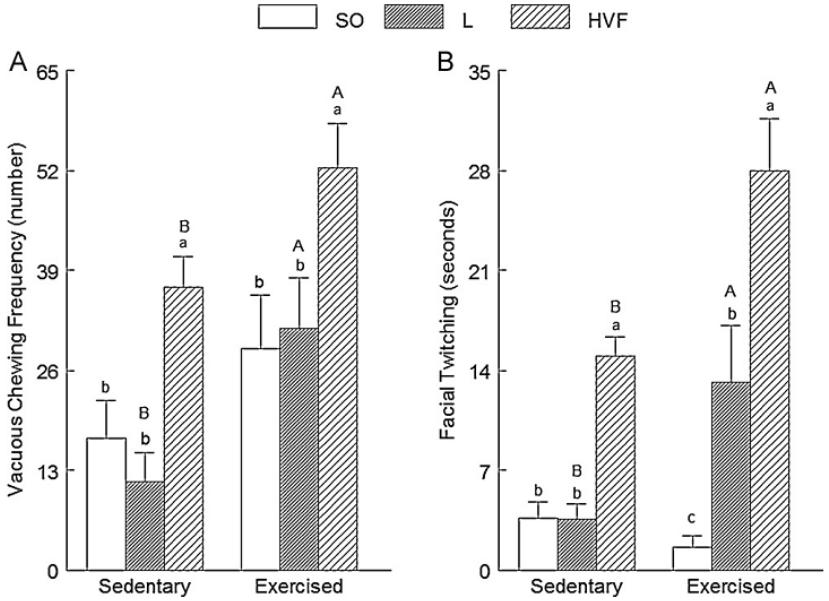

Fig. 1. Effect of diets enriched with soybean oil (SO), lard (L) or hydrogenated vegetal fat for 60 weeks and exercise (30 min of swimming/day in the last 12 weeks), on orofacial movements: vacuous chewing frequency (A) and facial twitching (B). Data are expressed as mean ± S.E.M. Lowercase indicates significant difference between the different diets and the same physical condition (exercise or sedentary) ($P < 0.05$); uppercase indicates difference between sedentary and exercised rats in the same diet ($P < 0.05$).

brain function closely related to movement disorders, depending on its balance [26,45–47]. In the present study, physical exercise started at 12 months of age exacerbated orofacial movements of L and HVF- fed rats. It is possible that exercise and *trans* FA previously incorporated into neuronal membranes have increased striatal dopamine levels, as well as modifying the functionality of transmembrane transporters and receptors, which reinforces the hypothesis that *trans* FA incorporation may modify brain functions related to movement control, especially in the striatal dopaminergic system. On the other hand, animal age is another factor that may modify the beneficial effects of exercise: at the end of the experiments animals were 15 months old. A previous study performed in

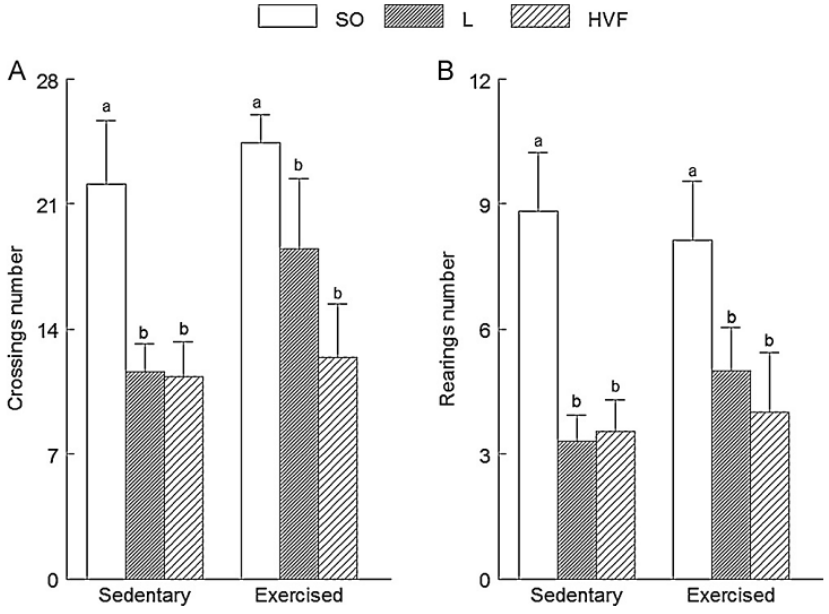

Fig. 2. Effect of diets enriched with soybean oil (SO), lard (L) or hydrogenated vegetal fat (HVF) for 60 weeks and exercise (30 min of swimming/day in the last 12 weeks), on locomotor (A) and exploratory (B) activities in an open-field task. Data are expressed as mean ± S.E.M. Lowercase indicates significant difference between the different diets and the same physical condition (exercise or sedentary) ($P < 0.05$).

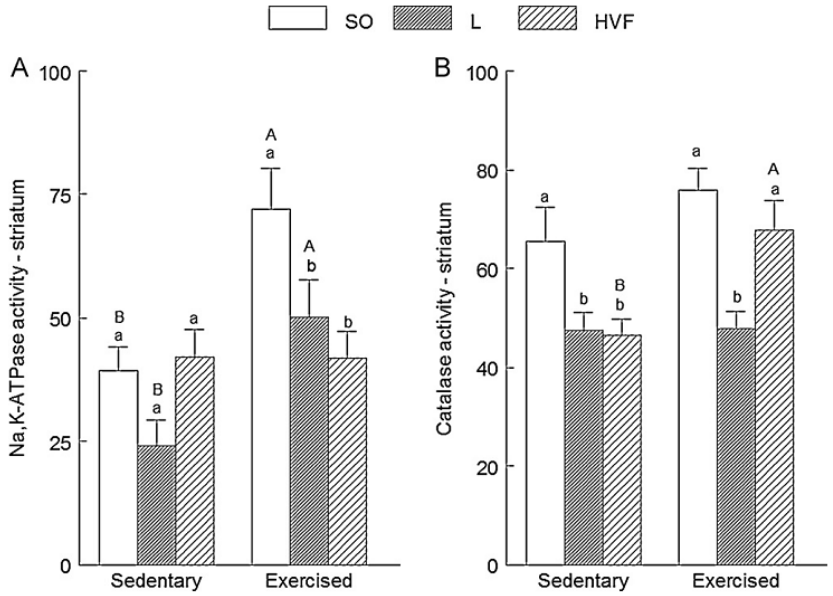

Fig. 3. Effect of diets enriched with soybean oil (SO), lard (L) or hydrogenated vegetal fat (HVF) for 60 weeks and exercise (30 min of swimming/day in the last 12 weeks), on Na^+K^+-ATPase activity (A) and catalase activity (B) in striatum of rats. Data are expressed as mean ± S.E.M. Lowercase indicates significant difference between the different diets and the same physical condition (exercise or sedentary) ($P < 0.05$); Uppercase indicates difference between sedentary and exercised rats in the same diet ($P < 0.05$).

our laboratory showed the effects of age on OD development [48], while other studies have reported that OD can occur spontaneously with aging [49], making this movement disorder phenomenologically similar to the animal model of drug-induced OD [50,51]. This relationship between involuntary orofacial movements and aging has also been reported in humans, where old people who have never received antipsychotic drugs develop spontaneous OD [52]. A recent study showed that the effect size was smaller when exercise was initiated in mature animals, confirming that adult rat brain remains dynamic and adapts to chronic exercise. Nevertheless, some brain areas appear to be more affected if physical activity is initiated earlier [53]. Different responses to physical exercise on locomotor behavior were also reported by Fabene et al. [54], when physical training started in old animals showed negative effects on motor behavior.

Different studies have shown that dopamine catabolism is an important generator of pro-oxidant metabolites and OS, which are closely related to antioxidant defenses [16,20,47,55,56]. A study performed by Abilio et al. [49] showed the protective effects of catalase in the development of movement disorder, when the increase of enzyme activity was able to reduce OD related to aging process as well as in an animal model. In this sense, catalase is an antioxidant enzyme that exerts a critical role in the development of OD [57]. Interestingly, in the present study exercise increased catalase activity in striatum of HVF-fed rats, but this apparent beneficial effect of exercise was not sufficient to reduce OD evocated by long time intake of *trans* FA. We believe that these findings are not contradictory, but indicate activation of regulatory mechanisms in the antioxidant defense system, whose function may be impaired by changes in the neuronal membranes properties. These results confirm previous studies of our group showing beneficial effects of exercise on recovery of catalase activity in striatum of rats with movement impairments [43]. So far, no study has shown the influence of *trans* FA on catalase activity in striatum, neither its effects on development of movement disorders. Thus, it is possible to relate lower OD and higher locomotor activity of SO-fed rats to greater catalase activity in striatum, which was maintained in exercised rats.

Furthermore, in this study we did not observe influence of different dietary FA on Na^+K^+-ATPase activity in striatum, but exercise was able to increase its activity in SO and L-fed rats. So far, we do not know if this effect can be considered as favorable, but it is interesting to note that the highest incorporation of *trans* FA was observed in HVF group, where exercise had no effect on this enzyme. Na^+K^+-ATPase plays a key role in cell function, regulating the exchange of Na^+ and K^+ between the cell and the intercellular space. Thus, an increase in membrane fluidity is related to greater activation of this enzyme, while an increase in that rigidity inhibits its action [58]. This leads us to hypothesize that a minimum but primary incorporation of *trans* FA in brain neuronal membranes can be sufficient to change striatal Na^+K^+-ATPase activity, compromising its functionality. In this sense, it was reported that EFA and cholesterol levels [59], as well as oxidative damage [60–63] might regulate Na^+K^+-ATPase activity. In addition, its activity appears to be influenced by hormones and neurotransmitters such as dopamine [64,65], which seems to be increased by *trans* FA incorporation as well as by physical activity, as mentioned above.

In conclusion, this study showed that prolonged dietary intake of HVF, as well as of lard in lower proportion, allows *trans* FA incorporation in brain neuronal membranes, facilitating the development of oxidative processes and movement disorders. Physical activity can impair this motor condition in old rats and increase catalase activity in a compensatory way, trying to restore the antioxidant status. Exercise also showed ability to increase Na^+K^+-ATPase activity in striatum of L and SO-fed rats, which can be closely related to minor and absent *trans* FA incorporation, respectively.

Further studies about the influence of saturated and *trans* FA consumption on development of movement disorders are needed. The possible relationship between dietary FA and susceptibility to movement disorders must be carefully investigated, especially in Western countries, where these intakes occur with greater frequency.

Acknowledgements

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de amparo à pesquisa do estado do Rio Grande do Sul (FAPERGS), and Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) for fellowships and financial support.

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CAPÍTULO III

4. DISCUSSÃO GERAL

Diversos benefícios sobre o SNC estão associados à prática regular de atividade física, no entanto, os mecanismos envolvidos são incertos. O BDNF é um fator amplamente estudado e relacionado aos efeitos positivos do exercício. Subsequente ao aumento de sua expressão, observa-se o aumento do número de células hipocampais (VAN PRAAG et al., 1999) e maior plasticidade cerebral (COTMAN; BERCHTOLD, 2002), beneficiando, deste modo, o aprendizado e a memória (OGONOVSZKY et al., 2005; RADAK et al., 2006). Estudos também demonstram que a prática regular de exercício físico pode elevar mecanismos de defesa antioxidante cerebral, promovendo uma resistência aos danos oxidativos (BANERJEE et al., 2003; RADAK et al., 2006; SOMANI; RAVI; RYBAK, 1995).

Sabe-se que o cérebro é mais sensível ao estresse oxidativo (EO), em comparação a outros órgãos e sistemas, por possuir uma ampla variedade de aminoácidos excitotóxicos, baixo nível de defesas antioxidantes e neurotransmissores auto-oxidáveis (HALLIWELL; GUTTERIDGE, 1999).

Sendo assim, o primeiro estudo desenvolvido nesta tese demonstrou efeitos benéficos do exercício físico sobre os distúrbios do movimento (movimentos de mascar no vazio) e a atividade locomotora, em um modelo de danos oxidativos induzidos por haloperidol (bloqueador pré- e pós-sináptico de receptores de dopamina). Constatou-se a relação destes movimentos com menor atividade da enzima catalase associada a elevados níveis de peroxidação lipídica (TBARS) em regiões cerebrais relacionadas ao controle motor, sendo esta peroxidação prevenida pelo exercício. Foi observado também um efeito modulador do exercício sobre a liberação de dopamina em presença do haloperidol (Figura 1A). Este estudo está de acordo com o de outros pesquisadores, no qual a atividade física foi capaz de normalizar os níveis de dopamina (AKIYAMA; SUTOO, 1999) e amenizar os sintomas de doenças envolvendo distúrbios motores como os da doença de Parkinson (BAATILE et al., 2000; MIYAI et al., 2000).

Considerando ainda a vulnerabilidade do SNC, o cérebro possui a segunda maior concentração de lipídeos, imediatamente após o tecido adiposo, podendo se

tornar particularmente sensível a modificações fisiológicas decorrentes de ácidos graxos (AG) provenientes da alimentação (CARRIÉ et al., 2000).

O exercício físico e a dieta compartilham características similares e, em muitos casos, complementares, podendo ser úteis na estimulação cerebral e no combate ou na prevenção de desordens neurológicas/neurodegenerativas. Neste contexto, o segundo e o terceiro estudos analisam a capacidade de incorporação cerebral de AG poliinsaturados (óleo de soja), saturados (banha) e *trans* (gordura vegetal hidrogenada, GVH), a partir da suplementação alimentar. Os possíveis efeitos desta incorporação sobre as regiões do córtex, hipocampo e estriado e a influência da associação de uma atividade física regular também foram investigados.

A partir destes experimentos foi possível observar que, após 15 meses de suplementação com os diferentes AG, houve uma pequena, mas relevante, incorporação do AG *trans* no cérebro dos animais alimentados com a banha e a GVH. O cérebro necessita de aporte adequado de AG para manter a sua integridade estrutural e, consequentemente, suas funções normais, principalmente por seu elevado teor de lipídeos de membrana (MARTEINSDOTTIR et al., 1998; UAUY; DANGOUR, 2006). Deste modo, a incorporação de AG *trans* aqui observada, pode estar interferindo no metabolismo de AG essenciais, modificando propriedades físicas das membranas e suas interações celulares, além de influenciar na atividade de enzimas, de receptores e transportadores transmembrana (BOURRE et al., 1989; CLANDININ; JUMPSEN, 1997).

Considerando os parâmetros de aprendizado e memória, nosso estudo mostrou efeitos positivos, principalmente nos animais que receberam a suplementação com óleo de soja, o qual é rico em ácidos graxos poliinsaturados n-6. Estes efeitos foram menos pronunciados nos animais suplementados com banha, enquanto não observados nos animais tratados com GVH (maior teor de AG *trans*). A associação com o exercício físico foi capaz de nivelar o aprendizado independentemente do tipo de AG consumido (Figura 2). Neste sentido, a expressão adequada de BDNF é fundamental para o bom funcionamento cerebral, enquanto níveis reduzidos deste importante fator foram mostrados em ratos alimentados com AG saturados (WU et al., 2003). De acordo com Wu et al. (2008), prejuízos de memória e cognição decorrentes de danos ao BDNF induzidos por trauma podem ser revertidos por uma integração adequada entre exercício e dieta, favorecendo a função e a manutenção dos circuitos neuronais. Isto é de extrema importância, visto

que estudos recentes relacionam disfunções ou reduções nos níveis deste fator neurotrófico com a fisiopatologia de diversas desordens neurodegenerativas como Alzheimer (YE;TAI;ZHANG, 2011), Parkinson (KARAKASIS et al., 2011), Huntington (GRIFFIOEN et al., 2011) e esquizofrenia (ZHOU et al., 2010).

No cérebro, a atividade da enzima Na^+K^+-ATPase é outro importante fator para a atividade neuronal e os processos de sinalização celular, através da manutenção do gradiente eletroquímico transmembrana (MOSELEY et al., 2007; STAHL; HARRIS, 1986). À atividade desta enzima, correlacionam-se benefícios de memória e aprendizado (DOS REIS et al., 2002; NAKAZATO et al., 2002; VASCONCELLOS et al., 2005). De acordo com isso, o estudo que desenvolvemos mostra que os animais suplementados com óleo de soja apresentaram uma elevada atividade da enzima Na^+K^+-ATPase nas regiões do córtex e hipocampo, mostrando aprendizado mais rápido e duradouro. O exercício não modificou significativamente a atividade da enzima no córtex, mas foi capaz de reduzir a atividade da mesma no hipocampo dos animais suplementados com óleo de soja e com banha. Mesmo assim, essa redução não comprometeu o desempenho dos animais exercitados, e este dado merece futuras investigações.

Outro fator importante a ser considerado, é que alterações no processo de industrialização dos alimentos levam a uma redução na ingestão de PUFAs, principalmente n-3, e a um aumento no consumo de n-6 e AG *trans* (BORSONELO; GALDURÓZ, 2008). Segundo Viola e Viola (2009), a razão entre o consumo de AG n-6/n-3 não deve ultrapassar 10:1. No presente estudo, a razão n-6/n-3 variou em torno de 9, 14 e 20 para óleo de soja, banha e GVH, respectivamente. De acordo com os dados observados no labirinto em cruz elevado, os animais suplementados com óleo de soja e exercitados apresentaram comportamento exploratório e de maior risco, denotando um menor grau de ansiedade. Observações comportamentais demonstram que uma dieta enriquecida com AG n-6 é capaz de aumentar o comportamento agressivo em roedores, enquanto uma enriquecida com n-3 é capaz de reduzir o estresse dos animais (IKEMOTO et al., 2001; RAYGADA; CHO; HILAKIVI-CLARKE, 1998). Por outro lado, níveis reduzidos de AG n-3 foram encontrados no plasma de pacientes que sofrem de distúrbios de ansiedade (GREEN et al., 2006).

Sabe-se que razões reduzidas n-6/n-3 são associadas à menor produção de citocinas pró-inflamatórias (FERRUCCI et al., 2006; KIECOLT-GLASER et al., 2011),

entre elas a interleucina 1 (IL-1). Comportamentos de ansiedade e estresse podem aumentar a secreção de glicocorticóides por estimulação da IL-1, ativando também a liberação de dopamina, serotonina e noradrenalina (LACOSTA; MERALI; ANISMAN,1998). Além disto, uma variação genética da expressão do BDNF pode ser relacionada a desordens de ansiedade humana (GORMANNS et al., 2011). Foi observado também que ratos com deficiências neste fator são mais agressivos e mais ansiosos. Por outro lado, a atividade física aeróbica regular de ratas prenhes foi capaz de manter os níveis do BDNF nos filhotes que sofreram separação materna, em comparação com os filhotes de ratas não exercitadas (UYSAL et al., 2011). Neste estudo, a separação materna foi considerada um modelo de estresse e ansiedade neonatal.

Sendo assim, infere-se que o exercício confere maior resistência para enfrentar situações estressantes do dia a dia, mesmo de forma indireta (Figura 2). Ainda, a concentração de n-3 observada no óleo de soja, a partir do presente estudo, possivelmente foi suficiente para manter uma fluidez apropriada das membranas cerebrais, favorecendo assim os efeitos do exercício sobre a ansiedade. Neste sentido, podemos sugerir a participação adequada de processos neurotróficos e antiinflamatórios discutidos acima. A elevada razão n-6/n-3 observada nas suplementações contendo AG saturados e, principalmente *trans,* pode estar relacionada à maior atividade pró-inflamatória. De acordo, pesquisadores correlacionam positivamente marcadores de inflamação no plasma de pacientes com a elevada ingestão de AG *trans* (LOPEZ-GARCIA et al., 2005). Pode-se encontrar uma razão mais rigorosa, 1:4 n-6/n-3, para o bom funcionamento das funções cerebrais (YEHUDA; CARASSO, 1993; YEHUDA et al., 2002). No entanto, de acordo com a eficácia do exercício aqui observada, conclui-se que a razão 9:1, verificada com a suplementação de óleo de soja, pode não ser a ideal, mas encontra-se dentro dos limites aceitáveis para um equilíbrio funcional do organismo, e pode ser considerada menos prejudicial em relação às demais avaliadas. Esta informação é relevante, porque estados ansiosos prolongados debilitam o sistema de defesa antioxidante (EREN; NAZIROGLU; DEMIRDAS, 2007; HOVATTA; JUHILA; DONNER, 2010) e são um passo importante para o desenvolvimento de desordens mais graves de humor, como a depressão (WILLNER, 2005).

Além dessas variáveis, este estudo relaciona a influência de AG *trans* suplementados na dieta e incorporados nos tecidos cerebrais com os distúrbios de

movimento e a atividade das enzimas Na^+K^+-ATPase e catalase na região do estriado. Os animais alimentados com GVH (0,30% *trans*) apresentaram um maior número (movimentos de mascar no vazio) e tempo (tremor facial) de movimentos discinéticos orofaciais. Estes parâmetros foram intensificados pela atividade física, o que ficou evidente também no grupo suplementado com AG saturado (0,20% *trans*) (Figura 1B). A região do estriado é particularmente sensível aos danos oxidativos porque possui uma quantidade elevada de monoaminas (LOHR; KUCZENSKI; NICULESCU, 2003). Assim, torna-se suscetível ao desenvolvimento de movimentos involuntários anormais (BURGER et al., 2005 a, b; DAWSON et al., 2000), quando estas aminas são oxidadas. O exercício moderado já demonstrou reduzir estes movimentos sobre um modelo animal de discinesia orofacial (TEIXEIRA et al., 2008), assim como intensificá-los quando ocorre uma sobrecarga de atividade física (TEIXEIRA et al., 2009). Na atual pesquisa, observou-se ainda um aumento na atividade da catalase pelo exercício físico no grupo GVH, justamente aquele com os distúrbios de movimentos mais acentuados. Estudos demonstram o papel crítico desta enzima sobre o desenvolvimento de discinesia orofacial e EO (ABILIO et al., 2004; FARIA et al., 2005). Tomados em conjunto, estes resultados sugerem que a maior atividade da enzima catalase encontrada no grupo *trans*-exercício, pode ser considerada uma resposta compensatória ou de sinalização de dano celular, como já discutido por Gomez-Cabrera et al. (2006) a partir de outro modelo experimental.

Relacionada à atividade da Na^+K^+-ATPase na região do estriado, nos grupos exercitados e suplementados com GHV ou banha, houve menor atividade da enzima em relação ao grupo exercício-óleo de soja. Isto significa que mesmo uma pequena incorporação do isômero *trans* pode ser capaz de modificar os tecidos cerebrais, possivelmente por aumentar sua rigidez e bloquear uma resposta enzimática satisfatória frente ao exercício físico. A Na^+K^+-ATPase é uma enzima bastante sensível ao EO (JAMME et al., 1995; MOREL et al., 1998) e a associação do exercício pode ser promissora na manutenção de sua atividade (LIMA et al., 2009) quando AG adequados fazem parte da composição cerebral.

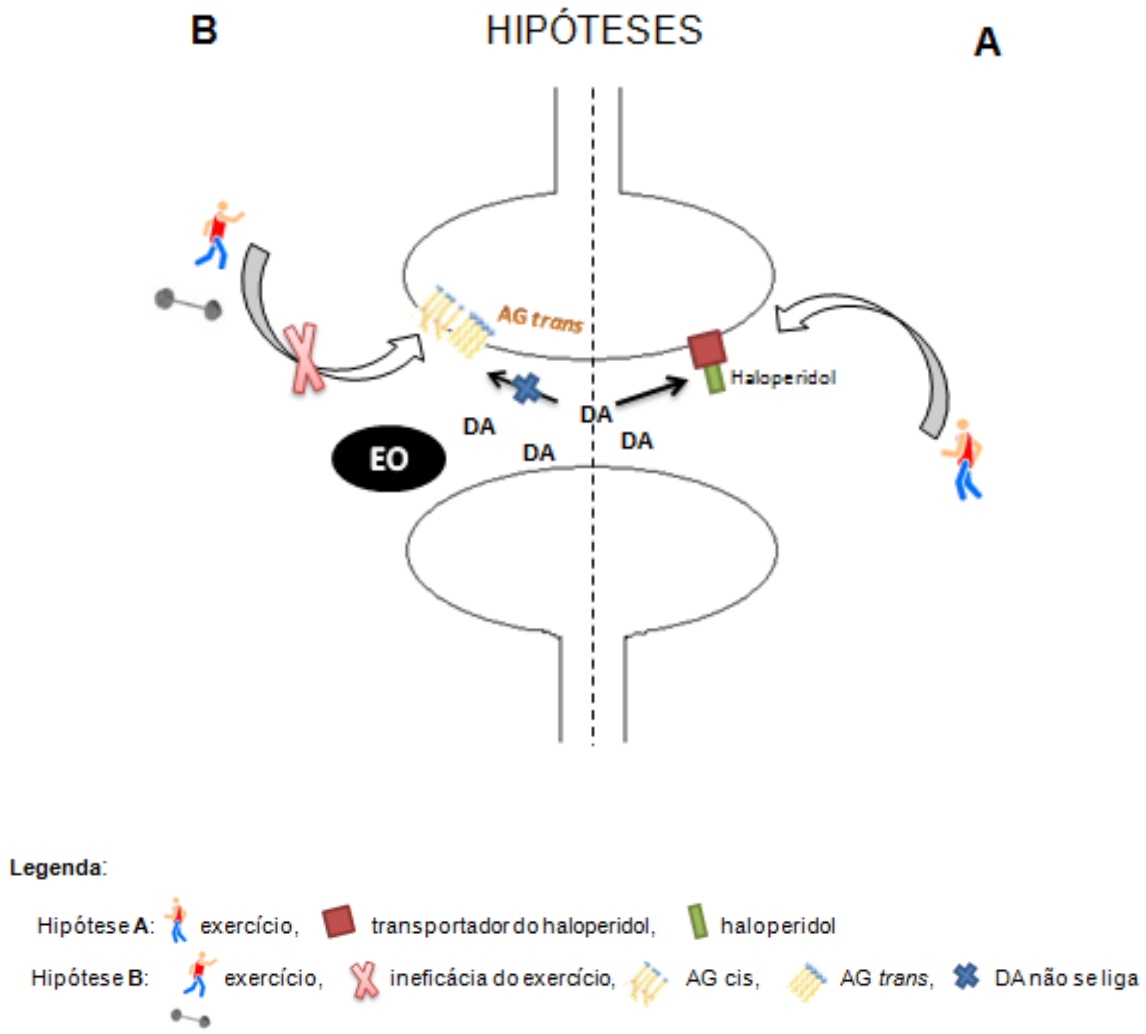

Figura 1. Possível mecanismo do efeito do exercício sobre discinesia orofacial (DO) induzida por: Hipótese (A) - haloperidol, ou Hipótese (B) - desenvolvida pelo consumo de AG *trans*.

Hipótese (A): O exercício físico reduz o EO, quando não existe dano ao tecido cerebral, e facilita a função do transportador de dopamina (DA), mesmo na presença do haloperidol, mantendo níveis reduzidos de DA na fenda sináptica e menor suscetibilidade ao EO e desenvolvimento de DO.

Hipótese (B): O elevado tempo de consumo de AG *trans* pode modificar características da membrana, prejudicando a função do transportador e dificultando o *clearance* de DA. Esta DA pode sofrer auto-oxidação, gerando metabólitos que contribuem para o desenvolvimento de EO e dos distúrbios de movimento. Ocorrendo o comprometimento dos tecidos cerebrais, o exercício se torna ineficiente ou prejudicial.

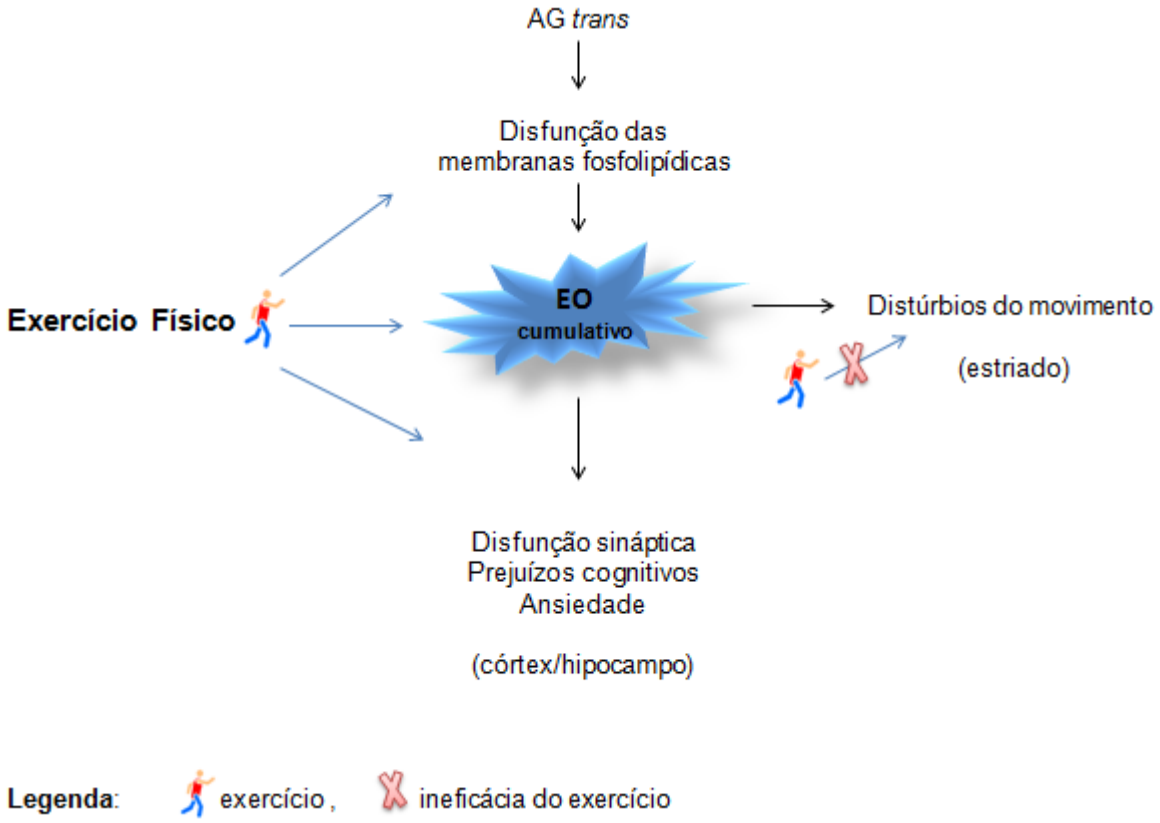

Figura 2. Possível mecanismo sobre os efeitos do exercício na restauração da plasticidade neuronal após danos à membrana induzidos pela incorporação de isômeros *trans*. Esta incorporação pode reduzir a fluidez dos tecidos cerebrais, prejudicando diversas funções, o que culminaria no acúmulo de espécies reativas e consequente desenvolvimento de EO. O EO está relacionado ao desenvolvimento de distúrbios do movimento, como a DO, além de gerar disfunção sináptica, prejuízo cognitivo e sintomas semelhantes ao da ansiedade. O exercício físico é capaz de restaurar os tecidos cerebrais das regiões do hipocampo e córtex, mais estreitamente relacionadas à memória e à ansiedade, impedindo o desenvolvimento do EO. Na região do estriado, mais relacionada às desordens do movimento, a disfunção provoca desenvolvimento intenso de DO e o exercício físico, tardiamente iniciado, não é capaz de evocar proteção.

5. CONCLUSÕES FINAIS

5.1 Conclusões Principais

- O haloperidol pode induzir EO pelo aumento do catabolismo de dopamina, prejuízos na ação da enzima catalase e elevação da peroxidação lipídica, culminando no desenvolvimento da discinesia orofacial;

- Uma atividade física moderada regular pode ser capaz de modular transportadores dopaminérgicos e de agir sobre parâmetros oxidantes/antioxidantes, prevenindo os distúrbios de movimento induzidos por haloperidol;

- Os AG *trans* provenientes da suplementação e/ou dieta podem ser incorporados ao cérebro, modificando a atividade das enzimas catalase e $Na^{+}K^{+}$-ATPase;

- A atividade física pode promover maior resistência ao desenvolvimento de ansiedade, beneficiando também os processos de aprendizado e memória, dependentemente do tipo de AG consumido na dieta (prejuízo da gordura *trans);*

- Reduzindo o limiar ao EO, a incorporação de AG *trans* possivelmente eleva a incidência de desordens do movimento;

- O exercício físico, iniciado tardiamente, não é capaz de prevenir o desenvolvimento de desordens do movimento intensificadas pelo consumo crônico de AG *trans*.

5.2 Conclusão Geral e Perspectivas

Este estudo mostrou, pela primeira vez, os benefícios do exercício físico sobre prejuízos motores induzidos por haloperidol, uma substância amplamente utilizada na clínica para tratar psicoses. Pela primeira vez, também, foram incorporados isômeros *trans* nos tecidos cerebrais a partir de suplementação pós-natal, quando as membranas já estão formadas, que podem estar relacionados com o desenvolvimento de distúrbios do movimento. Verificou-se que estes distúrbios são muito similares àqueles causados pelo tratamento com haloperidol e, de forma interessante, a incapacidade do exercício físico em promover benefícios nesta situação. A ausência de efeitos benéficos do exercício também foi constatada sobre a ansiedade, quando associado às dietas contendo AG *trans*.

Contudo, estudos mais aprofundados sobre receptores transmembrana, atividade enzimática, expressão de fatores neurotróficos e inflamação, decorrentes da incorporação de AG *trans*, fazem-se necessários. Além disso, análises da incorporação de AG em regiões específicas cerebrais, como hipocampo, córtex frontal e estriado, devem ser futuramente conduzidas.

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