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O CONTEÚDO DIETÉTICO DOS ÁCIDOS GRAXOS MODIFICA PARÂMETROS DE MEMÓRIA EM RATOS: ESTUDO COMPARATIVO ENTRE UMA DIETA BASEADA NO MEDITERRÂNEO *versus* DIETAS OCIDENTAIS

Santa Maria, RS
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Dissertação apresentada ao Curso de Pós-Graduação em Farmacologia, Área de Concentração em Neuropsicofarmacologia e Imunofarmacologia, da Universidade Federal de Santa Maria como requisito parcial para obtenção do título de **Mestre em Farmacologia.**

Orientadora: Prof.ª Dr.ª Marilise Escobar Burger

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Dedico esta dissertação à minha família.

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“A tarefa não é tanto ver aquilo que ninguém viu, mas pensar o que ninguém ainda pensou sobre aquilo que todo mundo vê.”

(Arthur Schopenhauer)

RESUMO

O CONTEÚDO DIETÉTICO DOS ÁCIDOS GRAXOS MODIFICA PARÂMETROS DE MEMÓRIA EM RATOS: ESTUDO COMPARATIVO ENTRE UMA DIETA BASEADA NO MEDITERRÂNEO *versus* DIETAS OCIDENTAIS

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O envelhecimento populacional aliado à alteração dos hábitos alimentares vem aumentando a incidência de comprometimento cognitivo e doenças neurodegenerativas, incluindo a doença de Alzheimer (DA), a qual constitui um grave problema de saúde pública, e representa cerca de 70% da demência na população idosa. Dessa maneira, a preservação da função cerebral e redução do risco de distúrbios neurológicos tornaram-se questões fundamentais para melhorar a qualidade de vida. Estudos recentes têm demonstrado que o tipo de alimentação ao longo da vida pode desfavorecer a suscetibilidade individual para o desenvolvimento de demência. A razão entre a ingestão diária de alimentos fontes de ácidos graxos poliinsaturados (AGPI) ômega-3 *(n-3)* e ômega-6 *(n-6)* têm sido relacionada à menores riscos de muitas doenças crônicas. Porém, é crescente o consumo crônico de alimentos processados, os quais possuem altos níveis de ácidos graxos *trans* (AGT), que podem causar mudanças estruturais nos fosfolípideos de membrana, afetando a neurotransmissão cerebral. O elevado consumo dos AGT tem sido relacionado a diversas doenças metabólicas, favorecendo também processos oxidativos cerebrais, o que pode estar relacionado ao desenvolvimento de doenças neuropsiquiátricas. Na tentativa de minimizar o prejuízo causado pelo consumo de gorduras *trans* (GT), esta têm sido substituída pela gordura interesterificada (GI), uma gordura plástica rica em ácidos graxos saturados (AGS) e baixo teor ou livre da configuração *trans*, ainda pouco conhecida quanto a suas ações sobre o SNC. O objetivo deste estudo foi avaliar comparativamente a influência do consumo de óleos e gorduras baseando-se na dieta mediterrânea *versus* dietas ocidentais, desde o desmame até a vida adulta, sobre os prejuízos de memória *per se*, como também sobre um modelo animal de déficit cognitivo em ratos *Wistar* machos. Para isso, a partir do dia pós-natal (DPN) 21, os animais foram alimentados com ração padrão suplementada com diferentes óleos ou gorduras (20%), resultando e três diferentes grupos experimentais: dieta mediterrânea (DM, com razão ideal entre AGPI *n-6/ n-3* 1:1), dieta ocidental 1 (DO-1, com AGT (30%) ou dieta ocidental 2 (DO-2, com GI (AGS-55%). No DPN 90 cada grupo experimental foi subdividido para receber solução salina (grupo controle) ou escopolamina (ESCO) (1mg/kg i.p), um indutor de déficit cognitivo, e submetidos à avaliações comportamentais, a fim de quantificar possíveis prejuízos cognitivos induzidos pela alimentação, e se estes seriam agravados pela ESCO. Após a última avaliação comportamental os animais foram anestesiados e eutanasiados para retirada do plasma, epidídimo e hipocampo. Citocinas pró-inflamatórias (IL- 1β, IL-6, TNF-α) no plasma e (IL-1β) no hipocampo, anti-inflamatória (IL-10) no plasma, além de imagens histológicas no hipocampo foram avaliados. Os resultados obtidos mostraram que os animais alimentados com a DM apresentaram melhor performance de memória e menores níveis de citocinas pró-inflamatórias, enquanto os grupos alimentados com DO-1 e DO-2 apresentaram memória prejudicada, a qual mostrou correlação positiva com as citocinas pró-inflamatórias plasmáticas, cujos níveis foram aumentados. Os grupos experimentais tratados com ESCO apresentaram maiores danos de memória,

independentemente da dieta. Cortes histológico do hipocampo mostraram que os animais alimentados com a DM apresentaram células neuronais regulares, enquanto os grupos alimentados com as dietas ocidentais (DO-1 e DO-2) mostraram camada neuronal reduzida e irregular. Tomados em conjunto, é possível sugerir que a prevalência dos diferentes tipos de AG na dieta podem facilitar o desenvolvimento de distúrbios neurológicos cognitivos. Além disso, no nosso entendimento, estamos demonstrando pela primeira vez, que a substituição da GT pela GI na dieta ocidental, não representa benefícios, considerando as doenças neuropsiquiátricas, e particularmente, aquelas que afetam a performance de memória. Deste modo é possível propor que as gorduras reconhecidas como "processadas" são capazes de exercer influências deletérias sobre parâmetros de memória, já que seu consumo crônico foi associado à aumentados níveis de citocinas pró-inflamatórias no plasma e hipocampo, alterando também a organização neuronal do hipocampo.

PALAVRAS-CHAVE: Dieta Mediterrânea, Ácidos Graxos *Trans*, Gordura Interesterificada, Doença de Alzheimer, Neuroinflamação.

ABSTRACT

THE DIETARY CONTENT OF FATTY ACIDS MODIFIES MEMORY PARAMETERS IN RATS: A COMPARATIVE STUDY BETWEEN A MEDITERRANEAN-BASED DIET *versus* WESTERN DIETS

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Population aging associated with altered eating habits has been increasing the incidence of cognitive impairment and neurodegenerative diseases, including Alzheimer's disease (AD), which is a serious public health problem and accounts for about 70% of dementia in the elderly population. Thus, the preservation of brain function and the reduction of the risk of neurological disorders has become an important issue to improving quality of life. Recent studies have shown that the type of lifelong feeding may disadvantage individual susceptibility to the development of dementia. The ratio between the daily intake of food sources of polyunsaturated fatty acids (PUFAs) omega-3 *(n-3)* and omega-6 *(n-6)* have been linked to lower risks of many chronic diseases. However, chronic consumption of processed foods is increasing, rich in *trans* fatty acids (TFA), which can cause structural changes in membrane phospholipids, affecting brain neurotransmission. The high consumption of TFA has been related to several metabolic diseases, favoring also cerebral oxidative processes, which may be related to the development of neuropsychiatric diseases. In an attempt to minimize the damage caused by the consumption of TFA, this has been replaced by interesterified fat (IF), a plastic fat rich in saturated fatty acids (SFA), low or free *trans* configuration and still little known their actions on the CNS. The objective of this study was to evaluate the influence of the consumption of oils and fats on the Mediterranean diet versus Western diets, from weaning to adulthood, on memory loss *per se*, as well as on an animal model of cognitive deficit in male *Wistar* rats. For this, after the postnatal day (PND) 21, the animals were fed a standard diet supplemented with different oils or fats (20%), resulting in three different experimental groups: Mediterranean diet (MD, with an ideal ratio of PUFA (WD-1, with TFA (30%) or western diet 2 (WD-2, with IF (SFA-55%). After, the animals was subdivided to receive saline (control group) or scopolamine (ESCO) (1mg / kg ip), an inductor of cognitive deficit, and submitted to behavioral evaluations of memory. 24 h after the last behavioral, the animals were euthanized to remove plasma and hippocampus to evaluated the levels of pro inflammatory cytokines, IL-1β, IL-6, TNF-α in the plasma and IL-1β in the hippocampus, anti-inflammatory (IL-10) in the plasma, besides histological images in the hippocampus were evaluated. The results obtained showed that MD-fed animals presented better memory performance and lower levels of pro inflammatory cytokines, whereas WD-1 and WD-2 fed groups had impaired memory, which showed a positive correlation with plasma pro inflammatory cytokines, whose levels were increased. Experimental groups treated with ESCO presented increase memory damage, regardless of diet. Histological sections of the hippocampus showed that MD-fed animals had regular neuronal cells, while groups fed the western diets (WD-1 and WD-2) showed reduced and irregular neuronal layer. Taken together, it is possible suggest that the prevalence of different types of FA in the diet may facilitate the development of cognitive neurological disorders. Moreover, in our understanding, we are demonstrating for the first time that the replacement of TFA by IF in the Western diet does not represent benefits, considering neuropsychiatric diseases, and

particularly, those that affect memory performance. Thus, it is possible to propose that fats recognized as "processed" are capable of exerting deleterious influences on memory parameters, since their chronic consumption was associated with increased levels of proinflammatory cytokines in the plasma and hippocampus, also altering the neuronal organization of the hippocampus.

KEY WORDS: Mediterranean Diet, Trans Fatty Acids, Interesterified Fat, Alzheimer's Disease, Neuroinflammation

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PRODUÇÃO CIENTÍFICA

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AA	Ácido Araquidônico
AG	Ácido Graxo
AGE	Ácidos Graxos essenciais
AGM	Ácidos Graxos Monoinsaturados
AGPI	Ácidos Graxos Poliinsaturados
AGS	Ácidos Graxos Saturados
AGT	Ácidos Graxos *Trans*
ALA	Ácido α-linolênico
DA	Doença de Alzheimer
DHA	Ácido Docosahexaenóico
DM	Dieta Mediterrânea
DO	Dieta Ocidental
DPN	Dia pós natal
EPA	Ácido Eicosapentaenóico
ESCO	Escopolamina
GI	Gordura Interesterificada
GT	Gordura *trans*
GVH	Gordura Vegetal Hidrogenada
IL-1β	Interleucina-1β
IL- 10	Interleucina-10
IL- 6	Interleucina-6
LA	Ácido Linoleico
n-3	Ômega 3
n-6	Ômega 6
SNC	Sistema Nervoso Central
TNF-α	Fator de necrose tumoral-α
TAG	Triacilglicerol

SUMÁRIO

APRESENTAÇÃO

Esta dissertação está estruturada em seções dispostas da seguinte forma: Introdução, Desenvolvimento, Objetivos, Produção Científica (Artigo), Conclusão e Referências. No item **INTRODUÇÃO** e **DESENVOLVIMENTO,** encontram-se considerações iniciais sobre o tema desenvolvido nesta dissertação. Os itens Materiais e Métodos, Resultados, Discussão e Referências encontram-se inseridos no artigo na seção **PRODUÇÃO CIENTÍFICA** e representam a íntegra deste estudo.

Ao fim, encontra-se o item **CONCLUSÃO**, no qual há comentários gerais dos resultados contidos no estudo. O item **REFERÊNCIAS** diz respeito somente às citações apresentadas no item **INTRODUÇÃO** e **DESENVOLVIMENTO**.

1 INTRODUÇÃO

Em decorrência do envelhecimento da população mundial está ocorrendo um aumento dramático na prevalência de condições crônicas, em particular o declínio cognitivo e a demência, que estão sendo, cada vez mais, reconhecidas como uma prioridade de saúde pública, visto que geram enormes problemas de ordem socioeconômica, principalmente devido à ausência de tratamentos efetivos (CAVANELLI, 2016; ARIDI, 2017; WORLD HEALTH ORGANIZATION, 2012).

O maior entendimento científico da demência, ao longo dos anos, vem demonstrando que essa doença crônica de aparecimento tardio pode resultar de um processo de implicações ao longo da vida, advindo da concorrência de múltiplos fatores de riscos e de proteção, associados ao estilo de vida (LIPNICK, 2016). Dessa forma, estudos visam indicar os diversos fatores de risco que podem elevar as chances de desenvolver essa enfermidade (COSKUN, 2012; WALKER, 2017). Muitos desses fatores são potencialmente modificáveis, o que pode constituir alvos para estratégias preventivas, como por exemplo, a nutrição, que representa uma via interessante para investigação (ARIDI, 2017).

Nesse sentido, existe um interesse crescente ao que se refere ao estilo de vida e aos componentes da dieta como possíveis fatores de prevenção da demência (ALZHEIMER'S ASSOCIATION, 2012, MORRIS, *et al.*, 2003). Diversos estudos têm demonstrado que alguns alimentos, como os peixes e alimentos com alto teor de AG n-3 (BILBUL, 2011; CHOULIARAS et al., 2010) e dietas com níveis elevados de ácidos graxos saturados (AGS) ou ácidos graxos *trans* AGT (HISSAGNA, 2012) podem alterar significativamente o risco de uma pessoa desenvolver essa doença (ARIDI, 2017). A composição da gordura dietética é um fator importante para a função e composição dos fosfolipídios de membrana, estando diretamente envolvida na neuropatologia da demência, como Doença de Alzheimer (DA) (MORRIS, 2003).

A ingestão equilibrada de AGPI n-6 e n-3 é importante para as funções cerebrais, uma vez que são os principais componentes das membranas fosfolipídicas, e o seu consumo equilibrado tem sido constantemente relacionado a um risco reduzido de declínio cognitivo e demência (SHEPPARD, CHEATHAM 2013). Por outro lado, nas últimas décadas, os hábitos alimentares da população têm se modificado drasticamente (CRAIG-SCHIMIDT, 2006), reduzindo o consumo de alimentos ricos em AG n-3, com conseqüente aumento do consumo

de alimentos industrializados, os quais são ricos em AGT (POPKIN, 1998; ALLISON et al., 1999). Experimentalmente, tal alteração têm sido relacionada ao desenvolvimento de danos oxidativos neurais, os quais foram relacionados à transtornos neuropsiquiátricos (TREVIZOL, 2015, TREVIZOL, 2015; KUHN, 2013; KUHN, 2015; ROVERSI, 2015; PASE; 2015). Assim, devido à preocupação com as conseqüências do consumo de AGT sobre a saúde, a indústria de alimentos têm substituído a GT pela gordura interesterificada (GI) (ENIG, 2010). A GI surge como uma alternativa para a preparação de alimentos processados com baixos teores de isômeros *trans* ou até mesmo com a ausência deles (RIBEIRO, 2007), contudo os seus efeitos sobre a saúde ainda são pouco estudados.

Portanto, avaliar a influência do consumo de dietas contendo diferentes AG ao longo da vida, torna-se especialmente importante, visto que essa é uma linha consolidada do laboratório Farmatox (UFSM), onde diferentes estudos já relataram o prejuízo dos AGT ou GI sobre diferentes patologias neuropsiquiátricas (TREVIZOL, 2015; PASE, 2015, 2017; DIAS, 2015; ROVERSI, 2015; D'AVILA, 2017; MILANESI, 2017). No entanto, até o momento, nenhum estudo comparativo envolvendo a influência do consumo destes óleos e gorduras processados e os óleos naturais (com razão ideal, 1:1, entre AGPI n-6 e n-3) em uma dieta normolipidica, sobre funções de memória e a neuroinflamação no SNC, foi realizado. Os resultados deste estudo poderão ser uteis para o desenvolvimento de estratégias relacionadas à prevenção de déficits cognitivos, alertando a indústria alimentícia e órgãos afins de que a produção de alimentos contendo gorduras processadas podem exercer prejuízos sobre as funções cerebrais.

2 DESENVOLVIMENTO

2.1 ÁCIDOS GRAXOS

Ácidos graxos (AG) são constituintes importantes das membranas fosfolipídicas neuronais e desempenham importantes funções no desenvolvimento e funcionamento do cérebro. As membranas do cérebro apresentam uma composição rica em lipídeos, cerca de 50% do seu peso total, dos quais 25% são ácidos graxos poli-insaturados (AGPI) de cadeia longa e destes, 15 % são de ácido docosaexaenóico (DHA) (LEVANT, 2006). Os AGP são fundamentais para as membranas neuronais, sendo responsáveis por aumentar a fluidez da membrana e a plasticidade sináptica (MITCHELL, 2003).

Estruturalmente, a maioria dos lipídios da dieta contém três ácidos graxos ligados a uma molécula de glicerol, conhecida como triacilglicerol (TAG) (BELL, 1997). Os TAG são formados por uma cadeia hidrocarbonada (2 a 20 ou mais átomos), contendo uma carboxila (COOH) em uma extremidade da cadeia e uma metila (CH3), na outra extremidade (MARSZALEK 2005).

A nomenclatura química convencional classifica os AG de acordo com o número dos átomos de carbono e quantidade das insaturações presentes na molécula (LEHNINGER; NELSON; COX, 2002). Quanto à extensão da cadeia, os AG podem ser classificados em cadeia curta, média ou longa. Aqueles de cadeia curta possuem cauda alifática de 6 a 12 carbonos, já os de cadeia longa têm mais de 12 carbonos e, por sua vez, os de cadeia muito longa têm com mais de 22 átomos de carbono. De acordo com as insaturações, os AG podem ser classificados em saturados (AGS, sem duplas ligações) ou insaturados (AGI). Este último ainda subdivide-se em AG monoinsaturado (AGMI, com uma dupla ligação) ou poliinsaturados (AGPI, com mais de uma dupla ligação) (LEHNINGER; NELSON; COX, 2014). As inúmeras duplas ligações de carbono presentes nos AGPI dificultam a interação molecular, conferindo característica líquida a estes AG quando em temperatura ambiente, uma importante propriedade física requerida para a manutenção do alto grau de flexibilidade da bicamada lipídica das membranas celulares (HULBERT, 2005; INNIS, 2007; TORRES, 1999).

Em mamíferos, os AGS, os AGMI e alguns AGPI podem ser obtidos através da dieta ou pela síntese "*de novo*" de AG através da enzima acetilcoenzima A, sendo considerados AG não essenciais (CARVALHO, 2003). Os AGMI da série n-9 podem ser sintetizados a partir da

dessaturação dos AGS e essa conversão é realizada pela Δ9 desaturase, uma enzima ativa em tecidos de mamíferos que introduz uma dupla ligação entre a posição n-9 – n-10 da cadeia dos AG (RATNAYAKE e GALLI, 2009). Alimentos de origem animal como leite, carne, queijo e manteiga e de origem vegetal como coco, palma e dendê, são fontes de AGS (CARVALHO, 2003). Já os AGMI e AGPI podem ser encontrados em alimentos como o azeite de oliva, óleo de canola e de soja e também em nozes, sendo o ácido oléico (C18:1) o seu principal representante (DUNCAN; SCHMIDT; GIUGLIANI, 2004).

Os AG *trans*, são AG insaturados compostos pelo menos uma dupla ligação apresentada na configuração *trans*, ou seja, quando os átomos de hidrogênio localizam-se em lados opostos da cadeia carbônica, formando uma molécula linear (MARTIN, 2007). Estes podem ser encontrados naturalmente em carnes e leites de animais ruminantes, entretanto, através da hidrogenação de óleos vegetais, formando a gordura vegetal hidrogenada, que foi pioneiramente inserida na produção de alimentos industrializados, sendo responsáveis pela melhor palatabilidade, consistência e maior prazo de validade dos alimentos (MOZZAFRIAN, 2009).

2.1.1 Razão ideal *n6/n-3* 1:1

Os ácidos graxos poli-insaturados (AGPI) da série *n-3* e *n-6*, que se diferenciam pela posição da primeira dupla ligação a partir do grupo metílico terminal da cadeia, são denominados ácidos graxos essenciais (AGE), por não serem sintetizados endogenamente pelos mamíferos (SOLFRIZZI, 2005). A razão entre a ingestão diária de alimentos fontes de AG *n-6* e *n-3* assume grande importância na nutrição humana, resultando em diversas recomendações que têm sido estabelecidas por autores e órgãos de saúde, em diferentes países (MARTIN, 2006).

De modo geral, os AGE n-6 são abundantemente encontrados nos óleos vegetais como girassol, milho e soja, tendo como principal representante o ácido linoléico (LA; C18:2 n-6), enquanto os AGE n-3 estão presentes principalmente nos peixes de águas frias e profundas, como sardinha e salmão, além de oleaginosas como nozes, castanhas e alguns óleos vegetais (linhaça e canola), possuindo como representante principal o ácido α-linolênico (ALA; C18:3 n-3) (SANGIOVANNI e CHEW, 2005; LARSSON, 2014).

Os AGE são os principais componentes das membranas fosfolipídicas e a sua ingesta da dieta reflete a composição das membranas fosfolipídicas neuronais (HAAG, 2003; YEHUDA, 2005), o que pode afetar importantes funções fisiológicas, dentre elas: a influência na resposta inflamatória e geração de radicais livres, fluidez e permeabilidade da membrana neuronal, entre outros (YEHUDA, 2002; YEHUDA, 2005). Os AG de cadeia longa, como os ácidos araquidônico (AA) e docosaexaenóico (DHA), desempenham importantes funções no desenvolvimento e funcionamento do cérebro (SIMONIAN, 1996).

Os AGPI n-6, como o AA, são precursores de eicosanóides com propriedade pró-inflamatória, a qual se relaciona a dor, inflamação e gênese tumoral (BORSONELO e GALDURÓZ, 2008). Já os AGPs n-3 são importantes mediadores de pró-resolvinas que incluem: resolvinas da série E sintetizadas a partir do ácido eicosapentaenoico (EPA); resolvinas da série D, sintetizadas a partir do DHA; e as neuroprotectinas/protectinas sintetizadas do DHA, que são consideradas antioxidantes nutricionais e influenciam a transdução de sinais, na atividade de alguns neurotransmissores, assim como os segundos mensageiros (YEHUDA, 2002; CHALON, 2001; NATALIE, 2009).

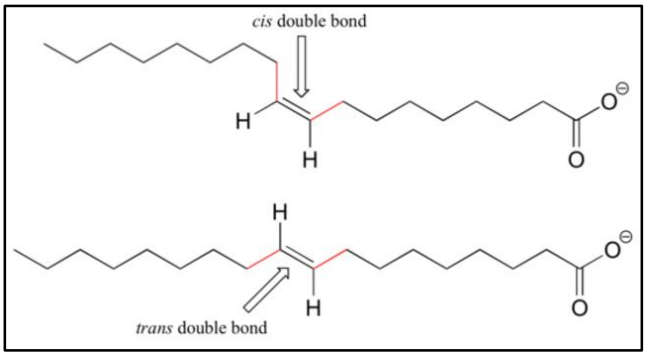
Estima-se que a razão de AGPI *n-6/n-3* na dieta das pessoas que viveram no período que antecedeu a industrialização, estava em torno de 1:1 a 2:1, devido ao consumo abundante de vegetais e de alimentos de origem marinha, contendo AGPI *n-3* (MARTIN, 2006). Porém, a mudança nos hábitos alimentares, ao longo das décadas, promoveu o aumento da relação dos AGE *n-6/n-3*, principalmente pela ingestão reduzida de AGPI *n-3* (AILHAUD, 2006). Atualmente, o consumo de alimentos apresentam uma razão de AGPI *n-6/n-3* superior à 20:1 (BEILHARZ, 2016; HOPPERTON, 2016).

No entanto, dados epidemiológicos ressaltam o papel protetor de certos nutrientes, quando a carência de AGPI *n-3* pode afetar funções cognitivas e facilitar o desenvolvimento de doenças neurodegenerativas cerebrais (GILLETTE-GUYONNET, 2007). Por ser altamente insaturado, o DHA atua influenciando as propriedades físicas das membranas cerebrais, as características dos seus receptores, as interações celulares e a atividade enzimática (YEUDA, 2002). Por essas evidências, que o AGPI *n-3* é um dos principais componentes dietéticos de estudos no tratamento do Alzheimer (SIMONIAN, 1996; HOPPERTON, 2016).

2.1.2 Gordura *Trans*

A GT é formada por AG insaturados, onde sua designação "*trans*" é referente à ordem da cadeia de átomos do AG, ou seja, os átomos de hidrogênio ligados aos carbonos insaturados estão em planos opostos, aparecendo como uma conformação molecular linear (Fig 1). Esses isômeros são, estruturalmente, similares às gorduras saturadas, sendo capazes de modificar as funções metabólicas das gorduras poliinsaturadas e competir com os AGE em vias metabólicas complexas (RIBEIRO, 2007).

Figura 1- Representação ácido graxo insaturado *cis* e ácido graxo insaturado *trans*



Fonte: (SODEBERG, 2015).

As fontes animal e vegetal, os AG geralmente possuem insaturações na forma *cis*, quando os hidrogênio da dupla ligação se encontram no mesmo lado da cadeia carbônica (MARTIN, 2007). Os AGT são naturalmente encontrados em baixos níveis em carnes e produtos lácteos pelo fato de ocorrer hidrogenação bacteriana no estômago dos ruminantes (KLONOFF, 2007).

Na década de 1960, a partir de campanhas de saúde pública direcionadas à diminuição do consumo de gorduras animais, a indústria alimentícia inseriu quantidades significativas de óleos vegetais parcialmente hidrogenados para serem usados no processamento de alimentos, dessa forma, iniciou-se o consumo de AGT (DOWNS; THOW, 2013).

Atualmente, a maior fonte de AGT na dieta são alimentos que contêm a fração industrial desta gordura, além dos óleos parcialmente hidrogenados (KLONOFF, 2007). No processo de

hidrogenação, a estrutura química do AG é modificada, fazendo com que estes fiquem com os átomos em alinhamento transversal, fornecendo para a molécula maior estabilidade à oxidação lipídica e alto ponto de fusão, propriedade muito importante para a indústria de alimentos (REMIG, 2010; STENDER; ASTRUP; DYERBERG, 2008).

O óleo de soja é um dos mais empregados para a hidrogenação, é composto por AGS e AGI, em que os principais saturados são o ácido palmítico (C16:0 em torno de 10 a 12%) e o esteárico (C18:0 em torno de 3 a 5%); já os principais insaturados são o ácido oleico (C18:1 em torno de 24%), o linoleico pertencente a família ômega-6 (*n-6*) (C18:2; 54%,) e o linolênico pertencente a família ômega-3 (*n-3*) (C18:3, 8.0%,) (MENGISTU, 2013). Durante o processo, o hidrogênio é borbulhado através da gordura a altas temperaturas na ausência de oxigênio e na presença do níquel como catalisador (RUIZ-JIMÉNEZ, 2007), dessa forma, os AGT são produzidos quando o óleo, líquido a temperatura ambiente, é convertido a gordura sólida através do referido processo químico (BHARDWAJ, PASSI, 2011).

A utilidade tecnológica da GT é diversa, gerando vantagens comerciais como: manutenção da estrutura, textura e estabilidade; aumenta o tempo de prateleira; realce do sabor e redução da oxidação do alimento; (MENAA, MENAA, TRÉTON, 2013). No entanto, os efeitos do consumo desses AGT têm causado controvérsia no que diz respeito aos aspectos da sua absorção e metabolismo (MANCINI e CHEMIN, 1996).

As principais preocupações com os efeitos dos AGT na saúde têm aumentado, pois evidências começaram a apontar que o aumento do consumo de GT estava relacionado com diversas co-morbidades, como doenças cardiovasculares, inflamação sistêmica, dislipidemias, diabetes, disfunção endotelial, (MOZAFFARIAN, ABDOLLAHI,2007; BARNARD, BUNNER, 2014) e, mais recentemente, com doenças neurodegenerativas. Com relação ao SNC, pesquisas já mostraram que o consumo de GT durante a gestação até a idade adulta (TREVIZOL, 2013) foi relacionado com danos oxidativos no cérebro e prejuízos comportamentais atrelados ao modelo animal de mania, nos filhotes (TREVIZOL, 2015a, TREVIZOL, 2015b, DIAS, 2015a, DIAS, 2015b). O consumo prolongado de GT permitiu a incorporação de AG do tipo *trans* nas membranas neurais cerebrais, facilitando a geração do estresse oxidativo, desordens do movimento e perda cognitiva (TEIXEIRA, 2012).

Alternativas visando a diminuição dessa GT nos alimentos processados já existem. A indústria alimentícia tem utilizado o processo de interesterificação de gorduras, resultando na GI, a qual vem sendo utilizada em diversos alimentos. Porém, alguns estudos tem levantado a hipótese que essa nova gordura pode possuir efeitos similares ou até mesmo mais prejudiciais que os processos de hidrogenação.

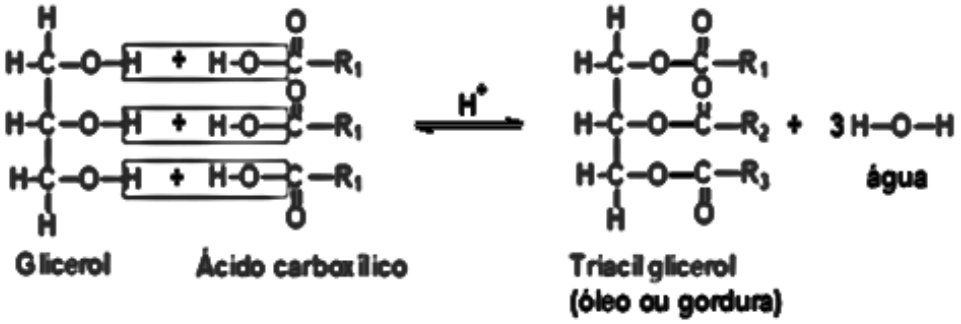
2.1.3 Gordura interesterificada

A gordura interesterificada (GI) é utilizadas em uma ampla gama de produtos alimentares e foram introduzidas como um substituto para as GT, que já são conhecidas como prejudiciais para saúde (MILLS, 2017). A interesterificação consiste em uma alternativa tecnológica ao processo de hidrogenação parcial, ela se caracteriza por um rearranjo dos AG na molécula do TAG, através de catalisadores (FARFA´N; 2013).

A interesterificação consiste em uma mistura de óleos vegetais totalmente hidrogenados ou de frações mais saturadas com óleos líquidos, em que a gordura resultante apresenta baixos teores ou ausência de AGT. Em contraste à hidrogenação, tal processo não promove a isomerização de duplas ligações dos AG e não afeta o grau de saturação deles, nem causa a isomerização das duplas ligações, pois os AG não são modificados, mas redistribuídos nas reações éster do glicerol, criando novas estruturas (RIBEIRO, 2007; FARFA´N, 2013).

Os produtos resultantes do processo em pauta mantêm o perfil de AG e o grau de saturação das misturas iniciais (KARABULUT, 2004; RODRIGUES e GIOIELLI, 2003). Entretanto, apresentam uma estereoquímica diferente dos TAG, resultando em novas características físico-químicas e propriedades nutricionais (KLINKESORN, 2004), pois o processo é realizado por troca ou rearranjo de AG dentro de um TAG (entre as posições sn-1, 2 e 3) ou entre TAGs (Fig.2), por qualquer produto químico ou enzimática (MILLS, 2017). A interesterificação de óleos e gorduras pode ser aplicada para: influenciar o comportamento na fusão, fornecendo consistência desejada; melhorar ou modificar o comportamento cristalino, facilitando os processos de produção; e para diminuir a tendência à recristalização, durante a vida útil do produto (RIBEIRO, 2007).

Figura 2 - Estrutura de triacilglicerol (TAG)

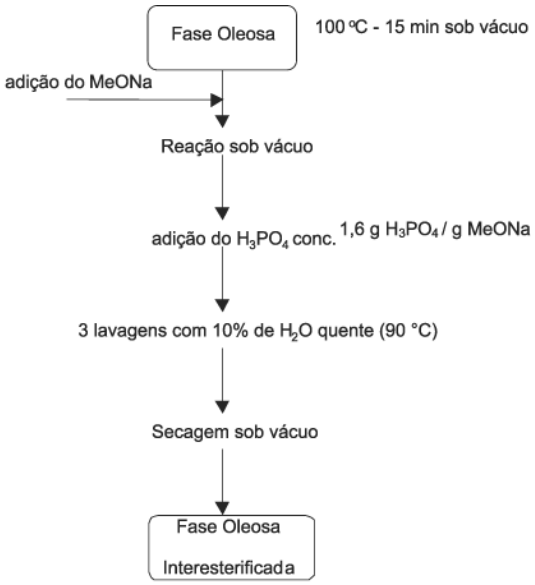


Fonte: (KODALI e LIST, 2006).

No processo enzimático de interesterificação, biocatalisadores, tais como lipases microbianas, são utilizados. Já no processo químico, é largamente utilizado devido à facilidade e baixo custo, o metóxido de sódio (MeONa), o qual é o catalisador empregado com maior freqüência (Fig.3) (GUSTONE, 1998).

Contudo, há diversas possíveis conseqüências nutricionais que poderiam ser esperadas da ingestão dessa gordura processada, por exemplo, a modificação de absorção do triglicerídeo, elevando a proporção colesterol (RIBEIRO, 2007). Além disso, nenhuma legislação exige atualmente a rotulagem da GI em produtos alimentares, portanto, as estimativas das taxas médias de consumo na população ainda são indisponíveis (MILLS, 2017).

Figura 3. Esquema da reação de interesterificação química



Fonte: (GRIMALDI, 2005).

Estudos sobre as implicações do consumo de GI à saúde ainda são escassos e a maioria relaciona a sua ingestão com distúrbios metabólicos (SUNDRAM; KARUPAIAH; HAYES, 2007; ENIG, 2010; ROBINSON, 2009; AFONSO, 2016; MAGRI, 2014). Até o momento, no melhor do nosso conhecimento, D'avila (2017) e Milanesi (2017) foram os primeiros estudos a relacionar a influência da GI sobre prejuízos de funções cerebrais, quando parâmetros comportamentais de depressão e drogadição foram experimentalmente avaliados. No entanto, as implicações dessa gordura sobre o SNC, são ainda muito pouco conhecidas.

2.2 DIETA: UM IMPORTANTE FATOR MODIFICÁVEL FRENTE AO RISCO DE DECLÍNIO DE MEMÓRIA E NEUROINFLAMAÇÃO.

Desde o início do século XXI, a preocupação com a ingestão de óleos e gorduras e sua relação com a saúde tem despertado o interesse de pesquisadores. Desde então diversos estudos têm tentado definir qual seria o tipo de AG mais prejudicial e em que quantidade essa ingestão poderia ser saudável (MOZAFFARIAN, 2006).

Nos países ocidentais, têm se observado um aumento do consumo de alimentos industrializados, os quais contêm quantidades significativas de AGS, monoinsaturados e poli-insaturados, bem como concentrações consideráveis de AGT (BAGGIO e BRAGAGNOLO, 2006; HULSHOF, 1999). Essa mudança nos hábitos alimentares, com o aumento do consumo de AGT representa uma perda no valor nutricional dos alimentos (bem como a perda de AGE) e o impacto dessa condição na saúde humana precisa ser monitorado (MONTEIRO, 2011).

Estudos recentes têm demonstrado que alguns alimentos da nossa dieta, como alimentos mediterrâneos, peixe e alimentos com alto teor de AGPI *n-3*, atrelados a outros fatores, como exercício, cigarro, traumatismo craniano, infecções, inflamações sistêmicas, atividade intelectual e exposição a pesticidas podem alterar significativamente o risco de uma pessoa desenvolver demência (BILBUL, 2011; CHOULIARAS, 2010). Considerando o alto consumo de AGT em uma dieta deficiente de AGE, ocorre a substituição destes pelos AGT nas membranas fosfolipídicas neurais, causando alterações na fluidez de membrana e nas respostas de seus receptores (GURR e HARWOOD, 1991; ROACH, 2004), além de um aumento nos marcadores de inflamação (MOZAFFARIAN, 2006).

A neuroinflamação vem sendo, cada vez mais, reconhecida como uma característica

importante na DA. Estudos têm mostrado níveis mais altos de marcadores neuroinflamatórios como citocinas e ativação de células gliais como astrocitos, microglia no cérebro de pacientes com DA (HOPPERTON, 2016). Além disso, pacientes com DA apresentam níveis plasmáticos mais altos de citocinas, como interleucina (IL-6 e IL-1β), enquanto que, em pacientes com DA moderada, a elevação do fator de necrose tumoral no soro (TNF-α) está associada ao declínio cognitivo. A principal origem dessa resposta inflamatória sistêmica está no tecido adiposo, o qual produz uma variedade de citocinas pró-inflamatórias e de quimiocinas, denominadas adipocinas como a IL-1, a IL-6, o TNF-α (CANCELLO e CLEMENT, 2006).

Nesse contexto, a mudança dos hábitos alimentares (SIMOPOLOUS, 2006), motivada principalmente pelo consumo de *fast-foods* e alimentos pré-prontos congelados e o aumento da incidência de desordens neuro-psiquiátricas (HAAG, 2003) têm despertado o interesse e o debate no meio científico. No entanto, tais observações carecem de estudos experimentais e clínicos, raramente encontrados na literatura. Tais observações foram o estímulo inicial para o desenvolvimento do presente estudo.

2.3 MEMÓRIA E APRENDIZADO

Aprendizagem é o processo pelo qual nós adquirimos um determinado conhecimento, enquanto a memória é o processo de aquisição, formação ou consolidação e evocação de informações adquiridas (Fig.4) (KANDEL, 2003; IZQUIERDO, 2011; IZQUIERDO, 2002). Para a formação de uma memória é necessário que ocorra um aprendizado e, as novas informações, podem ou não ser armazenadas de forma permanente para serem recuperadas ou evocadas quando necessário (BADDLEY & NAVARRO, 1999; IZQUIERDO, 2002)

A memória é o resultado de pelo menos três tipos de processamento distintos, mas relacionados entre si: aquisição, consolidação e evocação. A aquisição refere-se aos processos pelos quais novas informações aprendidas são tratadas e processadas por sistemas neurais específicos, quando encontradas pela primeira vez. A consolidação é o armazenamento de uma informação recém adquirida; e a evocação se refere aos processos que permitem a lembrança e o uso das informações retidas (DUDAI, 2004, IZQUIERDO e MCGAUCH, 2000).

Figura 4 - Fases da formação da memória. Aquisição, consolidação e evocação

Fonte: (QUILLFELDT, 2006).

As memórias podem ser classificadas de acordo com diversos fatores. As classificações mais usadas estão atreladas ao conteúdo e tempo de permanência (IZQUIERDO, 2002; QUILLFELDT, 2006). Quanto ao conteúdo armazenado, é dividido em: memória declarativa, associada a eventos e conhecimentos; e memória não declarativa, manifestada por meio de comportamentos motores ou sensoriais (IZQUIERDO, 2002; MÜLLER & SHUMANN, 2011; STICKGOLD, 2005; ROBERSTON, 2004). Quanto ao tempo de permanência, podemos separá-las em: memória de trabalho, na qual as informações estão disponíveis por segundos a minutos; memória de curta duração (minutos a poucas horas após aquisição); e memória de longa duração (dias, meses ou anos) (SQUIRE, 2004).

O hipocampo desempenha um papel fundamental na formação de memórias de curto e longo prazo (IZQUIERDO, 1998a). Diferentes áreas corticais aferentes e eferentes interagem com o hipocampo para regular a aquisição e o armazenamento de nova informação (IZQUIERDO E MEDINA, 1998).

De particular importância, recentes estudos do nosso grupo de pesquisa mostraram uma relação entre o consumo prolongado de GT (TREVIZOL, 2013) e GI (D'AVILA, 2017) com uma incorporação significativa de AGT e perda de AGE no hipocampo, respectivamente, resultando em uma redução na aprendizagem e no desempenho cognitivo.

2.4 DECLÍNIO COGNITIVO

O declínio cognitivo é uma característica comum de diversas síndromes neurológicas, como a demência, e está associado, principalmente, à perda de memória, influenciando na capacidade do indivíduo viver de forma independente (HAIDER, 2016).

A prevalência dos transtornos cognitivos, devido ao aumento da expectativa de vida e as terapias farmacológicas limitadas para o tratamento de doenças neurodegenerativas, é responsável por um importante problema de saúde pública, com consequente perda da qualidade de vida do idoso (MACHADO, 2012). O aumento da expectativa de vida e as terapias farmacológicas limitadas para o tratamento de doenças neurodegenerativas em todo o mundo (NORTON, 2014).

A demência é uma síndrome neurológica, geralmente crônica, caracterizada principalmente por uma progressiva e global perda da memória e da capacidade intelectual do indivíduo, prejudicando tanto o desenvolvimento das atividades de vida diária quanto o desempenho social e ocupacional do indivíduo (JANCA, 2006; FORLENZA, 2005). Inicialmente, ocorre diminuição na aquisição de novas informações, com piora progressiva de aprendizado e memória (SELKOE, 2004).

A Doença de Alzheimer (DA) é considerada a principal causa de declínio cognitivo em adultos, sobretudo em idosos, representando mais da metade dos casos de demência e atinge, aproximadamente, 36 milhões de pessoas em todo o mundo. Estima-se que a prevalência global aumente para cerca de 131,5 milhões de pessoas em 2050. (QUERFURTH e LAFERLA, 2010; WISNIEWSKI e GOÑI, 2014; World Alzheimer Report 2016). Nos primeiros estágios, o sintoma mais comum da DA é a dificuldade em recordar eventos recentes, o que se denomina perda de memória a curto prazo. À medida que a doença evolui, o quadro de sintomas pode incluir confusão, irritabilidade, alterações de humor, comportamento agressivo, dificuldades com a linguagem e perda de memória a longo prazo (World Alzheimer Report, 2016).

Além da idade, fatores genéticos e ambientais são apontados como fatores de risco para o aparecimento da DA. A deficiência de fatores neurotróficos, mitocondriais e no metabolismo energético, também o desenvolvimento de processo inflamatório e aumento na formação de espécies reativas são hipóteses que vem sendo propostas para a DA (HARDY e SELKOE, 2002; PRATICO, 2008).

Já é bem relatado que o sistema colinérgico tem importante papel nos processos de formação da memória (YAMAZAKI, 2005). O déficit colinérgico parece ser um dos principais elementos responsáveis pela perda de memória, típica da doença de Alzheimer (BATOOL, 2015).

Além disso, alguns estudos correlacionam o estresse oxidativo e a disfunção mitocondrial como fator central na etiologia da DA (COSKUN, 2012). Outro mecanismo que parece estar envolvido na patogênese dessa doença é a neuroinflamação que parece contribuir diretamente para a progressão da DA (KHANDELWAL al., 2011). A ativação da microglia

pode promover o processo neurodegenerativo através da liberação de citocinas pró-inflamatórias, tais como interleucina-1 (IL-1 beta) e fator de necrose tumoral (TNF-α), dentre outros produtos tóxicos que podem conduzir ao danos e eventual morte neuronal (NILLERT, 2017). Histopatologicamente, a DA se caracteriza pela maciça perda sináptica e pela morte neural observada nas regiões cerebrais responsáveis pelas funções cognitivas, incluindo o córtex cerebral e o hipocampo (SERENIK e VITAL, 2008).

Uma relação direta entre dieta e mudanças a nível de SNC vem sendo estabelecida (PASE, 2013, 2014). Vários estudos enfocaram o papel dos padrões alimentares na cognição, acumulando evidências de que combinações de alimentos e nutrientes podem atuar de forma sinérgica para proporcionar efeitos modificadores à saúde (CARACIOLLO, 2014; SHAO, 2017).

2.4.1 Sistema colinérgico

O sistema colinérgico desempenha um papel essencial nos processos de aprendizado e memória (WINKLER, 1995). Durante o envelhecimento, os neurônios colinérgicos sofrem uma moderada degeneração, resultando em uma hipofunção colinérgica e esta tem sido relacionada com os declínios cognitivos progressivos no envelhecimento (SCHLIEBS e ARENDT, 2011).

A síntese do neurotransmissor acetilcolina ocorre nos terminais nervosos a partir de dois precursores, colina e acetil-coenzima A, pela ação da enzima colina acetiltransferase. Após sua formação, a acetilcolina é liberada na fenda sináptica, onde poderá interagir com receptores muscarínicos ou nicotínicos (SCHLIEBS e ARENDT, 2006).

O aprendizado e a memória podem ser modificados por drogas que afetam a função colinérgica central (YAMAZAKI, 2005). A escopolamina (ESCO), um alcalóide natural presente em algumas espécies vegetais da *Solanaceae*, age antagonizando competitivamente a atividade do neurotransmissor acetilcolina, junto aos receptores muscarínicos periféricos e centrais, inibindo a despolarização das membranas adjacentes (BATOOL, 2015). O bloqueio dos receptores colinérgicos muscarínicos interfere no armazenamento de novas informações, quando o mecanismo envolvido nesse processo se pronuncia pela supressão da atividade neural excitatória (ZHANG, 2008; ATRI, 2004).

A importância da AChE nos déficits cognitivos vai além do controle dos níveis de

hidrólise da ACh. Evidências sugerem que a AChE poderia desempenhar um papel chave no desenvolvimento das placas senis, acelerando a agregação e a deposição do peptídeo β-amilóide (Aβ), uma das características neuropatológicas mais relevantes na DA (BENTLEY, 2011).

Ebert e Kirch no ano de 1998, observaram que as alterações de eletroencefalograma no sistema colinérgico influenciadas pela ESCO são semelhantes às encontradas nos pacientes portadores de demência. Ademais, a ESCO produz deficiências de memória similares observadas nos idosos, além de induzir comprometimento da memória em ratos adultos jovens, corroborando no desenvolvimento da demência, condição observada em idade avançada (BATOOL, 2015).

2.4.2 Modelo animal de déficit cognitivo

Os modelos animais são sistemas experimentais essenciais para identificar, compreender e investigar os mecanismos das doenças desde os primeiros estágios (WENTZELL e KRETZSCHMAR, 2010). O modelo animal de déficit cognitivo pode ser induzido pelo uso de ESCO, antagonista colinérgico não-seletivo, dos receptores muscarínicos. O bloqueio desses receptores interfere no armazenamento de novas informações, em que o mecanismo envolvido no referido processo se pronuncia pela supressão da atividade neural excitatória (ATRI, 2004).

O efeito amnésico, característico da administração de ESCO, torna-a uma ferramenta farmacológica interessante, uma vez que contempla o déficit cognitivo e a perda da memória característica de demência, bem como mimetiza sintomas da DA (DEIANA, 2009). Desta forma, tal droga é capaz de promover esses déficits poucos minutos após sua administração. No campo da neuropsicofarmacologia, o bromidrato de escopolamina, ainda vem sendo muito utilizado na indução de modelos de prejuízo cognitivo relacionado à idade ou à demência, característicos do déficit colinérgico (KLINKENBERG, 2010; HAIDER, 2016; SAFAR, 2016, LI, 2016, HWANG, 2017).

Nesse sentido, levando em consideração a etiologia multifatorial do declínio cognitivo, torna-se importante avaliar através deste modelo animal de DA a influência da ingestão crônica

de diferentes óleos e gorduras sobre alterações comportamentais de aprendizagem e memória em ratos.

3 OBJETIVOS

3.1 OBJETIVO GERAL

O objetivo deste estudo foi comparar a influência do consumo crônico de dietas normolipídicas, suplementadas com diferentes óleos e gorduras, baseados em uma dieta mediterrânea *versus* 2 dietas ocidentais, em ratos machos *Wistar* ao longo da vida, sobre parâmetros de aprendizagem e memória, além de parâmetros inflamatórios e histológicos.

3.2 OBJETIVOS ESPECÍFICOS

- Avaliar comparativamente a influência do consumo de diferentes padrões alimentares baseando-se na dieta mediterrânea (DM, rica em AGPI *n-6/n-3*, razão de 1:1), ocidental 1 (DO-1, com gordura *trans* (rica em AGT) e ocidental 2 (DO-2, com gordura interesterificada (rica em AGS), desde o DPN 21 até a idade adulta dos filhotes machos *Wistar* sobre comportamentos de diferentes tipos de memória (memória de trabalho, memória curta e longa de reconhecimento de objeto e memória espacial);
- Verificar a influência do consumo crônico da DM, DO-1 e DO-2 sobre parâmetros de memória (aquisição, consolidação e evocação), após indução de danos cognitivos induzidos por escopolamina, nos ratos *Wistar* adultos;
- Verificar a influência do consumo das DM, DO-1 e DO-2 sobre o parâmetro ponderal e relação entre massa total e massa de gordura corporal dos animais, monitorado durante o desenvolvimento e crescimento dos animais;
- Verificar a influência do consumo das DM, DO-1 e DO-2 sobre os níveis de citocinas pró-inflamatória e anti-inflamatória no plasma, além de citocina pró-inflamatória (IL-1β) no hipocampo;

- Avaliar a influência do consumo das DM, DO-1 e DO-2 sobre parâmetros histológicos no hipocampo.

4 PRODUÇÃO CIENTÍFICA

Os resultados, como também os itens Material e Métodos, Discussão e Referências incluídos nesta dissertação apresentam-se sob a forma de um artigo científico, no mesmo formato adequado para sua submissão para publicação

4.1 ARTIGO

Can the dietary fat type facilitate memory impairments in adulthood?

A comparative study between Mediterranean and Western-based diet in rats

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Running Title: Memory damages: Mediterranean vs. Western-diet fat

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Abstract

A balanced intake of fatty acids (FA) of both omega-6 (n-6) and -3 (n-3) series is essential for memory. The Mediterranean diet, rich in n-3 polyunsaturated FA (PUFA) and low n-6/n-3 PUFA ratio, has shown beneficial influences on health. Inversely, the Western diet contains saturated fats, including hydrogenated vegetable fat (HVF, rich in *trans* fat) and interesterified fat (IF), making the n-6/n-3 PUFA ratio high. Due to the health impairments caused by HVF, it has been replaced by IF in processed foods. We compared a Mediterranean diet (MD, balanced n-6/n-3 PUFA ratio) with Western diets 1 (WD1, rich in *trans* fat) and 2 (WD2, rich in IF) on memory process *per se* and following scopolamine (SCO) administration, which induces amnesia in rats. While MD exerted protective effects, WD1 and WD2 showed declined memory *per se*, showing higher susceptibility to SCO-induced memory deficits. In addition, WD1 and WD2 showed increased pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and decreased anti-inflammatory cytokines (IL-10) in plasma. IL-1β was higher in the hippocampus of WD1, which was reflected on histological assessments. Significant correlations between cognitive decline and inflammatory markers reinforce our hypothesis: MD-like fats may act preventively on cognitive loss in old age, while WD-like fats may facilitate this.

Key-words: Memory impairments; Inflammatory cytokines; Interesterified fat; *trans* fat; Scopolamine.

1. **Introduction**

The aging of society has increased the prevalence of cognitive deficits [1], which can occur congenitally, due to environmental factors such as toxicity and stress [2] as well as physiological factors, including inflammation and neurological disorders [3]. It is known that memory maintenance and cognitive performance are closely related to the intake of balanced foods rich in omega-6 (n-6) and omega-3 (n-3) fatty acids (FA), and whose deficiency has been associated to increased susceptibility to cognitive decline and development of dementia [4]. The Mediterranean diet is acknowledged by its benefits to health, mainly due to the adequate levels of polyunsaturated fatty acids (PUFA), presenting n-6/n-3 PUFA ratio about 1:1 [5,6]. On the other hand, changes in the eating habits of modern society have increased the consumption of processed foods, which are rich in *trans* fatty acids (TFA), saturated fatty acids (SFA) and n-6 PUFA [7,8] in detriment of n-3 PUFA, aggravating the imbalance of n-6/n-3 PUFA ratio [9], a recognized risk factor for the development of central nervous system (CNS) diseases [10]. In this sense, humans have evolved on a diet in which the omega-6/omega-3 essential fatty acids (EFA) ratio was about 1, while in current Western diets this ratio is approximately 15:1 [5].

Dietary TFA derive mainly from hydrogenated vegetable oils [11,12], whose consumption has already reached 1.7-8% of the world's dietary fat intake [13] through processed and fast food [14,12]. Experimental studies of our group have related the chronic consumption of hydrogenated vegetable fat (HVF), which is rich in TFA, to oxidative damages and TFA incorporation in different brain areas, and increased susceptibility to neuropsychiatric conditions [15,16,17,18,19,20,21,22,23,24,25]. Due

to the growing concerns about the nutritional impact of the Western diet and the consumption of TFA on health, the interesterification of FA has presently been the main method for replacement of *trans* isomers from foods, thus meeting international rules to reduce damages related to their consumption [26]. In this sense, interesterified fat (IF) is the current substitute for HVF in processed foods, even though it still is heavily present in processed foods in many countries. Such process consists of physical-chemical modifications of oils and fats [27], resulting in a redistribution of FA to triacylglycerol molecules. In contrast to hydrogenation, this process does not affect the saturation degree [26], nor does it cause isomerization of double bonds [28], which has been related to impairments to health [29,20,21,15,10].

A growing interest regarding a possible relationship between nutrition and cognitive health have led to the design and conduction of interventional studies [30]. In this context, the relationship between nutritional habits and cognitive functions has given incentive to promising investigations. It is known that the CNS is strongly regulated by different neurotransmitters and mediators such as cytokines, which are related to modulation and trafficking of immune cells in response to tissue damage [31]. In particular, microglia and astrocytes produce a wide range of proinflammatory factors such as interleukin-1beta (IL-1β); *Tumor necrosis factor alpha* (TNF-α), interleukin 6 (IL-6), and interleukin 10 (IL-10), which promote an inflammatory environment, leading to a blood-brain barrier deficiency [32]. In this sense, neuroinflammation is a complex process resulting from tissue injury due to the harmful stimuli originated directly in the CNS, or from the systemic circulation [33,31].

Considering that the type of dietary FA consumed during long periods is able to alter the phospholipid membrane, making the CNS more susceptible to the development of neurodegenerative diseases, the current study has been designed to

comparatively assess the influence of FA based on a Mediterranean diet (MD), with low n-6/n-3 PUFA ratio versus two different Western diets (WD1, rich in *trans* fat and WD2, rich in IF) on parameters of behavioral and inflammatory markers in plasma and hippocampus of adult rats exposed to an animal model of scopolamine-induced cognitive decline.

2. **Materials and Methods**

2.1 Animals

Thirty-six male *Wistar* rats (21 days old) from the breeding facility of Universidade Federal de Santa Maria (UFSM), RS, Brazil were kept in Plexiglas cages (four per cage) with free access to food and water in a room with controlled temperature (22°C±1 °C), on a 12 h-light/ dark cycle with lights on at 7 a.m. The experimental protocol was approved by the Animal Ethics Committee (n° 9373231116/2016), which is affiliated to the Council for the Control of Animal Experiments (CONCEA).

2.2 General procedures

Animals were randomly assigned to one of three experimental groups (n=12 for each group), which received chow (PUROLAB 22) enriched with 20% additional fat [10,17,34], from postnatal day 21 (PND21) until the end of the experiment (PND98). The different fats resulted in three personalized diets: a balanced diet (n6/n3 PUFA ratio, based on the Mediterranean diet- MD) and two different Western diets containing *trans* FA (Western diet 1- WD1) and interesterified fat (Western diet 2- WD2). After PND90, animals were submitted to behavioral tests, which started 30 min after scopolamine (SCO-1mg/kg; i.p.) or saline (NaCl, 0.9%) administration: Y Maze (YM-

PND90), Novel Object Recognition Test (NORT-PND91-92) and Morris Water Maze (MWM-PND93-97) observations. All experiments were carried out between 8 am and 5pm. After behavioral tests and metabolism monitoring, animals were anesthetized (isofluorane, inhalation solution) and euthanized by exsanguination (blood was collected by cardiac puncture in heparinized tubes). Their brains and epididymides were removed, kept on ice for further evaluations.

Experimental design

Figure 1[1]

2.3 Diet composition

The diets were isocaloric and normolipid, differing only by the quality of the fatty acids. All three diets consisted of standard chow (PUROLAB 22, Puro Trato Nutrição Animal Ltda), which was enriched with 20% additional fat: i) soybean oil (SO, rich in n-6 FA), (Camera®, Ijuí, Brazil), purchased from a local supermarket plus fish oil (FO, rich in n-3 FA), donated by Laboratório Tiaraju® (Santo Ângelo, Brazil); or ii) HVF (rich in TFA), (Primor®, Ijuí, Brazil), purchased from a local supermarket; or iii) interesterified fat (IF), donated by Triângulo Alimentos® (São Paulo, Brazil). Animals were fed with one of these diets from weaning (PND21) until the end of the experiment (PND98). Diets (Table 1) were prepared weekly throughout the experimental protocol, stored (-20°C) in daily portions [35], and given to the animals every 24 h. Animals' body weight and food consumption were monitored weekly.

Table 1. Composition of different diets (%)

[1] As figuras 1, 2, 3, 4, 5, 6 e 7, deste artigo, estão dispostas ao final da seção 4, entre as páginas 74-77. Estão organizadas desta maneira devido à adequação das normas da revista, a qual o artigo que integram foi submetido.

Constituents	MD	WD1	WD2
Ash	6.1 ± 0.1	6.0 ± 0.1	6.0 ± 0.3
Crude protein	20.0 ± 0.1	20.3 ± 0.1	21.3 ± 0.7
Fat	25.1 ± 0.1	27.6 ± 0.7	22.82 ± 0.1
Carboydrate	48.8 ± 0.1	46.5 ± 1.4	49.9 ± 1.0

Abbreviations: MD: Mediterranean diet; WD1: Western diet 1; WD2: Western diet 2.

2.3.1 Dietary Fatty acids

Moisture content of diets was obtained after oven-drying at 105°C until a constant weight [36] was reached. Ash content was determined at 550°C [36]. Crude protein was determined by the micro Kjeldahl method (N × 6.25) [36]. Fat content was determined by the gravimetric method [37]. Carbohydrates were estimated by difference. Dietary fats were extracted with chloroform-methanol according to Bligh and Dyer (1959) followed by saponification in methanolic KOH solution and esterification in methanol H_2SO_4 solution [38]. Methyl ester fatty acids were analyzed with a gas chromatographby Agilent Technologies (HP 6890) equipped with a DB-23 capillary column (60 m x 0.25 mm x 0.25 μm) and flame ionization detector. The temperature of the injector port was set at 250°C and the carrier gas was nitrogen (0.6 mL/min). After injection (1 μL, split ratio 50:1), the oven temperature was held at 150°C for 1 min, then increased to 240°C at 4°C/min and held at this temperature for 12 min. Standard fatty acid methyl esters (37-component FAME Mix, C 22:5n3 and PUFA no. 2 from Sigma, Saint Louis, MO, USA) were run under the same conditions and subsequent retention times were used to identify the fatty acids. Fatty acids were expressed as a percentage of the total fatty acids identified (Table 2).

Table 2. Fatty acids composition of the diets enriched with different fats (% of total fatty acids identified).

Fatty acids	MD	WD1	WD2
∑**SFA**	23.93	27.71	50.22
∑**MUFA**	25.69	34.70	32.34
∑**TFA**	0.29	16.19	0.045
∑**n-3**	16.60	0.47	0.43
∑**n-6**	31.56	12.73	14.53
n-6/n-3 PUFA ratio	1.90	26.95	34.10

Abbreviations: MD: Mediterranean diet; WD1: Western diet 1; WD2: Western diet 2; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; TFA: *trans* fatty acids; n-3: omega-3; n-6: omega-6.

2.4 Drugs and solutions

Scopolamine hydrobromide (SCO) was purchased from Sigma Aldrich (Munich, Germany). The drug was dissolved in saline solution (NaCl 0.9%) immediately before use, and injected intraperitoneally at a dose of 1 mg/kg [39,40].

2.5 Behavioral evaluations

2.5.1 Y-maze task

The Y-maze behavioral paradigm was carried out as previously described [41], 30 min after a single SCO (1mg/kg, ip) or saline (SAL) administration. The test relies on the innate tendency of rats to explore a novel environment. Each animal was

placed in the center of the Y-maze and allowed to explore freely through the maze during a 5-min session for the assessment of spontaneous alternating behavior. The sequence and the total number of arm entries in the apparatus were video recorded and monitored by the Any-Maze software. An arm entry was considered valid if all four paws entered the arm. Alternation was defined as three consecutive entries in three different arms (i.e., ABC or BCA). The percentage of spontaneous alternation was measured as an index of working memory using the following formula:

$$\left(\frac{Total\ alternation\ number}{Total\ number\ of\ entries - 2}\right) x\ 100$$

The total number of arm entries was used as locomotor index [42]. The apparatus was cleaned with alcohol solution 20% using a wet sponge and paper towel before the introduction of each animal.

2.5.2 Novel object recognition task (NORT)

The NORT paradigm is related to the natural motivation of animals to explore novelties, considered an innate instinct they use to recognize their environment [43], and a higher score implies a higher recognition rate, indicating better memory. Recognition memory was conducted in the arena floor covered with sawdust (from bedding material) during recognition memory training and test trials. On the first day, 30 min following administration ofa single dose of scopolamine (SCO - 1 mg/kg, i.p.) or SAL, rats were given one training trial being exposed to two identical objects (A1 and A2, double Lego toys) positioned in two adjacent corners, and were allowed to freely explore the objects for 5 min (training session). Tests of short-term memory

(STM) and long-term memory (LTM) were performed 1 and 24 h after the training session, respectively. Rats were allowed to explore the arena for 5min in the presence of two objects: the familiar Object A and a second novel Object B or C, which were placed in the same locations of the training session. All objects had similar textures, colors, and sizes, but distinctive shapes. The objects were cleaned with a 20% alcohol solution before the introduction of each rat; exploration was defined as sniffing or touching the object with the nose. A recognition index calculated for each animal was expressed by the ratio TN / (TF + TN) (TF = time spent exploring the familiar object; TN = time spent exploring the novel object).

2.5.3 Morris Water Maze Test (MWM)

This paradigm is performed to assess spatial memory and learning, as described by Morris (1981) [44]. Animals acquire information about spatial location and reach a hidden platform in a circular pool. Thus, a decrease in the time to reach the hidden platform suggests learning. The apparatus is a circular pool 150 cm diameter and 60 cm high, filled with water (23±2 °C) to a depth of 40 cm. The pool is divided into four equal quadrants (compass locations: NE, NW, SE, SW) and a movable escape platform made of black Plexiglas with 8 cm diameter located in the center of a fixed quadrant. The apparatus was placed in a room with numerous extra-maze clues, posters, and objects that remained constant throughout the experiment. Rats were submitted to four trial sessions per day (30 min intervals), for four consecutive training days. The first trial of each training day started 30min after a single SCO (1mg/kg, ip.) or SAL administration. During the performance of each trial session, animals were put in alternated quadrants of the pool and left free to find the platform in the designated quadrant. Once the rat located the platform, it was allowed to remain on it for 60 s; if an animal failed to reach the platform within 60 s, it was placed on the platform for 30

s. On the fifth day, a probe test (retrieval trial) was performed (30min after SCO (1mg/kg; i.p.) or SAL administration), where the platform was removed, and each rat was allowed to explore the pool for 60s. The time spent by the animal in the target quadrant searching for the hidden platform was regarded as the index of learning. All data obtained in the test were analyzed by the Any-Maze software.

2.5.4 Tissue Preparation

One day after the last behavioral assessment, 30 min after SCO (1mg/kg, ip) or saline administration, all animals were anesthetized (isoflurane, inhalation solution) and euthanized by exsanguination. Blood was collected by cardiac puncture in heparinized tubes and centrifuged at 1,300 g for 15 min for plasma. Brains were removed, maintained on ice for hippocampus dissection, according to Paxinos and Watson [45]. While plasma was used for TNF-α, IL-1β, IL-6 and IL-10, hippocampus was used for 1IL-1β quantification. In addition, representative brains in each group were fixed in 10 % formalin/saline for the histopathology assessment [46].

2.5.5 Estimation of Inflammatory markers

2.5.5.1 TNF-α, IL-1β, IL-6 and IL-10 quantified in plasma

Plasma was centrifuged at 16,000xg at 4 ºC for 10 min [47]. The supernatant obtained was used to determine the levels of TNF-α, IL-1β, IL-6 and IL-10 using an enzyme-linked immunoassay kit Proteintech TM (Rosemont, USA). All procedures were performed according to the manufacturer's instructions.

2.5.5.2 1IL-1β level quantified in the hippocampus

Tissues were placed in a 80mM PBS (pH 7.4) solution containing 0.5% Tween 20, 0.1 mM PMSF, 2mM EDTA and 0.1% BSA. After homogenization and centrifugation (16,000xg at 4ºC) for 10 min [47], supernatants were used to determine the IL-β level using an enzyme-linked immunoassay kit (Peprotech, Brazil), according to the manufacturer's specifications. Using a curve plotted from standard solutions to cytokine, results were expressed as ng cytokine/mg protein.

2.5.6 Qualitative histological examination of hippocampus

The samples of the hippocampus of each experimental group were dissected at the end of the experiment and fixed in 10% formaldehyde in phosphate buffer (pH 7.2) for 24h. The paraffin-embedded scar tissue specimens were sectioned (5μm), deparaffinized and stained with hematoxylin-eosin (HE) for microscopic evaluation. The stained tissue sections were examined using a microscope (Leica®, model DM2000, Germany) with a 20x objective equipped coupled to digital image capture camera (Leica®, model DFC295, Germany). The images generated by the camera were transferred to a microcomputer and the histological slide was analyzed by optical microscopy, magnification 200x. In the qualitative analysis, the microscopic images were analyzed for the hippocampal structure, the thickness of the neuronal layer, and for the structure and arrangement of neurons [46].

2.5.7 Statistical Analysis

Levene's test was applied to examine the homogeneity of the data. One-way ANOVA was used for body weight gain and epididymal weight data. Two-way ANOVA, 3 diets (MD/WD1/WD2) x 2 treatments (Saline/SCO) for YM, NORT and cytokine levels were performed. For MWM task, three-way ANOVA, 3 diets (MD/WD1/WD2) x 2

treatments (Saline/SCO) x 4 periods (sessions days) was used. This last factor was considered a repeated measure, and pair wise comparisons were used to compare the behavior at different time points. For all the tests, post-hoc Duncan's test was applied when appropriate. Data were expressed as mean ± S.E.M. and $P<0.05$ was considered statistically significant.

3. Results

3.1. *Body weight gain and epididymal fat ratio (epididymal weight/ bodyweight) (Table 3)*

Experimental groups showed similar body weight gain during the whole procedure. One-way ANOVA followed by Duncan's test showed that animals fed with WD1 and WD2 showed increased epididymal weight/ body weight ratio in relation to MD group.

Table 3.Influence of different diets on body weight and epidydimal fat ratio (weight epidydimal / weight body).

Groups	Body Weight (g)	EFW/BW Ratio
MD	313.66± 9.297	1.60 ± 0.06^{b}
WD1	302.83 ±16.01	1.99 ± 0.05^{a}
WD2	304.50 ± 11.36	2.00 ± 0.12^{a}

Abbreviations: MD: Mediterranean diet; WD1: Western diet 1; WD2: Western diet 2; EFW: epidydimal fat ratio; BW: weight body.

3.2. Behavioral assessments

3.2.1 *Influence of different diets on working memory assessed in the Y-maze task is shown in Figure 2.*

Two-way ANOVA of spontaneous alternation percentage in the Y-maze revealed a significant main effect of the diet [F(2,30)=19,75,*P*<0.000]. Post-hoc analysis showed that WD1 decreased spontaneous alternation percentage in relation to both MD and WD2 groups. SCO administration decreased this behavioral parameter only in WD2 group. Animals from WD1 and lower percentage of alternations in relation to MD group after SCO administration (Fig. 2).

3.2.3 *Time spent on the novel object recognition task (NORT) is shown in Figure 3.*

Two-way ANOVA of short-term memory in the NORT revealed a significant main effect of diet and drug [F(2,30)=22.04, *P*<0.000 and F(1,30)=30.86, *P*<0.000], respectively. Two-way ANOVA of long-term memory revealed a significant main effect of diet and diet X drug interaction [F (2,30) = 23.18, *P*<0.000 and 5.98, *P*<0.007], respectively.

Post-hoc test showed that both Western diets (WD1 and WD2) presented impaired short-term recognition memory (1h) *per se* in relation to animals fed with MD. SCO administration decreased short-term memory in MD and WD1 groups, whose recognition index was lower than their respective SAL-injected groups. After SCO administration, WD1 and WD2 groups showed a reduced short-term memory in relation to MD-fed animals (Fig. 3A).

Long-term recognition memory (24h) decreased *per se* inWD1 and WD2 in relation to MD group. SCO administration decreased long-term memory in WD1-fed group, which was lower than WD2 and MD groups (Fig. 3B).

3.2.4.*The Influence of different diets on spatial memory assessed in Morris Water-Maze task (MWM) is shown in Figure 4*

Three-way ANOVA with repeated measure (3 x 2 x 4) of latency to reach the platform revealed a significant main effect of diet and repeated measure [F(2,30)=7.61, P<0.05 and F(6,90)=16.79; P<0.000], respectively. Among SAL-injected groups, a comparison with the paired test indicates that MD-fed animals showed a shorter latency time to find the platform, which was observed on the 3rd and 4thtraining days, whereas WD1 and WD2-fed animals presented a shorter latency time to find the platform only on the last training day (day 4th) (Fig. 4A). In addition, among SAL groups, WD1 and WD2-fed rats spent more time to reach the original position of the platform in comparison to MD group.

Among SCO-injected groups, the paired test indicated that MD-fed rats showed lower latency to find the platform on days 3 and 4 in relation to the first training day, while WD1- and WD2-fed animals did not find the platform until the last training day (Fig. 4B). Furthermore, SCO treatment increased the time spent to reach the original position of the platform in MD-fed group in relation to its SAL-paired group, while no difference of this behavioral parameter was observed among different diets (Fig. 4C).

3.3. *Inflammation Markers*

3.3.1*Influence of different diets on cytokine levels assessed in plasma is shown in Figure 5*

Two-way ANOVA of plasma IL-1β levels revealed a significant main effect of diet, drug and diet X drug interaction [F(2,30)=262.85, *P*<0.000; F(1,30)= 28.82, *P*<0.000 and F(2,30)=6.79, *P*<0.01], respectively. Considering both SAL and SCO-injected groups,WD1 and WD2-fed rats showed higher plasma IL-1β levels in

comparison to MD group. In fact, regardless of SCO administration, WD1 group presented higher plasma IL-1β levels than WD2 group. SCO administration increased IL 1β in plasma of MD group only (Fig. 5A).

Two-way ANOVA of plasma TNF-α levels revealed a significant main effect of diet and drug [F(2,30) =504.2, *P*<0.000 and F(1,30)=14.49, *P*<0.01], respectively. Regardless of SCO administration, the post-hoc test showed that WD1-fed animals had higher plasma TNF-α levels in relation to both WD2 and MD groups, being this plasma marker higher in WD2 in comparison to MD group. SCO administration increased TNF-α levels in plasma of the three experimental groups (Fig. 5B).

Two-way ANOVA of plasma IL-6 levels revealed a significant main effect of diet and drug [F(2,30)= 207.10, *P*<0.000 and F(1,30)=7.23, *P*<0.014], respectively. Regardless of SCO administration, the post-hoc test showed that WD1-fed rats had higher levels of IL-6 than WD2 and MD groups, which were also significantly different from each other (Fig. 5C).

Two-way ANOVA of plasma IL-10 levels revealed a significant main effect of diet [F(2,30) =464.62, *P*<0.000]. WD1 and WD2 groups showed lower levels of IL-10 in comparison to MD, regardless of SCO administration. In fact, plasma IL-10 levels were similar between WD1 and WD2 groups in both SAL and SCO treatments (Fig. 5D).

3.3.2*Influence of different diets on cytokine levels in hippocampus is shown in Figure 6*

Two-way ANOVA of IL-1β levels in the hippocampus evidenced a significant main effect of diet [F (2,30) = 11.04, *P*<0.001]. The post-hoc test revealed that WD1-fed rats showed higher IL-1β levels in the hippocampus in relation to both MD and

WD2 groups, regardless of SCO administration. Interestingly, SCO administration was able to increase cytokine in this tissue only in WD1-fed group (Fig. 6).

3.3.3 *Correlations between behavioral parameters and inflammatory markers quantified in plasma / hippocampus of MD, WD1 and WD2-fed rats, which were subsequently exposed to SCO is shown in Table 4.*

Additional statistical analysis revealed a significant negative correlation between plasma IL-1β levels and short-term memory (r^2= 0.53, P=0.000), long-term memory (r^2= 0.51, P=0.000) in the NORT, and correct alternation % (r^2= 0.46, P=0.002) in the Y-Maze, and a positive correlation with hippocampal IL-1β levels (r^2= 0.28, P=0.008).

Interestingly, plasma IL-6 and TNF-α levels were negatively correlated with short-term memory (r^2=0.44, P=0.000 and r^2=0.35, P=0.002, respectively), long-term memory (r^2=0.50, P=0.000 and r^2=0.48, P=0.000, respectively) in the NORT, and with correct alternation % (r^2= 0.50, P=0.000 and r^2= 0.43, P=0.000, respectively) in the Y-Maze.

In addition, plasma IL-10 levels showed a positive correlation with short-term memory (r^2= 0.36, P=0.002), long-term memory (r^2= 0.53, P=0.000) in the NORT, and correct alternation % (r^2= 0.48, P=0.000) in the Y-Maze.

Hippocampal IL-1β levels were negative when correlated with long-term memory (r^2= 0.18, P=0.029) in the NORT.

Table 4.Correlations between behavioral parameters and inflammation markers quantified in plasma and hippocampus of the animals fed with MD-, WD1- and WD2- and subsequently exposed to SCO:

	Parameter	Correlations (r^2)	*P* value
Plasma			
IL 1β	Short-term memory	(-) 0.53	0.000
	Long-term memory	(-) 0.51	0.000
	Correct alternation %	(-) 0.46	0.002
	IL 1β (hippocampus)	(+) 0.28	0.008
IL 6	Short-term memory	(-) 0.44	0.000
	Long-term memory	(-) 0.50	0.000
	Correct alternation %	(-) 0.50	0.000
TNFα	Short-term memory	(-) 0.35	0.002
	Long-term memory	(-) 0.48	0.000
	Correct alternation %	(-) 0.43	0.000
IL 10	Short-term memory	(+) 0.36	0.002
	Long-term memory	(+) 0.53	0.000
	Correct alternation %	(+) 0.48	0.000
IL 1β **(hippocampus)**	Long-term memory	(-) 0.18	0.029

Abbreviations: IL 1β: interleukin-1beta; TNF α:*Tumor necrosis fator alpha*; IL 6: interleukin 6; IL 10: interleukin 10.

3.3.4 Histological aspects in the hippocampal brain area of MD, WD1 and WD2-fed rats exposed or not to SCO are shown in Figure 7.

Histological assessments allow us to observe that the MD group presented an intact hippocampal structure, normal distribution of neuronal cells with neurons arranged in a regular and clear boundary, with no abnormal cell structure and marked neuronal migration. On the other hand, while WD1 and WD2 groups showed reduced

neuronal layer thickness in comparison to MD group, MD-SCO group showed reduced thickness of the neuronal layer and decreased neuronal migration. Experimental groups fed with both Western diets (WD1 and WD1), and exposed to SCO showed an evident reduction in the thickness of the neural layer. In fact, independently of SCO exposure, WD2 group showed marked reduction in the neuronal layer thickness (Figure 7).

4. Discussion

This study was performed to compare Mediterranean diet *versus* two different Western diets on memory function as well its influences on scopolamine-induced cognitive impairments. We hypothesize that the consumption of healthy fats, i.e. from MD, throughout life is able to minimize the risks for- the development of senile dementia and/or Alzheimer's disease in old age, while the chronic consumption of synthetic FA, which is usual in Western countries, could favor the development of memory diseases commonly observed in this stage of life.

Our current findings showed a better memory performance of MD-fed animals, which was positively correlated to lower plasma levels of inflammatory markers together with higher plasma and hippocampal levels of anti-inflammatory cytokine. Our findings also allowed us to observe that both Western diets can facilitate the development of SCO-induced cognitive impairments together with increased levels of neuroinflammation markers in plasma and hippocampus of these animals. Interestingly, the chronic consumption of the three different diets was not related to differences in body weight gain among the experimental groups, however, both Western diets (WD1 and WD2) facilitated the increase of visceral fat in relation to the Mediterranean diet (MD).

One of the most relevant public health concerns is the exponential growth of elderly population, which may impact the onset of diseases characteristic of this life stage, including senile dementia and Alzheimer's disease [48,49,50,3]. For millions of people who are in the process of aging, dementia poses a frightening threat. It is estimated that by 2050, the incidence of these diseases can reach about 131.5 million people worldwide [51]. Based on these alarming data, efforts have been invested in the search for a better understanding of the pathophysiological aspects involved in memory disorders of old age, as well as treatment, prevention or retardation of their progress [52]. Recent research has shown that some environmental factors such as stress, sedentary lifestyle and poor eating habits are able to exert significant influences on the development of these diseases [3]. In this sense, a paradox exists between the balanced level of FA present in the Mediterranean diet (MD) and the high content of synthetically saturated fats present in the Western diet (WD), what has motivated the development of the current study. Here we seek to compare the impact of consumption of different FA present in the Mediterranean and Western diets on endogenous neuroinflammation markers, which can be reflected on the cognitive functions of rats, exacerbated or not by a memory loss inductor such as SCO.

Recent studies of our group have shown a significant relationship between dietary FA and molecular damages in the central nervous system (CNS) together with behavioral alterations, since these diets present qualitative differences in fat content [19,20,21,23,24,25,54], indicating that the nature of FA in foods exert a key role on neural functions. Thus, while a high intake of monounsaturated fatty acids (MUFA) appears to exert protection against senile dementia and Alzheimer's disease [54], observations concerning mechanisms underlying beneficial influences of the n-3/n-6 polyunsaturated FA (PUFA) on cognitive and memory performance have been shown

in animal models [55,56]. In the last decades, *trans* and interesterified fat consumption, here represented by WD1 and WD2, respectively, has been expanded in Western society, thus modifying the dietary n-3/n-6 PUFA ratio, which has increased from about 1:1 to15-20:1, thus increasing the risk of dementia and cognitive deficit development [55,57]. Many mechanistic processes underlying cognitive decline resulting from an inadequate diet have been proposed, which include oxidative damages, increased permeability of the blood-brain barrier, reduced brain neurotrophic factors, insulin insensitivity, as well as neuroinflammation development [10,19,21,24,25,56]. In a similar line of thinking, our current findings indicate that the prolonged consumption of foods rich in *trans* and interesterified fat (with high n-6/n-3 PUFA ratio), similarly to WD1 or WD2, may be related to memory impairments, as observed by the lower percentage of alternation in the Y-maze task after SCO exposure and by decreased short-term memory *per se* in the NORT. In fact, the previous SCO administration intensified cognitive loss (short- and long-term memory) of WD1 group in this last behavioral paradigm. Inversely, MD group, whose intake of n-6/n-3 PUFA ratio was adequate, showed preserved working memory following SCO exposure, as observed in the Y-maze task, while the novel object recognition task (NORT) was impaired only acutely (short-term memory), since long-term memory was not affected by SCO exposure. In order to expand our understanding about the influence of the different diets on long-term memory acquisition, the Morris paradigm, which assesses working memory, has been included in the current experiment. The findings indicated that MD *per se* evoked a faster learning, since this experimental group found the platform on the 3rd training day, inversely to what was observed with both WD1 and WD2-fed animals, which showed learning only on the last day of training. Interestingly for our hypothesis about susceptibility to memory impairments in old age, when animals were

exposed to SCO, both WD1 and WD2 groups did not find the platform, while MD animals found it on the 3rd training day, whose learning time was the same observed in MD-fed animals not exposed to SCO.

Of particular importance to the current outcomes, different protocols developed through representative animal models performed in our laboratory have shown that chronic consumption of *trans* fat in Western diets exerts deleterious influence on the development of neuropsychiatric disorders, indicating a facilitation for the development of movement disturbances [17], mania [16,18,19,20], anxiety and stress [25]; psychostimulant [22,23,24] and morphine addiction [58]; and memory impairments [21,34,57]. Some of these studies show a relationship between behavioral impairments and oxidative damages in important brain areas [15,16,18,19,20,21,22,24,34,57,58], which were also linked to a small but significant incorporation of *trans* FA into neural membranes of different brain areas of animals [16,19,20,21,23,24,34,56,59]. More recently, our growing concern regarding the deleterious influence of the consumption of synthetically saturated fats, which are common in the Western diet, culminated in two additional studies involving interesterified fat (IF). Innovatively, such studies showed that animals fed with IF from early life periods until adulthood showed increased incorporation of saturated FA (ΣSFA) and linoleic acid (LA- C18:2n6) in addition to the decreased incorporation of DHA in the hippocampus. This same brain area was related to oxidative damages and decreased BDNF and TrkB levels, what compromised the memory acquisition of these animals [53]. In another study, animals exposed to similar chronic IF consumption showed absence of morphine addiction and an increased level of kappa-opioid receptors in the spinal cord, which were reflected on reduced pain threshold [60]. Taken together, our previous studies indicate that lipid content replacement into brain membranes due to *trans* fat and IF intake, here

represented by WD1 and WD2, may affect the synaptic plasticity, modifying neurotransmission, thus favoring conformational modifications in membrane-bound proteins.

Additional outcomes of the current study showed that both WD1 and in minor proportion, WD2-fed animals presented increased plasma level of pro-inflammatory cytokines such as IL-1β, TNF-α and IL-6, together with reduced IL-10 levels, an anti-inflammatory cytokine, whose levels were not affected by SCO exposure, allowing us to hypothesize that the chronic consumption of these Western fats may facilitate the development of an environment conducive to imbalance, what consequently activates the glial cells. In fact, while dietary fats can exert deleterious influences on health, neural inflammatory processes may generate brain dysfunctions and lead to neurodegenerative diseases [61,62]. Furthermore, saturated FA, which are present in both WD1 and WD2, are acknowledged to activate toll-like receptors (TLR), particularly TLR4, which are expressed in glial cells, and are responsible for an increased inflammatory response [63]. More precisely, TLR are transmembrane receptors of the innate immune system able to recognize different ligands related to pathogens and tissue damages. Of particular importance to our current findings, TLR can be activated by saturated FA (SFA), but inhibited by n-3 polyunsaturated FA (PUFA), particularly by the docosahexaenoic acid (DHA) [64]. This inflammatory environment facilitates the recruitment of circulating immune cells whose transmigration to the brain is usually limited by the so-called blood-brain barrier (BBB), which may have its permeability affected by cytokines [65], as well as compromised by saturated fatty acids [66]. In this sense, systemic inflammation clearly increases cytokine concentrations in the CNS with consequent effects on behavior and cognition [61].

Besides increased proinflammatory cytokines in plasma of both WD1 and WD2-fed groups, our study also showed higher levels *per se* of IL-1β in the hippocampus of WD1-fed group, whose values were increased when these animals received SCO. Indeed, the administration of this muscarinic cholinergic receptor antagonist [66] is a relevant animal model of cognitive loss, inducing memory impairments in rodents. Interestingly, MD and WD2-fed animals showed no increasein hippocampal IL-1β, indicating that *trans* fat, which is present in WD1, can more easily activate the proinflammatory cytokines cascade in this brain area, which is affected in a greater proportion, given the memory disturbances here observed. Our current outcomes are consistent with other literature data, where the systemic inflammation clearly increased cytokine levels in the CNS, with consequent influences on cognition parameters [67], also inducing changes in the working memory and loss of cholinergic neurons in animals [61]. Our current findings showed an increase of inflammatory cytokines in plasma of both WD1 and WD2, although changes in hippocampal IL-1β was not observed in WD2 group, which may be explained by the fact that microglia initially overexpressed anti-inflammatory cytokines [60,61], what may have been sufficient to control the inflammatory process in this experimental group. In line with this, activated microglia can adopt two functional states: proinflammatory state and anti-inflammatory microglial activation. It is possible that, in the early stages of Alzheimer's disease, initial microglial activation may act in a protective form, i.e. anti-inflammatory [68]. In fact, a considerable line of evidences suggest that a systemic inflammatory process is able to exert harmful consequences on the CNS, especially when the brain presents increased vulnerability due to genetic predisposition, aging, neurodegenerative disease [61], what may also be influenced by environmental factors. As observed in our findings, the chronic consumption of both *trans* and

interesterified fat in WD1 and WD2 served as a factor that influenced on memory impairments, also elevating inflammatory cytokine levels in plasma. Inversely, animals that consumed balanced fish oil plus soybean oil through the MD, showed no compromising of memory or plasma levels of inflammatory cytokines. In addition, histological outcomes confirmed the Western diet-induced damages in the hippocampus, which were characterized by a decreased neuronal layer. Indeed, both WD1 and WD2 consumption were related to evident thickness reduction in the neural layer in comparison to MD. Considering SCO exposure, our findings showed a reduction of the thickness of the neuronal layer, independently of the diet.

Taken together, our findings revealed that prolonged consumption of different dietary FA, such Western diets, were sufficient to: i) cause memory impairments, as demonstrated in different behavioral paradigms; (ii) change proinflammatory cytokines levels in both plasma and hippocampus, which may be closely linked to learning and cognition; (iii) reduce the thickness of the neuronal layer in the hippocampus.

In conclusion, our current findings indicate that the dietary fats may be considered an environmental factor of great relevance for memory impairments and development of neuroinflammatory processes, which may be related to the onset of neurodegenerative diseases such as Alzheimer's disease. In fact, different types of fats present in foods may generate long chain PUFA that are easily incorporated into brain membranes, affecting their fluidity and neurotransmission, which together may affect memory and cognitive functions in adulthood. These data are even more relevant if we consider the current lack of data about IF, since this fat has replaced TFA in the Western diet aiming to reduce health damages. However, to the best of our understanding, we are showing for the first time that chronic consumption of both *trans* fat and IF is not safe, and they may favor the development of memory impairments,

especially in old age. Innovatively, our findings open a perspective to an understanding about eating characteristics, when the type of FA is more important than the amount consumed, what may indicate a predictability to cognitive and memory damage development. Considering that so far there is no direct treatment for memory loss and/or senile dementia, it is fundamental to find preventative strategies to minimize the risks of development of such diseases. The Mediterranean diet, balanced in n-3 and n-6 PUFA, is able to act preventively on the risk of cognitive decline, representing an economic and sustainable source of numerous beneficial effects, particularly on brain health.

Based on these findings, further studies are needed to confirm the relationships described here considering molecular targets involved in memory impairments and their reflex on public health. These outcomes combined will provide important insights about environmental causes related to cognitive impairments and dementia syndromes, adding perspectives of novel interventions to prevent the incidence and severity of these disorders.

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Katsiardanis K, Diamantaras AA, Dessypris N, Michelakos T, Anastasiou A, Katsiardani KP, Kanavidis P, Papadopoulos FC, Stefanadis C, Panagiotakos DB, Petridou ET Cognitive impairment and dietary habits among elders: the Velestino Study J Med Food 2013,16(4):343–50. Doi: 101089/jmf20120225

[2] Puri A, Srivastava P, Pandey P, Yadav RS, Bhatt PC Scopolamine induced behavioral and biochemical modifications and protective effect of Celastrus paniculatous and Angelica glauca in rats Int J Nutr Pharmacol Neurol Dis 2014, 4(3): 158–169. Doi: 104103/2231-0738132675

[3] Adami PVM, Galeano P, Wallinger ML, Quijano C, Rabossi A, Pagano ES, Olivar N, Toso CR, Cardinali D, Brusco LI, Do Carmo S, Radi R, Gevorkian G, Castaño EM, Cuello AC, Morelli L Worsening of memory deficit induced by energy-dense diet in a rat model of early-Alzheimer's disease is associated to neurotoxic Aβ species and independent of neuroinflammation Biochim Biophys Acta 2017, 1863:731–743. Doi: 101016/jbbadis201612014

[4] Sheppard KW, Cheatham CL Omega-6 to omega-3 fatty acid ratio and higher-order cognitive functions in 7- to 9-y-olds: a cross-sectional study Am J Clin Nutr 2013, 98(3):659-667 Doi: 103945/ajcn113058719

[5] Simopoulos AP The importance of the ratio of omega-6/ omega-3 essential fatty acids Biomed Pharmacother 2002, 56:365–79. Doi: 101016/S0753-3322

[6] Morris, MC, Tangney, CC, Wang, Y, Sacks, FM, Bennett, DA, Aggarwal NT MIND diet associated with reduced incidence of Alzheimer's disease Alzheimer's Dement 2015, 11:1007-1014. Doi: 101016/jjalz201411009

[7] Mayneris-perxachs J Diet and plasma evaluation of the main isomers of conjugated linoleic acid and trans-fatty acids in a population sample from Mediterranean north-east Spain Food Chem 2010, 123:296–305. Doi: 101186/1743-7075-8-65

[8] Baldini M, Pasqui F, Bordoni A, Maranesi M Is the Mediterranean lifestyle still a reality? Evaluation of food consumption and energy expenditure in Italian and Spanish university students. Public Health Nutr 2009, 12:148–155 Doi: 101017/S1368980008002759

[9] Ailhaud G, Massiera F, Weill P, Legrand P, Alessandri JM, Guesnet P Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity Prog Lipid Res 2006, 45:203–236. Doi: 101016/jplipres200601003

[10] Pase CS, Roversi Kr, Trevizol F, Kuhn FT, Dias VT, Roversi K, Vey LT, Antoniazzi CT, Barcelos RC, Burger, ME Chronic consumption of *trans* fat can facilitate the development of hyperactive behavior in rats Physiol Behav 2015, 139: 344-350 Doi: 101016/jphysbeh201411059

[11] Wolff RL, Precht D, Molkentin J Occurrence and distribution profiles of trans-18:1 acids in edible fats of natural origin ed: Dundee: The Oily Press, 1998 Sebedio JL, Christie WW Trans fatty acids in human nutrition, 1–33.

[12] Stender S, Astrup A, Dyerberg J Ruminant and industrially produced trans fatty acids: health aspects Food Nutr 2008, 52:1–8 Doi: 103402/fnrv52i01651

[13] Osso FS, Moreira AS, Teixeira MT, Pereira RO, Tavares do Carmo Md, Moura AS Trans fatty acids in maternal milk lead to cardiac insulin resistance in adult offspring Nutrition 2008, 24:727–732 Doi: 101016/jnut200803006

[14] Van de vijver LP, Kardinaal AF, Couet C, Aro A, Kafatos A, Steingrimsdottir L, Amorim Cruz JA, Moreiras O, Becker W, Van Amelsvoort JM, Vidal-Jessel S, Salminen I, Moschandreas J, Sigfússon N, Martins I, Carbajal A, Ytterfors A, Poppel G Association between trans fatty acid intake and cardiovascular risk factors in Europe: the trans fair study Eur J Clin Nutr 2000, 54:126–135 Doi: 101038/sjejcn1600906

[15] Dias VT, Trevizol F, Barcelos RCS, Kunh FT, Roversi K, Roversi Kr Lifelong consumption of trans fatty acids promotes striatal impaiments on Na^+/K^+ ATPase activity and BDNF mRNA expression in a animal model of mania Brain Res Bull 2015, 118:78-81 Doi: 101016/jbrainresbull201509005

[16] Dias VT, Trevizol F, Roversi Kr, Kuhn FT, Roversi K, Pase CS Trans-fat supplementation over two generations of rats exacerbates behavioral and biochemical

damages in a modelo f mania: Co-treatment with lithium Life Sci 2015,132:6-12 Doi: 101016/jlfs201504013

[17] Teixeira AM, Dias VT, Pase CS, Roversi K, Boufleur N, Barcelos RCS, Benvegnú DM, Trevizol F, Dolci GS, Carvalho NR, Quatrin A, Soares FA, Reckziegel P, Segat HJ, Rocha JB, Emanuelli T, Burger ME Could dietary trans fatty acids induce movement disorders? Effects of exercise and its influence on Na+K+-ATPase and catalase activity in rat striatum Behav Brain Res 2012, 226:504–510 Doi: 101016/jbbr201110005

[18] Trevizol F, Benvegnú DM, Barcelos RCS, Boufleur N, Dolci GS, Müller LG, Pase CS, Reckziegel P, Dias VT, Segat HJ, Teixeira AM, Emanuelli T, Rocha JB, Burger ME Comparative study between n−6, trans and n−3 fatty acids on repeated amphetamine exposure: a possible factor for the development of mania Pharmacol Biochem Behav 2011, 97:560–565 Doi: 101016/jpbb201011004

[19] Trevizol F, Roversi K, Dias VT, Roversi Kr, Pase CS, Barcelos RCS, Benvegnú, DM, Kuhn FT, Dolci GS, Ross DH, Veit JC, Piccolo J, Emanuelli T, Burger ME Influence of lifelong dietary fats on brain fatty acids and amphetamine-induced behavioral responses in adult rat Prog Neuropsychopharmacol Biol Psychiatry 2013, 45:215-222 Doi: 101016/jpnpbp201306007

[20] Trevizol F, Dias VT,Roversi K, Barcelos RCS, Kuhn FT, Roversi Kr, Pase CS, Golombieski R, Veit JC, Piccolo J, Emanuelli T, Rocha JB, Burger, ME Cross-generational trans fat intake modifies BDNF mRNA in the hippocampus: impact on

memory loss in a mania animal model Hippocampus 2015, 25:556-565 Doi: 101002/hipo22391

[21] Trevizol F, Roversi Kr, Dias VT, Roversi K, Barcelos RCS, Kuhn FT, Pase CS, Golombieski R , Veit JC, Piccolo J, Pochmann D, Porciúncula LO, Emanuelli T, Rocha JB, Burger ME Cross-generational trans fat intake facilities mania-like behavior: Oxidative and molecular markers in brain cortex Neuroscience 2015, 286:353-363. Doi: 101016/jneuroscience201411059

[22] Kuhn FT, Roversi Kr, Antoniazzi CTD, Pase CS, Trevizol F, Barcelos RCS, Dias VT, Roversi K, Boufleur N, Benvegnú DM, Piccolo J, Emanuelli T, Burger ME Influence of trans fat and omega-3 on the preference of psychostimulant drugs in the first generation of young rats Pharmacol Biochem Behav 2013, 110:58-65. Doi: 101016/jpbb201306001

[23] Kuhn FT, Dias VT, Roversi K, Vey LT, de Freitas DL, Pase CS,Roversi K, Veit JC, Emanuelli T, Burger ME Cross-generational trans fat consuption favors self-administration of amphetamine and changes molecular expression of BDNF, DAT, and D1/D2 receptors in hippocampus of rats. Neurotox Res 2015, 28:319-331. Doi: 101007/s12640-015-9549-5

[24] Kuhn FT, Trevizol F, Dias VT, Barcelos RCS, Pase CS, Roversi K, Antoniazzi CTD, Roversi Kr, Boufleur N, Benvegnú DM, Emanuelli T, Burger ME Toxicological aspects of trans fat consumption over two sequential generations of rats: Oxidative

damage and preference for amphetamine Toxicol Lett, 2015, 232:58-67. Doi: 101016/jtoxlet201410001

[25] Pase CS, Roversi Kr, Trevizol F, Kuhn FT, Schuster AJ, Vey LT, Dias VT, Barcelos RC, Piccolo J, Emanuelli T, Burger ME Influence of perinatal trans fat on behavioral responses and brain oxidative status of adolescent rats acutely exposed to stress. Neuroscience 2013, 247:242-252. Doi:101016/jneuroscience201305053

[26] Ribeiro M, Marques ACPR Abuso e Dependência da Anfetamina Projeto Diretrizes: Associação Médica Brasileira e Conselho Federal de Medicina, 2002 Disponível em: <http://wwwbibliomedcombr/diretrizes/pdf/abuso_anfetaminapdf> Acesso em: mar 2016

[27] Norizzah AR, Chong CL, Cheow CS, Zaliha O Effects of chemical interesterification on physicochemical properties of palm stearin and palm kernel olein blends Food Chem2004, 86:229-235. Doi: 101016/jfoodchem200309030

[28] Farfan M, Villalón MJ, Ortíz ME, Nieto S, Bouchon P The effect of interesterification on the bioavailability of fatty acids in structured lipids. Food Chem 2007, 139:571–577 Doi: 101016/jfoodchem201301024

[29] Mozzafarian D,Stampfer MJ Removing industrial trans fat from foods BMJ 2010, 340. Doi: 101136/bmjc1826

[30] Aridi YS, Walker JL, Wright ORL The Association between the Mediterranean Dietary Pattern and Cognitive Health: A Systematic Review Nutrients, 2017, 9:674, Doi:103390/nu9070674

[31] Kim YS, Lee KJ, Kim H Serum tumour necrosis factor-α and interleukin-6 levels in Alzheimer's disease and mild cognitive impairment. Psychogeriatrics 2017, 17(4):224-230. Doi: 101111/psyg12218

[32] Mosley RL, Hutter-Saunders JA, Stone DK, Gendelman HE Inflammation and adaptive immunity in Parkinson's disease Cold Spring Harb Perspect Med, 2012, 2(1):a009381 Doi: 101101/cshperspecta009381

[33] Li J, Gao L, Sun K, Xiao D, Li W, Xiang L, Qi J Benzoate fraction from Gentianarigescens Franch alleviates scopolamine-induced impaired memory in mice model in vivo J Ethno pharmacol 2016, 193:107–116. Doi: 101016/jjep201608001

[34] Teixeira AM, Pase CS, Boufleur N, Roversi K, Barcelos RCS, Benvegnu DM, Segat HJ, Dias VT, Reckziegel P, Trevizol F, Dolci GS, Carvalho NR, Soares FA, Rocha JB, Emanuelli T, Burger ME 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 Neuroscience 2011, 195:80-88. Doi: 101016/jneuroscience201108055

[35] Magri TPR, Fernandes FS, Souza AS, Langhi LG, Barboza T, Misan V, Mucci DB, Santos RM, Nunes TF, Souza SA, de Mello Coelho V, Tavares do Carmo Md

Interesterified fat or palm oil as substitutes for partially hydrogenated fat in maternal diet can predispose obesity in adult male offspring. Clin Nutr 2015, 14:242-248. Doi: 101016/jclnu201409014

[36] AOAC Official methods of analysis (18th ed) Gaithersburg, MD: Association of Official Analytical Chemists, 2005

[37] Bligh EG, Dyer WJ A rapid method of total lipid extraction and purification Can J Biochem Physiol 1959, 37:911-917. Doi: 101139/o59-099

[38] Hartman L, Lago BC A rapid preparation of fatty methyl esters from lipids Lab Pract 1973, 22:475-476 Doi: 101021/ac60235a044

[39] Cunha RLOR, Gouvea IE, Juliano L A glimpse on biological activities of tellurium compounds An Acad Bras Cienc 2009, 81:393-407. Doi: 101590/S0001-37652009000300006

[40] Harrison FE, Hosseini AH, Dawes SM, Weaver S, May JM Ascorbic acid attenuates scopolamine-induced spatial learning deficits in the water maze. Behav Brain Res 2009, 205:550-558. Doi: 101016/jbbr200908017

[41] Chu J, Giannopoulos PF, Diaz C, Golde TE, Pratico D Adeno-associated virus-mediated brain delivery of 5-lipoxygenase modulates the AD-like phenotype of APP mice Mol Neurodegener 2012, 7:57-67. Doi: 101186/1750-1326-7-1

[42] Hritcu L, Clicinschi M, Nabeshima T Brain serotonin depletion impairs short-term memory, but not long-term memory in rats Physiol Behav 2007, 91:652-657. Doi: 01016/jphysbeh200703028

[43] Heldt SA, Stanek L, Chhatwal JP, Ressler KJ Hippocampus-specific deletion of BDNF in adult mice impairs spatial memory and extinction of aversive memories Mol Psychiatry, 2007, 12(7):656-670Doi: 101038/sjmp4001957

[44] Morris RGM Spatial localization does not depend on the presence of local cues. Learn Motiv 1981, 12:239-260

[45] Paxinos G, Watson C The Rat Brain in Stereotaxic Coordinates, 2013, 7th ed Amsterdam: Elsevier

[46]Chen K, Sun Y, Diao Y, Ji L, Song D, Zhang T **α**7 nicotinic acetylcholine receptor agonist inhibits the damage of rat hippocampal neurons by TLR4/Myd88/NF-**κ**B signaling pathway during cardiopulmonary by-pass Mol Med Reports, 2017, 16:4770-4776

[47] Oliveira SM, Silva CR, Trevisan G, Villarinho JG, Cordeiro MN, Richardson M, Borges MH, Castro CJ, Jr Gomez MV, Ferreira J Antinociceptive effect of a novel armed spider peptide Tx3-5 in pathological pain models in mice Pflugers Arch 2016, 468(5):881-894 Doi: 101007/s00424-016-1801-1

[48] Rocca WA, Petersen RC, Knopman DD, Hebert LE, Evans DA, Hall KS, Gao S, Unverzagt FW, Langa KM, Larson EB, White LR Trends in the incidence and prevalence of Alzheimer's disease, dementia, and cognitive impairment in the United States Alzheimers Dement 2011, 7(1):80-93Doi: 101016/jjalz201011002

[49] Tom SE, Hubbard RA, Crane PK, Haneuse SJ, Bowen J, McCormick WC, McCurry S, Larson EB Characterization of Dementia and Alzheimer's Disease in an Older Population: Updated Incidence and Life Expectancy With and Without Dementia Am J Public Health 2015, 105(2):408-413Doi: 102105/AJPH2014301935

[50] Teixeira JB, Junior PRBS, Higa J, Filha MMT Doença de Alzheimer: estudo da mortalidade no Brasil, 2000-2009 Cad Saúde Pública 2015, 31(4):850-860Doi: 101590/0102-311X00144713

[51] Alzheimer's Disease International World Alzheimer Report 2016 London: Alzheimer's Disease International, 2016 Available from: hhttps://wwwalzcouk/research/WorldAlzheimer_Report_2016_pdf Accessed October 06, 2017

[52] Lipnicki DM, Sachdev PS, Crawford J, Reppermund S, Kochan NA, Trollor JN, Draper B, Slavin MJ, Kang K, Lux O, Mather KA, Brodaty H Risk Factors for Late-Life Cognitive Decline and Variation with Age and Sex in the Sydney Memory and Ageing Study. PLoS One 2013, 14:841-865. Doi: 101371/journalpone0065841

[53] D'avila LF, Dias VT, Vey LT, Milanesi LH, Roversi K, Emanuelli T, Burger ME, Trevizol F Toxicological aspects of interesterified fat: Brain damages I rats. Toxicol Lett 2017, 276:122-128. Doi: 101016/jtoxlet201705020

[54] Morris RGM Spatial Localization Does Not Require the Presence of Local Cues Learning and Motivation, 1981, 12(2):239–260 Doi: 101016/0023-9690(81)90020-5

[55] Beilharz JE, Kaakoush NO, Maniam J, Morris MJ The effect of short-term exposure to energy-matched diets enriched in fat or sugar on memory, gut microbiota and markers of brain inflammation and plasticity. Brain Behav Immun 2016, 57:304-313. Doi: 101016/jbbi201607151

[56] Pase CS, Roversi Kr, Roversi K, Vey LT, Dias VT, Veit JC, Maurer LH, Duarte T, Emanuelli T, Duarte M, Burger ME Maternal trans fat intake during pregnancy or lactation impairs memory and alters BDNF and TrkB levels in the hippocampus of adult offspring exposed to chronic mild stress. Physiol Behav 2017, 169:114-123. Doi: 101016/jphysbeh201611009

[57] Hopperton KE, Trépanier MO, Giuliano V, Bazinet RP Brain omega-3 polyunsaturated fatty acids modulate microglia cell number and morphology in response to intracerebroventricular amyloid-β 1-40 in mice J Neuroinflammation, 2016, 13:257. Doi: 101186/s12974-016-0721-5

[58] Roversi K, Pas CS, Roversi K, Vey LT, Dias VT, Metz VG Trans fat intake across gestation and lactation increases morphine preference in females but not in male rats: Behavioral and biochemical parameters. Eur J Pharmacol 2016, 788:210-217 Doi: 101016/jejphar201606031

[59] Teixeira AM, Pase CS, Boufleur N, Roversi K, Barcelos RCS, Benvegnú DM, Segat HJ, Dias VT, Reckziegel P, Trevizol F, Dolci GS, Carvalho NR, Soares FA, Rocha JB, Emanuelli T, Burger ME 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 Neuroscience, 2011, 195:80-88 Doi: 101016/jneuroscience201108055

[60] Milanesi LH, Roversi K, Antoniazzi CTD, Segat HJ, Kronbauer M, D'avila LF, Dias VT, Sarib MHM, Barcelos RCS, Maurer LH, Emanuelli T, Burger ME, Trevizol F Chronic consumption of interesterified fat modifies brain Opioid system and affects morphine-induced reward Effects in rats Food Chem Toxicol 2017, 110:25-32 Doi: 101016/jfct201709048

[61] Cunningham C Microglia and Neurodegeneration: The Role of Systemic Inflammation Glia, 2013, 61:71-90 Doi: 101002/glia22350

[62] Berger ME, Smesny S, Kim S-W, Davey CG, Rice S, Sarnyai Z, Schlögelhofer M, SchäferM R, Berk M, McGorry PD, Amminge RGP Omega-6 to omega-3 polyunsaturated fatty acid ratio and subsequent mood disorders in young people with at-risk mental states: a 7-year longitudinal study Transl Psychiatry 2017, 7(8):1220 Doi: 101038/tp2017190

[63] Misan V, Estato V, Velasco PC, Spreafico FB, Magri T, Santos RMAR, Fragoso T, Souza AS, Boldarine VT, Bonomo IT, Sardinha FLC, Oyama L, Tibirica E, Carmo MGT Interesterified fator palm oil as substitutes for partially hydrogenated fat during the perinatal period produces changes in the brain fatty acids profile and increases leukocyte–endothelial interactions in the cerebral microcirculation from the male offspring in adult life Brain Res 2015, 1616:123-133 Doi: 101016/jbrainres201505001

[64] Hwang DH, Kim JA, Lee JY Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid. Eur J Pharmacol 2016, 785:24-35 Doi: 101016/jejphar201604024

[65] Gualtierotti R, Guarnaccia L, Beretta M, Navone SE, Campanella R, Riboni L, Rampini P, Marfia G Modulation of neuroinflammation in the central nervous system: role of chemokines and sphingolipids Adv Ther 2017, 34(2):396-420Doi: 101007/s12325-016-0474-7

[66] Goverdhan P, Sravanthi A, Mamatha T Neuroprotective effects of meloxicam and selegiline in scopolamine-induced cognitive impairment and oxidative stress Int J Alzheimers Dis 2012, 974013 Doi: 101155/2012/974013

[67] Hein AM, Stutzman DL, Bland ST, Barrientos RM, Watkins LR, Rudy JW, Maier SF Prostaglandins are necessary and sufficient to induce contextual fear learning impairments after interleukin-1beta injections into the dorsal hippocampus Neuroscience 2007, 150:754-763 Doi: 101016/jneuroscience200710003.

[68] Fan Z, Brooks DJ, Okello A, Edison P An early and late peak in microglial activation in Alzheimer's disease trajectory *Brain*, 2017, 140:792–803 Doi: 101093/brain/aww349

Figure captions

Figure 1.Three experimental groups of rats were fed from weaning (PND21) until PND98 with diets enriched with different fats. After PND90, animals were treated with

saline or scopolamine (SCO- 1mg/kg/mL, i.p) 30 min before behavioral assessments, which were performed daily, sequentially.

Abbreviations: MD: Mediterranean diet; WD1: Western diet 1; WD2: Western diet 2; YM: Y-maze task; NORT: Novel object recognition task; WM: Morris Water Maze Test; SAL: Saline; SCO: Scopolamine.

Figure 2. Influence of different diets on working memory of ratsfed with different diets from PND21 and maintained on the same diet until the end of the experiment (PND98). Animals were assessed in the Y-maze test 30 minutes after a single injection of SCO (1mg/kg, i.p) or SAL (n = 6), and quantified as correct alternation percentage. Data are expressed as mean± S.E.M. Abbreviations: MD: Mediterranean diet; WD1: Western diet 1; WD2: Western diet 2; SAL: saline; SCO: scopolamine. Different lowercase indicates significant difference among the diets in the same treatment; *Indicates significant difference from SAL in the same diet (P< 0.05).

Figure 3. Influence of different diets on recognition index. Animals were fed with different diets from PND21 and maintained on the same diet until the end of the experiment (PND91). The behavioral test was performed 30 minutes after a single SCO (1mg/kg, i.p) or SAL (n=6) injection. Short-term memory (STM) and long-term memory (LTM) retention tests were performed 1h (A) and 24h (B) after training, respectively. Data are expressed as mean ± S.E.M. Abbreviations: MD: Mediterranean diet; WD1: Western diet 1; WD2: Western diet 2; SAL: saline; SCO: scopolamine. Different lowercase indicates significant difference among the diets in the same treatment; *Indicates significant difference from SAL in the same diet (P< 0.05).

Figure 4.Influence of different diets on spatial memory performance, which was observed in the Morris Water Maze task (MWM). Animals were fed with different diets

from PND21 and maintained on the same diet until the end of the experiment (PND91). 30 minutes before the test, animals received a single SCO (1mg/kg, i.p) or SAL (n = 6) injection. Analysis of escape latency of SAL-injected rats for four training days is shown in Fig.4A; Analysis of escape latency of SCO-injected rats for four days of training are shown in Fig.4B. Latency time to find the original location of platform on the test day are shown in Fig. 4C. Data are expressed as mean ± S.E.M. Abbreviations: MD: Mediterranean diet; WD1: Western diet 1; WD2: Western diet 2; SAL: saline; SCO: scopolamine. #Indicates significant difference from Day 1 (repeated measure in the same experimental group) ($P<0.05$); Different lowercase indicates significant difference among the diets in the same treatment; *Indicates significant difference from SAL in the same diet ($P< 0.05$).

Figure 5. Influence of different diets on plasma levels of IL-1β (A), TNF-α (B), IL-6 (C) and IL-10 (D) of rats fed with MD, WD1 or WD2 from PND21 until the end of the experiment (PND91) (n= 6). On PND91, one half of each dietary group received a single SCO (1mg/kg, i.p) or SAL (n = 6) injection. Data are expressed as mean ± S.E.M. Abbreviations: MD: Mediterranean diet; WD1: Western diet 1; WD2: Western diet 2; SAL: saline; SCO: scopolamine; IL1β: interleukin-1beta; TNF-α:*Tumor necrosis factor alpha*; IL 6: interleukin 6; IL 10: interleukin 10. Different lowercase indicates significant difference among the diets in the same treatment; *Indicates significant difference from SAL in the same diet ($P < 0.05$).

Figure 6. Influence of different diets on the hippocampal levels of IL-1β of rats fed with MD, WD1 or WD2 from PND21 until the end of the experiment (PND91) (n= 6). On PND91, one half of each dietary group received a single SCO (1mg/kg, i.p) or SAL (n = 6) injection. Data are expressed as mean ± S.E.M. Abbreviations: MD: Mediterranean diet; WD1: Western diet 1; WD2: Western diet 2; SAL: saline; SCO:

scopolamine; IL-1β: interleukin-1beta. Different lowercase indicates significant difference among the diets in the same treatment; *Indicates significant difference from SAL in the same diet (P< 0.05).

Figure 7. Histological sections of hippocampus stained with HE. Slides were observed by optical microscopy at 200µm. MD group: intact hippocampal structure, normal distribution of neuronal cells and marked neuronal migration (A). MD-SCO group: a reduction in the thickness of the neuronal layer and in the neuronal migration (B). WD1 (C) and WD1-SCO group: evident reduction in the thickness of the neural layer (D). WD2 (E) and WD2-SCO groups: marked reduction in neuronal layer thickness(F). MD: Mediterranean diet; WD: Western diet; SCO: scopolamine.

Figure 1. Experimental design

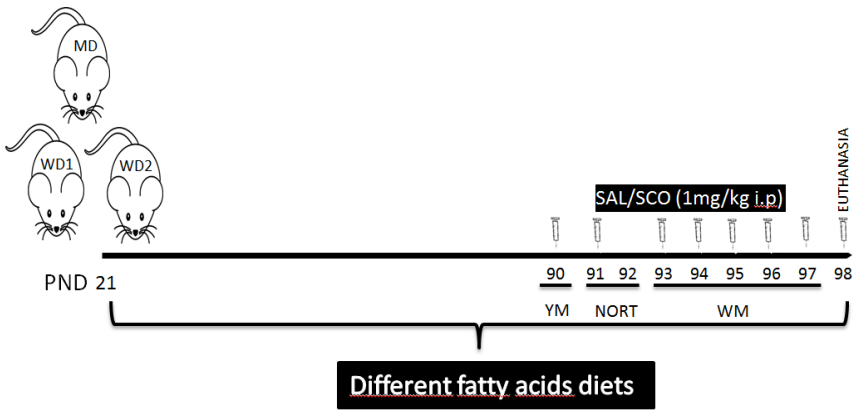


Figure 2. Spontaneous alternation percentage assessed in the Y-maze test

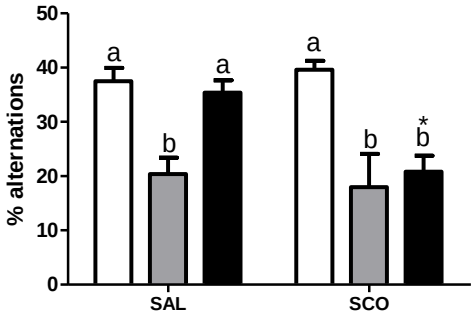


Figure 3. Time spent on the novel object recognition task (NORT)

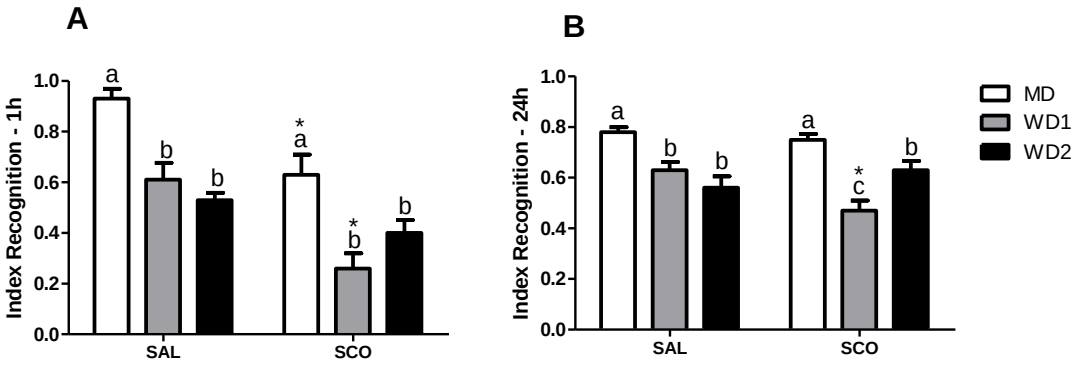


Figure 4. Influence of different diets on spatial memory performance was determined in Morris Water Maze task (MWM)

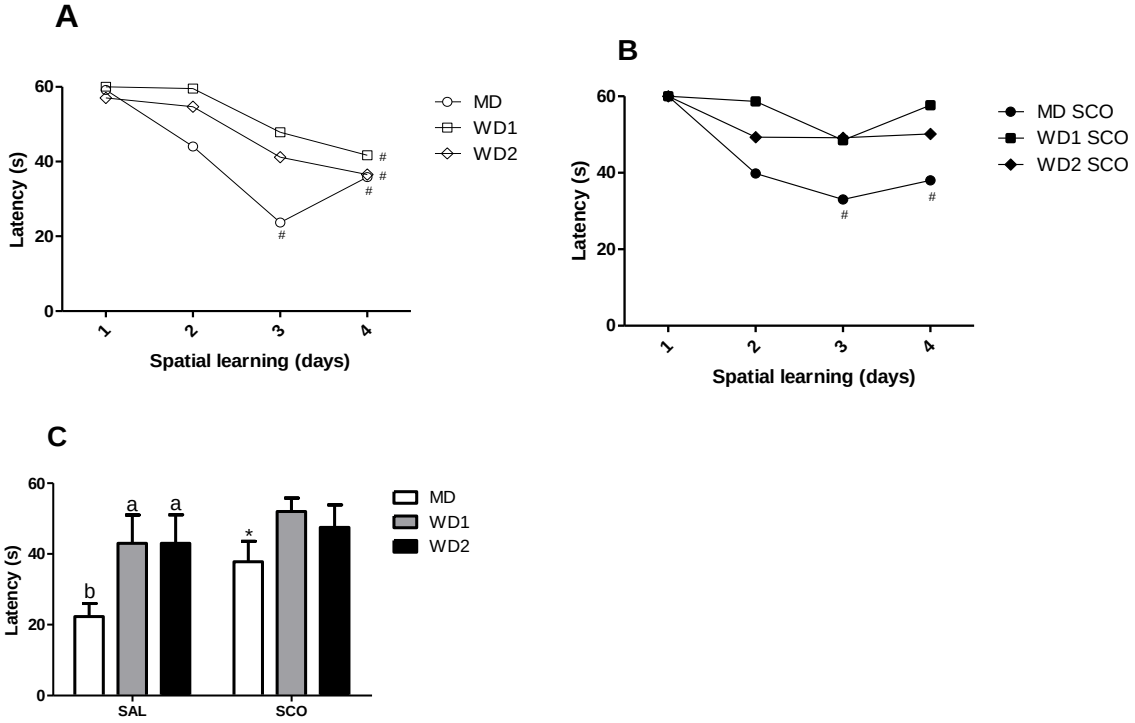


Figure 5. Influence of different diets on cytokine levels in plasma

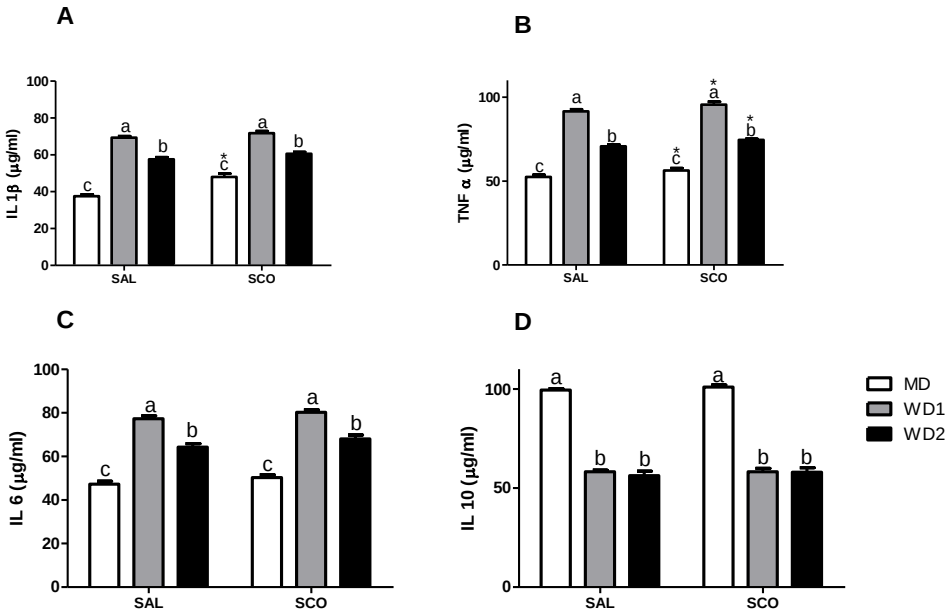


Figure 6. Influence of different diets on cytokine levels in hippocampus

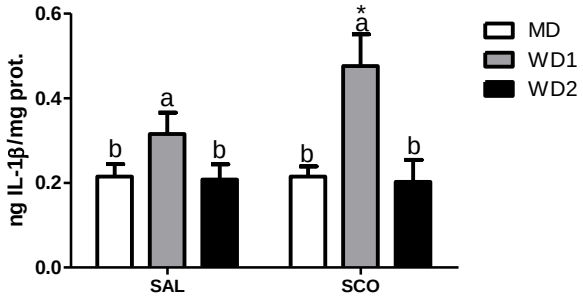
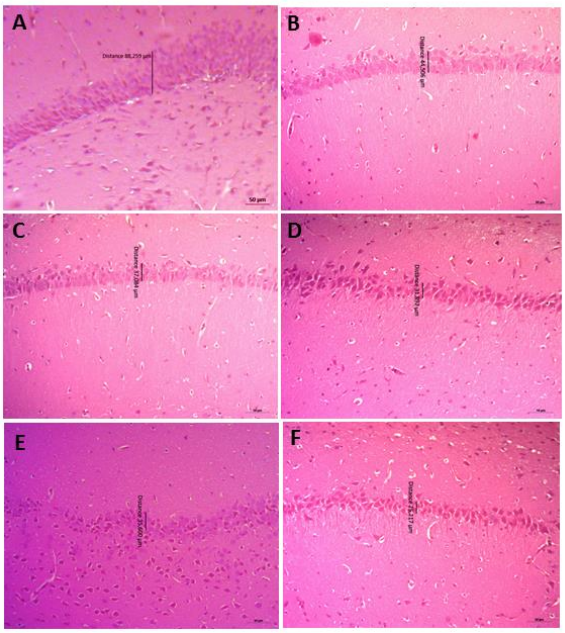


Figure 7. Histological aspects of hippocampal brain area of MD, WD1 and WD2-fed rats exposed or not to SCO



5 CONCLUSÃO

Através do presente estudo foi possível observar que o consumo de uma dieta normolipídica, somente diferenciada pela natureza das gorduras nela incorporadas, foi capaz de alterar parâmetros de aprendizagem e memória em ratos adultos, além de modificar a suscetibilidade ao déficit cognitivo induzido pela escopolamina. O consumo de dietas contendo as gorduras *trans* e interesterificada, baseadas em uma dieta ocidental, foram relacionadas à prejuízos de aprendizagem e memória de curto e longo prazo, aumento das citocinas pró-inflamatórias no plasma e hipocampo e redução de citocina anti-inflamatória no plasma.

Com base nestas evidências, este estudo permitiu concluir que a substituição da gordura vegetal hidrogenada (GVH) pela gordura interesterificada (GI) em busca de alimentos mais saudáveis pela indústria alimentícia, deve apresentar prudência, pois, até o momento, experimentalmente, é possível predizer que ambas exercem influências deletérias sobre funções cerebrais, afetando os parâmetros de memória, os quais foram correlacionados com a elevação de citocinas pró-inflamatórias, contrariamente ao que foi observado no grupo que recebeu a dieta baseada na mediterrânea, a qual apresentou níveis adequados de AGPI *n-6/n-3*. Esta, por sua vez, através de evidências comportamentais, mostrou que pode atuar preventivamente sobre os prejuízos de memória induzidos ou não pela ESCO, o que foi confirmado pelo maior nível da citocina antiinflamatória (IL-10) no plasma, além dos níveis reduzidos de citocinas pró-inflamatórias (IL-6, IL-1β e TNF-α) no plasma. Tais benefícios da dieta mediterrânea foram confirmados pela distribuição regular de células neuronais, observado no corte histológico do hipocampo, como também através das correlações positivas observadas entre a citocina anti-inflamatória (IL-10) e memória. Tomados em conjunto, o presente estudo poderá apresentar uma aplicação direta em saúde pública preventiva, servindo para uma modificando de hábitos alimentares em busca evitar maiores prejuízos de memória, especialmente durante o envelhecimento, evitando ou minimizando os problemas associados ao desenvolvimento de demências. Mais estudos são necessários para confirmar as relações descritas aqui, considerando os objetivos moleculares envolvidos nas deficiências de memória e seus reflexos sobre a saúde pública.

PERSPECTIVAS FUTURAS

A hipótese lançada neste estudo abre perspectivas para diversos protocolos que serão continuados pelo grupo de pesquisa. O grupo de trabalho vinculado ao laboratório Farmatox deverá dar continuidade à esta importante linha de pesquisa já estabelecida no grupo, a qual envolve a influência do consumo de diferentes AG da dieta durante o desenvolvimento e, também, ao longo da vida de ratos *Wistar* sobre diferentes modelos farmacológicos que afetam diferentes funções do SNC. Desse modo, investigar os mecanismos moleculares envolvidos em resposta a esses achados comportamentais, assim como estudos epigenéticos para avaliar a influência do consumo dos ácidos graxos sobre a prole, está e continuará sendo intenso objeto de pesquisa do laboratório.

REFERÊNCIAS

ABDOLLAHI, A. et al. Transcriptional network governing the angiogenic switch in human pancreatic cancer. **Proc Natl Acad Sci.,** v.104(31), p.12890-5, Jul. 2007.

AFONSO, M. S. **Efeito das gorduras interesterificadas sobre o desenvolvimento da lesão aterosclerótica em camundongos knockout para o receptor LDL**. 2016, 107 p. Tese (Doutorado em Ciências) – Universidade de São Paulo, São Paulo, SP, 2016.

AILHAUD, G. et al. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. **Prog Lipid Res.,** v.45, p**.** 203-236, Mar. 2006.

ALLISON, D. B.,et al. Estimated intakes of trans fatty and other fatty acids in the US population. **J Am Diet Assoc**., **cidade,** v. 99, p.166-174, Feb.1999.

ALZHEIMER'S DISEASE INTERNATIONAL WORLD ALZHEIMER REPORT, 2016 London: Alzheimer's Disease International, 2016 Available from: hhttps://wwwalzcouk/research/WorldAlzheimerReport2016pdf Accessed October 06, 2017.

ARCEGO D.M., et al. Early life adversities or hight fat diet intake reduce cognitive BDNF signaling in adult rats: Interplay of these factors changes these effects. **Int J Dev Neurosci**, v.50, p.16-25, 2016. Doi: 10.1016/j.ijdevneu.2016.03.001.

ARIDI, Y.S.; WALKER, J.L.; WRIGHT, O.R.L. The Association between the Mediterranean Dietary Pattern and Cognitive Health: A Systematic Review. **Nutrients**, v.9, p.674, 2017. Doi:10.3390/nu9070674

ATRI, A.S. et al. Blockade of central cholinergic receptors impairs new learning and increases proactive interference in a word pairedassociate memory task. **Behav. Neurosci**., v. 118, p. 223-136, 2004.

BADDELEY, A. D. **Memoria Humana**. Teoría y Práctica. Mc. Graw Hill Editora Madrid, p. 496, 1999.

BAGGIO, S.R.; BRAGAGNOLO, N. The effect of heat treatment on the cholesterol oxides, cholesterol, total lipid and fatty acid contents of processed meat products. **Food Chem**., v.95, p. 611-617, 2006.

BARNARD, N.D.; BUNNER, A.E.; AGARWAL, U. Saturated and trans fats and dementia: a systematic review. **Neurobiol Aging.**, v. 35, Suppl 2, p.65-73, Set. 2014. Doi:10.1016/j.neurobiolaging.2014.02.030.

BATOOL, Z.; et al. Repeated administration of almonds increases brain acetylcholine levels and enhances memory function in healthy rats while attenuates memory deficits in animal model of amnesia. **Brain Research Bulleti,** *2015.*

BEILHARZ, J.E.; KAAKOUSH, N.O.; MANIAM, J.; MORRIS, M.J. The effect of short-term exposure to energy-matched diets enriched in fat or sugar on memory, gut microbiota

and markers of brain inflammation and plasticity. **Brain Behav Immun** ,v.57, p.304-313, 2016. Doi: 101016/jbbi201607151.

BELL,S.J., BRADLEY, D. FORSE, R.A., BISTRIAN, B. The new dietary fats in health and disease. **J. Am. Diet. Assoc.,** Chicago, v.97, p.280-286, 1997.

BHARDWAJ, S.; PASSI, S.J.; MISRA, A. Overview of trans fatty acids: biochemistry and health effects. **Diabetes Metab Syndr.**, v.5, p. 161-164, 2011.
BILBUL, M. et al. Risk Profiles of Alzheimer Disease. **Can J. Neurol. Sci.**, v. 38, n.4, p.580-592, 2011.

BORSONELO, E.C.; GALDURÓZ, J.C.F. The role of polyunsaturated fatty acids (PUFAs) in development, aging and substance abuse disorders: Review and propositions. **Prostaglandins Leucotrienes & Essential fatty Acids**, v.78, p.237-245, 2008.

CANCELLO, R. et al. Increased Infiltration of Macrophages in Omental Adipose Tissue Is Associated With Marked Hepatic Lesions in Morbid Human Obesity. **Diabetes**, v. 55, n. 6, p.1554-1561, 2006.

CARACIOLLO, B., et al. Cognitive decline, dietary factors and gut–brain interactions. **Mechanisms of Ageing and Development**, v.136–137, p. 59-69, 2014.

CHALON, S.; et al. Polyunsaturated fatty caids and cerebral functions: focus on monoaminergic neurotransmission. **Lipids**, v.36, p.937-944, 2001.

CHOULIARAS, B.P. et al. Epigenetic regulation in the pathophysiology of Alzheimer's disease. **Prog. Neurobiol**., v.90, p. 498-510, 2010.

COSKUN, P. et al. A mitochondrial etiology of Alzheimer and Parkinson disease. **Biochim. Biophys. Acta**, 1820, p. 553-564, 2012.

CRAIG-SCHMIDT, M.C. World-wide consumption of trans fatty acids. **Atheroscler Suppl**. v.7(2),p.1-4, May. 2006.

CUMMINGS, J. L. Alzheimer's Disease. **New Engl J Med,**, v.351, p.56-67, 2004.

CURI, R.; et al. **Entendendo a gordura** – os ácidos graxos. 1 ed. São Paulo: Manole, 2002.

D'AVILA, L.F.; et al. Toxicological aspects of interesterified fat: Brain damages I rats. **Toxicol. Lett.** v.*276*, p.122-128, 2017. Doi: 10.1016/j.toxlet.2017.05.020.

DEIANA, S. et al. Methylthioninium chloride reverses cognitive deficits induced by scopolamine: comparison with rivastigmine. **Psychopharmacology**, v.202, p.53-65, 2009.

DHAKA, V.; GULIA, N; AHLAWAT, K. S.; KHATKAR, B. S. "Trans fats—sources, health risks and alternative approach - A review". **J Food Sci Technol**, v.48(5), p.534–541, 2011.

DOWNS, S.M, THOW, A.M, LEEDER, S.R. The effectiveness of policies for reducing dietary *trans* fat: a systematic review of the evidence. **Bull World Health Organ**., v.91, p.262–269, 2013. Doi: 10.2471/BLT.12.111468.

DUDAI, Y. The neurobiology of consolidation, or, how stable is the engram? **Ann Rev Psychol**, v.55, p.51-86, 2004.

DUNCAN, B. B; SCHMIDT, M. I.; GIUGLIANI, E. R. **Medicina ambulatorial**: condutas de atenção primária baseadas em evidências. 3 ed. Porto Alegre: Artmed: 2004.

ENIG, M.G. **Know Your Fats:** The Complete Primer for Understanding the Nutrition of Fats, Oils, and Cholesterol. Bethesda: Pr Later Printing Used edition, 2000.

FARFAN, M. et al. The effect of interesterification on the bioavailability of fatty acids in structured lipids. **Food Chem**., v. 139, p. 571–577, 2013.

FORLEZA, O.V. Pharmacological Treatment of Alzheimer's Disease. **Rev. Psiq. Clín**., v.32 (3); p.137-148, 2005.

GRIMALDI, R; GONÇALVES, L.A.G; ANDO, M.Y. Otimização da reação de interesterificação química do óleo de palma. Quím. Nova**, São Paulo, v.28, n.4, Jul/Ago. 2005.**

GURR, M.I.; HARWOOD, J.L. **Lipid. Biochemistry** – an introduction. 4 ed., Great Britain: Chaoman& Hall, 1991.

GUSTONE, F. D. Movements towards tailor-made fats. **Progr. Lipd. Res**., v.37, n.5, p.277-305, 1998.

GUYONNET, G.; et al. Task force on nutrition and cognitive decline with aging. **J Nutr Health Aging**. v.11(2), p.132-52, Mar. 2007.

HAAG, M., et al. Essential fatty acids and the brain. **Can J. Psychiatr.,** v. 48, p.195-203, 2003.

HAIDER,S.; TABASSUM, S.; PERVEEN, S. Scopolamine-induced greater alterations in neurochemical profile and increased oxidative stress demonstrated a better model of dementia: A comparative study **Brain Research Bulletin,** v.127, p.234-247, 2016. http://dx.doi.org/10.1016/j.brainresbull.2016.10.002

HARDY, J.; SELKOE, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. **Science,** v. 297, p.353-356, 2002.

HISSAGNA, V.M. et al. Trans fatty acids in Brazilian food products: a review of aspects related to health and nutrition labeling. **Rev. Nutr.** v.25, n.4, 2012.

HLAVACOVA, N. et al. Eplerenone, a selective mineralocorticoid receptor blocker, exerts anxiolytic effects accompanied by c hanges in stress hormone release. **J. Psychopharmacol**., v. 24, p. 779-86, 2010.

HOPPERTON, K.E.; TRÉPANIER, MO.; GIULIANO, V.; BAZINET, R.P. Brain omega-3 polyunsaturated fatty acids modulate microglia cell number and morphology in response to

intracerebroventricular amyloid-β 1-40 in mice. **J. Neuroinflammation**, v.13, p.257, 2016. Doi: 10.1186/s12974-016-0721-5.

HULBERT, A. J. et al. Dietary fats and membrane function: implication and metabolism disease. **Biol. Rev.,** v.80, p.155-169, Feb. 2005.

HULSHOF, K.F.A.M.; e cols. Intake of fatty acids in Western Europe wiyh emphasis on trans fatty acids: the TRANFAIR study. **Eur. J. Clin. Nutr**., v.53, p.143-157, 1999.

HWANG, D.H.; KIM, J.A..; LEE, J.Y. Mechanisms for the activation of Toll-like receptor 2/4 by saturated fatty acids and inhibition by docosahexaenoic acid**. Eur. J. Pharmacol.** v.*785*, p.24-35, 2016. Doi: 10.1016/j.ejphar.2016.04.024

INNIS, S. M. Fatty acids and early human development. **Early Hum. Dev.**, v. 83, p. 761-766, Dec. 2007.

IZQUIERDO, I. **Memória**. Porto Alegre: Artmed, 2002.

IZQUIERDO, I. et al. Different molecular cascades in different sites of the brain control memory consolidation. **Trends Neurosci**, v.29, p.496-505, 2006.

IZQUIERDO, I. et al. Mechanisms for memory types differ. **Nature,** v.393, p.635-636, 1998.

IZQUIERDO, I.; MCGAUGH, J. L. Behavioural pharmacology and its contribution to the molecular basis of memory consolidation. **Behav Pharmacol**, v.11, p.517-534, 2000.

JANCA, A. et al. WHO/WFN Survey of neurological services: a worldwide. **Neuro Sci.** p. 29-34, 2006.

KANDEL, E.R.; SCHWARTZ, J.H.; JESSEL, T.M. **Princípios da Neurociência**. São Paulo: Manole, 2003.

KARABULUT, I; TURAN, S; ERGIN, G. Effects of chemical interesterification on solid fat content and slip melting point of fat/oil blends. **Eur Food Res Technol.,** v.218, p.224-229, 2006. Doi:10.1007/s00217-003-0847-4.

KHANDELWAL, P. J.; HERMAN, A. M.; MOUSSA, C. E. Inflammation in the early stages of neurodegenerative pathology. **J Neuroimmunol**, v.238, p.1-11, 2011.

KLINKENBERG I, BLOKLAND A. The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. **Neurosci Biobehav Rev**., v.34, p.1307-50, 2010.

KLINKESORN, U.; H-KITTIKUN, A.; CHINACHOTI, P.; SOPHANODORA, P. Chemical transesterification of tuna oil to enriched omega-3 polyunsaturated fatty acids. **Food Chem,** v.87, p.415-421, 2004. Doi: 10.1016/j.foodchem.2003.12.021.

KLONOFF, D. C. "Replacements for Trans Fats—Will There Be an Oil Shortage?".**Journal of Diabetes Science and Technology**, v.1(3), p.415-422, 2007.

KODALI, G.R.; LIST, D.R. **Trans fat alternatives**. Champaign: AOCS press 2006.

KUHN, F.T., et al. Influence of trans fat and omega-3 on the preference of psychostimulant drugs in the first generation of young rats. **Pharmacol Biochem Behav**., v.110 p.58-65, 2013. Doi: 101016/jpbb201306001.

KUHN, F.T., et al. Cross-generational trans fat consuption favors self-administration of amphetamine and changes molecular expression of BDNF, DAT, and D1/D2 receptors in hippocampus of rats. **Neurotox Res.,** v.28, p.319-331, 2015. Doi: 101007/s12640-015-9549-5.

LEHNINGER, A. L; NELSON, D. L.; COX, M. M. **Lehninger: princípios de bioquímica**. 6 ed. São Paulo: Artmed, 2014.

LEIWU & DALI SUN. Adherence to Mediterranean diet and risk of developing cognitive disorders: An updated systematic review and meta-analysis of prospective cohort studies. **Scientific Reports**, v.7, p.41317., DOI: 10.1038/srep41317.

LEVANT, B.; RADEL, J. D.; CARLSON, S. E. Decreased brain docosahexaenoic acid during development alters dopamine-related behaviors in adult rats are differentially affected by dietary remediation. **Behavioural Brain Research**. v. 152, p. 49-57, 2004.

LI, J.; GAO,L.; SUN, K.; XIAO, D.; LI, W.; XIANG, L.;QI, J. Benzoate fraction from Gentiana rigescens Franch alleviates scopolamine-induced impaired memory in mice model in vivo. **Journal of Ethnopharmacology,** v.193, p.107–116, 2016. Doi: 10.1016/j.jep.2016.08.001.

LIPNICK, D.M., et al. Risk Factors for Mild Cognitive Impairment, Dementia and Mortality: The Sydney Memory and Ageing Study. JAMDA 2016.

MACHADO, R.M., et al. Omega-6 polyunsaturated fatty acids prevent atherosclerosis development in LDLr-KO mice, in spite of displaying a pro-inflammatory profile similar to *trans* fatty acids. **Atherosclerosis,** v.224, p.66-74, 2012. Doi: 10.1016/j.atherosclerosis.2012.06.059.

MAGRI, T.P.R., et al. Interesterified fat or palm oil as substitutes for partially hydrogenated fat in maternal diet can predispose obesity in adult male offspring. **Clin. Nutr.,** v.14, p.242–248, 2015. http://dx.doi.org/10.1016/j.clnu.2014.09.014.

MANCINI, J.; CHEMIM, S. **Implicações nutricionais dos ácidos graxos *trans*.** In: Seminários "Gorduras Modificadas com Baixos Teores de Ácidos Graxos *trans*: aspectos nutricionais e tecnológicos". São Paulo: Sociedade Brasileira de Óleos e Gorduras, 1996.

MARSZALEK, J. R.; LODISH, H. F. Docosahexaenoic acid, fatty acid-interacting proteins, and neuronal function: breastmilk and fish are good for you. **Annu. Rev. Cell Dev. Biol.**, v. 633, p. 21-57, Jul. 2005.

MARTIN, C. A. et.al. Ácidos graxos poliinsaturados ômega-3 e ômega-6: importância e ocorrência em alimentos. **Rev. Nutr.,** v.19, p.6, 2006.

MILANESI, L.H.; ET al. Chronic consumption of interesterified fat modifies brain Opioid system and affects morphine-induced reward Effects in rats. **Food Chem. Toxicol.,** v.110, p.25-32, 2017. Doi: 10.1016/j.fct.2017.09.048

MILLS, C.E; HALL, W.L; AND BERRY, S.E.E. What are interesterified fats and should we be worried about them in our diet? **British Nutrition Foundation Nutrition Bulletin**, v.42, p.153-158, 2017.

MITCHELL, S.A.; et al. The Apaf-1 internal ribosome entry segment attains the correct structural conformation for function via interactions with PTB and unr. **Mol Cell**, v. 11(3), p.757-771, 2003.

MONTEIRO, C.A. Increasing consumption of ultra-processed foods and likely impact on human health: evidence from Brazil. **Health Nutr.**, v.14(1), p.5-13, mês, 2011.

MORRIS, M. C, et al.. "Dietary Fats and the Risk of Incidence of Alzhemer Disease". **Arch Neurol.,** v.60, p.194-200, 2006.

MORRIS, R. Developments of a water-maze procedure for studying spatial learning in the rat. **J. Neurosci.** Methods, p.47-60, 1984.

MOZAFFARIAN, D. et al. Trans fatty acids and cardiovascular disease. J. Med., New Engl., v. 354 (15), p. 1601-1613, 2006.

MOZZAFARIAN, D.; STAMPFER M.J. Removing industrial *trans* fat from foods. **BMJ,** v. 340, 2010. Doi: 10.1136/bmj.c1826.

MÜLLER CP1, SCHUMANN G. Drugs as instruments: a new framework for non-addictive psychoactive drug use. **Behav Brain Sci.,** v.34(6), p.293-310, Dez. 2011. Doi: 10.1017/S0140525X11000057.

MÜLLER-OERLINGHAUSEN, B.; BERGHÖFER, A.; BAUER, M. Bipolar Disorder. **Lancet**, v.359, p.241–247, 2002.

NATALIE, J. B.; FLORISN. H. G. M.; BRENDON, J.M. Functionality at the end of a fatty acid chain chemical and biological routes to ω-hydroxylated fatty acids. **Lipid Technology**, v. 21(10), p. 216-219, 2009.

NILLERT, N., et al. Neuroprotective Effects of Aged Garlic Extract on Cognitive Dysfunction and Neuroinflammation Induced by Amyloid in Rats. **Nutrients**, p. 9-24, 2017.

NORTON, S. et al. Potencial para a prevenção primária da doença de Alzheimer: uma análise dos dados populacionais. **The Lancet Neurology,** v.13, n.8, p.788-794, 2014.

PASE, C.S., et al. Influence of perinatal trans fat on behavioral responses and brain oxidative status of adolescent rats acutely exposed to stress. **Neuroscience**, v.247, p.242-252, mês, 2013.

PASE, C.S., et al. Maternal trans fat intake during pregnancy or lactation impairs memory and alters BDNF and TrkB levels in the hippocampus of adult offspring exposed to chronic mild stress. **Physiol. Behav.**, v.169, p.114-123, 2017. Doi: 10.1016/j.physbeh.2016.11.009.

PASE, C.S., et al. Prolonged consumption of trans fat favors the development of orofacial dyskinesia and anxiety-like symptoms in older rats. **Int J Food Sci Nutr**, v.65(6), p.713–719, 2014.

PRATICO, D. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. **Trends. Pharmacol. Sci**, v.29, p.609-615, 2008.

QUERFURTH, H. W.; LAFERLA, F. M. Alzheimer's disease. **N. Engl. J. Med.,** v.363, p. 329-344, 2010.

QUILLFELDT, J. A. Behavioral Methods to Study Learning and Memory in Rats. In: ANDERSEN, M. L.; TUFIK, S. (org). **Animal Models as Tools in Ethical Biomedical Research**. 1 ed. São Paulo: Associação de Incentivo à Psicofarmcologia, 2010, p.227-269.

RACCHI, M. et al. Acetylcholinesterase inhibitors: novel activities of old molecules. **Pharmacol Res**, v.50, p.441-451, 2004.

RATNAYAKE e GALLI, et al. Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: a background review paper. **Ann Nutr Metab**., v.55(1-3), p.8-43, 2009. Doi: 10.1159/000228994.

REMIG, V. et al. *Trans* fats in America: A review of their use, consumption, health implications, and regulation. **J. Am. Diet. Assoc.**, v. 110, p. 585-592, Apr. 2010.

RIBEIRO, A.P.B. et al. Chemical interresterification: alternative to production of zero *trans* fats. Quím. Nova, São Paulo, v. 30, n. 5. 2007.

ROACH, C.; e cols. Comparison of *cis* and *trans* fatty acid containing phosphatidylcholines on membrane properties. **Biochemistry**, v.43, p. 6344-6351, 2004.

ROBINSON, M. J.; COBB, M. H. Mitogen-activated protein kinase pathways Curr. Opin. Cell Biol., v.9, p.180-186, 1997.

RODRIGUES JN, GIOIELLI LA. Chemical interesterification of milkfat and milkfat-corn oil blends. **Food Int Res.,** v.36, p.149-159, 2003. Doi: 10.1016/S0963-9969(02)00130-8.

ROVERSI, K. et al. Trans fat intake across gestation and lactation increases morphine preference in females but not in male rats: Behavioral and biochemical parameters. **Eur J Pharmacol**., v.788, p.210-217, 2016. Doi: 101016/jejphar201606031.

SAFAR, M.M, et al. Bone Marrow-Derived Endothelial Progenitor Cells Protect Against Scopolamine-Induced Alzheimer-Like Pathological Aberrations. **Mol Neurobiol**, v.53, p.1403–1418, 2016. DOI 10.1007/s12035-014-9051-8.

SANGIOVANNI, S. N; CHEW, E. Y. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. **Prog. Retin. Eye Res.**, v. 24, p. 87-138, Jan. 2005.

SCHLIEBS, R.; ARENDT, T. The significance of the cholinergic system in the brain during aging and in Alzheimer's disease. **J Neural Transm**, v.113, p.1625-1644, 2006.

SELKOE, D. J. Alzheimer’s disease is a synaptic failure. **Science**, v.298, p.789-779, 2002.

_____. Cell biology of protein misfolding: the examples of Alzheimer's and Parkinson's diseases. **Nat. Cell Biol.,** v.11, p. 61-1054, 2004.

SERENIKI, A.; VITAL, Maria A. B. F., Alzheimer's disease: pathophysiological and pharmacological features. Rev. Psiquiatr. Rio Gd. Sul**, Porto Alegre, v. 30 n.1. 2008.**
SHAO, A., et al. Optimal nutrition and the ever-changing dietary landscape: a conference report. **Eur J Nutr.** v. 56 p.1-21, 2017.

SHEPPARD, K.W AND CHEATHAM, C.L. Omega-6 to omega-3 fatty acid ratio higher-order cognitive functions in 7- to 9-y-olds: a cross-sectional study. **Am J Clin Nutr.** v. 98, n.3, p. 659-67. 2013, doi: 10.3945/ajcn.113.058719.

SIMONIAN, N.A.; COYLE, J.T. Oxidative stress in neurodegenerative diseases. **Ann Rev Pharmacol Toxicol**, v. 36, n.1, p.83-106, 1996.

SIMOPOULOS, A.P. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic disease. **Biomedicine&Pharmacotherapy**, v. 60, p. 502-507, 2006.

SOLFRIZZI, V, et al. Dietary fatty acids intake: possible role in cognitive decline and dementia. **Exp Gerontol.,** v.40, n.4, p.257-70, Apr. 2005.

SQUIRES, R.F.et al. Some properties of brain specific benzodiazepine receptors: new evidence for multiple receptors. **PharmacolBiochemBehav.**, v.10, p.825-830, 1979.

STENDER, S.; ASTRUP, A.; DYERBERG, J. Ruminant and industrially produced trans fatty acids: health aspects. **Food Nutr**., v.52, p.1-8, 2008. Doi: 103402/fnrv52i01651

SUNDRAM, K.;, KARUPAIAH; T. HAYES, K.C. Stearic acid-rich interesterified fat and trans-rich fat raise the LDL/HDL ratio and plasma glucose relative to palm olein in humans. **Nutr Metab.,** v.4, p.3, 2007.

TEIXEIRA, A.M. et al. Could dietary *trans* fatty acids induce movement disorders? Effects of exercise and its influence on Na+K+-ATPase and catalase activity in rat striatum. **Behav Brain Res.,** v.226, p 504-510, 2012.

TORRES, A. et al. Gender differences in cognitive functions and influence of sex hormones. **Actas Esp. Psiquiatr.**, v.34, n.6, p.408-415, Nov 2006.

TREVIZOl, F. et al. Cross-generational trans fat intake modifies BDNF mRNA in the hippocampus: impact on memory loss in a mania animal model. **Hippocampus.** v.25, p.556-565, 2015.

TREVIZOL, F. et al. Influence of lifelong dietary fats on brain fatty acids and amphetamine-induced behavioral responses in adult rat. **Prog Neuropsychopharmacol Biol Psychiatr**, v.45, p. 15-222, 2013.

TREVIZOL, F. et al. Cross-generational trans fat intake facilites mania-like behavior: Oxidative and molecular markers in brain cortex. **Neurosc**., v.286, p.353-363, 2015.

WALKER, Q. D.; RAY, R.; KUHN, C. M. Sex differences in neurochemical effects of dopaminergic drugs in rat striatum. **Neuropsychopharmacology,** v. 31, p. 1193–1202, Jun. 2006.

WENTZELL J. S. et al Amyloid precursor proteins are protective in *Drosophila* models of progressive neurodegeneration. **Neurobiol. Dis**. v.46, p.78–87, 2012.

WINKLER, J. et al. Essential role of neocortical acetylcholine in spatial memory**. Nature.** v. 375, p.484-487, 1995.

WISNIEWSKI, T.; GOÑI, F. Immunotherapy for Alzheimer's disease. **Biochem. Pharmacol.**, v.88, n.4, p.499-507, 2014.

WORLD ALZHEIMER REPORT, In: ALZEIMER'S Disease International. London. 2016. Disponível em: <https://www.alz.co.uk/research/world-report-2016> Acesso em: 20 fev. 2017.

WORLD HEALTH ORGANIZATION, 2012 ALZHEIMER'S ASSOCIATION, 2012. Alzheimer's diseases facts and figures. **Alzheimer's and Dementia**, v.8, n.2, p.131-168, 2012.

YAMAZAKI, T. et al. Effects of an aqueous extract of Puerariae flos (Thomsonide) on impairment of passive avoidance behavior in mice. **J Ethnopharmacol.,** v.100, p. 244-248, 2005.

YEHUDA, S. et al. Essential fatty acids and the brain: From infancy to aging. **Neurobiology of Aging,** v.26, p.98-102, 2005.

YEHUDA, S. et al. The role of polyunsaturated fatty acids in restoring the aging neuronal membrane. **Neurobiol. Aging**., p. 23-53, 2002.

ZHANG, M.; CAI, J. X. Neonatal tactile stimulation enhances spatial working memory, prefrontal long-term potentiation, and D1 receptor activation in adult rats. Neurobiol. Learn. Mem**., v. 89, p. 397-406, 2008.**